

STUDIES ON NEONATAL CALF DIARRHEA IN KUWAIT

By

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Abstract

Neonatal calf diarrhea, also known as calf scours, is a common disease affecting newborn calves. It causes significant economic losses due to morbidity, mortality, and therapy cost and decreased rates of development in affected calves. Multiple enteric pathogens are known to cause this disease including viruses, bacteria and parasites. *Cryptosporidium* spp. is a protozoan parasite usually responsible for gastrointestinal tract disease in human and calves. *E. coli* K99 was also incriminated as one of the important causes of neonatal diarrhea. Corona viral infection could also cause diarrhea in newborn calves. One hundred ninety-eight fecal samples collected from diarrheic calves were tested with immunochromatographic rapid test. Sixty one (30.8%) were positive for different pathogens either as single infection or co-infection with two or more enteric pathogens. *Cryptosporidium* was the highest 20.2% (40/198), followed by *E. coli* K99 at 17.2% (34/198), then Rotavirus 9.1% (18/198) and only 2 samples were positive for Coronavirus (1%).

Key words: Kuwait, Neonatal calf, *Cryptosporidium* species, *E. coli* K99, Rotavirus

Introduction

Generally, the calf morbidity and mortality rates are often high in dairy herds globally; raising animal welfare concerns and negatively affecting the farm economic efficiency (Baxter-Smith and Simpson, 2020). Neonatal calf diarrhea or the so called calf scours is a risky infectious disease affecting the newborn calves (Özkan *et al*, 2011). This is especially true during the first 28 days of their life (Khan and Zaman, 2007). Calf scours is a complicated syndrome owing to morbidity and mortality, as well as decreasing animal developmental rates (Zhu *et al*, 2011).

There are multiple enteric pathogens which are frequently reported as a cause of neonatal calf diarrhea such as viruses, rotavirus as coronavirus and bovine viral diarrhea (Izzo *et al*, 2011), bacteria as *Escherichia coli*; *E. coli* K99, *Clostridium perfringens* Type C, *Salmonella* spp. (Bartels *et al*, 2010) and parasites as *Cryptosporidium* spp. & *Eimeria* spp. (Kumar *et al*, 2010).

Co-infection in diarrheic calves was frequently noted, although in some instances a single pathogen may be the main cause (Izzo *et al*, 2011). The prevalence of each pathogen may differ according to farm location, farm

management, and herd size (Hur *et al*, 2013).

Cryptosporidium spp. is a protozoan parasite usually responsible for gastrointestinal disturbances in humans, animals (Gerace *et al*, 2019) and domestic and zoo birds (El-Shahawy and Elenien, 2015). Even *Cryptosporidium* oocysts were detected in different water sources together with other pathogenic parasites (El Shazly *et al*, 2007). The cryptosporidiosis infected calves may be asymptomatic or symptomatic becoming weak, lethargic and present with loose to watery stool that may be mild or risky with fatal dehydration (Razakandrainibe *et al*, 2018). Intestinal epithelium disturbance occurred after cryptosporidiosis infection led to prolonged mal-nutrition and decreased rates of development in infected calves due to mal-absorption and undigested milk fermentation in intestinal lumen (Fayer *et al*, 1998).

E. coli K99 was incriminated as one of the causes of neonatal diarrhea detected in the first 4 days of life, but rarely led to diarrhea in older calves or adult cattle (Foster and Smith, 2009). The corona viral infection in cattle was presented by three distinct clinical syndromes; a- Calf diarrhea in calves of 1 to 2 weeks ages, b- Winter dysentery with hemo-

rhagic diarrhea in adults, & c- Respiratory disease including bovine respiratory complex infection in both young and adult cattle (Liu *et al*, 2006). The virus is ingested from the contaminated environment by other infected calves or adult cattle where virus spike (S) protein facilitated its entry and pathogenesis by the ability of neutralizing antibody (Lin *et al*, 2000). The Rotavirus was the major viral causes of the diarrhea recognized in neonatal calves and was originally referred to as neonatal calf diarrhea virus (Geletu *et al*, 2021). Globally, it was detected as the well-known the most important diarrheic pathogen for children and others mammals (Alkan, 1998). However, the vaccinations for rotavirus over a decade ago, rotavirus infections resulted in > 200,000 deaths annually, mainly in low-income countries (Crawford *et al*, 2017).

Clostridium perfringens is a risky cause of enteric disease in domestic animals (Songer, 1996). *C. perfringens* type C more frequently cause enteritis in neonatal animals as calves, sheep, goats, particularly pigs (Songer and Uzal, 2005). Globally, it is an economically risk problem (Simpson *et al*, 2018). *Clostridium perfringens* bacteria are one of the most common causes of food poisoning (CDC, 2023).

This study aimed to evaluate the Rotavirus, Coronavirus, *E. coli* K99, *Cryptosporidium* species and *Clostridium perfringens* Type C prevalence in neonatal calf diarrhea in Sulaiyia Dairy Farms, Kuwait by the rapid test.

Materials and Methods

In this study, a total of 198 fecal samples were collected from diarrheic calves from November 2017 to October 2018 from ten different farms. The specimens were gathered in 48 hours of diarrheal onset from calves up to two months old. The fecal samples were aseptically collected directly from the rectum in sterile labeled plastic bottles kept in an ice box and submitted to the laboratory as soon as possible. All samples were carefully examin-

ed by commercial immunochromatography rapid test (Rainbow Calf Scour 5, Biox-Diagnostics, Belgium), according to the manufacturers' recommendations.

Results

Of 198, 61 (30.8%) were positive for different pathogens either as single agent or co-infection with two or more pathogens. *Cryptosporidium* species was the highest detected one in 20.2% (40/198), followed by *E. coli* K99 17.2% (34/198), then Rotavirus 9.1% (18/198) and only two samples were positive for Corona- virus (1%), but *Cl. perfringens* Type C was not detected.

The highest infection rate was in the age-group 1 (1-15 days old) at 70% where n=50. While, the lowest infection was in age-group 4 (45-60 days old) at 6.8% where n=44. The age-group 2 (16-30 days old) recorded the second highest infection rate at 28.5% where n=56. The age-group 3 (30-45 days old) recorded infection rate of 14.4% where n=48.

The co-infection with two or more pathogens was recorded in 31 samples (15.7%). The commonest co-infection pathogens were *Cryptosporidium* species and *E. coli* followed by *Cryptosporidium* species and Rota (8.0%, & 4.5%, respectively). Co-infection with more than two pathogens (*Cryptosporidium*, Rota and *E. coli*) was detected in two samples (1.0%). Co-infection with Rotavirus and *E. coli* was detected in four samples (2.0%). Of the 61 diarrheic calves 35 (57.3%) were two weeks old and 16 (26.3) were at age group two weeks- a month old. Older calves, up-to 45 days old and those of between 46 days to 60 days old, were less susceptible to infectious diarrhea with the rates of 11.5%, & 4.9%, respectively.

Ethical considerations: Ethical declared in animals' welfare were adopted (Animal Welfare, 2009: [https://www. regjeringen.no/ en/dokumenter/ animal-welfare-act/id571188/](https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/)

Detailed were given in tables (1 & 2) and figure (1).

Table 1: Prevalence of pathogens in fecal calves samples (n=198) by immunochromatography test.

No of positive samples with reference to each pathogen (percentage)				
<i>Cryptosporidium</i> species	Rotavirus	Coronavirus	<i>E. coli</i>	<i>Cl. perfringens</i>
40 (20.2)	18 (9.1%)	2 (1.0%)	34 (17.2%)	0.0

Table 2: Prevalence of different pathogens and co-infection in different age-groups

Pathogenic agent	Total	1-15 days (n= 50)		16-30 days (n= 56)		30-45 days (n= 48)		45-60 days (n= 44)	
		No	%	No	%	No	%	No	%
<i>Cryptosporidium</i> only	13	8	13.1	4	6.6	-	-	1	1.7
Rota only	3	3	4.9	-	-	-	-	-	-
Coronavirus only	2	-	-	-	-	-	-	2	3.3
<i>E. coli</i> only	12	6	9.8	4	6.6	2	3.3	-	-
<i>Cryptosporidium</i> + Rota	9	6	9.8	3	4.9	-	-	-	-
<i>Cryptosporidium</i> + <i>E. coli</i>	16	8	13.1	5	8.2	3	4.9	-	-
Rotavirus + <i>E. coli</i>	4	2	3.3	-	-	2	3.3	-	-
<i>Cryptosporidium</i> +Rota+ <i>E. coli</i>	2	2	3.3	-	-	-	-	-	-
Total	61	35	57.3	16	26.3	7	11.5	3	4.9

Discussion

Although significant progress was made in the field of cattle industry with herd management, animal welfare and care, feeding and nutrition, and using of bio-pharmaceuticals, calf diarrhea remained the challenging of issue due to disease multi-factorial nature (Palczynski *et al.*, 2021). Prevention and control of calf diarrhea must be based on a strong knowledge of the nature of the disease such as the various pathogens, co-pathogens, environmental variables, feeding and management during the calving period (Cho and Yoon, 2014).

In the current study, diarrhea was recorded at 30.8% (61/198) in calves up to two months old, with the highest rate recorded in the calves up to two weeks old 57.3% (35/61). Razaque *et al.* (2010), in Kuwait reported that diarrhea is the most common disease in neonatal calves with an incidence rate of 90.6% (678/749) in diseased calves. But, in Egypt, prevalence of diarrhea was at 16.7% (200/1200) of examined neonatal calves (El-Naker *et al.*, 2008). In another study, the peak incidence of diarrhea was reported within the first two weeks of age, and the case fatality rate was highest for diarrhea at 52% (Perez *et al.*, 1990). Waltner-Toews *et al.* (1986) in Canada estimated diarrhea rates between birth and weaning to be 20%.

The present study revealed that *Cryptosporidium* spp. (20.2%) was the most predominant cause of diarrhea in neonatal calves followed by *Escherichia coli* (17.2%), Rotavirus (9.1%) and Coronavirus (1%). Majeed *et al.* (2011), an outbreak of diarrhea in neonatal calves in Kuwait was studied and the results showed that *Cryptosporidium* spp. infection was at 38.3% and Rotavirus at 28.8% of

diseased calves. *Cryptosporidium* is a protozoan parasite causing diarrhea in neonates and young cattle and not easily to be treated, in the young animals (Diaz-Lee *et al.*, 2011).

Cryptosporidiosis was identified as the most frequent causative agent of calf diarrhea in Belgium (de Graaf *et al.*, 1999), in Saudi Arabia (Areeshi *et al.*, 2007), Switzerland (Uhde *et al.*, 2008), Jordan (Hijawi *et al.*, 2016) and in Egypt (Abdelaziz *et al.*, 2022). Ouakli *et al.* (2018) in Algeria reported that bovine cryptosporidiosis was highly endemic in ten farms and represented a veterinary public health concern that must be adequately tackled by the Veterinary Health Authorities and Policy Makers.

In the current study, most of the diarrhea cases caused by *Cryptosporidium* spp. were observed in the first 2-3 weeks. *Cryptosporidium* spp. was frequently detected in calves with or without diarrhea. Calves infected by *Cryptosporidium* ranged from one to three weeks after birth (Bhat *et al.*, 2012). Majeed *et al.* (2022) in Kuwait reported that calves less than one month of age (OR =4.32, P= 0.0001) was identified as the significant *Cryptosporidium* infection risk factor.

In the current study, the rotavirus was detected in 9% of the examined calves. Also, the present study showed that the majority of rotavirus diarrhea in cattle occurred in the first two weeks after birth. Diarrhea caused by rotavirus occurred in pre-weaned ruminants (about 2 weeks of age), as feeding on milk may provide a suitable survival environment for rotavirus and facilitates its infection of the intestinal epithelium.

Içen *et al.* (2013) in Turkey found that rotavirus was the main single cause of calf sco-

urs with a higher overall prevalence rate of 53.1% & 25%. Abdou *et al.* (2021) in Kuwait using rapid immunochromatography test, reported that the rotavirus infection in livestock was 10.23% (18/176) of calves less than 3 months old. They added that age was the RVA risk factor in cattle (P=0.00001), and that calves ≤ 14 days' old had the highest prevalence (9/47; 19.1%) followed by a rate of 9.8% (6/61) in calves aged 15-30 days old.

Ramani and Kang (2007) in India concluded that animal rotaviruses could be considered as zoonotic to humans due to the possibility of genetic reassortment, materialized by exchange of gene segments. Infections by bovine-human reassortants and the presence of several unusual strains in cases of infant diarrhea suggest that animal rotaviruses can be considered of having significant zoonotic impact

In the present study, *E. coli* K99 was detected in 17.2% of diarrheic neonatal calves. Foster and Smith (2009) reported that neonatal calves are most susceptible to *E. coli* K99 infection during first 4 days after birth and develop watery diarrhea if infected. Günes *et al.* (2004) and Ok *et al.* (2009) reported that *E. coli* was found in 45% of sick calves, while Içen *et al.* (2013) found that the prevalence of *E. coli* K99 in calves with diarrhea was 27.0 % as a single and co-infection. These variations in incidence rates between these studies might be due to different diagnostic techniques and tools being used, farm management practices and age of examined cattle. Saad *et al.* (2022) in Egypt reported that *Escherichia coli* were one of the main pathogenic bacteria found in meat and its products.

In this study, coronavirus was reported in only two 45-60 days old calves, 1% of sick calves, affected calves in the first 3 weeks after birth, and peak incidence occurred between 7th & 10th days. Clinical signs start about two days later and continued up to 3-6 days. This agreed with Amer (2018) in Egypt, who reported that scientists have identifi-

ed bovines like coronaviruses group, causing similar clinical signs in other domestic and wild ruminants serving as a reservoir(s) for cattle infection & vice versa. Meanwhile, in the present study, *Cl. perfringens* was not detected due to the national vaccination program applied by the Kuwaiti Authorities to control this infectious disease.

Conclusion

This study showed that cryptosporidiosis, *E. coli*, rotavirus and coronavirus are etiological agents of diarrhea in the neonatal calves.

Rapid and accurate diagnoses help in quick implementation of suitable interventions to decrease economic losses.

The rapid immunochromatographic assays proved to be the useful as a diagnostic tool in identifying, and characterizing *E. coli* isolated from calves, as well as diagnosing the Rotavirus, coronavirus, cryptosporidiosis and *Escherichia coli* K99.

Recommendation

The basic principle of zoonosis prevention is to proper diagnose and effective treatment of the animal reservoir hosts.

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Explanation of figures

Fig. 1: Prevalence of positive cases in different age-groups

