

# SEROPREVALENCE OF *LEPTOSPIRA* SPP. IN CATTLE AND DOGS IN MAHASARAKHAM PROVINCE, THAILAND

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## ABSTRACT:

**Background:** Leptospirosis is a zoonotic disease that is caused by the spiral-shaped bacterium *Leptospira* spp. It is distributed worldwide and classified as an emerging infectious disease in humans and animals. Many serovars are carried by a wide range of animals, all of them are capable of causing illness in humans. The objective of this study was to determine the seroprevalence of *Leptospira* spp. in cattle and dogs in Mahasarakham Province, Thailand

**Methods:** This study was conducted in Non Rasi sub-district, Borabue district, Mahasarakham province, Thailand. Blood samples were collected from 40 cattle and 55 dogs. All samples were tested by microscopic agglutination test (MAT).

**Results:** Five cattle (12.5%) were positive by MAT; all of them were identified as *Leptospira* serovar *sejroe* while 6 dogs (10.9%) were positive by MAT and all of them were *Leptospira* serovar *canicola*.

**Conclusion:** This result is considered as evidence-based information to indicate that several pathogenic serovars are circulating in the livestock and domestic animals in the study area. These animal reservoirs could also potentially contact humans in a fashion similar to rats and other rodents, which are the primary reservoirs. This could increase the risk of human leptospirosis in the study area.

**Keywords:** Leptospirosis, Seroprevalence, Cattle, Dog, Thailand

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## INTRODUCTION

Zoonotic-infectious diseases, are being transferred from animals to human, is considered as a serious problem in public health in worldwide. Leptospirosis is one of important tropical bacterial diseases which is easily being harbored by a wide range of mammals that eventually serves as reservoir host causing illness to human [1]. It is caused by a spiral shaped serovars of a spirochete bacterium known as *Leptospira interrogans*. *Leptospira* has more types of serovar and serogroup which varies depending on different location, different type and species of reservoirs with the wild and domestic animal affected.

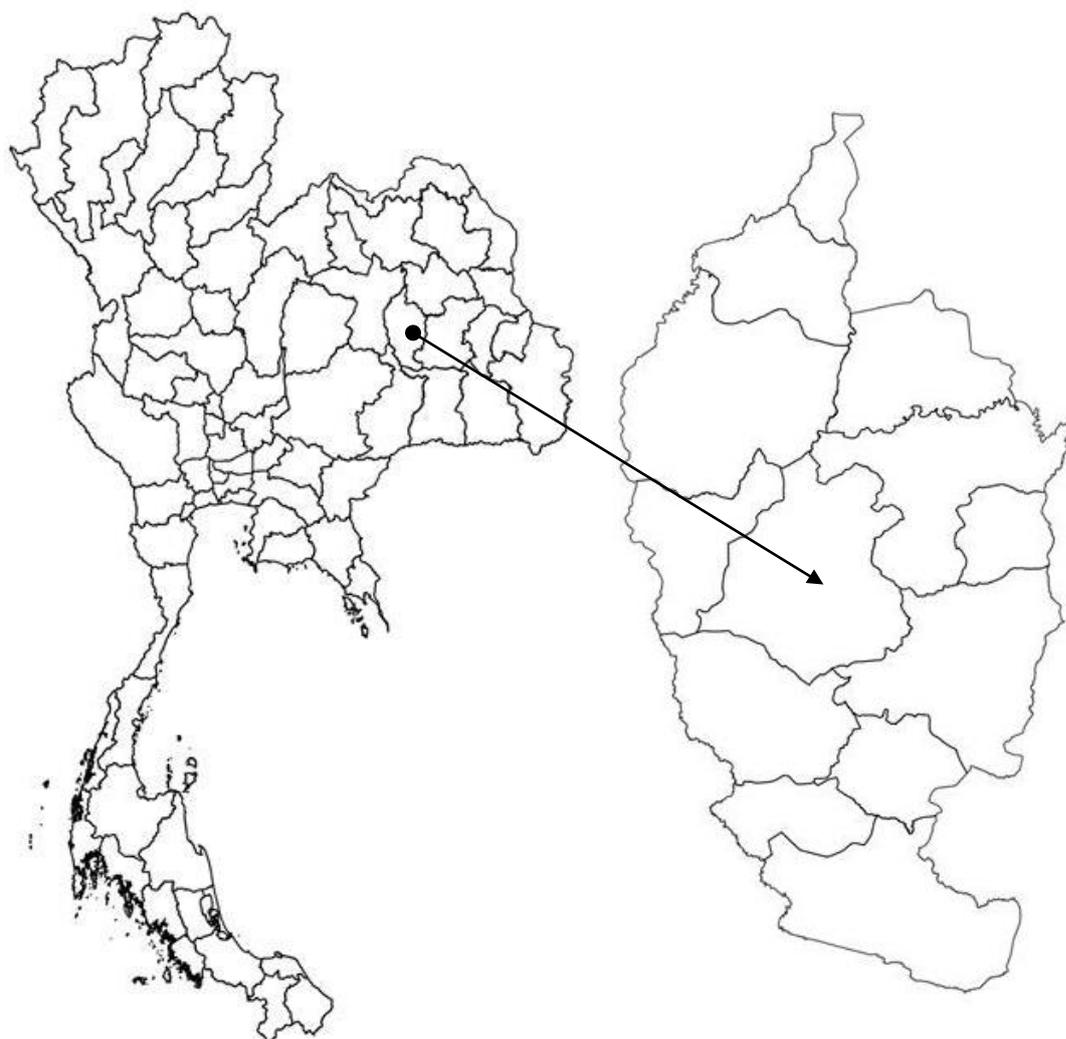
*Leptospira* serovars are generally adapted to one or more mammal hosts. The animals may or may not develop clinical signs at its onset but they

eventually show sub clinical sign in reservoirs which are difficult to detect from observation. Dogs are reservoirs hosts for serovar *Canicola* while pigs are mostly reservoirs for *Bratislava* and *Pomona*. Horses may maintain serovar *Bratislava* and rodents are mostly reservoir hosts for a number of *Leptospira* serovars, including members of the serogroups *Icterohaemorrhagiae*, *Grippityphosa* and *Sejroe*. Rats are considered as important reservoir hosts for serovars *Icterohaemorrhagiae* and *Copenhageni* in the serogroup *Icterohaemorrhagiae*, and cattle are the primary reservoir hosts for serovar *Hardjo* [2]. A previous research showed that antibodies against *L. interrogans* serovars *Ranarum*, *Sejroe*, and *Mini* in cattle in several provinces of Thailand [3]. As information on seroprevalence of *Leptospira* spp. among cattle and dog in Thailand are limited and have less published, therefore, it is important to study *Leptospira* spp. in Thailand to be able to develop vaccines controlling Leptospirosis in

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**Figure 1** Study area at Non Rasi sub-district, Borabue district, Mahasarakham province; Thailand

animal reduce its transmission to human.

Accurate identification and classification of *Leptospira* spp. are important steps for epidemiological seroprevalence study and public health surveillance because different serovars of *Leptospira* spp. can present different host specificities in the area of an epidemic and may not easily be associated with a particular clinical form of infection among animals and human [4].

Thailand is located in tropical region where Leptospirosis has been reported and considered as endemic disease. A study in Brazil summarized from several studies that incidences were influenced by environmental, cultural, social, occupational, and behavioral factors of people living in the disease-burden area [5]. The outbreaks of this disease have been reported all the year round [6]. The risk is higher in countryside where there is a large population of reservoir animals in agriculture or animal husbandry with which people interact with.

Disease surveillance data among human in Mahasarakham Province indicated they have more than 230 patients from Leptospirosis in the last 5 year (2010-2014) with 3 cases of death [7]. In 2015, during January 1 to July 3, Mahasarakham Provincial Health Office has reported 21 human cases with 2 deaths [8].

This study focused on livestock and domestic animals, such as cattle and dogs, which serves as reservoir of *Leptospira* spp affecting human because they are close to human and have high chance to spread this pathogen to human easily. It determined the seroprevalence of *Leptospira* spp. and serovars responsible for *Leptospira* spp. exposure in Non Rasi sub-district, Borabue district of Mahasarakham province, Thailand by using microscopic agglutination test (MAT) because it is a gold standard for determining serovar of Leptospirosis. This method is used since Leptospirosis diagnosis mostly relies on antibody detection which has been

widely used as the reference test for antibody detection. Information on infecting serovars obtained by MAT was used for epidemiological field study.

## MATERIALS AND METHODS

### Study site

Non Rasi sub-district, Borabue district, Mahasarakham province, Thailand was selected as our study sites. It is a low plain agricultural area [9]. People living in this area are mostly rice farmers who usually work on bare-foot which the information was observed and interviewed by researchers from Faculty of Veterinary, Mahasarakham University (unpublished information). Because of this, their feet became channel to transfer Leptospirosis to their bodies [10]. The study sites are shown in Figure 1.

### Animals / Sample collection

A total of 95 serum samples from non-leptospirosis vaccination cattle and dogs, (40 from cattle and 55 from dogs) were collected during July 2014 to January 2015. Samples were collected aseptically using sterile 10 ml syringe for cattle and 5 ml syringe for dogs. Each serum sample was separated by centrifugation of blood at 3,000 g for 10 minutes at room temperature. The serum samples were transferred into 1.5 mL sterile micro tube (Eppendorf) and were kept at -20 °C until further testing. These samples were examined at the veterinary laboratory center of Mahasarakham University Teaching Hospital, Faculty of Veterinary Sciences, Mahasarakham province, Thailand and the Veterinary Research and Development Center (upper northeastern region), Khon Kaen province, Thailand.

### Microscopic Agglutination Test (MAT)

Reference strains of *Leptospira interrogans* were used including the 24 serovars: *australis*, *autumnalis*, *ballum*, *bataviae*, *canicola*, *celledoni*, *cynopteri*, *djasiman*, *grippotyphosa*, *hebdomadis*, *icterohaemorrhagiae*, *javanica*, *louisiana*, *manhao*, *mini*, *panama*, *pomona*, *pyrogenes*, *ranarum*, *sarmin*, *sejroe*, *shermani*, *tarassovi* and *patoc*. Each serum sample was serially diluted in phosphate buffer solution (PBS) in a microtiter plate, starting from 1 with 50 dilution using 2-fold dilution (1 in 100, 200, 400, 800 and 1600). After that, 10 µL of

serum dilution was added to 10 µL of appropriate antigen on a microscopic slide and was placed in a petri dish incubated at temperature 30 °C for 90 minutes. The slide samples were examined under the dark-field microscope with one antigen control, positive and negative standard serum controls used each time. Titers 1:100 or greater were considered positive. The cut-point titer was determined as the highest serum dilution showing agglutination of at least 50% of the *Leptospira* spp.

## RESULTS

A total of 95 serum samples of cattle (40) and dogs (55) collected from Non Rasi sub-district, Borabue district, Mahasarakham province, Thailand. Eleven (11.58%) samples were positive by MAT test. Of 40 serum samples from cattle, 5 samples were positive (12.5%) to *Leptospira* serovar *sejroe* and 35 samples (87.5%) were negative to all serovars. While, 6 samples, out of 55 serum samples collected from dogs, were positive (10.9%) of *Leptospira* serovar *canicola* and 49 samples (89.1%) were negative to all serovar (Table 1).

## DISCUSSION

Results are shown that seroprevalence found from dog serum samples are *Leptospira* serovar *Canicola*. This is consistent to previous studies indicating that the most prevalent *Leptospira* serogroups present in domestic and livestock animal reservoirs in Thailand are *Leptospira* serovars *Bataviae*, *Canicola* and *Australis* [11].

Results of this study further support a serological survey of leptospirosis in cattle and buffalo in Kra Nuan district and Puay Noi district, Khon Kaen province by Chanepaiboon et al. in year 2000 [12].

This finding is significant in identifying accurate serovars that spread out in the area. This is also be evidence-based results of serovars that might be related to clinical manifestations with human and animals. In the future, planning an optimal program to control bovine leptospirosis and canine leptospirosis will prevent this most especially to avoid urinary shedding in animals exposed to an identical of leptospiral serovars.

## CONCLUSION

This study could confirmed that serovar *Seroj*

**Table 1** Number, percentage of positive serum and type of serovars among animal hosts by MAT tests

Animal species	Number of positive	Total samples	Percentage of positive	Serovar
Cattle	5	40	12.5	<i>Sejroe</i>
Dog	6	55	10.9	<i>Canicola</i>

among cattle and serovar *Canicola* among dogs are circulating the study areas. The findings would alarm on potentially risks of leptospirosis infection from infected cattle and dogs through surrounding human and animals.

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