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Prevalence of rotavirus (GARV) and coronavirus (BCoV) associated with neonatal diarrhea in calves in western Algeria

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PEER REVIEW

Peer reviewer

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Comments

The frequency of rota-coronavirus virus infection is quite variable among different geographical regions, the production type (dairy or beef herds), and the diagnostic techniques used. In this case report, authors make an attempt to deals with the prevalence of GARV and BCoV associated with neonatal diarrhea in calves in Western Algeria.
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ABSTRACT

Objective: To study the prevalence of bovine group A rotavirus (GARV) and bovine coronavirus (BCoV) in diarrheic feces from calves and the sensitive's parameters such as age group and sex.

Methods: Feces samples from 82 diarrheic dairy calves from farms around Tiaret (Western Algeria) were collected. These samples were tested by ELISA assay.

Results: The results showed that the prevalence of rotavirus and coronavirus infection are 14.63% (12.2% alone and 2.43% associated with bovine coronavirus) and 20.73% (18.3% alone and 2.43% associated with GARV), respectively.

Conclusions: The present study demonstrates that the both BCoV and GARV are involved in the neonatal calves' diarrhea, where the frequency of BCoV is clearly higher than that of GARV.

KEYWORDS

Calves, Diarrhea, Coronavirus, Rotavirus, Prevalence, ELISA

1. Introduction

Neonatal calf diarrhea is one of the most devastating diseases of the dairy industry worldwide[1]. It is defined as pathology having a complex multifactorial etiology, influenced by infectious, nutritional and environmental factors as well as management practices. Its suggested causes include toxins, bacteria, protozoa, viruses and management/environmental factors such as overfeeding, low temperature, poor hygiene, colostrum deprivation and individual animal susceptibility. Infectious agents, either singly or in combination, may be associated with field outbreaks with considerable economic burden[2].

The incidence risks of diarrhea in calves under 30 d old was reported by several studies and it varies between 15% and 20%[3]. According to the latter, the mortality risk is

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1.5%-8%. In Algeria, the epidemiological studies conducted to date showed that the syndrome came after each period of calving and it causes major economic loss directly through the morbidity, mortality and therapy and indirectly by poor growth problems after clinical diseases[4].

Rotavirus, bovine coronavirus (BCoV), *Escherichia coli* F5 (*E. coli*), and *Cryptosporidium* species are internationally recognized as the most important enteropathogens in acute diarrhea in young calves[5]. In calves, bovine group A rotavirus (GARV) and BCoV are the most commonly associated viruses with neonatal diarrhea and it is not unusual that both viruses can concomitantly infect calves[6].

Difficulties in the clinical diagnosis of infectious enteritis arise from frequent nonspecific clinical signs and lesions, the presence of asymptomatic infections, the involvement of multiple agents, and the interactions of intrinsic and extrinsic factors that predispose the host to infection[7]. However, many laboratory diagnostic tests based on the different methods such as direct electron microscopic test, ELISA, latex agglutination, polyacrylamide gel electrophoresis, reverse transcription polymerase chain reaction as well as immuno-electron microscopy. Among these methods, ELISA using monoclonal antibody as a sensitive, fast, and inexpensive method is used for the simultaneous detection of BCoV and GARV in the feces of diarrheic calves[8].

The high frequency and persistence of calves' neonatal diarrhea in farming has gained the interest of many researchers. Thus; the purpose of the present study is to report and estimate the prevalence of GARV and BCoV using ELISA assay in diarrheic feces from calves and the sensitive's parameters like age-group and sex in Western Algeria (Tiaret).

2. Material and methods

2.1. Feces samples

Eighty two samples of diarrheic feces were collected from both sexes of Holstein calves, aged less than 30 d old, which had not received prior treatment with antibiotics during calving period. In fact, these calves were issued from cows which were not vaccinated against the causative agent of neonatal diarrhea. They showed an important dehydration and had poor appetite, liveliness and suckling reflex, and characterized by a pasty-watery faeces. The studied calves were grouped in cowsheds with a bad hygiene. The population was stratified into four age-groups: group 1: ≤7 d, group 2: ≥8 d≤14 d, group 3: ≥15 d≤21 d and group 4: ≥ 22 d≤30 d.

Samples were collected in sterile flasks after cleaning of the anal area with a paper towel and beats by rectal stimulation with the index finger protected by disposable sterile plastic gloves. The collected samples were stored at −20 °C until analyzing.

2.2. ELISA test

A commercial indirect antigen-capture ELISA kit (rotavirus and coronavirus, Pourquier Institute, France) employing specific monoclonal antibodies was used to detect VP7 protein of GARV and S protein of BCoV in fecal samples. This assay is realized in the Veterinary Regional Laboratory of Tlemcen (Algeria). The ELISA procedure was performed according to the manufacturer instruction (Kit reference P 00603). After adding stop solution (0.5 mol/L of H_2SO_4), the optical density (OD) of each well was measured at 450 nm. Calculating the net OD of each sample and interpreting the results were performed as described by manufacturer instruction. Briefly, all samples were considered positive if the %E/P is upper or equal to 7%.

%E/P = 100×(OD of sample to test-OD of negative control sample)/(Average OD of positive control sample-OD of negative control sample)

The ELISA test is considered valid insofar as:

1. The positive control sample to a minimal average OD 450: 0.5 or OD 450=0.5.
2. OD 450 mean of the positive control sample/OD 450 of negative sample=5.

2.3. Statistical analysis

The contingency table was used at 5% significance to assess the differences among the proportions of fecal samples positive to GARV and BCoV variables such as age group and sex of the animals studied. The statistical analysis system program was used to perform the statistical calculations.

3. Results

3.1. Detection of enteropathogens

During this study, eighty two samples from diarrheic calves were studied. Briefly, 26 of the calves included in this study were 1-7 d old, and 13, 16 and 27 calves were 8-14, 15-21 and 22-30 d old, respectively.

BCoV was the dominant enteropathogen detected being present in 17 of 82 samples (20.73%). As mentioned in the Table 1, among the 17 positive samples, 15 calves (18.3%)

presented BCoV alone and 2 calves (2.43%) were associated with GARV. Likewise, GARV infections were detected in 12 calves (14.63%). GARV was the only agent detected in 10 calves (12.2%), the remainder of GARV (2.43%) infections is combined with BCoV (Table 1).

Table 1

Detection of BCoV and GARV in the studied diarrheic calves

Enteropathogen(s) detected	Calves (*n*=82)	
	Number	%
None	55	67.07
Coronavirus only	15	18.30
Rotavirus only	10	12.20
Coronavirus+rotavirus	2	2.43

3.2. Age distribution of infections

Table 2 summarizes the proportions of positive samples as for the two enteropathogens during the four weeks of life.

Table 2

Prevalence of BCoV and GARV at different age group.

Age group	Numbers of examined calves	Numbers of positive calves (%)	BCoV (%)	GARV (%)
1-7 d	26	9 (34.62%)	5 (19.23%)	5 (19.23%)
8-14 d	13	5 (38.46%)	2 (15.38%)	4 (30.77%)
15-21 d	16	1 (6.25%)	1 (6.25%)	0 %
22-30 d	27	12 (44.44%)	9 (33.33%)	3 (11.11%)
Total	82	27 (32.92%)	17 (20.73%)	12 (14.63%)

All the four investigated age-groups were positive to the enteropathogens. Nevertheless, the 22-30 d old calves showed higher frequency (44.44%) to enteropathogen infection compared to the other three age-groups. In fact this group is highly sensitive to the coronavirus infection (33.33%) while the fewer occurrences were recorded in the age-group 15-21 d old. In the case of GARV infection, the age-group 8-14 d old presented the highest frequency (30.77%).

3.3. Sex distribution of infections

Among the 82 collected faecal samples, 27 cases were found to have one of both viruses; 16 cases (59.26%) were male and 11 (40.74%) were female. A total of 17 cases were found to have BCoV using ELISA test; 13 were males and 4 females. On the contrary, 7 females and 5 males were affected by GARV.

4. Discussion

Diarrhea in the neonatal calf is a serious welfare problem and a cause of economic loss due to mortality, treatment costs and poor growth. Calf diarrhea is an example of a complex or multifactorial disease, resulting as it does from an interaction between the calf, its environment, nutrition and infectious agents[9]. Enteric infections may be caused by protozoa, bacteria and/or virus[10].

In the present study, BCoV was the predominant enteropathogen associated with calves' diarrhea. The incidence of this agent is 20.73% (17/82) either alone or in combination with GARV. Our result is in agreement with those reported by Brandão *et al*[11], Stipp *et al*.[10] in Brazil, AKam *et al*. in Algeria[4] and Izzo *et al*.[12] in Australlia, in which the incidence of BCoV are 22.22%, 19%, 18.48% and 21.6% respectively, though much higher to those reported by Reynolds *et al*.[13] in England (14%), Snodgrass *et al*.[14] in Scotland (4%), Perez *et al*.[15] in Costa (9%), De La Fuente *et al*.[16] in Spain (7.34%), Rai *et al*.[17] in India (11.76%) and Dash *et al*.[18] in India (4.76%). However the result reached in our work remains lesser when comparing to that formerly cited by Abraham *et al*.[19] in Ethiopia (38.9%).

On the other hand, GARV infections were detected at less prevalence rate (14.63%, *i.e.*12/82). This finding corroborates with the result noticed by Abraham *et al*.[19] (16.7%), Rai *et al*.[17] (15.68%), and Al-Robaiee and Al-Farwachi[20] (15.5%). Alfieri *et al*.[2] stated that the rate of diarrheic fecal samples infected in beef and dairy calves were 33% and 20.2%, respectively. While Zrelli *et al*.[21] recorded an average rate of 22.8%, Langoni *et al*.[22] showed an average rate of 25.1% and AKam *et al*.[4] cited a percentage of 21.84%. Interestingly, others reported an important rate of GARV infection such as Reynolds *et al*.[13] (42%), Snodgrass *et al*.[14] (50%), De La Fuente *et al*.[16] (42.7%), Izzo *et al*.[12] (79.9%) and Nourmohammadzadeh *et al*.[23] (34%). Nevertheless, Perez *et al*.[15] observed a prevalence of 7%. The differences in detection of enteropathogens may be attributed to the different diagnostic methods used[24], farm management practices exercised in different regions, hygienic status[8,24], environmental condition, and geographical locations (climate condition).

No enteropathogens were detected in the 55 (67.07%) sample calves. Negative results may occur because some cases of diarrhea might not be associated with infectious agents, and probably due to nutritional or other management factors, or because other non-investigated pathogens were involved, such as many other enteric bacterial (*E. coli* F5 and other enteropathogenic *E. coli*, and *Campylobacter etc.*), parasite (*Cryptosporidium parvum*) and virus (calicivirus, torovirus *etc.*).

Regarding age-group in this study, the frequency of infection is higher in age-group 22-30 d olds (44.44%). This age bracket is considered as the most susceptible to infection by BCoV (33.33%). While the frequency of infection by rotavirus is higher in age-group 8-14 d olds (30.77%), this age bracket is reported as the most susceptible to infection by rotavirus[2,25]. The 2- and 4-week-old calves were the most susceptible to GARV and BCoV infections,

which may be due to decreasing of passive immunity and the absence of the natural resistance against infection for both enteropathogens. The presence of BCoV infection in older calves (22–30 d) could be explained by the method of livestock farming of calves (using the grouping of the calves instead of individual stall). The 3–weeks–old calves are characterized by absence of GARV, this may be highlighted by an increased natural resistance against infection for this enteropathogen and small number of sample cooperated with 1 and 4 age–group, in spite of the absence of significant difference among the various age–groups.

The frequency of infection of the male (59.26%) is superior to the female (40.74%) for both enteropathogens. We can assume that the size of the male at birth induces dystocia, and consequently decrease colostrum absorption. The similar result is gathered by Clement *et al*[26], who noticed that the sensibility of males for diarrhea was double compared to females. Whereas, in our study the percentage of the females affected by rotavirus is superior to males. The comparable result is recorded by Hosso and Pandey[27], who revealed that among 23 calves positive for rotavirus, nine were males and 14 females.

The result of this study showed that both BCoV as well as GARV were involved in the neonatal calves diarrhea, though, the frequency of BCoV is higher in comparison with GARV. However, further investigations dealing with fecal samples analysis to identify the presence of other enteropathogens such as *E. coli* and *Cryptosporidium* are needed. Furthermore, the multifactorial etiology (bacterial, viral and protozoa agents) influenced by nutritional and environmental factors, as well as difficulties in the clinical diagnosis of neonatal diarrhea and failure of the treatment in young calves affected by diarrhea require to adopt the prophylactic measures such as hygiene, management and vaccination of dam cows by an immunization through the injection of either modified–live or inactivated vaccines. This will result in enhancement of antibody titres in the colostrum and milk of vaccinated cows for prevention of neonatal diarrhea caused by coronavirus and rotavirus.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Diarrhea is one of the main causes of calf mortality, morbidity and economic losses worldwide in the cattle industry and it is a complex multifactorial disease involving animal, environmental, nutritional and infectious agents.

Research frontiers

The object of the work is to evaluate the impact of these viral infectious agents in breeding farms in Western Algeria according to the clinical state and age distribution in younger dairy calves.

Related reports

Several techniques have been developed by detecting the viral particle (cell culture, electron microscopy, immune electron microscopy), viral protein (ELISA, latex agglutination, direct immunofluorescence), and viral genome (SS–PAGE, RT–PCR).

Innovations and breakthroughs

In the scientific literature only few published information on the epidemiology of rota–coronavirus virus infection in neonatal calves in Algeria were available. In this paper the authors conducted a study to throw light on the prevalence of rotavirus and coronavirus associated with neonatal diarrhea in calves in Western Algeria by using an ELISA assay.

Applications

This report could provide useful information for epidemiological study of these viral infections in Western Algeria.

Peer review

The frequency of rota–coronavirus virus infection is quite variable among different geographical regions, the production type (dairy or beef herds), and the diagnostic techniques used. In this case report, authors make an attempt to deals with the prevalence of GARV and BCoV associated with neonatal diarrhea in calves in Western Algeria.

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