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OCCURRENCE OF LANCEFIELD GROUP C STREPTOCOCCAL SPECIES IN STRANGLES CASES OF FOALS IN PUNJAB, PAKISTAN

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ABSTRACT

Three equine rearing districts of Punjab, Pakistan including Sargodha, Okara and Faisalabad were surveyed. The occurrence of strangles was highest in foals during February to the start of May compared to winter and summer seasons. Strangles-inflicted foals showed inability to eat, strangled breathing with roaring sounds, swollen submandibular and submaxillary lymph nodes, abscessation in the lymph nodes, hyperthermia and anorexia with general debilitating conditions. Morbidity and case fatality rates were 78 and 2%, respectively. Pus samples were collected from 70 foals showing burst lymph node abscesses and processed on blood agar. Typical growth showing pinpoint mucoid colonies, with beta hemolysis and catalase negative characteristics were subjected to API Strep 20 system. The isolates of streptococcus recovered from pus samples include *S. equi subspecies equi* (54%), *S. equi subspecies zooepidemicus* (11%), *S. dysgalactia subspecies equisimilis* (11%) and mixed isolates of *S. equisimilis* and *S. equi* (23%). Streptococcus species were found sensitive to Penicillin and Cefotoxime, whereas Gentamycine and Tetracycline were ineffective.

Key words: Strangles, foals, SDS-PAGE, M-protein, Streptococcus species.

INTRODUCTION

With the development of mechanical transport, the number of equines is decreasing in large cities of Pakistan but its importance is unequivocal in rural and northern areas of the country. In many parts of the Pakistan, donkeys, horses and mules are the only means of traveling in the remote areas (Ashraf, 2001). Equine population in the country was reported as 4.6 million heads, out of which 2.47 millions existed in districts Sargodha, Okara and Faisalabad of Punjab (Anonymous, 2006).

In equines, strangles is a disease commonly associated with fever, depression, anorexia (Sweeney *et al.,* 1989; Dalgleish *et al.,* 1993; Wallace *et al.,* 1995), purulent nasal discharge and abscessation of the lymph nodes of the head and neck (George *et al.,* 1983). Mortality and morbidity associated with this disease have been reported to be 3.6 and 62%, respectively in an outbreak in a standard bred farm in Kentucky, USA (Sweeney *et al*., 1989).

According to Gillespie and Timoney (1981), in strangles the involvement of *S. equisimilis* was not significant as equine pathogen, whereas Grant *et al.* (1993) concluded that some horses were infected with both *S. equi* and *S. equisimilis*. The incidence of strangles in young equine population distributed in the thickly populated areas of Punjab has been described in this paper. Moreover, pus samples from strangles affected foals were investigated to verify the previously concluded facts.

MATERIALS AND METHODS

Incidence of strangles

Three equine rearing Districts of Punjab, Pakistan including Sargodha, Okara and Faisalabad were surveyed throughout the year during January to December, 2005, each month, for new cases of strangles in horses and mules of ages from 9 months to 2 years (foals) and 2-5 years (adults). New cases of strangles during each month were included in the study and the incidence was calculated.

Collection of pus samples

From January to May 2005, a total of 70 foals (9 months to 2 years of age) with clinical signs of swollen submandibular lymph nodes and ruptured abscesses were approached in the equine rearing districts of Punjab including Sargodha, Okara and Faisalabad. Pus samples were collected in sterilized disposable plastic syringes and transported in ice packs to the laboratory for further processing.

Bacterial isolation and identification

Each pus sample was cultured separately on sodium azide blood agar containing 5% defibrinized sheep blood and 0.2% sodium azide and incubated at

37^0C for 24 hours under anaerobic conditions. Pure growth of typical mucoid beta hemolytic colonies were analyzed through Gram's staining, under microscope and were confirmed for streptococcus genus through catalase test. Mucoid, beta hemolytic, and catalase negative colonies containing Gram positive cocci were declared as pure streptococci and were further processed through API Strep 20 system (Bio Meriux®, France) for streptococci species confirmation. Biochemical tests including Voges Proskauer test, Hipurate test, Esculine test, Pyrolidonyl test, Alpha galactosidae test, Beta glucuronidase test, Beta galactosidase test, Alkaline phosphates test, Leucine amino peptidase test, Arginin Dihydrolase test and fermentation of sugars including D-ribose, L-arabinose, D-manitol, D-sorbitol, D-lactose, D-trehalose, Inuline, D-raffinose, Starch and Glycogen in API strep 20 kit system were used to confirm the streptococcal species.

Molecular comparison between indigenous *Streptococcus equi* and foreign strain CF32

M-protein of *Streptococcus equi* was procured following the standard method of Woolcock (1974) and was subjected to Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE, Timoney and Strickland, 1984). Band pattern observed though SDS-PAGE was compared with that of CF32 strain of *Streptococcus equi* already recovered (Galan and Timoney, 1987).

Optimization of modified Todd-Hewitt broth

Filtered and heat inactivated (56^0C) horse serum was dispensed in duplicate at a rate of 2, 5 and 10% in three test tubes containing 10 ml of modified Todd-Hewitt broth. Fresh culture of *S. equi* was inoculated in each of three test tubes. These tubes were incubated at 37^0C and count of streptococcal cells was conducted every 12 hours up to 96 hours.

Antibiotic sensitivity

Streptococcal species were subjected to susceptibility studies against eight antibiotics including Penicillin G, Streptomycin, Sulphamethoxazole, Gentamycine, Chloramphenicol, Cefotoxime, Tetracycline and Ampicillin using standard antibiotic discs and dispenser (Bioanalyse® France). For this purpose standard technique elaborated by Cappuccino and Sherman (2005) was followed.

RESULTS

The incidence of strangles and occurrence of streptococcus species in strangles-inflicted foals of 9 months to 2 years of age during four seasons around the year was recorded and it was found to be the highest during spring season (Mid of January to Start of May). Animals from 2 years to 5 years of age showed no clinical signs in any season of the year (Table 1).

Out of 70 pus samples, typical Streptococcus species were recovered from 55 samples (78.57%), 6 samples had non-streptococcus isolates (8.57%), whereas 9 samples (12.85%) were declared as sterile where no growth appeared even after 96 hours. Single isolate of *S. equi* was recovered in 54%, followed by mixed isolates of *S. equi* and *S. equisimilis* (23%), single isolates of *S. equisimilis* (11%) and single isolate of *S. zooepidemicus* (11%, Table 2).

Molecular analysis of M-protein extracted from *S. equi* revealed a pattern with bands 30 Kda, 41 Kda, 46 Kda and 60 Kda. An equal count ($4x10^{11}$/ml) was recorded after 24 hours in test tubes containing modified Todd-Hewitt broth with 5 and 10% horse serum at 37^0C. In broth with 2% horse serum, count was found as $4x10^9$/ml.

Each of the three streptococcal species were cultured and growth after 24 hours was subjected to

Table 1: Incidence of strangles during January to December 2005 in Punjab, Pakistan

Months	Adult horses/mules (2 – 5 years) (Cases in number)					Young foals (9 months-2 years) (Cases in million)				
	Sargodha	Okara	Fsd	Total	Incidence (%)	Sargodha	Okara	Fsd	Total	Incidence (%)
Jan*	1	-	0	1	0.000	0.03	0.01	0.01	0.05	2.00
Feb*	1	1	-	2	0.000	0.03	0.02	0.01	0.06	2.40
March*	-	-	1	1	0.000	0.04	0.01	0.01	0.06	2.40
April*	-	-	1	1	0.000	0.02	0.03	0.01	0.06	2.40
May*	-	-	-	-	0.000	0.01	0.02	0.00	0.03	1.21
June	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00
July	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00
Aug	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00
Sep	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00
Oct	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00
Nov	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00
Dec	-	-	-	-	0.000	0.00	0.00	0.00	0.00	0.00

* Strangles season.

Table 2: Streptococcal species isolated from pus samples in strangles cases of foals

No. (%) of isolates	Hemolytic character	Esculine hydrolysis	Fermentation test				Streptococcal Spp.	API #
			Ribose	Trehalose	Sorbitol	Lactose		
30 (54%)	Beta hemolytic	-ve	-ve	-ve	-ve	-ve	*S. equi subspecies equi*	0461007
6 (11%)	Beta hemolytic	-ve	-ve	+ve	-ve	-ve	*S. equisimilis*	0461017
13 (23%)	Beta hemolytic	-ve	-ve	-ve/+ve	-ve	-ve	*S. equi* & *S. equisimilis*	0461007 0461017
6 (11%)	Beta hemolytic	+ve	+ve	-ve	+ve	+ve	*S. zooepidemicus*	4463607

eight antibiotic culture sensitivity discs. From the results, it is clear that each of the three recovered streptococcal species was sensitive to Penicillin G and Cefotoxime and showed intermediate behavior against streptomycin and Sulphamethoxazole. Streptococcal species showed resistance against Gentamycine and tetracycline (Table 3).

DISCUSSION

A survey for the incidence of strangles in young as well as adult population of equines round the year was conducted to get an idea about the season in which this disease can affect animals and to confirm the age group usually inflicted with this disease. Incidence of strangles was found highest in foals of 9 months to 2 years of age during spring season (Mid of January to start of May).

Out of total samples, 23% streptococcal isolates contained mixed growth of *S. equi* and *S. equisimili* and six streptococcal isolates (11%) contained *S. equisimilis* alone, confirming the fact that horses are infected with both *S. equi* as well as *S. equisimilis* (Grant *et al*., 1993). M-proteins of locally isolated *S. equi* after analysis through SDS-PAGE showed a band pattern similar to that found in *S. equi* strain CF32 thus confirming the fact that *S. equi* is homogenous through out the world (Galan and Timoney, 1987).

From the observations made in this study, it is recommended that both *S. equi* as well as *S. equisimilis* should be considered for the development of a safe and effective vaccine against strangles. Optimization of already modified Todd-Hewitt broth was conducted by supplementing the broth with different concentrations of horse serum. From the experimentation, it was concluded that modified Todd-Hewitt broth supplemented with 5% horse serum is economical and efficient compared to broth dispensed with 2% and 10% horse serum for the mass scale growth of *S. equi*.

Antibiotic susceptibility of each of three streptococcal species revealed that in vitro Penicillin G and Cefotoxime were very effective against Streptococci. As far as the effects of antibiotics treatment in strangles is concerned, it is a different story because in early stages of infection the use of these antibiotics aborts the reaction and no immunity is developed and as treatment is withdrawn, the infection re-occurs (Harrington *et al*., 2002). In later stages, lack of sufficient vascularity at the site of infection and abscessation interferes with the antibiotic to combat with infection.

In the present study, it was seen that incidence of strangles continuously increased from January to April and then it started decreasing. In July, no new case was reported after the report of few cases in June. Incidence of strangles from the end of January to the start of May in foals of 9 months to 2 years of age was highest (2.56%) compared to the adult equines of age from 2 to 5 years, confirming the fact that once an animal is infected with strangles it attains life long immunity (Walker and Timoney, 2002).

Table 3: Antibiotic sensitivity of Streptococcal isolates recovered from strangles cases of foals

Streptococcal species	Antibiotic Sensitivity Discs (30 micro grams of antibiotic/disc)							
	Penicillin G	Streptomycin	Sulphamethoxazole	Gentamycine	Chloramphenicol	Cefotoxime	Tetracycline	Ampicillin
S. equi	S (30.5)	I (13. 5)	I (12 .3)	R (9.5)	I (13.5)	S (24.5)	R (14)	I (12)
S. equisimilis	S (30)	I (14)	I (11.5)	R (9)	R (12)	S (23)	R (13 .5)	R (11)
S. zooepidemicus	S (30 .6)	I (13.5)	I (13)	R (9)	I (14.9)	S (23.8)	R (13 .6)	R (11)

S = Sensitive, I = Intermediate, R = Resistant

Values in parentheses indicate zone diameter in mm.

Conclusions

A higher occurrence of *S. equi* was recorded in young foals compared to adults. *S. equi* alone and in conjunction with *S. equisimilis* were isolated in the laboratory. The isolates showed maximum sensitivity to penicillin and cefotoxime. Therefore, it may be recommended to prepare a bivalent vaccine for the control of strangles in foals.

REFERENCES

Anonymous, 2006. Economic Survey. Economic Affairs Division, Govt. Pakistan, Islamabad.

Ashraf, M., 2001 Some blood parameters of equines in strangles. PhD Thesis, Univ. Agri, Faisalabad, Pakistan.

Cappuccino. J. C. and N. Sherman, 2005. Microbiology-A laboratory Manual. 6th Ed., Pearson Education (Singapore), Indian branch, Dehli, India, pp: 280-285.

Dalgleish, R., S. Love, H. M. Pirie, M. Pirie and D. J. Taylor, 1993. An outbreak of strangles in young ponies. Vet. Rec., 132: 528-531.

Galan, J. E. G. and J. F. Timoney, 1987. Molecular analysis of the M-protein of *Streptococcus equi* and cloning and expression of the M-protein gene in *Escherichia coli*. Infec. Immunity, 55(12): 3181-3187.

Gillespie, J. H. and J. F. Timoney, 1981. Hagen and Bruner's Infectious Diseases of Domestic Animals. 7th Ed., Cornell Univ. Press, Ithaca, New York, USA, pp: 176-178.

George, J. S., J. S. Reif, R. K. Sheider, C. J. Small, R. P. Ellis, S. P. Snyder and A. E. McChesney, 1983. Identification of carriers of *Streptococcus equi* in a naturally infected herd. J. Amer.Vet. Med. Assoc., 183(1): 80–84.

Grant, S. T., A. Efstratiou and N. Chanter, 1993. Laboratory diagnosis of strangles and the isolation of atypical *Streptococcus equi*. Vet. Rec., 133: 215-216.

Harrington, D. J., I. C. Sutcliffe and N. Chanter, 2002. The molecular basis of *Streptococcus equi* infection and disease. Microbes and Infection, 4: 501–510.

Sweeney, C. R., C. E. Bensen, W. H. Robert, D. A. Meirs, S. O. Birmingham, S. C. Whitehead and D. Cohen, 1989. Description of an epizootic and persistence of *Streptococcus equi* infection in horses. J. Amer. Vet. Med. Assoc., 194(9): 1281-1286.

Timoney, J. F. and K. L. Strickland, 1984. Lysogeny and immunologically reactive proteins of *Streptococcus equi*. Vet. Rec., 115: 148-149.

Walker, J. A. and J. F. Timoney, 2002. Construction of a stable non-mucoid deletion mutant of the *Streptococcus equi* Pinnacle vaccine strain. Vet. Microbiol., 89: 311–321.

Wallace, F. J., D. Emery, A. W. Cripps and A. J. Husband, 1995. An assessment of mucosal immunization in protection against *Streptococcus equi* (strangles) infection in horses. Vet. Immunol. Immunopathol., 48: 139-154.

Woolcock, J. B., 1974. Purification and antigenecity of an M-like protein of *Streptococcus equi*. Inf. Immunity, 10(1): 116-122.