Full Article

A Case-Control Study of Association between Diarrhea in Newborn Calves and Infection with *Rotavirus* and *Coronavirus* in Some Industrial Dairy Herds of Mashhad Aarea, Iran in 2008

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ABSTRACT

A 1:1 matched case-control study of calves under 1 month of age was carried out by weekly visits to 7 dairy farms in Mashhad from May 2008 to October 2008. Fecal samples were collected from a total of 112 calves with clinical signs of diarrhea and from 112 matched animals without clinical signs of diarrhea as assessed by a scoring system. The samples were investigated for the presence of *Rotavirus* and *Coronavirus* by a commercial antigen capture ELISA test. *Rotavirus* antigen was detected in 29.5% and 17% of diarrheic and non-diarrheic calves, respectively and *Coronavirus* antigen was detected in 2.7% and 1.8% of diarrheic and non-diarrheic calves, respectively. Among diarrheic calves *Rotavirus* was the most common in the third week of life and *Coronavirus* was detected only in some cases of second week of life. The excretion of *Rotavirus* in the feces of scouring calves was significantly higher than in non-diarrheic calves (P value =0.03, odds ratio = 1.9 (1.05 – 3.76)), but there was not any significant association between shedding of *Coronavirus* in the feces and diarrhea (P value =0.66, odds ratio = 1.4 (0.24 –9.05)). These results indicate that in these industrial dairy farms in Mashhad, infections by *Rotavirus* can be considered as an important cause for newborn calf diarrhea.

Keywords: *Rotavirus, Coronavirus,* matched case-control study, newborn dairy calves, diarrhea, Iran.

INTRODUCTION

Diarrhea is one of the most serious diseases of neonatal calves. It causes major economic losses due to mortality, poor growth and the costs of treatment. It is a complex, multifactorial disease that is affected by the intrinsic characteristics of the calf, its nutritional and immunological status, management of the herd, environment and various infectious agents (Bendali *et al* 1999b). Calves are at the greatest risk of developing diarrhea in the first month of life and the incidence of

diarrhea decreases with age (Frank & Kaneene 1993, Bendali *et al* 1999b).

Coronavirus and *Rotavirus* are the most common viruses involved in neonatal calf diarrhea (Almeida *et al* 1978, Dea *et al* 1980, Reynolds *et al* 1984, Carman & Hazlett 1992) and it is not unusual for both viruses to concomitantly infect calves (Torres-Medina *et al* 1985, De La fuente *et al* 1998). *Rotavirus* genus belongs to the *Reoviridae* family and viruses in this genus are classified serologically in seven distinct groups (A–G) and most of rotaviruses detected in clinical specimens of domestic animals including calves, belong to group A (Flewett & Woode 1978, Theil *et al* 1995) although isolates belonging to groups B and C have been described (Lucchelli *et al* 1992). *Coronavirus* genus belongs to the Coronaviridae family and Bovine *Coronaviruses* is the cause of severe diarrhea, especially in neonatal calves and is associated with winter dysentery (WD) in adult cows, along with severe diarrhea (Cho *et al* 2001, Parreno *et al* 2001, Jeong *et al* 2005). A previous study in beef calves describes that Bovine *Coronavirus* infection was more frequent among calves of up to 30 days of age (Quinn 2002). After an agent has been identified, sometimes it is difficult to determine whether it is responsible for the diarrhea in a population, because most of the agents are also found in healthy calves (Saif & Smith 1985, Fagan *et al* 1995). Since no documented information were available on *Rotavirus* and *Coronavirus* infection status and *its* association with diarrhea among newborn calves in industrial dairy farms around Mashhad (Iran), we devised a matched case-control study to investigate the relationship between diarrhea and excretion of *Rotavirus* and *Coronavirus* in feces and also to determine the prevalence of these viruses in diarrheic newborn calves in some industrial dairy farms in the area.

MATERIALS AND METHODS

Study population. The study was done in the Mashhad area, the capital city of the Khorasan Razavi province. A convenient sample of 7 dairy farms was selected in Mashhad (Table 1). The breed of cows in all of these farms was Holstein/Friesian. In all farms calves were separated from their dams after they received colostrum and were housed in individual pens until weaning.

Sampling technique. All farms were visited weekly from May 2008 to October 2008 (A 6 months period: time available for authors) and fecal samples were collected. In each visit, all calves under 1 month age with sign of diarrhea were sampled, provided that they had not received prior treatment with antibiotics. Fecal consistency was scored on a 4-point scale as described by Larson et al. (1977). An animal with a score of 3 or 4 was considered a case of diarrhea and an animal with a score of 1 or 2 was considered as control.

For each case calf, a corresponding calf with normal feces (with a fecal score of 1 or 2), closest in age (with a maximum age difference of 4 days) on the same farm was sampled as control. Whenever an animal was sampled, a form was completed with relevant information. At the end of six months of sampling, 112 pairs of calves were sampled; each pair was consisted of a diarrheic calf and a non-diarrheic calf.

Samples from control animals were discarded whenever they became diarrheic within one week of sampling and, consequently, the match (consisting of one diarrheic and one control calf) was not used in the analysis. Fecal samples were collected directly from the rectum by stimulating the anus with fingers and all samples were transported on ice to the virology laboratory of school of veterinary medicine of Ferdowsi university of Mashhad at the same day. Samples were stored at -20°C until processing.

ELISA test. All the fecal samples were tested for the presence of *Rotavirus* and *Coronavirus* by a commercial antigen capture ELISA kit (Duo Digestive Easy ELISA Kit. BIO K 347, Bio-X Diagnostics, Jemelle, Belgium). The ELISA was performed according to the manufacturer's instructions.

Optical densities were measured with an ELISA reader (ELx800™ Absorbance Microplate Reader) at

450 nm filter absorbance, according to the recommendations in the test procedure. Samples giving values equal to or greater than 0.15 were accepted as positives and values less than 0.15 as negatives.

Statistical analysis. A conditional logistic regression (CLR) for matched sets was performed for *Rotavirus* and also *Coronavirus* separately. The analysis consisted of an l-to-l matching with two covariates (parity of the dam and gender of the calf). Predictive (independent) variables were Rotavirus and Coronavirus. We entered 2 covariates in our analysis (genus and parity of the dam) because we had not used them in matching and theses factors can have a role as confounding factors.

The chi-squared test was used to assess the significant difference of shedding of *Rotavirus* in the feces among different age groups of calves.

P values less than 0.05 was considered to be statistically significant and data were analyzed with SAS statistical software (Version 9.2).

RESULTS

Fecal samples were collected from 112 pairs of calves, in each pair one calf was diarrheic and another one was non-diarrheic. There were calves which shed *Rotavirus* in their feces in all farms but there were calves which shed *Coronavirus* in their feces in only 2 dairy farms (Figure1).
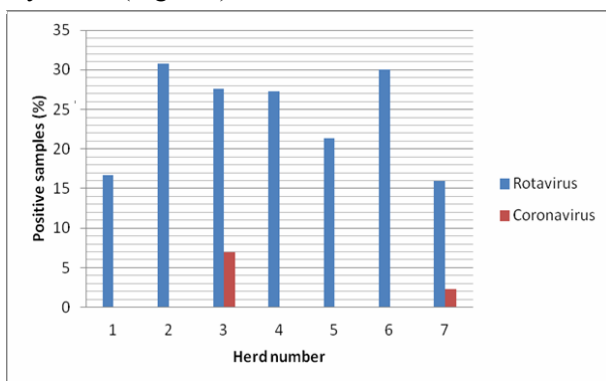

Figure 1. Frequency of *Rotavirus* and *Coronavirus* infection in all calves in different herds.

The average age of a scouring calf was 12.98 ±8.04 days (median 11 days with a range of 1 to 30 days) and for control animals, 13.46 ±8.06 days (median 12 with a range of 2 to 30 days). Among all animals sampled (224 calves include 112 diarrheic and 112 non-diarrheic animals), 52 calves were positive for *Rotavirus* (prevalence of *Rotavirus* shedding in feces in all calves: 23.21%) and 5 calves were positive for *Coronavirus* (prevalence of *Coronavirus* shedding in feces in all calves: 2.28%). *Rotavirus* infection was detected in 33 out of 112 diarrheic (prevalence in diarrheic calves: 29.5%) and 19 out of 112 non-diarrheic calves (prevalence in non-diarrheic calves: 17%). *Coronavirus* infection was detected in 3 out of 112 diarrheic (prevalence in diarrheic calves: 2.7%) and 2 out of 112 non-diarrheic calves (prevalence in non-diarrheic calves: 1.8%). Of 112 pairs of calves sampled, 28 (25%), 48(42.85%), 14 (12.5%) and 22 (19.64%) pairs were in the age groups 1-7, 8-14, 15-21 and 22-30 days old, respectively.
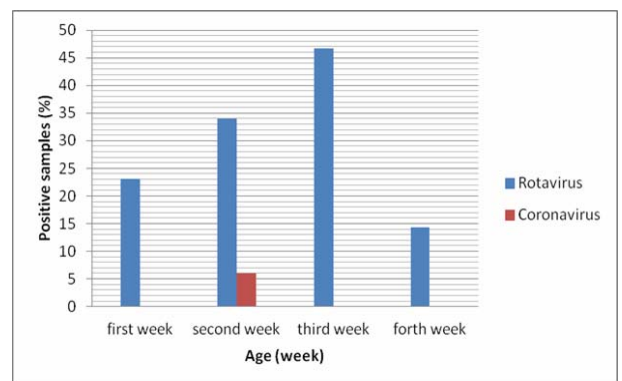

Figure 2. Age distribution of *Rotavirus* or *Coronavirus* positive samples among diarrheic calves.

Table 2 shows the distribution of matched pairs in newborn diarrheic (case) and non-diarrheic (control) calves for *Rotavirus* and *Coronavirus*. Age distribution of *Rotavirus* positive diarrheic calves revealed that *Rotavirus* was the most common in the third week of life (46-66%) and *Coronavirus* was detected only in some cases of second week of life (figure 2), although most of the samples were from calves in age group 8-14 days old.

Table1. Some characteristics of dairy farms in this study.

Farms	Average total population	Average milking cow number	Average number of calves under 1 month age (male- female)	Number of samples taken from diarrheic calves
1	1439	515	23-24	18
2	1333	451	20-22	13
3	1606	568	26-28	29
4	1567	504	18-20	11
5	635	182	10- 10	15
6	407	157	11-13	5
7	1149	405	22-16	21

Comparisons between diarrheic and non-diarrheic calves using CLR for *Rotavirus* and *Coronavirus* showed significant differences in odds ratios for the presence or absence of *Rotavirus*.

Table 2. Distribution of matched pairs in newborn diarrheic (case) and non-diarrheic (control) calves for *Rotavirus* and *Coronavirus*

	Number of Pairs with Case + Control +	Number of Pairs with Case + Control -	Number of Pairs with Case - Control +	Number of Pairs with Case - Control -	Total number of pairs
Rotavirus	7	26	12	67	112
Coronavirus	0	3	2	107	112

The excretion of *Rotavirus* in the feces of scouring calves was significantly higher than in non-diarrheic calves (OR 1.9) but there was not any significant association between shedding of *Coronavirus* in the feces and diarrhea (Table 3).

Table 3. Results of the conditional logistic regression model to match pairs consisting of the diarrheic case and non-diarrheic matched control.

virus	Odds ratio	95% CI for odd ratio	p-value
Rotavirus	1.9	1.05 to 3.76	0.0339*
parity	0.8	0.69 to 0.97	0.0230*
gender	1.2	0.95 to 1.73	0.0972
Coronavirus	1.4	0.24 to 9.05	0.6697
parity	0.8	0.71 to 0.99	0.0421*
gender	1.3	1.00 to 1.80	0.0459*

*: statistically significant

However, we found no difference in morbidity between male and female calves but there was a difference in morbidity rate between calves of primiparous heifers and cows with a higher parity and the risk of diarrhea was more in primiparous cows in compare with cows with a higher parity.

DISCUSSION

This study reports the detection rates of *Rotavirus* and *Coronavirus* infection in newborn non-diarrheic and diarrheic calves in different age groups (1-7, 8-14, 15-21 and 22-30 days) and also the association between shedding of each of these two viruses in the feces and diarrhea in all of newborn calves based on a matched case control study because it has been shown that the main value of matching is the enhanced efficiency in controlling confound subsequent stratified analysis (Thrusfield 2005). The detection rate of *Rotavirus* in newborn diarrheic calves in this study (29.5%) is lower than those found in neonatal diarrheic calves in other countries such as England (Reynolds *et al* 1986), Spain (De la Fuente *et al* 1998), Sweden (De Verdier & Svensson 1998), Turkey (Gumusova *et al* 2007), Switzerland (Luginbuhl *et al* 2005, Uhde *et al* 2008) but is higher than those found in other studies that were performed in Argentina (Bellinzoni *et al* 1990), Costa

Rica (Perez *et al* 1998), Bangladesh (Alam *et al* 1999), Sweden (Bjorkman *et al* 2003), Turkey (Erdogan *et al* 2003), Brazil (Langoni *et al* 2004), and Brazil (Alfieri *et al* 2006). There were not any significant differences in shedding of *Rotavirus* in the feces among calves of different age groups. The highest prevalence of *Rotavirus* shedding among diarrheic cases was in age group 15-21 days (46.66%) and the lowest prevalence of *Rotavirus* shedding was in age group 22-30 days (14.28%). In contrast, Erdogan et al., 2003 reported that *Rotavirus* was most common in the first week of life and that there was an invert association between the age of calves and the presence of Rotavirus in feces.

 Coronavirus infection was detected in 3 out of 112 diarrheic (prevalence in diarrheic calves: 2.7%). The relatively low prevalence of bovine *Coronavirus* agrees with the results of other studies in Sweden (Bjorkman *et al* 2003), and Japan (Kirisawa *et al* 2007). In contrast bovine *Coronavirus* appeared to be of more importance in most of other studies in countries such as England (Reynolds *et al* 1986), Ethiopia (Abraham *et al* 1992), Spain (De la Fuente *et al* 1998 & Perez *et al* 1998), France (Bendali *et al* 1999a), Switzerland (Uhde *et al* 2008), and Brazil (Stipp *et al* 2009). One of the possible reasons for low prevalence of *Coronavirus* in the present study is that samples were not collected during winter, the season in which the prevalence of calf diarrhea caused by *Coronavirus* is higher than other seasons (Radostits 2007).

 The distribution and occurrence of enteropathogens in the feces of diarrheic and non-diarrheic calves varies depending on the geographic location, the farm, the age and type of calves being examined and the extent to which the diagnostic laboratory is capable of isolating or demonstrating the pathogens. On the other hand, the role of other infectious agents in diarrhea in different countries and regions and the sensitivity and specificity of used tests in each study can explain the observed differences in prevalence of Rotavirus or Coronavirus among a variety of studies. There is good evidence that *Rotavirus* is a primary pathogen causing acute diarrhea in neonatal calves (Torres-Medina *et al* 1985, Reynold

et al 1986, Snodgrass *et al* 1986, Busato *et al* 1998, Haschek *et al* 2006) and the present study showed that there was a significant difference in excretion of rotavirus in the feces of diarrheic and non-diarrheic calves. This is in agreement with previous reports (Reynolds *et al* 1986, Waltner-Toews *et al* 1986, Viring *et al* 1993, Busato *et al* 1998, De Verdier & Svensson 1998, Perez *et al* 1998, Bjorkman *et al* 2003, Erdogan *et al* 2003, Alfieri *et al* 2006, Kirisawa *et al* 2007).

 In the present study there was not a significant association between the presence of *Coronavirus* in feces and diarrhea and this is similar to the results of other studies. (Busato *et al* 1998, Bjorkman *et al* 2003; Erdogan *et al* 2003) but in some other studies there was a significant association between shedding of *Coronavirus* in feces and diarrhea (Reynolds *et al* 1986, Perez *et al* 1998, Bendali *et al* 1999a, Stipp *et al* 2009).There was a difference in morbidity rate between calves of primiparous heifers and cows with a higher parity in this study and it has shown in some studies previously that in beef calves born alive of primiparous cows have a higher mortality and morbidity due to diarrhea than calves born by older cows (Olsson *et al* 1993, Clement *et al* 1995) possibly because of a higher risk of failure of transfer of passive immunity in heifers that can also be the case for dairy heifers.The results of this study indicate that *Rotavirus* can be considered as a cause of newborn calf diarrhea in these industrial dairy herds in Mashhad and important preventive approaches for infection with either Rotavirus or Coronavirus can be considering of hygienic principles and adequate closrul intake.

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