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SOME OF THE RISK FACTORS OF NILI-RAVI BUFFALO (*BUBALUS BUBALIS*) NEONATAL CALF MORTALITY IN PAKISTAN

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ABSTRACT

This study was carried out to investigate neonatal calf mortality (NCM) in 864 Nili-Ravi buffalo calves born during 1993-2000 at the Livestock Production Research Institute (LPRI), Bahadurnagar, Okara, Pakistan. The overall mortality in buffalo neonates was 9.4%. The NCM in relation to birth weight, sex and age of neonatal calf showed non-significant difference. The highest mortality was recorded during 2^{nd} week of age (35.0 and 39.0%) and summer season (11.5 and 12.3%) in male and female buffalo neonatal calves, respectively. Mortality was also high in calves born to first parity dams (11.3%). Morbidity and mortality due to various disease conditions showed highly significant (P = 0.0001) difference. Neonatal calf diarrhoea (NCD) rendered the highest morbidity (16.6%) and mortality (5.2%), followed by pneumonia and pneumono-enteritis. Mange (3.4%), navel-ill (3.1%) and umbilical hernia (1.6%) lead morbidity only. Relative risk of morbidity (two and seven times) and mortality (three and eight times) was more in calves with intermediate and low immunoglobulins (Igs) concentration, respectively as compared to calves having high Igs concentration (P = 0.000).

Key words: Neonatal calf mortality, Nili-Ravi buffaloes, risk factors.

INTRODUCTION

Livestock play a vital role in the economy of Pakistan by providing essential items of human diet like milk and meat and other byproducts which account for 49.1% of agriculture value added and about 11.4% of the GDP. Buffalo population in the country is estimated as 25.5 million heads out of a total 134.1 million heads of livestock in the country and it contributes 67 and 28% of 28624 and 1810 thousand tones milk and meat production, respectively in the country (Anonymous, 2005). In view of increasing demand for milk and meat, more and more emphasis is being placed on the improvement of health and productive potentials of buffaloes. This objective can be achieved by increasing the number of calves successfully reared per dam in a given year. Neonatal calf mortality (NCM) is a major dilemma that makes this goal difficult (Khan and Khan, 1996a). Reduction in calf mortality can be achieved only by identifying and targeting its specific causes (Khan and Khan, 1995).

It is estimated that 20% NCM can reduce net profit by 38% (Radostits *et al.,* 2000). The NCM varies from 7 to 51% throughout world (Umoh, 1982; Khan and Khan, 1996a). The NCM in the first month of age is accounted for 84% of the total mortality (Jenny *et al.,* 1981) and is particularly high in the third week of life (Khan and Khan, 1996a). Mortality in neonatal calves has mostly been attributed to infectious agents like enteropathogenic *Escherichia coli* (EPEC), salmonella species, rotavirus, coronavirus, and cryptosporidium (Snodgrass *et al.,* 1986; Bendali *et al.,* 1999). Other important causes of NCM include immunodeficiency, seasonal effects, difficult parturition and faulty management conditions (Khan and Khan, 1996b). Moreover, the health and nutritional status of the dam can also influence calf mortality. Neonatal calf diarrhea (NCD) is a major cause of NCM accounting for 24 to 63% in buffalo calves in Pakistan (Khan and Khan, 1996a). Diarrhea in infancy results in future reduction of meat volume (20%) and dairy production (18%) (McDowell *et al.,* 1995). Delayed growth, reproductive development and the percentage of infertility is twice high in cattle having diarrhea in infancy. In addition the general, non-specific resistance of animal is reduced, facilitating the other diseases. Since work on the factors affecting NCM is limited in Pakistan, the objective of this study was to identify the major causes and risk factors contributing to NCM in Nili-Ravi buffalo calves in Pakistan.

MATERIALS AND METHODS

This study was carried out on 864 Nili-Ravi buffalo neonatal calves, comprising 416 females and 448 males, born alive from 1993 to 2000 at the Livestock Production Research Institute (LPRI), Bahadurnagar, Okara, Pakistan. Prior and post parturition (2–3 days), each dam was monitored closely and it was ensured that the neonatal calf had ingested colostrum within the specified period (first 10–12 hours of life). After parturition, the neonates were allowed to stay with their dams for four days and later transferred into separate sheds. Thereafter, whole milk @ 10% of the body weight was fed to calves through bottle. At the age of two weeks, in addition to milk, supplements of calf starter ration, green fodder and salt lick were also provided.

Parity of dam, sex and birth weight of calves were recorded. All dams and calves were kept under identical feeding and management conditions. The dams were housed together in sheds covered on three sides with the fourth side open. In the winter, the open side was covered with curtains to protect dams and calves from the cold. Dams were offered seasonal green fodder (*Trifolium alexandrinum* - Barseem; *Brassica compestris* –Sarson; *Zea mays* – Maize and *Sorghum vulgare* – Sorghum). Wheat straw (*Triticum aestivum*) was mixed with green fodder. No concentrate or fodder was provided in the shed at night, however, water was available *ad libitum*. The dams were vaccinated against foot and mouth disease and haemorrhagic septicemia and dewormed twice a year.

Blood samples without anticoagulant were collected randomly from 200 neonatal calves 24–36 hours after colostrum feeding. Serum was separated and stored at –20˚C for the determination of immuno-globulins (Igs) by glutaraldehyde coagulation test (Blom, 1982). Briefly, to prepare test reagent, 25% glutaraldehyde solution (4 mL) was mixed in distilled water (6 mL). In a separate test tube, test reagent (50 µL) and serum (0.5 mL) were mixed and results were recorded for complete (within 15 minutes), incomplete (15-59 minutes) or negative (after one hour) coagulation indicating high (>1.7 g/dL), intermediate (0.41-1.69 g/dL) or low (<0.4 g/dL) Igs concentration, respectively.

The health of all neonatal calves under study was monitored twice daily up to neonatal life (30 days). Clinical signs of any disease were recorded. Diagnosis of the disease conditions was based on the parameters as described earlier (Radostitis *et al.,* 2000). Morbidity and mortality due to disease condition was calculated. To determine relationship of mortality with season, year was divided into four seasons i.e., summer (May 1 to September 15), autumn (September 16 to November 14), winter (November 15 to February 15) and spring (February 16 to April 30). Data collected were subjected to an appropriate test (Chi-square test or analysis of variance) on personal computer using the Minitab statistical software package (Anonymous, 2003).

RESULTS

The overall mortality in buffalo neonates was 9.4% (Table 1). Data analysis revealed non-significant difference in mortality between male and female buffalo neonates ($\chi 2$ value = 0.181; *P* = 0.671). The NCM in relation to birth weight in male and female neonates showed non-significant difference (Table 2). The highest mortality was recorded during 2nd week of age with non-significant difference between male and female neonates (Table 3). Though the mortality was high during summer but statistical analysis revealed non-significant difference ($\chi 2$ value = 4.723; *P* = 0.193 (Table 4). The mortality in relation to parity also showed non-significant difference (Table 5).

Table 1: Prevalence of mortality among buffalo neonates in relation to sex

Sex	Total birth	Mortality	
		n	%
Male	448	40	8.9
Female	416	41	9.9
Overall	864	81	9.4

$\chi 2$ value = 0.181; P = 0.671

Table 2: Birth weight (kg) in relation to sex and survival/mortality of buffalo neonates

Survival Status	Male		Female	
	n	**Mean (± SD)**	***n***	**Mean (± SD)**
Survived	408	37.5 ± 3.8a	375	36.6 ± 2.3a
Died	40	37.2 ± 2.4a	41	35.7 ± 1.9a

Values in a row and column with same letters differ non-significantly (P>0.05).

Morbidity ($\chi 2$ value = 176.218; *P* = 0.000) and mortality ($\chi 2$ value = 17.950; *P* = 0.0001) due to various disease conditions showed highly significant difference. NCD rendered the highest morbidity (16.6%) and mortality (5.2%), followed by pneumonia and pneumono-enteritis (Table 6). Mange, navel-ill and umbilical hernia lead morbidity only.

Relative risk of morbidity was two and seven times more in calves with intermediate and low Igs concentrations as compared to calves having high Igs concentration ($\chi 2$ value = 27.111; *P* = 0.000). Similarly, calves with intermediate and low Igs concentrations were three and eight times more liable to mortality as compared to calves having high Igs concentration (Table 7).

DISCUSSION

Number of calves successfully reared in a given year is a measure of success in dairy farming. However, NCM is a major obstacle adversely affecting the survival of calves. According to an estimate, 20% calf mortality results in 38% reduction in net profit (Radostitis *et al.,* 2000), causing considerable economic

Table 3: Mortality in neonates in relation to age among buffalo neonatal calves

Week	Male (n = 40)		Female (n = 41)		χ2 value	P value
	n	%	*n*	%		
1	11	27.5	10	24.4	0.060	0.807
2	14	35.0	16	39.0	0.065	0.799
3	9	22.5	8	19.5	0.071	0.790
4	6	15.0	7	17.1	0.047	0.829

Week-wise mortality overall (χ2 value = 6.116; df = 3; P = 0.106) and also with sex (male χ2 value = 2.737, DF = 3, P-Value = 0.434 and female χ2 value = 3.619, DF = 3, *P*-Value = 0.306) differ non-significantly.

Table 4: Mortality in buffalo neonates in relation to season

Season	Male			Female			χ2 value	P value
	Births	Died		Births	Died			
		n	%		*n*	%		
Summer	192	22	11.5	203	25	12.3	0.054	0. 816
Autumn	113	7	6.2	82	6	7.3	0.084	0.772
Winter	96	7	7.3	109	8	7.3	0.000	0.990
Spring	47	4	8.5	22	2	9.0	0.005	0.942

Overall season-wise mortality also differ non-significantly (χ2 value = 4.723; df = 3; *P* = 0.193).

Table 5: Neonatal calf mortality in relation to parity of dams

Parity	Male			Female			Overall		
	Births	Died		Births	Died		Births	Died	
		n	%		*n*	%		*n*	%
1	104	11	11.0	100	12	12.0	204	23	11.3
2	89	8	9.0	92	8	10.4	181	16	8.8
3	76	7	9.2	72	7	9.7	148	14	9.5
4	64	5	7.8	43	4	9.3	107	9	8.4
5	41	3	7.3	28	3	10.7	69	6	8.7
6	21	2	9.5	31	3	9.7	52	5	9.6
7	22	2	9.1	21	2	9.5	43	4	9.3
8	13	1	7.7	13	1	7.7	26	2	7.7
9	13	1	7.7	10	1	10.0	23	2	8.7
10	5	0	-	6	-	-	11	-	-
Overall	448	40	8.9	416	41	9.9	864	81	9.4

Overall parity-wise mortality up to 6th revealed non-significant difference (χ2 value = 0.838; df = 5; *P* = 0.975). Data in above 6th parities could not be analysed because these parities were having expected counts less than 1.0, therefore, Chi-Square approximation probably became invalid.

losses directly to the farmer and indirectly to the country and also results in of milk and meat supplies. In the present study, the occurrence of NCM in buffalo neonates was 9.4%. However, a much higher mortality (13 and 34%) has been reported earlier (Sharma *et al.,*1984; Bhullar and Tiwana, 1985). The differences could be due to variations in environmental factors and management conditions prevailing at the respective sites of these studies. Moreover, some studies might have specifically concentrated on farms with high mortality.

In the present study, non-significant difference in mortality between female and male neonates was recorded which has also been reported by others (Umoh, 1982; Maarof *et al.,* 1987). Reports on higher mortality in female than in male neonates (Sharma et *al.,* 1984; Thurmond, 1986) or vice versa (Debnath *et al.,* 1990) are also available. At LPRI, no discrimination of sex is carried out, both male and female neonates are reared together under similar management conditions, therefore, the difference in mortality in relation to sex was not recorded.

Table 6: Morbidity and mortality of buffalo neonates (n = 864) due to disease conditions

Disease condition	Morbidity		Mortality	
	n	%	*n*	%
Diarrhea	143	16.6	45	5.2
Pneumonia	93	10.8	15	1.7
Pneumo-enteritis	74	8.6	21	2.4
Mange	29	3.4	-	-
Navel-ill	27	3.1	-	-
Umbilical hernia	14	1.6	-	-
Data Analysis				
$\chi 2$ value	176.218		17.950	
P value	0.000		0.000	

Table 7: Relationship of immunoglobulins with mortality in buffalo neonatal calves (n = 200)

Parameters		Immunoglobulin concentration (g/dL)		
		> 1.7	**0.41-1.69**	**< 0.41**
Calves tested		145	25	30
Morbidity	No	14	5	20
	%	10.0	20.0	66.7
	Relative risk	1	2.0	6.7
Mortality	No	7	4	11
	%	4.8	16.0	36.7
	Relative risk	1	3.3	7.7

Data analysis by Chi-square revealed a significant difference in morbid ($\chi 2$ value = 27.111; df = 2; P = 0.000) and died ($\chi 2$ value = 18.427; df = 2; P = 0.000) neonates at various concentrations of immunoglobulins.

In the present study, NCM was the lowest during 1st week and increased in 2nd week of life (Table 3). This might be the effect of more frequent occurrence of diarrhea due to enteropathogenic *E. coli* (54.5%) and salmonella spp. (13.64%) in buffalo neonates during this period as compared to other weeks of neonatal life (Khan and Khan, 1997). Attaching and effacing *E. coli* may cause haemorrhagic colitis with blood in faeces of diarrheic calves at about 2 weeks of age with increased frequency (Stordeur *et al.,* 2000). The results of the present study are also substantiated by another study (Bendali *et al.,* 1999) by reporting 52% of diarrhea during the first week and only 15% after the second week of life.

Buffalo NCM is mainly attributed to NCD, pneumonia and pneumo-enteritis (Sharma *et al.,* 1984, Bhullar and Tiwana, 1985). In the present study, NCD rendered the highest mortality (5.2%), followed by pneumo-enteritis (2.4%) and pneumonia (1.7%). NCD has a complex etiology. Many infectious agents and non-infectious factors contribute to the disease process (Khan and Khan, 1996a). Enteropathogenic *E. coli* is the major pathogen found in the faeces of diarrheic neonates (Khan and Khan, 1997). Rotavirus was reported to be second in prevalence in NCD, followed by coronavirus, salmonella spp. and cryptosporidium (Reynolds *et al.,* 1986). Various risk factors associated with increased incidence of diarrhea in neonatal calves include immaturity and lack of vigor of the calf at birth, the presence of intrapartum hypoxemia and acidosis from a difficult birth if it occurs and failure to acquire sufficient colostral immunity (Radostitis *et al.,* 2000).

The role of enteropathogenic *E. coli* in buffalo NCD is an established factor (Hafiz et al., 1994; Khan and Khan, 1997). Enteropathogenic *E. coli* adheres to the mucosa, proliferates in intestinal lumen and produces a potent enterotoxin, stimulating excessive fluid secretion (Khan, 1994). This loss of fluid from intestinal mucosa causes the principal sign (diarrhea) which often leads to dehydration. As a result of dehydration, acid-base imbalance, excessive loss of water and electrolytes and hypovolumea take place consequently resulting into shock and death (Radostitis *et al.,* 2000). According to Hudson and Johnson (1986), as much as 15 per cent loss of fluid can result in death.

In the present study, relative risk of morbidity and mortality was significantly higher in calves with intermediate and low Igs concentration as compared to calves having high Igs concentration. In this regard, White and Andrews (1986) reported that calves without adequate circulating Igs are nine times more likely to die and four times as likely to become ill than calves with adequate circulating Igs. The major predisposing factor for the development of diarrhea and other infectious diseases is the failure of the calf to acquire adequate passive immunity from colostrum as bovine calves are born agammaglobulinaemic and neonate ingest colostrum and absorb colostral Igs within 6-8 hours after birth to acquire protection against septicaemic and enteric collibacillosis (Khan and Khan, 1996a). Colostrum is not only an important source of Igs that the calf needs for passive immunity; it is also an important source of nutrients, vitamins, minerals, energy and proteins, which the calf needs to survive and prosper (Khan and Khan, 1996b). Colostrum must be fed to the neonate as early as possible after birth to maximize the acquisition of passive immunity. Maximum absorption of Igs occurs within first 6-8 hours after birth, therefore, this is the best time for colostrum feeding. The neonate may become hypo- or agammaglobulinaemic due to late feeding of colostrum, low concentration of Igs in colostrum or less quantity of colsotrum, early loss of absorption of Igs from the intestine and malabsorption. During absorption process, enteropathogenic *E. coli* and other gram negative organisms also gain entrance into the intestine if the environment/surroundings is not clean and not disinfected properly and bind to the intestinal receptors to whom Igs had to attach for absorption into the circulation. This is the possible mechanism by which malabsorption of Igs occurs and leads to NCD, ultimately death.

In conclusion, in the present study, NCM in buffalo neonates was 9.4%. Diarrhea rendered the highest morbidity and mortality, followed by pneumonia and pneumono-enteritis. Relative risk of morbidity (two and seven times) and mortality (three and eight times) was higher in calves with intermediate and low Igs concentration, respectively as compared to calves having high Igs concentration.

REFERENCES

Anonymous, 2003. Minitab Statistical Software, Minitab Release 13.20, Minitab Inc, USA.

Anonymous, 2005. Pakistan Economic Survey, 2004–2005, Govt. of Pakistan, Finance Division, Economic Adviser's Wing, Islamabad, Pakistan.

Bendali, F., H. Bichet, F. Schelcher and M. Sanaa, 1999. Pattern of diarrhoea in newborn beef calves in south-west France. Vet. Res., 30: 61-74.

Bhullar, M. S. and M. S. Tiwana, 1985. Factors affecting mortality among buffalo-calves. Indian J. Anim. Sci., 55: 599-601.

Blom, J. Y., 1982. The relationship between serum immunoglobulin values and incidence of respiratory diseases and enteritis in calves. Nord. Vet. Med., 34: 276-284.

Debnath, N. C., B. K. Sil, S. A. Selim, M. A. M. Prodhan and M. M. R. Howlader, 1990. A retrospective study of calf mortality and morbidity on smallholder traditional in Bangladesh. Prev. Vet. Med., 9: 1-7.

Hafiz, M. A. H., A. Khan, M. Z. Khan, M. A. Sabri and N. A. Naz, 1994. Bacteriology of neonatal calf diarrhea. Buffalo J., 10: 177-183.

Hudson, D., and J. Johnson, 1986. Management of diarrhoea in neonatal buffalo calves. Modern Vet. Pr., 67: 132-136.

Jenny, B. F., G. E. Cramling and T. M. Glaze, 1981. Management factors associated with calf mortality in South Carolina dairy herds. J. Dairy Sci., 64: 2284-2289.

Khan, A., 1994. Studies on the bacterial aetiopathology of mortality in buffalo and cow neonates, Ph D Thesis. Dept. Vet. Pathol., Univ. Agri. Faisalabad, Pakistan.

Khan, A. and M. Z. Khan, 1995. Epidemiological aspects of neonatal calf mortality in the Nili-Ravi buffalo (*Bubalus bubalis*). Pakistan Vet. J., 15: 163-168.

Khan, A. and M. Z. Khan, 1996a. Neonatal calf mortality in Pakistan. I: Prevalence and factors influencing mortality in buffalo and cow neonates. Buffalo J., 12: 219-229.

Khan, A. and M. Z. Khan, 1996b. Neonatal calf mortality in Pakistan. III: Immunoglobulins in relation to mortality in buffalo and cow neonates. Buffalo J., 12: 243-252.

Khan, A. and M. Z. Khan, 1997. Bacteria isolated from natural cases of buffalo and cow neonatal calf diarrhoea, pneumonia and pneumo-enteritis. Vet. Archiv, 67: 161-167.

Maarof, N. N., K. N. Tahir and R. A. Mahmood, 1987. Factors affecting mortality rate among Friesen calves in Iraq. Indian J. Anim. Sci., 57: 647-650.

McDowell, R. E., J. C. Wilk, S. K. Shah, D. S. Balain and G. H. Metry, 1995. Potential for commercial dairying with buffalo. North Carolina State University, pp: 64.

Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff, 2000. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9th Ed. W.B. Saunders, London, UK.

Reynolds, D. J., J. H. Morgan, N. Chanter, P. W. Jones, J. C. Bridger, T. G. Debney and K. J. Bunch, 1986. Microbiology of calf diarrhoea in southern Britain. Vet. Rec., 119: 34-39.

Sharma, M. C., N. N. Pathak, N. N. Hung, N. H. Lien and N. V. Vuc, 1984. Mortality in growing Murrah buffalo calves of Veitnam. Indian J. Anim. Sci., 54: 998-1000.

Snodgrass, D. R., H. R. Terzolo, D. Sherwood, I. Campbell, J. D. Menzies and B. A. Synge, 1986. Aetilogy of diarrhoea in young calves. Vet. Rec., 119: 31-34.

Stordeur, P., B. China, G. Charlier, S. Roels and J. Mainil, 2000. Clinical signs, reproduction of attaching effacing lesions, and enterocyte invasion after oral inoculation of an O118 enterohaemorrhagic *Escherichia coli* in neonatal calves. Microbes Infect., 2: 17-24.

Thurmond, M. C., 1986. Epidemiological approaches used in herd health practice to investigate neonatal calf mortality. Prev. Vet. Med., 4: 317-328.

Umoh, J. U., 1982. Relative survival of calves in a university herd in Zaria, Nigeria. British Vet. J., 138: 507-514.

White, D. G. and A. H. Andrews, 1986. Adequate concentration of circulating colostral proteins for market calves. Vet. Rec., 119: 112-114.