Helminthic infections associated with the use of raw wastewater for agricultural purposes in Beni Mellal, Morocco

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عدوى الديدان الناجمة عن استعمال المخلفات السائلة الخام للأغراض الزراعية في بني ملاّل بالمغرب

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خلاصة: أردنا في هذا البحث أن نحدِّد المخاطر المحتملة التي تنجم عن استعمال المخلفات السائلة غير المعالجة لأغراض زراعية، وبصفة خاصة انتقال عدوى الديدان بين الأطفال. فاخترنا عينة عشوائية مكوَّنة من 1343 طفلاً، جاء 740 طفلاً منهم من خمس مناطق تستعمل المخلفات السائلة في أعمال الزراعة، بينما اختير 603 طفلاً من أربع مناطق شاهدة لا تستعمل فيها هذه المياه. فتبيَّن أن معدل انتشار عدوى الديدان كان أعلى بدرجة يعتد بها إحصائياً بين أطفال المناطق التي تستعمل المخلفات السائلة وذلك بالمقارنة بالمناطق الشاهدة. ووجد ارتباط بين الخصائص السلوكية وإمدادات المياه العامة وبين معدلات العدوى المرتفعة. ونوصي بشدة بتوفير المعالجة الكافية للمخلفات السائلة، إلى جانب التثقيف الصحي للجمهور.

ABSTRACT We aimed to determine the possible risks associated with raw wastewater use for agricultural purposes, particularly the transmission of helminthic infections among children. In a randomly-selected sample of 1343 children, 740 of them were from five regions using raw wastewater for agriculture, while 603 were from four control regions that do not use such water. The prevalence of helminthic infection was found to be significantly higher among children in regions using wastewater, compared to control regions. Behavioural characteristics and public water supply were found to be associated with higher infection rates. Adequate treatment of wastewater as well as public health education are highly recommended.

Infections à helminthes associées à l'utilisation des eaux usées brutes dans l'agriculture à Beni Mellal (Maroc)

RESUME Notre but était de déterminer les risques éventuels associés à l'utilisation des eaux usées brutes dans l'agriculture, en particulier la transmission d'infections à helminthes chez les enfants. Dans un échantillon randomisé de 1343 enfants, 740 provenaient de cinq régions où l'on utilise les eaux usées dans l'agriculture, tandis que 603 venaient de quatre régions témoins où ces eaux ne sont pas utilisées. On a constaté que la prévalence des helminthiases était significativement plus élevée chez les enfants des régions utilisant les eaux usées par rapport aux régions témoins. Les caractéristiques comportementales et l'approvisionnement public en eau étaient associés à des taux d'infestation plus élevés. Un traitement approprié des eaux usées ainsi qu'une action d'éducation sanitaire sont vivement recommandés.

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Received: 27/06/99; accepted: 05/08/99

Introduction

The use of wastewater for irrigation in agriculture has been practised for many years in arid zones of industrialized as well as developing countries. This has been dictated by several factors, some of which are: the decreasing availability of water resources for irrigation resulting from increasing demand for potable water in urban agglomerations; the high cost of artificial fertilizers, and the realization that nutrients in wastewater can increase crop production; and social acceptance of the practice [*1*].

In Morocco, a country with semi-arid to arid climate, the volume of wastewater generated was 370×10^6 m^3 in 1990, and is expected to reach 500×10^6 m^3 by the year 2000 and 900×10^6 m^3 by the year 2010. In 1993, a national survey revealed that more than 7200 hectares were irrigated with raw wastewater in the vicinity of urban centres for the production of cereals, fruits and vegetables.

Wastewater reuse may lead to public health risks of transmission of enteric diseases following consumption of raw vegetables. It may also result in the transmission of endemic parasitic diseases that mostly strike children [*2*]. A World Bank report of 1986 indicated that the main public health risk associated with wastewater reuse in agriculture was due to the waterborne and foodborne transmission of helminths, particularly nematodes [*3*]. The World Health Organization (WHO) has pointed to the need to study the transmission of intestinal parasites in children living in areas where wastewater reuse is practised [*4*]. A literature search indicated that there are few well designed epidemiological studies and there was a need for further studies, especially those that include control groups that use unnpolluted water [*5*].

The objectives of our epidemiological study were to assess the possible impact of raw wastewater use in agriculture on the occurrence of intestinal parasitic diseases among children living in such areas, and the possible influence of other risk factors on this important public health problem.

Subjects and methods

Beni Mellal is located in central Morocco and has a semi-arid to arid climate. The climate is characterized by temperate winters, hot and dry summers, and a mean rainfall of 350–400 mm. The urban area has a population of 210 000; it produces 15 500 m^3 of wastewater per day. Untreated wastewater is used to irrigate approximately 600 hectares of agricultural land.

As regards parasitic helminths, data on occurrence of these diseases among schoolchildren are generally representative of the situation in the community as a whole [*6*]. Therefore, our study focused on children attending primary schools. They are the most suitable study group because schools are easily accessible, and disease occurrence is usually highest among this age group. The data collected on this age group may be used to assess the health of children and the general community. We surveyed 740 schoolchildren from five schools in an area where wastewater reuse for agriculture is practised. A control group consisted of 603 children from five schools located in an area that uses unpolluted water for irrigation. Demographic, hygiene and behaviour-contact data were collected for all children using a specially designed questionnaire.

Faecal samples were collected as follows. Children were given sterile flasks the day before sampling of faeces. They were instructed on the proper way to introduce the faeces inside the flask. For very young children, the parents were given the instructions instead. Fresh faeces were obtained in the morning and brought back to school where they were collected by the researchers.

The concentration technique used was the formaldehyde–ether method recommended by WHO [7]. This method is suitable for concentrating both helminthic ova and larvae, as well as protozoan cysts. One gram of faeces was suspended in 10 mL of 10% formaldehyde solution and mixed with a glass rod. The suspension was passed through a funnel covered with a gauze pad into a centrifuge tube. Then 3 mL of ether were added and the suspension was mixed for 1 minute. The tubes were centrifuged for 1 minute at 4000 RPM. After discarding the supernatant, the sediment was examined by sampling a drop with a Pasteur pipette and depositing it on a glass slide. The ova were identified according to the key proposed by the WHO [7].

The data were analysed using *STATITCF*. Frequency distribution tables were constructed. Percentage prevalence and intensity of infection (expressed as number of eggs per gram of faeces) attributed to different intestinal helminthic infections were estimated using standard formulae suggested by WHO [*7,8*]. Statistical significance tests (chi-squared and Fisher exact tests) were used, as appropriate, to test the possible effect of exposure to wastewater use for irrigation, as well as selected demographic, behavioural and hygiene variables on the occurrence of single and mixed intestinal helminthic infections among children in the study [*9*]. The 5% level of significance was used.

Results

In both the exposed and the control areas, the children's age range was 7–14 years, with a mean age of 9.3 years in the exposed area and 10.1 years in the control area. A study of the sex ratio did not show any significant differences between the two areas; the sex ratio was 1.3 in the exposed area compared to 1.4 in the control area.

Table 1 shows the prevalence of intestinal helminthic infections caused by five parasites (*Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Hymenolepis nana* and *Taenia saginata*). In the

Table 1 **Percentage prevalence of intestinal helminthic infections among children by exposure, Beni Mellal, Morocco, 1998**

Helminth	Exposed group (n = 740)	Non-exposed group (n = 603)	χ^2	P-value
Ascaris lumbricoides	20.5	3.8	82.02	< 0.001
Trichuris trichiura	0.4	0.3	(1.00)	> 0.05
Enterobius vermicularis	5.2	1.0	18.75	< 0.001
Hymenolepis nana	7.2	0.6	33.80	< 0.001
Taenia saginata	0.5	0.0	(0.13)	> 0.05
Total	30.8	5.6	134.07	< 0.001

Values between brackets are calculated using the two-tailed Fisher exact test.

areas of wastewater use, 30.8% of the children were infected with helminthic parasites, as compared to only 5.6% among children living in the control areas. The difference between the two areas was highly significant ($P < 0.001$). The highest infection was due to *A. lumbricoides*, while the lowest was due to *T. trichiuria*. For *A. lumbricoides*, *H. nana* and *E. vermicularis*, the increase in prevalence in the areas of wastewater use was significantly higher, but this was not the case with *T. trichiura* and *Taenia saginata*.

Children in the study sample suffered low-intensity infection with *A. lumbricoides* and *T. trichura*. The parasite load of *Ascaris* infection, as expressed by number of eggs per gram of faeces (epg), was much higher among children living in wastewater-exposed areas (18.3 epg) versus unexposed areas (2.3 epg); but the load of *Trichuris* infection in the two areas was similar (0.09 epg and 0.07 epg respectively). We compared these loads with the thresholds established by WHO [*8*]. It is clear that for the two parasites (*A. lumbricoides* and *T. trichiura*), the infections can be classified as light-intensity infections (< 5000 epg and < 1000 epg respectively).

While mixed infections by two or three parasites was observed among 2.9% of the wastewater-exposed children, none occurred among children living in the control area. Among the children infected by two or three parasites, 40.9% were infected by *A. lumbricoides/E. vermicularis*, 27.3% by *A. lumbricoides/H. nana*, 18.2% by *E. vermicularis/H. nana*, 9.1% by *A. lumbricoides/E. vermicularis/H.nana* and 4.5% by *T. trichiura/E. vermicularis* (Table 2).

In the area exposed to wastewater irrigation, *A. lumbricoides*, *T. trichiura*, *E. vermicularis*, *H. nana*, and *Taenia saginata* affected 21.1%, 0.4%, 6.0%, 8.5%, and 2.5% of boys, and 19.7%, 0.3%, 4.2%, 5.5%, and 0.1% of girls respectively. No statistically significant sex difference was found, however, except for *Taenia saginata* (Table 3). Similarly, other demographic variables such as age, family size, and educational level and profession of the parents did not have any statistically significant impact on the occurrence of intestinal parasitic infection in the children studied, as shown in Table 3.

The three hygiene factors under study included: the source of drinking water, the presence of a toilet (latrine) at home and hand-washing. As regards the water source, about two-thirds of the families used private wells inside their homes, while the other third used public wells and fountains. No statistically significant difference was found between the two water sources, except for the prevalence of infection by *A. lumbricoides*. As regards the other two factors, hand-washing before meals and

Table 2 **Multiple (mixed) intestinal parasitic infections among children exposed to wastewater irrigation, Beni Mellal, Morocco, 1998**

Parasites causing mixed infection	Exposed group No.	%
Enterobius vermicularis/ Hymenolepis nana	4	18.2
Ascaris lumbricoides/ E. vermicularis	9	40.9
A. lumbricoides/ H. nana	6	27.3
Trichuris trichiura/ E. vermicularis	1	4.5
A. lumbricoides/ E. vermicularis/H. nana	2	9.1
Total	22	100

Table 3 **Percentage prevalence of intestinal helminthic infections by selected demographic variables among exposed children, Beni Mellal, Morocco, 1998**

Demographic variable	*Ascaris lumbricoides*	*Trichuris trichiura*	*Enterobius vermicularis*	*Hymenolepis nana*	*Taenia saginata*
Sex					
Male (*n* = 431)	21.1	0.4	6.0	8.5	2.5
Female (*n* = 309)	19.7	0.3	4.2	5.5	0.1
χ^2	0.21	(1.00)	1.20	2.53	6.36
P-value	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05
Age (years)					
7 (*n* = 136)	19.1	0.7	8.0	8.0	0.7
8 (*n* = 170)	22.9	0.5	4.7	5.8	1.1
9 (*n* = 119)	27.7	0.0	5.0	5.0	0.0
10 (*n* = 103)	21.3	0.0	6.7	7.7	0.0
11 (*n* = 94)	19.1	1.0	4.2	9.5	1.0
12 (*n* = 81)	17.2	0.0	1.2	7.4	0.0
13–14 (*n* = 37)	0.0	0.0	5.4	10.8	0.0
χ^2	12.18	NV	5.60	2.95	NV
P-value	> 0.05		> 0.05	> 0.05	
Family size					
Small (*n* = 75)	22.6	0.0	2.6	10.6	0.0
Intermediate (*n* = 398)	21.1	0.5	4.0	6.5	0.5
Large (*n* = 267)	19.1	0.3	7.8	7.4	0.7
χ^2	0.62	NV	5.87	1.62	NV
P-value	> 0.05		> 0.05	> 0.05	
Father's education					
Illiterate (*n* = 319)	20.3	0.3	5.9	5.6	0.9
Educated (*n* = 421)	20.6	0.4	4.7	8.5	0.2
χ^2	0.01	0.06	0.53	2.27	0.62
P-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Mother's education					
Illiterate (*n* = 639)	20.5	0.3	5.3	7.3	0.6
Educated (*n* = 101)	20.7	0.9	4.9	6.9	0.0
χ^2	0.00	(0.36)	0.02	0.02	(1.00)
P-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Father's occupation					
Farmer (*n* = 600)	20.6	0.5	5.0	7.8	0.6
Other (*n* = 140)	20.0	0.0	6.4	5.0	0.0
χ^2	0.03	(1.00)	0.46	1.35	(1.00)
P-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Mother's occupation					
Farmer (*n* = 38)	18.4	0.0	5.2	7.8	0.0
Housewife (*n* = 702)	20.6	0.4	5.2	7.2	0.5
χ^2	0.11	(1.00)	0.00	0.02	(1.00)
P-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Values between brackets are calculated using the two-tailed Fisher exact test. *NV = not valid*

Table 4 **Percentage prevalence of intestinal helminthic infections according to selected variables of sanitation among exposed children, Beni Mellal, Morocco, 1998**

Hygiene variable	*Ascaris lumbricoides*	*Trichuris trichiura*	*Enterobius vermicularis*	*Hymenolepis nana*	*Taenia saginata*
Source of water					
Private ($n = 495$)	17.1	0.6	5.4	8.4	0.2
Public ($n = 245$)	27.3	0.0	4.8	4.8	1.2
χ^2	10.40	(0.55)	0.10	3.12	(0.11)
P-value	< 0.01*	> 0.05	> 0.05	> 0.05	> 0.05
Toilet at home					
Yes ($n = 266$)	16.5	0.4	5.9	5.9	0.4
No ($n = 474$)	24.0	0.3	4.1	9.7	0.7
χ^2	3.15	(1.00)	1.07	3.77	(1.00)
P-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Hand-washing					
Yes ($n = 24$)	12.5	0.0	4.1	4.1	0.0
No ($n = 716$)	20.8	0.4	5.3	7.4	0.5
χ^2	0.98	(1.00)	0.06	0.36	(1.00)
P-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Values between brackets are calculated using the two-tailed Fisher exact test.
**Significant*

the presence of a toilet at home had no statistically significant effect on the prevalence of intestinal helminthic infection (Table 4).

Only 37 out of 740 children were found to have been in contact with wastewater. Among these children, 35.1% were infected with *A. lumbricoides*, as compared to 19.7% of those that had not been in contact with wastewater ($P < 0.05$). As regards infections with the other four parasites, no statistically significant differences were observed between the two groups (Table 5). Concerning the contact of children with wastewater-irrigated soils, there was a significant increase in infections caused by *A. lumbricoides* ($P < 0.001$), *E. vermicularis* ($P < 0.05$) and *H. nana* ($P < 0.001$) (Table 5).

Discussion

Epidemiological studies are important for identifying the etiologic factors that may play a role in risk assessment and in decision-making. In our study, risk assessment was based on the determination of the excess risk associated with the reuse of wastewater for irrigation. To that end, two groups were compared; a group that was exposed to wastewater and another (control group) that was not exposed to wastewater. Differences in prevalence of infections between the exposed and control population provide a measurement of the risk associated with wastewater-irrigated agricultural crops.

Prevalence of ascariasis was five-fold higher in children living in areas where wastewater irrigation was used compared

Table 5 **Percentage prevalence of intestinal parasitic infections according to contact with wastewater and wastewater-irrigated land among exposed groups, Beni Mellal, Morocco, 1998**

Variable	*Ascaris lumbricoides*	*Trichuris trichiura*	*Enterobius vermicularis*	*Hymenolepis nana*	*Taenia saginata*
Wastewater					
Yes ($n = 37$)	35.1	0.0	0.0	2.7	0.0
No ($n = 703$)	19.7	0.4	5.5	7.5	0.5
χ^2	5.08	(1.00)	2.17	1.22	(1.00)
P-value	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Wastewater-irrigated land					
Yes ($n = 430$)	26.0	0.4	6.7	9.5	0.6
No ($n = 310$)	12.9	0.3	3.2	4.1	0.3
χ^2	19.07	(1.00)	4.47	7.60	(0.64)
P-value	< 0.001*	> 0.05	< 0.05	< 0.001*	> 0.05

Values between brackets are calculated using the two-tailed Fisher exact test.
**Significant*

to the control group. In a similar study carried out in India, it was found that 47% of sewage-farm workers were positive for ascariasis, as compared to only 13% for control groups. This difference was significant ($P < 0.01$) [*3*]. Another study reported that the prevalence of ascariasis was 3%, 16%, 30% and 8% among maintenance workers, wastewater treatment operators, wastewater irrigation workers and a control group respectively [*10*]. Srivastava and Pandey reported that 20.4% of sewage-farm workers were positive for *Ascaris* infection [*11*]. In Europe, the use of raw sewage for agricultural purposes has caused epidemics of ascariasis in the past [*12*]. At present, the risk of spreading ascariasis by sewage has been significantly reduced but the potential exists, if sewage is not properly treated [*12*]. Another example of epidemic spread of ascariasis by wastewater-irrigated vegetables has been reported in Jerusalem [*13*].

The factors responsible for the easy spread of ascariasis are: the simple life cycle of the parasite, with stages developing quickly; the high reproductive potential of the female *Ascaris* worm, which produces approximately 240 000 eggs per day, i.e. about 65 million eggs during its lifetime [*12*]; long survival time of *Ascaris* eggs in the environment; the high resistance of the eggs to physical and chemical agents, which makes the infective stages vulnerable to heat and dryness only [*10*]; and the few natural predators or embryo-destroying agents such as fungi [*14*].

In Morocco, several studies have shown that trichuriasis affects less than than 2% of the populations examined [*15,16*]. No case of trichuriasis was observed following an epidemiological study of 300 people in Beni Mellal, Morocco in 1996 [*17*]. Wastewater from the city of Beni Mellal was found to contain 8.8 *A. lumbricoides* eggs/L and only 3.4 *T. triciuria* eggs/L [*18*]. This could partially explain the lower prevalence of trichuriasis in our study of wastewater-exposed areas in Beni Mellal. Wastewater reuse for irrigation did not lead to an increase in the prevalence of trichuriasis in Chile [*5*] and similar results were obtained in India [*11*] and in Europe [*12*].

Among the helminthic infections, only two parasites have eggs that can be immediately infectious to man after being shed in the faeces. These are the pinworms (*E. vermicularis*) and the dwarf tapeworm (*H. nana*). All the other excreted helminths require a distinct latent period, either because their eggs must develop into an infectious stage in the environment outside the body, or because these parasites have one or more intermediate hosts through which they must pass in order to complete their life cycle [*3*]. WHO has reported that *E. vermicularis* and *H. nana* are generally transmitted directly from person to person, but they could persist long enough and present a health risk when wastewater is reused for agricultural purposes [*4*]. In our study, we noted that these infections were higher among children living in the wastewater-exposed areas.

As regards *Taenia saginata*, endemic infection is common under European conditions where indirect transmission is the predominant mode. This is largely attributable to sewage [*19*]. The eggs of all taenids are sensitive to desiccation and temperature. In temperate zones, such as those where the summer and winter air temperatures range from 10 °C to 20 °C, and −20 °C to −10 °C respectively, survival, as measured by infectivity, is in the order of 100–200 days and > 200 days respectively [*20*]. In India, only 0.9% of sewage-farm workers were positive for taeniasis [*11*]. Helminthic infections requiring an intermediate host can be prevented not only by improved excreta disposal practices, but also by proper cooking of beef meat [*10*]. In Beni Mellal, as in all Morocco, meat is generally well cooked, preventing the population from acquiring *Taenia saginata*.

We found that the prevalence of helminthic diseases was not influenced by factors such as sex, age, family size, and the educational level and profession of the parents of the children studied. Similarly, in India no significant differences between males and females among sewage-farm workers were noted [*11*].

WHO has noted that human behaviour may influence the prevalence and intensity of intestinal infections [*21*]. In our survey, we found that the major behavioural factors which played a role in disease transmission were: children playing in areas treated with raw wastewater; drinking water from public wells; and consuming crops irrigated with wastewater.

Acknowledgements

The authors cordially thank the Ministries of Public Health and National Education for their collaboration. We also wish to extend our deep thanks to all the staff at the Beni Mellal schools for their cooperation and support.

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In view of the regional water scarcity, the Regional Office and the WHO Regional Centre for Environmental Health Activities (CEHA), Jordan have paid special attention to water conservation and wastewater reuse. As part of the activities of the Inter-Agency Task Force (IATF) on Land and Water Resources, special focus is placed on wastewater reuse. As a follow-up to the recommendations of the IATF regional consultation on the use of low quality water for sustainable agriculture, held in December 1997, the Food and Agriculture Organization of the United Nations (FAO) prepared a draft manual for wastewater users on farms. The Regional Office and CEHA technically reviewed this manual and are providing a chapter on the health aspects. Also, the Regional Office and CEHA are collaborating with FAO on the preparation of an assessment report for the status of wastewater reuse and establishment of a wastewater network in the Region.

Source: The work of WHO in the Eastern Mediterranean Region. Annual Report of the Regional Director. 1 January–31 December 1998. *Page 88.*