Seroprevalence of *Toxoplasma gondii* Infection in Goats by the Indirect Haemagglutination, Immunofluorescence and Immunoenzymatic Tests in the Region of Uberlândia, Brazil

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A comparative study of the indirect haemagglutination (IHA), immunofluorescence (IFAT) and immunoenzymatic (ELISA) tests was carried out to determine the prevalence of Toxoplasma gondii *antibodies in goats. One hundred seventy-four serum samples were obtained from four goat herds from the region of Uberlândia, State of Minas Gerais. The distribution of the animals, according to their origin, was as follow: 71 from herd I; 39 from herd II; 37 from herd III; and 27 from herd IV. Serum samples were analyzed by IHA, IFAT and ELISA, considering the reactivity of the serum samples at dilution ≥ 1:64 as cut off titer for the three tests. A global seroprevalence of 18.4% was observed, with significantly higher positivity rate in the herd II (66.7%) and older animals (> 36 months). A high and significant positive correlation was found between the titers obtained by the IHA versus IFAT, IHA versus ELISA, and ELISA versus IFAT. Therefore, it can be concluded that the three analyzed tests have shown to be highly concordant and appropriate for epidemiological surveys of* Toxoplasma *infection in goats. Although the seroprevalence of* T. gondii *infection in goats is relatively low in this region as compared to other regions of the country, adequate management might be useful and essential to control the infection in the goat herds.*

Key words: *Toxoplasma gondii* - goat - seroprevalence - indirect haemagglutination - indirect immunofluorescence - ELISA - Brazil

Toxoplasmosis, an infection caused by *Toxoplasma gondii* Apicomplexa protozoan, is widespread in humans and other animal species, having already been reported in many countries and different climates. Intermediate hosts of the parasite become infected by ingestion of sporulated oocysts, cyst-contaminated meats, specially from pig and sheep, contact with free tachyzoites or congenitally by placentary via (Pepin et al. 1997).

Goats infected by *T. gondii* represent an important source of human infection due to ingestion of meat and milk from infected animals. Such fact is extremely important concerning the disease control and mainly for public health, since the consumption of goat milk is elevated in children with allergy to cow milk. *T. gondii* tachyzoites were already isolated from vaginal mucosa, saliva, nasal secretion and urine from experimentally infected goats (Dubey 1980), and the excretion of tachyzoites in the milk of naturally infected goats was also reported by Chiari and Neves (1984). Furthermore, an epidemiological survey carried out in the region of Belo Horizonte, MG, showed that there was a statistically significant correlation between positive serology for *T. gondii* in humans and ingestion of goat milk (Chiari et al. 1987).

Serodiagnosis has been a more full and adequate tool to diagnose *Toxoplasma* infection in both man and animals, using various serological tests as indirect haemagglutination (IHA) (Nieto & Melendez 1998), indirect immunofluorescence (IFAT) (Van der Puije et al. 2000), enzyme-linked immunosorbent assay (ELISA) (Hashemi-Fesharki 1996).

Serological analysis using IFAT and ELISA has been widely employed in order to detect herds contaminated by *Toxoplasma*, including swine and sheep (Van der Puije et al. 2000). A comparative study of the ELISA, IFAT and HAI tests for the detection of specific antibodies to *T. gondii* in dogs showed that serodiagnosis of canine toxoplasmosis have to be based on the combination of serological tests, particularly IFAT and ELISA (Silva et al. 1997).

Due to increasing exploration of goat herds and the risk for public health by ingestion of contami-

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nated meat and milk, the development of reliable and sensitive tests for diagnosing this zoonosis becomes very important. Thus, the aim of this study was to evaluate the HAI, IFAT and ELISA tests to determine the seroprevalence of *T. gondii* infection in goats in the region of Uberlândia, MG.

MATERIALS AND METHODS

Animals and serum samples - A total of 174 goats were randomly selected from four herds in the region of Uberlândia, codified as I, II, III and IV. The herds I and II came from the periurban region, and the herds III and IV came from the rural area. The distribution of the animals, according to their origin, was as follow: 71 from herd I; 39 from herd II; 37 from herd III; and 27 from herd IV. The age ranged from 7 months to 10 years, with 14 animals from 0 to12 months, 104 from 12 to 36 months and 56 animals older than 36 months. Serum samples were collected following centrifugation at 500 g for 10 min of 5 ml of blood samples obtained from the jugular vein of goats, and all sera were stored a -20ºC until being analyzed for *Toxoplasma* antibodies.

Serological tests - First, a screening was done using three serological tests (IHA, IFAT and ELISA), with serum dilutions at 1:32 and 1:64. The reactive sera at dilution of 1:64 were retested, in duplicate, at serial twofold dilutions from 1:64 to the end-point titer.

An *IHA* was performed using a commercial kit, HAP Toxoplasmose (Salck Ind. Com. Prod. Biológicos Ltda., São Paulo, Brazil), according to the manufacture instructions. All reactive serum samples by the screening (1:64) were also retested after treatment with 2-mercaptoethanol (2-ME) in order to verify the presence of IgM antibodies (Camargo et al. 1978). Thus, a higher or two-titer decrease observed in the treated serum samples was indicative of IgM presence or active infection.

The *IFAT* for detecting IgG antibodies to *T. gondii* was similar to that used for diagnosis of human infections (Camargo 1964). Briefly, microscopic slides containing formolized *T. gondii* tachyzoites were incubated with goat serum samples for 30 min at 37°C. In parallel, positive and negative sera previously determined by IHA, were included as controls of reaction. An isothiocyanate fluorescein (FITC) labeled rabbit IgG anti-goat IgG (prepared as described by Clark & Shepard 1963) was used as secondary antibody and the optimum titer (1:50) was determined by block titration with positive and negative serum controls. After incubation for 30 min at 37°C, the slides were washed in 0.01M phosphate-buffered saline (PBS), pH 7.2, and then mounted in buffered glycerol pH 8.5. The preparations were examined by epifluorescent microscope (Olympus, Mod BH2, Tokyo, Japan).

An *ELISA* was carried out for the detection of IgG antibodies to *T. gondii* as described by Mineo et al. (1980), with some modifications. Briefly, polystyrene microtiter plates (Interlab, São Paulo, Brazil) previously coated with $1x10^5$ *Toxoplasma* tachyzoites/well were washed three times in PBS containing 0.1% Tween 20 (PBS-T). Goat serum samples were added (50 µl/well) and incubated at 37°C for 45 min. Positive and negative serum controls previously determined by conventional serological tests (i.e., IHA and IFAT) were included on each plate. Washings in PBS-T were made between the steps of the reaction. Further, peroxidase-labeled rabbit IgG anti-goat IgG (prepared as described by Wilson & Nakane 1978) was added (100 µl/well) at 1:3,000 dilution in PBS-T for 1 h at 37°C. Next, enzyme substrate consisting of 0.03% hydrogen peroxide and ortophenylenediamine (OPD - Merck, Germany) in 0.1M citrate-phosphate buffer, pH 5.0 was added (50 µl/well) and incubated for 10-15 min at room temperature. The reaction was stopped by adding (25 µl/well) 2N H_2SO_4 and the absorbance was determined in ELISA reader (Titertek Multiskan Plus, Flow Laboratories, USA) at 492 nm. The cut off was established as the mean absorbance values of negative controls plus three standard deviations (Richardson et al. 1983).

Positivity criteria for serological assays - The determination of the cut off titers for IHA, IFAT and ELISA were made based on reports about seroprevalence of *T. gondii* in sheep and goat, where at least one or two of these tests were used. Thus, serum samples that showed reactivity at dilution ≥ 1:64 were considered positive samples (O'Donoghue et al. 1987, Hashemi-Fesharki 1996, Nieto & Melendez 1998).

Statistical analysis - As the results followed a non-Gaussian distribution, antibody titers were transformed in $\log_2$ in order to determine the geometric means (GM) with 95% confidence intervals (CI). Differences between the prevalence of *T. gondii* IgG antibodies in different herds and ages obtained by the three serological assays were analyzed using the differences between two proportions by Z statistic. The correlation coefficients between the titers obtained by the IHA, IFAT and ELISA reactions were determined using Spearman's correlation test.

RESULTS

Table I shows the frequencies of antibodies to *T. gondii* in goats as determined by the IHA, IFAT and ELISA reactions. From a total of 174 goat serum samples, 33 (19%) were positive in the IHA

test with the following distribution: 3/71 (4.2%) in the herd I, 26/39 (66.7%) in the herd II, 3/37 (8.1%) in the herd III and 1/27 (3.7%) in the herd IV. Statistically significant differences were found only in the herd II ($p < 0.0001$). From the 33 positive serum samples retested after treatment with 2-mercaptoethanol, 10 (30.3%) had specific IgM antibodies, and 9 from these samples (90%) were coming from the herd II. By IFAT, 34/174 (19.5%) serum samples were positive, thus distributing in the different herds: 3 (4.2%) in I, 27 (69.2%) in II, 2 (5.4%) in III and 2 (7.4%) in IV. Similarly to the IHA test, no statistically significant differences were found in the herds I, III and IV, except for the herd II ($p < 0.0001$). In the same way, 34/174 (19.5%) goat serum samples were positive in ELISA with frequencies of 4.2%, 71.8%, 5.4% and 3.7% in the herds I, II, III and IV, respectively. Again, only the herd II demonstrated significantly higher frequencies of positive sera ($p< 0.0001$).

The results based on age distribution showed a significantly higher seroprevalence of *T. gondii* antibodies (41.1%, 42.9% and 44.6%, respectively in IHA, IFAT and ELISA) in older animals (> 36 months) as compared with younger ones ($p < 0.0001$) (Table I).

Table II demonstrates the distribution of antibody titers to *T. gondii* determined by IHA, IFAT and ELISA in goat sera from the different herds. The antibody titers varied from 64 to 4096 in the IHA test, and those more frequently observed were from 256 to 2048 (GM: 7.3; 95% CI: 7.3-9.1). The distribution of antibody titers obtained by IFAT demonstrated titers varying from 128 to 8,192, with the most frequent titer of 512 (GM: 8.7; 95% CI: 8.6-10.4). The antibody titers observed by ELISA varied from 64 to 4,096, with the titer of 2,048 found in higher frequency (GM: 8.7; 95% CI: 8.6-10.5).

As demonstrated in the Figure, significant correlations were found between the antibody titers obtained by IHA *versus* IFAT ($r = 0.6512$; $p < 0.0001$), IHA *versus* ELISA ($r = 0.6332$; $p < 0.0001$), and particularly between IFAT *versus* ELISA ($r = 0.7480$; $p < 0.0001$).

TABLE I

Seroprevalence of *Toxoplasma gondii* in goats as measured by IHA, IFAT and ELISA

		Positive samples[a] (%)		
	n	IHA	IFAT	ELISA
Herds				
I	71	3 (4.2)	3 (4.2)	3 (4.2)
II	39	26 (66.7)[b]	27 (69.2)[b]	28 (71.8)[b]
III	37	3 (8.1)	2 (5.4)	2 (5.4)
IV	27	1 (3.7)	2 (7.4)	1 (3.7)
Age (mo)				
0-12	14	0 (0)	0 (0)	0 (0)
12-36	104	10 (9.6)	10 (9.6)	9 (8.6)
> 36	56	23 (41.1)[b]	24 (42.9)[b]	25 (44.6)[b]
Total	174	33 (19)	34 (19.5)	34 (19.5)

a: positive samples in IHA, IFAT and ELISA (titer ≥ 64); *b*: $P < 0.0001$

TABLE II

Distribution of antibody titers to *Toxoplasma gondii* determined by the IHA, IFAT and ELISA tests in 36 positive serum samples of goats coming from four herds (I, II, III and IV) of the region of Uberlândia, MG. The titer 64 was considered cut off for the three tests

Herd	Sample no.	Antibody titers		
		IHA	IFAT	ELISA
I	1	256	512	128
	2	1024	1024	2048
	3	256	128	64
II	4	128	512	256
	5	512	512	2148
	6	256	1024	2048
	7	4096	2048	1024
	8	64	512	512
	9	1024	4096	2048
	10	128	512	4096
	11	256	256	256
	12	512	2048	2048
	13	64	8192	2048
	14	512	4096	2048
	15	128	512	256
	16	256	1024	512
	17	1024	512	2048
	18	2048	4096	2048
	19	1024	2048	2048
	20	1024	2048	2048
	21	256	512	2048
	22	512	1024	256
	23	512	256	2048
	24	2048	512	1024
	25	2048	4096	4096
	26	1024	1024	4096
	27	1024	4096	4096
	28	1024	8192	2048
	29	512	8192	4096
	30	NR	128	512
	31	NR	NR	64
III	32	512	1024	512
	33	2048	4096	4096
	34	64	NR	NR
IV	35	128	256	512
	36	NR	128	NR

NR: non-reactive

Seroprevalence of *Toxoplasma* infection in goats of Uberlândia was determined based on positive results obtained by the three tests (IHA, IFAT and ELISA). Thus, from a total de 174 goats, 32 (18.4%) were seropositive in all tests, and the distribution according to the herds was: I (4.2%), II (66.7%), III (5.4%) and IV (3.7%). Only 4 out of 174 serum samples showed discordant results, that is, were positive in at least one test.

When analyzing the distribution of antibody titers to *T. gondii* obtained by the three tests in the different herds, the titers varied from 64 to 2,048 (GM: 8.3; 95% CI: 7.2-9.7) in the herd I; 64 to 8,192 (GM: 9.0; 95% CI: 9.0-10.1) in the herd II; 64 to 4,096 (GM: 5.8; 95% CI: 4.6-11.2) in the herd III; and 128 to 512 (GM: 3.9; 95% CI: 1.8-9.2) in the herd IV. Antibody titers were significantly higher in the herd II as compared to the other herds ($p < 0.05$).

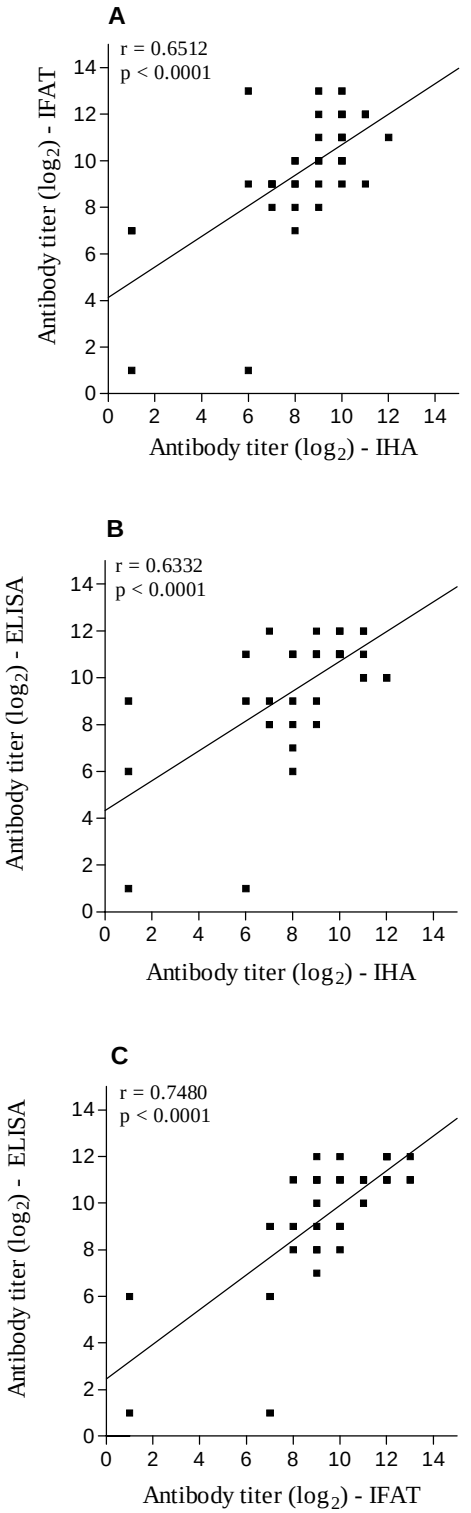
DISCUSSION

Toxoplasmosis in goats has been widely studied due to its importance to public health, since the dissemination of the parasite for man can occur either through the direct contact with domestic animals or by consuming products of animal origin. Thus, evaluation of serological tests becomes important in order to use sensitive and specific tests in serological surveys, since the majority of the infected animals are usually asymptomatic.

In the present study, the frequencies of anti-*T. gondii* antibodies detected by IHA (19%), IFAT (19.5%) and ELISA (19.5%) in goats of Uberlândia, revealed high indexes of positive and negative concordant results (97.7%). Also, high correlation coefficients among the three tests, particularly between IFAT and ELISA. Accordingly, Voller et al. (1976) described the high sensitivity and specificity of such techniques. Similar results were obtained in IFAT and ELISA for the detection of anti-*T. gondii* antibodies in experimentally infected ovine (Uggla & Nilsson 1987, O'Donogue et al. 1987)

In serological surveys on toxoplasmosis in sheep of South Australia, O'Donoghue et al. (1987) found significant correlation between the positivity rates obtained by IFAT (7.4%) and ELISA (9.2%). When using IFAT and ELISA for detecting anti-*T. gondii* antibodies in small ruminants (732 sheep and 526 goats) in Ghana, Africa, Van der Puije et al. (2000) demonstrated that ELISA was both highly sensitive (92%) and specific (91%) when compared to IFAT, which was used as a reference test.

When analyzing the antibody titers obtained in our study, high titers were predominantly found in IFAT and ELISA as compared to the IHA test, what



Correlation between the antibody titers obtained by IHA *versus* IFAT (A), IHA *versus* ELISA (B), and IFAT *versus* ELISA (C) in 36 positive serum samples of goats coming from four herds of the region of Uberlândia, MG.

is justified for greater sensitivity of the former techniques (Voller et al. 1976). High antibody titers were significantly found in the herd II, thus increasing the antibody titer mean determined in this region. High values of antibody titers can be ascribed to active toxoplasmic infection as well as reactivation of infection due to immunosupressor conditions (Robert et al. 1981).

The concordant results of high positivity by the three tests in the herd II suggest poor manage conditions, as contaminated food, presence of cats, infected male, and the origin of the animals (Dubey 1996).

In the same way, the three techniques revealed a higher frequency of anti-*T. gondii* antibodies in older animals, similarly to the findings of Nieto and Melendez (1998) that found 6.7% positivity by IHA in goats older than four years of age and no positivity in animals younger than 12 months of age. Such fact is due to increasing opportunities of exposure to several sources of *Toxoplasma* infection with age. In our study, no positivity was found in animals between 0-12 months of age. It is noteworthy that all animals included in this age range were older than seven months and thus had not maternal passive immunity remaining.

The presence of positive IgM samples mainly found in the herd II suggests recently acquired or active infection, demonstrating that these animals can constitute an important source of transmission to man, since they are able to present *T. gondii* tachyzoites in their body fluids, as milk.

The 18.4% seroprevalence of *T. gondii* found in goats of Uberlândia was lower than those obtained by several authors in goats from different regions of the world. Prevalence values of 28.9% in the State of Bahia, Brazil (Gondim et al. 1999), 31% in Uganda (Bisson et al. 2000), 63.3% in Canary Islands (Rodriguez-Ponce et al. 1995), and 92.4% in the metropolitan region of Belo Horizonte, MG, Brazil (Chiari et al. 1987) were already reported. On the other hand, our prevalence results were similar to the 19.3% level of infection found in Iran (Hashemi-Fesharki 1996) and higher than the 5.9% prevalence reported in the region of Lara State, Venezuela (Nieto & Melendez 1998).

Although the prevalence of *T. gondii* antibodies in goats from our region is lower than those mentioned regions, the infection can not be considered under control due to various factors: (1) it is an important zoonosis that causes abortion in humans and congenital abnormality in children (Dubey 1996); (2) the consumption of raw or undercooked goat meat contaminated with cysts (Pepin et al. 1997) or goat milk containing *T. gondii* tachyzoites (Chiari & Neves 1984) constitute an important source of human infection; (3) the several sources of infection for the goat herd as vertical transmission (Dubey 1996); (4) the economic losses that toxoplasmosis can lead to the herd due to abortion and stillbirths as well as pneumonia and changes in the reproductive and neural systems of susceptible animals (Dubey 1990); (5) the rearing of extensively managed goats, that is, under non-confined conditions can facilitate the contamination of feed with cat feces (Pepin et al. 1997). Therefore, caprine toxoplasmosis deserves special attention of the public health organizations in order to advise the population on the real situation of the infection in the country through frequent serological surveys, since this disease often occurs as subclinical form in man and animals.

Our data demonstrate that the serological assays studied in the present investigation could be useful, since the utilization of reliable methods for determining the prevalence of *T. gondii* infection in goats will allow the adequate management for the control of infection on the goat herds.

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