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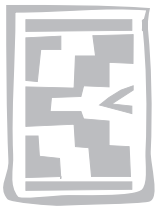

Seroprevalence of *Toxoplasma gondii* infection in goats and sheep in Zimbabwe

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ABSTRACT

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Seroprevalence rates of *Toxoplasma gondii* anti-antibodies in adult goats and sheep from different parts of Zimbabwe were determined. A total of 225 (67.9 %) of the 335 serum samples tested were positive for anti-*T. gondii* IgG antibodies with the indirect fluorescent antibody test. There were differences in antibody seroprevalences among communal land goats from the different agro-ecological zones (Natural regions IIb and III: 80 and 96.7 %, respectively; Natural region IV: 65.9 %; Natural region V: 45 %; and Natural region III had a significantly higher seroprevalence than IV and V. The highest seroprevalences found in Natural regions II b and III are likely to be linked to the existence of more households and hence the possibility of a higher concentration of domestic cats that increases the chances of environmental contamination with their faeces harbouring *T. gondii* oocysts. The seroprevalence rate in sheep from a large commercial farm (10 %) was significantly lower than that of sheep reared under the communal grazing system (80 %). Overall, significantly higher proportions of seropositive animals had antibody titres of 1:50 (34.2 % of 225) and 1:100 (44 % of 225) as compared to the 9.8 % and 12 % with antibody titres of 1:200 and ≥ 1:400, respectively.

Keywords: Goats, IFAT, IgG antibodies, public health implications, sheep, *Toxoplasma gondii*, Zimbabwe

INTRODUCTION

Zimbabwe has a high population of cattle and goats in comparison to the small population of sheep (Central Statistical Office 1999; 2002). The Central Statistical Office livestock census figures are given in Table 1. Small ruminants are mostly reared by farmers in communal land areas in the drier agro-ecological regions of the country [Natural regions III, IV and V]. These regions are characterised by low annual rainfall (< 650 mm) and very little pasture (overgrazed) and are mostly suitable for semi-extensive farming. Small-holder farmers in the intensive-farming Natural region II b (annual rainfall: 700–1 050 mm) also keep goats among other livestock on very limited pasture. All these areas rely on a communal free-range livestock grazing system (Moyo, O'Keefe & Sill 1993; Muir 1994) in which animals are kept in pens overnight and share communal grazing fields owned by the state during the day. Goats are a valuable resource for improving food security in dry areas in Zimbabwe. Ninety-nine percent of goats in Zimbabwe are managed under the traditional husbandry system in which they are wholly dependent on rangelands for nutrition, with no supplementary feeds during the dry season (Sibanda 1993). The indigenous Matabele

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and Mashona breeds are the predominant type of goats in these communal land areas (Chifamba, Khombe & Sithole 1993). Most sheep on commercial properties are found around Harare in Natural region II a (wet area, with adequate pasture) and are reared under extensive grazing systems.

Toxoplasmosis is a common cause of abortion and mortality in sheep and goats in many parts of the world (Dubey & Towle 1986; Dubey & Beattie 1988). Sheep and goats from different parts of the world have also shown high prevalences of antibodies against *Toxoplasma gondii* as they are kept permanently on pasture (Tenter, Heckeroth & Weiss 2000). Among the ruminants, *Toxoplasma* tissue cysts are mostly found in sheep and goats and rarely in farmed deer, cattle and water buffalo (*Bubalis bubalis*) (Dubey & Beattie 1988; Tenter *et al*. 2000). Cattle seem to be able to reduce the number or even eliminate the parasite cysts from their tissue (Sanger, Chamberlain, Chamberlain, Cole & Farrel 1953; Catár, Bergendi & Holková 1969; Dubey & Thulliez 1993). The most important source of *T*. *gondii* infection for strict herbivores such as sheep and goats kept mostly on pasture is the oocysts shed by cats in their faeces (Blewett & Watson 1983).

The only documented study on *T*. *gondii* seroprevalence in small ruminants in Zimbabwe was by Pandey & Van Knapen (1992) who reported seroprevalence rates of 9.2 % and 10 % in adult sheep and goats, respectively, using an indirect ELISA test. The purpose of the present study was to determine the prevalence of *T*. *gondii* infection in adult domestic small ruminants from different agro-ecological zones (Natural regions) of Zimbabwe.

MATERIALS AND METHODS

Sampling sites

The study was carried out on adult goats and sheep from six districts shown in Fig. 1. The number of samples collected from each district is given in Table 2.

Sample collection

The majority of the serum samples were collected during 1999–2000 from three communal land areas of Zimbabwe with the highest goat population. Cluster sampling was used to select the goats to sample. Animals over a year old were bled from the jugular vein into plain Vacutainer tubes. Blood was transported on ice to the laboratory where the serum was separated and kept at –20 °C until used. A few samples were collected from sheep and goats that were culled at the University of Zimbabwe campus, in suburban Harare and from sheep and goats slaughtered at a small ruminant abattoir close to Harare during 2000–2001.

Immunoassay

Sera were screened for anti-*T*. *gondii* IgG antibodies using the indirect fluorescent antibody test (IFAT). Cell culture-derived parasites of the Danish *T. gondii* strain SSI 119 (Work 1968; Pettersen 1977) were used as antigen. Multi-well slides coated with whole parasite antigen were incubated with test sera for 45 min at room temperature in a moist chamber. After incubation, they were washed three times for 5-min periods in neutral phosphate buffered saline (PBS) and air dried. Each well was covered with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-goat (DAKO), at a dilution of 1:50, incorporating 0.01 % Evans Blue dye and incubated for 45 min. The slides were then washed as described above and mounted in buffered glycerol. Each sample was tested in duplicate, and positive and negative controls were included on each slide. Fluorescence at a serum dilution of $\geq$ 1:50 was considered as positive. The optimum concentration of the conjugated secondary antibody and the cut-off titre were determined by back titration using known negative and positive sera.

Statistical analysis

Serological results for the different districts were compared using the Chi-square test. A probability (*P*) value of < 0.05 was considered significant.

TABLE 1 Livestock census, Zimbabwe (summarised from 1999 and 2002 reports of the Central Statistical Office)

Animal species	Commercial farms (2002)	Communal lands (1999)
Beef cattle	708 036	3 975 429
Dairy cattle	50 731	–
Goats	13 840	4 339 123
Sheep	34 307	250 185
Pigs	97 996	139 867

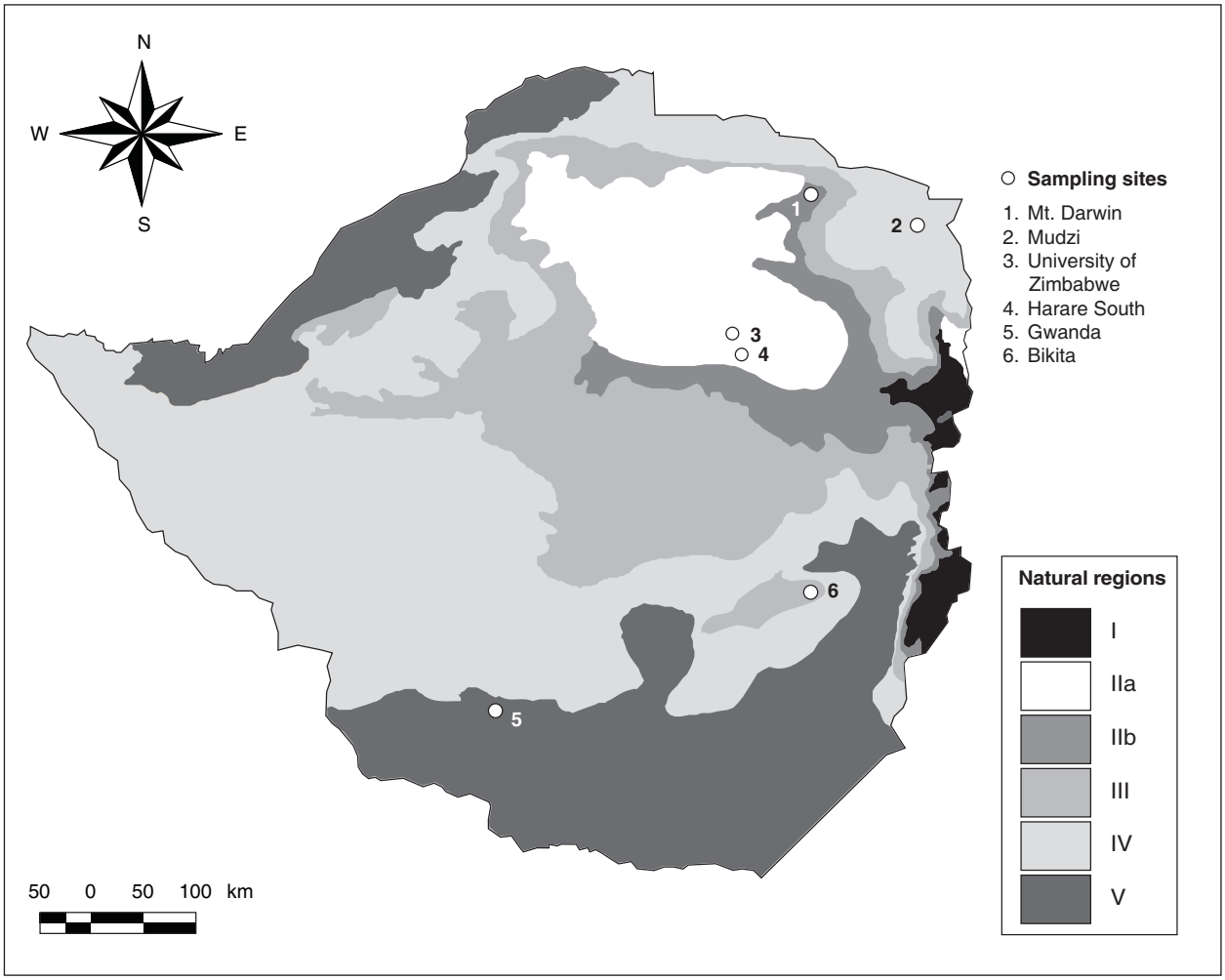

FIG. 1 Map of Zimbabwe showing the centres of the districts from where the sheep and goats sampled originated

TABLE 2 Prevalence of anti-*Toxoplasma gondii* antibodies in goats (and some sheep) from different natural regions of Zimbabwe in an IgG IFAT

Natural region	District	Sampling site	Host	Sample	Positive (%)	Distribution of positives according to titre (%)			
						Antibody titre			
						1:50	1:100	1:200	1:400
IIa	Harare	Commercial farm	Sheep	10	1 (10)	1 (100)	0	0	0
IIa	Harare	University	Sheep	3	2 (66.7)	0	0	0	2 (100)
			Goats	1	1 (100)	0	0	0	1 (100)
IIb	Mt. Darwin	Subsistence	Goats	20	16 (80)	12 (75)	2(12.5)	1 (6.25)	1 (6.25)
III	Bikita	Subsistence	Goats	91	88 (96.7)	20 (22.7)	44 (50)	8 (9.1)	16(18.2)
IV	Mudzi	Subsistence	Goats	91	60 (65.9)	26 (43.3)	25 (41.7)	7 (11.7)	2 (3.3)
V	Gwanda	Subsistence	Goats	109	49 (45)	12 (24.5)	27 (55.1)	6 (12.2)	4 (8.2)
			Sheep	10	8 (80)	6 (75)	1 (12.5)	0	1 (12.5)
Gwanda subtotal				119	57 (47.9)	18 (31.6)	28 (49.1)	6 (10.5)	5 (8.8)
Total				335	225 (67.2)	77(34.2)	99 (44)	22 (9.8)	27 (12)

TABLE 3 Comparison of *Toxoplasma gondii* seropositivity rates in small ruminants from different communal land districts using the Chi-square test

Districts compared	Degrees of freedom	Chi-square value	*P* value
Mt. Darwin/Bikita	1	7.74	0.05
Mt. Darwin/Mudzi	1	1.50	0.22
Mt. Darwin/Gwanda	1	6.00	0.01
Bikita/Mudzi	1	28.36	1.00927×10^{-7}
Bikita/Gwanda	1	51.43	7.41478×10^{-13}
Mudzi/Gwanda	1	4.64	0.03
Commercial farm/Gwanda (sheep only)	1	9.90	0.002

TABLE 4 Comparison of seropositive small ruminants at each individual antibody titre using the Chi-square test

Titres compared	Degrees of freedom	Chi-square value	*P* value
50/100	1	2.75	0.10
50/200	1	30.55	3.24441×10^{-8}
50/400	1	24.04	9.44304×10^{-7}
100/200	1	49.00	2.55963×10^{-12}
100/400	1	41.14	1.41499×10^{-10}
200/400	1	0.51	0.48

RESULTS

The seroprevalence results are summarized in Table 2. The south-western district of Bikita had the highest percentage of positive animals (96.7) followed by Mt. Darwin (80). Prevalences of 47.9 % and 65.9 % occurred in Gwanda and Mudzi, respectively. Seroprevalence in the Natural region III district of Bikita was significantly higher than that found in the Natural region IV and V districts of Mudzi and Gwanda, respectively. That of Mudzi district (Natural region IV) was also significantly higher that that of Gwanda district (Natural region V) (Table 3). There were no significant differences between seroprevalences in the Natural regions IIb and III districts of Mt. Darwin and Bikita, respectively and between Mt. Darwin and the Natural region IV district of Mudzi. The prevalence observed in the sheep from the commercial farm (Natural region IIa) was the lowest (10 % of 10) compared to that found in sheep from Gwanda (80 % of 10) and from goats from the other sampling sites. Most of the positive animals had antibody titres of 1:50 (34.2 %) and 1:100 (44 %) whilst only 22 (9.8 %) and 29 (12 %) had antibody titres of 1:200 and $\geq$ 1:400, respectively. The three animals that tested positive at the University pens had antibody titres of $\geq$ 1:400. There were no significant differences in the overall proportion of animals positive at the 1:50 and 1:100 titres and at the 1:200 and 1:400 titres but there were significant differences with the other titre comparisons (Table 4).

DISCUSSION

Previous work has shown that there is a high correlation in results obtained in *T. gondii* seroprevalence studies in sheep and goats using the modified agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA), IFAT and indirect haemagglutination test (IH) (Seefeldt, Kirkbride & Dubey 1989; Figueiredo, Silva, Cabral & Mineo 2001).

In this study, a high overall anti-*T. gondii* antibody seroprevalence (67.2 %) in adult sheep and goats in Zimbabwe was determined. Pandey & Van Knapen (1992) found low prevalences of *Toxoplasma* infection of 9.2 % and 2.9 % in adult sheep and goats, respectively from different parts of Zimbabwe using an indirect ELISA (cut-off titre: > 1:64) but the actual origin of the animals sampled is not stated. Neighbouring Botswana had a seroprevalence of 10 % in the sera of farmed goats with a history of abortion collected in 1994–1996 using the IHAT (Binta, Mushi, Raborokgwe & Ndebele 1998). The lower national prevalence in Botswana could be because it has a semi-desert climate with extensive grazing systems. It has also been demonstrated that *Toxoplasma* oocysts only sporulate where there is sufficient humidity (Dubey 1986) and can only survive short periods of dehydration (Frenkel 2000).

Differences in *T. gondii* infection prevalences were found in animals reared under different natural regions and managemental conditions in the pres-

ent study. Though the number of sheep examined was low as compared to that of goats, there is some indication that *T. gondii* seroprevalence is significantly lower in the sheep that originated from the commercial farm (10 %) as compared to that of sheep from communal land areas (80 %) (P = 0.002, χ^2 = 9.9). Animals on commercial farms in Natural region II a are reared on large tracts of land where wild cats are usually absent and domestic cats are restricted to and around farmhouses.

The generally high prevalences in goats and sheep from the overgazed communal land villages are probably due to the fact that in these regions there is animal, crop and human pressure on the land and animals basically graze around households where there is likely to be the highest concentration of domestic cat faeces. Natural regions IIb and III had higher prevalences than regions IV and V, probably because they receive a higher rainfall, are more arable and have more households per unit area, and therefore probably have a higher percentage of domestic cats that increase the chances of contamination of the environment with *Toxoplasma* oocysts. Van der Puije, Bosompem, Canacoo, Wastling & Aknomori (2000) found significantly lower seroprevalences in the drier Guinea savannah of Ghana as compared to the wetter coastal savannah and the forest zones.

The serum samples from the sheep and goats of the University of Zimbabwe were convenient. Though their number is small, they were included because the results obtained demonstrate the high exposure to *T. gondii* at a site which is frequented by a large number of feral cats. A high seroprevalence in an urban setting was also found in goats from urban Kampala, Uganda (Bisson, Maley, Rubaire-Akiiki & Wastling 2000). Binta *et al*. (1998) also detected higher prevalences in the mining towns of Selibe-Phikwe (46.2 %) and Jwaneng (22.9 %) in Botswana as compared to a national seroprevalence of 10 %.

Six of eight Zimbabwean toxoplasma isolates characterized in a related study (Hove, unpublished results) originated from slaughter sheep and goats; parasite isolation being successful in those animals that had antibody titres of ≥ 1:400. Although *T. gondii* has been isolated from seronegative hosts, the level of success in isolation has been shown to increase in proportion to increasing levels of anti-*T. gondii* antibody titres (Waldeland 1976; Dubey, Thulliez & Powell 1995; Lind, Haugegaard, Wingstrand & Henriksen 1997). Overall, there were no significant differences between the 1:50 and 1:100 titre groups and 1:200 and 1:400 titre groups in this study as indicated by the insignificant differences in the proportion of animals that were positive at each titre. However, the distribution of positives with the lower titres of 1:50 and 1:100 varied considerably among some districts but there were limited differences among districts at the higher titres (P values not shown). These titre relationships could be very useful in future sampling strategies.

The study demonstrated a very high prevalence of anti-*T. gondii* antibodies in goats and sheep sera from communal land areas of Zimbabwe. There is a high potential of transmission of *T. gondii* to humans if goat meat or mutton is consumed under-cooked as seroprevalence is usually indicative of presence of tissue cysts in the meat of animals. However, Rivieré (1993) found that generally most goat meat consumers in Zimbabwe did not ask for tender meat from young animals to roast but preferred meat from older animals for stewing. If this is the case, then stewing should render *T. gondii* tissue cysts unviable. Goat milk is traditionally not consumed in Zimbabwe but there are projects promoting the consumption of milk from Saanen goats crossbred with local breeds. It would be interesting to determine the prevalence of *T. gondii* antibodies and the actual parasites in milk and other tissue fluids of these goats, as infected milk (with tachyzoites) could be of high risk to babies, expectant mothers and the immunocompromised.

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