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FREQUENCY of ROTAVIRUS and CORONAVIRUS in NEONATAL CALVES in KARS DISTRICT*

Hidayet Metin ERDOĞAN** Ahmet ÜNVER*** Vehbi GÜNEŞ** Mehmet ÇİTİL**

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Summary: This study was aimed at determining the frequency of rotavirus and coronavirus in neonatal calves in Kars district. A two stage stratified random sampling strategy was used to select localities and farms. The study involved weekly visits to the farms and clinical examination of neonatal calves. Faecal samples were collected from diarrhoeic and non-diarrhoeic calves between September 2001 and July 2002 for the detection of rotavirus and coronavirus using latex agglutination and ELISA tests, respectively. Incidence of diarrhoea was 28.8% in neonatal calves. The farm prevalence of rotavirus and coronavirus in neonatal calves was determined to be 31.1% and 2.2%, respectively. The incidence rates (animal-year/100) of rotavirus and coronavirus were found to be 4.5 and 0.2 in all herds and 8.9 and 2.3 in affected herds, respectively. Coronavirus was detected in one diarrhoeic calf (1%) while rotavirus was found in 19% of samples examined. Rotavirus was present in 26.9% of diarrhoeic calves and in 4.9% non-diarrhoeic calves. Calves diagnosed as diarrhoeic were more likely to be positive for rotavirus (Odd Ratio=6.8, P=0.008). Mean age of rotavirus positive cases was 6.5 days with the range of 1-19. Rotavirus was the most common in the first week of life and as the calves grew older rotavirus positivity decreased (P<0.001). Calves born in crowded herds (P=0.03) or grouped in large numbers (P=0.01) were more likely to be positive for rotavirus and as the number of calves in the herd increased the risk of rotavirus infection also increased (P=0.009). The results indicate that rotavirus and coronavirus should also be considered when evaluating the aetiology and epidemiology of neonatal calf diarrhoea in Kars.

Key words: Rotavirus, coronavirus diarrhoea, neonatal calves, Kars

Kars Yöresindeki Neonatal Buzağılarda Rotavirus ve Coronavirus Yaygınlığı

Özet: Bu çalışmada Kars yöresinde neonatal buzağılarda rotavirus ve coronavirusun yaygınlığının belirlemesi amaçlandı. Bölge ve çiftliklerin seçiminde iki aşamalı kotalı tesadüfi örnekleme metodu kullanıldı. «alışma kapsamında çiftliklere haftalık ziyaretler yapılarak neonatal buzağıların klinik muayeneleri gerçekleştirildi. Latex aglitünasyon ve ELISA kullanılarak rotavirus ile coronavirusun belirlenmesi için Eylül 2001 ile Temmuz 2002 tarihleri arasında ishalli ve ishalsiz buzağılardan dışkı örnekleri toplandı. Neonatal buzağılarda ishal insidansı % 28,8 olarak bulundu. Neonatal buzağılarda rotavirus ve coronavirusun çiftlik prevalansının sırasıyla %31,1 ve %2,2 olduğu belirlendi. Rotavirus ve coronavirusun insidans oranları (hayvan-yıl/100) tüm sürülerde sırasıyla 4,5 ve 0,2, etkilenen sürülerde ise 8,9 ve 2,3 olduğu bulundu. Coronavirus sadece bir ishalli buzağıda (%1) belirlenirken, rotavirus muayene edilen örneklerin % 19'unda bulundu. Rotavirus ishalli buzağıların % 26,9'unda ve ishalsiz buzağıların ise % 4,9'unda bulundu. İshalli olarak teşhis edilen buzağılarda rotavirus pozitivitesi daha yüksekti (Odds Ratio=6.8, P=0.008). Rotavirus yönünden pozitif vakaların yaşları 1-19 günler arasında değişti ve ortalama 6,5 günlüktü. Rotavirus çoğunlukla hayatın ilk haftası içinde görülürken, buzağılar yaşlandıkça rotavirus görülme oranı azaldı (p<0,001). Kalabalık sürülerde(P=0.03) doğan veya çok sayıda bir arada tutulan (P=0.01) buzağılarda rotavirus yönünden pozitif olma oranı daha yüksekti ve gruptaki buzağı sayısı arttıkça rotavirus görülme oranı da artmıştı (P=0.009). Sonuçlar rotavirus ve coronavirus'lerinde Kars yöresindeki neonatal buzağı ishallerinin etiyolojisi ve epidemiyolojisinin değerlendirilmesinde kayda alınması gerektiğini göstermektedir.

Anahtar Sözcükler: Rotavirus, Coronavirus, ishal, neonatal buzağı, Kars.

INTRODUCTION

Diarrhoea is one of the most important clinical problems of calves in all over the world where cattle husbandry is practiced. It inflicts considerable economical losses to the farmers and national economy at large through deaths, loss of genetic materials, treatment and poor performance later in life[1-4]. This is especially important where agriculture and livestock raising are the key economical activities as in Kars.

Epidemiological and aetiological studies have disclosed that bacteria, virus, parasite, nutritional and environmental factors, and management practices are implicated in the development of diarrhoea in calves as either primary cause or predisposing factors. Major micro-organisms and parasites implicated in neonatal calf diarrhoea as primary cause include *E. coli (enterotoxigenic- ETEC, verotoxigenic-VTEC), Campylobacter spp., Yersinia spp., Salmonella spp., Clostridium perfringens,* Rotavirus, Coronavirus, Gastrointestinal helmints, *Cryptosporidium spp.*, and *Eimeria spp.*[1-7].

Infections of neonatal calves with rotavirus and coronavirus have worldwide distribution and these viruses have been proved to play an important role in neonatal diarrhoea in variety of mammals especially at first weeks of their lives[8]. The overall prevalence rates of infection with rotavirus and coronavirus varied among studies and have been estimated within the range of 16%-80% and 11%-81%, respectively[1,9-13]. Studies carried out in different parts

* Funded by Scientific and Technical Research Council of Turkey, Project code: VHAG-1777
** Kafkas University Faculty of Veterinary Medicine, Department of Internal Medicine, Kars-TURKEY
*** Kafkas University Faculty of Veterinary Medicine, Department of Microbiology, Kars-TURKEY

of Turkey have found the prevalence of rotavirus and coronavirus infection in diarrhoeic calves very from 0 to 53%[14-18] and 13 to 18%, respectively[4,16].

Previous studies carried out on the aetiology of calf diarrhoea in Kars district revealed that bacterial (*E. coli, Campylobacter jejuni, and Salmonella typhimurim*) and parasitic (*Cryptosporidium parvum, Eimeria spp and Toxocara vitulorum*) agents were implicated in calf diarrhoea[6,7]. However, there has been no study investigating the presence of rotavirus and coronavirus in neonatal calves in Kars.

An epidemiological survey was, therefore, designed to determine the status of rotavirus and coronavirus in neonatal calves.

MATERIALS and METHODS

Farm selection: Seven localities (Çağlayan, Dikme, Kümbetli, Boğazköy, Çakmak, Başgedikler, Kars-Central) and 45 dairy farms within the District were selected using a two-stage stratified random sampling (simple lottery) strategy.

Study Design: This survey was carried out between September 2001 and July 2002 and involved weekly visits to the farms. All neonatal calves present at the time of visits were examined for any clinical abnormalities and faecal samples were collected from diarrhoeic neonatal calves. Where possible, for each diarrhoeic calf one non-diarrhoeic calf was also sampled.

Laboratory analyses: Faecal samples were analysed for the presence of rotavirus by means of a commercial latex agglutination test (Virotect-Rota, Omega Diagnostics, Alloa, Scotland, UK) and corona virus using commercial ELISA test (Bio-X Diagnostics, Marche-en-Famenne, Belgium). The tests were carried out and interpreted as instructed by manufacturers.

Data Analyses: Two estimates of the frequency of rotavirus and coronavirus in neonatal calves were calculated in this study; the farm level prevalence and the within herd incidence rate. To calculate the within herd incidence rate, the mean number of dairy cattle present (a total of 1052 dairy cattle) and number of calves born between September 2001 and July 2002 (a total of 624 calves) were used to estimate the number of animals at risk. The within herd incidence rate of rotavirus and coronavirus was calculated separately for affected herds and for all herds[19]. All data were numerically coded and entered on to a database (Microsoft access 2000) and analysed using Epi info 6[20]. Proportions obtained were compared using Yates corrected chi-square test and chi-square for trend. Kruskall-Wallis test was used to compare the means. The significance level was set at $p<0.05$[20].

RESULTS

A total of 624 calves born to 1052 dairy cattle between September 2001 and July 2002, were followed up on 45 dairy farms in 7 localities. Of the neonatal calves examined, 28.8% (180/624) had diarrhoea syndrome and 147 (104 diarrhoeic and 43 non diarrhoeic) of these calves were faecal sampled for the presence of rotavirus and coronavirus.

The proportion of farms affected by rotavirus and coronavirus was 31.1% and 2.2%, respectively. The within herd incidence rates of rotavirus and coronavirus for all herds and affected herds were 4.5 (28/624) and 8.9 (28/314), and 0.2 (1/624) and 2.3 (1/44), respectively (Table 1).

Coronavirus was detected in only one diarrhoeic calf (1%, 1/104) while the proportion of neonatal calves positive for rotavirus was 19% (28/147). Rotavirus was present in 26.9% (26/104) of diarrhoeic calves and in 4.9% (2/43) non-diarrhoeic calves. Calves diagnosed as diarrhoeic were more likely to be positive for rotavirus (Odd Ratio=6.8, P=0.008) (Table 2).

The frequencies of rotavirus in neonatal calves in different localities are given in the table 3. The proportion of rotavirus positive animals was the lowest in Başgedikler (9.7%, 3/31) and the highest in Kümbetli (36.4, 4/11). However, the differences between the localities were not statistically significant (P=0.47) (Table 3).

Table 1. Farm prevalence and incidence rate of rotavirus and coronavirus infection in neonatal calves.
Tablo 1. Neonatal buzağılarda rotavirus ve coronavirus enfeksiyonlarının çiftlik prevelansı ve insidans oranları.

Rotavirus	Farm Prevalence	Incidence rate (animal-year/100)	
	%	All herds	Affected herds
Rotavirus	17/45 (31.1)	28/624 (4.5)	28/314 (8.9)
Coronavirus	1/45 (2.2)	1/624 (0.2)	1/44 (2.3)

Table 2. Relationship between diarrhoea and presence of rotavirus
Tablo 2. Rotavirus varlığı ile ishal arasındaki ilişki.

		Diarrhoeic	Non-diarrhoeic	OR (95% CI)
Rotavirus	Present	26	2	
	absent	78	41	
Rotavirus	Present	28/104 (26.9)	2/43 (4.9)	6.8 (1.46-43.9)*

*P= 0.008

Mean age of neonatal calves positive for rotavirus was 6.5 days with the range of 1-19. Age distribution of rotavirus positive cases revealed that rotavirus was the most common in the first week of life and that there was an inversely proportional relationship between the age of calves and rotavirus positivity, as the calves grew older rotavirus positivity decreased. (P<0.001) (Table 4).

Investigation of the effect of stock density on the presence of rotavirus revealed that farms with higher herd size were more likely to have rotavirus (P=0.03). This was also the case for calves (P=0.01) (Table 5a).

Number of calves born between September 2001 and July 2002 was categorised as herds of <10 calves, 11-20 and >20. It was noted that as the number of calves in the herd increased the prevalence of rotavirus increased (P=0.009) (Table 5b).

Table 3. Regional frequency of rotavirus infection in neonatal calves.
Tablo 3. Neonatal buzağılarda rotavirus enfeksiyonlarının bölgesel yaygınlığı.

Localities	Overall frequency (%)	Frequency in Diarrhoeic cases(%)	Frequency in Non-diarrhoeic cases (%)
Çağlayan	3/14 (21.4)	3/10 (30)	0/4 (0)
Dikme	4/17 (23.5)	3/13 (23.1)	1/4 (25)
Kümbetli	4/11 (36.4)	4/9 (44.4)	0/2 (0)
Boğazköy	2/15 (13.3)	2/13 (15.4)	0/2 (0)
Çakmak	5/23 (31.7)	4/16 (25)	1/7 (14.3)
Başgedikler	3/31 (9.7)	3/20 (15)	0/11 (0)
Kars-Central	7/36 (19.4)	7/23 (30.4)	0/13 (0)
Kars-Central	28/147 (19)	26/104 (26.9)	2/43 (4.9)

Table 4. Age distribution of rotavirus positive cases.
Tablo 4. Rotavirus pozitif olguların yaş dağılımı.

Age	Rotavirus positive	Percentage	OR*
0-7[R]	18/28	64.3	1.00
8-14	7/28	25	0.19
15-21	3/28	10.7	0.07
>21	0/28	0	0.00

[R] Reference value. X^2 for trend. OR adds ratio. P<0.001

Table 5a. Relationship between herd size and farm prevalence of rotavirus in neonatal calves.
Tablo 5a. Neonatal buzağılarda sürü büyüklüğü ile rotavirus'ların çiftlik prevelansı arasındaki ilişki.

	Dairy cattle herds Mean (range)	Dairy calf herds Mean (range)
Rotavirus present	31.3 (7-100)	19.7 (7-44)
Rotavirus absent	18.6 (6-50)	12.7 (4-25)
X^2	4.4	6.4
P value	0.03*	0.01*

X^2chi square valuse. * significant difference between the means in the same column.

Table 5b. Relationship between herd size and farm prevalence of rotavirus in neonatal calves
Tablo 5b. Neonatal buzağılarda sürü büyüklüğü ile rotavirus'ların çiftlik prevelansı arasındaki ilişki.

	Herd size	Rotavirus present	Rotavirus absent	OR*	P valuex
	>10[R]	2	14	1.00	
Dairy calves	11-20	10	11	6.36	
	>21	5	3	11.67	0.009

[n] number of animals, [R] reference category, [x] x^2 for trend, OR, Odds ratio.

DISCUSSION

This study provided descriptive epidemiological data on coronavirus and especially rotavirus in neonatal calves in Kars district. It added valuable information to the previous studies in the district in terms of the aetiology of calf diarrhoea[6,7].

Rotavirus and coronavirus are ubiquities in the farm environment and their role, alone or in conjunction with other enteropathogens, in the aetiology of diarrhoea have well been established[8]. The present study revealed that there was a positive relationship between diarrhoea and existence of rotavirus. This is in agreement with the previous reports[9,10] and may suggest that rotavirus may have played a causative role in the development of neonatal calf diarrhoea in the district.

This study also revealed that a considerable proportion of farms were affected. The farm prevalence figure for rotavirus obtained in this study was higher than that found for Canadian herds (19%)[21] but lower than those of Ohio herds (63.8%)[22]. The incidence rates determined for rotavirus and coronavirus in this study were 8.9% and 2.3%, respectively. These figures were comparable to those reported from other parts of the world[10-13]. As for diarrhoeic cases, the proportion of rotavirus positive samples (25%) determined in this study fell within the overall frequency rates reported from other parts of the world[10-13] and Turkey[4,14-18] Interestingly, only one diarrhoeic calf was positive for coronavirus (1%) this is considerably lower than the figures reported from other parts of the world[1,11,12] and Turkey[4,16]. The differences in farm prevalence, incidence rate and proportion of positive samples among the studies may be attributed to different diagnostic methods used and farm management practices exercised in different regions.

Mean age of rotavirus positive calves in this study was similar to that of 6.1 days reported by McNulty et al.[23] but lower than that of 12.7 days and 13.6 reported

by Burgu et al.[14] and Alkan[16]. It is well known that rotavirus is commonly encountered in calves under 10 days old. This was also supported by inversely proportional relationship between age of calves and presence of rotavirus. Although contradicting to the findings of Garcia et al.[24] and Eskiizmirliler et al[4] a significant proportion of cases (64.7%) were less than 1 week old and the positivity of rotavirus decreased to none as calves grew older. Age predisposition of calves has been associated with immune state of the calves in first week of their life[25].

Larger number of dairy cattle and calves born to them in the herd was positively associated with farm prevalence of rotavirus. Higher stock density has already been associated with increased risk of diarrhoea in calves[3]. This relationship may be explained by the factors required for an epidemic to occur such as the presence of the infectious agent in a sufficient quantity (infectious pressure), sufficient proportion of susceptible animals with close contact, and host resistance[26]. Healthy adult cattle and calves have been reported to excrete rotavirus in their faeces[8] contributing to infectious pressure in crowded herds through contaminated farm environment. Animals are more likely to be in close contact and expose to infectious agents in larger herds or groups and are exposed to more stress. Infectious pressure, close contact with a contaminated environment and weak host resistance due to stress and other presumptive factors associated with crowded herding are important factors for an epidemic to occur in herds where calves and their dams are housed together as in Kars[27].

This study identified some descriptive epidemiological data on the presence of rotavirus and coronavirus in neonatal calves but more detailed studies are required to understand the dynamics of rotavirus and coronavirus infection in Kars.

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