ISOLATION OF *STREPTOCOCCUS EQUI* DURING STRANGLES SURVEILLANCE IN PENINSULAR MALAYSIA

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ABSTRACT. Strangles is an extremely contagious bacterial infection specific to equine species(horses, mules and zebras). A nationwide screening of *S. equi* was conducted among horses following an isolation of *Streptococcus equi* subsp. equi (*S. equi*) from a horse. All horses were monitored for the presence of respiratory signs, nasal discharge and submandibular swelling. This paper reports the isolation of *S. equi* from horses during a nationwide survey from August 2010 to December 2010.

From August 2010 to December 2010 our laboratory received 2,825 nasal swabs, 9 guttural pouch flushes, 1 submandibular swab and 1 submandibular abscess. The samples were subjected to conventional bacterial isolation and identification. *Streptococcus equi*-positive samples were also confirmed by detecting the M-gene (SeM) of the bacteria by using PCR.

Two nasal swabs from two horses and one submandibular abscess from a horse were positive for *S. equi* by culture and subsequently by PCR. Surveillance for *S. equi* should be continued for the control of the strangles. PCR can be carried out in parallel to bacterial culture to increase the detection rate of carriers and shedders.

Keywords: submandibular abscess, strangles, *Streptococcus equi*

INTRODUCTION

Strangles is a highly infectious upper respiratory disease affecting horses of all ages, but most commonly in horses less than five years of age (especially in weanling foals or yearlings) and different breeds. Foals less than 4 months of age are usually protected by colostrum-derived passive immunity (Timoney, 1977).The causative agent, *Streptococcus equi* subsp. equi (*S. equi*) infects the upper respiratory tract. The disease got its name, strangles, from the swelling of the lymph node under the jowl and around the throatlatch area which restricts the horse's ability to breathe, creating 'strangled' breathing sound. *S.equi* is maintained in the horse population by carrier horses but does not survive for more than 6-8 weeks in the environment. Although the organism is not very robust, the infection is highly contagious. Transmission is either by

direct or indirect contact of susceptible animals with a diseased horse. Direct contact includes contact with a horse that is incubating strangles or has just recovered from the infection or with an apparently clinically unaffected long-term carrier. Indirect contact occurs when an animal comes in contact with a contaminated stable (buckets, feed, walls, and doors) or pasture environment (grass, fences, usually from water troughs) or through flies (Timoney, 1993). Susceptible horses develop strangles within 3-14 days of exposure (Timoney, 1993). Animals show typical signs of a generalized infectious process (depression, inappetence, and fever 39-39.5°C). This is accompanied by nasal discharge (initially mucoid,rapidly thickening and purulent), catarrh and coughing. A slight but painful swelling between mandibles develops with swelling of the submandibular lymph node. If left untreated, pus-filled lymph node develops. Laboratory diagnosis of strangles is usually by isolation and identification of *S. equi*. Polymerase chain reaction (PCR) is a rapid and sensitive test that can be used as an alternative to culture.

After an isolation of *Streptococcus equi* subsp. equi (*S. equi*) from a horse at our laboratory, a nationwide screening of *S. equi* was conducted among horses in Malaysia from August 2010 to December 2010. All horses were monitored for the presence of respiratory signs, nasal discharge and submandibular swelling.

This paper reports the isolation of *S. equi* from horses during a nationwide survey from August 2010 to December 2010.

MATERIALS AND METHOD

Samples for isolation and identification of *S. equi*

During a nationwide screening for *S. equi* among horses from August 2010 to December 2010, our laboratory received 2,825 nasal swabs, 9 guttural pouch flush, 1 swab from mandibular abscess, 1

Table 1. Type and number of samples according to state

	Perak	Penang	KL	Pahang	Trengganu	Selangor	Johore	Brunei	Total
Case	75	7	17	11	13	19	1	1	144
Nasal swab	2117	201	145	141	114	83	20	4	2,825
Guttural pouch flush	9								9
Submandibular abscess swab	1								1
Submandibular swab		1							1
Wound swab	1								1
Organ	10								10

Table 2. Biochemical reaction to differentiate between equine and other streptococci

Streptococcus sp.				
Gram stain	positive cocci/coccobacilli			
Catalase	negative			
6.5% salt broth	no growth			
	Trehalose	Sorbitol	Lactose	Maltose
S. equi subsp. equi	-	-	-	+
S, equi subsp. zooepidemicus	-	+	+	+(-)
S. dysgalactiae subsp. equisimilis	+	-	v	+

submandibular swab and 1 wound swab. The number of samples according to states is shown in Table 1. The samples were subjected for isolation and identification of *S. equi* (Table 1).

Collection of samples

Nasal swabs of 50 cm. long with 2 cm. cotton tip were introduced via the ventral meatus to the nasopharnygeal and were gently rotated to sample the mucosa. The swabs were removed and placed into amies charcoal transport media and delivered directly to the laboratory for testing.

Isolation and identification

Nasal swabs, guttural pouch flush and submandibular abscess were cultured onto 5% blood agar (BA) plates and incubated at 37°C under 5-10% CO_2. The BA plates were examined for haemolytic, medium sized, mucoid, dew-drop like colonies resembling *S. equi*. For purity, suspected colonies were subcultured onto fresh BA plates and incubated under the same condition. Biochemical tests, such as gram stain, catalase test and growth in 6.5% sodium chloride broth were carried out. *Streptococcus equi* should appear as gram positive cocci/coccobacilli in pairs or short chains, catalase negative and its inability to grow in 6.5% sodium chloride broth. It is further differentiated from other streptococci, based on its inability to ferment, trehalose, sorbitol, lactose except maltose. The biochemical reactions to differentiate between species of equine and other streptococci is shown in Table 2. As a positive control, a reference culture from Japan was included in the test.

Polymerase Chain Reaction

The DNA of *S. equi* was extracted by using boiling method. Briefly, a pure colony of *S. equi* from BA plate was picked and added to 50 µl. dH_20. The mixture was boiled for 10 min. The boiled mixture was cooled at room temperature for 5 min and

Table 3. Number of sample positive for *S. equi*

Type of sample tested	No. of sample	No. of sample positive by culture and PCR
Nasal swab	2825	2
Guttural pouch flush	9	
Submandibular abscess swab	1	1
Submandibular swab	1	
Wound swab	1	
Organ	10	

centrifuged at 16,000 g for 30 sec. The supernatant was collected for PCR.

A total of 20 µl PCR reaction mix was prepared in a buffer containing 2 mM MgCl, 0.2 mM dNTP, 0.5 units of Taq polymerase, 7.5 mM forward primer, 7.5 mM reverse primer and 2 µl of test sample. The sequences of the primers were 5'-TGCATAAAGAAGTTCCTGTC-3' and 5'-GATTCGGTAAGAGCTTGACG-3' (Timoney and Artiushin, 1997). The expected PCR product is 679 bp.

RESULTS

Of 2,825 nasal swabs, 9 guttural pouch flushes, 1 submandibular swab, and 1 submandibular abscess tested, 2 nasal swabs and one submandibular abscess were positive for *S. equi* by using culture method and PCR method (Table 3). Only those cultured positive were subjected to PCR. The samples were from three horses.

The PCR product of *S. equi* from the positive samples is shown in Figure 1. First lane is the 100 bp ladder. Lane 3 is

Figure 1. PCR product of *S. equi.* Lane 1, ladder; Lane 2, 7 and 8, *S. equi* from samples; Lane 3 and 4, positive and negative control respectively; Lane 5, *S. equisimilis*; Lane 6, *S.equi* (broth)

the positive control from Japan. Lanes 2, 7 and 8 are from the three cultures-positive cases.

DISCUSSION

Method of isolation and identification and of *S. equi* and the PCR method to confirm the bacteria were described in the materials and methods section. Bacterial culture is the gold standard to diagnose strangles. However in persistent or chronic shedders the number of bacteria present is much lower than what is found in a horse with an active infection and showing clinical signs of disease, therefore are more difficult to detect. Improved testing methods are required.

PCR is a sensitive test detecting DNA from both living and dead *S. equi* bacteria and could be more than three times more sensitive than bacterial culture. By using pure colony from BA plates, this study showed that PCR detecting the M-gene (SeM) of *S. equi* can be conducted in our laboratory. However, further study need to be conducted to evaluate the PCR test for detecting *S. equi* from clinical samples so that the test can be routinely used for detecting carriers and shedders.

The present study indicates that the isolation rate of *S. equi* from the samples received was low (3 of 2,847 samples or 0.1%). The *S.equi*-positive samples were from three horses. In the first horse, *S. equi* was isolated from submandibular abscess. The abscess was drained, flushed, cleaned and dressed with antibiotic gel. Twenty-five ml of penicillin was given daily for a week. Eventually, the abscess dried and the horse recovered.

In the second horse, *S. equi* was isolated from nasal swab. The horse had no clinical sign or lesion. However, after diagnosed positive for *S. equi,* the horse was treated daily with penicillin for a week. The horse was later euthanised. Post-mortem investigation revealed that the lungs, heart and kidney were normal in texture, colour and consistency. However, the liver and spleen were moderately enlarged, swollen with thickened edges. The parenchymas of the organs were normal and no evidence of abscess. Most of the lymph glands examined were numerous and smaller in size except for the anterior mediastinal lymph glands, which were grossly enlarged. No obvious lesion was observed when the lymph glands were cut. The cerebral and cerebellum of the brain was normal.

In the third horse, *S. equi* was also isolated from nasal swab. The horse was inappetance for a day and had fever for awhile. After being diagnosed positive for *S. equi*, the horse was treated with 25 ml penicillin daily, for a week. The horse eventually recovered.

On the basis of our data, we suggest that the surveillance for *S. equi* must be continued for the control of the strangles. PCR can be carried out in parallel to bacterial culture to increase the detection rate of carriers and shedders.

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