

## Serology Study of *Streptococcus equi* in Saudi Arabia

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**Abstract:** *Streptococcus equi* which is a Gram-positive bacteria causes strangles a highly contagious disease that affects the respiratory system of the horse. The disease is characterized by fever, nasal discharges and enlargement of the lymph nodes in the head and upper neck region. The aim of this study was to determine the prevalence of *S. equi* infection among horses residing on horse farms in the main regions of the Kingdom of Saudi Arabia. To achieve this aim, field visits were made to horse farms in Al-Ahsa, Dammam, Jubail, Riyadh, Jeddah, Hafr Al-Baten, Al-Baha and Al-Tayf. A total of 103 farms that contained 1343 horses were investigated. Farm owners and managers were questioned regarding the presence of signs similar to strangles. In the meantime, serum was collected from 181 horses. This initial survey indicated no horses had clinical signs of strangles. However, serologic work detected antibody titer against *S. equi* in 52 horses (>28%) of which 12 horses had highly significant titer. These horses were located in several locations around Saudi Arabia. It is very likely that the disease has been underdiagnosed.

**Key words:** Horses, *Streptococcus equi*, serology, underdiagnosed, Saudi Arabia

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### INTRODUCTION

Strangles is a highly contagious respiratory disease of horses which is characterized by fever, nasal discharges and inflammation of lymph nodes in the head region. *Streptococcus equi*, the causative agent is a Gram-positive, ovoid or spherical in shape, a member of Lancefield Group C streptococcus (Hardie, 1986). The disease has a world-wide distribution according to the Equine Disease Quarterly. Several outbreaks have been reported from Ireland, South Africa, Sweden, Switzerland and the United Arab Emirates among two quarantined horses recently imported from Argentina and Uruguay. Equidae is the only animal group affected by *S. equi*. The large distribution of horses around the world makes the disease world wide in distribution. Foals <6 months are more susceptible to the disease due to the lack of acquired immunity (Fallon, 1969). However, any age animal can be affected unless a vaccination program has been used or earlier exposure has occurred. Inhalation and ingestion are the most common route of infection. The disease can be transmitted via direct oral or nasal contact or by the aerosol route. It also can be transmitted by indirect contact through transfer of purulent discharge in feed, water, water bucket, bedding, handlers, flies, veterinarians and other animals.

Morbidity rate is extremely high and may reach 100%. However, the mortality rate is low usually and does not exceed 10%. Long term carriage of *S. equi* has been reported in which the organism remains in the guttural

pouch for 3 years (Newton *et al.*, 1997). Clinical manifestation includes elevated body temperature of >40°C, depression, anorexia and restlessness. Nasal discharge is serous in the beginning but becomes mucopurulent and eventually purulent. The intermandibular region is painful with some swallowing difficulties and head extension due to lymphadenitis of regional lymph nodes. Local edema may develop. External rupture of affected lymph nodes may occur in 1-2 weeks as well as internal rupture of lymph nodes into the pharynx. Additional complications are guttural pouch empyema that might result in chronic nasal discharges or acute severe upper airway obstruction, Horner's syndrome purpura hemorrhagica and spread of the infection to the lungs, liver, kidney, brain and mesenteric mediastinal, periorbital and perivertebral lymph nodes leading to signs due to involvement of these lymph nodes and organs may then occur (Sweeney *et al.*, 1987).

Very little information regarding strangles in the Kingdom of Saudi Arabia is available. The aim of this study was to document strangles in horses if any using clinical and serologic approaches.

### MATERIALS AND METHODS

**Clinical evaluation:** One hundred and three horse farms located in Al-Ahsa, Dammam, Jubail, Riyadh, Jeddah, Hafr Al-Baten, Al-Baha and Al-Tayf were included in the study during the year of 2007. A survey investigation of signs of strangles such as fever, depression, nasal secretion,

Table 1: Horse farms investigated in various regions of Saudi Arabia

Geographic source	No. of farms	No. of positive farms	No. of horses	Positive horses	Horses with high titer
Al-Ahsa	34	9	267	14	2
Dammam	12	10	85	20	6
Jeddah	12	2	80	14	3
Jubail	6	0	16	0	0
Riyadh	26	1	625	4	1
Tayf	2	0	130	0	0
Baha	3	0	20	0	0
Hafr Al-Baten	8	0	120	0	0
Total	103	22	1343	52	12

enlargement of area under the mandibles and the upper neck region was conducted with the owners. In addition, a total number of 1343 horses were examined. Examination of horses included complete history taking, physical examination for the presence of signs of depression, nasal secretion, enlargement of lymph nodes in the head and upper neck areas. Finally, blood samples were collected from 181 animals (Table 1). The serum was separated and stored at -20°C for future serologic work.

**Serology:** The examining wells were coated in triplicate with 0.025 µg rec SeM in 100 µL of 0.06M carbonate buffer pH 9.2 for approximately 1h after which they were washed three times with PBS-T. The reaction was blocked with 4% non-fat milk then washed three times with PBS-T. About 100 µL serum diluted 1:200, 1:400 in PBS-T were added in triplicate and incubated for 1 h at 37°C. Then, samples were washed three times with PBS-T. The horse radish peroxidase conjugated Protein G diluted in PBS-T was added and incubated for 1 h at 37°C. After that samples were washed three times with PBS-T then 100 µL substrate solution were added to each well. The mixture was incubated 5 min at room temperature after which the reaction was stopped by adding 50 µL 2M sulfuric acid. Readings were taken at Odsat490nm.

## RESULTS

Detailed history taking failed to detect evidence of strangles among horses residing on 103 horse farms that are located in Al-Ahsa, Dammam, Jubail, Riyadh, Jeddah, Hafr Al-Baten, Al-Baha and Al-Tayf (Table 1). Physical examination of 1343 horses showed no signs of depression, nasal secretion, enlargement of lymph nodes in the head and upper neck areas (Table 1). No signs of active disease were detected.

Serologic results showed antibody titer against *S. equi* exists in several horses residing on farms in Riyadh (one farm), Jeddah (two farms), Dammam (ten farms) and Al-Ahsa (nine farms) (Table 1). At least, 52 (>28% morbidity rate) horses were positive for strangles. These horses were in Riyadh (4 horses), Jeddah

Table 2: Titers of serum antibody to SeM and Se18.9 in 12 horses previously screened at a 1:200 serum dilution

Horse No.	SeM	Se18.9
7	>1:12,800	>1:12,800
11	1:6,400	1:3,200
12	>1:12,800	1:1,600
58	>1:12,800	>1:12,800
60	>1:12,800	1:3,200
70	>1:12,800	1:3,200
90	1:1,600	1:800
102	1:3,200	1:800
104	1:6,400	1:800
108	1:1,600	1:800
165	1:1,600	1:6,400
167	>1:12,800	1:1,600

(14 horses), Dammam (20 horses) and Al-Ahsa (14 horses). The antibody titer was found significantly high in 12 horses located in Riyadh (1 horse), Dammam (6 horses), Jeddah (3 horses) and Al-Ahsa (2 horse) (Table 2). The antibody titer against SeM ranged between 1:1,600 to >1:12,800 while antibody titer against Se18.9 ranged between 1:800 to >1:12,800.

## DISCUSSION

The survey study that included careful questioning of owners on important clinical signs that are highly indicative of strangles such as nasal secretion and enlargement of lymph nodes in the head and the upper neck region has been unrewarding. Despite the fact that most of the horse owners were closely associated with their horses none of these signs were described. In a earlier study, strangles has not been detected among examined horses in Saudi Arabia despite reaching out to different regions of the country (Al-Ghamdi, 2008). Therefore, additional and more specific testing for strangles are required in order to be sure that no horses carry the disease.

In this study, horses that showed seroconversion had no clinical evidence of the disease which may explain the failure in detecting the disease in the earlier research. The serologic assay was based on two antigens that are highly conserved and specific to *S. equi* SeM and Se18.9 (Kelly *et al.*, 2006; Tiwari *et al.*, 2007). The serum titer was significantly high against both antigens in several horses which indicates active immune response and possibly recent infection.

## CONCLUSION

The morbidity rate according to the serology is >28% which is significant figure. Prior to this research, strangles has never been reported in Saudi Arabia. It is very likely that clinical cases may recover unobserved by owners. Typical clinical signs that are detected in cold climates may not be necessary seen in hot climates. Nonetheless

close attention has to be made to detect any suggestive clinical changes of strangles. It might be unnecessary to perform serologic tests at this point unless changes in the performance of horses and respiratory signs were noticed, then strangles has to be part of differential diagnosis. Other conditions such as guttural pouch tympany and empyema were miss-diagnosed with strangle (Al-Ghamdi, 2006). The other striking point in this research was that strangles positive horses were distributed in the main regions of the country. Such distribution complicates the epidemiology and control of the disease. It is known that horses travel very frequently in the country for many reasons such as racing, breeding and sale. Therefore, additional effort is required to educate owners in prevention measurements of strangles.

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