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Sero-Prevalence of *Toxoplasma Gondii* Infection and Associated Risk Factors in Animals Presented to Sholla and Akaki-Kality Veterinary Clinics, Addis Ababa

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Keywords: toxoplasmosis, veterinary clinics, sero-prevalence, risk factors.

I. Introduction

Toxoplasma gondii infections are prevalent in humans and animals worldwide (Dubey and Beattie, 1988). Felids are the key animal species in the life cycle of this parasite because they are the hosts that can excrete the environmentally-resistant stage, the oocyst. Humans become infected postnatal by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease. It is unknown whether the severity of toxoplasmosis in immune-competent hosts is due to the parasite strain, host variability or other factors. Recently, attention has been focused on genetic variability among *T. gondii* isolates from apparently healthy and sick hosts. It has been 100 years since the discovery and naming of *T. gondii*. The parasite was first found in laboratory animals (Dubey, 2007). Its medical importance remained unknown until 1939 when *T. gondii* was identified conclusively in tissues of a congenitally-infected infant in New York City, USA (Wolf *et al*., 1939), and its veterinary importance became known when it was found to cause abortion storms in sheep in 1957 in Australia (Hartley and Marshall, 1957).

Although infection does not clinically affect cattle, transmission of infection to humans from tissue cysts when eating raw or undercooked beef should not be discounted. Toxoplasmosis may be important in Ethiopia where raw or partially cooked meat is regarded as a delicacy (Bekele and Kasali, 1989). In Ethiopia, there are documented reports on serological survey of Caprine toxoplasmosis by Teshale and his colleagues in Central and Southern Ethiopia (Teshale *et al.,* 2006). The serological survey results on toxoplasmosis by Negash and his associates further confirm the presence of *T. gondii* infection in sheep and goat population in Ethiopia (Negash *et al.,* 2004). The results of a questionnaire survey in Debre Birhan and the surrounding area revealed that abortion was the major cause of lamb loss during 12 months studied period (Getachew and Tilaye, 2002). In addition, the Sero-prevalence, assessment of its zoonotic importance and identification of factors associated with Sero-prevalence was documented in Nazareth town, Ethiopia (Negash *et al*., 2008).

Toxoplasmosis is recognized as disease of great economic importance since it causes heavy losses through abortion, stillbirth, neonatal mortality, encephalitis and pneumonia particularly in sheep and goats (Radostits *et al*., 2007 and Singh and Msolla, 1994). If animals are important in the epidemiology of human toxoplasmosis it is well to have information concerning serological study in those hosts (Morris *et al*., 2007). In the present paper, we summarize

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information on serological prevalence of *T. gondii* infection in different animals species presented Veterinary Clinics and economic impact of the disease in the study area.

II. Materials and Methods

a) Study Area

The study was conducted in Addis Ababa which lies at an altitude of 2000-3000 meters above sea level. The mean annual rainfall is 1800 mm with a bimodal pattern. There are dry and rainy seasons in the area. The long rainy season extends from June to September, contributes about 84% of the total annual rainfall while the dry season lasts from October to February. The short rainy season lasts from March to May. The study was conducted from November 2011-March 2012 in Sholla and Akaki-Kality Veterinary Clinics, Addis Ababa. The mean annual minimum and maximum temperature are 14^0C and 21^0C respectively with an average rate of 17^0C the mean relative humidity is 61.3% (CSA, 2009).

b) Study Population

The study included all animals which came to Sholla and Akaki-Kaliti Veterinary Clinics and consisted of bovine, ovine, caprine, swine, camel and equine species regardless of their age, breed and disease case. Blood sample for swine and camel was taken from Addis Ababa Abattoir Enterprise.

c) Study Design

A cross-sectional study was conducted from October 2011 to March 2012 to determine the prevalence toxoplasmosis among animals coming to Sholla and Akaki-Kaliti Veterinary Clinics for various health problems. After reviewing daily patient flow (case) to the clinic, the expected patient population in the study period was taken as a sampling frame. A simple random sampling method was used to collect blood sample from different animal species.

d) Sample Size Determination

The required sample size for the study animals was determined by the formula given by (Thrusfield, 1995) assuming 95% of confidence interval and at 5% desired precision. This was estimated with the assumed toxoplasmosis prevalence of 34.5% based on previous study by (Getachew and Tilaye, 2002) by taking the average prevalence of 34% (sheep) and 35% (goat) in Debre Birhan and the surrounding areas. Accordingly the desired sample size was 347.

e) Study Methodology

i. *Serum collection and serological examination*

Approximately 5ml of blood was taken from jugular vein of each study animal but for swine it was taken directly from heart in abattoir and the serum was separated and stored at deep freezer until tested. *Toxoplasma gondii* antibody was determined by the Slide Agglutination Test using a commercial kit (HUMA TEX TOXO, Human Gesellsfür Biochemica und Diagnostoica mbH Max-Plank-ring21.65205 Wiesbaden. Germany). This method is quick, simple and requires smaller quantity of reagents. Agglutination reactions are more sensitive than immuno-precipitation tests. The tests are simple and have an added advantage of easily readability (Chauhan and Agarawal, 2006).

Comparable assessment of slide agglutination test shows that as sensitive as, and a more specific than latex agglutination test. The predictive value of a negative slide agglutination test is less than the latex agglutination test but produced results within minutes, although, quantitative results is not comparable to other assays. Slide agglutination presents a rapid alternative to the latex agglutination test as a screening assay toxoplasmosis, although patients at risk of life threatening infection require detailed serological examination using additional methods (Dunford and Johnson, 1991). In this test a clean dry glass with 6 cells was taken and a drop of antigen suspension was placed over the middle of area of each 6 cell and one drop of positive control serum (goat) in one of cell while the negative control serum was placed on the other cell and one drop of test serum was placed on the rest 4 cells. Then mixed with separate disposable sticks and spreader the fluid over the entire area of the particular cell the slide was tilted back and forth of 4 minutes so that rotates slowly inside the cell. Finally it was observed for clamping (agglutination) by naked eye and magnifying lenses in comparison with the two controls (positive and negative). The negative result was identified as negative control result which did not form agglutination (homogenous appearance) but distinct agglutination was indicator for positive toxo-Ab of at least 4 IU/ml similar with positive control.

ii. *Questionnaire Survey*

A pre-tested structural questionnaire was prepared to animal owners with respect to the case they brought which included both open ended and closed ended questions. The questions was concerned with hygiene, environment, management, nutrition, reproductive disorder history and nervous signs, ownership of cats, purpose of cat keeping as well as the mechanism of cat feces disposal. In addition, the habit of exposure of raw meat and milk were important questions that gave useful information for epidemiology of human toxoplasmosis.

f) Data Analysis

All data obtained from the study were entered into Ms Excel 2007 data sheet and analyzed using STATA 11, statistical software programme. The Sero-prevalence was calculated later on by dividing the sera were found positive to slide agglutination test to the total sample size multiplied by 100. The risk factors associated with toxoplasmosis were determined using

percent values and using Pearson's Chi-square ($\chi 2$). A statistically significant association between variables was said to exist if the calculated level of significance is less than 5% ($p<0.05$) at 95% confidence level. The strength of associations between the exposure to the risk factors and sero-positivity is measured using odds ratio (Wasserthiel-smaller, 1995).

III. Results

a) Sero-prevalence

The overall Sero-prevalence rate of the test result were found in 126 of the 347(36.31%) animals (table 1) (6 different species of animals) examined for slid agglutination test (CI=31.22, 41.40, 95% level of confidence). Sero-prevalence by origin, age, species, management system, hygiene, reproductive abnormality, cat ownership as well as associated clinical findings is not significant while a statistically significant difference($p<0.05$). Sero-prevalence among males than females being observed. Higher prevalence was observed in males and females (table 5).

b) Risk Factors Associated with Sero-positivity

Factors closely related to the natural history of toxoplasmosis are presence of cats, origin, history of abortion or neonatal mortality or births of weak lambs and reproductive abnormalities, management practices, hygiene and clinical finding. These factors and its association with sero-positivity ($p>0.05$) is explained in (table 2,3,4,6,7,8,9, 10). Breed was not included in the analysis since most farmers in the area had local breeds.

c) Result of the Questionnaire Survey

A questionnaire survey was conducted on 100 livestock owners revealed that during the previous months lamb loss amounted to 60% (30% abortion, 12% stillbirth and 18% neonatal mortality). Birth of weak lambs amounted to 20% while reproductive abnormalities were 31% (17% dystocia, 8% retained fetal membrane and 6% endometritis). Seventy two respondents confirmed the presence of cats in their house hold kept for clearing rodents. Only 10% of interviewed individuals reported disposal of cat feces by burying in the ground. Thirty percent of them reported the disposal of cat feces on the backyard or grazing land, which increased the risk of exposure to toxoplasma oocyst and cats had close contact with most family members. The survey also showed that 83% of the interviewed people had a history of consumption of raw or under cooked meat. Sixty five percent of them had animals with poor hygienic management in their grazing area and drinking water. Fifty four percent of these owners informed that their livestock had a contact with dead animal carcass, which is improperly disposed. Thus further had contract the infection through ingestion of oocysts from these areas is a probable source of toxoplasmosis. In addition livestock owners revealed that there were wild cats coming to the grazing area of their livestock which act as a definitive host.

Table 1: Overall relative prevalence of toxoplasmosis in different species

Species	No. of animals examined	Positive	Prevalence (%)
Ovine	194	71	55.91
Caprine	40	15	11.53
Bovine	44	11	12.68
Swine	40	19	11.53
Equine	20	7	5.76
Camel	9	3	2.59
Total	347	126	100.00

χ^2= 1.2429 P=0.537

Table 2: Association between prevalence and origin of animals

Origin	No of examined	No of positive
Sholla vet clinic	74(21.33%)	28(22.22%)
Akaki Kaliti	215(61.96%)	75(59.22%)
A.A Abattoir Enterprise	58(16.71%)	23(18.25%)
Total	347(100.00%)	126(100.00%)

χ^2=0.5445 P=0.762

Table 3: Association between Sero-prevalence and reproductive loss

Reproductive loss	No of examined	No. positive (%)
Present	269(77.52)	95(75.40)
Absent	78(22.48)	31(24.00)
Total	347(100.00)	126(100.00)

χ^2=0.5126 p = 0.474

Table 4: Association between prevalence and cat ownership

Cat ownership	No of examined	No. positive (%)
Present	113(32.56)	35(27.78)
Absent	234(67.44)	91(72.22)
Total	347(100.00)	126(100.00)

χ^2=2.0645 p=0.151

Table 5: Association between Sero-prevalence and sex

Sex	No of examined	No. positive (%)
Male	170(48.99)	76(60.32)
Female	177(51.01)	50(39.68)
Total	347(100)	126(100.00)

χ^2=10.1556 p =0.001

Table 6: Prevalence on the basis of age

Age	No of examined	No. positive (%)
Old	106(30.55)	34 (26.98)
Adult	149(42.94	56(44.44)
Young	92(26.51)	36(28.57)
Total	347(100.00)	126(100.00)

χ^2= 1.2429 p = 0.537

Table 7: Prevalence on the basis of sex and species

Species	Sex	No of examined	No. Positive	Prevalence (%)
Ovine	Male	76	36	47.37
	Female	118	35	29.66
Caprine	Male	29	9	31.03
	Female	11	6	54.55
Bovine	Male	26	8	30.77
	Female	18	3	16.67
Swine	Male	31	18	58.06
	Female	9	1	11.11
Equine	Male	7	4	57.14
	Female	13	3	23.08
Camel	Male	1	1	100.00
	Female	8	2	25.00

Table 8 : Association between prevalence and management system

Management	No of examined (%)	Positive (%)
Extensive	228(65.71)	147(66.72
Semi intensive	119(34.29)	74(33.48
Total	347(100.00)	221(100.00)

χ^2=0.1771 p=0.674

Table 9: Association between prevalence and hygienic condition

Hygiene	No of examined (%)	Positive
Poor	151(43.52)	55(43.65)
Good	196(56.46)	71(56.35)
Total	347(100.00)	221 (100.00)

χ^2=0.00125 p=0.969

Table10: Association between prevalence and clinical finding

Clinical Finding	No of examined (%)	Positive (%)
Respiratory	116(33.43)	38(30.16)
GIT	69(19.88)	20(15.87)
Nervous	2(0.58)	2(1.59)
Skin and Mucosal	38(10.95)	16(12.70)
Metabolic	1(0.29)	1(0.79)
Poisoning (Toxicosis)	1(0.29)	0(0.00)
Normal	103(29.68)	44(34.92)
Traumatic Wound	3(0.86)	2(1.59)
Reproductive	14(4.03)	3(2.38)

IV. Discussion

The overall Sero-prevalence of 126(36.1%) in the 347 study animals of different six species .Of which ovine (56.35%), caprine (11.9%), bovine (8.73%), swine (15.08%), equine (5.56%) and camel (2.38%) lies midway between the three previous studies in Ethiopia. The overall prevalence of 36.6% in sheep in this study lies between the three previous studies in Ethiopia. Bekele and Kassali, Getachew and Tilaye, and Tamiru reported Sero-prevalence of 22.9%, 33% and 54.7% in sheep, respectively. This is in agreement with studies in other African countries with prevalence rates ranging from 11.5% to 34% (Deconinck *et al.,* 1996). In goats the overall Sero-prevalence in this work was higher than the results of the two previous studies in Ethiopia with prevalence of 11.9% and 26.7% but less than those reported from other African countries with prevalence rates ranging from 31.9% to 63% (Tamiru, 2000). On the other hand, the finding agrees with recent study reports of Getachew and Tilaye of 35% in goats (Getachew and Tilaye, 2002). In bovine the overall Sero-prevalence (25%) out of 44 cattle examined. Thought number of animals studied were not proportional relative to previous studies, it is higher prevalent than the reports of Bekele and Kasali (2002) who reported a prevalence of 6.6%. In swine although those animals serum was taken from Addis Ababa abattoir and its number of sample size was not proportional relative to other species constitute the largest Sero-prevalence which is 47.5%. This result agrees with published reports in other parts of the world, the sero-positive prevalence in swine is 22% with a range of 0-97 % (Radostits *et al.,* 2007). The overall Sero-prevalence in equine species (horse and donkey) was 35%. The overall Sero-prevalence in camel (*Camelus dromedarius*) species was 33.3% which is high. This result revealed that a higher Sero-prevalence when compared with previous studies in three ecologically different areas of Sudan (22.2%) (Khali *et al.,* 2007).

In the present study, no statistically significant difference in Sero-prevalence was noted among different origin, species, age groups, management system, hygienic condition, reproductive abnormalities, cat ownership and the associated clinical finding. This seems contradictory with the established facts, however, it is difficult to made firm conclusion as number of study animals is low in proportion with these factors. The odd ratio of the four factors (management system, reproductive abnormality, hygienic condition and cat ownership) is explained in figure 2. The prevalence would have been significantly higher in warm and moist areas than in cold or hot dry areas, increased with age, in extensive (small holder) management system than intensive type, prevalent in cat ownership and a major cause of abortion and neonatal mortality. The disagreement of the assessment of risk factors associated with sero-positivity to *T. gondii* in addition to the above reasons could be due to lack of the specificity of the serological test used. The slight variation in the results of Sero-prevalence observed can be attributed to variation in ecological conditions as most animals came from different areas to Addis Ababa. Variation may also be due to the diagnostic technique utilized (Assadi-Rad *et al*., 1995). Even though the test used (slide agglutination test) was not done in Ethiopia, the results showed a higher Sero-prevalence than the previous studies it is evidenced that it is more sensitive and the result is valued in all species.

The results of the questionnaire survey indicated that economic loss due to abortion, stillbirth, neonatal mortality and related reproductive abnormality are important in the study area. It is also important that the inclusion of the questionnaire survey on ownership

of cats and purpose of cat keeping as well as the mechanism of cat feces disposal and the habit of raw meat and milk consumption. As oocysts are essential in the life cycle of *T. gondii* and in which both domestic cats and other felids may shed oocyst. These can contract the infection through contamination of grazing area as well as close contact with human (domestic cats) leading its public health hazard in addition to the owners habit of raw or under cooked meat as tissue cysts are the end stage of the parasite waiting to be eaten by animals and human (Morris *et al.*, 2007).The results of questionnaire survey based on its economic impact agrees with previous studies by Getachew and Tilaye in 2002 (abortion 30%, stillbirth 12%, neonatal mortality 18%, dystocia 17%, retained fetal membrane 8% and endometritis 6%). Seventy two (72) percent of the interviewed individuals had cats in their premises and kept for clearing rodents only together with livestock and almost all fed cats raw or under cooked meat. In general the maintenance of the cycle is achieved among the intermediate hosts, definitive host (cat) and environment (contaminated by infective stage of the parasite).

The Sero-prevalence in this study was significantly high both from public health and economic perspectives. Toxoplasmosis is a disease of economic importance as it is a major cause of abortion, stillbirth and neonatal mortality in sheep and goats (Getachew and Tilaye, 2002). Ovine abortion and neonatal mortality due to *T. gondii* are important problems in New Zealand, Australia, Canada, United States and the United Kingdom; in countries they are second in importance only to Chlamydia (Radiostits *et al*., 2007). Several studies conducted so far indicated that *T. gondii* infection in humans is widely distributed in most tropical countries (Negash, 2000). The high Sero-prevalence in the study animals and the results of the questionnaire survey ensured that Toxoplasmosis in Addis Ababa suggests a high risk to humans. The recent study in Adama town of Ethiopia is an evidence for its prevalence in that of 65% people examined for anti-*Toxoplasima gondii* antibodies by the MDAT, serologic evidence of Toxoplasmosis was found in 60% (39/65) (Negash *et al.,* 2008).

Statistical analysis revealed that there is significant association between males and females with males having higher Sero-prevalence than females. This is in agreement with findings by Getachew and Tilaye observed in goats (Getachew and Tilaye, 2002). This could be due to the fact male animals are stressed due to transport from different ecological areas and as most of them were kept for feedlot and breeding purpose for long time.

V. Conclusion and Recommendations

In general, the Sero-prevalence survey conducted in this study showed that toxoplasmosis is a widespread and well established infection among the six species (ovine, caprine, bovine, equine swine and camel) two veterinary clinics (Sholla and Akaki Kality) and Addis Ababa Abattoir Enterprise. The significance of toxoplasmosis as a disease of zoonotic importance and its economic impact was demonstrated. Therefore, prevention efforts should focus on educating cat owners about the importance of collecting cat feces in litter boxes, spaying cats, reducing the numbers of feral cats, cooking all meats, and promoting rigorous hand hygiene, reducing the numbers of wild rats is also important for control of toxoplasmosis. We think that further studies should be conducted to determine whether any host reservoirs exist amongst domestic and wild animals in this area, in which the disease was previously not found. This study will be the basis for further studies that will deepen our knowledge of the epidemiology of *T. gondii*. More extended studies are required to determine the sero-prevalence rates among populations of wild rats and other wild animals in difference areas, and the implications of *T. gondii* prevalence on both animal and human health.

Conflict of interest

The authors have no declared any conflict of interest

VI. Acknowledgements

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