

INVESTIGATION OF TOXOPLASMOSIS IN PREGNANT WOMEN AND SLAUGHTERED ANIMALS

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SUMMARY

A total of 21 women with complicated pregnancy (abortion, premature labor and genital disorders) together with 51 with normal pregnancy from Beni-Suef Governorate hospitals were investigated for detection of anti-toxoplasma antibodies level by ELISA (IgM & IgG). Tissues (diaphragm, masseter & thigh muscles) from slaughtered animals (60 cattle, 97 sheep and 26 camels) were collected from Beni-Suef abattoir for detection of *Toxoplasma* cyst (bradyzoites) using bio-assay in mice. The results showed that all controls (normal pregnancy) were IgM negative, however 19 (37.25%) were IgG positive. As to the women with complicated pregnancy 3/21 or 14.28% were IgM positive and 15/21 or 71.42% were IgG positive. The epidemiological survey revealed that the risk factors associated with *Toxoplasma* infection in humans were contact with cat, eating raw or undercooked meat and other uncertain factors.

Concerning meat, *Toxoplasma* cysts (bradyzoites) were detected only in sheep meat at a rate 15.46% but failed detection in bovine and camel meat. However, as *T. gondii* was principally detectable in sheep meat, the consumption of such meat harbors a potential infection risk for humans.

INTRODUCTION

Toxoplasmosis caused by the intracellular parasite *Toxoplasma gondii* is a zoonotic infection of cosmopolitan distribution. Serologic evidence indicates that human infections are common in many parts of the world (Zuber and Jacquier 1995). Although adult-acquired toxoplasmosis is usually mild to asymptomatic, the disease can be severe in the immunocompromised, leading to encephalitis (Franzen et al.1997). In addition, the influence of sex-and pregnancy-associated hormones on the severity of *T. gondii* infection is of particular public health interest due to the ability

of this parasite to cause congenital disease when infection occurs during pregnancy (Roberts et al. 2001).

Congenital infections usually result as a consequence of acute maternal infection. This infection can cause premature delivery, spontaneous abortion or stillbirth (Wong and Remington, 1994). Neonates may manifest chorioretinitis, hydrocephaly, microcephaly, cerebral calcification and psychomotor retardation (Kopecky et al. 2001). Most congenitally infected children will not exhibit any symptoms until later in life (Wong and Remington 1994).

Before conception *Toxoplasma* infected women do not transmit the infection to their fetuses later, with rare exceptions,. However after conception the infection can pass across the placenta to their fetuses.

Maternal infections early in pregnancy are less likely to be transmitted to the fetus than infections in late pregnancy, but early fetal infections, when they do occur, are more likely than later infections to be severe (Francis and Joynson, 1993).

Humans get infected either by consuming inappropriately prepared cyst-containing meat or by ingesting oocysts originating from cat feces (Wyss et al. 2000).

It has been reported that cattle are more resistant to toxoplasmosis than sheep. Congenital disease due to *T. gondii* infection is rarely reported in cattle whereas the parasite is a major cause of abortions and neonatal mortality in sheep (Ciamek Ghazae 2005).

It is believed that sheep remain chronically infected for the whole life. Undercooked meat from infected sheep is an important source of infection to man. In contrast cattle are thought to harbor fewer parasite cysts, which may not persist for the lifetime of the host. Therefore, cattle are believed to pose less risk for human (Esteban-Redondo et al. 1999).

Management of toxoplasmosis requires serological monitoring of infected individuals, as the organism is not readily available for culture (Krogstad,1985). Quantitative testing for the presence of *Toxoplasma* IgG can be useful to determine prior infection and indicate reactivation of the infection. Accurate diagnostic information is important, particularly during pregnancy, as treatment with spiramycin can reduce the risk to the fetus (Remington and McLeod, 1981).

Infected individuals with the *Toxoplasma* organism may not exhibit detectable levels of IgG antibody in the early stages of infection. IgG levels begin to rise 1 or 2 weeks after infection. Peak levels are reached in 6 to 8 weeks, then gradually decline over a period of months or even years.

Low levels of IgG are generally detectable for life. Detectable levels of IgM antibody appear immediately before or soon after the onset of symptoms (Krogstad, 1985). IgM levels normally decline within 4 to 6 months, but may persist at low levels for up to a year (Jeannel, 1990).

The aim of this work was to investigate toxoplasmosis among pregnant women (either normal pregnancy or complicated one) and to assess the risk of meat of slaughtered animals for toxoplasmosis.

MATERIAL AND METHODS

1-Women study groups:

Cases were selected among women attending the outpatient clinics and inpatient department of obstetrics in Beni- Suef Governorate hospitals. One blood sample was collected from 72 cases selected based on their history of pregnancy. The cases include 21 pregnant women suffering from complicated pregnancy (abortion, premature labor and genital & health disorders). Also 51 blood samples were collected from healthy women with a history of normal pregnancy attending the outpatient clinics for routine gynecologic checking. All women were interviewed using a structured questionnaire including the number of pregnancies, number of abortions in any, eating raw or undercooked meat, drinking unpasteurized milk and contact with cats or other domestic animals living

nearby.

2-Tissue samples from slaughtered animals

About 25 gm of fresh samples from each of diaphragm, masseter and thigh muscles were obtained from 60 cattle, 97sheep and 26 camels slaughtered at Beni Suef abattoir. The tissue samples from each animal were minced together and digested by pepsin-hydrochloric acid technique. The presence of *Toxoplasma* cyst in tissue samples was determined by bioassay in mice according to Dubey and Beattie, (1988). The inoculated mice were examined serologically by IHAT. Positive mice were killed 4 weeks post inoculation and impression smears from liver and brain were examined pathologically for the presence of tissue cysts.

3-Serological tests:

a. ELISA:

The sera obtained from women were tested for the presence of specific IgM and IgG antibodies to *Toxoplasma* using ELISA kit obtained from EUROGENETICS N.V. Transportstraat 4.3980 Tessenderlo, Belgium. The test was done according to Welch et al. (1980).

b-Indirect Haemagglutination Test (IHAT):

For examination of blood of the inoculated mice by the suspension of digested meat from different animals. The test was done according Janitschke, (1991).

RESULTS

Table (1): Positive results of ELISA for IgM and IgG antibodies for 72 pregnant women.

Trimester	Cases (n=21)*		Cases (n=51)**	
	Positive		Positive	
	IgM	IgG	IgM	IgG
First	1 (4.76%)	8 (38.95%)	0 (0%)	5 (9.8%)
Second	1 (4.76%)	5 (23.8%)	0 (0%)	6 (11.76%)
Third	1 (4.76%)	2 (9.52%)	0 (0%)	8 (15.68%)
Total	3 (14.28%)	15 (71.42%)	0 (0%)	19 (37.25%)

*Women with complicated pregnancy.

**Women with normal pregnancy.

Table (2): Risk factors associated with toxoplasmosis in the study groups according to the result of ELISA IgG.

Factor	Cases (n=15)	Cases (n=19)
Contact with cat	7 (46.66%)	6 (31.57%)
Consuming raw or under-cooked meat or raw milk	4 (26.66%)	3 (15.78%)
Other factors	2 (9.52%)	10 (52.63%)

Table (3): Results of IHAT for toxoplasma in inoculated mice by digested muscles of different animals.

Animal examined	Number of specimen	IHAT of inoculated mice
Cattle	60	0 (0.0%)
Sheep	97	15 (15.46%)
Camel	26	0 (0.0%)

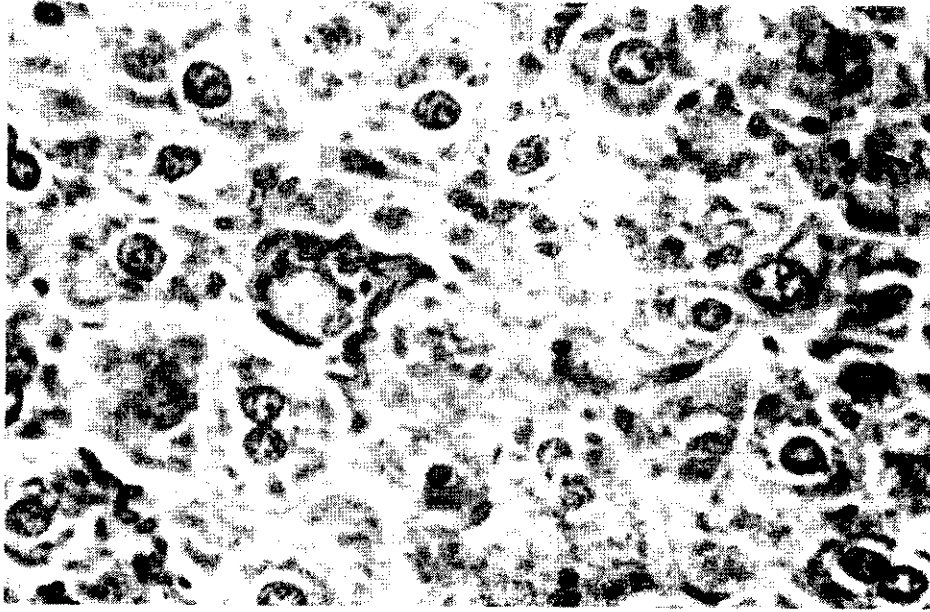


Fig. (1): Brain of infested mice showing individual cerebral cells with bradyzoites of *Toxoplasma gondii*.

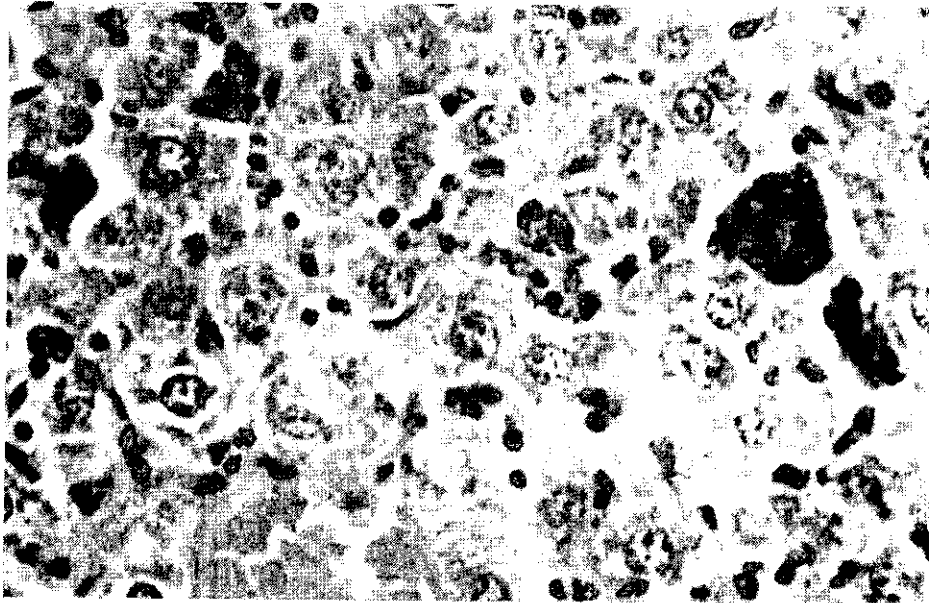


Fig. (2): Liver of infested mice showing intercellular *Toxoplasma* cysts.

DISCUSSION

Table (1) showed that all controls (women with normal pregnancy) were IgM negative while 19 (37.25%) were ELISA IgG positive. As to the women with complicated pregnancy 3/21 or 14.28% were IgM positive and 15/21 or 71.42% were IgG positive. The results revealed a significant difference between the complicated cases and controls. This result was in accordance with Leon et al. (1996), Zhang et al. (1997) and Kumar et al. (2004). The seroprevalence of toxoplasmosis in pregnant women, on worldwide scale, varies from 7 to 51.3% and in women with abnormal pregnancies and abortions the seroprevalence varies from 17.5 to 53.3% (Singh, 1998).

Routine serologic diagnosis of toxoplasmosis provides high sensitivity, but specificity varies depending on the test used and false-positive IgM antibody test results have been reported by Hofgartner et al. (1997). In addition, even true positive results must be interpreted with caution (Liesenfeld et al. 1997) because IgM antibodies may persist for year after acute infection (Bobic et al. 1991). Diagnosis of primary infection with *T. gondii* in early pregnancy could be improved by determination of anti-toxoplasma IgG avidity, which has the ability to discriminate between recent and prior infections (Jenum et al. 1997).

Although, the percentage of IgG positive women with normal pregnancy in the third trimester and

in the first one in women with complicated pregnancy was the highest, the difference compared with other trimesters was of low significance. These results are consistent with those in previous studies conducted by Abdel-Hafez et al. (1986).

The gestational age during which the maternal infection occurs is important. Although, the frequency of fetal infection is higher when maternal infection occurs later in the pregnancy (e.g., third trimester), (Robert-Gangneux et al. 1999) the sequelae are more severe when maternal infections occur early in the first trimester (Gagne 2001).

The presence of elevated levels of Toxoplasma-specific IgG antibodies indicates that infection has occurred at some point but does not distinguish between an infection acquired recently and one acquired in the distant past. The presence of a high Toxoplasma-specific IgM antibody titer combined with a high IgG titer probably indicates an acute infection within the previous 3 months. A low-to-medium IgM titer and a high IgG titer might indicate an acute infection 3-6 months previously, but IgM antibodies have been detected as long as 18 months after initial infection (Wilson and McAuley 1999).

The diagnosis of abortion, stillbirth, premature labor, or neonatal death due to Toxoplasma infection needs careful and laborious evaluation. The use of ELISA (IgG & IgM) is a must (Soliman et

al. 2001).

The risk factors in the current study that are most strongly associated with toxoplasmosis in women with abnormal pregnancy were contact with cat (46.66%) followed by eating raw or under cooked meat (26.66%) while in case of women with normal pregnancy, contact with cat and eating raw or undercooked meat represent 31.57% and 15.78% of the risk factors associated with toxoplasmosis respectively as shown in table (2). Direct and frequent contact with infected cats undoubtedly increase the chance for human infection with *Toxoplasma gondii*. This finding was reported by Frenkel and Ruiz (1980) and Bobic et al. (1998). The evidence of human infection with *Toxoplasma* by ingestion of raw or undercooked beef, pork and mutton is well established by Knaus (1975), Arias et al. (1996) and Samad et al. (1997). In contrast Camargo et al (1995) found no correlation between toxoplasmosis and consumption of raw meat, milk and eggs. Also Dar et al. (1997) found that there is no statistical correlation between *Toxoplasma* infection and consumption of raw meat, milk or proximity of cat and other animals. The possibility of *Toxoplasma* infection may exist through contamination of food by filth, flies and cockroaches (Wallace 1972). The bad habit of licking the fingers during handling and processing foods may constitute additional factor in the transmission of *Toxoplasma* (Scott 1978). Such possibilities of transmission in our study

represent 20% in women with complicated pregnancy and 52.63% in women with normal pregnancy.

Although *Toxoplasma* infections are associated either with eating contaminated meat or with ingesting oocysts passed in the feces of cats, no laboratory test exists that can determine the origin of a *Toxoplasma* infection in a specific person and whether it was associated with food borne, cat borne, or soil borne transmission. Epidemiologic studies of the transmission of toxoplasmosis have been hindered by an inability to determine the origin of isolated infections (James and Hughes 2000).

In the present work, *Toxoplasma* cyst was detected in sheep meat at a rate of 15.63% but failed to be detected in cattle and camel tissues as shown in table(3). The failure of detection of *Toxoplasma* cyst in beef was recorded by Munday (1970) who proved that, although circulating antibodies to *Toxoplasma gondii* have been repeatedly detected in cattle by variety of serological methods, the parasite has usually eluded isolation. Also Esteban-Redondo et al. (1999) found the same result. On the contrary, few records of successful isolation of *Toxoplasma* cyst from diaphragm (Carter et al. 1969), retina (Mayer and Boechringer 1967) and bovine meat (Wyss et al. 2000) were reported. Concerning sheep, the appearance of positive mice infected with their meat digests af-

ter 2 weeks post infection was an indication that the mutton constitutes a health hazard to humans especially for those handling or eating raw or insufficiently cooked meat. Such fact must be put in consideration when processing sheep meat especially in sausage or hamburger. Our results are in accordance with findings of Waldland (1976), Tizord et al.(1979) and Wyss et al. (2000). Also Esteban-Redondo et al. (1999) were able to detect *Toxoplasma* cyst in tissues of sheep by bioassay in mice and PCR following oral infection with sporulated oocysts while the cyst could not be detected in bovine meat. However as *Toxoplasma gondii* was principally detectable in sheep meat, the consumption of this meat harbors a potential infection risk for humans. In contrast, the lack of any parasite detectability in bovine and camel meat reported in this work allows to consider this infection source as neglectable. Sheep are more susceptible to toxoplasmosis than cattle because these animals are reared outdoors, which put them at greater risk of environmental exposure than animals reared indoors (Cook et al. 2000).

Since contaminated meat is a significant infection source to man, it is particularly beneficial to ensure continuous surveillance of *T. gondii* in animal species destined for human consumption. Certain precautions diminish the possibility of acquiring toxoplasmosis, and they should be observed by high-risk groups, such as nonimmune pregnant women and immunodeficient patients.

These individuals should not eat undercooked meat, should wash their hands after handling raw meat, and should avoid contact with material that is potentially contaminated with cat feces.

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البحث في مرض التوكسوبلازما موزيس في السيدات الحوامل وفى الحيوانات المذبوحة

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تم فحص ٢١ سيدة تعاني من مشكلات في الحمل بالإضافة الى ٥١ سيدة بصحة جيدة ولتعانى من مشكلات في الحمل باستخدام إختبار الأليزا لتحديد نوع الأجسام المضادة. أيضاً تم فحص ١٨٢ حيوان مذبوح (٦٠ من الماشية ، ٩٧ من الأغنام ، ٢٦ من الجمال) يأخذ عينات من الحجاب الحاجز وعضلات من كل من الفك والفخذ وفحصها باستخدام إختبار الهضم الصناعي. وقد أظهرت النتائج ان السيدات اللاتي كن يعانين من مشكلات فى الحمل كانت نسبة الأجسام المناعية من نوع جى ٧١.٤٢٪ بينما كانت النسبة ١٤.٢٨٪ للنوع ام. أما بالنسبة للسيدات اللاتي كن يتمتعن بحمل طبيعى فكانت نسبة الأجسام المناعية من نوع جى ٢٧.٢٥٪ بينما لم يتم تحديد أى نسبة للنوع ام.

بالنسبة للحيوانات المذبوحة أمكن عزل حويصلات التوكسوبلازما من لحوم الغنم فقط بنسبة ١٥.٤٦٪ هذا وقد أظهرت الدراسة ان هناك بعض العوامل التي من شأنها ان ترفع احتمالية الإصابة بالتوكسوبلازما فى الإنسان منها درجة القرب وملامسة القطط وأيضاً تناول لحوم بصورة نيئة أو غير مطهية جيداً. وقد إنتهت الدراسة الى بعض الإرشادات للوقاية والسيطرة على المرض فى الإنسان.