STATUS OF STRANGLES DISEASE IN PAHANG: SAMPLES RECEIVED BY EASTERN ZONE VETERINARY LABORATORY (PAHANG) FROM 2018 TO 2022

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ABSTRACT. Strangles is a bacterial infection caused by *Streptococcus equi sp*, which is the major infectious respiratory disease in horses. It is highly contagious, and the infection can be spread by horse to horse or by humans and other environmental factors such as feed containers and drinking troughs. Naturally in the equine industry, horses are regularly moved to and from competitions or between breeding farms, making it easy to spread and infectious. Strangles is economically important and related to the productivity of equine farms significantly. A study was carried out on the records of strangles in equines diagnosed from 2018 to June 2022 by the Bacteriology Section of the Eastern Zone Veterinary Laboratory (Pahang). The study aims to understand the current status of strangles disease from horse farms/premises in Pahang. A total of 175 nasal swab samples were taken from all districts in the state. All data cases were compiled and analysed. This study showed the distribution of samples received that were negative for strangles disease throughout the year (2018 to June 2022). Thus, it shows that the Eastern region, especially Pahang is moving towards strangles-free region. Good management practices such as farm management, surveillance programs, continuous monitoring are needed to ensure the health status of horses in the Eastern region.

Keywords: Disease, horse, strangles, *Streptococcus equi sp*, infection

INTRODUCTION

Streptococcus equi is a gram-positive bacterium that causes infectious respiratory disease in horses. It produces high morbidity and low mortality in susceptible equine populations (Timoney, 2004). Strangles is an infectious disease caused by *Streptococcus equi*. It is a highly contagious disease transmitted directly by contact with infected horses or indirectly by contact with pastures, feed buckets, water troughs, grooming equipment, and others (Neamat & El Damaty, 2016). Other than that, flies also act as vectors that can spread the bacteria from horse to horse. The survival of the organism in the environment depends on the temperature and humidity, whether extreme heat, cold, or exposure to sunlight, and must be protected within mucoid secretions to survive (Bonnie, 2022).

The first occurrence of strangles was reported by Jordanus Ruffus in 1251, and the causative agent *Streptococcus equi* was identified by Schutz in 1888 (Andrew, 2016). Meanwhile, cases of strangles are rarely reported in Malaysia. The last case reported was in August 2010. This case was detected in one horse that entered the Horse Racing Competition in Ipoh, Perak. The horse had shown clinical signs such as high fever. Veterinary officers conducted inspections and laboratory tests, and later found that the horse was positive for strangles (Khoo *et al.*, 2011). Department of Veterinary Services (DVS) had succeeded in exercising control by prohibiting the movement of horses temporarily (DVS, 2022).

The severity of the disease varies greatly depending on the animal's immune status. Older horses often exhibit a mild form of the disease characterized by nasal discharge, small abscesses, and rapid disease recovery. In contrast, younger

horses are more likely to develop severe lymph node abscessation that subsequently opens and drains (Corinne *et al*., 2005). Repeated exposure to the bacteria can permanently weaken the immune system, which becomes a common risk to matured horses (Fleur *et al*., 2019). The first clinical sign of strangles in horses is fever (increase in temperature), followed by difficulty to swallow, depression, anorexia, nasal discharge, decreased appetite/reluctant to eat, swelling of lymph nodes that can obstruct the airway puss secreted from an abscess, and abscess ruptures in 7 days and 4 weeks (Figure 1). On top of that, the major clinical signs are lymphadenopathy and severe lymph node abscessation. Abscesses of the lymph nodes at the thoracic inlet can cause severe tracheal compression, asphyxia, and death.

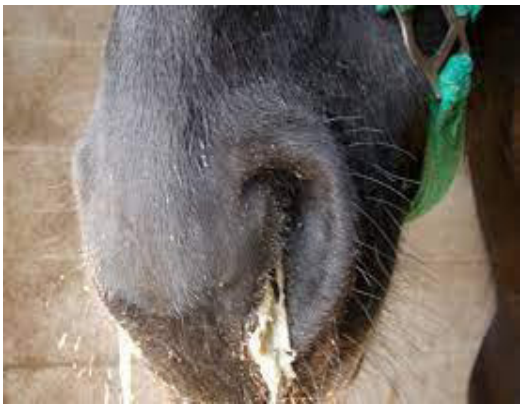

Figure 1. Clinical sign of strangles in horses (Source: The Horse.com)

The main objective of this study is to update the current status of strangles disease in the Eastern Region especially in Pahang. By understanding the current status, this study can assert that equines in the Eastern region are safe and the region is going towards strangles-free status. Several factors and methodologies must be considered before declaring an area free from strangles. Current data include reports from veterinary services, surveillance records, and diagnostic results indicating no recent outbreaks of strangles. Other than that, the region must also comply with International Standards such as World Organisation for Animal Health (WOAH) that provides guidelines on the declaration of disease-free status. This includes requirements for continuous surveillance, reporting, and transparency. According to WOAH, there should be evidence of no cases reported over a significant period (2 years for strangles), continuous and systematic surveillance, and comprehensive biosecurity measures to prevent reintroduction. This study is a preliminary study based on the diagnostic testing (culture) that has been conducted on equines in Pahang to ensure the accuracy of the disease status.

MATERIALS AND METHOD

Samples

From 2018 to June 2022, a total of 175 samples of nasal swabs from various horses from farms in Pahang were sent to Eastern Zone Veterinary Laboratory (Pahang) [MVZT (Pahang)] for laboratory examination. Samples of suspected cases of strangles were examined to identify the microbial. All detailed information about each horse were obtained from the records at MVZT (Pahang). Preliminary data were collected from the DVS system, *Sistem Maklumat Makmal* (SIMMAK) (DVS, 2022). All data of the samples were subjected to descriptive analysis and presented in graphical form. All samples were collected from a few districts in Pahang from the year 2018 until June 2022.

Isolation and Identification of Bacteria

Isolation and identification of *Streptococcus equi* (strangles) were conducted following standard

procedures established by DVS. The standard procedure is recognized under Malaysian Standard ISO 17025:2017 (JSM, 2022) in MVZT (Pahang). For bacterial isolation, samples were processed in the biosafety cabinet level 2 (BSL 2). The specimen sample was cultured aseptically into Blood Agar and MacConkey Agar. The inoculum was streaked to obtain a single colony. After inoculation, the agar was put into an anaerobic jar with carbon dioxide (5 % CO^2) gel and incubated at 37 °C for 48 hours. After incubation, the plate was examined for typical *Streptococcus equi* subsp. *equi* colonies. The colonies of bacteria that appeared in the culture were chosen, and then proceeded for gram-staining and catalase tests. For the staining process, the sample was smeared on a glass slide and heat fixed. Then, the slide was stained with crystal violet dye followed by iodine solution, which formed a complex with the dye. Then it was decolourized with acetone, and subsequently applied withn dilute carbol fuchsin and bloat dry. The purpose of gram-staining is to differentiate between gram-positive and gram-negative bacteria based on their cell wall constituent by the cell colour - red or violet. *Streptococcus equi sp* are gram-positive bacteria which retain crystal violet-iodine and appear purple due to the thick peptidoglycan layer in their cell walls; they are also spherical-shaped, or cocci bacteria arranged in chains. Meanwhile, the catalase test was conducted to test the presence of catalase, an enzyme that breaks down harmful substances like hydrogen peroxide into water and oxygen. The indication of *Streptococcus equi sp* is catalase negative where no bubbling is seen when hydrogen peroxide is added. *Streptococcus equi sp* are not detected (negative) if there is no indication which refers to positive in strangles after the test in laboratory.

RESULTS AND DISCUSSION

A total of 175 nasal swab samples were collected and examined from horses for identification and isolation of *Streptococcus equi sp*. The characteristics of positive infection from *Streptococcus equi sp* are beta-hemolytic, medium-sized, mucoid, dew-drop-like colonies *S.equi* on blood agar and MacConkey agar (Neamat-Allah *et al*., 2016). Other than that, the appearance of *Streptococcus equi sp* colonies is small, greyish white in colour, haemolytic and glistening after 18 – 48 hours incubation, the colonies appear big after further incubation, dew drop with a wide zone of haemolysis (beta-haemolysis). Meanwhile, there is no growth in MacConkey agar. Gram staining characteristics are gram positive cocci or coccobacilli in pairs or shorts. For biochemical tests, catalase test characteristics are negative with no bubbling seen. Lancefield grouping is a serological method for classifying streptococci into one of 20 groups based on the presence of polysaccharides and teichoic acid antigen in the bacterial cell wall. The interpretation for Lancefield group test *Streptococcus equi sp* is agglutinates at group C (Anzai *et al*., 1997). Then, a sugar test was conducted to see if the microbe can ferment the carbohydrate trehalose, maltose, lactose, and sorbitol as carbon sources. For sugar test, *Streptococcus equi sp* appears negative in trehalose, lactose, sorbitol but positive in maltose (has reaction with acid and gas) (Microbiologyinfo.com, 2022). Based on this finding, no positive samples were recorded based on the characteristic of positive *Streptococcus equi sp.*

Table 1 below shows the total strangles sample received in MVZT (Pahang) from 2018 to June 2022. Meanwhile, based on the graph in Figure 2, the fluctuation trend of cases received is observed. In 2020, the number of cases

received in MVZT (Pahang) was high because the surveillance program was conducted during that time compared to 2021, where no surveillance program was conducted due to COVID-19 pandemic. The number of samples is highest based on the types of work in a certain district in Pahang which are import, followed by monitoring, surveillance, animal movement, and diagnostic as shown in Table 2. For import and animal movement from one area to another area, the horses need to be tested for strangles disease to avoid infection to other animals. Pekan district recorded the highest samples, with a total of 128 samples, because of the horse-riding activity near beach for tourism attraction and royal polo sport followed by Kuantan (21), Bentong (12), Maran (5), Cameron Highland (5), Temerloh (3), and Raub (3).

Table 1. Total strangles samples received in MVZT (Pahang) from 2018 to June 2022

Year	Number of samples	Positive sample
2018	38	0/38
2019	25	0/25
2020	86	0/86
2021	12	0/12
2022	14	0/14

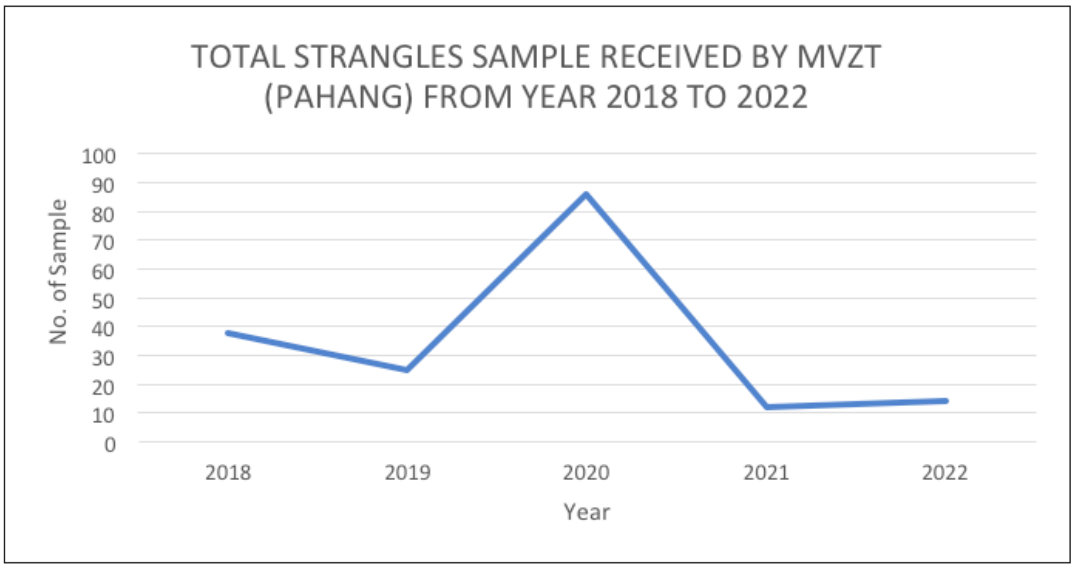

Figure 2. Total strangles samples received in MVZT (Pahang) from 2018 to June 2022

Table 2. Types of work and number of samples based on district

Item/ District	Pekan	Kuantan	Bentong	Maran	Cameron Highland	Temerloh	Raub
Import	50	0	0	0	0	0	0
Monitoring	36	0	2	0	0	0	0
Animal movement (PH)	25	0	0	0	0	0	0
Surveillance	5	21	10	5	5	3	1
Diagnostic	12	0	0	0	0	0	0
Total	128	21	12	5	5	3	1

Strangles is a disease that easily spreads and affects the economy through reduced horse performance and long-period treatment (Duran,2021). The movement of an animal from farm to farm and the procedure for import or export can be the cause of spreading the strangles disease. Hence, monitoring and surveillance must be done regularly to control the spread of this disease (DVS, 2011).

A few methods to control the transmission of *Streptococcus equi* are to immediately stop/ restrict all movement of suspected/affected horses, quarantine the horse (DVS, 2011), and then monitor by taking at least 3 nasal swabs weekly from all suspected or recovered cases and tested for *S.equi* by bacterial culture and Polymerase Chain Reaction (PCR) (Corrine *et al*., 2005). Carriers of *S.equi* were identified by culture and PCR testing of lavage fluid from the nasopharynx/nasal swabs after recovery from acute disease and in post mortem findings. PCR increases the detection rate of *S.equi* and it is three times more sensitive than cultures and DNA sequence of *S.equi* SeM gene (Arias, 2013). Ivens *et al.* (2011) mentioned that sequencing of the surface protein (SeM) gene is a useful tool for the elucidation of strangles epidemiology at regional and national level.

Corrine *et al*. (2005) mentioned that using antibiotic and non-steroidal anti-inflammatory medications can reduce fever, pain, and inflammatory swelling of abscesses and encourage the horse to eat and drink. Besides that, personnel should use proper protective clothing when dealing with infected animals and always keep strict hygiene in the farm area (Osman *et al*., 2021). Manure and waste feed from infectious animals should be removed or composted in an isolated area (Fridberg *et al.*, 2023).

However, further study needs to be conducted to evaluate the PCR or RT-PCR test to detect *S.equi* from laboratory samples, so that the PCR method can be routinely used for detecting carriers and shedders (Khoo *et al*., 2011).

CONCLUSION

The study provides preliminary data and background information on the current status of strangles disease in Pahang. All samples indicate negative results for strangles disease from 2018 to June 2022. Information obtained in this study regarding the status of strangles disease in Pahang could inform future policy regarding the

disease. Further study and surveillance programs need to be conducted to ensure the data are accurate. Declaring a region to be free from strangles requires robust evidence, continuous surveillance, and adherence to international guidelines. Future research should focus on extensive sampling and periodic testing to ensure the continued absence of the disease. Collaboration with international organizations and transparent reporting will strengthen the credibility of such a declaration. Bacteriological screening for the disease detection of horses especially the identification of *Streptococcus equi sp* (Strangles) is important to ensure the health status of the equines in Pahang. For future diagnostic test in Regional Laboratory, the use of PCR is highly sensitive and specific to detecting bacterial DNA. Disease outbreaks can be controlled when every animal (horses especially) proceeds to disease screening after import from overseas (compulsory term).

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ACKNOWLEDGEMENT

The authors would like to thank the State Department of Veterinary Services Malaysia for their contributions in submitting samples to the laboratory and all staff from Regional Eastern Veterinary Laboratories.