

Prevalence of *Coronavirus* and *Rotavirus* in dairy calves in north-central of Algeria

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Abstract

Coronavirus and *Rotavirus* have been reported as the two main pathogens implicated in neonatal gastroenteritis in calves. The aim of this study is to provide descriptive and analytical epidemiological data and to evaluate the influence of infection with these two viruses on the onset of diarrhea in calves less than 60 days old. In our study, we included 35 dairy cattle farms in the north-central part of Algeria from January 2019 to December 2021. We analyzed 152 samples using the *Copro-ELISA* test, which revealed a prevalence of 10.53% and 9.87% for *Rotavirus* and *Coronavirus*, respectively. The analysis of the results shows that infection is strongly linked to the animal's age, with the peak of virus excretion occurring at around two weeks of age. Thus, 53% of positive cases of *Bcov* were recorded in calves less than a week old, and the highest prevalence of rotavirus (17.02%) was in young cattle aged between 8 and 15 days. Infection by one of these viruses was significantly dependent on the calving season ($p < 0.05$), as the prevalence was high in winter calving. In this study, we found that the infection by another enteropathogen, such as *Cryptosporidium* and *E.coli* F5, significantly increased the infection by the viral agents. However, the combination of the two viruses was also encountered in 12% of cases. Our study also showed that both viruses caused acute diarrhea in calves (RR = 5), as *Rotavirus* was only detected in diarrhoeic calves, where it caused severe dehydration ranging from 6 to 8% in 50% of cases.

Keywords: Algeria, calf, *Coronavirus*, *Rotavirus*, dehydration, neonatal gastroenteritis

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Introduction

Neonatal gastroenteritis in calves has a variable etiology. Few epidemiological data describing the actual involvement of the main pathogens are available in dairy cattle farms in central Algeria, but its importance for human health is not negligible; the transmission of certain enteropathogens such as *Cryptosporidium*, *E.coli* F5, and *Rotavirus* has been demonstrated in humans and could have a bovine origin. In addition, *Rotavirus* is the leading aetiological agent of infectious diarrhea in children (Stripen, 2013).

At least seven groups of *Rotavirus* have been described (A to G), and a BRADFORD study shows that *Rotavirus* groups A and B are present in equivalent proportions in calves: in 94% and 81% respectively of the individuals in this study, the proportion of sick calves infected by *Rotavirus* was 40-50%, compared with 10-20% in healthy calves (Bradford et al., 2008).

Bovine Coronavirus is widespread in cattle herds, causing economic losses for dairy and beef herds worldwide. The virus has been detected on every continent and the serological incidence (>90%) suggests that most cattle are exposed to *BCoV* during their lives. Moreover, the presence of *BCoV* in the lungs was the second highest incidence after that of bovine *Herpesvirus* (Kapil et al., 2008), *BCoV* can be subdivided into *BCoV* and *BRCoV*, subdivided into *BCoV*-induced calf diarrhea (*BCoV*-CD) and winter dysentery (*BCoV*-WD) (Melanie et al., 2010).

The aim of this study is to identify and provide epidemiological data on the two enteropathogenic viruses, *Rotavirus* and *Coronavirus*, implicated in neonatal diarrhea in calves, and to study their distribution according to several zootechnical parameters in order to determine the risk factors and also to assess the pathogenic role of each virus and its physiopathogenic effect in diarrhoeic calves.

Materials and Methods

Area and period of study: We sampled animals during the different seasons to study the influence of climatic and environmental conditions on the occurrence of the disease. This research involved five (05) regions of northern Algeria: Blida, Medea, Tizi-Ouzou and Bouira, and the willaya of Setif. These regions are considered the most important in dairy milk production, with the Setif region heading the list with an annual production of 287,325,000 liters in 2017, followed by the willaya of Tizi-Ouzou with 178,785,000 liters (Mard, 2018). Our study was carried out between January 2019 and December 2021. During that time, we sampled animals during the various calving seasons in order to study the influence of climatic and environmental conditions on the onset of the disease. We programmed (2 visits/month/farm), one at the beginning and the other towards the end of the month, using a monitoring form and technical sheets for each farm to ensure the recording of information concerning cases of neonatal diarrhea and the factors favoring their appearance. A case of calf diarrhea is defined as the emission of more than 2/3 of feces by a sick calf in liquid form, persisting for more than 24 hours before the age of two months. Our data recording was based

on clinical reports, farmers' declarations, and veterinary surgeons' reports.

Study population: Our research concerned dairy herd farms in the five regions described above. We included 35 farms with a herd ranging in population size from 5 to 250 dairy cows, mainly of the following breeds: Montbéliarde, Prim'holstein, Alpine brunette, and Fleckvieh. In our study we targeted calves less than 60 days old and which had not received treatment for diarrhoea. The barn is stanchion-railed or semi-stanchion-railed, with milk production of 15-25 liters/cow/day. Feeding is essentially based on concentrated feed and green forage. The majority of farms use artificial insemination (over 80%), while the remainder use natural breeding. There is no synchronization of calving, which can significantly influence the quality of information provided in our survey when collecting epidemiological data. The practice of drying off is well known among breeders, with 76% of farms practicing it. In our study population, 13 farms (37%) use individual stalls for calves, while the remainder use group pens for calves of different ages.

Samples collection: The feces were collected in sterile plastic bottles as soon as they were emitted after excitation of the anal orifice. Our procedure began by cleaning the anal region with toilet paper and, if necessary, exciting the anal orifice with the index finger of the gloved right hand, immediately after collection, the samples were labeled with an indelible pen and then transported under cold cover in an isothermal cooler (at around 4°C) to the Animal Production Biotechnology and Health (APBH) research laboratory in the veterinary science department of the university of SoukAhras, Algeria, then we froze the samples (-20°C) until the day of analysis by *Cp-ELISA*. Each sample is accompanied by an information sheet covering animal identification, clinical conditions and degree of dehydration, calving season, as well as rearing conditions and practices. We collected 152 fecal samples during the survey period, including 113 from diarrhoeic calves and 39 from symptomatically healthy calves.

Practical laboratory techniques - Copro-ELISA test: We used a tetravalent ELISA kit (BIO-K 348 ELISA, Bio-X Diagnostics, Rochefort, Belgium) in which the cups (96) of the microplates are alternately sensitized by specific antibodies in order to capture the antigens of *Rotavirus*, *Coronavirus*, *E.coli* F5 and *Cryptosporidium* spp eliminated in the feces of infected and those of healthy animals. We began by thawing the sample on the bench at room temperature to avoid denaturation of the different virus glycoproteins. Then we proceeded to dilute with the Buffer solution, volume/volume: 0.5 ml sample + 0.5 ml diluent, then followed the various steps recommended by the manufacturer. We used a tetravalent ELISA kit comprising four microplates and sometimes two microplates of 96 wells, where each microplate can test for the presence of the four antigens in 11 fecal samples thanks to the sensitivity of the wells in the microplate alternately by specific antibodies against *Rotavirus* in

the first line (A) followed by a negative control line (B) sensitized by non-specific antibodies, followed by line (C) which is sensitized by anti-Coronavirus antibodies and then a negative control line (D). Line (E) was sensitized with antibodies to *E. coli* attachment factor F5, followed by a negative control line (F) sensitized with non-specific antibodies. The two last lines of the microplate are for *cryptosporidium spp.*, line (G) is sensitized with specific antibodies followed by the negative control line (H). The test is only validated if the positive control wells give a positive reaction with color changing and an optical density $\geq 6\%$. The optical density of the other samples is calculated using the following formula:

$$\text{Val (ue)} = \frac{\text{Delta OD sample}}{\text{Delta OD positive control}} \times 100$$

Use the following prevalence table to determine the status of the samples: *Rotavirus*: Val $\geq 6.00\%$; *Coronavirus*: Val $\geq 7.00\%$; *E. coli* F5: Val $\geq 6.00\%$; *Cryptosporidium*: Val $\geq 6.00\%$.

The results obtained were processed using Microsoft Office Excel 2007 software. Descriptive statistics were used to calculate the prevalence rates of infection by one of the targeted viruses, as well as their distribution according to the age classes of the calves, the farms of origin, the calving season, and the degree of dehydration. With regard to the analytical epidemiology aspect, we used the Chi-square (X^2) test of dependence with a significance level of 5% ($p < 0.05$) in order to establish the link between the different variables associated with the presence of viruses in the calves of the study population. The relative risk (RR) was also calculated to show the pathogenic effect of infection with one of the viruses and their association with the appearance of diarrhea in the calves included in the target population. With regard to the analytical epidemiology aspect, we used the Chi-square (X^2) test of dependence with a significance level of 5% ($p < 0.05$) in order to establish the link between the different variables associated with the presence of viruses in the calves of the study population. The relative risk (RR) was also calculated to show the pathogenic effect of infection with one of the viruses and their association with the appearance of diarrhea in the calves included in the target population.

Results

Individual and herd prevalence: In the target population, 9 farms were affected, resulting in a herd prevalence of 25.71% (22.30% - 29.13%). Among 152 samples, 16 were positive, indicating an individual prevalence of 10.53% (9.82% - 11.24%) (Table 1). The tetra-variant ELISA test identified bovine Coronavirus (BCoV) on 7 of the surveyed farms, with an estimated herd prevalence of 20% (13.44% - 26.56%). Furthermore, BCoV was detected in 15 calves across five study regions, yielding an individual prevalence of 9.87% (8.43% - 11.31%) (Table 1).

Prevalence of Coronavirus and Rotavirus according to age: The analysis of Coronavirus distribution by age

revealed a significantly higher prevalence in calves during the first week of life, with 53.33% of positive cases detected in this group (8/15). The second age group, calves aged 8 to 15 days, accounted for 26.66% of the positive cases (4/15). The lowest prevalence was observed in calves aged between 22 and 30 days, with only one positive case detected in a diarrhoeic calf aged 24 days. No cases of Coronavirus infection were found in calves older than 30 days (Table 2).

From this study, it is evident that Coronavirus infects calves from the earliest days after birth, particularly during the immune gap period (8 to 15 days), when calves are highly susceptible to neonatal infections. Furthermore, suboptimal husbandry practices during the perinatal and postnatal periods likely play a critical role in preventing infectious diseases in newborn calves. The prevalence of Rotavirus was highest in calves aged 8 to 15 days, with 8 out of 47 samples testing positive, corresponding to a prevalence of 17.02% (16.01% - 18.03%). In the first age group (1 - 7 days) and the fourth age group (22 - 30 days), the prevalence was 11.1% (10.1% - 12.11%). Calves aged 16 to 21 days had a lower prevalence of 5.41% (4.40% - 6.41%), and no cases were reported in calves older than 30 days (Table 2). The age-related susceptibility to Rotavirus was confirmed statistically using the chi-square test, which demonstrated a significant difference in prevalence across age groups ($p < 0.05$). Rotavirus exclusively affected calves under 30 days old, likely due to their compromised immune status from inadequate colostral immunity transfer, compounded by the involvement of other enteropathogens during this critical period, when they are highly susceptible to neonatal infections.

Coronavirus and Rotavirus prevalence according to the calf sex: Of the 98 samples collected from male calves, 13 tested positive for Coronavirus, resulting in a prevalence of 13.27% (12.49% - 14.04%). In contrast, only 2 out of 54 samples from female calves were positive, indicating an estimated prevalence of 3.70% (2.93% - 4.47%) (Table 3).

As illustrated in the table, 86.67% (13/15) of all Coronavirus-positive cases were identified in males, while the 2 cases in females accounted for just 13.3% (2/15) of the total. This difference in prevalence based on sex is statistically significant ($p < 0.05$). The prevalence of Coronavirus is very high among males compared with females, so in the population included in our survey, calves (males) are four times more likely (RR = 4.33) to be infected with Coronavirus than females. The dependence of the infection on the gender of the calves could be explained by the size and weight of the males at the time of calving, which is greater than that of the females and consequently increases cases of dystocia, making the male calves more susceptible in the post-natal period.

Our study indicates that the prevalence of Rotavirus is slightly higher in female calves. We found eight positive samples among 54 tested calves under 60 days old, resulting in a prevalence of 14.81% (14.28% - 15.35%). In comparison, the prevalence in male calves was estimated at 8.16% \pm 0.54, with eight positive samples identified from a population of 98 calves (Table 3). The prevalence data reveal that both sexes

are equally affected by Rotavirus, with laboratory tests confirming 8 infected individuals from each gender, representing 50% of all positive cases. Statistically, the difference in prevalence between males and females is

not significant ($p > 0.05$), suggesting that gender does not influence susceptibility to Rotavirus infection during the first two months of age.

Table 1 Individual prevalence of *Coronavirus* and *Rotavirus* by study region.

Area	Sample length	<i>Coronavirus</i>		<i>Rotavirus</i>	
		Positive cases	Prevalence % (CI to 95% \pm 1.44)	Positive cases	Prevalence % (CI to 95% \pm 0.71)
Medea	42	0	0.00%	4	9.52%
Blida	32	5	15.63%	2	6.25%
Bouira	13	0	0.00%	1	7.69%
Tizi-Ouzou	25	2	8.00%	2	8.00%
Setif	40	8	20.00%	7	17.50%
Total	152	15	9.87%	16	10.53%

CI to 95%: confidence interval with risk of error $\alpha = 5\%$

Table 2 *Coronavirus* and *Rotavirus* prevalence according to the calve's age.

Age range	Sample length	Positive cases <i>Coronavirus</i>	Prevalence (CI to 95% \pm 1.2)	Positives cases <i>Rotavirus</i>	Prevalence (CI to 95% \pm 1.01)
1-7 days	36	8	22.22%	4	11.11%
8-15 days	47	4	8.51%	8	17.02%
16-21 days	37	2	5.41%	2	5.41%
22-30 days	18	1	5.56%	2	11.11%
31-45 days	6	-	0.00%	-	0.00%
46-60 days	8	-	0.00%	-	0.00%
Total	152	15	9.87%	16	10.53%

CI to 95%: confidence interval with risk of error $\alpha = 5\%$ / $\chi^2 = 19$; $p = 0.001$

Table 3 *Coronavirus* and *Rotavirus* prevalence according to the calve's gender.

Gender	Sample length	<i>Coronavirus</i>			<i>Rotavirus</i>		
		Positive cases	Prevalence (%) (CI to 95% \pm 0.77)	Rate (%) by flat positive cases (n = 15)	Positive case	Prevalence (%) (CI to 95% \pm 0.54)	Rate (%) by flat positive cases (n = 16)
Male	98	13	13.27%	86.67%	8	8.16%	50.00%
Female	54	2	3.70%	13.33%	8	14.81%	50.00%
Total	152	15	9.87%	100.00%	16	10.53%	100.00%

$\chi^2 = 6.66$; $p = 0.01$

$\chi^2 = 1.6$; $p = 0.2$

CI to 95%: confidence interval with risk of error $\alpha = 5\%$

Effect of season on *Coronavirus* and *Rotavirus* infection: Regarding the frequency of *Coronavirus* infection by calving season, our research indicates that the highest prevalence occurs in autumn, with an estimated rate of 18.18% (17.13% - 19.23%). This is followed by winter at 11.36% (10.31% - 12.42%), spring at 6.98% (5.92% - 8.03%), and no infections reported during the summer (Table 4).

These findings suggest that the incidence of *Coronavirus* infection in dairy calves under 60 days old is significantly influenced by seasonal factors, as confirmed by the Chi-square test, which indicates a dependency between season and *Coronavirus* infection ($p < 0.05$). In terms of *Rotavirus*, our findings reveal that 62.50% (10/16) of positive cases were detected during the winter calving season, while 37.50% (6/16) were identified in spring. Notably, no cases were reported among calves born in autumn or summer (Table 4).

***Coronavirus* pathogenic role in the occurrence of diarrhea:** During our research, the CpAg-ELISA test allowed the *Coronavirus* to be identified in 14 diarrhoeic calves, either 93.33% of all the positive cases detected (14/15). However, the infection was only found in one (1) non-diarrhoeic calf aged 18 days (third

age group), representing 6.67% of the *Coronavirus* cases detected in our survey (Table 5). According to the relative prevalence, calves less than 60 days old included in our study were almost 5 times more likely to have diarrhea (RR = 4.83) after infection with bovine *Coronavirus* (Bcov). However, asymptomatic shedding was detected in only one case (1/15). The chi-square test assessed the pathogenic effect of the *Coronavirus* on the onset of diarrhea in calves, particularly those less than 30 days old ($p = 0.0008$).

The pathogenic effect of the *Coronavirus* was also established by the evaluation of the degree of dehydration induced in diarrhoeic calves. We found that the *Coronavirus* induces second degree dehydration (6 to 8%) in 50% (7/14) of the diarrhoeic calves found positive for the *Coronavirus* and first degree dehydration (<5%) in 42.86% (6/14) of cases. In comparison, 3rd degree dehydration (>8%) was recorded in just one case (1/14), representing 7.14% of the diarrhoea population infected with the *Coronavirus* (Table 6).

The above table shows that *Coronavirus* induced dehydration of less than 5% in 15.79% (15.06% - 16.52%) and dehydration between 6 and 8% in 11.86% (11.14% - 12.59%) of the infected calves. However, it causes severe dehydration (more than 8%) in 6.25%

(5.52% - 6.98%) of the diarrhoeic calves, despite the fact that the majority of the diarrhoeic calves (11.50% vs 0.88%; 13/113 vs 1/113) affected by the *Coronavirus* presented first and second degree dehydration (between 5% and 8%) the chi-square test did not show a significant difference between the different prevalences according to the degree of dehydration ($\chi^2 = 4.42$, $p = 0.01$). Based on the above results, we found that *Coronavirus* causes significant morbidity in diarrhoeic calves less than 30 days old, although mortality remains low as severe dehydration was only reported in a handful of affected calves.

Rotavirus pathogenic effect on the occurrence of diarrhea: Our research did not record any cases of asymptomatic viral excretion, as all cases of *Rotavirus* infection were detected in diarrhoeic calves, with the virus occurring in $14.16\% \pm 1.58\%$ of the diseased population (either 16 infected calves out of 113 with diarrhea), irrespective of the age of the animal, however, with a higher prevalence in sick subjects aged between 8 and 15 days 22.86% ($13.24\% - 16.39\%$), the chi-square test shows a dependency between *Rotavirus* infection and the appearance of diarrhea in young calves less than 30 days old ($p < 0.05$) (Table 7).

Among the population covered by our study (152 calves), *Rotavirus* caused diarrhea in 10.53% of the subjects examined (16/152) and is consequently an integral part of the etiology of the disease, with a rate of 14.16%. Infected calves had a 4 greater risk ($RR = 4$)

of suffering from diarrhea during the first month post-natal. Our analyses show that *Rotavirus* is one of the causes of neonatal diarrhea without asymptomatic carriage in calves less than 60 days old.

The pathophysiological effect of rotavirus in diarrhoeic calves is illustrated in the following prevalence table, which shows the degree of dehydration caused by the virus (Table 8). The results above show that *Rotavirus* causes significant dehydration in diarrhoeic calves, with 50% of infected calves showing second-degree dehydration of between 6 and 8%, the second part of the sick population (50%) had severe dehydration estimated at over 8%, and we did not observe any cases of first-degree dehydration (<5%).

Combining two viruses: The tetravalent AgCp-ELISA detected *Coronavirus* isolated in 8 samples with a rate of 53.33% (8/15) of positive cases. Diarrhea caused by *Rotavirus* (*Rotavirus* isolated) was observed in 7.89% (7.46% - 8.33%) of the population included in our survey, either 12 calves out of 152 examined. An association with *Coronavirus* was observed in two (2) samples, representing a prevalence of $1.32 \pm 0.43\%$ (2/152), so the association of two viruses always caused diarrhea in calves less than 30 days old, complicated the clinical picture in sick calves and induced moderate dehydration (6% to 8%) to severe dehydration (>8%)

Table 4 *Coronavirus* and *Rotavirus* prevalence according to the season.

Season	Sample length	<i>Coronavirus</i>			<i>Rotavirus</i>		
		Positive cases	Prevalence (%) (CI to 95% ± 1.05)	Rate (%) by flat positive cases (n = 15)	Positive cases	Prevalence (%) (CI to 95% ± 1.05)	Rate (%) by flat positive cases (n = 16)
Autumn	11	2	18.18%	13.33%	0	0.00%	0.00%
Winter	88	10	11.36%	66.67%	10	11.36%	62.50%
Spring	43	3	6.98%	20.00%	6	13.95%	37.50%
Summer	10	0	0.00%	0.00%	0	0.00%	0.00%
Total	152	15	9.87%	100.00%	16	10.53%	100.00%

$\chi^2 = 15.13$; $p = 0.001$

$\chi^2 = 18$; $p = 0.004$

CI to 95%: confidence interval with risk of error $\alpha = 5\%$

Table 5 Distribution of *Coronavirus* cases according to the clinical condition of the calves (n=15)

Age range	<i>Bcov</i> (+)				
	Sample length	D	Prevalence (CI to 95% ± 0.2)	ND	Prevalence (CI to 95% ± 2.9)
1 - 7 days	8	8	100.00%	0	0.00%
8 - 15 days	4	4	100.00%	0	0.00%
16 - 21 days	2	1	50.00%	1	50.00%
22 - 30 days	1	1	100.00%	0	0.00%
31 - 45 days	0	0	0.00%	0	0.00%
46 - 60 days	0	0	0.00%	0	0.00%
Total	15	14	93.33%	1	6.67%

CI to 95%: confidence interval with risk of error $\alpha = 5\%$

D: Diarrhoeic calves / ND: Non-Diarrhoeic calves

(93.33% vs 6.67% ; $\chi^2 = 18.6$; $p = 0.0008$ / $RR = \text{risque relatif} = 4.83$)

Table 6 Degree of *Coronavirus*-induced dehydration in diarrhoeic calves

Degree of dehydration	Sample length	<i>Bcov</i> (+)	Prevalence (%) (CI to 95% ± 0.73)	Rate (%) by flat positive cases (n=14)
1st (0 - 5%)	38	6	15.79%	42.86%
2nd (6 - 8%)	59	7	11.86%	50.00%
3rd (> 8%)	16	1	6.25%	7.14%
Total	113	14	12.39%	100.00%

CI to 95%: confidence interval with risk of error $\alpha = 5\%$

Diarrhoeic calves ; n = 113: / Diarrhoeic *Coronavirus*-infected calves = 14 / $\chi^2 = 4.42$; $p = 0.01$

Table 7 Occurrence of *Rotavirus* according to calf clinical status

Age range	Sample length	Diarrhoeic sample	Positive case (<i>Rotavirus</i> +)	Prevalence D (%) (CI to 95% \pm 1.58)
1 - 7 days	36	27	4	14.81%
8 - 15 days	47	35	8	22.86%
16 - 21 days	37	28	2	7.14%
22 - 30 days	18	13	2	15.38%
31 - 45 days	6	4	0	0.00%
46 - 60 days	8	6	0	0.00%
Total	152	113	16	14.16%

CI to 95%: confidence interval with risk of error $\alpha=5\%$

(Diarrhoeic : 14,16% vs Non Diarrhoeic : 0.00% ; $\chi^2 = 4.76$; $p = 0.02$ / $RR = \text{risque relatif} = 1.4$)

Table 8 Degrees of dehydration caused by *Rotavirus* in diarrhoeic calves.

Degree of dehydration	Sample length	Bcov (+)	Prevalence (%) (CI to 95% \pm 0.73)	Rate (%) by flat positive cases (n = 16)
1st (0 - 5%)	38	0	0.00%	0.00%
2nd (6 - 8%)	59	8	13.50%	50.00%
3rd (> 8%)	16	8	50.00%	50.00%
Total	113	16	14.16%	100.00%

CI to 95%: confidence interval with risk of error $\alpha=5\%$

Discussion

In our research, we collected samples from calves on farms spread across five provinces (wilayas) in order to obtain a representative overview of the dairy basins in the north of the country. Regarding age, we included calves less than 60 days old, which are susceptible to infection, and age groups where calves are susceptible to several neonatal infections. Our laboratory results show that *Coronavirus* and *Rotavirus* are incriminated in gastroenteritis in calves less than 60 days of age, using the CpAg-ELISA test, which gives results with a sensitivity and specificity that exceed 97% (Beauty *et al.*, 2014).

We found that viral diarrhea (*Coronavirus* and *Rotavirus*) is more prevalent on farms in the central-northern region. This may be accounted by the greater number of cattle in the northern part of the country included in our survey, as well as the density of farms (more than 30 dairy cows) declared infected during the period covered by our study and the grouping of calving, which increases the contamination by infectious agents, particularly viruses.

Regarding the seasonal effect, we found a high prevalence in winter calves, which could be explained by the fact that during winter calves are inside the barn, which favors contamination, and low temperatures (generally between 6°C and 10°C, and sometimes even dropping to below zero in our study region) have a very important role in the conservation of viral virulence.

With regard to age, it should be noted that a calf's immunity in the first few weeks depends on colostrum immunity, which means the quality, quantity, and timing of the colostrum it has taken. If these conditions are not met, the calf will be immunocompromised and susceptible to the agents responsible for diarrhea.

In addition, between 2 and 3 weeks of age, passive immunity is extinguished, while active immunity has not yet been established, which is why we chose this period for sampling.

In addition, the cows had not been vaccinated against the agents responsible for diarrhea, and

colostrum lacked specific antibodies against these agents.

As for the coronavirus, it attacks the mature cells of the intestinal villi and the immature cells of the crypts, which are responsible for the renewal of villi cells. This is why the diarrhea and the dehydration caused by the coronavirus are severe.

Rotavirus only attacks mature intestinal villi and does not attack crypt cells, which allow the intestine to renew itself more easily, which is why diarrhea and dehydration, in this case, are not severe.

The pathogenic role of viral agents was nevertheless clearly demonstrated in our study, as *Rotavirus* was only detected in diarrhoeic calves, and 93% of *Coronavirus* cases were detected in diarrhoeic calves in our survey. These results are in accordance with studies by Khelef *et al.* (2007) in northern Algeria and by Bendali (1999) and Quillet (2005) in France, which indicate the involvement of viruses in the occurrence of calf neonatal gastroenteritis.

Coronavirus was declared the first pathogenic agent in the etiology of neonatal gastroenteritis in calves in Costa Rica by E. Pérès *et al.* (1998) with a prevalence of 9% in agreement with our results (9.87%); however, Gomez *et al.* (2017) in northern Canada detected the *Coronavirus* in 64% of diarrhoeic calves and 46% of healthy calves in a population of 143 calves using RT-PCR testing, and using the same diagnostic method Ivana *et al.* (2015) tested 101 fecal samples from calves belonging to dairy herds in the central region of Croatia where they detected *Coronavirus* (Bcov) in 78.8% of diarrhoeic calves, while Lanz Uhde (2008) and Izzo (2011) estimated the prevalence of *Coronavirus* to be between 0 and 7.4% in non-diarrhoeic calves and between 3.4 and 40% in diarrhoeic calves.

However, for veal calves in Brazil, 93 fecal samples were analyzed using the semi-nested PCR technique by Lorenzetti *et al.* (2013), which revealed the presence of bovine *Coronavirus* (BCoV) in 31 samples, with a prevalence of 33.3%.

The differences between the studies could be explained, at least in part, by the size of the study

population, the difference in the type of test used to detect BCoV, in particular, the sensitivity and specificity of each method, which play a decisive role in detecting the virus, and also by the different types of livestock and farm management practices.

In conclusion, Although our research shows a high prevalence of *Coronavirus* and *Rotavirus* in diarrhoeic calves, as well as in symptomatically healthy calves, their presence is clearly associated with diarrhea in young cattle. They induce severe dehydration, which often complicates their clinical picture and decreases the therapeutic success rate. As these two viruses occupy a primordial place in calf pathologies and are zoonotic, further in-depth research using different molecular techniques is required to determine the different genotypes and also to trace the epidemiological cycle of these two enteropathogenic viruses.

Conflicts of interest: There were no conflicts of interest that may have biased the work reported in this study.

References

- Athanassious R, Marsolais G, Assaf R, Dea S, Descoteaux JP, Dulude S and Montpetit C 1994. Detection of bovine coronavirus and type A rotavirus in neonatal calf diarrhea and winter dysentery of cattle in Quebec: evaluation of three diagnostic methods. *Can Vet J*. 35: 163-169.
- Bendali F, Bichet H, Schelcher F and Sanaa M 1999. Pattern of diarrhoea in newborn beef calves in south-west France. *Vet Res*. 30: 61-74.
- Bertoni E, Aduriz M, Bok M, Vega C, Saif L, Aguirre D, Cimino RO, Mino S and Parreno V 2020. First report of group A rotavirus and bovine coronavirus associated with neonatal calf diarrhea in the northwest of Argentina. *Trop Anim Health Prod*. 52: 2761-2768.
- Boileau MJ and Kapil S 2010. Bovine coronavirus associated syndromes. *Vet Clin North Am Food Anim Pract*. 26: 123-146.
- Gomez DE and Weese JS 2017. Viral enteritis in calves. *Can Vet J*. 58: 1267-1274.
- Izzo MM, Kirkland PD, Mohler VL, Perkins NR, Gunn AA and House JK 2011. Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Aust Vet J*. 89: 167-173.
- Kapil S, Lamm CG, Mcvey DS 2008. Detection of bovine respiratory Coronavirus in beef cattle. In: *Proceedings of the 27th annual meeting of the American Society of Virologists*. Cornell University, Ithaca, NY, 9-1.
- Khelef D, Akam A, Kaidi R, Touati K 2007. Enquête épidémiologique sur les 4 principaux agents responsables de diarrhées chez les veaux dans des élevages du centre et de l'est de l'Algérie. 26ème congrès maghrébin vétérinaire.
- Lojkić I, Krešić N, Šimić I and Bedeković T 2015. Detection and molecular characterisation of bovine corona and toroviruses from Croatian cattle. *BMC Vet. Res*. 11: 202.
- Lorenzetti E, De R, Leme R, Ribeiro J, Rodrigues V, Souza A, Alfieri A, Amauri and Alfieri A 2013. Neonatal diarrhea by bovine coronavirus (BCoV) in beef cattle herds. *Semina Ciências Agrárias*. 34: 3795-3800.
- Mard 2018. Minister of Agriculture and Rural Development. <http://madrp.gov.dz/>.
- Omoruyi BE, Nwodo UU, Udem CS and Okonkwo FO 2014. Comparative diagnostic techniques for cryptosporidium infection. *Molecules*. 19: 2674-2683.
- Perez E, Kummeling A, Janssen MM, Jimenez C, Alvarado R, Caballero M, Donado P and Dwinger RH 1998. Infectious agents associated with diarrhoea of calves in the canton of Tilaran, Costa Rica. *Prev Vet Med*. 33: 195-205.
- Quillet J-M, Assié S, Beaulny M, Lepeule J and Seegers H 2006. Agents pathogènes mis en évidence chez des veaux lors de gastro-entérites néonatales dans les troupeaux bovins de Vendée. *Bulletin de l'Académie vétérinaire de France*. 159: 37.
- Smith BP. 2009. Large animal internal medicine. 4th ed. In: Mosby Elsevier, St. Louis, Mo.
- Striepen B 2013. Parasitic infections: Time to tackle cryptosporidiosis. *Nature*. 503: 189-191.
- Uhde FL, Kaufmann T, Sager H, Albini S, Zanoni R, Schelling E and Meylan M 2008. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. *Vet Rec*. 163: 362-366.
- Vlasova AN and Saif LJ 2021. Bovine Coronavirus and the Associated Diseases. *Front Vet Sci*. 8: 643220.