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RESEARCH ARTICLE

HAEMORRHAGIC SEPTICAEMIA: TREND OF SEROPOSITIVE CASES IN MALAYSIA FROM 2015 TO 2022

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ABSTRACT

Haemorrhagic septicaemia (HS) is an acute disease caused by the bacteria *Pasteurella multocida* serotypes B:2 and E:2. This disease causes acute septicaemia in cattle and buffaloes. The objective of this paper is to study HS trends in the country based on serodiagnostic cases sent to the Veterinary Research Institute (VRI), Ipoh from 2015 to 2022 using the ELISA technique. Between 2015 and 2022, a total of 261 cases were received at VRI. Of the 3576 samples, 85.9% (3070) were from cattle, 11.0% from buffalo, 2.4% from sheep, 0.4% from goats, and 0.3% from rabbits. The IgG antibodies of *P. multocida* were detected in 13.5% (16/118 cases) in 2015; 17.4% (12/69 cases) in 2016; 4.8% (2/42 cases) in 2017; 20% (1/5 cases) in 2018; zero detection in 2019 (2 cases) and 2020 (8 cases); 33.3% (3/9 cases) in 2021; and 36.4% (4/11 cases) in 2022. Based on the geographic distribution of seropositive cases, Perak reported the highest antibody detection with 33 cases, followed by Kedah and Terengganu with 2 cases each, and Kelantan with 1 case. Over the period of study, cattle contributed 94.2% of positive cases, whereas buffalo and rabbits contributed 1.9% and 3.8%, respectively. Hence, the trend of seropositive cases has been shown to be unstable over the past 8 years. Post-vaccination monitoring and detailed screening of naïve herds are important to provide valuable epidemiological information about the disease.

KEYWORDS

serodiagnostic, antibodies, post-vaccination, epidemiological, disease

1. INTRODUCTION

Haemorrhagic septicaemia (HS) is a major infectious disease affecting cattle and buffaloes, not only in Malaysia but also across many regions of Asia. The disease is caused by *Pasteurella multocida* serotype B:2, which triggers significant respiratory tract lesions (Hussain et al., 2014), and can advance to acute septicemia (Annas et al., 2015). Numerous outbreaks have been reported in bovine populations throughout Asia, emphasizing the widespread nature and serious impact of this disease (Rafidah et al., 2010). This highly contagious and economically significant disease affects domestic and wild ruminants worldwide, leading to substantial financial burdens in the cattle industry due to high mortality rates and consequent impact on animal productivity, particularly in tropical and subtropical regions (Shaukat and Khan, 2011). The disease is characterised by a sudden onset of fever, swelling of the throat and neck and extensive haemorrhaging, often resulting in rapid death (Ahmed and Lema, 2006).

Haemorrhagic septicaemia (HS) is a significant economic disease affecting cattle and buffalo populations. Buffaloes are particularly vulnerable, experiencing higher morbidity and mortality rates compared to other susceptible species (Jindal et al., 2002). Studies indicate that crossbred cattle exhibit the highest morbidity, followed by buffaloes and indigenous cattle; however, mortality rates and the proportion of affected cases are greatest in buffaloes (Bardhan et al., 2020). The economic burden of HS in Cambodia was estimated at \$375 per animal, with bi-annual vaccination providing a benefit of \$951.58 per household (Kawasaki et al., 2015). In India, research has shown that morbidity accounts for approximately 23% of losses, while mortality contributes to 77%, highlighting the substantial economic impact of the disease (Singh et al., 2014).

In Karnataka, a primary survey of affected farms revealed losses of \$275, \$284, and \$415 per head for indigenous cattle, water buffaloes, and crossbred cattle, respectively. The total estimated loss was around \$23.89 million annually under a high-incidence scenario (Govindaraj et al., 2017). Conversely, analyses based on outbreak data from 2007–2011 estimated that losses were approximately ₹6816 (\$80) per cattle head and ₹10,901 (\$130) per buffalo, with national scale estimates reaching ₹5255 crores (~\$800 million) (Singh et al., 2014). The variation in estimates is likely due to differing data sources; primary surveys tend to provide more localized loss estimates, whereas outbreak data can reflect broader epidemiological patterns. These differences underscore the importance of data type in economic assessments of HS (Govindaraj et al., 2017; Singh et al., 2014).

In Malaysia, HS has historically been a major concern, particularly among cattle and buffaloes, which are crucial to the country's agriculture and economy (Mohd and Ismail, 2018). Despite the availability of effective vaccines and improved management practices, HS outbreaks persist, indicating potential gaps in vaccination coverage, disease surveillance, and control measures. The epidemiology of HS is influenced by various factors including climate, animal husbandry practices, and livestock movement (Tan and Lee, 2017).

Recent studies have suggested that climate conditions such as high humidity and fluctuating temperatures significantly impact the incidence and spread of HS (Lim and Ng, 2020). Additionally, the effectiveness of vaccination programs and the level of herd immunity are critical factors in controlling the disease (Mahmud and Faruk, 2019). Nevertheless, the frequency of HS outbreaks suggests on going challenges in managing the disease effectively.

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Advances in ELISA technology have increased its utility in epidemiological investigations, enabling quick and cost-effective screening of large populations for HS antibodies (Hungerbühler et al., 2024). This methodological approach not only aids in early disease identification but also provides valuable data on the prevalence and distribution of *P. multocida* Type B:2 circulating throughout livestock herds (Shome et al., 2024).

This study aims to assess the trend of seropositive cases of HS in Malaysia from 2015 to 2022, using ELISA as a diagnostic tool. By analysing data on seropositive cases collected across different states, this study seeks to identify temporal and geographical patterns in the prevalence of HS. Serological surveillance is critical in determining the prevalence and spread of HS. Understanding the trends in seropositive cases is crucial for developing targeted immunisation campaigns and implementing biosecurity measures to reduce disease occurrence (Yousuf et al., 2019). By examining these trends, we aim to contribute valuable insights into the current status of HS in Malaysia and provide recommendations for future research directions and disease management strategies.

2. MATERIAL AND METHODS

2.1 Blood serum collection

Blood serum samples were obtained from various regions across Malaysia between 2015 and 2022. The samples were derived from cattle, buffaloes, sheep, goats, and rabbits, originating from both smallholder and commercial farms. Upon collection, all samples were transported to the Veterinary Research Institute (VRI), Ipoh for diagnostic evaluation.

2.2 Antibody detection

The samples were stored in the Immunoassay Section, VRI at a temperature of 2°C until analysis. An in-house ELISA was employed to identify immunoglobulin G (IgG) antibodies specific to *P. multocida* Type B:2. For antibody identification, the ELISA unit (EU) percentage was calculated using the Agilent BioTek 800 TS (Agilent Technologies Inc., USA) microplate reader. Samples exhibiting an EU value exceeding 45% were deemed seropositive for HS, while those with an EU value of 45% or lower were grouped as seronegative.

3. RESULT

Between the years 2015 and 2022, a total of 261 cases were submitted for serum antibody detection against *P. multocida* using the ELISA method. The distribution of cases showed significant variation over the years, with the highest number recorded in 2015 (115 cases, 44.1%), followed by a substantial decrease in subsequent years. In 2016, 69 cases (26.4%) were reported, dropping to 42 cases (16.1%) in 2017, 5 cases (1.9%) in 2018, and only 2 cases (0.8%) in 2019. The number of cases remained low in 2020 (8 cases, 3.1%), 2021 (9 cases, 3.4%), and 2022 (11 cases, 4.2%).

The state of Perak accounted for the majority of cases, with 186 out of 261 cases (71.2%) occurring in this state. Kedah followed with 12.3% (32 cases), while Terengganu contributed 6.5% (17 cases), and Penang reported 5.0% (13 cases). Less frequent occurrences were noted in Sabah and Johor (3 cases each), Kelantan, Selangor, and Pahang (2 cases each), and Negeri Sembilan (1 case).

From 2015 to 2022, a total of 3576 blood serum samples were submitted for HS antibody detection. Perak accounted for 80.2% (2869/3576) of these samples, indicating a disproportionately high contribution compared to other states. Kedah contributed 9.2% (331 samples), Terengganu 3.3% (118 samples), Penang 2.5% (88 samples), and Kelantan 1.7% (60 samples). Selangor accounted for 1% (36 samples), with Sabah, Johor, Pahang, and Negeri Sembilan each contributing less than 1% of the total samples.

Regarding species distribution, 3070 out of 3576 serum samples (85.9%) were from cattle, reflecting the predominant role of cattle in HS surveillance. The largest number of cattle serum samples was submitted in 2015 (1712 samples). Buffalo samples accounted for 11.0% (392 samples), followed by sheep with 2.4% (87 samples), goats with 0.45% (16 samples), and rabbits with 0.3% (11 samples).

Over the 8-year period, a total of 38 seropositive cases were detected. In 2015, 16 seropositive cases were identified, all from Perak. The following year, 12 cases were reported (9 from Perak, 2 from Kedah, and 1 from Kelantan). The number of seropositive cases continued to decline, with 2 cases (1 each from Perak and Terengganu) in 2017, and only 1 case in Perak in 2018. No seropositive cases were detected in 2019 or 2020. In 2021, 3 cases (all in Perak) were reported, followed by 4 cases in 2022 (3 from Perak and 1 from Terengganu) as shown in Figure 1.

From this study, cattle contributed 94.2% of positive cases, whereas buffalo and rabbits contributed 1.9% and 3.8%, respectively as shown in Figure 2. The overall seropositive rate of 1.06% (38/3576) is consistent with a declining trend in HS seropositivity.

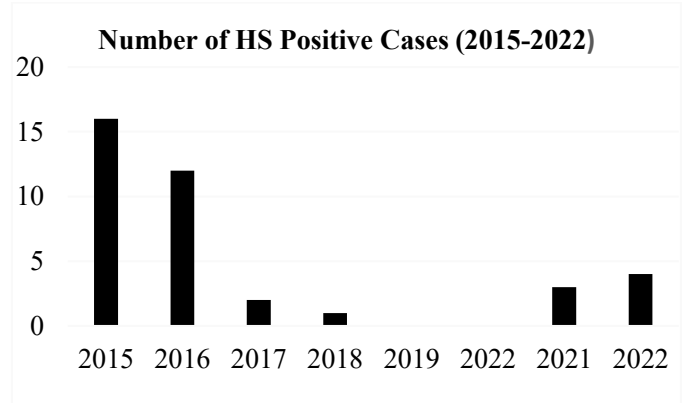


Figure 1: Total number of HS-positive cases from 2015-2022

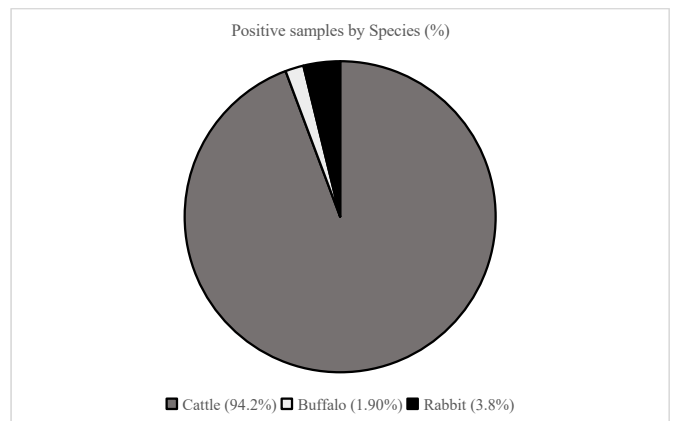


Figure 2: Total number of HS-positive samples according to species.

4. DISCUSSION

The study demonstrates the efficacy of indirect ELISA in detecting HS in animal species. The highest seroprevalence was recorded in Perak, with 33 out of 38 cases (86.8%) being seropositive. Notably, Perak accounted for the majority of seropositive cases, with 71.2% of all recorded cases from 2015 to 2022 occurring in this region. The disproportionately high number of cases in Perak may be attributed to factors such as the region's large livestock population, higher density of farming operations, and environmental conditions conducive to disease transmission. The consistently high rate of HS antibody detection in Perak indicates the need for more intensive control and prevention efforts in the area to manage the disease.

The findings underscore the necessity of targeted interventions in high-risk region such as Perak, including enhanced vaccination coverage, improved biosecurity measures, and continued disease trend monitoring. The differences in seropositivity rates between states also emphasise the need for a region-specific approach to HS control, with focused resources directed toward high-risk areas. The seropositive cases in other states, such as Kedah, Kelantan, and Terengganu, were sporadic, possibly reflecting localised outbreaks or lower levels of disease circulation in these regions. In contrast, states such as Sabah, Johor, Pahang, and Negeri Sembilan submitted relatively few samples, indicating either a lower prevalence of the disease or reduced surveillance activities in these areas. It is noteworthy that no seropositive cases were recorded in 2019 and 2020, which could indicate a temporary improvement in HS control measures during this period.

Based on the result, cattle recorded 94% (98 samples out of 104) of seropositive samples against HS organisms from 2015-2022 whereas 2% seropositive samples were in buffaloes. The high prevalence of cattle samples reflects the economic importance of cattle farming in Malaysia, as well as the species' known susceptibility to HS. Buffaloes, although less frequently sampled, are also highly susceptible to HS (Rafidah et al., 2010). Buffaloes are more susceptible to HS (Ahmad et al., 2018 and Hasnan et al., 2022). Previous research demonstrated the increased vulnerability of buffaloes to HS compared to cattle, as well as higher mortality rates in buffaloes during outbreaks (De Alwis 1999). Similarly, observed a

significantly higher mortality rate in buffaloes than in cattle during HS outbreaks (Bisht et al., 2006). Further reported that juvenile buffaloes and cattle are more prone to HS infection, with higher morbidity (21.19%), mortality (95.25%), and case fatality rates compared to older animals (Khan et al., 2006). A previous study identified the susceptible young age group of animals for hemorrhagic septicaemia as ranging from 6 months to 2 years (Almoheer et al., 2022). In endemic places, outbreaks are more likely to occur during the rainy season, when the organism can spread more readily, and young animals are more vulnerable to sickness (Chanda et al., 2024).

The detection of seropositivity in rabbit samples in 2018 was a result of research and development work related to Haemorrhagic Septicaemia vaccine production. According to Katoch et al. 2015, capsular typing of *P. multocida* isolates from rabbits has shown that to date, pasteurellosis in rabbits is mainly caused by the capsular type A and to a lesser extent by capsular type D strains. While, capsular type B has not yet been reported to be associated with pasteurellosis in rabbits. *P. multocida* capsular type B is mainly responsible for haemorrhagic septicaemia (HS) in ruminants, which is a major disease of cattle and buffaloes causing devastating epidemics with high morbidity and mortality in Asia, Africa and some countries of southern Europe and the Middle East (Farooq et al., 2011). Hence, the seropositivity in rabbits observed in this study cannot be taken as an indication of natural Haemorrhagic Septicaemia infection at the field level.

The study also emphasises the influence of hot and humid weather on the spread of HS, with elevated ambient temperatures promoting the growth of *P. multocida* Type B:2 (Hajikolaie et al., 2008). The analysis hot and humid weather is a major contributory factor for HS outbreaks (Shivachandra et al., 2011). Additionally, the spread of HS tends to accelerate during extended dry periods followed by heavy rainfall, with transmission facilitated by high humidity and rainy weather (Abdulkadir et al., 2024). In general, increased susceptibility to HS outbreaks was observed in crossbred animals as they are more susceptible to heat stress during hot and humid climates (Malik et al., 2020 and Hasnan et al., 2022).

Furthermore, the study underscores surveillance gaps in the control and management of HS in Malaysia, attributed to factors such as underreporting, inconsistent data, and inadequate reporting mechanisms. One of the most notable gaps in HS surveillance in Malaysia is the underreporting of cases. Often, outbreaks, particularly in rural areas, go unreported due to a lack of resources and trained personnel. The actual burden of the disease may therefore be underestimated. Inadequate disease reporting mechanisms and data-sharing systems also contribute to inconsistent monitoring, hindering effective control measures. Inadequate vaccination coverage, particularly among small-scale farmers, contributes to sporadic outbreaks and challenges surveillance efforts. Many small-scale farmers lack access to vaccination programs, or are unaware of their importance, leading to gaps in herd immunity (Mustafa et al., 2013).

Finally, the study emphasises the crucial role of vaccination in controlling HS, particularly during the onset of new outbreaks. Various types of vaccines including broth bacterin, oil adjuvant vaccines, double emulsion vaccines, and live vaccines, have been developed for HS control. However, challenges such as the loss of maternal immunity after the 60th day of life, combined with delays in immunisation, contributes to the heightened susceptibility of young calves to HS (Mahmood et al., 2007). Apart from that, post-vaccination monitoring in Malaysia involves both serological and clinical assessments to determine vaccine efficacy and overall herd immunity. Serological monitoring includes tests like ELISA to detect antibody titres in vaccinated animals. These tests assess whether animals have developed sufficient immunity against HS. Additionally, regular follow-up visits and epidemiological studies are conducted to assess the vaccination program's success and to detect any signs of vaccine failure or disease outbreaks despite vaccination efforts.

5. CONCLUSION

The analysis of HS in Malaysia spanning from 2015 to 2022 provides valuable insights into the trend of seropositive cases nationwide. The data illustrates fluctuations in the seropositive cases, with noticeable peaks in specific years, likely associated with environmental and management variables. These findings highlighted the persistent threat of HS to livestock, particularly cattle and buffalo, posing substantial economic and health risks to the agricultural sector. Based on current trends of HS research and control in Malaysia, several crucial recommendations can be proposed for the future studies aimed at enhancing comprehension, prevention, and management of this disease in cattle and buffaloes. These include molecular epidemiology and genomic studies, vaccine development and optimisation, strengthening diagnostic capacity and

disease reporting, as well as socioeconomic studies and farmer education. Robust surveillance, vaccination initiatives, and enhanced diagnostic tools are imperative for controlling and diminishing the prevalence of HS. Continuous research and collaboration with veterinary services are pivotal in establishing improved prevention and management strategies in the future.

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DECLARATION OF INTERESTS

The authors affirm that there are no financial, personal, or professional relationships with any individuals, institutions, or organizations that could in any way influence or create a bias in the findings, interpretations, or conclusions presented in this study. This work has been conducted with the utmost integrity, ensuring that all results are based purely on objective scientific inquiry and evidence.

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