

Serological survey of antibodies to *Toxoplasma gondii* in food animals from São Paulo state, Brazil

Pesquisa sorológica de anticorpos anti-*Toxoplasma gondii* em animais de produção do Estado de São Paulo, Brasil

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Summary

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, with the infection rate in those animals being an important risk predictor of human disease, high in Brazil and São Paulo State. Looking for this public health problem, we tested serum samples from cattle, goat, sheep and chicken from the State of São Paulo, Brazil, for IgG antibodies to *Toxoplasma gondii* by enzyme-linked immunosorbent assay (ELISA). Antibodies to *Toxoplasma gondii* were found in 31.00% (62/200) of sheep, 17.00% (34/200) of goat and 11.00% (22/200) of cattle, without positive sample in chicken. Despite differences in feeding habits of each species, the rate of infection of tested animals could be better attributed to livestock management methods, which improvement could reduce infection.

Key-words

Toxoplasma gondii.
Food animals.
Seroprevalence.
ELISA.

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Recebido para publicação: 29/10/2002
Aprovado para publicação: 06/05/2003

Introduction

Infection by the protozoan parasite, *Toxoplasma gondii*, is widespread in humans and many other species of warm-blooded animals¹. Although the course of disease is generally benign, it can cause significant morbidity and mortality in the developing fetus² and in immunocompromised individuals, including humans with Acquired Immunodeficiency Syndrome - AIDS³ or submitted to cancer chemotherapy⁴.

Among livestock, sheep and goat are more widely infected with *Toxoplasma gondii* than cattle and chicken¹. This parasite is a major cause of abortion, with significant economic

losses to sheep and goat breeders⁵. The infection does not usually cause clinical symptoms in cattle⁶ and in chicken⁷.

Recent studies showed that a small percentage of affected individuals acquire infection in the uterus, but the majority becomes exposed to *Toxoplasma 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^{8,9}. *Toxoplasma gondii* have been demonstrated in mutton^{10,11} including in Brazil¹², goat meat¹³, beef^{6,11} and chicken¹⁴.

Although *Toxoplasma gondii* is found in most parts of the world¹,

there have been relatively few recent reports on small ruminants^{15,16,17}, cattle^{16,17,18} and chicken^{7,14} in Brazil. Epidemiological surveys still remain the most useful way of assessing the relative importance of different sources of *Toxoplasma gondii* infection in humans. Since contaminated meat is a significant infection source to man⁸, it is particularly beneficial to ensure continuous surveillance of *T.gondii* prevalence in animal species destined for human consumption.

Material and Method

Sera were collected during 1999 to 2000 from a total of 785 food animals from São Paulo State, Brazil: 200 being from extensive breed cattle slaughtered at abattoirs in the city of Taquaritiba, 200 from semi-intensive breed goat from farms of the Botucatu region, 200 from extensive breed sheep slaughtered at abattoirs in the city of São Manuel and 185 from intensive breed chicken slaughtered at abattoirs in the city of Botucatu. 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 *Toxoplasma gondii* tachyzoites salt soluble antigen was prepared from infected mouse peritoneal fluid as described elsewhere¹⁹, 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. ELISA was performed as described elsewhere²⁰, using high protein binding-certified microplates (Sigma®) coated with 100 ml/well of *Toxoplasma 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) was measured in a microplate reader (Labsystems Multiskan MS). In each plate, we included positive, usually obtained from an experimentally infected animal from the same species, negative and threshold controls, all previously determined by Indirect Immunofluorescence Assay (IFA). 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 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 sera were tested in duplicate, with reproducibility inter and intra tests higher than 99.00%.

Results and Discussion

The ELISA results were shown both as frequency of infection in the Table 1 and also as their reactivity distribution in the Figure 1. We found 31.00% of seropositivity in sheep, 17.00% in goat, 11.00% in cattle, without positive sample in chicken with a 95.00% confidence interval for each measure, which was significant higher in sheep than in goats and cattle, and absent in chicken. Their reactivity distribution shows clearly the expected bimodal distribution of infected and non infected animals.

The results of this survey would suggest that small ruminants play a more important role as source of toxoplasmosis than cattle, as

discussed elsewhere^{16,17}. Nonetheless, despite the lower seroprevalence detected in cattle, the Brazilian consumption of beef is much greater than that of mutton or goat meat and this thus increases the importance of cattle 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 *Toxoplasma gondii* oocysts shed by cats⁹. 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 *Toxoplasma gondii* oocysts in pasture and water than goats, which are supplied with better water and food quality under semi-intensive management. Although, 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, otherwise an 39.00 seropositivity was found in chicken with extensive and free living management¹⁴. Our data showed that, out of the three infected species, the lowest seroprevalence occurred in

cattle, but in view of the typical preference of Brazilians for beef, bovine protein cannot be ruled out as a significant source of human infection, including as processed products¹¹. The high infection rate in sheep might have local implications because, in the State of São Paulo, mutton is recently becoming more popular as source of animal protein, especially in barbecue steaks, turning into a potential source of human toxoplasmosis of increasing importance. We emphasize the care in adequate goat's milk pasteurization, usually in batch preparation, due to the *Toxoplasma gondii* infection in goats, reared to produce milk, due to the risk of acquired toxoplasmosis among infants, since goat's milk is now being introduced in the State of São Paulo as an alternative dairy food for infants allergic to cow's milk.

These data suggest that it is possible to significantly reduce the risk of *Toxoplasma gondii* infection in livestock using intensive farm management with adequate measures of hygiene, confinement, and prevention.

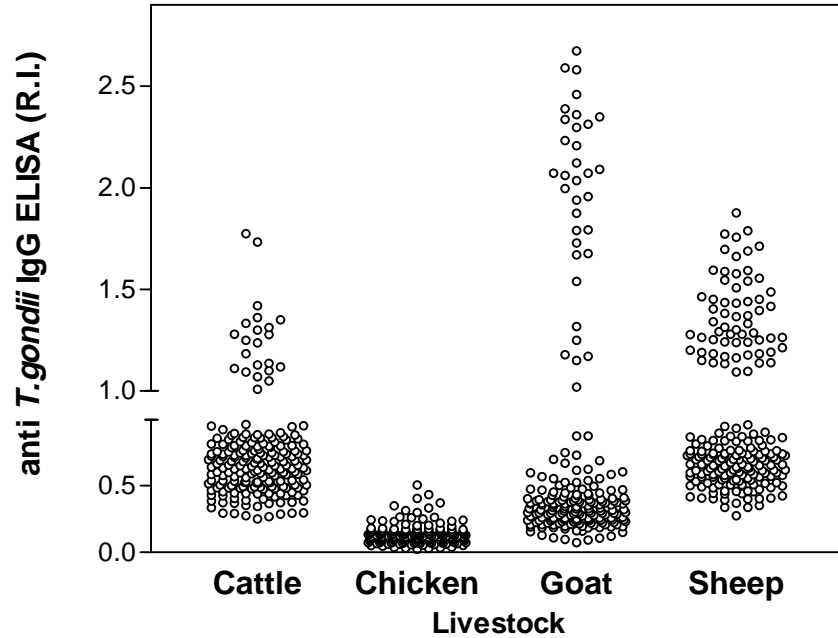
Acknowledgements

We are grateful to Hélio Langoni, Ph.D. M.V.Sc., for the supply of goat blood samples from the Laboratory for Diagnosis of Zoonosis of UNESP, Botucatu, SP, and the technical assistance of Roselaine P. A.

Table 1

Frequency of seropositivity for *T.gondii* among livestock from São Paulo State, Brazil. Comparison between frequencies by c2 test showed that the highest frequency was found in sheep, with cattle and goat with similar intermediate frequencies and chicken with the lowest seropositivity, São Paulo, 2002

Livestock	Management	Seropositivity(n/total)	I.C.95.00%	Statistics (c ²)
Cattle	Extensive	11% (22/200)	7.0 - 16.2 %	
Chicken	Intensive	0 (0/185)	0 - 1.9 %	Lowest(p<0.001)
Goat	Semi-intensive	17% (34/200)	12.1 - 23.0 %	
Sheep	Extensive	31% (62/200)	24.9 - 37.7 %	Highest(p<0.005)

Figure 1Distribution of anti-*Toxoplasma gondii* in livestock, showing typical bimodal distribution in infected species

Cardoso. This work was part of the Master in Parasitology Program of L.R. Meireles, supported by FAPESP (98/13323-0). A.J. Galisteo Jr. is an

undergraduate student sponsored by FAPESP (98/01681-0). This work was partially supported by FAPESP (99-04926-6) and LIMHCFMUSP-49.

Resumo

A toxoplasmose é uma das infecções parasitárias mais prevalentes no homem e no rebanho, e a sua transmissão tem sido usualmente atribuída à ingestão de carnes cruas ou mal cozidas, de animais de produção infectados, sendo que a taxa de infecção destes animais pode ser um importante índice preditivo da doença humana, de alta prevalência no Brasil e no estado de São Paulo. Estudando este aspecto de Saúde Pública, nós testamos amostras séricas de bovinos, caprinos, ovinos e frangos de corte de matadouros do Estado de São Paulo, para a presença de anticorpos IgG anti-*Toxoplasma gondii* por um ensaio imunoenzimático de fase sólida (ELISA). Nós encontramos anticorpos específicos em 31,00% (62/200) dos ovinos, 17,00% (34/200) dos caprinos e 11,00% (22/200) dos bovinos testados, sem positividade em 185 amostras de frangos de corte. Apesar de diferenças em hábitos alimentares de cada espécie, estas taxas de infecção foram menores em criações intensivas, relacionadas ao tipo de manejo da criação, o que pode reduzir a taxa de infecção por este parasita no rebanho.

Palavras-chave

Toxoplasma gondii.
Animais de produção.
Soroprevalência.
ELISA.

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