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Seroprevalence of *Toxoplasma gondii* in water buffaloes (*Bubalus bubalis*) in South-West of Iran

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Abstract. The prevalence of antibodies to *Toxoplasma gondii* was conducted in 300 buffaloes from Ahvaz, Kouzestan province, south - west of Iran. Blood sera were screened using a Modified agglutination test (MAT) incorporating 2-mercaptoethanol. Positive reactions in sera dilutions above 1:25 were considered as indicative for the presence of *T. gondii* antibodies. The overall prevalence of infection in the animals was 14.33% with titers of 1:25 in 21, 1:50 in 12, 1:100 in 6, 1:200 in 2 and 1:400 in 2. The prevalence was different in relation to the sex with buffaloes with 19.7% and 7% in females and males respectively. These results indicate that *T. gondii* infection in water buffaloes of Khouzestan is relatively high and consumption of buffalo meat may be a risk factor for humans in Ahvaz, South - West of Iran.

INTRODUCTION

Toxoplasmosis has the highest human incidence amongst the parasitic zoonoses that is caused by the protozoan parasite *Toxoplasma gondii* (Dubey & Beattie, 1988). Infection with the parasite is ubiquitous throughout the world. Approximately one - third of the human world population is infected with the protozoan parasite *T. gondii* (Dubey & Beattie, 1988; Dubey, 2008). The parasite is responsible for approximately 20% of all deaths attributed to food - borne pathogens in the US, and the Centers for Disease Control estimate that 50% of all human exposures to *T. gondii* are food - borne (Mead *et al*., 1999). Less than 20% of individuals develop clinical signs including fever, lymph node enlargement or intraocular inflammation (Holland, 2003). Also, *T. gondii* can cause significant morbidity and mortality in the developing fetus (Remington & Desmonts, 1990) and in immunocompromised individuals, including humans with Acquired Immunodeficiency Syndrome, AIDS, or submitted to cancer chemotherapy (Israelski & Remington, 1993).

Infection with the parasite in human occurs congenitally by transmission of the tachyzoite during primary infection of the mother; by ingestion of food or water contaminated with oocysts in the faeces of infected felids; or by ingestion of raw or undercooked meat containing the bradyzoite in tissue cysts (Dubey, 1996). Details concerning both asymptomatic and symptomatic infection and the risks of transmission from animals to humans in the USA were recently reviewed by Dubey & Jones (2008).

Toxoplasma gondii has been studied most intensively among the coccidian due to its zoonotic nature. Epidemiological surveys still remain the most useful way of

assessing the relative importance of different sources of *T. gondii* infection in humans. Serological studies have found evidence of widespread *T. gondii* infection in meat producing animals (Astrid *et al*., 2000; Chandrawathani *et al*., 2008; Santos *et al*., 2009). Also, *T. gondii* is widely prevalent in human and animals in Iran (Ghorbani & Samii, 1973; Ghorbani *et al*., 1983; Hoghooghi-rad & Afraa, 1993; Hashemi-fesharki, 1996; Sharif *et al*., 2007; Hamidinejat *et al*., 2008).

In Khouzestan province, water buffaloes, an important animal in tropical areas, are as food animals. Little is known about the epidemiology of *T. gondii* infection in water buffaloes especially in Iran. Thus a survey was conducted, to determine the seroprevalence of *T. gondii* infection in buffaloes from Ahvaz, Khouzestan province, in Iran as buffalo meat in this area is commonly consumed by humans.

MATERIALS AND METHODS

Blood samples were collected from 300 water buffaloes (105 males and 195 females) at the slaughterhouse in Ahvaz, the centre of the Khuzestan province, South-West of Iran, from December 2007 to June 2008. Males and females were selected by random sampling. The female group was divided in two age groups (74 heifers and 121 cows). Buffaloes did not have any apparent clinical signs at sampling. Sera were tested by the modified agglutination test (MAT) using formalin treated whole tachyzoites prepared in the Pasteur Institute of Iran and incorporating 2-mercaptoethanol as described by Desmonts & Remington (1980) and Gilot-Fromont *et al*. (2009). All sera reactive at 1:25 were considered as positive.

In the present study, the prevalence of infection was correlated with sex and age of the animals and the statistical significances of their association or independence were analyzed by Chi-square and Fisher's exact tests, with a confidence level of 95%. Analyses were done with spss 16 software for Windows, with a probability (p) value <0.05 as statistically significant (Altman, 1991).

RESULTS

A total of 43 of 300 (14.33%) buffalo sera had antibodies to *T. gondii* with titers of 1:10 in 79, 1:25 in 43, 1:50 in 11, 1:100 in 9, 1:200 in 4 and 1:400 in 3. The prevalence was different in relation to the sex of buffaloes as it was 6.66% and 28.57% in females and males respectively ($P<0.05$). Detailed results are shown in Tables 1 and 2.

DISCUSSION

Toxoplasma gondii is one of the most common parasitic infections world-wide with an estimated prevalence in 1–2 billion people (Chang, 1996; Dubey, 2008). The prevalence and impact of *T. gondii* on human health are highly variable geographically and sources of infection vary greatly in different human populations with differences in culture and eating habits (Garcia *et al*., 2006). Seroprevalence in the human population ranges from 0 to 90% (Dubey & Beattie, 1988; Dubey, 2008).

Table 1. Prevalence of *T. gondii* antibodies in water buffaloes

	Number of Positive	Number of Negative	Total
Male	30 (28.57%)	75 (71.42%)	105
Female	13 (6.66%)	182 (93.33%)	195
Total	43 (14.33%)	257 (85.66%)	300

Table 2. Distribution of *T. gondii* antibodies in female water buffaloes

	Number of Positive	Number of Negative	Total
Cow	4 (3.41%)	113 (96.58%)	117
Heifer	9 (11.53%)	69 (88.46%)	78
Total	13 (6.66%)	182 (93.33%)	195

Several epidemiological studies reported a broad range of serologic prevalence of *T. gondii* antibody titers among different localities of Iran. According to Assmar *et al.* (1997), 51.8% seropositivity was detected in more than 13000 serum samples collected from 12 provinces in Iran. High prevalence of *T. gongii* infection (68.3%) among the people in south of Tehran, was also reported by Salahi-Moghaddam & Hafizi (2009).

Transmission occurs often following ingestion of sporulated oocysts, or bradyzoites within cysts present in the tissues of numerous food animals (Dubey, 2008; Gilot-Fromont *et al*., 2009).

There is insufficient data on buffalo toxoplasmosis in the world, and few new reports on buffalo toxoplasmosis from Iran. For example Navidpour & Hoghooghi-rad (1998) reported anti-*Toxoplasma gondii* antibodies from various areas of the Khoozestan province in Iran by the IFA test in 8.8% buffaloes. Previous studies on toxoplasmosis were often designed without prior consideration of the related species, *Neospora caninum*. Thus, it was timely that a survey in buffaloes from Ahvaz region in Iran was conducted to determine the seroprevalence of *T. gondii* infection in this animal which are commonly used for meat consumption and producing milk in this area.

Since direct observation of cysts in tissues was not a suitable diagnostic method to be carried out on live animals, the serological techniques appear to be the method of choice. The MAT was considered as one of the most specific and sensitive tests for the detection of *T. gondii* antibodies in animals (Dubey & Beattie, 1988; Dubey *et al*., 1995; Dubey & Jones, 2008; Gilot-Fromont *et al*., 2009).

The prevalence of anti-*T. gondii* antibodies in buffaloes varies in different parts of the world. For example the seroprevalence in Brazil is 3.85%, in India 10-24%, in Indonesia 0%, in Vietnam 10% and in Malaysia 11% (As reviewed by Dubey & Beattie, 1988).

The results of the present survey on *T. gondii* infection in buffalo showed the seroprevalence of 14.33% and this prevalence increased statistically in heifers and also was higher in male buffaloes than females. The highest titer of MAT in the present study was 1:400 shows that buffalo may not be a suitable host for *T. gondii*. This result was less than the seroprevalence of toxoplasmosis in sheep, goats, cattle, birds and humans in this area (Hohgooghi-Rad & Afraa, 1993).

Present study suggests that buffaloes are similar to cattle for *T. gondii* infection because the antibody response to *T. gondii* in buffaloes does not persist for life and decreased with increase in age. These findings were similar to findings in cattle. Dubey *et al.* (1985) showed that antibody titers were lower in cows than in calves in all tests. In one steer where viable cysts where present more than 3 years after experimental infection, the serological titer for the Modified Agglutination Test (MAT) decreased from 1:8000 to 1:20 after 19 months (Dubey & Thulliez, 1993). In the present study approximately all of the male buffaloes were under two years old, so the prevalence of infection was statistically higher than in females. On the other hand prevalence of infection was higher in heifers (under two year old females) than cows ($P>0.05$).

In our study, the seroprevalence of *T. gondii* antibodies in buffaloes may be due to the fact that cats were extensively distributed throughout the area. A high prevalence of toxoplasmosis within hot and humid environments compared to cold and dry ones is attributed to the longer viability of *T. gondii* oocysts under humid conditions (Fayer, 1981) that exists in the area of this study.

In conclusion, several epidemiological studies found a significant association between toxoplasmosis and consumption of meat by human, (Bobic *et al*., 1998; Baril *et al*., 1999). Although these results do not provide an estimate of the percentage of infected buffalo meats, the consumption of buffalo meat especially from young animals may be one of the sources of infection for human in Ahvaz. Thus, meat and other edible parts of animals should be

cooked thoroughly before consumption. Freezing meat before cooking can reduce the risk of infection but does not kill all tissue cysts. The tissue cysts are relatively resistant to changes in temperature and remain infectious in refrigerated carcasses or minced meat for up to 3 weeks, i.e. Tissue cysts also survive freezing at temperatures between 21 and 28°C for longer than a week.

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