Veterinary and Animal Science 5 (2018) 7–9

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Sero-prevalence of *Toxoplasma gondii* in sheep in different geographical regions of Nepal

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ARTICLE INFO

Keywords:
ELISA
Seroprevalence
Toxoplasma gondii
Nepal

ABSTRACT

The present study was conducted to investigate the prevalence of *Toxoplasma gondii* in sheep in Nepal. Blood samples were collected from 235 sheep from three districts of three different eco-zones namely, mountainous Jumla (88), hilly Pokhara (62) and plain/terai Chitwan (85). The samples were tested by using commercial ELISA kit. The overall prevalence of *T. gondii* infection in sheep was 36.17% (CI: 30.29–42.49%). The region wise prevalence showed highest in Chitwan (57.65%; CI: 47.04–67.60%), followed by Pokhara (32.94%; CI: 23.88–43.48%) and Jumla (9.41%; CI: 4.85–17.49%). Prevalence of *T. gondii* in Jumla was significantly lower than Pokhara and Chitwan ($p < 0.05$) but no significant difference in seroprevalence was encountered between Pokhara and Chitwan ($p > 0.05$). Similarly, no significant difference ($p > 0.05$) in prevalence of *T. gondii* was found in sex and age groups ($p > 0.05$). The result showed that *T. gondii* parasite is widely spread in the studied geographical regions of Nepal.

Introduction

Sheep play a vital role in livelihood upliftment of Nepalese rural farmers. They are a source of income and provide meat, clothing and manure to the farmers (Rauniyar, Upreti, Gavigan, & W.J. Parker, 2000). Increasing incidence of disease in sheep is a major constraint in the sheep production system of Nepal. *Toxoplasma gondii* is infectious disease of sheep which results in still birth, abortion, and neonatal death causing severe economic losses (Buxton, Thomson, Maley, Wright, & Bos, 1991; Hussein et al., 2011). There is dearth of information regarding Toxoplasmosis in Nepal. Few studies in human toxoplasmosis have been reported before (Rai et al., 1994, 1999; Rai, Sharma, Shrestha, & Pradhan, 2011). Out of 302 samples Rai et al. (1994) reported an overall 57.9% positive rate of human toxoplasmosis from the samples collected from Chitwan and Mustang districts of Nepal. In another study by Rai et al. (1999) seroprevalence was found to be 65.3% out of 404 samples collected from Achham and Dang District of Nepal. Rai et al. (2011) also reported the first case of congenital toxoplasmosis from Nepal in a 53 days old full term male baby. There is dearth of information regarding animal toxoplasmosis in Nepal. Toxoplasmosis in goats was reported to be 29.56% in Sunsari District of Nepal (Sah, Talukdher, Alam, Rahman, & Singh, 2017). Moreover, this study is the first one which documents toxoplasmosis in the sheep of Nepal. Based on the prevalence of toxoplasmosis in human and goat populations in Nepal, we hypothesize that *T. gondii* may also present in sheep. The main objective of the study was to investigate the prevalence of *T. gondii* in sheep of different geographical regions of Nepal.

Materials and methods

A cross-sectional study was carried out in different sheep farms and its vicinity to find the seroprevalence of *T. gondii* in the sheep of the study area. A total of 235 blood samples were collected using convenient cluster sampling methods; from Chitwan (n = 85), Pokhara (n = 62) and Jumla (n = 88).

Chitwan is located in the south of Nepal with the average temperature range from 30.7 °C to 17.4 °C and average relative humidity from 83.65% to 70.52%. Pokhara is located in the western part of Nepal. The average temperature in Pokhara ranges from 26.47 to 15.32 °C and the average relative humidity ranges from 82.34% to 64.14% (Source: analyzed from raw data collected between 1967 and 2012 by Department of hydrology and Metrology, Nepal). Jumla is situated in mid-western part of Nepal with an annual average rainfall of 1343.0 mm. The average temperature differs from 18 °C to 30 °C in summer and in winter from −14°C to 8 °C (RAP 3, 2016).

Three and a half milliliters of blood was collected from the jugular vein from the selected sheep using sterile 10 ml syringes and needles and kept in a vial without anticoagulant to extract serum. The vial was tilted side by side gently 4–5 times and was taken to the laboratory

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https://doi.org/10.1016/j.vas.2018.01.001
Received 20 June 2017; Received in revised form 18 November 2017; Accepted 5 January 2018
Available online 10 January 2018

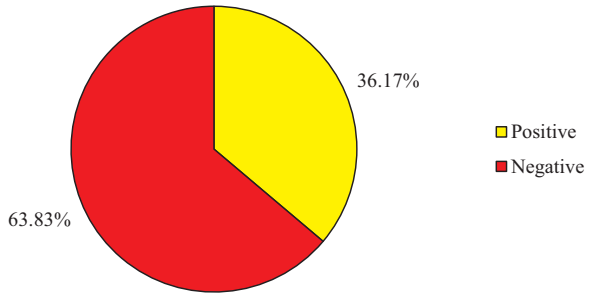

Fig. 1. Results of ELISA test for anti-Toxoplasma antibody detection in sheep.

within 2 h for centrifugation. Centrifugation was carried out at 3000 rpm for 15 min to separate the serum from the remaining clot. Then the serum was poured into a sterile 3 ml serum vial and stored at −20 °C until the test was conducted. The ELISA test was conducted using ID Screen Toxoplasmosis Indirect Multispecies manufactured by IDVet, France with wells coated with P30 *T gondii* antigen to detect the anti-Toxoplasma antibody.

The statistical analysis of the data was performed using software R version 3.0.0 (R Core team, 2015). The significance of the difference of the prevalence among different age groups and between sexes was tested using a Chi-square test.

Results and discussion

Out of the 235 serum samples tested for the presence of antibody against *T. gondii,* 85 samples (36.17%, CI: 30.29–42.49%) were found to be positive using ELISA (Fig. 1). This is the first data available on *T. gondii* in sheep for this country. A recent study by Sah et al. (2017) in goats showed the prevalence of toxoplasmosis to be 29.56% (159/47) in the Sunsari district of Nepal. The difference in the prevalence within the country may be due to the different geographical and climatic conditions, and the diagnostic test used. Different serological tests (modified agglutination test, ELIZA, immunosorbent agglutination assay, indirect fluorescent antibody test and indirect haemagglutination assays, Dye test) have been developed (Liu, Wang, Huang, & Zhu, 2015) to detect *T. gondii.* However, differences in the sensitivity and specificity, makes comparison very difficult between the studies.

With respect to prevalence according to sex, 12 males (14.12%; CI: 8.26–23.07%) and 73 females (85.88%; CI: 76.93–91.74%) were found to be seropositive for *T. gondii* (Fig. 2). Thus higher prevalence was observed in ewes than in rams. However, this difference was not statistically significant ($p > 0.05$). Ragozo et al. (2008), van der Puije (2000), Sah et al. (2017) reported the prevalence to be higher in females when compared to males. According to Sah et al. (2017) higher prevalence of Toxoplasmosis in female was possibly due to the management system of Nepal in which only 'high quality' males are kept for mating and the majority of males were sold and/or culled, whereas females are kept in the farm for longer period of time for breeding.

The relationship between age and sheep toxoplasmosis showed that

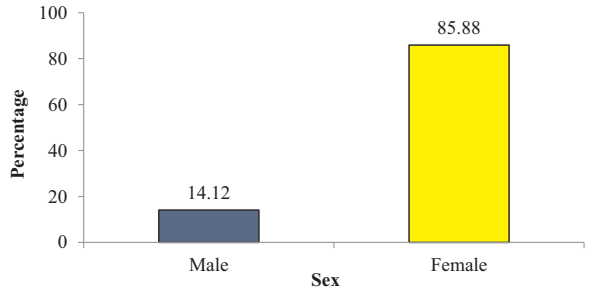

Fig. 2. Seroprevalence of *T. gondii* in sheep according to sex.

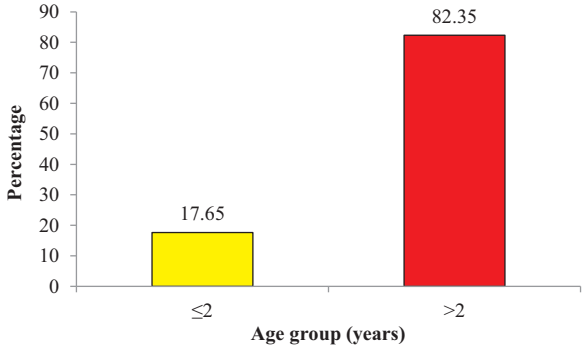

Fig. 3. Age wise seroprevalence of *T. gondii* in sheep.

the prevalence was higher in sheep of more than 2 years of age (82.35%; CI: 72.90–89.00%) and lower in age group of 2 or less than 2 years of age (17.65%; CI: 11.00–27.10%), though the difference was not statistically significant ($p > 0.05$) (Fig. 3).

Animals acquire *Toxoplasma* infectionwith higher likelyhood as their age progresses through ingestion of infective oocysts from the environment (Figliuolo et al., 2004; van der Puije, Bosompem, Canacoo, Wastlaing, & Akanmori, 2000).

The seroprevalence of *Toxoplasma gondii* was found to be significantly lower at Jumla (9.41%; CI: 4.85–17.49%) when compared to Pokhara (32.94%; CI: 23.88–43.48%) and Chitwan (57.65%; CI: 47.04–67.60%) ($p < 0.05$). However seroprevelance findings between Pokhara and Chitwan were not significantly different when compared to each other ($p > 0.05$) (Fig. 4).

Chitwan being at low altitude, and having a subtropical climate (hot and humid), had a higher prevalence. The probable reasons for high seroprevalence are semi-intensive sheep farming, humid conditions in the study area at low altitude, the presence of feral cats, and open feed storage with access to cats). Similar findings was found in Mexico by Caballero-Ortega, Palma, García-Márquez, Gildo-Cárdenas, and Correa (2008) who revealed that the highest prevalence of *T. gondii* was present at low altitude. Similarly, higher prevalence of *Toxoplasma gondii* in Pokhara can be attributed to the high rainfall and semi intensive sheep farming. A 10 year study in France revealed the relationship of *T. gondii* prevalence with temperature and rain (Afonso, Thulliez, & Gilot-Fromont, 2006). The risk of acquiring infection was enhanced when the weather was moist and warm. Oocyst survival increases in moist conditions during longer periods of hot weather (Afonso et al., 2006; Frenkel, Ruiz, & Chinchilla, 1975). The results for Jumla revealed the lowest prevalence, and this may be due to high altitude and the arid environment. Similar findings were found in the work of Vollaire, Radecki, and Lappin (2005) who showed *T. gondii* prevalence in cats to be highest in arid regions of the country.

Conclusion

As an outcome of this study, the overall seroprevalence of toxoplasmosis in sheep in Nepal was found to be 36.17% using the ELISA

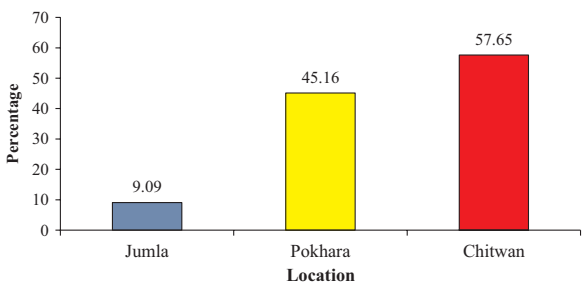

Fig. 4. Seroprevalence of *T. gondii* in sheep according to eco-zones.

test. Region wise prevalence was estimated to be 57.65%, 45.16% and 9.09% at Chitwan, Pokhara and Jumla respectively. Since Toxoplasma is a transmissible parasite it is likely that the infection, or toxoplasmosis, may also be prevalent in the human beings in the studied area. Animal and human health practitioner effort should be integrated to develop national action plan for the prevention, control and eradication of Toxoplasmosis in Nepal, to safeguard both animal and human health.

Acknowledgement

This study was supported through a grant from the USAID Livestock Innovation Lab for adapting livestock systems to climate change, Colorado State University, USA. We acknowledge the livestock farmers from our study area for their participation and cooperation in this study.

Ethical approval

Approval for this research was granted from Nepal Veterinary Council (NVC) which is the national veterinary statutory body of Nepal.

Conflict of interest

The authors have no conflict of interest regarding this work.

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