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# Diarrhea outbreak associated with coronavirus infection in adult dairy goats

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## Abstract

**Background:** Infection by coronaviruses cause gastrointestinal disease in many species. Little is known about its prevalence and importance in goats.

**Objective:** Identify the etiology, demographics, and clinical features of an outbreak of diarrhea in adult goats.

**Hypothesis:** Bovine coronavirus (BCoV) PCR would detect viral material in feces of goats in the herds involved in the diarrhea outbreak.

**Animals:** Twelve herds with 4 to 230 adult goats were affected. Goats sampled for fecal PCR were  $\geq 1$ -year-old: 25 from affected herds and 6 from a control herd.

**Methods:** This is a cross-sectional descriptive study of an outbreak of diarrheal disease in adult goats. BCoV PCR primers for the spike (S) or nucleocapsid (N) proteins were used to test fecal material from affected goats. The N protein sequencing and phylogenetic analysis was performed. Herd records and owner surveys were used to characterize morbidity, clinical signs, and treatment.

**Results:** In 2 affected herds 18/25 of animals had at least 1 positive BCoV PCR test. Goats from affected herds were significantly more likely to be PCR positive than the control herd (OR 8.75, 95% CI 1.11-104,  $P = .05$ ). The most common clinical signs were change in fecal consistency (19/20) and decreased milk production (14/15). Phylogenetic analysis of the N protein showed this virus was closely related to a bovine-like coronavirus isolated from a giraffe.

**Conclusions and Clinical Importance:** Bovine coronavirus primers detected nucleic acids of the N and S proteins in feces of goats in affected herds. Coronavirus shedding frequency was temporally associated with the outbreak.

## KEYWORDS

caprine, infectious disease, PCR assays, viral disease

**Abbreviations:** BCoV, bovine coronavirus; CoV, coronavirus; EM, electron microscopy; N, nucleocapsid; S, spiked.

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## 1 | INTRODUCTION

Coronaviruses (CoVs) are enveloped, positive-stranded RNA viruses with a tropism for the gastrointestinal and respiratory tracts causing diseases in a wide range of hosts.<sup>1</sup> In cattle, CoV is associated with neonatal calf diarrhea,<sup>2</sup> winter dysentery in adult cattle<sup>2</sup> and outbreaks of respiratory disease.<sup>3,4</sup> Bovine-like CoVs are associated with diarrhea outbreaks in adult captive wild ruminants<sup>5</sup> and CoVs are identified in diarrhetic sheep.<sup>6-8</sup> Little is reported about CoV in goats. It is not an important pathogen in neonatal diarrhea in goats.<sup>8-10</sup> There is variable seropositivity rates (1%-43.1%) to bovine coronavirus (BCoV) in adult goats.<sup>11-13</sup> Molecular detection of BCoV occurs in 4.5% goats comingled with cattle but is not associated with disease.<sup>14</sup> PCR detection of BCoV is reported in captive wild ruminants with diarrhea.<sup>5</sup>

Animal exhibitions present a risk for disease transmission, because of comingling different herds and species. Biosecurity practices by individual herds vary with many having poor protocols, increasing the risk of disease transmission.<sup>15</sup> Disease outbreaks associated with livestock exhibitions are reported,<sup>16,17</sup> as well as frequent anecdotal accounts. Dispersal of animals after an event can make outbreak investigations difficult.

Our objectives were to identify common clinical signs in adult dairy goats in an outbreak of diarrheal disease and to determine the relevance of BCoV positive PCR in affected herds. We hypothesized that BCoV PCR could detect viral antigen in goat feces and a greater frequency of positive results would occur in the affected herds.

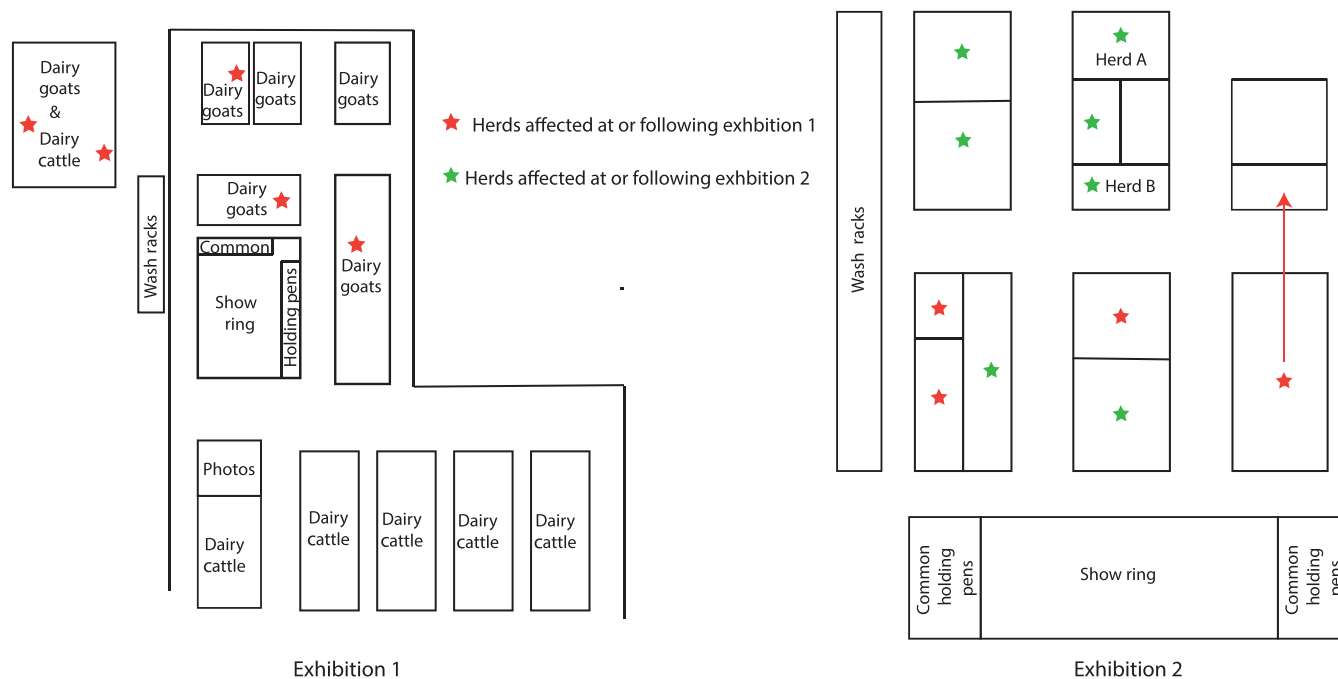
## 2 | MATERIALS AND METHODS

### 2.1 | Outbreak population and survey

The outbreak involved dairy goat herds that attended 2 exhibitions between July 24th and August 3rd, 2017 in Northern California. Twelve herds were identified as having adult goats with diarrhea, by contacting exhibitors using the fairs' entry lists. Five herds were affected after the first exhibition, 7 additional herds were affected after the second (Figure 1). Exhibition 1 had >30 goat herds in attendance and shared wash racks, barn airspace and movement corridors with dairy cattle (Figure 1). Exhibition 2 had 13 herds in attendance, 4 were affected herds from the first fair, and the facility only housed goats (Figure 1). Exhibition 2 required an entry veterinary inspection, but animals developing diarrhea during the fair remained on the premises. A survey was sent to the 12 herds reporting diarrheal cases regarding clinical signs, animals affected, treatments used and outcomes of affected animals.

### 2.2 | Diagnostic workup group

Two of the 12 affected herds (herd A and herd B), both affected after exhibition 2 (Figure 1), submitted fecal samples to Laboratory A (California Animal Health and Food Safety Laboratory, University of California, Davis, California) for fecal culture, *Salmonella* spp. PCR, clostridial toxin testing, fecal egg count for trichostrongyles, *Coccidia*, and *Cryptosporidium*. Two samples were submitted for electron



**FIGURE 1** Facility configuration and location of affected herds at 2 consecutive exhibitions associated with outbreaks of adult goat diarrhea. Diagram illustrates proximity of affected herds as well as shared facilities such as movement corridors; show rings, holding pens and wash racks at the 2 exhibitions. The 2 herds (herds A and B) that were followed extensively were affected after exhibition 2

microscopy (EM). Feces were tested for CoV using PCR with BCoV primers. These samples were submitted within 3 days of the start of the outbreak from clinically affected animals (3 from herd A and 2 from herd B).

After the initial positive BCoV PCR results on the clinically affected animals, investigators initiated longitudinal fecal testing of all adult animals (>1 year of age) from the 2 herds. Feces or rectal swabs were submitted to Laboratory B (Real-Time PCR Research and Diagnostics Core Facility, University of California, Davis, School of Veterinary Medicine, Davis, California) for PCR for the nucleocapsid (N) protein gene of BCoV. Herd A had 21 and herd B had 4 adult animals. These were samples of convenience because of accessibility to the authors. Weekly sampling for BCoV PCR at Laboratory B was performed for 4 weeks starting 17 days after the start of the outbreak. Herd health records from both herds were analyzed for clinical signs, duration of illness and recurrence of disease.

### 2.3 | Control group

Fecal sample/rectal swabs were collected from 6 adult (>2-years-old) dairy breed goats from a herd that had no animal movement in >6 months. The samples were collected at the same time as the initial sampling of herds A and B.

### 2.4 | PCR analysis

BCoV PCR testing at the Laboratory A was performed as described.<sup>18</sup> In brief, RNA extraction was performed according to the manufacturer's specifications (MagMAX-96 Viral RNA Isolation Kit, Life Technologies, Carlsbad, California). Total RNA was converted to complementary DNA (cDNA). The samples were assayed for the presence of BCoV by real-time PCR detection of an 84 base-pair product of the spiked (S) glycoprotein gene (the primers were provided by Dr Kathy Kurth, Wisconsin Veterinary Diagnostic Laboratory, Madison, Wisconsin). The samples' amplification condition was: 50°C for 10 minutes, 95°C for 10 minutes, 2-cycle repeat of 40 cycles of 15 seconds at 95°C, and 60°C for 1 minute. An internal quality control (XIPC) was used for each sample, each batch had positive and negative PCR controls and extraction controls.

For the longitudinal surveillance of herds A and B feces/rectal swabs from adult goats were submitted to laboratory B. Nucleic acids were purified using a QIAcube extraction system and QIAamp 96 DNA QIAcube HT kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. A eukaryotic 18 seconds rRNA (Life Technologies, Carlsbad, California) was used for extraction control. cDNA was synthesized using a Quantitect Reverse Transcription Kit (Qiagen, Hilden, Germany): RNA (10 µL) was digested with 1 µL of gDNA WipeOut Buffer for 5 minutes at 42°C, then 0.5 µL of Reverse Transcriptase, 2 µL RT buffer, 0.5 µL RT Primer Mix, 0.5 µL 20 pmol Random Primers (Invitrogen, Carlsbad, California) and 5.5 µL RNase free water were added and incubated at 42°C for 40 minutes. The

samples were inactivated at 95°C for 3 minutes, chilled, and 60 µL of water was added.

For BCoV N protein gene detection the forward primer is vBCoV-826f (CCCAATAACAATGCACTGTTCA), the reverse primer is vBCoV-919r (CACTAGTTCCAAGTTTTAACATTTCTCC), and the probe is UPL#119 (TTGGTGGT) for a 93 base-pair (bp) amplicon were designed using Primer Express software (Applied Biosystems, Waltham, Massachusetts). The qPCR system was validated using 10-fold dilutions of cDNA testing positive for the target gene and a standard curve plotted. Amplification efficiency was calculated using the slope of the standard curve and the formula  $E = 10^{1/-s} - 1$ . For validation, the efficiency must be >90%. Each qPCR reaction (12 µL) contained a final concentration of 400 nM for each primer and 80 nM for the probe, TaqMan Universal PCR Mastermix (Applied Biosystems, Waltham, Massachusetts) and 5 µL of the diluted cDNA. The samples were run on an ABI PRISM 7900 HTA FAST real-time PCR sequence detection system (Applied Biosystems, Waltham, Massachusetts). The amplification conditions were 2 minutes at 50°C, 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C and 1 minute at 60°C.

### 2.5 | Sanger sequencing

A 440 bp amplicon was generated using universal primers designed for N protein gene sequencing (CoV-forward (GCAAAGTCTACTTCTCAG C), CoV-reverse (TCCCGAGCCTTCAATATAGTA). Advantage 2 polymerase (Takara Bio, Kusatsu, Japan) was used for PCR according to the manufacturer's recommendations. The amplicon was submitted for Sanger sequencing (College of Biological Sciences UC DNA Sequencing Facility, University of California, Davis, Davis, California). Sequences were analyzed using the Sequence Scanner Software 2 from Life Technologies. Other CoV genomes were identified using NCBI Blast.

The evolutionary history was inferred using the Neighbor-Joining method and a bootstrap consensus tree was inferred from 500 replicates. The evolutionary distances are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGAX.

### 2.6 | Data analysis

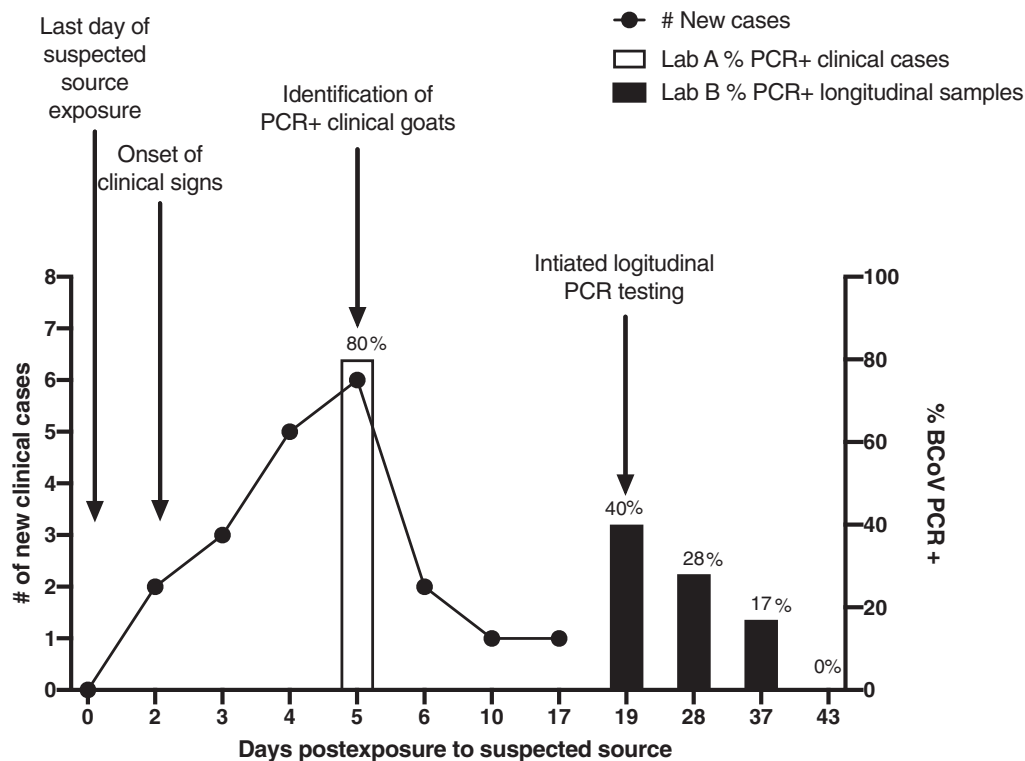
Clinical signs and demographics were reported as population proportions. BCoV PCR and clinical signs were reported as percentages and population proportion at each timepoint. Chi-square tests compare the frequency of PCR positive tests and a Student's *t* test compare Cq values. Statistical analysis was performed using GraphPad Prism Version 8. A *P*-value of <.05 was significant.

## 3 | RESULTS

Twelve herds reported animals with alterations in fecal consistency, ranging from soft-formed to watery. Herd size ranged from 4 to

230 adult animals. All 12 herds had affected adult females of dairy breeds: Nubian (3/12), Toggenburg (2/12), Alpine (2/12), Mixed including Alpine, La Mancha, Nubian, Saanen, Nigerian Dwarf, and crossbred (5/12). The survey had a response rate of 75% (9/12). Some herds

reported affected animals that did not attend exhibitions, including adult males (2/9) and weaned kids (>4 months of age; 4/9). The reported morbidity ranged from 15% to 90%. One death was reported. Other clinical signs reported included: acute drop in milk production



**FIGURE 2** Temporal association of the number of new daily clinical case and prevalence of fecal PCR positivity for BCoV in adult dairy goats in affected herds with exhibition-associated diarrhea. Animals began exhibiting clinical signs 2 days after returning from exhibition with the highest number of new cases 5 days after returning (line graph with black dots). BCoV was detected in fecal material from clinically affected animals via PCR for the S glycoprotein gene of BCoV ( $n = 5$ , 80% PCR+, open column). Positivity rate of PCR for N protein gene of BCoV testing feces from all animals >1 year in 2 affected herds revealed decreasing prevalence of BCoV over time after the outbreak.  $N = 25$  animals for days 19 to 28,  $n = 23$  for day 37, and  $n = 24$  for day 43 (black columns)

|                                                                       | Herd A      | Herd B    | Total       |
|-----------------------------------------------------------------------|-------------|-----------|-------------|
| Affected goats/total goats (%)                                        | 17/21 (81%) | 3/4 (75%) | 20/25 (80%) |
| Age median (range)                                                    | 2 (1-8)     | 2 (2-4)   | 2 (1-8)     |
| Female                                                                | 18          | 4         | 22          |
| Male                                                                  | 3           | 0         | 3           |
| <b>Clinical signs</b>                                                 |             |           |             |
| Change in fecal consistency                                           | 16          | 3         | 19          |
| Acute decrease in milk production (lactating females only, $n = 15$ ) | 12          | 2         | 14          |
| Anorexia                                                              | 6           | 2         | 8           |
| Fever                                                                 | 2           | 1         | 3           |
| Cough                                                                 | 1           | 1         | 2           |
| No clinical signs                                                     | 4           | 1         | 5           |
| Relapse cases                                                         | 4           | 1         | 5           |

**TABLE 1** Summary of demographics and frequency of clinical signs of 2 dairy goat herds with coronavirus associated diarrhea

Note: Changes in fecal consistency was the most common clinical finding, followed by decreased milk production in lactating animals.

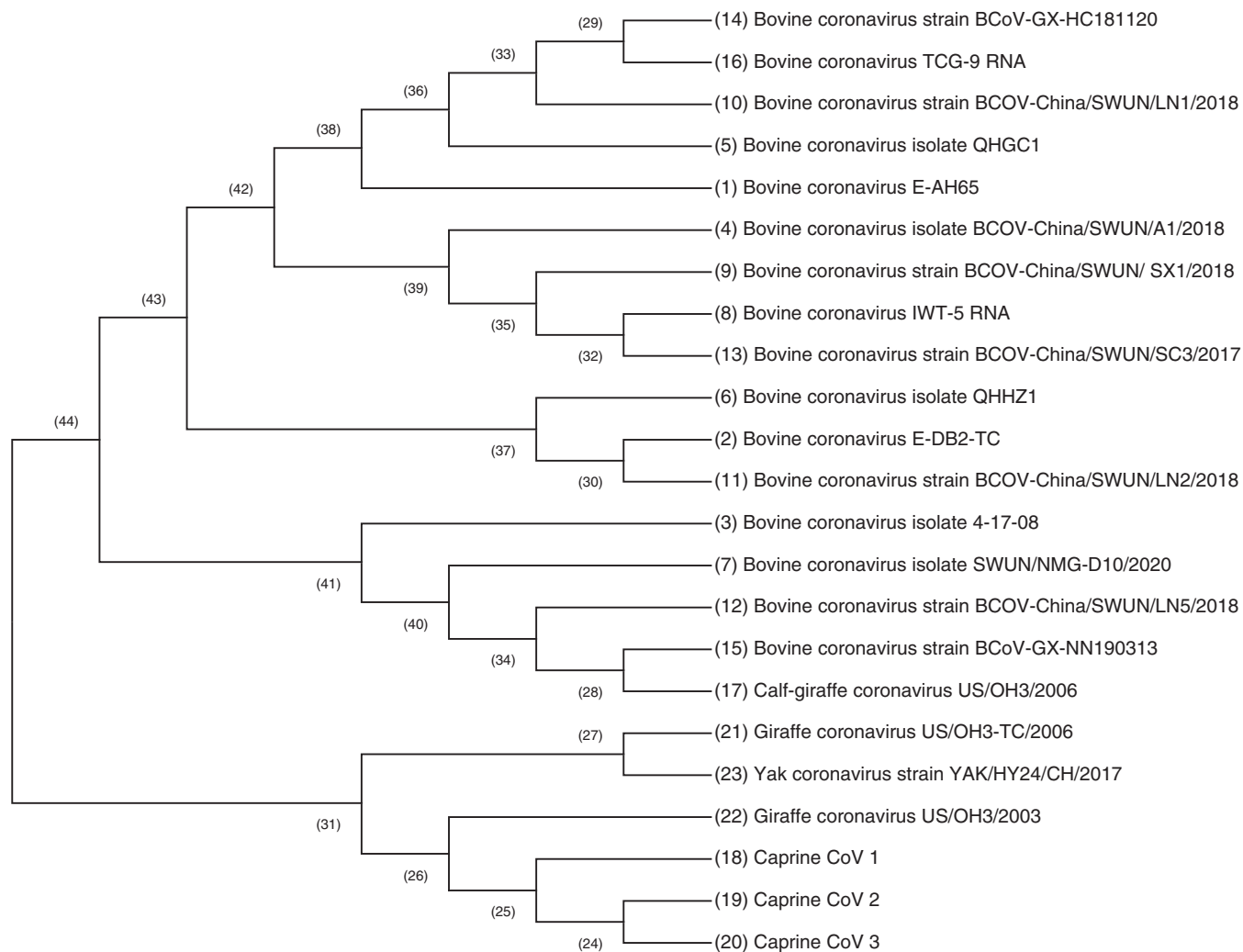
(9/9), anorexia (9/9), fever (5/9), and cough (3/9). Owner-reported treatments included antibiotics (oxytetracycline, penicillin and sulfadimethoxine), anti-inflammatories (flunixin meglumine), *Clostridium perfringens* C & D antitoxin, probiotics, and B-vitamins.

In the initial workup, feces from 5 clinically affected individuals in herd A and B were culture negative for enteropathic bacteria, PCR negative for *Salmonella* spp., ELISA negative for clostridial toxins, and negative for roundworms and *Cryptosporidium* at Laboratory A. All samples were positive for *Coccidia* with 350 to 1000 oocysts/g. Electron microscopy failed to detect viruses in 2 samples. Herd A had 2/3 and B had 2/2 samples PCR positive for an 84 bp product of the S glycoprotein gene of BCoV.

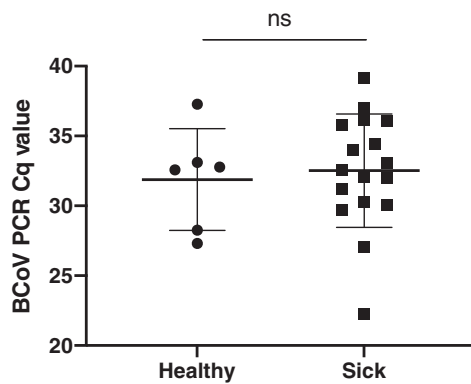
Seventeen days after the first clinical case, longitudinal herd surveillance was initiated showing 40% (10/25) of sampled animals (feces or rectal swab) were positive for a 93 bp product of the N protein

gene of BCoV (Figure 2). In the control herd 0% (0/6) were PCR positive. Animals from the affected herds were 8.75 more likely to be PCR positive than control animals (OR 8.75, 95% CI 1.11-104.0,  $P = .05$ ). Weekly sampling of the 2 affected herds showed a decreasing prevalence of PCR positive animals: day 26: 28% (7/25), day 35: 17.4% (4/23), day 41: 0% (0/24; Figure 2). The missing samples at days 35 and 41 failed PCR quality control. In total 2.8% (3/106) of BCoV N protein gene PCR tests failed quality control. The cumulative prevalence of BCoV PCR positivity was 72%: 66.7% (14/21) goats in herd A and 100% (4/4) animals in herd B. Seven animals exhibited intermittent shedding being PCR positive on multiple nonconsecutive samplings.

Health records from herds A and B are summarized in Table 1. The median age was 2-years-old (range, 1-8 years). Herds A and B were comprised of 18 lactating and 2 dry multiparous does, 2 nulliparous yearlings, and 3 bucks. The prevalence of sick animals in the



**FIGURE 3** Phylogenetic analysis of coronavirus (CoV) N protein amplicons from 3 goats in the diarrhea outbreak. The amplicons were compared to other CoV identified by NCBI Blast based on a comparison of a 440 bp sequence of the N protein gene. The 3 goats analyzed were all from herd A. The amplicons were most closely related to a bovine-like CoV amplicon from a diarrhetic giraffe. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the sequences analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances are in the units of the number of base substitutions per site



**FIGURE 4** BCoV PCR Cq values as a proxy for viral load do not correlate with whether the animal was sick or healthy during the outbreak. Cq values are not necessarily from sample taken while sick animals were exhibiting clinical disease. Each dot represented the Cq value from a positive test, some animals may be represented by more than 1 dot if they were positive at more than 1 time point. Bars represent mean  $\pm$  SD. Total positive tests:  $n = 21$  (healthy  $n = 6$ , sick  $n = 15$ )

2 herds was 80% (20/25), 83.3% in lactating females (15/18). The most common clinical signs were change in fecal consistency 95% (19/20), decrease in milk production 93.3% (14/15), anorexia 40% (8/20), fever 15% (3/20) and cough 10% (2/20). Treatments included sulfadimethoxine, flunixin meglumine, di-tri-octahedral smectite, and *C. perfringens* C & D antitoxin. Treatment of affected goats ranged from no therapy to all therapies based on severity of clinical signs. In most cases the disease was self-limiting and short lived (1-5 days). Five animals (25%, 5/20) experienced a relapse in clinical signs after apparent clinical recovery. The relapse cases included change in fecal consistency 100% (5/5) and drop in milk production 40% (2/5).

Phylogenetic analysis of a 440 bp N protein sequence from 3 goats revealed that amplicon more similar between the 3 individuals than CoV in the NCBI Blast Database (Figure 3). The outbreak isolates were most related to a bovine-like CoV US/OH3/2003, an enteric isolate from a diarrhetic giraffe<sup>5</sup> (Figure 3).

Sick animals were no more likely than healthy animals to be BCoV PCR positive (OR 0.58, 95% CI 0.048-5.65,  $P > .99$ ). There was no correlation between the PCR Cq value, as a viral load proxy, and being a sick or healthy animal in an affected herd ( $P = .74$ ; Figure 4).

## 4 | DISCUSSION

Coronavirus has not been reported in association with diarrheal disease in adult goats. During this outbreak, 12 affected herds were identified with more than 300 affected animals, with morbidity ranging from 15% to 90%. Affected herds attended 1 of 2 exhibitions over a 10-day period. Exhibitions result in the comingling of animals from different sources and often different species providing an opportunity for infectious pathogens to transmit between herds and possibly species.<sup>16,17</sup> A survey of exhibitors attending exhibition 1 indicates poor biosecurity practices are common.<sup>15</sup> Practices at exhibitions result in

multiple points for possible disease transmission between herds. At exhibition 2 animal movement required the use of narrow aiseways flanked by affected herds and both exhibitions had common-use wash racks and holding pens. Similar to winter dysentery this outbreak occurred when animals were comingled in a high-density environment.<sup>3,4,19</sup>

Change in fecal consistency and decreased milk production were the most common clinical signs, however, cough was noted in 3 herds. BCoV is associated with coincident respiratory and gastrointestinal disease.<sup>3</sup> Further work is needed to determine if CoV is involved in respiratory disease in goats. Additionally, although commonly known pathogens were ruled out as the cause of diarrhea in the initial 5 animals, we cannot exclude the possibility of an unidentified pathogen(s) or comorbidity contributing to the clinical signs.

Goats involved in this outbreak were tested for BCoV via PCR because the transmission pattern and clinical signs were reminiscent of winter dysentery<sup>2</sup> and equine CoV.<sup>20</sup> Onset of clinical signs and PCR positivity were consistent with experimental infection in cattle showing onset of clinical signs 3 to 16 days and viral nucleic acid detection 3 to 18 days after inoculation.<sup>21</sup> Molecular detection of viral nucleic acids for the S glycoprotein and the N protein genes in this outbreak strengthens the association with CoV. The detection of CoV using BCoV primers suggests that BCoV PCR is useful for the detection of CoV in goats and may be more sensitive than EM, which failed to detect virus in 2 PCR positive samples. This is consistent with findings in cattle which show PCR to be more sensitive than EM for detection of CoV.<sup>22,23</sup> It would have been preferable to perform EM on more samples to look for whole virus.

The present study provides evidence that this outbreak was associated with CoV, however, the longitudinal testing occurred after the peak of clinical disease (Figure 2); sampled animals may or may not have been exhibiting clinical signs at the time. This likely affected the correlation between symptomatic and asymptomatic animals and Cq values, although viral load and clinical disease does not correlate in PCR positive horses in equine CoV outbreaks.<sup>20</sup> Additionally, sampling more affected and unaffected herds/individuals would have been preferable and would have provided greater insight as to the significance of CoV in this outbreak. Despite the limited sampling, animals from the affected herds were significantly more likely to be BCoV positive than the control herd. Additionally, the shedding frequency was much higher than is reported in surveilled clinically normal goats.<sup>14</sup> Our results also indicate viral shedding may occur in animals that never exhibited clinical signs. Subclinical shedders present challenges to control of transmission, as a potential source of exposure. There was a strong temporal association between the outbreak and the frequency of CoV shedding in the 2 affected herd, but further work is needed to determine the definitive cause. Given the wide host range and ability of CoVes to be zoonotic,<sup>24,25</sup> good hygiene and biosecurity is important when dealing with animals shedding CoV.

## ACKNOWLEDGMENT

No funding was received for this study. The authors thank all the farms that participated in the survey and spoke with the authors about their experience during the outbreak.

**CONFLICT OF INTEREST DECLARATION**

Fauna L. Smith and Joan D. Rowe are the owners of Herd B and Herd A, respectively. Meera C. Heller is the owner of the control herd. No other authors have a conflict of interest.

**OFF-LABEL ANTIMICROBIAL DECLARATION**

Off-label antimicrobial use was reported by herd owners in the survey: sulfadimethoxine, oxytetracycline and penicillin. Dosages were not reported. In the herd records of Herd A and B some animals received sulfadimethoxine at a dosage of 55 mg/kg PO once, then 27.5 mg/kg PO q24h for 4 days.

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION**

Authors declare no IACUC or other approval was needed. The investigation was done as part of a clinical workup of the affected herds in the face of an outbreak of diarrhea. The control herd was a client/author owned herd that was used to try to help the attending clinicians determine if the PCR positive results were significant at the time.

**HUMAN ETHICS APPROVAL DECLARATION**

Authors declare human ethics approval was not needed for this study.

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