Toxoplasmosis: the innocent suspect of pregnancy wastage in Duhok, Iraq

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داء المقوَّسات: المتهم البريء في ضياع الحمل في دهوك، العراق

علاء هاني رزاق، شونم أحمد ويس، علي يحيى سعيد

الخلاصة: بُغْيَةَ تحديد دور المقوَّسات في فقدان الأجنَّة، وفي السوابق الولادية السيئة، قام الباحثون باختبار 310 نساء، فقدت 77.4٪ منهن واحداً أو أكثر من الأجنة مع وجود بيِّنة على العدوى. وقد أُجريت الدراسة في دهوك، شمال العراق، من تموز/يوليو 2002 وحتى أيلول/سبتمبر 2003. وأُجري الفحص لجميع النسوة لتحرِّي الأضداد من الغلوبولينات المناعية M للمقوَّسات، باستخدام المعايرة الومضانية المناعية المرتبطة بالإنزيم، فوجد 3 نسوة فقط (0.97٪) إيجابيات الاختبار. كما أُجري على 187 امرأة اختبار تراص اللاتكس، فكان 55 منهن إيجابيات الاختبار. ثم أُجري التحليل الهيستولوجي المرضي على 9 حوامل من إيجابيات اختبار تراص اللاتكس، فلم توجد أيَّة بيِّنة على العدوى بالمقوَّسات. وتدلُّ هذه النتائج على أن مساهمة المقوَّسات في ضياع الأجنة في إقليمنا أمرٌ مبالغ به إلى حد كبير.

ABSTRACT To identify the true contribution of toxoplasmosis to fetal loss and bad obstetric history, we tested 310 women, 77.4% of whom had had single or multiple fetal loss, for evidence of infection. The study was conducted in Duhok, northern Iraq, from July 2002 till September 2003. All the women were examined for the presence of toxoplasma-specific IgM antibodies by enzyme-linked immunofluorescent assay; only 3 (0.97%) tested positive. We also tested 187 of the women by latex agglutination test; 55 tested positive. Histopathological examination was done for 9 pregnant women who tested positive by the latex agglutination test but we found no evidence of toxoplasma infection. The results indicate that the contribution of toxoplasmosis to fetal loss in our region is greatly overestimated.

La toxoplasmose : suspectée à tort dans les cas de grossesses interrompues à Duhok (Iraq)

RÉSUMÉ Afin d'identifier la contribution réelle de la toxoplasmose à la perte foetale et aux mauvais antécédents obstétricaux, nous avons effectué des tests chez 310 femmes, dont 77,4 % avaient eu une ou plusieurs perte(s) fœtale(s), à la recherche de preuves d'une infection. L'étude a été réalisée à Duhok (nord de l'Iraq) de juillet 2002 à septembre 2003. Toutes les femmes ont été examinées à la recherche d'anticorps IgM anti-toxoplasme par la méthode immuno-enzymatique ; seules 3 (0,97 %) avaient un test positif. Nous avons également réalisé le test d'agglutination au latex chez 187 femmes ; 55 avaient un test positif. Un examen histopathologique a été effectué pour 9 femmes enceintes qui avaient un test d'agglutination au latex positif mais nous n'avons trouvé aucune preuve d'infection à toxoplasme. Ces résultats indiquent que la contribution de la toxoplasmose à la perte fœtale dans notre région est largement surestimée.

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Introduction

Humans are hosts to over 70 species of protozoa. Our knowledge of parasitic infection extends into antiquity, and descriptions of parasites and parasitic infections are found in the earliest writings and have been demonstrated in archaeological material [*1*]. Toxoplasmosis, caused by *Toxoplasma gondii*, is found worldwide and is one of the most common parasitic infections of man and other warm-blooded animals. Nearly one third of humanity has been exposed to *T. gondii* [*2*]. This parasite is able to develop in a wide variety of vertebrate hosts but its definitive host is the house cat and certain other Felidae [*3*].

Most human infections are benign: in adults and children past the neonatal period, the disease is usually asymptomatic. Nevertheless, a generalized infection probably occurs. When symptoms are seen, they are most frequently mild and the disease picture simulates infectious mononucleosis [*3*]. This nearly totally benign picture differs in 3 situations: pregnant women, neonates and immunocompromised patients [*3–5*]. Among women infected during pregnancy, 40%–60% give birth to infected infants. The later in pregnancy that infection occurs, the more likely it is that the fetus will be infected but the less severe the illness will be [*6*].

Diagnosis of toxoplasma infection is seldom made by recovery of the parasite; usually it is done by serological tests, and for proper diagnosis the algorithm illustrated in Figure 1 should be followed [*3*].

Most gynaecologists working in the general hospital in Duhok, a small city in northern Iraq, consider toxoplasmosis a primary cause of cases with bad obstetric history. They rely on positive results of serum agglutination tests. Patients who test positive will be subjected to long-term and expensive therapy with anti-toxoplasma drugs, and because the antibody titre (detected via the latex agglutination test) does not come down for months (or even years) some gynaecologists extend the period of therapy for the whole pregnancy, thus subjecting pregnant women and their fetuses to the side-effects of these drugs. In addition, such long-term therapy exerts an economic burden on families who may already be financially overburdened. Antitoxoplasma drugs are prescribed in huge quantities in this region, to the extent that from 1 pharmacy in 1 year about 1500 packs of spiramycine were sold—the price of each pack of 20 tablets is about US$ 10 (personal communication). Nearly all the prescriptions were dependent upon a positive agglutination test.

Another factor that increased our interest in this subject was the widespread public view about the deleterious effects of toxoplasmosis which created a type of panic reaction in the population. We often had women coming to check for toxoplasma antibodies without a doctor's referral, without being pregnant and even before marriage.

For these reasons we aimed to identify the true contribution of toxoplasmosis to bad obstetric history by comparing the agglutination test, the enzyme-linked immunofluorescent assay for toxoplasma-specific IgM antibodies, and histopathological findings in the placenta. In our opinion, differentiation between active toxoplasmosis and past infection is important so that treatment can be limited to those having acute infection. This will definitely reduce the incidence of unnecessary therapy.

Methods

This study was carried out on 310 women who had been referred to the laboratory by a gynaecologist, most after testing positive

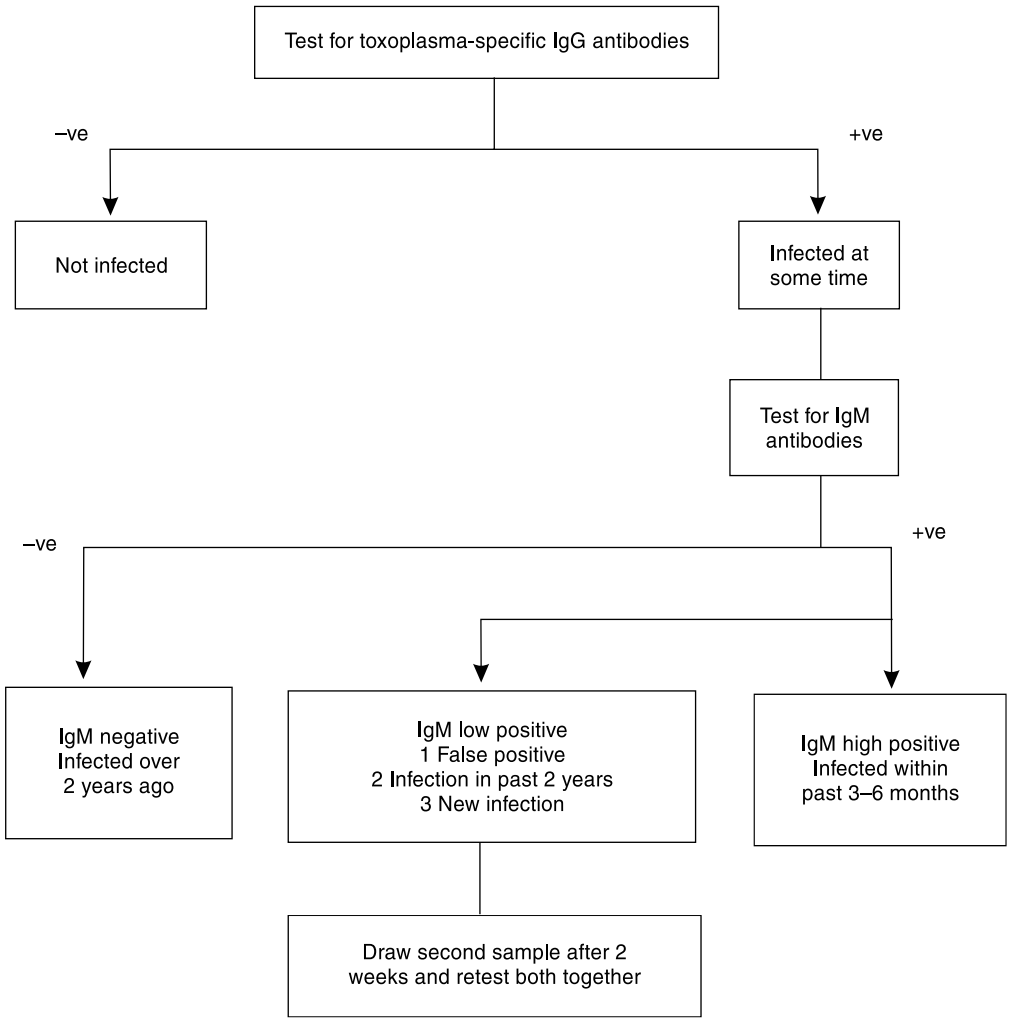

Figure 1 **Algorithm for testing for toxoplasma infection**

by the latex agglutination test. For every patient a full history was taken by a gynaecologist on our team covering age, gravidity, parity and the fate of every pregnancy, as well as the health of her live-born children. The study was conducted during the period July 2002–September 2003.

We carried out a latex agglutination test (Toxocell Latex, Biokit, Barcelona) for detection of antibodies to *T. gondii* on 187 patients (59.4%). The other 23 women refused to have the agglutination test, some because their gynaecologist had not requested it or had specifically requested immunofluorescent assay, others because it would be a trouble to them. The antibody titre was also estimated by serial serum dilution. For all the participants, specific anti-

toxoplasma IgM antibody testing was done using an enzyme-linked immunofluorescence technique (Vidas Toxo IgM, bioMerieux, Marcy-l'Etoile, France).

Histopathological examination of the placenta (after the delivery) was carried out for 9 pregnant women who tested positive for toxoplasma antibodies by the latex agglutination test. Five samples were taken from each placenta, 4 randomly and 1 near the cord attachment. These were fixed in 10% formalin and paraffin embedded sections produced. Then multiple sections were taken from each and the slides were stained with haematoxylin and eosin [*7*]. The slides were thoroughly and carefully examined for histopathological evidence of toxoplasmosis such as the presence of granuloma [*8,9*]. The slides were double-checked by a senior pathologist in 7 of the 9 cases.

Results

Three hundred and ten women participated in this study. Age range was 15–46 years (mean 26.9 years). The majority of the participants, 201 women (64.8%), were in their twenties; 12 were < 20 years, 90 were 30–39 years and 7 were ≥ 40 years.

There was no history of fetal loss in 70 (22.6%) of the women. Only 2 had had babies with congenital abnormalities (1 with congenital heart disease, the other with neural tube defect). At the time the tests were done, 185 (59.7%) were pregnant. Residence was rural for 82 women (26.5%); the others were from urban areas.

The toxoplasma agglutination test was done for 187 women (59.4%) and all tested positive, with titres ranging from 1:20 to 1:640 (Table 1). Enzyme-linked immunofluorescent assay to detect specific anti-toxoplasma IgM was done for all the participants. The results were positive for only 3 (1.0%). One of these had a history of fetal loss of an anencephalic baby and the other 2 gave a history of a single fetal loss.

Histopathological examination of the placenta was done for 9 patients. All of them gave a positive reaction in the latex agglutination test and all were negative for IgM using the enzyme-linked immunofluorescent technique. Histopathological evidence of toxoplasmosis infection was not detected in any of them (Figure 2).

Table 2 gives a summary of the results of the 3 different tests used.

Discussion

Toxoplasmosis acquires its importance for 2 reasons. First, it can cause fetal infection if it is acquired during pregnancy, with un-

Table 1 **Detection of *Toxoplasma gondii* antibodies by the latex agglutination test in 187 women**

Group	No. of women testing positive						Total
	Titre						
	1:20	1:40	1:80	1:160	1:320	1:640	
Pregnant (***n*** = 132)	30	40	50	6	5	1	132
Not pregnant (***n*** = 55)	10	20	17	5	2	1	55
Total	40	60	67	11	7	2	187

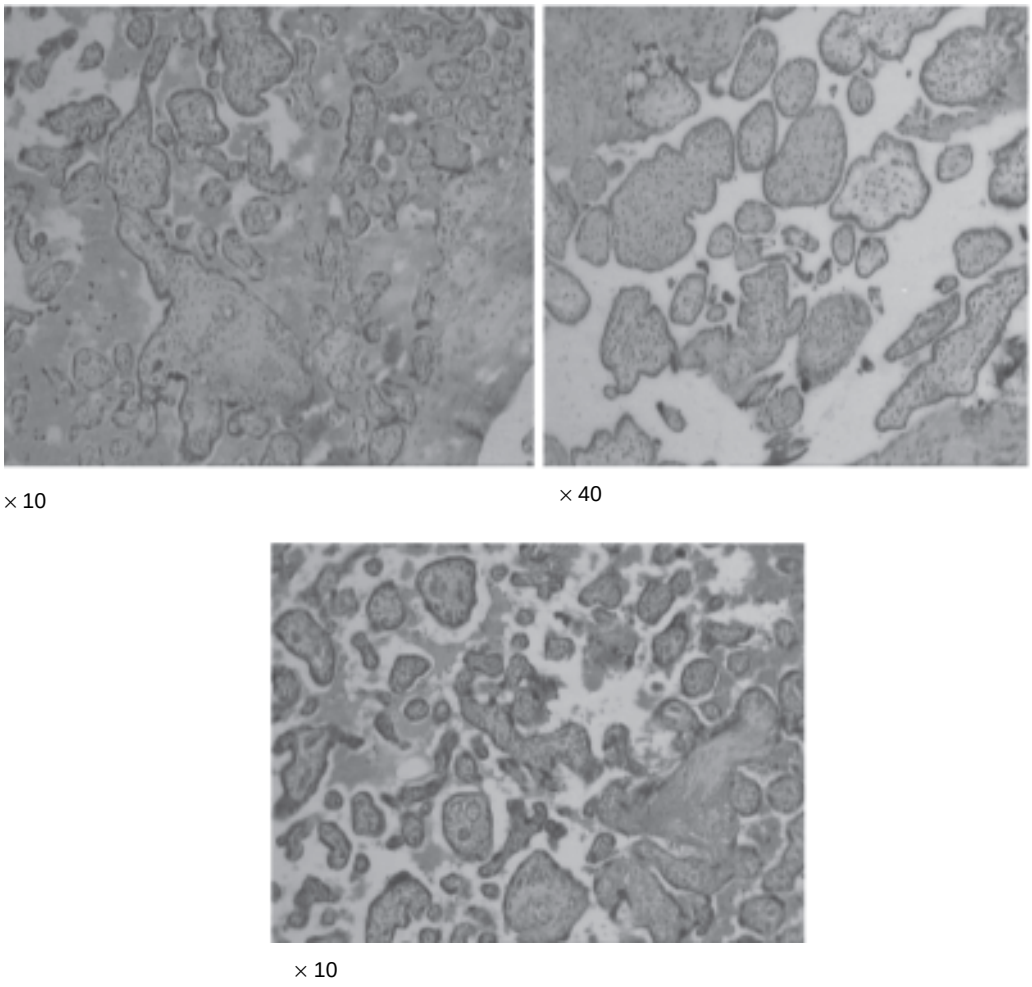

Figure 2 **Histopathological sections of placental specimens from three different patients who had a positive latex agglutination test (there was no histopathological evidence of toxoplasmosis in these sections)**

predictable manifestations in the fetus and neonate [*10,11*]. Second, it is an important cause of morbidity and mortality among immunocompromised patients [*12,13*]. It is not surprising, therefore, that there is great interest in this infection. Overall seropositivity for *T. gondii* among women of childbearing age has been reported as 20%–25% in the UK to 50%–60% in Belgium and 70%–80% in France [*14*]. The dilemma which arises is how to differentiate between past exposure to the parasite and acute active disease.

Despite the limitations, serum testing is still used for the diagnosis [*15*]. Commercial kits for detection of toxoplasma anti-

Table 2 **Summary of the results for three different tests for *Toxoplasma gondii* infection**

Patients	Latex agglutination test (*n* = 187)		Specific antitoxoplasma IgM ELIFA (*N* = 310)		Histopathological examination of the placenta[a] (*n* = 9)	
	Not tested	Positive	Negative	Positive	Not tested	Negative
Not pregnant (***n*** = 125)	70	55	122	3	–	–
Pregnant (***n*** = 185)	53	132	185	0	176	9

[a]Histopathological examination of the placenta was done after birth. All the 9 women had given birth to normal children.
ELIFA = enzyme-linked immunofluorescent antibody.

bodies are increasingly being used. In general, it is agreed that in most cases a positive IgG titre is sufficient to establish that a patient has been infected with *T. gondii*, but a negative IgM result virtually rules out a recently-acquired infection, unless sera are tested so early that an antibody response has not yet developed or is undetectable [*16,17*]. Thousands of studies have been conducted in different parts of the world on the detection of seropositivity and detection of active disease, utilizing different serological tests.

In our study, all 187 women tested by latex agglutination test were positive for *T. gondii* antibodies. This could be explained in 2 ways. First, seropositivity for toxoplasmosis is high in this region because it is an agricultural area and cats and other animals are usually kept in or near homes. Second (the more probable explanation), most of these women had already tested positive by the latex agglutination test and were then referred to the laboratory in which the study was conducted because it is the only available laboratory unit in the region in which the specific antitoxoplasma IgM antibody test is done using the enzyme-linked immunofluorescent technique.

High seropositivity has, however, been reported in this region of Iraq. Al-Doski studied 320 persons in Duhok province and found that 134 were positive by latex agglutination test [*18*]. Al-Sim'ani reported a seropositivity of 39.33% by the latex agglutination test and 45.33% by the indirect haemagglutination test in nearby Mosul province [*19*]. Despite this high seropositivity, only 3 women (1.0%) out of the 310 patients in our study were positive for IgM by enzyme-linked immunofluorescent assay. These results are very similar to the finding of Rai et al., who studied 345 pregnant Nepalese women and found an overall prevalence of 55.4% by the latex agglutination test; only 3.0% of the women testing positive had toxoplasma IgM antibodies by IgM enzyme-linked immunosorbent assay [*20*]. Similarly, Singh studied sera from 2343 patients who had had single or multiple fetal losses in the United Arab Emirates and he found that only 3 were positive for IgM from this very large sample [*21*]. Another study in the eastern region of Saudi Arabia confirmed a high prevalence of IgG antibodies (25%) but low prevalence of active disease [*22*]. Also in our study, histopathological examination of 9 placental

specimens from patients testing positive by the latex agglutination test failed to demonstrate any evidence of toxoplasmosis.

For all these reasons, we found it unwise to attribute most cases of abortion and bad obstetric history to toxoplasmosis while ignoring the long list of possible causes of abortion, of which genetic and chromosomal abnormalities alone are responsible for about half the cases [*14*].

In our opinion, there are 2 explanations for gynaecologists in this region attributing abortion and bad obstetric history to toxoplasmosis resultant on a positive latex agglutination test. There is a lack of availability of techniques to investigate other possible causes, particularly chromosomal and genetic analysis. Also, even when these investigations are done, many abortions remain obscure in their etiology, therefore attributing abortion to a particular disease—even on an infirm basis—will give a vindication to the gynaecologist and satisfy the patient in that "a cause has been identified".

We conclude from this study that the contribution of toxoplasmosis to bad obstetric history is greatly overestimated and the true prevalence of active toxoplasmosis is much lower than previously thought. This finding should direct gynaecologists to seek other causes of bad obstetric history.

Recommendations

- We strongly recommend the use of enzyme-linked immunofluorescent assay for detection of specific antitoxoplasma IgM antibodies and diagnosis of active toxoplasmosis.
- The latex agglutination test can be useful as a screening test but when the result is positive, it should be followed by a test for specific antitoxoplasma IgM antibodies.
- Treatment should be limited to those who are IgM positive.

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