Pakistan J. Zool., vol. 47(1), pp. 161-167, 2015.

Seroprevalence and Associated Risk Factors of Toxoplasmosis in Sheep and Goats in Pothwar Region, Northern Punjab, Pakistan

Nisar Ahmad,[1#] Zubaria Iqbal, Muhammad Mukhtar,[2] Muhammad Mushtaq,[2] Khalid Mehmood Khan[2] and Mazhar Qayyum[1*]

[1]Department of Zoology and Biology, Pir Mehr Ali Shah-Arid Agriculture University, 46300, Murree Road, Rawalpindi, Pakistan.

[2]Barani Livestock Production Research Institute, Kharimurat, Fateh Jhang, Pakistan

Abstract.- Present study was designed to find out the prevalence and associated risk factors of *Toxoplasma gondii* infection in sheep and goats present in northern parts of Punjab province, Pakistan. For this purpose serum samples collected from 413 sheep and 419 goats were tested for detection of anti-*Toxoplasma*-IgG and IgM antibodies by using enzyme linked immunosorbent assay. The results showed that overall prevalence of *T. gondii* antibodies in sheep and goats was 18.16% (75/413) and 14.32% (60/419) respectively. Prevalence of IgG antibodies was 15.49% (64/413) in sheep and 11.93% (50/419) in goats while IgM antibody prevalence was 3.39% (14/413) and 2.86% (12/419) in sheep and goats respectively. Infection was more common in female and older animals. Other statistically significant risk factors in sheep were poor hygienic conditions (OR = 4.91, $p<0.01$), presence of cats (OR = 2.08, $p<0.05$), extensive farming practice (OR = 2.19, $p<0.05$), flock size larger than 50 individuals (OR = 4.24, $p<0.01$) and pregnancy (OR =2.50, $p<0.05$). Similarly statistically significant risk factors in goats were poor hygienic condition (OR = 2.06, $p<0.05$), usage of outdoor water source (OR = 2.06, $p<0.01$), presence of cats (OR = 2.03, $p<0.05$), extensive farming practice (OR = 2.25, $p<0.05$) and flock sized larger than 30 (OR = 4.24, $p<0.05$) and 50 (OR = 6.82, $p<0.01$) individuals. Seroprevalence was significantly high in salt range sheep as compared to other breeds of sheep (OR = 5.51, $p<0.01$). Results indicate that *T. gondii* infection is widely prevalent in sheep and goats of northern Punjab, Pakistan and may have important consideration for livestock industry and public health.

Key words: Seroprevelance, *Toxoplasama gondii*, sheep, goats, serology, Pothwar region.

INTRODUCTION

Toxoplasmosis is a widely distributed parasitic infection in small ruminants caused by a protozoan *Toxoplasma gondii*. It is not only responsible for economic losses due to abortion and neonatal deaths in infected animals, but also have zoonotic significance as it may be transmitted to humans via contaminated meat and milk (Dubey, 1996; Pal *et al*., 1995,1996; Ghoneim *et al*., 2009). Sheep and goats may acquire infection by grazing on contaminated pastures and/or through drinking oocyst contaminated water of cat origin (Vesco *et al*., 2007). In small ruminants, toxoplasmosis is a leading source of abortion, still births and neonatal deaths, however, sub-clinical infection may also occur in adult animals (Buxton, 1990; Hassig *et al*., 2003). Congenital transmission occurs when female animals get infection during pregnancy and acute infection may cross placenta and multiply in fetal tissue resulting in abnormal development and abortion. Prevalence rates vary greatly in both species in different parts of the world mainly due to difference in farming practices and environmental conditions (Dubey and Beattie, 1988; Tenter *et al*., 2000).

Epidemiological studies of toxoplasmosis have been carried out in many countries owing to its importance in livestock sector (Figliuolo *et al*., 2004). In Pakistan, breeding of sheep and goats is an important source of milk and meat production. Apart from commercial husbandry practices, these animals are also reared by poor farmers because of their low cost maintenance and short term return. Studies conducted in southern parts of the country have confirmed the prevalence of *T. gondii* in sheep and goats (Ramzan *et al*., 2009; Lashari and Tasawar, 2010; Tasawar *et al*., 2011). No such report of *T. gondii* infection in small ruminants exists for northern Punjab (Pothwar Plateau) which has unique geography and climate as compared to

This paper is a part of PhD thesis of first author

* Corresponding author: mazhar_dr@yahoo.com

0030-9923/2015/0001-0161 $ 8.00/0

other parts of the country. Therefore, keeping in view the importance of the infection, a sero-epidemiological study was conducted to work out the seroprevalence of *T. gondii* infection and associated risk factors in sheep and goats raised in northern Punjab, Pakistan.

MATERIALS AND METHODS

Study area and animal management

This study was carried out in Pothwar region, Pakistan. Pothwar plateau is located in northern parts of the Punjab province between 32°-30´ to 34° northern latitudes and 71°-45´ to 73°-45´ eastern latitudes. It occupies a total area of 23,160 sq kilometers and has a total human population of 7.5 millions. It includes Islamabad capital territory along with four districts namely Rawalpindi, Attock, Chakwal and Jhelum. Agriculture is mainly dependent on rainfall which averages 370-500 mm annually. Most of the rainfall occurs in northwestern parts while southwestern parts are usually arid. Sheep and goats grazing in Pothwar region of Pakistan follow extensive and semi-extensive farming practices. Sheep and goat are generally kept separate but they closely follow each other sharing the grazing lands. The flocks of sheep and goats are taken out in the morning for grazing along the communal lands, roadsides and land along foot-hills and brought back to their kraal in the evening.

Study animals, sampling and serum preparation

Sheep and goats were randomly selected from different farms located in Pothwar region. The area was divided into 20 clusters and 20-25 samples were taken from each cluster for each species. A total of 831 small ruminants (413 sheep and 419 goats) were screened for the presence of *Toxoplasma* specific IgG and IgM antibodies between September, 2011 and December, 2012. Three breeds of sheep (Salt Range, Awassi and Afghani) and three breeds of goats (Beetal, Jattal and Teddy) along with their crosses were included in the study. About 3-4 ml blood samples were collected from jugular vein in 5 ml vacutainers without anticoagulant. Blood samples were immediately transported to PMAS-Arid Agriculture University, Rawalpindi. Serum was removed by centrifugation at 3000 rpm for 15 minutes. All the serum samples were stored at –20^{o}C until used for *Toxoplasma*-specific IgG and IgM antibodies detection. Age was determined from animal record kept at farms. Additional data regarding management practice, source of water and presence of cats etc was obtained through surveys and interviews.

Enzyme-linked immunosorbent assay (ELISA)

Toxoplasma-specific IgG-antibodies were measured by a commercial ELISA Kit (ID Screen *Toxoplasmosis* Indirect ® (ID-VET Company, France) according to manufacturer's instruction. Results were also interpreted according to the instructions of the manufacturer.

Statistical analysis

Odds ratios were calculated by using SPSS statistical package by comparing infected and non-infected animals for working out different determinants of *T. gondii* infection such as breeds, sex, age, management practice, source of water, presence of cats, reproductive status and flock size.

RESULTS

In current study, out of total 413 sheep sampled, 75 were found positive giving an overall prevalence of 18.16%. *Toxoplasma*-specific IgG antibodies were found in 64 (15.49%) while IgM antibodies were found in 14 (3.39%) sheep. Three sheep were positive for both IgG and IgM antibodies. Overall seroprevalence in goats was 14.32% (60/419). IgG antibodies were found in 50 (11.93%) while IgM antibodies were found in 12 (2.86 %) sheep. Two goats were positive for both IgG and IgM antibodies. No significant difference was observed according to seroprevalence in both species (Table I).

Analysis of the data collected from the questionnaires and interviews revealed that likelihood of infection increased with age in both species. Female animals of both species were more likely to be infected as compared to males (OR 1.85 and 1.70 in sheep and goats respectively). Further statistically significant risk factors in sheep were poor hygienic conditions (OR = 4.91, $p<0.01$), presence of cats (OR = 2.08, $p<0.05$), extensive

Table I.- Number of IgG and IgM positive and negative sheep and goats.

Species	Total	IgG (-) and IgM (-) (A)	IgG (+) and IgM (-) (B)	IgG (-) and IgM (+) (C)	IgG (+) And IgM (+) (D)	Total IgG (+) E=(B+D)	Total IgM (+) F=(C+D)	Overall Positive (E+F)-D
Sheep	413	338	61	11	3	64	14	75
Goats	419	359	48	10	2	50	12	60

Table II.- Risk factors analysis of *T. gondii* infection in sheep.

Factors	Category	N	+ve	Prevalence % (95% C.I.)	Odds Ratio (95% C.I.)	p– value
Age	<12	44	4	9.09 (3.59 - 21.16)	Reference	
	13-24	174	16	9.20 (5.74 - 14.42)	1.01 (0.32 - 3.20)	>0.05
	25-36	138	35	25.36 (18.84 - 33.22)	3.40 (1.13 - 10.18)	<0.05
	>36	57	20	35.09 (24.00–48.06)	5.41 (1.69–17.29)	<0.01
Sex	Male	156	20	12.82 (8.45 – 18.97)	Reference	
	Female	257	55	21.40 (16.83 – 26.82)	1.85 (1.06 – 3.23)	<0.05
Breeds	Afghani	97	9	9.28 (04.96–16.70)	Reference	
	Awassi	135	16	11.85 (07.43–18.38)	1.31 (0.56–3.11)	>0.05
	Salt Range	111	40	36.04 (27.71–45.30)	5.51 (2.51–12.11)	<0.01
	Awassi× Afghani	70	10	14.29 (07.95–24.34)	1.63 (0.62–4.25)	>0.05
Hygienic condition	High	112	8	7.14 (3.66–3.46)	Reference	
	Moderate	155	27	17.42 (12.26–24.16)	2.79 (1.21–6.39)	<0.05
	Low	146	40	27.40 (20.81–35.14)	4.91 (2.19–10.98)	<0.01
Source of water	Indoor	217	33	15.21 (11.04–20.59)	Reference	
	Outdoor	196	42	21.43 (16.26–27.69)	1.52 (0.92–2.52)	>0.05
Presence of cats	No	100	11	11.00 (06.25–18.63)	Reference	
	Yes	313	64	20.45 (16.35–25.26)	2.08 (1.05–4.12)	<0.05
Management	Intensive	94	12	12.77 (7.46 – 21.00)	Reference	
	Semi–intensive	179	29	16.20 (11.52 – 22.30)	1.32 (0.64 – 2.73)	>0.05
	Extensive	140	34	24.29 (17.94 – 32.02)	2.19 (1.07 – 4.50)	<0.05
Flock Size	<10	47	7	14.89 (7.40 – 27.68)	Reference	
	11–30	138	17	12.32 (7.84 - 18.84)	0.80 (0.31 - 2.08)	>0.05
	31–50	174	28	16.09 (11.37 - 22.27)	1.10 (0.45 - 2.69)	>0.05
	>50	54	23	42.59 (30.33 – 55.83)	4.24 (1.61 – 11.15)	<0.01
Reproductive Status (Females)	Non-Pregnant*	121	18	14.88 (9.62 – 22.30)	Reference	
	Pregnant	69	21	30.43 (20.85 – 42.08)	2.50 (1.22 – 5.13)	<0.01
	Lactating	67	16	23.88 (15.27 – 35.33)	1.80 (0.85 – 3.81)	>0.05

*Non-pregnant = neither pregnant nor lactating

farming practice (OR = 2.19, p<0.05), flock size larger than 50 individuals (OR = 4.24, p<0.01) and pregnancy (OR = 2.50, P<0.01) (Table II). Similarly statistically significant risk factors in goats were poor hygienic condition (OR = 2.06, p<0.05), usage of outdoor water source (OR = 2.06, p<0.01), presence of cats (OR = 2.03, p<0.05), extensive farming practice (OR = 2.25, p<0.05) and flock sized larger than 30 (OR = 4.24, p<0.05) and 50 (OR = 6.82, p<0.01) individuals (Table III). No statistically significant breed wise difference of seroprevalence was observed in goats. However, seroprevalence was significantly high in salt range sheep as compared to other breeds of sheep (OR = 5.51, p<0.01).

DISCUSSION

Seroprevalence of *Toxoplasma*-specific IgG antibodies can be determined by various diagnostic

Table III.- Risk factors analysis of *T. gondii* infection in goats.

Factors	Category	N	+ve	Prevalence % (95% C.I.)	Odds Ratio (95% C.I.)	p– value
Age	<12	30	3	10.00 (03.46–25.62)	Reference	
	13-24	181	15	8.29 (5.09 - 13.23)	0.81 (0.22 - 3.00)	>0.05
	25-36	137	23	16.79 (11.46 - 23.93)	1.82 (0.51 - 6.49)	>0.05
	>36	71	19	26.76 (17.85–38.05)	3.29 (0.89–12.11)	<0.05
Sex	Male	153	16	10.46 (6.54 – 16.31)	Reference	
	Female	266	44	16.54 (12.56 – 21.47)	1.70 (0.92 – 3.13)	<0.05
Breeds	Jattal	144	16	11.11 (06.96–17.29)	Reference	
	Beetal	161	22	13.66 (09.20–19.82)	1.27 (0.64–2.52)	>0.05
	Teddy	55	11	20.00 (11.55–32.36)	2.00 (0.86–4.64)	>0.05
	Beetal× Jattal	59	11	18.64 (10.74–30.37)	1.83 (0.79–4.23)	>0.05
Hygienic condition	High	138	14	10.14 (06.14–16.30)	Reference	
	Moderate	159	23	14.47 (09.84–20.78)	1.50 (0.74–3.04)	>0.05
	Low	122	23	18.85 (12.90–26.70)	2.06 (1.01–4.21)	<0.05
Source of Water	Indoor	259	28	10.81 (07.59–15.18)	Reference	
	Outdoor	160	32	20.00 (14.54–26.87)	2.06 (1.18–3.58)	<0.01
Presence of cats	No	142	13	9.15 (05.42–15.03)	Reference	
	Yes	277	47	16.97 (13.01–21.84)	2.03 (1.06–3.89)	<0.05
Management	Intensive	93	8	8.60 (4.42 – 16.06)	Reference	
	Semi–intensive	143	20	13.99 (9.24 – 20.62)	1.73 (0.73 – 4.10)	>0.05
	Extensive	183	32	17.49 (12.67 – 23.65)	2.25 (0.99 – 5.11)	<0.05
Flock Size	<10	42	2	4.76 (1.31 – 15.79)	Reference	
	11–30	175	18	10.29 (6.61 - 15.68)	2.29 (0.51 - 10.29)	>0.05
	31–50	143	25	17.48 (12.13 - 24.53)	4.24 (0.96 - 18.69)	<0.05
	>50	59	15	25.42 (16.05 – 37.79)	6.82 (1.47 – 31.69)	<0.01
Reproductive Status (Females)	Non-Pregnant*	89	16	17.98 (11.38 – 27.23)	Reference	
	Pregnant	72	16	22.22 (14.17 – 33.09)	1.30 (0.60 – 2.83)	>0.05
	Lactating	105	12	11.43 (06.66 – 18.92)	0.59 (0.26 – 1.32)	>0.05

*Non-pregnant = neither pregnant nor lactating

techniques in sheep and goats (Masala *et al*., 2003). There are incredible variations in behavior of different livestock species in different diagnostic tests. Moreover, there is no worldwide typical *Toxoplasma gondii* reference material available against which different diagnostic tests can be standardized. In the current study enzyme-linked immunosorbent assay was used which is appropriate for detection of *Toxoplasma*-specific IgG and IgM antibodies (Ghazaei, 2005; Vesco *et al*., 2007).

Results of our serologic study show that *T. gondii* infection is widely present in sheep and goats in northern Punjab, Pakistan. Previously, *T. gondii* infection in sheep is also reported from southern parts of the country (Ramzan *et al*., 2009; Lashari and Tasawar, 2010; Tasawar *et al*., 2011) and neighboring countries like Iran, India and China (Ghorbani *et al*., 1983; Sharma *et al*., 2008; Zhao *et al*., 2011). *T. gondii* infection in sheep is also reported from Egypt, Zimbabwe, Serbia, Brazil, South Africa and Saudi Arabia (Maronpot and Botros, 1972; Hove *et al*., 2005; Klun *et al*., 2006; Romanelli *et al*., 2007; Samra *et al*., 2007; Sanad and Al-Ghabban, 2007). On the other hand prevalence in goats is also reported from Uganda, Thailand, Saudi Arabia, and Egypt (Bisson *et al*., 2000; Jittalpalapong *et al*., 2005; Sanad and Al-Ghabban, 2007; Barakat *et al*., 2009). Seroprevalence differs significantly in different areas due to difference in climate, hygienic conditions and farm managements (Zhao *et al*., 2011). Also it differs because of difference in serodiagnostic tests used (Vesco *et al*., 2007)

We found an increase in the prevalence of infection with age in both species. Increase in prevalence of the disease in older animals is due to exposure of animals to the risk factors for longer period of time than the younger ones (O'Donoghue

et al., 1987; Puije *et al*., 2000). Similar findings in sheep and goats are also reported by Vesco *et al*. (2007), Lashari and Tasawar (2010) and Tasawar *et al*. (2011). It is also reported previously that susceptibility to infection from protozoan parasites is found greater in female animals as compared to males (Alexander and Stinson, 1988). We also observed higher prevalence of *T. gondii* infection in female animals which may be explained by the fact that immunity in females is reduced by various factors such as pregnancy, nutrition and lactation (Martin, 2000; Kelly *et al*., 2001). We also found significant higher seroprevalence in pregnant sheep (OR = 2.50, P <0.01) and non-significant higher seroprevalence in pregnant goats (OR = 1.30, P>0.05). Higher prevalence of toxoplasmosis in female goats is also reported by Tasawar *et al*. (2011). Current study also revealed unusual high prevalence in salt range sheep as compared to other breeds of sheep. However, it was observed that salt range sheep in the region was mostly overcrowded and were managed under extensively farming practice which may increase the risk of infection.

No statistically significant difference of seroprevalence was observed in sheep and goats from different districts which suggest uniformity of the climate and management practices in the study area. Analysis of other risk factors showed outdoor water source was a putative risk factor for goats but not for sheep (Tables II and III). This may be explained by the facts that goats are mostly infected by drinking contaminated water. Chances of getting infection through food are low as they usually browse the leaves which are far from the ground. On the other hand sheep graze close to the ground and have better chances of getting infection via both food and water (Waldeland, 1976).

Presence of cats also significantly contributed in increasing the likelihood of infection in both sheep and goats in present study. Cats are the definitive hosts of the parasite and play a vital role in infecting other animals by shedding oocysts in the environment (Lopes *et al*., 2010). A study from Poland reported that the presence of free-roaming cats is an important risk factor for the transmission of the infection in goats (Neto *et al*., 2008). Similar findings have also been reported in other studies in livestock animals (Puije *et al*., 2000; Chaudary *et al*. 2006). Lopes *et al*., 2010; Ahmad and Qayyum, 2014,

Seroprevalence was also higher in animals kept under poor hygienic conditions. Poor hygienic condition may favor the food and water to be contaminated with cat faeces, hence increase the likelihood of infection in livestock animals (Hove *et al*., 2005). Current study also found higher seroprevalence in livestock animals raised in extensive and semi-intensive management system. Extensive management in sheep and goats presents greater risk of *T. gondii* infection. Moreover, the use of bulk feed or pasture also poses threat of getting toxoplasmosis. Both practices render animals to come closer in contact with oocysts shed by wild and domestic felids. As compared to extensive or semi-intensive management, animal raised intensively are usually caged and get little chance to ingest oocysts contaminated food and water (Anderlini *et al*., 2011). Similar findings have been reported in Ghanaian and Brazilian sheep raised extensively (Puije *et al*., 2000; Lopes *et al*., 2010). Increase in prevalence of toxoplasmosis in extensive management is also found more recently in sheep and goats from northeastern China (Wang *et al*., 2011).

Current study also found significant increase in seroprevalence in animals which were kept in larger flocks. Although toxoplasmosis cannot be transferred from animal to animal however, higher seroprevalence in larger flock may be explained by the fact that the larger flock sampled in the study were mostly managed under semi-intensive or extensive system of management which increases the risk of getting toxoplasmosis as compared to intensive system of management. Similar findings have been reported in cattle and sheep in Serbia (Klun *et al*., 2006). More recently, increase in prevalence of *T. gondii* infection with flock size is also reported in a Brazilian study (Anderlini *et al*., 2011).

In summary, the results of current study confirm the presence of *Toxoplasma gondii* infection in sheep and goats in Pothwar region of Punjab. This verification of seroprevalence in sheep and goats indicated that toxoplasmosis is widely present and may cause economic losses in livestock in the form of abortion and neonatal deaths. Further

investigations are needed to find out zoonotic impact of the parasite and to explore the economic losses due to this infection.

ACKNOWLEGMENTS

We are grateful to Higher Education Commission of Pakistan for providing financial assistance to first author (Mr. Nisar Ahmad) under Indigenous PhD fellowship programme and farm managers at private animal farms and Livestock Production Research Institute, Kharimurat (LPRI), Fateh Jhang for providing experimental facilities.

REFERENCES

AHMAD, N. AND QAYYUM, M., 2014. Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan. *J. Infect. Dev. Countr.*, **8**: 1022-1028.

ALEXANDER, J. AND STINSON, W.H., 1988. Sex hormones and the course of parasitic infection. *Parasitol, Today*, **4**: 189-193.

ANDERLINI, G.A., MOTA, R.A., FARIA, E.B., CAVALCANTI, E.F., VALENCA, R.M.B., JUNIOR, J.W.P., ALBUQUERQUE, P.P.F. AND NETO, O.L.S., 2011. Occurrence and risk factors associated with infection by *Toxoplasma gondii* in goats in the state of Alagoas, Brazil. *Rev. Soc. Brasil. Med. Trop.* **44**: 157-162.

BARAKAT, A.M.A., ABDELAZIZ, M.M. AND FADALY, M., 2009. Comparative diagnosis of toxoplasmosis in Egyptian small ruminants by indirect hemagglutination assay and ELISA. *Glob. Vet.*, **3**: 9-14.

BISSON, A., MALEY, S., RUBAIRE-AKIIKI, C.M. AND WASTLING, J.M., 2000. The seroprevalence of antibodies to *Toxoplasma gondii* in domestic goats in Uganda. *Acta Trop.*, **76**: 33-38.

BUXTON, D., 1990. Ovine toxoplasmosis – A review. *J. R. Soc. Med.*, **83**: 509-511.

CHAUDHARY, Z.I., AHMED, R.S., HUSSAIN, S.M.I. and SHAKOORI, A.R., 2006. Detection of *Toxoplasma gonidi* infection in butchers and buffaloes by polymerase chain reaction and latex agglutination test. *Pakistan J. Zool.* **38:** 333-336

DUBEY, J.P. AND BEATTIE, C.P., 1988. *Toxoplasmosis of animals and man.* CRC Press, Boca Raton, Florida, pp. 61-80.

DUBEY, J.P., 1996. Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. *Vet. Parasitol.*, **64**: 65-70.

FIGLIUOLO, L.P.C., KASAI, N., RAGOZO, A.M.A., DEAULA, V.S.O., DIAS, R.A., SOUZA, S.L.P. AND GENNARI, S.M., 2004. Prevalence of anti-*Toxoplasma gondii* and anti-*neospora caninum* antibodies in ovine from Sao-Paulo state, Brazil. *Vet. Parasitol.*, **123**: 161-166.

GHAZAEI, C., 2005. Serological survey of antibodies to *Toxoplasma* in Ardabil Iran. *Int. J. Vet. Med.*, **2**: 45-50.

GHONEIM, N.H., SHALABY, S.I., HASSANAIN, N.A., ZEEDAN, G.S.G., SOLIMAN, Y.A. AND ABDALHAMED, A.M., 2009. Detection of genomic *Toxoplasma gondii* DNA and anti-*Toxoplasma* antibodies. *Life Sci. J.*, **6**: 54-60.

GHORBANI, M., 1983. Animal toxoplasmosis in Iran. *J. Trop. Med. Hyg.*, **86**: 73-80.

HASSIG, M., SAGER, H., REITT, K., ZIEGLER, D., STRABEL, D. AND GOTTSTEIN, B., 2003. *Neospora caninum* in sheep: a flock case report. *Vet. Parasitol.*, **117**: 213–220.

HOVE, T., LIND, P. AND MUKARATIRWA, S., 2005. Seroprevalence of *Toxoplasma gondii* infection in domestic pigs reared under different management systems in Zimbabwe. *Onde. J. Vet. Res.*, **72**: 231-237.

JITTAPALAPONG, S., SANGVARANOND, A., PINYOPANUWAT, N., CHIMNOI, W., KHACHAERAM, W., KOIZUMI, S. AND MARUYAMA, S., 2005. Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Satun Province, Thailand. *Vet. Parasitol.*, **127**: 17-22.

KELLY, A., MESSINGHAM, N., ELIZABETH, A.H. AND KOVACS, J., 2001. Estrogen restores cellular immunity in injured male mice via suppression of interleukin-6 production. *J. Leuk. Biol.*, **70**: 887-895.

KLUN, I., DJURKOVIC, O.D., RADIVOJEVIC, S.K. AND NIKOLIC, A., 2006. Cross sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. *Vet. Parasitol.*, **135**: 121-131.

LASHARI, M.H. AND TASAWAR, Z., 2010. Seroprevalence of toxoplasmosis in sheep in Southern Punjab, Pakistan. *Pak. Vet. J.*, **30**: 91-94.

LOPES, W.D.Z., SANTOS, T.R., DE-SILVA, R.D.S., ROSSANESE, W.M., DE'SOUZA, F.A., RODRIGUES, J.D.F., MENDONCA, R.P., SOARES, V.E. AND DA'COSTA, A.J., 2010. Seroprevalence of and risk factors for *Toxoplasma gondii* in sheep raised in the Jaboticabal microregion, São Paulo State, Brazil. *Res. Vet. Sci.*, **88**: 104-106.

MARONPOT, R.R. AND BOTROS, B.A.M., 1972. *Toxoplasma* serologic survey in man and domestic animals in Egypt. *J. Egypt. Publ. Hlth. Assoc.*, **47**: 58-67.

MARTIN, J.T., 2000. Sexual dimorphism in immune function: the role of prenatal exposure to androgens and estrogens. *Eur. J. Pharmacol.*, **405**: 251-261.

MASALA, G., PORCU, R., MADAU, L., TANDA, A., IBBA,

B., SATTA, G. AND TOLA, S., 2003. Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy. *Vet. Parasitol.*, **117**: 15–21.

NETO, J.O.A., AZEVEDO, S.S., GENNARI, S.M., FUNADA, M.R., PENA, H.F.J., ARAUJO, A.R., BATISTA, C.S.A., SILVA, M.L., GOMES, A.A., PIATTI, R.M. AND ALVES, C.J., 2008. Prevalence and risk factors for anti-*Toxoplasma gondii* antibodies in goats of the Serido Oriental micro region, Rio Grande do Norte state, Northeast region of Brazil. *Vet. Parasitol.*, **156**: 329- 334.

O'DONOGHUE, P.J., RILEY, M.J. AND CLARKE, J.F., 1987. Serological survey for *Toxoplasma* infections in sheep. *Aust. Vet. J.*, **64**: 40-45.

PAL, R. A., QAYYUM, M. AND YASEEN, M., 1995. A serological antibody survey for *Toxoplasma gondii* in twin cities of Rawalpindi and Islamabad. *J. Pak. med. Assoc*., **45**:188-189.

PAL, R. A., QAYYUM, M. AND YASEEN, M., 1996. Seroprevalence of antibodies to *Toxoplasma gondii*, with particular reference to obstetric history of patients in Rawalpindi-Islamabad, Pakistan. *J. Pak. med. Assoc*., **46**:56-58.

PUIJE, W.N.A., BOSOMPEM, K.M., CANACOO, E.A., WASTLING, J.M. AND AKANMORI, B.D., 2000. The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanian sheep and goats. *Acta Tropica*, **76**: 21-26.

RAMZAN, M., AKHTAR, M., HUSSAIN, I., MUHAMMAD, F., HISZCZYNSKA-SAWICKAA, E., HAQ, A.U. AND MAHMOOD, M.S., 2009. Seroprevalence of *Toxoplasma gondii* in sheep and goats in the area of Rahim Yar Khan (Punjab). *Trop. Anim. Hlth. Prod.*, **41**: 1225-1229.

ROMANELLI, P.R., FREIRE, R.L., VIDOTTO, O., MARANA, E.R.M., OGAWA, L., DE'PAULA, V.S.O., GARCIA, J.L. AND NAVARRO, J.L., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Parana State, Brazil. *Res. Vet. Sci.*, **82**: 202–207.

SAMRA, N.A., MCCRINDLE, C.M., PENZHORN, B.L. AND CENCI-GOGA, B., 2007. Seroprevalence of toxoplasmosis in sheep in South Africa. *J. S. Afr. Vet. Assoc.*, **78**: 116-120.

SANAD, M.M. AND AL-GHABBAN, A.J., 2007. Serological survey on toxoplasmosis among slaughtered sheep and goats in Tabouk, Saudi Arabia. *J. Egypt Soc. Parasitol.*, **37**: 329-340.

SHARMA, S., SANDHU, K.S., BAL, M.S., KUMAR, H., VERMA, S. AND DUBEY, J.P., 2008. Serological survey of antibodies to *Toxoplasma gondii* in sheep, cattle and buffaloes in Punjab, India. *J. Parasitol.*, **94**: 1174-1175.

TASAWAR, Z., LASHARI, M.H., HANIF, M. AND HAYAT, C.S., 2011. Seroprevalence of *Toxoplasma gondii* in domestic goats in Multan, Punjab, Pakistan. *Pak. J. life Sci.*, **9**: 24-27.

TENTER, A.M., HECKERROTH, A.R. AND WEISS, L.M., 2000. *Toxoplasma gondii*: from animals to human. *Int. J. Parasitol.*, **30**: 1217–1258.

VESCO, G., BUFFALANO, W., LA'CHIUSA, S., MANUCUSO, G., CARACAPPA, S., CHIANCA, A., VILLARI, S., CURRO, V., LIGA, F. AND PETERSEN, E., 2007. *Toxoplasma gondii* infections in sheep in Sicily, southern Italy. *Vet. Parasitol.*, **146**: 3-8.

WALDELAND, H., 1976. Toxoplasmosis in sheep. *Toxoplasma gondii* in muscular tissue, with particular reference to dye test titers and haemoglobin type. *Acta Vet. Scand.*, **17**: 403–411.

WANG, C.R., QIUA, J.H., GAOA, J.F., LIUA, L.M., WANGA, C., LIUC, Q., YAND, C. AND ZHUA, X.Q., 2011. Seroprevalence of *Toxoplasma gondii* infection in sheep and goats in northeastern China. *Small Ruminant Res.*, **97**: 130–133.

ZHAO, G.H., ZHANG, M.T., LEI, L.H., SHANG, C.C., CAO, D.Y., TIAN, T.T., LI, J., XU, J.Y., YAO, Y.L., CHEN, D.K. AND ZHU, X.Q., 2011. Seroprevalence of *Toxoplasma gondii* infection in dairy goats in Shaanxi Province, Northwestern China. *Parasite Vector*, **4**: 47-51.

(Received 13 August 2014, revised 27 August 2014)