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Detection of fecal coronavirus antigen in diarrheic calves of high- and average-producing Holstein dairy cows

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Abstract: From January to December 2009, 661 fecal samples from natural cases of diarrheic calves were taken by veterinary staff from the Department of Large Animal Internal Medicine of Shiraz Veterinary School and veterinary practitioners in Fars Province, Iran. The samples were taken from 267 diarrheic calves of high-producing dairy cows (HPDCs) and 394 diarrheic calves of average-producing Holstein dairy cows (APDCs). Fecal samples were collected and submitted for the laboratory diagnosis of coronavirus antigens. Herd selection was based on geographical location and density of cattle in the region. All herds had HPDC and APDC coronavirus-infected diarrheic calves in their population. The rate of coronavirus infection in diarrheic APDC calves in the northern region was highest when compared to other geographical locations. It was also found that diarrheic coronavirus-infected HPDC calves in the northern region of Fars Province were at much lower risk of diarrhea than APDC calves in the same region ($P < 0.05$). Diarrheic coronavirus-affected APDC Holstein calves of younger dams (>2 to 3 years) showed a higher rate of infection when compared to diarrheic HPDC coronavirus-infected calves in the same age group ($P < 0.05$). It was also found that the proportion of infected coronavirus diarrheic APDC calves decreased with the increased parity of their dams. There was no difference among the occurrence of coronavirus infection in diarrheic HPDC and APDC calves of different herd size groups.

Key words: Coronavirus, diarrheic calves, dairy cows

1. Introduction

Diarrhea in newborn farm animals, especially calves under 30 days of age, is one of the most familiar types of disease complexes that large-animal clinicians face in practice (1). It is a notable cause of economic loss in cattle herds. The problem is a complex, multifactorial disease that is influenced by the inherent characteristics of the calf, its nutritional and immunological conditions, management of the herd, environment, and various infectious agents (2). Calves are at significant risk of developing diarrhea in the first month of life and the incidence of diarrhea decreases with age (2,3). Viral diarrheas are the origin of high mortality among children and animals, including many mammalian and avian species (4–6). Coronaviruses are species in the genera of animal viruses that are members of subfamily *Coronavirinae* in the family *Coronaviridae* (7). They are enveloped viruses with a positive-sense single-stranded RNA genome and chiefly infect the upper respiratory and gastrointestinal tract of mammals and birds (7). Coronaviruses also cause a spectrum of diseases in farm animals and domesticated pets, some of which can be dangerous and are a risk to the farming industry (8–12). Only 4 bovine coronavirus isolates have been fully sequenced and, hence, the knowledge about the genetics of the virus is still insufficient (13). Reports (2,14–16) indicate that the coronavirus is an important cause of diarrhea in calves accounting for between 4% and 26% of isolates, although it is also associated with healthy calves (17,18). Scours caused by the coronavirus happen in calves that are over 5 days of age (5–21 days) (1). Some cases are observed as late as 3 weeks of age (19). Previous breeding policies for dairy cattle have been very useful in producing a rapid genetic gain to achieve industry targets and raise interest (20). This form of selection may additionally affect other systems, such as the immune system. The differences in genetic potential and immunity (specific and innate immunity) and other unknown factors in diarrheic calves of high-producing dairy cows (HPDCs) and average-producing dairy cows (APDCs) among Holsteins may vary and affect the level of coronavirus infection in diarrheic calves. In one instance, Kawakami et al. (21) reported that calf diarrhea in the early lactation period would be caused partly due to the immaturity of leukocyte innate immunity. The innate immune system comprises the immediate host defense mechanisms that respond to infection, and differences in the magnitude and rapidity of

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this response are known to influence the susceptibility to pathogens and their clearance. The aim of this study was to investigate the occurrence of the coronavirus in fecal samples of diarrheic calves of HPDC and APDC Holsteins while considering factors such as geographical location, parity of dams, and herd size.

2. Materials and methods

From January to December 2009, based on a random cluster sampling method, 661 fecal samples of diarrheic calves were taken by veterinary staff from the Department of Large Animal Internal Medicine of Shiraz Veterinary School and veterinary practitioners in Fars Province, Iran. The samples were taken from 267 diarrheic calves of HPDC (average 305-day milk production was approximately 7340 kg per cow) and 394 diarrheic calves of APDC (average 305-day milk production was approximately 3800 kg per cow) Holsteins. The fecal samples were collected directly from the rectum in sterile glass bottles, chilled, and submitted for laboratory diagnosis. The fecal samples were obtained on the first day of the onset of diarrhea from untreated calves up to 35 days of age. The fecal consistency was scored on a 4-point scale (22). For this study, a score of 3 or 4 indicated the presence of diarrhea, and a score of 1 or 2 indicated the absence of diarrhea. The median age of the studied calves was 13 days and the age at which the calves were first fed colostrum was almost the same. All cows were never vaccinated against coronavirus infection in the study. Herd selection was based on geographical location and density of cattle in the region. Samples were collected based on 5% of the herd population in 4 geographical regions: the north, west, east, and south of Fars Province, Iran. The herds were stratified to small (50–100 cows), medium (101–200 cows), and large (>200 cows) sizes. The coronavirus antigen detection ELISA test kit (Bovine Coronavirus ELISA Kit, BIO K 344, Bio-X Diagnostics S.P.R.L., Belgium) was used in accordance with the manufacturer's instructions. The test used a Sandwich ELISA method. An ELISA microtiter plate reader (BDSL Immunoscan PLUS) was set at 450 nm and optical densities were read. Parity of the dam was also recorded.

Data were computed using Epi Info Version 6.04. The true prevalence was calculated using the following formula (23): True prevalence = (apparent prevalence + specificity – 1) /(sensitivity + specificity – 1). Statistical analysis for 2-way tables were tested using the 1-tailed Fisher exact test with a value of 0.05.

3. Results

The apparent and true prevalence of the coronavirus infection in HPDC and APDC diarrheic calves are shown in Table 1. The rates, risk and odds ratio, and results of the 1-tailed Fisher exact probability test of coronavirus infection in the 4 different geographical locations (north, west, east, and west) of HPDC and APDC diarrheic calves are shown in Table 2. The odds ratio compared the relative odds of coronavirus infection in the diarrhea of HPDC and APDC calves. An odds ratio of less than 1 in coronavirus-infected diarrheic calves indicated a more probable occurrence of diarrhea in APDC calves. The risk ratio compared the probability of coronavirus infection in diarrheic calves of HPDC and APDC groups rather than the odds. All herds had HPDC and APDC coronavirus-infected diarrheic calves in their population. The rate of coronavirus infection in diarrheic APDC calves in the northern region was highest when compared to other geographical locations. It was also found that the diarrheic coronavirus-infected HPDC calves in the northern region of Fars Province were at much lower risk of diarrhea than APDC diarrheic calves in the same region ($P < 0.05$). The results of the infection rate in coronavirus-infected diarrheic Holstein calves (HPDC and APDC) from different age groups of Holstein dams are shown in Table 3. Diarrheic coronavirus-affected APDC Holstein calves of younger dams (>2 to 3 years) showed a higher rate of infection when compared to diarrheic HPDC coronavirus-infected calves ($P < 0.05$) in the same group. It was also found that the proportion of coronavirus-infected diarrheic APDC calves decreased with increased parity of their dams. There was no difference among the occurrence of the coronavirus infection in diarrheic HPDC and APDC calves of different herd size groups (Table 4).

Table 1. Mean apparent and true prevalence of coronavirus fecal infection in HPDC (n = 267) and APDC (n = 394) diarrheic calves.

Groups	Apparent prevalence	True prevalence
HPDC	0.1	0.113
APDC	0.123	0.143

HPDC: High-producing dairy cows; APDC: average-producing dairy cows.

4. Discussion

Apparent prevalence, although helpful as a consistent index, may underestimate the true prevalence of disease. The difference between true and apparent prevalence shows the accuracy of the diagnostic test used to assess the prevalence of coronavirus infection. Our study showed that the diarrheic APDC Holstein calves in the northern region experienced more episodes of coronavirus infection than in other regions. There are 3 distinct climatic regions in Fars Province, Iran. The mountainous areas of the north and northwest have moderate cold winters and mild

Table 2. Epidemiologic parameters in 4 different geographical locations (north, west, east, and west of Fars Province) of HPDC and APDC coronavirus-affected Holstein diarrheic calves.

Geographical location	Number of calves	Seropositive	Seronegative	Rate	Risk ratio	Odds ratio	P
North	HPDC: 72	n = 6	n = 67	0.06	0.4	0.36	0.04*
	APDC: 94	n = 16	n = 78	0.17	(0.15–1.06)†	(012–1.04)†*	
South	HPDC: 58	n = 8	n = 50	0.13	1.26	1.31	NS
	APDC: 92	n = 10	n = 82	0.10	(0.53–3.02)	(0.48–3.54)	
East	HPDC: 69	n = 11	n = 58	0.15	2.09	2.29	NS
	APDC: 105	n = 8	n = 97	0.07	(0.88–4.93)	(0.87–6.04)	
West	HPDC: 68	n = 10	n = 58	0.14	1.16	1.19	NS
	APDC: 103	n = 13	n = 90	0.12	(0.54–2.5)	(0.49–2.9)	

†: 95% confidence interval; HPDC: high-producing dairy cows; APDC: average-producing dairy cows; *: P < 0.05; NS: nonsignificant.

Table 3. Epidemiological parameters of coronavirus-affected HPDC and APDC diarrheic Holstein calves in dams of different ages.

Years	Number of calves	Seropositive	Seronegative	Rate	Risk ratio	Odds ratio	P
>2	HPDC: 96	n = 8	n = 88	0.08	0.51	0.47	0.05*
	APDC: 142	n = 23	n = 119	0.16	(0.24–1.1)†	(0.2–1.1)†	
3	HPDC: 82	n = 11	n = 71	0.13	1.1	1.11	
	APDC: 125	n = 15	n = 110	0.12	(0.53–2.29)	(0.48–2.55)	NS
^{3}4	HPDC: 89	n = 10	n = 79	0.11	1.01	1.02	NS
	APDC: 127	n = 14	n = 113	0.11	(0.47–2.19)	(0.43–2.41)	

†: 95% confidence interval; HPDC: high-producing dairy cows; APDC: average-producing dairy cows; *: P < 0.05; NS: nonsignificant.

Table 4. Epidemiological parameters of coronavirus-affected HPDC and APDC diarrheic Holstein calves of different herd size groups.

Herd size	Number of calves	Seropositive	Seronegative	Rate	Risk ratio	Odds ratio	P
Small	HPDC: 81	n = 11	n = 70	0.13	1.06	1.07	NS
	APDC: 141	n = 18	n = 123	0.12	(0.52–2.1)†	(0.47–2.4)†	
Medium	HPDC: 88	n = 10	n = 78	0.11	0.82	0.8	
	APDC: 116	n = 16	n = 100	0.13	(0.39–1.72)	(0.34–1.86)	NS
Large	HPDC: 98	n = 8	n = 90	0.08	0.62	0.58	NS
	APDC: 137	n = 18	n = 119	0.13	(0.28–1.37)	(0.24–1.41)	

†: 95% confidence interval; HPDC: high-producing dairy cows; APDC: average-producing dairy cows; NS: nonsignificant.

summers. The central regions have relatively rainy mild winters and hot dry summers. The third region, located in the south and southeast, has moderate winters with very hot summers. The absorption of immunoglobulins (Igs) may be affected by the environment in which the calf was born (24). Intense cold (25,26), but not moderate cold (27,28), lowers the absorption of Igs by calves. The effects of ambient temperature outside the thermoneutral range for calves may involve direct effects on intestinal absorption and transport (27) as well as the ability of the calf to stand and nurse (25,26). Bovine coronavirus is moderately sensitive to heat (29). Seasons have an important effect on the calf mortality as well as on the absorption of Igs in neonatal calves (30). In temperate climates the mean serum IgG1 concentrations were lowest in winter-born calves and increased during the spring and early summer (31). As most of the fecal samples of APDC calves in the northern region were taken during the winter, it seemed that cold temperatures were stressful for calves and probably affected colostrum composition and Ig content in APDC coronavirus-affected calves. It was also noted that under the same conditions, diarrheic coronavirus-infected APDC calves in the northern region of Fars Province were at a much higher risk than HPDC calves ($P < 0.05$). It is most likely that the level of stress experienced by these calves was probably higher than in HPDC calves. These stress factors were unknown but may involve those that negatively affect specific and innate immune defenses (nonspecific immunity). Stress is commonly considered to restrain the immune system and may be responsible for an increase in the occurrence of disease in the presence of a pathogen. It has been stated that the pituitary adrenocorticotropic hormone travels through the blood to the adrenal cortex, where cells of the zona fasciculata secrete glucocorticoids (32), with cortisol being the principal glucocorticoid in swine and cattle (33). Stress hormones released in response to the activation of the hypothalamic–pituitary–adrenal axis (CRF, ACTH, and cortisol) have been indicated to have an effect on aspects of the immune system (34). It has been shown that incubation of cattle and porcine immune cells with cortisol suppresses lymphocyte proliferation, interleukin-2 production and neutrophil function (35–37). The role of possible different genetic compositions of the HPDC and APDC coronavirus-affected diarrheic Holstein calves on the level of stress experienced in the same environment may be another explanation for differences in the immune system condition. The stress responsiveness of an animal has also been shown to be affected by genetics. Studies by Sutherland et al. (38,39) demonstrated numerous breed effects on different immune elements. Genetic differences between breeds of food-producing animals are known to play a leading part in disease resistance, and a few studies have described breed-dependent differences in the prevalence of mastitis (40). Blecha et al. (41) noted that Angus and Brahman × Angus cattle responded immunologically differently to shipping stress. Angus calves had higher total IgG and IgM titers against pig red blood cells when compared with Simmental calves (42). Large White pigs had greater poststress ACTH levels after exposure to an unfamiliar environment than Meishan pigs (43). Another possible factor leading to higher stress in APDC diarrheic calves may be higher attention given to the HPDC calves in the same environmental conditions. It has been stated that one of the factors that can play a part in the quality (particularly Ig content) of colostrum is parity of the dam (44,45). This was also demonstrated in our study and the rate of coronavirus infection in diarrheic APDC Holstein calves belonging to cows between >2 and 3 years old was higher than in other age groups. In dams between >2 and 3 years old, the rate of coronavirus infection in HPDC was lower than in APDC calves ($P < 0.05$), showing that parity can affect the prevalence of the infection in this age group. This may also be related to the better quality of colostrum in dams of HPDC calves, their better specific and nonspecific immunity, and the higher colostrum yield in HPDC dams.

In conclusion, our results revealed that there are differences in the coronavirus infection of diarrheic HPDC and APDC calves. The rate of coronavirus infection in diarrheic APDC Holstein calves can be affected by geographical location and parity of the dam. The lower rate of coronavirus infection in HPDC diarrheic Holstein calves found in this study could be due to the better specific and nonspecific immunity (possibly related to genetic composition) in HPDC calves and higher colostrum yield in HPDC dams.

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