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Review articles

SEROLOGY, MOLECULAR AND BACTERIOLOGY OF *LEPTOSPIRA* SPP. AMONG HUMANS AND ANIMALS IN SOUTHEAST ASIA FROM 2011-2021: A REVIEW

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SUMMARY

Leptospirosis is a bacterial disease caused by pathogenic *Leptospira* spp. As a zoonotic disease, it can be passed from animals to humans, causing infection in humans. However, a comprehensive compilation of serovars' diagnosis and distribution among infected hosts is still lacking. This review summarised these aspects within Southeast Asia to guide future studies and prevention efforts in the region. The database search was conducted from January 2011 to January 2021 using Scopus, PubMed, and Google Scholar. Studies on leptospirosis from all 11 Southeast Asia countries (Brunei, Burma/ Myanmar, Cambodia, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, Timor Leste, and Vietnam) were obtained during this search. Data related to leptospirosis, including serovar types, human and animal infections, and diagnostic methods, were extracted from relevant studies. The 155 studies reported in Southeast Asia originated from Cambodia, Indonesia, Laos, Malaysia, Philippines, Thailand, and Vietnam. A total of 155 studies consisted of multiple tests: 87.1% (135/155) studies employed serological tests, with 71% (110/155) studies that reported serovars; 65.2% (101/155) studies used molecular tests; and 30.3% (47/155) studies utilised culture methods. The presence of circulating serovar within these countries indicates an endemic re-emerging disease. This review highlights the potential for various host species to become infected and act as carriers of leptospirosis. The importance of circulating serovars in epidemiological studies should be further investigated. There is an urgent need for intervention and the development of improved diagnostic strategies, particularly in low-income countries.

Keywords: Leptospirosis, Southeast Asia, Serology, Molecular, Isolation

INTRODUCTION

Leptospirosis has become a significant and re-emerging health problem around the world. This similar public health issue is also prevalent in Southeast Asia countries and is acknowledged as an endemic illness. Outbreaks in Southeast Asia are closely associated with the tropical climate, which facilitates the survival of pathogenic *Leptospira* spp. (Munoz-Zanzi et al., 2020). Surveillance data from 11 Southeast Asian nations indicates a notable incidence of leptospirosis. Between 1970 and 2008, this region reported among the highest global disease burdens, with an estimated morbidity exceeding 50 cases per 100,000 population annually (Costa et al., 2015).

The broad range of symptoms in infected humans can vary from no symptoms to mild symptoms such as fever, headache, conjunctival suffusion, myalgia, and jaundice, to severe complications including pulmonary haemorrhage, kidney failure, and meningitis (Gancheva, 2017; Bhatia et al., 2015; Mendoza et al., 2013). In Southeast Asia, leptospirosis is an undifferentiated fever, which shares common symptoms with diseases like dengue, hantavirus, malaria, rickettsioses, and influenza. The similarity in symptoms among these diseases can lead to underdiagnosis and misdiagnosis, contributing to fatalities due to delayed treatment. Clinical signs vary depending on immunity, severity, stage of infection, and infecting serovar (Ellis, 2015).

A confirmed diagnosis of leptospirosis depends on laboratory tests, including serological, molecular, and culture. Serological testing using MAT is the gold standard for determining serovar-specific antibodies in hosts (Budihal & Perwez, 2014). Understanding the epidemiological distribution of serovars helps detect disease patterns and provides insights into outbreaks in highly affected regions. The serovars' diversity reflects *Leptospira* spp. interspecies interaction potentially influences variation among animals. Previous research indicates that specific serovars are host-specific, highlighting the endemic nature of leptospirosis (Bonhomme & Werts, 2022).

Environmental and social changes in Southeast Asia over the past decade have significantly impacted leptospirosis epidemiology and transmission. These changes include alterations in landscapes. Livestock practices, urbanisation, and the evolution of *Leptospira* spp. have resulted in the emergence of new strains (Levett & Picardeau, 2021). Additionally, a new *Leptospira* spp. (*L. kirschneri* serogroup Pomona) has been reported in symptomatic dogs (Bertasio et al., 2020). Isolation and identification of *Leptospira* spp. from clinical samples help accurately diagnose leptospirosis, while molecular techniques are used to detect the presence of *Leptospira* spp.

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Preventing leptospirosis outbreaks relies on vaccination, yet challenges persist due to a limited understanding of circulating serovars in endemic areas (Guerra, 2013). The commercially available vaccine currently includes serovar Australis, Autumnalis, Canicola, Copenhageni, Hebdomadis, Icterohaemorrhagiae, Lai, Linhai, Mozdok, and Pomona in humans, while Canicola, Grippotyphosa, Hardjo Icterohaemorrhagiae, Pomona, and Bratislava in animals, but there are over 300 serovars documented (Xu & Ye, 2018; Klaasen et al., 2014; Srivastava, 2006). Therefore, it is crucial to identify the serovars that typically result in illness within a specific geographic area for developing future vaccines. However, research on serovar present among animals and humans in Southeast Asia remains scarce. Additionally, while different serovars show specificity for certain host species among animals, many undiscovered mammals can potentially carry serovars asymptomatically or are susceptible, often overlooked. Diagnosing leptospirosis in hosts poses challenges, and little is known about standard diagnostic practices for each host species. Moreover, this review comprehensively studied the serovar distribution and diagnostic practices conducted from 2011 to 2021 in Southeast Asia. The result can help raise public awareness about exposure risks and prevention strategies against infected animals.

Geographical leptospirosis studies in Southeast Asia

The geographically selected studies of leptospirosis among humans and animals in Southeast Asia were defined as Brunei, Burma/ Myanmar, Cambodia, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, Timor Leste, and Vietnam (Figure 1).

Sources of studies were obtained from the Scopus,

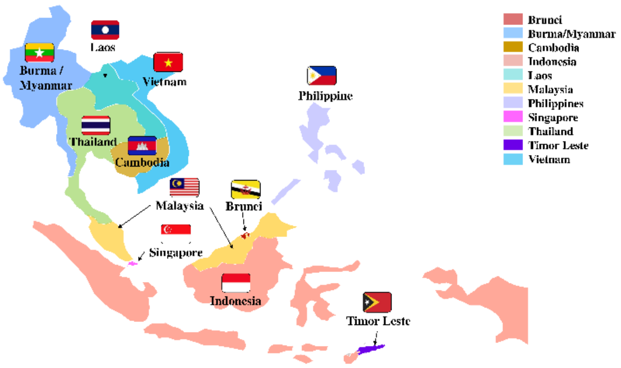

Figure 1. Map of the Southeast Asia countries.

PubMed and Google Scholar. The search keywords inclusive were "leptospirosis" OR "*Leptospira*" AND "country name" from January 2011 until January 2021. Data analysis steps were initiated with the search for studies on leptospirosis or *Leptospira* spp. in those countries, whereby unrelated titles were excluded. Secondly, related titles were screened for abstracts from each database. The eligibility of journals was included in studies related to leptospirosis or *Leptospira* spp. and laboratory diagnostic methods. The data report for publication was manually included in Excel to remove duplicates from three different databases. The included journals were further classified into two categories; diagnostic methods and serovars present.

Studies were selected based on their reporting of serovar types involving humans or animals in leptospirosis research and the use of diagnostic methods such as serology (e.g., MAT), molecular techniques (e.g., PCR), and bacterial isolation and identification. All the included studies must have been conducted in Southeast Asia countries and related to leptospirosis with a period of publishing from January 2011 until January 2021.

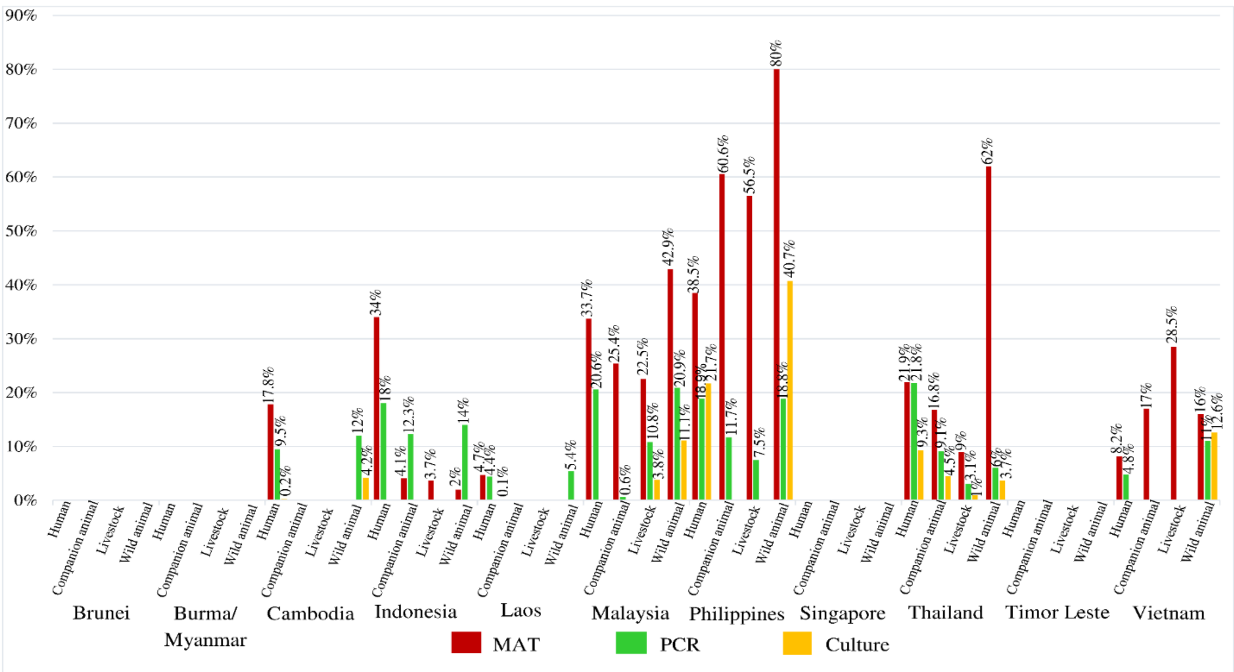

Figure 2. Accumulative numbers of positive detection and isolation of leptospirosis in humans and animals in Southeast Asia.

Prevalence of leptospirosis among Southeast Asia

Overall, a total of 1836 papers were reviewed from three types of databases, including Scopus (n=443), PubMed (n=488), and Google Scholar (n=945). Out of these, only 155 studies (Supplementary Data Table 1) met the criteria for leptospirosis in Southeast Asia. The distribution of these studies by country was as follows: 5.8% (9/155) studies in Cambodia, 15.5% (24/155) studies in Indonesia, 5.8% (9/155) studies in Laos, 36.1% (56/155) studies in Malaysia, 12.3% (19/155) studies in the Philippines, 20% (31/155) studies in Thailand and 5.8% (9/155) studies in Vietnam.

Among the 155 studies related to leptospirosis, 20% (31/155) involved multiple hosts. Of these studies, which varied in sample sizes (Figure 2), the laboratory diagnostic tests used for leptospirosis in humans and animals in Southeast Asia were 87.1% (135/155) used serological methods, 65.2% (101/155) used molecular methods and 30.3% (47/155) used culture methods. Additionally, 87.1% (135/155) of the studies used MAT, but only 71% (110/155) of the studies were recorded to state the positive serovars in hosts (Supplementary Data Table 2).

All studies were analysed and categorised into humans, companion animals, livestock and wild animals. Humans infected with leptospirosis included travellers, patients, occupational groups, and communities. Companion animals were specifically cats and dogs. Livestock consisted of ruminants such as cattle, sheep, goats, buffalo and swine. Wild animals comprised animals that lived on their own, such as wild rats, feral boars, bats,

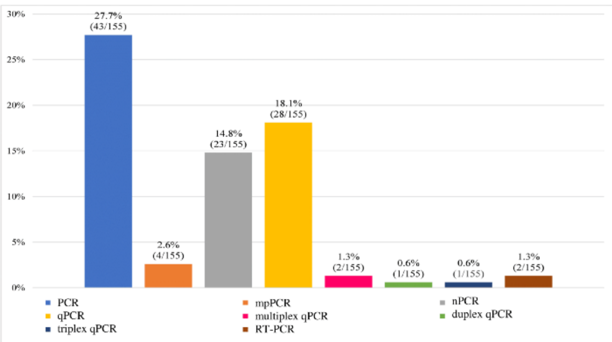

Figure 3. The types of Polymerase Chain Reaction (PCR) methods used for detection of leptospirosis in Southeast Asia region.

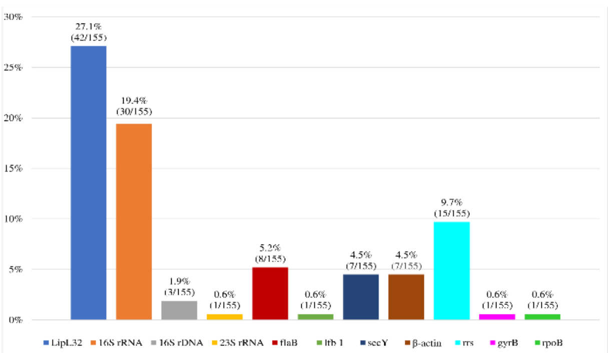

Figure 4. The types of primers used for PCR of leptospirosis in Southeast Asia region.

squirrels, mongooses, monkeys, and others. The study employed multiple molecular techniques, namely conventional PCR, multiplex PCR (mpPCR), nested PCR (nPCR), real-time PCR (qPCR-single, duplex, triplex, multiplex), reverse transcriptase PCR (RT-PCR) (Figure 3), which were utilized along with specific primers (Figure 4).

Microscopic Agglutination Test (MAT) in Southeast Asia

Humans

In Southeast Asia, MAT, PCR, and culture methods are commonly utilized to diagnose *Leptospira* spp. infection. The documented studies showed that seropositive by MAT in human cases within Southeast Asia was prevalent in the Philippines (38.5%), Indonesia (34%) and Malaysia (33.7%). These percentages varied due to differences in total population among the countries. Climate change and increased occupational exposure to animals have contributed to the risk of leptospirosis infection in Southeast Asia (Bradley & Lockaby, 2023; Dhewantara et al., 2022; Schønning et al., 2019; Garba et al., 2018; Villanueva et al., 2016a). This region experiences a tropical climate known for intense rainfall and frequent monsoons, which have led to more frequent instances of flooding. The occurrence of leptospirosis is often associated with flooding, as it creates optimal conditions for *Leptospira* spp. survival and disease transmission (Chadsuthi et al., 2021; Naing et al., 2019). The environment may not be the primary source of *Leptospira* spp., but it plays a role in infection dissemination. Consequently, these countries experience higher reported cases during the rainy season. For instance, a study in Wajo district, Indonesia, found communities exposed to floodwaters testing positive for leptospirosis (Syamsuar et al., 2018).

Leptospirosis is associated with occupational activities that involve potential exposure to infected animals and contaminated environments. The article search showed that workers such as sanitation workers, shelter caretakers, dog handlers, plantation workers, wet market workers, abattoir workers, farmers, rangers, military personnel and town service workers are at high risk of exposure. Insufficient personal protective equipment (PPE) increases the risk of *Leptospira* spp. entering through wounds. For instance, in Malaysian palm oil farms, high rat populations have led to transmission of *Leptospira* spp. to workers handling thorny fruit (Mohd Ridzuan et al., 2016). Similarly, slaughterhouse workers in the Philippines face heightened exposure to infected animal fluids (Tabo et al., 2018). In Indonesia, analysis of farmer blood serum and livestock urine has revealed a significant occupational association with leptospirosis (Pawitra et al., 2021).

The low seroprevalence in human leptospirosis cases could be attributed to underreporting. Medical practitioners might overlook leptospirosis symptoms, resembling common diseases in Southeast Asia such as dengue, meningitis, influenza, or hepatic disease (Ilham et al., 2020). Although medical history provided by patients is essential, patients may not always accurately describe their past clinical experiences. Therefore, even though obtaining a follow-up sample between 7 to 14 days after the initial sample is challenging, it remains necessary to confirm the infection. The limited sample size is causing difficulty in assessing the *Leptospira* spp. Seroprevalence in epidemiological studies can be affected by small sample numbers that may inadequately represent disease burdens in studies area. A study indicates that low prevalence is attributed to small sample size (Ngbede et al., 2012), while others have noted that small sample result in seronegative findings (Grimm et al., 2020). Some study designs use small sample sizes due to cost or time constraints, yet many fail to justify these limitations (Vasileiou et al., 2018). Small sample sizes may reduce the ability to detect leptospirosis within populations, necessitating carefully interpreting the result as it can affect the data quality (Ersbøll & Ersbøll, 2003). The availability of different host species may also influence variation in sample sizes. For example, studies involving wildlife animals face challenges in trapping due to their elusive behaviours and low population densities in some species (Branco & Redgrave, 2020; Gil-Sánchez et al., 2020).

Animals

Antibodies against leptospirosis were prevalent in companion animal populations, with high detection rates in the Philippines (56.5%), Vietnam (28.5%), and Malaysia (22.5%). Reported seropositivity among livestock has risen in the Philippines (60.6%), followed by Malaysia (25.4%). Poor hygiene and vaccination practices by smallholder farmers, due to high costs and lack of information on animal vaccination, have contributed to leptospirosis outbreaks among livestock (Villanueva et al., 2016a, 2016b). Vaccines currently available may not cover all strains of *Leptospira* spp., necessitating the development of locally specific vaccines (Mgode et al., 2021). Vaccinated dogs may remain susceptible to other serovars. This shows that the vaccination can be less effective due to the absence of serovars prevalent in those particular regions or countries. While the speculation that cats may be resistant to leptospirosis and there is currently no vaccination available for cats, the finding of antibodies detected without symptoms underscores the importance of further investigation. Wild animals in the Philippines (80%), Thailand (62%), and Malaysia (42.9%) exhibit high MAT positivity, likely due to extensive exposure to pathogenic *Leptospira* spp. in their free-living habitats. The attraction of wild animals such as rats to areas with poor food waste management increases environmental contamination through *Leptospira* spp. shedding.

Serovar distribution in humans

This review examines the serovar distribution of *Leptospira* spp. in humans across Southeast Asia, where humans are incidental hosts infected through contaminated environments or direct contact with animals. In Cambodia, serovars such as Autumnalis, Rachmati, and Sejroe were detected (Nagai et al., 2020; Hem et al., 2016). Infections with hoarseness and diarrhoea are likely contracted from a history of swimming in pools in leptospirosis-endemic areas (Nagai et al., 2020). Serovar Sejroe was more commonly found in communities in Kampong Cham, Cambodia, where residents frequently come into contact with remaining water bodies during and after the rainy season (Hem et al., 2016).

In Indonesia, the most identified serovar was Bataviae, particularly in flood-prone areas with high rat populations, which are the major source of contamination (Manyullei et al., 2021; Syamsuar et al., 2018; Gasem et al., 2015). Infection with Bataviae led to severe pulmonary haemorrhage causing pneumonia, along with other symptoms including fever, dry cough, dyspnoea, icteric sclerae, gum bleeding, calf tenderness, and conjunctival suffusion (Gasem et al., 2015).

In Laos, humans were infected with a range of serovars, including Canicola, Copenhageni, Hebdomadis, Mwalok, Krematos, and Poi, all of which are classified as pathogenic *Leptospira* spp. Individuals infected with the serovars Krematos and Poi in Laos experienced symptoms such as an enlarged spleen and congested bulbar conjunctivae (Yaita et al., 2013).

Serovar Sarawak (IMR LEP 175) was the main serovar in Malaysia, found in various regions and affecting high-risk groups, such as sanitation workers, army personnel, dog handlers, zoo rangers, market workers, cattle farmers, food handlers, town service workers, oil planter worker (Philip et al., 2021; Jeffree et al., 2020; Goh et al., 2019; Nadia et al., 2019; Rahman et al., 2018; Samsudin et al., 2018; Zainuddin et al., 2017; Ridzuan et al., 2016; Suut et al., 2016; Thayaparan et al., 2015). These individuals are particularly vulnerable due to their close contact with animals and water sources. The presence of wild animals in farms, zoos, and shelters also poses a significant risk for leptospirosis transmission.

Serovars Poi was frequently reported in the Philippines, especially in post-flood areas and high-risk occupational groups (Tabo et al., 2018; Gloriani et al., 2016a; Anacleto et al., 2014). An infected individual presented with both icteric and non-icteric acute kidney injury (AKI) after being infected with serovar Poi (Anacleto et al., 2014).

Serovar Canicola was commonly detected in humans in Thailand, associated with symptoms of fever, severe myalgia, mild headache and acute hepatitis (Rodriguez-Valero et al., 2018; Saitoh et al., 2015; Calvo-Cano et al., 2014). This suggests potential exposure to infected dogs, which are primary carriers of this serovar. In contrast, serovar Icterohaemorrhagiae was more commonly identified in southern Thailand, likely due to the variety of host species in the region (Chusri et al., 2012).

In Vietnam, several serovars, including Hebdomadis, Pomona, and Saxkoebing, were reported in locations such as Thao Binh, Ha Tinh, and Can Tho, while Bataviae and Hurstbridge were more commonly found in the Mekong delta (Tran et al., 2021; Loan et al., 2015). Additionally, Australis and Autumnalis were detected in patients who had been camping in Hue in central Vietnam (Mishima et al., 2013).

The diversity of serovars in the region is likely influenced by both local geographical and demographic factors, as the survey was conducted in the Mekong Delta, situated by the river, while Hue is located in a mountainous forest region.

Serovar distribution in animals

Leptospirosis is a significant concern in Southeast Asia, especially within livestock populations, where various serovars have been reported. Serovar such as Hardjo, Icterohaemorrhagiae, Bangkinang, and Hebdomadis have been detected in cattle across Indonesia (Widiasih et al., 2021; Suprayoga et al., 2021; Noach & Noach, 2020). Serovar Hardjo, in particular, is commonly serovar maintained by cattle, where it has adapted over time, resulting in relatively low pathogenicity in this host. However, infection with Hardjo can result in production losses, including decreased milk output, premature births, and abortion in cattle (Ellis, 2015). In Indonesia, Hardjo infections are mainly linked to contaminated water sources, which pose a risk to cattle and other livestock in the region. Similarly, serovar Harjo was also reported in cattle in Malaysia and Vietnam (Mai et al., 2021; Abdul Rahman et al., 2020; Daud et al., 2018a).

In the Philippines, serovar Hardjo has also been reported in buffalo, where these non-vaccinated animals living in contaminated environments are particularly vulnerable to leptospirosis (Villanueva et al., 2018). In contrast, serovar Poi is more commonly associated with cattle and pigs in the Philippines (Tabo et al., 2018; Gloriani et al., 2016b). Antibodies of this serovar have been exhibited in abattoir workers who are regularly exposed to infected animal fluids in slaughterhouses (Tabo et al., 2018). With years of exposure, these workers are at high risk of contracting leptospirosis from these animals, indicating that livestock can be a source of infection in humans.

In Thailand, serovars such as Shermani, Ranarum, and Sejroe have been detected across five livestock species, including cattle, water buffalo, sheep, goats, and pigs (Chadsuthi et al., 2017; Pumipuntu & Suwannarong 2016; Suwancharoen et al., 2013; Wongpanit et al., 2012). The similar serovars detected in water buffalo and cattle suggest that transmission may occur due to shared pastures and water sources. Additionally, serovar Sejroe has been reported in humans, livestock and rodents in Thailand since 2000, indicating the potential for cross-species transmission (Wangroongsarb et al., 2002; Suwancharoen et al., 2000). Other serovar, like Tarassovi, have been detected in cattle and buffalo in Thailand, as well as in buffalo in the Philippines and pigs in Vietnam. Serovar Hebdomadis has also been detected in cattle and buffalo in Vietnam and in cattle in Indonesia (Mai et al., 2021; Noach & Noach, 2020).

Similar serovars in multiple countries suggest that shared environmental factors, livestock management practices, and possibly cross-border animal movement contribute to the spread of leptospirosis. Moreover, both humans and livestock can become infected from the same source, such as wild animals (e.g., rats) or companion animals, emphasising the need for a One Health approach to address the issue. This cross-species transmission highlights the interconnectedness of human, animal, and environmental health, essential for effectively preventing the spread of leptospirosis in Southeast Asia.

Serovars Bataviae and Icterohaemorrhagiae have been commonly reported in companion animals across Indonesia, Malaysia and Thailand (Rahman et al., 2021; Goh et al., 2021; Alashraf et al., 2020a; Alashraf et al., 2020b; Goh et al., 2020; Goh et al., 2019; Alashraf et al., 2019; Mulyani et al., 2017; Lau et al., 2017; Khor et al., 2016; Lau et al., 2016). The frequent occurrence of these serovars is likely due to high-risk exposure to common reservoir hosts, particularly rats, which are the primary carriers of both serovars. The presence of rats is associated with risk factors for leptospirosis in cats and dogs, likely due to their hunting habits. Reported cases of leptospirosis in sheltered animals can be attributed to food provided for companion animals in shelters, which attract rats. Indirect transmission through exposure to contaminated environments should also be considered, as companion animals are free roaming. In addition, serovar Bataviae, which is not covered by vaccines, poses a risk to companion animals. Both serovars Bataviae and Icterohaemorrhagiae are virulent and responsible for causing renal manifestation (Sharun et al., 2019; Bakoss et al., 1965). Natural behaviours such as urination and faecal marking facilitate the transmission of leptospirosis since companion animals live close to humans (Neilson, 2003; Simpson, 1997). Infected companion animals, even asymptomatic ones, can potentially transmit leptospirosis to humans, as evidenced by similar serovar found in humans, dogs and cats (Alashraf et al., 2020a). As a result, companion animals may act as intermediaries, spreading leptospirosis to humans.

Studies in this review showed that Icterohaemorrhagiae can be found in both vaccinated and non-vaccinated companion animals, as the antibodies produced by the vaccine may still be present after vaccination (Goh et al., 2022; Martin et al., 2014). While serovar Canicola is known as a common serovar in dogs, this serovar reported only in Indonesia, Malaysia and Thailand, with its lower prevalence in dogs is possibly related to limited exposure to source of infection (Altheimer et al., 2020; Goh et al., 2019; Mulyani et al., 2017; Benacer et al., 2017; Lau et al., 2016; Pumipuntu & Suwannarong, 2016). In contrast, serovar Tarassovi was reported in both cats and dogs in Indonesia, but only Bataviae and Icterohaemorrhagiae were reported in dogs (Mulyani et al., 2018; Mulyani et al., 2017). The small sample size of cats evaluated in that study could explain the absence of serovar Bataviae and Icterohaemorrhagiae. It cannot be ruled out that unexamined cats were infected with these serovars if a larger sample of cats had been included.

Despite the absence of studies on cat seroprevalence in the Philippines between 2011 and 2021, serovar Manilae is a serovar commonly found in dogs in the country (Villanueva et al., 2018; Gloriani et al., 2016b). Moreover, the serovars reported in companion animals in Vietnam were varied, including serovar Hardjo, Patoc and Castellonis (Mai et al., 2021). This variation may result from exposure to infected cattle and swine, as the study was conducted in areas where livestock have the same serovars.

Anti-leptospiral has been detected in wild animals across Southeast Asia of various types of species such as Geoffroy's side-neck turtle, crested porcupine, Weddell's saddle-back tamarin (*Leontocebus weddelli*), emperor tamarin (*Saguinus imperator*), captive neotropical felids, black-tailed prairie dogs, fox squirrel, hispid cotton rats and feral swine (Aliaga-Samanez et al., 2021; Coppola et al., 2020; Oliveira et al., 2016; Montiel-Arteaga et al., 2015; Ullmann et al., 2012). This reveals their potential role as a reservoir for pathogenic *Leptospira* spp. Wild animals are recognised as reservoirs of *Leptospira* spp., often carrying the bacteria asymptomatically.

In Malaysia, serovar Sarawak (IMR LEP 175) has been frequently identified in wild animals, including rodents, squirrels, bats, mongooses, and orangutans (Nadia et al., 2019; Thayaparan et al., 2015; Thayaparan et al., 2014; Benacer et al., 2017). In contrast, serovars Copenhageni and Pomona are commonly reported in the Philippines (Gloriani et al., 2016b; Villanueva et al., 2016b; Zamora & Gloriani 2015). In Thailand, serovar Ballum was detected in feral boars, likely reflecting their interactions with other wild animals in captivity and their ground-feeding behaviour, which increases the risk of leptospirosis exposure in contaminated environments (Prompiram et al., 2019). Serovar Louisiana has been found in Vietnam in rats (Mai et al., 2021; Loan et al., 2015). These findings highlight the diversity of serovars and animal species involved in leptospirosis transmission across Southeast Asia. Despite the region's shared tropical climate, which links most leptospirosis cases to flooding and the rainy season, the presence of infected wildlife plays a critical role in human transmission.

The studies reviewed here highlight several limitations in the existing literature. First, many studies focus on a single species without considering reservoir animals, with only a few including multiple hosts. A more comprehensive approach that involves humans, animals, and the environment (e.g., water and soil) would provide a clearer understanding of transmission dynamics. Such studies would allow for comparisons of serovars across humans, animals and the environment, helping to identify transmission routes in specific regions. Therefore, the interactions between hosts in leptospirosis transmission required further investigation. Secondly, many studies reviewed that use MAT do not report or interpret either serovar panels, seropositivity, or the prevalence of each serovar. Lastly, surveillance studies conducted in humans in hospitals may not accurately represent the situation, as the infected patients might not be from the area of origin. Therefore, future studies are suggested to include information on the origin of infection to identify and record cases in that specific area accurately.

Polymerase Chain Reaction (PCR) in Southeast Asia

Humans

High rates of PCR-positive human cases in Thailand (21.8%) and the Philippines (18.9%) show that PCR effectively detects *Leptospira* spp. DNA early in infection. However, these high prevalence rates may be attributed to poor community hygiene practices, especially basic soap hand-washing, which occur due to a lack of awareness of preventing leptospirosis (Dung et al., 2022; Peltzer & Pengpid, 2014). Moreover, limited knowledge about signs and symptoms of leptospirosis leads to delays in individuals seeking timely medical care, particularly in low-income or rural areas where people with mild symptoms like fever often may not seek healthcare promptly.

Animals

Leptospira spp. DNA was reported at high levels in both companion animals (10.8%) and wild rats (20.9%) in Malaysia. Leptospirosis in companion animals was underestimated due to the perceived low prevalence (Lau et al., 2016), resulting in inadequate research in other Southeast Asia countries. In reported studies, animal handlers, caretakers or owners were found to have antibodies against *Leptospira* spp. in their blood, likely due to unknowingly being exposed to infected animals (Benitez et al., 2021; Alashraf et al., 2020a; Goh et al. 2019). This situation poses transmission risks, as cats and dogs in the same study were reported to have antibodies against the same leptospiral serovars. Despite these carriers showing no symptoms, cats and dogs have been identified as shedding *Leptospira* spp. in their urine (Goh et al., 2021; Alashraf et al., 2020b). This underscores the need for increased awareness. PCR's sensitivity allows the detection of *Leptospira* spp. even in carrier animals, preventing further spread to humans and other animals (Martins et al., 2012). The prevalence of this DNA in livestock (11.7%) was high in the Philippines. Leptospirosis in the livestock industry causes economic loss since it reduces the productivity of animals (Carvalho et al., 2024; Tiwari et al., 2013). The risk factors associated with the prevalence of leptospirosis in livestock in the Philippines may be linked to factors such as age, animal groups, species and sources, farming practices, drinking water sources, and interaction with other animals (Tabo et al., 2018; Villanueva et al., 2016a).

Types of PCR

Over time, researchers have developed various types of PCR methods, including conventional PCR, multiplex PCR (mpPCR), nested PCR (nPCR), real-time PCR (qPCR-single, duplex, triplex, multiplex), reverse transcriptase PCR (RT-PCR). The choice of PCR method depends on factors like availability, cost, and purpose of the study. Conventional PCR was the most used technique among Southeast Asia countries, as indicated by a total of 27.7% of studies conducted across Cambodia, Indonesia, Malaysia, Philippines and Thailand. Conventional PCR, valued for its cost-effectiveness and simplicity in DNA amplification, gained widespread use in animal and human diagnosis across Southeast Asia. Moreover, qPCR has been reported in Vietnam and Laos. Although its benefits include rapid results, high sensitivity, reduced contamination risk, and elimination of the need for gel electrophoresis, qPCR requires expertise and is costlier to perform (Pinto et al., 2022; Schreier et al., 2013).

Types of primer

The widely applied primers in Southeast Asian studies were LipL32 (27.1%) and 16S rRNA (19.4%). This preference for 16S rRNA is due to its conserved gene, which has a low mutation rate compared to other genes (Bartoš et al., 2024; Janda & Abbott, 2007). According to a report, 16S rRNA has been confirmed as a stable gene (Beltrán et al., 2022). While 16S rRNA does not differentiate between classes of *Leptospira* spp., the predominance of LipL32 as a primer is justified by its abundance in the outer membrane of pathogenic *Leptospira* spp. (Di Azevedo & Lilenbaum, 2021). Various other primers have been utilised, including rrs, 16S rDNA, 23S rRNA, SecY, gyrB, Ifb1, flaB, and rpoB (Philip et al., 2021; Gunawan et al., 2020; Jeffree et al., 2020; Tantibhedhyangkul et al., 2020; Rozo et al., 2020; Blasdell et al., 2019; Koizumi et al., 2019; Obusan et al., 2019; Schönfeld et al., 2019; Kudo et al., 2018; Woods et al., 2018; Tubalinal et al., 2018; Azhari et al., 2018; Kurilung et al., 2017; Della Rossa et al., 2016; Iwasaki et al., 2016; Loan et al., 2015; Saitoh et al., 2015; Jittimanee & Wongbutdee, 2014; Cosson et al., 2014; Goarant et al., 2014; Mueller et al., 2014; Roque et al., 2012).

Isolation and Identification of leptospirosis in humans and animals

Isolating *Leptospira* spp. through culture definitively confirms disease presence. Culture provides valuable information for genomic studies, identifying serovars, and studying virulence for vaccine development in prevention and epidemiology studies (Duan et al., 2020; Xu & Ye, 2018; Marquez et al., 2017; Koizumi & Watanabe, 2009). Despite its low sensitivity, culture has 100% specificity (Adler, 2015). The Philippines reported the highest human isolation rate at 21.7%, with Malaysia leading among companion animals (3.8%), Thailand among livestock (4.5%), and the Philippines among wild animals (40.7%).

However, the low success rate or negative results in isolating *Leptospira* spp. in other studies may be influenced by the type of sample collected during different phases of infection. *Leptospira* spp. exhibit varying presence in different bodily fluids across infection stages. Leptospires are typically more abundant in the blood during the early stages of infection and may transition to the urine as the infection progresses (second week) (Al-Orry et al., 2016; Sykes et al., 2022). Therefore, the timing of sample collection is critical to ensure accurate detection of the bacteria. Samples collected too early or too late may not contain sufficient leptospires for detection, leading to false-negative results. Contamination is a significant factor that inhibits the growth of *Leptospira* spp. It can generally be reduced by media of specimen filtration using a 0.45 µm membrane filter and subsequent subculturing into fresh media supplemented with 5-Fluorouracil (Gorman et al., 2022; Nervig & Ellinghausen, 1978). Fastidious *Leptospira* spp. require selective media for growth. Researchers developed EMJH (Johnson & Gary, 1963; Johnson & Harris, 1967), Fletcher (Lim, 2011), and Korthof (Terpstra, 2003) media to mimic a suitable environment that supports *Leptospira* spp. growth. Moreover, pathogenic *Leptospira* spp. are challenging to culture due to their fastidious nature, requiring high concentration (10^6 above) and media adaptation (Narkkul et al., 2020). Studies show Javanica grows better in EMJH than Korthof (Villanueva et al., 2014, 2010). Media preparation is crucial due to this bacterium are sensitivity to pH and nutrient levels. Other factors that could contribute to success may be antibody reactions or antibiotic administration before the laboratory test, limiting the ability to culture. Further identification was approached by molecular or serology. Data indicates various methods, including molecular sequencing, phylogenetic analysis, MLST, PFGE, BLAST, RAPD-PCR, LAMP, WGS and MLVA, while serology involves hyperimmune sera. *L. interrogans, L. borgpetersenii, L. kirschneri, L. kmetyi, L. weilli,* and *L. noguchii* has been identified as the predominant *Leptospira* spp. in Southeast Asia.

CONCLUSION

This review highlights diagnostic methods used among Southeast Asia countries. Serovars circulating in humans and animals are diverse within Southeast Asia. This information provides a better understanding of potential circulating serovars among host infected in the region. Including local serovars in the MAT panel is recommended for future studies. Leptospirosis diagnosis in Southeast Asia is still underreported. To address this issue, active surveillance can help determine the current situation and prevalence of leptospirosis incidence and burden in the country. The combination of serological, molecular, and identification techniques, including MAT, PCR, and bacterial isolation, could help confirm the diagnosis of leptospirosis. Additionally, future developments of methods that are highly sensitive, specific, economical, rapid, and easy to conduct are encouraged.

CONFLICT OF INTEREST

None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Supplementary Data

Table 1. Studies on leptospirosis in Southeast Asia.

COUNTRIES	REFERENCES
CAMBODIA	Chheng et al., 2013; Cosson et al., 2014; Hem et al., 2016; Ivanova et al., 2012; Koizumi et al., 2022; Kudo et al., 2018; Mueller et al., 2014; Nagai et al., 2020; Rozo et al., 2020.
INDONESIA	Anwar et al., 2020; Chu et al., 2017; Gasem et al., 2016; Gasem et al., 2020; Goarant et al., 2014; Gunawan et al., 2020; Khariri, 2019; Kutsuna et al., 2015; Lokida et al., 2016; Manyullei et al., 2021; Mulyani et al., 2018; Mulyani et al., 2017; Noach & Noach, 2020; Pawitra et al., 2021; Punjabi et al., 2012; Ristiyanto et al., 2018; Riyadi & Sunarno, 2019; Schönfeld et al., 2019; Sumanta et al., 2015; Suprayoga et al., 2021; Syamsuar et al., 2018; Tunjungputri et al., 2017; Widiasih et al., 2021; Widjajanti et al., 2017.
LAOS	Chansamouth et al., 2016; Cosson et al., 2014; Dittrich et al., 2018; Dittrich et al., 2015; Mayxay et al., 2015; Tanganuchitcharnchai et al., 2012, Ter et al., 2021, Weiss et al., 2016, Woods et al., 2018, Yaita et al., 2013.
MALAYSIA	Abdul Rahman et al., 2020; Affendy et al., 2020; Alashraf et al., 2020a; Alashraf et al., 2020b; Alashraf et al., 2019; Alia et al., 2019; Atil et al., 2020; Azhari et al., 2018; Benacer et al., 2013; Benacer et al., 2016; Benacer et al., 2017; Blasdell et al., 2019; Daud et al., 2018a; Daud et al., 2018b; Goh et al., 2019; Goh et al., 2020; Goh et al., 2021; Hin et al., 2012; Ilham et al., 2020; Jeffree et al., 2020; Khor et al., 2016; Latifah et al., 2012; Lau et al., 2016; Lau et al., 2017; Mohamad Ikbal et al., 2019; Mohd Taib et al., 2020; Mohd-Taib et al., 2020; Nadia et al., 2019; Neela et al., 2019; Ng et al., 2021; Philip et al., 2020; Philip et al., 2021; Pui et al., 2017; Rafizah et al., 2013; Rahman et al., 2018; Rahman et al., 2021; Rakesh et al., 2020; Rao et al., 2021; Rao et al., 2020; Ridzuan et al., 2016; Sabri et al., 2019; Samsudin et al., 2018; Sara et al., 2020; Shafei et al., 2012; Suut et al., 2018; Suut et al., 2016; Tan et al., 2014; Thayaparan et al., 2014; Thayaparan et al., 2015a; Thayaparan et al., 2015b; Thayaparan et al., 2016; Yong & Koh, 2013; Yusof et al., 2019; Zainuddin et al., 2017; Zin et al., 2019.
PHILIPPINES	Anacleto et al., 2014; Chavez et al., 2019; Gloriani et al., 2016a; Gloriani et al., 2016b; Iwasaki et al., 2016; Kitashoji et al., 2015; Obusan et al., 2019; Roque et al., 2012; Tabo et al., 2018; Tomacruz et al., 2019; Tubalinal et al., 2018; Van Dijck et al., 2016; Villanueva et al., 2016b; Villanueva et al., 2018; Villanueva et al., 2014; Zamora & Gloriani 2015.
THAILAND	Altheimer et al., 2020; Boonsilp et al., 2011; Calvo-Cano et al., 2014; Chadsuthi et al., 2017; Christen et al., 2015; Chusri et al., 2012; Della Rossa et al., 2016; Hinjoy et al., 2019; Hinjoy et al., 2017; Jittimanee & Wongbutdee, 2014; Krairojananan et al., 2020; Kurilung et al., 2017; Luvira et al., 2019; Ngasaman et al., 2020; Paungpin et al., 2020; Prompiram et al., 2019; Pumipuntu & Suwannarong, 2016; Rodriguez-Valero et al., 2018; Saitoh et al., 2015; Schmidhauser et al., 2013; Sonthayanon et al., 2013; Sprißler et al., 2019; Suwancharoen et al., 2013; Suwancharoen et al., 2016; Tantibhedhyangkul et al., 2020; Temeiam et al., 2020; Thongdee et al., 2019; Van et al., 2017; Wongbutdee & Jittimanee, 2016; Wongpanit et al., 2012; Yatbantoong & Chaiyarat, 2019.
VIETNAM	Koizumi et al., 2019; Lee et al., 2017; Lee et al., 2019; Le-Viet et al., 2019; Loan et al., 2015; Mai et al., 2021; Mishima et al., 2013; Tran et al., 2021; Van Hiep et al., 2014.

Supplementary Data

Table 2. The serovar distribution detected in humans, companion animals, livestock and wild animals in Southeast Asia region.

HUMANS			
COUNTRIES	**SEROVAR PANEL**	**SEROPOSITIVE**	**REFERENCES**
CAMBODIA	Australis, Autumnalis, Bataviae, Canicola, Castellonis, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Mini, Panama, Pyrogenes, Sejroe, Sermani, Sermin, Tarassovi	Autumnalis, Canicola, Djasiman, Icterohaemorrhagiae, Javanica, Louisiana, Pyrogenes, Rachmati, Sarmin, Sejroe, Tarassovi	(Nagai et al., 2020; Hem et al., 2016)
INDONESIA	Andaman, Australis, Autumnalis, Ballum, Bangkinang, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae,	Bangkinang, Bataviae, Bataviae, Canicola, Djasiman, Hebdomadis, Icterohaemorrhagiae, Mini, Rama, Robinsoni, Sejroe	(Manyullei et al., 2021; Riyadi, & Sunarno, 2019; Schönfeld et al., 2019; Syamsuar et al., 2018; Lokida et al.,

	Javanica, Kremastos, Manhao, Mini, Patoc, Poi, Pomona, Pyrogenes, Rachmati, Rama, Robinsoni, Sarmin, Semaranga, Tarassovi		2016; Gasem et al., 2016; Kutsuna et al., 2015)
LAOS	Not stated	Canicola, Copenhageni, Hebdomadis, Kremastos, Mwalok, Poi	(Dittrich et al., 2015; Yaita et al., 2013)
MALAYSIA	Australis, Autumnalis, Ballum, Bangkinang, Bataviae, Biggis, Birkini, Canicola, Celledoni, Copenhageni, Coxi, Cynopteri, Djasiman, Fugis, Grippotyphosa, Hardjobovis, Hardjoprajitno, Hebdomadis, HOSHAS, Icterohaemorrhagiae, IMR LEP 1(Melaka), IMR LEP 115 (Terengganu), IMR LEP 175 (Sarawak), IMR LEP 22 (Lai), IMR LEP 27 (Hardjobovis), IMR LEP 803/11 (Copenhageni), Javanica, Jonsis, Lai, Malaya, Malaysia, Panama, Patoc, Pomona, Pyrogenes, Sejroe, Semaranga, Shermani, Tarassovi, Whitcombi	Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Copenhageni (IMR LEP 803/11), Copenhageni, Cynopteri, Djasiman, Fugis, Grippotyphosa, Hardjo, Hardjobovis (IMR LEP 27), Hardjoprajitno, Hebdomadis, Icterohaemorrhagiae, Javanica, Lai, Langkawi, Malaysia, Melaka (IMR LEP 1), Panama, Patoc, Pomona, Pyrogenes, Sarawak (IMR LEP 175), Sejroe, Shermani, Tarassovi, Terengganu (IMR LEP 115)	(Philip et al., 2021; Jeffree et al., 2020; Goh et al., 2019; 2020; Alashraf et al., 2020a; Sara et al., 2020; Nadia et al., 2019; Neela et al., 2019; Ab Rahman et al., 2018; Daud et al., 2018a; Samsudin et al., 2018; Zahiruddin et al., 2017; Mohd Ridzuan et al., 2016; Suut et al., 2016; Thayaparan et al., 2015; Rafizah et al., 2013; Shafei et al., 2012)
PHILIPPINES	Andaman, Anhoa, Australis, Autumnalis, Ballum, Bataviae, Canicola, Carlos, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Javanica, Losbanos, Louisiana, Manhao 3, Manilae, Mini, Panama, Patoc, Poi, Pomona, Pyrogenes, Ratnapura, Saopaulo, Sarmin, Sejroe, Semaranga, Shermani, Tarassovi	Andamana, Anhoa, Bataviae, Canicola, Copenhageni, Hurstbridge, Icterohaemorrhagiae, Javanica, Losbanos, Manilae, Patoc, Poi, Pomona, Pyrogenes, Ratnapura, Saopaulo, Sejroe, Semaranga, Tarassovi	(Tabo et al., 2018; Baterna et al., 2017; Dijck et al., 2016; Iwasaki et al., 2016; Gloriani et al., 2016b; Kitashoji et al., 2015; Anacleto et al., 2014; Mendoza et al., 2013)
THAILAND	Australis, Autumnalis, Ballico, Ballum, Bataviae, Bratislava, Canicola, Castellonis, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Manhao, Mini, Panama, Patoc, Poi, Pomona, Pyrogenes, Rachmati, Ranarum, Sarmin, Sejroe, Shermani, Tarassovi, Wolffi	Australis, Autumnalis, Bratislava, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Sejroe, Shermani	(Luvira et al., 2019; Rodriguez-Valero et al., 2018; Calvo-Cano et al., 2014; Chadsuthi et al., 2017; Saitoh et al., 2015; Schmidhauser et al., 2013; Chusri et al., 2012)
VIETNAM	Australis, Autumnalis, Bataviae, Bratislava, Canicola, Castellonis, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Hurtbridge, Icterohaemorrhagiae, Javanica, Louisiana, Mini, Panama, Patoc, Pomona, Pyrogenes, Saxkoebing, Shermani, Tarassovi, Tonkini, Vughia, Wolffi	Australis, Autumnalis, Bataviae, Canicola, Copenhageni, Cynopteri, Hardjo, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Javanica, Louisiana, Panama, Patoc, Pomona, Pyrogenes, Saxkoebing, Tonkini	(Tran et al., 2021; Loan et al., 2015; Van Hiep et al., 2014; Mishima et al., 2013)
LIVESTOCK			
COUNTRIES	**SEROVAR PANEL**	**SEROPOSITIVE**	**REFERENCES**

INDONESIA	Australis, Ballum, Bangkinang, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Manhao, Mini, Pomona, Pyrogenes, Rachmati, Rama, Robinsoni, Sarmin, Tarassovi	Hardjo, Icterohaemorrhagiae, Bangkinang, Hebdomadis, Mini	(Widiasih et al., 2021; Suprayoga et al., 2021; Noach & Noach, 2020)
MALAYSIA	Australis, Autumnalis, Ballum, Bangkinang, Bataviae, Biggis, Birkini, Canicola, Celledoni, Copenhageni, Coxi, Cynopteri, Djasiman, Fugis, Grippotyphosa, Hardjobovis, Hardjoprajitno, Hebdomadis, HOSHAS, Icterohaemorrhagiae, IMR LEP 1(Melaka), IMR LEP 115 (Terengganu), IMR LEP 175 (Sarawak), IMR LEP 22 (Lai), IMR LEP 27 (Hardjobovis), IMR LEP 803/11 (Copenhageni), Javanica, Jonsis, Lai, Malaya, Malaysia, Panama, Patoc, Pomona, Pyrogenes, Sejroe, Semaranga, Shermani, Tarassovi, Whitcombi	Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjobovis, Hardjoprajitno, Hebdomadis, Icterohaemorrhagiae, Javanica, Lai, Malaysia, Melaka (IMR LEP 1), Patoc, Pomona, Pyrogenes, Sarawak (IMR LEP 175), Tarassovi, Terengganu (IMR LEP 115)	(Abdul Rahman et al., 2020; Daud et al., 2018b)
PHILIPPINES	Australis, Autumnalis, Canicola, Copenhageni, Cynopteri, Grippotyphosa, Hardjo, Hebdomadis, Hurtsbridge, Icterohaemorrhagiae, Losbanos, Louisiana, Manilae, Mini, Patoc, Poi, Pomona, Pyrogenes, Ratnapura, Saopaulo, Sejroe, Semaranga, Shermani, Tarassovi	Autumnalis, Copenhageni, Cynopteri, Hardjo, Hebdomadis, Hurstbridge, Icterohaemorrhagiae Strain Ictero No. 1, Icterohaemorrhagiae Strain RGA, Los Banos, Louisiana, Manilae, Mini, Patoc, Poi, Pomona, Pyrogenes, Ratnapura, Saopaulo, Sejroe, Semaranga, Shermani, Tarassovi	(Villanueva et al., 2018; Tabo et al., 2018; Gloriani et al., 2016a; Villanueva et al., 2016b)
THAILAND	Australis, Autumnalis, Ballum, Bataviae, Bratislava, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Patoc, Pyrogenes, Ranarum, Sarmin, Sejroe, Shermani, Tarassovi	Autumnalis, Ballum, Bataviae, Bratislava, Canicola, Cynopteri, Djasiman, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Min, Mini, Pomona, Pyrogenes, Ranarum, Sarin, Sejroe, Shermani, Tarassovi	(Yatbantoong & Chaiyarat, 2019; Chadsuthi et al., 2017; Pumipuntu & Suwannarong, 2016; Suwancharoen et al.,2013; Wongpanit et al., 2012)
VIETNAM	Australis, Autumnalis, Bataviae, Bratislava, Canicola, Castellonis, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Mini, Panama, Patoc, Pomona, Pyrogenes, Saxkoebing, Shermani, Tarassovi, Vughia, Wolffi	Canicola, Hebdomadis, Castellonis, Patoc, Australis, Autumnalis, Bataviae, Bratislava, Canicola, Grippotyphosa, Icterohaemorrhagiae, Javanica, Panama, Patoc, Pyrogenes, Tarassovi, Australis, Autumnalis, Javanica, Mitis, Tarassovi, Patoc, Pyrogenes	(Mai et al., 2021; Lee et al., 2019; Lee et al., 2017; Van Hiep et al., 2014)
COMPANION ANIMALS			
COUNTRIES	**SEROVAR PANEL**	**SEROPOSITIVE**	**REFERENCES**

INDONESIA	Australis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Rachmati, Tarassovi	Bataviae, Canicola, Celledoni, Cynopteri, Icterohaemorrhagiae, Pyrogenes, Rachmati, Tarassovi	(Mulyani et al., 2019; Mulyani et al., 2017)
MALAYSIA	Andaman, Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Lai, Malaysia, Patoc, Pomona, Pyrogenes, Shermani, Tarassovi	Australis, Autumnalis, Ballum, Bataviae, Canicola, Hardjo, Hardjobovis, Icterohaemorrhagiae, Javanica, Lai, Malaysia, Pomona	(Abdul Rahman et al., 2021; Goh et al., 2021; Alashraf et al., 2020b; Goh et al., 2020; Goh et al., 2019; Alashraf et al., 2019; Benacer et al., 2017; Lau et al., 2016; 2017; Khor et al., 2016)
PHILIPPINES	Australis, Autumnalis, Canicola, Copenhageni, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Losbanos, Manilae, Patoc, Poi, Pomona, Pyrogenes, Ratnapura, Semaranga, Tarassovi	Autumnalis, Manilae, Patoc	(Villanueva et al., 2018; Gloriani et al., 2016a)
THAILAND	Anhoa, Australis, Autumnalis, Ballum, Bataviae, Bratislava, Broomi, Canicola, Celledoni, Coxi, Cynopteri, Djasiman, Grippotyphosa, Haemolytica, Hebdomadis, Icterohaemorrhagiae, Javanica, Khorat, Louisiana, Manhao, Mini, Paidjan, Panama, Patoc, Pomona, Pyrogenes, achmati, Ranarum, Sarmin, Saxkoebing, Sejroe, Shermani, Tarassovi	Anhoa, Autumnalis, Bataviae, Canicola, Celledoni, Copenhageni, Djasiman, Icterohaemorrhagiae, Patoc, Sejroe	(Altheimer et al., 2020; Sprißler et al., 2019; Pumipuntu & Suwannarong, 2016)
VIETNAM	Australis, Autumnalis, Bataviae, Bratislava, Canicola, Castellonis, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Mini, Panama, Patoc, Pomona, Pyrogenes, Shermani, Tarassovi, Vughia, Wolffi	Castellonis, Hardjobovis, Hebdomadis, Javanica, Louisiana, Patoc	(Mai et al., 2021)
WILD ANIMALS			
COUNTRIES	**SEROVAR PANEL**	**SEROPOSITIVE**	**REFERENCES**
MALAYSIA	Australis, Autumnalis, Ballum, Bangkinang, Bataviae, Biggis, Birkini, Canicola, Celledoni, Copenhageni, Coxi, Djasiman, Fugis, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, HOSHAS, Icterohaemorrhagiae, Javanica, Jonsis, Lai, IMR LEP 1(Melaka), IMR Lepto 115 (Terengganu), IMR LEP 175 (Sarawak), Malaya, Panama, Patoc, Pomona, Pyrogenes, Sejroe, Shermani, Tarassovi, Whitcombi	Australis, Autumnalis, Bataviae, Celledoni, Copenhageni, Fugi, Hardjoprajitno, Hebdomadis, Icterohaemorrhagiae, Javanica, Lai, Langkawi, Patoc, Pomona, Pyrogenes, Sarawak (IMR LEP 175), Tarassovi, Terengganu (IMR LEP 115)	(Nadia et al., 2019; Suut et al., 2018; Thayaparan et al., 2013; 2014; 2015)
PHILIPPINES	Australis, Autumnalis, Canicola, Shermani, Copenhageni, Cynopteri, Grippotyphosa, Hardjo, Hebdomadis, Hurtsbridge, Icterohaemorrhagiae, Losbanos, Louisiana, Manilae, Mini, Patoc, Poi, Pomona, Pyrogenes,	Autumnalis, Canicola, Copenhageni, Copenhageni, Hebdomadis, Icterohaemorrhagiae, Poi, Pomona	(Gloriani et al., 2016a; Villanueva et al., 2016; Zamora & Gloriani, 2015)

	Ratnapura, Saopaulo, Sejroe, Semaranga, Tarassovi		
THAILAND	Autumnalis, Ballum, Bataviae, Bratislava, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Patoc, Pomona, Pyrogenes, Ranarum, Sarmin, Sejroe, Shermani, Tarassovi	Autumnalis, Ballum, Bataviae, Bratislava, Canicola, Celledoni, Cynopteri, Djasiman, Hebdomadis, Javanica, Manhao, Mini, Pomona, Pyrogenes, Ranarum, Shermani, Tarassovi	(Prompiram et al., 2019)
VIETNAM	Australis, Autumnalis, Bataviae, Bratislava, Canicola, Castellonis, Celledoni, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Hurtbridge, Icterohaemorrhagiae, Javanica, Louisiana, Mini, Panama, Patoc, Pomona, Pyrogenes, Saxkoebing, Shermani, Tarassovi, Tonkini, Vughia, Wolffi	Copenhageni, Cynopteri, Icterohaemorrhagiae, Javanica, Louisiana, Pomona	(Mai et al., 2021; Loan et al., 2015)

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