Serologic Survey of Antibodies to *Toxoplasma gondii* in Ardabil, Iran

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ABSTRACT

Toxoplasmosis is one of the most prevalent parasitic infections of humans and livestock. Its transmission to humans is usually attributed to ingestion of undercooked or raw meat from infected livestock. The toxoplasmosis infection rate in livestock is an important predictor of human disease risk. In Iran's Ardabil state, there is a high rate of toxoplasmosis infection among livestock. Serum samples from cattle, goat, sheep, and chicken in the region were tested for IgG antibodies to *Toxoplasma gondii* by enzyme-linked immunosorbent assay (ELISA). Antibodies to *T gondii* were found in 30% (60/200) of sheep, 15% (30/200) of goats, and 9% (18/200) of cattle; no positive samples were found in chicken. Infection rates of tested animals can be attributed to livestock management methods rather than differences in feeding practices. Improved livestock management methods could therefore reduce infection rates.

INTRODUCTION

Infection by the protozoan parasite *Toxoplasma gondii* is widespread in humans and many other warm-blooded animals. Although the course of the disease is generally benign, it can cause significant morbidity and mortality in the developing fetus and in immunocompromised individuals, including people with HIV/AIDS or those undergoing cancer chemotherapy.

Among livestock, sheep and goats are more widely infected with *T gondii* than cattle and chicken.[1,4] This parasite is a major cause of abortion, causing significant economic losses to sheep and goat breeders. The infection does not usually cause clinical symptoms in cattle or chicken.[5,7]

Recent studies[8,9] showed that a small percentage of affected individuals acquire *T gondii* infection in utero, but the majority become exposed to *T gondii* by ingestion of undercooked or raw meat containing tissue cysts, ingestion of oocysts shed by infected cats, or consumption of contaminated drinking water or fresh vegetables. In Iran, *T gondii* have been found in mutton from sheep, goat meat, beef, and chicken.[10]

Although *T gondii* is found in most parts of the world, there have been relatively few reports of *T gondii* infection in small ruminants, cattle, and chicken in Iran.[10,11] Epidemiological surveys still remain the most useful way to assess the relative importance of different sources of *T gondii* infection in humans. Since contaminated meat is a significant source of infection in humans, it is particularly important to ensure continuous surveillance of *T gondii* prevalence in animal species destined for human consumption.

MATERIALS AND METHODS

Serum samples were collected from a total of 750 food animals from Ardabil State,

Table 1. Frequency of *Toxoplasma gondii* Seropositivity Among Livestock from Ardabil, Iran

Livestock	*Management method*	*Seropositivity rate (n/total)*	*Statistical significance*
Cattle	Extensive*	9% (18/200)	($P < 0.001$)
Chicken	Intensive†	0% (0/150)	($P < 0.001$)
Goats	Semi-intensive‡	15% (30/200)	($P < 0.005$)
Sheep	Extensive	30% (60/200)	($P < 0.005$)

$\chi^2 = 69.5$
*Breeding herd is maintained over a large open area.
†Breeding herd is kept outside as well as in housing.
‡Breeding herd is kept solely in housing.

Iran. Two hundred animals were cattle from breeding herds maintained over a large area of land (*extensive management method*) and slaughtered in Ardabil; 200 animals were goats from Ardabil-region farms that kept breeding herds outside as well as in housing (*semi-intensive management method*), 200 animals were sheep managed extensively and slaughtered in Ardabil, and 150 were chickens kept solely in housing (intensive management method) and slaughtered in Ardabil.

Blood samples from cattle, sheep, and chicken were collected immediately after slaughter; goat blood was collected by venipuncture. Serum was separated by centrifugation at 1000 g for 10 minutes, mixed with phosphate buffered glycerol in a 1:1 ratio (volume/volume) at pH 7.2, and stored at –20˚C until use. *T gondii* RH strain tachyzoites salt-soluble antigen was prepared from infected mouse peritoneal fluid as described elsewhere, with the exception of an additional step of mammalian cell exclusion by adhesion to sterile pre-packed Sephadex G50 columns (Radim).[10,11] The antigen was adjusted to 1 mg protein/mL and stored at -70˚C until use. ELISA was performed as described elsewhere[10,12] using high protein binding-certified microplates (Sigma) coated with 100 mL/well of *T gondii* antigen 10 mg/mL. A serum sample, diluted 1:100 in phosphate-buffered saline plus 0.5 mL Tween 20 per liter, was added to each well. Bound IgG was detected with species-specific anti-IgG peroxidase conjugate. Optical density was measured in a microplate reader. A positive control was included in each plate assay, usually obtained from an experimentally infected animal from the same species; negative and threshold controls, all previously determined by Indirect Immunofluorescence Assay (IFA), were also included. The threshold control, obtained from the dilution of the positive serum of known IFA titer in standard negative serum, was used to clearly distinguish reactive from nonreactive serum samples in multiple plate assays. The absorbance of the threshold serum was taken to be the lowest level of identifiable positive reaction. The reactivity index (RI) of the samples was defined as the ratio of the average absorbance of the samples to the average absorbance of the threshold serum, being positive when RI equals 1.0. All serum samples were tested twice, with inter- and intratest reproducibility above 99%.

RESULTS

Table 1 shows the frequency of *T gondii* infection among livestock in Ardabil, Iran. The seropositivity rate was 31% in sheep, 17% in goats, and 11% in cattle; no infection was found in chickens (95% confidence interval for each measure, which was significantly higher in sheep than in goats and cattle, and absent in chicken). Chi-square testing showed the highest frequency of infection was in sheep, with cattle and goats having similar intermediate frequencies, and chickens the lowest seropositivity rates. The reactivity distribution (Figure 1) clearly shows the expected bimodal distribution of infected and noninfected animals.

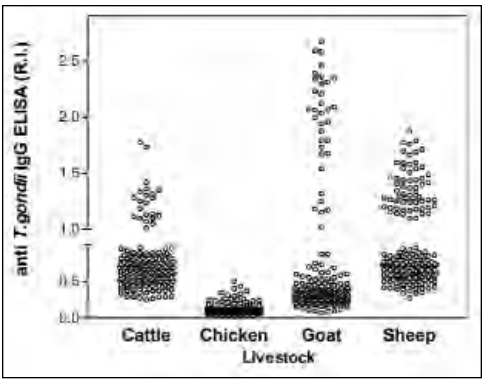

Figure 1. Distribution of anti-*Toxoplasma gondii* infection in livestock, showing typical bimodal distribution in infected species.
ELISA, enzyme-linked immunosorbent assay. RI, reactivity index.

DISCUSSION

The results of this study suggest that small ruminants play a more important role as a source of toxoplasmosis than do cattle, an observation confirmed by previous studies.[10,11] In Iran, consumption of sheep meat (mutton) is much greater than is consumption of beef or goat meat, thus increasing the importance of sheep as a source of local toxoplasmosis infection.

Our current understanding of the epidemiology of toxoplasmosis leads us to believe that herbivores acquire infection by ingestion of grass and water contaminated with *T gondii* oocysts shed by cats. The differences in rate of infection could be attributed to both differences in susceptibility to *T gondii* or differences in livestock management methods. Since sheep are bred under extensive management, it is more likely to be exposed to *T gondii* oocysts in the pasture and in water than goats, which are supplied with better water and food quality under semi-intensive management methods. Lower seropositivity rates in cattle compared with those in sheep may be attributed to differences in susceptibility, since both species are bred under extensive management. On the other hand, the absence of *T gondii* infection in chickens can also be attributed to management method, as these animals are bred in a highly intensified management method. Our data showed that of the three infected species, the lowest seroprevalence occurred in cattle, but in view of the typical preference for beef (and high use of processed beef products), bovine protein cannot be ruled out as a significant source of human infection. The high infection rate in sheep might have local implications because in the State of Ardabil and Iran generally, mutton is a more popular source of animal protein and thus is an increasingly important potential source of human toxoplasmosis.

CONCLUSION

Reducing the high *T gondii* infection rate in sheep might have an impact on human health because in Iran sheep meat is a more popular source of animal protein and thus is an important source of human toxoplasmosis. The data from the present study suggest that it is possible to significantly reduce the risk of *T gondii* infection in livestock using intensive farm management with adequate measures of hygiene, confinement, and prevention.

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