



World Health Organization  
Regional Office for the Eastern Mediterranean

# Operational Research in Tropical and Other Communicable Diseases

Final Report Summaries 2001–2002

*Implemented during 2002–2003*

**RESULTS PORTFOLIO 2**  
Small Grants Scheme

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# FOREWORD

The WHO Regional Office for the Eastern Mediterranean supports operational research projects in the area of communicable diseases. A main feature of this research activity is the active collaboration between the control programmes of the ministries of health and researchers from academic institutions. Together they target critical national health problems by turning them into research priorities and devising solutions.

This document summarizes the outcomes of two rounds of the Small Grants Scheme for operational research in tropical and other communicable diseases during 2001 and 2002. It is the second issue in a series that is planned to be produced every biennium to disseminate research results to national control programmes and the international scientific community.

It is hoped that these research results will be translated into policy and practice, thereby achieving progress towards equity in health and avoiding the unnecessary waste of limited resources that would result from the duplication of research funding or the ineffective implementation of control measures.

Dissemination of the results also aims at stimulating interest in operational research among control programmes and strengthening their collaboration with researchers from academia in conducting research. Such contributions would ensure the rational use of limited resources in solving major public health problems.

However, this publication is more than a documentation of research results. It is also a call for action: a plea that these recently produced results are utilized and implemented in order to face the challenges of communicable disease control.



Hussein A. Gezairy MD, FRCS  
Regional Director for the Eastern Mediterranean

# PREFACE

The WHO Regional Office for the Eastern Mediterranean together with the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) are jointly supporting the Small Grants Scheme (SGS) for operational research in tropical and other communicable diseases. The scheme, which had initially focused on tropical diseases since its inception in 1992, was expanded to include other communicable diseases in 2002. It aims to identify needs and problems, and to devise solutions related to the prevention and control of communicable diseases. It also seeks to strengthen health research capacity in the Eastern Mediterranean Region by providing the necessary tools to enable countries to identify their national health problems and devise solutions for communicable disease control.

The research portfolio presented here represents a departure from the first issue of the final report summaries series. Rather than being restricted to tropical diseases, such as leishmaniasis, filariasis, malaria, onchocerciasis, schistosomiasis and tuberculosis, other priority communicable diseases are now embraced, including HIV and sexually transmitted diseases, vaccine-preventable diseases, haemorrhagic fevers, brucellosis, meningitis and echinococcosis. Further, a list of articles originating from the projects supported by the scheme and published in indexed journals is included. This list includes articles accepted for publication in a special issue of the *Eastern Mediterranean Health Journal* on tropical disease research. In connection with this, support in scientific writing was provided for manuscripts originating from the projects supported during 2000–2001.

From proposal development until publication, each research project has witnessed beneficial cooperation between researchers from control programmes and academia, on one hand, and the staff of the Division of Communicable Disease Control in the Regional Office, on the other. This publication provides numerous examples of the fruits of this cooperation and is a testament to the important contribution that researchers are making to communicable disease control in the Eastern Mediterranean Region.



Dr Zuhair Hallaj

Director, Communicable Disease Control

# Brucellosis

## Diagnosis

### Lebanon

Development and validation of a high-throughput assay for rapid diagnosis of human and animal brucellosis using real-time polymerase chain reaction

Lebanon  
Beirut

Study period:  
September 2002–  
August 2004

Small Grants  
Scheme  
(SGS) 2002 No. 160

Principal Investigator  
Dr Mireille Kattar  
Department of Pathology  
and Laboratory Medicine  
American University of Beirut  
Beirut, Lebanon  
e-mail:  
mk33@aub.edu.lb

### Abstract

Culture is considered the gold standard for diagnosis of brucellosis. However, it requires at least 1–2 weeks for completion and carries a risk of laboratory-acquired infection. Serological assays may be difficult to interpret in endemic regions. A study was therefore carried out to develop a real-time polymerase chain reaction (PCR) assay on the LightCycler instrument for the laboratory diagnosis of human brucellosis. The assay targets the 16S-23S internal transcribed spacer region that displays 100% sequence homology among *Brucellae*. The assay was then clinically validated with human specimens derived from active, relapsing and treated brucellosis patients and other conditions where brucellosis was initially suspected. Two other assays targeting conserved regions of 2 outer membrane porin genes, omp25 and omp31, were also developed.

**Results** The assay detected 100% of 24 consecutive clinical isolates of *Brucellae* and showed 100% analytical specificity when tested on 14 reference strains and clinical isolates of common Gram-negative and Gram-positive bacterial pathogens. The analytical sensitivity was 4 genome-copies in a background of 100 ng of genomic human DNA. The clinical sensitivity, specificity, and positive and negative predictive values of the assay were comparable to culture. The 2 relapsing cases were PCR-positive and all treated patients were PCR-negative.

**Conclusion** The study developed and

validated a real-time PCR assay for the rapid diagnosis of human brucellosis.

### Background

Human brucellosis is endemic in many regions including countries from the Eastern Mediterranean Region. The two species commonly implicated in human disease are *Brucella abortus* and *Brucella melitensis*. *Brucella abortus* is epizootic in cattle, whereas *Brucella melitensis*, a more virulent species, mainly infects goats and sheep. Brucellosis is usually transmitted to humans through consumption of contaminated and untreated milk products or by direct contact with infected animals. In Lebanon, brucellosis is a reportable disease and the most common zoonosis [1]. In 1997, the incidence of the disease in Lebanon was 8.1/100.000 according to surveillance data of the Ministry of Public Health [2]. An epidemiologic study in 1997 and 1998, reported a seroprevalence of 15% among 1732 tested blood donors in Northern Lebanon [3]. A study of 597 individuals in high-risk occupations e.g. butchers, farmers, laboratory technicians, slaughterhouse workers and veterinarians, in 10 regions of Lebanon, showed a seroprevalence of up to 50% in some groups, with a regional variation ranging from 3.4% to 34% [4].

The laboratory diagnosis of brucellosis relies on serological and culture methods. Serological tests suffer from lack of standardization of antigen preparations and assay methodologies, resulting in lack of reproducibility and variable diagnostic performance, especially in endemic areas. Moreover, they may not be useful in chronic and relapsing cases because titres may remain high in the absence of clinical conditions. Culture is considered the gold standard in the laboratory diagnosis of brucellosis. The sensitivity of blood culture ranges from 40%–90%, whereas in focal brucellosis, culture may miss up to 70% of cases.

## Conclusions and implications of the study

■ This study developed and validated a real-time polymerase chain reaction (PCR) assay for the rapid diagnosis of human brucellosis that showed an excellent analytical performance. Though comparable in sensitivity to previously reported conventional assays, this assay is fast, can be completed in less than 2 hours and minimizes the risk of contamination.

■ The assay can be applied retrospectively to archival tissues in cases where tissue was submitted for pathologic examination but not for culture.

■ The assay can distinguish *Brucella abortus* from *B. melitensis*, the 2 most common human pathogens among *Brucellae* and showed that all isolates were *B. melitensis*.

Recent studies have shown that molecular assays could provide a rapid, sensitive and specific alternative for the diagnosis of brucellosis. However, they are usually developed in-house and require postamplification handling of the polymerase chain reaction (PCR) product, with a risk of contamination. Moreover, they have been used mainly in the diagnosis of acute brucellosis, and their diagnostic performance in focal, chronic and relapsing cases has not been properly assessed. In addition, there are no reliable studies on the identification of *Brucella* species in the Middle East. Therefore, a study was carried out to develop a rapid and safe high-throughput real time PCR assay on the LightCycler instrument for the diagnosis of human brucellosis and species identification of *Brucella* species.

### Materials and methods

A real time PCR assay using hybridization probe technology was developed on the LightCycler instrument for diagnosis of human brucellosis. The assay targets 3 conserved regions: 16S-23S internal transcribed spacer region that displays 100% sequence homology among *Brucellae*; 25 kDa outer membrane protein gene, omp25, in both *B. melitensis* and *B. abortus*; and 31 kDa outer membrane protein gene, omp31, that is detected in *B. abortus*. It was initially validated using 24 consecutive clinical isolates of the *Brucella* species and 14 strains of common Gram-positive and Gram-negative bacterial pathogens. The assay was then applied to 195 whole blood patient samples accrued over 7 months: 14 with active brucellosis, 5 with treated brucellosis and 176 with other defined etiologies where brucellosis was initially suspected.

### Main study findings

The assays detected 100% of 24 consecutive local clinical isolates of *Brucellae* and showed 100% analytical specificity when tested on 14 reference strains of common Gram-positive and Gram-negative bacterial pathogens. All 3 assays displayed a reproducible linear quantitative range over 6–7 log<sub>10</sub> copy number with an analytical sensitivity of 4 genome copies for internal transcribed spacer segment (ITS) and 30 genome copies for both omp25 and omp31, in the background of 100 ng of genomic human DNA.

The clinical sensitivity, specificity, positive and negative predictive values were 86%, 100%, 100% and 98.9%, respectively. All assays were positive when tested on DNA extracted from 3 blocks of formalin fixed paraffin-embedded tissue derived from 2 adult female patients with culture-proven brucellosis. One patient had a chronic tuboovarian abscess that mimicked malignancy. The other patient had acute

chorioamnionitis with premature rupture of membranes at the 23rd week of gestation.

### Conclusions and recommendations

The study developed and validated a highly sensitive and specific real time PCR assay for the rapid diagnosis of human brucellosis. The assays can be applied retrospectively to archival tissues in cases where tissue is only submitted for pathologic examination not for culture. The 3 assays combined can distinguish *B. abortus* from *B. melitensis*, the 2 most common human pathogens among *Brucellae*. The study also showed that infections in this study population are caused by *B. melitensis*.

### References

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# Brucellosis

## Epidemiology

### Egypt

Integrated approach  
for controlling  
brucellosis in an  
endemic rural area  
in Gharbeya  
Governorate:  
community-based  
and case-control  
studies

Egypt  
Gharbeya Governorate

Study period:  
November 2002–January  
2004

Small Grants  
Scheme  
(SGS) 2002 No. 82

Principal Investigator  
Dr Azza El Sherbini  
Tanta Fever Hospital  
Ministry of Health and  
Population  
Tanta, Egypt  
e-mail:  
azza\_el\_sherbini@hotmail.com

### Abstract

A cross-sectional community-based survey was conducted in 2 villages in Gharbeya Governorate among 616 villagers and their 350 livestock animals to determine the seroprevalence of *Brucella* antibodies among humans and animals. Another hospital-based case-control study was also carried out in Tanta Fever Hospital on 149 cases and 301 controls to identify risk factors for brucellosis, and determine the most suitable laboratory tests for its diagnosis.

**Results** The seroprevalence of brucellosis among humans was 0.0% and 1.7% compared to 0.0% and 16.0% among animals, in villages I and II, respectively. Significant risk factors for brucellosis among humans were: admission to a fever hospital within the previous year, having sheep as livestock, high risk occupation (farmers and butchers), having aborted animals and younger age. Rose Bengal panel test was comparable to tube agglutination test for diagnosis of brucellosis.

**Conclusion** Despite the low reported seroprevalence rate of *Brucella* antibodies

among humans and its focal distribution in animals, more emphasis should be given to brucellosis control according to WHO recommendations, including animal vaccination.

### Background

Brucellosis control requires multidisciplinary collaboration between veterinary, medical and other personnel in active surveillance at the district level, and integrated disease monitoring at the provincial, national and intercountry levels. A definitive diagnosis of brucellosis is based on culture of brucellosis strains from different samples, mainly blood. Diagnosis relies mainly on serology, particularly the standard tube agglutination test (TAT).

An increasing rate of admission of brucellosis has been noticed among febrile patients in fever hospitals during recent years in Gharbeya Governorate. This governorate is one of the highest for reporting brucellosis in Egypt. Therefore, this study aimed at determining the seroprevalence of, and study the risk factors for, brucellosis, and to assess the diagnostic performance of different laboratory tests. It also examined whether the specificity of the standard TAT in diagnosis of active brucellosis could be enhanced by adding 2-mercapto ethanol (ME) and to compare the results with the rapid and easy Rose Bengal panel test (RBPT).

### Materials and methods

**Community-based study** The study was carried out in 2 villages in Tanta area: Mansheit El-Aokaf and Kafr Sobtas. A total of 98 families were randomly selected from both villages, consisting of 616 villagers and 350 livestock animals. A standardized questionnaire was completed for each individual including information on demographic data, risk factors for brucellosis and demographic data for the livestock animals. A 5 cc

## Conclusions and implications of the study

■ The seroprevalence of brucellosis among humans in a rural endemic area in one of the high burden governorates in Egypt was unexpectedly low in comparison to earlier reports. This could be explained by better brucellosis control among animals or improved hygiene measures.

■ The results of this study and those of earlier reports indicate that animal brucellosis is not evenly distributed but occurs as foci in different areas in Egypt. Predilection of infection for certain species varies across studies; in this study sheep, particularly pregnant breeds, reported the highest rates of infection.

■ WHO recommendations for controlling/eradication of brucellosis among animals in low prevalence areas are to test and slaughter if there are adequate means of survey. Otherwise, mass vaccination is recommended. Accordingly, the implementation of the latter strategy ought to be considered.

■ For serological tests of brucellosis, the following is recommended: introduction of Rose Bengal panel test as a rapid, sensitive and specific test for brucellosis in all fever hospitals; increase the seropositive level of standard tube agglutination test (TAT) to 1/320; and routine testing of C-reactive protein to assess activity of brucellosis. The addition of 2-mercapto ethanol to TAT and testing for blocking antibodies for brucellosis by Coombs test, proved to be unreliable in brucellosis diagnosis and are not recommended.



blood sample was taken from each individual and the livestock animals, and transported immediately to the Tanta Fever Hospital and the Animal Health Research Institute for human and animal laboratory tests, respectively. Sera were separated and stored in deep freeze at  $-70^{\circ}\text{C}$  until tested. Human sera were tested by TAT, while animal sera were tested by RBPT and buffered acidified plate antigen (BAPA) as screening tests, and then TAT and Rivanol tests for confirmation. Public health promotion was conducted in each house during the field work.

**Case-control study** The case-control study was conducted for brucellosis in the Tanta Fever Hospital over a 1 year period (1 January–31 December, 2003) during which 149 brucellosis patients were enrolled. The control cases (301) were upper respiratory infections, common cold, bronchitis, urinary tract infections, typhoid fever and other infections. Data from cases and controls were collected in a standardized data collection form including the following information: potential risk factors for infection, clinical features, complete blood picture, urine and stool analysis, Widal test, C-reactive protein, liver enzymes, alanine aminotransferase and aspartate aminotransferase. Sera were tested by RBPT and then TAT for all cases and controls. Positive cases with TAT were tested again after adding ME. The procedure was the same as for TAT except that the same dilutions were prepared in 0.85 % NaCl (sodium chloride) containing ME 0.05 mol/litre.

### ■ Main study findings

**Community-based study** The seroprevalence of brucellosis was 0.0% and 1.7% among humans (TAT titre 1/160), and 0.0% and 16% among animals (TAT titre 1/40) in villages I and II, respectively. About one third of families in village II had animals infected with brucellosis. There was a significant difference between the 2 villages regarding the seroprevalence of brucellosis, animal species, animal age distribution, and the number of pregnant and aborted animals. In both villages, no animal vaccination was reported as it is not one of the obligatory vaccinations delivered by the veterinary health authorities, which include Rift Valley fever and foot and mouth disease vaccines.

Sheep had the highest rate of brucellosis infection among the livestock (20%) followed by cows (16%), buffaloes (14%) and goats (12%), although this was not statistically significant. All aborted animals were negative for *Brucella* antibodies in the 4 laboratory tests (a rate of abortion of 1.6% and 4.4% in villages I and II, respectively). However, pregnancy, particularly in sheep, was significantly associated with brucellosis infection. The different diagnostic tests used with the animals (BAPA, RBPT and Rivanol test) had a very good concordance with TAT (kappa value 0.9). They

recorded a sensitivity of 100%, a specificity of 98.8%, a positive predictive value of 84% and a negative predictive value of 100%.

**Case-control study** Univariate analysis revealed the following risk factors for brucellosis: high risk occupation (farmers and butchers), spring season, ingestion of several items of dairy product, previous admission to a fever hospital within a year, history of having an aborted animal and drinking fresh milk. Additionally, buffaloes, sheep and goats were significantly associated with brucellosis. Prolonged fever and arthritis were significant clinical signs for brucellosis, and among the nonspecific tests, the positive Widal test (titre 1/160) and C-reactive protein test were significantly associated with brucellosis. RBPT showed very good concordance with TAT for diagnosis of *B. abortus* and *B. melitensis* (kappa value = 1.0) with 100% sensitivity, specificity, positive predictive value and negative predictive value. All cases were positive for *B. abortus* and *B. melitensis* antibodies with a high titre of 1/640 encountered in 93% and 92% of cases, respectively.

Titre was reduced 1 to 6-fold after adding ME to TAT, denoting the presence of IgM antibodies in all cases. Prozone phenomenon was present in 9.4% of cases at titre 1/160 using TAT; however RBPT was positive in these cases. Coombs test was negative in all cases and controls. Logistic regression revealed the following significant risk factors: previous admission to a fever hospital within a year (odds ratio (OR): 6.5), having sheep (OR: 6.2), high risk occupation (OR: 4.4), history of aborted animal (OR: 3.5), and younger age (OR: 1.04).

### ■ Conclusions and recommendations

The study reported a low seroprevalence of brucellosis among humans and the presence of foci of brucellosis among livestock in an endemic governorate. Sheep had the highest rate of infection among livestock. Risk factors for brucellosis were previous admission to a fever hospital, having sheep, a history of having an aborted animal and high risk occupation.

Raising the awareness of health care providers about the different clinical presentations, particularly arthritis, and control of animal infection, particularly sheep, through an integrated policy is recommended. In this regard, mass vaccination should be considered and is recommended in the 2 high burden Governorates of Gharbeya and Menoufeya. It is also recommended to introduce RBPT as a rapid, sensitive and specific test for brucellosis in all fever hospitals, and increasing the seropositive level of TAT to 1/320.

# Crimean-Congo Haemorrhagic Fever

## Epidemiology

### Islamic Republic of Iran

Epidemiology of Crimean-Congo haemorrhagic fever infection in Sistan and Baluchestan Province, Islamic Republic of Iran

Islamic Republic of Iran  
Sistan and Baluchestan Province

Study period:  
September 2002–May 2004

Small Grants Scheme  
(SGS) 2002 No. 90

Principal Investigator  
Dr Kouros Holakouie Naieni  
School of Public Health and Institute of Health Research  
Teheran University of Medical Sciences  
Teheran, Islamic Republic of Iran  
e-mail:  
holakoik@hotmail.com

#### Abstract

Crimean-Congo Haemorrhagic Fever (CCHF) is one of the most widespread haemorrhagic fevers. Since 1999, the disease has been epidemic in the northern parts of Sistan and Baluchestan Province of the Islamic Republic of Iran. A study was therefore undertaken in this area to determine the seroprevalence and risk factors for CCHF infection.

Using probability proportional to size cluster sampling, 310 subjects were selected from Zahedan and Zabol Districts in the northern part of Sistan and Baluchestan Province. The subjects were interviewed, and a blood sample taken from those who consented, at the start of the study (in spring) and 6 months later.

**Results** In the first round, 18 out of 285 consenting subjects were positive using IgM and IgG capture ELISA (8 IgG positive, 7 IgM positive, 4 borderline IgM positive, and 1 positive for both). None of the subjects had a history of clinical signs or symptoms of CCHF. Considering only positive IgG and IgM subjects (14 subjects), seroprevalence was 4.68%, while 12 out of 14 definitely IgG or IgM positive subjects were females. In adjusted analysis of risk factors, the significant risk factors for infection were age above 30 years, female

sex, being a housewife, level of education below 5 years and a history of slaughtering. History of travel to rural areas was inversely related to the risk of infection. Of the 208 initially seronegative individuals who consented to be followed up at 6 months, 18 had seroconverted while none had developed full blown disease. Therefore, in this study, the incidence of infection in the region is 8.7%, but disease incidence is very low (0.0%). None of the subjects who had history of febrile illness in the interval between the two rounds of the study, were positive by IgG capture ELISA test.

**Conclusion** Most CCHF infections are subclinical in this region. The epidemiologic characteristics of the infection in the region are different from other parts of the world, which may be due to living conditions and the social practice of having small animal husbandries in the home.

#### Background

In April 1999 and 2000, 90 suspicious cases were reported in the Islamic Republic of Iran. Of these, 17 out of the 27 serologically confirmed were reported from Sistan and Baluchestan Province [1]. In another report from 2000–2002, 81 of 222 suspected cases from various Iranian provinces were confirmed positive. Of these, 49.4% of positive cases were from Sistan and Baluchestan Province. In order to have a true picture of the disease burden, the role of subclinical cases and the extent of under-reporting should be adequately studied.

*Hyalomma* ticks, the disease vector, particularly *H. Marginatum*, are widespread in the Islamic Republic of Iran, with the highest rates of infestation in sheep and goats. However, the number of confirmed CCHF patients with a positive history of tick bite is surprisingly low. Most cases have no history of recent tick bite. Another curious feature in the epidemiology of the disease is the presence of many urban cases, especially among urban housewives. These findings indicate that the epidemiology of CCHF needs to be further elucidated.

### Conclusions and implications of the study

■ The seroprevalence of CCHF has not changed in the study area during the last few years. Moreover, although 8.7% of seronegative individuals subjected to follow-up turned seropositive after 6 months, none developed the full blown disease. These findings indicate that most infections are subclinical and disease incidence is very low in the region.

■ 12 out of 14 cases (85.7%) were women (female/male = 6/1). This result was contrary to the expectation of occupational infection, which would be more prevalent in men.

■ The chance of infection increased with age, as the likelihood of contact with risk factors increases.

■ Educational level has an inverse relationship with the likelihood of infection. The likelihood of infection is higher for those with an education level of 5 years or less. This is attributed to the relationship between improvement in healthy behaviour and education level.

■ Slaughtering is a significant risk factor for CCHF infection. This finding is in accordance with the results of studies in other parts of the world.

The main objectives of this study were to determine the seroprevalence of CCHF in the study area, to study the different modes of transmission and the determinants of CCHF infection, and to estimate the incidence of Crimean-Congo haemorrhagic fever in Sistan and Baluchestan Province.

## Materials and methods

The study design involved a survey with follow-up. It was a hybrid design combining elements of two basic designs, a cross-sectional and a prospective cohort design, back to back.

The cross-sectional phase was designed to determine the seroprevalence of CCHF infection in Zahedan and Zabol Districts in the northern part of Sistan and Baluchestan Province. To determine the seroprevalence of infection and the prevalence of disease (seroprevalence and clinical condition), IgM and IgG capture ELISA were used. IgM is detectable mostly within the first 4 months of the disease and is usually used for detection and/or confirmation of ongoing or recent infection, while IgG remains detectable for a long time after infection.

A questionnaire was completed during an interview for every individual and a blood sample was taken. Both cases and controls were asked retrospectively about the determinants of infection during the 4 months prior to the study. Blood samples were transferred to the local laboratory where sera were separated and frozen. The frozen sera were transferred to the Pasteur Institute of Iran (by air under cold-chain regulations) for IgG and IgM capture ELISA tests.

Subjects that had a positive IgM or IgG capture ELISA test results, with or without clinical history, were selected as cases. Seronegative subjects (subjects with a negative IgM and IgG ELISA test result) without a clinical history of haemorrhagic fever were considered as a control.

Study subjects, were randomly selected using a modification of the classical probability proportional to size cluster sampling. Seronegative controls identified during the cross-sectional phase were subjected to follow-up for a 6-month period for development of clinical signs and symptoms of the disease that were further tested using serological tests.

## Main study findings

Of the 285 blood samples, 14 were positive in ELISA tests for detection of anti-CCHF IgG and IgM: 8 samples were IgM positive and 7 were IgG positive, with 1 of the samples positive for both IgG and IgM. In addition, 4 subjects were borderline positive for IgM. Therefore, the seroprevalence of CCHF infection in the study population was 4.68% (95% CI: 1.92% to 7.45%).

The significant risk factors for infection were female sex (OR = 4.91, 95% CI: 1.08 to 22.37), travel history (OR = 7.56, 95% CI: 0.97 to 59.30), age above 30 years (OR = 3.08, 95% CI: 1.04 to 9.09), education level ( $P = 0.007$ ) and history of slaughtering of livestock ( $P = 0.010$ ). History of travel to a rural area was inversely related to the chance of infection.

Of 310 interviewed subjects, 4 had a history of tick bite within the past 4 months and none of these were seropositive (one was borderline positive for IgM). Only 2 of the interviewed subjects had a history of contact with a CCHF case and neither of these was seropositive.

Of 285 enrolled in the first round, 249 were successfully followed up in the second round. Of these, 22 (8.8%) had a history of febrile disease in the interval between the first and second visits. Of these 22 patients, 5 (2%) had signs and symptoms compatible with CCHF (nose, gum and/or rectal bleeding, and history of travel and/or contact with livestock). The serum samples of 21 consenting febrile subjects were tested at the Pasteur Institute of Iran, and none was found to be positive.

Of 208 initially seronegative subjects that consented to be retested in the follow-up phase, 18 seroconverted. Therefore the incidence of infection was 8.7% (8.3% in Zahedan and 9.1% in Zabol). However, the disease incidence in this group was 0.0% as none developed the overt clinical condition. This indicates that most CCHF infections are subclinical in this region.

## Conclusions and recommendations

The seroprevalence of CCHF has not changed in the study area over the last few years, and most infections are subclinical. These findings highlight the importance of subclinical infections in the epidemiology of the disease and their role in the transmission of infection. Unexpectedly, housewives were found to be at a significantly higher risk of infection. This finding is of importance in programming future health promotion activities and changes the previously-held view that risk groups are mostly occupational. The other findings that increasing likelihood of infection is associated with older age, involvement in slaughtering and low educational level are consistent with findings in other countries.

## References

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# Cystic Echinococcosis

## Control

Islamic Republic of Iran

Community-based approach to hydatid disease control among nomads in Khuzestan Province, Islamic Republic of Iran

Islamic Republic of Iran  
Khuzestan Province

Study period:  
November 2002–March 2004

Small Grants Scheme  
(SGS) 2002 No. 143

Principal Investigator  
Dr Abdollah Rafiei  
Ahwaz Medical Science University  
Ahwaz, Islamic Republic of Iran  
e-mail:  
Abdollahrafiei@hotmail.com

### Abstract

Cystic echinococcosis is one of the most geographically widespread helminth zoonoses, especially in rural areas. A study was undertaken to determine the extent of spread of human cystic echinococcosis among nomads in Khuzestan and to evaluate the effectiveness of health education in increasing community knowledge regarding the disease. For the study, 3513 blood samples were randomly collected from the community, the serum was tested using the enzyme-linked immunosorbent assay (ELISA) technique for antibody detection against purified antigen B from *Echinococcus granulosus* hydatid cyst. Households were interviewed using a questionnaire to evaluate their knowledge of hydatid disease. Three different types of educational activity were undertaken in the community and the increase in knowledge at 3 and 12 months post-education was compared to baseline levels.

**Results** A very low level of knowledge about hydatid disease was found in the community. However, the health education of treated individuals in Izeh city produced a significant increase in knowledge. The prevalence of human cystic echinococcosis among nomads in Khuzestan was 13.78%, while prevalence in the four surveyed cities was 1.94% in

Behbahan, 12.42% in Shoush, 17.33% in Masjed-Soleyman and 18.23% in Izeh.

There was no significant association between cystic echinococcosis seropositivity and age or sex, except for a significantly higher prevalence among females in the 31–50 age group. Similarly, cystic echinococcosis seropositivity was not associated with dog ownership, dog numbers or home slaughtering of sheep. However, it was significantly associated with occupation, shepherds being at a significantly higher risk of infection.

**Conclusion** This study confirms the high endemicity of human cystic echinococcosis in the nomadic population in Khuzestan. It also reports the effectiveness of health education in increasing the knowledge of populations at risk, and subsequently, their preventive behaviour.

### Background

Cystic echinococcosis is an important but neglected public health problem in the Islamic Republic of Iran, especially in rural and nomadic communities. Human cystic echinococcosis has been reported from different parts of the country. The prevalence of hydatidosis ranges from 2%–20% in the intermediate hosts and 3.3%–63.3% in sheepdogs, and 20% of these sheepdog infections have been reported from Khuzestan Province, in the south-western part of the Islamic Republic of Iran [1].

Half the Iranian population live in rural areas as farmers, ranchers and shepherds. Given that sheep act as intermediate and dogs as definitive hosts of *Echinococcus granulosus*, hydatidosis poses both a human health risk and an economic loss to the country. A study was therefore undertaken to determine the extent of spread of human cystic echinococcosis among nomads in Khuzestan and to test the feasibility of promoting community participation in preventive activities by evaluating the effectiveness of health education in increasing community knowledge about hydatidosis.

### Conclusions and implications of the study

- The entire Iranian nomadic population is at high risk of cystic echinococcosis infection due to lifestyle, occupation (mainly as shepherds) and lack of public health facilities.
- Unexpectedly, the rate of seropositivity among people practicing home slaughtering of sheep was not significantly higher than those who do not practice it. This could be attributed to immunity conferred by home slaughtering.
- Nomads in Khuzestan were unaware of the modes of disease transmission and their many risks of infection: dogs are fed with contaminated viscera, there is often no access to potable water and personal hygiene is very low.
- The health education undertaken in the study, especially face-to-face education in the nomad's language, was very effective. The sustained gain in knowledge 1 year post-education suggests that the information gained has been consolidated in the community and has influenced behaviour. This health education approach could therefore be a successful way to prevent infection.

## Materials and methods

Household members were interviewed regarding the determinants of infection and their knowledge of the disease was evaluated using a structured questionnaire. At 3 and 12 months following the baseline questionnaire, two nomad communities in Izeh were tested on their knowledge about cystic echinococcosis following treatment.

**Blood sample collection** Blood samples were collected from nomad populations in four cities: Izeh, Shoush, Behbahan and Masjed-Soleyman. Venous blood samples (5 ml) were taken from randomly selected family members. All blood samples were transferred to the laboratory on ice, and sera were collected and stored at  $-70\text{ }^{\circ}\text{C}$  until tested.

**Antigen preparation** Sheep hydatid cyst fluid was collected from naturally infected sheep at Ahwaz abattoir and a hydatid cyst fluid extract enriched with the lipoprotein antigen B was prepared. The presence of this antigen was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The antigen was then assayed for protein content by the BioRad protein assay.

**Serological testing** All serum samples were tested for cystic echinococcosis antibodies by microplate enzyme-linked immunosorbent assay (ELISA). A positive control group consisted of serum samples from surgically proven Iranian patients ( $n = 120$ ), while a negative control group consisted of blood donors or randomly selected blood samples from endemic healthy people, who had no cystic lesions ( $n = 300$ ). The positive/negative cut-off point (optical density) value was calculated as the mean + 2 standard deviation of the endemic healthy individuals.

**Educational materials** Three different types of educational activity were done: distribution of coloured leaflets written in Persian with simple illustrations, face-to-face education, and an educational programme for 120 students in Masjed-Soleyman and Izeh. The educational activities were designed to describe cystic echinococcosis and the causative parasite, modes of parasite transmission between animals and people, the most common symptoms, a brief description of diagnostic tests, treatment and preventive measures.

## Main study findings

3513 screened nomads were given the 10-question test to evaluate their knowledge before any educational activity. The average baseline test score was 3.5 indicating a very low level of knowledge, although most recognized hydatid cysts in infected animals and knew which animals transmit the infection. The average knowledge scores achieved in the Izeh nomad community increased significantly at 3 and 12 months post-test. This was coupled with the adoption of preventive measures by the community against infection.

Serum samples taken from 3446 nomads in Khuzestan recorded a seropositivity rate of 13.78%. Of the seropositive people, 3.1% had high ELISA optical density values ( $> 0.3$ ) and the mean optical density of seropositive samples was  $0.272 \pm 0.058$ . The prevalence of cystic echinococcosis seropositivity in the four surveyed cities was 1.94%, 12.42%, 17.33% and 18.23% in Behbahan, Shoush, Masjed-Soleyman and Izeh, respectively. There was no significant association between cystic echinococcosis seropositivity and age or sex, except for significantly higher rates among females aged 31–50 years old. Similarly, literacy was not a significant determinant of infection. Only 10 nomads (0.29%) reported history of surgery for cystic echinococcosis.

A total of 2863 dogs were kept by the community; approximately 0.8 dogs per person. Dogs were used for home and livestock guarding and were usually allowed within tents and houses. Despite this, there was no significant association between cystic echinococcosis seropositivity and dog ownership or number of kept dogs. The study also failed to report any significant association between cystic echinococcosis seropositivity and home slaughtering. The only significant determinant of cystic echinococcosis seropositivity was occupation of household, with a significantly higher risk among shepherds.

## Conclusions and recommendations

This study confirmed the high endemicity of human cystic echinococcosis in the nomad population of Khuzestan. Due to similar living and cultural conditions in other Iranian nomadic communities and rural areas, high cystic echinococcosis prevalence is expected to prevail in these communities as well. Health education was found to be effective in increasing knowledge, which had a positive impact on community behaviour.

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# HIV/AIDS

## Infection Control

Egypt

Evaluation of risk perception and precautions taken by health care workers for HIV infection in haemodialysis units

Egypt

Gharbia, Menoufia, Dakahlia and Kafr El-Sheikh Governorates

Study period:  
September–December 2002

Small Grants Scheme  
(SGS) 2002 No. 184

Principal Investigator  
Dr Ibrahim Kabbash  
Department of Public Health  
Faculty of Medicine  
Tanta, Egypt  
e-mail:  
Kabbash-ia@Maktoob.com

### Abstract

A cross-sectional study was conducted in 32 haemodialysis units to evaluate both the perceptions of the risks of human immunodeficiency virus (HIV) infection by health care workers and the precautions taken to address these risks. Data were collected using two pretested and structured instruments: the first measured the knowledge and attitudes of the health care workers; the second was an observation checklist for the performance of nurses. A total of 317 health care workers were enrolled in the study.

**Results** Previous exposure to needle puncture was reported by 48.9% of health care workers in government haemodialysis units and 47.6% in private units. Compared to private health care workers, government health care workers have significantly higher rates of screening for HIV and hepatitis viruses. However, a significantly higher percentage of private health workers reported previous vaccination for hepatitis B virus and the availability of facilities needed for infection control.

A significantly higher proportion of workers in government units had good knowledge of bloodborne infections, universal blood precautions and safe disposal of contaminated items, and recognized asymptomatic HIV patients as a risk of HIV infection in

haemodialysis units. The mass media was the main source of information for health care workers. In contrast to previous training, experience had a limited influence on knowledge. Despite the good knowledge observed, the performance of health care workers was deficient in universal blood precautions, and this was worse in private compared to government units. Years of experience and previous training had limited effect on this. Most of the deficient aspects of performance were in the afternoon and night shifts. Protocols for post exposure prophylaxis of needle puncture were mostly not available or inaccessible.

**Conclusion** Health care workers and patients at haemodialysis units are at increased risk for bloodborne infections.

### Background

Three major outbreaks of HIV infection in haemodialysis units have occurred in Egypt. The first was in 1990 and resulted in 95 cases of infection in Cairo and Fayoum Governorates. The second was in 1993, resulting in 72 cases of HIV infection being diagnosed in haemodialysis units in Gharbia Governorate. The third occurred again in Cairo, with 51 cases of HIV infection. Practices involving the sharing of syringes among patients were observed at the centres involved in these outbreaks. A study was therefore undertaken to evaluate both the perceptions of health care workers on the risk of HIV infection among haemodialysis patients and the precautions they take to avoid infection.

### Materials and methods

A cross-sectional study was conducted in 32 randomly selected haemodialysis units in the main cities of the Nile delta region, including Tanta, Mahalla El-Koubra, Mansoura, Shebin El-Koum and Kafr El-Sheikh. These cities are located in the Governorates of Gharbia, Menoufia, Dakahlia and Kafr El-Sheikh. Data were collected using 2 structured and pretested instruments: a self-administered

## Conclusions and implications of the study

The results of the study revealed a high level of knowledge regarding bloodborne infections, especially HIV. However, the practices of health care workers during care of haemodialysis patients were not safe enough to prevent occupational infections.

The defects in practice were attributed to insufficient qualifications, especially in private haemodialysis units, insufficient dissemination of infection control guidelines and supervision of their implementation, and a lack of regular in-service training

The main study recommendations are to: train health care workers before employment and provide continuous in-service training regarding bloodborne infections, especially on HIV and universal blood precautions; ensure wide-scale dissemination of guidelines for universal blood precautions, needle stick injuries and infection control in the form of wall charts and handouts; and ensure that medical and paramedical curricula contain updated information on bloodborne infections, especially HIV.

questionnaire evaluating the knowledge and attitudes of nurses and physicians towards infection with HIV and other bloodborne pathogens between patients and from patients to health care workers; and an observation checklist filled in by field workers evaluating the performance of nurses during haemodialysis sessions, that included one section to record the performance of the haemodialysis procedure by the nurse and another section to check the environmental aspects of the haemodialysis unit under investigation. The checklist covered all situations that may carry the risk of infection for both patients and health care workers. Each nurse's performance was recorded for three patients.

### ■ Main study findings

Of the 317 health care workers enrolled in the study, 33 were physicians, 5 were head nurse supervisors and 279 were nurses. The majority of workers in government haemodialysis units were nurses with diplomas in nursing (81.3%), while more than one third of personnel working in the private sector were unqualified. Almost half the health care workers in both sectors had reported needle puncture accidents during the last year.

Government health care workers had significantly higher rates of screening for HIV and hepatitis viruses compared to private health care workers (80% and 59.8%, respectively). They also had significantly higher knowledge levels about bloodborne infections, equipment liable to be a source of infection in the unit and modes of HIV transmission. In addition, they had significantly higher knowledge about the risk of infection from HIV asymptomatic subjects and about universal precautions and safe waste disposal. There was no significant difference between the government and private sectors regarding the knowledge of the possibility of HIV infection in haemodialysis units and on the importance of wearing gloves and changing them when serving more than one patient. Compared to public sector health workers, a significantly higher percentage of private health workers reported previous vaccination for hepatitis B virus and availability of facilities needed for infection control.

The practice of health care workers varied significantly between sectors. A significantly higher percentage of private health care workers had adequate practices in relation to washing the skin at the site of vascular access and applying sterile bandage over the site of a venous or arterial puncture. However, sharing needles was practiced by a significantly higher percentage of health care workers in the private sector (8.1% compared to 3.4% in government sector).

Government health care workers were significantly more adherent to universal blood precautions compared to private health care workers. Among

government workers, 72.2% and 87.3% were found to wear gowns and gloves, respectively compared to 39.2% and 52.7% of private workers, respectively. Hand washing or washing other body surfaces immediately if contaminated by blood was practiced by 62.4% of government workers compared to 44.6% of private sector workers. Avoidance of touching surfaces with ungloved hands and thorough washing of hands after changing of gloves was not done by a considerable percentage of health care workers in both groups (49.3% and 23.4%, respectively among government workers compared to 35.1% and 8.1%, respectively among private sector workers).

Regarding waste disposal, use of double plastic bags and the proper labeling of wastebaskets at place of collection was done significantly more often in government health care units than in private ones. Disposal of needles in puncture proof containers was also deficient in both private and government units (47.3% and 31.2%, respectively). Private health care workers were significantly more adherent to discarding used needles unbroken and sending them for incineration, and in maintaining the sanitation of the dialysis machine.

**Environmental hygiene** There was no significant difference between government and private health care units regarding environmental hygiene. However, some aspects were found to be deficient in both sectors.

**Experience** Health care workers with shorter experience (< = 5 years) had significantly higher knowledge regarding modes of HIV transmission. On the other hand, asymptomatic HIV patients were recognized as a risk of infection significantly more often by nurses with longer than those with shorter experience. Health care worker practices that were associated with longer experience included applying sterile bandages on the vascular access site and wearing gloves. Health care workers with shorter experience more frequently washed the skin at the site of vascular access and were more likely to discard used needles uncapped.

**Training** Previous training was found to have a significant impact on knowledge of HIV, universal blood precautions and methods of safe disposal. It also had a significant effect on washing the skin at the site of vascular access and wearing of gloves.

### ■ Conclusions and recommendations

There is great discrepancy between the knowledge and beliefs of health care workers in haemodialysis units and their practices. This places them and their patients at an increased risk of bloodborne infection. There is an urgent need to ensure the adherence of health care workers to infection control guidelines.

# HIV/AIDS

## Infection Control

### Lebanon

Adherence to universal precautions among laboratory personnel in Lebanon

Lebanon Nationwide

Study period:  
September 2002–  
July 2003

Small Grants Scheme  
(SGS) 2002 No. 182

Principal Investigator  
Dr Joseph Kahhaleh  
Lebanese Health Society  
Mount-Lebanon, Lebanon  
e-mail: jkahlaleh@hotmail.com

### Abstract

A national cross-sectional study was conducted in 2003 throughout Lebanon to evaluate the degree of adherence of clinical laboratories to universal precautions and prevention of occupational risk of transmission of bloodborne or body fluid associated pathogens.

Data were collected using 3 pretested and structured instruments: an observation list of practices, supplies and equipment; a questionnaire for laboratory directors/owners on available supplies, personnel and the implementation of universal precautions; and a self-administered questionnaire evaluating the knowledge, attitudes and practice of laboratory personnel.

Results Compared with the results of a baseline study conducted in 1993, the study reported that the profile of laboratory personnel has become more specialized. Adherence to universal precautions has witnessed significant progress over the past 10 years. Despite this, the knowledge and beliefs of laboratory personnel about universal precautions had limited impact on their practices.

Conclusion The discrepancy between what laboratory personnel know, believe in and practice has decreased significantly during 1993–2003, but could be further improved.

### Background

Health care workers are potentially exposed to blood borne pathogens. As a result, they are at increased risk of acquiring human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus, all of which can be transmitted through percutaneous injury. A study in 1993 found that health care workers, particularly laboratory technicians, do not adhere to universal precautions and practices for the prevention of occupational risk of transmission of bloodborne or body fluid associated pathogens.

Since then, training and curriculum modification has been undertaken for laboratory personnel in the country. In order to assess the degree of adherence to universal precautions, reassess the knowledge, attitudes and practice of laboratory technicians regarding these precautions, evaluate achievements, identify gaps and needs, and plan future interventions, a national study was conducted in 2003.

### Materials and methods

A cross-sectional study was conducted among a countrywide representative sample of laboratory personnel. A two-stage cluster sampling of Lebanese laboratories was carried out, in which 96 laboratories out of a total of 183 were randomly selected.

All laboratory directors and 288 technicians (at least 3 technicians per laboratory) were included in the study. The information was collected by 3 pretested and structured instruments: (1) observation of practices, equipment and supplies (observation list); (2) interview on available supplies and knowledge regarding universal precautions (by the laboratory owners/directors), (3) survey of the knowledge, attitudes and practices of laboratory technicians regarding

### Conclusions and implications of the study

■ Despite the relatively high level of knowledge of laboratory personnel in Lebanon about their occupational hazards, this was not reflected in their practices.

■ An increase in positive practices was observed in 2003 compared to 1993. This includes: wearing protective gloves (by 7.5%); disposal of needles and syringes in special containers; supervision of waste disposal; availability of autoclaves; and reduction in use of pipettes by mouth.

■ Though the management of residual blood in blood vacutainers or other containers has improved, appropriate decontamination of spills by technicians decreased from 91.5% in 1993 to 65.8% in 2003.

■ Pretest counselling services were absent. This issue needs to be addressed because technicians dealt directly with patients in 38% of laboratories.

■ The deficiencies identified indicate the need to organize regular training for laboratory personal on universal precautions.



universal precautions (self-administered questionnaire by technicians).

## ■ Main study findings

The majority (87.3%) of directors were medical doctors with postgraduate training in laboratory medicine. The rest were either pharmacists, or had a Bachelor of Science degree or high school qualifications with training on the job. Of the laboratory technicians, 87.3% had a technical professional degree or higher and only 9.5% had a high school and/or training on the job experience.

**Knowledge** One third of laboratory personnel reported attendance at educational sessions, and 20.3% had, in addition, training on HIV testing. Laboratory personnel had acquired their knowledge mostly from physicians, medical journals and books, lectures or from the media. They knew that, while working, they should take protective measures by wearing laboratory gowns (98.6%) or gloves (98.2%). Almost all of them (97.3%) knew that they should dispose of used needles and syringes in special containers. Their level of knowledge about modes of transmission of HIV, hepatitis B and hepatitis C, was 90.1%, 92.3% and 88.3%, respectively.

Half the laboratory personnel considered heat to be the most effective method for deactivation of HIV, 24.3% did not agree and 23.2% did not know. Almost all (97.3%) knew that blood or other contaminated materials must be disposed in special containers. This was in contradiction with field observations in 58.9% of visited laboratories. Moreover, only 31.5% of laboratories had the list of universal precautions posted, and some personnel did not know about the existence of such a list.

**Beliefs, attitudes and practices** Almost all laboratory personnel (98.2%) do not work with reusable syringes, and the majority (88.3%) reported a change in their practices due to knowledge of HIV or hepatitis. The changes reported were becoming more cautious when performing tests on any body fluid (84.2%), sampling (75.7%), or dealing with patients (48.7%). On the other hand, only 1 (0.4%) reported that he had stopped performing HIV or hepatitis tests. A few (11%) displayed behavioural laxity inside the laboratory, including eating, drinking, smoking or using pipettes by mouth.

There was a significant difference between what was observed in laboratories (75.3%) and what was reported (93.7%) regarding the disposal of contaminated syringes and needles in special boxes. Most of the technicians believed that proper disinfection of all materials (90.1%) and wearing a white coat (96.4%) or gloves (94.1%) are important measures for prevention of disease transmission in the health care setting. Less than half (44.1%) believed that virus testing should be in specialized laboratories,

while 16.6% believed that pregnant technicians should not perform blood testing. Gloves were reported to be available by 95% of technicians and 92.6% of laboratory directors. However, technicians were observed to wear gloves or coats in only 37% and 86.3% of laboratories, respectively.

**Sterilization and disinfection** The majority of laboratories (87.7%) have an adequate liquid disinfectant present on the bench. While 90.4% of laboratories have autoclaves, only 89.7% of laboratory directors reported their use. Most laboratories reported sterilizing materials for repeated usage by autoclaving (78.1%), boiling (12.3%), washing (28.8%) or disinfectant (26%). While 41.2% of laboratories dispose of their contaminated waste separately after autoclaving, they do not know its outcome thereafter. One third of laboratories dispose laboratory materials as noncontaminated waste; one quarter by incineration and 14.7% by dumping.

**Blood spillage** It was observed that spillage of blood on the floor or bench was handled mainly by pouring disinfectant (65.8% of laboratories) or by cleaning with dry napkins. Residual blood in vacutainer tubes was disinfected before disposal with normal waste in only 37% of laboratories, while 30.1% separate it inside the laboratory and dispose it later with normal waste, 17.8% pour the blood in the sink and only 4.1% dispose of it with the regular garbage. Handling of broken blood samples was more conservative; 71.2% of the laboratories wash and disinfect the blood, while the rest clean with dry napkins without disinfection.

**Technician/patient relationship** Technicians deal directly with patients in 38% of laboratories, while in the rest they receive blood samples collected in tubes. In 83.6% of laboratories, the blood is taken in a special room; while in only 9.9% of laboratories technicians give results directly to patients. Regarding confidentiality, 78.3% of technicians would inform only the laboratory physician or the treating physician of results, 37.4% would inform laboratory technicians or others and 24.3% would inform nursing staff. Only 12.2% would keep the results confidential. Less than half of laboratories keep separate registries for HIV testing or hepatitis testing. Almost all of them retest the blood in cases of a positive result for an HIV test.

## ■ Conclusions and recommendations

The discrepancies between what technicians know, believe and practice, significantly decreased during 1993–2003, but could be further improved. Health authorities should supervise the registration and licensing of laboratories and ensure the adherence of personnel to universal precautions and the prevention of occupational risk of transmission of bloodborne or body fluid associated pathogens.

# HIV/Tuberculosis

## Epidemiology

### Islamic Republic of Iran

Human immunodeficiency virus (HIV) and HIV-associated tuberculosis infection, and their risk factors in incarcerated injecting drug users in the Islamic Republic of Iran

Islamic Republic of Iran  
Teheran Province

Study period:  
December 2002–  
April 2004

Small Grants  
Scheme  
(SGS) 2002 No. 37

Principal Investigator  
Dr Behnam Farhoudi  
Research Center of the  
Penitentiary Organization of  
Teheran Province, Teheran,  
Islamic Republic of Iran  
e-mail: b\_farhoudi@yahoo.com

#### Abstract

Injecting drug users (IDUs) are considered to be at high risk of human immunodeficiency virus (HIV) and tuberculosis infection. 369 newly-admitted IDU prisoners and 371 existing IDU inmates were investigated for HIV infection and HIV-associated tuberculosis. Both groups were interviewed using a pretested questionnaire that included questions about the risk factors for these infections.

Results Of 369 newly-admitted IDU prisoners, 22.0% were infected with HIV and 11.4% were coinfecting with tuberculosis and HIV. Duration of imprisonment, age at first drug injecting, duration of drug injecting, lower education level and a history of drug injecting in prison were significant risk factors for HIV infection. Of the 371 existing IDU inmates, 24.0% were HIV positive and 14.6% were coinfecting with tuberculosis and HIV. Frequent sharing of injecting equipment, drug injecting in prison and tattooing outside prison were all risk factors for HIV infection. Duration of drug injecting, history of drug injecting inside prison and a negative history of sex with a sex worker were all significant risk factors for HIV/tuberculosis coinfection.

Conclusion The high prevalence of HIV and HIV-associated tuberculosis infection demands

a well planned programme to prevent both diseases in this high risk group.

#### Background

HIV seroprevalence is generally several times higher in prisons than in the general population. The prisoner population in some prisons in the Islamic Republic of Iran has a high prevalence of injecting drugs, the main route of HIV transmission in the country. People infected with both *Mycobacterium tuberculosis* and HIV have an annual risk of developing active tuberculosis similar to the estimated lifetime risk of those infected by *M. tuberculosis* alone. The risk is even higher among IDUs with a history of imprisonment. In view of this situation, and given the current lack of information on HIV and HIV-associated, this study aimed at determination of the prevalence of these infections and their risk factors in incarcerated injecting drug users.

#### Materials and methods

The study was conducted in a big prison in the Islamic Republic of Iran. It enrolled 369 consenting newly-admitted IDU prisoners and 371 consenting IDU prisoners who had been in the prison for at least 1 week and who had evidence of drug injecting on physical examination and/or from self-reporting.

After counselling about HIV testing, the study participants submitted 2 blood samples for HIV antibody testing using enzyme-linked immunosorbent assay (ELISA), and if ELISA positive, Western blot, and received a PPD (purified protein derivative) skin test using the Mantoux procedure. For HIV seronegative prisoners an induration of 10 mm or greater and for HIV seropositive prisoners an induration of 5 mm or greater were considered positive. Those with suggestive symptoms of tuberculosis were examined to rule out tuberculosis through sputum examination and, if

### Conclusions and implications of the study

■ Almost one quarter of injecting drug users (IDUs) in prison were found to be HIV-infected. The fact that 5.7% of newly-admitted prisoners and 21.3% of existing inmates had started to inject in prison highlights the need to intensify harm reduction activities in prisons.

■ The association between HIV infection and duration of imprisonment may be explained by prison circumstances in which high risk drug use occurs. Enforced abstinence, the lack of sterile injecting materials and the high price of drugs, lead to the neglect of precautions. In this situation, a very high level of risk of infection exists from the occasional sharing of drug injecting equipment.

■ Preventing the spread of HIV in correctional facilities will also protect the general population as most prisoners are imprisoned frequently and usually for relatively short periods. Most of these prisoners have unprotected sexual intercourse once they are released, which puts their sexual partners at a high risk of HIV infection.

■ Implementing risk reduction programmes within prisons must be a priority.

necessary, X-ray. The study subjects were then interviewed according to a pretested questionnaire that included information about the potential risk factors of HIV and tuberculosis infections.

## ■ Main study findings

**Newly-admitted prisoners** Of the 369 IDUs, 81 (22.0%) were HIV positive and 51.9% of these (42 of the 81) were coinfecting with tuberculosis, while only 23.6% (67 of the 284) of the HIV-negative group were infected with tuberculosis. Of those without any imprisonment history (69 inmates), 4 (6%) were HIV positive. All subjects were males, with a mean age of  $30.7 \pm 7.7$  years (range 18–57 years). The mean duration of imprisonment during the last 10 years was  $27.0 \pm 30.1$  months. The mean age of starting drug injecting was  $25.0 \pm 6.7$  years (range 11–55 years). About one fifth had shared injecting equipment in the last event of drug injecting, one third had a history of ever injecting inside prison and 5.7% had started to inject inside prison. The mean duration of drug injecting was  $24.0 \pm 50.4$  months; 71.8%, 83.5% and 87.5% had injected during the previous 1, 6 and 12 months, respectively; and 20.3% and 64.0% injected once and more than once per day, respectively.

In terms of sexual activity, 32.5% lived with their wives and all these had at least 1 sexual contact with their wives during the year previous to incarceration, while 53.9% had at least 1 sexual contact (with their wives or otherwise) during the previous 12 months, but only 17% had used a condom during the last occasion of sexual intercourse.

Coinfection was more frequent among those who were unemployed (12.1%) than employed (8.0%), and among the divorced (19.5%) compared to those of other marital status (about 10%). Risk factors of HIV infection were: younger age, age at first drug injecting, lower education level, tattooing inside prison, frequent sharing of drug injecting equipment, frequent drug injecting, using a handmade instrument for drug injecting, duration of drug injecting, drug injecting inside prison and duration of imprisonment. The logistic regression model that best explained the distribution of HIV infection included age at first drug injection (16–20 and 26–30 years versus those over 36 years), lower education level, duration of drug injecting and duration of imprisonment. Existing inmates 89 of the 371 (24.0%) IDUs were HIV positive and among these, 54 (60.7%) were coinfecting with tuberculosis. The frequency of tuberculosis infection for HIV negative IDUs was 39.3% (110 of 280). All the subjects were males, with a mean age of  $35.9 \pm 9.1$  years (range 18–71 years). The mean duration of imprisonment during the last 10 years was  $59.0 \pm 45.9$  months. The mean age of starting drug injecting was  $28.1 \pm 8.9$  years (range

10–66 years). About 43% had shared their injecting equipment on the last occasion of drug injecting. More than half had a history of ever injecting inside prison and 21.3% had started to inject inside prison. The mean duration of drug injecting was  $31.0 \pm 47.8$  months; 28.6%, 47.7% and 58.2% of the 371 had injected during the previous 1, 6 and 12 months respectively; and 19.1% and 35.0% of the 371 injected once per day and more than once per day, respectively. In terms of sexual activity, 33.2% were married and 81.3% of these had at least 1 sexual contact with their wives during the previous year, 39.1% had at least 1 occasion of sexual intercourse during the last year and 24.5% had used condom at last sexual intercourse.

Coinfection was more frequent among the employed (14.9%) than the unemployed (9.5%), among the divorced (20.8%) compared to those of other marital status and among those aged 35–39 years (20.8%) in comparison to other age groups (range 7.4%–16.7%), although this was not statistically significant. Risk factors for HIV infection among this group were: divorce, duration of imprisonment, duration of drug injecting, frequent drug injecting, sharing drug injecting equipment, drug injecting inside prison, sharing injecting equipment at the last occasion of drug injecting, using handmade instruments for drug injecting and tattooing inside prison. The logistic regression model that best explained the distribution of HIV infection included frequent sharing of injecting equipment, drug injecting inside prison and tattooing outside prison.

**All prisoners** Of the 740 prisoners, 170 (23.2%) were HIV positive. Of these, 96 (56.5%) were coinfecting with tuberculosis. In univariate analysis, HIV/tuberculosis coinfection was significantly associated with age, duration of drug injecting, frequency of drug injecting, frequency of needle sharing, history drug injecting inside prison, history of using a handmade instrument for drug injecting and negative history of sex with a sex worker. The logistic model that best explained the distribution of HIV/tuberculosis coinfection included duration of drug injecting, history of drug injecting inside prison and negative history of sex with a sex worker.

## ■ Conclusions and recommendations

This study reported a high prevalence of HIV risk behaviour and a high prevalence of HIV infection among IDUs. This will have major epidemiological impact if prevention and control programmes are not intensified among this high risk group in society. The high prevalence of HIV/tuberculosis coinfection suggests the need for an aggressive and well-planned programme of antituberculosis chemoprophylaxis.

# Cutaneous Leishmaniasis

## Epidemiology

Epidemiological study in a new focus of cutaneous leishmaniasis for a control programme close to the Silk Road in north-east Islamic Republic of Iran

Islamic Republic of Iran  
Sabzvar county, Khorasan province, north-east Islamic Republic of Iran

Study period:  
September 2001–  
November 2002

Small Grants  
Scheme  
(SGS) 2001 No. 34

Principal Investigator  
Dr Mohammad Reza  
Yaghoobi-Ershadi  
Department of Medical  
Entomology and Vector  
Control  
School of Public Health and  
Institute of Public Health  
Research  
Teheran University of  
Medical Sciences  
Teheran, Islamic Republic  
of Iran  
e-mail:  
rezatr12@yahoo.com

## Islamic Republic of Iran

### Abstract

An outbreak of cutaneous leishmaniasis close to the Silk Road in Sabzvar county prompted this study during 2001–2002. This region was not previously considered an endemic zone of leishmaniasis. The rural district most affected was Jovein. Examination of 543 schoolchildren reported a rate of 9.4% for scars and 6.1% for ulcers. A study of prevalence among 807 inhabitants of four villages reported a rate of 10.4% for scars and 3% for active lesions.

A total of 12 849 sandflies, representing 7 species, were present in the study area. *Phlebotomus papatasi* was active from April to late October with two peaks of activity in June and August. Natural leishmaniasis infection was found in *P. papatasi*, *P. caucasicus* and *Sergentomyia sintoni*. *P. papatasi* was susceptible to DDT, permethrin and propoxur. *Rhombomys opimus*, *Meriones libycus* and *Nesokia indica* were present around the villages. *R. opimus* was found to be infected with *Leishmania*. Parasites from humans, *P. papatasi* and *R. opimus* were isolated and characterized as *L. major* using random amplification of polymorphic DNA (RAPD) polymerase chain reaction (PCR).

**Conclusion** An epidemic of zoonotic cutaneous leishmaniasis was reported in the area, with *R. opimus* as the main animal reservoir host and *P. papatasi* as vector among rodents, and probably among humans.

### Background

Recently a new focus of cutaneous leishmaniasis was found in villages located in Sabzvar county near the Silk Road in the north-east part of the country. A preliminary survey showed a rate of 9.4% for ulcers in November 2000. Following the establishment of the Sarakhs free zone and the opening of the Silk Road, economic development has started in the area. Frequent non-immune visitors to the study area are exposed to the bites of infected sandflies during their active season. The epidemiological features of cutaneous leishmaniasis were unknown in the area, so an in-depth epidemiological study was required as the basis for the future implementation of control measures.

### Materials and methods

The investigation was carried out in four villages in the rural districts of Jovein and Davarzan, located on a plain in Sabzvar county, Khorasan province, in the north-east of the Islamic Republic of Iran. Buildings in the villages were made of sun-dried bricks and girders, with mud or wooden roofs covered by reeds, and many of them were newly constructed. The villages are close to railway lines and a sugar refinery, and rodent burrows can be easily identified in the area. Farming is the main occupation of the inhabitants and some keep cows and sheep.

807 inhabitants and 543 schoolchildren from Jovein district, and 970 inhabitants from Davarzan district, were interviewed and clinically examined for the presence of ulcers or scars.

Rodents were baited with cucumber and dates and caught using 40 live traps at the end of the high season of infection. Impression smears were prepared from the ears of each rodent, stained using the standard Giemsa method, and examined under a microscope. Samples

## Conclusions and implications of the study

■ A new focus of zoonotic cutaneous leishmaniasis was identified in Jovein and Davarzan rural districts of Sabzvar county, north-east Islamic Republic of Iran. *R. opimus* was the reservoir host and *P. papatasi* was the vector among rodents and probably among humans.

■ The outbreak of zoonotic cutaneous leishmaniasis was attributed to the construction of buildings near the rodent colonies, the habit of sleeping in yards during summer, lack of good environmental sanitation, construction of bedrooms and sitting rooms with mat-like roofs close to stables, existence of manure in some villages and the expansion in beetroot cultivation, a characteristic vegetation for *Gerbillidae* rodents.

■ Health authorities were recommended to take strong measures to control the epidemic and prevent its spread to other villages and neighbouring countries such as Turkmenistan, Uzbekistan and Kazakhstan.

from patients and infected rodents were injected subcutaneously at the base of the tail of inbred (BALB/c) mice. Parasites were re-isolated from infected mice and cultured in NNN media plus LIT medium. PCR technique was used for the identification of parasites.

Sandflies were collected using 30 sticky traps once a month from indoor (bedrooms, stables, warehouses) and outdoor (rodent burrows) fixed sites, throughout the active season. For species identification, the sandflies were mounted in Puri's medium and identified after 24 hours using the keys of Theodor and Mesghali [1]. They were then mounted and segregated by sex.

Collection of live sandflies was performed using funnel traps, CDC light traps at rodent burrows and with aspirators inside houses. Blood-fed females collected indoors were kept alive for 3–4 days to allow blood digestion and were then dissected. Parasites were later re-isolated from infected mice and cultured in NNN biphasic medium with penicillin. All female sandflies were identified by the morphology of the pharyngeal armature and of the spermatheca [1]. RAPD PCR technique was used for identification of parasites.

The susceptibility status of *Phlebotomus papatasi* to DDT, permethrin and propoxur was studied in the field surveys, following the WHO standard method.

## ■ Main study findings

Jovein rural district A rate of 10.4% was reported for scars and 3% for active lesions, being highest in the 5–9 years age group and lowest in the 0–4 years age group. The prevalence of ulcers was 3%, the most highly infected age group being 0–4 years, while the lowest rate was among those aged 20–24 years.

A prevalence of 9.4% and 6.1% was reported for scars and ulcers, respectively, among schoolchildren. The highest prevalence of active lesions of 50% was found among the 15 years age group, while the lowest prevalence of 4% was found among the 9 years age group.

Davarzan rural district A prevalence rate of 0.9% for scars was reported in this district. There were no ulcers among the residents during November and December coincident with the appearance of new cases in the area. There was no significant difference in the prevalence of scars by sex. The overall scar rate among the schoolchildren screened was 0.3% and none had ulcers.

Of 56 small mammals captured from Jovein, the predominant rodent species was *Rhombomys opimus* (80.4%). The other three rodent species caught were *Nesokia indica* (12.5%), *Meriones libycus* (3.6%) and *Hemiechinus auritus* (3.5%). Infection with *L. major* was found in 15.6% of the *R. opimus* caught.

8726 adult sandflies were collected and identified

in Jovein. Six species were identified indoor: *Sergentomyia sintoni* (64.6%), *P. papatasi* (32.4%), *P. caucasicus*; *P. ansarii*; *S. grekovi* and *S. sumbarica*. In rodent burrows, *S. sintoni* (74.3%), *P. caucasicus* (8.9%), *P. papatasi* (8.5%), *P. ansarii*, *P. sergenti* and *S. grekovi* were collected.

A total of 4123 adult sandflies were collected and identified in Davarzan. Four species were identified indoor: *S. sintoni* (76.3%), *P. papatasi* (14.7%), *P. sergenti* (8.7%) and *S. grekovi* (0.3%). In rodent burrows, *P. sergenti* (46.8%), *S. sintoni* (44.4%), *P. papatasi* (8.7%), *S. sumbarica* (0.04%) and *S. grekovi* (0.04%) were collected.

*P. papatasi*, *P. caucasicus*, *P. sergenti*, *P. ansarii*; *P. keshishiani* and *S. sintoni* were collected in the vicinity of rodent burrows in Jovein and were dissected. The results of the dissections showed that *P. papatasi* (4.5%), *P. caucasicus* (2.3%) and *S. sintoni* (2.8%) were infected with leptomonads. Infections were seen in the gut, oesophagus and head indicating that the promastigotes in *P. papatasi* are in metacyclic form and can migrate to the foregut, and can therefore be transmitted by the mouthparts of this sandfly. Dissected indoor flies were all negative for infection.

Examination of 4 isolates from humans, 4 from *P. papatasi* and 5 from *R. opimus* were characterized as *L. major*. Insecticide susceptibility tests showed that *P. papatasi* is susceptible to DDT, permethrin and propoxur in the area.

## ■ Conclusions and recommendations

A new focus of zoonotic cutaneous leishmaniasis was identified in some villages in Jovein and Davarzan rural districts, in Sabzvar county, north-east Islamic Republic of Iran. The outbreak of zoonotic cutaneous leishmaniasis in the study villages seems to be related to environmental conditions favouring the breeding of rodents. Sabzvar county is located on a transit road to Turkmenistan, Uzbekistan and Kazakhstan with a moderate amount of truck traffic that will increase in the future. The south of Sabzvar county is also vulnerable to the disease and new epidemics may occur in different foci in the coming years. These results emphasize the need to destroy gerbils with zinc phosphide mixed with wheat grains and vegetable oil at a radius of 500 metres once a month during May, June, July and September every other year. Passive and active case detection, and rapid treatment of patients are also recommended.

## ■ References

[1] Theodor O and Meshghali A. On the *Phlebotominae* of Iran. *Journal of Medical Entomology*, 1964, 39:285–300.

# Cutaneous Leishmaniasis

## Epidemiology

### Pakistan

Epidemiology of cutaneous leishmaniasis in Larkana district of Sindh province with particular reference to phlebotomine sandflies

Pakistan  
Larkana district, Sindh province

Study period:  
November 2001–  
December 2002

Small Grants  
Scheme  
(SGS) 2001 No. 67

Principal Investigator  
Dr Birjees Mazher Kazi  
Public Health Division  
National Institute of Health  
Islamabad, Pakistan  
e-mail:  
kazis@isb.paknet.com.pk

### Abstract

As a result of cutaneous leishmaniasis outbreaks in Larkana and Dadu districts of Sindh Province during 2001–2002, a study was designed to assess the prevalence of the disease in two villages in Larkana district with particular reference to the population dynamics of phlebotomine sandflies in the area. A household survey was carried out in March 2002 immediately before the sandfly season, whereby household members were screened for scars and active lesions of cutaneous leishmaniasis and were interviewed regarding the determinants of infection. All suspected cases were clinically and microscopically confirmed. Entomological studies were carried out between January and October 2002.

**Results** The incidence of cutaneous leishmaniasis in the area was 36.44 per 1000 population. Out of a total of 89 residents in both villages infected with the disease, 78.65% had active lesions and 21.34% had scars. The lesions were typical of zoonotic cutaneous leishmaniasis: wet, large in size and mostly ulcerated. The mean size of the lesions was 2.8 centimetres.

A total of 1232 *Phlebotomus* sandflies were collected from the study villages. Two species were identified: *P. papatasi* (53.32%) and *P. (Paraphlebotomus) alexandri* (46.7%). *Phlebotomus* species were found from April through October. The peak activity of the dominant sandfly, *P. papatasi*, was in May. *P. alexandri* showed the same pattern of seasonal activity as *P. papatasi* except that the number of flies caught inside houses was slightly more than *P. papatasi*, and there was a second small peak in July, declining thereafter and coming to an end in October.

The proportions of *P. papatasi* and *P. alexandri* were significantly higher in light trap than sticky trap collections.

**Conclusion** Transmission of zoonotic cutaneous leishmaniasis in the Larkana district of Sindh province seems to occur mainly in rural settlements between mid-August and end September, and the only species involved in disease transmission is *P. papatasi*.

### Background

In January 2002, an outbreak of zoonotic cutaneous leishmaniasis was reported from two districts (Larkana and Dadu) of Sindh province with an estimated 11 700 cases in both districts. This study was conducted in Taluka Warah in Larkana district, where the highest number of cases was reported during the outbreak in 2002. Previous records do not document any detailed study on phlebotomine sandflies in this region. The only study is that of Lewis (1967) [1] which lists the *Phlebotomus* and *Sergentomyia* species of Pakistan.

### Materials and methods

The survey was conducted in March 2002, immediately before the sandfly season, in two selected villages of Larkana district: Gul Mohammad Tunio and Junani. The data collection form included information on the number of residents in each house, their sociodemographic characteristics, type of house construction, availability of electricity, domestic animals kept, number of patients with active lesions or scars due to cutaneous leishmaniasis, and size and duration of active lesions. All cases of cutaneous leishmaniasis recorded were clinically and microscopically confirmed.

Entomological studies were carried out between January and October 2002. These studies were undertaken to investigate the sandfly biology with particular reference to sandfly fauna, species composition, the seasonal and relative abundances of phlebotomine

## Conclusions and implications of the study

■ Both sandfly species *P. papatasi* and *P. alexandri* collected from the study area are predominantly exophilic as the highest number of both species were caught outdoors.

■ Phlebotomine sandfly fauna from this area of Larkana district have been studied for the first time and shown to be strictly Mediterranean.

■ Transmission of zoonotic cutaneous leishmaniasis in the Larkana district of Sindh province seems to occur mainly in rural settlements between mid-August and end September, and the only species involved in disease transmission is *P. papatasi*.

sandflies, and natural infections. Three houses were randomly selected from each village. Outdoor and indoor sandflies were collected using CDC light traps and sticky paper traps. All sandflies belonging to genus *Phlebotomus* were identified for species and dissected for natural infection with *Leishmania* parasites in the field.

## ■ Main study findings

A total of 89 cutaneous leishmaniasis cases were detected in the two study villages (35 in Gul Mohammad Tunio and 54 in Junani). The number of cases was estimated to be 36.44 per 1000 population in both villages. However, the number of cases per 1000 population was significantly higher in Gul Mohammad Tunio (51.23/1000) than in Junani (30.85/1000). Males were more commonly infected (56.6%) than females (41.1%). Out of 89 residents of both villages infected with cutaneous leishmaniasis, 70 (78.65%) had active lesions and 19 (21.34%) had scars.

Fifty-five percent (55%) of the cases had single lesions, 23.59% had 2 lesions and 21.34% had 3 or more lesions on different body parts. Most lesions were on the legs (30.34%), followed by feet (23.6%), arms (18%) and face (17.98%). Other body parts with fewer active lesions included the neck, back of the palm, fingers and ears. The lesions were typically wet, large in size and mostly ulcerated. Such lesions are typical of zoonotic cutaneous leishmaniasis. The mean size of the lesions was 2.8 centimetres.

A total of 1232 (1072 in light trap and 160 in sticky trap collections) *Phlebotomus* sandfly species were collected from the study villages between January and October 2002. Two species were caught: *P. papatasi* (53.32%) and *P. (Paraphlebotomus) alexandri* (46.7%).

*Phlebotomus* species were found from April to October. The peak activity period of the dominant sandfly, *P. papatasi*, was in May inside houses when the average temperature is between 30 °C and 35 °C and mean relative humidity is above 50%. There was a sharp decline in activity in June when the average temperature is above 40 °C. Activity started to increase again in July, with a second peak in August, and thereafter slowly declined. The number of sandflies caught outdoors was significantly higher than indoors, indicating that the fly is highly exophilic and may be exophagic. Since blood meal analysis could not be done, the extent of zoophilia and anthrophilia could not be ascertained. *P. alexandri* displayed the same pattern of seasonal activity as *P. papatasi*, except that the number of flies caught inside houses was slightly more and there was a second small peak in activity in July, declining thereafter and almost coming to an end in October.

The proportions of *P. papatasi* and *P. alexandri*

were significantly higher in light trap than sticky trap collections. However, the flies caught from sticky papers placed in rodent burrows showed the highest activity of *P. papatasi* in March, May, June and August, in contrast to the catches made in CDC light traps when the peak activity was in May. *P. alexandri* showed peak activity in May, June and August.

All *Phlebotomus* sandflies collected in CDC light traps, both indoors and outdoors, during August, September and October were dissected for natural infection with leishmania promastigotes. These months were chosen because the highest number of parous flies are found when the density is decreasing. A total of 400 female *P. papatasi* and *P. alexandri* were dissected but none were found to be naturally infected. Based on the fact that the vector of zoonotic cutaneous leishmaniasis is only *P. papatasi*, except in a few areas where *P. salehi* has also been found to be naturally infected, it is assumed that *P. papatasi* is the most probable vector in the transmission of the disease in this area.

Further studies are required to determine the blood meals of *Phlebotomus* species to understand their host preferences, and to establish natural infections in wild-caught flies. Study of rodents is also needed in order to confirm the reservoir of infection.

## ■ Conclusions and recommendations

Based on the results of this study, the transmission of zoonotic cutaneous leishmaniasis in the Larkana district of Sindh province seems to occur mainly in rural settlements. The seasonal changes in sandfly population suggest that the disease is transmitted between mid-August and end September when the sandfly population is declining and maximum parous flies are found. Since *P. papatasi* is mainly exophilic as well as exophagic, humans are at maximum risk outdoors. As most people in these villages sleep outdoors, the likelihood is that transmission takes place outdoors. Although no naturally infected *P. papatasi* was found during the study, on the basis of circumstantial evidence, *P. papatasi* can be considered the only species involved in disease transmission in this area.

## ■ References

[1] Lewis DJ. The Phlebotomine sandflies of West Pakistan (Diptera: Psychodidae). *Bulletin of the British Museum (Natural History)*, 1967, 19:1–57.

# Cutaneous leishmaniasis

## Epidemiology

### Sudan

The epidemiological components of cutaneous leishmaniasis outbreaks in Tuti Island: a field and simulation approach

Sudan  
Tuti Island, Khartoum and  
El-Tarajma El-Ghaba, Nile  
State

Study period:  
October 2002–2004

Small Grants  
Scheme  
(SGS) 2002 No. 195

Principal Investigator  
Dr Muntaser Ibrahim  
Department of Molecular  
Biology  
Institute of Endemic  
Diseases  
University of Khartoum  
e-mail:  
mibrahim@iend.org

### Abstract

To try to understand the cyclical nature of leishmaniasis epidemics and identify the various factors governing the initiation and build-up of outbreaks, 2 surveys were conducted in two endemic areas for cutaneous leishmaniasis: one is Tuti Island in the middle of Khartoum, where a previous outbreak took place in the mid-1980s and the other is El-Tarajma village north of Shendi in Nile State. Schoolchildren were subjected to leishmanin skin test (LST) and clinical examination and were asked about their past history of active disease and treatment. A model and computer program were used to simulate different scenarios for possible future epidemics based on these data.

Results Unlike Tuti Island, there was still active transmission in El-Tarajma. Epidemiologically, it seemed that the disease in El-Tarajma was in a similar phase to that of Tuti Island in 1996, both in leishmanin and clinical data. Comparing parameters in Tuti Island 1996 to that of 2003, allowed a rate of decline of LST positivity to be inferred, a decisive factor in the initiation of the build-up phase of the epidemic.

### Background

Cutaneous leishmaniasis is one of the most common endemic diseases in Sudan. There have been several reported outbreaks of the disease in Sudan, the last being in Khartoum State in 1985 and involving at least 10 000 cases. Following the original epidemic, a study has shown that about 30% of the examined individuals from Tuti Island had sub-clinical infections as proven by a positive leishmanin skin test in the absence of scars or previous history of active skin lesion. By contrast, during the epidemic of the 1980s, 80% of patients from Tuti Island and other parts of Khartoum were active parasitologically confirmed cases. The nature of cutaneous leishmaniasis outbreaks in terms of timing and factors governing their initiation and build up is not well understood. A study was therefore carried out to develop a model to simulate the population structure, vector and parasite density, immunity and outbreaks, which could be used to predict future outbreaks and optimize interventions measures.

### Materials and methods

The study was conducted in 2 areas. One, Tuti Island, lies at the junction of the Blue and White Niles in Khartoum. The island is covered with dense vegetation and is characterized by a hot dry season followed by a rainy season from July to September. The vectors and reservoir hosts of cutaneous leishmaniasis exist in this area. It is believed that the first epidemic in Khartoum State started on the island. The number of active cases has declined gradually over the years but sporadic cases still occur, indicating that the island is still endemic for the disease. According to the last census (1996), the population size was 20 000. The other study area was El-Tarajma El-Ghaba, north of Shendi in Nile State with a population of 5000,

## Conclusions and implications of the study

Two epidemiological situations were identified in the study: One in Tuti Island where the disease is endemic but with progressive decline in transmission as evident from the distribution of scars and leishmanin skin test (LST) positivity in the different age groups in 2003 as compared to 1996. The rate of decline of leishmanin was estimated as 2.02% per year in the age group below 20. In El-Tarajma, the situation appears to be different with more clinical cases and features of an outbreak in its peak either in the ascending or descending phase. It may not be easy to differentiate between these phases without longitudinal follow-up data.

Simulation showed an exponential rise in incidence and scar rate given a population natural increase of 15% every 5 years.

An increase in the population size due to immigration could possibly lead to the initiation of an outbreak. This happened in Tuti Island in 1998 due to incoming agricultural labour from western Sudan. Another decisive factor is the intensity of transmission (the vector density and the number of infective bites), but these could not be investigated in this study.



where an outbreak of cutaneous leishmaniasis was reported in 1978. It is believed to be the original focus from which the disease spread along the Nile.

**Clinical examination** A cross-sectional survey of schoolchildren was conducted whereby children and young adults aged 20 years or younger were clinically examined and interviewed about their past history of cutaneous leishmaniasis and its treatment. The number, distribution, type and size of lesion and sporotrichoid spread were recorded for patients with active disease. Specimens were cultured on Novy-MacNeal-Nicolle (NNN) media, and collected for polymerase chain reaction (PCR) to detect *Leishmania* DNA. Patients were treated with ketoconazole in case of 5 lesions or more, sporotrichoid leishmaniasis or if the lesions were on the face. Patients with <5 lesions were reassessed and secondary infections were treated with antibiotics and/or gentian violet.

**Leishmanin skin test (LST)** Leishmanin skin test was performed with intradermal injection of 0.1 ml of *L. major* antigen (Pasteur Institute, Teheran) in the volar aspect of the left forearm. Antigen diluent without antigen was injected as control at least 10cm from the test site. The reaction was read 48–72 hours later using the ballpoint pen technique. An induration of >5mm at the site of the test and a negative reaction at the control site was considered a positive one.

**Simulation and modelling** The maximum likelihood approach was used to evaluate the contribution of each factor included in the model. The model was based on related models that are extensions of the classical Macdonald-Ross model for malaria transmission.

## ■ Main study findings

A total of 910 individuals were enrolled, 433 from El-Tarajma and 477 from Tuti Island. They were screened by LST, and examined for active cutaneous lesions and scars. Their age ranged from 1–20 years. Overall, 321 and 422 were read for LST from the 2 areas, respectively. There was a significant strong positive correlation between the LST positivity and age in all conducted surveys whether in El-Tarajma in 2003, or in Tuti Island both in 1996 and 2003.

The situation in El-Tarajma in 2003 was comparable to that of Tuti Island in 1996 regarding scars and active lesions. A scar rate of 22.6% in El-Tarajma in 2003 was reported, compared to 25.2% and 10.4 % in Tuti Island in the 1996 and 2003 surveys, respectively. Similarly, the prevalence of active lesions was 3.6% in El-Tarajma compared to 2.1% and 0.6% in Tuti Island in 1996 and 2003, respectively.

The rate of decline in leishmanin positivity, a marker of immunity and exposure, was 2.02% per year based on the present data from Tuti Island. The loss of immunity was found to be the variable that best explained the variation in a regression model. This

rate was used in equations to estimate the time required to reach a critical point of prevalence, which was estimated at between 5%–10% according to previous observations.

The basic reproductive number ( $R_0$ ) was calculated from the Anderson model (Stanford Research Institute) for horizontal transmission to start an outbreak with modification.

Different values of  $R_0$  were used to simulate different scenarios and the following assumptions were made:

1) The effect of the disease on host fitness and life span is negligible, as the disease is mostly self limited, with a spontaneous shift in immune response from T-helper lymphocytes 2, which enhance the humoral response, to T-helper lymphocytes 1, which enhance cell-mediated immune response (represented by leishmanin conversion). The decisive factor in the model is the availability of the non-immune host carriers. This should be in the simplest terms proportional to the number of infected at their peak minus the natural mortality in a generation time plus the new births. The sand fly density was considered of minor significance as it is mostly related to major climatic factors such as rainfall.

2) The other assumption is the loss of parasite virulence during the build-up of the immunity in the population and the gradual increase in virulence of the parasite population while the immunity is declining (virulence being a function of loss of immunity). The hypothesis was that a critical point is reached where a certain parasite clone is required to generate the next outbreak. This point was simulated by substituting for  $R_0$  by different values. The results matched some of the true epidemiological situations with intervals of 10–20 years between outbreaks.

Simulating the scar rate as a measure of the disease incidence using a population natural increase of 15% every five years (estimated rate for the general population of the country) indicated an exponential rise in incidence and scar rates.

## ■ Conclusions and recommendations

A progressive rate of decline of leishmanin over time is due to immunological saturation of the older population coupled with reduced transmission intensity in the new born. Conversely, there is a rise in virulence of the parasites due to repeated passage in a new pool of non-immune and semi immune individuals. The parasite virulence will reach a critical level depending upon the availability of a certain number of susceptible hosts in which repeated passage could take place. The parasite population structure will turn from clonal into semi-clonal with more diverse strains circulating in the population.

# Visceral Leishmaniasis

## Control

Could infections in wild canids limit the potential effectiveness of insecticide-impregnated dog collars for reducing *Leishmania infantum* transmission in the Islamic Republic of Iran?

Islamic Republic of Iran  
Kalaybar Region,  
north-west Islamic Republic  
of Iran

Study period:  
September 2002–  
March 2003

Small Grants  
Scheme  
(SGS) 2001 No. 73

Principal Investigator  
Dr Abdolsamad  
Mazloumi Gavgani  
Leishmania Research Unit  
Immunology and  
Parasitology Dept  
Faculty of Medicine  
Tabriz University of Medical  
Sciences  
Tabriz, Islamic Republic of  
Iran  
e-mail: mazloumi@tbzmed.ac.ir

## Islamic Republic of Iran

### Abstract

This study was carried out to evaluate the extent of visceral leishmaniasis infection in jackals and foxes, and its impact on the effectiveness of insecticide-impregnated dog collars in nine treated and control villages during 2000–2002 in north-west Islamic Republic of Iran.

Wild canids were trapped, and examined clinically and serologically for visceral leishmaniasis, and bone marrow aspirates were taken for parasite isolation and identification. In addition, a household survey was conducted to collect information regarding wild and domestic canids in 40 endemic villages.

**Results** In response to a questionnaire, 39% of villagers claimed to own at least one dog. Everyone claimed to have seen foxes in their village, 42% to have seen jackals and wolves, and 82% to have seen stray dogs. The number of red foxes captured from the four study villages was 21 including 8 foxes from two treatment villages and 13 foxes from two control villages.

Two (9.5%) foxes were seropositive including 1 positive by direct agglutination test (DAT) and enzyme-linked immunosorbent assay (ELISA) and dipstick, and 1 positive by DAT and ELISA but not dipstick. None of the seropositive or seronegative foxes showed overt clinical signs of canine visceral leishmaniasis. The seropositive foxes were older in age and were both caught in control

villages (2 out of 14 foxes). All foxes caught in treatment villages were seronegative.

**Conclusion** Seropositive foxes were captured only in villages where dogs did not receive insecticide-impregnated collars suggesting a protective effect of these collars on domestic and wild canids.

### Activities carried out in the framework of the project

Dr Orin Courtenay, Department of Biological Sciences, University of Warwick, Coventry, United Kingdom, trained Iranian field staff to initiate the fieldwork on this project.

### Background

In countries endemic for zoonotic visceral leishmaniasis, targeting domestic dogs may not be sufficient to control the disease. However, even if stray dogs are culled, the effectiveness of either collars or lotions on domestic dogs will have limited effect where infected sylvatic canids play an important reservoir role.

This study was carried out in order to test the hypothesis that infection in wild canids could have reduced the effectiveness of impregnated dog collar intervention in nine treatment villages during the period 2000–2002. By 2002, transmission has been reduced in domestic dogs in the nine treatment villages compared to the nine control villages for two consecutive years.

### Materials and methods

In the rural communities of Kalaybar, veterinary technicians and a number of local health workers were trained to live-trap and take samples from wild canids. Training included identifying suitable trap locations, animal handling, blood sampling, animal marking and animal release. The 20 live-traps (imported from the United Kingdom) were then positioned

## Conclusions and implications of the study

■ The prevalence of fox infection in this study (9.5%) is similar to reported infection prevalence in red foxes and jackals from elsewhere in the Islamic Republic of Iran and is not significantly different from infection in domestic dogs.

■ No seropositive canids were identified in treated villages suggesting a protective effect of these collars on both domestic and wild canids.

■ The effectiveness of insecticide-impregnated dog collars needs further validation by conducting xenodiagnostic studies to evaluate transmission in the vector and investigating the impact on a larger sample size of wild canids.

on the periphery of three villages (Abdorrazzag, Haddadan and Galeh-kandi) to capture foxes on the way to and from the villages.

Traps were set and checked each day. They were set at night in summer and during the day in winter, and regularly checked. Captured foxes were anaesthetized and clinically examined for visceral leishmaniasis. The presence of anti-*L. infantum* antibodies (Ab) was measured by DAT, ELISA and dipstick serological antibody test, and clinical diagnosis was made by physical examination. Bone marrow aspirates were taken for parasite detection, isolation and identification. Information was collected on age, sex and weight, and the foxes were permanently marked for individual identification using a mechanical tattoo.

A questionnaire was designed to seek information about wild and domestic canids, and distributed to 2000 households in 40 villages.

### ■ Main study findings

A total of 1872 completed questionnaires were received out of 2000 distributed to 40 villages (50 questionnaires each). Of respondents, 727 (39%) claimed to own at least one dog. All dog owners claimed to have seen foxes in their villages, and 42% claimed to have seen jackals. Foxes and jackals were also regularly seen in the hills, as well as wolves (46%). Hunting of wild animals is illegal and few admitted that they had hunted them (6%), even though the majority owned poultry and livestock (88%) that are at risk of predation by wild canids. Stray dogs had been seen by 82% of respondents, including 22% who had seen “plenty”, with 43% always reporting them to a health worker, 3% to the environment office and 15% preventing them entering the village.

On 26 occasions between September 2002 and February 2003, 21 red foxes were captured from four study villages located 5km–10km apart, including 8 foxes from two treatment villages and 13 foxes from the two control villages. Of captured foxes, 12 (57%) were male and 48% were older than one year, indicating a population replacement rate of 65% per year, corresponding to an annual per capita mortality rate of around 40%. The age structure of the population was comparable between the intervention and control villages, and to fox populations elsewhere. These data suggest a healthy birth rate and steady supply of young *Leishmania*-naïve foxes into the endemic study population.

Two (9.5%) foxes were Ab seropositive, including 1 fox positive by all three diagnostic tests and 1 positive by DAT and ELISA but not dipstick. Both the seropositive foxes were in the older age group. None of the seropositive or seronegative foxes showed overt clinical signs of progressive canine visceral leishmaniasis. Both seropositive foxes were caught

in control villages (2 out of 14 foxes), compared to none caught in the treatment villages.

The prevalence of fox infection in this study (9.5%) was similar to reported prevalence rates in red foxes and jackals from other Iranian regions. No jackals were trapped in the four villages.

### ■ Conclusions and recommendations

Due to the difficulty in trapping wild canids, the sample size achieved in this study was too small to assess the effect of the intervention on fox infection rates. However, seropositive foxes were captured only in villages where dogs did not receive insecticide-impregnated collars. It is likely that these foxes were exposed to *Leishmania* after the start of the intervention 3 years earlier (in March 2000). The similarity of infection prevalence in domestic dogs and foxes in this region suggests that fox infection represents a “spill-over” from infectious dogs.

These preliminary findings are encouraging, but need further validation using a larger sample size of wild canids, and assessing the relative contribution of wild and domestic canid populations to vector transmission.

# Visceral Leishmaniasis

## Drug Studies

Pentostam-resistant  
visceral  
leishmaniasis in  
eastern Sudan:  
Incidence and  
parasite  
characterization

Sudan  
Gedaref State, eastern  
Sudan

Study period:  
November  
2001–February 2003

Small Grants  
Scheme  
(SGS) 2001 No.109

Principal Investigator  
Dr Maowia Mukhtar  
Department of Molecular  
Biology  
Institute of Endemic  
Diseases  
University of Khartoum  
Khartoum, Sudan  
e-mail:  
mmukhtar@iend.org

## Sudan

### Abstract

A longitudinal study was carried out in a kala-azar endemic region in eastern Sudan to determine the incidence of failures of sodium stibogluconate (SbV, Pentostam) treatment. The study recruited 820 consenting confirmed visceral leishmaniasis patients. All patients were treated with Indian Pentostam at a dose of 20 mg/kg for at least 28 days. Patients were followed for active and passive detection of relapse.

Results 22 patients were identified as relapsed patients. Parasites were isolated from their lymph node aspirates, thereby recording a 2.7% incidence of clinical resistance to Pentostam treatment. All isolates were typed as *Leishmania donovani* based on polymerase chain reaction (PCR) amplification of parasite kDNA compared with reference strains. Six parasites showed in vitro resistance to Pentostam using murine J774 macrophage/amastigote testing method. The resistant isolates had different restriction profiles when their amplified kDNA PCR products were digested with Alu1 restriction enzyme, indicating that the resistance was mediated by different parasite clones.

### Background

Emergence of Pentostam-resistant strains of *Leishmania donovani* in different kala-azar endemic areas in Asia and Africa is becoming a serious health problem. Lack of response to the initial

course of pentavalent treatment is usually 10% in most areas, but this rate seems to be increasing [1]. This study was undertaken to characterize and determine the diversity of Pentostam-resistant *Leishmania* isolates from eastern Sudan and to determine the incidence of Pentostam resistance in the area.

### Materials and methods

A longitudinal study was done in a kala-azar endemic area in Gedaref State, eastern Sudan, for which 820 consenting visceral leishmaniasis patients were recruited. Kala-azar patients were diagnosed based on their direct agglutination test reactivity, and confirmed by detection of parasites in lymph node aspirates. All patients were treated with Indian Pentostam at the dose of 20 mg/kg for at least 28 days. Patients were followed for active and passive case detection to determine the incidence of relapse. Test of cure was done by parasite detection in lymph node aspirate. Those who failed to clear their parasites after a complete treatment course and patients who developed visceral leishmaniasis symptoms after completing their treatment course were defined as relapsed patients.

Parasites were isolated by inoculating lymph node aspirate of the relapsed patients into biphasic media (NNN) consisting of a solid-phase agar mixed with defibrinated rabbit blood (10%) and a liquid phase of RPMI 1640 supplemented with 10% fetal calf serum. Primary cultures were incubated at 26 °C for 4 days and then grown in RPMI 1640 containing 25mM Hepes pH 7.4, 10% heat inactivated fetal calf serum, streptomycin and penicillin at 5IU/ml in 25 ml culture flasks.

Characterization of the isolates: kDNA polymerase chain reaction DNA was extracted from cultured parasites by Chelex extraction method and polymerase chain reaction (PCR)

## Conclusions and implications of the study

- Clinical resistance to Indian Pentostam treatment at the dosage of 20 mg/kg for 20 days in eastern Sudan is less than 3%.
- Parasites isolated from patients who did not respond to Pentostam treatment were typed as *Leishmania donovani* based on PCR amplification of parasite kDNA using AJS/DBY primers. Similar typing profiles were obtained using isoenzyme profiles.
- Relative resistance to Pentostam was shown by 30% of the parasites isolated from resistant patients when tested in vitro using J774 macrophage infection system. The in vitro resistance was less than 1% among treated patients.
- The resistance was mediated by different parasite clones as shown by the restriction fragment length polymorphism analysis.

was carried out using genus-specific primers for minicircle kinetoplast DNA (kDNA) (AJS3, 5'ggggTTggTgTAAAATAgggC-3' and DBY 5'CCAGTTTCCCgCCCCggAg-3') as described by Smyth et al [2]. The amplification was run for 35 cycles on a Perkin Elmer PCR machine at annealing temperature of 64 °C for 1 minute.

Restriction fragment length polymorphism (RFLP) 13 µl of amplified PCR products were digested with 1 IU of Alu1 restriction enzyme in a total volume of 20 µl. The digestion was done at 37 °C for 4 hours. The restriction profile was determined by electrophoresis separation of 10 µl of the digested product on 2% agarose using 80 volts, 100 mA current. *in vitro* sensitivity testing of the isolates to Pentostam Monolayers of cultured murine macrophage cell line J774 were prepared by aliquoting 1 ml/well of growing cells into 24-well tissue culture plates containing 13 mm diameter glass cover slips. The plates were incubated overnight at 37 °C in 5% CO<sub>2</sub> atmosphere. Growing cells were infected by stationary-phase promastigotes at a ratio of 10:15 promastigotes/macrophage. Infected cultures were incubated overnight at 37 °C in 5% CO<sub>2</sub> atmosphere. Excess promastigotes that did not infect the macrophages were washed with RPMI 1640 and 1 ml of Indian Pentostam (diluted at 1:10 in RPMI 1640 media) was added to two wells, while 1 ml of RPMI 1640 was added to the remaining two wells and used as control. Each parasite isolate was tested in four separate wells. The plates were re-incubated for 3 days at 37 °C in 5% CO<sub>2</sub> atmosphere. The cover slips were then removed, air dried, fixed in 70% methanol and stained with 10% Giemsa stain. One hundred macrophages were counted under a light microscope with a 100x oil immersion lens. The percentage of infected cells and the number of parasites/cell were counted.

### ■ Main study findings

The study recruited 820 visceral leishmaniasis patients. The age of patients ranged from 4 to 60 years. Of those patients who were treated with complete courses of Pentostam, 22 were identified as relapsed patients based on detection of parasite in their lymph node aspirates. Patients relapsed within 2–6 months post-Pentostam treatment. The age of relapsed patients ranged from 4 to 25 years, with adults comprising about 40% of all treated patients. All patients were negative for HIV antibodies and had no pulmonary tuberculosis.

PCR results showed that all isolates were identified as *L. donovani* based on the amplified minicircle band 800 bp. The results of RFLP analysis showed the presence of common profile patterns in all *Leishmania* isolates with band sizes of 290 and 580 bp. Comparison with the sensitive isolates showed a

band size of 316 bp in the resistant isolates. A band size of 750 bp was detected in both resistant and sensitive isolates. There was no difference in the electrophoresis patterns between the resistant isolate pairs before and after treatment (VLR4B-VLR4A, VLR5B-VLR5B and VLR6B-VLR6A).

Six out of the 22 *Leishmania* isolates from relapsed patients showed relative *in vitro* resistance to Pentostam compared with sensitive field isolates and *L. donovani* reference strain (LD 1S). A significant difference was detected regarding parasite survival in the presence of Pentostam between resistant and sensitive reference isolates.

### ■ Conclusions and recommendations

Clinical resistance to Indian Pentostam treatment at the dosage of 20 mg/kg for 20 days in eastern Sudan is less than 3%, while *in vitro* resistance was less than 1% among treated patients. Parasites isolated from patients who did not respond to Pentostam treatment were typed as *L. donovani*. Resistance was mediated by different parasite clones.

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- [2] Smyth AJ et al. Rapid and sensitive detection of *Leishmania* kinetoplast DNA from spleen and blood samples of kala-azar patients. *Parasitology*, 1992, 105:183–92.

# Lymphatic Filariasis

## Epidemiology

### Egypt

Impact of the Egyptian control programme for elimination of filariasis on the indices of *Wuchereria bancrofti* transmission by mosquitoes

Egypt  
Filaria endemic villages

Study period:  
November 2001–  
June 2002

Small Grants  
Scheme  
(SGS) 2001 No. 102

Principal Investigator  
Dr Reda Ramzy  
Research and Training  
Centre on Vectors of  
Diseases  
Faculty of Science  
Ain Shams University  
Cairo, Egypt  
e-mail:  
reda\_m@egyptonline.com

### Abstract

Bancroftian filariasis is focally endemic in the Nile delta of Egypt where the vector mosquito, *Culex pipiens*, is extremely abundant, and most endemic villages are characterized by low rates and intensities of infection. The ultimate goal of the Lymphatic filariasis elimination programme is to reduce blood microfilaraemia to levels at which transmission by the vector mosquito cannot be sustained, thereby interrupting the cycle of the disease. Therefore, a study was carried out to evaluate the impact of the filariasis elimination programme on the indices of *Wuchereria bancrofti* transmission by mosquitoes.

A total of 34 microfilaraemic subjects were selected based on the results of a blood survey carried out 6 months before mass drug administration (MDA) of diethylcarbamazine/albendazole (DEC/ALB) in filaria-endemic villages. Venous and capillary blood samples were taken 11 months after the intake of the first annual dose of DEC/ALB, and 3, 6 and 9 months after having received the second annual dose of the drug regimen. Blood samples were stained and microscopically examined for the presence and number of microfilaria (MF).

Field-collected *Culex pipiens* larvae were reared to maturity and unfed females were exposed to selected volunteers, after which 20–30 blood-engorged females were dissected and examined for the presence and number of MF. To estimate the infectivity rate of mosquitoes, remaining females were maintained on a carbohydrate diet then dissected for the presence and number of infective *W. bancrofti* larvae (L3).

Results 93.3% and 96.7% of subjects had cleared their microfilaraemia, as determined by both membrane filtration and thick blood smear, respectively, 11 months after the second round of MDA. Median intensity of MF infection, decreased from 10 MF/ml and 3 MF/50  $\mu$ l to 4 MF/ml and zero MF/50  $\mu$ l, following two cycles of MDA.

Eleven months after the first round of MDA, overall rates of MF ingestion and development to infectivity in *Culex pipiens* were 2.4% (19/781) and 0.5% (14/2722), respectively. Nine months following the second round of MDA these numbers fell to 0.0% (n = 1310) and 0.01% (3/3337), respectively (P < 0.005).

**Conclusion** The transmission cycle of the filarial parasite by mosquitoes is seriously impaired by the administration of annual single doses of a combined regimen of DEC/ALB.

### Background

Egypt is one of the first countries to implement a nationwide programme to eliminate lymphatic filariasis as a public health problem. The programme is based on 4–5 years of mass drug administration (MDA) of single doses of albendazole (ALB) (600 mg) in combination with diethylcarbamazine (DEC) (6 mg/kg). However, so far there is no information regarding the effects of such therapy on individuals with low-level microfilaraemia, and on the uptake and maturation of filarial parasites in vector mosquitoes.

Field studies implemented in other regions of the world indicate that low level microfilaraemia could initiate a resumption of transmission after very efficient control programmes where *Aedes* species are vectors [1]. Eradication of microfilaraemia has been achieved in areas of *Anopheles* transmission [1]. However, the situation in endemic areas where *Culex* species are vectors is less clear, and ought to be investigated.

Accordingly, a study was carried out to evaluate the effects of annual single doses of a combined regimen of diethylcarbamazine/albendazole (DEC/ALB), when administered to individuals with

### Conclusions and implications of the study

■ This study clearly demonstrated that the administration of two rounds of DEC/ALB resulted in the clearance of microfilaraemia from more than 96% of the studied microfilaraemic subjects, and that mosquitoes failed to ingest microfilariae and develop the infective stage from these treated subjects.

■ The transmission cycle of the filarial parasite by mosquitoes is seriously impaired by the administration of annual single doses of a combined regimen of DEC/ALB.

■ It is recommended to sustain a high coverage rate of mass drug administration of combined DEC/ALB in order to eliminate lymphatic filariasis as a public health problem.

low-level microfilaraemia in lymphatic filariasis-endemic villages in Egypt, on the parameters of parasite transmission by *Culex pipiens*.

## Materials and methods

A total of 34 microfilaraemic subjects were studied. These were selected based on records from a blood survey carried out 6 months before the Ministry of Health and Population initiated MDA of DEC/ALB in filaria-endemic villages. This group of subjects with low-level microfilaraemia represents a significant percentage (60%) of all microfilaraemic residents in endemic localities in Egypt. The criteria for their inclusion in the study were: low microfilaraemia level (1–74 MF/ml) before treatment, participation in the national lymphatic filariasis elimination programme (having received the first dose of treatment), and voluntary agreement to provide night blood and exposure to mosquito bites. Both venous and capillary blood was taken from selected individuals 11 months after they had received the first annual dose of DEC/ALB, and 3, 6 and 9 months after having received the second annual dose of the drug regimen. Volunteers were exposed to mosquitoes at the same time intervals.

**Blood collection and processing** Venous blood samples (2 ml) were collected in vacutainers between 22:00 and 24:00 from selected subjects within one week before volunteering for mosquito feeding. Samples were refrigerated overnight, after which blood samples were processed by filtration of 1 ml on nitrocellulose membranes, 5 µm pore size (Nuclepore, Pleasanton, CA, USA), then stained with Giemsa and microscopically examined for the presence and number of microfilaria (MF). Immediately before exposure to mosquitoes, a 50 µl finger-prick blood sample was thick-smear on a glass slide, methanol fixed and air-dried. Giemsa stained smears were examined for the presence and number of MF.

**Mosquito feeding experiments** Field-collected *Culex pipiens* larvae were reared to maturity in an indoor insectary at 27 °C and 70%–80% relative humidity. Emerging mosquitoes were maintained on a sugar diet until shortly before feeding. Starving females, 3–5 days old (approximately 200/cage), were transported to the field and exposed for 30 min to selected volunteers, between 22:00 and 24:00.

To estimate the rate of MF uptake by mosquitoes, 20–30 blood-engorged females were cold-killed immediately after blood feeding and their midgut dissected. Midgut content was smeared in a drop of saline solution and microscopically examined for the presence and number of MF. To estimate the infectivity rate of mosquitoes, the remaining females (about 100) were maintained on a carbohydrate diet for 12 days (the extrinsic incubation period of the parasite). Females surviving the extrinsic incubation period

were cold-killed and their body parts separately dissected (head, thorax and abdomen) for the presence and number of infective *W. bancrofti* larvae (L3).

## Main study findings

**Human microfilaraemia** Of 34 cases selected for low-level microfilariae (1–74 MF/ml), 30 different subjects were available for examination at each time point. Of these, 93.3% and 96.7% had cleared their microfilaraemia, as determined by both membrane filtration and thick blood smear, respectively, 11 months after the second round of MDA. Median intensity of MF infection had decreased from 10 MF/ml and 3 MF/50 µl to 4 MF/ml and zero MF/50 µl, following two cycles of MDA.

**Mosquito infection** A total of 19 469 adult female mosquitoes were used in the study for assessment of rates of MF ingestion ( $n = 4970$ ) and for measuring mosquito infectivity rates ( $n = 14\ 499$ ) at determined intervals. Eleven months after the first round of MDA, overall rates of MF ingestion and development to infectivity were 2.4% (19/781) and 0.5% (14/2722), respectively. Nine months after the second round of MDA these numbers fell to 0.0% ( $n = 1310$ ) and 0.01% (3/3337), respectively ( $P < 0.005$ ).

## Conclusions and recommendations

The transmission cycle of the filarial parasite by mosquitoes is seriously impaired by the administration of annual single doses of a combined regimen of DEC/ALB. It is recommended to sustain high MDA coverage rates to eliminate filariasis as a public health problem in Egypt.

## References

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# Malaria

## Drug Studies

### Sudan

Characterization and control of the dry season *Plasmodium falciparum* gametocyte reservoir in an area of marked seasonal transmission in Sudan

Sudan  
Gedaref State, eastern Sudan

Study period:  
October 2001–  
February 2004

Small Grants  
Scheme  
(SGS) 2001 No. 58

Principal Investigator  
Dr Safaa Nurain  
Department of Zoology  
Faculty of Science  
University of Khartoum  
Khartoum, Sudan  
e-mail:  
safaagamal@hotmail.com

#### Abstract

A large proportion of inhabitants who contract malaria during the wet season in areas of marked seasonal transmission retain chronic submicroscopic asymptomatic infections throughout the dry season. A study was therefore carried out in 2 villages in Gedaref State to evaluate the effectiveness of gametocytocidal drugs in eliminating gametocytes among inhabitants before the start of the transmission season.

Parasitological and entomological surveys were conducted during the dry, pretransmission and transmission seasons of 2001 and 2002. In the intervention village, all inhabitants harbouring gametocytes during the pretransmission season received a schizontocidal drug (chloroquine) and a gametocytocidal drug (primaquine), while asymptomatic parasite carriers in the control village did not receive antimalarial treatment. Detection of subpatent parasitaemia was performed by polymerase chain reaction (PCR) and reverse transcription (RT)-PCR was used for the detection of gametocytes among those with subpatent parasitaemia.

**Results** During the dry season of 2001, the entomological inoculation rate was zero and low mosquito density was reported during the transmission season of 2002. Overall, 40% and 28.8% of the studied

population harboured subpatent infections in the intervention and control villages, respectively, in 2001. In 2002, subpatent parasitaemia existed in only 6% and 4% of the population in the intervention and control villages, respectively. RT-PCR showed a significant reduction in the prevalence of gametocyte carriage in the intervention village from 12.4% during the pretransmission season of 2001 to 8% during the pretransmission season of 2002, compared to a constant figure of 10% in the control village during the same period.

In addition, there was a significant increase in the parasite prevalence in the control compared to the intervention village in the transmission season of the 2002 (12% and 3.8%, respectively).

**Conclusion** Targeting the gametocyte reservoir by administering gametocytocidal drugs during the pretransmission season has a significant impact in reducing malaria burden in areas with seasonal malaria transmission.

#### Background

In central and eastern Sudan, malaria is a disease of the short rainy season. The rest of the year remains dry and almost malaria transmission-free. The source of the human malaria parasites that give rise to the cyclic malaria outbreaks in these areas is maintained by clusters of susceptible human carriers who thus represent a source of long term infection. Longitudinal parasitological surveys have demonstrated that a large proportion of inhabitants who contract malaria during the wet season retain chronic submicroscopic asymptomatic infections throughout the dry season. However, no *Anopheles* mosquitoes are seen during this time of the year. The parasites that persist during the dry season most probably seed the next transmission, following annual rain and the resurgence of mosquitoes.

A study was therefore carried out to evaluate the effectiveness of gametocytocidal drugs administered during the pretransmission season in eliminating gametocytes among

### Conclusions and implications of the study

- During the dry season of areas with seasonal malaria transmission, up to 40% of the population harbour subpatent parasitaemia. Of these, 10%–12% are gametocyte carriers.
- Targeting the gametocyte reservoir by administering gametocytocidal drugs during the pretransmission season has a significant impact in reducing malaria burden in the short transmission season.
- Other interventions such as other antimalarial drugs or combinations should be evaluated in order to devise the most cost-effective intervention for the control of the gametocyte reservoir and hence malaria transmission in these areas.
- To study the efficacy of chloroquine, *crt* is the target gene to be examined since *mdr* detects a low level of mutant alleles. In the transmission season of 2001, chloroquine resistance was high in the intervention village. Sulfadoxine/pyrimethamine was therefore recommended as the first line drug in 2002 due to the reported high sensitivity in the intervention village.



inhabitants before the start of the next transmission season.

## Materials and methods

Taiba and Abu Elnaja villages in Gedaref State were selected as intervention and control villages, respectively. A baseline parasitological survey was carried out in October 2000 in both villages whereby all inhabitants with microscopically diagnosed *P. falciparum* and clinical symptoms were treated. Dry season survey (April 2001–2002) A parasitological survey and molecular screening were carried out in the study villages to identify people with asymptomatic sub-patent *P. falciparum* infection. Inhabitants found to harbour parasites detectable by microscopy and to have malaria symptoms were treated. Malaria parasite-negative samples were examined by polymerase chain reaction (PCR) to detect subpatent *P. falciparum* infections. All PCR-positive samples were examined for the presence of gametocytes using reverse transcription (RT)-PCR. Pretransmission season survey and intervention (August 2001–2002) A workplan similar to that of the dry season was carried out and inhabitants who harboured subpatent gametocytes and/or asexual infection were treated with chloroquine and primaquine. This was followed by a post-intervention survey (transmission seasons, October 2001–2002). Parallel entomological surveys were carried out at the same time as the above parasitological surveys.

Dot blot technique was used for the detection of drug mutant alleles. In the 2001 transmission season, 2 genes: *crt* and *mdr* were examined for typing chloroquine resistance alleles, and *dhfr* gene for typing sulfadoxine/pyrimethamine (SP) resistance alleles.

## Main study findings

**Entomological survey** During the dry season of 2001, the entomological inoculation rate was zero since no infected mosquitoes were captured in the 2 villages. Mosquito density started to increase in the pretransmission season. Low mosquito density was reported during the transmission season of 2002 and no density was recorded during the dry and pretransmission season of that year.

**Detection of subpatent parasitaemia during the dry season (2001–2002)** In 2001, 40% of the studied population harboured subpatent infection in the intervention village compared to 28.75% in the control village. These rates were significantly lower in 2002, when subpatent parasitaemia was reported in 6% and 4% of the population in the intervention and control villages, respectively.

**Gametocyte carriage rates (2001–2002)** 11.9% and 4.3% of the population in the intervention

village were gametocyte carriers as detected by Pfs 18s rRNA and Pfs25, respectively during 2001. In the control village, 7.4% of the subpatent infections harboured gametocytes as detected by Pfs25 and only 3.7% as detected by Pfs 18s rRNA. RT-PCR showed a significant reduction in the prevalence of gametocyte carriage in the intervention village from 12.4% during the pretransmission season of 2001 to 8% during the pretransmission season of 2002. The prevalence of gametocyte carriage remained constant at 10% in the control village during the same period. Parasitological surveys in 2000–2002 The parasite rate reported during the transmission season of 2000 was 0.99% and 0.69% in the intervention and the control villages, respectively. During the dry season of 2001, the rate was very low in the intervention village (0.35%), while no parasite was detected in the control village. During the pretransmission season, no parasite was detected in the intervention village and a low rate was reported in the control village (0.28%). In the transmission season of 2001, the prevalence increased in both the control and the intervention villages recording figures of 3.9% and 3.3%, respectively. During the dry and the pretransmission seasons of 2002, no cases were recorded in the intervention village, whereas rates of 2.5% and 0.34% were detected in the control village. During the transmission season of 2002, many clinical cases were encountered in the control village 32/266 (12%), whereas only 12/312 (3.8%) were reported in the intervention village. Most of the positive cases were either febrile only or febrile with other symptoms during the transmission seasons of 2001 and 2002, while afebrile cases were only encountered during the dry seasons of both years. Detection of drug mutant alleles during the transmission season (2001–2002) The mutant alleles detected by *crt* in both the intervention and the control village were 66.7% and 90.9.7%, respectively, whereas *mdr* detected only 32.26% and 8.7% in the intervention and control villages, respectively. In the transmission season of 2002, a low level of mutant alleles was detected by the *dhfr* gene (3.57% and 12.5% in the intervention and the control villages, respectively).

## Conclusions and recommendations

The study reported the effectiveness of gametocytocidal drugs in the elimination of the gametocyte reservoir during the dry season. This will reduce the malaria burden in the transmission season in areas with seasonal transmission. The study also reported high rates of subpatent infection and gametocyte carriage in these areas.

# Malaria

## Drug Studies

Y e m e n

Rational use of  
antimalarial drugs in  
Hajjah

Yemen  
Hajjah

Study period:  
January–May 2002

Small Grants  
Scheme  
(SGS) 2001 No. 132

Principal Investigator  
Dr Ahmed Abdo-Rabbo  
Department of  
Pharmacology and  
Therapeutics  
Faculty of Medicine and  
Health Sciences  
Sana'a University  
Sana'a, Yemen  
e-mail:  
abdorabbo@y.net.ye

### Abstract

The objectives of the study were to assess prescribing rationality, to describe treatment in households and to identify the available antimalarial drugs in Yemen. Drug use rationality was assessed using WHO drug use indicators, a household survey and recording the available antimalarial drugs at public health facilities, and private community pharmacies and drug stores. The study was carried out in randomly selected public and private health facilities, and households in three districts of Hajjah governorate.

**Results** Several patterns of irrational drug use were identified including inadequate laboratory diagnosis in public health facilities (21.2%), informal prescriptions (over 50%), and badly written prescriptions.

Patient and drug information were unlikely to be stated in prescriptions. Other patterns of irrational drug use in public and private health facilities, respectively, were: polypharmacy (2.7, 4 drugs per patient), high incidence of prescribing antimalarial drugs by brand names (33%, 64%), and misuse and overuse of injectable antimalarial drugs

(18.4%, 33.5%). Inappropriate self-medication practices were also identified. Antimalarial drugs including the newer types are available under different brand names and can be obtained over-the-counter.

**Conclusion** This study reported common patterns of irrational use of antimalarial drugs. This baseline information is necessary for designing future interventions to promote the rational use of drugs in Yemen.

### Background

In many malaria-endemic areas, a majority of the population does not have ready access to antimalarial drugs or to reliable and consistent information about malaria treatment and prevention. Moreover, available drugs are frequently obtained from informal sources. They can also vary in quality, may be partially or completely ineffective against local parasite strains and are often used in inappropriate dosage.

There is very little data on prescribing practices in Yemen for endemic diseases, in general, and for malaria, in particular. Accordingly, this study was carried out to assess the current situation regarding prescribing rationality of antimalarial drugs, to identify the available antimalarial drugs at different health facilities and to collect information regarding the knowledge, attitudes and practice of the community concerning treatment of malaria in households.

### Materials and methods

The study was conducted in three malaria-endemic districts in Hajjah governorate, namely Hajjah, Haradh and Abbs, and at the different levels of health facility, two hospitals, six health centres and nine health units, as well as private health facilities. The approximate number of prescriptions collected were 120 from each public hospital, 60 from each health centre, 50 from each health unit and 60 from each private health facility.

### Conclusions and implications of the study

■ This study reported inadequate laboratory diagnosis, mainly as a result of inadequate/insufficient diagnostic equipment and experienced personnel. Over-prescription of injections is mainly due to lack of appropriate training for health workers, lack of public education and patients' demand.

■ It is important to limit the importation and use of antimalarial drugs to those recommended in the National Standard Treatment Guidelines and National Malaria Programme Treatment Protocols. The way towards rational prescribing of antimalarial drugs is to prescribe the minimum number of drugs per consultation and the correct antimalarial drug(s) at the correct dosage, frequency and length of time, with minimum injections. Also, in order to prescribe economically, it is important to prescribe one antimalarial drug at a time, using the generic name from the national list of essential drugs.

■ Although self-medication is meant for prevention or treatment of disease, it may result in health hazards and economic loss. It is important to find ways of using self-medication to strengthen primary health care through educating providers and consumers in how to avoid the misuse of drugs. In order to reduce the incidence and duration of illness in the community, people should be informed about alternatives to self-medication i.e. seeking advice from a qualified source.

Information related to prescribing practices, availability of antimalarial drugs and malaria treatment in households was also collected. Evaluation of rational drug use was based on the core drug use indicators of the WHO Action Programme on Essential Drugs (WHO/DAP) and the International Network for Rational Use of Drugs (INRUD).

The antimalarial drugs officially registered by the Supreme Board of Drugs and Medical Appliances (SBDMA) as well as those available in private pharmacies, drug stores and public health facilities were investigated by visiting the department of registration at the SBDMA, nine private pharmacies and drug stores (three from each district) and the studied health facilities. Data collectors checked the availability of antimalarial drugs at the public health facilities and recorded their results in a data collection form. Antimalarial drugs registered by the SBDMA available in private pharmacies and drug stores were recorded with information on the name of the product (generic and brand), form, strength, packing and price.

Information related to malaria home management was collected using a questionnaire that was distributed to 179 households (about 60 households from each district). The prescribed and non-prescribed antimalarial drugs found in households were recorded.

## ■ Main study findings

Laboratory examinations for malaria patients were performed for 21.2% of those cases managed at public health facilities; and all patients attending private health facilities. Formal prescription sheets were used for 47.4% of malaria prescriptions collected in public hospitals and 47.3% at health centres, compared to 28.5% in private health facilities. In the health units, registration books are used instead of prescription sheets.

In public versus private health facilities, patient information collected included, respectively, name (98.1%, 100%), sex (21.5%, 6.6%), age (62.1%, 90.7%), address (52.6%, 17.2%) and diagnosis (62.7%, 57.6%). Written information for prescribed antimalarial drugs, such as strength, dose, frequency and duration of administration was more frequently provided in private health facilities compared to public hospitals and health centres, and much less frequently in health units.

The mean number of antimalarial drugs per prescription was 1.1 in public health facilities compared to 1.2 in private health facilities. The highest number of antimalarial drugs in one prescription (n=3) was found in Algomhory public hospital, Mabian health centre and in the private health facilities in Haradh town and Hajjah town.

The percentage of antimalarial drugs prescribed by generic name in public health facilities (67.1%)

was significantly higher than that of the private sector (35.8%). In public health facilities, antimalarial drugs were prescribed in the form of tablets (47.8%), oral liquids (16.9%) and injections (17.2%), while 18.1% were prescribed without mentioning dosage form. The rate of injections was significantly higher in the private sector. Chloroquine was more frequently prescribed in the public (77.7%) compared to the private (55.3%) sector, while quinine was only prescribed in the public sector (1.8%). On the other hand, sulfadoxine/pyrimethamine was more commonly prescribed in the private (27.4%) than in the public (17%) sector, as were primaquine, artemether and halofantrine. Most antimalarial drugs were registered by the SBDMA and were also available in private pharmacies. These drugs are produced by different local and foreign manufacturers under generic or different brand names for the same drug. Chloroquine is the main antimalarial drug imported and locally produced. There are 52 products of chloroquine in different forms and strength available from different manufacturers. A great discrepancy was also observed in the price of different brand names for the same antimalarial drug.

There are four types of antimalarial drugs in different dosage forms on the National Essential Drugs List. Some of the essential antimalarial drugs or their dosage forms were not available at public health facilities, or were available at a lower level. Chloroquine tablets were available in all public health facilities, while chloroquine syrup was out of stock in some health units and injections were found in some health centres but not in one of the two public hospitals. Primaquine was the antimalarial drug found least at health facilities.

An average of 1.3 antimalarial drugs per prescription were used by patients according to formal prescriptions, while the highest number of antimalarial drugs in one prescription (n = 3) were found in Haradh and Abbs households. The mean of non-prescribed antimalarial drugs found in all households surveyed was 0.04, ranging from 0 to 1. Antimalarial drugs found in households were in the form of tablets (43.6%), oral liquids (13.9%) and injections (42.5%). The most frequent type found was chloroquine, and the least frequent was halofantrine.

## ■ Conclusions and recommendations

Common patterns of irrational use of antimalarial drugs were identified in this study. In view of the limited health resources, rational drug use emerges as a cost-effective intervention for reducing malaria burden in endemic countries.

# Malaria

## Drug Studies

Quality of  
antimalarial drugs  
available in Yemen

Yemen  
Lahej Governorate

Study period:  
September 2002–  
November 2003

Small Grants  
Scheme  
(SGS) 2002 No. 187

Principal Investigator  
Dr Ahmed Abdo-Rabbo  
Abdulla  
Department of  
Pharmacology and  
Therapeutics  
Faculty of Medicine and  
Health Sciences  
Sana'a University  
Sana'a, Yemen  
e-mail:  
abdorabbo@y.net.ye

Yemen

### Abstract

A study was carried out to assess the quality of antimalarials (chloroquine and sulfadoxine/pyrimethamine) available in Yemen and to determine whether quality is related to the levels of the distribution chain at which samples were collected. Four samples of each antimalarial product were collected from each of the levels of the distribution chain. Of the four samples, one was kept with the research team, two were tested at the drug quality control laboratories in Sana'a and Aden, and the fourth was sent to the Centre for Quality Assessment of Medicines in Potchefstroom, South Africa, for analysis. Quality indicators measured were the content of the active ingredient and dissolution rate (for tablets only) in comparison to standard specifications for these products in the relevant pharmacopoeia.

**Results** Several substandard products within the drug distribution chain were identified. These included high and low failures in the

ingredient content for chloroquine tablets and chloroquine syrup. There was some dissolution failure for chloroquine tablets and high dissolution failures were found at most of the collection points for sulfadoxine/pyrimethamine tablets, mostly with the dissolution of pyrimethamine. No clear relationship between the quality of products and the level of the distribution chain was observed. Similarly, there was no difference between locally manufactured and imported products.

**Conclusion** Substandard antimalarial drugs circulate within the drug distribution chains in Yemen. This appears to be due to noncompliance with good manufacturing practice guidelines by manufacturers in the production of these antimalarials.

### Background

Malaria tops the list of diseases causing high morbidity and mortality in Yemen, with about half the population at risk of malaria. Treatment failure, ascribed to resistance, may also be due to low quality, yet in most countries the quality of antimalarials is rarely independently evaluated, and the local capacity for independent drug quality assurance is worst where the disease burden is highest. Poor quality of antimalarials poses a problem to malaria control and hence to public health, especially in countries where there is little or no drug regulatory infrastructure. A study was therefore conducted to assess the quality of commonly used antimalarial drugs in Yemen and to evaluate the performance of the national reference laboratories.

### Materials and methods

Chloroquine phosphate 250 mg tablets, chloroquine base 50 mg/5 ml syrup and sulfadoxine/pyrimethamine 500 mg/25 mg tablets were sampled and evaluated. Samples were collected at various levels of the drug distribution chain in the public and private sector in Lahej Governorate such as medical stores, teaching hospitals, district hospitals, health centres, health units and private sector

## Conclusions and implications of the study

■ The study identified several problems of substandard products within the drug distribution chain. These included high and low failures in ingredient content for chloroquine tablets (CQT) and chloroquine syrup (CQS), some dissolution failure for CQT and high sulphadoxine/pyrimethamine tablets (SPT) dissolution failures, mainly attributed to pyrimethamine at most collection points. These findings have serious implications not only on the reduced therapeutic effectiveness of antimalarial drugs but also on the development of drug resistance.

■ No clear relationship between the quality products and the level of the distribution chain was observed. Similarly there was no difference between locally manufactured and imported products.

■ Regarding the evaluation of the national reference laboratories performance, there was no significant difference between the national laboratories in Sana'a and Aden and that of the international laboratory CENQAM in South Africa regarding CQS, CQT or SP ingredient content, and CQT dissolution. On the other hand, there was a significant difference between them regarding the SP dissolution whereby 30% of SP tested were fulfilling the USP criteria in CENQAM international laboratory compared to all tested products in the national laboratories.

■ Testing for initial quality should be given high priority. Routine testing of new supplies as well as monitoring and supporting manufacturers' and suppliers' Good Manufacturing Practice (GMP) compliance is recommended.

pharmacies. A supervisor was chosen to coordinate sample collection following the standard sampling protocol, coding method and packaging for all health facilities involved in the study, i.e. standard operating procedures on collection of samples. The total number of samples collected from private community pharmacies/drug stores and the public drug distribution chain, as well as different health facility levels, were 25 chloroquine tablets, 15 chloroquine syrup samples and 10 sulfadoxine/pyrimethamine tablets. Four samples from each batch were collected from each private community pharmacy/drug store and each level of the public drug distribution chain. Of these samples, one was kept with the research team, one was tested at the Sana'a drug quality control laboratory, one was tested at the Aden drug quality control laboratory and one was sent to the Regional Office and then to the Centre for Quality Assurance of Medicine (CENQAM) in Potchefstroom, South Africa, for analysis. Samples were analysed according to pharmacopoeial specifications to assess the quality of the products. Tests were done on the content of the active pharmaceutical ingredient for tablets and syrup, and on the dissolution and content for tablets.

### ■ Main study findings

**Chloroquine syrup** was found in all health facilities except one district medical store, one health centre/rural hospital and four health units, and was found expired in three health facilities. Chloroquine tablets were found in all studied health facilities. In two facilities, the available chloroquine was from two different manufacturers in each. Sulfadoxine/pyrimethamine was not found in all studied health units as it is not officially supplied according to Yemen's treatment guidelines and essential drugs list.

**Chloroquine syrup** The percentage of chloroquine phosphate contents in syrup in all studied samples tested at the three reference laboratories ranged from 90.7% to 116.2%. For chloroquine syrup, only one product had some high content failures, i.e. sample content above the upper limit of specification. The highest ingredient content failure (116.2%) was recorded at Tor-Albaheh rural hospital/health centre and was manufactured by Yedco (Yemen). **Chloroquine tablets** The percentage of chloroquine contents in chloroquine phosphate tablets for all studied chloroquine tablet samples analysed at the three reference laboratories ranged from 89.5% to 110.3%. Most failures were low i.e. sample contents below the minimum recommended levels for the products. The lowest percentage failures in ingredient content were recorded for three products at Al-Raga health unit in Tor-Albaheh District, the central medical store in Toben District and the regional medical store in Aden. The three products were manufactured by Yedco.

The dissolution rate for all studied chloroquine tablet samples ranged from 73.1% to 113.3%. In addition, two products had high content failures. The highest ingredient content failures (107.7% and 110.3%) were found in Akan health unit and Al-Mosimir rural hospital in Al-Mosimir District, and were manufactured by Yedco and Pharmed (the Netherlands), respectively. Chloroquine tablet dissolution failures were recorded for two products, one at Al-Fioosh health unit in Toben District manufactured by Alkaloida Chemical Company Ltd. (Hungary), the other found at Radfan rural hospital in Al-Habilin District, manufactured by Yedco.

**Sulfadoxine/pyrimethamine tablets** The sulfadoxine/pyrimethamine contents in all sulfadoxine/pyrimethamine tablet samples analysed at the three reference laboratories ranged from 93.0% to 102.9% for sulfadoxine and from 91.4% to 104.5% for pyrimethamine. None of the tested products had low or high content failure i.e. sample contents were found to be within the recommended level for the products. The dissolution rate for all studied sulfadoxine/pyrimethamine tablet samples ranged from 85.7% to 105.1% for sulfadoxine and 22.9% to 106.7% for pyrimethamine. High sulfadoxine/pyrimethamine tablet dissolution failures were found at most collection points. Only three products complied with the criteria for both active ingredients. The first product was found at the central medical stores, Lahej Governorate, the second at Eskander private drug store in Toben District manufactured by Intas Pharmaceuticals Ltd., (India) and the third at Al-Ikhlās private drug store in Al-Mosimir District, manufactured by Roche (Switzerland).

There was no significant difference between the national drug quality control laboratories in Yemen and CENQAM regarding the active ingredients for chloroquine tablets, chloroquine syrup and sulfadoxine/pyrimethamine tablets, or for chloroquine tablet dissolution. On the other hand, there was significant difference between them regarding sulfadoxine/pyrimethamine tablet dissolution; only 30% of sulfadoxine/pyrimethamine tablets tested by CENQAM fulfilled United States Pharmacopeia (USP) criteria, compared to 100% in the national drug quality control laboratories.

### ■ Conclusions and recommendations

Substandard antimalarial drug products exist in different districts and at different levels of the distribution chain in Lahej Governorate, Yemen.

National regulatory authorities should give more emphasis to the supervision of manufacturing and distribution channels, and monitoring and supporting manufacturers and suppliers to improve manufacturing practice compliance, procurement, storage and distribution policies.

# Malaria

## Drug Studies

### Sudan

Deployment of antimalarial drug combinations in control of malaria in an area of markedly seasonal and unstable transmission in Sudan

Sudan  
Gedaref State

Study period:  
Phase 1: November  
2002–December 2003

Small Grants  
Scheme  
(SGS) 2002 No. 110

Principal Investigator  
Dr Hayder Giha  
Department of Biochemistry  
Faculty of Medicine  
University of Khartoum  
Khartoum, Sudan  
e-mail:  
hgeha@hotmail.com

#### Abstract

Use of drug combinations is a strategy adopted for better cure of malaria, delay of development of resistance and reduction of transmission. A randomized clinical trial was therefore carried out in Daraweesh and Kajara, 2 neighbouring villages in eastern Sudan, to assess the efficacy of 2 combinations of antimalarial drugs: chloroquine (CQ) plus sulfadoxine/pyrimethamine (SP) and CQ plus dihydroartemisinin (DHA).

**Results** Most of the patients ( $n = 52$ ) in the 2 villages who developed uncomplicated *Plasmodium falciparum* malaria during the malaria season of 2002 were enrolled in the study, and half were randomly allocated to each of the 2 combinations of treatment. Adequate clinical response and parasite clearance was found to be 64% and 69.6% in the CQ/SP and CQ/DHA treatment groups, respectively. The corresponding rate of treatment failure in both groups was mainly due to parasite resistance type RI, with low parasitaemia and mild, vague or no symptoms. The recrudescence was proved by genotyping and the prevalence of gametocytaemia after

treatment was significantly lower in the CQ/DHA group ( $p = 0.02$ ).

**Conclusion** The study revealed a lower than expected efficacy of both SP and DHA in parasite clearance in this area.

#### Background

A single control strategy for all African countries, or even for all districts in a single country, is very unlikely to be successful. This is because the epidemiology of malaria varies considerably from place to place and consequently drug resistance patterns and the magnitude of protective immunity also vary. This necessitates regional data collection and analysis followed by adoption of a suitable strategy. Among the new methods in malaria control is the use of drug combination in the management and control of the disease. Drug combination is believed to increase the effectiveness of individual drugs in a synergistic manner, and to halt the chances of development of separate resistance to each drug.

There is evidence that chloroquine (CQ) resistance in Sudan is spreading widely and that for sulfadoxine/pyrimethamine (SP), is increasing (unpublished data). Unless much greater control efforts and strategies are adopted, the spread of highly resistant parasite types will have disastrous consequences. Artemisinin has been proven to be very efficacious in resolution of malaria symptoms, with potent anti-parasitic and transmission blocking activities. Logically, artemisinin should be conserved for severe malaria, as a standby for or alternative to quinine; however, there is a current trend for its use in combination with other antimalarials for treatment of uncomplicated malaria.

A study was therefore undertaken to test the efficacy of dihydroartemisinin (DHA) in combination with CQ versus SP in combination with CQ in the treatment of uncomplicated *Plasmodium falciparum*

### Conclusions and implications of the study

- Despite the small sample size, the unexpectedly high failure rate of treatment made the results of this study highly informative.
- A very high frequency (>80%) of pfprt and pfmdr1 mutations was reported from the same region (Gedaref State) in 2003. Therefore, the ~70% parasitological effectiveness of both combinations could be attributed to an additive effect to CQ, or stands for the efficacy of SP and DHA alone.
- The implications of the above findings is important because Sudan was planning to adopt drug combination therapy for the treatment and control of malaria at the time of conducting the study, and the 2 arms of the suggested combination were SP and artesunate. Starting with a 30% failure rate for each arm seems perturbing.
- In areas hyperendemic for malaria, the efficacy of artemisinin derivatives in parasite clearance would be difficult to assess, since children carry the malaria parasites throughout most of their lives irrespective of the clinical features. While it is easier in this setting to report such a relatively high rate of parasite resistance to artemisinin derivatives with high confidence, this might not have a clinical significance per se. However, such a low level might act as a parasite reservoir and influence parasite transmission in the long term.

malaria. Deployment of drug combination with transmission blocking components is expected to suit this epidemiological setting, in which there is a seasonal pattern of malaria transmission in conjunction with a low entomological inoculation rate.

## Materials and methods

A randomized clinical trial was carried out in 2 neighbouring villages, Daraweesh and Kajara, in Gedaref State, eastern Sudan. *Anopheles arabiensis* is the sole malaria vector and the entomological inoculation rate in the region is very low. Malaria transmission is highly seasonal, peaking during October/November with considerable variation in incidence from year to year. The estimated sample size was 50 and 100 individuals for each of the 2 types of combination. Only half of the minimum sample size was enrolled during the transmission season 2002–2003 (phase 1). These included most of the patients who developed malaria during the malaria season in the 2 villages. However, more cases will be enrolled during the transmission season of 2004 (phase 2).

The health team was available in the villages on a daily basis; individuals complaining of symptoms suggestive of malaria had blood smears (thin and thick blood films) and blood taken on filter paper. The former were stained with Giemsa and read under ordinary microscopy. Parasite and gametocyte counts were performed for positive slides. Febrile patients with positive blood smears were randomly allocated to either of the 2 treatment regimes: CQ plus SP, with CQ in a dose of 10 mg–10.5 mg/kg/day over 3 days and SP in a single dose of 1.25 mg/kg; or CQ plus DHA, with CQ given as above and DHA in an adult dose of 120 mg once on the first day, and 60 mg/daily for 6 days, while for children the dose was adjusted according to age as per the packed leaflet (COTECXINCHINA).

Patients were investigated for malaria (clinical and parasitological) on days 0, 1, 2, 3, 7, 14, 21 and 28, according to the WHO protocol for in vivo studies of drug resistance. Clinical information was recorded in follow-up sheets and later entered in a database programme. Blood smears, blood samples (5 ml) and filter papers were always taken.

Parasite DNA was prepared from filter papers, and a limited number of parasite isolates were genotyped to distinguish true recrudescences (resistance) from new infection. Genotyping of the parasite DNA samples was carried out using the *P. falciparum* MSP-1 gene Block 2-specific primer pairs, the *P. falciparum* MSP-2 gene IC1 and FC27 primer pairs.

## Main study findings

The age range of the patients was 4–47 years, with a mean age ( $\pm$ SD) of 16.9  $\pm$ 10.6 years. Overall, 26

patients (50%) were treated with CQ plus DHA, while the others were treated with CQ plus SP. Four patients were withdrawn from the study, 1 on day 3 and the other 3 on day 14; 1 was treated with CQ/SP and 3 with CQ/DHA. All were successfully treated, but were excluded from the analysis. In the CQ/SP treated group ( $n = 25$ ), 64% (16/25) of the patients achieved adequate clinical response and complete parasite clearance, while 36% (9/25) had resistant parasites. Of these, no patients had early treatment failure, 7 had late treatment failure, 1 patient had parasitaemia with RI and 1 patient had parasitaemia with RII-type resistance. Of the 9 patients, only 6 had malaria symptoms during follow-up. In the CQ/DHA treated group ( $n = 23$ ), 69.6% (16/23) of the patients had adequate clinical response with complete parasitaemia resolution, and a resistance rate of 30.4% (7/23). Of these, none had early treatment failure, 3 patients had late treatment failure with RI resistant parasites and 4 patients had asymptomatic infection with RI-type of parasites. The efficacy of both combinations was comparable, with no clinical and/or parasitological significant difference between the treated groups. The serial blood samples (parasite DNA) that were obtained from the 16 patients with recurrent parasitaemia were genotyped at 2 loci: MSP1 (K1, MAD20, RO33) and MSP2 (3D7/IC, FC27). Genotyping revealed that all parasites seen after diagnosis (during follow-up) had all or some of the alleles seen before treatment (D0). The differences were mainly due to the reduction in clone numbers in the samples of the follow-up. The overall gametocyte carriage at the time of diagnosis was 19.2% (10/52); 13.5% (7/52) in the group that was treated later with CQ/DHA and 5.8% (3/52) in the group that was treated with CQ/SP. Thereafter, during the 28 days of the follow-up, gametocytes were detected in 81 blood samples; 53 (65.4%) from patients treated with CQ/SP and 28 (34.6%) from patients treated with CQ/DHA. ( $p = 0.020$ , McNemar test). The peak of gametocytaemia in both groups occurred at day 7 of the follow-up, which accounted for 22.6% and 25% of all gametocyte carriage in the CQ/SP and CQ/DHA treatment groups, respectively.

## Conclusions and recommendations

The addition of SP or DHA to CQ for management of uncomplicated malaria in this area improved the previously known efficacy of CQ in parasite clearance and symptoms resolution. Additionally, the advantage of DHA over SP in gametocyte clearance is clearly shown in this area for the first time. Finally, the study revealed a lower than expected efficacy of both SP and DHA in parasite clearance in this area, a fact that will have consequences for the control programme in the long term.

# Malaria

## Epidemiology

### Sudan

Epidemiology of malaria among displaced people in Khartoum State, Sudan

Sudan  
Jebel Awlia and Cartoon  
Kassala camps, rural  
Khartoum

Study period:  
November 2001–  
December 2002

Small Grants  
Scheme  
(SGS) 2001 No. 36

Principal Investigator  
Dr Intisar El-Fadil Saeed  
Afhad University for Women  
Omdurman, Sudan  
e-mail:  
intisarsaeed@yahoo.com

#### Abstract

A cross-sectional study was carried out among displaced people in Khartoum State to determine risk factors for malaria and determinants of mortality from malaria by verbal autopsy among displaced populations. A total of 856 households were visited and the head of each household interviewed. Data regarding sociodemographic characteristics, history of malaria, treatment-seeking behaviour and mortality attributed to malaria during the previous year were collected in a pretested questionnaire.

**Results** Two-thirds of the study population reported having at least one episode of malaria during the previous year. The frequency of malaria attacks was found to be associated with ethnic origin (tribe), language, education, water supply and food expenditure. The majority (85.5%) of those reporting previous malaria attacks were local language speakers. The majority (70.4%) of illiterate respondents had history of malaria attacks during the previous year. Approximately half the respondents (50.2%) delayed seeking treatment at health facilities.

A total of 81 (9.6%) deaths due to malaria were reported among visited households. Fever, diarrhoea, vomiting and headache were reported as the most prevalent symptoms before death. Of 81 families with deaths due to malaria, 76.5% reported a malaria attack

in the family during the previous year. The study revealed a higher mortality among those with poor knowledge, the fairly educated and local language speakers. The mortality rate of 11.6% among those obtaining water by cart was significantly higher than the 7.5% rate among those obtaining it from a well. Mortality was similar in both sexes and according to age. Housing conditions, crowding and food expenditure of the family did not influence the mortality rate.

**Conclusion** The determinants of malaria morbidity and mortality among displaced populations are residence, ethnic group, language, educational level, level of knowledge and source of water supply.

#### Background

In Sudan, malaria accounts for 25.7% of total hospital admissions and 15.9% of total deaths [1]. Displaced populations in southern Sudan suffer from a high incidence and prevalence of malaria. Moreover, chloroquine-resistant malaria aggravates the problem. Measuring malaria mortality is very difficult since in many parts of rural Africa as many as 90% of deaths from the disease occur at home and are not registered in any formal way [2]. Therefore, verbal autopsy has been adopted as a tool to measure mortality in infants and children. Information regarding the determinants of malaria morbidity and mortality among displaced populations is deficient. Hence, this study was carried out to determine the risk factors for contracting malaria, the mortality rate by verbal autopsy and the determinants of malaria mortality among displaced populations in Khartoum State.

#### Materials and methods

There are about two million displaced people in Sudan. Of these, around 400 000 are resident in four official camps in Khartoum State. The study was carried out in two of these, namely Jebel Awlia and Cartoon Kassala camps. A cross-sectional study was carried out in 272

### Conclusions and implications of the study

- Malaria episodes and mortality from malaria were significantly higher among those speaking a local language and those with a low level of knowledge. This is probably because local language speakers depend primarily upon self-medication using traditional remedies and consequently delay seeking treatment. Conveying health education messages in local languages is therefore recommended.
- The majority of the study population believed malaria is a serious disease and identified mosquito bites as the cause of malaria. However, more than 40% of the population delayed seeking treatment due to economic constraints and use of traditional remedies.
- Only a small proportion (18.8%) of the study population owned bednets due to the price being unaffordable for the majority.
- The study found that malaria deaths were reported more among those who obtained water from carts. Provision of safe water supply should be ensured for these populations.



households in Jebel Awlia camp and 584 households in Cartoon Kassala camp. The head of each household was interviewed according to a questionnaire that collected demographic, social and behavioural data. Information about malaria attacks and mortality attributed to malaria during the previous year was also recorded. Summation scores for knowledge, attitudes and practices as well as treatment-seeking behaviour were developed and categorized into adequate or inadequate using a median cut off level.

## ■ Main study findings

Females constituted 85.7% of those interviewed. Housing conditions differed greatly between the two camps, being worse in Jebel Awlia camp.

The population of Jebel Awlia camp obtained water from public hand pumps, whereas in Cartoon Kassala camp, 69.2% obtained water from carts and the rest from public hand pumps. Only 0.9% of households kept water for more than a week. The dominant (66.4%) tribes in the two camps are from southern Sudan, the rest being mainly from western Sudan. The main languages spoken in Jebel Awlia camp (77.2%) and Cartoon Kassala camp (72.9%) were Dinka and Dinka plus Arabic. Health services were provided free of charge by health centres for 55.5% of respondents in Jebel Awlia camp and 40.1% of respondents in Cartoon Kassala camp.

More than half the respondents had good knowledge about malaria, although this was significantly higher among inhabitants of Jebel Awlia camp compared to Cartoon Kassala camp. In contrast, attitudes and treatment-seeking behaviour in Cartoon Kassala camp were significantly better than in Jebel Awlia camp. The majority of the population believed malaria is a serious disease and fever was identified by 83.8% as the most common symptom associated with malaria. Other symptoms (rigors, vomiting, diarrhoea and headache) were mentioned more frequently by inhabitants of Jebel Awlia camp. Chloroquine was mentioned as a malaria treatment by 72.2% of the respondents.

Mosquito bites were identified as the cause of malaria by 72.8% of respondents at Jebel Awlia camp and 79.1% at Cartoon Kassala camp. Suitable mosquito breeding sites were correctly described by 86.7% of respondents. The principal method used to keep mosquitoes away described by respondents in both camps was burning herbs (47%) followed by use of bednets (18.8%), spraying (14.6%) or other methods (mixture of burning herbs plus spraying, or oil).

Health centres were cited as the first resort for treatment by 23.5% of respondents in Jebel Awlia camp and 63.9% in Cartoon Kassala camp. Delays of 2 or more days in seeking treatment at health

services were mentioned by 430 (50.2%) of all respondents, while 6.2% stated that they never attend health services. Reasons for delay in seeking treatment were waiting for improvement (24.4%), waiting for the effect of traditional remedies (20.6%) and lack of money (18.5%). Incorrect chloroquine dosage was cited by (28.7%) of respondents.

The frequency of malaria attacks was significantly associated with language, tribe, education and food expenditure. Local language speakers reported the highest frequency (85.5%) of malaria attacks during the last year and were at a 2.71 fold increased risk for contracting malaria compared to those speaking Arabic plus local languages, while southern tribes were at a two-fold increased risk compared to other tribes. Economic constraints and illiteracy were also significant risk factors for malaria attacks. Knowledge, attitudes and practices had no significant influence on malaria attacks, but respondents who obtained water from carts were at a five-fold increased risk for contracting malaria compared to those getting water from wells.

Death due to malaria was reported by 9.6% of respondents in both camps. This figure is rather high and can be explained by the inclination to diagnose most febrile conditions as malaria since the point prevalence at the time of the survey during the transmission season did not exceed 3%. Fever, diarrhoea, vomiting and headache were reported as the most prevalent symptoms before death. There was a significantly higher risk of reported mortality from malaria among those obtaining water from carts.

Only 37.7% reported adequate health-seeking behaviour. This group recorded a lower mortality rate (8.4%) compared to those with inadequate health seeking behaviour.

## ■ Conclusions and recommendations

Risk factors for malaria morbidity and mortality in displaced population camps are: residing in Jebel Awlia camp, illiteracy, belonging to a southern tribe, speaking a local language, inadequate knowledge regarding malaria and unsafe water supply. For reduction of mortality among displaced populations it is recommended to provide affordable health services and to encourage early treatment-seeking at health facilities. It is also recommended to deliver educational programmes in local languages and to provide safe water supply.

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# Malaria

## Epidemiology

### Sudan

Stratification of Khartoum urban area by the risk of malaria transmission

Sudan  
Khartoum urban area

Study period:  
January 2002–  
March 2003

Small Grants  
Scheme  
RBM 2001 No. 1

Principal Investigator  
Dr El-Fatih Malik  
National Malaria Control  
Programme  
Federal Ministry of Health  
Khartoum, Sudan  
e-mail:  
malarsud@hotmail.com

### Abstract

A cross-sectional community-based study was undertaken to stratify Khartoum urban area by the risk of malaria transmission. Surveys were conducted in the last week of January, May and September 2002, whereby 8092 individuals residing in 2000 households were surveyed. The collected information consisted of clinical, parasitological, entomological, meteorological and environmental variables.

**Results** The overall prevalence of malaria was very low as indicated by parasite, spleen and fever rates (0.21%, 0.17% and 0.97%, respectively). Access to health services was found to be very high, but only 3.8% of households kept antimalarial drugs at home. The majority of the population (63%) seek care from the public sector, 24.8% from the private sector and the rest from nongovernmental organizations. The use of protective measures was very low, with 10% of households having mosquito nets, 5% having screened windows, and 1.8% having both. Overall results for the Khartoum urban

area found that Droshab, Umbada, Halfya, Bahry, Elmukhtar, and Greef E. zones were at higher risk of getting malaria. Five of these zones are in Greater Bahry.

**Conclusion** People living in Greater Bahry have a greater burden of malaria with a seasonal pattern of transmission extending from January to May. They use less protective measures compared to Khartoum or Omdurman.

### Background

Khartoum State is divided by the Blue and White Nile into 3 main areas: Khartoum, Greater Bahry and Omdurman. The 3 areas differ in their malaria vulnerability due to differences in topography, agriculture and socioeconomic activities. Malaria constitutes a public health problem in this area, mainly due to *Plasmodium falciparum*. *Anopheles arabiensis* is considered to be the sole vector.

Efficient and cost-effective interventions need to be developed for each location separately in order to target high-risk zones. A study to stratify Khartoum urban area according to the risk of malarial transmission was therefore undertaken to facilitate the planning of effective preventive and control measures.

### Materials and methods

A cross-sectional community-based study was conducted whereby surveys were carried out in the last week of January, May and September 2002 in each of the 15 zones of Khartoum urban area. During each of the 3 surveys, 300 individuals were enrolled from each zone. Each zone was divided into 3 sectors, with 1 sector covered in each period. Data were collected by trained personnel in 3 teams: community, entomological and environmental survey teams.

The household-based data were collected by a trained social worker, public health officer, sanitary officer, 2 laboratory technicians and a medical doctor.

## Conclusions and implications of the study

■ This study reported low malaria prevalence in Khartoum urban area, which could be attributed to the Khartoum malaria-free project that was launched in May 2002. The project was the product of a partnership between WHO, the Federal Ministry of Health and Khartoum State Government. This indicates that Khartoum is now hypoendemic and no longer a mesoendemic zone as previously categorized.

■ The relatively small number of anopheline mosquitoes collected throughout the survey, with zero sporozoite rates, explains and confirms the low prevalence. By contrast, the considerable number of culicine mosquitoes detected in almost every house surveyed, has raised suspicion of malaria and thus many febrile cases have been wrongly diagnosed as malaria. The possibility of the coexistence of other illnesses transmitted by this vector cannot be ruled out.

■ Though breeding sites were detected everywhere, anopheline mosquitoes were only found in zones adjacent to agricultural areas, with none being detected in Greater Omdurman, which has very few irrigated areas.

■ The use of protective measures seems to be very limited in this area, with only a minority of households found to have nets or screened windows. Protective measures, particularly insecticide-treated nets, should be promoted and their distribution strengthened.

Information was collected through history taking and by clinical examination. The entomological and environmental surveys were conducted by an entomology technician and a public health officer, respectively. Overall, 450 houses were surveyed for adult mosquitoes, and identification of the anopheline species was carried out using the Gilles identification keys.

Thick and thin blood film were stained with 5% Giemsa stain for 30 minutes and 100 field were examined at 10 x 100 magnification by experienced microscopists for the presence of trophozoite stage, gametocytes and for species identification. The density was estimated by counting the number of asexual stages associated with 200 white blood cells.

### ■ Main study findings

**Households survey** 8092 individuals residing in 2000 households in the 15 Khartoum urban zones of Khartoum State were surveyed. Their age ranged from 1–95 years, 62.2% were females and 37.4% were males. 10% of households had mosquito nets, 5% had screened windows, and 1.8% had both. Access to health services seemed to be very high, with 97.9% of households being within 5 km from the nearby health facilities. This may explain why only 3.8% of households keep antimalarial drugs at home. The majority of the population (63%) seek care at the public sector, 24.8% at the private sector and the rest at nongovernmental organizations.

There was significant difference between the 15 urban zones and between the 3 greater Khartoum areas regarding the screening of windows, use of mosquito nets, access to services, presence of antimalarial drugs in the home and preference for health sectors. Greater Bahry and its 6 urban zones had a significantly lower number of houses using mosquito nets or having antimalarial drugs in the home.

One quarter of the population reported a positive history of malaria within the last month prior to the survey, suggesting a high incidence of malaria. By contrast, the overall prevalence of malaria was very low as reflected by parasite (0.21%), spleen (0.17%) and fever (0.29%) rates, with no significant difference between those aged 2–9 years and the older age groups regarding the parasite and spleen rates. Greater Bahry showed the highest parasite and spleen rates, but the difference from the other greater Khartoum areas was not statistically significant. In fact, Greater Bahry contained almost 60% of households with detectable parasite, 65% of those with enlarged spleen, 50% of the gametocytes detected and a greater rate (9.7%) of those with a history of being treated for malaria.

Mapping of the 15 urban zones revealed that both a high incidence and high prevalence of malaria were superimposed in the following zones: Droshab,

Umbada, Halfya and Greef E, indicating that these are high-risk malaria zones. Multivariate logistic regression analysis showed that the significant risk factors for contracting malaria in the month prior to the survey were: age <5 years, female gender, region (Greater Bahry and Omdurman having significantly higher risk than Khartoum) and season (a higher risk during May and September compared to January). **Entomological survey** In addition to the 196 outdoor permanent (mainly irrigated areas) and 132 outdoor temporary (mainly broken water pipes and stored water) breeding sites found near or within residential areas, indoor breeding was found in 2.8% of the surveyed households, with a significant difference between the 15 urban zones. Greater Khartoum showed a higher rate of indoor breeding, almost double that of Greater Bahry. There was no significant seasonal variation regarding indoor breeding sites.

Anopheline mosquitoes were detected in 6 (4.0%), 5 (3.3%) and 21 (14%) houses in the January, May and September surveys, respectively (overall 7.1%). Droshab and Kalakla zones reported adult mosquitoes in all 3 surveys, while Haj Yousif zone reported them in the May and September surveys. Halfya zone reported the highest numbers of household with mosquitoes (60% of houses), but only in the September survey.

Interestingly, nuisance mosquitoes (*Culex*) were identified in 14 zones during the January survey, in 9 zones in the May survey and in all zones during the September survey. All anopheline mosquitoes were identified as *Anopheles arabiensis*. Dissection of sporozoite rate using saline and Giemsa stain revealed no sporozoites.

**Environmental survey** During 2002, the upper temperature in Khartoum State ranged from 28.2 °C to 41.9 °C and the lower temperature from 13.7°C to 27.8 °C. The relative humidity varied between 17.7% and 61.8% reaching the highest point in August and the lowest in May.

### ■ Conclusions and recommendations

The recorded frequency of malarial attacks during the month prior to the surveys was 25.5% indicating a high malaria burden in the area, with a point prevalence of 0.2%. However, there are serious doubts about the former rate due to a clear tendency to diagnose any febrile illness as malaria. The study found that the Droshab, Halfya, Bahry, Elmukhtar, Greef E (in Greater Bahry) and Umbada (in Greater Omdurman) urban zones were at higher risk for malaria in the Khartoum urban area. The Khartoum Malaria-Free Project should give greater emphasis to increasing the use of insecticide-impregnated bednets by the community and better malaria diagnosis. More attention should be given to areas with a high malaria risk.

# Malaria

## Epidemiology

### Morocco

#### Malaria in Morocco: Evaluation of the current level of the transmission

Morocco  
Al Hoceima, Chaouen and  
Taounate, northern region  
and Khouribga, central  
region

Study period:  
January–November 2002

Small Grants  
Scheme  
(SGS) 2001 No. 42

Principal Investigator  
Dr Mohammed Lyagoubi  
Institut National D'Hygiene  
Rabat, Morocco  
e-mail:  
mlyagoubi@hotmail.com

#### Abstract

The malaria transmission level of *Plasmodium vivax* was monitored at four high-risk districts in north and central Morocco. Intensive mosquito collections were carried out using the two classic sampling techniques of daytime resting catches and light trap catches each month during the active malaria transmission season from May to October 2002. A parasitological survey was conducted and all *Anopheles sergentii* and *Anopheles labranchiae* females were tested for the presence of two phenotypes of *P. vivax* (PVK210 and PVK247) antigen by enzyme-linked immunosorbent assay.

**Results** Four species were identified in these regions: *An. labranchiae*, *An. sergentii*, *An. cinereus*, and *An. claviger*. *An. cinereus* was most abundant followed by *An. sergentii*, then *An. labranchiae*. No *P. vivax* antigen was detected in 1347 analysed mosquitoes. Parasitological investigation of 2665 out of 4343 blood samples found that they were all negative for *P. vivax*.

**Conclusion** This study suggests the interruption of malaria transmission in the residual focus of malaria in Morocco and recommends the use of ELISA assays for routine surveillance and prevention of malaria resurgence in these areas.

Activities conducted within the framework of the project

The ELISA assay was introduced in the Laboratory of Entomology, Institut National D'Hygiene, Rabat, Morocco.

#### Background

In 1998, Morocco initiated a programme for the elimination of the locally transmitted malaria, *Plasmodium vivax*. Malaria control activities resulted in regression of the number of cases so that no cases were registered in 2001. However, the frequent atypical clinical forms of *P. vivax*, its long latent period and the presence of the vector in high-risk areas increase the probability of malaria transmission in these areas. It was therefore important to confirm the interruption of transmission and monitor the situation by mass screening for sporozoites of the *Anopheles* mosquito. The use of fast immunological techniques, especially enzyme-linked immunosorbent assays (ELISA), has widely replaced manual dissection for detecting and determining mosquito sporozoites [1]. The main advantage of circumsporozoite enzyme-linked immunosorbent assays (CS-ELISA) is that it is species-specific and can detect sporozoites in pooled samples. Processing pooled specimens is a rapid, efficient and economic method for determining sporozoite rates, especially in areas of low malaria endemicity where dissections may not be conducted routinely [1].

The present study was conducted in a residual focus of malaria in Morocco, using CS-ELISA assays. The aim was to monitor the level of malaria transmission by measuring sporozoite infection rates of anopheline mosquitoes for *P. vivax*.

#### Materials and methods

**Study area** The study was conducted in the north region (Al Hoceima, Chaouen and Taounate) and central region (Khouribga), regions of Morocco which are classified, according to epidemiological data, as high-risk zones. **Parasitological survey** (January–October 2002) A malaria

### Conclusions and implications of the study

■ *An. labranchiae* and *An. sergentii* are present, at considerable densities, during the malaria transmission season that extends from May to November in Morocco. *An. sergentii* prevails in mountain villages, whereas *An. labranchiae* is more abundant in valley villages.

■ The negative results obtained by CS-ELISA and parasitological investigation suggest the absence of sporozoites circulation in *Anopheles* mosquitoes, thereby providing evidence about the efficiency of the malaria elimination strategy adopted by the Ministry of Health since 1998.

■ The CS-ELISA assay could be a useful surveillance tool to prevent resurgence of malaria. Its integration in the entomological surveillance of the National Malaria Control Programme is therefore recommended.

prevalence survey, parallel to the entomological survey, was conducted in the eight study villages. Entomological survey (May–October 2002) Mosquitoes were collected by daytime resting catches and light trap catches during the active malaria transmission season. Using a torch and a suction tube, the mosquitoes were collected while resting on walls, clothes and other areas in houses. Light trap catches were carried out using four CDC light traps in each village.

All collected mosquitoes were first stored alive in paper cups covered with netting and placed in a cold box, and then killed and stored individually in a polypropylene micro centrifuge tube with silica gel, which was refrigerated.

**Anopheline identification and conservation**  
The species of the adult female anopheline mosquitoes collected were identified using the keys developed by et al (2000) [2]. All *An. labranchiae* and *An. sergentii* females were bisected into head-thorax and abdomen. The head-thorax was kept dry and stored in the refrigerator (+4°C) until specific ELISA tests [1] were done for the presence of CS antigens for two *P. vivax* phenotypes (PVK210 and PVK247) [3].

**Enzyme-Linked Immunosorbent Assay (ELISA)**  
The collected mosquitoes were divided into pools according to species, collection site, collection date and method. Pooled mosquitoes were triturated in 50 µl blocking buffer (BB) with Igepal 630 (IG-630) using a motorized pestle in a 1.5 ml polypropylene microcentrifuge tubes. The pestle was cleaned between samples by high-speed passes in beakers of phosphate-buffered saline-Tween 20 and then dried to prevent contamination. Aliquots of mosquito triturate were frozen for later analysis. Subsequently, 96-well microtitre plates were incubated overnight with 50 µl of each monoclonal antibody (MAb) and covered at room temperature. The plate contents were aspirated and the wells were filled with BB before addition of the horseradish peroxidase-conjugated MAbs for the two *P. vivax* phenotypes (0.05µg/50 µl BB/well for PVK210 and 0.01µg/50µl BB/well for PVK247). Plates were read at an optical density of 450 nm using an ELISA plate reader. Positive and negative controls were run on each plate.

## ■ Main study findings

Parasitological survey of 2665 out of 4343 inhabitants of the eight villages could not detect a single case of malaria.

The 5646 mosquitoes collected consisted of the following species: 4295 *An. cinereus* (76%), 1203 *An. sergentii* (21%), 144 *An. labranchiae* (3%) and 4 *An. claviger* (0.07%). *An. cinereus* was the species most frequently found in the four districts, being found

in the following proportions: Al Hoceima (83%), Chaouen (74%), Khouribga (16%) and Taounate (63%). *An. sergentii* accounted for the following proportions: Al Hoceima (16%), Chaouen (25%), Khouribga (1%) and Taounate (32%). The rate of *An. labranchiae* was significantly different between the four districts, being more abundant in Khouribga (83%), than in Taounate (5%), and rare in Al Hoceima (0.5%) and Chaouen (0.5%). *An. claviger* appeared sporadically in Chaouen and Taounate (0.1% in each district) and was absent elsewhere.

*An. sergentii* mosquitoes were collected during the study period in increasing densities from May to September, while *An. labranchiae* was encountered from May to July but in low numbers. Daytime resting catches were irregular, negative or often composed solely of *An. cinereus*.

A total of 302 mosquito pools containing from 1 to 5 specimens were prepared from the 1347 *An. labranchiae* and *An. sergentii* collected, and were tested for the presence of CS antigen by ELISA. Absorbance values for the 302 samples in comparison to the control group showed the absence of infection for the two *P. vivax* phenotypes (PVK210 and PVK 247) in the mosquitoes collected.

## ■ Conclusions and recommendations

The negative results obtained by CS-ELISA and parasitological investigation suggest the absence of sporozoite circulation in *Anopheles* mosquitoes and therefore the interruption of malaria transmission in these areas. CS-ELISA assay could be a useful tool in the surveillance and prevention of resurgence of malaria in Morocco and other countries with comparable epidemiological situations.

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# Malaria

## Epidemiology

Oman

Determination of criteria for enhancing early malaria diagnosis among expatriates seeking medical care in Muscat Region, Oman

Oman  
Muscat Region

Study period:  
October 2002–January 2004

Small Grants  
Scheme  
(SGS) 2002 No. 9

Principal Investigator  
Dr Salim Al-Wahaibi  
Department of  
Environmental Health  
and Malaria Eradication  
Ministry of Health  
Muscat, Oman  
e-mail:  
enmal898@omantel.net.om

### Abstract

In the present consolidation phase of the malaria eradication programme in Oman, the most important activity is early case detection, whether active or passive. In this phase, private clinics are expected to play an important role in passive case detection. To increase their participation in case detection, 68 private clinics in Muscat were selected to determine criteria by which to identify those expatriates who should be examined for malaria.

Results Of 9366 collected blood slides, 91 were positive for malaria. Of these, 63 were expatriates, mainly from the Indian subcontinent. Study of expatriate malaria cases found that *Plasmodium vivax* malaria responded well to chloroquine therapy, and that more than 96% of malaria cases complained of actual fever (fever at presentation or during the previous 4 days) or recent fever (fever during the previous 4 weeks), while a considerable number gave history of chills, rigors, nausea, vomiting, body ache and headache. Most *P. vivax* cases were relapses, with no recent travel history to home countries. The majority of patients were from rural areas in their home countries and were not highly educated. However, all cases of *P. falciparum* gave history of fever and recent travel. Thrombocytopenia was a constant finding and improved progressively with malaria treatment.

Another survey to detect active cases among expatriates living in labour camps found that of 2856 slides examined, only one was positive for *P. vivax*, indicating that

asymptomatic expatriates are rarely positive for malaria parasites.

Conclusion Expatriates with actual or recent fever should be examined for malaria regardless of other signs and symptoms, especially those with past history of malaria, thrombocytopenia, or who have lived in known endemic areas in their home countries.

### Background

The malaria eradication programme in Oman is currently in its consolidation phase during which epidemiological surveillance is of the utmost importance. All malaria cases should be early detected and radically treated to prevent complications and resurgence of malaria in the country.

During the last 3 years, about 600 malaria cases have been detected each year, nearly all of them were imported. In 2002, expatriates represented 26.3% of the total population in Oman, 48% of these residing in Muscat. Most are from the Indian subcontinent and prefer to seek medical care at private clinics where many malaria cases are not detected. As antimalarial drugs are not on sale in private pharmacies, malaria cases that are not reported or detected are not treated and hence remain a reservoir of infection. This represents a threat to the malaria eradication programme in Oman, increasing the risk of malaria resurgence. These cases should be detected and radically treated early enough to prevent transmission.

The objectives of this study were to promote the participation of private clinics in Muscat in early case detection by identifying criteria by which to select those individuals most likely to be malaria carriers for blood examinations for malaria. These criteria may be applicable to other regions in Oman and those countries of the Gulf Cooperation Council that have similar epidemiological situations.

### Conclusions and implications of the study

- The major criteria for enhancing early diagnosis of malaria among expatriates seeking medical care in the private sector in Oman are: history of actual or recent fever; thrombocytopenia; past history of malaria during the previous 2 years; and history of living in malaria endemic areas in their homeland.
- The results of this study should be implemented in the private sector throughout Oman to promote early malaria case detection.
- Expatriates should be educated about the importance of early diagnosis and management of malaria and about possible complications.
- Vector control measures should be tightly supervised, especially in areas with a large expatriate population.

## Materials and methods

Of 124 private clinics and 56 private medical complexes in the Muscat Region, 68 clinics were selected representing the different *wilayats* (districts) of Amerat, Baushar, Muscat, Muttrah and Seeb, and different levels of care. The clinics were requested to prepare thin and thick smears from all suspected malaria cases among expatriates, which were collected by the Department of Environmental Health and Malaria Eradication laboratory staff. They were also instructed to notify and refer suspected malaria cases to the Department for history taking, clinical examination and investigations for total leukocyte and platelet counts, and haemoglobin, glucose, urea and creatinine levels.

All detected malaria cases were treated as per the national drug policy, as follows: *P. vivax* patients received first line antimalarial drugs (chloroquine and primaquine); and *P. falciparum* patients were admitted to hospital and treated with second line antimalarial drugs (quinine for 2–3 days, sulfadoxine/pyrimethamine single dose and primaquine single dose). All *P. vivax* cases were given primaquine for 14 days as per the results of glucose-6-phosphate dehydrogenase test.

**Labour camp survey** A survey was carried out in 25 labour camps to collect blood slides from expatriate labourers. Slide examination and positive case management were conducted in the same manner as with the private clinics.

**Field investigations of malaria cases** In line with the national policy, each patient's residence was checked for larvae and adult mosquitoes. In addition, the results of entomological surveys in the area during the last 3 months and vector control measures were reviewed. This was done in order to exclude introduction of malaria before classifying these cases as imported, as most of the *P. vivax* cases detected among expatriates were relapses of primary attacks acquired in their home countries.

## Main study findings

About 0.97% of the slides collected by the 68 private clinics were positive for malaria (91 out of 9366 slides); 28 were Omanis and 63 were expatriates. Most of the latter were from the Indian subcontinent and were *P. vivax* cases. More cases were detected in Baushar, Muttrah and Seeb *wilayats*, respectively, and in the months of June, July and October.

The *P. vivax* strain predominant in the Indian subcontinent responds to chloroquine therapy. These cases relapse 6 months–1.5 years after the primary attack, and rarely after 2 years, as they are not given hypnozoitocidal drugs in their home countries. Actual or recent fever was present in all cases of malaria, along with many other symptoms. Asymptomatic expatriates were very rarely positive for malaria,

indicating that malaria relapses almost always give rise to signs and symptoms. About 80% of imported *P. vivax* cases were diagnosed within 6 months of arrival in Oman, while about 95% of *P. falciparum* cases were diagnosed during the first months of arrival.

Early case detection and prompt management prevented malaria complications in all species. Laboratory investigation revealed that 18% of cases were anaemic, 17% had abnormal blood glucose levels, 13% had abnormal leukocyte counts, 3.6% had abnormal blood urea levels, 22.5% had abnormal creatinine levels and 35.2% had an enlarged spleen. Platelet counts were below normal levels in 76% of examined *P. vivax* cases and some had severe thrombocytopenia. However, platelet count increased progressively and within a very short time after starting malaria treatment. No cases gave history of blood transfusion, indicating that induced malaria may not be a problem among expatriates living in Oman.

## Conclusions and recommendations

The major criteria that enhance early diagnosis of malaria among expatriates seeking medical care in private institutions in the Muscat Region are: history of actual or recent fever; thrombocytopenia; past history of malaria during the previous 2 years; and living in known endemic areas in their homeland. These identified criteria should be communicated to the private sector countrywide in order to promote early case detection, and thereby prevent resurgence of malaria in Oman.

# Malaria

## Epidemiology

### Egypt

Towards the development of a georeferenced database for malaria transmission risk management in Egypt using remote sensing and GIS

Egypt  
All governorates

Study period:  
January 2002–  
September 2003

Small Grants  
Scheme  
(SGS) 2001 No. 43

Principal Investigator  
Dr Ali Hassan  
Institute of Environmental  
Studies and Research  
Ain Shams University  
Cairo, Egypt  
e-mail:  
alihassan\_eg@yahoo.com

### Abstract

A geographic information systems (GIS)-based malaria management database was developed to help in preparation and planning for malaria surveillance and control under the current rapid environmental changes in Egypt. It brings together disease surveillance (30 years worth of data), vector distribution and bionomics, insecticide resistance, environmental and socioeconomic data about malaria. The frequency distribution of malaria occurrence over the last three decades was used to classify the governorates of Egypt into two clusters of high and low malaria risk for each decade. GIS and statistics were combined to analyse the collected data and to generate hypothetical risk maps for malaria.

**Results** The database was used to generate an updated map of anopheline vectors in Egypt. Using GIS, spatial association between *Anopheles sergentii* and *Plasmodium falciparum* was proven. Between 1971 and 1981, 9 governorates were classified as low-risk areas and 26 were classified as high-risk. During the following two decades, only Fayoum governorate was classified as a high-risk area. Risk predictors included malaria parasites, anopheline vector distribution and hydrogeology. GIS was then used to generate malaria risk maps for each decade. Spatial operations were also used to map high-risk areas based on spatial association between

predetermined hydrology and soil features alone or in combination with vector distribution data.

**Conclusion** To assist disease management, a surveillance map for malaria was generated.

### Activities conducted within the framework of the study

Ministry of Health staff and three junior scientists were trained on GIS, vector-borne diseases, field surveys and statistics. Technical support is being given to the Ministry of Health to assist in maintaining and updating the database.

### Background

The geographic distribution and diversity of anopheline mosquitoes in Egypt were last reported in 1988 [1]. However, major land use and other environmental changes are taking place in the country, mainly related to large water resources development projects such as Toshka, East Owinat, Halayeb and Al Salam Canal.

The objectives of this study were to develop a georeferenced database related to malaria in Egypt; to study whether anopheline mosquito vectors exist in areas of new water resources development projects; and to develop a preliminary malaria receptivity (risk) map for better surveillance and preparedness in Egypt.

### Materials and methods

The available literature was reviewed to collect data on the distribution of *Anopheles* mosquitoes in Egypt, malaria parasites, vector bionomics and the susceptibility of *Anopheles* mosquitoes to insecticides.

Entomological surveys were carried out in two main areas of mega-projects for water development: South of Aswan (Toshka) and North Sinai (Al Salam irrigation project). Mosquito surveys were

## Conclusions and implications of the study

■ The GIS database developed provides the first digitally archived data on malaria in Egypt.

■ In Fayoum, large waterlogged areas where thick emergent vegetation cover exists make it very difficult to reach and control mosquitoes with conventional methods. Environmental and water management techniques should therefore be adopted such as planting of trees with high evapotranspiration potential to lower the water table, water table lowering using engineering methods and mass vegetation clearance. This will require intersectoral collaboration.

■ Although only Fayoum governorate was identified as a high-risk area for malaria, other areas in Egypt should be considered. Aswan is located in southern Egypt where it shares borders with malaria-endemic countries. Cairo should also receive more attention, as it is home to a large number of people from malaria-endemic countries who stay for education and religious purposes.



also carried out in South Sinai and in Hurghada-Al Quseir sector and Shalatin area of the Red Sea governorate. In addition, surveys were carried out around Port Safaga, along the Red Sea coast.

Historical data on malaria incidence collected through active and passive surveillance from 1971 to 2001, and the type of malaria parasites recorded in each governorate between 1980 and 2001 were provided by the Ministry of Health.

Environmental and landscape features were also collected, including: annual mean high and low temperatures; relative humidity and rainfall; the geological, soil and hydrogeology map of Egypt; and vegetation data. Normalized Difference Vegetation Index (NDVI) images were used to map areas of vegetation and to test the association with malaria occurrence.

Socioeconomic data such as the population census, average household size, crowding index and sanitary conditions index were obtained from the latest report prepared by the Central Agency for Public Mobilization and Statistics (CAPMAS, 2001).

These former input data (maps or files) were converted to shapefile (*ArcView* format) or imported to *ArcView*. GIS and statistics were combined to analyse the collected data and to generate “hypothetical risk maps” of malaria. Based on malaria incidence during the periods 1971–1981, 1982–1991 and 1992–2001, governorates were grouped into two categories of “malaria risk” (high and low) using cluster analysis.

## ■ Main study findings

The mosquito fauna in Toshka was represented by *Culex pipiens*, *Cx. prexiguus* and *Cx. deserticola*. In the area of the Al Salam irrigation project, *An. pharoensis*, a major malaria vector, was repeatedly collected along the west–east transect from the east delta to the west of North Sinai, where Al Salam Canal passes. Along the Red Sea coast, between north of Hurghada and Al Quseir (a 350 km stretch), *An. multicolor* was the only anopheline species encountered. It was collected close to the south extension of Port Safaga for the first time in this town. Moreover, *An. sergentii*, reported during the 1980s from Al Quseir, was not found although the search was carried out at the same site. In that site, this *An. sergentii* was replaced by *Cx. deserticola*, which was also reported in the Red Sea governorate for the first time.

In South Sinai governorate, no anopheline mosquitoes were collected during the survey period although several sites were searched. Only *Cx. pipiens* was collected from and around urban development areas, nature reserves and wastewater treatment ponds where white storks, carriers of West Nile virus, exist.

Maps of the current spatial distribution of *An. pharoensis* and *An. sergentii*, the main vectors of malaria in Egypt, showed that the former is more widely distributed than the latter indicating more ecological plasticity. Most of the governorates where *An. sergentii* was not reported are agricultural and located within the Delta and Valley, indicating that this species favours desert environs. Maps of the co-distribution of these two main malaria vectors may direct attention to governorates with higher priority for surveillance based on a higher entomological risk related to a mix of efficient vectors.

The currently available data was used to generate a map of the spatial distribution of all 11 *Anopheles* mosquito species currently present in Egypt. The highest *Anopheles* species diversity exists in North and South Sinai governorates (6 species) followed by Aswan, Ismailia and El Wadi El Gedid (5 species).

Malaria risk maps for three decades showed that high-risk governorates are clustered within the Nile Delta and Valley. Low-risk governorates are mainly the frontier governorates dominated by desert landscapes and paucity of water. The risk maps generated for the second and third decades indicated that malaria had declined tremendously in the country, persisting in Fayoum only. This was attributed to the hydrogeological characteristics of this governorate that may be reflected in the distribution and abundance of malaria vectors. The maps indicated that the hydrogeological unit and soil type characterizing Fayoum are found in other areas of Egypt. However, through intersection within the GIS, Fayoum was identified as the only area of Egypt where both features are spatially associated. Accordingly, malaria risk in Egypt could be predicted based on features of the physical environment and information on vector distribution.

## ■ Conclusions and recommendations

This study provides the basic framework for developing an early warning system for malaria in Egypt through monitoring parasite prevalence rates and vector distribution, and mapping these data in relation to environmental variables such as hydrogeology and soil. Managing malaria in Fayoum requires more than the conventional methods as it relates to the physical features of the area.

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# Malaria

## Knowledge, Attitudes and Practice

Impact of a training programme in malaria education on teaching skills of rural health workers (behvarzes) in Sistan va Baluchestan province, Islamic Republic of Iran

Islamic Republic of Iran  
Sistan va Baluchestan Province

Study period:  
October 2002–  
December 2003

Small Grants Scheme  
(SGS) 2002 No. 116

Principal Investigator  
Dr Fatemeh Rakhshani  
Zahedan School of Health  
Zahedan University of  
Medical Sciences  
Zahedan, Islamic Republic  
of Iran  
E-mail:  
rakhshanif@yahoo.com

### Islamic Republic of Iran

#### Abstract

A quasi-experimental study was conducted in rural areas of Sistan va Baluchestan Province to evaluate the impact of a malaria training programme on the teaching skills of primary health workers (behvarzes). The intervention and control groups both consisted of 32 behvarzes. A two-day workshop was held to increase the intervention group's knowledge of the malaria education process and their teaching skills in community malaria education. Their knowledge and behaviour were then assessed at 3 and 6 months post-intervention. A questionnaire with 63 open-ended questions and a checklist with 19 assessed behavioural items were designed and used to assess knowledge and a range of active teaching behaviours.

**Results** There was significant improvement in the knowledge and active teaching behaviour of the intervention group at three and six months follow-up after workshop attendance compared to baseline scores, and in comparison to the control group, regarding the following: knowledge of malaria (+32% increase in score); knowledge of assessment of educational needs (+27%); knowledge of the correct areas of learning required (+40%); knowledge of proper content for malaria education (+22%); knowledge of correct teaching methods (+42%); knowledge of

evaluation of education (+30%); and active teaching skills (+48%). The only significant determinant of knowledge was the educational level of the health workers.

**Conclusion** The training programme was effective in improving the knowledge and teaching behaviour of behvarzes about malaria education.

#### Activities conducted within the framework of the study

A manual for primary health care workers was developed as an educational aid to enhance teaching skills in malaria education. This manual, entitled "*Manual of active teaching skills in malaria education*," has been posted on the web site of the Regional Office, in the section for educational materials originating from Small Grants Scheme projects. Publication of this manual in Farsi could be an important educational resource for behvarzes working in malaria endemic areas in the Islamic Republic of Iran. The adaptation of this manual for other malaria endemic countries in the Region could also be assessed.

#### Background

Health education is a process that can enable people in developing countries to take control of their lives by gaining the knowledge to prevent or reduce the risk of disease.

Health educators need to possess a good ability to transfer their knowledge to different populations. Future health educators will need skills in developing programmes that respond to rapid changes in families and societies, and be able to address health and social problems. Health educators have a major role in helping people achieve and maintain malaria-preventive behaviour in endemic areas. They must also be skillful in gathering information about behaviour, identifying those that foster well-being and those that hinder it, and identifying health education needs. Effective health

### Conclusions and implications of the study

- The training programme was effective in improving the teaching behaviour of the primary health care workers (behvarzes), and should therefore be made available to all health workers engaged in malaria education.
- The study reported the effectiveness of a training programme to improve the teaching behaviour of behvarzes in malaria education. Teaching instructors of health workers about these methods is therefore recommended.
- There is a need to reconsider the educational curriculum of health care practitioners, especially rural health workers, and improvement of education quality through emphasis on learner-centered teaching methods.
- Appropriate and innovative methods for assessing the quality of health education delivered to the community should be introduced and implemented by national control programmes in malaria endemic countries.

instruction hinges on two interrelated issues: what should be taught and how.

A study was therefore undertaken to evaluate the impact of a malaria training programme on the teaching skills of primary health workers (behvarzes) in Sistan va Baluchestan Province.

## Materials and methods

A quasi-experimental study was conducted in rural areas of Sistan va Baluchestan Province. The intervention and control groups consisted of 32 behvarzes selected from poor communities. A two-day workshop was held to increase the intervention group knowledge and teaching skills in community malaria education. The levels of knowledge and teaching behaviours of both groups were assessed prior to the intervention, then three and six months after the intervention. A questionnaire with 63 open-ended questions and a checklist with 19 assessed behavioural items were designed to assess knowledge and a range of active teaching behaviour.

## Main study findings

**Knowledge of malaria education** There was a suboptimal level of knowledge regarding malaria education in both groups before the intervention. The level of knowledge increased by 32% after 6 months. **Knowledge of assessment of educational needs** Participants were asked to design three questions to assess each area of the knowledge and behaviour of learners. Their ability to design correct questions was very low at baseline, especially on behaviour. An overall 27% post-intervention increase in this ability was recorded, with the greatest increase (33%) observed in the knowledge of behaviour assessment.

**Knowledge of the identification of the different learning areas** Prior to the intervention, participants could hardly differentiate between the three areas of knowledge, attitudes and behaviour. After 6 months, the knowledge of participants of the different learning areas showed a 40% increase, with the greatest increase in the identification of attitudes (43%) and behaviour (40%).

**Knowledge of proper content for malaria education** Proper content should be determined through needs assessment, taking into consideration the learning areas, the learner's educational level and other factors. The description of different kinds of malaria parasites with their often confusing names can induce boredom in an illiterate audience, as has often been the case in community malaria education. Health workers should use educational materials that focus on the malaria preventive behaviour of the audience. Knowledge of proper content for malaria education was low in both study groups prior to the training. Following the workshop, this knowledge

reached 60% in the intervention group, a moderate level and less than expected. At the end of 6 months, the knowledge of the health workers of proper content had increased by 22%, with the greatest increase in knowledge of the malaria vector (30%). This limited improvement in knowledge might be explained by the presence of conflicting statements in the educational materials and in a bias in the interpretation of the answers of open-ended questions.

**Knowledge of correct teaching methods** The knowledge of health workers of teaching methods reached 56% after the workshop, an increase of 42%. In addition to the traditional lecture method, which remains effective, the instructors should be able to select and implement a variety of other methods during educational sessions.

**Knowledge of evaluation of education** Evaluation of the learning process during and at the end of a class allows the instructor to assess progress in learning, and to identify the strengths and weaknesses of the experience. Health workers had little information about evaluation methods before the intervention. However, a significant increase in knowledge about these methods was recorded afterwards (30%), with the highest increase in knowledge of behavioural evaluation methods.

**Active teaching skills** The majority of malaria education sessions were done as traditional lectures. During the workshop, health workers participated in role-playing sessions on active teaching. An increase of 48% in the teaching skills of health workers was seen at 6 months post-intervention. This was mainly attributed to the application of active teaching methods.

**Determinants of knowledge** There was no significant association between the knowledge of health workers in the intervention group and their age, sex or years of experience. On the other hand, there was a significant association between knowledge and the educational level of the health workers.

## Conclusions and recommendations

The training programme was effective in improving the teaching behaviour of the primary health care workers. The results of this study can be applied to improve community health education in the Islamic Republic of Iran, especially in malaria education. Moreover, the possibility of adapting the manual of active teaching skills that was produced by this study for use in other malaria endemic countries in the Region should be assessed.

# Malaria

## Molecular Entomology

Islamic Republic of Iran

Investigation of sibling species of the *Anopheles maculipennis* complex in Islamic Republic of Iran

Islamic Republic of Iran  
north-west and central provinces

Study period:  
October 2001–2002

Small Grants Scheme  
(SGS) 2001 No. 28

Principal Investigator  
Dr Mohammad Ali Oshaghi  
Department of Medical Entomology and Vector Control  
School of Public Health and Institute of Health Research  
Teheran University of Medical Science  
Teheran, Islamic Republic of Iran  
e-mail:  
oshaghi@lycos.com

### Abstract

Mosquitoes of the *Anopheles maculipennis* complex have been incriminated as the primary malaria vector in the north and central regions of the Islamic Republic of Iran. With the possible reintroduction of the *Plasmodium* species due to increased travel to and from neighbouring countries where malaria is endemic, accurate identification of mosquito species will be essential for preventive measures. For this purpose, PCR was used to identify species composition of the complex in 9 north-west and central provinces of the country.

Results *An. maculipennis* complex in the area consists of only two species: *An. maculipennis*, Meigen and *An. sacharovi*, Faver. Sequences for the second internal spacer (ITS2) of the ribosomal DNA were obtained from 21 specimens and compared with the ITS2 sequences previously published in GenBank. Comparison showed that the specimens were identical to either species of *An. maculipennis* s.s. or *An. sacharovi*, confirming the results of species-specific PCR assays.

Conclusion This study is the first record based on molecular and morphological tools of the distribution of *An. sacharovi* and *An. maculipennis* s.s in the north-west and central

regions of the Islamic Republic of Iran. The results provide a better understanding of the epidemiology of malaria and its control.

### Background

Previous reports concerning members of the *An. maculipennis* complex in the Islamic Republic of Iran, particularly in the north (Gilan and Mazandaran provinces) recorded the presence of five members of the complex: *An. maculipennis*, *An. sacharovi*, *An. melanoon*, *An. messeae* and *An. subalpinus*. Other studies reported the geographical distribution of *An. maculipennis* s.l. in various provinces of the country. However, these reports did not identify which sibling species of the complex are present in those provinces, and no further studies have been carried out to confirm which of the member species are present. Moreover, little is known about their current distribution.

Although malaria has been eradicated from north and central regions of the country, major ecological and social changes, such as increased travel to and from the south-east corner of the country and neighbouring countries where malaria is endemic, could result in the reintroduction of malaria in these regions. Among five reported species in the Islamic Republic of Iran, *An. sacharovi*, *An. messeae* and *An. maculipennis* are capable of transmitting malaria, but they exhibit different vector capacities. Given this, and the high risk of resurgent malaria transmission in the region, correct vector identification is essential to be able to assess the potential risk of malaria and to devise appropriate control or monitoring strategies.

### Materials and methods

Mosquito collection from the field was carried out in nine provinces of the semi-arid and mountainous regions of the north-west and central provinces of the country. These were West Azerbaijan,

## Conclusions and implications of the study

■ PCR assay and sequence data showed that only two members of the *Anopheles maculipennis* complex exist in north-west and central regions of the Islamic Republic of Iran: *An. sacharovi* and *An. maculipennis* s.s.

■ PCR-based identification of mosquito complex species proved to be an appropriate methodology. It is easy to apply in practice, works on all developmental stages of both sexes, and only a small amount of tissue is needed, which facilitates further extensive studies on the same specimen. Early reports on species composition of these taxa in the Islamic Republic of Iran was based on the morphological character of eggs, which is not highly reliable.

■ The presence of two important malaria vectors (*An. sacharovi* and *An. maculipennis*) of the Maculipennis group, and the presence of other important malaria vectors such as *An. superpictus*, *An. pulcherrimus* and *An. dthali* in the studied areas is highly important for the malaria epidemiology and vector control programmes in the region.

Kermanshah, Zanjan, Teheran, Isfahan, Chahar-Mahal va Bakhtiary, Fars, Markazi, and Koh-Keloyeh va Boyer-Ahmmad provinces. Based on the results of previous studies on the distribution of the species in each province, 2–5 villages were selected for sample collection. Sample collection was performed during two seasonal sessions, October–early December 2001 and June–early October 2002. To take into account different ecological and biological factors, different techniques were used to collect adult mosquitoes from the inside and outside of stables and houses, and from humans and animals. All breeding places were also checked for larvae. The specimens were then pinned for further morphological identification using standard keys for larvae and adult *Anopheles* species.

Individual blood-fed live females were placed into cups to lay eggs. Eggs were reared in an insectary to produce larvae.

DNA was extracted from specimens following the phenol-chloroform extraction protocol. Diagnostic PCR was carried out using the 5.8S rDNA as universal forward primer, whereas other six species-specific primers were used as reverse primers. To sequence the second internal spacer (ITS2) region, PCR amplification of ITS2 was carried out using the 5.8SF and 28SR primers of Collins and Paskewitz [1], which were complementary to the conserved regions of the 5.8S rDNA and 28S rDNA.

Sequence data were obtained following PCR purification with the Qiagen PCR purification kit, and cycle sequencing reactions were read by an ABI 377 automated sequencer (PE Biosystems). Following sequencing, the template DNA was dried and retained at -70°C for future reference. Sequences were edited and aligned using Sequencer version 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan) and Clustal X software packages [2]. Similarity with other sequences in GenBank was assessed using FASTA search [3].

### ■ Main study findings

The diagnostic PCR and sequencing results showed that only two species of the *An. maculipennis* complex, including *An. maculipennis* s.s. and *An. sacharovi* are present in the north-west and central parts of the Islamic Republic of Iran. Specific PCR products for the two species of the complex were 410 bp for *An. maculipennis* s.s. and 180 bp for *An. sacharovi*. Geographical distribution of these species in different provinces was as follows: both species of *An. maculipennis* s.s. and *An. sacharovi* were present in the mountainous West Azerbaijan and Zanjan provinces, with rates of *An. sacharovi* at 80% and 40% respectively; in Teheran, Isfahan, Chahar-Mahal va Bakhtiary and Koh-Keloyeh va Boyer-Ahmmad provinces only *An. maculipennis* s.s. was found; in

Fars province only *An. sacharovi* was found; none of the *maculipennis* sibling species were found in Kermanshah and Markazi provinces. In addition to *maculipennis* s.l., other anopheline species found in the study area included *An. superpictus*, *An. multicolor*, *An. pulcherrimus*, *An. apoci*, *An. sergentii*, *An. turkhudi*, *An. claviger* and *An. dthali*.

DNA sequences were generated for 26 specimens of *An. maculipennis* s.s. and *An. sacharovi* from the north-west and central regions. Inclusive of primers (43 bp), the ITS2 fragment lengths were 472 and 494 nucleotides for *An. maculipennis* s.s. and *An. sacharovi*, respectively. Percentage AT content was 49.36% in *An. maculipennis* s.s. and 52.23% in *An. sacharovi*, which is concordant with 40%–50% AT values reported for other mosquitoes of subgenus *Anopheles*, including members of the *maculipennis* and *quadrimaculatus* group.

Total sequence divergence between the species was 26.37%. Comparing the sequence data with published ITS2 sequences in FASTA [3] showed that *An. sacharovi* sequences share 100% similarity with entry Z83198 [4] and 99.35% similarity with entry AF436062. The latter is erroneously identified as *maculipennis*, which is indeed a *sacharovi*. A similar situation was found for the *An. maculipennis* s.s. sequences that share 100% similarity with AF455820, AF342714, AF342713 and AF342715, 99.788% similarity with Z50104, 99.364% similarity with AF455819, and 99.153% similarity with AF455818 [4].

Sequences generated in this study were submitted in GenBank under the following accession numbers: AY114205 to AY114211 for *An. sacharovi* and AF536337, AY137789, AY137792, AY137793, AY137794, AY137799, AY137800, AY137801, AY137805 and AY137806 to AY137816 for *An. maculipennis* s.s.

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# Malaria

## Molecular Entomology

### Islamic Republic of Iran

The role of a novel genetic variant of *Anopheles culicifacies* on malaria transmission in south-eastern Islamic Republic of Iran

Islamic Republic of Iran  
South-eastern districts

Study period:  
November 2001–  
December 2002

Small Grants  
Scheme  
(SGS) 2001 No. 108

Principal Investigator  
Dr Navid Dinparast Djadid  
Department of  
Biotechnology  
Pasteur Institute of Iran  
Teheran, Islamic Republic  
of Iran  
e-mail:  
navid@institute.pasteur.ac.ir

#### Abstract

This study was designed to examine the genetic composition of *Anopheles culicifacies* species complex based on the identification of a novel genetic variant (named species X) identified in Zeineddini, Baluchistan Province, during 1995–1997. Field and laboratory experiments included two rounds of mosquito collection, followed by morphological identification, characterization of rDNA-ITS2 region by universal and specific primers to species A and B, RAPD and SSR analysis of their genome, and cloning and sequencing of selected ITS2 and RAPD fragments.

**Results** A new genetic variant of *An. culicifacies* was detected in Koutij, near Kerman Province, confirming the presence of this species in a new area of Baluchistan Province, and perhaps in Kerman Province as well. On the other hand, detection of two other specimens in Zeineddini, collected during the second peak, confirmed that this new genetic variant of *An. culicifacies* was still present in its first identified distribution area. Cloning and sequencing RAPD fragments in both species A and X revealed a common repeated region, partially present in different loci of other organisms. Through comparison with the sequence of the *An. gambiae* genome,

the functions of genes and gene complexes can be revealed.

**Conclusion** The present study confirmed the presence of a novel genetic variant, or species X, of *An. culicifacies* in south-eastern Islamic Republic of Iran.

#### Background

*Anopheles culicifacies* is an efficient vector of human malaria from the Islamic Republic of Iran to southern China, Afghanistan, India, Pakistan and Sri Lanka. It is known to consist of at least four sibling species: A, B, C and D. In India, this nominal taxon consists of the sibling species A, B, C, D and E, distinguished by their chromosome morphology.

*An. culicifacies* (probably species A) has been reported as the main vector of malaria in Baluchistan Province, south-eastern Islamic Republic of Iran. *An. culicifacies* specimens collected during two peaks of malaria in Zeineddini village in Ghasreghand district, Baluchistan Province, provided ecological and molecular evidence for the presence of a different genetic population morphologically related to *An. culicifacies*. It was concluded that this new genetic variant of *An. culicifacies* may be responsible for the maintenance and transmission of malaria during the second peak in Ghasreghand, and this needed further investigation.

The main objectives of the current study were to identify the extent of genetic variation within *An. culicifacies* populations in Baluchistan Province, and to compare the pattern of malaria transmission with the geographical distribution of the different genetic variants.

#### Materials and methods

Mosquito collection was carried out in Chah-e-Hashem in Iranshahr district, Pishin in Sarbaz district, Zeineddini, Sarbouk and Koutij in Nikshahr district,

### Conclusions and implications of the study

■ *An. culicifacies* species A is prevalent in Baluchistan Province, south-eastern Islamic Republic of Iran. Other species prevalent are *An. stephensi*, *An. fluviatilis*, and *An. dthali*. However, probably due to environmental and geographical conditions, mainly drought, the density of each species varied in the different study areas.

■ A new genetic variant of *An. culicifacies* was identified in Koutij, confirming the presence of the species in a new area of Baluchistan Province aside from Zeineddini where it was previously identified. Its existence in Koutij, near Kerman Province, raises the suspicion that it also exists in that Province.

■ Cloning and sequencing of a RAPD fragment confirmed the inverted repeated nature of this fragment, and more interestingly, the presence of repeats inside the sequence, which was found, when comparisons were done with GenBank data, to be common in other organisms such as *Drosophila melanogaster*, mice and humans. Characterization of this and other RAPD or SSR-amplified products seems to be essential for providing new insights into different parts of the genome of anopheline vectors.

and Ouraky in Chahbahar district during April–May and October–November 2002.

Mosquitoes were locally identified by morphological key, and different body parts from each mosquito, together with a provided information sheet, were sent to the main laboratory of the malaria research group of the Biotechnology Department, Pasteur Institute of Iran. The maximum numbers of mosquitoes caught per collection site were around 200–250. All mosquito specimens and the information on the information sheets were double-checked, after which DNA extraction was done. PCR analysis of rDNA-ITS2 region was first amplified by universal primers and selected specimens (PCR products) were sent to Germany (GATC Biotech AG) for sequencing.

Cloning and sequencing of selected RAPD fragments was carried out using the Inst/Aclone PCR Product Cloning Kit number K1214 (MBI Fermentas, Inc.). RAPD and SSR analysis of selected specimens, mainly those designated species X, was performed using a set of primers. SSR primers were used to amplify genome between two satellite-containing regions from selected specimens collected in each study area.

All amplified fragments were manually scored followed by drawing the standard graph based on the size and migration of MWM fragments, and plotting and measuring the correct size of each PCR product. The size, initiation and ending sequence of ITS2 in each species was determined by comparison with the previous work of Djadid on malaria vectors from Africa (*An. gambiae* species complex) to the Indian subcontinent (*An. culicifacies*, *An. fluviatilis* and *An. maculipennis* species complexes, and *An. stephensi* and *An. pulcherrimus*). All reproducible RAPD and SSR bands in the range of 100–3000 bp were included in the scoring and subsequent analysis.

Every single sequence in PCR products was checked with the original signals followed by Blast with NCBI, GenBank and Fasta. Analysis of the sequence, their reading frames and secondary RNA structures was performed on GenRunner, Megaline and RNAdraw programs.

## ■ Main study findings

The diagnostic PCR and sequencing results along with *An. culicifacies*, *An. superpictus*, *An. stephensi* and *An. fluviatilis* were identified in Zeineddini, while six anopheline species were identified in Sarbouk: *An. culicifacies*, *An. turkhudi*, *An. dthali*, *An. stephensi*, *An. fluviatilis* and *An. superpictus*. In the other study areas, *An. culicifacies* (species A), *An. stephensi* and *An. fluviatilis* were more abundant.

Anopheline specimens collected in the first round from animal shelters in Koutij, near the border with Kerman Province, allowed the identification of a specimen (KT123) of *An. culicifacies* that is about

98% similar to the previously recorded new variant of *An. culicifacies* (GenBank accession number AF402296) from Zeineddini.

In the second round of mosquito collection, two specimens (ZD903, ZD922) collected from a single shelter pit in Zeineddini had different patterns with the combination of species A and B-specific primers and 100% identity with species X (GenBank accession number AF402296). In other study areas, all *An. culicifacies* specimens were identified as species A, having a nearly identical sequence with *An. culicifacies* species A, submitted to GenBank (AF402297) by Djadid.

Construction of the secondary structure of RNA in species X of *An. culicifacies* revealed its differences and similarities with other published sequences. It was shown that, like other animals, it has a similar stem region, which contains a putative ribosomal processing site in many organisms.

Screening of RAPD and SSR primers was performed on selected specimens, especially the three *An. culicifacies* species X specimens. None of the primers could produce a specific and diagnostic fragment. However, AB1 primer produced a single fragment in different specimens of *An. culicifacies* collected in all study areas, including the three specimens related to species X. Subsequently, the single fragment in ZD922 and an individual related to species A have been selected for cloning and sequencing. The successful cloning of two PCR products was compared with the size of the plasmid and then re-amplified with AB1-01 primer followed by the sequencing of this fragment in species X. The sequence of this fragment showed the correct amplification by having the primer sites in both ends of the sequence. Interestingly, the Blast search on GenBank data revealed partial homology of this sequence with a repeat containing region in *Drosophila melanogaster* chromosomes (2R, 3R and X), *Mus musculus* (chromosomes 1, 3, 5, 6, 8), human genome (chromosomes 3, 11, 14, 16, 17, 21, 22) and other organisms.

## ■ Conclusions and recommendations

Molecular tools for genome analysis confirmed the presence of a novel genetic variant or species X of *An. culicifacies* in the Islamic Republic of Iran.

These findings suggest a further DNA-level study of the five members of *An. culicifacies* species complex that were detected from India, in order to define and provide a nearly complete picture of the different species within this complex and their role in transmission. This could be achieved through collaborative work with Afghanistan, India, Oman, Pakistan and Sri Lanka.

# Malaria

## Morbidity

### Sudan

Malaria and pregnancy in an area of seasonal and unstable transmission in eastern Sudan

Sudan  
New Halfa,  
eastern Sudan

Study period:  
June 2001–May 2002

Small Grants  
Scheme  
(SGS) 2001 No. 81

Principal Investigator  
Dr Gehad ElGhazali  
Department of Biochemistry  
Faculty of Medicine  
University of Khartoum  
Khartoum, Sudan  
e-mail:  
gelghazali@hotmail.com

#### Abstract

A one-year prospective community-based study of malaria during pregnancy was conducted in an area of seasonal and unstable malaria transmission in a village near New Halfa town in eastern Sudan. The study aimed to investigate the morbidity pattern of *Plasmodium falciparum* malaria in pregnant women. An antenatal care clinic was set up and 86 pregnant were enrolled at a mean gestational age of 22.2 weeks (9.1) and were followed every 2 weeks until 6 weeks following delivery. In parallel, 89 non-pregnant women were enrolled as a control group.

**Results** The incidence of falciparum malaria was significantly higher among pregnant as compared to non-pregnant women, 17.4% (15/86) and 5.6% (5/89) respectively, and this was not statistically different between primigravidae or multigravidae.

The mean haemoglobin concentration in the infected women was lower than non-infected mothers, but this was not statistically significant (9.1 (1.3) g/dl and 9.5 (0.6) g/dl, respectively,  $p = 0.069$ ). The mean birth weight was 2.727 kg for babies born to infected mothers, compared to 2.959 kg for babies

born to non-infected mothers; and the difference was statistically significant.

**Conclusion** In areas of seasonal and unstable malaria transmission, malaria affects all parities and can lead to significant reduction in birth weight despite early diagnosis and treatment.

#### Background

In areas where malaria transmission is seasonal and unstable, both the mother and her fetus can suffer from the most severe consequences of the infection. In these areas, it is believed the degree of acquired immunity of the women prior to pregnancy is likely to be low or poorly developed and the epidemiological profile and clinical pattern of disease are different from highly endemic areas. While the epidemiology of malaria during pregnancy has been extensively studied in highly endemic areas, very little data are available from areas with seasonal and unstable malaria. The present study was conducted to investigate the morbidity pattern of malaria during pregnancy in the eastern Sudan and to evaluate the impact of early diagnosis and treatment on the pregnancy outcome of infected mothers.

#### Materials and methods

The study was performed in Elhara Eloula village, New Halfa area, eastern Sudan, with a population of 1841 individuals (2001 census). The area is mesoendemic for falciparum malaria with peak transmission following the rainy season.

The predominant malaria parasite species is *P. falciparum*. *Anopheles arabiensis* is the sole malaria vector in the area. Bednets and malaria chemoprophylaxis are not used in the area.

An antenatal clinic supervised by an obstetrician was initiated in the health centre in order to enrol and follow-up all

### Conclusions and implications of the study

■ In areas of seasonal and unstable malaria transmission in eastern Sudan, malaria infection is significantly higher in pregnant as compared to non-pregnant women. In contrast to studies conducted in areas where malaria transmission is stable, the infection rate was not significantly different between primigravidae and multigravidae.

■ Malaria infection had a negative impact on pregnancy outcome manifested by low birth weight, maternal anaemia and preterm labour. However, the impact on the mothers was less pronounced and this could be attributed to early diagnosis and treatment.

■ The low birth weight of babies born to infected mothers is attributed to the fact that *P. falciparum*-infected erythrocytes may persist in the placenta despite adequate therapy. The parasite sequestration in the placenta is considered to be the main pathological mechanism. Placental infection leads to thickening of the syncytiotrophoblasts, abnormal utero/placental blood flow and placental insufficiency.

■ This study provides further evidence for the crucial need to ensure adequate use of insecticide-treated bednets and effective chemoprophylaxis for pregnant women in order to reduce malaria morbidity and mortality in endemic areas.



pregnant women in the village after obtaining their consent to participate in the study. Pregnant women were requested to present to the antenatal clinic once every 2 weeks until 6 weeks after delivery. Their obstetric history was recorded in detail, including last menstrual period, gravidity, parity, history of abortion, and preterm labour. At the time of the enrolment they were divided into low or high-risk patients according to their obstetric history (repeated abortion, preterm labour or history of pre-eclampsia/eclampsia). Initially and at every visit, women were asked about symptoms suggestive of malaria. Obstetric and physical examinations were done. The pregnancy and its duration were calculated from the last menstrual period and confirmed by ultrasound. For controls the ultrasound was performed to exclude pregnancy. Free medical care, including free medication, was provided, so it is unlikely that the women sought medical advice anywhere else. As a control group, for each pregnant woman, the nearest non-pregnant neighbour that matched age and socioeconomic level was asked to participate in the study. None of the pregnant women or their controls were shown to have G-6-phosphate dehydrogenase deficiency or haemoglobin S.

Parasitological and detailed medical examinations were carried out in cases presenting to the medical staff complaining of fever or giving a recent history of fever (within the past 3 days). Blood was taken by finger prick from each subject, and thick and thin blood films were prepared and stained with Giemsa. The number of asexual parasites per 200 leukocytes was counted, and parasite densities (asexual parasites/ $\mu$ l) calculated. The haemoglobin concentration was estimated by the haematic acid method.

Due to high chloroquine resistance in the area, falciparum infections were treated with quinine 10 mg/kg body weight 3 times/day for 7 days. Every visit, pregnant women were supplied with ferrous sulfate (200 mg/1 tablet/day) and folic acid (0.5 mg/1 tablet/day). Deliveries were conducted by trained midwives and the birth weight was recorded. High-risk patients were advised to deliver in New Halfa hospital.

Abortion was defined as expulsion of dead fetus before 28 weeks. Premature labour means labour before completing 37 weeks of gestation. Anaemia was defined as haemoglobin less than 8 g/dl and severe anaemia as haemoglobin less than 5 g/dl. Perinatal death means death from 28 weeks *in utero* until the age of 1 week. Low birth weight was defined as birth weight less than 2.5 kg.

### ■ Main study findings

Of 209 women enrolled, 175 women (86 pregnant and 89 non-pregnant) completed the study. The mean gestational age of pregnant women at the time of enrollment was 22.2 (9.1) weeks.

**Incidence of malaria infection** All infections occurred during the transmission season following the rainy season and were due to *P. falciparum*; no *P. vivax* or *P. malariae* were detected. There was a significant difference in the incidence of infection between pregnant and non-pregnant women (17.4% versus 5.6%, respectively,  $p = 0.014$ ). Malaria infections were detected in 22.2% of primigravidae, 8.3% of secundigravidae and 17% of multigravidae, and this was not statistically significant. Eleven (73.3%) of the 15 infections were in the third trimester, while only 26.7% were in the second trimester and none were in the first trimester. The mean gestational age of the infections was 29.9 (0.8) weeks. Most of the infections (66.6%) were detected by presence of the asexual stage of *P. falciparum* in peripheral blood smear and infected women were symptomatic at presentation. There was no significant correlation between the level of parasitaemia and age or parity.

**Malaria and haemoglobin level** In spite of the non-significant difference in haemoglobin levels at enrollment, there was high tendency for the mean haemoglobin levels near delivery to be lower among infected compared to non-infected pregnant women (9.1 g/dl (1.3) and 9.5 g/dl (0.6) respectively,  $p = 0.069$ ). Only one of the infected pregnant women presented with severe anaemia.

**Pregnancy outcome** The mean birth weight of babies born to malaria-infected women was significantly lower than that of babies born to non-infected women (2.72 (0.26) versus 2.95 (0.05),  $p < 0.001$ ), but did not significantly differ by parity.

One maternal death was reported as a complication of septicaemia following obstructed labour. Four perinatal deaths occurred, all in the non-infected group, and no clear reason was identified. One of the non-infected women aborted at 10 weeks gestational age. Four of the pregnant women (4/86) delivered prematurely, 2 in the infected group (13.3%) and 2 (2.8%) in the non-infected group.

**Treatment response** All the infected women responded satisfactorily to quinine therapy, all were symptom-free by the third day and their blood films were negative on days 7, 14, 21 and 28.

### ■ Conclusions and recommendations

In areas of unstable and seasonal malaria transmission, pregnant women are at significantly higher risk of infection and of giving birth to low birth weight babies despite early diagnosis and treatment. They are also at higher risk for anaemia at delivery and premature labour. Ensuring adequate use of insecticide-treated bednets and effective chemoprophylaxis for pregnant women is therefore recommended in order to reduce malaria morbidity and mortality in endemic areas.

# Malaria

## Morbidity and Mortality

### Sudan

Determinants of complicated malaria and mortality from malaria in Khartoum urban area

Sudan  
Khartoum

Study period:  
March 2002–April 2004

Small Grants  
Scheme  
RBM 2002 No. 1

Principal Investigator  
Dr Wasailat Ibrahim  
Malaria Surveillance  
Department  
National Malaria Control  
Administration  
Federal Ministry of Health  
Khartoum, Sudan  
e-mail:  
WASAILAT@hotmail.com

### Abstract

A prospective case-control hospital-based study was undertaken in hospitals in Khartoum State to identify the determinants of severe malaria morbidity and mortality from malaria. During a 1-year period, 431 admitted patients, clinically diagnosed as severe complicated malaria cases, were enrolled, while 332 uncomplicated malaria patients were randomly selected from the outpatient clinics of the same hospitals as a control group. A pretested questionnaire was used to collect information on various determinants of malaria morbidity and mortality. Patients were also physically examined and relevant laboratory tests were performed.

Results Complicated malaria cases recorded a significantly longer duration between onset of symptoms and initiation of treatment, significantly longer total duration of illness, a significantly shorter time interval since the last malaria attack in the family and a significantly longer waiting time at health facilities. The majority of patients seek care initially at a health centre or hospital, with few seeking care in the private sector. General practitioners are the main health care providers. Seeking initial care at a hospital was found to be a

protective factor against progression from uncomplicated malaria cases to complicated ones. The significant risk factors for complicated malaria were: living in suburban/rural area; history of travelling to a hyperendemic area; and delay in initiation of treatment for more than 2 days. Significant clinical criteria for identifying complicated malaria in the field or remote settings are rigors, dehydration, pallor and jaundice. The case fatality rate for complicated cases was 10.7%. The significant predictors of mortality among these were: female gender; higher educational levels; longer duration of illness; negative history of malaria; and reluctance or delay in administering the proper antimalarial drug on admission.

Conclusion These results indicate the need to raise the awareness of the community about insecticide impregnated bednets and timely access to treatment, and to ensure the adherence of health care providers to the national control programme guidelines in the management of complicated malaria.

### Background

The malaria burden has increased in Khartoum State during the last decade due to environmental and biological changes, together with population displacement, leading to high morbidity and mortality. To date there has been no accurate information regarding the extent of morbidity and mortality due to malaria. In addition, there is a need to identify the determinants of morbidity and mortality from malaria to facilitate the planning of appropriate interventions in order to reduce malaria mortality.

### Materials and methods

A prospective case-control hospital-based study was carried out in Khartoum State hospitals including Ibrahim Malik, Khartoum Paediatric, Khartoum Teaching, Ahmed Gasim Omdurman Paediatric, Omdurman Teaching and some other hospitals. During a 1-year period (September 2002–2003), 431 admitted patients, clinically diagnosed as severe complicated malaria cases, were enrolled,

## Conclusions and implications of the study

■ A negative blood film for malaria parasite was reported in 22.7% of patients diagnosed as malaria patients based on clinical judgement. This could be explained by a tendency of physicians to diagnose any febrile illness as malaria, by a low sensitivity of the microscopic examination due to inadequate performance or other factors, or by the severity of the disease.

■ Risk factors for complicated malaria are: suburban/rural residence; history of travelling to hyperendemic areas; and delay in initiation of treatment by more than 2 days. Raising the awareness of the community about protective measures such as insecticide impregnated bednets and the importance of timely access to treatment is therefore recommended.

■ The case fatality rate of complicated cases was 10.7%. The significant predictors of mortality among complicated cases were: female gender; higher educational levels; duration of illness; negative history of malaria; and reluctance or delay in administering the proper antimalarial drug on admission. These results indicate the need to ensure the adherence of health care providers to national control programme guidelines in the management of complicated malaria.

and 332 uncomplicated malaria patients were randomly selected from the outpatient clinics of the same hospitals as a control group. A pre-tested questionnaire was used to collect information regarding sociodemographic characteristics, health seeking behaviour, clinical data, treatment outcome, and other determinants of malaria morbidity and mortality. A knowledge summation score was developed based on knowledge of malaria symptoms, transmission, treatment, prevention and control. The median cut-off point was used to categorize patients into those having adequate or inadequate knowledge. Patients were also physically examined and relevant laboratory tests were performed. From each patient, thick and thin blood films were prepared and stained with 10% Giemsa stain for 10 minutes and 100 fields of Giemsa stained thick blood film were examined for the presence of malaria parasites. Thin blood films were used for identification of species where appropriate.

### ■ Main study findings

**Malaria diagnosis and management** Of the 431 patients clinically diagnosed as having complicated malaria, 274 (63.67%) were found to be confirmed cases, compared to 227 (68.4%) of the 332 patients clinically diagnosed as having uncomplicated malaria. Of the 763 study subjects, 89 (11.7%) were managed based on clinical features without laboratory confirmation. Despite a negative blood film for malaria parasite in 173 (22.7%) patients, 169 of these (97.7%) were managed as malaria patients. In addition, 15 (2.0%) did not receive antimalarial drugs despite positive blood films. There was very poor agreement between clinical and microscopic diagnosis of malaria.

Clinical examination and laboratory investigation were significantly more performed in those diagnosed as complicated malaria cases. In addition to blood film examination for malaria parasite, few patients were subjected to the following tests: complete blood picture, erythrocyte sedimentation rate, blood sugar, renal and liver functions. Of these, anaemia, increased white blood cell count and abnormal renal function tests were significantly associated with complicated malaria.

**Malaria history and health care accessibility** The duration between onset of symptoms and initiation of treatment was significantly longer in complicated compared to uncomplicated malaria patients. This was also true for the total duration of illness. The time interval since last malaria attack in the family was significantly shorter in complicated compared to uncomplicated cases. A significantly longer waiting time at health facilities was reported by complicated compared to uncomplicated cases. On the other hand, there was

no significant difference between both groups regarding the number of malaria attacks during the previous year, time interval since last attack, distance between household and health facility, and time interval for follow-up visit.

**Health seeking behaviour** The majority of patients seek care initially at a health centre or hospital (32.6%, 54.3% of uncomplicated cases, versus 46.7%, 43.3% of complicated cases), with few doing so in the private sector. Seeking initial care at a hospital was found to be a protective factor against the progression from uncomplicated to complicated cases of malaria. General practitioners were the main health care providers in almost 70% of cases, with the rest being specialists and medical assistants. No significant difference was found between uncomplicated and complicated cases regarding the specialty of the health care provider.

**Determinants of complicated malaria** Multivariate analysis found that the significant risk factors for complicated malaria were: suburban/rural residence (2.2-fold); history of travelling to hyperendemic areas (6.7-fold); and delay in initiation of treatment for more than 2 days (1.6-fold). There was increased risk for those under-5 years, with lower educational levels and with inadequate knowledge of malaria, but this was not statistically significant.

**Case fatality rate for complicated malaria** The case fatality rate for complicated cases was 10.2% for clinically diagnosed cases and 10.7% for laboratory confirmed cases. The highest case fatality rate for confirmed cases was recorded in Ibrahim Malik Hospital (14.6%) and the lowest in Omdurman Hospital (8.1%), with no significant difference between hospitals.

**Determinants of mortality from complicated malaria** Multivariate analysis found that the significant predictors of mortality from confirmed complicated malaria were: female gender (2.6-fold); duration of illness (1.1-fold for each day); negative history of malaria (2.7-fold); and reluctance or delay in administering the proper antimalarial drug on admission (5.6-fold). Lower educational levels were a significant protective factor. There was an increased risk for those under-5 years of age but this was not statistically significant.

### ■ Conclusions and recommendations

The risk factors for complicated malaria identified in this study highlight deficiencies in the health care system including a lack of protective measures, especially for those travelling to hyperendemic areas, and delays in the initiation for treatment for more than 2 days.

# Malaria

## Vector Behaviour

### Sudan

Biting behaviour of malaria vectors in relation to community sleeping behaviour and usage of insecticide treated bednets in Sudan

Sudan  
Eddamar, Medani,  
Kassala, Malakal areas  
and Nyala city

Study period:  
September 2003–  
March 2004

Small Grants  
Scheme  
(SGS) 2002 No. 135

Principal Investigator  
Mr Elsamani Ibrahim  
and Mr Osama  
Seidahmed  
National Malaria Control  
Programme  
Federal Ministry of Health  
Khartoum, Sudan  
e-mail:  
osamamekki@yahoo.co.uk

#### Abstract

A cross-sectional study was carried out in Sudan during September 2003 to March 2004 to study the association between the biting times of the malaria vector(s) and the sleeping times of communities of 5 different epidemiological sites. The biting cycle of *Anopheles gambiae sensu lato* was studied using human landing collection and the physiological age of the hourly-collected biters for parity. The knowledge, attitudes and practices (KAP) of household members regarding sleeping habits and insecticide treated bednets (ITNs) were also evaluated.

**Results** The peak biting cycle of *An. gambiae s.l.* was observed between 20:00–21:00 and 24:00–01:00. Although no significant hourly variation existed, hourly-dissected females showed a predominance of nulliparous females in age composition throughout the night. Children under-5 years of age sleep before 22:00, in contrast to other age groups that sleep after 22:00. Other household members-related data were collected.

**Conclusion** In comparison to other age groups, children under-5 years are probably protected by early sleeping if they use ITNs. Other protective measures are needed to protect those above-5 years who go to sleep later.

#### Background

Malaria is one of the most serious vector-borne diseases, affecting millions of people in the tropics. In Sudan, malaria leads to 30%–40% of total outpatient attendance with an annual number of cases of 7.5 million and 35 000 deaths (National Malaria Administration, unpublished data, 2000). The majority of cases are infected with *Plasmodium falciparum* although other plasmodia occur. *Anopheles arabiensis* is considered to be the main vector throughout the country, while *An. gambiae* and *An. funestus* are mainly restricted to the humid southern region [1].

To address the pending crisis brought about by a lack of adequate intervention methods, insecticide treated bednets (ITNs) were introduced as the method of choice for the reduction of transmission risk. It has been extensively observed that the use of ITNs provides adequate protection against malaria infection, particularly in children.

Large-scale implementation and sustainable use of ITNs needs to overcome several obstacles linked to human behaviour and vector biting behaviour. One of these is the effectiveness of the intervention in taking the various sleeping habits of the different populations in the country into consideration. ITNs are more likely to affect late biters than early biters. People may be bitten before going under the bednet as many mosquitoes are active after sunset when people gather together to relax or watch television. The peak biting time of the local vector might be expected to be an important determinant of ITN effectiveness. Therefore, a study was carried out to investigate the association between the biting time of the malaria vector(s) and the sleeping hours of the community.

### Conclusions and implications of the study

- The distribution of parous females among the 2 different periods of main activity (pre-bedtime and bedtime exposure) differed between children under-5 years and other age groups.
- The early sleeping habits of children under-5 are an important factor in preventing the older biters (which are probably infective) from reaching them. Pre-bedtime exposure of all age groups to the bites of *Anopheles gambiae sensu lato* showed that younger biters (which are probably non-infective) are predominant during this period of biting.
- People in the different epidemiological sites have a tendency to sleep before 01:00, although there are variations between different age groups, reflecting differences in culture and lifestyle between the study areas. Generally, the older household members go to sleep later, while the younger members go to sleep earlier.
- Bednets have been traditionally used to protect from the nuisance caused by nocturnal biting insects.

## Materials and methods

A cross-sectional community-based study was conducted in 5 regions representing the different ecological strata of Sudan: the desert fringe in the northern part of the country was represented by Eddamar; the irrigated sector was represented by Medani; Kassala in the eastern Sudan represented poor savannah with a short rainy season; Nyala city was selected to cover the border areas between poor savanna and wet savanna; and the rich wet savannah area was represented by Malakal. These areas also represent different social and cultural dimensions, and the transmission season varies from 3–6 months, starting from June. The main city and a nearby village were selected in each area for mosquito and household surveys.

**Night biting collection** At least 100 night biting anophelines were collected in each ecological region over 10 nights. Indoor and outdoor human landing catches were carried out from 19:00–07:00 to observe the vector-biting cycle. The working team were provided with antimalarial prophylaxis. Using aspirators and hourly cups 2-person teams collected mosquitoes by exposing body extremities, especially legs and feet. The teams worked in 2-hour shifts.

**Mosquito processing** Mosquitoes were taken to the laboratory, killed and counted. They were morphologically identified using taxonomic keys, and the abdomens of 464 mosquitoes were dissected to determine parity. All mosquitoes were then desiccated over silica gel and kept at room temperature until being further processed to detect sporozoites using enzyme linked immunosorbent assay (ELISA).

**Household surveys** In each study area, 100 housewives were randomly selected, using systematic sampling technique, from the city and 50 were selected from a neighbouring village, giving an overall total of 750. A structured and pretested questionnaire was used to collect household information regarding age, residence and occupation of the housewife, family size, socioeconomic status, sleeping times of different age groups, where people sleep (outdoors or indoors) and the presence of, beliefs about, and affordability of ITNs.

## Main study findings

**Mosquito surveys** A total of 827 female anophelines were sampled from night biting catches in the study areas. In addition, *Culex* spp., sand flies and Aedines were observed. The investigated anophelines belonged to the species *An. gambiae sensu lato* (n = 812), *An. pharoensis* (n = 14) and *An. coustani* (n = 1). The essential features of the biting cycle of *An. gambiae s.l.* seem to be an initial period of moderate activity reaching its peak at 21:00–22:00. A decrease in biting behaviour at 22:00–23:00 occurs followed by a crescendo of feeding reaching a peak at 24:00–1:00, but with

considerable activity continuing until shortly before dawn.

Nulliparous biters were predominant throughout the night hours. However, there was no significant difference in the age-composition of the hourly-collected mosquitoes between nulliparous and parous females. Excluding data from the Nyala region (4.9%), the parity rates in all the study regions ranged between 22.0%–34.3%.

**Household surveys** There was significant difference in sleeping hours before 22:00 (for those under-5) and before 23:00 (for older age groups) in the 5 areas studied and between children <5 years, children at primary school age, young people after primary school age and adults, especially between children <5 years and other age groups. However, there was no significant difference between them regarding sleeping after 22:00 for those under-5 and after 23:00 for older age groups.

Usage and perception of bednets also differed significantly between the study areas, with a range of 20%–89.3% of interviewed households using bednets regularly. Of the interviewed mothers, 52%–88.9% agreed that bednets are protective measures against malaria. However, 44.3% of respondents said they were using nothing to protect themselves against malaria and only 14.5% said they were using ITNs. Regarding protective measures against mosquito bites, no clear finding was obtained as to whether respondents were using ITNs, insecticide spraying, smoking, other methods or nothing.

## Conclusions and recommendations

The early sleeping habits of children under-5 are an important factor in preventing the older biters (which are probably infective) from reaching them. Pre-bedtime exposure of all age groups to the bites of *An. gambiae s.l.* showed that younger biters (which are probably non-infective) are predominant during this period of biting. These findings accord with transmission of malaria being at its lowest during the early hours of the night when individuals usually engage in social activities and are therefore not under bednets. A comprehensive intervention package is urgently needed that will explore in greater detail and more frequently the behavioural aspects of people and vectors in relation to ITNs, in order to prevent long-term behavioural changes that may lead to a failure in malaria control.

## References

[1] Petrarca V et al. Cytogenetics of *Anopheles gambiae* complex in Sudan with special reference to *An. arabiensis*: relationship with East and West African populations. *Medical and Veterinary Entomology*, 2000, 14(2):149–164.

# Malaria

## Vector Control

### S o m a l i a

Community participation in malaria vector control using larvivorous fish in Somaliland

Somalia  
North-west Zone  
(Somaliland)

Study period:  
February 2002–2003

Small Grants  
Scheme  
(SGS) 2001 No. 7

Principal Investigator  
Dr Abdurahman  
Mohamed  
Primary Health Care  
Ministry of Health  
Hargeisa, Somalia

### ■ Abstract

An intervention study of larvivorous fish introduction and community participation was done in Kallabeydh village of Gabiley district, in the North-west Zone of Somalia, to evaluate the effectiveness of the naturally available larvivorous fish, Tilapia (*Oreochromis spilurus spilurus*) and community participation as a method for malaria vector control in Somalia. A knowledge, attitude, behaviour and practice (KABP) survey regarding malaria and its control was conducted prior to fish introduction in the village.

Results Tilapia have significant resistance towards chlorine in water up to a concentration of 1 mg/litre. Tilapia were shown to be voracious larvae eaters in the laboratory setting.

Tilapia were introduced into 25 berkits (reservoirs). After only one month, the number of larvae was reduced by more than 50% on average (from 16.5% to 78.6%).

The KABP survey revealed that malaria is not among the top five major diseases in the village. In the pre-intervention phase, larvivorous fish were not mentioned as a malaria control method, but in the post-

intervention phase, it was the most commonly stated malaria vector control, in addition to mosquito nets and insecticide spraying. Moreover, the KABP of the community was significantly improved by the intervention.

Conclusion Introduction of larvivorous fish is a feasible and efficacious vector control method that is acceptable to the community. However, its sustainability needs consideration and dedication from officials and the community.

These results indicate that community participation should be an integral part of any vector control strategy.

### ■ Background

Larvivorous fish are naturally found in several streams at the foot of the Gollis range of mountains that run along the coast of the Red Sea. Three species of larvivorous fish are available in these areas, the most common and efficient species regarding larvae-eating capacity is Tilapia (*Oreochromis spilurus spilurus*).

The objective of the study was to test the feasibility, applicability, acceptability and community participation in introducing larvivorous fish as a malaria vector control; to determine the chlorine resistance of the fish; and to evaluate its larvae-eating capacity in the laboratory and in the berkits.

### ■ Materials and methods

The intervention study was initiated in February 2002 in Kallabeydh village, Gabiley district. A knowledge, attitudes, behaviour and practice (KABP) survey regarding malaria and its control was conducted prior to fish introduction in the village.

A total of 84 Tilapia fish (*Oreochromis spilurus spilurus*) were collected from the four water streams in the Sahil region. The fish were introduced in two fishponds in Hargeisa to determine their sensitivity to chlorine in water and study their larvae-eating capacity and some of their characteristics.

## Conclusions and implications of the study

■ Tilapia larvivorous fish (*Oreochromis spilurus spilurus*) survive chlorinated water up to a concentration of 1 mg/litre and therefore can be used in normally chlorinated piped water, which usually contains a concentration of less than 1 mg/litre. This means that the fish can be applied in the urban water tanks as an alternative method of mosquito control.

■ Tilapia fish, which are naturally available in several streams in Somaliland and are well-adapted to the environment, have a very high larvae-eating capacity, and are therefore considered a very efficient malaria vector control method, especially in many malaria endemic areas where the only existing breeding sites are man-made water reservoirs.

■ Berkit owners found the fish beneficial because of their tadpole-eating rather than larvae-eating capacity. This is due to superstitious beliefs related to tadpoles and frogs. These beliefs could be an enabling factor for the sustainability of fish introduction.

■ Community acceptance and participation in larvivorous fish introduction was far better than expected. These results emphasize the need to implement this successful intervention on a wider scale and to ensure its sustainability.

The chlorine sensitivity of Tilapia was tested by collecting fish from the breeding pond and putting them in buckets of water covered with mosquito nets. Ten fish were selected and put in pairs into 10 litre buckets of water containing different concentrations of chlorine, while one pair were put into normal non-treated water. The conditions of the fish were followed over 24 hours.

In March 2002, meetings and discussions were held with community members in Kallabeydh village. The objectives of these meetings were to: inform the elders and community leaders about the study; explain its objectives and details; become acquainted with the leaders of Kallabeydh; observe the views and comments of the elders on the study; request full collaboration and support for the different aspects of the study; train some young people on larvorous fish introduction to enable them to continue the activity in the future; explain the benefits of the fish including mosquito/malaria control, nutritional value, cleaning of the berkits etc.; and to inform the berkit owners about the benefits of larvorous fish. Checking for the presence of larvae in the berkits and contact with the community happened at least once every week. Introduction of fish into the berkits The nearest natural location where fish were available was in the stream at Lafaruug village in Sahil region, around 148 km east of Kallabeydh. The fish were transported in plastic containers, one-third filled with water, with 18 fish in each container. As the number of fish in this stream is very high, the fish were collected in locally made fishnets. The fish were collected from Lafaruug village in September 2002 and transferred in jerry cans to larger basins, kept overnight without food, and then transported in the early morning to Kallabeydh.

**Methodology of introduction** During each introduction a short discussion was held with the owners of the berkits about the fish concerning their acceptance of introduction, its benefits, the care required, dangers to the fish and plans to ensure sustainability. After determination of larvae density, the fish were taken from the containers into buckets, the number of fish to be introduced into the berkit was counted and the fish put slowly into the water in the presence of the owner. On average, one fish was introduced in every 3–4 m<sup>2</sup> of water.

**Pre-intervention larval density assessment** Long-tailed dippers with two litre capacity and an opening of 30 cm diameter were introduced into the four corners of each berkit i.e. four dippers per berkit. The collected larvae were transferred into buckets containing 2 litres of water. Larvae were then taken from the main bucket using a plastic dipper of 30 ml capacity; the number of larvae in each of these dippers was counted and classified into anopheline and culicine larvae and their stage determined.

**Post-intervention larval density assessment** One month after the introduction of the fish into the berkits, a second assessment of larvae density was carried out to evaluate the impact of the intervention. The procedure was carried out with the same methodology done in the pre-intervention stage.

### ■ Main study findings

All fish introduced in a chlorine concentration up to 1 mg/litre remained alive and were very calm, active and swimming normally after 24 hours of introduction. The fish tested during the study ate more than 83% of the total larvae given to them, within 24 hours (range 83–94%). The average reduction of larvae in the berkits was 52.8% (ranging from 16.5% to 78.5%).

The significant reduction of larvae in the berkits is mainly attributed to the voracious larvae-eating capacity of the fish. Other factors, such as temperature and humidity can influence the gonadotrophic cycle of mosquitoes, but there were no significant climatic differences between September and October when the larvae assessments took place.

Following community participation in the study, there was a significant increase in the KABP of the population, regarding their knowledge of malaria, its mode of transmission, protective measures and the importance of larvorous fish. The pre- and post-intervention KABP surveys reported, respectively, that: 56.2% and 66% of the community knew about malaria; 23% and 30.5% reported an episode of malaria, but only 9% had had malaria during the previous year; 19.1% and 23% reported that another person in their family had had a malaria episode during the previous year; 5% and 8% reported a person who had died due to malaria within the last 10 years and; 16% and 44% knew at least one malaria control method. The two main traditional treatments mentioned were camel milk and inducing diarrhoea through local laxatives. Fish introduction was acceptable to 59% and 83% of the community.

### ■ Conclusions and recommendations

These results suggest that larvorous fish can be a feasible, cost-effective and acceptable vector control method, especially in malaria-endemic areas where the only existing breeding sites are man-made water reservoirs.

# Malaria

## Vector Control

### Islamic Republic of Iran

Stability and wash resistance of locally made mosquito bednets treated with pyrethroids against malaria vector *Anopheles stephensi*

Islamic Republic of Iran  
Teheran

Study period:  
September 2002–  
July 2003

Small Grants  
Scheme  
(SGS) 2002 No. 27

Principal Investigator  
Dr Hassan Vatandoost  
Department of Medical  
Entomology  
School of Public Health and  
Institute of Health Research  
Teheran University of  
Medical Sciences  
Teheran, Islamic Republic  
of Iran  
e-mail:  
hvatandoost@yahoo.com

#### Abstract

The resistance of locally made insecticide-treated bednets (ITNs) impregnated with different pyrethroids was evaluated after washing, with or without shaking. Four pyrethroids were tested: permethrin, deltamethrin, lambda-cyhalothrin and cyfluthrin. Three detergents frequently used in the Islamic Republic of Iran were used for washing.

**Results** With increasing number of washings, the washing machine detergent Rakht reduced the efficacy of deltamethrin and lambda-cyhalothrin insecticides, with or without shaking, but had no effect on permethrin and cyfluthrin. Use of hand washing soap and the hand washing detergent Shoma reduced the efficacy of deltamethrin and cyfluthrin, but increased the insecticide effect of lambda-cyhalothrin. Washing bednets impregnated with permethrin with Shoma had no effect on mosquito mortality, but mortality was increased on washing with soap.

**Conclusion** Lambda-cyhalothrin is the most stable and resistant pyrethroid to detergents and washing, while deltamethrin is the least resistant.

#### Background

Insecticide-treated bednets (ITNs) are

the main vector control method promoted by the Roll Back Malaria programme. The residual effect of pyrethroid insecticides remains for 6–12 months if the bednets are not washed. This residual effect depends on the type and quantity of the insecticide. However, the extent of removal of insecticide molecules by washing and/or their detoxification by soap or detergents has not been properly studied. This lack of information explains the reported discrepancy in wash resistance of the different pyrethroids. Results of bioassay in different parts of the world also vary greatly due to differences in the formulations of insecticides, species of *Anopheles*, susceptibility level of the mosquito, time of exposure, texture of the bednet and type of test. In the Islamic Republic of Iran, the Ministry of Health and Medical Education has decided to promote community use of pyrethroid impregnated bednets. Accordingly, a local factory was established in collaboration with nongovernmental organizations to provide ITNs to the population living in malaria endemic areas. Consequently, the study was undertaken to provide information on the extent of physical removal and/or detoxification of insecticide molecules on locally made ITNs through washing them with different locally-manufactured detergents.

#### Materials and methods

The following calculations were made: the surface area of the bednet; the amount of water necessary to saturate the bednet (water retention capacity of the wet fabric); and the amount of insecticide necessary to treat the bednet. Pyrethroid insecticides were used at the recommended dosages. These consisted of suspension concentrate formulation of deltamethrin (10%) (Aventis), emulsifiable concentrate formulation of permethrin (10%) (Aventis), suspension concentrate formulation of

### Conclusions and implications of the study

■ Pyrethroid insecticide-treated bednets (ITNs) are efficacious for 6–12 months without washing. Washing, with or without shaking, using different detergents or soap will generally affect the efficacy of insecticides.

■ Lambda-cyhalothrin is the most stable and resistant pyrethroid to detergents and washing, while deltamethrin is the least resistant. Permethrin and cyfluthrin have intermediate stability.

■ Insecticide efficacy is significantly affected by the number of washings, but not greatly influenced by shaking.

■ Washing resistance of ITNs depends on several factors such as washing process and type of soap or detergent. Bioassay results also depend on insecticide formulations, species of *Anopheles*, mosquito susceptibility, time of exposure, texture of bednet and type of test. All these factors vary greatly across countries. Therefore, malaria endemic countries should conduct their own studies on wash resistance in order to develop national guidelines on the treatment of ITNs.



lambdacyhalothrin (2.5%) (Zeneca) and emulsifiable oil in water formulation of cyfluthrin (5%) (Bayer).

Locally made detergents were used including a washing machine powder called Shoma, a hand washing detergent called Rakht and a hand washing soap called Golnar. Locally made nylon bednets were impregnated with recommended pyrethroid dosages. Bioassay test A bioassay test with WHO cones (Lin method) was used to determine the efficacy of pyrethroids on the ITNs.

**Washing without shaking** ITNs were introduced in 1 litre beakers containing 0.5 litres of de-ionized water, with 2 grams/litre detergent and left for 20 minutes without shaking. The water was maintained at 30°C during exposure to detergent and rinsing. Then the ITN samples were removed and rinsed twice for 10 minutes in clean water.

**Washing with shaking** ITNs were introduced in 1 litre glass bottles containing 500 millilitres de-ionized water, with 2 grams/litre detergent. The bottles were immediately introduced into a water bath at 30°C and shaken for 20 minutes at 155 movements per minute. They were then rinsed twice during 10 minutes at the same agitation speed.

In both tests, the pH of the washing bath was measured and comparison made at baseline, and after 1, 2 and 3 washings. The test was carried out at 25°C ± 2°C and 70%–80% relative humidity. The percentage mortality was recorded after a 24-hour recovery period on a report form. If the control mortality was between 5%–20%, then the percentage mortality was corrected using Abbott's formula. If the control mortality exceeded 20%, the results were recorded and the test repeated. Knock down time (KD50) was recorded at regular intervals following exposure, ending when about 80% of mosquitoes were knocked down, and stopping after 60 minutes. In addition, the 24-hour mortality was recorded.

## ■ Main study findings

**Hand washing powder (Rakht)** There was no significant difference in the 24-hour mosquito mortality by washing bednets impregnated with permethrin (10%) once or more, with or without shaking (72.4% ± 5.9% without washing and 82.5% ± 5% after 3 washings without shaking, compared with 51.7% ± 6.6% to 68.4% ± 6.2% with shaking, respectively). However, washing bednets impregnated with deltamethrin (10%), with or without shaking, resulted in a significant reduction of the 24-hour mortality rate after washing once or more (48.3% ± 6.6% without washing to 11.9% ± 4.2% after 3 washings, and 47.3% ± 6.7% and 13.3% ± 4.4%, without and with shaking, respectively). Washing bednets impregnated with lambdacyhalothrin (2.5%) significantly reduced mortality after 3 washings, with or without shaking (79.3% ± 5.3% without washing to 56.4% ± 6.7% after 3 washings, and 83.1% ± 4.9% to 37.3% ±

6.3%, without and with shaking, respectively). The efficacy of bednets impregnated with cyfluthrin 5% was not significantly affected by washing, with or without shaking.

**Hand washing soap** Washing bednets impregnated with permethrin (10%) with soap once or more, without shaking, resulted in a significant increase in mosquito mortality from 59.3% ± 6.4% to 93.3% ± 3.2%, respectively. Similarly, there was an increase in the mortality rate with washing with soap once, with shaking, from 67.3% ± 6.5% to 94.8% ± 2.9%, respectively. Washing bednets impregnated with deltamethrin (10%) resulted in a significant reduction in the mortality rate after 2 washes, without shaking (54.3% ± 6.5% to 20.7% ± 5.3%), compared to one wash, with shaking (54.2% ± 7.2% to 25.5% ± 5.9%). On the other hand, washing bednets impregnated with lambdacyhalothrin (2.5%) increased mortality after one washing, with or without shaking (from 91.7% ± 3.6% and 89.5% ± 4.1%, without and with shaking, respectively to 100% for both methods after one washing). Washing bednets impregnated with cyfluthrin (5%) resulted in a significant reduction of the mortality rate from 80% ± 5.2% to 63.3% ± 6.2% after one wash, without shaking, and from 76.7% ± 5.4% to 67.2% ± 6.2% after 2 washings, with shaking.

**Machine washing powder (Shoma)** There was no significant reduction in the mosquito mortality by washing bednets impregnated with permethrin (10%) once or more, with or without shaking. However, washing bednets impregnated with deltamethrin (10%) resulted in a significant reduction in mortality after 2 washings, without shaking. On the other hand, washing bednets once, with shaking, produced a significant reduction in the mortality rate from 88.3% ± 4.1% to 17.2% ± 5%. Washing bednets impregnated with lambdacyhalothrin (2.5%) increased mortality after one washing, with or without shaking (from 91.8% ± 3.5% and 95% ± 2.8%, without and with shaking, respectively, to 100% for both methods after one washing). Washing bednets impregnated with cyfluthrin (5%) resulted in a significant reduction of the mortality rate from 96.5% ± 2.4% to 60.3% ± 6.4% after 3 washings, without shaking, and from 83.9% ± 4.7% to 33.3% ± 6.1% after one washing, with shaking.

## ■ Conclusions and recommendations

Lambdacyhalothrin is the most stable and resistant pyrethroid to detergents and washing, while deltamethrin is the least resistant. Permethrin and cyfluthrin have intermediate stability. Insecticide efficacy is significantly affected by the number of washings, but not greatly influenced by shaking. This information is crucial to the development of guidelines for the treatment of mosquito nets.

# Malaria

## Vector Control

Oman

Can larvivorous fish be an efficient vector control for malaria eradication in Oman?

Oman  
Barka Wilayat, South  
Batinah Region

Study period:  
October 2002–  
January 2004

Small Grants  
Scheme  
(SGS) 2002 No. 16

Principal Investigator  
Dr Mohammed Abdul  
Razzak Hamza  
Department of  
Environmental Health and  
Malaria Eradication  
Ministry of Health  
Muscat, Sultanate of Oman  
e-mail: Dehme@mail.com

### Abstract

A study was done to evaluate the effectiveness of three local fish in Oman, *Tilapia galilaea*, *Aphanius dispar* and *Rasbora daniconius*, as vector control measures in irrigation tanks, permanent potential breeding places of *Anopheles*. In the coastal wilayat (district) of Barka, 324 tanks were randomly selected for fish release and 276 tanks were selected in which no vector control measures were applied as a control. All vector control measures were suspended for 2 months prior to introducing the fish in the selected tanks. All tanks were checked twice a month for larvae, state of tanks, fish survival and climatic variation.

**Results** *T. galilaea* had been introduced in 100% of selected tanks by the end of June, while *A. dispar* had been introduced by the end of July. *R. daniconius* had survived in only 38% of tanks by the end of July. The frequency of tanks positive for larvae was 5.7% and 8% with *T. galilaea* and *A. dispar*, respectively, significantly lower than the rate in the control group (44.7%) and the pre-intervention baseline rate (32%). The mean number of larvae was 12, 15, 37 and 71.5 in the tanks containing *T. galilaea*, *A. dispar*, *R. daniconius* and the control tanks, respectively.

**Conclusion** Biological vector control in irrigation tanks using local fish (*T. galilaea*, *A. dispar*) may be effective during the

consolidation and maintenance phases of malaria eradication in Oman and could be used as a supplementary control measure in other settings.

### Activities conducted within the framework of the project

A meeting was held in the Sport Club of Barka Wilayat on 22 December 2002, under the auspices of His Excellency, *Wali* (governor) of Barka attended by the Consultation Council representative of the *Wilayat*, directors of related sectors (governmental and private), community leaders, and medical, paramedical and field staff. The principal investigator explained the objective, methodology and importance of the project and the community's role in supporting it.

### Background

The malaria eradication programme in Oman is currently in its maintenance phase. Semi-permanent and permanent potential breeding places of mosquitoes are no longer submitted to the regular chemical larviciding that was carried out during the attack phase of malaria eradication. Alternative mosquito control measures should therefore be implemented.

The use of fish as a biological agent to control mosquito breeding in semi-permanent and permanent water bodies has been advocated under the bioenvironmental control strategy for malaria control. The three locally available species in Oman (*A. dispar*, *R. daniconius*, and *T. galilaea*) have all the characteristics suitable for investigation. They are small sized, with high reproduction rates and tolerance to pollution, salinity, temperature fluctuation and transportation. Also they originate from the region to be studied. However, preference for mosquito larvae over other types of food located in water needs to be fully evaluated. A study was done to evaluate the effectiveness of these three local fish in Oman as vector control

### Conclusions and implications of the study

- Both *A. dispar* and *T. galilaea* are very effective as malaria vector controls when they are established in irrigation tanks. *T. galilaea* can survive and multiply in irrigation tanks, with over 93% of fish established within 2 months of release, while 91% of *A. dispar* are established within 4 months.
- Larvivorous fish are cost-effective in the long run compared to larviciding, and have the advantage of being environmentally-friendly.
- *T. galilaea* and *A. dispar* can survive and multiply under all climatic conditions throughout the year in Oman.
- *T. galilaea* can live in tanks with organic materials or algae, while *A. dispar* need cleaner water.
- *R. daniconius* cannot be established in irrigation tanks under some environmental and climatic conditions.

measures in irrigation tanks, which are permanent potential breeding places of *Anopheles*.

## Materials and methods

The intervention study was carried out in 8 villages in Barka Wilayat from which 600 irrigation tanks with an average 20m<sup>2</sup> surface area were randomly selected. As a control, 276 of the 600 tanks were selected where no vector control measures were taken, while 324 were selected for fish release: 113 for *T. galilaea*, 110 for *A. dispar* and 101 for *R. daniconius*. Prior to the start of the study, the tanks in Barka Wilayat had been the subject of different larviciding strategies. All such vector control measures in the selected tanks were suspended from December 2002. Geographical reconnaissance of the tanks was updated and field workers were trained on filling data collection forms and methods of measuring temperature and relative humidity. The tanks were checked for the presence and densities of larvae prior to the intervention.

**Fish catching, transport and release** (January–February 2003) Three types of local fish were used in the study: *A. dispar* obtained from wadis (valley streams) in the Batinah Region, both *A. dispar* and *R. daniconius* taken from falajes (traditional manmade underground water channels used for irrigation) in the interior of Oman (Dakhliya Region) and *T. galilaea* gathered from wadis in Quriyat Wilayat, Muscat governorate.

The fish were collected using handmade nets about 4m<sup>2</sup> with small holes, put in thermocol boxes (10 litre capacity) with water taken from the same collection sites, and immediately released in a special large tank, after adjustment of the water temperature. Within 2 days, the fish were transported to the field and released at a rate of 5 fish per square meter of water surface. During May–September, oxygen injection was carried out during fish transportation. Each experiment tank was checked once every 2 weeks to determine presence and density of *Anopheles* larvae.

**Fish density determination** The presence and number of fish in each tank were checked periodically every 2 weeks; 5 fixed spots were checked using a special net to calculate the average number of fish. An average number of 4 fish per square meter surface area or less indicates an inability of the fish to survive in the tank. When this occurred, fish were re-introduced.

**Laboratory investigations of the larvae-eating capacity of the fish** To examine the larvae-eating capacity of the fish, different species of fish were put in different boxes filled with 2 litres of water with increasing numbers of larvae. The ability of the fish to eat larvae when no other food sources were available and in the presence of other food sources was tested. After 24 hours the remaining

larvae in the boxes were counted and the fish dissected to detect the remains of mosquito larvae in the gut.

## Main study findings

*T. galilaea* and *A. dispar* proved to be very effective vector control methods for tanks, despite the long time needed to become established. The average larvae density per tank was 12, 15, and 71.5 in tanks with *T. galilaea*, *A. dispar* and the control tanks, respectively. *T. galilaea* survived and multiplied in the tanks in considerable quantity, refuting some doubts about its ability to survive in tanks. There was a significant difference between experimental and control tanks regarding larval positivity (5.37% for *T. galilaea*, and 8% for *A. dispar*, compared to 44.7% for the control tanks), and significant reduction of larval positivity in the experimental tanks compared to the baseline pre-intervention rate (32%). Laboratory investigation found that both *T. galilaea* and *A. dispar* are very efficient larvae eaters whether other food sources are available or not.

Mean larval positivity was significantly lower in the tanks using *T. galilaea* or *A. dispar* compared to those using *R. daniconius* and the control group. However, there was no significant difference between *T. galilaea* and *A. dispar* regarding mean larval positivity. Significant predictors of the larval positivity of tanks using *T. galilaea* were time and humidity. Time was the only significant predictor of larval positivity using *A. dispar*. Larval positivity using *R. daniconius* decreased with time and humidity but increased with temperature, although this was not statistically significant. In the absence of larvivorous fish intervention, larval positivity increased significantly with time, humidity and temperature, and this was statistically significant.

*R. daniconius* failed to establish itself in tanks, and therefore cannot be used as an effective vector control method. The study found that *An. culicifacies* and *An. stephensi* breeding increased during August–October and decreased during the cold months, December–January.

Apart from their nutritional value, larvivorous fish proved to be cost-effective.

## Conclusions and recommendations

During the consolidation and maintenance phase of a malaria programme, fish can be used as a vector control method in breeding places where other measures have been suspended. Countries with a similar geographical, ecological, climatic and epidemiological situation can use *T. galilaea* and *A. dispar* as a potent vector control measure in wadis, lakes and tanks.

# Measles and Rubella

## Immunization Status

### Yemen

Immune status of  
measles and rubella  
in urban and rural  
Sana'a Governorate,  
Yemen

Yemen  
Sana'a Governorate

Study period:  
September 2002  
–February 2004

Small Grants  
Scheme  
(SGS) 2002 No. 78

Principal Investigator  
Dr Talal A Sallam  
Department of Microbiology  
Faculty of Medicine and  
Health Sciences  
Sana'a University  
Sana'a, Yemen  
e-mail:  
talalsallam2000@yahoo.com

### Abstract

A study was undertaken to investigate the susceptibility to measles and rubella in Yemen. A total of 1394 subjects were enrolled from urban and rural areas of Sana'a Governorate. These consisted of schoolchildren, pregnant women, children aged 18 months–6 years and adults aged 19–85 residing in Sana'a city. The collected data included age, sex, measles and rubella vaccination status, participation in catch-up measles campaigns and history of measles. Blood samples were collected, and the serum separated and tested by enzyme-linked immunosorbent assay (ELISA) for detection of measles and rubella IgG, and determination of antibody titres.

**Results** Overall, 11.7% of subjects did not have antibodies to measles virus. Susceptibility to measles decreased significantly with age; males were significantly more susceptible to measles than females; rural schoolboys had significantly higher susceptibility than urban schoolboys; and IgG levels were significantly higher in rural compared to urban schoolgirls. Only 13.6% reported history of measles and only 33.3% of those who recalled their measles vaccination status reported they had received measles vaccine.

Overall, 14.6% of subjects were susceptible to rubella virus, including 4.6% of females in

childbearing age and 7.1% of pregnant women. Susceptibility decreased significantly with age, while rubella IgG levels increased significantly. There was a significantly higher susceptibility in rural residents compared to urban residents (14.8% and 7.1%, respectively). However, IgG levels were significantly higher in rural compared to urban areas, and in females aged 9–12 years compared to males.

**Conclusion** The study reported a high prevalence of susceptibility to measles and rubella. Catch-up measles immunization campaigns involving children aged 9 months–15 years are essential to prevent virus circulation in this community. Rubella vaccination should be seriously considered and should include a policy to target females of childbearing age.

### Background

As in many other developing countries, measles remains a major cause of morbidity and mortality among children in Yemen. Until 2000, the measles immunization programme was based on 1 dose of live measles vaccine at 9 months. Catch-up immunization campaigns were implemented during 2001 and 2002 to immunize children under 15. This was meant to provide an extra dose of vaccine to children who had previously been immunized and to immunize those who had missed immunization. The impact of these immunization campaigns on the control of measles in Yemen has not been previously investigated.

Rubella vaccination is not part of the expanded programme of immunization in Yemen. In view of the limited data on rubella in Yemen, the status of rubella immunity remains to be fully investigated. This will help to assess the need for inclusion of the rubella vaccine in the expanded programme of immunization. A study was therefore undertaken to investigate the status of susceptibility to measles and rubella in Yemen.

## Conclusions and implications of the study

■ The study reported that 11.7% of subjects were susceptible to measles, including 55% of children less than 5 years of age. This indicates that many children of this age are still unprotected. The possible reasons could be vaccine efficacy, interference of maternal antibodies with the vaccine and/or low vaccination coverage. Further investigation is warranted.

■ Catch-up immunization campaigns for measles involving children aged 9 months–15 years is essential in this community.

■ The comparable IgG levels in vaccinated and unvaccinated subjects suggest that the unvaccinated have acquired their IgG through natural infection with measles virus.

■ A rubella susceptibility of 14.6% was reported by this study. However, this remains relatively low in the absence of rubella vaccination. While the susceptibility rate of 4.6% among females of childbearing age is relatively low, a rate of 7.1% among pregnant women exposes them to the risk of rubella infection.

## Materials and methods

The urban area of Sana'a city and the rural Arhab district located 60 km from Sana'a city were selected for the study. A total of 1394 subjects were enrolled from the 2 areas, consisting of: schoolboys and schoolgirls randomly selected from primary and secondary schools; pregnant women attending a maternity hospital in Sana'a city or maternity health care centres in Arhab district; children aged 18 months–6 years from Sana'a city randomly selected by household survey; and adults aged 19–85 residing in Sana'a city. The urban subjects ( $n = 1017$ ) consisted of 273 schoolboys, 90 schoolgirls, 120 children aged 18 months–6 years, 149 pregnant women and 385 adults. The rural subjects ( $n = 377$ ) consisted of 147 schoolboys, 144 schoolgirls and 86 pregnant women. The collected data included age, sex, measles and rubella vaccination status, participation in catch-up measles campaigns and history of measles.

Blood samples were collected, and the sera separated and tested using enzyme-linked immunosorbent assay (ELISA) to detect measles and rubella IgG and their levels. The absorbance of calibrator and samples were determined by a microplate ELISA reader. All samples with optical density (OD450) cutoff value plus 15% of this value were considered positive. Samples with OD450 < cutoff value minus 15% of this value were considered negative. All samples with a reading falling within the range of negative and positive readings were regarded as equivocal. Determination of measles antibody levels was achieved by a formula provided by the manufacturer. Determination of rubella antibody levels was performed using OD450 and IgG levels of the two calibrators provided with the kit.

## Main study findings

Of 1394 total study subjects, 636 (45.6%) were males and 756 (45.2%) were females. Excluding subjects with missing data or equivocal results, 1207 of the remaining 1369 were found to be measles seropositive (88.2%) with an overall measles susceptibility rate of 11.7%. There was a trend of decreasing susceptibility and increasing IgG levels with age. Males were significantly more susceptible than females with a two-fold increased risk of infection. In pregnant women around the time of delivery, 8 of 223 (3.6%) were susceptible. However, the immune pregnant women recorded high IgG levels. There was significant difference in the susceptibility rates between urban and rural schoolboys, and IgG levels also differed significantly between urban and rural schoolgirls.

Of 1089 subjects who were able to recall history of measles infection, 148 (13.6%) reported a positive history of measles. The latter group recorded significantly higher IgG levels compared to those with

negative measles history. Only 338 of 1014 (33.3%) who recalled their measles vaccination status reported that they had been vaccinated. IgG levels were comparable among subjects with both positive and negative history of vaccination for measles.

A total of 1275 subjects were investigated for rubella immune status. Subjects with missing data or equivocal results were excluded. Of 1253 eligible subjects, 183 were rubella IgG negative, giving an overall rubella susceptibility rate of 14.6%. Of 459 females of childbearing age, 21 (4.6%) were susceptible, while 8 (7.1%) out of 113 pregnant women tested around time of delivery were susceptible. The susceptibility rate decreased significantly with age, while rubella IgG levels increased significantly. There was a significant difference in antibody levels between various age groups below 9 years of age. On the other hand, there was no significant difference between males and females regarding their susceptibility to rubella (13.8% and 15.5%, respectively) or their rubella IgG levels. However, the difference in IgG levels between males and females differed significantly in the 9–12 years age group. There was a significantly higher susceptibility rate in rural compared to urban subjects (14.8% and 7.1%, respectively), with a two-fold increased risk among individuals living in rural compared to urban areas.

## Conclusions and recommendations

The study reported high overall measles and rubella susceptibility rates. The rubella susceptibility rate was comparable to that of measles despite the fact that rubella vaccination is not included in the expanded programme of immunization in Yemen. Catch-up measles immunization campaigns involving children aged 9 months–15 years seem to be essential to prevent virus circulation in this community. This is in line with the Ministry of Public Health and Population strategy to conduct a nationwide catch-up campaign for children in this age group. Rubella vaccination should be seriously considered in Yemen and a vaccination policy should target females of childbearing age.

# Meningitis

## Epidemiology

### Pakistan

Study of etiology and antibiotic susceptibility of infective agents of acute bacterial meningitis in Rawalpindi and Islamabad

Pakistan  
Rawalpindi and  
Islamabad

Study period:  
October 2002–June 2004

Small Grants  
Scheme  
(SGS) 2002 No. 126

Principal Investigator  
Dr Birjees Mazher Kazi  
National Institute of Health  
Islamabad, Pakistan  
e-mail:  
kazis@isb.paknet.com.pk

### Abstract

Meningitis is caused by a large number of bacterial and viral agents. However, 3 bacterial pathogens share the main burden of disease: *Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (SPN). During the period December 2003–June 2004, 529 cerebrospinal fluid (CSF) samples from suspected cases of acute bacterial meningitis in Rawalpindi and Islamabad were screened for the presence of bacteria by culture method and direct antigen detection using Wellcogen kit.

Results 97.5% and 97.3% of the 529 CSF samples were culture and antigen negative, respectively, while only 13 samples (2.5%) were positive for bacterial growth. Of the 529 samples, Hib was found in 1 (0.19%), SPN in 1 (0.19%) and *N. meningitidis* was not isolated from any. Other bacteria isolated from the 529 samples were *Enterobacter* spp. in 3 (0.57%), *Acinetobacter* spp. in 3 (0.57%),

*Staphylococcus aureus* in 2 (0.38%), *Escherichia coli* in 2 (0.38%) and *Klebsiella pneumoniae* in 1 (0.19%). The maximum number of infections occurred during March and April. Most isolates showed resistance to a wide range of antibiotics.

**Conclusion** Bacteria were identified in only 2.5% of meningitis cases, indicating that viral infections are the main cause of meningitis in Rawalpindi and Islamabad. Hib and SPN contribute to only 15.4% of acute bacterial meningitis cases, while the remaining are caused by a wide range of bacteria. Bacterial isolates showed a high level of antibiotic resistance.

### Background

*Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (SPN) are known to cause acute bacterial meningitis in children over one month of age. *N. meningitidis* is the only organism which can cause epidemic meningitis. In Pakistan, different figures for the incidence of suspected meningococcal meningitis were reported: 6.15 per 100 000 population for the period 1998–2000 [1] and 26 per 100 000 in 1997 from Sindh province [2]. However, none of these studies investigated the causative serotypes of *N. meningitidis*. Further, the incidence of Hib and SPN invasive disease is either not reported or data is not published. Therefore, a study was conducted to isolate, identify and characterize bacteria from the cerebrospinal fluid (CSF) of acute bacterial meningitis cases in Rawalpindi and Islamabad, and to determine the relative frequency of *N. meningitidis*, its serotypes, Hib and SPN. The study also sought to determine the prevalence of meningitis among admitted suspected cases, including the age and sex-specific prevalence, the seasonal trends of meningitis infection and the antibiotic susceptibility pattern of the isolates.

## Conclusions and implications of the study

■ Contrary to the belief that *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (SPN) are the main causes of acute bacterial meningitis among children in developing countries, *Enterobacter* spp. and *Acinetobacter* spp., were isolated from 23% of cases, whereas Hib and SPN were isolated from only 7.7% of cases.

■ This reported figure of 2.5% of cerebrospinal fluid (CSF) culture positive cases for bacteria is far too low compared to the 22% figure previously reported from Pakistan. However, some of the studies claiming to isolate Hib and SPN organisms did not perform proper bacteriological analysis and their main emphasis was on the interpretation of results based on clinical data.

■ The absence of *Neisseria meningitidis* isolate from cases of meningitis in this area coincides with the absence of reports of epidemics throughout the country during the study period. Meningococcal meningitis is a notifiable disease under the Disease Early Warning System (DEWS) within the National Institute of Health Epidemic Investigation Cell and no cases were reported to DEWS countrywide. Moreover, the Hajj pilgrims receive the quadrivalent vaccine covering *N. meningitidis* strains A, C, Y and W135; and no cases were reported during the Hajj season as well.

■ The wide range of antibiotic resistance of the 13 isolates is an indicator of the antibiotic load in the society and its effect on environmental bacteria.

## Materials and methods

A prospective, hospital-based surveillance study was conducted in 3 large referral hospitals, 1 from Rawalpindi (Rawalpindi General Hospital) and 2 from Islamabad (Pakistan Institute of Medical Science: Main Hospital and the Children's Hospital). The Microbiology Department, Public Health Laboratories Division, National Institute of Health (NIH), Islamabad, was the study coordinating centre and reference laboratory for the project. One microbiologist in each hospital was designated as the focal person for the study and was responsible for sending the CSF samples of suspected meningitis cases to the NIH.

Inclusion criteria for study subjects were any patient reporting to the hospital with signs and symptoms of meningeal irritation. Exclusion criteria were: previous intake of antibiotics during the last 7–10 days; lumbar puncture yielding haemorrhagic spinal fluid with positive xanthochromia; and previous refrigeration of CSF samples. Each enrolled patient was subjected to a detailed clinical history, lumbar puncture, and CSF examination and culture. CSF examination included the following parameters: physical (colour, turbidity, coagulum, xanthochromia); chemical (protein and glucose); microscopic (white blood cell count, gram stain); and direct antigen detection of specific antigens of *N. meningitidis*, SPN and Hib using Wellcogen kit (Remel, UK) containing meningitis panel A, B, C, Y and W135; Hib; SPN, group B streptococci; and *Escherichia coli* (KI). Centrifuged samples of spinal fluid were cultured on chocolate and blood agar at the hospital and NIH laboratories. Identification of isolates Culture isolates were identified at the NIH using WHO laboratory methods for the diagnosis of meningitis caused by *N. meningitidis*, Hib and SPN. Gram-negative rods were identified biochemically.

**Antibacterial susceptibility** Modified Kirby-Bauer method was used for determining antibacterial susceptibility of the isolates.

**Serogrouping of meningococcal isolates** Serogrouping of all meningococcal isolates was performed using a standard set of antiserum from Remel, UK.

**Blood cultures** Blood cultures were performed for patients for whom spinal fluid could not be obtained. Quality assurance protocol as recommended by WHO was strictly followed in the study.

## Main study findings

Of the 529 suspected meningitis cases, 13 (2.5%) samples yielded single bacterial isolates. Of the 529 samples, 112 were also tested for the presence of antigens using the Wellcogen antigen detection kit; 3 of these (2.6%) samples showed positive results with this kit, of which 2 were also culture positive and 1 positive for SPN antigen in CSF but culture negative.

Of the 13 culture positive CSF samples, Hib was isolated in 1 (7.7%), SPN was found in 1 (7.7%) and *N. meningitidis* was not isolated from any sample. Other bacteria that were isolated from the 13 culture positive CSF samples were *Enterobacter* spp. in 3 (23%), *Acinetobacter* spp. in 3 (23%), *S. aureus* in 2 (15.4%), *E. coli* in 2 (15.4%) and *Klebsiella pneumoniae* in 1 (7.7%).

In terms of seasonal variation, the maximum number of infections occurred during March (23%) and April (38%), when humidity is generally less. Only 15% of cases were seen in December. Of the children under the age of 5 years who were culture positive for bacteria, 54% were male and 46% were female. One strain of *E. coli* was resistant to 7 out of 8 commonly used antibiotics. Patients from whom the resistant bacterial species were isolated were less than 5 years old, and 1 case was a 9 months old baby with *Acinetobacter* spp. infection that was resistant to 6 out of the 7 antibiotics tested in vitro and in another case, *Enterobacter* spp. was resistant to the 8 antibiotics tested in vitro.

## Conclusions and recommendations

Failure to isolate any pathogen from 97.5% of suspected meningitis cases could be explained either by previous antibiotic intake before admission or by the low incidence of laboratory confirmed bacterial meningitis. These results suggest that the predominant form of acute meningitis in Rawalpindi and Islamabad is aseptic meningitis of viral origin. The bacterial isolates have shown a high incidence of antibiotic resistance to a wide range of antibiotics pointing towards another emerging Public Health problem.

## References

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# Schistosomiasis

## Drug Studies

### Egypt

Evaluation of the prophylactic effect of artemether on human schistosomiasis: a randomised controlled trial

Egypt  
Kafr El Sheikh  
Governorate

Study period:  
February 2003–2004

Small Grants  
Scheme  
(SGS) 2002 No. 39

Principal Investigator  
Dr Taha El-Khoby  
Ministry of Health and  
Population  
Egypt  
e-mail:  
telkhoby@yahoo.com

### Abstract

A double-blind randomised controlled trial was conducted in 2 primary schools in Kafr El Sheikh Governorate to evaluate the prophylactic effect of artemether chemotherapy on *Schistosoma mansoni* and to study the safety and efficacy of combined praziquantel and artemether versus praziquantel. A total of 913 schoolchildren were randomised into 1 of 2 treatment groups. The first group received 2 doses of praziquantel (40 mg/kg), 4 weeks apart, and the second group received combined praziquantel and artemether. Artemether was given every 3 weeks following the second dose of praziquantel and covering most of the transmission season from April to August. Three stool samples were collected: at the beginning of the study, 4 weeks following the first dose of praziquantel and at the beginning of the new school year in October 2003.

**Results** There was a significant difference between the 2 groups regarding the prevalence and incidence of infection which were halved in the artemether group and significantly reduced in the first grade schoolchildren. This effect could have only been achieved with 5 annual rounds of

praziquantel. The percentage reduction of the incidence of infection in the combined regimen group was significantly higher than that of the other group, indicating that infection among the combined drug therapy group was lower than among the praziquantel group. Artemether was palatable and no side effects were reported.

**Conclusion** These results provide evidence about the safety and efficacy of the combined regimen praziquantel/artemether and its prophylactic effect against reinfection, which could be beneficial for interruption of transmission in endemic areas.

### Background

For more than 2 decades, praziquantel has played a key role in morbidity control. However, rapid reinfection limits the success of control programmes and reliance on a single drug carries a potential risk of developing resistant strains. Artemether, a well-known antimalarial drug, has been shown to be effective against the juvenile stages of the three schistosome species: *Schistosoma japonicum*, *S. mansoni* and *S. haematobium*. Since artemether blocks the development of adult worms, it could theoretically wipe out parasite transmission. Using 2 drugs, praziquantel and artemether, acting against different stages of the parasite will improve control measures and may lead to higher rates of cure. Artemether could ideally be an additional tool to control schistosomiasis in nonendemic malaria areas e.g. Egypt, Brazil and China. In Egypt, the national schistosomiasis control programme has succeeded in reducing the force of *S. mansoni* infection in the Nile delta. However, foci with high transmission levels remain. In addition, schistosome isolates showing reduced susceptibility to praziquantel have been reported. This situation calls for additional control measures, preferably affecting disease transmission. A study was therefore undertaken to evaluate the prophylactic

## Conclusions and implications of the study

■ The strategy of annual mass praziquantel chemotherapy has been adopted to control *Schistosoma mansoni* morbidity in Egypt. As a result of this strategy, infection indices have been stabilized at low levels after successive annual rounds. However, infection indices remain high among schoolchildren enrolled in the first grade who have not received the drug, indicating persistent transmission. It is therefore expected that cessation of praziquantel control activities will raise infection indices to high levels. Consequently, using a drug such as artemether for premature infection will affect disease transmission.

■ Taking into consideration the difficult application of snail control measures for interruption of disease transmission in Egypt, due to the high cost and complex water channel network, the tested combined regimen should be considered as a measure for interruption of *S. mansoni* transmission. This should be preceded by a study of the cost-effectiveness of this approach through a large scale community study.

■ The repeated drug dosage within a short duration calls for increasing community participation to ensure high drug compliance. Studying the effectiveness of less frequent regimens is therefore recommended.



effect of artemether chemotherapy on *S. mansoni* and to study the safety and efficacy of a combined regimen of praziquantel and artemether versus praziquantel alone.

## Materials and methods

A double-blind randomised controlled trial was conducted in 2 primary schools in Kafr El Sheikh Governorate, School 15 (El Rouse) and School 36 (Abo Okada), in which 913 schoolchildren in grades 1 to 5 were enrolled. The data collection form included the following information: child's name, class, identification number, sex, residence, body weight, compliance to stool sample collection and treatment, and the results of stool analysis and treatment.

Stool samples were collected in April to determine baseline prevalence and intensity of infection. Another sample was collected in May, 4 weeks after the first dose of praziquantel, for the assessment of cure. To assess the combined therapy regimen, a third stool sample was collected at the beginning of the school year in October 2003. Pre-labelled wide-mouthed tight proof cups were distributed to all children and collected the next day. Collected samples were transferred to the central laboratory at the High Institute of Public Health. Stool samples were kept overnight at 4°C and 2 slides each of 43.7 mg were prepared from each sample. The Kato-Katz slides were examined microscopically and *S. mansoni* eggs were counted in every positive slide.

**Praziquantel treatment** According to the policy of the Ministry of Health and Population, mass chemotherapy with praziquantel was offered free of charge to all participants, irrespective of stool examination result. At the baseline, to ensure that almost every participant was free of infection, 2 doses of 40 mg/kg each were given within 4 weeks. In October 2003, children received praziquantel chemotherapy as a single oral dose according to the policy of the Ministry of Health and Population. Almost all of the children tolerated the drug with no obvious side effects, while less than 1% experienced minor complaints such as vomiting and headache.

**Artemether treatment** Children were offered 5 doses of 6 mg/kg each of artemether as a single oral dose: the first dose in May, the second in June, 2 doses in July and the fifth in August. The drug was very safe and no complaints were recorded. It was also more palatable than praziquantel. The children were reached during the summer vacation by house-to-house visits. This necessitated community participation in the form of regular meetings with community leaders, doctors at rural health units, the clergy and parents. Mass campaigns were conducted during Friday prayers at local mosques to motivate parents to adhere to treatment.

## Main study findings

The baseline prevalence in the 2 treatment groups was comparable (30.2% and 27.3%), as was the geometric mean egg count (GMEC) (59.8 and 57.6 eggs per gram). First grade schoolchildren had a significantly higher prevalence compared to older children (56.2% versus 17.0% and 50.0% versus 14.3% in the artemether and praziquantel groups, respectively). This younger age shift in the peak prevalence was observed in areas under repeated drug pressure to control the infection, where children of the fifth grade had received about 5 cycles of praziquantel chemotherapy. The impact of repeated drug therapy was confirmed by the low GMEC for almost all children. School 36 had a fairly high prevalence of *S. mansoni* infection among children enrolled in grades 1–5, which could be attributed to the lower socioeconomic status of the children compared to those enrolled in the other school.

**Praziquantel cure rate** The overall cure rate of praziquantel was 81.9%. The very high cure rate (94.7%–100.0%) observed among children enrolled in School 15 was due to a small number of positive children as well as the low intensity of infection. As for the high prevalence school, the lowest cure rate (72.8%) was observed for grade 1 (highest prevalence and GMEC) and the highest (92.3%) for grade 5 (lowest prevalence and GMEC).

**Combined drug therapy** The prevalence of *S. mansoni* infection in the praziquantel/artemether group was nearly half that of the praziquantel group in all grades. This difference was observed in both the low prevalence school (2.1% versus 4.3%) and the high prevalence school (6.7% versus 11.6%). The overall percentage reduction in prevalence for the praziquantel/artemether group was significantly higher than that for the praziquantel group (77.8% and 57.5%, respectively), and this was invariably true across all grades. Only within the combined regimen group, there was no significant difference in the percentage reduction of prevalence across grades. This might be explained by the protective effect of artemether against reinfection and its effectiveness against premature worms. This was confirmed by the significantly lower incidence of infection among the praziquantel/artemether group compared to the praziquantel group (0.6% versus 3.1% and 2.8% versus 6.5% in the low and high prevalence schools, respectively).

## Conclusions and recommendations

The combined praziquantel/artemether regimen proved to be an effective and safe regimen for schistosomiasis control. Furthermore, its prophylactic effect against reinfection could be beneficial for the interruption of transmission in endemic areas.

# Schistosomiasis

## Epidemiology

### Yemen

Application of a school questionnaire for schistosomiasis screening in Yemen

Yemen  
Kafr El Sheikh  
Governorate

Study period:  
August 2002–July 2003

Small Grants  
Scheme  
(SGS) 2002 No. 23

Principal Investigator  
Dr Abobakar Ahmed Ba-  
amer

Department of Community  
Medicine

Faculty of Medicine  
Hadramout University for  
Science and Technology  
Hadramout, Yemen

e-mail:  
aabaamer@maktoob.com

### Abstract

A study was undertaken to test the applicability of a school questionnaire for rapid screening of communities for schistosomiasis in Yemen. The questionnaire was distributed to all primary schools in 6 randomly selected districts in Abyan Governorate. The diagnostic performance and cost of the questionnaire and reagent strips were compared to conventional methods.

Results 7818 schoolchildren were interviewed in 101 schools. Positive answers for blood in urine were reported in 75 schools (74%), for blood in stool in 71 schools (70%) and for having schistosomiasis in 64 schools (63%). The questionnaire identified 5 schools with prevalence in the range of 20%–49% for *Schistosoma haematobium*, and the same prevalence in 1 school for *S. mansoni*. The results obtained by questionnaire were comparable to those obtained by other methods in 5 out of 6 schools in the case of urinary schistosomiasis but not for intestinal schistosomiasis.

The reagent strip proved to have high sensitivity (79.7%), specificity (92.2%), and positive (74.2%) and negative (94.2%) predictive values. For its low cost, easy

application and ability to give semiquantitative results, it is a feasible approach for rapid screening for urinary schistosomiasis. The average cost of implementing screening for one school is US\$ 31 for the questionnaire, US\$ 135.2 using the reagent strip method, US\$ 474.7 using filtration and US\$ 591.7 for the Kato-Katz method.

Conclusion The questionnaire and reagent strip are both cost-effective tools for rapid screening of communities for urinary schistosomiasis.

### Background

Yemen is one of the countries with the highest burden of schistosomiasis infection in the Eastern Mediterranean Region. However, there is no comprehensive information on disease distribution across the country and the reported prevalence rates vary considerably across different parts of the country. The prevalence of *S. mansoni* ranged from 8% in Hajjah Governorate to 35% in Ma'rib Governorate and up to 76.3% among schoolchildren in Sada'a Governorate. Similarly, the prevalence of *S. haematobium* varied from 10% among schoolchildren in Sana'a, to 49% and 52% among schoolchildren in Sada'a and Hajjah, respectively, and up to 59% in Khemir in northern Sana'a.

Accordingly, there was a need for a reliable and simple means for the rapid diagnosis of schistosomiasis in endemic communities in order to provide a detailed map of the distribution of the disease in the country. This would enable proper planning for adequate control measures. A study was therefore undertaken to test the applicability of a schistosomiasis school questionnaire for rapid screening of endemic communities.

### Materials and methods

All 137 primary schools present in 6 randomly selected districts out of the 11 districts of Abyan Governorate were screened for schistosomiasis using the

## Conclusions and implications of the study

■ The single enquiry about blood in urine by health workers or teachers can be a sensitive and specific tool for rapid screening of communities for urinary schistosomiasis. However, it is not a reliable diagnostic tool for intestinal schistosomiasis.

■ The questionnaire identified 5 schools with urinary schistosomiasis with prevalence in the range of 20%–49%. As the questionnaire is known to underestimate the true prevalence by 20%, the 5 localities of these schools are expected to have a prevalence of 50% or higher, and are therefore in need of control measures.

■ Urine reagent strips proved to be a highly sensitive and specific rapid diagnostic tool for *Schistosoma haematobium*. In view of the semiquantitative nature and low cost of this method, its implementation by the control programme is recommended. Teachers can be also trained to use this tool for early detection of cases.

■ The estimated cost of implementing the questionnaire for one school was US\$ 31, which was at least 4 times cheaper than the reagent strip, and 15 times cheaper than the filtration method.

questionnaire. For questionnaire validation and cost comparison, 100 children were randomly selected from each of the 6 schools. The tested questionnaire was based on the questionnaire used in Kilosa, Tanzania. It was translated and adapted to the local context, and used after pretesting and making the necessary changes.

The questionnaire contained structured questions about 7 signs and symptoms including blood with urine, blood with stool and 7 diseases including schistosomiasis. It also collected identification data including school name and location, class, age and sex. In the original questionnaire, questions were asked about the presence of blood in urine or stool during the last month in order to estimate the prevalence of schistosomiasis in communities. For this study, questions were additionally asked by health workers about the presence of blood in urine or stool while taking urine and blood samples. The questionnaire was administered to schoolchildren by teachers.

The urine and stool samples were collected between 10:00 and 14:00 hours and were tested for microhaematuria by reagent strips (Uriscan, GEN 9SG, YD Diagnostics, Republic of Korea), for *S. haematobium* eggs in urine by filtration method using polycarbonate membrane filters and Swinnex filter holders (Millipore, United States of America) and for *S. mansoni* eggs in stool by modified Kato-Katz method. In evaluating the diagnostic performance of the questionnaire and the urine reagent strip, the gold standard was urine filtration for *S. haematobium* and modified Kato-Katz method for *S. mansoni*.  
**Cost estimation** The cost of the different diagnostic methods were estimated and compared. The following expenses were calculated: local travel; training; office work including communication, stationary, printing, photocopying, data management and reporting; and supplies and equipment including laboratory investigations. The comparison was based on estimating the cost of each method for 1 school.

## ■ Main study findings

The teachers interviewed 7818 schoolchildren from the third (2729), fifth (2436), and seventh (1987) years, as well as from the first year (666) in schools where there was no seventh year. Schoolchildren reported positive answers for blood in urine in 75 of the 101 schools (74%), positive answers for blood in stool in 71 schools (70%) and positive answers for having schistosomiasis in 64 schools (63%). Of the 101 schools, 70 (69.3%) were classified as belonging to the low prevalence category (1%–19%) for both species, while 5 (5%) were classified as having moderate prevalence (20%–49%) for *S. haematobium*, while only 1 (1%) was classified as having moderate prevalence for *S. mansoni*.

Questionnaire validation 580 randomly selected children from the 6 selected schools were subjected to laboratory investigation to detect microhaematuria by reagent strip, ova in urine by filtration method and ova in stool by modified Kato-Katz method. In terms of diagnostic performance, the question on current presence of blood in urine had a sensitivity of 73.6%, a specificity of 80.9%, a positive predictive value of 50.9% and a negative predictive value of 91.9%. Similarly, the urine reagent strip proved to be a sensitive and specific diagnostic test for rapid screening for *S. haematobium* in endemic communities (sensitivity of 79.7%, specificity of 92.2%, positive predictive value of 74.2% and negative predictive value of 94.2%). Enquiry about the presence of blood in stools was much less reliable in diagnosing *S. mansoni*, with a low sensitivity (36.2%) and positive predictive value (31.8%), but a high specificity (85.5%) and negative predictive value (87.8%).

**Cost** The average cost per child was: US\$ 0.31 for the questionnaire, US\$ 1.35 for the reagent strip, US\$ 4.75 for the urine filtration method, and US\$ 5.92 for the modified Kato-Katz method. Comparison between the costs of different diagnostic methods at school level showed that the questionnaire was approximately 4 times cheaper than the urine reagent strip method, 15 times cheaper than the urine filtration method and 19 times cheaper than the Kato-Katz stool smear method. The cost of filtration or Kato-Katz methods will be less than the calculated cost if both methods are implemented together at the same time, because the costs of local travel, training and office work can be combined.

## ■ Conclusions and recommendations

The questionnaire proved to be a reliable diagnostic method for rapid screening of communities for *S. haematobium* but not for *S. mansoni* infection. A locally-available reagent strip is another reliable, simple and cheap method, which can be implemented after short training of health workers or teachers for quick identification of urinary schistosomiasis infection at school or primary health care level.

Both questionnaire and reagent strip are much less costly than the other conventional diagnostic methods. Their implementation by the control programme will make better use of limited resources to provide a detailed map of the distribution of the disease in the country, prioritize control activities and monitor the effectiveness of control measures.

# Schistosomiasis

## Epidemiology

### Morocco

*Bulinus truncatus* as an intermediate host for *Schistosoma haematobium* in Agadir Province

Morocco  
Agadir Province

Study period:  
September 2002–  
April 2004

Small Grants  
Scheme  
(SGS) 2002 No. 203

Principal Investigator  
Dr Abdelrrahmane  
Zekhnini  
Laboratory of Water and  
Environment  
Faculty of Sciences  
Ibn Zohr University  
Agadir, Morocco  
e-mail:  
abd.zekhnini@caramail.com

### Abstract

A study was undertaken to determine the role of *Bulinus truncatus* in the transmission of *Schistosoma haematobium* in Agadir Province. Asrar Kiss and Massa (Tassila) water stations, 2 neighbouring schistosomiasis foci in the District of Aït Baha, were selected as the study area. Snails were collected, counted and measured, then transferred to laboratories where their offspring were studied for the characteristics of snail infestation with *Schistosoma* miracidia and to evaluate the effect of desiccation on the snail population.

Results *B. truncatus* was detected in the majority of the foci in the area, but could not be detected in Targa n'Touchka. Both intermediate hosts, *B. truncatus* and *Planorbarius metidjensis*, coexisted in Tagharabout. Study of the evolution of different age groups of the *Bulinus* snail demonstrated 2 peaks, indicating the existence of 2 annual generations. The first generation started in January at Massa and in June at Asrar Kiss, while the second started in August and November in the 2 stations, respectively. The results of the experimental snail infestation revealed that the snail population

in Asrar Kiss recorded significantly higher mortality rates compared to those from Massa station (37% and 20%, respectively), significantly higher prevalence of infestation (36.5% and 3.75%, respectively) and a significantly longer patent period (21 and 12 days, respectively). On the other hand, the mean number of emitted cercariae per snail for the two populations was  $2360 \pm 1772$  and  $1226 \pm 1792$ , respectively.

Desiccation caused the death of 3 out of 11 snails. The mean number of living sporocysts was significantly lower in the experimental compared to the control group (13.5 and 103.9, respectively). On the other hand, the mean number of degenerated sporocysts was 25.1 in the control group compared to 82 in the experimental group.

### Conclusion

*B. truncatus* is the main intermediate host for *S. haematobium* in the District of Aït Baha, Agadir Province. Desiccation has a negative effect on *B. truncatus* and the development of the parasite.

### Background

In Agadir Province, *Bulinus truncatus* and *Planorbarius metidjensis* are implicated in the transmission of *Schistosoma haematobium*. Previous studies by the research team confirmed the role of *P. metidjensis* as an intermediate host for *S. haematobium* in south Morocco. These studies have shown that *P. metidjensis* has 3 annual generations, that complete development of larval stages is taking place and that desiccation produces a significant reduction in the production of cercariae. However, *B. truncatus* remains the intermediate host in numerous sites, especially in the District of Aït Baha. A study was therefore conducted to determine the role of *B. truncatus* in the transmission of *S. haematobium* in Aït Baha.

The objectives of this study were to identify new habitats of *B. truncatus* in relation to the presence of human cases of schistosomiasis in the Agadir Province, determine the characteristics

## Conclusions and implications of the study

- *Bulinus truncatus* is the main intermediate host for *Schistosoma haematobium* in the District of Aït Baha, Agadir Province.
- The coexistence of *B. truncatus* and *Planorbarius metidjensis* in Tagharabout focus is reported for the first time. The public health importance of this finding is that both species can ensure complete development of *S. haematobium* larval stages.
- *B. truncatus* was not detected in Targa n'Touchka during 2003. This was attributed to chemical control measures taken in 2002 which resulted in the complete disappearance of the snail for more than a year.
- The existence of *B. truncatus* in Asrar Kiss station allows the complete development of *S. haematobium* larval stages, unlike its existence in Massa station, where it seems resistant to the parasite. This finding is confirmed by the absence of schistosomiasis from the population of Massa, despite frequent contact with water sources.
- Desiccation of the environment has a negative effect on *B. truncatus* and the development of the parasite. This could limit the development of the parasite in several water sources in Aït Baha District that are temporary sources which dry out for a long period of the year.

of infection in the snail population and evaluate the effect of desiccation on snail populations of *B. truncatus* and the parasite (sporocyst development and cercariae shedding).

## Materials and methods

The study was conducted in the water points of neighbouring schistosomiasis foci in Aït Baha District. The 2 stations of Asrar Kiss, in Aït Baha, and Massa (Tassila), close to the dam of Youssef Ibn Tachfine, were selected as the study area due to the permanent presence of water.

**Snail collection in the field** The snails were collected, counted and measured for further categorization into: newborn, height < 1mm, juvenile (1 mm–2.5 mm), pre-adult (2.6 mm–3.5 mm) and adult: (> 3.5 mm).

**Laboratory studies** Snails collected in the field were transferred to the laboratories under isothermal conditions and then placed in large containers filled with water brought from the same station of origin together with pieces of lettuce. The containers were artificially aerated and the room was kept at 24–26 °C and illuminated. Upon reaching the juvenile stage (2 mm height), the offspring of the snails were then used for laboratory work. The miracidia used for snail infestation were obtained from the urine of children, after diluting the urine and hatching of *Schistosoma* eggs.

To study the characteristics of infestation, the snails were placed in Petri dishes 35 mm in diameter filled with water obtained from the station. Two miracidia were added in each box for 4 hours and then the snails were placed in breeding containers, 50 snails per container. On the 36<sup>th</sup> day post-infestation, the snails were again put in the Petri dishes for a cercarial emission test. This consisted of placing the snails under intense light for 4 hours, which resulted in cercarial emission. Snails that did not shed cercariae were tested for a second time after 3 days. Counting of the emitted cercariae took place until the death of the snails.

The effect of desiccation was studied using infested snails on the first day of the patent period (control group) and for 10 days (experimental group). Soil collected from Asrar Kiss station was sterilized (120 °C over 24 hours) and distributed in Petri dishes (100 gm of soil per Petri dish). The snails were then placed in the soil that had been saturated with water (45 ml of water), 6 snails per Petri dish. The dishes were covered with a perforated lid to allow good aeration, and hydration was maintained at its original state each morning by adding an equal volume of evaporated water. This was determined by comparing the volumes of the experimental and control Petri dishes. Soil humidity varied daily from 100% to 50%–60%. After 10 days, the surviving snails were

sacrificed and the soft parts fixed for 3 days at 4 °C, dehydrated by alcohol and tertiary butanol, and then paraffin sections were prepared and stained. The snails subjected to this experiment were compared to the 6 control groups.

## Main study findings

*B. truncatus* was detected in the majority of the foci of the study area. In Massa District it was detected in Youssef Ibn Tachfine, Tassila, Tagharabout and Aït Ouadrim, while in Aït Baha District it was detected in Aït Moussa, Asrar Kiss and Tanalt, but could not be detected in Targa n'Touchka. Tagharabout represented a special focus where both intermediate hosts, *B. truncatus* and *P. metidjensis*, coexisted together. *Physa acuta* was encountered in all studied foci. Other snails such as *Ancylus fluviatilis* and *Lymnaea truncatula* also coexisted with the *Bulinus* snail.

Studying the evolution of the different age groups of the *Bulinus* snail demonstrated 2 peaks, indicating the existence of 2 annual generations. However, these generations differed between the 2 stations. The first generation starts in January at Tassila and in June at Asrar Kiss, while the second starts in August and November in the 2 stations, respectively.

The results of the experimental infestation of snails were different for the 2 stations. The snail population from Asrar Kiss recorded significantly higher mortality rates compared to those from Massa station (37% and 20%, respectively), significantly higher prevalence of infestation (36.5% and 3.75%, respectively) and a significantly longer patent period (mean of 21 and 12 days, respectively). The mean number of emitted cercariae per snail for the 2 populations was  $2360 \pm 1772$  and  $1226 \pm 1792$ , respectively.

Desiccation caused the death of 3 out of 11 snails. The mean number of living sporocysts was significantly lower in the experimental compared to the control group (13.5 and 103.9, respectively (-87%,  $p < 0.001$ )). The mean number of degenerated sporocysts was 25.1 in the control group compared to 82 in the experimental group (+227%,  $p < 0.001$ ).

## Conclusions and recommendations

*B. truncatus* is the main intermediate host for *S. haematobium* in the region of Aït Baha, Agadir. Desiccation has a negative effect on *B. truncatus* and development of the parasite. This could limit the development of the parasite in several water sources in Aït Baha District that are temporary sources which dry out for a long period of the year.

# Schistosomiasis

## Epidemiology

### Yemen

Schistosomiasis,  
soil-transmitted  
helminthiasis and  
nutritional status in  
schoolchildren of  
Sahar District,  
Sada'a Governorate,  
Yemen

Yemen  
Sada'a Governorate

Study period:  
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Principal Investigator  
Dr Yahia Raja'a  
Department of Community  
Medicine  
Faculty of Medicine and  
Health Sciences  
Sana'a University  
Sana'a, Yemen  
e-mail:  
yahiarajaa@yahoo.com

### Abstract

A cross-sectional study was undertaken in Sahar District, Sada'a Governorate, to estimate the prevalence and intensity of schistosomiasis and other intestinal parasites, and to determine the nutritional status of schoolchildren. For the study, 557 pupils in 15 randomly selected primary schools underwent urine and stool analysis, and anthropometrical measurements.

**Results** The prevalence of schistosomiasis was 5.6%; *S. haematobium* accounted for 3.3% and *S. mansoni* for 2.3%. Ova of *Ascaris lumbricoides* were found in only 2 cases and no other soil-transmitted helminths were detected. Other intestinal parasites found included: *Entamoeba histolytica* (6.4% prevalence), *Hymenolepis nana* (2.4%) and *Taenia saginata* (0.2%). The intensity of *Schistosoma* and *Ascaris* infections was low.

According to WHO standards, anthropometric measurements indicated stunted growth in 50.9% of pupils, underweight in 48.7% and wasting in 4.5%. There was no significant association between *Schistosoma* infection and nutritional status, whether by international or Yemeni norms.

**Conclusion** The study reported low prevalence and intensity of schistosomiasis and soil-transmitted helminthiasis infection in Sahar District, Sada'a Governorate, and a

lack of association between infection status and nutritional deficiency.

### Background

Sada'a governorate is an endemic area for both *S. haematobium* and *S. mansoni* infections. Both species were reported in rural areas surrounding Sada'a city, with prevalence rates in 1982 of 48% and 8% for *S. mansoni* and *S. haematobium*, respectively [1]. In 1999, higher rates were reported for both species: 76.3% and 49%, respectively [2]. As a result of this high endemicity, the Ministry of Public Health and Population established a schistosomiasis control unit in the Health Office and encouraged nongovernmental organizations to work in the area. As a result, 4 campaigns (including targeted deworming, selective treatment and focal mollusciciding) have been implemented in the area since 1999. To assess the impact of these control activities on the prevalence and intensity of schistosomiasis and other intestinal parasites, 5 years after their implementation, this study was carried out. It also sought to determine the nutritional status of schoolchildren in Sahar, a subtle morbidity of these infections.

### Materials and methods

Sahar District is the rural area surrounding the city of Sada'a, and lies 250 km from Sana'a. A cross-sectional study was undertaken in Sahar in which 15 out of 78 primary schools were randomly selected. All children of the third year classes present on the day of the survey were included in the study. Two teams visited 2 schools daily. A total of 557 children were examined, and 548 urine samples and 540 stool samples were collected. Information was obtained for 530 pupils including urine and stool samples, and anthropometrical

## Conclusions and implications of the study

■ The study reported low prevalence and intensity of schistosomiasis and soil-transmitted helminthiasis infection in Sahar District, Sada'a Governorate. This is attributed to annual health education campaigns, case-finding and treatment of schoolchildren, and snail control over the previous 5 years. This strategy has resulted in the reduction of prevalence rates of *S. mansoni* and *S. haematobium* in the study area from 76.3 and 49%, respectively, in 1999, to 2.3% and 3.3% in 2003.

■ The WHO recommended treatment strategy for low prevalence schistosomiasis areas is targeted treatment of school-age children twice during primary schooling (once on entry, again on leaving), and access to praziquantel therapy. Improvements in sanitation and access to clean water, appropriate health education and environmental measures (snail control) should be promoted in all epidemiological situations.

■ For low prevalence soil-transmitted helminthiasis areas, such as the study area, selective treatment of school-age children and the community in health facilities is the recommended strategy.

measurements.

Filtration and modified Kato -Katz techniques were used for urine and stool analysis. Two digital Seca scales, to the nearest 100 g, and 2 stadiometers, to the nearest 1 mm, were used to measure the weight and height of the children. Anthropometrical measurements were processed using Epi Info Version 6 (nutritional anthropometry). Drugs were dispensed the next day for all children who tested positive.

### ■ Main study findings

The mean age of pupils was  $10.1 \pm 1.3$  years, and three quarters of pupils were boys. *S. haematobium* eggs were identified in 18 stool samples (3.3%), whereas *S. mansoni* eggs were identified in 12 stool samples (2.3%). *Schistosoma* eggs, either *mansoni* or *haematobium*, were seen in the samples of 30 pupils (5.6%). No mixed *Schistosoma* infection was identified. Ova of *Ascaris lumbricoides* were found in 2 cases only and no other soil-transmitted helminths were seen. Other intestinal parasites included *Entamoeba histolytica* (prevalence of 6.4%), *Hymenolepis nana* (2.4%) and *Taenia saginata* (0.2%). The intensity of *Schistosoma* and *Ascaris* infection was low, with geometric mean egg count (epg) of 0.16 epg/10 ml urine for *S. haematobium*, 0.18 epg for *S. mansoni* and 0.08 epg for *Ascaris lumbricoides*.

Anthropometric measurements denoted stunted growth in 50.9% of pupils, underweight in 48.7% and wasting in 4.5% of pupils, according to WHO standards. The mean height in boys aged 7 and 14 years ranged from  $116.70 \pm 10.8$  cm to  $147.92 \pm 17.06$  cm. In girls, the mean height ranged from  $118.60 \pm 2.26$  cm to  $137.00 \pm 0.00$  cm in these age categories. The mean weight in boys aged 7 and 14 years ranged from  $20.22 \pm 3.03$  kg to  $35.26 \pm 8.98$  kg. In girls of these ages, the mean weight ranged from  $21.10 \pm 0.71$  kg to  $26.80 \pm 0.00$  kg. There was no significant association between *Schistosoma* infection and nutritional status, whether by international norms or by those derived from non-infected children of the same cohort (Yemeni norms).

### ■ Conclusions and recommendations

The study reported low prevalence and intensity of schistosomiasis and soil-transmitted helminthiasis infection in Sahar District, Sada'a Governorate. This is attributed to annual campaigns in line with WHO recommendations for the control of schistosomiasis and soil-transmitted helminthiasis in high burden areas. Since a considerable part of the burden of schistosomiasis and soil-transmitted helminthiasis involves subtle morbidity that particularly affects children, the strategy applied provides the opportunity to prevent nutritional deficiencies as indicated by the

lack of association between infection status and nutritional deficiency.

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# Sexually Transmitted Diseases

## Epidemiology

Yemen

Sexually transmitted diseases and reproductive health of women in Sana'a, Yemen

Yemen  
Sana'a

Study period:  
April 2003–2004

Small Grants Scheme  
(SGS) 2002 No. 19

Principal Investigator  
Dr Iman AlWazer  
Althawra Teaching Hospital  
Sana'a, Yemen

### Abstract

A cross-sectional study was carried out to determine the prevalence of sexually transmitted diseases in Sana'a, Yemen and to study the association between sexually transmitted diseases and reproductive health. At 8 health facilities delivering gynaecology and obstetrics health services in Sana'a, 200 women were interviewed and underwent clinical examination and laboratory investigations. Women were categorized based on their poor or good reproductive health status and were then compared.

**Results** The mean age of women was 28.8 ±7.2 years and 52% were illiterate. Contraceptive methods had been used by 61.5% of women, 8% were in a polygamous marriage and 50% had a past history of sexually transmitted disease. Almost 50% of women that had attended the health facilities had at least 1 sexually transmitted disease and 23.5% were categorized as having poor reproductive health. The prevalence of sexually transmitted diseases was 18.5% for trichomoniasis, 46.5% for bacterial vaginosis, 5% for gonorrhoea, 28% for cervical erosions/ulcers and no cases of syphilis. The prevalence of human immunodeficiency virus (HIV) was 1%. Hepatitis B and hepatitis C markers were prevalent in 4.5% and 1% of

cases, respectively, and were more frequent in women with poor reproductive health. There was a significant association between sexually transmitted diseases and the reproductive health statuses of women.

**Conclusion** The results of this study confirmed that sexually transmitted diseases are frequent in women attending health facilities in Sana'a and are significant determinants of their reproductive health.

### Background

In developing countries, sexually transmitted diseases are mainly or not at all notified by health officials. Patients usually seek care from pharmacists, private sectors or traditional healers. This leads to underestimation of the health impact of these diseases in most of these countries [1].

To date, there is no reliable information on the prevalence of sexually transmitted diseases in Yemen and the majority of cases are undiagnosed. However, the observations of health care providers suggest an increasing burden of sexually transmitted diseases in the community, particularly among women seeking family planning services. Therefore, a study was conducted to determine the prevalence of the common sexually transmitted diseases influencing reproductive health in Yemen, and to study the association between sexually transmitted diseases and the reproductive health of women in Sana'a governorate.

### Materials and methods

A cross-sectional study was conducted in 8 out of the 20 health facilities delivering gynaecology and obstetrics care in Sana'a city. Patients were categorized based on their poor or good reproductive health status and were compared. Subjects suffering from primary or secondary infertility and any other symptom (discharge, irregular menstruation, dyspareunia, back pain, abdominal pain and dysmenorrhoea) were labelled as

### Conclusions and implications of the study

- Almost 50% of women that attended the gynaecology and obstetric health facilities had at least 1 sexually transmitted disease and 23.5% were categorized as having poor reproductive health.
- Improving sexually transmitted disease treatment for patients seeking care in primary health care settings may help reduce the burden of sexually transmitted diseases in the population and also prevent the spread of HIV.
- There is a significant association between sexually transmitted diseases and the reproductive health statuses of women.
- The scope of reproductive health must be broadened beyond family planning and maternity care to include prevention and treatment of reproductive tract infections, safe motherhood and reproductive health education. The approach of reproductive health care services should be comprehensive, whereby several aspects of reproductive health are integrated into primary health care facilities. The emphasis on prevention and early detection is clearly less resource intensive than dealing with the severe consequences of reproductive tract infection.



suffering from poor reproductive health; the others were labelled as having good reproductive health.

After obtaining their informed consent, each participant was interviewed by a female physician using a standardized pre-tested questionnaire that elicited information on sociodemographic characteristics, sexual behaviour, history of past genital infection and reproductive history. All patients underwent proper physical and clinical investigations, as well as laboratory investigations. Syndromic diagnosis was made on the first visit and confirmed after laboratory results.

### ■ Main study findings

Most of the women (60.5%) were between 20–30 years old. 52% were illiterate, and only 3% had a university degree. Overall, 39.5% of the women belonged to a low/very low social class. Most of the women (81.5%) were housewives, and 94.5% were married, the rest being divorced/separated or widowed.

There was no significant association between age, education, occupation, or residence and the reproductive health status of these women. Interestingly, a significantly higher proportion of patients with poor reproductive health belonged to a high social class (45.7%) compared to those in lower social classes (19.8%, 18.5% and 14.3% for medium, low or very low social class, respectively). High social class was a significant risk factor for poor reproductive health.

Almost 50% of women that attended the health facilities had at least 1 sexually transmitted disease and poor reproductive health was prevalent in 23.5% of the women. Women with poor reproductive health were more likely to be in a polygamous marriage (14.9%), (OR = 2.8 (0.87–8.88),  $p > 0.05$ ). Contraceptive methods were uncommon, with the majority of cases (61.5%) not using any type of contraceptive method. 19.5% used intrauterine loop and 12.5% used oral contraceptive pills. Tubal ligation and injection were used by 2.5 % and 0.5%, respectively. Only 3.5% of husbands used condoms. Women using contraceptive methods were significantly protected against poor reproductive health.

A past history of operation was detected in 31 (15.5%) of the patients. Of these, 10 (5%) had had a caesarean section and 3% a laparoscopy. Ovarian cyst removal had been performed in 1.5%, and other less frequently experienced operations included herniotomy, myomectomy and ectopic pregnancy. Women operated upon were at a 4-fold increased risk for poor reproductive health.

Vaginal discharge was the common presenting complaint in 73.5% of patients. The other frequent

symptoms were back pain, lower abdominal pain (pelvic pain) and painful intercourse (dyspareunia) (71.5%, 61% and 52.5%, respectively). Symptoms such as irregular menstruation, dysmenorrhoea and frequent micturation were reported in 20%, 22% and 31%, respectively. All these signs and symptoms were significantly more frequent among women with poor reproductive health. External genital ulcer lesions were reported in only 2% of women, while bacterial vaginosis was reported in 46.5% and trichomonas vaginosis in 18%. Cervical erosions/ulcers and gonorrhoea were reported in 28.9% and 5% of cases, respectively, and no cases had syphilis. The prevalence of HIV was 1%, while the prevalence of hepatitis B and hepatitis C markers was 4.5% and 1%, respectively, with a higher prevalence among women with poor reproductive health. There was a significant association between sexually transmitted disease status and the reproductive health status of women. Having 2 or 3 sexually transmitted diseases or more than 3 sexually transmitted diseases was significantly associated with an increased risk for poor reproductive health (2.8 and 8-fold, respectively).

### ■ Conclusions and recommendations

The study highlighted the burden of sexually transmitted diseases and its negative impact on the reproductive health of women attending the gynecology and obstetric health facilities in Sana'a. Improving sexually transmitted disease treatment for patients seeking care in primary health care settings may help to decrease the sexually transmitted disease burden in the population and also prevent the spread of HIV. Early recognition and prompt treatment will prevent complications such as pelvic inflammatory diseases and its sequelae. It reduces the potential risk factors for infertility and poor reproductive health, and offers a unique opportunity for targeted education about HIV prevention in the community.

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# Tuberculosis

## Control

### Islamic Republic of Iran

Comparison of daily and three times a week home visit of patients with pulmonary tuberculosis in Zahedan

Islamic Republic of Iran  
Zahedan

Study period:  
November 2001–  
May 2003

Small Grants  
Scheme  
(SGS) 2001 No. 63

Principal Investigator  
Dr Masoud Salehi Shaban  
Zahedan University of  
Medical Sciences  
Zahedan, Islamic Republic  
of Iran  
e-mail:  
shahstan@yahoo.com

#### ■ Abstract

WHO and the International Union Against Tuberculosis and Lung Disease recommend the use of the directly observed treatment, short course (DOTS) strategy to control tuberculosis. The direct observation of treatment component is intended to address patient non-compliance in tuberculosis control.

In the Islamic Republic of Iran, DOTS is carried out by the daily visit of health workers to patient homes. However, daily visits are sometimes difficult and may be too ambitious. Visiting three times a week would be more practical with no shortcomings for the course of treatment. A study to compare the treatment outcome of patients treated by daily home visits DOTS compared to thrice-weekly home visits DOTS was therefore carried out.

A randomized clinical trial of 140 new cases of sputum-positive pulmonary tuberculosis was conducted whereby 70 patients were assigned to either daily home visits or thrice-weekly home visits.

The cure rates (84.3%, 85.7%) and treatment-completion rates (7.1%, 4.3%) in each group, respectively, were comparable.

**Conclusion** These results demonstrate that it is possible to decrease the number of health worker visits to tuberculosis patients' houses without negative impact on the success of treatment. Moreover, it is more practical and cost-saving.

#### ■ Background

Ninety-five percent (95%) of tuberculosis cases occur in developing countries, where few resources are available to ensure proper treatment and where human immunodeficiency virus (HIV) infection may be common. Patient compliance with treatment is commonly poor, and non-completion of a course can lead to relapse, possibly with drug-resistant bacilli.

WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) recommend the use of the directly observed treatment, short course (DOTS) strategy to control tuberculosis, and further recommend that no tuberculosis treatment (short course) containing rifampicin should be given without direct observation of treatment [1].

In rural and suburban areas, there is substantial economic burden and time-cost involved in traveling to a clinic (or patients' home) daily for 2 months for treatment. Patients can be reluctant to attend repeatedly because of the stigma related to tuberculosis in most countries. It can also interfere with patients' lives by restricting their access to work or due to the high costs involved, which may cause them to discontinue treatment.

The treatment is carried out by the daily visit of health workers to patient homes. But as these daily visits are not easy to do, there is always a need to convince the patient of their necessity. If the patient is female, the daily visit by a male health worker is not appropriate from a cultural perspective.

Another problem is the limitation of having to stay at home to be visited by the health worker. Further, the Islamic Republic of Iran has more national holidays than other countries.

As a result, daily visits by health workers seem difficult and too ambitious. However,

### Conclusions and implications of the study

- There was no additional benefit in terms of cure, completion of treatment and sputum conversion rates in daily home visit DOTS compared to thrice-weekly DOTS.
- The results of this study demonstrated that it is possible to decrease the number of health worker visits to tuberculosis patients' houses without hindering the success of treatment.
- In the Islamic Republic of Iran, the DOTS strategy uses health workers daily visits to patients' houses. This is costly, and expenses can be halved by thrice-weekly visits.
- These results need to be validated by a wider scale study before drawing strong recommendations that would influence the policy and practice of the national tuberculosis control programme.

visiting three times a week could be more practical with no shortcomings for the course of the treatment.

## Materials and methods

A randomized controlled trial was conducted in a district health centre in Zahedan, the capital of Sistan va Baluchestan province.

One hundred and forty (n = 140) newly diagnosed smear-positive tuberculosis cases were randomly assigned to either daily or thrice-weekly visits by health workers for direct observation of treatment during the intensive phase. The physicians, laboratory technicians, researchers and statistical adviser were all blinded about the treatment groups.

Apart from the differences in direct observation of treatment, patients in the two groups received the same case management approved by WHO and the national tuberculosis control programme, including a staggered supply of free drugs (2 months of isoniazid, rifampicin, pyrazinamide and ethambutol, followed by 4 months of isoniazid and rifampicin).

The doctor, health educator, laboratory technicians and other regular staff of the diagnostic centre provided health education and monitored the clinical aspects of care, side-effects, sputum conversion and culture, following the same procedure for all patients. The enrolment officer had no role in care provision. Outcome assessment was by laboratory examination of sputum by technicians unaware of treatment allocation.

The outcome measures used were cure and cure plus treatment completion (treatment success rate), as defined by the IUATLD and WHO.

Randomization codes were broken only when analysis started, and the identity of each group was revealed only after preliminary outcome analysis. Analysis of results was on an intention-to-treat basis.

The chi-square test was used for comparison of outcomes between each treatment strategy. The student t-test was used for comparison of continuous variables.

## Main study findings

Of the 140 patients enrolled in the trial, no eligible patients refused to participate. The number of patients enrolled in each group was 70. There was little difference in the distribution of demographic factors of enrolled patients by trial group. There was no significant difference between the cure rates and the treatment success rates for daily DOTS (91.4%) compared to thrice-weekly DOTS (90%).

## Conclusions and recommendations

The results of the study revealed that it is possible to decrease the number of health worker visits to tuberculosis patients' houses without hindering the

success of the treatment. Moreover, it is more practical and cost-saving.

The main study limitation was that disease relapse was not investigated due to time constraints. Therefore, a broader study taking into account the relapse rate is recommended.

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# Tuberculosis

## Control

### Sudan

Community and individual factors influencing the health-seeking behaviour of tuberculosis defaulters in Sudan

Sudan  
Abu-Anga, Gedaref State  
and Mayo Farm

Study period:  
March 2001– May 2002

Small Grants  
Scheme  
(SGS) 2001 No. 111

Principal Investigator  
Dr Imad Obeid-Allah  
National Tuberculosis  
Control Programme  
Federal Ministry of Health  
Khartoum, Sudan  
email:  
imadamin@hotmail.com

#### Abstract

A hospital-based prospective cohort study was conducted to study the extent of defaulting in tuberculosis treatment and its determinants in 3 different areas: Abu-Anga, Gedaref State and Mayo Farm. During March–May 2001, 409 new adult pulmonary tuberculosis patients registered in the three areas were interviewed regarding potential determinants of defaulting. The cohort was followed up until completion of treatment.

Results 107 pulmonary tuberculosis patients were identified as defaulters (defaulter rate of 26.2%), and this was not significantly different between sexes. However, the social impact of the disease was more pronounced in females mainly due to stigma and financial dependency. Significant predictors of defaulting were: marital status (divorced and widowed); rural residence; being on wages; inadequate knowledge about the disease; source of information (inexperienced nurses); family; financial or work problems; severe illness; being informed about diagnosis using the stigmatizing Arabic terms for tuberculosis, *sul* or *daran*; and seeking care initially at nongovernmental organization clinics. The main reasons for refusing hospitalization among defaulters were stigma, boredom and indirect cost of illness (loss of work or income).

Conclusion More than one quarter of pulmonary tuberculosis patients default on treatment, which has a serious impact on tuberculosis control in Sudan.

#### Background

Tuberculosis is a major public health problem in Sudan. The estimated number of positive pulmonary tuberculosis patients was 27 356 in 2001, with an incidence of 86 per 10 000 population. One of the main problems in the management of tuberculosis patients is non-compliance with treatment and a high rate of defaulting. It has been reported that the defaulter rate is high in areas where high tuberculosis prevalence is coupled with limited resources. The objective of this study was to determine the extent of defaulting in tuberculosis treatment and its determinants in Sudan.

#### Materials and methods

A prospective cohort study was conducted in three different areas: Abu-Anga hospital (urban area), Gedaref hospital (rural area) and Mayo Farm (displaced populations). New adult pulmonary tuberculosis patients registered in the three areas during March–May 2001 were interviewed using a standardized questionnaire including information on sociodemographic characteristics, clinical data, health education, stigma, knowledge and health-seeking behaviour. The cohort was followed up until completion of treatment for the last patient included in the study.

The defaulters to treatment were identified at the end of this period. A defaulter was defined as a patient who completed at least 1 month of treatment and recorded after at least 2 months of treatment interruption. Both defaulters and non-defaulters were compared regarding the determinants of defaulting.

### Conclusions and implications of the study

- More than one quarter of tuberculosis patients default on treatment in Sudan, which has a serious impact on disease control.
- In order to achieve better treatment outcomes more attention should be given to accessibility, and the social and psychological profile of patients.
- Nurses and nongovernmental organizations should receive more attention from national tuberculosis control programmes. There is a need to organize regular health education sessions and training programmes for this important sector of health workers in close contact with tuberculosis patients.
- The results of this study indicate the need to educate tuberculosis patients and their families about the seriousness of the disease and the importance of adherence to treatment.
- This study highlighted the impact of stigma on defaulting and indicates the need to identify less stigmatizing approaches in order to reduce non-compliance/defaulting rates and achieve better treatment outcomes.

## ■ Main study findings

Of 409 pulmonary tuberculosis patients, 107 were identified as defaulters at the end of their treatment period, (defaulter rate of 26.2%). There was no significant difference between the three settings regarding the rate of defaulting: Abu Anga (28.8%), Gedaref (24.6%) and Mayo Farm clinic (25.3%). However, data analysis based on patient initial residence identified rural residence as a significant risk factor for defaulting. Although almost two-thirds of defaulters were males, this reflected the male to female ratio of pulmonary tuberculosis patients, with no significant association between gender and defaulting rates (27.8% in males and 23.6% in females). However, the social impact of the disease was more pronounced in females, mainly due to stigma and financial dependency. Divorced and widowed patients recorded a significantly higher defaulter rate (41.7%) compared to single (23.1%) or married patients (25.8%). Patients on wages were also at a significantly higher risk of defaulting (40.8%) compared to others (23.7%). Significantly higher defaulter rates were recorded among tradesmen, those in clerical occupations, students and the unemployed, while lower rates were recorded among farmers, housewives and technical workers. Surprisingly, patients with limited housing facilities recorded significantly lower defaulter rates (24.2%) compared to more advantaged groups (39%). Only 2.8% of patients were ranked as having a high socioeconomic status, which was not a significant determinant of defaulting. A limited proportion of patients in both groups correctly identified measures of prevention and treatment of tuberculosis such as the importance of early treatment and seeking care in chest hospitals, and the importance of adherence to treatment and improving nutrition. They also identified several healthy habits such as avoidance of droplet dispersion or spitting, avoidance of overcrowding and close contact with patients, avoidance of sharing utensils and the importance of proper ventilation. However, there was a significant difference between both groups regarding knowledge of the mode of disease transmission. Commonly stated causes of tuberculosis by defaulters were contaminated air, exposure to cold air draft, drug addiction and casual sexual relations.

There was a significant difference between defaulters and non-defaulters regarding the source of information. Most defaulters obtained information from nurses (44%), chest hospitals (15%) and relatives (8.4%), while most non-defaulters obtained information from chest hospitals (36%), the media (30%), pharmacists (28%) and other partners (22%), with only 13.7% obtaining it from nurses. Defaulters recorded significantly higher rates of financial problems (2.1-fold), family or work problems (3-fold), and severe

illness (5-fold).

Interestingly, the terms used when diagnosis was told to the patient appeared to be a significant determinant of defaulting. Defaulting was significantly associated with use of Arabic terms for tuberculosis such as *sul* (32.85%) or *daran* (50%), while being non-significantly associated with vague or inaccurate terms such as *lung* (19%) or gastrointestinal infection (17%).

Health-seeking behaviour was also significantly different between defaulters and non-defaulters; while the majority of defaulters (84%) sought care initially at nongovernmental organization clinics, followed by governmental health services, a significantly lower proportion of non-defaulters visited nongovernmental organization clinics (68.5%), with a higher proportion seeking initial care at governmental health services, or other health-care providers such as general practitioners, pharmacists and even traditional healers.

The main reasons for refusing hospitalization among defaulters were stigma, boredom and indirect cost of illness through loss of work or income. While 76.4% of defaulters and 54% of non-defaulters claimed that their working ability was impaired by hospitalization, a significantly higher proportion of defaulters (20.8%) had to stop working due to their illness compared to non-defaulters (13.8%). There was no significant difference between the two groups regarding their readiness to inform their parents of their illness, to inform their spouses or to inform their brothers and sisters. Defaulters were more willing to inform other relatives (91.6%) compared to non-defaulters (80.8%,  $p < 0.05$ ). Interestingly, a significantly higher percentage of defaulters (86.9%) tended to accept their illness compared to non-defaulters (68.9%), while it was mainly non-defaulters who expressed negative feelings such as denial, sadness and fear. Moreover, family members of defaulters were more supportive to patients compared to those of non-defaulters.

## ■ Conclusions and recommendations

A high defaulter rate for tuberculosis treatment was reported in this study. The risk factors for defaulting reported in this study should be the subject of an intervention study to reduce the burden of the disease in this community.

# Tuberculosis

## Control

### Egypt

A multicountry study in 7 Eastern Mediterranean countries: Egypt, Islamic Republic of Iran, Iraq\*, Pakistan, Somalia, Syrian Arab Republic and Yemen

#### Case-finding in tuberculosis patients: diagnostic and treatment delays and their determinants

Egypt  
Nationwide

Study period:  
October 2002–July 2004

Small Grants  
Scheme  
(SGS) 2002 No. 61

Principal Investigator  
Dr Sahar Shaker Soliman  
National Tuberculosis  
Control Programme  
Cairo, Egypt  
e-mail:  
saharsoliman@hotmail.com

#### Abstract

A cross-sectional study was conducted during 1 year (2002–2003) in randomly selected directly observed treatment, short-course (DOTS) centres nationwide. Using a structured and pretested questionnaire, 802 new smear-positive patients were interviewed regarding their health seeking behaviour and other determinants of delay in getting timely and appropriate care. The aim was to assess delays in diagnosis and treatment of new smear positive pulmonary tuberculosis patients in DOTS areas and their determinants.

**Results** The median diagnostic delay and total delay were 42 and 44 days (average 55.9 and 57 days), respectively, of which the median patient delay, defined as the duration between onset of symptoms and seeking health care, amounted to 12 days only (average 24.3 days). The median system delay, defined as the duration between seeking health care and treatment, was 18 days (average 33.6 days). The private sector was the first choice for 65% of patients, but chest facilities were responsible for diagnosing 93% of patients. Being in debt, illiteracy, high degree of stigma, high crowding index, high cost of health services, time to reach health facility, seeking care at a nonspecialized health facility and visiting more than 1 health care provider before diagnosis were determinants of different types of delay.

**Conclusion** Delayed management of tuberculosis patients was mainly attributed to

late diagnosis within the health care system. Increasing awareness among health care providers about the signs and symptoms of tuberculosis is therefore recommended.

#### Background

The global targets of the directly observed treatment, short-course (DOTS) strategy are to achieve 70% case detection and 85% cure rates by 2005. According to the WHO global report in 2001, DOTS programmes have successfully treated 80% of all registered new smear-positive patients, but detected only 27% of the estimated tuberculosis cases in the world in 2000. The report indicated that the target of 70% case detection might not be reached until 2013, unless interventions are able to increase the case detection rate. The situation in Egypt is similar. In 2000, the case detection rate was 51% while the treatment success rate was 87%. A study of case-finding activities was therefore carried out, with an in-depth analysis of various types of delay and their determinants. The goal was to identify gaps in case-finding activities under DOTS.

#### Materials and methods

Within the framework of a multicountry study, a cross-sectional study was conducted in a representative sample of chest facilities implementing DOTS in Egypt. New smear positive pulmonary tuberculosis cases were randomly selected using cluster sampling technique with probability proportional to size method. 802 newly diagnosed smear positive adult pulmonary tuberculosis cases were included in the study. Patients were retrospectively interviewed according to a pretested questionnaire including information about sociodemographic characteristics, access to health services, health seeking

#### Conclusions and implications of the study

■ The main health seeking behaviour with onset of symptoms is to visit a health care provider of patients. However, the majority of patients seek care at the private sector rather than the National Tuberculosis Control Programme where most of patients are finally diagnosed. The main challenge facing the National Control Programme is therefore to integrate DOTS strategy within the private sector.

■ The first action after suspecting tuberculosis disease is to request a sputum smear examination with or without X-ray (99.7%).

■ Half the patients are treated within 2 months from the onset of symptoms. This was mainly attributed to health care system rather than patient factors. The significant risk factors for delay were being illiterate, living at a far distance from the health facility, high cost of health services and visiting more than 1 health care provider before diagnosis.

behaviour and other determinants of delay in getting timely and appropriate care. Patients with longer delays were categorized as cases, while the others were considered to be controls. The median value was used as a cut-off point for the different types of delay. Both groups were compared regarding the determinants of longer delay.

**Definitions** Diagnostic delay is the time interval between onset of symptoms and pulmonary tuberculosis diagnosis. Treatment delay is the time interval between pulmonary tuberculosis diagnosis and onset of treatment. Health care system delay is the time interval between seeking care at a health care provider and pulmonary tuberculosis treatment. Total delay is the time interval between onset of symptoms and pulmonary tuberculosis treatment. This duration is therefore the sum of two durations: patient delay (determined by health seeking behaviour) and health care system delay.

### ■ Main study findings

More than half the newly diagnosed tuberculosis patients were aged 35 years old, with a male predominance (male to female ratio = 2.1).

Cough was invariably reported by almost all patients, followed by fever and loss of weight. Cough alone or with any other complaint was reported as the main symptom motivating patients to seek health care. The mean socioeconomic score and knowledge were significantly lower in females compared to males, while no significant difference was found regarding stigma and satisfaction with care.

**Health seeking behaviour before diagnosis** All 802 patients went to health care providers. Pharmacies were used by 83 patients, self-medication by 62 patients and traditional medicine by 32 patients. Before diagnosis, half the patients spent \$US 8.46, with an average of \$US 21 being spent per patient. The private sector was the first choice for medical consultation for 65% of patients, the second choice for 10% and the third choice for only 5% of patients. The primary health care centres and chest facilities (hospitals and clinics) were the first choice for 14.5% of patients and a second choice for 48.6%. Patients first seek care mainly at chest specialists (35.4%), internists (19.3%) and general practitioners (25.9%). Half the patients visited 1 health care provider before diagnosis, with a mean of 1.8 (1.6), ranging from 0–17. Accessibility, confidence in obtaining a cure and advice from others were the main reasons for the first choice of the health facility.

A high level of Tuberculosis stigma was recorded among the patients. The most important source of information about tuberculosis for patients was the health staff of chest facilities (45%), followed by the campaign of the Ministry of Health and Population (20%). More than two-thirds of patients had the correct

information about the disease being contagious and curable, on the duration of treatment and on tuberculosis drugs.

**Initial tuberculosis diagnosis** The majority of patients were diagnosed in chest facilities (93%). Diagnosis was mainly done by chest specialists (99.3%). Sputum examination was done to almost all patients (801) and X-ray to 773 (96%).

**Accessibility to public health facilities** Almost two-thirds of tuberculosis patients could reach the chest facilities providing treatment within less than half an hour.

### ■ Types of delay

The mean patient-related diagnostic delay was 24.3 days and approximately half of the patients took 12 days to seek health care. The significant risk factors for patient related diagnostic delay were: being in debt (3.6-fold increased risk) and high degree of stigma (1.2-fold increased risk) while female sex and living in a rural area were protective, with patients being more motivated to seek health care.

The mean diagnostic delay duration was 55.9 days and approximately half the patients were diagnosed within 42 days. The mean treatment delay was 1.2 days and approximately half the patients were immediately treated following diagnosis.

The mean health-care system delay was 33.6 days with a median of 18 days.

**Total delay** The mean duration between onset of symptoms and treatment was 57 days for all patients and approximately half the patients were treated within 44 days. The significant risk factors for total delay were being illiterate (2.76-fold increased risk), time to reach health facility being more than half an hour and more than an hour (1.73-fold and 1.75-fold increased risk compared to those reaching the health facility in less than half an hour), high cost of health services and visiting more than 1 health care provider before diagnosis (2.55-fold increased risk). Being females or a student were protective factors, motivating patients to seek health care earlier than others.

### ■ Conclusions and recommendations

The long time interval between the onset of symptoms and treatment reported in this study was mainly attributed to delay within the health care system rather than patient delay. Involving the private sector in DOTS implementation seems to be the main challenge facing the National Tuberculosis Control Programme in Egypt.

# Tuberculosis

## Control

### Islamic Republic of Iran

A multicountry study in 7 Eastern Mediterranean countries: Egypt, Islamic Republic of Iran, Iraq\*, Pakistan, Somalia, Syrian Arab Republic and Yemen

#### Case-finding in tuberculosis patients: diagnostic and treatment delays and their determinants

Islamic Republic of Iran  
Nationwide

Study period:  
January–December 2003

Small Grants Scheme  
(SGS) 2002 No. 132

Principal Investigator  
Dr Mahshid Nasehi  
National Tuberculosis Control Programme  
Ministry of Health and Higher Education  
Teheran, Islamic Republic of Iran  
e-mail:  
mnasehi@yahoo.com

#### Abstract

A cross-sectional study was conducted in a representative sample of district tuberculosis coordination units throughout the Islamic Republic of Iran to study the extent and determinants of delay in diagnosis and treatment of pulmonary tuberculosis patients. Using a structured and pre-tested questionnaire, 800 new smear-positive patients aged 15 years and older were interviewed regarding their health seeking behaviour and other determinants of delay in getting timely and appropriate care.

**Results** The mean diagnostic delay, defined as the interval between onset of symptoms and diagnosis, was 114 days, and approximately half the patients were diagnosed within 88 days. The mean time interval between diagnosis and treatment, was 3.1 days and approximately half the patients were treated within one day of diagnosis. The mean total duration between onset of symptoms and treatment was 126.6 days (total delay) and approximately half the patients were treated within 91 days. Health care system delay was significantly longer than patient delay, and this was significantly longer among patients seeking care in the private sector. The significant risk factors for total delay were: older age (> 35 years), non-Iranian nationality, smoking, a positive history of chronic pulmonary disease, economic

constraints, visiting several health care providers before diagnosis, and having a negative sputum smear result for acid fast bacilli on entering the health system. Fever as the first symptom, having a sputum smear and X-ray examination, and having multiple symptoms at the first visit to the health sector were all associated with reduced risk for total delay.

**Conclusion** To reduce health care system delay and subsequently total delay, health care providers, especially physicians in the private sector, should be regularly trained on national tuberculosis control guidelines.

#### Background

One of the main objectives of tuberculosis control programmes is to reduce tuberculosis transmission in the community through early detection of sputum smear-positive pulmonary tuberculosis cases and rapid administration of a full course of treatment. Delay in diagnosis and starting effective treatment increases the risk of transmission as well as morbidity and mortality from tuberculosis. A study was therefore conducted to assess the extent of this delay and to investigate the factors affecting the different components of delay from the onset of symptoms to the start of treatment in newly diagnosed sputum smear-positive adult pulmonary tuberculosis patients in the Islamic Republic of Iran, a country with a well established, decentralized tuberculosis control programme.

#### Materials and methods

As part of a multicountry study in the Eastern Mediterranean Region, a nationwide cross-sectional study was conducted in a representative sample of district tuberculosis coordination units in the Islamic Republic of Iran. Using a structured and standardized questionnaire, 800 newly diagnosed sputum smear-positive pulmonary tuberculosis patients aged 15 years and

### Conclusions and implications of the study

- The mean total delay was 126.6 days and approximately half the patients were treated within 91 days and this was mainly attributed to health care system delay rather than patient delay.
- The significant risk factors for total delay were: older age (> 35 years), non-Iranian nationality, smoking, a positive history of chronic pulmonary disease, economic constraints, visiting several health care providers before diagnosis, and having a negative sputum smear result for acid fast bacilli on entering the health system. These identified risk factors should be the target of any future intervention programme to reduce the delay in getting timely care for tuberculosis patients.
- 67% of cases seek initial care in the private sector. A significantly longer health care system delay was reported among patients seeking care with the onset of symptoms in the private sector compared to those seeking care in the public sector. Integrating tuberculosis re-training courses into the national continuous medical education programme for private physicians is therefore recommended.



older were interviewed regarding their health seeking behavior and other determinants of delay in getting timely and appropriate care.

**Definitions** Diagnostic delay is the time interval between onset of symptoms and pulmonary tuberculosis diagnosis. Treatment delay is the time interval between pulmonary tuberculosis diagnosis and onset of treatment. Health care system delay is the time interval between seeking care at a health care provider and pulmonary tuberculosis treatment. Total delay is the time interval between onset of symptoms and pulmonary tuberculosis treatment. This duration is therefore the sum of two durations: patient delay (determined by health seeking behaviour) and health care system delay.

### ■ Main study findings

Of the 800 study subjects, 395 (49.4%) were males, with a median age of 45 years (range 15–90). The mean total delay was 126.6 with a median of 91 days. The median diagnostic delay was 88 days; mean (SD) of 123 (114), ranging from 3–728 days, and the median treatment delay was 1 day; mean (SD) of 3.11(5.5), reaching up to 66 days.

Health care system delay was significantly longer than patient delay; mean (SD) of 75 (90.9), with a median of 42 days, ranging from 1–583 days, versus 51.1 (74.4) a median of 24 days, ranging from 1–393 days, respectively.

The median number of visits before diagnosis was 3 (0–41), and 67% of cases sought initial care in the private sector. A significantly longer health care system delay was reported among patients seeking care with the onset of symptoms in the private sector compared to those seeking care in the public sector.

### ■ Types of delay

**Patient-related diagnostic delay** The significant risk factors for patient-related diagnostic delay were: non-Iranian nationality (2.6-fold increased risk), smoking (1.7-fold increased risk), self-medication or visiting a traditional healer as the first action (2.1-fold increased risk), hope of self cure (2.2-fold increased risk) and economic constraints (2.5-fold increased risk).

**Total diagnostic delay** The significant risk factors for diagnostic delay were: age > 35 years (1.01-fold increased risk for each year); non-Iranian nationality (2.5-fold increased risk); smoking (2.01-fold increased risk), positive history of chronic pulmonary disease (3.3-fold increased risk), economic constraints (2.2-fold increased risk), seeking care at several health care providers before diagnosis (1.9-fold increased risk) and having a negative sputum smear result for acid fast bacilli on entering the health system (3-fold increased risk). Fever as the first symptom and performance of a sputum smear or chest X-ray

examination at first attendance of the health facility were associated with reduced risk for diagnostic delay.

**Health care system delay** The significant risk factors for health care system delay were a positive history of chronic pulmonary disease (4.8-fold increased risk) and having a negative direct microscopy for acid fast bacilli during the course of the disease (11.7-fold increased risk). Fever as the first symptom, a fewer number of symptoms at first attendance of the health facility and performance of a sputum smear examination or chest X-ray at the first attendance of the health facility were associated with reduced risk for health care system delay.

**Total delay** The significant risk factors for total delay were: older age (> 35 years) (1.01-fold increased risk), non-Iranian nationality (2.3-fold increased risk), smoking (1.9-fold increased risk), a positive history of chronic pulmonary disease (3.1-fold increased risk), economic constraints (2.4-fold increased risk), seeking care at several health care providers before diagnosis (1.9-fold increased risk) and having a negative sputum smear result for acid fast bacilli on entering the health system (3-fold increased risk). Fever as the first symptom, presenting with a cough at the first attendance of the health facility and performance of a sputum smear or chest X-ray examination at first attendance of the health facility were associated with reduced risk for diagnostic delay.

### ■ Conclusions and recommendations

The long time interval between onset of symptoms and treatment reported in this study was mainly attributed to health care system delay rather than patient delay. Integrating tuberculosis re-training courses into the national continuous medical education programme for private physicians is highly recommended.

# Tuberculosis

## Control

### Pakistan

A multicountry study in 7 Eastern Mediterranean countries: Egypt, Islamic Republic of Iran, Iraq\*, Pakistan, Somalia, Syrian Arab Republic and Yemen

Case-finding in tuberculosis patients: diagnostic and treatment delays and their determinants in Pakistan

Pakistan  
Karachi

Study period:  
January–December 2003

Small Grants Scheme  
(SGS) 2002 No. 47

Principal Investigator  
Dr Mubina Agboatwalla  
Health Oriented Preventive Education (HOPE)  
Karachi, Pakistan  
e-mail:  
agboat@gerrys.net

#### Abstract

A cross-sectional study was conducted in 3 chest clinics of the National Tuberculosis Control Programme in Karachi. A total of 844 patients with sputum-positive tuberculosis above the age of 15 years were enrolled in 2003 to assess the extent and determinants of delay in diagnosis and treatment of new positive pulmonary tuberculosis cases detected in directly observed treatment, short-course (DOTS) areas.

**Results** The mean total delay calculated as the time interval between the onset of symptoms and initiation of treatment was 100.7 days. Patient delay calculated as the time period from the onset of symptoms to seeking advice from a health care provider contributed a mean of 9.9 days, only 10% of this delay. The health care component, calculated as the time from seeking health care to diagnosis, was a mean of 52.75 days. This component was chiefly contributed by private health care providers. The significant risk factors for total delay were living in suburban areas, inadequate satisfaction with care, suboptimal knowledge regarding tuberculosis, seeking care at several health care providers before diagnosis and being diagnosed at health facilities other than those of the national tuberculosis control programme.

**Conclusion** The health system is chiefly responsible for the long delay between onset

of symptoms and treatment of tuberculosis patients in this community.

#### Background

Pakistan has a low case detection rate of 6% compared to an overall case detection rate in the Eastern Mediterranean Region of 24% and the global DOTS case–detection rate target of 70%. As Pakistan contributes 44% of tuberculosis cases in the Eastern Mediterranean Region, the regional case detection rate can only be improved with improvement in the case detection rate in Pakistan. Such an improvement can only be achieved by investigating the determinants of delay in case-finding. The delay may occur at the level of the patient or at the level of the health system. A study was therefore conducted to assess the extent and determinants of delay in diagnosis and treatment of pulmonary tuberculosis patients in Pakistan.

#### Materials and methods

As a part of a multicountry study carried out in 7 countries of the Eastern Mediterranean Region, a cross-sectional study was conducted in 3 DOTS implementing centres in Karachi: Nazimabad and Malir chest clinics, and the Ojha Institute of Chest Diseases. Using a pre-tested and standardized questionnaire, 844 newly-diagnosed smear-positive pulmonary tuberculosis cases aged more than 15 years were consecutively enrolled and interviewed. The following information was collected: sociodemographic characteristics; knowledge concerning tuberculosis; and health seeking behaviour and its determinants. Patients with longer delays were compared to others with less delay. The median cut-off was used to categorize patients as cases or controls.

### Conclusions and implications of the study

■ The mean total delay was 100.7 days and approximately half the patients were treated within 97 days. Health care system delay was significantly longer than patient delay.

■ The significant risk factors for total delay were: living in suburban areas, inadequate satisfaction with care, suboptimal knowledge regarding tuberculosis, seeking care at several health care providers before diagnosis, and consulting health facilities other than those of the national tuberculosis control programme.

■ Almost three quarters of patients seek advice at homeopathic practitioners with the onset of symptoms compared to 9.1% who seek care from health care providers. In addition, the first medical consultation for nearly 90% of tuberculosis patients is with a private general practitioner in the neighbourhood. Intervention to involve both sectors in case detection activities and directly observed treatment, short-course (DOTS) implementation is highly recommended.

**Definitions** Diagnostic delay is the time interval between onset of symptoms and pulmonary tuberculosis diagnosis. Treatment delay is the time interval between pulmonary tuberculosis diagnosis and onset of treatment. Health care system delay is the time interval between seeking care at a health care provider and pulmonary tuberculosis treatment. Total delay is the time interval between onset of symptoms and pulmonary tuberculosis treatment. This duration is therefore the sum of two durations: patient delay (determined by health seeking behaviour) and health care system delay.

### ■ Main study findings

73.5% of patients were between 15–35 years, with a mean of 30.1 years. Males constituted 49.4% of cases, with a male to female ratio of 0.98. Previous exposure to a tuberculosis patient was reported by 93.4% of patients. Cough and fever were the predominant symptoms present in all cases. Cough accompanied by another symptom was the most common symptom that prompted patients to seek health care (86%).

**Health seeking behaviour** While 59.7% of patients started self-medication with the onset of symptoms, 73.2% took medicines directly from the medical store. Homeopathic practitioners were consulted by 74.2% of patients, while only 9.1% of patients consulted health workers. However, the number of patients consulting health care providers increased when the symptoms did not subside. Patients consulted several different private health care providers before finally reaching the chest clinic with a mean of 5 health care providers, while only 1.5% patients consulted 2 doctors before they were referred to the National Tuberculosis Control Programme clinic. Chest clinics could be reached within half an hour for 25% of patients.

**Initial tuberculosis diagnosis** Diagnosis of tuberculosis was mainly made by chest specialists at tuberculosis clinics (81.3%). Internists made the diagnosis in 13% cases, while private general practitioners diagnosed only 5.7% of cases.

### ■ Types of delay

The mean patient related diagnostic delay was 9.9 days and approximately half the patients took 9 days to seek health care. The significant risk factors for delay were: lower occupational levels (2.4, 1.6 and 3.9-fold for workers, students and the unemployed, respectively, in comparison to those in technical/professional occupations), living in suburban areas (1.6-fold), dissatisfaction with care (1.1-fold) and a higher cost of illness (2.8-fold).

The mean health system delay was 49 ( $\pm$ 30.5) days with a median of 56 days.

The mean diagnostic delay was 96.3 days and

approximately half the patients were diagnosed within 91 days.

The mean duration between diagnosis and treatment was 4.2 days. The duration before initiating treatment was significantly longer among patients diagnosed in the private sector compared to those diagnosed in National Tuberculosis Control Programme centres.

**Total delay** The mean duration between onset of symptoms and treatment was 3.3 (1.13) months, with a median of 3.2 months. The significant risk factors for total delay were living in suburban areas, inadequate satisfaction with care, suboptimal knowledge regarding tuberculosis, an increased number of health care providers before diagnosis and consulting health facilities other than those of the National Tuberculosis Control Programme.

### ■ Conclusions and recommendations

The long time interval between onset of symptoms and treatment reported in this study was mainly attributed to health care system delay rather than patient-related delay. These results indicate a dire need to integrate the private health sector within the mainstream public health intervention of the DOTS strategy. An important step in this context would be to allow the private sector access to the central laboratory for sputum microscopy and for patient registration and supervision through the National Tuberculosis Control Programme.

# Tuberculosis

## Control

A multicountry study in 7 Eastern Mediterranean countries: Egypt, Islamic Republic of Iran, Iraq\*, Pakistan, Somalia, Syrian Arab Republic and Yemen

Case-finding in tuberculosis patients: diagnostic and treatment delays and their determinants

Somalia  
North, South and Central regions

Study period:  
September 2002–  
March 2004

Small Grants  
Scheme  
(SGS) 2002 No. 115

Principal Investigator  
Dr Ismail Adam Abdilahi  
Hargeisa Tuberculosis  
Hospital  
Hargeisa, Somalia,  
e-mail:  
isadam33@hotmail.com

## S o m a l i a

### Abstract

A cross-sectional study was conducted in five accessible tuberculosis centres implementing directly observed treatment, short-course (DOTS) strategy for tuberculosis control in north, south and central Somalia, in order to determine the extent and determinants of delay in diagnosis and treatment of pulmonary tuberculosis patients. Using a structured and pretested questionnaire, 809 new smear-positive patients were interviewed regarding their health seeking behaviour and other determinants of delay in getting timely and appropriate care.

**Results** The mean diagnostic delay, defined as interval between onset of symptoms and diagnosis, was 76.6 days. Patient factors constituted the main component of delay rather than health system factors. The mean treatment delay, defined as the time interval between diagnosis and treatment, was 4.5 days and approximately half the patients were treated within 2 days of diagnosis. The mean total delay, defined as the duration between onset of symptoms and treatment was 79.5

days for all patients and approximately half of all patients were treated within 2 months. The significant risk factors for total delay were living in suburbs, and seeking care from nonspecialized individuals and from more than one health care provider before diagnosis.

**Conclusion** A long time interval between onset of symptoms and treatment was reported mainly attributed to patient rather than health care system factors.

### Background

The global targets of the directly observed treatment, short-course (DOTS) strategy are to achieve 70% case detection and 85% cure rates by 2005. According to the WHO global report in 2001, DOTS programmes have successfully treated 80% of all registered new smear-positive patients, but detected only 27% of the estimated tuberculosis cases in the world in 2000. The report indicated that the target of 70% case detection might not be reached until 2013, unless interventions are able to increase the case detection rate. The situation in Somalia is similar, with a case detection rate of 34% and a treatment success rate of 86% in 2000.

A study was therefore done of case-finding activities, with an in-depth analysis of the various types of delay and their determinants. The goal was to identify gaps in case-finding activities under DOTS.

### Materials and methods

Within the framework of a multicountry study, a cross-sectional study was conducted in all accessible centers implementing DOTS in Somalia including Mercy Mogadishu and Jawhar in south and central Somalia, and Hargeisa, Borama and Borao in north Somalia. 809 newly diagnosed smear positive pulmonary tuberculosis patients aged over 15 were interviewed according to a structured and pretested questionnaire.

## Conclusions and implications of the study

■ The main health seeking behaviour with onset of symptoms is to visit a health care provider (63.8%). Unlike most countries of the Region, the majority of patients seek care at the national tuberculosis control programme rather than the private sector. Almost 90% of patients are initially diagnosed at the tuberculosis centres.

■ The first action after suspicion of tuberculosis disease is to request a sputum smear examination (96%). Diagnosis is rarely based on X-ray examination only (1.2%), and referral accounts for 0.4% of cases.

■ Half the patients are treated within 2 months of the onset of symptoms. This is mainly attributed to patient rather than health care system factors. The significant risk factors for delay are living in suburbs, and seeking care of nonspecialized individuals and more than one health care provider before diagnosis.

■ The use of tuberculosis health services is directly dependent upon the availability and accessibility of tuberculosis facilities in the community. The challenge is to expand DOTS services to inaccessible populations. This could be achieved by integrating tuberculosis programmes into other existing health services at all levels, and by involving outreach community workers and other agencies working in health services.

The questionnaire included information regarding the various delay durations and the factors that might influence the health seeking behavior and other potential determinants of delay in getting timely health care. Patients with longer delays were compared to those with shorter delays. The median value was used as a cut-off point for the different types of delay. **Definitions** Diagnostic delay is the time interval between onset of symptoms and pulmonary tuberculosis diagnosis. Treatment delay is the time interval between pulmonary tuberculosis diagnosis and onset of treatment. Health care system delay is the time interval between seeking care at a health care provider and pulmonary tuberculosis treatment. Total delay is the time interval between onset of symptoms and pulmonary tuberculosis treatment. This duration is therefore the sum of two durations: patient delay (determined by health seeking behaviour) and health care system delay.

### ■ Main study findings

More than two-thirds of newly diagnosed tuberculosis patients were aged < 35 years old, with a male to female ratio of 2.5.

Cough was reported by almost all patients, followed by fever and chest pain. Cough with any other complaint was the main symptom motivating patients to seek health care. The mean socioeconomic score was significantly lower for women than men, while there were no significant differences between them regarding knowledge of the disease, stigma or satisfaction with care.

A high level of stigma regarding tuberculosis was recorded among tuberculosis patients, and only one-third of patients had previously heard about tuberculosis. The main source of information about the disease was identified as friends/relatives by half the patients. Only 8.4% of patients identified the Ministry of Health campaign as their main source of information.

Most patients were satisfied with the availability of tuberculosis services. However, there was suboptimal satisfaction with health service coverage, workload and waiting time. The reasons given by 61.5 % of patients for attending tuberculosis centres were confidence in obtaining a cure and the availability of services in these centres.

**Health seeking behaviour before diagnosis** The majority of patients seek care at a tuberculosis centre rather than with the private sector as their first action (76.2% versus 17%, respectively) and second action (70.8% versus 20.8%, respectively). This can be explained by the provision of free services by these facilities throughout the country.

**Initial tuberculosis diagnosis** The majority of patients were diagnosed at tuberculosis centres (89.6%). The first action after suspicion of tuberculosis

was to request a sputum smear examination (96%), while X-rays were infrequently performed, due to the unavailability of X-ray machines.

**Access to public health facilities** More than half the patients could reach the health facility in less than 30 minutes, 20.4% in 30 minutes to 1 hour and 21.6% in more than one hour.

### ■ Types of delay

The mean patient-related diagnostic delay was 69 days and approximately half took 53 days to seek health care. The significant risk factors for patient-related diagnostic delay were living in suburbs and rural areas (2 and 4.8-fold increased risk, respectively, compared to urban areas), and inadequate satisfaction with care. A high level of stigma was found to motivate patients to seek health care earlier.

The mean diagnostic delay was 76.6 days and approximately half the patients were diagnosed within 58 days. The mean treatment delay was 4.5 days and approximately half were treated within 2 days of diagnosis. The mean health care system delay was 19.5 days with a median of 7 days.

**Total delay** The mean duration between onset of symptoms and treatment was 79.5 days for all patients and approximately half the patients were treated within 60 days. The significant risk factors for total delay were living in suburbs (2.2-fold increased risk compared to urban areas), seeking care from nonspecialized individuals (2.2-fold increased risk compared to a health care provider), and visiting more than one health care provider before diagnosis (1.5-fold increased risk). A high degree of stigma was protective.

### ■ Conclusions and recommendations

The long time intervals between onset of symptoms and treatment reported in the study were mainly attributed to patient-related diagnostic delay rather than delay within the health care system. Detection, follow-up and treatment of tuberculosis among people living in suburbs and rural areas, as well as women and poor people, should be improved by integrating the tuberculosis control programme into other existing health services at all levels, and increasing community awareness through health education using appropriate channels.

# Tuberculosis

## Control

A multicountry study in 7 Eastern Mediterranean countries: Egypt, Islamic Republic of Iran, Iraq\*, Pakistan, Somalia, Syrian Arab Republic and Yemen

Case-finding in tuberculosis patients: diagnostic and treatment delays and their determinants

Syrian Arab Republic  
Nationwide

Study period:  
November 2002–  
January 2004

Small Grants  
Scheme  
(SGS) 2002 No. 38

Principal Investigator  
Dr Fadia Maamary  
National Tuberculosis  
Control Programme  
Ministry of Health  
Damascus, Syrian Arab  
Republic  
e-mail:  
npsyria@mail.sy

## Syrian Arab Republic

### Abstract

A cross-sectional study was conducted in all tuberculosis centres implementing directly observed treatment, short-course (DOTS) strategy for tuberculosis control across the Syrian Arab Republic, in order to determine the extent and determinants of delay in diagnosis and treatment of pulmonary tuberculosis patients. Using a structured and pre-tested questionnaire, 800 new smear-positive patients were interviewed regarding their health seeking behaviour and other determinants of delay in getting timely and appropriate care.

**Results** The mean diagnostic delay, defined as the interval between onset of symptoms and diagnosis, was 77.6 days. Patient factors constituted the main component of delay, rather than health system factors. The mean treatment delay, defined as the time interval between diagnosis and treatment, was 2.9 days and approximately half the patients were treated within 1 day of diagnosis.

The mean total delay, defined as the duration between the onset of symptoms and treatment was 80.4 days and approximately half the patients were treated within 57 days. The significant risk factors for total delay were living at a far distance from the health facility, a high degree of stigma, and seeking care from nonspecialized individuals and from more than one health care provider before diagnosis.

**Conclusion** A long time interval between onset of symptoms and treatment was reported, mainly attributed to patient rather than health care system factors. The identified determinants of delay duration, and the information obtained concerning different barriers to timely health care, should be used to rectify gaps and to plan future interventions to improve case-finding in this community.

### Background

The global targets of tuberculosis control are to achieve 70% case detection and 85% cure rates by 2005. According to the WHO global report in 2001, directly observed treatment, short-course (DOTS) programmes in 2000 successfully treated 80% of all registered new smear-positive patients, but detected only 27% of the world's estimated tuberculosis patients. The report indicated that the target of 70% case detection might not be reached until 2013, unless interventions are implemented to increase the case detection rate.

The situation in the Syrian Arab Republic is similar, with an estimated incidence of 38 per 100 000 in 2000, a case detection rate of 32% and a treatment success rate of 79% under complete DOTS coverage. Therefore, improving case-finding activities tops the list of challenges facing disease control.

A study was therefore undertaken of case-finding activities, with an in-depth analysis of the various types of delay and their determinants. The goal was to identify gaps in case-finding activities under DOTS in order to assist in the planning of future interventions.

### Materials and methods

Within the framework of a multicountry study, a cross-sectional study was conducted in all tuberculosis centres implementing DOTS in the country (total  $n = 13$ ). 800 newly diagnosed smear positive pulmonary tuberculosis patients were interviewed according to a structured

## Conclusions and implications of the study

■ The main health seeking behaviour with onset of symptoms is to visit a health care provider (92.1%). The majority of patients seek care at the private sector rather than the national tuberculosis control programme (2.9%). However, almost half the patients are finally diagnosed in the tuberculosis centres.

■ The first action after suspecting tuberculosis disease is to request sputum smear examination and X-ray (95.3%). Diagnosis is rarely based on sputum smear examination only (0.6%), and referral accounts for 2.3% of cases.

■ Half the patients are treated within 2 months of the onset of symptoms. This is mainly attributed to patient rather than health care system factors. The significant risk factors for delay are living at a far distance from the health facility, a high degree of stigma, and seeking care from nonspecialized individuals and more than one health care provider before diagnosis.

and pre-tested questionnaire. Patients with longer delay were compared to those with shorter delay. The median value was used as a cut-off point for the different types of delay.

The questionnaire included information regarding various delay durations and the factors that may influence health seeking behaviour and accessibility to timely and appropriate care.

**Definitions** Diagnostic delay is the time interval between onset of symptoms and pulmonary tuberculosis diagnosis. Treatment delay is the time interval between pulmonary tuberculosis diagnosis and onset of treatment. Health care system delay is the time interval between seeking care at a health care provider and pulmonary tuberculosis treatment. Total delay is the time interval between onset of symptoms and pulmonary tuberculosis treatment. This duration is therefore the sum of two durations: patient delay (determined by health seeking behaviour) and health care system delay.

## ■ Main study findings

More than two-thirds of newly diagnosed pulmonary tuberculosis patients were aged < 35 years old, with a male to female ratio of 1.8. Cough was reported by almost all patients, followed by fever and weight loss, and was the main symptom motivating patients to seek health care. Females recorded significantly lower socioeconomic status and higher stigma than males, while there was no significant difference in knowledge of the disease or satisfaction with care.

**Health seeking behaviour before diagnosis**  
The main health seeking behaviour with the onset of symptoms was to visit a health care provider (92.1%). The majority of patients seek care at the private sector (79.1%) or public hospitals (17.9%), rather than the national tuberculosis control programme (2.9%).

Patients first seek care mainly at chest specialists (50%), followed by general practitioners (29.2%) and specialists in internal medicine (25.7%). The majority of patients visited 1 health care provider before diagnosis, but 42% visited more than 1 and up to 5 health care providers. Most patients (90.3%) were living within half an hour from the health facility. Almost all patients had previously heard about the disease, mainly from Ministry of Health campaigns (51.7%), followed by information from a sick relative or friend (27%). A considerable percentage of patients had inadequate knowledge regarding the presence of a vaccine for the disease, duration of treatment and types of drugs.

**Initial tuberculosis diagnosis** Almost half the patients were diagnosed in tuberculosis centres (48.5%). Diagnosis was mainly done by chest specialists (68.1%), specialists in internal medicine (20.5%) and general practitioners (9.3%). The first

action after suspicion of tuberculosis was to request sputum smear examination and X-ray (95.3%). Diagnosis was rarely based on sputum smear examination only (0.6%), and referral accounted for 2.3% of cases. Reasons for consulting the national tuberculosis control programme were free services (30.5%), confidence in obtaining a cure (27.6%) and accessibility (22%).

## ■ Types of delay

The mean patient related diagnostic delay was 52.7 days and approximately half the patients took 31 days to seek health care. The significant risk factors for delay were: inadequate knowledge regarding the disease (a 1.1-fold increased risk); seeking care at a nonspecialized individual who is not a health care provider (5.7-fold increased risk compared to seeking care at a health care provider); and seeking care from more than one health care provider before diagnosis (1.2-fold increased risk).

The mean diagnostic delay was 77.6 days and approximately half the patients were diagnosed within 55 days. The mean treatment delay duration between diagnosis and treatment was 2.9 days and approximately half the patients were treated within one day of diagnosis. The mean health care system delay duration between seeking health care in the health system and treatment was 27.6 days with a median of 15 days.

**Total delay** The mean duration between onset of symptoms and treatment was 80.4 days and approximately half the patients were treated within 57 days. The significant risk factors for total delay were living at a far distance from the health facility (2.5-fold increased risk); high degree of stigma (1.2-fold increased risk), seeking care at a nonspecialized individual who is not a health care provider (3.6-fold increased risk); and seeking care from more than one health care provider before diagnosis (2.0-fold increased risk).

## ■ Conclusions and recommendations

The long time intervals between onset of symptoms and treatment reported in this study were mainly attributed to patient-related diagnostic delay rather than delay within the health care system. The main study recommendations are to: increase awareness in the community about chest symptoms, and the availability of free diagnostic and therapeutic services; educate public and private health care providers about national tuberculosis control guidelines; and increase collaboration between both public and private sectors.

# Tuberculosis

## Control

### Yemen

A multicountry study in 7 Eastern Mediterranean countries: Egypt, Islamic Republic of Iran, Iraq\*, Pakistan, Somalia, Syrian Arab Republic and Yemen

#### Case-finding in tuberculosis patients: diagnostic and treatment delays and their determinants

Yemen  
Nationwide

Study period:  
September 2002– April  
2004

Small Grants  
Scheme  
(SGS) 2002 No. 214

Principal Investigator  
Dr Amin Al-Absi  
National Tuberculosis  
Control Programme  
Sana'a, Yemen  
e-mail:  
amin940@hotmail.com

#### Abstract

A cross-sectional study was conducted in all tuberculosis centres implementing directly observed treatment, short-course (DOTS) strategy for tuberculosis control across Yemen, to determine the extent and determinants of delay in diagnosis and treatment of pulmonary tuberculosis patients. Using a structured and pre-tested questionnaire, 598 new smear-positive patients were interviewed regarding their health seeking behaviour and other determinants of delay in getting timely and appropriate care.

**Results** The mean diagnostic delay, defined as the interval between onset of symptoms and diagnosis, was 57.4 days. Patient factors constituted the main component of delay, rather than health system factors. The mean treatment delay, defined as the time interval between diagnosis and treatment, was 1.7 days and approximately half the patients were treated immediately following diagnosis.

The mean total delay, defined as the duration between onset of symptoms and treatment was 59.2 days for all patients and approximately half the patients were treated within 35 days. The significant risk factors for total delay were female sex, spending over 30 minutes to reach the health facility, and a high degree of stigma.

**Conclusion** A long time interval between onset of symptoms and treatment was reported, mainly attributed to patient rather than health care system factors.

#### Background

The global targets of tuberculosis control are to achieve 70% case detection and 85% cure rates by 2005. According to the WHO global report in 2001, directly observed treatment, short-course (DOTS) programmes in 2000 successfully treated 80% of all registered new smear-positive patients, but detected only 27% of the world's estimated tuberculosis patients. The report indicated that the target of 70% case detection might not be reached until 2013, unless interventions are implemented to increase the case detection rate. In Yemen, the case detection rate in 2000 was 60.1%, while the treatment success rate was 79%. Although this case detection rate is relatively higher than other countries of the Region, it is still below the global target.

A study was therefore undertaken of case-finding activities, with an in-depth analysis of the various types of delay and their determinants.

#### Materials and methods

Within the framework of a multicountry study, a cross-sectional study was conducted in all tuberculosis centres implementing DOTS in the country. 598 newly diagnosed smear positive pulmonary tuberculosis patients were enrolled using the cluster sampling technique based on probability proportionate to size sampling. Patients with longer diagnostic or treatment delays were compared to those with shorter delays. The median was used as a cutoff point for the different types of delay. **Definitions** Diagnostic delay is the time interval between onset of symptoms and pulmonary tuberculosis diagnosis. Treatment delay is the time interval between pulmonary tuberculosis diagnosis and onset of treatment. Health care system delay is the time interval between seeking care at a health care

#### Conclusions and implications of the study

■ The main health seeking behaviour with onset of symptoms is to visit a health care provider (88.8%). The majority of patients initially seek care at public or private health facilities (31.6% and 32.1%, respectively) but an increasing proportion then seek care at tuberculosis centres. The majority of patients (63.1%) are initially diagnosed in tuberculosis centres.

■ The first action after suspecting tuberculosis disease is to request a sputum smear examination (72.6%). Diagnosis is rarely based on chest X-ray only (1.6%), and referral accounts for 2.4% of cases.

■ Half the patients are treated within 35 days from the onset of symptoms. This is mainly attributed to patient rather than health care system factors. The significant risk factors for delay are female sex, spending more than 30 minutes to reach the health facility and high degree of stigma.



provider and pulmonary tuberculosis treatment. Total delay is the time interval between onset of symptoms and pulmonary tuberculosis treatment. This duration is therefore the sum of two previously calculated durations: patient delay (determined by health seeking behaviour) and health care system delay.

### ■ Main study findings

More than two-thirds of all newly diagnosed tuberculosis patients were aged < 35 years old, with a male to female ratio of 1.36.

Cough was reported by almost all patients, followed by fever and chest pain. Cough with any other complaint was the main symptom motivating patients to seek health care. The mean socioeconomic score was significantly lower in females compared to males, while there was no significant difference between them regarding knowledge of the disease, stigma or satisfaction with care.

**Health seeking behaviour before diagnosis**  
The first health seeking behaviour for 88.8% of patients was to visit a health care provider, while the second was to self medicate, take traditional medicine or seek advice from a health worker at home. Before diagnosis, half the patients had spent US\$ 8, with an average amount spent of US\$ 20. At first, the majority of patients seek care at public (31.6%) or private (32.1%) health facilities, but afterwards an increasing proportion seeks care at tuberculosis centres. Patients first seek care mainly at chest specialists (34.7%), specialists in internal medicine (38.0%) and general practitioners (25.2%). Half the patients visited 1 health care provider before diagnosis, with a mean of 1.16 (0.92), ranging from 0–6.

A high level of tuberculosis stigma was recorded among tuberculosis patients, and the majority of patients (80%) had previously heard about tuberculosis. The source of information was friends/relatives with the disease (39%), friends and relatives (27.1%), and Ministry of Health campaigns (23.9%).

Half the patients were satisfied with the availability of services and the promptness of action from health care providers, but there was suboptimal satisfaction with the availability of free drugs, coverage of health services, workloads and waiting times. Almost 60% of patients reported that their reasons for consulting the tuberculosis centres were their accessibility and confidence in getting a cure.

**Initial tuberculosis diagnosis**  
The majority of patients were diagnosed in the tuberculosis centres (63.1%), and diagnosis was mainly done by chest specialists (66.6%). The first action after suspicion of tuberculosis was to request sputum smear examination (72.6%), but X-rays were infrequently performed.

**Access to public health facilities** More than

half the patients were able to reach the health facility in less than 30 minutes, 29.9% in 30 minutes to 1 hour and 19.2% in more than 1 hour.

### ■ Types of delay

The mean patient-related diagnostic delay was 39 days and approximately half the patients took 28 days to seek health care. The significant risk factors for patient-related diagnostic delay were: female sex (2-fold increased risk) and inadequate knowledge regarding the disease (1.1-fold increased risk of delay for each unit of poor knowledge).

The mean diagnostic delay was 57.4 days and approximately half the patients were diagnosed within 35 days. The mean treatment delay was 1.7 days and approximately half the patients were immediately treated. The mean health care system delay was 20 days with a median of 4 days.

**Total delay**  
The mean duration between onset of symptoms and treatment was 59.2 days and approximately half of the patients were treated within 35 days. The significant risk factors for total delay were: female sex (2.3-fold increased risk); spending more than half an hour to reach the health facility (1.8); and high degree of stigma (1.7).

### ■ Conclusions and recommendations

The long time interval between onset of symptoms and treatment reported in this study was mainly attributed to patient-related diagnostic delay rather than delay within the health care system. Detection, follow-up and treatment of cases should be improved by: integrating the tuberculosis programme into other existing health services at all levels; involving outreach community workers and other agencies working in health service provision; and increasing community awareness through health education, using appropriate channels.

# Tuberculosis

## Epidemiology

### Pakistan

Prevalence of  
pulmonary  
tuberculosis in  
Karachi juvenile  
prison, Pakistan

Pakistan  
Karachi

Study period:  
January–October 2002

Small Grants  
Scheme  
(SGS) 2001 No. 26

Principal Investigator  
Dr Shah Sharaf Ali  
Sindh AIDS Control  
Programme  
Ministry of Health  
Karachi, Pakistan  
e-mail:  
sharafshah@cyber.net.pk

### Abstract

Three hundred and eighty-six (386) juvenile detainees were enrolled in a study to determine the prevalence of pulmonary tuberculosis and possible risk factors.

**Results** The prevalence of tuberculosis among juvenile prison inmates was 3.9%, significantly higher than the estimated 1.1% prevalence among the general population. These inmates represent a potential source of infection for the whole community.

The study identified poor adherence of diagnosed patients to anti-tuberculosis treatment. It also highlighted the high vulnerability of prison inmates to tuberculosis due to the presence of highly infectious cases together with overcrowding, poor ventilation and an overall unhealthy living environment.

Poor health care facilities in prisons, leading to inadequate diagnosis and treatment of tuberculosis infection were also implicated.

It is highly recommended to introduce a tuberculosis control programme in the prison environment, ensuring regular screening of prisoners for tuberculosis infection, and early and effective management of cases. Special care should be given to those reporting a

positive family history for tuberculosis. There is also a need to ensure that all prisoners who are receiving directly observed treatment, short course (DOTS) in prison must continue to do so until treatment is completed, including after release.

### Background

Prison inmates are at increased risk for pulmonary tuberculosis. Factors such as overcrowding, malnutrition and limited access to health care services put prison inmates at a high risk for pulmonary tuberculosis. Children and adolescents are even more vulnerable. There is an increasing recognition that the high risk of tuberculosis in settings such as prisons, detention centres for asylum seekers, penal colonies and prisoner of war camps poses a problem for those imprisoned and for the wider society.

Pakistan ranks sixth among the 22 countries with high tuberculosis burden in the world. It also ranks first among the countries of the Eastern Mediterranean Region. However, there is limited data regarding the prevalence of tuberculosis among prison inmates in general and juvenile detainees in particular. Therefore, this study was conducted in order to determine the prevalence and risk factors of pulmonary tuberculosis among juvenile detainees.

### Materials and methods

The city of Karachi was selected for the study because it is the most populous city of the country, with a population of more than 10 million. It has inhabitants from all parts of the country. The Karachi juvenile prison population reflects the characteristics of the city population, with inmates from all parts of the country and even from neighbouring countries.

The medical officer and technicians of the prison were trained in laboratory, clinical diagnosis and management of tuberculosis using the directly observed treatment, short course (DOTS) strategy

## Conclusions and implications of the study

■ The prevalence of pulmonary tuberculosis among the juvenile prison inmate population is 3.9%, significantly higher than the general population. This is attributed to overcrowding, poor ventilation and malnutrition. A family history of tuberculosis and poor health services in the prisons are also implicated.

■ Poor compliance with the anti-tuberculosis regimen appeared to be responsible for treatment failure of cases diagnosed outside the prison, and is explained by the economic burden and lack of knowledge regarding the consequences of non-adherence to treatment.

■ Two week training of technicians for acid-fast bacilli (AFB) staining and microscopy may not be sufficient to independently carry out staining and microscopy for AFB testing.

■ Language barriers seem to interfere with proper history taking, sputum collection and adherence to treatment.

■ A tuberculosis control programme should be introduced in the prison environment, ensuring regular screening of prisoners for tuberculosis infection, and early and effective management of the cases. Special care should be given to those reporting a positive family history for tuberculosis who are more likely to be infected.

at the Ojha Institute of Chest Diseases, Karachi for two weeks. The technicians and dispenser of the prison also received training in sputum microscopy at the Institute.

The medical officer examined all juvenile inmates clinically and obtained family and past history of illness through administering a structured questionnaire after obtaining their informed consent. Prisoners were suspected of tuberculosis infection on the grounds of clinical findings, past history of diagnosis of tuberculosis infection and family history of the illness. Individuals identified as suspected cases for tuberculosis infection were investigated for acid-fast bacilli (AFB) in their sputum. Three specimens of sputum were collected and examined by microscopy using the Ziehl-Neelson method of staining.

### ■ Main study findings

A total of 386 male, unmarried, juvenile detainees were recruited for the study, 90% were in the age group 16–19 years, ranging from 15 to 23 years, with a mean age of 17.7 years. The average family size was 8, ranging from 1 to 25. All were imprisoned in 10 barracks, averaging 35 prisoners per barrack and ranging from 10 to 59 prisoners per barrack. 92% were from Pakistan, mostly from Karachi, while the rest were from Bangladesh, India and Myanmar.

In 25% of cases, the father of the inmate was a labourer, while 19% of fathers were self-employed, 13% were working in public and private services, 24% had other professions and the rest were unemployed or dead. Almost half the inmates were labourers, 17% were self-employed, 13% worked in services and the rest had other professions. Sixty percent (60%) of prison inmates were illiterate, while none had secondary school or a higher education.

More than half the inmates were serving an imprisonment period of 1 to 6 months, 20% between 6 and 12 months, 16% more than 1 year and 11% were serving less than 1 month. Thirteen percent (13%) of inmates had earlier records of imprisonment.

Forty-two percent (42%) of prison inmates were smokers, and 21% were drug users. Among the drug users, 83% inhaled drugs, while only 1% had history of injecting drugs.

Nine prison inmates had been diagnosed with tuberculosis in the past. Three had received 6 months treatment, but were symptomatic at the time of clinical examination. One of these 3 cases was also positive for AFB test. Of the other 6 previously diagnosed inmates, 2 had not received any treatment and 4 had received irregular treatment, while all were symptomatic for tuberculosis infection for more than a month. Among these 6 patients, 3 had complaints of cough, fever, weight loss and haemoptysis. One had a history of fever and cough, while the other 2

were suffering from chronic cough.

Forty-eight (12%) inmates had one or more complaints of cough, fever, weight loss and haemoptysis for more than one month; while 73 (20%) were initially suspected of tuberculosis infection on the basis of clinical symptoms, family history and past diagnosis of tuberculosis infection. Among these 73, 48 cases had clinical signs and symptoms suggestive of tuberculosis infection, 33 cases had a family history of tuberculosis infection and 8 cases had symptoms suggestive of tuberculosis as well as a family history of the disease.

Of 73 suspected cases, sputum was obtained from 19 (26%), while the rest had saliva and blood in their specimens and no sputum. Among the 19 whose sputum was tested for AFB, 5 (2.6%) showed AFB on microscopy.

On the basis of AFB smear results, earlier diagnosis of the disease, and signs and symptoms strongly suggestive of tuberculosis infection, 15/386 (3.9%) prison inmates were identified as tuberculosis cases and selected for the DOTS regimen. Three prison inmates who were negative for AFB smear and had no history of earlier diagnosis were selected for DOTS regimen mainly on the clinical judgement of the treating physician.

Although there was an elevated risk for contracting tuberculosis infection among the illiterate, Karachi residents, those with unemployed parents, smokers and drug users, none was statistically significant. On the other hand, there was a 7-fold increased risk among those reporting a positive family history of tuberculosis and this was statistically significant.

### ■ Conclusions and recommendations

The study reported a 3.9% prevalence of tuberculosis among this prison inmate population, which was significantly higher than that of the general population (1.1%). This high prevalence could be attributed to overcrowding, poor ventilation and malnutrition in the prison environment. A family history of tuberculosis and poor health care facilities in prisons leading to inadequate diagnosis and treatment of tuberculosis are also implicated.

It is recommended to introduce the tuberculosis control programme in prisons, ensure regular screening of prisoners for tuberculosis infection, and early and effective management of cases.

# Tuberculosis

## Epidemiology

### Iraq

Prospective follow-up study of primary school children with positive tuberculin skin test and their contacts in Iraq

Iraq  
Nationwide

Study period:  
April–November  
2002

Small Grants  
Scheme  
(SGS) 2001 No. 4

Principal Investigator  
Dr Waqar Al-Kubaisy  
Community Medicine  
Department  
College of Medicine  
Baghdad, Iraq

### Abstract

A study was conducted in the 16 Iraqi governorates in 2002 whereby the household contacts of 215 primary school children who tested positive in a tuberculin skin test (TST) survey in 2000 were investigated for tuberculosis infection and disease. The objectives were to assess the impact of contact screening on case-finding, to estimate the incidence of tuberculosis infection and disease, and to identify the risk factors associated with a positive TST case among household contacts.

All participants < 15-years old including index cases were subjected to TST. Chest radiography was performed for children with a positive TST and all adult members.

Three consecutive sputum smear examinations for acid-fast bacilli were performed for every adult member.

Results 144 new cases of tuberculosis were detected. The incidence of tuberculosis disease among household contacts of positive TST index cases was 14.3%. Diseased household contacts were significantly older than healthy household contacts (mean age  $37.1 \pm 14.7$  and  $18 \pm 16.2$  years, respectively). Significant association was found between tuberculosis disease and the crowding index, body mass index and smoking.

Conclusion These results revealed a deficiency in the control programme regarding chemoprophylaxis of positive TST individuals during the prevalence survey and highlighted the need for an awareness programme to prevent household contacts from acquiring *Mycobacterium tuberculosis* infection.

### Background

Globally, Iraq ranks 44<sup>th</sup> among countries in tuberculosis burden and 7<sup>th</sup> among the countries of the Eastern Mediterranean Region. The mandatory screening programme for all new primary school students throughout Iraq reported that 2.3% were positive by tuberculin skin test (TST) in 2000. A study was therefore designed to screen all household contacts of students with a positive TST in 2000 in order to assess the impact of contact screening on case-finding, to estimate the incidence of tuberculosis infection and identify the risk factors associated with tuberculosis.

### Materials and methods

The household contacts of 215 primary school children with positive TST ( $\geq 10$ mm) results during the national tuberculin test survey in 2000 (NTS) were traced.

Each participant was interviewed using a structured questionnaire, collecting information on sociodemographic, clinical characteristics, and risk factors for tuberculosis. Confirmation of previously positive TST results was carried out. Clinical examination was done for all 834 household members. Household contacts aged < 15 years old were first given a TST, then chest X-ray was performed for those with positive TST results. Chest X-ray and three consecutive sputum smear examinations for the detection of acid-fast bacilli (AFB) were carried out for household members older than 15 years. Tuberculosis case definition was: positive chest X-ray and one or more sputum smear-positive results; negative chest

## Conclusions and implications of the study

■ The incidence of new cases of tuberculosis was 14.3% among household contacts, which was significantly higher than reported rates in other countries. Many factors are implicated in these high figures such as non-adherence to the tuberculosis control guidelines, inadequate health education, and increased poverty and undernourishment during the sanction period.

■ Almost 80% of tuberculosis cases in the community are attributed to household contacts. Moreover, 7.2% of contacts developed tuberculosis per year in Iraq during the sanction era.

■ Tuberculosis infection or disease is higher among female than male household contacts since it is mainly related to the degree of social interactions, which vary by gender.

■ All grandfathers and 50% of grandmothers of tuberculosis-infected children had the disease, with the highest rates among the mothers and fathers. This could be explained by the close contact that children have with their grandparents.

X-ray, but at least two positive sputum smear results; or negative three consecutive direct sputum smear results but strong evidence of positive chest X-ray associated with characteristic clinical features.

In accordance with the national tuberculosis control programme (NTP) guidelines, chemoprophylaxis was given to all participants younger than 15 years old (index case and household members) showing positive TST while household contacts confirmed as tuberculosis cases were given anti-tuberculosis drugs under the NTP.

### ■ Main study findings

The study involved 205 out of 215 TST-positive children during the 2000 national tuberculin survey. They were retested by TST to confirm the previous positive results and 191 (93%) children were confirmed positive (TST  $\geq 10$  mm). The tuberculosis infection rate was higher in males (93.6%) than females (92.8%), among children younger than 10 years old (93.6% compared to 88% for older children), among children with a low body mass index (BMI), among children with illiterate mothers, or living in extended families and households with high crowding indices.

A total of 834 household contacts of 195 index children were investigated. Their mean age was  $21.96 \pm 17.4$  years ranging from 1 to 80 years. Of these household contacts, 146 (17.5%) were diagnosed as tuberculosis cases.

Determinants of tuberculosis disease among household contacts were older age ( $> 15$  years), low BMI, current smoking and diabetes mellitus.

Diagnostic clinical criteria for tuberculosis disease were: fever, cough, pallor, weakness and chest pain. The presence of lymph nodes was not diagnostic of the disease.

A significantly higher rate of fever (61.1%), cough (51%) and pallor (62.5%) was found among children with TST indurations of 15–20 mm compared to those with indurations of 10–14 mm. In addition, induration size of 15 mm and more was significantly associated with low BMI levels.

The cumulative incidence of tuberculosis disease among contacts of index cases was 16.7%, with 14.3% new cases detected among this group. Accordingly, 79.6% of cases are attributed to household contacts, and 7.2% of contacts develop tuberculosis per year.

These figures are significantly higher than those reported from other countries. This could be attributed to non-adherence to tuberculosis control guidelines regarding contact tracing and investigation, inadequate health education, increased poverty and its effect on nutrition and health-seeking behaviour as a result of sanction, poor accessibility to medical care for diagnosis and treatment, and the emergence of anti-tuberculosis drug resistance.

### ■ Conclusions and recommendations

Although NTP guidelines recommend that household contacts of tuberculosis patients should be given chemoprophylaxis, this study suggests that it is not routinely performed as indicated by the relatively high frequency of the disease among household contacts. These results confirm that screening household contacts is useful in identifying new tuberculosis cases and may help in early diagnosis of tuberculosis. This study reveals the deficiency of the control programme regarding contact tracing and highlights the need for an awareness programme to prevent household contacts from acquiring *M. tuberculosis* infection. Strengthening supervision of contact investigation and chemoprophylaxis is therefore recommended.

# Tuberculosis

## Gender Differences

### Syrian Arab Republic

Gender differences and tuberculosis: prospects for better control in the Syrian Arab Republic

Syrian Arab Republic  
Nationwide

Study period:  
November 2001–2002

Small Grants  
Scheme  
(SGS) 2001 No. 97

Principal Investigator  
Dr Hyam Bashour  
Department of Community  
Medicine  
Faculty of Medicine,  
Damascus University  
Damascus, Syrian Arab  
Republic  
e-mail:  
bashourh@mail.sy

#### Abstract

A study was carried out to: determine whether gender is related to tuberculosis diagnosis, compliance and/or treatment outcomes of tuberculosis patients; identify tuberculosis patients' knowledge, beliefs and attitudes towards tuberculosis; and identify gender-related factors and/or barriers that may influence patient compliance with tuberculosis treatment and utilization of health care services.

The study enrolled 552 newly diagnosed smear-positive patients attending 14 tuberculosis centres nationwide from 2 January 2002 to 31 July 2002. Patients were interviewed at recruitment then followed up until the end of treatment. Patient compliance and treatment outcomes were recorded from patient treatment cards.

**Results** Presentation of symptoms did not differ between males and females. Mean number of days from onset of symptoms to reporting to a health facility was significantly longer among males (63.61) than females (40.02).

Significant differences between males and females were noted in places attended for care.

Gender did not seem to greatly determine knowledge and attitudes. Compliance with

treatment was better among females than males, but this was not statistically significant. The treatment success rate was higher among females than males (90.1% vs. 85.6%,  $p = 0.2$ ). Multivariate analysis found that male sex was a significant predictor of a negative treatment outcome. Other significant determinants of treatment outcome were educational level, and adherence to sputum examination and treatment.

**Conclusion** The study identified gender differences in relation to tuberculosis. Despite cultural factors, females had more positive indicators. Gender sensitive interventions are needed to rectify any inequalities in tuberculosis diagnosis, compliance and treatment between males and females.

#### Background

Sex/gender inequalities have long been identified as a major determinant that can lead to delay in diagnosis, poor access to health care, lack of treatment compliance and poor treatment outcomes. Health-seeking and treatment behaviour of men and women suffering from tuberculosis is largely determined by how he or she and those around perceive the symptoms, regard the diagnosis, accept the treatment and adhere to it. Gender may influence each of these and affect detection of the disease and its outcome. In the Syrian Arab Republic, the case notification rate suggests that tuberculosis may be less frequent among females. In 2001, the male to female ratio for smear positive cases was 1.8:1. There is no previous research in the Syrian Arab Republic that has examined gender-related differences in connection with diagnosis, compliance and treatment outcome of tuberculosis.

Therefore, a study was conducted to determine whether sex/gender is related to tuberculosis diagnosis, compliance and/or treatment outcome; identify tuberculosis patient (both men and women) knowledge, beliefs and attitudes towards tuberculosis; and identify gender-related factors and/or barriers that may

### Conclusions and implications of the study

- Male cases outnumbered female ones. Female patients were more socioeconomically disadvantaged than males.
- Females outnumbered males in early diagnosis and treatment, and treatment compliance, while defaulting was significantly greater among males.
- Gender disparity exists in patterns of health-seeking behaviour, stigma and barriers to health care utilization.
- Tuberculosis patients were generally satisfied with the quality of services provided.
- Male sex proved to be a significant predictor of negative treatment outcome, as was low educational level, and non-adherence to sputum examination and treatment.
- Health education should be emphasized in the national tuberculosis control programme, and the psychosocial implications of tuberculosis should be given due attention. Health education should be culturally sensitive, taking into account perceptions of tuberculosis.

influence patient compliance with tuberculosis treatment and utilization of health care services.

## Materials and methods

**Setting** All 14 tuberculosis centres in the Syrian Arab Republic.

**Subjects** A total of 552 patients were enrolled in the study out of a total of 800 new smear-positive patients registered in all tuberculosis centres during the recruitment period (2 January 2002 to 31 July 2002).

**Design** A prospective study was carried out, in which patients were interviewed at point of recruitment when diagnosed as new cases of smear-positive tuberculosis. The cohort of patients was followed up until the end of the treatment period. The treatment compliance of patients and their treatment outcomes were recorded from the patient treatment cards.

## Main study findings

Most of the study subjects were young, 20.7% had no formal education and only 11.8% had a higher level of education. There were significant differences between the sexes in age distribution, marital status, level of education and occupation but not regarding the presentation of symptoms.

Significant differences between males and females were noted in connection to the places they usually go to seek care; a higher proportion of males (81%) sought care at hospitals while a higher proportion of females (66.4%) sought care at private physicians. Only 3.1% of all study subjects sought care at pharmacies.

Symptomatic males took longer time to seek medical care; the delay in seeking care being significantly longer among males (mean delay 63.6 days vs. 40 days for females). Due to this delay, more severe cases occurred among males, explaining the fact that more males attended hospital services than females. Stigma did not seem to prevent women from seeking care. Furthermore, perception of gender issues relating to care-seeking behaviour did not affect the actual behaviour of women.

Active case finding led to similar proportions of males and females in close contact with study subjects. Compliance with treatment and treatment supervision schedules did not significantly differ between males and females, in spite of the higher proportion of women who had to obtain permission to attend the health centers and needed to have a companion with them.

Most patients were satisfied with the care provided at the health centres. However, some felt that the waiting time was quite long (12.4% of females compared to 10.9% of males,  $p > 0.05$ ).

Although not statistically significant, a positive treatment outcome was more frequently reported

among female study subjects (a treatment success rate of 89% in females compared to 82% in males). This is not surprising because women tended to comply more with treatment despite the difficulties.

The failure and defaulter rates were higher in males than females (3.7% and 8.7% compared to 2.3% and 5.8%, respectively). On the other hand, the mortality rate was comparable in the two sexes (1.4% in males vs. 1.7% in females).

Using multivariate analysis, male sex proved to be a significant predictor of a negative treatment outcome after controlling for other confounding variables. Other significant determinants of treatment outcome were educational level and adherence to sputum examination and treatment.

The study identified barriers for women to the utilization of health services in the context of culture and gender. However, these barriers did not influence treatment outcomes. Women were more compliant with treatment and had a higher treatment success rate. It can be concluded that stigma, lack of knowledge and perception of gender roles play little role in relation to tuberculosis care-seeking behaviour. This may be due to the seriousness of tuberculosis and education about the curability of the disease. More important is that tuberculosis services are only provided by tuberculosis health facilities under the national programme.

## Conclusions and recommendations

Gender sensitive interventions are needed to rectify any gender inequalities in tuberculosis diagnosis, compliance and treatment. These interventions need to be appropriate to local settings. A gender-sensitive approach to tuberculosis policy and control may lead to the greater effectiveness of national tuberculosis control programmes.

# Tuberculosis

## Gender Differences

### Pakistan

Studying gender perspectives in knowledge, attitudes and practices concerning tuberculosis in Pakistan's Sindh province

Pakistan  
Sindh province

Study period:  
December 2001–  
November 2002

Small Grants  
Scheme  
(SGS) 2001 No. 47

Principal Investigator  
Dr Mubina Agboatwalla  
Health Oriented Preventive  
Education (HOPE)  
Sindh, Pakistan  
e-mail:  
agboat@gerrys.net

### Abstract

A cross-sectional study was conducted in both urban and rural areas of Karachi in which 200 households, 100 urban and 100 rural, were randomly selected and 754 family members, aged 20 years and older, were interviewed. Data was collected using semi-structured questionnaires and focus group discussions.

**Results** Overall knowledge about tuberculosis is deficient in the general population. This includes predisposing factors for tuberculosis, its contagious nature, signs and symptoms, and preventive measures. Females' knowledge, especially rural females, was extremely deficient. Rural females were, in general, not allowed to leave the house unaccompanied and had less exposure to information. Interestingly, rural males were the most knowledgeable, which can be attributed to the health education messages they receive through the public health facilities they attend.

Most preventive health programmes, especially the tuberculosis control programme, are implemented through government health facilities. By contrast, urban respondents had more contact with private doctors, who generally lack awareness of tuberculosis control initiatives and do not have time to impart health education messages. Only in

the case of rural men where doctors were an important source of information about tuberculosis. The role of media (radio, television and newspapers) was limited.

**Conclusion** This study reported a low level knowledge and a high degree of stigma regarding tuberculosis. Tuberculosis is strongly perceived to be an extremely dangerous disease.

### Dissemination of research results

On 5 November 2002, a workshop was jointly organized by HOPE, the Sindh provincial tuberculosis control programme and the World Health Organization. The meeting had wide participation from government, tuberculosis programme officers, public health professionals, nongovernmental organizations and media representatives. The study results were also disseminated by two press releases in the *International News and The Dawn* newspapers in Karachi.

### Background

Pakistan has the sixth highest tuberculosis burden globally and accounts for 44% of the tuberculosis burden in the Eastern Mediterranean Region. Being a leading infectious disease, tuberculosis kills more women than all cases of maternal mortality combined. In 1998, 750 000 women died due to tuberculosis. As tuberculosis mainly affects women in their reproductive years, it has a major impact on children and families.

Health-seeking behaviour generally differs between men and women. Due to inadequate information, women are misinformed about their health and fail to recognize the early symptoms of the disease. However, the human element in tuberculosis control has often been overlooked. For successful tuberculosis control and success of the directly observed treatment, short course (DOTS) programme, it is important to

## Conclusions and implications of the study

- Overall knowledge about tuberculosis is deficient in the general population especially rural females.
- Rural males were the most knowledgeable, which can be attributed to the health education messages they receive through public health facilities.
- There is serious need to intensify the health education component of the tuberculosis control programme. There should also be more involvement of the private sector, especially in urban areas. More intensified efforts are needed by the media, not only to promote health education, but also to help in de-stigmatization of the disease.
- Policy-makers should take into account the constraints expressed by most females during their regular visits to health facilities and administration of tuberculosis medicines under supervision. To reduce defaulter rates, especially in rural areas, alternative means such as involving Lady Health Workers should be considered. Private doctors should also be involved in DOTS implementation.



women to discover their beliefs and knowledge about tuberculosis. This project therefore aimed at studying gender differences in knowledge and attitudes regarding tuberculosis in urban and rural communities in Sindh.

### Materials and methods

A cross-sectional study was conducted in Baldia Town, an urban site in Karachi and in Tando Jam, a union council in Hyderabad Division, a rural site. A total of 754 study subjects, 20 years of age and older, and residing 200 randomly selected households, 100 urban and 100 rural, were enrolled. Data was collected using semi-structured questionnaires and focus group discussions.

### Main study findings

The overall knowledge of the signs and symptoms of tuberculosis was generally deficient, especially in rural females. Only 21% of respondents mentioned cough as the predominant symptom of the disease, while 5.6% mentioned blood in sputum and 4.9% mentioned prolonged fever as symptoms. Only 9.8% of rural females cited germs as causing tuberculosis, compared to 30% of urban females, while only 6.3% of rural females and 23.1% of rural males knew that sputum testing was diagnostic of tuberculosis. The contagious nature of the disease was mentioned by 37%–38% of males. Sharing utensils was considered a major means of its spread. Married women with tuberculosis were expected to sleep away from their husbands and children, and to keep their clothing and utensils separate. Between 14% and 20% of rural and urban men said that completing tuberculosis treatment helped to prevent other family members developing the disease, while only 7%–9% of females were aware of this. Most females generally considered tuberculosis a dangerous disease and a death penalty. Men, however, considered it dangerous but curable.

The stigma associated with tuberculosis and social isolation by the community were linked to perceptions of the dangerous nature of the disease. Tuberculosis in young girls was considered to have a negative impact on their marriage prospects. Married women with tuberculosis, especially in rural areas, expected to be treated badly by their in-laws and to be expelled from the house. More female than male respondents feared social isolation and rejection by friends and colleagues.

A disparity was found in the health-seeking behaviours in urban and rural areas, with urban respondents attending private clinics and rural respondents, especially males, attending government public hospitals. This health-seeking behaviour explains the better knowledge level of rural males. In urban areas, men and women in the slum areas attend mainly unqualified private health care providers

and since programmes like DOTS are not implemented through the private system, many may not be aware of such strategy. In the rural areas, most public health programmes are implemented at government hospitals where the doctors are involved in public health programmes and provide health education to all patients. Since rural males generally attend these hospitals, their knowledge is much better than their urban counterparts. Rural females have an overall low exposure to information and are not allowed to freely attend health facilities unaccompanied.

In terms of the DOTS strategy, it was found that 30% of urban females and 70% of rural females were not agreeable to regular visits to health facilities for supervised drug administration. Nearly 80% of rural respondents, but only 30% of urban respondents, agreed to have an outsider coming to the house to administer medicines and on the condition that the health worker is a woman.

### Conclusions and recommendations

The study reported a low overall level of knowledge about tuberculosis. An intensified health education programme needs to be implemented to raise community awareness about the disease. This should involve the private sector, especially in the urban areas. There is dire need to de-stigmatize the disease. Social isolation and rejection by family, friends and colleagues, as well as misconceptions about its contagious nature, lead to the idea that tuberculosis is a disease to be feared. A concentrated effort is required by the media, doctors and health workers to remove these misconceptions, so that the stigma associated with tuberculosis can be removed. Policy-makers, while planning the DOTS strategy, should also take into account the constraints expressed by most women during their regular visits to health facilities for supervised drug administration. To reduce the defaulter rate, alternative approaches such as involving Pakistan Lady health Workers or female community workers should be considered.

# Tuberculosis

## Gender Differences

Egypt

Gender differences  
in utilization pattern  
and outcome of  
respiratory  
tuberculosis in  
Alexandria

Egypt  
Alexandria

Study period:  
January–December 2002

Small Grants  
Scheme  
(SGS) 2001 No. 32

Principal Investigator  
Dr Mohamed I Kamel  
Department of Community  
Medicine  
Faculty of Medicine,  
Alexandria University  
Alexandria, Egypt  
e-mail:  
kamelm@dataxprs.com.eg

### Abstract

The aim of this study was to gain more insight into the gender differences in the health-seeking and illness behaviour of tuberculosis patients, and to explore the factors that influence men and women's decisions to seek medical care and to stay on regular treatment until they are cured. All newly diagnosed pulmonary tuberculosis cases (total 334 cases) who commenced on anti-tuberculosis therapy (through the DOTS programme) during the period from November to December 2001, at the seven chest facilities in Alexandria, were included in the study. The distribution of the various study variables among male and female tuberculosis cases was studied and the cohort was subjected to 8 months follow-up to study the impact of these factors on treatment outcome. Their records were followed-up monthly to study treatment compliance.

**Results** Male cases outnumbered female cases with an overall male to female ratio of 2.2:1. Female patients were more socioeconomically disadvantaged than males. The pattern of symptoms was different between the sexes, and relapse was

significantly higher among female patients, as well as the mean duration of hospital admission. Moreover, women seemed to have different health-seeking behaviour compared to men.

Stopping treatment was encountered among 15% of tuberculosis patients. Defaulters were significantly more encountered among males than females (10.7% compared to 9.7%). Stopping treatment was mainly due to minor side-effects in females, while in males it was due to the time and money spent. Non-compliance was recorded in 47.6% of cases. Females tended to be more compliant to health education and medical examination compared to males.

Tuberculosis was perceived by the patients to affect marital relationships and women's reproductive health. Limited understanding and knowledge of tuberculosis was found to a significant degree among females.

The treatment success rate at the end of follow-up period was 94%, with no statistically significant difference between males and females. The failure rate, which was encountered among 4.8% of patients, was higher in males than females. Patient satisfaction with quality of care provided was the only predictor of treatment outcome. To a great extent, tuberculosis patients were satisfied with the quality of services provided with no significant association with gender.

**Conclusion** Gender disparity exists in the health-seeking and illness behaviour of tuberculosis patients, but does not seem to influence the treatment outcome.

### Background

Gender analysis in health takes the emphasis away from questions of organic/biological causality and concentrates on explaining the differential constraints experienced by women and men in access to health and health care.

The aim of this research was to gain more insight into the gender differences in health-seeking and illness behaviour of tuberculosis patients, and to explore and describe the factors that influence men and women's decisions to seek medical care and to stay on regular treatment until they are cured.

## Conclusions and implications of the study

■ Male cases of tuberculosis outnumbered female ones. Female patients were more socioeconomically disadvantaged than male patients.

■ Female patients outnumbered male patients regarding early diagnosis and treatment, treatment compliance, duration of hospital admission and disease relapse. Limited knowledge of tuberculosis was more encountered among female patients, while defaulters were more encountered among male patients.

■ Gender disparity was found in the pattern of symptoms, health-seeking behaviour and sputum submission.

■ The tuberculosis patients were generally satisfied with the quality of services provided, and this was the only determinant of treatment outcome. No significant gender difference was observed regarding patient satisfaction with care, and gender did not prove to be a significant predictor of treatment outcome.

■ Health education should be emphasized in the tuberculosis control programme and the psychosocial implications of tuberculosis should be given due attention. Health education should be culturally sensitive, taking into account perceptions of tuberculosis.

## Materials and methods

A cross-sectional study was conducted at the seven chest facilities in Alexandria whereby all newly diagnosed pulmonary tuberculosis patients ( $n = 334$ ), during the period November–December 2001, were enrolled. Study subjects were interviewed regarding their health seeking and illness behavior and their records were monthly checked for treatment compliance. This was followed by a cohort study to determine the impact of the study factors on treatment outcome.

## Main study findings

Male tuberculosis cases outnumbered female cases with an overall male to female ratio of 2.2:1. By contrast, among children there was female predominance with a female to male ratio of 2.9:0.9.

Female patients were more socioeconomically disadvantaged than males. Less than half of females (46.6%) were illiterate, the majority of them (93.2%) were unemployed and 81.6% were supported by other family members, either their husband or father. Moreover, the mean family size and mean crowding index of female patients were significantly higher than males.

The pattern of symptoms was also different between sexes. Women tended to present the disease in a more severe form than men do. More women than men suffered clinical manifestations of tuberculosis such as fever, chest pain, purpura and urinary incontinence. This is likely to be due to a higher rate of progression from infection to disease among females compared to males. However, symptoms of cough and expectoration were found less among females than males. This may be associated with a shorter diagnostic and treatment delay. Moreover, poor diagnosis at the interview was encountered more among females. Disease relapse was significantly higher among females than males (16.5% compared to 12.1%). The indications of hospitalization were either for treatment of emergencies such as haemoptysis, uncontrolled diabetes, the need for further investigations and patients unable to take supervised treatment on an ambulatory basis. The mean duration of hospital admission was significantly higher for females than for males.

Women seemed to have different health-seeking behaviour compared to men. More female patients than males tried several treatment possibilities before reaching the health facility. A significantly higher percentage of females were using traditional herbal medicines, or sought treatment from the private sector first.

In spite of the greater access to and control over household financial resources, the ability to travel alone and relative freedom from domestic responsibilities, men had a longer delay to tuberculosis

diagnosis and treatment compared to women.

Stopping treatment was encountered among 15% of patients. Defaulters were more significantly encountered among males than females (10.7% compared to 9.7%). Less than a tenth of females stopped treatment in spite of minor side-effects compared to 8.7% of males. More males (6.9%) than females (3.9%) stopped treatment due to spending too much time and money.

More than half of the studied sample were compliant with the prescribed regimen. Females tended to be more compliant to health education and medical examination compared to males. Despite the fact that factors which determine compliance (personal, cultural, operational, institutional, structural and environmental) tend to work against women, they were, nevertheless, more compliant than men.

To a great extent, the tuberculosis patients were satisfied with the quality of services provided. No significant gender difference was observed as regard patient satisfaction with the quality of services provided.

Tuberculosis was perceived by the patients to affect marital relationships and to diminish marriage prospects for young patients. Tuberculosis was also more significantly perceived by females to affect women's reproductive health, lead to severe complications during pregnancy, and to affect pregnancy outcome and breast-feeding. Limited understanding and knowledge of tuberculosis was encountered among females to a significant degree.

Significantly fewer females (6.8%) had a positive sputum test at the end of the follow-up period compared to males (10.4%).

The treatment success rate at the end of the follow-up period was 94%, with no statistically significant difference between males and females. Failure rate was encountered among 4.8% of patients, and was higher in males (5.6%) than females (2.9%), although this difference was not statistically significant. Patient satisfaction with quality of care provided was the only predictor of treatment outcome among the patients.

The insights gained by this study will hopefully benefit the planning of effective gender-sensitive interventions and policies for better tuberculosis control.

# Tuberculosis

## Knowledge Attitudes and Practice

Iraq

Tuberculosis KAP study among health care workers and tuberculosis patients in Iraq

Iraq  
Nationwide

Study period:  
November 2001–2002

Small Grants  
Scheme  
(SGS) 2001 No. 3

Principal Investigator  
Dr Hashem Dhafer  
National Tuberculosis  
Control Programme  
Ministry of Health  
Baghdad, Iraq

### Abstract

A cross-sectional study was carried in 250 primary health care centres in order to evaluate the knowledge, attitudes and practice (KAP) of tuberculosis patients and health care workers (HCWs) regarding tuberculosis. A total of 500 patients and 500 HCWs were randomly selected and interviewed using pre-tested structured questionnaires.

**Results** The frequency of patients with optimum knowledge of tuberculosis was 64.4%, but 54.8% reported negative attitudes and practice towards tuberculosis due to the high degree of stigma.

Similarly, there was a high degree of optimum knowledge of HCWs regarding tuberculosis. HCW knowledge was determined by age, job duration and qualifications. HCW practice towards those suspected of having tuberculosis was not satisfactory, with only 38.2% responding correctly as per the recommended guidelines.

The two most important sources of patient information about tuberculosis were their physicians and mass media (television). Education, training and supervision by the national tuberculosis control programme (NTP) had a good impact on the knowledge of tuberculosis among both patients and HCWs.

**Conclusion** Knowledge is not the only determinant of health-seeking behaviour and compliance to treatment among tuberculosis patients, as stigma was the main barrier to proper and timely health-seeking behaviour. The study found poor adherence of HCWs to NTP guidelines regarding tuberculosis suspects, and highlighted a major cause of low case detection in the community.

### Background

There is growing evidence that the psychosocial aspect of tuberculosis in patients and their families needs more attention. Problems in case detection and case holding could be solved not only through a clinical approach but also through community participation.

A study was carried out to evaluate the knowledge, attitudes and practice (KAP) of two sectors of the population involved in tuberculosis control: tuberculosis patients and health care workers (HCWs). The aim was to identify the factors leading to a better understanding of tuberculosis as a social and medical problem, with the ultimate goal of improving the quality of case detection and better treatment outcomes for tuberculosis patients in Iraq.

### Materials and methods

A cross-sectional study was carried out during a one-year period in which a random sample of 500 tuberculosis patients and 500 HCWs were enrolled from randomly selected primary health care (PHC) centres and interviewed according to a pre-tested questionnaire regarding their KAP concerning tuberculosis. The interviewees were selected from 250 out of 975 PHC centres (404 rural and 541 urban) by random sampling from each centre of 2 tuberculosis patients and 2 HCWs to be involved in the study.

Two questionnaires were designed, one for HCWs and the other for tuberculosis patients. Every question was rated and a total score obtained for patient

### Conclusions and implications of the study

■ Almost two-thirds of tuberculosis patients recorded optimum knowledge regarding the disease without significant difference between rural and urban areas. This is probably attributable to NTP, mass media and other educational activities that are delivered equitably across the country.

■ In spite of the relatively adequate knowledge of patients regarding tuberculosis, there was a high degree of stigma related to the disease (54.8%) and delayed health-seeking behaviour, indicating that a complexity of factors determines behaviour in addition to knowledge, such as culture and resources.

■ The results of this study provide baseline information upon which behavioural models would be built in order to modify the behavior of the community regarding health-seeking and treatment completion.

■ Health care workers had a high level of knowledge regarding tuberculosis that was not reflected on their practice. This could be attributed to the shortage of supplies during sanction, which hindered them from translating knowledge into practice.

and HCW KAP. The median value was used as a cut-off to categorise KAP into optimal or suboptimal.

### ■ Main study findings

Patients A majority of patients presented with cough (83.8%), followed by fever (56.6%), chest pain (44.4%) or hemoptysis (30.2%). Most patients recognized tuberculosis as a highly infectious (80.2%) but curable (90%) disease. The mode of transmission was reported to be through cough (61%), breathing (55.4%), spitting (26.8%), touch (20.6%), kissing (20.8%) or food (15.2%).

More than half the patients believed the disease would never be cured if treatment was interrupted, 27% believed the patient would die and only 3.6% that the patient would develop anti-tuberculosis drug resistance. Patients gained information from their physicians (27.5%), the mass media (23.2%) and their life experience (16%).

More than half the respondents attributed their delayed health-seeking behaviour to their fear of being diagnosed with tuberculosis, while 32.8% attributed it to their economic status and only 5.8% to ignorance or not being worried about their health. Advice was sought first from a traditional healer rather than health facilities, whether public or private, by 20.6% of respondents.

Almost all patients did not buy anti-tuberculosis drugs from private pharmacies and these recorded significantly higher levels of optimum knowledge. Only 43.6% reported encouraging family members to undergo tuberculosis investigation, and these recorded significantly higher levels of optimum knowledge (91.3%). First suspicion of tuberculosis was mainly raised by physicians (71.4%) and less frequently by families (10.8%).

HCWs The majority of HCWs (87.8%) had a diploma degree, while only 10.6% were graduates of secondary nursing school. Optimum knowledge of HCWs was significantly related to older age, longer job duration, and higher qualification. Almost all HCWs reported that active pulmonary tuberculosis is the most infectious type.

Most HCWs (97.5%) recognized that tuberculosis affects mainly the lungs, while afflictions to the kidneys (55.6%), bones (66.8%) and abdomen (50.4%) were also reported. Only 87.4% of HCWs mentioned that tuberculosis is caused by bacteria.

The mode of tuberculosis transmission was correctly answered (through the respiratory tract) by 491 (98.2%) of respondents, while 93% knew that tuberculosis is a health problem in Iraq. Continuous close contact with an infected person was the main risk factor for contracting infection mentioned by 418 (83.6%), while overcrowding (80.8%), humidity (68.4%) and under nutrition (67.8%) were also cited. Only 1.4% mentioned tuberculosis of "another organ"

and 0.4% reported contaminated food as major source of infection.

Almost all HCWs (466, 93.2%) mentioned that tuberculosis should be suspected in the presence of a cough for more than 3 weeks, while 71% mentioned hemoptysis and 70% night sweats. Half the HCWs mentioned loss of appetite, chest pain, loss of weight and general weakness as the most diagnostic symptoms for tuberculosis. Active pulmonary tuberculosis was defined correctly (patient with 2 or 3 sputum smear-positive results) by 78.8%, with only 19% reporting that active pulmonary tuberculosis could be detected only by chest X-ray. Case relapse was correctly defined (patient initially cured who returns back with sputum smear-positive) by 77.6%, and the duration of anti-tuberculosis treatment course for patients with newly diagnosed active pulmonary tuberculosis was correctly answered (6 months treatment) by 87%, although a few answered 9 (6.6%) or 2–3 months (4.4%). The need to investigate household contacts was mentioned by 90.2%.

Those HCWs (38.2%) who asked for three consecutive sputum smear examinations had significantly higher rates of optimum knowledge, while 76.4% referred suspects to specialists and 5.4% prescribed anti-tuberculosis drugs themselves. The need for daily supervision during the intensive phase (first 2 months) of anti-tuberculosis drug treatment was correctly mentioned by 87%.

### ■ Conclusions and recommendations

These results call for the need to organize an awareness programme to de-stigmatize the disease and for regular training of HCWs on the national tuberculosis control programme (NTP) guidelines.

# Tuberculosis

Private sector

Islamic Republic of Iran

Tuberculosis  
detection in private  
laboratories,  
Teheran, Islamic  
Republic of Iran

Islamic Republic of  
Iran  
Teheran

Study period:  
September 2002–  
June 2004

Small Grants  
Scheme  
(SGS) 2002 No. 85

Principal Investigator  
Dr M Reza Masjedi  
National Research Institute  
of Tuberculosis and Lung  
Diseases  
Teheran, Islamic Republic  
of Iran.  
e-mail: mrmasjedi@nritld.ac.ir

## Abstract

A study investigated the participation of the private sector in detection, diagnosis and management of suspected tuberculosis cases. All cases referred to private laboratories for acid-fast bacilli examination during 2003 were enrolled and the total number of referred cases and the positivity rate were calculated. Positive cases were followed-up to confirm registration with the Ministry of Health and Medical Education.

**Results** A total of 9037 cases were enrolled in the study, 44.9% females and 55.1% males. The age of most referred patients was 15–25 years and > 65 years, and the majority were Iranian (98.6%). In total, 637 cases had positive results (7.1%), of which 531 (5.9%) cases were direct smear positive and 489 (5.4%) culture positive. Only a small proportion of cases were registered with the Ministry of Health and Medical Education, indicating that greater cooperation should exist between the public and private sectors in the detection and management of tuberculosis cases.

**Conclusion** A larger than expected proportion of tuberculosis patients are detected and managed by the private sector, indicating that closer cooperation should be developed between the public and private sectors.

## Background

In many high tuberculosis burden countries a large proportion of case detection and treatment is performed by the private sector [1]. This sector includes physicians, pharmacists, nurses and, in particular, laboratories which have an essential role in the process. Usually cases detected by this sector are treated within the sector and are therefore not registered with the national tuberculosis programme.

Unfortunately, the national tuberculosis programme has no effective mechanism for observing private sector activities in tuberculosis detection and treatment. It is assumed that the difference between estimated and registered cases is due to two factors: cases that have not been diagnosed and cases that have been diagnosed but not reported.

To investigate this issue, private laboratories were selected as a proxy for evaluating tuberculosis detection in the private sector. The study also followed-up those cases with positive results reported by the private sector to evaluate collaboration with the public health system in the registration and treatment of tuberculosis patients.

## Materials and methods

All laboratories involved in the detection of acid-fast bacilli in the urban area of Teheran municipality were included in the study (Pasteur, Danesh, Zarifi, Ghazi Saeed). All cases referred to these 4 laboratories for acid-fast bacilli detection during 2003 were enrolled in the study. Initially, the laboratory manager and staff were interviewed to estimate the approximate rate of specimen referral to their laboratories for acid-fast bacilli detection, and to estimate the expected rate of admission. Each laboratory was also evaluated by looking at previously performed quality control results or by observing methods of examination and

## Conclusions and implications of the study

■ During the study period, 9037 cases were referred to private laboratories in Teheran, compared to the 9479 cases referred to government laboratories. Therefore, private laboratories performing acid-fast bacilli examination play a major role in tuberculosis diagnosis in Teheran.

■ The positivity rate for direct smear examination was 5.9%, compared to 2.2% reported by government laboratories. According to the reference laboratories, the quality of the four private laboratories under study was in accordance with the standards proposed by the Ministry of Health and Medical Education and the results are therefore highly reliable.

■ The majority of cases are not referred to, or are not registered by, the Ministry of Health and Medical Education, hence are missed from the National Tuberculosis Programme. Establishing means of collaboration between the private and public sector is therefore recommended.

other laboratory evaluation guideline-based criteria. These guidelines were established by the Islamic Republic of Iran reference laboratory for tuberculosis laboratory quality control. Subsequently, the participating laboratory personnel were instructed about the required information, method of data collection and inclusion criteria of the study. The questionnaire was presented and the staff were trained to gather information from patients or the referring laboratory. Each laboratory was contacted weekly to register the obtained data. The inclusion criteria were all specimens, including sputum, bronchoalveolar lavage, gastric lavage and tissue, referred for direct Ziehl-Neelson staining, culture in Lowenstein Jensen medium and antibiogram type specification. Thereafter, all cases with a positive direct smear culture or pathology results were registered for follow-up. The follow-up procedure included referral to the nearest health centre to the patient's residential address to confirm the registration of the case in the district tuberculosis registration notebook, and for unregistered cases, referral to the physician in charge of the patient to confirm that the patient is under treatment. The addresses were also checked to detect any new or different addresses. All unregistered cases (treated in the private sector or lost) were reported to the Ministry of Health and Medical Education as cases missing from the health system network.

### ■ Main study findings

A total of 9037 of suspected active tuberculosis cases referred to the 4 private laboratories in Teheran were enrolled in the study. They consisted of 4036 (44.9%) females and 4959 (55.1%) male, aged 1–99 years with two distinct peaks at 15–25 years and > 65 years. Regarding nationality, 8885 (98.6%) cases were Iranian and 123 (1.4%) were Afghan.

The main specimens referred were sputum (6670, 73.8%), bronchoalveolar lavage (888, 9.8%), urine (395, 4.4%), pleural fluid (301, 3.3%), and gastric washing (222, 2.5%). Examination results revealed 637 (7.1%) total (direct smear and culture) positive cases consisting of 311 (49%) female and 324 (51%) male cases. Of these, 598 (97.4%) were Iranian and 16 (2.6%) were Afghan.

Of the total positive results, 531 (5.9%) cases were direct smear positive. The rate of positive specimens (direct smear and/or culture) was significantly higher in the age groups 16–25 years and > 65 years, in females compared to males (7.7% and 6.5%, respectively), in Afghans compared to Iranians (13% versus 6.7%, respectively) and in September compared to other months of the year (11%).

Regarding the assessment of the registration of positive cases by the Ministry of Health and Medical Education, the study found that of the total positive

cases, 272 (42.7%) of the patients had stated their residential address. These were sent to the Ministry of Health and Medical Education for confirmation of registration. Of these, only 3 (0.007%) had been registered. The remaining 57.3% who did not provide a residential address were followed-up either by telephone or by contacting their physicians who were unwilling to give the requested information. However, contacting patients was highly informative: 58 patients were contacted, of whom 45 (77%) cooperated and only 5 (11%) stated that they were referred to the Ministry of Health and Medical Education for registration. The inability to contact 330 positive cases was mainly due to the lack of appropriate information and their inaccessibility. As a whole, a total of 8 (of 637 positive cases) were registered with the Ministry of Health and Medical Education, i.e. 1.2% of the total positive cases.

According to data given by the Ministry of Health and Medical Education, 9479 cases were referred to governmental laboratories in the Teheran urban area during the study period. Of these, 208 (2.2%) were positive, including 7850 (82.8%) Iranian and 1629 (17.2%) Afghan. Of the positive results, 164 (78.8%) were Iranian and 44 (21.2%) were Afghan, with no significant difference in the rate of positive results between the two nationalities (2.1% and 2.7%, respectively).

### ■ Conclusions and recommendations

A larger than expected proportion of tuberculosis patients are detected and managed by the private sector. The majority of positive cases are not referred to the Ministry of Health and Medical Education, or at least are not registered. While many of these cases can be assumed to be treated by the private sector after diagnosis, a considerable number may have left the treatment cycle and are therefore "missing" from the national tuberculosis programme. These alarming findings call for the urgent need to implement a programme ensuring collaboration between the private and public sectors to avoid "loosing" smear positive cases and hence achieving better tuberculosis control.

### ■ References

[1] Uplekar M et al. Tuberculosis patients and practitioners in private clinics in India. *The international journal of tuberculosis and lung disease*, 1998, 2(4):324–329.

# Tuberculosis

## Public-Private Mix

### Pakistan

Does providing free sputum microscopy service to general practitioners help in their case

notification to the national tuberculosis control programme?

Pakistan  
Karachi

Study period:  
May–October 2002

Small Grants  
Scheme  
(SGS) 2001 No. 61

Principal Investigator  
Dr Javid Khan  
Pulmonary Section  
Department of Medicine  
Agha Khan University  
Pakistan  
e-mail: javaid.khan@aku.edu

### Abstract

A study was carried out to assess the impact of providing free sputum microscopy services to private practitioners on their case notification to the national tuberculosis control programme. It also evaluated the practices of private practitioners in diagnosis and treatment of tuberculosis.

A pre-tested questionnaire was administered to all private practitioners working in a densely populated area of Karachi, Pakistan. For the first 3 months, the practitioners were asked to fill tuberculosis notification cards and their response was recorded. An incentive was then provided to the practitioners for the next three months in the form of free sputum microscopy. They were asked to fill sputum microscopy referral forms for this period.

**Results** A total of 103 private practitioners participated in the study. Provision of free sputum microscopy did not improve the tuberculosis notification rate in the community. Half the practitioners felt that provision of free sputum microscopy alone was not enough and it should be supplemented with free chest

radiographs and blood tests. Severe deficiencies existed in the diagnosis and treatment of tuberculosis by practitioners. Sputum microscopy was employed less often than chest radiography and tuberculin tests in the diagnosis of tuberculosis. Antituberculosis prescribing did not conform to WHO or national tuberculosis guidelines in a large majority of cases. Only 23% of patients were referred to a government tuberculosis centre and only 22% of practitioners kept a record of their tuberculosis patients.

**Conclusion** Private practitioners in Pakistan do not generally rely on sputum for diagnosis of tuberculosis. Therefore, provision of free microscopy did not lead to an improvement in tuberculosis case notification by the practitioners.

### Background

Pakistan has an extensive private health sector, and nearly 80% of tuberculosis patients initially report to private practitioners for their diagnosis and treatment.

Effective linkages between private practitioners and the provincial tuberculosis control programme have yet to develop along organized lines. Private practitioners are currently diagnosing and treating a significant proportion of tuberculosis patients in Karachi, which houses nearly a third of the population of Pakistan's southern province of Sindh.

In order to make the DOTS strategy more comprehensive, it is imperative to involve the private sector in tuberculosis control. A study to assess the impact of free sputum microscopy services on tuberculosis case notification by private practitioners to the national tuberculosis programme (NTP) was therefore conducted. The prescribing practices of the practitioners were also reviewed.

### Materials and methods

The study was conducted in Karachi between May and October 2002. A private sector hospital located in a densely populated area of the city was equipped

## Conclusions and implications of the study

■ The study failed to show any positive impact of the provision of free sputum microscopy on tuberculosis notification in the private sector. Private practitioners were not familiar with NTP or WHO tuberculosis management guidelines. They relied more on chest radiographs and tuberculin tests for tuberculosis diagnosis, and less on sputum microscopy. Therefore, the provision of free sputum microscopy was not seen as an incentive by these practitioners.

■ The majority of the practitioners prescribed an inappropriate combination of antituberculosis medications, particularly in the continuation phase. These inappropriate regimens may lead to an increase in the prevalence of multidrug resistant tuberculosis.

■ Only 22% of practitioners kept a record of their patients, which meant that tracing treatment defaulters was not possible in the community. The role of good record keeping is crucial to the success of an effective tuberculosis control programme.

■ This study was one of the few projects ever carried out in Pakistan in the context of public/private collaboration in tuberculosis control. However, given the current situation, the gaps in the knowledge and practices of private practitioners ought to be addressed first.

■ The situation warrants better collaborative efforts between private practitioners and public health services.



to provide sputum microscopy facilities. All medically qualified private practitioners practising in a radius of 2 km were identified. An attempt was made to develop linkages with the nearest government diagnostic and treatment centre.

Prior to the start of the study, all private practitioners in the designated area were invited to attend an awareness workshop on tuberculosis control. This was facilitated by investigators from the tuberculosis programme, including a provincial level tuberculosis control coordinator. All the practitioners were requested to complete a previously prepared self-administered questionnaire to collect information on the number of suspected tuberculosis patients seen every month and the diagnostic, treatment and referral practices of the doctors. All the practitioners present at the workshop completed and returned the questionnaire, while those unable to attend were contacted personally at their clinics and requested to fill the questionnaire. A total of 120 private practitioners completed the questionnaire. Anonymity was optional and confidentiality guaranteed. During the workshop, the private practitioners were oriented about the need to follow the national guidelines for the treatment and control of tuberculosis (NTP guidelines), and WHO recommendations for tuberculosis control.

Effective case management and follow-up using the DOTS strategy through a government diagnostic and treatment centre was planned for all cases. However, during the period of the study, no paramedics or other government outreach workers were available to provide DOTS at the nearby facility.

The study was in two stages, each lasting three months. In the first stage, practitioners were asked to fill tuberculosis notification cards on all suspected tuberculosis patients. These were collected every fortnight by a field assistant especially employed for this purpose. In the second stage, free sputum microscopy was provided at a local hospital. Practitioners were invited to a seminar to inform them about the availability of this service and to re-emphasize the importance of tuberculosis control and case notification. Those who did not participate were delivered letters by hand informing them about this service. They were again requested to fill tuberculosis notification cards on all suspected tuberculosis patients, which were collected every fortnight by the field officer.

At the end of the study, a questionnaire was sent to all the practitioners. They were asked to make recommendations for tuberculosis control in Pakistan. Those unable to fill any tuberculosis notification card were asked reasons for not doing so.

### ■ Main study findings

A total of 103 private practitioners participated in

the study. On average, each examined 4 to 5 tuberculosis patients per month. When diagnosing patients with tuberculosis, 70% felt confident in making a diagnosis themselves, 23% referred the tuberculosis suspects to a government tuberculosis centre and 7% to a specialist clinic. Following diagnosis, 50% preferred to treat tuberculosis patients themselves, 23% referred them to a government tuberculosis centre, 22% to a private consultant and 6% considered it appropriate to let patients decide where to be treated. Only 22% of practitioners kept a record of their tuberculosis patients.

In the first stage of the study, 51 (50%) of practitioners filled tuberculosis notification cards. In the second stage, after providing the incentive of free sputum microscopy, only 35 (34%) filled referral cards for sputum acid fast bacilli (AFB). Of 68 (66%) who did not fill referral cards, 32 felt they were too busy to fill cards, 5 did not agree with the idea of the referral card and 31 said that they did not see any patient during the three-month period. When asked for further suggestions to improve tuberculosis control, 52 (50%) felt provision of free sputum microscopy facility alone was not enough and that it should be supplemented with free chest radiographs and blood tests. Monetary incentives were suggested by a few and 18 (17%) suggested holding regular medical education programmes.

Questionnaire data was also used to evaluate the practice of private practitioners in diagnosing and treating tuberculosis patients. To establish diagnosis, chest radiography was used by 96%, tuberculin test by 63% and sputum microscopy by 48%, while 25% thought that clinical examination itself would suffice. When asked to write the prescription for a 60 kg man recently diagnosed with smear-positive pulmonary tuberculosis, 83% gave the 4-drug regimen of isoniazid, rifampicin, pyrazinamide and ethambutol for the initial phase. Of these, 80% used individual drugs, while 20% gave all 4 drugs in a fixed dose combination regimen. Incorrect regimens were given by 17% of physicians, including 2, 3 and 5-drug regimens, while 11 different regimens were prescribed for the continuation phase. Only 20 (21%) prescribed a correct regimen in accordance with the NTP guidelines.

### ■ Conclusions and recommendations

The study failed to show any positive impact of free sputum microscopy on tuberculosis notification in the private sector which relies mainly on chest radiography and tuberculin test rather than sputum microscopy for case diagnosis. Gaps in the knowledge and practices of private practitioners ought to be addressed first to establish collaboration between private practitioners.

# Tuberculosis

## Public-Private Mix

### Yemen

Involvement of the private sector in tuberculosis control in Lahej Governorate, Yemen

Yemen  
Lahej Governorate

Study period:  
September 2002–  
December 2003

Small Grants  
Scheme  
(SGS) 2002 No. 201

Principal Investigator  
Dr Abdel Nasser Ayyash  
Ahmed

Lahej Health Office  
National Tuberculosis  
Control Programme  
Lahej, Yemen  
e-mail:  
binalihm@yahoo.com

### Abstract

An intervention study was conducted among private health care workers delivering care to tuberculosis patients. The intervention consisted of: training private sector health care workers and providing them with free sputum microscopy and drugs; health education in both the public and private sector; and tight supervision by the national tuberculosis control programme (NTP) of the private sector.

**Results** The pre-intervention baseline survey revealed a lack of coordination between the private and public health sectors in tuberculosis control activities, and the nonadherence of the private sector to NTP guidelines for management of tuberculosis cases. Post-intervention evaluation showed that the intervention had a significant impact on the adherence of trained personnel to NTP guidelines in the diagnosis and treatment of tuberculosis cases.

There was a significant increase in the number of cases detected after the intervention (167 in the 2nd and 3rd quarters of 2003 compared to 136 for the same period in 2002). In addition, the treatment success rate significantly increased after the intervention (90% compared to 77% beforehand). There was also an improvement in other treatment outcome parameters such as death, failure and defaulter rates, but this was not statistically significant.

**Conclusion** The study reported a beneficial effect from involving private practitioners in tuberculosis control activities. Collaboration between private and public sectors in tuberculosis control activities has a positive impact on the number of tuberculosis cases detected and their treatment outcome.

### Background

Tuberculosis is a health problem in Yemen with an estimated incidence of smear positive cases of 48/100 000 population in 2001. However, the treatment success and case detection rates (75% and 40%, respectively, in 2001) are still below regional targets. Lahej Governorate is located in the south-west of Yemen and has a population of 850 000 and a surface area 11300 km<sup>2</sup>. It is divided into 14 districts; 30% of which are urban and the rest are rural. Public health facilities cover the district capitals and 60% of the rural areas.

Directly observed treatment, short course (DOTS) was implemented in Lahej in 1997 in the district capitals and then expanded to other district public health facilities. However, the detection rate of smear positive cases has increased and treatment outcomes are still sub-optimal due to stigma and because many cases live at far distances from public health facilities. A considerable proportion of patients are managed in the private health facilities in the district capitals and rural areas. Consequently, tuberculosis control cannot be achieved without the involvement of the private sector in control activities. A study was therefore undertaken to evaluate the impact of involvement of the private sector in tuberculosis control activities.

### Materials and methods

An intervention study was conducted among private health care workers delivering care to tuberculosis patients.

The study included a pre-intervention phase to collect baseline information

## Conclusions and implications of the study

- Involving private practitioners in tuberculosis control activities is essential for effective disease control. This can be achieved through the intervention evaluated by this study.
- The intervention consisted of: training private health care workers and providing them with free sputum microscopy and drugs; health education in both public and private sectors; and tight supervision by the national tuberculosis control programme (NTP) of the private sector.
- It is important that the provision of free sputum microscopy and drugs to the private sector should be conditional on their collaboration with the NTP, including referral of patients for confirmation of diagnosis, registration and follow-up, and the supervision of private sector control activities.
- The intervention had a significant impact on the adherence of the trained personnel to NTP guidelines, and on the number of tuberculosis cases detected and their treatment outcome.

during the 2nd and 3rd quarters of 2002. Cases detected during the last quarter of 2002 ( $n = 79$ ) were randomly allocated to either the public (29) or private (50) sector for management. The intervention consisted of: training private sector health care workers and providing them with free sputum microscopy and drugs; health education in both public and private sectors; and tight supervision of the private sector by the national tuberculosis control programme (NTP).

15 physicians were trained on the DOTS strategy, how to identify suspected tuberculosis cases, health education, tuberculosis treatment and follow-up of smear positive cases. 25 nurses were trained on the administration of free anti-tuberculosis drugs to patients under DOTS, registration, defaulter tracing and health education. 10 laboratory technicians were trained on sputum staining and reading, and registration of examined cases. The trained personnel was then placed under supervision by district tuberculosis coordinators and governorate tuberculosis coordinators throughout the first quarter of 2003. **Post-intervention evaluation** To evaluate the impact of the intervention on the trained personnel, they were placed under supervision by district tuberculosis coordinators during May–August 2003 and by governorate tuberculosis coordinators and governorate laboratory supervisors during June–September 2003. The impact of the intervention on tuberculosis control was assessed using 3 indicators: case detection rate, smear conversion rate and treatment outcome.

### ■ Main study findings

**Pre-intervention baseline survey** The survey found a lack of coordination between the private and public health sectors in tuberculosis control activities. In addition, it found that the private sector does not follow NTP guidelines for management of tuberculosis cases. The majority of health care workers in the private sector are not trained on tuberculosis control which leads to misdiagnosis and improper treatment of detected cases. Tuberculosis patients are not recorded by the private sector or referred to public health facilities. Moreover, as they are not subjected to follow-up, treatment results are not available.

Many patients in the private sector default from treatment due to the cost of purchasing anti-tuberculosis drugs, lack of supervision by the NTP on the private sector; and lack of tracing of defaulters, which was also deficient in the public sector. Many tuberculosis patients also default because of the distance of their residence from public health facilities. Inadequate health education was also observed in both sectors.

The number of cases detected and the smear conversion rates in public health facilities during the first three quarters of 2002 in Lahej Governorate were

collected. The number of detected cases ranged between 63–70 cases, and the smear conversion rate from 79%–81%. The treatment outcomes for cases detected in 2001 were a treatment success rate of 73%–76% and a defaulter rate of 13%–18%. These indicators did not exist for the private sector due to inadequate tuberculosis control activity. **Post-intervention** The intervention had a significant impact on the adherence of the trained personnel to NTP guidelines in the diagnosis and treatment of tuberculosis cases. There was a significant increase in the number of cases detected before and after the intervention (167 in the 2<sup>nd</sup> and 3<sup>rd</sup> quarters of 2003 compared to 136 for the same period in 2002). A significant increase in the number of detected cases can therefore be predicted for subsequent years. The prediction equation used is: number of cases detected in a particular year =  $78 + 29.5 \text{ year}$ , where the regression coefficient = 29.5. This is the increase in the number of cases detected per year in the 2nd and 3rd quarters if the intervention is sustained. This means that 226 cases will be detected during these 2 quarters if the intervention is sustained for 5 years. The treatment success rate was significantly higher after the intervention (90% compared to 77% beforehand). Additionally, there was an improvement in other treatment outcome parameters such as death, failure and defaulter rates, but this was not statistically significant.

### ■ Conclusions and recommendations

This study reported the beneficial effect of involving private practitioners in tuberculosis control activities. It is important that the provision of free sputum microscopy and drugs to the private sector should be conditional on their collaboration with the NTP, including the referral of patients for confirmation of diagnosis, registration and follow-up, and the supervision of private sector control activities.

# Vector Control

## Insecticide Resistance

### Egypt

Bioassays and biochemical assays for detection of insecticide resistance in field populations of *Culex pipiens* from Egypt

Egypt  
Qalubiya, Sharkiya and Assiut Governorates

Study period:  
August 2002–  
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Principal Investigator  
Dr Abdel Baset Zayed  
Zoology Department  
Faculty of Science  
El Azhar University  
Assiut, Egypt  
email:  
a\_zayed70@hotmail.com

### Abstract

Bioassays and biochemical assays were used to determine the mechanisms and levels of insecticide resistance in *Culex pipiens* in filariasis endemic areas of three governorates in Egypt. The susceptibility of different field populations of *Culex pipiens* adults and larvae was measured and compared to laboratory reared colonies.

**Results** Bioassay results of *Culex pipiens* larvae in Qalubiya showed susceptibility to malathion only and resistance to other insecticides. In Sharkiya, larvae were also susceptible to malathion, temephos and chlorpyrifos, but resistant to the remaining insecticides. In Assiut, the larvae population seemed to be resistant to all insecticides. Bioassay results of adult *Culex pipiens* populations in Qalubiya showed their susceptibility to most insecticides used except for DDT. The Sharkiya adult population was susceptible to all the pyrethroids tested, while resistant to other insecticides. The Assiut adult population was also susceptible to most pyrethroids and resistant to permethrin, organophosphate and carbamate insecticides, as well as DDT.

There were significantly higher levels of acetylcholinesterase in Qalubiya and Sharkiya adult and non specific esterase in the 3 larvae populations compared to the laboratory colony. A significantly higher level of glutathion S-transferase was observed in Sharkiya and Assiut adult populations compared to Qalubiya. The microplate assays of elevated nonspecific esterases and acetylcholinesterase confirmed

the bioassay results.

**Conclusion** The study reported the resistance of *Culex pipiens* to organophosphate insecticides. Microplate assay is a suitable, and simple method for the detection of insecticide resistance and can be used to confirm bioassay results.

### Background

Dose-mortality bioassays used to detect insecticide resistance require a big sample size of insects and do not efficiently detect resistance at low prevalence rates or estimate the frequency of resistance genes in populations. However, the Centers for Disease Control and Prevention (CDC) has developed a simple biochemical assay in which several detoxified enzymes can be detected from one single mosquito. The biochemical assay is based on the determination of elevated levels of these enzymes which would indicate tolerance or resistance to various insecticides. Using this biochemical assay, a study was conducted to determine the resistance status of *Culex pipiens*, the main vector for filariasis and arboviruses (such as the Rift Valley fever and West Nile fever viruses) to several insecticide groups.

### Materials and methods

*Culex pipiens* larvae were collected by standard methods from three different areas: Ezbit Zaki, Benha district, Qalubiya Governorate; El Adelia village, Sharkiya Governorate; Abo Khars village, Abo Teeg, Assiut Governorate. Mosquito larvae were transported to a laboratory and reared to adult stage. Emerged female mosquitoes were offered guinea pig blood meals and allowed to oviposit (F1). The hatched larvae and emerged adults were used in the bioassays and biochemical assays.

**Insecticide resistance detection methods** Susceptibility tests were

### Conclusions and implications of the study

■ The study reported resistance of *Culex pipiens* to organophosphate insecticides, possibly due to the extensive use of these compounds in agriculture and public health vector control in the last four decades in Egypt.

■ Environmental management and biological control techniques should be used to reduce the probability of developing Vector resistance to pesticides.

■ Biochemical assay is a simple technique that allows the detection of several detoxifying enzymes responsible for resistance to insecticides using a single mosquito. This technique can also be easily used to confirm the results of standard bioassays and to detect the particular mechanisms of resistance against various classes of insecticides.

performed using WHO test kits for measuring insecticide resistance. Bioassays were performed according to standard methodology. A portion of hatched larvae (F1) was used in larval bioassays and their susceptibility to malathion, temephos, bromophos, fenitrothion, fenthion and chlorpyrifos insecticides was studied using WHO recommended diagnostic doses. Four replicates, each with 25 larvae, were used in each bioassay test and the percentage mortality was counted after 24 hours of exposure.

Adult bioassay was carried out using 1–3 days-old unfed females of the first generation. Insecticide impregnated papers with WHO recommended diagnostic doses of test insecticides were used. The latter included permethrin, deltamethrin, cyfluthrin, lambdacyhalothrin, malathion, fenitrothion, propoxur, bendiocarb and DDT. For each bioassay test four replicates with a total of 74 to 127 unfed females were used. Exposure time was one hour for all insecticides and percentage mortality was measured after 24 hours of exposure. Biochemical assay was performed using the CDC method to detect insecticide resistance by measuring the titre and frequency of detoxifying enzymes such as esterase, altered acetylcholinesterase, glutathion S-transferase and the oxidase content of individual adult mosquitoes.

### ■ Main study findings

Bioassays In Qalubiya, *Culex pipiens* larvae were susceptible to malathion only and resistant to all other insecticides. In Sharkiya, larvae were also susceptible to malathion, temephos and chlorpyrifos but resistant to the remaining insecticides used. The Assiut larvae population seemed to be resistant to all insecticides used, with intermediate levels of resistance to malathion, temephos and chlorpyrifos.

Adult Qalubiya population was susceptible to most insecticides used except DDT. The Sharkiya adult population was susceptible to all the pyrethroids tested (cyfluthrin, deltamethrin, lambdacyhalothrin and permethrin) while resistant to other insecticides. Similarly, the Assiut adult population was susceptible to most pyrethroid insecticides used (cyfluthrin, deltamethrin and lambdacyhalothrin) and resistant to only permethrin. However, this population was resistant to organophosphate and carbamate insecticides, as well as DDT. The obtained data on adult knock down were similar to the susceptibility status as measured by percentage mortality, particularly for pyrethroids. In general, the Qalubiya adult population showed more resistance to pyrethroid insecticides and the ability to knock down this population was significantly less than that of the other two populations.

**Biochemical assay** The levels of elevated nonspecific esterase showed significant differences between the colony strain and the three field larvae populations.

There were significantly higher levels of acetylcholinesterase in the Qalubiya and Sharkiya adult populations compared to the laboratory colony (23.9% and 4.4% excess enzyme levels for each population, respectively). A significantly higher level of glutathion S-transferase was observed in Sharkiya and Assiut compared to Qalubiya adult populations. Mosquitoes from both populations had levels exceeding those of the laboratory colony at 84.8% and 50%, respectively.

Insensitive acetylcholinesterase is an indicator of resistance to organophosphates and carbamate insecticides, while elevated nonspecific esterases indicate resistance to organophosphates, carbamates and pyrethroids. Elevated levels of glutathion S-transferase indicate DDT and organophosphate resistance. The high titre of the elevated nonspecific esterase and insensitive acetylcholinesterase in the Qalubiya larvae population may explain resistance to bromophos and fenthion. The microplate assays of elevated nonspecific esterases and acetylcholinesterase confirmed bioassay results.

The bioassay results indicated that the three examined populations were resistant to DDT. Previous results have shown that glutathion S-transferase is the major detoxifying enzyme responsible for DDT resistance in many insects. However, the levels of this enzyme were high in only two larvae populations from Sharkiya and Assiut. This could be explained by a different mechanism of resistance in Qalubiya or the existence of a mechanism that was not detected by the assay method.

### ■ Conclusions and recommendations

The results obtained from microplate assays confirm those of standard bioassays regarding resistance of *Culex pipiens* to organophosphate insecticides, possibly due to the extensive use of these compounds in agriculture and vector control over the last four decades in Egypt. It is therefore recommended to withhold using organophosphate insecticides for mosquito control in Egypt. Environmental management and biological control techniques should be used whenever possible.

Microplate assay is a suitable and simple technique and has the capacity to detect several detoxifying enzymes, which may be responsible for resistance to insecticides. This technique can also be used to confirm the results of ordinary bioassays and to detect the particular mechanisms of resistance used against the various classes of insecticides.

# List of Publications

## Articles originating from the small grants scheme supported projects during the period 1992–2002

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51. Parvez SD and Al-Wahaibi SS. Comparison of three larviciding options for malaria vector control.
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Abdul Razzak Al Sanhoury St. P.O. Box 7608 Nasr City, Cairo 11371, Egypt.  
Tel.: (202) 670 25 35 Fax.: (202) 670 24 92/4  
E-mail: [tdr@emro.who.int](mailto:tdr@emro.who.int) Web Site: [www.emro.who.int/tdr](http://www.emro.who.int/tdr)