

SEROLOGICAL PREVALANCE OF LEPTOSPIRAL INFECTION IN CAPTIVE MALAYAN PORCUPINESS (*Hystrix brachyura*)

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SUMMARY

Leptospirosis is recognised as one of the leading zoonotic diseases and rodents have been implicated as one of the natural reservoirs of the disease. The Malayan porcupines (*Hystrix brachyura*) which are also a rodent could possibly be a carrier of leptospiral organisms. This study was conducted to determine the serological prevalence of leptospiral infection among captive Malayan porcupines and to disclose the possibility of porcupines as a reservoir for leptospiral infection. Fifty serum samples were obtained from the Malayan porcupines kept in captivity at the Wildlife Conservation Centre, Sungai Dusun, Malaysia. The microscopic agglutination test (MAT) was performed on the serum samples to detect the presence of agglutinating antibodies to a panel of 16 *Leptospira* serovars (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Djasiman, Hardjobovis, Hardjoprajitno, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Sejroe). From the serological test, 18% (n=9/50) of the Malayan porcupines tested had leptospiral antibodies to serovars Javanica (8%), Hurstbridge (4%), Ballum (2%), Celledoni (2%) and Hardjoprajitno (2%). It is seen that this study disclosed a high prevalence of leptospiral infection in the Malayan porcupines tested and indicated that the Malayan porcupines could possibly be a source of leptospirosis to other animals including humans and that they might play an important role in the epidemiology of leptospiral infection in the country.

Keywords: Leptospirosis, Malayan porcupines, Hystrix brachyura, serological prevalence.

INTRODUCTION

Porcupines, one of the rodent species, are herbivores and usually nocturnal in nature. Out of the 4 species of porcupines found in Malaysia, the Malayan porcupines (*Hystrix brachyura*) is bred commercially. There are over 40 well established Malayan porcupine farms in Malaysia.

Rodents particularly rats are important reservoirs for *Leptospira*. The role of the Malayan porcupines in maintaining leptospirosis is not known. A study in Canada by Mitchell *et al.* (1966) reported that *Leptospira pomona* was isolated from a porcupine trapped from a farm following an outbreak of leptospirosis. This indicated that porcupines just like rats could possibly harbor leptospires and be a source of infection to other animals and humans. Thus, the objectives of this study were to determine the serological prevalence of *Leptospira* infection in a group of Malayan porcupines and to identify the leptospiral serovars that might be infecting the animals.

MATERIALS AND METHODS

Animals

Prior to the start of the study, permission to handle the porcupines for blood sampling was obtained from the Department of Wildlife and National Parks Peninsular Malaysia.

Fifty Malayan porcupines (*Hystrix brachyura*) (age group, 43 adults and 7 juveniles) kept in captivity at the Wildlife Conservation Centre, Sungai Dusun, Malaysia were selected for this study.

All animals were observed to be overtly healthy based on their general behaviour and physical conditions. These porcupines were kept as parent stock for breeding farms nationwide. They were kept in pairs or individually in metal wire pen with dimension of 40 × 36 × 28 inches and fed twice a day with fruits, vegetables, tubers and concentrates.

Each porcupines were restrained using a trap net, weighed and anaesthetised with Zoletil[®] 50 (Virbac) (Tiletamine-Zolezepam) at a dosage of 5 mg/kg given intramuscularly at the hindlimb. An eye ointment (Terramycin) was applied to both eyes of animals to prevent desiccation. A piece of towel was also used to cover the eye area to prevent or reduce stress on the animal. About 3 ml of blood samples were obtained from the femoral artery of each porcupine and stored into a plain tube. All blood samples were temporarily kept in an icebox for transportation, then centrifuged at 10,000 rpm for 2 min to separate the serum from the blood clot before transferring the serum into a 1.5 ml Eppendorf vial. The serum samples were kept at -20^oC until ready to be used for the microscopic agglutination test (MAT) as described by Cole *et al.* (1973) against a panel of 16 leptospiral serovars.

The Microscopic Agglutination Test (MAT)

Sixteen *Leptospira* serovars consist of; Australis (Ballica), Autumnalis (Akiyami A), Ballum (Mus 127), Bataviae (Swart), Canicola (Hond Utrecht IV), Celledoni (Celledoni), Djasiman (Djasiman), Hardjobovis (Sponselee), Hardjo (Hardjoprajitno), Hebdomadis (Hebdomadis), Hurstbridge (BUT 6), Icterohaemorrhagiae (RGA), Javanica (Veldrat Batavia 46), Pomona (Pomona), Pyrogenes (Salinem) and Sejroe (M 84) were used as

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antigens in the MAT. The antigens were cultured in Johnson-Seiter liquid medium for a period of 7 to 10 days and adjusted to a cell density of 1.5×10^8 leptospir/ml based on MacFarland Standard. The MAT performed in this study is a modification of the method described by Cole *et al.* (1973). Each serum sample was initially diluted (1/10) with phosphate-buffered saline (PBS) pH 7.2 in a test tube. Twenty five microlitres of PBS were placed in each well of the microtitre plate (Grenier, Germany) and equal volume of the diluted serum sample was placed in the first row (Row A) of the plate. The diluted serum sample (now 1/20) was then serially diluted (two fold) from Row A to Row H using a hand-held microdiluter (Dynatech, Malaysia). Next, 25 μ l of the antigen were added to each well. Thus, each would contain an equal volume of the diluted serum sample and antigen. For each serum sample tested there would be eight dilutions ranging from 1/40 to 1/5120 after the addition of the antigen. The plate was incubated for 2 hr at 37°C before examining it for evidence of agglutination. The test was read by transferring a drop from each well onto a glass microscope slide and examined by darkfield microscopy at a magnification of 200X. A positive reaction was regarded as one in which 50% or more of the antigens were agglutinated. The titre end point was taken as the last well in which 50% or more agglutination was observed. To date, serological study on leptospirosis has not been conducted on porcupines in Malaysia and no cut-off titre has been set for porcupines to consider whether MAT positive or not. Hence, the choice of minimum titre of 1/40 was based on previous study done in determining the serological prevalence of leptospiral infection in wild rats based on MAT by Mohamed-Hassan *et al.* (2010). The prevalence rate was then determined based on positive MAT divided by the total serum samples and converted into percentage (%).

RESULTS

Based on the MAT, 9 (18%) of the 50 serum samples were positive to one of the following serovars: Javanica, Ballum, Celledoni, Hardjoprajitno and Hurstbridge. All seropositives were from adult porcupines in which the highest serum titre was found to be 1/40. The highest prevalence of leptospiral infection in the porcupines was due to serovar Javanica at 8% (4/50), followed by Hurstbridge at 4% (2/50) and Ballum, Celledoni and Hardjoprajitno at 2% each (1/50). All serum samples were tested negative against Australis, Autumnalis, Bataviae, Canicola, Djasiman, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes and Sejroe.

DISCUSSION

Porcupines are classified as Rodentia and are similar to rats which are known to be the most important reservoir hosts for *Leptospira* in the wild (Bahaman and Ibrahim, 1988). The Malayan porcupines have not been investigated and are not known to be infected with *Leptospira* and whether they posed similar threat to the communities and livestock.

From this study, there is possibility that the Malayan porcupines could be carriers of leptospiral organisms as porcupines are rodents similar to rats. To date, no study has been carried out on porcupines to implicate them as the source of leptospirosis to livestock and humans in Malaysia. Thus, further research is required to look into its association with humans. Exposure of porcupine farmers and handlers to urine-contaminated environment from the animals put them at risk at contracting leptospirosis.

Based on the results, the serological prevalence of *Leptospira* in the Malayan Porcupines was determined to be 18%. This value is considerably high and comparable to the prevalence in rats. Mohamed-Hassan *et al.* (2010) reported a serological prevalence of 17.9% (30/168) of leptospiral infection in wild rats in Kelantan and Terengganu. Similarly, a study by Siti Aminah (2006) also reported a similar serological prevalence of 18.1% (6/33) of leptospiral infection among wild rats caught in Kuala Lumpur.

In this study, Javanica was the major serovar detected in the serum samples with 8% (4/50) prevalence. The same serovar was found to be the dominant serovar in the rats examined by Siti Aminah (2006). Besides that, positive MAT titres were found only among adult Malayan porcupines. No positive MAT was seen on juvenile porcupines tested. This could be possibly due to that they have not been exposed to the *Leptospira*.

From the findings, it is suggestive that the Malayan porcupines could possibly be carriers of leptospiral organisms and thus, the farmers as well as handlers are at risk of contracting leptospirosis from the Malayan porcupines. Extra precautions should be taken during handling of the porcupines or their carcasses in preparation of their meat for human consumption. Farmers and handlers are advised to wear protective clothing such as boots and gloves whenever handling such animals or during cleaning their cages or pens. It is also important to wash or shower after being exposed to urine from these porcupines or environment contaminated by them.

CONCLUSION

From this study, it is seen that the Malayan porcupines were infected with leptospiral infection. To date, no study has been carried out on porcupines to implicate them as the source of leptospirosis to humans in Malaysia. Thus, further research is required to determine their role in the epidemiology of leptospiral infection, particularly now with more porcupine farms being established. Exposure of infected porcupines to farmers and handlers would put them at risk of contracting leptospirosis.

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CONFLICT OF INTEREST

None of the authors have any potential conflicts of interest to declare.

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