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The seroprevalence of bovine babesiosis in Malaysia

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Abstract. One hundred sera of Malaysian cattle were used in this seroprevalence study for bovine babesiosis. All sera were obtained from the Serological Unit of the Veterinary Research Institute (VRI), Ipoh, Perak. The sera were tested using a Veterinary Medical Research & Development (VMRD) commercial Indirect Immunofluourescent Antibody Test (IFAT) kit. The results showed that 17.0% were found to be positive for *Babesia bovis*, 16.0% for *Babesia bigemina*, and 9.0% for both *B. bovis* and *B. bigemina* infections.

INTRODUCTION

Babesiosis is a tick-borne disease caused by several species of protozoa from the genus *Babesia*. Bovine babesiosis is a cattle disease caused by *Babesia bigemina* and *Babesia bovis*. *Babesia divergens*, *Babesia major*, *Babesia jakimovi*, *Babesia ovata*, *Babesia occultans* are other species of bovine babesiosis. The main vector is a one-host tick of the Ixodidae family. For *B. bigemina*, the common vector is *Boophilus annulatus* while the vector for *B. bovis* is *Boophilus microplus* (Losos, 1986).

Bovine babesiosis can be found wherever the tick vectors exist, but is most common in the tropics and subtropics. *Babesia bovis* and *B. bigemina* are particularly important in Asia, Africa, Central and South America, southern Europe, and Australia
(Spickler & Roth, 2008).

In Malaysia, there had been several outbreaks of bovine babesiosis disease since 1996 (World Organization for Animal Health Report, 2008). In 1996, there were 29 outbreaks and in 1997, 15 outbreaks and 17 cases were reported with 17 deaths in Malaysia. In 1998 there were 3 outbreaks, 4 cases and 4 deaths were recorded in the country. In 1999, there were 15 outbreaks with 11 cases reported and 11 deaths. There was a great increase in cases in 2001 where 263 cases were reported with 8 outbreaks.

Serological testing with Indirect Immunofluorescent Antibody Tests (IFATs) is useful in diagnosing for *Babesia* sp. infections, particularly for chronic infections (Telford & Spielman, 1998). IFAT is both specific and sensitive and is the current recommended serologic method (Chisholm *et al.*, 1978). So far, it still remains the method of choice for serological detection for a number of infectious diseases, including babesiosis (World Organization for Animal Health Report, 2008).

Information on bovine babesiosis in Malaysia is crucial because the prevalence rate of babesiosis should be known and fully understood. The present paper reports on the seroprevalence rate of bovine babesiosis in Malaysia by using IFAT, and to compare the infection rates among local and imported breeds of cattle.

MATERIALS AND METHODS

Collection of blood samples

One hundred cattle sera were collected from all the states in Malaysia. The sera were then kept in the serum bank of the Serological Unit of the Veterinary Research Institute (VRI), Ipoh, Perak. The sera were kept at -6°C until analysed using the Indirect Immunofluorescent commercial test kit from Veterinary Medical Research & Development (VMRD).

Indirect Immunofluoroscent Antibody Test (IFAT)

(a) Testing materials

Cattle sera were collected from various breeds, which included mix, Brahman X, yellow cattle, cross, local, and Australian. The cattle sera were obtained from the commercial, smallholder or government farms.

Fluorescent antibody substrate slides contained 10 or 12 wells spotted with an antigen. Serum Diluting Buffer (Catalog No: 210-93-3B) was used to dilute samples to working dilution, by adding phosphate buffered saline (PBS), 1% BSA, preserved with 0.09% sodium azide. Anti-Immunoglobulin Conjugate was used to detect bound antibody on the slide. To detect babesiosis, the anti-immunoglobulin conjugate was FITC-labeled anti-IgG or IgM conjugate. Rinse buffer, pH 9.0 (Catalog No: 210-90-RB) was used for washing off unbound antibodies and conjugates. Mounting Fluid (Catalog No: 210-92-MF), made by mixing FA Rinse Buffer, pH 9.0, and glycerol in 50:50 (VMRD) was used to enhance visualization of fluorescence.

(b) Testing Methodology

Before starting the IFAT, the sera and slides were warmed to room temperature before removing from the foil pouch. Diluted sera were then placed into designated wells, the FA substrate slides which contained antigen were incubated 30°C to prevent drying. This procedure allowed the antigen-antibody binding to occur. The slides were then rinsed and soaked in Rinse Buffer to remove unbound antibody. The Anti-Bovine IgG1, 2 FITC Conjugate was then added onto the designated wells, which was fluorescein-labeled and reactive to human IgG. The slides were incubated again, washed, dried with blotter, mounted with Mounting Fluid to enhance visualization of fluorescence and viewed under a fluorescence microscope at 400x magnification. In a positive sample, the antibody will bind and permit the fluorescein-labeled antibody to form secondary binding. The cells will fluoresce and can be observed under microscope. For a negative sample, no antibody will bind to it and no fluorescein coloured will be shown under fluorescence microscope (Coons *et al.*, 1941).

Assay Procedure

The slides were warmed to room temperature before removing from the foil pouch. The sera were diluted in buffer with pH 7.2. 20µl and the diluted sera were placed onto the designated wells. The slides were incubated at 37°C for 30 min in a humid chamber.

After 30 min, the diluted sera in the slide were gently rinsed in FA slide chambers with the FA rinse buffer, pH 9.0 using a warm bottle and soaked for 10 min in the slide chambers with the FA rinse buffer. The slides were then drained and dried around wells by pressing blotter (included in pouch) to front surface. Then, 20 µl FITC-labeled anti-IgG conjugate were placed on the wells and incubated, rinsed and soaked using the same procedure that mentioned previously. The slides were drained and dried back and the edges of slides were dried using the paper towels. The slides were mounted using a mounting fluid which was a mixture of glycerol and FA rinse buffer, pH 9.0 at the ratio 1:1. The slides were observed for the presence of babesiosis with a fluorescence microscope at 400 x magnification. Positive samples will shine under the fluorescent microscope while the negative samples will not show any fluorescent colour under the microscope. Significance difference

between samples were analyzed by Chi-square statistical analysis.

RESULTS

By using IFAT, the positive slides will be indicated by organisms with fluorescence staining and vice-versa for negative slides. The animals indicated as 'positive' in the IFAT showed that IgG antibodies against *B. bovis* and *B. bigemina* are present in the body.

For *B. bovis*, the positive samples obtained in this study were 17 (17.0%). The highest prevalence rate in Malaysia was Perlis, with 9 positive samples. On the other hand, the lowest prevalence rates were Penang, Perak, Negeri Sembilan, Pahang, Terengganu, Kelantan and Sarawak, with no positive samples (Fig. 1). By using Chi-square analysis, χ^2, there was significant difference between samples from every state ($\chi^2 = 63.036$, P = 0).

The positive samples for *B. bigemina* were 16 (16.0%). Again, the highest prevalence rate was Perlis with 9 positive samples. The states of Penang, Negeri Sembilan, Pahang, Terengganu, Kelantan, and Sarawak were negative for bovine babesiosis. By using Chi-square statistical analysis, there was significant difference between samples from every state ($\chi^2 = 56.566$, P = 0).

From this study, mixed infections occurred, where the cattle is infected simultaneously with *B. bovis* and *B. bigemina*. Again, Perlis showed the highest prevalence rate of infection, with 9 positive samples. The states of Penang, Perak, Selangor, Negeri Sembilan, Pahang, Terengganu, Kelantan, and Sarawak recorded not a single positive sample. There was significant difference between samples getting from every state after analyzed by Chi-square statistical analysis ($\chi^2 = 65.516$, P = 0).

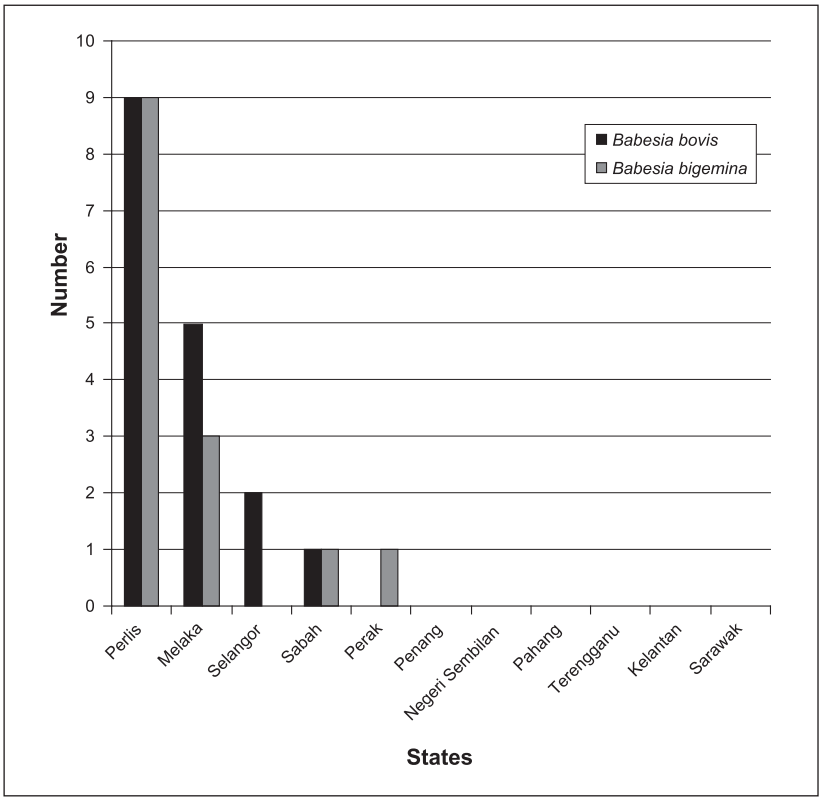

Figure 1. Comparison of Babesia bovis and Babesia bigemina in Malaysia

From a total of 67 local breed samples, 16 were positive for *B. bovis* infection as compared to none for the 10 imported, and one from 23 of the unknown breed. There was significant difference between the cattle breeds for the positive result after analyzed by Chi-square statistical analysis ($\chi^2 = 6.906$, P = 0.032).

For *B. bigemina* infection there were 15 positives for local breed; none for imported and one for cattle of unknown breeds . There was significant difference between the infected irrespective of breeds after analyzed by Chi-square statistical analysis ($\chi^2 = 6.263$, P = 0.044).

For mixed infections, there were 12 positives for local breed, none for imported and one for cattle of unknown breeds. By using Chi-square analysis, there was no significant difference between the samples irrespective of breeds ($\chi^2 = 4.445$, P = 0.108).

DISCUSSION

IFAT was used to test for the bovine babesiosis because it is a widely used method for detecting the antibodies of *B. bovis*. IFAT has been used for detection of antibodies to *B. bigemina*, but serological cross-reactions make species diagnosis difficult. Hence, for *B. bigemina*, Enzyme-linked Immunosorbent Assay (ELISA) is the common serological test used (World Organization for Animal Health Report, 2008).

The diagnosis of bovine babesiosis by blood smear examination during the acute phase is limited not only by the low parasitemias (generally observed at the beginning of parasite multiplication), but also limits the number of samples that can possibly be examined in a day (Gonçalves Ruiz *et al.*, 2001).

The sera obtained from all states in Malaysia were kept in the Serum Bank in the Serological Unit, VRI at temperatures around -6°C. The sera were selected from all states in Malaysia except Kedah and Johor.

Premunity is the main concept for babesiosis infection. The presence of babesial antigen in the form of living parasites inside the vertebrate host was considered to be a prerequisite for good immunity (Lumsden & Dawes, 1997). Cattle that have been infected may have antibodies which are detectable after infection for many years and for some time after the parasite has been eliminated. Thus the presence of antibodies will show that an animal has been infected and may be immuned. This can help to define the epizootiological balance in the population and to determine control strategies for the diseases (Seifert, 1996). Enzootic stability is an epidemiological concept where the rate of transmission of *Babesia* is sufficient to infect the majority of calves before calfhood resistance is lost (Mahoney & Ross, 1972).

In the present study, single and mixed infections were high thus indicating a stable endemic situation in Malaysia (Goff *et al.*, 2002). In general, *B. bigemina* transmission rates are higher when compared with *B. bovis*. This is because there are more ticks that carry it and therefore the endemic stability is more likely to develop to *B. bigemina* than to *B. bovis* in regions where both are present (Bock *et al.*, 2005). However, the results of the present study showed almost similar results for both *B. bigemina* (16%) and *B. bovis* (17%) infection rate instead of higher transmission rate in *B. bigemina*. This may be due to the poor specificity of *B. bigemina* test in IFA test (World Organization for Animal Health Report, 2008). Also, the serological detection of *B.* bigemina infections is difficult due to lack of universally acceptable purified antigen (Singh *et al.*, 2008).

The cattle population of Perlis for the year 2007 was 797,641 (Malaysian Veterinary Services Reports, 2007). The high density of cattle in a small state like Perlis may explain the high prevalences for both single and mixed infections for the State. In addition to that, the management of tick problem might not have been

handled well and may be the cause for the the cattle to be seriously infected by *Babesia* species. Besides, *Babesia* inoculation rate is directly related to intensity of infestation and to infection rate. In Perlis, the rainfall appeared to be the climatic factor that influenced the intensity of infestation of *B. microplus* (Quintão-Silva & Ribeiro, 2003). The maximum rainfall and mean rainfall amount in Perlis in 2008 was 267.2mm and 10.72mm, respectively (Suhaila & Jemain, 2008). Although it may not receive the highest rainfall among all States, the amount of rainfall may play an important role in transmission of babesiosis.

The positive results revealed there was lack of management of tick in the local breed. There are several chemicals that are well-known and are highly recommended for control such as dipping vat, spray, spray race or spray-dip machine, hand dressing, acaricides and other methods (Drummond, 1983). However, the control strategies were not carried out properly. Hence, the prevalence rate of bovine babesiosis was high in local breed.

On the other hand, usually imported cattle are well cared for. They are given good food and provided with de-tick programme to make sure the imported breed of cattle are in good shape. Usually de-ticking is done 3 weeks once. Thus, there were no infections of *Babesia* species in cattle. Moreover, imported cattle are vaccinated for tick fever with a live attenuated cryopreserved vaccine of *B. bovis* and *B. bigemina* (Chandrawathani, 2000). Thus, the number of bovine babesiosis found in imported cattle is less, or even none as observed in the present study.

The most common method of tick control in Malaysia is by chemical dipping or spraying. Pour-on formulations are also available in the market, especially for dairy cattle farmers. Imidocarb is used as treatment in some cases (Chandrawathani, 2000). Thus, in controlling tick problem, the above methods should be carried out more frequently.

In this study, the percentages of *Babesia* species infections are different from the research done by Chandrawathani *et al*. (1993). In 2000 (Chandrawathani, 2000), there were 17% of *B. bovis*, 16% of *B. bovis* and 13% of mixed infections of both *Babesia* species, compared with in 1993, 72% of the animals surveyed had a mixed infection of *B. bovis* and *B. bigemina*. This is because the control measures such as regular de-ticking and prophylactic treatment was done more frequently than in 1993 (Chandrawathani *et al.*, 1993). However, there were some cases of bovine babesiosis reported in Malaysia until today.

In 1993, a study was carried out to determine the prevalence of antibodies to *B. bovis* in cattle in Indonesia. The prevalence rate (96%) indicated that certain areas surveyed were endemic for *B. bovis* (Sukanto *et al.*, 1993). Hence, there was reason for Sabah to have 2 reported cases of *B. bovis* infection in that study. This high prevalence rate of *B. bovis* in Indonesia may contribute to the distribution of this protozoan disease to neighboring country, such as Malaysia.

Two cases each of *B. bovis* and *B. bigemina* infections were found in Selangor in this study. However, compared with a survey done by Noor Azian *et al.* (2008), the number of infected cattle is reduced drastically from 55.4% of *B. bigemina* infection. This may be due to the control methods being carried out more efficiently to eradicate the tick population.

In conclusion, bovine babesiosis is still an important issue to take note. Tick fever will reduce yield and cause losses to farmers. Hence, proper management of ticks in cattle must be carried out. In Malaysia, control strategies such as regular de-ticking and prophylactic treatment are common. Synthetic pyrethroid and organophosphate are also part of chemical control of tick problems. However, this study is just a preliminary study of bovine babesiosis. So, further studies should be done on larger samples to validate the present observation.

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