

PREVALENCE OF GROUP A BOVINE ROTA VIRUS IN NEONATAL CALVES IN PUNJAB, PAKISTAN

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ABSTRACT

Neonatal calf diarrhea is one of the main economic losses to dairy industry in developing countries. Study was planned to assess the prevalence of rotavirus diarrhea in Pakistan. Fecal samples (n=200) from cattle and buffalo diarrheic calves, age < 3 months were collected aseptically from ten districts of Punjab province selected on the basis of agro-ecological zones (canal fed, rain fed, desert and Pothohar). Commercially available ELISA kit was used for rotavirus detection. Results revealed 6% positivity including 3% from cattle calves and 4% from buffalo calves. District wise analysis showed higher prevalence (4%) in Lahore as compared to Faisalabad district (2%). Statistically significant (P < 0.05) correlation was found between diarrhea and age groups of calf showing < 1 month susceptible for diarrhea, similarly significant association was found among gender, housing type, feeding pattern and season. We could not establish any correlation between species. Statistical analysis showed significant correlation between diarrhea incidences and source of water as canal water, underground and tap water. The present findings revealed rotavirus infection in calves in Pakistan that needs to be addressed using vaccine and improving livestock management.

Key words: Neonatal diarrhea, Rotavirus, ELISA, Punjab, Pakistan.

INTRODUCTION

Rotavirus is known enteric pathogen, present all over the world, infecting animals and humans (Yamamoto *et al.* 2011). Poor hygienic conditions are considered predisposing factor of neonatal diarrhea, leading to severe dehydration followed by high mortality rate in developing countries (Alam *et al.* 2011). The disease occurs in two forms i.e. acute and sub-acute forms. Body fluid loss results in dehydration and loss of conditions results in cold ears and limbs, presence of a gap between inner lid and eyeballs well assunken eyes (Constable 2004; Lanz 2008).

Rotavirus is a member of the family *Reoviridae* of the viruses having eleven segmented RNA genome. Six structural proteins i.e. VP1, VP2, VP3, VP4, VP6 and VP7 and six non-structural proteins that range from NSP1-NSP6 are coded by the genome (Estes and Kapikian, 2007; Greenberg and Estes, 2009). On the basis of serological assays rotaviruses are categorized into seven different groups (A-G) on the bases of capsid protein VP4 and VP7 (Estes and Kapikian, 2006; Dennehy, 2008). Rotavirus serotype A is well documented to be the chief causative agent of acute gastroenteritis in infants (Dennehy, 2008). Based on molecular differences a total of 25 different G and 33 P genotypes have been presently described (Collins *et al.* 2010; Esona *et al.* 2009; Abe *et al.* 2011). G6, G8 and G10 are the most frequently found genotypes in cattle,

linked with P1, P5 or P11 (Fukai *et al.* 1999; Ghosh *et al.* 2008).

Rotavirus outbreaks devastatingly affect the buffalo and cattle calves population around the globe, neonatal mortality fluctuates from 9 to 64% which accounts for 84% of total mortality at first month of age (Jenny *et al.* 1981; Umoh *et al.* 1982; Khan *et al.* 1991). The maximum percentage of the mortality was 35 to 39 which was recorded at the age of two weeks and was 11.3% higher in the calves of the dams of first parity. (Zaman *et al.* 2006). Therefore, the present study was designed to explore the prevalence of bovine rotavirus among population of calves in Punjab.

MATERIALS AND METHODS

Samples for this study were collected from public and private sector farms of large ruminants from 10 districts of the Punjab selected on the basis of agro-ecological zones including canal fed area (Lahore, Faisalabad, Okara, Sahiwal, Sargodha, and Multan), Pothohar area: (Chakwal), rain fed area (Bhakkar and Bahawalnagar) and desert (Bahawalpur). The inclusion criterion for farm selection was on the basis of disease incidence in the farm and consent of the owner or public and private farm to study the diarrheic animals. Animal husbandry practices including the housing type provided to calves; open or closed, feeding pattern of diarrheic calves; dam colostrum or artificial milk and source of drinking water provided to calves; tap water, canal or

underground water, during early days of life were analyzed in this study for some association with rotavirus and overall diarrhea incidences.

A total of 10 samples (n=10) from each diarrheic, buffalo and cattle calves from all mentioned districts were collected. Rectal swabs and fecal material from live calves and morbid materials from dead calves were collected aseptically and transported to Quality Operations Lab (QOL), University of Veterinary and Animal Sciences, Lahore. Samples were processed for the presence of Bovine rotavirus as described by Shahrabadi and Sedeh 2010. Direct sandwich enzyme linked immune-sorbent assay (ELISA) was performed for antigen detection using commercial Rotavirus detection kit (Cypress Diagnostics, Hulshout, Belgium) according to the instructions provided by the manufacturer.

The SPSS 16 version software was applied on the epidemiological data collected in the current study. Chi square and odd ratio was used to analyze the data for association of rotavirus with risk factors like age, species, gender and season.

RESULTS

Epidemiological information reveals that rotavirus distribution is associated with some of factors like species, age, gender, feeding pattern, housing type, season and source of water. The findings in present study indicates that less than 22 days age is susceptible for calves to be infected with rotavirus and other diarrhea causing agents summarized in Table-1. The results indicate that among the rotavirus positive cases equal numbers were reported in calves with more than 22 days age group and less than 22 days age (50%). The number of diarrhea cases due to other agents remains high in calves with age less than 22 days (82.9%) in comparison of calves with age more than 22 days (17.0%). Statistically significant ($P < 0.05$) correlation was found between diarrhea and age groups of calf; age less than 1 month was more susceptible for diarrhea. Species-wise distribution of rotavirus cases in different geographical areas was not considered to be a risk factor for the

rotavirus or diarrhea incidences. The table-1 also indicates that among the cases in cattle highest numbers were reported in other agents of diarrhea category (50.5%) as compared to buffalo calf (49.4%). Hence the p-value remains insignificant reveals that there was no statistically significant association between diarrhea and species of calf. The gender remained a risk factor as male affected by rotavirus (58.3%) and with other agents (84.0%) were more in number as compare to female affected with rotavirus (41.7%) and other agents (15.9%). The results reveals significant association between diarrhea cases and gender of calf (chi square = 5.16, $P < 0.05$, CI = 95%, OR = 0.26). Male calves were on higher risk in all districts. Similarly, the housing type of calves showed some association with diarrhea cases as more cases were observed among rotavirus cases (83.3%) and other agents (51.5%) from calves rearing in open housing type as compared to close housing type where rotavirus and other agents cases (16.7%, 48.4%) were less in number. Overall, significant p-value indicates that there is statistically significant association between diarrhea cases and housing type.

The feeding pattern of calves indicates that diarrhea cases were associated with dam and artificial feeding to calves. Calves with artificial feeding showed higher rotavirus positivity (66.7%) than those fed from dam (33.3%) resulting significant p-value (Chi-square=8.17, $P < 0.05$, OR=0.19). Seasonality based analysis revealed significantly lower incidences (chi square = 4.2, $P < 0.05$, OR = 4.49) in summer season (16.2%) in comparison of winter season (83.3%). Statistical analysis showed significant correlation (chi square = 7.22, $P < 0.05$) with diarrhea incidences between calves offered canal water (41.7%) and calves offered underground and tap water (25% each).

District wise analysis showed 4% prevalence in Lahore district (3% in cattle calves and 5% in buffalo calves) and 2% in Faisalabad district (2% in cattle calves and 2% in buffalo calves).

Overall rotavirus prevalence was 6% from Punjab.

Table 1. Demographical factors associated with rotavirus distribution.

Factors	Parameter	Diarrhea		Chi-Square	p-value	Odd Ratio (OR)	Confidence Interval (CI-95%)
		Rotavirus	Others				
Age of calf	< 22 days	6 (50.0%)	156 (82.9%)	7.97	0.004	0.20	0.06-0.67
	>22 days	6 (50.0%)	32 (17.0%)				
Species	Cattle Calf	5 (41.7%)	95 (50.5%)	0.35	0.55	0.69	0.21-2.28
	Buffalo Calf	7 (58.3%)	93 (49.4%)				
Gender	Male	7 (58.3%)	158 (84.0%)	5.16	0.02	0.26	0.07-0.89
	Female	5 (41.7%)	30 (15.9%)				
Housing	Open type	10 (83.3%)	97 (51.5%)	4.5	0.03	4.6	1.0007-21.9

Type	Close type	2 (16.7%)	91 (48.4%)				
Feeding Pattern	Dam Feeding	4 (33.3%)	136 (72.3%)				
	Artificial Feeding	8 (66.7%)	52 (27.6%)	8.17	0.004	0.19	0.05-0.66
Season	Winter	10 (83.3%)	99 (52.6%)	4.2	0.03	4.49	0.9-21.07
	Summer	2 (16.7%)	89 (47.3%)				
Source of Water	Tap water	3 (25.0%)	12 (6.38%)				
	Canal water	5 (41.7%)	134 (73.4%)	7.22	0.02		
	Underground water	4 (25.0%)	42 (22.3%)				
Districts	Lahore	8 (66.7%)	12 (6.38%)	58.16	0.00001		
	Faisalabad	4 (33.3%)	16 (8.51%)				
	Others	0	160 (85.1%)				

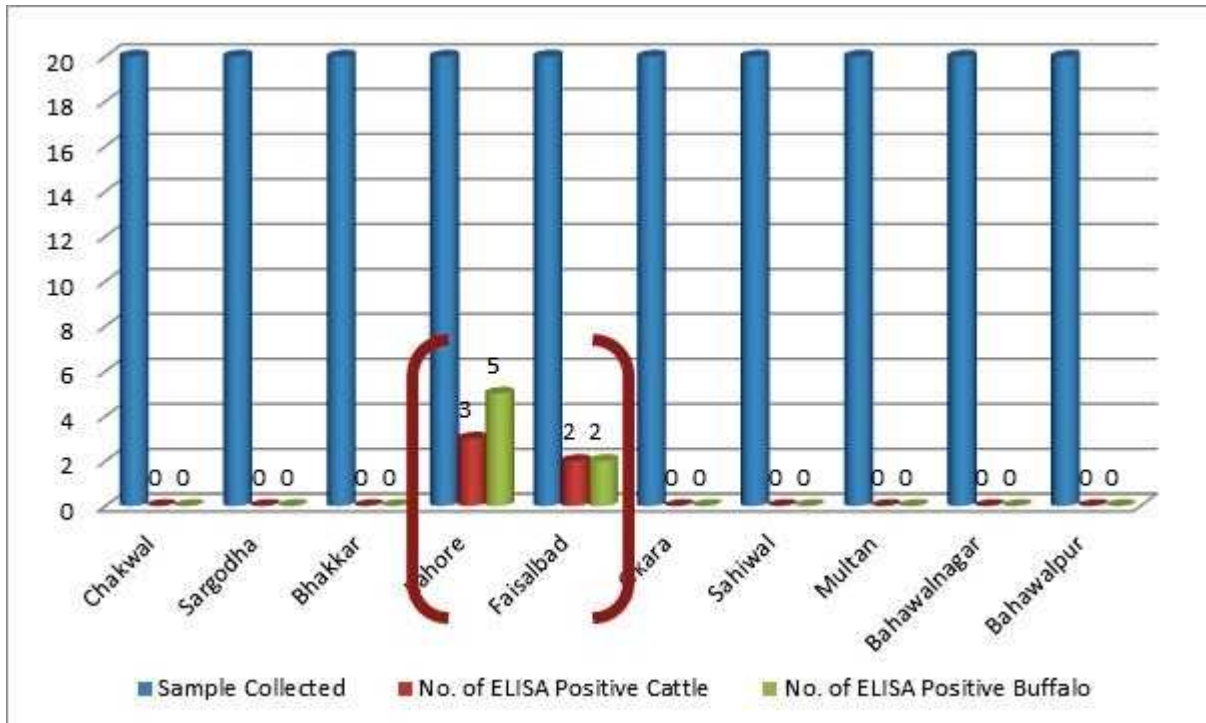


Fig: 1 Graphical presentation of positive Ag-capture ELISA Results

*Lahore – 3 Cattle Calf and 5 Buffalo Calf, Faisalabad – 2 each

DISCUSSION

Neonatal calf diarrhea is a prime disease affecting newborn calves and considered as important factor responsible for mortality (Lanz, 2008), leading to economic losses due to the treatment cost, diagnostic, and reduced growth performance (Constable 2004). Calves are more prone to diarrhea during early days of age after birth. In developing countries like Pakistan where livestock raising is the source of income for more than 8.0 million rural family's income (Economic Survey of Pakistan 2014-2015). Moreover, due to insufficient resources and unhygienic condition, animals suffer badly with neonatal calf mortality. Calf mortality at early ages varied from 8.7 to 64%, worldwide. As reported by Martin and Wiggins (1973) that 20% mortality at a livestock farm drastically reduces 38% farm

profits. Another study revealed that 84% neonatal calf mortality is accounted during first month (Jenny *et al.* 1981). Specifically, maximum mortality was recorded during 3rd week of first month after birth (Umoh. 1982). Mortality ratio in buffalo calves was found higher (39.8%) than that of cattle calves (29.1%) (Afzal *et al.* 1983). More or less similar findings were recorded in current study of higher percentage positivity in buffalo calves as compared to cattle calves, but statistically these results are not in accordance to the study of Niture *et al.*, 2010. Among numerous viral, bacterial and parasitic causative agents bovine rotavirus is leading cause of neonatal calf diarrhea in domestic animals. The cause of neonatal calf mortality is specifically related to Group A bovine rotaviruses (Estes and Kapikian 2007).

In present study, the rate of diarrhea among calves of less than 22 days age is significant in

comparison of increase in age as during the 1st month of life the calves are at highest risk of developing diarrhea, and the risk decreases with increasing age (Bendali *et al.*, 1999; Garcia *et al.*, 2000). In current study the significant findings on increased diarrheic cases among males proves the importance of female calves in the livelihood of poor people in comparison of male calf which was neglected and infected at early age with infectious agents. The open housing type showed more diarrhea cases in present study in comparison of close housing type where less number of animal/herd contact is observed. The present findings are in accordance of the study of Trotz-Williams *et al.* (2008b) where more infectious diarrheic cases were observed in open housing type.

The outcome that calves born during the winter months have the greatest risk of developing diarrhea is consistent with results from the study of Frank and Kaneene (1993), but is opposite to the results from Swedish studies (Svensson *et al.*, 2003, 2006). The increased risk of developing diarrhea during the winter months might be due to higher levels of causative agents involved in infection during this season because of higher animal density per housing unit, low ambient temperature, and higher humidity of inside air. A cool, humid environment favors the survival of numerous infectious agents for prolonged periods (Gulliksen *et al.*, 2009).

The antibodies of rotavirus in the colostrums and circulating blood are only important during the early days. After this the gut antibodies are the most important and feeding the small amounts of colostrums for a longer period may be beneficial to prevent rotavirus infection (Murphy *et al.*, 1999). The current findings are in accordance with these statements and increase diarrheic cases were observed in calves without colostrums feeding.

Source of water is important for calf health. The present study reveals that calves fed on canal water showing more diarrhea cases in comparison of underground and tap water. The canal water in the districts under study is usually more turbid, with low BOD and more bacterial contamination due to mixing of sewage water.

By using Cypress Diagnostics, Belgium kit; twelve samples were recorded as positive for bovine rotavirus from collected 200 diarrheic samples. According to the current results, the prevalence of bovine rotavirus was shown to be 6% in Punjab which included 4% from Lahore and 2% from Faisalabad districts. These results are higher than the reported 2% prevalence by (Khan *et al.* 2009) in Lahore. While the 6% prevalence of present study is much lower than 16.83% that was detected by Dash *et al.* 2011) and these results in accordance with 7.25% of another study by Manuja *et al.* 2008.

Conclusion: The present study concluded the prevalence of bovine rotavirus in diarrheic calve population in Punjab (6%). Healthy calves added in livestock will improve production level of this industry and finally support the national economy of country as most of the rural families generate their income through this sector. Therefore, measures should be adopted in future to minimize disease burden in this population and further studies are required regarding immunization.

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