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Comparative Seroprevalences of Bovine Toxoplasmosis and Neosporosis in Five States in Malaysia

[1]W.A. Rahman, [1]V. Manimegalai, [2]P. Chandrawathani, [2]B. Premaalatha and [2]C.M. Zaini

[1]School of Biological Sciences, Universiti Sains Malaysia, Penang, 11800, Malaysia
[2]Veterinary Research Institute, Jalan Sultan Azlan Shan, Ipoh, 3400, Perak, Malaysia

Abstract: A study was conducted to determine the prevalences of *Toxoplasma gondi* and *Neospora caninum* in Malaysia. One hundred and sixteen adult cattle sera were randomly selected from 17 farms in five different states of Malaysia. All serum samples were tested by Flourescent Antibody Test (IFAT) using specific species conjugates. Results showed that 3.5% was positive for *Neospora caninum* while *Toxoplasma gondii* showed a positive of 2.6%, meaning that cattle in the country are more susceptible to *Neospora caninum* than *Toxoplasma gondii*.

Key words: Seroprevalences · Bovine Toxoplasmosis · Neosporosis · Malaysia

INTRODUCTION

According to Dubey and Beattie [1] the only definitive host for toxoplasmosis is feline, while humans act as an intermediate hosts. The organism has a worldwide distribution and is a general cause of infertility, stillbirth and abortion in animals and man [2]. The parasite can cause problems to pregnant woman and immunodeficiency individuals [3].

Infections in small ruminants do not only result in significant reproductive losses, but also has implication for public health. Consumption of infected meat can facilitate zoonotic transmission [4].

Neospora caninum is a protozoan parasite which closely resembles *T. gondii*. *N. caninum* is a canine coccidian parasite [5, 6]. *Neospora* has been found worldwide and is the most common cause of abortions in cattle in many areas. The organism was first identified in 1988 as a cause of abortion in dogs and, shortly after; a *Neospora*–like organism was described as causing factor in inducing abortions in dairy cows. Now the causative organism in inducing abortion in cattle is known to be the same species but a different strain in dogs [7]. Cattle are the natural intermediate host for *N. caninum* [8] and also has been recommended as a source of such a transmission [9].

There are several tests used to detect toxoplasmosis and neosporosis in animals such as Enzyme-linked immunosorbent assay (ELISA), Indirect Fluorescent Antibody Tests (IFAT) and isolation of organism. The IFAT has been widely used for both *N. caninum* and *T. gondii* as a serological tests for the diagnosis of infection in cattle. Positive IFAT results mean that the cows are exposed to the disease but do not necessarily indicates infection status at the time of the test.

Many studies have not been conducted on the seroprevalence of *T. gondii* and *N. caninum* in animals in Malaysia and some surveys were done a long time ago [10, 11, 12]. The objective of this study is to determine the seroprevalences of *N. caninum* and *T. gondii* in Malaysian cattle using IFAT.

MATERIALS AND METHODS

Serum Samples: Bovine serum samples from Veterinary Research Institute (VRI), Serum Bank consisting of 116 adult cattle sera randomly selected from 17 farms in five different states in Malaysia (Perak, Johor, Melaka, Terengganu and Sabah). All samples were tested by IFAT using species specific conjugates (from VMRD).

Indirect Fluorescent Antibody Test (IFAT)

Materials: Fluorescent antibody substrate slides with 12 wells were used for the detection of antibody against *T. gondii* and *N. caninum*. Other testing materials used were positive or negative control sera, serum diluting buffer, anti-immunoglobulin conjugate, rinse buffer and mounting fluid.

Corresponding Author: W.A. Rahman, School of Biological Sciences, Universiti Sains Malaysia, Penang, 11800, Malaysia. E-mail: arawahab@usm.my.

Procedure: The slides were warmed under room temperature after being removed from foil pouch. The sera were diluted in a serum dilution plate in a portion 1:20 with a serum diluting buffer solution at pH 7.2. Then 10-50 µl (depending on well size) diluted sera from each sample placed on the designated wells. Later the slides were incubated in a humid chamber at 37°C for 30 minutes. After that, the slides were tenderly rinsed in fluorescent antibody rinse buffer using a wash bottle and then soak inside the same buffer solution for 10 minutes. Then the slides were drained and dried around the wells by pressing blotter to remove excessive water from the slides.

After that 10 µl of FITC-labeled anti-IgG or IgM conjugate was added on the wells. Then the wells again were incubated in humid chamber at 37°C for 30 minutes. The wells were then rinsed as mentioned earlier with fluorescent antibody rinse buffer. The slides were drained and the back and edges of the slides were dried using paper towels. The stained surface was not allowed to rinse trough water or dry. Before viewing the slides under fluorescent microscope at 400X magnification the slides more mounted with fluorescent antibody mounting fluid per well and covered with cover slip. This was to have a better image of the slide under fluorescent microscope. The existence of bright fluorescent cytoplasmic bodies under microscope indicated the presence of antibody in the sera.

RESULTS

The prevalence of *T. gondii* infection in the cattle is shown in Table 1. Three of 116 serum samples were found positive for *T. gondii* with percentage of 2.59, from the state of Melaka. Meanwhile four samples (3.44%) were found to be positive for *N. caninum* (Table 2). Figure 1 showed the comparison of seroprevalence of *T. gondii* and *N. caninum* infection among cattle in five different states in Malaysia.

Table 1: Seroprevalence of toxoplasmosis in five states in Malaysia

State	No. Examined	No. Positive	Percentage
Johor	20	0	0.00
Melaka	25	3	2.59
Perak	21	0	0.00
Sabah	20	0	0.00
Terengganu	30	0	0.00
Total	116	3	2.59

Table 2: Seroprevalence of neosporasis in five states in Malaysia

State	No. Examined	No. Positive	Percentage
Johor	20	1	0.86
Melaka	25	1	0.86
Perak	21	1	0.86
Sabah	20	1	0.86
Terengganu	30	0	0.00
Total	116	4	3.44

This figure shows the total number of seropositive for both parasites in these five different states. The total seropositive for *T. gondii* is obtained from slaughterhouse of Melaka; mean 3 samples were shown positive out of 116 samples. Meanwhile for *N. caninum* there was 4 positive samples out of 116 samples.

DISCUSSION

In the present study 3 out of 116 sera (2.6%) of cattle were positive for the presence of IgG antibodies to toxoplasmosis infection. These studies showed lower prevalence than the prevalence studies by Singh [10], Rajamanickam *et al.* [12], Normaznah *et al.* [13] and Chandrawathani *et al.* [14]. High seroprevalence values were found in Spain (41%, Moreno *et al.* [15]), Poland

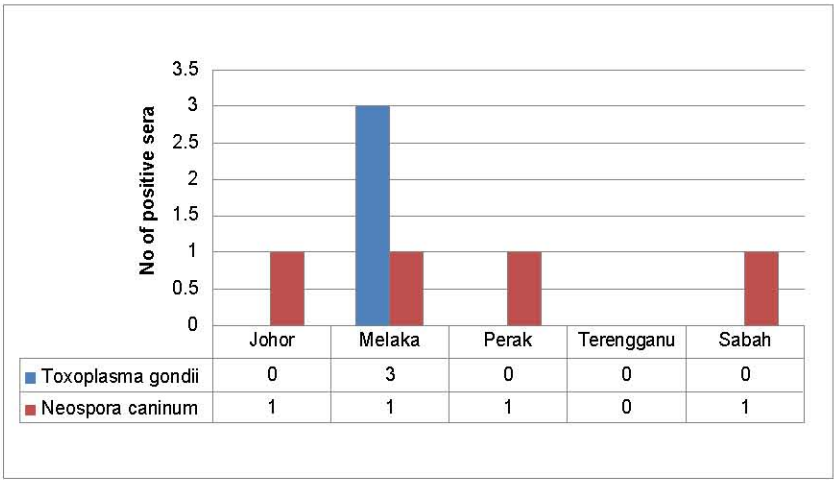

Fig. 1: Comparison of seroprevalence of toxoplasmosis and neosporasis among cattle in five states in Malaysia

(53.8%, Sroka [16], Serbia (76.3%, Klun *et al.* [17]) and Brazil (71%, Santos *et al.* [18]). Lower prevalence of infection in cattle also reported in some countries such as Tanzania (3.6%, Luuk *et al.* [19]), France (7.8%, Gilot-Fromont *et al.* [20]) and Malaysia (7.9% in local cattle and 4% in yellow cattle, Chandrawathani *et al.* [14]). Cats are common pets kept in most families in Asia and often found in public places. An increase in the number of cats, especially stray cats, causes the increase in the number of toxoplasmosis cases in both animals and human. The cats can form a chain in transmitting this disease to human through cattle when the meat and raw milk are consumed (Gargia *et al.* [21]).

Concerning neosporosis infection 4 out of 116 sera (3.44%) of cattle were positive for the presence of IgG antibodies to. This results showed lower prevalence than those of cattle on small scales farms in the northeast Thailand (37.5-70%) reported by Kashiwazaki *et al.* [22] and in Taiwan (44.9%) by Ooi *et al.* [23] who used IFAT for the detection of IgG antibodies in cattle. The reasons for the disparity in the prevalence of antibodies against *N. caninum* in Malaysia and other 2 location are unknown. Difference in seroprevalance in cattle in different countries may reflect different climatic factors. Cattle were significantly more infected by *N. caninum* than other ruminants. The likelihood of being seropositive to *Neospora* was more than three times higher in cattle than the other species [24].

Serological tests have been used exclusively for the diagnosis of *N. caninum* infection in live animals [6, 25] and IFAT and several ELISAs have been industrial for the purpose. Selection of cut-off values of existing IFAT and ELISA protocols have been carried out to detect a specific humoral response in cattle; yet whether or not these tests identify chronically infected animals with the same sensitivity is anonymous. The IFAT considered as the gold-standard test for the serology of *N. caninum* infection in cattle and dogs [6, 25]. A positive IFAT result at the cut-off of 1:200 is generally considered to be indicative of infection [26]. The 1:200 serum dilutions in the IFAT has also been used in a validation of a commercially available monoclonal antibody-based comperative-inhibition ELISA [27].

In conclusion, although the overall animal prevalences of toxoplasmosis (2.59%) and neosporosis (3.44 %) were low and cases were widespread, the overall prevalence was 6.03%. The parasite is normally found to be ubiquitous in warm moist environments and both toxoplasmosis and neosporasis are consistently considered to be a health hazard to human particularly to those exposed because of their respective occupations.

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