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PREVALENCE AND HAEMATO-BIOCHEMICAL STUDIES OF STRANGLES (*STREPTOCOCCUS EQUI*) AFFECTED HORSES IN PAKISTAN

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ABSTRACT

The study was carried out to find the prevalence of *Streptococcus equi serovar equi* (*S. equi*) in nasal discharges and pus from sub-mandibular lymph nodes in horses at Lahore and Sargodha districts of Punjab province. A total of 194 nasal swabs and 56 pus samples were collected and examined from horses clinically diagnosed for strangles; 113 (45.2%) were found positive for *S. equi.* A higher prevalence (87%) of *S. equi* was recorded in horses with less than two years of age. The haemato-biochemical parameters were also measured at 1st, 2nd, 3rd and 4th week following the onset of infection. Total WBCs, mean segmented neutrophils count (MSNC) and monocytes were significantly increased ($P<0.05$) while the concentration of lymphocytes was significantly decreased ($P<0.05$) in infected horses whereas the concentration of total serum protein, serum globulin and fibrinogen was significantly increased ($P<0.05$), while the concentration of serum albumin was significantly decreased ($P<0.05$). It is concluded that horses ≤ 2years of age are highly susceptible than adult horses while increased total serum proteins, serum globulin and fibrinogen along with decreased serum albumin were important indicators of infection by *S. equi* in horses.

Key words: Strangles*; Streptococcus* species; prevalence; haemato-biochemical analyses.

INTRODUCTION

Strangles is an infectious malady of equidae characterized by upper respiratory tract infection, dyspnoea, anorexia, regional suppurative lymphadenitis and causes high morbidity and low mortality. Strangles is named from the air restriction in late stages of the disease where the horse breathes as if it is being strangled because of the restriction of the trachea due to swollen lymph nodes. It is considered to be one of the top three most significant and feared respiratory diseases in horses (Natarajan and Langohr, 2003). It accounts for close to 30% of all equine infections reported worldwide, making it the most frequently encountered single horse illness (Harrington *et al.,* 2002).

Strangles infected horses, naturally and experimentally, depicts a great deal of change in hematological parameters, such as total and differential leukocytic counts (Hamlen *et al.,* 1994). The signs become apparent after an incubation period of 3 to 8 days, and the clinical course usually lasts 3 to 4 weeks (Nara *et al.,* 1983). Marked fever (103-106°F) develops during the acute phase and may subside until the abscess formation in lymph nodes; this is the time when a second wave of fever may develop. Affected horses become anorexic, depressed, and develop bilateral, serous to mucoid nasal discharge within 24 hours of fever. The discharge becomes mucopurulent as the disease progresses, and a moist cough may develop in some cases. Plasma fibrinogen concentration and leukocyte counts usually increase at this time. The sub-mandibular lymph nodes are involved most oftenly and become enlarged, firm, and painful. The retropharyngeal lymph nodes may also be affected and if become markedly enlarged, they may induce dysphagia. The abscessed lymph nodes typically rupture within 7 to 10 days after the onset of clinical signs and in uncomplicated cases; convalescence period may be of 1 to 2 weeks thereafter (Reed, 2004).

Changes in hematological parameters, such as total and differential white cell counts have been recorded in horses naturally and experimentally infected with *S. equi* (Hamlen *et al*., 1994). This study describes the prevalence of *S. equi* and its effect on various blood parameters and serum protein values in horses showing clinical signs of strangles at Lahore and Sargodha districts of Punjab, Pakistan.

MATERIALS AND METHODS

Prevalence: A total of 250 horses which were declared positive for strangles on the basis of clinical signs at Lahore and Sargodha districts of Punjab province during January 2009–December 2009 were included in the present study. Fever, cough, purulent nasal discharge, enlargement of sub-mandibular lymph nodes, increased respiration rate, loss of appetite, and abnormal auscultation of the trachea and thoracic cavity were the main clinical signs (Merchant and Packer, 1983).

Samples Collection: A total of 194 samples of nasal discharge were collected using sterile cotton swabs, and 56 pus samples from affected sub-mandibular lymph

nodes were collected aseptically using sterile disposable syringes, from clinically strangles affected animals. These samples were processed at the Medicine & WTO Laboratories, University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan.

Bacterial isolation and identification: Samples were cultured on blood agar plates and incubated at 37°C for 24 h under anaerobic conditions (Jorm, 1990). Typical beta haemolytic streptococci-like colonies were detected on blood agar and identified by colony characteristics, Gram-staining, and biochemical tests, including catalase. Isolates identified as *S. equi* fermented salicin and sucrose but not sorbitol, lactose, inulin, trehalose, raffinose, or manitol. The isolates hydrolysed starch but not aesculin (Quinn *et al.,* 1994).

Haemato-biochemical analyses: A 5 ml blood sample was collected directly from the jugular vein of *S. equi* affected horses (n=20) and healthy horses (n=20) from each age group in sterilized plastic bottles without anticoagulant for measuring the total serum proteins, serum albumin, serum globulin and 5 ml in other tube coated with anticoagulant, EDTA @ 1 mg/ml of blood for fibrinogen and various blood parameters. Total serum proteins, serum albumin, serum globulin, and fibrinogen were estimated using a serum chemistry analyzer while haematological values were estimated by haematological analyzer.

Statistical analysis Finally the data on prevalence of the disease was analyzed by Chi-square test using statistical software package STATA 9.1 (College Station T×77845, USA) while data on haematological and biochemical examination was analyzed with one way ANOVA. The difference between diseased and healthy animal along different weeks was tested by Tukey's test. A P-value <0.05 was used to reject the null hypothesis that the model is not significant.

RESULTS AND DISCUSSION

Prevalence: Data on the prevalence of *S. equi* are shown in table 1, 2 and 3. Out of 250 horses 113(45.2%) horses tested positive for *S. equi*. The number of *S. equi* isolates were significantly higher (P<0.05) in pus samples collected from sub-mandibular lymph nodes as compared to nasal discharge samples. The difference in number was found significant (P<0.05) among horses of different age groups. The highest prevalence of strangles was recorded in horses less than 2 years as compared to those having age more than 2 years. Our results are consistent with those of Ijaz *et al.* (2010) who reported that equines under 2 years of age were highly susceptible than adult equines in Pakistan. The Results of present study also correlate with the findings of Timoney (1993) who also reported that horses of all ages may be affected, but the disease is most common and most severe in young horses. Sweeny (1987) and Piche (1984) observed that yearlings and young adults are most at risk, followed by weanlings and then adults. Typically, yearlings are most severely affected with a longer duration of clinical signs (Piche 1984). During the breeding season, nursing mares brought of suckling foals may introduce *S. equi* in this manner. Findings of our study are also in line with the findings of Sweeny, (1990) who reported that infection occurs primarily in 1 to 5 years old horses, but is not restricted to age groups. Our results were also broadly consistent with the findings of Manzoor *et al.* (2008) who reported 54% infection in foals in Punjab, Pakistan. Our findings are also in agreement with the results of Sweeney *et al.* (1989) who found rates of *S. equi* infections of the upper respiratory tract and lymph nodes (strangles) in horses to be 47.5% for 1-year-old horses, and 37.5% for foals. *S. equi* was isolated from nasal, pharyngeal, or lymph node specimens in 31 (60.8%) of 51 sick horses. Our results also correlate with the study of Hamlen *et al.,* (1994) who reported that foals were highly susceptible to developing strangles following *S. equi* exposure as shown by attack rates of 86% (19/22) and 91% (10/11) respectively. Similarly our study also correlates with the findings of Manzoor *et al.* (2008) who recorded incidence of strangles in foals of 9 months to 2 years of age and it was found to be the highest during the spring season (Mid of January to Start of May).

Haemato-biochemical values of horses: Data on the haematology of *S. equi* is shown in table 4. The results of present study has revealed a significant increase (P< 0.05) of total WBCs, MSNC, and basophils in strangles affected horses, while a non significant difference was observed (P>0.05) among values of lymphocytes, eosinophils, basophils, erythrocytes, hemoglobin and packed cell volume. Our results were in line with the findings of Higgins and Snyder (2006) who reported pronounced leukocytosis with neutrophilia. Our results were also broadly consistent with the findings of Hamlen *et al.* (1994) who reported that the total leukocyte count increases quickly in horses in the first week post infection, whereas this increase was delayed into the 2nd week. Other blood parameters including total eosinophilic count, total basophilic count, total erythrocyte count, packed cell volume and hemoglobin concentration remained non significant (P>0.05) during the four weeks post infection. Gomez (1990) also recorded hematologic changes which include leukocytosis with counts up to 30,000/µL, a segmented neutophil count that may be in excess of 25,000/µL. The result of this study was also in line with of Ijaz *et al.* (2011) who also reported total WBCs, MSNC, basophils, lymphocytes, eosinophils, basophils, erythrocytes, hemoglobin and packed cell volume in strangles affected equines in Pakistan. Higgins and Snyder (2006) also

reported leukocytosis and neutrophilia. Our findings were also in the same line with the finding of Hamlen *et al.* (1994) who reported that strangles cases had lower mean PCV and hemoglobin concentration than non infected animals during weeks 4, 6 and 10 of the outbreak. The erythrocytes count was also lower in positive cases compared to non infected animals during weeks 6 and 10. Collins (1999) reported that a complete blood count proved to be a useful adjunctive test to support a diagnosis of *S. equi* and may help differentiate horses with acute *S. equi* infection from those with acute viral infection. Golland *et al.* (1995) reported that hematological data was recorded for 8 horses. Leukocytosis with a marked neutrophilia was present in 7 horses, with total nucleated cell counts of up to 23.2×10^9 cells/L. This was typically followed by the appearance of band neutrophils and marked lymphocytosis. Seven horses were anemic (mean packed cell volume 0.32L/L). Our findings are in agreement with the results of Canfield *et al.* (2000) who reported that all experimentally infected horses with *S. equi* showed a consistent leukocytosis as a consequence of a mature neutrophilia. These changes developed within two days of infection and in some individuals persisted up to 35 days. Neutrophilia in all the horses were mature and an increase in band neutrophils was not detected in any of the horses. Other abnormalities in individual horses included a mild lymphocytosis and a monocytosis.

In the present study, total serum protein, serum globulin, and fibrinogen were significantly increased ($P<0.05$), whereas the value of serum albumin significantly decreased ($P<0.05$) in strangles affected horses (Table 5). Our study correlates with the study of Radostits et al. (2000) who reported that hyperfibrinogenaemia is characteristic of both acute and chronic phase of disease. Similarly hyperproteinemia attributable to a polyclonal gammaglobulinaemia is characteristic of chronic abscess. Fibrinogenaemia has also been reported by Higgins and Snyder (2006). Gomez (1990) also recorded fibrinogen level of 6.0mg/dL. Our findings of fibrinogen correlate with the finding of Golland *et al.* (1995) who recorded hyperfibrinogenaemia was present in 2 horses, with values of 7 and 8 g/L. The results of the present study are in complete agreement with the findings of Taylor and Wilson (2006) who reported elevated concentration of globulin and fibrinogen and anemia of chronic inflammation are typical findings. Collins (1999) reported that plasma fibrinogen concentration frequently prove to be a useful adjunctive test to support a diagnosis of *S. equi* and may help differentiate horses with acute *S. equi* infection from those with acute viral infection.

Table 1: Overall prevalence of Strangles in nasal discharge and pus samples of sub-mandibular lymph nodes of horses

Age Groups	n	Number positive for *S. equi*								Total (%)
		January	February	March	April	May	Jun	July	Aug-Dec	
<1year	50	02	09	11	13	07	02	01	00	45(90)
1-2year	50	01	08	15	12	02	04	00	00	42(84)
2-3year	50	00	03	05	03	03	00	00	00	14(28)
3-4year	50	00	01	02	03	02	00	00	00	08(16)
4-5year	50	00	01	00	02	01	00	00	00	04(08)
Total	250	03	22	33	33	15	06	01	00	113(45.2)

Chi-square analysis showed significant difference in prevalence of strangles among all age groups (Chi-square value 122.02, P- value 0.0001)

Table 2: Prevalence of Strangles in nasal discharge of horses

Age Groups	n	Number positive for *S. equi*								Total (%)
		January	February	March	April	May	Jun	July	Aug-Dec	
<1year	33	01	04	08	09	04	02	01	00	29(87.88)
1-2year	37	01	04	11	09	01	04	00	00	30(81.08)
2-3year	35	00	00	03	01	02	00	00	00	06(17.14)
3-4year	45	00	01	01	02	02	00	00	00	06(13.33)
4-5year	44	00	01	00	01	01	00	00	00	03(06.82)
Total	194	02	10	23	22	10	06	01	00	74(38.14)

Chi-square analysis showed significant difference in prevalence of strangles among all age groups (Chi-square value 122.02, P- value 0.0001)

Table 3: Prevalence of Strangles in pus samples of sub-mandibular lymph node of horses.

Age Groups	n	Number positive for *S. equi*								Total (%)
		January	February	March	April	May	Jun	July	Aug-Dec	
<1year	17	01	05	03	04	03	00	01	00	16(94.12)
1-2year	13	00	04	04	03	01	00	00	00	12(92.31)
2-3year	15	00	03	02	02	01	00	00	00	08(53.33)
3-4year	05	00	00	01	01	00	00	00	00	02(40.00)
4-5year	06	00	00	00	01	00	00	00	00	01(16.67)
Total	56	01	12	10	11	05	00	01	00	39(69.64)

Chi-square analysis showed significant difference in prevalence of strangles among all age groups (Chi-square value 122.02, P- value 0.0001).

TABLE 4: Haematological values of healthy and *S. equi* positive horses (Mean ± SE)

Haematological Parameters	n		Post Infection Weeks							
	Healthy	Diseased	1st		2nd		3rd		4th	
			Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Total white blood cell count ($x10^9$/L)	20	20	*11.78 ±0.17a	*14.99 ±0.22a	*10.71 ±0.16b	*14.07 ±0.19b	*09.93 ±0.09c	*12.80 ±0.16c	**11.16 ±0.19b	**11.91 ±0.16d
Erythrocytes count in (X 10^{12}/L)	20	20	**6.27 ±0.03a	**5.97 ±0.04ab	**6.13 ±0.05ab	**6.03 ±0.04a	**6.07 ±0.06b	**5.91 ±0.05ab	**6.02 ±0.04b	**5.87 ±0.04b
Mean segmented Neutrophils count ($x10^9$/L)	20	20	*04.54 ±0.07a	*07.07 ±0.11a	*04.72 ±0.08a	*06.98 ±0.07a	*03.96 ±0.05b	*05.08 ±0.08b	*03.78 ±0.07b	*04.55 ±0.07c
Haemoglobin concentration (g/L)	20	20	**123.47 ±0.61a	**109.45 ±0.21a	**109.89 ±0.20d	**100.67 ±0.11c	**115.73 ±0.23c	**099.12 ±0.20d	**117.24 ±0.17b	**104.61 ±0.17b
Packed cell volume (%)	20	20	**35.67 ±0.24b	**29.14 ±0.14c	**34.89 ±0.17c	**32.98 ±0.06a	**34.88 ±0.17c	**30.67 ±0.17b	**36.45 ±0.07a	**28.10 ±0.09d
Lymphocyte ($x10^9$/L)	20	20	**02.89 ±0.05c	**02.71 ±0.05c	**03.48 ±0.06b	**03.98 ±0.07b	**03.98 ±0.04a	**04.76 ±0.08a	**02.93 ±0.05c	**02.56 ±0.04c
Monocyte ($x10^9$/L)	20	20	**00.36 ±0.018a	**00.53 ±0.021a	**00.38 ±0.020a	**00.45 ±0.014b	**00.38 ±0.016a	**00.49 ±0.011ab	**00.36 ±0.015a	**00.47 ±0.011b
Eosinophil ($x10^9$/L)	20	20	**00.53 ±0.016a	**00.48 ±0.010a	**00.46 ±0.010c	**00.32 ±0.022b	**00.51 ±0.014ab	**00.45 ±0.011a	**00.48 ±0.007bc	**00.50 ±0.014a
Basophil($x10^9$/L)	20	20	*00.05 ±0.004a	*00.43 ±0.012b	*00.03 ±0.003b	*00.48 ±0.010a	*00.04 ±0.003ab	*00.20 ±0.007c	**00.01 ±0.001c	**00.07 ±0.003d

Mean in a row followed by the same letter were not significantly different at P≤0.05, by Tukey HSD test.
* indicates significant difference (p<0.05) among healthy and diseased groups
** indicates non-significant difference (p>0.05) between healthy and diseased group

TABLE 5: Serum protein values of healthy and *S. equi* positive horses (Mean ± SD)

Age groups	N		Proteins (g/L)							
	Healthy	Diseased	Total serum protein		Serum albumin		Serum globulin		Fibrinogen	
			Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
<1 year	20	20	64.9a ±03.4	82.7b ±03.3	33.8a ±02.2	25.4b ±02.9	32.9a ±01.8	37.1b ±03.4	03.4a ±01.0	07.2b ±02.3
1-2 year	20	20	66.5a ±03.1	80.9b ±05.7	36.3a ±02.4	27.1b ±03.9	34.5a ±03.0	36.7a ±04.5	03.8a ±01.0	06.7b ±02.4
2-3 year	20	20	62.7a ±01.2	79.3b ±05.3	34.7a ±04.4	24.8b ±04.4	33.2a ±06.5	35.2a ±04.0	03.5a ±01.1	06.3b ±02.2
3-4 year	20	20	63.6a ±05.8	78.8b ±06.4	45.3a ±05.3	26.2b ±04.5	34.4a ±03.2	36.7a ±02.7	03.7a ±0.70	05.7b ±01.0
4-5 year	20	20	64.1a ±04.1	78.5b ±04.7	34.5a ±04.4	28.4b ±05.4	33.7 ±04.4	32.9a ±01.9	03.6a ±01.4	05.1b ±0.70

Mean in a row followed by the same letter were not significantly different at P≤0.05, by Tukey HSD test.

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