SHORT COMMUNICATION

Serological survey of antibodies to *Toxoplasma gondii* in Ardabil State, Iran

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Toxoplasmosis is one of the most prevalent parasitic infections of man and livestock, and its transmission has usually been attributed to ingestion of undercooked or raw meat from infected livestock. The infection rate in those animals is an important risk predictor of human disease in Ardabil State in Iran. Infection by the protozoan parasite, *Toxoplasma gondii,* is widespread in humans and many other species of warm-blooded animals. Although the course of disease is generally benign, it can cause significant morbidity and mortality in the developing foetus and in immunocompromised individuals, including humans with Human Immunodeficiency Virus/ Acquired Immuno-deficiency Syndrome (HIV/AIDS) or submitted to cancer chemotherapy (Remington, 1990).

Among livestock, sheep and goat are more widely infected with *T. gondii* than cattle and chicken. This parasite is a major cause of abortion, with significant economic losses to sheep and goat breeders. The infection does not usually cause clinical symptoms in cattle or in chicken (Dubey & Beattie, 1998).

Recent studies (Tenter, 2000; Dubey & Beattie, 1998) showed that a small percentage of affected human individuals acquire infection in the uterus, but the majority becomes 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. *T. gondii* have been demonstrated in mutton including in Iran, goat meat, beef and chicken. Although *T. gondii* is found in most parts of the world, there have been relatively few recent reports on small ruminants, cattle and chicken in Iran. Epidemiological surveys still remain the most useful way of assessing the relative importance of different sources of *T. gondii* infection in humans. Since contaminated meat is a significant infection source to man, it is particularly beneficial to ensure continuous surveillance of *T. gondii* prevalence in animal species destined for human consumption (Tenter, 2000; Dubey & Beattie, 1998).

In this study, sera were collected from a total of 750 food animals from Ardabil State, Iran: 200 being from extensive breed cattle slaughtered at abattoirs, 200 from semi-intensive breed goat from farms, 200 from extensive breed sheep slaughtered at abattoirs and 150 from intensive breed chicken slaughtered at abattoirs. Cattle, sheep and chicken blood were collected during slaughter, immediately after killing, and goat blood was collected by venipuncture. Serum was separated from clot by centrifugation at 1000g for 10 min, mixed (1:1, v/v) with phosphate buffered glycerol, pH 7.2, and stored at -20°C until use. RH strain *T. gondii* tachyzoites salt soluble antigen was prepared from infected mouse peritoneal fluid as described elsewhere, except for one step of mammalian cell exclusion by adhesion to sterile pre-packed Sephadex G50 columns. The antigen was adjusted to 1mg protein/ml and stored at -70°C until use (Gorbani *et al.,* 1978; Hoghooghi-Rad *et al.*, 1993; Venkatesan & Wakelin, 1993). ELISA was performed as described elsewhere, using high protein binding-certified microplates (Sigma®) coated with 100 ml/ well of *T. gondii* antigen 10 mg/ml. Serum sample, diluted 1:100 in PBS-T, was added to each well and bound IgG detected with species-specific anti IgG peroxidase conjugate, with optical density (OD) measured in a microplate reader.

In each plate, positives usually obtained from an experimentally infected animal from the same species, negative and threshold controls were included, all previously determined by Indirect Immunofluorescence Assay (IFA). The threshold control, obtained from the dilution of the positive serum of known IFA titre in standard negative serum, was used to clearly distinguish reactive from non-reactive serum samples in multiple plate assays; the absorbance of the threshold serum was taken as 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 by the average absorbance of the threshold serum, being positive when RI $\geq$ 1.0. All serum were tested in duplicate, with reproducibility inter and intra tests higher than 99.0% (Gorbani *et al.,* 1978; Hoghooghi-Rad *et al.*, 1993; Venkatesan & Wakelin, 1993).

The ELISA results were shown both as frequency of infection (Table1), and also as their reactivity distribution (Figure1). Their reactivity distribution showed clearly the expected bimodal distribution of infected and non-infected animals. Comparison

between frequencies by χ^2-test showed that the highest frequency was found in sheep, with cattle and goat with similar intermediate frequencies and chicken with lowest seropositivity.

The results of this survey would suggest that small ruminants play a more important role as source of toxoplasmosis than cattle, Nonetheless, despite the lower seroprevalence detected in cattle, consumption of mutton in Iran is much greater than that of beef or goat meat and thus increases the importance of sheep as source of local infection. Our current understanding of the epidemiology of toxoplasmosis leads us to think that herbivores acquire infection by ingestion of pasture and water contaminated with *T. gondii* oocysts

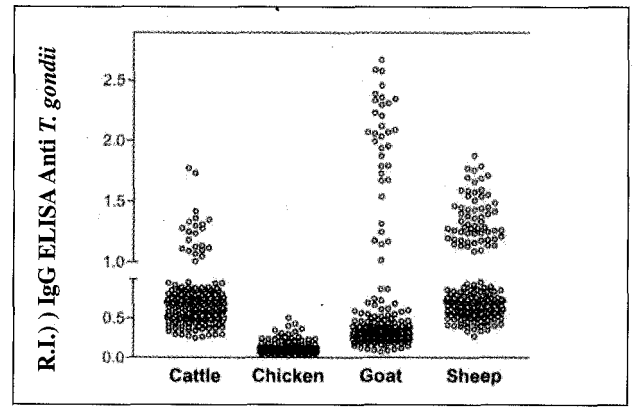

Figure 1: Anti-*Toxoplasma gondii* showing typical bimodal distribution in infected species

Table 1: Frequency of seropositivity for *gondii* among livestock from Ardabil state, Iran

Livestock	Management	seropositivity(n/total)	Statistic
Cattle	extensive	9%(18/200)	
Chicken	intensive	0(0/150)	Lowest(p<0.001
Goat	semi-intensive	15(30/200)	
Sheep	extensive	30%(60/200)	Highest(p<0.005

shed by cats. The differences in rate of infection could be attributed both to differences in susceptibility to *T. gondii* or to differences in management methods. Since sheep is bred under extensive management, it is more likely to be exposed to *T. gondii* oocysts in pasture and water than goats, which are supplied with better water and food quality under semi-intensive management.

The lower seropositivities in cattle samples compared to 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 infection in the chickens can also be attributed to the management method, as these animals are bred in highly intensified management. Findings from this study showed that, out of the three infected species, the lowest seroprevalence occurred in cattle, but in view of the typical preference for beef, bovine protein cannot be ruled out as a significant source of human infection, including processed products. The high infection rate in sheep might have local implications because, in the State of Ardabil and Iran in general, mutton is more popular as source of animal protein, therefore regarded as a potential source of human toxoplasmosis.

These data 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.

References

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