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RESEARCH ARTICLE

# Prevalence of Four Enteropathogens with Immunochromatographic Rapid Test in the Feces of Diarrheic Calves in East and Southeast of Turkey

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**ABSTRACT**

In this study, fecal specimens taken from 192 diarrheic and 14 healthy calves (2-40 days old) were examined for the presence of bacterial and parasitic agents. Fecal samples from diarrheic calves with the four immunochromatographic rapid tests were 92.7% positive for four enteropathogens. The individual prevalence was 25, 21.8, 9.4 and 2.1% for Rotavirus, *Cryptosporidium parvum*, *E. coli* K99 and Coronavirus, respectively. Concomitant infections caused by two agents were 15.6% for Rotavirus+Cryptosporidium, 1.0% for Rotavirus+Coronavirus, 5.2 % for Cryptosporidium+*E. coli* K99, and 7.3% for Rotavirus+*E. coli* K99. Besides concomitant infections caused by three agents were 3.1% for Cryptosporidium +Rotavirus+*E.coli* K99 and 1.0%, Cryptosporidium+Rotavirus+Coronavirus. In addition one calf (1.0%) was infected by combination of four agents as Cryptosporidium, Rotavirus, Coronavirus, and *E. coli* K99. The calculated individual prevalence was 56.9% for Rotavirus, 47.8% for *C. parvum*, 26.0% for *E. coli* K99 and 5.2% for Coronavirus. However, 88 samples were positive in smear detection for Cryptosporidium while 92 were positive in rapid test. As a result of this study it can be concluded that multiple etiologies of diarrhea can be seen and this can help in the development of a specific treatment and preventative measures for practitioners in east and southeast of Turkey.



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## INTRODUCTION

Neonatal calf diarrhea is a common disease affecting the newborn calf worldwide (Achá *et al.,* 2004; Khan and Zaman, 2007; Özkan *et al.,* 2011). Calf diarrhea is a complex syndrome with a complex etiopathogenesis, causing important economic losses due to morbidity and mortality, treatment costs, and reduced growth rates in affected calves (Zhu *et al.,* 2011). The pathogens most commonly incriminated in neonatal calf scours include viral (rotavirus and coronavirus), protozoal (*Cryptosporidium parvum, coccidia*) and bacterial pathogens (enterotoxigenic *Escherichia coli* K99 and *Salmonella* spp) (Bartels *et al.,* 2010; Kumar *et al.,* 2010; Izzo *et al.,* 2011). In addition to influence of environment, management and physiological and immunological conditions and nutritional factors such as improper diet or feeding practices, or poor quality milk replacer trigger diarrhea (Aydın *et al.,* 2001; Millemann, 2009).

Epidemiologic studies of both beef and dairy calves have implicated *Escherichia coli* K99 as the major cause of neonatal diarrhea occurring in the first 4 days of life; however, it rarely leads to diarrhea in older calves or adult cattle (Foster and Smith, 2009). Rotavirus was one of the first identified viral causes of diarrhea, and was initially known as neonatal calf diarrhea virus. Subsequently, it has been found throughout the world and has been identified as a significant pathogen of children and most other mammals (Alkan, 1998; Foster and Smith, 2009). Classically, rotavirus diarrhea is thought to be primarily a malabsorptive diarrhea, but recent evidence indicates that there is also a toxin-mediated secretory component as well. Coronavirus typically affects calves in the first 3 weeks after birth, and peak incidence occurs between 7th

and 10th days. The virus is ingested from the environment, which is contaminated by other calves or adult cattle (Torres-Medina *et al.,* 1985).

Clinical signs begin approximately 2 days later and continue for 3 to 6 days (Torres-Medina *et al.,* 1985). Among protozoan, *Cryptosporidium spp.* is frequently seen in calves with or without diarrhea. Calves infected by cryptosporidium have ranged from one to three weeks after birth (Bhat *et al.,* 2012).

Several diagnostic methods are used to detect enteropathogenic agents. Diagnosis is done through collecting feces of animals suffering from diarrhea by a rectal swab or collecting intestinal contents (Busato *et al.,* 1998) and using laboratory diagnostic tests such as direct electron microscopic test, Enzyme-linked immunosorbent assay (ELISA), latex agglutination, polyacrylamide gel electrophoresis (PAGE), reverse transcription polymerase chain reaction, and also immuno-electron microscopy (Cho *et al.,* 2010).

The aim of this study was to investigate the prevalence of Rotavirus, Coronavirus, *E. coli* K99 and Cryptosporidium in neonatal calves diarrhea in dairy farms in east and southeast of Turkey by rapid test.

## MATERIALS AND METHODS

In this study, 206 fecal samples from 192 diarrheic calves (from nine different farms within 24-48 h of onset of clinical signs from non-treated calves up to 2-40 days after birth) and 14 heathy calves were collected directly from the rectum in sterile plastic bottles and submitted to the laboratory in the same day. All the faecal samples were tested for the presence of Rotavirus, Coronavirus, Cryptosporidium and *E. coli* K99 by a commercial immunochromatography rapid test (Rainbow Calf Scour 4, Bio-X Diagnostics, Belgium), (Klein *et al.,* 2009) following manufacturer's instructions. After 10 minutes, the results were read. In the test red lined strip, yellow lined strip, blue lined strip, green lined strip were corresponds to rotavirus, coronavirus, *E. coli* attachment factor K99 and *C. parvum* respectively. Moreover, fecal samples were also tested for the presence of Cryptosporidium. Smears were made from non-concentrated faecal samples on glass slides for oocysts detection and oocysts were detected microscopically using Ziehl-Neelsen staining method.

## RESULTS AND DISCUSSION

From November 2010 to November 2011, 206 fecal samples were collected from 9 farms. The analyses of the fecal samples with the immunochromatographic rapid test were positive for in 92.7% of the fecal samples in diarrheic calves. In healthy group (n=14) enteropathogens were detected as Cryptosporidium in one sample and rotavirus in two samples. The prevalence of individual or concomitant agents is shown in Table 1. Also the results of enteropathogens detection in the calves with different life durations are shown in Table 2. In addition, 88 samples were positive in smear detection for Cryptosporidium while 92 were positive in rapid test.

Diarrhea in neonatal calves is one of the major problems in Turkey and also in many other countries, but there have been few studies, carried out for the pathogens causing this problem (Erdoğan *et al.,* 2003; Luginbühl *et al.,* 2005). Diagnostic testing for the etiologic agent responsible for diarrhea can only be performed in the laboratory because clinical signs do not allow differentiation of the causal microorganisms (Nussbaum *et al.,* 1998). The rapid detection of these pathogens should assist in treatment management. Although electron microscopy, virus culture on cell monolayers, PAGE, ELISA, and flotation techniques complemented by staining allow detection of rotavirus, coronavirus, *E. coli*, and *Cryptosporidium,* these methods are quite complicated require specialized equipment, and a regular time period (García *et al.,* 2000). They are, nevertheless, considered the gold standard for comparisons among different methods. In addition a new diagnostic method has been developed based on lateral immuno-chromatography test for Rotavirus, Coronavirus, *E. coli* f5 (K99), Cryptosporidium detection (Cho *et al.,* 2012). It has the advantage of not requiring special equipment or expertise and, therefore, it is suitable for small laboratories and field research (Klein *et al.,* 2009).

**Table 1:** Detection of enteropathogen(s) in the diarrheic dairy calves studied (n: 192) through immune-chromatic test in the faecal material.

| Enteropathogen(s) detected | Number | % |
|---|---|---|
| None | 14 | 7.3 |
| Cryptosporidium only | 42 | 21.8 |
| Rotavirus only | 48 | 25.0 |
| Coronavirus only | 4 | 2.1 |
| *E. coli* K99 only | 18 | 9.4 |
| Rotavirus+Cryptosporidium | 30 | 15.62 |
| Cryptosporidium+*E. coli* K99 | 10 | 5.2 |
| Rotavirus+Coronavirus | 2 | 1.0 |
| Rotavirus+*E. coli* K99 | 14 | 7.3 |
| Cryptosporidium+Rotavirus+*E. coli* K99 | 6 | 3.1 |
| Cryptosporidium+Rotavirus+Coronavirus | 2 | 1.0 |
| Cryptosporidium+Rotavirus+Coronavirus+*E. coli* K99 | 2 | 1.0 |

The most farms which included in this study have gaps in management and overcrowding, contaminated lots, calving heifers and cows together, cold temperatures. All this stressful to the newborn calf caused to increase its exposure to infectious agents.

In a study (De Graaf *et al.,* 1999) done for diarrheic calves in Belgium it is estimated that the prevalence of *E. coli*, Rotavirus, Coronavirus and *C. parvum* as 4, 20, 8 and 31%, respectively. In a recent Swiss study on diarrheic calves, the prevalence for these enteropathogens was 6, 59, 8 and 55%, respectively (Uhde *et al.,* 2008). For a similar study in Germany, these prevalence were 3, 43, 46 and, 0% respectively (Luginbühl *et al.,* 2005). In another study (Bartels *et al.,* 2010) performed among young dutch dairy calves the prevalance of enteropathogen were obtained as 2.6% for *E. coli*, 17.7% for Rotavirus, 3,1% for Coronavirus and 27.8% for Cryptosporidiosis. According to the prevalance of enteropathogen in Brazil were 25.1% for Rotavirus, 21.3% for Cryptosporidiosis, and 34.9% for *E. coli* (Langoni *et al.,* 2004). The results of present study, with detection rates of Rotavirus 25%, Cryptosporidiosis 21.8%, *E. coli* 9.4% and Coronavirus 2.1%, respectively were similar to the results obtained by the previously researcher (Luginbühl *et al.,* 2005; Bartels *et al.,* 2010).

Erdoğan *et al.* (2003) 19% for Rotavirus, 1% for Coronavirus, Gülyaz *et al.* (2010) indicated 27.1% for Rotavirus were determined in different survey about

**Table 2:** Detection of four enteropathogen(s) in the different age-groups of diarrheic calves

| Enteropathogen(s) detected | Age of diarrheic calves (Days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-10 (n=60) | | 10-20 (n=60) (n=60) | | 20-30 (n=54) (n=60) | | 30-40 (n=18) (n=60) | |
| | No. | % | No. | % | No. | % | No. | % |
| None | 4 | 6.6 | 2 | 3.3 | 4 | 7.4 | 4 | 22.2 |
| Cryptosporidium only | 6 | 10 | 18 | 30 | 14 | 25.9 | 4 | 22.2 |
| Rotavirus only | 20 | 33.3 | 12 | 20 | 12 | 22.2 | 4 | 22.2 |
| Coronavirus only | - | - | - | - | 4 | 7.4 | - | |
| *E. coli* K99 only | 10 | 16.6 | 6 | 10 | 2 | 3.7 | - | |
| Cryptosporidium+Rotavirus | 10 | 16.6 | 14 | 23.3 | 4 | 7.4 | 2 | 11.1 |
| Cryptosporidium+*E. coli* K99 | - | - | 2 | 3.3 | 6 | 11.1 | 2 | 11.1 |
| Rotavirus+Coronavirus | - | - | - | - | 2 | 3.7 | - | - |
| Rotavirus+*E. coli* K99 | 6 | 10 | 2 | 3.3 | 4 | 7.4 | 2 | 11.1 |
| Cryptosporidium+Rotavirus+*E. coli* K99 | 4 | 6.6 | 2 | 3.3 | - | - | - | - |
| Cryptosporidium+Rotavirus+Coronavirus | - | - | 2 | 3.3 | - | - | - | - |
| Cryptosporidium+Rotavirus+Coronavirus+*E. coli* K99 | - | - | - | - | 2 | - | - | - |

neonatal diarrhea of calves in Turkey. In the present study only Rotavirus 25% and with concomitant agent were 53.1% in diarrhea and 14.3 % in healthy calves in this study. The importance of Rotavirus in the etiology of diarrhea is confirmed. The results obtained for Rotavirus in this study are higher than the previous studies while Coronavirus results were similar (Erdoğan *et al.,* 2003; Gülyaz *et al.,* 2010). *Cryptosporidium* is an important protozoan parasite that causes diarrhea in neonates and young bovine as this protozoan is hard to treat and can be found in animals with or without diarrhea (Díaz-Lee *et al.,* 2011). The prevalance of cryptosprodium 21.8% and with concomitant agent were 47.8% in calves with diarrhea and 7.1% in healthy calves in this study. Díaz-Lee *et al.* (2011) reported prevalence of cryptosprodium as 49.8% with Ziehl-Neelsen staning methods in calves with diarrhea in Chile while Soltane *et al.* (2007) reported as 86.7% in Tunisia. Also Aydın *et al.* (2001) stated this ratio 5.94% in Kars district and Gül *et al.* (2008) reported as 13.2% in Van district in Turkey. This study demonstrated that the prevalence of Cryptosporidium in dairy calves was higher than the studies performed by Aydın *et al.* (2001) and Gül *et al.* (2008) in Turkey while it was in agreement with Díaz-Lee *et al.* (2011) and lower than Soltane *et al.* (2007). It may be related to better overall management on the farms as a result of good hygiene practices. The diarrhea syndrome has a complex aetiopathogenesis, because of various infectious agents. Güneş *et al.* (2004) reported that *E. coli* O157 was detected in the 5.8% faeces of the diarrheaic calves by ELISA. However, De La Fuente *et al.* (1998) determined that *E. coli* f5 was detected in the 11.9% faeces of the diarrheaic calves by antigen ELISA. Ok *et al.* (2009) reported that *E. coli* K99 was detected not only as 13.4% in the diarrheaic calves but also as 5.6% in the healthy calves by ELISA. The prevalance of *E. coli* K99 concomitant agent were 27.0% in calves with diarrhea. In this study the percentage of *E. coli* K99 in diarrhoeic calves was higher than the results reported by some researchers (Güneş *et al.,* 2004; Ok *et al.,* 2009). These differences in incidence rates among the studies may be attributed to different diagnostic methods used farm management practices exercised in different regions and related to aging of calf.

**Conclusion:** As a result, findings in this study show that rotavirus, coronavirus, cryptosporidiosis and *E. coli* play a role in the aetiology agents of diarrhea in the neonatal calves. Identification of the possible causative agent in outbreaks of diarrhea is important because it allows targeted preventative measures such as vaccination and identification of possible risk factors or sources of infection. The multiple etiologies of diarrheas and the importance of microbiological diagnosis relating to the research of different enteropathogens of diarrheas in calves can help better understanding of their etiology, epidemiology and the development of a specific treatment and preventative measures for practitioners in east and southeast of Turkey. The rapid immunochromatographic assays test kit may be useful as a diagnostic tool in identification and characterization of *E. coli* isolated from calves. Also it is worth applying to field diagnosis for Rotavirus, Coronavirus, Cryptosporidiosis and *E. coli* K99.

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