PREVALENCE OF *SALMONELLA* AND *ESCHERICHIA COLI* ASSOCIATED WITH DIARRHEA IN BUFFALO AND COW CALVES

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ABSTRACT

Calf diarrhea caused by *Salmonella* and *Escherichia coli* was the particular focus in the current study. A total 150 calves up to 6 months of age suffering from diarrhea were examined. Fresh fecal and blood samples were collected. Fecal samples were cultured on McConkey and on S-S agar for isolation of bacteria. Bacterial colonies were identified by using an API 20 E Kit. Overall prevalence of *Salmonella* and *E. coli* was 33.3%. Prevalence of *Salmonella* and *E. coli* was 18.6% and 14.6%, respectively. Occurrences of diarrheal disease due to various risk factors like age, sex were recorded for determining the effect of these risk factors.

Keywords: buffalo, *Bubalus bubalis*, cow, *Bos taurus, Escherichia coli, Salmonella*, diarrhea

INTRODUCTION

Major causes of mortality of neonatal calves were conditions like diarrhea and pneumonia (Khan *et al*., 2009, Shimizu and Nagatoma 1978). The neonatal calf mortality in the first month of age is more than 80% of the total mortality in calves (Jenny *et al*., 1981). *Salmonella* and *Escherichia coli* are known to be the most common and economically important agents (Acha *et al*., 2004; House *et al*., 1978). *Salmonella typhimurium* is a major cause of calf morbidity and mortality in the United States and in Europe (Tsolis *et al.,* 1999; Rice *et at.,* 1997). Khan *et al*. (2009) found the prevalence of calf diarrhea due to *Salmonella* was up to 16.3%. Calf diarrhea is a clinical sign of disease that may have many causes but *E. coli* has been considered as the primary bacterial cause in calves (Yamamoto and Nakazawa, 1997). The morbidity and mortality rates associated with many outbreaks of gastrointestinal diseases caused by *Escherichia coli* have highlighted the threat that these organisms pose to public health (Paton *et al*., 1998). The prevalence of enterohemorrhagic *Eschrechia Coli* O157:H7 in the feces of dairy calves and feedlot cattle is low. *Eschrechia coli* have been isolated from 0.3 to 2.2% of fecal samples collected from healthy calves or cattle in the United States, the United Kingdom, Germany, and Spain (Blanco *et al.*, 1993).

MATERIALS AND METHODS

Selection of site: The research was conducted at different private livestock farms and civil veterinary hospitals of district Jhelum. Total duration of the study was of three months.

Animals: A total number of 150 cow and

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buffalo calves under six months of age, suffering from diarrhea were studied for determination of *Salmonella* and *E. coli*.

Collection of samples: A fecal sample was collected directly from the rectum of each of the calves under six months of age. Then the fecal material was shifted to sterilized plastic containers and again those containers were placed in zip bags to prevent entry of foreign particles. Fecal material was kept in contact with ice to avoid further changes in fecal material.

Blood: Blood samples from the same individuals were collected from the jugular vein after applying disinfectants and immediately shifted into anticoagulant coated vaccutainer tubes.

Bacterial culture: One gram of fecal material was dissolved in 10 ml of autoclaved normal saline and cultured on MacConkey agar with the help of safety buds. Then it was incubated in an incubator for 24 h.The next day the colorless colonies were again cultured on S-S agar by dissolving those colorless colonies first in 5 ml autoclaved normal saline and then culturing on S-S agar with safety buds.

The API 20E commercial kit was used for the identification of *E. coli* and *Salmonella* (Popovic *et al*., 2004; API 20E Kit Biomerieux, France).

Blood parameters: Following blood parameters were compared according to Benjamin (1979): **(a) Total leukocyte count:** Total leukocyte counts were performed with the help of leukocyte diluting pipette. **(b) Differential leukocytic count: The** Giemsa stain blood smear method, was preferred for differential leukocyte count. **(c) hemoglobin estimation:** Sahli's method was used to determine the Hb level. **(d) Erythrocyte sedimentation rate:** Erythrocyte sedimentation rate was determined with a Wintrobe hematocrit tube. The upper level of sedimenting erythrocytes

sas read in millimeters on the left scale after the time interval of 8-24 h for cow and buffalo calves. **Seasonal effect:** The effect of season, environment, temperature, rainfall and humidity was observed. **Risk factors:** The effects of age, sex, breed, diet, managemental practices and vaccination were also studied.

Statistical analysis: The data collected were tabulated and analyzed statistically by chi-square test (Steel *et al.,* 1997).

RESULTS

Of the total of 150 cow and buffalo calves under six months of age studied with the signs of diarrhea in district Jehlum, the total number of *E.coli* positive samples was 22, and 28 were *Salmonella* positive. The prevalence of *E. coli* was 14.6%, and that of *Salmonella* was 18.6% The data were analyzed by chi-square test and value of P was greater than 0.05, which means no significant difference was found. The positive samples of *Salmonella* and *E. coli* in 1-30 day, 31-90 day, 91-180 day age groups were 3, 11, 14 for *Salmonella* and 2, 6, 14 for *E. coli*. Thus the prevalence of *Salmonella* and *E. coli* in 1-30 days, 31-90 days, and 91-180 were 2%, 7.33%, 9.33% for *Salmonella* and 1.33%, 4%, 9.33% for *E. coli*, respectively.

The value of P showed was less than the level of significance so there was no difference for the values of different age groups. The prevalence of positive bacterial samples in the age groups of 1-30 days 31-90 days 91-180 days was noted 14%, 23% and 63% for *E. coli* and 11%, 29% and 60% for *Salmonella*, respectively. The total number of positive samples in male calves was 18 and the prevalence was 12%, and in the case of female calves, the total number of positive samples was

32 and the prevalence was 21%.

Out of the total number of 150 diarrheic calves, the number of positive samples for cow calves was 31 and that for buffalo was 19. The prevalence of *Salmonella* and *E. coli* in cow and buffalo calves was 21% and 12%, respectively. The P value for the test was greater than 0.05 and data showed non-significant. Total numbers of positive samples of *Salmonella* and *E. coli* in cow calves was 0, 17, 14 for 1-30 days, 31-90 days, 91-180 days and for *Salmonella* were 6, 0, 13 for 1-30 days, 31-90 days, and 91-180 days. The prevalence of bacteria (*Salmonella* and *E. coli*) was 0, 11.33%, 9.33% for cow calves and 4%, 0, 8.67% for buffalo calves for the different age groups.

The prevalence in males in the 1-30 day age group was 0, that in those in the 31-90 day group was 5.33%, and that in those in the 91-180 daygroup was 7.33%. Similarly in females in the 1-30 daygroup, the prevalence was 4%; in the 31-90 daygroup, it was 5.33% , and in the 91-180 daygroup, it was 11.33%. The prevalence of *E. coli* in male calves was 0 %, 6%, 4.66% for the 1-30 day, 31-90 day, and 91-180 day age groups, and that for *Salmonella* was 0%, 0% and 3.33% for the 1-30 day, 31-90 day, 91-180 day groups. The prevalence of *E. coli* in female calves was 2%, 2%, 3% for the 1-30 day, 31-90 day, and 91-180 day, and that for *Salmonella* was 1%, 3.330% and 5.33% for the 1-30 day, 31-90 day, 91-180 daygroups as shown in Figure 1.

Blood parameters varied in the 1-30 day, 31-90 day, 91-180 day age groups as shown in Table 1. Total leukocyte count values in these groups were 9%, 19%, 13% respectively. Similarly, hemoglobin values were 14%, 13%, 14% erythrocyte sedimentation rates were 11%, 8%, 19%, neutronphil values were 19%, 35%, 26%, eosinophil values were 6%, 11%, 19%, basophil values were 13% 9%, 19%, lymphocyte values were 37%, 38%, 59%, monocyte values were 16%, 8%, 10% for the three age groups, respectively. The effect of season was observed for three months (July, August, and September). The prevalence rate was high in August and least in July. Rainfall increased the number of bacterial positive samples.

DISCUSSION

The prevalence of diarrhea due to bacteria and *E. coli* in overall calves was 33.3% in the present study. Although that is very high, a similar prevalence was also seen in Mozambique. In Mozambique, Baule *et al.* (1995) reported an overall prevalence of diarrhoeic calves as high as 36% but this percentage includes values of prevalences of diarrhoea from other farms not included in the present study. This might be due to an outbreak in a particular area in those days or due to outbreaks among different farms as sampling was done from certain private livestock farms as well. Among bacteria, enterotoxigenic *Escherichia coli* and *Salmonella* are known to be the most common and economically most important agents that cause diarrhea in young calves (House *et al.*, 1978).

The values according to different age groups showed that total leukocytes were greater in age group of 31-90 days, hemoglobin in the younger group of 1-30 days, erythrocyte sedimentation was greater in the 91-180 day age group, neutrophils were higher in the 31-90 day group, eosinophils, basophils and lymphocytes were greater in the oldest age group (91-180 days) and monocytes were highest in the youngest age group. In the current study, the average of the total leukocyte count value increased in the cow as compared to the buffalo calves. Similarly, the average of
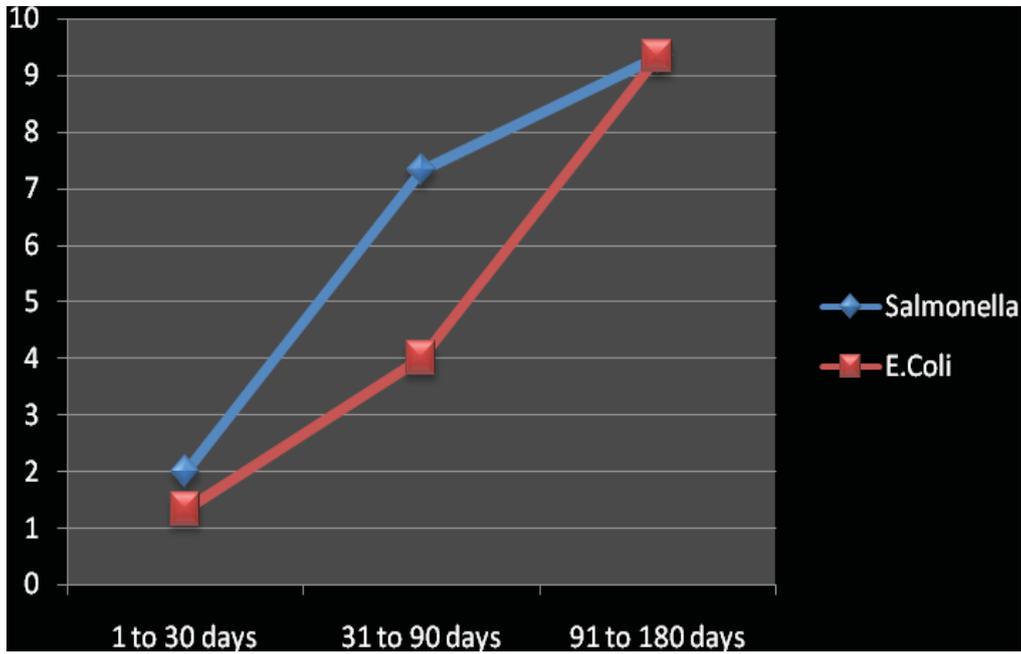
Figure 1. Prevalence of *Salmonella* and *E. coli* in calves in different age groups.

Table 1. Average values of blood parameters in cow calves according to different age groups.

Age	TLC	Hb	ESR	N	E	B	L	M
1-30 days	9%	14%	11%	19%	6%	13%	37%	16%
31-90 days	19%	13%	8%	35	11%	9%	38%	8%
91-180 days	13%	14%	19%	26%	19%	19%	59%	10%

hemoglobin was higher in the cow calves as compared to the buffalo calves, but the averages of erythrocyte sedimentation rate and neutrohils were higher in the buffalo calves than in the cow calves. Again. as far as basophiles, eosinophils and monocytes are concerned, the average values were greater in the cow calves than in the buffalo calves, but lymphocytes values were higher in buffalo calves than in cow calves.

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