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Case report

Cryptosporidium parvum and other enteric pathogens in scouring neonatal dairy calves from the Al Ain region, United Arab Emirates

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ABSTRACT

Faecal specimens from 36 scouring neonatal calves from two dairy farms located in the Al Ain region of the UAE were screened with pathogen-specific antigen ELISA for *Cryptosporidium parvum*, *Escherichia coli* K99, rotavirus, and coronavirus. Additionally, faecal smears were stained with modified-acid-fast for *Cryptosporidium* oocysts, and the VITEK 2 system plus Gram's stain used to identify bacteria isolated from the faecal samples. Farm management practices were also evaluated during a farm visit. Of the 36 calves, 29, 13, 5, and 6 were positive for *C. parvum*, *E. coli* K99, bovine coronavirus, and rotavirus antigens respectively, while 27 were positive for *Cryptosporidium* oocysts. In various combinations, mixed infections were detected in 20/36 calves. This is the first report of *C. parvum*, *E. coli* K99, *Salmonella* spp., rotavirus, and coronavirus in ≤14-days-old scouring neonatal dairy calves from the UAE. Molecular characterization of these pathogens and nationwide epidemiological calf scour studies are recommended.

1. Introduction

Neonatal calf scours is a serious animal health problem that is responsible for significant economic losses attributed to considerable morbidity and calf mortality. A report of the United States Department of Agriculture compiled by the National Animal Health Monitoring System in the US cited a mortality rate of 57.0% in pre-weaned scouring dairy calves (USDA, 2008) and another from South Korea reported a mortality rate of 53.4% (Hur et al., 2013). In a Norwegian study on the economic impact of neonatal calf scours in dairy farms, financial losses to the tune of USD $10 million were estimated (Østerås et al., 2007).

Of the broadly diverse pathogens that are causally linked to neonatal calf diarrhoea, *Cryptosporidium parvum* is the most ubiquitously seen (Nydam et al., 2001; Cho and Yoon, 2014; Lombardelli et al., 2019). Further still, various species of enteropathogenic bacteria including but not limited to virulent *Escherichia coli* (Foster and Smith, 2009), toxigenic *Clostridium perfringens* (Rings, 2004), and *Salmonella* spp. (Tsolis et al., 1999) have also been causally linked to calf scours. Other important enteropathogenic agents of neonatal calves include bovine rotavirus (Dhama et al., 2009) and coronavirus (Cho and Yoon, 2014) with the two often reported in calves aged between 1-day-old to 2-weeks-old (Schultze et al., 1991). Besides, bovine virus diarrhoea virus (Bolin et al., 1985) and a number of novel viruses like bovine norovirus, torovirus, and nebrovirus may also be associated with neonatal calf scours (Cho and Yoon, 2014).

In the United Arab Emirates (UAE), there is a general lack of livestock disease research which is in turn reflected by the paucity of peer-reviewed country-specific literature on infectious livestock diseases. For example, it is quite remarkable that there is no single retrievable country-specific literature on neonatal calf diarrhoea in the UAE despite the many large commercial dairy properties that are spatially distributed in the different Emirates or regions of the country. The present pilot study was therefore done to assess the comparative role of *Cryptosporidium spp* and other enteric pathogens in neonatal calf scours at two dairy farms located in the Al Ain region, Emirate of Abu Dhabi, UAE.

2. Case Presentation

This study was conducted at two large commercial dairy farms located in the Al Ain region, UAE. Around the 28/2/2020, the resident veterinarian for the two dairy farms reported calf scour-associated deaths to the Veterinary Medicine Department (VMD), United Arab Emirates University (UAEU). The referring veterinarian indicated that several neonatal calves aged ≤14-days-old had died after presenting with signs of severe diarrhoea. By his initial report, at least 15 neonatal

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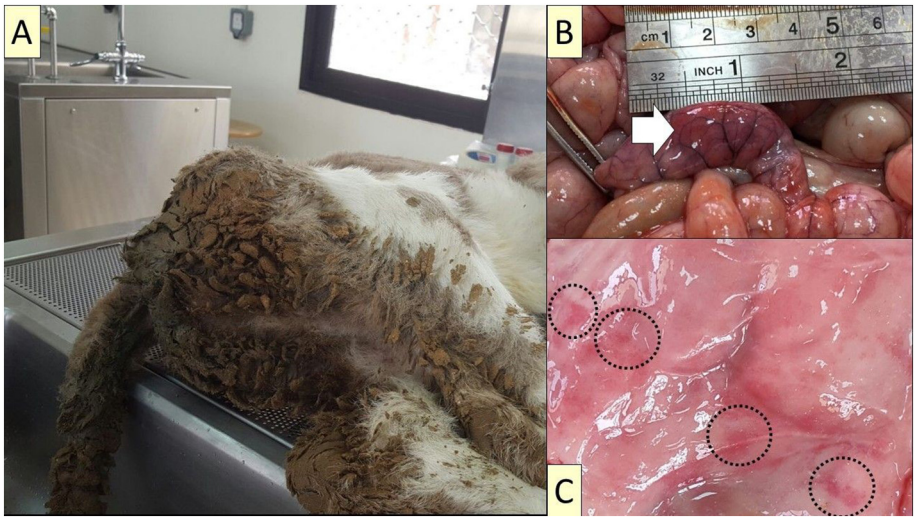

Fig. 1. Extensive soiling of the hindquarters (A) consistent with severe diarrhoea is seen in the 11-day-old male Chalorais (Calf #1), along with macroscopic changes suggestive of severe enteritis characterized by markedly congested loops of the small intestine (white arrow; B) associated with a mosaic network of necroulcerative mucosal lesions (inside broken circles; C) suggestive of enteritis. It is noteworthy that intestinal contents of this calf tested positive for *C. parvum* antigens and characteristic acid-fast staining oocyts morphologically consistent with *Cryptosporidium* were seen in faecal smears.

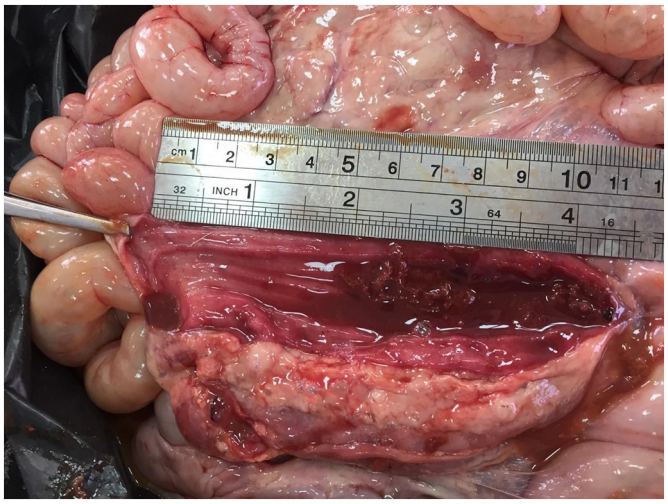

Fig. 2. Gross pathological changes suggestive of severe necrohaemorrhagic colitis in the spiral colon of an 11-day-old female Holstein-Friesian calf (Calf #6) that tested positive for *Cryptosporidium parvum*, and *Escherichia coli* K99 antigens. In addition, both *E. coli* and *Salmonella* were cultured from intestinal contents collected during necropsy examination.

calves had already died while several others were manifesting severe scours. The first carcass submission (Calf #1) was a 12-day-old male Chalorais that died 8 days into an episode of severe scours despite treatment with intravenous electrolytes and other medications. Once received, a detailed necropsy examination was conducted and various biological specimens collected for ancillary testing. Subsequently, six additional neonatal calf carcasses (Calves #2, #3, #4, #5, #6, and #7) were also received for necropsy and scour screen investigations. These were all Holstein-Friesians aged less than 2-weeks-old. Meanwhile, a farm visit was undertaken to gather more information and additional biological specimens for diagnostic testing. During the visit, a careful appraisal was made of the calf pens including floor type and proximity of the pens housing the sick and healthy calves. Rectally derived faecal samples were collected from the all the 29 scouring calves.

Once in the laboratory, the faecal and intestinal samples were screened for antigens to *C. parvum* and the other enteric pathogens using a commercial faecal antigen ELISA test (PrioCHECK® Calf Enteritis Ag Faeces Kit; Thermo Fisher Scientific). This kit simultaneously detects specific antigens from *C. parvum*, *E. coli* K99, bovine rotavirus (Group A), and bovine coronavirus and the tests were performed according to the kit manufactures' instructions. In addition, faecal smears were also stained by the modified acid-fast (MAF) stain and examined microscopically for *Cryptosporidium* oocysts. Meanwhile, faecal samples from all the 36 calves were cultured for *E. coli*, *Salmonella*, and *C. perfringens* based on laboratory protocols described by Cheesbrough (2006). Briefly, the faecal samples were inoculated into a nutrient broth and incubated at 37 °C for 24 h. After the initial incubation, the samples were streaked onto Mac-Conkey agar after which the lactose-fermenting colonies were further streaked onto EMB agar for *E. coli* confirmation. Following the initial culture, the pure colonies isolated on MacConkey agar that turned out to be pink-lactose fermenters were identified as *E. coli* while the colourless fermenters were identified as *Salmonella* spp. Additionally, these results were further corroborated by Gram's staining plus additional testing with the VITEK 2 system (Biomerieux Diagnostics, USA) done according to the manufacturer's instructions. For the anaerobic *C. perfringens* culture, the samples were introduced into glass tubes filled with anaerobic cooked meat medium and placed in a sealed anaerobic jar and incubated at 37 °C for 24–48 h. *Clostridium perfringens* colonies were identified by their growth under anaerobic conditions along with Gram's staining characteristics. All the Gram's stained slides were examined under oil immersion objective of an Olympus microscope.

As verified during the farm visits, the calf pen floor type at both farms was made up of loose “desert sand” that visibly bore calf manure in most pens. The farm manager attributed the ongoing calf scour outbreak to recent climatological events that included a severe sandstorm in early January 2020 that was followed by light rains. Rain is unusual in the UAE as weather conditions are typically characteristic of harsh desert climate. Coupled with the fact that the pens housing individual calves were in close proximity, the possibility of easy cross-pen spreading was considered high. In respect to Calf #1, the carcass showed sunken eyes consistent with severe dehydration while the hindquarters were extensively soiled with large amounts of foul smelling faeces (Fig. 1A). In addition, segments of the jejunum and ileum (Fig. 1B) along with the large intestine were congested and contained large amounts of greyish watery contents. The mucosal surface of the jejunum and ileum (Fig. 1C) as well as the spiral colon had focally extensive areas of congestion/hyperaemia along with numerous multifocal to diffusely distributed mucosal ulcerations. Among other gross lesions, the other six calves (Calves #3, #4, #5, #6, and #7) also demonstrated sunken eyes consistent with dehydration, soiling of hindquarters indicative of previous bouts of diarrhoea, and morphological lesions suggestive of enteritis. In addition, Calf #6 demonstrated gross pathological lesions that were morphologically suggestive of severe necrohaemorrhagic enterocolitis (Fig. 2) while Calf #7 also showed gross lesions suggestive of severe necrotizing enterocolitis. In addition

Table 1
Summary of test data obtained from pathogen-specific faecal antigen ELISA, faecal culture, and modified acid fast stain done on faecal samples from 36 scouring neonatal dairy calves.

Calf/sample no.	Calf age (days)	Faecal antigen capture ELISA				Modified acid fast stain	Faecal culture
		C. parvum	*E. coli* K99	Rotavirus	Coronavirus	*Cryptosporidium oocysts*	
***1**	9	+	−	+	−	+	*E. coli, C. perfringens*
***2**	12	+	+	−	−	+	*E. coli, C. perfringens*
***3**	12	+	−	−	−	+	*E. coli*
***4**	9	+	+	−	−	+	*E. coli*
***5**	10	+	−	−	−	+	*E. coli, Salmonella*
***6**	12	+	+	−	−	+	*E. coli, Salmonella*
***7**	8	+	+	−	−	+	*E. coli, Salmonella*
8	7	−	−	−	−	+	*E. coli*
9	10	+	+	−	+	−	*E. coli*
10	12	+	+		+	+	*E. coli*
11	12	+	−	−	−	+	*E. coli*
12	10	−	+	+	−	−	*E. coli*
13	13	+	−	−	+	+	*E. coli*
14	7	+	+	−	−	+	*E. coli*
15	14	+	−	−	−	−	*E. coli*
16	9	−	−	−	−	−	*E. coli, Salmonella*
17	8	−	+	−	−	+	*E. coli, Salmonella*
18	12	+	+	−	−	+	*E. coli*
19	8	−	+	−	+	+	*E. coli*
20	9	+	−	−	−	+	*E. coli*
21	12	+	−	+	−	+	*E. coli*
22	8	+	−	−	+	+	*E. coli*
23	11	+	+	−	−	+	*E. coli*
24	9	+	+	+	−	+	*E. coli*
25	10	−	−	−	−	−	*E. coli*
26	6	+	−	−	−	+	*E. coli*
27	6	+	−	−	−	−	*E. coli*
28	7	+	−	−	−	+	*E. coli*
29	7	−	−	−	−	−	*E. coli*
30	7	+	−	−	−	−	*E. coli*
31	9	+	−	−	−	+	*E. coli*
32	10	+	−	+	−	+	*E. coli*
33	10	+	−	−	−	+	*E. coli*
34	8	+	−	−	−	+	*E. coli*
35	7	+	−	−	−	−	*E. coli*
36	7	+	−	+	−	+	*E. coli*

The calves indicated in bold fonts and bearing an asterisk (#1–#7) were carcass submissions for which full necropsy examinations were performed.

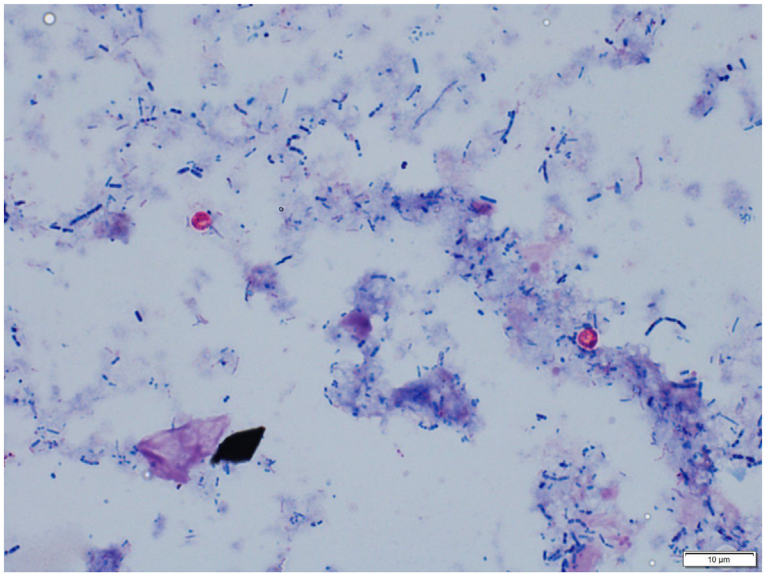

Fig. 3. A faecal smear from a 12-day-old male Chalorais calf (Calf #1) showing two acid fast coccoid bodies morphologically consistent with *Cryptosporidium* spp. The same faecal specimen also tested positive for *C. parvum* antigens by faecal antigen capture ELISA.

to these lesions, Calf #7 also had severe fibrinous peritonitis along with fibrinous synovitis in the right carpal joint.

The summarized test data indicate that 29, 13, 5, 6 of the faecal samples taken from 36 calves and tested by faecal antigen ELISA were positive for *C. parvum*, *E. coli* K99, coronavirus, and rotavirus antigens respectively (Table 1). In addition, *Salmonella* spp. was cultured from five, and *C. perfringens* from two out of the 36 faecal samples tested. When the MAF stain results were evaluated, 27 of the 36 samples were positive for *Cryptosporidium* oocyts. Presented in Fig. 3 is a photomicrograph of the faecal smear showing a few characteristically coccoid acid-fast bodies morphologically consistent with *Cryptosporidium* oocysts. When mixed infections are considered, eight calves had a *C. parvum- E. coli* K99 infection, five had a *C. parvum*-rotavirus infection, four had a *C. parvum*-coronavirus infection, two demonstrated a *C. parvum-E. coli* K99-coronavirus mixed infection, while one had a *C. parvum-E. coli* K99-rotavirus infection. Besides, three calves tested positive for *Salmonella* spp. and *C. perfringens* was cultured from two faecal samples collected postmortem. Of the *Salmonella* spp-positive samples, one calf was also positive for *C. parvum*, while the second one was positive for both *Salmonella* spp. and *E. coli* K99, while *Salmonella* spp. was the only pathogen detected in at least one calf. *Escherichia coli* was isolated from all the 36 faecal samples, and a mixture of *E. coli Salmonella* spp. was cultured from only three samples, and *C. perfringens* from only two specimens collected postmortem. It is noteworthy that only 13 of the faecal samples tested by the faecal ELISA antigen test were positive for *E. coli* K99.

3. Discussion and Conclusions

To the authors' knowledge, this is the first report of neonatal calf scours in the UAE in which *C. parvum* along with *E. coli* K99, *Salmonella* spp., rotavirus, and coronavirus infections are reported. All the 36 calves tested during the diagnostic investigations were aged between six and 14-days-old. As such, all the calves tested were “neonatal” and the test agents above were, therefore, of diagnostic significance. As was demonstrated during the present investigations, the majority of the scouring calves tested positive for *C. parvum* which underscores the apparent diagnostic significance of the protozoan parasite in the calf scour outbreak. Elsewhere, numerous investigators have reported on the role of *C. parvum* in scouring neonatal calves (Nydam et al., 2001; Nydam and Mohammed, 2005; Cho and Yoon, 2014; Lombardelli et al., 2019). In respect to pathogenesis, infection with *C. parvum* is associated with intestinal villous atrophy and loss, which ultimately leads to severe calf scours (Nydam and Mohammed, 2005).

While infections with *C. parvum* alone were recorded, however, mixed infections of the protozoan parasite with the other four test pathogens were more frequently encountered. As has been demonstrated elsewhere, single infections of *E. coli* K99 (Foster and Smith, 2009; Cho and Yoon, 2014), *Salmonella* (Tsolis et al., 1999; Cho and Yoon, 2014), bovine rotavirus (Dhama et al., 2009; Cho and Yoon, 2014), and bovine coronavirus (Cho and Yoon, 2014) may causally be associated with enteritis leading to calf scours. In respect to mixed infections, *C. parvum* was variably detected along with *E. coli* K99, *Salmonella*, bovine rotavirus, and bovine coronavirus. Such mixed infections are not uncommon and are usually believed to exacerbate the severity of calf scours when they occur (Cho and Yoon, 2014). It should be noted that together with *C. parvum*, these pathogens are largely environmental in origin and are usually perpetuated in populations of susceptible calves via the faeco-oral route of transmission. While the exact source of the enteric pathogens was not definitively established in the present calf scour outbreak, one could speculate that environmental contamination through asymptomatic pathogen shedding by adult cattle at the farm may have played a role. As the light rains that fell just prior to the outbreak resulted in flooding of some of the calf pens, it is probable that favourable environmental conditions created by the unusual climatological event permitted the proliferation of the enteric pathogens in the calf pens. Consistent with this hypothesis, the hygiene in the calf pens, as verified during the farm visit, was rather unimpressive. Besides, evidence of faeco-oral transmission was seen in form of substantial amounts of sand that were present in the pre-ruminant stomachs of most of the seven dead calves brought in for necropsy examination.

In the present case report, *E. coli* was isolated from all the 36 faecal samples while the *E. coli* K99 antigen was only detected in 13 specimens. While *E. coli* K99 is an important cause of neonatal calf scours, other virulence factors including but not limited to F41, intimin, *STa*, *Stx1*, *Stx2* have also been reported in *E. coli* isolates from scouring neonatal calves (Barigye et al., 2012; Ngeleka et al., 2019). While majority of the *E. coli* isolates from a bovine calf intestine are certainly normal flora, some of the K99-negative isolates cultured during the present study could have well been carrying other non-K99 virulence factors. As such, future studies will need to focus on a broader range of *E. coli* virulence factors so more useful data is collected in this regard. The other enteric pathogens detected during this study were *Salmonella* spp., rotavirus and coronavirus. However, these findings are not surprising since these pathogens are universally reported in scouring calves worldwide (Barigye et al., 2012; Cho and Yoon, 2014). As time had passed between the time of death and necropsy examination, the *C. perfringens* isolated from intestinal contents of two dead calves most likely reflect post-mortem proliferation of this organism.

In summary, the present study has documented *C. parvum,* as well as *E. coli* K99, *Salmonella* spp., rotavirus, and coronavirus in scouring neonatal dairy calves from the UAE. More extensive and nationwide studies will need to be done so the epidemiology of neonatal calf scours in the country may be better understood. In future studies, detailed molecular characterization of the various *E. coli* virotypes is also recommended as this will ensure that vaccines used against neonatal calf diarrhoea in the country incorporate regionally and nationally relevant *E. coli* virulence factors.

Ethical Statement

This study was fully compliant with the ethical requirements for animal welfare.

Declaration of Competing Interest

All authors of this manuscript declare there are no financial and personal relationships with other people or organizations that could inappropriately influence the intellectual work presented in this paper.

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