Seroprevalence of toxoplasmosis in sheep in South Africa

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ABSTRACT

Serum samples from 600 sheep were collected from 5 different provinces randomly chosen in South Africa. Two sheep abattoirs (representing formal slaughter of sheep) and 1 rural location (representing informal slaughter of sheep) per province were also selected randomly. The serum samples were tested for anti-*Toxoplasma gondii* IgG antibodies using 2 different serological tests: an indirect fluorescent antibody (IFA) test and an enzyme-linked immunosorbent assay (ELISA) test available as a commercial kit. This study provides the first published data on seroprevalence of toxoplasmosis in sheep in South Africa, although positive titres have been found previously in wild felids, ferrets, chinchillas and a dog. Data on seroprevalence in sheep is considered important because consumption of mutton is universally considered to be a source of zoonotic transfer to humans. Seroprevalence in humans in South Africa was previously found to be 20 % and it is postulated that this may be linked to the informal slaughter and consumption of mutton. During this study, the overall national seroprevalence per province in sheep was found to be 5.6 % (IFA) and 4.3 % (ELISA), respectively. This is lower than in other countries, possibly because South Africa has an arid climate. Differences in seroprevalence in different areas studied suggested an association with the climate and a significant correlation ($P > 0.05$) was detected between the prevalence of *T. gondii* and the minimum average temperature. The seroprevalence was found to be significantly higher ($P < 0.01$) in sheep originating from commercial farms (7.9 %) than in rural sheep in the informal sector (3.4 %). Also, sheep managed extensively had a seroprevalence of 1.8 %, which was significantly lower ($P < 0.05$) than the seroprevalence in sheep under semi-intensive or intensive management systems (5.3 %). An incidental finding of interest was the considerable movement of sheep to abattoirs and mutton after slaughter. The highest consumption of mutton was in the Western Cape Province (29.9 %) while the highest concentration of sheep is found in the Eastern Cape Province (30.1 %).

Key words: seroprevalence, sheep, toxoplasmosis, veterinary public health.

Abu Samra N, McCrindle C M E, Penzhorn B L, Cenci-Goga B **Seroprevalence of toxoplasmosis in sheep in South Africa**. *Journal of the South African Veterinary Association* (2007) 78(3): 116–120 (En.). Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan with a large number of intermediate hosts, including all warm-blooded animals (mammals and birds) and humans, and felids are its definitive hosts[5]. Serological surveys have been done in various parts of the world and show that more than a third of the human population have antibodies against *T. gondii*. This high seroprevalence in humans demonstrates its importance as a zoonotic disease. This has even more relevance now than when most seroprevalence studies were originally carried out, because of the current high risk of toxoplasmosis for HIV positive people[5]. Ingestion of undercooked mutton is regarded as an important source of infection for toxoplasmosis in humans[16]. In South Africa a human seroprevalence of 20 % was detected in 1978 by Jacobs and Manson[8].

Despite the fact that there is some literature on the seroprevalence of toxoplasmosis in wild felids, chinchillas, ferrets, free-ranging wild felids, cheetah and a dog[1,3,4,12,20,21], there were no data on the seroprevalence in sheep in South Africa, although these data are available for many other countries[2,6,7,11,14,16,18,22]. Data on seroprevalence in sheep are considered important because consumption of mutton is universally considered to be a source of zoonotic transfer to humans[16]. The estimated worldwide seroprevalence of toxoplasmosis in livestock is; cattle 9 %, sheep 30 % and goats 15 %[5]. Although the infection in animals is often asymptomatic, abortion storms in sheep have also been decribed worldwide[6].

Sheep farming, for both wool and mutton, occupies approximately 590 000 km^2 of South Africa, which represents 53 % of all agricultural land[10]. The prevalence of *T. gondii* in humans in South Africa is likely to be related to the prevalence of seropositive cases in sheep as it is elsewhere in the world[16]. The actual distribution of toxoplasmosis in sheep in South Africa was not known, but it was important to study the situation, because of the high consumption of mutton in this country[10]. The aim of this study was to investigate the seroprevalence of *T. gondii* in sheep in South Africa.

MATERIALS AND METHODS

Five of the 8 provinces of South Africa were chosen randomly to undertake this study[17]. The provinces were KwaZulu-Natal, Eastern Cape, Western Cape, Gauteng and Free State. A list of sheep abattoirs was obtained from the Red Meat Abattoir Association. In every province 2 abattoirs were randomly selected for sampling. The sheep sampled at abattoirs represented the formal or commercial sector. One state veterinary district was selected per province from a list obtained from the National Department of Agriculture, so as to sample animals belonging to small-scale sheep farmers (representing the informal marketing sector). At the end of the study, a total of 600 serum samples was collected. Extra animals were sampled to compensate for problems encountered while transporting or handling the sera.

The origins of the sampled sheep were traced using records supplied by the abattoir managers. Traceability comprised both backward tracing to the location of the farm of origin as well as forward tracing to the location of the butchers that bought the carcasses for retail distribution and sale to consumers. It was presumed that most of the sheep from the informal sector would be

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slaughtered and consumed close to the rural area where they were located.

Information on proportional mutton consumption per province in South Africa was obtained from the Shoprite/Checkers Supermarket Chain (D Diemont, Division Meat Markets, pers. comm., 2006).

Blood collection

Blood (*c.* 4 m*ℓ*) was collected from the jugular vein of sheep at abattoirs (prior to slaughter) and on rural farms. The blood samples were transported on ice to the laboratory. After clotting, the samples were centrifuged at 3000 *g* for 10 min and the serum decanted. The serum specimens were stored at –20 °C before analysis in the Serology Laboratory of the Department of Tropical Disease, University of Pretoria, Onderstepoort.

Serological analysis

Two serological tests were used for the detection of antibodies to *T. gondii:* the enzyme-linked immunosorbent assay (ELISA) test and the indirect fluorescent antibody (IFA) test. The agreement between the 2 tests was calculated by using the kappa value, as κ = (OP–EP/(1–EP)[17].

For the ELISA test, a commercial enzyme immuno-assay (EIA) test kit (CHEKIT-Toxotest, IDEXX Laboratories, Dr Bommeli AG, Switzerland) was used. The IFA test slides contain the whole *T. gondii* tachyzoites fixed in formalin, distributed in 12 wells (Diagnostic & Technical Services, Randburg, South Africa). One negative and 1 positive control were used on each slide. The conjugate used was rabbit anti-sheep immunoglobin G (IgG), with affinity for purified antibody and fluorescently labelled (Kirkegaard & Perry Laboratories, Maryland, USA). Fluorescence at serum dilutions of 1:64 was considered positive. This was the titre used as standard dilution for the IFA test by the majority of similar studies[11,15,18,22].

Data were entered into Microsoft Excel® and then transferred to Epi-Info (Version 3.3.2., CDC Atlanta, 2005) for statistical analysis. Quantitative data were analysed for significant correlations, with province of origin, climate temperatures and management systems.

RESULTS

Sampling

A total of 600 samples was collected. Of these, 278 originated from abattoirs and 322 were collected from rural farms. Traceability: the origin of the sheep at abattoirs and their destination after slaughter, are shown in Table 1.

Table 1: **Origin of sheep and destination of meat.**

Location of abattoir	Origin of sheep	Destination of mutton
Ladysmith (KZN)	Ladysmith district (KZN)	Ladysmith Butchery (KZN)
Cato Ridge (KZN)	Summerville(EC) Zastron (FS)	Durban Butchery (KZN)
Krugersdorp(G)	Gauteng	Central Meat Market, Krugersdorp (G) Seemans, Randburg (G) Broederstroom Butchery (G)
Bukeret (Pretoria) (G)	Mpumalanga (MP)	Johannesburg Butcheries (G)
Graaff-Reinet (EC)	Graaff-Reinet (EC)	Graaff-Reinet Butcheries (EC)
Port Elizabeth(EC)	Grahamstown (EC)	Port Elizabeth Butcheries (EC)
	Steytlerville (EC)	
East London (EC)	Port Elizabeth (EC)	East London Butcheries (EC)
Paarl (WC)	Piketberg (WC) Malmesbury (WC) Moorreesburg (WC)	Cape Town Butcheries (WC)
Bethulie (FS)	Colesberg (NC) Bethulie (FS)	Bethulie Butchery (FS) Durban Butcheries (KZN)
Brandfort	Brandfort (FS)	Brandfort Butchery (FS)

Table 2: **Serological results from the ELISA test.**

Province	IFA Prevalence per province in %	ELISA Prevalence per province in %
KwaZulu-Natal	3.6	6.3
Eastern Cape	5.4	7.8
Western Cape	4.0	6.0
Gauteng	6.0	6.0
Free State	2.7	2.7

According to the Shoprite/Checkers Supermarket Chain, mutton consumption in various provinces of South Africa was highest in the Western Cape (29.9 %), followed by KwaZulu-Natal (24.1 %), Gauteng (21.1 %) and Eastern Cape (12.6 %). The lowest mutton consumption was found in the Free State (6 %) (D Diemont, Division Meat Markets, Shoprite/Checkers, pers. comm., 2006).

Analysis

The results of the serological tests are summarised in Table 2.

Using the ELISA test, the seroprevalence shows some variation between provinces, with a range of 2.7 % to 6.0 %. The mean seroprevalence for all the samples (n = 600) was 4.3 %.

Using the IFA test the seroprevalence per province also varied, with a range of 2.7 % to 7.8 %. The mean seroprevalence for South Africa, using the IFA test on the same serum samples (n = 600) was 5.6 %.

The agreement between the IFA test and the ELISA test was calculated as 77 %, using the Kappa Test[17].

The highest overall seroprevalence among sheep tested was found in commercial sheep (7.9 %, IFA), sampled at abattoirs, while the lowest occurred in sheep from rural farms (3.4 %, IFA). A highly significant difference ($P < 0.01$) between these 2 groups was found, with an Odds Ratio of 0.4 (Table 3).

The seroprevalence of antibodies

Table 3: **Association between different farm types.**

Type of sample	IFA		ELISA	
	Pos*	Neg*	Pos*	Neg*
Abattoir samples	22	257	15	264
Rural samples	11	309	7	313
Total	33	566*	22	577*

Key: Pos = positive tests; Neg = negative tests, *Difference because 10 sera were insufficient volume for 2 tests to be done.

Table 4: **Seroprevalence of anti-*Toxoplasma gondii* antibodies in sheep sampled from different climatic areas in South Africa.**

Province	Min average temperature (°C)	Max average temperature (°C)	% Infection
Gauteng	10.4	24.3	6
KwaZulu-Natal	14–17	25	6.3
Western Cape	8–14	23–25	6
Eastern Cape	14–17	20–23	7.75
Free State	8	20–23	2.7

Table 5: **Analysis of minimum mean daily temperature.**

Minimum mean daily temperature (°C)	ELISA		IFA	
	Pos*	Neg*	Pos*	Neg*
8–10	5	278	9	274
10–14	10	157	12	167
14–17	7	143	12	150
Total	22	578	33	567

Key: Pos = positive tests; Neg = negative tests.

against *T. gondii* found in sheep sampled in the 5 different provinces, with a different minimum average and maximum average temperature, is shown in Table 4.

A significant correlation ($P > 0.05$) was detected between the seroprevalence of IgG in sheep and the minimum average temperature (Table 5).

Sheep managed intensively had a seroprevalence of 1.8 %, which was significantly lower ($P < 0.05$) than the seroprevalence in sheep under semi-intensive management (5.3 %) (Table 6).

DISCUSSION

This study has shown the overall seroprevalence of *T. gondii* in sheep in South Africa to be 5.6 % (IFA test) and 4.3 % (ELISA). In neighboring countries, such as Zimbabwe, an overall seroprevalence of 67.7 % was found in adult sheep and goats[7] and in Botswana a seroprevalence of 10 % was detected in goats[2]. Compared with the data from Zimbabwe, *T. gondii* seroprevalence in South Africa is very low and closer to that of Botswana, which, like South Africa, is an arid country.

The consumption of mutton in South Africa is highest in the Western Cape, followed by KwaZulu-Natal and Gauteng (D Diemont, Division Meat Markets, Shoprite/Checkers, pers. comm., 2006). The high consumption of mutton in these 2 provinces is possibly due to cultural differences in meat consumption patterns of South African populations in different provinces[8].

The highest number of sheep in South Africa is present in the Eastern Cape (30.1 %), Northern Cape (25.3 %) and the Free State (20.4 %)[10]. There is no agreement between the consumption of mutton per province and the number of sheep per province: the Western Cape with the highest consumption of mutton has the lowest number of sheep, while the Eastern Cape which has the highest number of sheep has a consumption of only 12.6 %. Considering this information, the trace-back of sheep at the abattoirs can be explained. KwaZulu-Natal receives sheep for slaughtering from the Eastern Cape and the Free State, which are the 2 provinces with the highest numbers of sheep.

The study done on the seroprevalence of *T. gondii* infection in sheep and goats in Zimbabwe showed that sheep and goats originating from commercial farms had an 8 times lower seroprevalence (10 %) compared with sheep and goats originating from rural areas (80 %)[7]. It was expected that a similar situation would be seen in South Africa and therefore the difference in seroprevalence between rural and commercial farms was examined. As mentioned in the results, there was a significantly higher seroprevalence in commercial then in rural farms ($P < 0.01$). Only KwaZulu-Natal showed a higher level in rural communities: 8.2 % of the rural sheep population was seropositive compared with 4 % of sheep from commercial farms. This province would fit the picture given by the study done in Zimbabwe by Hove *et al.*[7], while the other provinces show the opposite picture; Eastern Cape (1.9 % rural and 11.5 % urban), Western Cape (2 % rural and 10 % urban) and Gauteng (3 % rural and 9.8 % urban).

The different seroprevalences in the 5 provinces studied could be due to the variations in temperature and humidity in South Africa, ranging from a dry climate in the northwest to a temperate climate in the centre and a humid tropical climate along the southern and eastern seaboard. The analysis of the minimum average daily temperatures in the 5 study areas revealed a significant correlation between the seroprevalence of *T. gondii* and this climatic factor ($P < 0.05$). The Free State, with the lowest seroprevalence, has a minimum average daily temperature of 8.6 °C, while the Eastern Cape, with the highest prevalence, has a minimum average daily temperature of 13 °C. Eastern Cape has the highest percentage of infected sheep, followed by KwaZulu-Natal, Western Cape and Gauteng. The lowest prevalence of infected sheep was found in the Free State. The coastal areas of the Eastern and Western Cape and KwaZulu-Natal have a mild and humid climate, giving good conditions for sporulation of *T. gondii* oocysts. This may explain the lowest percentage (2.7 %) of infected sheep found in the Free State, which comprises mainly grassland with a semi-arid climate, with the low minimum average temperatures, likely to be unfavourable for the sporulation of oocysts.

Previous epidemiological studies have found infections in sheep to be more prevalent in cool, moist areas than in hot, dry areas[13]. Hove *et al.*[7] found similar results in the study done in Zimbabwe: a significantly higher seroprevalence in the Mt Darwin and Bikita districts of Zimbabwe, which receive a higher rainfall, compared to the more arid Mudzi and Gwanda districts.

Table 6: **Comparison between different farm management systems.**

Farm management system	IFA		ELISA	
	Pos*	Pos*	Neg*	Neg*
Extensive	10	5	273	268
Semi-intensive	23	17	305	299
Total	33	22	578	567

Key: Pos = positive tests; Neg = negative tests, *Difference because 10 sera were insufficient volume for 2 tests to be done.

Similar studies have also been done by Van der Puije *et al.* who justified the higher prevalence found in the wet coastal savanna and the humid forest zones of Ghana, as a factor responsible for the higher prevalence in these areas, especially if compared with the drier Guinea savanna of Ghana[22]. As variations in humidity are also correlated to the seroprevalence of *T. gondii,* the relatively low rainfall in southern Africa might justify the much lower overall prevalence of *T. gondii* in sheep in South Africa. South Africa has an average annual rainfall of 464 mm, compared with a world average of 860 mm[19] and, in addition, South Africa is regularly hit by droughts, with the most recent one having occurred in 2004.

Another factor that increases the risk of exposure of sheep to *T. gondii* is the management system. Plant *et al.* suggested that the prevalence of infection was even more influenced by management factors than by environmental factors[14]. In their study, a higher prevalence was recorded in sheep kept under more intensive systems of management and they did not find a positive association between the seroprevalence of *T. gondii* in different geographical areas. Previous epidemiological studies have found that infections are more prevalent in sheep kept under intensive or semi-intensive systems of management[15,23]. Van der Puije *et al.* associated the higher seroprevalence of *T. gondii* in sheep to the intensive and semi-intensive farming system in Ghana[22]. An association between management and prevalence was also observed in Norway[16] and in the USA[15]. In Tasmania, Munday concluded that the seroprevalence of *T. gondii* was related to the management system[9]. A similar trend was found in the current study. The seroprevalence was high in the coastal areas and in the urban areas (Gauteng) where sheep-management practices were more intensive compared with the Karoo area and the Free State, which are characterised by their vast, extensive grazing areas. In a semi-intensive or intensive farming system in South Africa, the sheep are housed together entirely or partly during the day. A possible explanation for the higher prevalence in these systems, could be that domestic cats are more probably living near the food stores on such farms, where they are used for controlling rodents. For this reason sheep under semi-intensive or intensive farming conditions might be more exposed to *Toxoplasma* oocysts shed by cats. An experimental study was done by Plant *et al.* where an outbreak of ovine congenital toxoplasmosis was simulated by feeding sheep grain contaminated with cat faeces. Their study confirmed that in an intensive farming system, where cats are commonly kept on farms, feed could easily be contaminated with oocysts and be responsible for a rapid spread of infection in a flock[14].

CONCLUSIONS

The seroprevalence of *T. gondii* in sheep in South Africa is lower than expected.

South Africans consume a relatively large amount of mutton, especially members of the Muslim community[8]. Informal slaughtering is frequently practiced in rural areas. It is estimated that about 30 % of sheep in South Africa are slaughtered informally among the rural communities mainly composed of subsistence or small-scale farmers. In these communities, many family members participate in the slaughter process, the cleaning of the carcass or the preparation of the meat. Each person involved has a risk of becoming infected with *T. gondii.* In rural areas, the lack of running water and the habit of eating with the hands, further increases the risk of becoming infected with *T. gondii*. It is thus fortunate that, although that risk of exposure to the parasite in mutton is higher in the rural areas, the seroprevalence of toxoplasmosis was found to be lower than in commercial sheep slaughtered at abattoirs.

The consumption of undercooked mutton and handling of raw meet during the informal slaughter process is possibly the main source of *T. gondii* infection for humans in South Africa. The contact with cats is a lower risk factor to South Africans, because the number of feral cats in this country is relatively low. Informal interviews with state veterinaries and animal health technicians, as well as personal observations indicated that feral cats are less common in South Africa than in Europe. Cats kept as pets mainly live in good hygienic conditions in urban areas and are therefore not considered as a high risk factor. However, in order to prove this statement it would be very interesting to obtain more information on the seroprevalence of *T. gondii* in domestic and feral cats and the role they play in the epidemiology of toxoplasmosis in South Africa.

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REFERENCES

1. Bigalke R D, Tustin R C, Du Plessis J L, Basson P A., Mc Cully R M 1966 The isolation of *Toxoplasma gondii* from ferrets in South Africa. *Journal of the South African Veterinary Medical Association* 37: 243–247
2. Binta M G, Mushi E Z, Raborokgwe M, Ndebele T R 1998 The prevalence of antibodies to *Toxoplasma gondii* in goats with history of abortion. *Zimbabwe Veterinary Journal* 29: 47–50
3. Brink J D, De Wet J S, Van Rensburg A J 1975 A serological survey of toxoplasmosis in the Bloemfontein area. *South African Medical Journal* 49: 1441–1443
4. Du Plessis J L, Bigalke R D, Gurnell T O 1967 An outbreak of toxoplasmosis in chinchillas in South Africa. *Journal of the South African Veterinary Medical Association* 38(1): 79–83
5. Dubey J P 2004 Toxoplasmosis – a waterborne zoonosis. *Veterinary Parasitology* 126 (1-2): 57–72
6. Dubey J P, Towle A 1986 Toxoplasmosis in sheep. *Miscellaneous Publication – Commonwealth Institute of Parasitology* (*United Kingdom*), no. 10, CAB International, Slough
7. Hove T, Lind P, Mukaratirwa S 2005 Seroprevalence of *Toxoplasma gondii* infection in goats and sheep in Zimbabwe. *Onderstepoort Journal of Veterinary Research* 72(4): 267–272
8. Jacobs M R, Mason P R 1978 Prevalence of *Toxoplasma* antibodies in southern Africa. *South African Medical Journal,* 53: 619–621
9. Munday B L 1975 Prevalence of toxoplasmosis in Tasmanian meat animals. *Australian Veterinary Journal* 51: 315–316
10. National Department of Agriculture (NDA) 2003 Animal production-livestock numbers. Directorate Agricultural Statistics; http://www.nda.gov.za
11. O'Donoghue P J, Riley M J, Clarke J F 1987 Serological survey for *Toxoplasma* infections in sheep. *Australian Veterinary Journal* 64(2): 40–45
12. Penzhorn B L, Stylianides E, Van Vuuren M, Alexander K, Meltzer D G A, Mukarati N 2002 Seroprevalence of *Toxoplasma gondii* in free-ranging lion and leopard populations in southern Africa. *South African Journal of Wildlife Research* 32(2): 163–165
13. Plant J W, Freeman P, Saunders E 1982 Serological survey of the prevalence of *Toxoplasma gondii* antibodies in rams in sheep flocks in New South Wales. *Australian Veterinary Journal* 59: 87–89
14. Plant J W, Richardson N, Moyle G G 1974 *Toxoplasma* infection and abortion in sheep associated with feeding of grain contaminated with cat faeces. *Australian Veterinary Journal* 50: 19–21
15. Riemann H P, Willadsen C M, Berry L J, Behymer D E, Garcia Z V, Franti C E, Ruppanner R. 1977 Survey for *Toxoplasma* antibodies among sheep in Western United States. *Journal of the American Veterinary Medical Association* 171(12): 1260–1264
16. Skjerve E, Waldeland H, Nesbakken T, Kapperud G 1998 Risk factors for the presence of antibodies to *Toxoplasma gondii* in Norwegian slaughter lambs. *Preventive Veterinary Medicien* 35(3): 219–222
17. Thrusfield M V 2005 *Veterinary Epidemiology* (3rd edn). Blackwell Science, Oxford, UK

18. Trees A J, Crozier S J, Buxton D, Blewett D A 1989 Serodiagnosis of ovine toxoplasmosis: an assessment of latex agglutination test and the value of IgM specific titres after experimental oocyst-induced infections. *Research in Veterinary Science* 46(1): 67–72
19. Tyson P D 1986 *Climatic change and variability in southern Africa*. Oxford University Press, Cape Town, South Africa
20. Van Heerden J & Van Rensburg I B J 1979 Toxoplasmosis in a dog. *Journal of the South African Veterinary Association* 50(3): 211–214
21. Van Rensburg I B J, Silkstone M A 1984 Concomitant feline infectious peritonitis and toxoplasmosis in cheetah (*Acinonyx jubatus*). *Journal of the South African Veterinary Association* 55: 205–207
22. Van Der Puije W N A, Bosompem K M, Canacoo E A, Wastling J M, Akanmori B D 2000 The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Tropica* 76(1): 21–26
23. Waldeland H 1976 Toxoplasmosis in sheep. The reliability of a microtitre system in Sabin and Feldman's Dye Test. *Acta Veterinaria Scandinvica* 17: 426–431