



Document heading

doi:10.12980/JCLM.1.2013C254

© 2013 by the Journal of Coastal Life Medicine. All rights reserved.

Seroprevalence of *Toxoplasma gondii* infection in domestic animals of Mohmand agency, Pakistan

Mudassir Shah¹, Muhammad Zahid^{1*}, Aftab Alam Sthanadar¹, Pir Asmat¹, Ayesha Kausar¹, Abdul Hamid Jan²

¹Department of Zoology, Islamia College University Peshawar, KP, Pakistan

²Department of Zoology, University of Peshawar, KP, Pakistan

PEER REVIEW

Peer reviewer

Dr. Muhammad Nasir Khan Khattak, Assistant Professor, Zoology Department Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan.

Tel: 0092–3319258072

E-mail: mnasir43663@gmail.com

Comments

This article reflects a pure research work in the back ward area of Mohmand agency, Pakistan. In my view this article is acceptable and needs no modifications.

Details on Page 74

ABSTRACT

Objective: To determine the prevalence of toxoplasmosis among domestic animals of Mohmand Agency, Pakistan.

Methods: Indirect haemagglutination test was used to detect *Toxoplasma gondii* (*T. gondii*) antibodies in the sera.

Results: Out of 384 domestic animals, 124 (32.29%) were seropositive for antibodies of *T. gondii*. *T. gondii* antibodies were detected in 56 out of 104 examined goats (53.84%). The seropositivity in male and female goats were 20 (38.46%) and 36 (69.23%) respectively. The seropositivity of *T. gondii* in sheep was 36% ($n=100$) while the prevalence in female sheep was higher (41.66%) as compared to male sheep (30.76%). A total of 8 out of 48 male cows were detected seropositive (16.66%) and 8 out of 32 female cows were detected seropositive (25%). Lowest prevalence was detected in buffaloes which was 16% ($n=100$). Out of 52 male buffaloes 4 were detected seropositive for *T. gondii* antibodies (7.69%) while in female buffaloes was 25% ($n=48$). The seroprevalence of *T. gondii* antibodies was higher in goats and lower in buffaloes.

Conclusions: The findings of the present study indicate that *T. gondii* infection is high in domestic animals in Mohmand agency, Pakistan, which has implications for public health in this region. Essential control strategies and suitable measures should be carried out rationally in this region, in order to lower the risk of exposure to *T. gondii*.

KEYWORDS

Antibodies, Domestic animals, Seroprevalence, *Toxoplasma gondii*

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite which is worldwide in distribution[1]. The definitive hosts of *T. gondii* are felids like cats while warm blooded animals act as an intermediate host[2]. The sexual reproduction of the *T. gondii* occurs only in cat which is the transmission vector and faeces are the main source for transfers of infection. Cats shed the oocysts in their faeces and transmit the infection to intermediate host. In animals the infection is transmitted by ingestion of food or water contaminated with cat faeces or by eating oocyte infected

meat while humans get infection by eating tissue cysts in undercooked or uncooked meat or even can be transmitted from mother to foetus[3]. Although it is commonly recommended to avoid contact with cats by pregnant women but the contribution of this risk factor in the disease spread is controversial. Regardless of the few studies relating exposure to cats as a minor source of disease while several other studies have failed to identify a significant risk factor for toxoplasmosis[4].

The symptoms of the disease are flu-like mild illness; characterized by fever, headache, fatigue or even no signs but the people with deficient immune system (HIV infected

*Corresponding author: Muhammad Zahid, Department of Zoology, Islamia College University Peshawar, KP, Pakistan.

Tel: 0092–345909285

E-mail: mzahidsafi75@yahoo.com

Foundation Project: Partly supported by Higher Education Commission Pakistan under the National Research Programme for Universities (NRPUS) with grant number 840.

Article history:

Received 15 May 2013

Received in revised form 18 Jun, 2nd revised form 23 Jun, 3rd revised form 29 Jun 2013

Accepted 20 Jul 2013

Available online 28 Aug 2013

persons) or pregnant women may suffer from serious illness starting from diarrhoea, pneumonia and liver diseases to weight loss and infection of central nervous system, even death in severe infection. In the latent stage, the disease remains asymptomatic ranging from few days to several months but emerges as flu-like mild symptoms in the early acute stage. The disease can relapses on the persons whose immune system is very weak (HIV infected persons or transplant receipts)[5,6].

About 33% of world's population is infected with *T. gondii* but the frequency differs according to the geographical areas. Prevalence of infection is 25% in Japan, 20%–30% in USA, Finland 50%, Netherland and Italy 60%, and Poland 50%–60%. Incidence can be as higher as 80%[3]. Seroprevalence of *T. gondii* infection of 17.4% was found in young school children in Islamabad, Pakistan[7]. The prevalence rate in Dera Ghazi Khan, Pakistan was detected to be 29.5%[8].

Since the disease is latent for long period and is lethal in its acute case for humans, and the domestic animals are the main source of disease transmission to humans, thus there is a need to develop methods to timely detect the disease. The data about the incidence and prevalence of the disease in the domestic animals will be highly helpful to divert the attention to take the necessary actions for the control and management of the disease. Furthermore, animal's toxoplasmosis may be a risk factor for infection to human population in the area. Therefore the current project was planned to detect the prevalence of toxoplasmosis in domestic animals in the Mohmand Agency and the connected regions.

2. Materials and methods

2.1. Serum collection

This study was carried out to determine the seroprevalence of *T. gondii* in domestic animals in Mohmand Agency, Pakistan. The animals (buffaloes, cows, goats and sheep) were chosen by random sampling method to investigate the seroprevalence of anti-*T. gondii* antibodies. A total of 384 samples were collected including 104 goat, 100 sheep, 80 cows, and 100 buffaloes from different regions (Khazeena, Nawagai, Chamarkan, Ulai and Ghalanai) in Mohmand Agency. Approximately 5 mL of blood was collected from jugular vein through disposable syringe and was transferred to the collecting tubes containing anticoagulant. The samples were centrifuged at 3500 r/min for five minutes at room temperature for the extraction of serum. The collected serum was stored in eppendorff tubes at -20 °C until further analysis.

2.2. Serological examination

The commercial Indirect Hemagglutination Antibody (IHA) test kits were used according to manufacturer protocol

(SERFIB, France) for detection of antibodies of *T. gondii*.

A serial two fold dilution was prepared starting from 1:40, up to 6th dilution. A drop of sensitized red blood cells was distributed in the each well containing diluted serum. While unsensitized and sensitized blood was used as positive and reagent control. The samples were mixed well and the plate was allowed to stand for 2 h before reading. All sera reactivated at $\geq 1:80$ were considered as positive.

2.3. Statistical analysis

The results were expressed in percentages. The values between different sex groups were analysed by using Chi Square test for Windows (Release 16.0 standard version). The P value < 0.05 was considered as statistically significant.

3. Results

A total of 384 domestic animals from different localities (Khazeena, Nawagai, Chamarkan, Ulai and Ghalanai) of Mohmand Agency were examined for the presence of *T. gondii* antibodies by using IHA. Out of 384 only 124 (32.29%) domestic animals were detected seropositive for *T. gondii* at dilution $\geq 1:80$. Seroprevalence of *T. gondii* infection was detected in goats, sheep, cows and buffaloes (Table 1). The seroprevalence of *T. gondii* varied in sex groups, ranging from 16% to 53.84% (Table 2).

Table 1

Seroprevalence of *T. gondii* in domestic animals.

| Type of animals | No. examined | No. positive | Prevalence (%) |
|-----------------|--------------|--------------|----------------|
| Goats | 104 | 56 | 53.84 |
| Sheep | 100 | 36 | 36.00 |
| Cows | 80 | 16 | 20.00 |
| Buffaloes | 100 | 16 | 16.00 |
| Total | 384 | 124 | 32.29 |

Table 2

Seroprevalence of *T. gondii* of different sex in goats, sheep, cows and buffaloes.

| Type of animals | Male n (%) | Female n (%) | Prevalence (%) |
|-----------------|------------|--------------|----------------|
| Goats | 20 (38.46) | 36 (69.23) | 56 (53.84) |
| Sheep | 16 (30.76) | 20 (41.66) | 36 (36) |
| Cows | 8 (16.66) | 8 (25) | 16 (20) |
| buffaloes | 4 (7.69) | 12 (25) | 16 (16) |

Toxoplasma gondii antibodies were detected in 56 out of 104 (53.84%) examined goats. Out of 104 examined goats, 52 were male and 52 were female. Out of 52 male, 20 were detected seropositive (38.46%). In 52 examined female, 36 were detected seropositive (69.23%). The seroprevalence of toxoplasmosis was higher in female as compared to male goats (Table 2). The seroprevalence of *T. gondii* antibodies was significantly higher in female goats with titers ranging from 1:80 to 1:160.

Toxoplasma gondii antibodies were detected in 36 out of

100 examined sheep (36%). A total of 16 out of 52 examined male were detected seropositive (30.76%). A total of 20 cases out of 48 female were tested seropositive (41.66%). High prevalence was also seen in female as compared to male sheep (Table 2).

A total of 8 out of 48 male cows were detected seropositive (16.66%) and 8 out of 32 female were detected seropositive (25%) (Table 2). High specific antibody levels were most frequent at $\geq 1:80$ titer.

Lowest prevalence was found in buffaloes. A total of 100 buffaloes were examined for seroprevalence of *T. gondii* antibodies. Buffaloes were divided into two groups of male and female. Out of 52 male, 4 were detected seropositive for *T. gondii* antibodies (7.69%). A total of 12 out of 48 (25%) female buffaloes were detected seropositive for *T. gondii* antibodies (Table 2). The seroprevalence of *T. gondii* antibody levels were most frequent at $\geq 1:80$.

4. Discussion

In this study, we examined the seroprevalence of *T. gondii* infection in domestic animals in Mohmand agency, Pakistan. 32.29% of the 384 tested domestic animals were found to be seropositive for *T. gondii*, which is higher than 9.2% reported from Guangxi but is lower than 46.4% from Xinjiang, China^{9,10}.

In the present study, seroprevalence for *T. gondii* in sheep was extremely low 36% as compared to 48.6% from Greece¹¹ and 46.2% from Brazil¹² and 44.1% from West Indies¹³. The 36% seropositivity rate found in sheep in present study is higher than 4.4% from Northeastern China¹⁴. This might possibly be due to the difference in climatic conditions. In our study, seroprevalence for *T. gondii* antibodies are widespread in animal population which show resemblance with others work¹⁵.

Among the domestic animals the highest prevalence of toxoplasmosis is present in goats 53.84% which is similar to 35.5% from Malaysia¹⁵. 53.84% Prevalence of toxoplasmosis in sera of goats in present study is higher than 35.5% from Malaysia, 30.7% Greece, 30.6 % Brazil, 31% Mexico and 42.8% West Indies¹³. The prevalence rate of toxoplasmosis in goats is similar to 52.8% reported from Romania^{11,13,15–18}.

In the present study seroprevalence of toxoplasmosis in sheep (36%) and goats (53.84%) is extremely higher than Pakistan (11.2% sheep, 25.4 % goats)¹⁹, and Iran (6.7% sheep, 4.6% goats)²⁰ but is lower than reported from Brazil (60.8 % sheep, 81.8% goats)²¹. In our study seroprevalence of *T. gondii* in cows in Mohmand agency, Pakistan is extremely high 20% compared to the 2.3% in China²².

The prevalence rates were different from different localities suggestive of different exposure to infection. It is therefore not possible to compare prevalence data of studies which used different serological tests with variable sensitivity and specificity. The contrast in results may be due to the use of different serological tests with variable

sensitivity. Toxoplasmosis is more common in those areas where the environment is warm and humid as compared to dry and cold areas²³. In the present study lowest prevalence of 16% is detected in buffaloes which is similar to the work done in Punjab²⁴.

The seroprevalence of *T. gondii* may be high in areas where people eat undercooked meat, do not wash vegetables and fruits and the people who have interaction with dogs and cats or other domestic animals or have direct contact with the soil. The consumption of municipal water also increases the prevalence of *T. gondii*³.

Toxoplasmosis is commonly found in both male and female domestic animals in Mohmand agency, Pakistan, but with an increased frequency in females as compared to males. It may be due to the fact that female cattle have more contact with the cats and dogs which are definitive hosts of *T. gondii*. These infected animals are the potential risk factors for the spread of toxoplasmosis to human population of the area. Therefore measures should be taken to control and prevent toxoplasmosis in all domestic animals in the study region.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was a part of a project, financially supported by Higher Education Commission Pakistan under the National Research Programme for Universities (NRPUS) with grant number 840.

Comments

Background

Toxoplasma gondii is an obligate intracellular protozoan parasite which is worldwide in distribution. The definitive hosts of *T. gondii* are felids like cats while warm blooded animals act as an intermediate host. About 33% of world's population is infected with *T. gondii* but the frequency differs according to the geographical areas.

Research frontiers

The study was carried out to determine the seroprevalence of *T. gondii* in domestic animals in Mohmand Agency, Pakistan. The animals (buffaloes, cows, goats and sheep) were investigated for the presence of anti-*Toxoplasma gondii* antibodies by using IHA.

Related reports

In this study, the seroprevalence of *T. gondii* infection in domestic animals in Mohmand agency, Pakistan was 32.29 %

of the 384 tested domestic animals which is higher than 9.2 % reported from Lv et al., 1994 but is lower than 46.4% by Mi et al., 2007. This may be due to interaction with definitive host and mud houses.

Innovations and breakthroughs

This study was carried out for the seroprevalence of toxoplasmosis in different domestic animals. The seroprevalence of *Toxoplasma gondii* antibodies was higher in goats and lower in buffaloes.

Applications

This study indicates that *T. gondii* infection is high in domestic animals in Mohmand agency, Pakistan, which has implications for public health in this region. Essential control strategies and suitable measures should be carried out rationally in this region, in order to lower the risk of exposure to *T. gondii*.

Peer review

This is an excellent study in which the researchers evaluate the prevalence of *Toxoplasma gondii* in domestic animals. The results are interesting and suggest that a high prevalence of toxoplasma in domestic animals may be a high risk factor for human beings.

References

[1] Cenci-Goga BT, Rossitto PV, Sechi P, McCrindle CME, Cullor JS. Toxoplasma in animals, food, and humans: An old parasite of new concern. *Foodborne Pathog Dis* 2011; **8**: 1-12.

[2] Carrada-Bravo T. Toxoplasmosis: Parasitosis reemergente del Nuevo milenio. *Rev Mex Patol Clin* 2005; **52**: 151-162.

[3] Shah M, Zahid M, Pir A, Alam A, Kausar A. Seroprevalence of *Toxoplasma gondii* infection in goats and sheep of district Mardan, Pakistan. *Int J Biosci* 2013; **3** (7): 1-8.

[4] Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenun PA, et al. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European research network on congenital toxoplasmosis. *BMJ* 2000; **321**: 142-147.

[5] Negash T, Tilahun G, Medhin G. Seroprevalence of *Toxoplasma gondii* in Nazareth town, Ethiopia. *East Afr J Public Health* 2008; **5**: 211-214.

[6] Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. *Int J Parasitol* 2008; **38**: 1257-1278.

[7] Sadaruddin A, Agha F, Anwar F, Ghafoor A. Seroepidemiology of *Toxoplasma gondii* infection in young school children in Islamabad. *J Pak Med Assoc* 1991; **41**: 131-134.

[8] Tasawar Z, Nawaz S, Lashari MH, Aziz F, Hayat CS. Seroprevalence of human toxoplasmosis in Dera Ghazi Khan, Punjab. *Gomal J Med Sci* 2011; **9**: 82-85.

[9] Lv YC, Cui JZ. Survey of *Toxoplasma gondii* infection in pigs and cattle in Guangxi Province, China. *J Anim Sci Vet Med* 1994; **3**: 26.

[10] Mi XY, Ba YCH, Li WC. Epidemic investigation of *Toxoplasma*

gondii infection in pigs, cattle and sheep in Xinjiang, China. *J Vet Parasitol* 2007; **15**: 22-24.

[11] Tzanidakis N, Maksimov P, Conraths FJ, Kiossis E, Brozos C, Sotiraki S, et al. *Toxoplasma gondii* in sheep and goats: seroprevalence and potential risk factors under dairy husbandry practices. *Vet Parasitol* 2012; **190**(3-4): 340-348.

[12] Silva AF, Oliveira FC, Leite JS, Mello MF, Brandão FZ, Leite RI, et al. Immunohistochemical identification of *Toxoplasma gondii* in tissues from modified agglutination test positivesheep. *Vet Parasitol* 2013; **191**(3-4): 347-352.

[13] Chikweto A, Kumthekar S, Tiwari K, Nyack B, Deokar MS, Stratton G, et al. Seroprevalence of *Toxoplasma gondii* in pigs, sheep, goats, and cattle from Grenada and Carriacou, West Indies. *J Parasitol* 2011; **97**: 950-951.

[14] Yang N, Li H, He J, Mu M, Yang S. Seroprevalence of *Toxoplasma gondii* infection in domestic sheep in Liaoning Province, Northeastern China. *J Parasitol* 2013; **99**(1): 174-175.

[15] Chandrawathani P, Nurulaini R, Zanin CM, Premaalatha B, Adnan M, Jamnah O, et al. Seroprevalence of *Toxoplasma gondii* in pigs, goats, cattle, dogs and cats in Peninsular, Malaysia. *Trop Biomed* 2008; **25**(3): 257-258.

[16] Neto JO, Azevedo SS, Gennari SM, Funada MR, Pena HF, Araújo AR, et al. Prevalence and risk factors for anti-*Toxoplasma gondii* antibodies in goats of the Seridó oriental microregion, Rio Grande do Norte state, Northeast region of Brazil. *Vet Parasitol* 2008; **156**(3-4): 329-332.

[17] Alvarado-Esquivel C, García-Machado C, Vitela-Corrales J, Villena I, Dubey JP. Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Durango State, Mexico. *Vet Parasitol* 2011; **183**(1-2): 43-46.

[18] Iovu A, Györke A, Mircean V, Gavrea R, Cozma V. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania. *Vet Parasitol* 2012; **186**(3-4): 470-474.

[19] Ramzan M, Akhtar M, Muhammad F, Hussain I, Hiszczyńska-Sawicka E, Haq AU, et al. Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Trop Anim Health Prod* 2009; **41**: 1225-1229.

[20] Kamani J, Mani AU, Egwu GO. Seroprevalence of *Toxoplasma gondii* infection in domestic sheep and goats in Borno state, Nigeria. *Trop Anim Health Prod* 2010; **42**: 793-797.

[21] Costa DG, Marvulo MF, Silva JS, Santana SC, Magalhães FJ, Filho CD, et al. Seroprevalence of *Toxoplasma gondii* in domestic and wild animals from the Fernando de Noronha, Brazil. *J Parasitol* 2012; **98**: 679-680.

[22] Yu J, Xia Z, Liu Q, Liu J, Ding J, Zhang W. Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (*Bubalus bubalis*) in the People's Republic of China. *Vet Parasitol* 2007; **143**: 79-85.

[23] Dubey JP. *Toxoplasmosis of animals and humans*. Boca Raton, New York: CRC Press, Inc; 2010, p. 1-313.

[24] Sharma S, Sandhu KS, Bal MS, Kumar H, Verma S, Dubey JP. Serological survey of antibodies to *Toxoplasma gondii* in sheep, cattle and buffaloes in Punjab. *India J Parasitol* 2008; **94**(5): 1174-1175.