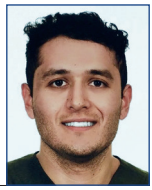
Prevalence and risk factors associated with bovine neonatal diarrhoea in lactating calves in the state of Veracruz, Mexico



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Abstract

Bovine neonatal diarrhoea (BND) is a problem of great relevance in cattle herds, resulting in a decline in productivity and performance in production units, and causing great economic losses. The objective of the study was to determine the prevalence of BND in the state of Veracruz. In total, 300 lactating calves were sampled. Samples were analysed for Rotavirus, Coronavirus, *Escherichia coli* and *Cryptosporidium* spp. Differences between groups were determined by chi-square and risk factors were determined by Odds Ratio. The general prevalence of three geographical areas sampled was 85%. Regarding the analysed pathogens, *Cryptosporidium* spp. Had the highest prevalence with 76%, followed by *E. coli* with a prevalence of 42%. Two pathogens were present in coinfections with a 32% prevalence, unlike diarrhoea caused by one, three or four pathogens. Calves that consume standing water after birth present a higher risk of infection. It is necessary to establish sanitary programmes to reduce the risk of infection, help to avoid economic losses, and improve the productivity of the herd. BND related pathogens Rotavirus, Coronavirus, *Escherichia coli* and *Cryptosporidium* spp. are present in the sampled areas.

Key words: *coronavirus; Cryptosporidium spp.; Escherichia coli; immunochromatography: calves; rotavirus*

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Introduction

Diarrhoea in calves is a problem of great relevance since it results in a decrease in productivity and performance of cattle herds around the world. The presence of diarrhoea can be caused by various factors; however, the most common is the infection of internal pathogens that can be bacterial, viral or protozoan (Suarez et al., 2018). Once they enter the body, they colonise the intestinal epithelium, causing problems of malabsorption, dehydration and even death of the animal, due to atrophy or destruction of microvilli as a consequence of the invasion of infectious agents (Carter et al., 2021). The economic losses caused by these agents are presented as expenses in veterinary assistance, workers training, inputs and pharmacological treatments, reaching figures of US$ 33.46 per calf per year (Tiranti et al., 2015; Dahmani et al., 2020). Bovine Neonatal Diarrhoea (BND) is recognised as a syndrome that causes diarrhoea in calves no older than 30 days of age, and it has also been called undifferentiated diarrhoea since it can be caused by various infectious agents, though mainly *Escherichia coli* k99, Rotavirus (BRoV), Coronavirus (BCoV) and *Cryptosporidium parvum* (Brunauer et al., 2021). The presence of these agents may vary depending on geographical area, type of animal health management, and the climate present in the livestock production unit (LPU) (Dahmani et al., 2020). There are also risk factors specific to the individual that can predispose infection towards the mother or the calf, such as the immunity of the mother, immunity of the calf, nutritional status of the calf, quality of the colostrum administered, overcrowding, and poor hygiene or sanitisation in animal pens (Al Mawly et al., 2015). On the other hand, in Mexico there is little information about the zoosanitary animal status in LPUs regarding BND in calves. Therefore, the objective of this study was to determine the prevalence of pathogens causing BND (*Cryptosporidium* spp., Rotavirus, Coronavirus and *Escherichia coli*) in the centre, north and south of the state of Veracruz, Mexico, in order to implement prevention measures and zoosanitary control at the LPU level.

Materials and methods

Type of study

A cross-sectional epidemiological study with convenience sampling was performed.

Sample size and study location

Sample size was estimated using the Win Episcope V.2.0 program for the estimation of an infinite population proportion with a $CI_{95\%}$ and an error of 5%. The total sample size was 300 lactating calves from one to 15 days of age, regardless of sex or breed. The study was carried out at ten sampling sites distributed in north (Nautla, Ozuluama, Tamiahua, Tuxpan), central (Ixhuacán de los Reyes, Naolinco, Medellín) or south Zone (Sayula de Alemán, Isla, Las Choapas) of the state of Veracruz, with average temperatures of 22.7°C and a relative humidity of 69.4% (INAFED, 2020).

Ethics Committee

The Bioethics and Animal Welfare Commission of the Universidad Veracruzana's Facultad de Medicina Veterinaria y Zootecnia approved the protocol dated October 25, 2020.

Collection and processing of samples

Faeces samples were taken directly from the rectum with a latex glove and were identified with the animal's number, date of birth, number and/or name of the mother. The rapid immunochromatography technique was used through a commercial diagnostic kit "Rainbow Calf

Scours" (BioX Diagnostics) for the antibodies against Rotavirus, Coronavirus, *Escherichia coli* and *Cryptosporidium* spp. Detection, following the manufacturer's instructions.

Data collection

A general and individual survey were applied for each sampled animal. The study variables in the calves were: age, sex, housing (cage or corral), deworming control, previous presented parasitism, frequency of veterinarian attendance, known episodes of diarrhoea, and cleaning habits.

Analysis of data

Descriptive statistics were used to obtain absolute and relative frequencies. The chi-square test with Yates correction ($\chi 2$) was used to compare the occurrences of infection between the analysed varia-

Table 1. Prevalence of Bovine Neonatal Diarrhoea in calves from livestock production units of geographical areas of the state of Veracruz

Geographical Area	*N*	Positives	Prevalence (%)	$CI_{95\%}$
North	100	92	92.0	84.8 - 96.4
Centre	100	87	87.0	79.9 – 93.6
South	100	78	78.0	68.6 – 85.6
Total	300	257	85.0	81.1 – 89.4

CI = 95% Confidence interval

Table 2. Prevalence of Bovine Neonatal Diarrhoea in lactating calves from the municipalities studied in the state of Veracruz

LPU	Municipality	*N*	Positives	Prevalence (%)	$CI_{95\%}$
		North Zone			
1	Nautla	20	19	95.0	75.1 - 99.8
2	Tuxpam	24	23	95.0	76.8 - 99.7
3	Ozuluama	30	26	86.0	68.3 - 95.6
4	Tamiahua	26	24	92.0	73.4 - 97.8
		Central Zone			
5	Medellín	40	32	80.0	63.8 - 90.3
6	Naolinco	30	27	90.0	76.4 -98.8
7	Ixhuacán	30	28	93.0	76.4 - 98.8
		South Zone			
8	Isla	30	22	73.0	53.8 - 87.0
9	Sayula	30	24	80.0	60.8 - 91.6
10	Choapas	40	32	80.0	63.8 - 90.3
TOTAL		300	257	85.0	81.1 - 89.4

CI: 95% Confidence interval

bles. The risk factors associated with bovine neonatal diarrhoea were evaluated by univariate analysis of the variables of interest and by logistic regression analysis, taking the results of the method used as the dependent variable. Odds ratio (OR) values were obtained for each parameter evaluated. The significance level was taken at 5% and all statistical analyses were performed using STATA® V17.0 software.

Results

Bovine Neonatal Diarrhoea was frequent in all three geographical areas sampled (north, centre and south). Calves corresponding to LPUs in the north had the highest prevalence, followed by the central zone and the lowest prevalence were the calves from the south (Table 1).

The prevalence of BND by municipality was relatively high, ranging from 73% to 95% (Table 2). The LPUs in the north

Table 3. Univariate analysis of risk factors associated by pathogen to Bovine Neonatal Diarrhoea in lactating calves in the state of Veracruz, Mexico

Variable	*N*	Positives	O.R.	$CI_{95\%}$	*P* > z	S.E.	Z
***Cryptosporidium* spp.**							
Age 1-15 days	229	130	1.01	0.59 - 1.74	0.94	0.27	0.06
Still water	229	97	5.06*	2.39 - 10.6	<0.01	1.92	4.26
Colostrum 30 – 60 min	229	83	0.87	0.50 - 1.50	0.62	0.24	-0.49
Escherichia coli							
Age 1-15 days	161	99	1.52	0.96 - 2.42	0.07	0.35	1.81
Still water	161	76	3.24*	1.95 - 5.40	0	0.84	4.54
Colostrum 30 – 60 min	161	67	1.53	0.95 - 2.47	0.07	0.37	1.78
Rotavirus							
Age 1-15 days	126	77	1.36	0.85 - 2.18	0.18	0.32	1.32
Still water	126	42	0.85	0.53 - 1.39	0.53	0.21	-0,62
Colostrum 30 – 60 min	126	55	1.63*	1.01 - 2.62	0.04	0.39	2.02
Coronavirus							
Age 1-15 days	117	66	0.98	0.61 - 1.56	0.94	0.23	-0,07
Still water	117	36	0.71	0.43 - 1.17	0.18	0.18	-1,32
Colostrum 30 – 60 min	117	52	1.68*	1.04 - 2.71	0.03	0.41	2.13

O.R: Odds ratio; CI: 95% Confidence interval. S.E: Standard Error; *statistically significant

stand out due to the hydrological and demographic characteristics of the region, since most are surrounded by water basins such as rivers and lagoons, presenting an extremely hot climatic condition.

Analysis of the risk factors in relation to the four pathogens studied and the variables of choice (age at the second week of life, still water, colostrum administered 30 to 60 min) are presented in Table 3. In the case of *Cryptosporidium* spp. and *E. coli*, it was observed that the consumption of still water was a significant risk factor. For *Cryptosporidium* spp., it was five times more probability that the animals would get sick, and similarly for *E. coli* three times more likely. For the viral agents, Rotavirus and Coronavirus, the significant risk factor was colostrum consumption of 30 to 60 min after birth (Table 3).

Discussion

Bovine Neonatal Diarrhoea was a frequent finding in the study areas, with a high prevalence in virtually all municipalities. In particular, the high temperatures in the sampled municipalities and the climatic conditions similar to a warm humid-subhumid tropic, with abundant rains in summer and high humidity, are a fundamental factor for the presence of sick animals due to diarrhoea (Brainard et al., 2020). In a previous study in the centre of Veracruz, LPUs of the municipalities that were at sea level presented a much higher prevalence; these results correspond to the results reported here as most of the LPUs of the sampled municipalities are at sea level, and this favours high temperatures and higher humidity (Castelan-Hernández et al., 2011; Urie et al., 2018). However, our results differ from a study in the central zone of Veracruz state (Romero et al., 2012) that reported a lower prevalence of general diarrhoea of 12.5% caused by *Cryptosporidium* spp. Nonetheless, the diagnostics performed were for this parasite alone and 40% of calves with diarrhoea tested negative, so it possible that other agents were responsible such as those considered in the present study.

Age is s relevant factor considered in production and health management, as diarrhoea in calves is more frequent in young animals because their immune system is not yet prepared for environmental agents, and the age between the first and second week after birth predisposes them to be more susceptible to BND pathogens (Dahmani et al., 2020). In a study conducted on a dairy farm in China, approximately 30% of calves tested positive for one or more pathogens, with a consistency of liquid or semi-liquid faeces, which decreased as the animal aged (Monney et al., 2020). Similar findings have been reported in Colombia, where the presence of diarrhoea decreased as the calf grew (Pardo and Oliver, 2012). The predisposition to diarrhoea in newborn calves is due to the placental characteristics of the mother, which do not allow the passage of immunoglobulins during gestation, resulting in hypogammaglobulinemic offspring post-birth (Al-Alo et al., 2018). Therefore, colostrum consumption is one of the most important variables in the neonatal condition of the calf in the first hours of life, as it acts as a protective barrier against agents in the environment and in the mother.

Our findings suggest that colostrum administration could be a risk factor for BND by Rotavirus and Coronavirus, however the mechanism is not clear as colostrum quality was not measured and practices in each LPU could vary. The purpose of maternal colostrum is to help promote the development and growth

of the suckling calf by providing growth factors, nutrients, proteins, fats, and antibodies present in the mother (Akagami et al., 2020). Colostrum and its quality should be considered when evaluating the presence of diseases in calves (Fontes et al., 2017) as could be vital to decreasing morbidity and mortality levels in the first six months of growth. When it contains good levels of nutrients and immunoglobulins, colostrum makes the calf competent to handle the environment and mitigates the impact of external factors predisposing to the presentation of diarrhoea (Schogor et al., 2020). Calves consuming colostrum with good levels of post-birth IgG have lower mortality rates, increase their weight gain, and are more productive in dairy herds, thanks to the correct transfer of passive immunity (Williams et al., 2014). Colostrum administration functions as a protective factor, though other variables such as the physiological, nutritional, and management conditions in the mother should be evaluated since these factors may be involved in the quality of the colostrum consumed by the calf (Schogor et al., 2020) and this will be reflected in the productive development of the calf.

Regarding the factors predisposing to infection with a causal agent of diarrhoea in calves, the origin of water for consumption in neonates predisposes them to become ill more easily (Sischo et al., 2000), as observed in the present study with the presence of *E. coli* and *Cryptosporidium* spp., both opportunistic pathogens that take advantage of moisture conditions and water sources to colonise the animal's enteric tract. Is reported that *E. coli* and *C. parvum* are the most common pathogens in the presence of bovine neonatal diarrhoea (Lombardelli et al., 2019). However, the presence of these pathogens can vary depending on environmental factors, internal management on holdings, and the type of production (Brunauer et al., 2021). Our findings concur with other studies where the most frequent pathogen was *Cryptosporidium* spp., followed by *E. coli* in young animals, Rotavirus and Coronavirus were also found but in a lesser presence (Abuelo, 2016). *C. parvum* is one of the pathogens that, in most cases, is associated with diarrhoea due to its affinity with stagnant water, poor bedding, poor hygiene in production, and is of great importance due to its zoonotic potential (Caffarena et al., 2020). As for the association of study variables, the consumption of stagnant water was identified as a risk factor for calves, as calves consuming still water were more likely to become ill due to the presence of a BND-causing pathogen. Tropical conditions also predispose to the presence of pathogens, as found in Colombia by Siabato et al. (2022), where *Cryptosporidium* was found to be the causal pathogen of diarrhoea using the same immunochromatographic kit.

Finally, sanitary parameters and zoosanitary management given to the animals in production are variables determining the presence of diarrhoea in calves, as management practices and worker behaviour towards animals in LPUs make it easier for the animal to become infected with pathogens causing LPU diarrhoea (Bertoni et al., 2020; Monney et al., 2020).

Conclusions

Bovine neonatal diarrhoea is a pathological entity of great importance present in calves from LPUs sampled in the the central, northern, and southern regions of the state of Veracruz. The presence of these pathogens represents a potential risk to animal and public health, especially *Cryptosporidium* spp., given its higher presence. However, it is recommended

that the management conditions within holdings be examined, and the quality of colostrum be evaluated through serology to measure its IgG levels and correlate it with the presence of diarrhoea in neonates.

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Prevalencija i čimbenici rizika povezani s neonatalnim proljevom goveda u teladi koja se hrani majčinim mlijekom u državi Veracruz, Meksiko

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Neonatalni proljev goveda (BND) predstavlja problem od velikog značenja u stadima goveda, jer je posljedica smanjenje produktivnosti i učinkovitosti u proizvodnim jedinicama, prouzrokujčeći velike ekonomske gubitke. Cilj je ove studije bio utvrditi prevalenciju BND-a u državi Veracruz. Uzorkovano je 300 teladi hranjenih majčinim mlijekom. Uzorci su analizirani s komercijalnim kompletom

Rainbow Calf Scours (rotavirus, koronavirus, *Escherichia coli* i *Cryptosporidium* spp.). Razlike između skupina su utvrđene metodom X2, a čimbenici rizika su utvrđeni omjerom izgleda (OR). Općenita prevalencija u tri uzorkovana geografska područja bila je 85 %. S obzirom na analizirane patogene, *Cryptosporidium* spp. je imao najveću prevalenciju sa 76 %, nakon čega je slijedila *E. coli* s prevalencijom od 42 %. Dva patogena bila su prisutna u koinfekcijama s 32 % prevalencije, za razliku od proljeva uzrokovanog jednim, tri i četiri patogena. U slučaju OR, otkriveno je da je telad koja konzumira stajaću vodu (OR=2,2) nakon teljenja u većem riziku od infekcije. Potrebno je utvrditi sanitarne programe za smanjenje rizika od infekcije, što bi uvjetolo smanjenju ekonomskih gubitka i poboljšanju produktivnost stada. Patogeni povezani s BND-om, rotavirus, koronavirus, *Escherichia coli* i *Cryptosporidium* spp. prisutni su u uzorkovanim područjima.

Ključne riječi: *koronavirus, Cryptosporidium spp., Escherichia coli, imunokromatografija, telad, rotavirus*