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Lateral flow assay (LFA) detection of co-infection of predominant enteric pathogens from LFA positive *Bovine rotavirus* samples

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Abstract

The terms "white scour" or "milk scour" are frequently used to describe *Rotavirus* diarrhoea in calves, piglets, foals, and lambs. When calves are 1 to 2 weeks old, the *Bovine Rotavirus* typically causes diarrhoea in them. This study was conducted to detect *Bovine Rotavirus* in diarrhoeic calves using diagnostic techniques, namely Lateral Flow Assay (LFA). A total of 50 faecal samples were collected from diarrhoeic calves in the Junagadh region. The Anigen Rapid BoviD-5 Ag Test Kit used in the study is manufactured by BioNote, Inc. (South Korea). In this study, four faecal samples that tested positive for *Bovine Rotavirus* using the Lateral Flow Assay (LFA) were further screened for co-infection with other predominant enteric pathogens, including *Giardia*, *E. coli* K99, *Coronavirus*, and *Cryptosporidium*. Notably, 2 out of 4 samples (50%) also tested positive for *Bovine Coronavirus* antigen, suggesting a significant level of viral co-infection of *Bovine Rotavirus* and *Bovine Coronavirus* among diarrhoeic calves. All LFA-positive samples contained *Bovine Rotavirus*, with half also positive for *Bovine Coronavirus*. No other pathogens were detected, indicating common *rotavirus–coronavirus* co-infection in diarrhoeic calves.

Keywords: *Bovine rotavirus*, *Bovine coronavirus*, diarrhoea, lateral flow assay, co-infection

1. Introduction

This disease is most commonly seen in young animals, while older animals tend to be less susceptible. This decreased sensitivity with age is likely due to changes in their physiology and/or the development of immunity from previous exposures (Estes and Kapikian, 2007)^[5]. A variety of bacteria, protozoa, and viruses have been identified as potential causes of neonatal gastroenteritis, acting either individually or in combination (Snodgrass *et al.*, 1990)^[16]. Among bacteria and protozoa, *Escherichia coli* and *Cryptosporidium* spp. are the leading causes of diarrhoeal diseases. While *Rotavirus* are the primary viral agents responsible for severe gastroenteritis in both humans and animals, other viruses such as *Coronavirus* and *Parvovirus* also commonly contribute to diarrhoea (Lucchelli *et al.*, 1992)^[9].

Group A *Bovine Rotavirus* is a major cause of neonatal calf diarrhoea, a significant economic disease in calves that leads to high mortality rates. Globally, this condition results in annual losses of around \$9.5 million USD (Niture *et al.*, 2009; Silva *et al.*, 2012)^[12, 13]. *Bovine Rotavirus* infection in neonatal calves leads to a mortality rate of approximately 5–20%. On average, the infection has an incidence and prevalence rate ranging from 30% to 40% (Dhama *et al.*, 2009; Swiatek *et al.*, 2010)^[4, 17]. Three countries in the subcontinent (India, Bangladesh and Pakistan) account for more than 30% of all *Rotavirus* related deaths worldwide (Miles *et al.*, 2012)^[10]. In India, prevalence of *Rotavirus* is between 10% to 52% of diarrhoeal cases in calves younger than one month old (Khatter and Pandey, 1986; Singh and Pandey, 1990; Singh *et al.*, 1993; Jindal *et al.*, 2000; Nataraju *et al.*, 2009)^[14, 15, 7, 11].

2. Materials and Methods

2.1 Sample collection from calves

A total of 50 faecal samples or rectal swabs were collected from diarrhoeic calves. The samples were collected following the standard method described by Yilmaz (2016) [18]. An aliquot of each faecal sample was diluted in phosphate-buffered saline (PBS, pH 7.2). A 10% (w/v) faecal suspension was prepared using PBS. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4°C to remove coarse debris and bacteria. The resulting clarified supernatant was stored at -20°C until further use for RNA extraction.

2.2 Lateral flow assay test (LFA)

The Anigen Rapid BoviD-5 Ag Test Kit used in the study is manufactured by BioNote, Inc. (South Korea).

Table 2.1: Components of lateral flow assay test kit (Anigen Rapid BoviD-5 Ag Test Kit)

Sr. No.	Reagent	10 Tests/ kit
1	BoviD-5 Ag Test Device	10
2	Assay diluent tube	10
3	Disposable swab	10
4	Disposable dropper	10

2.3 Principle

The Anigen Rapid BoviD-5 Ag Test Kit is a solid-phase immunochromatographic assay designed for the rapid and qualitative detection of *Cryptosporidium* antigen, *Rotavirus* antigen, *Coronavirus* antigen, *Escherichia coli* K99, and *Giardia lamblia* antigen in calf faecal samples.

2.4 Procedure of the Test

- Stool samples were collected using the sample collection swab.
- The swab was placed into a tube containing the test solvent.
- The swab was mixed thoroughly until the sample dissolved in the test diluent.
- The tube was left undisturbed for about one minute to allow large particles to settle at the bottom.
- Control devices were removed from their packaging and placed on a flat, dry surface.
- Using the supplied disposable dropper, the extracted sample was aspirated.
- Four drops of the sample were slowly added into the wells using the dropper.
- The timer was started as the sample flowed across the result window. If the sample did not appear within one minute, an additional drop of the prepared sample was added to the sample well.
- Test results were interpreted at 10 minutes, and readings taken after 20 minutes were disregarded.

2.5 Interpretation of the Result

- **Negative Result:** Only the control ("C") line appeared in the result window, indicating a negative outcome.
- **Positive Result:** Both the test ("T") line and the control ("C") line appeared in the result window, indicating the presence of Rotavirus antigen.
- **Invalid Result:** If the control ("C") line did not appear, the result was considered invalid, and the sample was retested.



Fig. 2.1: Components of lateral flow assay test kit (Anigen Rapid BoviD-5 Ag Test Kit)

3. Results and Discussion

In this study, four faecal samples that tested positive for *Bovine Rotavirus* using the Lateral Flow Assay (LFA) were further screened for co-infection with other predominant enteric pathogens, including *Giardia*, *E. coli* K99, *Bovine Coronavirus*, and *Cryptosporidium*. The results are summarized in the above table. All four samples confirmed positive for *Rotavirus*, as expected, since the selection was based on LFA-positive results. Notably, 2 out of 4 samples (50%) also tested positive for *Bovine Coronavirus* antigen, suggesting a significant level of viral co-infection among diarrhoeic calves. None of the samples tested positive for *Giardia*, *E. coli* K99, or *Cryptosporidium* (Fig. 3.1).



Fig 3.1: Lateral flow assay test shows positive result for *Rotavirus* with *Corona virus* and negative for *Giardia*, *E.coli* K99 and *Cryptosporidium*

Table 3.1: Lateral flow assay (LFA) detection of co-infection of predominant enteric pathogens from LFA positive *Bovine Rotavirus* samples

Enteric pathogens	No. of samples tested	No. of samples positive	Prevalence (%)
<i>Giardia</i> Ag	4	0	0.00
<i>Escherichia coli</i> K99 Ag	4	0	0.00
<i>Bovine Coronavirus</i> Ag	4	2	50
<i>Cryptosporidium</i> Ag	4	0	0.00

The results of present study were similar with Zhu *et al.* (2011) [19] tested 28 diarrhoeic faecal samples, the method

detected 2 samples co-infected with both viruses (*Bovine Coronavirus* & *Bovine Rotavirus*), 26 samples positive for group A *Bovine Rotavirus* BRV, and 2 samples negative for both. Sakli *et al.* (2019) analyzed 96 faecal samples from diarrhoeic calves. Out of them 4 cases of both BRV and BCoV presence (4.16%).

However, the result was in contrast to Brunauer *et al.* (2021) [2] investigated multiple enteropathogens, including *Bovine Rotavirus* (BRV), *Bovine Coronavirus* (BCoV), *Escherichia coli* K99 (EPEC), and *Cryptosporidium* spp. (Crypto), which are common causes of diarrhoea in calves during their first 30 days of life. The analysis included 41 studies (with 94 sub-studies) from 21 countries assessing mixed infections in calves worldwide. The highest pooled global prevalence was found for BRV-Crypto co-infections at 6.69%, followed by BRV-BCoV at 2.84%, and BRV-EPEC at 1.64%. Irehan *et al.* (2025) [6] analyzed 85 faecal samples from calves. The results showed 24 samples positive for BRV, 13 samples positive for BCoV, and 25 exhibiting mixed infections of both viruses. Immunochromatographic tests identified antigens of *E. coli* K99 in 3 samples, *Cryptosporidium* spp. in 12 samples, and *Giardia* spp. in 28 samples.

The presence of co-infection with *Bovine Coronavirus* and *Rotavirus* in 50% of cases is noteworthy, as these two viral agents are frequently implicated in multifactorial calf diarrhoea (Cho & Yoon, 2014) [3]. Co-infections can exacerbate disease severity, prolong viral shedding, and reduce the efficacy of treatment and vaccination (Al Mawly *et al.*, 2015) [1]. The lack of detection of protozoal (*Giardia*, *Cryptosporidium*) and bacterial (*E. coli* K99) pathogens in this subset may be attributed to the small sample size or differences in the regional epidemiological distribution of pathogens.

These findings underline the importance of routine screening for multiple enteric pathogens, especially in *Rotavirus*-positive calves, to guide effective management and control strategies. Broader surveillance using larger sample sizes is warranted to better understand the pattern and prevalence of co-infections in neonatal calf diarrhoea.

4. Conclusion

Rotavirus plays a crucial role as a major etiological agent of diarrhoea in bovine calves, leading to significant economic losses in both dairy and meat industries. For the detection of *Bovine Rotavirus* antigen, diarrhoeic samples should be screened using LFA. The findings indicated that 2 samples co-infected with both viruses (*Bovine Coronavirus* & *Bovine Rotavirus*).

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Conflict of Interest

Not available

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Not available

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