Serological Survey and Factors Associated with Toxoplasma gondii Infection in Domestic Goats in Myanmar

Saw Bawm,¹ Wint Yi Maung,¹,² Myat Yee Win,¹,² May June Thu,¹,³ Hla Myet Chel,¹
Tin Aye Khaing,¹,⁴ Soe Soe Wai,¹ Lat Lat Htun,¹ Tin Tin Myaing,⁵ Saruda Tiwananthagorn,⁶
Makoto Igarashi,⁷ and Ken Katakura⁸

¹Department of Pharmacology and Parasitology, University of Veterinary Science, Nay Pyi Taw 15013, Myanmar
²Livestock Breeding and Veterinary Department (Head Quarter), Nay Pyi Taw 15011, Myanmar
³Unit of Risk Analysis and Management, Research Centre for Zoonosis Control, Hokkaido University, Sapporo 060-0818, Japan
⁴Nay Pyi Taw Development Committee, Nay Pyi Taw 15011, Myanmar
⁵Myanmar Veterinary Association, Yangon 11011, Myanmar
⁶Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
⁷National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
⁸Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

Correspondence should be addressed to Saw Bawm; bestshadow@gmail.com

Received 5 December 2015; Accepted 18 January 2016

Copyright © 2016 Saw Bawm et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Goat farming is important for the livelihood of millions of rural people because it contributes to food security and creation of assets. However, infection of goats with Toxoplasma gondii could be a source of parasite transmission to humans. The information on T. gondii infection of goat was not reported yet in Myanmar. A total of 119 goat serum samples were collected from three cities in the central region of Myanmar for T. gondii antibody survey. With the occurrence value obtained in this first study, a second one, more complete, with larger number (162) of animals and properties, was carried out and the risk factors and prevalence were determined. In both studies the samples were analyzed by the LAT. Of these, 32 (11.4%) samples were showed to be positive. The infection was associated with the presence of cats at the farm (odds ratio [OR] = 4.66, 95% confidential interval [CI] = 1.03–21.06), farming with different animal species (sheep, cattle, and pigs) (OR = 4.33, 95% CI = 1.57–11.94), and farming without good management practices (OR = 0.23, 95% CI = 0.06–0.83). This is the first T. gondii prevalence study in goats in the country.

1. Introduction

Toxoplasma gondii is a zoonotic protozoan parasite, and the meat of infected food animals (pigs, goats, sheep, or poultry) is one of the most important potential sources of human toxoplasmosis [1]. In humans, transplacental transmission of the parasite in pregnant women often leads to death or damage to the central nervous system of the fetus [2]. In Myanmar, serological studies showed that the prevalence of T. gondii infection in humans was 31.8–41.0% [3–5], but infection sources are poorly understood.

Infection of domestic animals, including cattle, pigs, sheep, goats, and chickens, with T. gondii occurs widely around the world, resulting in insignificant reproductive and economic losses [2]. Ingestion of undercooked meat and unpasteurized milk and cheese from infected goats are potential sources of infection in humans [6–8]. In the central region of Myanmar, farming of small ruminants, especially goats (Capra hircus), is of economic importance. However, the prevalence of T. gondii infection among domestic animals is unknown in Myanmar. This study aimed to determine the seroprevalence of T. gondii antibodies in domestic goats in...
the central region of Myanmar and assess risk factors for infection.

2. Materials and Methods

2.1. Study Areas and Study Animals. In an initial study, blood samples were collected from 119 goats on eight farms near three cities, Nay Pyi Taw (located within 19°75'N and 96°13'E), Mandalay (located within 21°97'N and 96°08'E), and Pyin Oo Lwin (located within 22°03'N and 96°47'E), of Myanmar in January 2009. For a cross-sectional study in June 2013, blood samples were collected from 162 goats on 15 farms in Pyawbwe Township (located within 20°35'0''N and 96°4'0''E). The sample size in Pyawbwe was determined by taking into account the assumed prevalence of anti-*T. gondii* antibodies (20.7%), which was estimated from the ratio of positive samples in the initial study with an exact binomial confidence interval of 95% [9]. The total goat population in the municipality of Pyawbwe was 24,500 goats according to Pyawbwe Township Livestock Breeding and Veterinary Department in the year of 2012.

In these study areas, average herd size is 10–30 goats per farm and they were raised under semi-intensive system. Stratified systematic sampling method was used for sample collection. A questionnaire was used to record the general characteristics of the goats including age, sex, and breed (Kalahari or Jamnapari). In addition, environmental data were recorded, including the presence of cats on the farm, farming with other animals (sheep, cattle, pigs, and chickens), and farming with good management practices (good hygiene, proper feeding, regular vaccination, and deworming).

This project was approved by the Ministry of Livestock, Fisheries and Rural Development and University of Veterinary Science, Myanmar. Informed consent was obtained from all owners of the goats.

2.2. Blood Sampling and Serological Examination. Blood samples were collected from the jugular vein of goats and dropped on blood sampling paper (Nobuto Strip, ADVANTEC®, Tokyo, Japan). The filter paper strips were air-dried and kept in 4°C with a desiccant until use. Serum components were eluted from the strip by soaking the blood-absorbed area in 400 µL of 0.01 M phosphate buffered saline (PBS), pH 7.4, for 60 min at room temperature, in accordance with the manufacturer’s instructions, to make a solution equivalent to a 10-fold dilution of the serum. The eluent was further diluted with PBS and tested using a Latex Agglutination Test (LAT) kit (Toxocheck-MT; Eiken Chemical, Tokyo, Japan). The LAT was carried out in a 96-well microplate with twofold dilutions from 1:16 to 1:4,096. The plate was shaken for 2 min and then incubated at room temperature overnight. The test was considered positive reaction when a layer of agglutinated latex beads was formed in a well at dilutions of 1:64 or higher [10].

2.3. Statistical Analysis. Statistical analysis was performed using Epi Info software version 7.1.2 (Centers for Disease Control and Prevention: http://www.cdc.gov/epiinfo/7/). Yates’ corrected Chi-square test was used for comparison of the frequencies among groups. The difference in prevalence among groups was analyzed statistically by Chi-squared independence. Risk was expressed as an odds ratio (OR) with 95% confidence interval (CI). Statistical significance was set at a *p* value of <0.05.

3. Results and Discussion

The initial survey (2009) in Mandalay, Nay Pyi Taw, and Pyin Oo Lwin showed occurrence of *T. gondii* infection that ranged from 4.7% to 20.7% (Table 1). In the second study (2013) in Pyawbwe, the seroprevalence was 11.8%. In total, anti-*T. gondii* antibodies were detected in 32 of 281 (11.4%) goats (Table 1), and reciprocal titers ranged from 64 to 2,048, although no obvious peak of titers was observed (Table 2).

Risk factors associated with seropositivity against *T. gondii* infection in Pyawbwe are shown in Table 3. Statistical analysis using Chi-square test revealed that the presence of anti-*T. gondii* antibodies in goats was associated with the presence of cats on the farm (*p* = 0.038, OR = 4.66, 95%, and CI = 1.03–21.06), farming with different animal species

| City           | Sampling time | Number of samples (number of farms) | Number of positive |
|----------------|---------------|-------------------------------------|--------------------|
| Mandalay       | Jan 2009      | 50 (2)                              | 5 (10.0%)          |
| Nay Pyi Taw    | Jan 2009      | 40 (3)                              | 3 (4.7%)           |
| Pyin Oo Lwin   | Jan 2009      | 29 (3)                              | 6 (20.7%)          |
| Pyawbwe        | June 2013     | 162 (15)                            | 18 (11.8%)         |
| Total          |               | 281 (23)                            | 32 (11.4%)         |

| Titer     | Number of positive | % of positive |
|-----------|--------------------|--------------|
| <1:32     | 215                | 76.5         |
| 1:32      | 34                 | 12.1         |
| 1:64      | 8                  | 2.9          |
| 1:128     | 9                  | 3.2          |
| 1:256     | 3                  | 1.1          |
| 1:512     | 3                  | 1.1          |
| 1:1,024   | 6                  | 2.1          |
| 1:2,048   | 3                  | 1.1          |

(There were 7 farms out of 15 farms that were showed to be positive with at least one animal.)
Table 3: Factors associated with *T. gondii* infection in goats in Pyawbwe City.

| Category                                      | Sample size | Number of positive (%) | Odds ratio (95% CI) | p   |
|-----------------------------------------------|-------------|------------------------|---------------------|-----|
| Age of animal (years)                         |             |                        |                     |     |
| 0-1                                           | 9           | 1 (11.1)               | —                   |     |
| 1-2                                           | 102         | 10 (9.8)               | 1.15 (0.13–10.16)   |     |
| >2                                            | 51          | 7 (13.7)               | 0.68 (0.24–1.91)    | 0.550|
| Sex of animal                                 |             |                        |                     |     |
| Male                                          | 56          | 5 (8.9)                | —                   |     |
| Female                                        | 106         | 13 (12.3)              | 0.70 (0.24–2.08)    | 0.522|
| Breed                                         |             |                        |                     |     |
| Jad ni (Kalahari)                             | 7           | 2 (28.6)               | —                   |     |
| Htein san (Jamnapari)                         | 155         | 16 (10.3)              | 3.47 (0.62–19.40)   | 0.134|
| Presence of cats                              |             |                        |                     |     |
| Yes                                           | 107         | 16 (15.0)              | 4.66 (1.03–21.06)   | 0.038|
| No                                            | 55          | 2 (3.6)                | —                   |     |
| Farming with different species (sheep, cattle, pigs, and poultry) | 36 | 9 (25.0) | 4.33 (1.57–11.94) | 0.003|
| No                                            | 126         | 9 (7.1)                | —                   |     |
| Good management practices                     |             |                        |                     |     |
| Yes                                           | 70          | 3 (4.3)                | —                   |     |
| No                                            | 92          | 15 (16.3)              | 0.23 (0.06–0.83)    | 0.016|

(such as sheep, cattle, and pigs) (p = 0.003, OR = 4.33, and 95% CI = 1.57–11.94), and management without good practices (poor hygiene, lack of proper feeding, regular vaccination, and deworming) (OR = 0.23; 95% CI = 0.06–0.83). Age, sex, and breed of the goats presented no association with infection (Table 3).

A number of recent epidemiological studies showed the worldwide spread of *T. gondii* in goats. In Myanmar, the present study indicated that the seroprevalence of *T. gondii* in goats was 11.4%. In the neighboring countries, it was 27.9% in Thailand [11], 61.0% in Bangladesh [12], 41.3% in India [13], 25.4% in Pakistan [14], and 3.8–30.8% in China [15, 16]. The difference in the seroprevalence rates in these Asian countries could be due to differences in diagnostic techniques, sample size, age of the animals, and climatic and management variations from one region to another.

The risk factors for *T. gondii* infection in goats have been analyzed in different countries. In Brazil, risk factors were detected as age over 36 months, use of a pen, pure breed animals, the presence of cats alongside the herd, intensive or semi-intensive management systems, and a lack of mineral supplementation [17, 18]. In Greece, risk factors comprised intensive or semi-intensive management, feeding concentrate, and providing water from the public supply [19]. Furthermore, access of stray cats to animals’ water and the presence of wild felids were potential risk factors for seroprevalence of *T. gondii* in dairy sheep flocks in Italy [20] and Ethiopia [21], respectively. Taken together, the presence of cats, a large flock size, and the method of disposing of aborted fetuses are potential risk factors for *T. gondii* infection in goat and sheep. The present study also showed that one of the risk factors was the presence of cats on the farm, and this result was in agreement with those from previous studies.

Cats act as the final host in the life cycle of *T. gondii* and excrete oocysts in their feces that can be a source of infection for humans and other animals [2]. Oocysts eliminated by cats remain infective for months to years in moist, shaded, and temperature-regulated environmental conditions [22]. In this study almost all farms had different animal species (sheep, cows, oxen, pigs, and poultry) living in the same environment and via the movement of those animals the infection and transmission could be facilitated. The feces and urine of animals, waste food, and water supplied to animals might contribute to the maintenance of viability of oocysts. In addition, farming without good management practices was also identified as a risk factor in this study. Poor hygiene conditions and animals management implicate an increased risk for *T. gondii* infection. Studies on the seroprevalence of *T. gondii* infection in humans and other domestic animals are necessary to better understand the epidemiology of *T. gondii* in Myanmar.

Livestock are a key asset for farmers in Myanmar, and more than half of the national sheep and goat population were found in the central region, the studied area. In order to fight poverty in Myanmar it is important to develop goat related industries and *T. gondii*, an important zoonotic pathogen, needs to be controlled once this coccidian could be present in the meat, milk, and cheese of infected goats [6–8].

4. Conclusions

This is the first report on the seroprevalence of *T. gondii* infection in domestic goats in Myanmar that found a prevalence value of 11.4% and presence of cats on the farms, farming with different animal species, and farming without...
good management practices presented association with the infection.

Conflict of Interests
The authors declare that they have no conflict of interests.

Acknowledgments
The authors are grateful to Dr. Ryo Nakao of Hokkaido University and Dr. Naoaki Yokoyama of Obihiro University of Agriculture and Veterinary Medicine for valuable suggestions. The authors also thank all the staff of LBVD and veterinary officers for helping with sample collection in Myanmar. This study was supported in part by Global Center of Excellence Program for International Collaboration Centers for Zoonosis Control and JSPS KAKENHI Grant nos. 22-kyoudou-5 of National Culture, Sports, Science and Technology of Japan and a Centers for Zoonosis Control and JSPS KAKENHI Grant for excellence program for international cooperation.

Myanmar. This study was supported in part by Global Collaboration Centers for Zoonosis Control and JSPS KAKENHI Grant nos. 22-kyoudou-5 of National Culture, Sports, Science and Technology of Japan and a Cooperative Research Grant (22-kyoudou-5) of National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan.

References

[1] J. L. Jones and J. P. Dubey, “Foodborne toxoplasmosis,” Clinical Infectious Diseases, vol. 55, no. 6, pp. 845–851, 2012.

[2] J. P. Dubey, “The history of Toxoplasma gondii—the first 100 years,” The Journal of Eukaryotic Microbiology, vol. 55, no. 6, pp. 467–475, 2008.

[3] B. T. E. Chan, R. N. Amal, M. I. Noor Hayati et al., “Seroprevalence of toxoplasmosis among migrant workers from different Asian countries working in Malaysia,” Southeast Asian Journal of Tropical Medicine and Public Health, vol. 39, no. 1, pp. 9–13, 2008.

[4] U. T. Nyunt, “Report on laboratory diagnosis of toxoplasmosis in Myanmar at the National Health Laboratory (1995–2004),” Myanmar Journal of Current Medical Practice, vol. 9, pp. 120–124, 2005.

[5] H. Andiappan, V. Nissapatorn, N. Sawangjaroen et al., “Comparative study on Toxoplasma infection between Malaysian and Myanmar pregnant women,” Parasites & Vectors, vol. 7, article 564, 2014.

[6] J. J. Sacks, R. R. Roberto, and N. F. Brooks, “Toxoplasmosis infection associated with raw goat’s milk,” The Journal of the American Medical Association, vol. 248, no. 14, pp. 1728–1732, 1982.

[7] F. Mancianti, S. Nardoni, C. D’Ascenzi et al., “Seroprevalence, detection of DNA in blood and milk, and genotyping of Toxoplasma gondii in a goat population in Italy,” BioMed Research International, vol. 2013, Article ID 905326, 6 pages, 2013.

[8] J. P. Dubey, S. K. Verma, L. R. Ferreira et al., “Detection and survival of Toxoplasma gondii in milk and cheese from experimentally infected goats,” Journal of Food Protection, vol. 77, no. 10, pp. 1747–1753, 2014.

[9] M. Thrusfield, Veterinary Epidemiology, Blackwell Science, London, UK, 3rd edition, 2007.

[10] U. Opel, W. A. G. Charleston, W. E. Pomroy, and M. Rommel, “A survey of the prevalence of Toxoplasma infection in goats in New Zealand and a comparison of the latex agglutination and indirect fluorescence tests,” Veterinary Parasitology, vol. 40, no. 3–4, pp. 181–186, 1991.

[11] S. Jittapalapong, A. Sangvaranond, N. Pinyopanuwat et al., “Seroprevalence of Toxoplasma gondii infection in domestic goats in Satun Province, Thailand,” Veterinary Parasitology, vol. 127, no. 1, pp. 17–22, 2005.

[12] M. Rahman, M. T. A. Azad, L. Nahar et al., “Age-specificity of Toxoplasma gondii seroprevalence in sheep, goats and cattle on subsistence farms in Bangladesh,” The Journal of Veterinary Medical Science, vol. 76, no. 9, pp. 1257–1259, 2014.

[13] H. Singh, A. K. Tewari, A. K. Mishra et al., “Detection of antibodies to Toxoplasma gondii in domesticated ruminants by recombinant truncated SAG2 enzyme-linked immunosorbent assay,” Tropical Animal Health and Production, vol. 47, no. 1, pp. 171–178, 2014.

[14] M. Ramzan, M. Akhtar, F. Muhammad et al., “Seroprevalence of Toxoplasma gondii in sheep and goats in Rahim Yar Khan (Punjab), Pakistan,” Tropical Animal Health and Production, vol. 41, no. 7, pp. 1225–1229, 2009.

[15] C. R. Wang, J. H. Qiu, J. F. Gao et al., “Seroprevalence of Toxoplasma gondii infection in sheep and goats in northeastern China,” Small Ruminant Research, vol. 97, no. 1–3, pp. 130–133, 2011.

[16] G.-H. Zhao, M.-T. Zhang, L.-H. Lei et al., “Seroprevalence of Toxoplasma gondii infection in dairy goats in Shaanxi Province, Northwestern China,” Parasites & Vectors, vol. 4, no. 1, article 47, 2011.

[17] A. C. A. V. Carneiro, M. Carneiro, A. M. G. Gouveia et al., “Seroprevalence and risk factors of caprine toxoplasmosis in Minas Gerais, Brazil,” Veterinary Parasitology, vol. 160, no. 3–4, pp. 225–229, 2009.

[18] J. O. A. Neto, S. S. Azevedo, S. M. Gennari 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,” Veterinary Parasitology, vol. 156, no. 3–4, pp. 329–332, 2008.

[19] N. Tzanidakis, P. Maksimov, F. J. Conraths et al., “Toxoplasma gondii in sheep and goats: seroprevalence and potential risk factors under dairy husbandry practices,” Veterinary Parasitology, vol. 190, no. 3–4, pp. 340–348, 2012.

[20] B. T. Cenci-Goga, A. Ciampelli, P. Sechi et al., “Seroprevalence and risk factors for Toxoplasma gondii in sheep in Grosseto district, Tuscany, Italy,” BMC Veterinary Research, vol. 9, article 25, 2013.

[21] E. Z. Gebremedhin, A. Agonafir, T. S. Tessema et al., “Seroepidemiological study of ovine toxoplasmosis in East and West Shewa Zones of Oromia Regional State, Central Ethiopia,” BMC Veterinary Research, vol. 9, article 117, 2013.

[22] J. L. Jones and J. P. Dubey, “Waterborne toxoplasmosis—recent developments,” Experimental Parasitology, vol. 124, no. 1, pp. 10–25, 2010.