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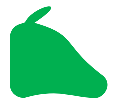
Article



Molecular follow-up study on the presence of bovine respiratory coronavirus in feedlot cattle in Northwest Mexico

Soila Maribel Gaxiola Camacho [a]

Gilberto López Valencia [b]

Enrique Trasviña Muñoz [b]

Nohemí Castro Del Campo [a]

José Carloman Herrera Ramírez [b]

Francisco Javier Monge Navarro [a*]

[a] Universidad Autónoma de Sinaloa. Facultad de Medicina Veterinaria y Zootecnia. Blvd. San Ángel 3886, Mercado de Abastos, San Benito. 80260 Culiacán Rosales, Sinaloa, México.

[b] Universidad Autónoma de Baja California. Instituto de Investigaciones en Ciencias Veterinarias. Mexicali, Baja California, México, México.

* Corresponding author: fmongenavarro@uas.edu.mx

Abstract:

In Mexico, studies on bovine respiratory coronavirus (BCoV) in feedlot cattle are scarce, and its presence has only been reported in feedlot cattle operations in the northwest of the country. The objective of this work was to conduct an epidemiological-molecular study in stabled feedlot cattle to establish the prevalence of BCoV and update the available information on its presence in the Northwest region of Mexico. Using quantitative real-time polymerase chain reaction (qRT-PCT) targeting a fragment of the gene that encodes for the spike protein of BCoV, 154 RNA samples from nasal exudate collected from asymptomatic animals (n=100) and sick (n= 54) were tested, from which 95 resulted positive for a general prevalence of 61.7 % and an average of 1 x 10^5 viral particles/ml of nasal exudate. Of them, 33 (21.4 %) belonged to the group of sick animals and 62 (40.3 %) to asymptomatic animals.

The prevalence of BCoV established in this work demonstrates an increase of 23.7 percentage points above the prevalence of 38.0 % obtained in a previous study, making evident the necessity to introduce vaccination programs that confer protection against BCoV and help to control its expansion on the feedlot cattle of the region. It is also necessary to carry out ecological-epidemiological studies to establish the possible causes that favor the rapid spread of this virus among the feedlot cattle of Northwestern Mexico.

Keywords: Bovine respiratory coronavirus, Stabled feedlot cattle, qRT-PCR, Prevalence, Viral load.

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Introduction

The bovine coronavirus (BCoV) is an enveloped virus with a single-stranded RNA genome and positive polarity of approximately 31 kilobases (kb) belonging to the Betacoronavirus genus of the Coronaviridae family(1). BCoV can cause respiratory and gastrointestinal disease in livestock, shedding the virus through nasal secretions and feces. BCoV is a pathogenic virus associated with calf diarrhea syndrome and winter dysentery in adult cattle. It is recognized as one of the viruses contributing to the development of bovine respiratory disease complex (BRD) in cattle of all ages(2). BCoV is easily transmitted within the herd and between herds in a region by direct contact between animals or indirectly through contaminated personnel or utensils(3). The presence of disease associated with the BRD has a strong impact on the beef cattle industry due mainly to the costs derived from cases of illness or death of cattle, decrease in weight gain, and decrease in carcass value and costs for medications and veterinary services. Preventive measures for BRD are based on vaccination programs and the administration of metaphylactic antibiotics to reduce the risk of co-infections from bacterial origin(4).

The presence of BCoV is frequently reported in domestic feedlot and dairy cattle populations worldwide, with prevalence rates varying from 2 to 90 % depending on the country or region(1). In Mexico, infections caused by BRD have been reported in most feedlot cattle operation regions(5); however, to date, studies of the presence of BCoV in the beef cattle industry in Mexico are extremely scarce, and the presence of BCoV has only been reported in beef cattle operations in the northwest of the country, where 38 % of animals positive to

the virus were identified through molecular tests[6]. Infections caused by BCoV negatively affect the economy of the agricultural sector due to decreased productivity, trade, and food security[7]. Additionally, the plasticity of coronaviruses to infect and cause disease in a wide variety of animal species, along with their high capacity for replication and mutation, makes BCoV a threat to animal health and even public health due to the potential for mutation and adaptation to multiple animal species, including humans[8]. Every year, around 400,000 head of cattle are introduced to the northwest region of Mexico from breeding ranches located in any of the country's 23 cattle-producing states; 95 % of these cattle are destined to feedlots in the municipality of Mexicali where they are finished, slaughtered, and marketed in Mexico and abroad[5]. The presence of BCoV and other pathogens associated with BRD has been previously reported for this region, however, there are no updated reports of the situation of BCoV in northwest Mexico. Based on the above, the objective of this work was to carry out an epidemiological-molecular study of BCoV in stabled feedlot cattle, to establish the prevalence of this pathogenic virus, and to update the information available on its presence in the feedlots from the northwest region of Mexico.

Material and methods

Ethical approval and informed consent

All animal handling and sampling procedures were carried out following the guidelines of the approved local official standards and procedures for the care of animals, including NOM051-ZOO-1995: Humane care of animals during mobilization and NOM-024- ZOO-1995: Animal health stipulations and characteristics during the transportation of animals, and NOM-033-ZOO-1995: Humanitarian care and animal protection during the slaughter process.

Location of the study

The present study was conducted in a feedlot cattle farm with a total confinement capacity of 15,000 head of cattle located in the Mexicali Valley, Baja California, in Northwest Mexico. Processing of nasal exudate samples and qRT-PCR tests were conducted at the Diagnostic Laboratory Unit (ULADI) of the Institute for Research in Veterinary Sciences (IICV) at the Universidad Autonoma de Baja California (UABC).

Nasal exudate samples

Sampling sessions were carried out during February and March of 2023. One hundred and fifty-four (154) heads of cattle, including 130 females and 24 males, with an average weight of 325 kg and 30 +/-3 d of arrival into the feedlot, were selected using the convenience sampling method based on the availability of animals to participate in the study. The animals were divided into sick (n= 54), which presented signs compatible with BRD, including fever ≥38.5 ºC, rapid and shallow breathing, nasal and ocular discharge, salivation, and loss of appetite; and asymptomatic (n= 100) who did not show any of the signs associated with BRD mentioned above. The classification as sick and/or asymptomatic animals was made based on the diagnostic conclusion of the veterinarian officer of the feedlot. From the 154 animals included in the study, a sample of deep nasal exudate was collected using a 15 cm long polyester sterile swab with a brush tip. Also, for each sampled animal, a questionnaire was applied and included the identification number from the National System for Individual Identification of Livestock (SIINIGA), weight in kg, sex, state of origin, presence of clinical signs, and rectal temperature at the time of sampling. This information was used to define the study groups.

RNA extraction from nasal exudate

The Aurum Total RNA Mini Kit (Bio-Rad, Mexico) reagents were used to extract RNA from nasal exudates following the manufacturer's instructions. The RNA extracted from the nasal exudates was reconstituted in a final volume of 20 µl using the RNA elution solution supplied with the reagents and stored frozen at -80 °C until molecular testing.

Oligonucleotides for the BCoV spike protein gene

A qRT-PCR platform was designed from the sequence of the gene that codes for the BCoV spike protein (GenBank reference number: OP037402.1). The sequence is 4,092 nt long and is located between positions 23,628 and 27,719 of the genome for the bovine coronavirus isolate SDSU/2020/06/R, published on July 25, 2023. From that sequence, a fragment of 78 bp located between positions 25,672 and 25,750 was selected as the target for the qRT-PCR. Oligonucleotides were designed using the GeneRunner program version 6.1 (http://www.generunner.net/) and the OligoCalc tool version 3.2 (http://biotools.nubic.northwestern.edu/OligoCalc.html). The characteristics of the

oligonucleotides and amplified product of the qRT-PCR platform are described in Table 1. The oligonucleotides were synthesized by Synbio Technologies (New Jersey, USA), packaged and shipped lyophilized, and reconstituted with molecular biology grade water to obtain an initial standard concentration of 100 micromolar (µM) and a final working concentration of 10 µM.

Table 1: Characteristics of oligonucleotides, amplicon, and synthetic DNA control used in the qRT-PCR-BCoV platform from the spike protein gene of the bovine coronavirus GenBank: OP037402.1

Oligonucleotide: BCoVF1
Sequence: 5'- CGTAGTTGCTATAGTGGTCG -3'
Start position: 25,672 Length: 20 pb Tm: 52 °C GC: 50 %
Oligonucleotide: BCoVR1
Sequence: 3'- GATATTCCGAAATAGCAATGC-5'
Start position: 25,729 Length: 21 pb Tm: 49 °C GC: 38 %
Product: 78 pb Tm amplicon: 79.5 °C
BCoV synthetic DNA control sequence:
ATGGTAATCTCTACGGTTTTAGAGACTACTTAACAAATAGAACTTTTATGA
TT**CGTAGTTGCTATAGTGGTCGTGTTTCAGCGGCCTTTCATGCTAACT**
CTTCCGAACCAGCATTGCTATTTCGGAATATCAAATGCAATTACGTTTT
TAATAACACTCTTTCACGACAGCTGCAACCTATTAA
Product: 184 pb

The 78 bp amplicon is shown in bold, the oligonucleotides are shown in bold and underlined, and the 53 bp sequences flanking the amplicon are in capital letters and without bold.

Positive control for the -qRT-PCR-BCoV platform

The positive control consists of a synthetic DNA molecule that corresponds to the 78 bp amplicon obtained using the oligonucleotides already described, plus the addition of a 53 bp fragment at each end of the amplification sequence to produce a 184 bp molecule of synthetic DNA (Table 1.). The use of positive controls confirms the validity of the results for the BCoV platform in field samples and is required for the generation of the calibration curve for the BCoV-RT-PCR platform[9]. The positive control used for the qRT-PCR-BCoV platform was synthesized by Synbio Technologies (New Jersey, USA) and supplied lyophilized at a concentration of 0.2 mg and reconstituted in 1 ml molecular biology grade water.

Calibration curve for the qRT-PCR-BCoV platform

For the quantification of the amplified DNA, a calibration curve was constructed using the positive synthetic DNA control in logarithmic serial dilutions from 10^7 to 10^1. By extrapolating the Ct value of an unknown sample with the calibration curve, it was able to determine the number of amplicons produced in a sample[10]. The results were expressed as genomic equivalents (GE) per ml of nasal exudate. Considering that the 78 bp amplification sequence is not repeated in the rest of the coronavirus genome used as a reference for this work, one GE is considered equal to one viral particle amplified and detected by the qRT-PCR-BCoV platform.

Protocol for qRT-PCR-BCoV

For the qRT-PCR-BCoV test runs, the qPCRBIO SyGreen 1-Step Go Lo-ROX master mix from PCR Biosystems (Pennsylvania, USA) was used to generate complementary DNA (cDNA) from the RNA samples in the same tube as the PCR reaction was performed. Briefly, each 10 µl reaction contained 5 µl of master mix, 10 ng of RNA extracted from each of the 154 samples, 0.5 µl (400 nM) of each of the BCoVF1 and BCoVR1 oligonucleotides, and 3.0 µl of molecular biology grade water. Additionally, positive control reactions containing 5 µl of master mix, 10 ng of the synthetic DNA positive control 0.5 µl (400 nM) of each of the BCoVF1 and BCoVR1 oligonucleotides, and 3.0 µl of molecular biology grade water were included in each test run. Negative controls contained master mix without DNA or RNA, and molecular biology grade water alone were also included in each test run. Samples were tested in duplicate, and positive and negative controls were in triplicate. The tests were performed in a CFX96 thermal cycler (Bio-Rad, Mexico). Denaturation, hybridization, and extension parameters used in the qRT-PCR-BCoV platform were calculated using the Protocol AutoWriter tool of the Maestro software package from the CFX96 thermocycler. PCR conditions start with a cycle of 50 °C for 10 min for reverse transcription of the RNA, followed by a cycle of 3 min at 95 °C for transcriptase inactivation, then, 40 cycles starting with denaturation at 95 °C for 10 sec, hybridization at 52.8 °C for 10 sec and an extension cycle at 72 °C for 8 sec. Additionally, in each run the dissociation curve analysis (Tm) was included from 65 °C to 95 °C with increments of 0.5 °C, to confirm that the amplification curves correspond to the Tm for the 78 bp amplicon established at 79.5 +/- 1.0 °C and compare them with the Tm obtained by the synthetic DNA positive control and each of the 154 nasal swab RNA samples tested.

Interpretation of results

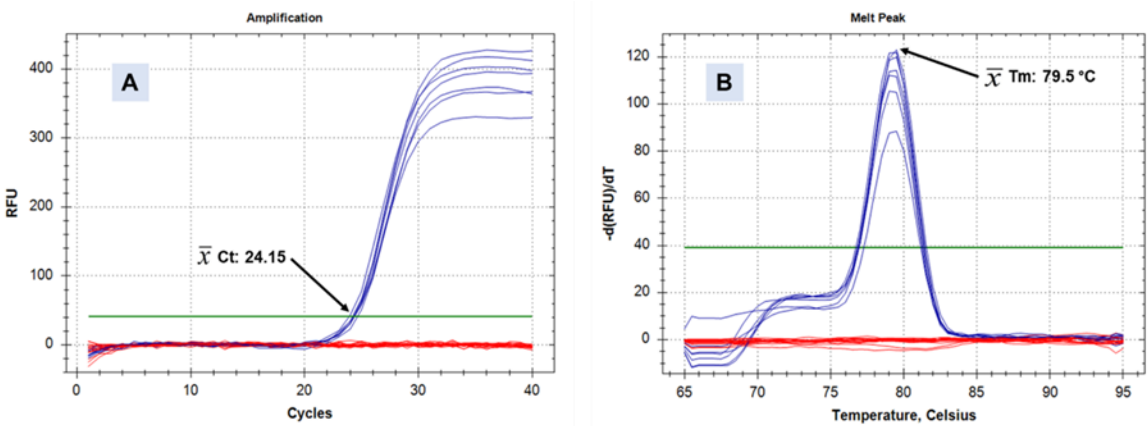
The results of the qRT-PCR-BCoV tests were considered positive when a sample developed an amplification curve above the cut-off value that the CFX96 Maestro package calculates for each run in a maximum of 40 cycles and which corresponds to 10 times the standard deviation of the average fluorescence index generated by all samples during the first 10 cycles of each run; and when the amplification curve develops a Tm of 79.5 +/- 1.0 °C. The results were considered negative when a sample failed to develop an amplification curve above the cut-off value in a maximum of 40 cycles, nor did it agree with the established Tm of 79.5 +/- 1.0 °C.

Results

Standardization of the qRT-PCR-BCoV platform

The analysis of the amplification and dissociation curves calculated by BioRad's Maestro software confirms that under the CFX96 thermal cycler conditions described, the optimal combination of reagents to obtain maximum amplification of the 78 bp fragment of the spike protein gene occurs by mixing the oligonucleotides at a concentration of 400 nM with 10 ng of template RNA or 10 ng of synthetic DNA positive control, 5 µl of master mix and, 3.0 µl of molecular biology grade water in a total reaction volume of 10 µl. Applying these conditions, the synthetic DNA positive control developed an amplification curve above the control line with an average threshold cycle (Ct) of 24.15, while the negative controls showed no evidence of amplification in 40 cycles. The analysis of the Tm for the positive control of synthetic DNA showed an average temperature of 79.4 °C (Figure 1), parameters that confirm the validity of the qRT-PCR-BCoV platform for the test and analysis of the RNA extracts obtained from the 154 nasal exudate samples.

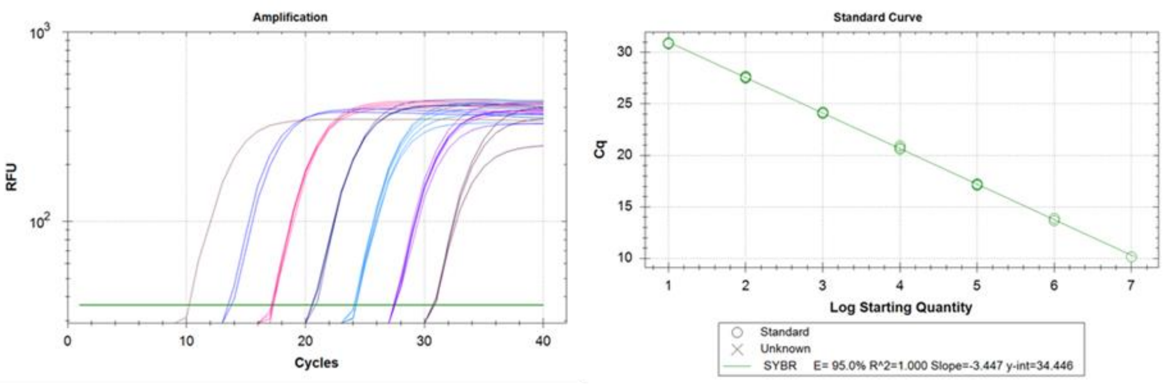
Figure 1: Amplification (A) and dissociation (B) curves obtained from the optimization of oligonucleotides, template RNA, and master mix of the qRT-PCR-BCoV platform using synthetic control DNA



qRT-PCR-BCoV calibration curve

To develop a quantitative molecular platform for the detection of bovine coronavirus, a calibration curve was constructed using the synthetic positive control DNA in logarithmic dilutions from 10^7 to 10^1 GE. The fluorescence index of each log dilution was plotted by the BioRad's Maestro software resulting in a calibration curve with an efficiency of 95 %, amplitude of 3.447 cycles between the log dilutions without dispersion among dilutions expressed with an R2 of 1.0, and range detection between 1 x 10^1 and 1 x 10^7 GE, allowing the calculation of the number of GE amplified and detected in each sample based on the Ct of each particular reaction (Figure 2.). It is important to mention that when applying the qRT-PCR-BCoV calibration curve, one GE corresponds to one viral particle.

Figure 2: Calibration curve for the qRT-PCR-BCoV platform using synthetic DNA control in logarithmic serial dilutions from 1 x 10^1 to 1 x 10^7 EG



Results of the qRT-PCR-BCoV in nasal swab samples

Using the qRT-PCR-BCoV platform, 154 samples of RNA extracted from nasal exudate from feedlot cattle were tested in duplicate, where 95 developed an amplification curve above the threshold line established by the CFX96 Maestro software and were considered positive for bovine coronavirus for a general prevalence of 61.7 %. Of the samples that resulted positive, 33 (21.4 %) belonged to the group of animals classified as sick and showed signs associated with BRD. The 62 (40.3 %) remaining samples belonged to the group of animals classified as asymptomatic and did not show signs of BRD at the time of sampling (Table 2.). The average Tm of the positive controls in the test runs was 79.4 °C and was considered valid for a positive result in the qRT-PCR-BCoV. The average Ct of the positive controls during the test runs was 24.15 cycles, equivalent to 1 x 10^4 genomic equivalents in 10 ng of RNA template per RT-PCR reaction. Similarly, the average Tm of the test samples with a positive result from both groups of animals was 79.38 °C, and the average Ct was 34.60 cycles, equivalent to 1 x 10^5 viral particles/ml of nasal exudate, with a Ct range between 30.87 and 35.95 cycles, equivalent to 5.8 x 10^6 and 6.5 x 10^4 viral particles/ml of nasal exudate, respectively.

Table 2: Results of qRT-PCR-BCoV tests on nasal swab samples

Group	Positive	Negative	Total
Sick	33 (21.4 %)	22 (14.3 %)	54 (35.7 %)
Asymptomatic	62 (40.3 %)	37 (24.0 %)	100 (64.3 %)
Total	95 (61.7 %)	59 (38.3 %)	154 (100 %)

Sick animals presented fever ≥38.5 ºC, rapid and shallow breathing, nasal and ocular discharge, salivation, and loss of appetite, based on the diagnostic conclusion of the veterinarian officer of the feedlot.

Results of the qRT-PCR-BCoV by group of animals and place of origin

The analysis of the questionnaire used to define the study groups indicates that the cattle included in the present study originated, in alphabetical order, from the states of Baja California, Durango, Jalisco, Michoacán, Nayarit, Sonora and Zacatecas, being the states of Jalisco, Sonora and Zacatecas where most of the livestock came from, accumulating 83.3 % (136/154) of animals sampled and tested with the qRT-PCR-BCoV platform. Of them, Sonora

was the state with the highest number of positive cases, with 64.5 % (20/31), followed by Jalisco with 63.2 % (36/57) and Zacatecas with 58.3 % (28/48) positive specimens to the qRT-PCR-BCoV platform (Table 3.).

Table 3: Results of the qRT-PCR-BCoV tests by place of origin and study group

	Positive		Negative		
Origin	**Sick**	**Asymptomatic**	**Sick**	**Asymptomatic**	**Total**
Baja California	1	1	1	3	6
Durango	2	1	0	0	3
Jalisco	8	28	4	17	57
Michoacán	1	2	0	1	4
Nayarit	1	2	0	2	5
Sonora	6	14	7	4	31
Zacatecas	14	14	10	10	48
Total	33	62	22	37	154

Sick animals presented fever ≥38.5 ºC, rapid and shallow breathing, nasal and ocular discharge, salivation, and loss of appetite, based on the diagnostic conclusion of the veterinarian officer of the feedlot.

It is important to mention the high number of samples from the group of asymptomatic animals that resulted positive for the qRT-PCR-BCoV platform with 65.3 % (62/95) cases, since these animals did not show any signs of respiratory disease during the sampling. Also, a group of animals classified as sick were diagnosed with respiratory disease by the feedlot veterinarian, resulting in 37.3 % (22/59) cases with a negative test for bovine coronavirus.

Discussion

Currently, BCoV infections are widely distributed throughout the world in different livestock populations, causing significant losses to the economy of the farm industry dedicated to the production of meat and milk. The role that BCoV plays as part of BRD has been a subject of intense debate since the 1990s[11]. However, at present, the role that BCoV plays as part of BRD is widely recognized in the development of respiratory tract infections and the decrease in the rate of weight gain in the feedlot[12]. Prevalence rates have increased in all places where the presence of BCoV has been reported, mainly due to the large quantity of infectious virus shed through nasal secretions and feces, to the presence of asymptomatic carriers that constantly shed viruses through the respiratory or digestive route and function as a source of infection for both susceptible livestock and other animal species [13], and to the fact that the immune response is not always capable of efficiently containing the infection of BCoV or completely eliminate the virus from recovered sick animals[14].

In this work, a quantitative real-time PCR platform was developed and applied for the detection of a fragment of the gene that encodes the BCoV spike protein in RNA samples extracted from the nasal exudate of feedlot cattle. The qRT-PCR-BCoV platform resulted in a highly sensitive, quantitative molecular diagnostic tool with high levels of repeatability and reproducibility capable of detecting up to 10 viral particles in a test reaction containing 10 ng of RNA template (Figure 2).

The prevalence of 61.7 % established for BCoV in this work demonstrates a large increase of 23.7 percentage points over the prevalence of 38.0 % obtained in a previous study conducted by our group on the same livestock farm[6]. According to the information provided by the veterinary staff of the feedlot, the overall animal management, preventive medicine, feeding and housing protocols of the recently arrived animals remain the same as from 5 yr ago when the previous study was conducted, suggesting an increase in the infection rate of cattle in the states of origin of the livestock, which added to the stress received from the long distance transportation journeys, it manifests as a high rate of sick animals with signs associated with BRD shortly after arrival to Baja California feedlots.

Compared to other studies conducted in the American continent, the prevalence obtained in this work is higher than the reported in Canada, where a study carried out using nasal exudate samples from feedlot cattle upon arrival resulted in a prevalence of 45.2 % for BCoV[15]; and similar to the reported in the United States in feedlot cattle at the initiation of treatment for BRD, reporting a 62.8 % BCoV prevalence in nasal swabs samples[16], but below to the 100% prevalence to BCoV reported in a study conducted also in the USA to detect viral pathogens in calves during a BRD outbreak, where it was found that all the samples analyzed by molecular techniques reacted positive for BCoV[17]. The results of the present work are also similar to those reported in Brazil, in a study carried out to determine the frequency of viruses present in calves diagnosed with BRD, and where a prevalence of 56.0 % was established for BCoV without evidence of infection with any of the other virus associated to BRD[18].

In Europe, the studies on BCoV in feedlot cattle mostly report prevalence rates lower than those obtained in this work. Slovenia reports a prevalence of 12.03 % for BCoV, where BCoV turned out to be the second most frequently detected virus in cattle diagnosed with BRD[19]. Ireland reports a lower prevalence than our work, with a 22.9 % prevalence for BCoV, resulting in the most frequently detected virus[20]. Reports from Switzerland indicate a slightly lower prevalence than that obtained in this study, with 53.5 % of positive animals, where again, BCoV was the most frequently identified virus[21]. In Italy, in a study carried out to evaluate the impact of respiratory stress in beef calves transported over long distances, they found a general prevalence of 70.12 % for BCoV, higher than that reported in this work and where BCoV was also the most frequently identified pathogen[22].

Reports of BCoV in the Asian continent indicate prevalence rates considerably lower than those obtained in this work. Japan reports a BCoV prevalence of 21.2 % for the period between 2016 and 2018 in healthy cattle and in cattle with evidence of BRD[2], while China reports a prevalence of 15.26 % for BCoV in nasal exudate samples from feedlot cattle[1]. In Australia, in feedlot cattle with BRD-associated disease, a BCoV prevalence of 72.0 % was reported[23], that prevalence is higher than the prevalence reported in this work but lower than the 40.1 % prevalence of BCoV reported previously in that same country in animals shedding the virus through nasal secretions[24].

It is important to highlight the fact that from the prevalence reports mentioned in the Americas, Europe, Asia, and Australia, the frequency where BCoV occupies the first or second position as the most frequent pathogen identified associated with BRD has been increased recently, displacing other virus associated with BRD and becoming the most frequently identified virus in feedlot cattle worldwide.

The qRT-PCR-BCoV platform developed in this work was used to establish the number of GE present in RNA extracted from nasal exudate samples, that is, the number of RNA copies coding for the 78 nt fragment of the BCoV spike protein gene. The results of the qRT-PCR tests for asymptomatic and sick animals indicate an average of 1×10^5 viral particles/ml of nasal exudate, with a range of 5.8×10^6 to 6.5×10^4 viral particles/ml of nasal exudate. The nasal exudate viral loads reported in this work match the viral loads excreted through nasal exudate reported as sufficient to produce BCoV transmission from infected animals with or without signs to susceptible exposed animals[22]. In the same way, when the GE data (viral loads) present in the nasal exudate are compared with the presence of clinical signs or the exposure of healthy susceptible animals to infected animals, it can be used as evidence of active viral replication within the animals of a feedlot, making possible to establish the magnitude of the infection in the feedlot, as well as to initiate biosecurity strategies for animals that are close to movement within the farm, for transportation or sale[13].

Regarding the high number of asymptomatic animals with a positive result in the qRT-PCR-BCoV, was discarded the possibility of the protective effect of vaccination, given that the feedlot where the present study was conducted does not vaccinate their cattle against bovine coronavirus. In any case, vaccines commercially available against bovine coronavirus fail to induce sterilizing immunity, that is, they are incapable of eliminating the totality of the virus from the host or preventing subsequent episodes of infection. The main goal of the existing bovine coronavirus vaccines is to elicit immunological protection for both enteric and respiratory diseases[25,26]. Given the latter, the continuous presence of coronavirus in infected or vaccinated cattle is expected to produce a positive result in the qPCR tests. Another possible explanation for why several asymptomatic animals showed a positive result on the qRT-PCR-BCoV is that they were recently infected by asymptomatic carrier animals

shedding the virus through nasal secretions and feces during transport or at the premises and were in a phase of viral proliferation with no apparent signs of disease[27,28].

Concerning the group of animals with a negative result in the qRT-PCR-BCoV and showing signs of respiratory disease, it is suggested that those animals would be infected with one or more of the other viruses associated with BRD, such as IBR, BVDV types 1 and 2, PI3, or BRSV. Although evidence of vaccination against these viruses is part of the feedlot biosecurity protocols, vaccines do not provide 100 % protection against disease and sometimes cattle get infected even when vaccinated mainly due to different factors including lack of efficacy and efficiency of vaccines in the field, inappropriate formulation of vaccine, inappropriate time of vaccination, maternal antibodies, and the condition of the animals[26]. Moreover, while viruses associated with BRD (IBR, BVDV, PI3, and BRSV) have been widely studied for decades and recognized as important components in the development of respiratory disease in cattle, new, emerging, or unrecognized viral agents have not been detected because they are not routinely considered until recently. The emergence of bovine rhinitis A virus, bovine influenza D virus, and bovine Nidovirus has been reported from feedlot cattle diagnosed with bovine respiratory disease and asymptomatic cattle[29,30]. Any of those viruses have been previously reported in feedlots from Mexico and might be another cause of disease associated with BRD.

Conclusions and implications

The qRT-PCR platform for BCoV developed in this study demonstrated to be a fast, accurate, and sensitive molecular diagnostic tool for the detection of BCoV in nasal exudate samples from feedlot cattle and can be used for diagnosis and epidemiological surveillance of BCoV and other pathogenic agents associated with BRD. Considering the increase of 23.7 percentage points in the prevalence obtained for BCoV over 5 yr in the same geographic region, it is evident the need to introduce new-generation vaccines that confer protection against this virus, along with other biosecurity strategies to control the expansion of BCoV infection in the feedlot cattle operations of the region. It is urgent to carry out eco-epidemiological studies to establish the possible causes that favor the rapid spread of this disease among the feedlot cattle population of Northwestern Mexico.

Conflicts of interest

The authors have no conflict of interest to declare regarding this publication.

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