Animal Reservoirs for *Leptospira* spp. in South-East Asia: A Meta-Analysis

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Abstract

Background and Aim: Leptospirosis is a zoonotic disease responsible for high morbidity worldwide. This review is intended to identify other possible animal reservoirs of *Leptospira* spp. within human surroundings, which may improve the prevention and control of the disease.

Methods: A systematic search was performed for the relevant titles, abstracts and keywords in PubMed, Scopus and Google Scholar in March 2018 based on the PICO strategy; which returned 1226 studies. Screening of abstracts had shortlisted 71 studies and data extraction was conducted for 15 studies which had been accepted after review of the full text. Only studies done in South-East Asia were considered. Meta-analysis of the accepted studies was done to compute the composite prevalence of each animal group using random effects model.

Results: The articles were analysed from the viewpoint of the study settings and the prevalence of *Leptospira* spp. in types of animal with the animals being grouped into five major groups, based on taxonomy and likelihood of human contact. It was found that all animal groups have statistically significant value of pooled prevalence, with a range between 8.17% (95% CI: 4.80% - 12.39%) for sheep and goats, to as high as 27.28% (95% CI: 1.24% - 69.69%) for carnivores. The prevalence for other animal groups are 17.95% (95% CI: 7.77% - 31.18%) for rodents, 19.24% (95% CI: 10.65% - 29.65%) for pigs, and 24.90% (95% CI: 15.45% - 35.71%) for ruminants.

Conclusion: Meta-analysis showed that other groups of animals, particularly carnivores, pigs, and large ruminants are just as culpable as rodents in being the reservoir for *Leptospira* spp. These other groups of animals may also play a vital role in the transmission and overall dynamics of human leptospirosis.

Keywords: Animal reservoirs, Leptospirosis, Prevalence, South-East Asia

Introduction

Leptospirosis is a zoonotic disease with high morbidity in tropical countries with incidence ranging from 10 to 100

cases per 100,000 individuals.¹ It is caused by a spirochete bacterium in the genus *Leptospira* spp. and transmitted to humans through animal urine or contaminated soil or water. In 2015, it was estimated that there were 1.03

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million cases (95% CI:434,000-1,750,000) annually with 58,900 deaths (95% CI:23,800-95,900) globally.² Almost half of the cases (48%; 95% CI: 40 – 61%) and more than two fifths of deaths (42%; 95% CI 34-53%) were estimated to occur in adult males with the age of 20-49 years.² The highest estimates of disease morbidity and mortality were observed in South Asia, Southeast Asia, Tropical Latin America, Central America, Oceania, East Sub-Saharan Africa, and Caribbean and Andean regions.²

The epidemiology of leptospirosis has been extensively studied in urban communities, where rats are thought to be the main vector of human leptospirosis in urban settings.^{3,4} For high risk occupational exposure, cattle and pigs are thought to be the animal reservoir for the disease.⁵ Further, an increasing number of studies revealed that other animals can also be the reservoir.^{6,7} Hence, this review is intended to identify the possible animal reservoir of *Leptospira spp.* within human surroundings. Understanding the source and animal reservoir of leptospirosis may provide insights into the transmission process that can ultimately be utilised to prevent and control this disease. The objectives of this systematic review are to identify animal reservoirs for leptospirosis, determine the prevalence of *Leptospira spp.* in animal reservoirs and identify high-risk animal reservoirs for human leptospirosis.

Methods

Search Method

Systematic search was performed for the relevant titles, abstracts and keywords in the journal databases of PubMed, Google Scholar and Scopus on March 2018 based on the PICO strategy (Figure 1). A total of 1226 studies were retrieved; keywords and terms entered were (leptospirosis OR leptospira OR zoonotic OR “Weil’s Disease” OR “field fever” OR “rat catcher’s yellows” OR “pretibial fever” OR Asia) AND (“animal reservoir” OR rat OR rodent* OR mouse OR mice OR pig* OR cattle OR livestock* OR pet OR dog* OR cat) AND (prevalence OR incidence) without restrictions on the publication date. Only original research, published articles and articles written in the English language were selected. Screening of abstracts had shortlisted 71 studies and data extraction was conducted. However, only 15 studies had been accepted after review of the full texts. The other 56 articles were excluded due to them being: (1) conducted outside of the South-East Asia region; (2) involving wild animals; (3) utilising methods of seropositivity detection other than the Microagglutination Test (MAT); and (4) having a small sample size of less than 30 individual animals.

Data Analysis

The studies included for meta-analysis are in principal

homogenous in terms of the methodology, the method of *Leptospira spp.* detection, and the setting. Heterogeneity may still be present and is likely due to systematic bias and is tested by chi squared with $p < 0.01$ denoting level of significance. The quantification of the degree of heterogeneity was done using the I^2 statistic, which represents the percentage of the total variability across studies. To quantify the degree of heterogeneity,² value of more than 75% is considered as highly heterogenous but is still included in the meta-analysis, as it is assumed that the studies included were answering similar research questions. Studies were assessed for systematic bias by scrutinizing the funnel plot. A study that falls outside of the funnel is considered as having high bias and may not be included in the meta-analysis. For each group of animals, at least 5 studies were included for the calculation of the prevalence. Analyses were performed using MedCalc Statistical Software version 18.2.1.⁸ Random effects model was used to calculate the prevalence of *Leptospira spp.* in each group of animals.

Results

Settings

A total of 15 articles exploring the prevalence of *Leptospira spp.* among animals from various settings in South-East Asia were analyzed (Table 1). The prevalence ranges were between 0.6% to 92.45%, being dependent on the MAT cut-off point used. Common animals studied in these articles were rodents, pigs, cattle, buffaloes, sheep, goat and carnivores such as cats, dogs, and civets. The studies were conducted in 15 South-East Asian countries, whereby 5 (33.33%) were in Thailand, followed by Malaysia (4; 26.67%), Vietnam (3; 20.00%), Philippines (2; 13.33%), and Indonesia (1; 6.67%). Animal samples were either obtained from livestock or captured strays. The majority of studies obtained samples from livestock or animal farms (9; 60.00%), while 3 (20.00%) were obtained from captured rodents around the residential area or from stray animals. Others include abattoirs (2; 13.33%) and one (6.67%) each from animals captured in the forest or shrubs, places of worship, veterinary clinic and a National Service camp.

Types of Animal

Ten studies researched a single animal whereas the remaining looked into leptospirosis in multiple animals. The most studied animal was pigs (8; 53.33%). Four of these studies were focused on pigs alone whereas the other four studies also included other farm animals such as cattle, buffalo, sheep and goat. A total of 9219 samples from pigs were taken in the studies. Rodents and buffaloes were the second most studied animal (5; 33.33%) with 2185 and 2441 samples respectively. Cattles were studied in four (26.67%) researches with 4314 samples. Three

studies (20%) looked into dogs, two studies (13.33%) had sheep while cats were researched in one study (6.67%). One study also included other animals such as squirrel, reptiles, shrews, birds, ox and other mammals.⁹

Microscopic Agglutination Test

MAT cut-off titres differed in the 15 included studies. The cut-off titres used were as low as 1:20, up to 1:400. Three studies conducted in Vietnam, Thailand and the Philippines used MAT cut-off titre of 1:20 to determine the seroprevalence in rats and dogs. One study conducted in Thailand among livestock used a cut-off titre of 1:50. Majority of the studies used cut-off titres of 1:100 and only two studies used cut-off titres of 1:400. In those 15 studies, different sets of reference antisera were used to identify different serovars depending on the local endemicity. The different number of antigens panel used ranges from 10

to 39 antisera. The most common panel used is by WHO/FAO/OIE which can be tested against 23 serovars.

Prevalence

It was found that there were a total of 13 groups of different animals; they are then placed into larger groups (total of 5 groups) based on similar taxonomy and likelihood of contact with humans. *Leptospira spp.* prevalence in pigs ranged from 8.2% to 73.4%, while rats/rodents' prevalence was from as low as 6.7% up to 92.5%. Large ruminants (buffaloes, cattle, ox) had a prevalence range from 3.8% to 40.5%, while the prevalence in small ruminants like sheep and goats were between 4.4% and 27.9%. Carnivores (dogs, cats, civets) on the other hand had 8.8% to 89.1% prevalence. There were few studies on other animals including reptiles (14.0%), squirrels (5.0%), shrews (0.8%), bats (0.7%) and birds (0.6%).

Table 1. Summary of studies included in the analysis

	Author and year	Study design & settings	Method of detecting <i>Leptospira spp.</i>	Type of animal	No. of sample	No. of positive samples	Prevalence of <i>Leptospira</i>
1.	Lee et al. ¹⁰	Cross-sectional. Slaughterhouses from 5 provinces Vietnam	MAT $\geq$ 1:100	Pigs	1959	160	8.17%
2.	Chadsuthi et al. ¹¹	Cross-sectional. Thailand. National passive surveillance data on leptospirosis infection in livestock.	MAT $\geq$ 1:100	Buffaloes Cattle Pigs	432 3648 3138	107 1026 356	24.8% 28.1% 11.3%
3.	Loan et al. ¹²	Cross-sectional. Farms in 12 Mekong Delta provinces, Vietnam	MAT $\geq$ 1:20	Rat	241	44	18.26%
4.	Suwanchaoen et al. ¹³	Cross-sectional. Livestock, 36 provinces in Thailand.	MAT $\geq$ 1:50	Cattle Buffalo Pig Sheep Goat	9288 1376 1898 1110 516	918 419 205 52 41	9.88% 30.45% 10.80% 4.68% 7.95%
5.	Niwetpathomwat et al. ¹⁴	Cross-sectional. 10 sow farms in 4 provinces in central Thailand	MAT $\geq$ 1:100	Pigs	400	40	10%
6.	Meeyam et al. ¹⁵	Out-patient department of the small animal hospital, faculty of Veterinary Medicine, Chiang Mai University, Thailand	MAT $\geq$ 1:20	Dogs	210	23	11%

7.	Bahaman et al. ¹⁶	Cross-sectional. Blood samples from animals in government farms, small holdings & animals sent for slaughter in abattoir in West Malaysia	MAT ≥ 1:40	Total Cattle Water buffaloes Goats Pigs Sheep	3377 1378 429 657 869 44	862 558 133 29 139 3	25.5% 40.5% 31% 4.4% 16% 6.8%
8.	Smith et al. ⁹	Cross-sectional. Animal species were trapped in forest localities, rice field areas, areas of scrub and cultivation and in several towns and villages, Malaysia	MAT ≥ 1:100	House rats and mice Rats of scrub and cultivation Forest ground rats Forest tree rats Tree shrews Squirrels Shrew Carnivore Bats Other mammals Birds Reptiles Ox Goat Buffalo Pig Dog Cat	393 701 522 147 101 237 44 57 295 25 173 116 52 61 34 47 51 68	26 42 50 1 3 1 2 9 4 5 1 16 2 17 5 9 6	6.6% 6% 9.6% 1% 3% 0.8% 5% 8.8% 0.7% 20% 0.6% 14% 3.8% 27.9% 14.7% 10.6% 17.6% 8.8%
9.	Villanueva et al. ¹⁷	Cross-sectional. Intensive-type farm in the Philippines	MAT ≥ 1:40	Rat Water buffalo	10 170	3 81	30% 48%
10.	Villanueva et al. ¹⁸	Cross-sectional. Metro Manila, the Philippines	MAT ≥ 1:20	Rat	106	98	92.45%
11.	Mohamed-Hassan et al. ¹⁹	Cross-sectional. National Service Training Centres in Kelantan and Terengganu, Malaysia	MAT ≥ 1:20 MAT ≥ 1:40	Rat	168 168	30 14	17.9% 8.3%
12.	Al-Khleif et al. ²⁰	Cross-sectional. Farms in provinces of Bali, Indonesia	MAT ≥ 1:100	Pig	484	131	27.07%
13.	Jittapa-lamong et al. ²¹	Cross-sectional. Around Buddhist monasteries in a district located in the center of Bangkok, Thailand	MAT ≥ 1:100	Dog	230	205	89.13%
14.	Boqvist et al. ²²	Cross-sectional. Pig farms in the Mekong delta, Vietnam	MAT ≥ 1:100 MAT ≥ 1:400	Pig	424 424	311 124	73.35% 29.25%
15.	Benacer et al. ²³	Cross-sectional. Urban rats from 3 KL sites, Malaysia	MAT ≥ 1:400	Rat	300	20	6.7%

Figure 1. Search method

Figure 2. Prevalence of Leptospira in rodents

Figure 3. Prevalence of Leptospira in pigs

Figure 4. Prevalence of Leptospira in cattle and buffaloes

Figure 5. Prevalence of Leptospira in sheep and goats

Figure 6. Prevalence of Leptospira in carnivores

Meta-Analysis

Meta-analysis was done to determine the pooled prevalence of Leptospiral infection. Animals were grouped according to taxonomy or likelihood of human contact. Rodents were considered together as one group and includes all species of rats and mice. Domestic pigs meant for human consumption made up the second group, while the third group consisted of ruminants like cattle and buffaloes. Next, sheep and goats formed another group, while cats, dogs and other carnivores such as civet were considered together in a group (denoted as carnivores) since these species share similarities in terms of contact with humans (as pets or as stray animals) and taxonomy.

The prevalence of *Leptospira* in rodents serves as a control for the comparison with other reservoir animals. Six studies on rodents were identified, including one study which had four different species of rodents analysed, and were considered separately in this analysis.^{9, 12, 17-19, 23} Range of prevalence were from as high as 92.45%¹⁸ to as low as 0.68%.⁹ Tests of heterogeneity showed that Chi-squared was 484.15 ($p < 0.01$), with high heterogeneity ($I^2 = 98.35\%$). Random effects model showed prevalence was 17.95% (95% CI: 7.77%-31.18%) (Figure 2). The next group of animals analysed was pigs. Eight studies for pigs included^{9-11, 13, 14, 16, 20, 22} with range of prevalence were from as high as 73.35%²² to as low as 8.17%.¹⁰ Tests of heterogeneity showed that Chi-squared was 871.55 ($p < 0.01$), with high heterogeneity ($I^2 = 99.20\%$). Via random effects model, the prevalence was 19.24% (95% CI: 10.65%-29.65%) (Figure 3). In Figure 4, there were four studies concerning cattle labelled as (i)^{9, 11, 13, 16} and five studies for buffaloes, labelled as (ii).^{9, 11, 13, 16, 17} Range of prevalence were from as high as 47.65%¹⁷ to as low as 3.85%.⁹ Tests of heterogeneity showed that Chi-squared was 1334.86 ($p < 0.01$), with high heterogeneity ($I^2 = 99.40\%$). Via random effects model, the prevalence was 24.90% (95% CI: 15.45%-35.71%).

There were two studies concerning sheep denoted with (i)^{13, 16} and three studies for goats labelled as (ii) (Figure 5).^{9, 13, 16} Range of prevalence were from as high as 27.87%⁹ to as low as 4.41%.¹⁶ Tests of heterogeneity showed that Chi-squared was 34.10 ($p < 0.01$), with high heterogeneity ($I^2 = 88.27\%$). Via random effects model, the prevalence was noted to be 8.17% (95% CI: 4.80%-12.39%). For carnivores, which consisted of cats, dogs and civets, they were analysed together in a Forest plot (Figure 6). Three studies were identified which had carnivores in its sampling; including one study which had three different types of carnivores analysed, and were considered separately in this analysis.^{9, 15, 21} Range of prevalence in carnivores were from as high as 89.13%²¹ to as low as 8.82%.⁹ Tests of heterogeneity showed that Chi-squared was 444.42 ($p < 0.01$), with high heterogeneity ($I^2 = 99.10\%$). Via random effects model, the prevalence was 27.28% (95% CI: 1.24%-69.69%).

Discussion

In most studies, the animals were sampled from livestock or animal farms. The risk of contracting leptospirosis for these animals is different compared to wild animals. Livestock animals live in a controlled environment with clean surroundings, as compared to the wilds. These animals are also well-fed and do not have to fend for themselves from the weather elements and predators; unlike the wilds or stray animals. Thus, livestock animals had less risk to be exposed to Leptospiral infection and became a reservoir. Pets are also considered as animals with low risk of Leptospiral exposure; even lower than that of livestock. Meeyam et al. had taken sera samples from dogs brought to out-patient department of animal hospital in Chiang Mai, Thailand, and found that presence of *Leptospira* antibodies was only 10.95%.¹⁵ On the contrary, another study conducted among stray dogs around Buddhist monasteries in the centre of Bangkok found, found that the presence of *Leptospira* antibodies was 89.13%.²¹ The specific settings in which the studies were conducted in play a pivotal role in determining the prevalence of *Leptospira* in animals studied, and thus a degree of heterogeneity is expected.

Most studied animals were domestic mammals and rodents as these animals have been established as reservoir hosts for leptospirosis. This finding is similar to study done in Pacific Islands which found that cattle, pigs, dogs and rodents may play significant roles in disease transmission to humans.²⁴ Although other animals can also carry *Leptospira*, domestic mammals and rodents carry the highest risk of infecting humans as they are in close contact with humans.²⁵ Farm animals like pigs, cows and cattle are managed by human workers from birth to slaughter, and dogs and cats are among the most favoured pets among humans. Additionally, rodent infestations are common in sewers and public places.

Microscopic agglutination test (MAT) is the World Health Organisation (WHO) gold standard test used to diagnose leptospirosis.²⁶ MAT detects both IgM and IgG, but cannot differentiate between current, recent, or past infections. It uses a specific panel of antigens for each test depending on the region of sample collection, availability of antigens and cost-effectiveness. An incomplete panel may be responsible for causing false negative results. In areas where endemic strains are unknown or poorly characterised, low seroprevalence may be due to a lack of utilising the correct panel of antigens.²⁷ Different cut-off points or titres used in those mentioned studies may affect the seroprevalence in animals. There is no established cut-off point on MAT titre for some of animals. The 1: 100 titre recommended by the World Organization for Animal Health (OIE) is considered positive for international trade, but lower MAT titres may indicate previous exposure to *Leptospira*.²⁸ The decision

to set a low cut-off point at 1:20 may be due to the fact that serum antibody levels often decline to undetectable levels in chronic leptospirosis infections such as in rat and cattle. Leptospire within the proximal renal tubules, genital tract and mammary glands of animal have been shown to be protected from circulating antibodies which allows persistence and multiplication in these areas.²⁹ A low titre is appropriate in a population in which exposure to leptospirosis is uncommon, but if exposure is frequent, as in most tropical countries, a higher cut-off titre is necessary.³⁰

There are variability and wide ranges in prevalence of *Leptospira* spp. within each group of animals. This could be affected by the grouping of animals in the analysis. Some studies broadly categorized different species of similar types of animals as one distinct group; e.g. *Rattus rattus* (black rat), *Rattus norvegicus* (brown rat) and *Mus musculus* (house mice) are all considered as “rats” without any differentiation. Moreover, different species/sub-species of the same group of animals harbour different and distinct serovars of leptospire. As an example, different rodent species may be reservoirs of distinct serovars, with rats generally being hosts for serovars of the serogroups *Icterohaemorrhagiae* and *Ballum*, and house mice are usually hosts for serogroup *Ballum*. Domestic animals are also potential distinct hosts; with dairy cattle usually harboring the serovars *hardjo*, *pomona*, and *grippotyphosa*; pigs could be hosting *pomona*, *tarassovi*, or *bratislava*; sheep usually are hosts for *hardjo* and *pomona*; and dogs may harbor *canicola* serovar.³¹ These different types of serovar which are distinct and unique to each animal might influence the sensitivity and specificity of the MAT test in detecting *Leptospira*.

Different geo-location and weather in each study setting might change the level of *Leptospiral* endemicity, thus requiring different MAT titre values used as cut-off points for *Leptospiral* detection at each setting to reflect the varying endemicity. Prevalence of leptospirosis is considerably higher in warm-climate countries than in temperate ones; due to longer survival of leptospire in warmer, more humid environment. Many tropical countries are agricultural countries. Therefore, there are greater risk for exposure of the human population to infected animals, either livestock, domestic pets, or wild/feral animals. The disease is markedly seasonal, with peak incidence occurring during summer or fall in temperate regions, where temperature is the limiting factor in the survival of leptospire, and during rainy seasons in warm-climate regions, where rapid desiccation would otherwise prevent survival.³² Different level of expertise among the researchers/lab technicians from all research groups and difference in the reliability of the laboratory machines/apparatus being used in the studies to analyse data could also influenced the final reported prevalence,

which is also a reflection of the availability of laboratory diagnosis and facility.³²

Conclusion

Apart from rodents which are traditionally known for *Leptospiral* transmission, other groups of animals, particularly carnivores, pigs and large ruminants are also identified as the high-risk animal reservoirs. Thus, rodent control alone is insufficient and focus should also be given to these other groups of animals in preventing human leptospirosis.

Conflict of Interest: None

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