Pertanika J. Trop. Agric. Sci. 41 (1): 477 - 484 (2018)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Short Communication

Seroprevalence of *Neospora Caninum* in Sheep and Goats of Gua Musang District in Kelantan, Malaysia

Than Kyaw[1]*, Athirah Mohd Mokhtar[1], Bee Lee Ong[1], Chee Hock Hoe[1], Abd Rahman Aziz[1], Erkihun Aklilu[1] and Suratan Kamarudin[2]

[1]*Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Pengkalan Chepa, Locked Bag 36, 16100 UMK, Kota Bharu, Kelantan Malaysia*

[2]*Department of Veterinary Services, Universiti Malaysia Kelantan, Kubang Kerian, 16150 UMK, Kota Bharu, Kelantan, Malaysia*

ABSTRACT

Exposure of *Neospora caninum* parasite in sheep and goats in Kelantan was first reported in March 2016. Out of 10 districts surveyed in Kelantan, Gua Musang was the only district with seropositive animals, suggesting that there might be potential infection of this parasite in sheep and goats in this area. Therefore, a cross-sectional study was conducted from May 2015 to February 2016 to investigate the prevalence and impact of *N. caninum* in sheep and goats in that area. A total of 311 sheep and goat blood samples from 37 farms and 10 animals from each farm were collected. A questionnaire on the risk factors (abortion history, presence of dogs and closeness to the cattle farms) was developed for analysis. Serological test was done using a commercial ELISA kit. Seroprevalence was found to be 0.32% (1/311). Although the results i.e. presence of stray dogs (32.4%; 12/37), abortion history (48.6%; 18/37) and closeness to the cattle farms (27%; 10/37) were rather high, the very low seroprevalence showed that these risk factors were not related to neospora infection. The results suggested that *N. caninum* was not the cause of reproductive failure in sheep and goats in Gua Musang.

Keywords: Gua Musang, Malaysia, *Neospora caninum*, prevalence, sheep and goats

ARTICLE INFO

Article history:
Received: 23 March 2017
Accepted: 04 July 2017

E-mail addresses:
than@umk.edu.my (Than Kyaw),
tirahmok@gmail.com (Athirah Mohd Mokhtar),
beelee@umk.edu.my (Bee Lee Ong),
hcheehock@umk.edu.my (Chee Hock Hoe),
abdrahman@umk.edu.my (Abd Rahman Aziz),
erkihun@umk.edu.my (Erkihun Aklilu),
suratan@rocketmail.com (Suratan Kamarudin)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

It is well recognised that *Neospora caninum*, which occurs worldwide, is an important protozoan disease in ruminants. Economic losses due to neosporosis in cattle by reproductive failure, mainly of abortion (Dubey, 1996, p. 28, p. 46; Dubey, 2003, p. 4, pp. 9–10; Dubey & Schares, 2011, p. 94) have been well documented. Similarly, abortion due to neosporosis in sheep and goats naturally (Corbellini, Colodel, & Driemeier, 2001; Kobayashi et al., 2001; Masala et al., 2007; Howe et al., 2008; Ezatpour et al., 2015) and experimentally (McAllister et al., 1996; O'Handley, Liddell, Parker, Jenkins, & Dubey, 2002) have also been reported. Neosporosis can also cause economic losses by reduced weight gain in beef cattle (Barling et al., 2000) and reduced milk yield in dairy cattle (Hernandez, Risco, & Donovan, 2001) but economic losses due to neosporosis have not been reported in sheep and goats. In Southeast Asia, most of the studies on neospora parasite were done on cattle. In this region, seroprevalence of *N. caninum* varied widely from 5.5% in Thailand (Kyaw et al., 2004, p. 255) to 41% in Vietnam (Duong et al., 2008). In Indonesia, seroprevalence in Bali cattle was 5.5% (Damriyasa et al., 2008), while it was 24% in dairy cattle (Sardjana et al., 2015). In a study in the Philippines six out of 17 (35.3%) aborting cows and 10 out of 63 (15.9%) non-aborting cows were seropositive to *N. caninum* (Konnai et al., 2008). Neospora seropositivity was also found to be not related to whether the animal was a heifer or cow (Kyaw et al., 2004, p. 255). IN addition, it has been reported that seropositivity of cattle herd was related to the presence of farm dogs (Arunvipas et al., 2012), while Kyaw et al. (2004) reported no significance difference of neospora seropositivity between herds with dogs or without herds. Although transplacental infection was reported in Thailand (Kyaw et al., 2005) and Malaysia (Chea et al., 2004), no report was found for neospora-related abortion in cattle.

In Malaysia, a few studies on prevalence of *N. caninum* antibodies in cattle have been conducted (Cheah, 2004; Rahman, Manimegalai, Chandrawathani, Premaalathan, & Zaini, 2011), including isolation of the parasite from a newborn calf (Cheah et al., 2004). However, there has been no study of neospora infection in sheep and goats in Malaysia. It was, therefore, considered important to study whether reproductive failure such as abortion in sheep and goats is related to neospora infection.

Neospora antibodies in sheep and goats in Malaysia was first reported in 2016 (Kyaw et al., 2016, p. 43). The study was conducted in 10 districts of Kelantan, and it was found that neospora antibodies in animals had a prevalence of 1.1% (5/472) and a farm prevalence of 6% (3/50). According to the report, all of the seropositive animals and farms were from Gua Musang district only. In Gua Musang, animal prevalence and farm prevalence were found to be 10% (5/50) and 60% (3/5), respectively. These results may indicate possible potential infection of the neospora parasite in sheep and goats in that particular district and the need for more extensive exploration, including studying the association of important risk factors. Therefore, this study was conducted to observe the seroprevalence of neospora antibodies in a representative sheep and goat population in Gua Musang and the possible association of seropositivity and common risk factors i.e. – the presence of dogs on the farm, abortion history and closeness to the cattle farms.

MATERIALS AND METHODS

Sample and Data Collection

The sheep and goat population in Gua Musang that was studied numbered 7,558 (sheep=3,971; goats=3,587); some of these animals were reared on mixed farms (Department of Veterinary Services, 2012, pp. 41–47). The number of animals in farms varied from three to 30 animals. A cross-sectional study with convenience sampling method was used. This was because some farms were too far in location and it was difficult to arrange for a visit by both the researcher and assistants by the Department of Veterinary Services, Kelantan, to collect samples. A total of 311 blood samples were collected from the jugular veins of the animals (ewes and does) from 37 farms, with approximately 10 animals from each farm being sampled. The calculation was based on the method by Naing et al. (2006). For farms having fewer than 10 ewes or does, blood samples were collected from all the animals. The sera were separated and stored at -20°C until further analysis.

During the blood sample collection, the responses via questionnaire to the three risk factors i.e. the presence of dogs or stray dogs on the farm, abortion histor, and closeness of sheep and goat farms to nearby cattle farms were recorded to investigate whether these potential risk factors were associated with neospora seropositivity. Closeness to nearby cattle farms was arbitrarily fixed at a distance of three km in the same area.

Serological Test

Sera were tested for *N. caninum* antibodies using a commercial ELISA test kit (IDEXX *Neospora* X2 Ab Test) according to the manufacturer's instructions. Briefly, the ELISA kit kept at 4°C was thawed at room temperature for 30 min. The test sera stored at -20°C were thawed at room temperature for 30 min. Ninety µl of sample diluents was dispensed into each well of the microplate. Ten µl of the test sera was dispensed in each well. The positive and negative control was dispensed into the appropriate wells. The final dilution of the mixture was 1:10 dilution. The wells were mixed gently by tapping the plate. The plate was covered with aluminium foil and incubated for 60 min at 37°C. After incubation, each well was washed three times with 200 µl of wash solution. Following the final wash, the plate was firmly tapped against absorbent material to remove liquid content. Then, 100 µl of the conjugate solution was dispensed in each well. The plate was covered with aluminium foil and incubated for 60 min. After incubation, the same washing step was done. After that, 100 µl of TMB substrate solution from the kit was dispensed in each well. The plate was covered with an aluminium foil and incubated at room temperature for 15 min. Finally, 100 µl of stop solution was dispensed in each well. The result was read at the wavelength of 450 nm using an absorbance reader (Bio-Tek, USA). The sensitivity and specificity of the test kit were 93.7% and 95.3%, respectively. The results were expressed as S/P ratio based on a positive and negative control serum according to the manufacturer's procedure. The optical density readings ≥40% were positive, while ≥30% and <40% were suspect and <30% readings were negative.

$$S/P\ \% = 100 \times [(Sample\ A450 - NC\text{x})/(PC\text{x} - NC\text{x})$$

where

PCx = positive control, and NCx = negative control

Statistical Analysis

Descriptive statistics were used. Risk factor analysis was not done as there was only one seropositive animal.

RESULTS

Seropositivity

Only one animal from one farm was seropositive. Seroprevalence, therefore, was 0.32% (1/311) and farm prevalence was 2.7% (1/37). The seropositive farm was a goat farm that had only three animals. There was also only one animal suspected of being seropositive as it gave an optical density reading in the range of 30%-40%.

Risk Factors

The occurrence of all three risk factors was high (Table 1). Thirty-two per cent of the sheep and goat farms had stray dogs. Only one farm had its own dogs. The seropositive farm was a goat farm that had only three female goats of over one year of age. The farm had stray dogs. It had no abortion history.

Table 1
Potential risk factor information of 37 sheep and goat farms in the study

Risk Factors	No. of Farms	%	Remarks
Presence of farm dogs (or) stray dogs	12	32.4	Only one farm had a dog. The rest (11 farms) had stray dogs.
Abortion history	18	48.6	Farms with abortion history were high.
Nearby cattle farms	10	27	Neospora serostatus of nearby cattle farms was not known.

DISCUSSION

The seroprevalence results obtained in the present study conducted in Gua Musang provided a very low percentage (0.32%) compared to that obtained from previous studies (10%). According to the other researchers, seroprevalence of neospora in sheep and goats differed depending on geographic location and range from 0.6% in New Zealand (Reichel, Ross, & McAllister, 2008) to 63% in Jordan (Abo-Shehada & Abu-Halaweh, 2010), while in goats it was from 0.4% in Poland (Czopowicz et al., 2011) to 23.6% in the Philippines (Konnai et al., 2008). The exposure and infection may also depend on the other risk factors.

The dogs are considered a main source of neospora transmission as they are definitive hosts (McAllister et al., 1998). The reason why the prevalence in the present result was lower than that reported in the previous report (Kyaw et al., 2016, p. 43) might been because the spread of the parasite was sporadic. Also, the opportunity of contamination of feed or water by the faeces of the infected definitive host might have been low as most of the farms were intensively reared. On the other hand, the frequency and the number of stray dogs that could get access to the farm might also have been very low. In addition, the dog(s) that had accessed the farms, if at all, might not have been infected with neospora. Although it might be difficult to collect samples from stray dogs, it would be meaningful to do so if serology for neospora antibody were detected in the animals. Since information on the serostatus or prevalence of neospora in cattle in Kelantan is lacking, it is probable that the cattle farms situated close to the sheep and goat farms may not be infected.

The number of farms with high occurrence of abortion indicated that most of the sheep and goat farms in Gua Musang had reproductive problems. This study concluded that the neospora parasite might not have been the cause of any reproductive failure as seroprevalence of *N. caninum* was very low. The abortions might have been due to other abortifacients rather than the neospora parasite. It is recommended to explore the real cause of abortion in sheep and goats in Gua Musang.

ACKNOWLEDGEMENT

This study was supported by the Fundamental Research Grant Scheme (FRGS; R/FRGS/A06.00/00675A/001/2014/00143) awarded by the Ministry of Education, Malaysia. The authors thank the Department of Veterinary Services, Kelantan, for their kind help in sampling the blood samples from the sheep and goat farms. Without their help this research could not have been completed.

REFERENCES

Abo-Shehada, M. N., & Abu-Halaweh, M. M. (2010). Flock-level seroprevalence of, and risk factors for, *Neospora caninum* among sheep and goats in northern Jordan. *Preventive Veterinary Medicine, 93*(1), 25–32. doi:10.1016/j.prevetmed.2009.08.004

Arunvipas, P., Inpankaew, T., & Jittapalapong, S. (2012). Risk factors of *Neospora caninum* infection in dogs and cats in dairy farms in Western Thailand. *Tropical Animal Health and Production, 44*(5), 1117–1121.

Barling, K. S., McNeill, J. W., Thompson, J. A., Paschal, J. C., McCollum III., F. T., Craig, T. M., & Adams, L. G. (2000). Association of serologic status for *Neospora caninum* with postweaning weight gain and carcass measurements in beef calves. *Journal of American Veterinary Medical Association, 217*(9), 1356–1360.

Cheah, T. S. (2004). *Seroepidemiology, diagnosis, isolation and characterization of Neospora caninum among cattle in Malaysia*. (Doctoral Dissertation). School of Graduate Studies, Universiti Putra Malaysia.

Cheah, T. S., Mattsson, J. G., Zaini, M., Sani, R. A., Jakubek, E. B., Uggla, A., & Chandrawathani, P. (2004). Isolation of *Neospora caninum* from a calf in Malaysia. *Veterinary Parasitology, 126*(3), 263–269. doi:10.1016/j.vetpar.2004.08.012

Corbellini, L. G., Colodel, E. M., & Driemeier, D. (2001). Granulomatous encephalitis in a neurologically impaired goat kid associated with degeneration of *Neospora caninum* tissue cysts. *Journal of Veterinary Diagnostic Investigation, 13*(5), 416–419. doi:10.1177/104063870101300509

Czopowicz, M., Kaba, J., Szalus-Jordanow, O., Nowicki, M., Witkowski, L., & Frymus, T. (2011). Seroprevalence of Toxoplasma gondii and *Neospora caninum* infections in goats in Poland. *Veterinary Parasitology, 178*(3), 339–341. doi:10.1016/j.vetpar.2011.01.039

Damriyasa, I. M., Schares, G., & Bauer, C. (2010). Seroprevalence of antibodies to *Neospora caninum* in *Bos javanicus* ('Bali cattle') from Indonesia. *Tropical Animal Health and Production, 42*(1), 95–98. doi:10.1007/s11250-009-9390-z.

DVS. (2012). *Bancian ternakan negeri Kelantan*. Department of Veterinary Services, Jabatan Perkhidmatan Veterinar Negeri Kelantan.

Dubey, J. P. (1996). A review of *Neospora caninum* and neosporosis. *Veterinary Parasitology, 67*(12), 1–59.

Dubey, J. P. (2003). Review of *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology, 41*(1), 1–16. doi: 10.3347/kjp.2003.41.1.1

Dubey, J. P., & Schares, G. (2011). Neosporosis in animals – The last five years. A review. *Veterinary Parasitology, 180*(1), 90–108. doi:10.1016/j.vetpar.2011.05.031

Duong, M. C., Alenius, S., Huong, L. T. T., & Bjorkman, C. (2008). Prevalence of *Neospora caninum* and bovine viral diarrhoea virus in dairy cows in Southern Vietnam. *Veterinary Journal, 175*(3), 390–394.

Ezatpour, B., Alirezaei, M., Hassanvand, A., Zibaei, M., Azadpour, M., & Ebrahimzadeh, F. (2015). The first report of *Neospora caninum* prevalence in aborted and healthy sheep from west of Iran. *Compararative Clinical Pathology, 24*(1), 19–22. doi:10.1007/s00580-013-1846-x

Hernandez, J., Risco, C., & Donovan, A. (2001). Association between exposure to *Neospora caninum* and milk production in dairy cows. *Journal of American Veterinary Medical Association, 219*(5), 632–635. doi:10.2460/javma.2001.219.632

Howe, L., West, D. M., Collett, M. G., Tattersfield, G., Pattison, R. S., Pomroy, W. E., … & Williamson, N. B. (2008). The role of *Neospora caninum* in three cases of unexplained ewe abortions in the southern North Island of New Zealand. *Small Ruminant Research, 75*(2), 115–122. doi:10.1016/j.smallrumres.2007.08.001

Kobayashi, Y., Yamada, M., Omata, Y., Koyama, T., Saito, A., Matsuda, T., … & Matsui, T. (2001). Naturally-occurring *Neospora caninum* infection in an adult sheep and her twin fetuses. *Journal of Parasitology, 87*(2), 434–436. doi:10.1645/0022-3395(2001)087[0434:NONCII]2.0.CO;2

Konnai, S., Mingala, C. N., Sato, M., Abes, N. S., Venturina, F. A., Gutierrez, C. A., … & Ohashi, K. (2008). A survey of abortifacient infectious agents in livestock in Luzon, the Philippines, with emphasis on the situation in a cattle herd with abortion problems. *Acta Tropica, 105*(3), 269–273. doi:10.1016/j.actatropica.2007.12.004.

Kyaw, T., Athirah, M. M., Kalthum, H., Abd Rahman, A., Aklilu, E. Hoe, C. H., … & Chandrawathani, P. (2016). *Neospora caninum* antibody detection in sheep and goats in Kelantan. In *52nd Annual Scientific Conference, Malaysian Society of Parasitology and Tropical Medicine*. Grand Season Hotel, Kuala Lumpur.

Kyaw, T., Suwimonteerabutr, J., Virakul, P., Lohachit, C., & Kalpravidh, V. (2005). Seronegative conversion in four *Neospora caninum*-infected cows, with a low rate of transplacental transmission. *Veterinary Parasitology, 131*(1), 145–150. doi:10.1016/j.vetpar.2005.04.025

Kyaw, T., Virakul, P., Muangyai, M., & Suwimonteerabutr, J. (2004). *Neospora caninum* seroprevalence in dairy cattle in central Thailand. *Veterinary Parasitology, 121*(3), 255–263. doi.org/10.1016/j.vetpar.2004.01.014

Masala, G., Porcu, R., Daga, C., Denti, S., Canu, G., Patta, C., & Tola, S. (2007). Detection of pathogens in ovine and caprine abortion samples from Sardinia Italy, by PCR. *Journal of Veterinary Diagnostic Investigation, 19*(1), 96–98. doi:10.1177/104063870701900116

McAllister, M. M., Dubey, J. P., Lindsay, D. S., Jolley, W. R., Wills, R. A., & McGuire, A. M. (1998). Dogs are definitive hosts of *Neospora caninum. International Journal of Parasitology, 28,* 1473–1478.

McAllister, M. M., McGuire, A. M., Jolley, W. R., Lindsay, D. S., Trees, A. J., & Stobart, R. H. (1996). Experimental neosporosis in pregnant ewes and their offspring. *Veterinary Parasitology, 33*(6), 647–655. doi:10.1177/030098589603300603

Naing, L., Winn, T., & Rusli, B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences, 1*, 9–14.

O'Handley, R., Liddell, S., Parker, C., Jenkins, M., & Dubey, J. P. (2002). Experimental infection of sheep with *Neospora caninum* oocysts. *Journal of Parasitology, 88*(6), 1120–1123. doi:10.1645/0022-3395(2002)088[1120:EIOSWN]2.0.CO;2

Rahman, W. A., Manimegalai, V., Chandrawathani, P., Premaalathan, B., & Zaini, C. M. (2011). Comparative seroprevalence of bovine Toxoplasmosis and Neosporosis in five states in Malaysia. *Global Veterinaria, 6*(6), 575–578.

Reichel, M. P., Ross, G. P., & McAllister, M. M. (2008). Evaluation of an enzymelinked immunosorbent assay for the serological diagnosis of *Neospora caninum* infection in sheep and determination of the apparent prevalence of infection in New Zealand. *Veterinary Parasitology, 151*(2), 323–326. doi:10.1016/j.vetpar.2007.11.002

Sardjana, I. K. W. (2015). Neosporosis in cattle: Preliminary study in Batu-Malang region, Indonesia. *Pinnacle Agricultural Research and Management, 3*(1), 487-491.