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Journal Articles

SEROLOGICAL DETECTION OF *Leptospira* spp. IN EQUINE POPULATIONS ACROSS FIVE DISTRICTS OF KELANTAN, MALAYSIA

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SUMMARY

Leptospirosis is a zoonotic disease caused by spirochetes of the genus *Leptospira*, which can infect a wide range of animal species, including horses. In Malaysia, information on equine leptospirosis is scarce, particularly in Kelantan, which has the country's largest horse population. Therefore, this study aimed to determine the serological detection of *Leptospira* spp. in horses in Kelantan, Malaysia. A total of 50 horses from eight stables across five districts were sampled, with complete demographic data recorded. Serum samples were tested using the microscopic agglutination test (MAT) against six leptospiral serovars namely Icterohaemorrhagiae, Pomona, Grippotyphosa, Bataviae, Australis, and Hardjobovis. Two horses tested positive, resulting in a serological detection of 4% (95% CI: 0.5%–9.4%). The serovars detected were Icterohaemorrhagiae and Pomona, both with titres of 1:100. These results are notable, as most horses were managed intensively in stables, and none had received leptospirosis vaccination, which is currently unavailable for equines in Malaysia. The detection of *Leptospira*-seropositive horses highlights their susceptibility to infection and raises concerns regarding potential zoonotic transmission. In conclusion, the presence of *Leptospira* spp. in horses emphasises the need for further studies and improved biosecurity to reduce risks to both animals and humans.

Keywords: Horses, Kelantan, Leptospira spp., leptospirosis, serological detection

INTRODUCTION

Leptospirosis is a widespread zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira* (Adler & de la Peña Moctezuma, 2010). It affects a broad range of mammalian hosts, including humans, livestock, and companion animals, leading to significant public health and economic impacts worldwide (Haake & Levett, 2015). Transmission occurs through direct contact with the urine of infected animals or indirectly via contaminated water or soil (Ellis, 2015). Horses, although often considered incidental hosts, can develop subclinical infections or present with clinical signs such as fever, jaundice, recurrent uveitis, and reproductive losses (Verma et al., 2013).

In Malaysia, leptospirosis is recognized as an important emerging and re-emerging zoonotic disease, with human outbreaks frequently reported in both urban and rural settings (Benacer et al., 2016b). However, there is a scarcity of data regarding its occurrence in equine populations. This gap in epidemiological information may lead to an underestimation of the disease burden, potentially posing risks to equine health, the equine industry, and individuals in close contact with horses.

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Kelantan, located in the northeastern region of Peninsular Malaysia, has one of the highest horse populations in the country due to active equestrian, recreational, and ceremonial uses, with native ponies widely kept in the East Coast states including Kelantan (Darmansah et al., 2017). Management practices in the region, ranging from intensive stable housing to semi-intensive systems, may influence exposure risk to *Leptospira* spp.

The microscopic agglutination test (MAT) remains the gold standard for serological detection of leptospiral antibodies and identification of infecting serovars (OIE, 2021). Understanding the seroprevalence and serovar distribution in horses is essential for clarifying their epidemiological role and developing targeted preventive measures. This study aimed to determine the serological detection of *Leptospira* spp. in horses from Kelantan, Malaysia, while also documenting demographic and management factors associated with infection risk.

MATERIALS AND METHODS

Study area and population

This cross-sectional study was conducted in five districts of Kelantan, Malaysia namely Kota Bharu, Bachok, Tanah Merah, Pasir Puteh, and Machang. A total of 50 horses from eight different stables were recruited, representing various management systems (intensive and semi-intensive). Complete demographic information, including sex, age, breed, vaccination status, deworming status, and clinical history, was recorded for each animal.

In this study, 'intensive management' referred to horses housed predominantly in stables, with all feed and water provided manually. 'Semi-intensive' systems refer to horses that are stabled overnight but allowed access to outdoor paddocks or grazing areas during the day. All 50 horses recruited from the eight stables were privately owned. Before blood collection, informed verbal and written consent was obtained from all respective stable owners. The distribution of horses was inherently uneven across the districts due to the availability and varying sizes of local stables.

Sample collection

Blood samples were collected aseptically via jugular venipuncture into plain vacutainer tubes. The samples were allowed to clot at room temperature before centrifugation at 3,000 rpm for 10 minutes to obtain serum. Serum aliquots were stored at –20 °C until analysis. Blood samples were collected aseptically via jugular venepuncture into plain vacutainer tubes between February, 2022 until July 2022.

Serological testing

The microscopic agglutination test (MAT) was performed according to the guidelines of the World Organisation for Animal Health (OIE, 2021). Six pathogenic leptospiral serovars were used as live antigens: *L. interrogans* serovars Icterohaemorrhagiae, Pomona, Grippotyphosa, Bataviae, and Australis, and *L. borgpetersenii* serovar Hardjobovis. Serum samples were initially screened at a dilution of 1:50, followed by serial two-fold dilutions for positive reactions. Agglutination of ≥50% of leptospiral cells at a dilution of 1:100 or higher was considered seropositive.

Data analysis

Serological detection was calculated as the proportion of positive samples among the total tested, with a 95% confidence interval (CI). Demographic variables were summarised descriptively, and results were presented in tables for clarity.

RESULTS

The demographic details of the sampled horses are summarised in Tables 1 and 2.

Table 1. Stable distribution by district

District	Stable	Number of Horses
Kota Bharu	A	5
Bachok	B	11
Bachok	C	3
Bachok	D	7
Tanah Merah	E	1
Tanah Merah	F	7
Pasir Puteh	G	2
Machang	H	14
	Total	50

Horses ranged in age from 4 months to 5 years and comprised both local and imported breeds. The majority were managed under intensive stable systems, with no history of leptospiral vaccination, as such vaccines are not available for equines in Malaysia. Clinical signs were observed in 11 horses, which included colic (n = 1), diarrhoea with weight loss (n = 1), incoordination (n = 4), melanoma (n = 1), rectovaginal tear (n = 1), reduced performance (n = 1), severe dermatological lesions (n = 1), and Wobbler syndrome (n = 1).

A portion of the population (n=24) had received standard equine core and non-core vaccines (including Tetanus, Equine Encephalomyelitis, West Nile Virus, Equine Influenza, Equine Herpesvirus, and Strangles); however, none had a history of leptospiral vaccination, as such vaccines are not available for equines in Malaysia.

Serological detection of Leptospira spp.

Out of the 50 horses tested, 2 were seropositive for *Leptospira* spp., yielding an overall serological detection of 4% (95% CI: 0.5%–9.4%). The positive samples reacted to *L. interrogans* serovar Icterohaemorrhagiae (n = 1) and *L. interrogans* serovar Pomona (n = 1), both with titres of 1:100.

A descriptive breakdown of the two seropositive horses revealed that both originated from the same facility in Bachok (Stable B) and were maintained under an intensive management system. Both individuals were adult mares and presented as healthy with no observable clinical signs at the time of sampling. Regarding breed, one was a KKB crossbreed positive for serovar Icterohaemorrhagiae, and the other was a Thoroughbred positive for serovar Pomona.

Due to the extremely low number of seropositive cases detected (n=2), basic statistical approaches to assess potential associations or trends between demographic variables and seropositivity were not conducted, as the sample size lacked the statistical power required for meaningful interpretation.

DISCUSSION

Leptospirosis in Malaysia has been actively reported in livestock such as cattle, goats, and sheep (Abdul-Rahman et al., 2021a; Sabri et al., 2019), as well as in companion animals like dogs and cats (Abdul-Rahman et al., 2021b; Rahman et al., 2021; Alashraf et al., 2020). The pathogen has also been detected in environmental samples including water and soil, rodents, and stray animals (Lea et al., 2025; Abdul-Rahman et al., 2023; Goh et al., 2021; Alashraf et al., 2019; Benacer et al., 2016a). However, there is a notable lack of information regarding leptospirosis in horses, with the most recent Malaysia-based report dating back to 1979 (Joseph, 1979). This gap underscores the urgent need for updated epidemiological data on equine leptospirosis in Malaysia.

This study identifies an overall serological detection of 4% (95% CI: 0.5% - 9.4%) for *Leptospira* spp. in horses from Kelantan, Malaysia. This result is much lower than that reported in other regions, such as the 45.9% (273/595; 95% CI: 41.9% - 49.9%) recorded in southern Brazil (Da Silva et al., 2020) or the 57.4.6% (513/894; 95% CI: 54.1%

Table 2. Demographic summary of horses

Category	Subcategory	Details
Sex	Colt	1
	Gelding	15
	Mare	32
	Stallion	2
Age	Colt (n=1)	4 months
	Gelding and Mare (n=47)	Adult
	Stallion (n=2)	5 months & 5 years
Breed	Arabian	24
	Arabian cross	2
	KKA[1]	4
	KKB[2]	8
	Paddy	6
	Thoroughbred	6
Vaccination[3]	Yes	24
	No	26
Deworming	Yes	35
	No	15
Management	Intensive	35
	Semi-intensive	15
Health	Clinical signs	11
	Healthy	39

[1]KKA – Kuda Kelantan A grade (cross between Thoroughbred and Paddy); [2]KKB – Kuda Kelantan B grade (cross between Thoroughbred and Paddy); [3]Equine core and non-core vaccines (Tetanus; Eastern/Western Equine Encephalomyelitis; West Nile Virus; Equine Influenza; Equine Herpesvirus; Strangles)

- 60.6%) reported in Goiás, Brazil (Romanowski et al., 2023). Such variation is likely due to differences in environmental factors, local epidemiology, sample size, recruitment criteria and management practices.

The detection of serovars Icterohaemorrhagiae and Pomona aligns with global trends. In North America, Pomona and Grippotyphosa are among the most frequently reported in horses (Divers, 2022), while in Brazil, Icterohaemorrhagiae and Australis are also common (Romanowski et al., 2023). These serovars are often associated with incidental hosts, suggesting sporadic environmental exposure rather than sustained transmission cycles.

The serovars used in this study were those most commonly applied in routine diagnostic testing. However, expanding the MAT panel to include a wider range of serovars especially those relevant to the local environment could improve the detection rate and provide a broader epidemiological perspective (Ellis, 2015).

Interestingly, a detailed breakdown of the positive cases reveals that both seropositive horses originated from the same facility (Stable B), were adult mares, and were maintained under an intensive management system. While the extremely small number of positive cases (n=2) mathematically precludes formal statistical analysis for risk associations, a qualitative assessment provides valuable epidemiological insights. Intensive housing systems are generally presumed to offer better biosecurity; however, they can paradoxically increase exposure risk if the stable environment becomes contaminated. The detection of serovar Icterohaemorrhagiae typically maintained by rodent reservoir hosts alongside serovar Pomona suggests that feed storage areas or shared water sources within Stable B may have been compromised by local wildlife or synanthropic rodents. Furthermore, the absence of clinical signs in both mares suggests that such environmental exposure resulted in subclinical or mild, transient infections. This highlights a critical vulnerability in intensive setups: once a pathogen enters the micro-environment, the close confinement of animals and shared resources can facilitate silent, localized transmission.

While the microscopic agglutination test (MAT) remains a valuable epidemiological tool, it detects only exposure and cannot confirm active infection. Even though a 1:100 titre is considered low, it remains indicative of past exposure or an early stage of infection (OIE, 2021). Such low titres should not be disregarded, particularly in endemic areas where continuous environmental exposure is possible. In subclinical or early cases, antibody levels may rise over time, and paired serum samples would help confirm recent infection (Ellis, 2015). Furthermore, culture and molecular detection methods are required to confirm the carrier state, though these are often challenging due to the fastidious nature of *Leptospira* spp. (Adler & de la Peña Moctezuma, 2010).

It is important to note that in this study, paired serum sampling, bacterial culture, and molecular detection were not performed. This was primarily because the work was designed as a pilot study for equine leptospirosis, and no clinical cases consistent with the disease had been reported in the study area. Nevertheless, the possibility of subclinical infection remains, especially given the environmental resilience of *Leptospira* spp. and their potential to circulate in equine populations without clear clinical evidence (Ellis, 2015).

The clinical signs reported in these horses did not align with the classical presentation of equine leptospirosis, such as equine recurrent uveitis (ERU), abortion, or acute renal and hepatic disease (Divers and

Chang, 2009). Instead, the signs were nonspecific and varied, which could indicate incidental exposure without active disease, or the presence of other underlying conditions. Recruiting horses that present with clinical signs suggestive of leptospirosis in future studies would likely increase the detection rate and strengthen the association between seropositivity and clinical disease.

In Malaysia, leptospirosis is a recognised but underreported zoonosis. Outbreaks in humans are often linked to flooding and occupational exposure (Benacer et al., 2016b). The finding of seropositive horses, particularly in intensively managed facilities, raises the possibility that equines may have a role in local transmission dynamics, which is an aspect that remains underexplored.

Additionally, commercial vaccination protocols for horses in Malaysia do not include *Leptospira* spp., which raises concerns when seropositive cases are identified. This gap in preventive measures highlights the need for greater awareness among veterinarians, horse owners, and equine facility managers regarding the potential risk of infection, even in the absence of typical clinical signs.

Given the zoonotic nature of leptospirosis, seropositive horses may represent a potential occupational risk for stable workers and veterinarians. Therefore, preventive strategies such as improved stable hygiene, rodent control, and periodic screening should be incorporated into equine management protocols.

This study has several limitations that should be considered. Primarily, the small sample size (n=50) restricts the statistical power of the analysis and limits the generalizability of the findings across the broader equine population in Malaysia. Furthermore, precise GPS coordinates for the sampled stables were not recorded during this initial phase, precluding detailed spatial epidemiological analysis or mapping of the exposure risk. Additionally, as a pilot study conducted in a region without prior reported clinical cases, paired serum sampling, bacterial culture, and molecular detection methods were not performed. Consequently, the MAT results indicate exposure but cannot definitively confirm active infection or carrier status. Finally, the cross-sectional design captures prevalence at a single point in time, limiting our ability to track seasonal variations in exposure risk. Furthermore, detailed environmental variables such as specific water sources, drainage systems, or prior exposure to flooding were not recorded during this initial sampling phase, limiting our ability to map specific environmental risk factors.

CONCLUSION

This study demonstrated the presence of seropositive horses for *Leptospira* spp. in the investigated population, indicating potential exposure and an underappreciated role of equines in the epidemiology of leptospirosis. The detection of antibodies in apparently healthy horses suggests that subclinical infections may be common, posing risks of zoonotic transmission to stable workers, veterinarians, and other animals. The identification of specific serovars provides valuable insights for targeted surveillance and potential vaccine development tailored to equine populations in the region.

From an epidemiological perspective, these findings underscore the need for integrated control measures, including routine serological monitoring, improved stable hygiene, and risk communication to stakeholders. Future research should focus on larger-scale longitudinal studies to determine the dynamics of infection, molecular characterization of circulating strains, and assessment of environmental sources of contamination. Additionally, evaluating the efficacy of vaccination and management interventions will be critical in reducing the prevalence of leptospirosis and mitigating its public health impact.

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ETHICAL STATEMENT

This study was approved by ethical committee Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, approval number UMK/FPV/ACUE/PG/001/2023.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this article.

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