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ABSTRACT. The serodiagnosis of bovine anaplasmosis was conducted on sera samples of various breeds of cattle and buffaloes of mixed ages, from randomly chosen farms from 13 different states in Malaysia received by VRI in 2012. The serum samples were tested for the presence of antibodies to *Anaplasma marginale* by Competitive Enzyme-Linked Immonusorbant Assay (c-ELISA). Out off 455 sera samples, 353 (77.6%) tested positive for bovine anaplasmosis.

INTRODUCTION

Anaplasma marginale are obligate intracellular protozoans parasitizing erythrocytes of higher vertebrates, mostly cattle. *Anaplasma marginale* is distributed worldwide in the tropical and subtropical regions which include Asia, United States of America, Europe, Africa and Australia (Ristic, 1960). Recently this pathogen has been classified into two different families: Anaplasmataceae and Rickettsiaceae with both belonging to the Order Rickettsiales. *Anaplasma* spp. is proven to be transmitted by ticks and currently there are about 20 species of ticks that have been identified worldwide which act as vectors for the transmission as reviewed by Ewing, 1981. In general, *A. marginale* can be transmitted by tick vectors which include *Boophilus* spp., some selected *Dermacentor* spp., *Ixodes ricinus* and *Rhipicephalus* spp.

This study was carried out to determine the seroprevalence of anaplasmosis caused by *A. marginale* in cattle throughout Malaysia as current information in this area is lacking. In this study, the detection of *A. marginale* is done via competitive enzyme-linked immunosorbent assay (c-ELISA); a diagnostic tool proven to be very sensitive and specific for the detection of Anaplasma-infected animals (Visser *et al.* 1992, Knowles *et al.* 1996; Strik *et al.* 2007). It is proven to be more sensitive in carrier-cattle-detection and is specific with well-characterized cross-reactivity between *A. marginale* and *A. centrale.* As there is no vaccination for anaplasmosis, this is an excellent tool for screening and is suitable for seroprevalence study of large herds of cattle or buffaloes rapidly and cheaply. Information on seroprevalence is necessary for formulating government

policies on control and treatment of this disease in order to aid farmers improve their management and increase their profits.

MATERIALS AND METHODS

Serum samples were obtained from 13 states in Malaysia namely, Kedah, Pahang, Johor, Penang, Terengganu, Perlis, Kelantan, Selangor, Melaka, Perak, Negeri Sembilan, Sabah and Sarawak. All samples used in this study were from buffaloes and cattle of Fresian Sahiwal, Brahman, Kedah-Kelantan, Mafriwal, Kobe, and cross breeds which were obtained randomly from smallholder and commercial farms. All samples were submitted to the Veterinary Research Institute (VRI) in Ipoh in 2012 for diagnostic tests and were stored in a serum bank at -6°C in the Serology Unit prior to use. The samples were randomly selected for the purpose of the seroprevalence study. The c-ELISA kit (VMRD Inc., Pullman,WA) used in this study consisting of reagents and plates, were kept refrigerated at a temperature between 2°C and 7°C till used. The specificity is 98% and sensitivity is 95% of this test respectively. Statistical analysis conducted on the data was the 95% confidence interval based on individual optical densities of samples tested.

RESULT

In this study, 77.6% (CI = 61.1-85.2%) of the 455 sample tested to be positive

Table1: Percentage of positive Bovine Anaplasmosis in 13 states in Malaysia.

State	Number Of Samples	Positive Results	Seroprevalence % (95% CI)
Kedah	37	27	73 (65.5-88.1)
Pahang	20	20	100 (86-100)
Johor	43	34	79.1 (65.0-89.3)
Penang	35	27	77.1 (61.2-88.9)
Terengganu	20	19	95.0 (77.7-99.7)
Perlis	21	18	85.7 (65.8-96.2)
Kelantan	33	28	84.9 (64.4-89.0)
Selangor	20	19	95.0 (77.7-99.7)
Sabah	49	29	59.2 (45.0-72.2)
Sarawak	26	16	61.5 (42.0-78.5)
Perak	63	57	90.5 (81.2-96.0)
Melaka	44	28	63.6 (48.7-76.8)
Negeri Sembilan	44	31	70.4 (55.8-82.5)
Overall	**455**	**353**	**77.6 (61.1-85.2)**

with bovine anaplasmosis as shown in Table 1. The lowest seroprevalence rate was 59.2% as seen in Sabah. The highest seroprevalence was Pahang, where all samples examined were positive for the bovine anaplasmosis infection followed by Terengganu (95%) and Selangor (95%).

DISCUSSION

In this study, c-ELISA was shown to be useful for the detection of *A. marginale* in bovine. The serum samples were obtained from 13 different states in Malaysia (Kedah, Pahang, Johor, Penang, Terengganu, Perlis, Kelantan, Selangor, Perak, Melaka, Negeri Sembilan, Sabah and Sarawak). The c-ELISA test indicated that anaplasmosis infections are prevalent in all 13 states. The highest seroprevalence in this study was recorded in Pahang as all of the samples (100%) were tested positive for bovine anaplasmosis. Similarly high seroprevalence was seen in Pahang (CI = 86-100). Medium seroprevalence were seen in Kedah, Terengganu, Perlis, Kelantan, Selangor and Perak (CI = 65-85). Low prevalence were seen in Penang, Sabah, Sarawak, Melaka and Negeri Sembilan (CI = 45-65). In this study, although the percentage of seroprevalence is high, the number of samples being small will require the consideration of the confidence interval to assess the severity of the problem. Thus a loose categorization of the states is done with low, medium and high seroprevalence. Previous study by Rahman *et al.* (2012) showed that Terengganu also has recorded the highest prevalence rate (100%) for the same parasite. However, no data was reported for Pahang. In this study, Terengganu and Selangor are two of the states which recorded a high prevalence rate (95%). In addition, Perak also has recorded a high prevalence, 90.5% for bovine anaplasmosis. Coincidentally, all those three states were located in the East Coast of Peninsular Malaysia (Perak and Selangor is in the West Coast, please revise). The reasons for the highest prevalence of bovine anaplasmosis from those three states are not clear and need a further investigation.

Samples from Pahang, Selangor, Terengganu and Perak had more than 90% seroprevalence indicating the importance of this disease in major cattle and buffalo rearing areas. This indicates the seroprevalence to be high and seriously affecting the cattle population. As Malaysia has a hot wet tropical climate with an endemic tick population, it is clear that anaplasmosis is highly prevalent. This may affect the health and productivity of animals, leading to other secondary infections due to poor immunity as established by the protozoan infections.

However, several studies from various regions indicated that there are a variety of factors that could contribute to the high prevalence rate in cattle and buffaloes. The prevalence and incidence of *A. marginale* was shown to be highest in regions where the tropical cattle fever tick *Rhipicephalus microplus* is endemic (Lincoln *et al.* 1987; Mekonnen *et al.* 2002).

According to Aguirre *et al* (1994), all different stages of these ticks preferentially feed on cattle, and each can efficiently acquire and transmit *A. marginale*. This high vectorial capacity of *R. microplus* contributed in most cases of anaplasmosis infection in subtropical and tropical regions and this high incidence represents a severe constraint on animal health and production (Graham & Hourrigan, 1977). Several studies from other regions also indicated that improper practices of acaricide also contribute to the high level of anaplasmosis in cattle and buffaloes (Nolan 1981; Solomon 1988; Mekonnen *et al*. 2002).This is due to the the use of an acaricide at the incorrect concentration and this is one of the prime causes of tick control failure at communal dipping tanks (Jonsson 1997). Some farmers were reported to increase dip concentration of acaricide that would undoubtedly have lead to a higher selection pressure for tick resistance (Spickett 1998).

Based on this study, Sabah and Sarawak both recorded the lowest percentage of prevalence, 59.2% and 61.5% respectively. A similar observation also was obtained from the previous study, where Sabah recorded the lowest percentage of bovine anaplasmosis, at 28% (Rahman *et al.* 2012). In Malaysia, Sabah is one of the main producers of cattle and buffaloes and because of that, more efforts are probably being focused in Sabah especially in the herd management which might contribute to the lowest percentage of bovine anaplasmosis. Although Sabah has recorded the lowest percentage in this study, we found that there is a rise in seroprevalence when compared with the latest study by Rahman *et al.* (2012). The rise of the infection might due to the importation of new infected breeding cattle as Sabah is in an attempt to become the main producer of bovine products in Malaysia. There was an increased of cattle and buffalo populations in Sabah from 2006 to 2011; 87122 to 96988 for cattle and 40929 to 45088 of buffalo (Ministry Of Agriculture And Agro-Based Industry Malaysia, 2012). The increase in number of cattle and buffalo populations in Sabah might be associated with increased infections. In addition, in Malaysia, all imported animals are required to be quarantined for a certain period before releasing the animals to the farms. However, the animals during quarantine only will be subjected to clinical examinations and laboratory tests as and when deemed required by the Quarantine Officer in charge or by any competent Veterinary Officer (Department of Veterinary Services, Malaysia). This practice is likely to contribute to an increase in infection because not all of the quarantined animals were tested serologically or conventionally by blood smears as clinical signs are not evident most of the time. However, further investigation is needed to validate this based on clinical signs in relation to seroprevalence as well as indications by conventional methods of diagnosis. As for Sarawak, no comparison could be made as no data were obtained or reported in previous study.

CONCLUSION

The results of this study indicated that the anaplasmosis infection in cattle in Malaysia is currently high and may affect the bovine population significantly in reducing general immunity, cause anemia and open the doorway to other infections. Therefore, further investigation is needed because the epidemiological knowledge of anaplamosis in Malaysia is still lacking in order to formulate effective methods of control and eradication of this disease. Current study is focusing on investigation of the factors that might contribute to the high infection rates in Pahang. In addition, the association of herd management and parasite burden also should be investigated. As this is a tick borne infection, bionomics and current information on vector distribution as well as control of ticks is essential for the successful control of this disease. Awareness on latest information for farmers will help alleviate the negative effects of anaplasmosis among farmers.

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