# RAPID ETIOLOGICAL DIAGNOSIS OF NEONATAL CALF DIARRHEA WITH IMMUNOCHROMATOGRAPHIC TEST KITS IN ESME DISTRICT OF USAK

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## ABSTRACT

This study was carried out for rapid etiological diagnosis of neonatal calf diarrhea by using immunochromatographic test kits in the Eşme district of Uşak. The animal material of the study consisted of 100 1-28 days old neonatal calves of different breeds and genders in the Eşme district of Uşak. Stool samples were taken from calves with diarrhea as a result of clinical examination. When stool samples were examined by a rapid diagnostic test, none of the disease factors sought in the study were found in 10 (10%) of 100 calves, while one or more disease factors were detected in 90 (90%) of calves. Rotavirus was detected in 27 (27%) calves, Escherichia coli 14 (14%) calves, Coronavirus in 8 (8%) calves, Clostridium perfringens in 19 (19%) calves, Cryptosporidium spp. in 17 (17%) calves, Rotavirus + Coronavirus in 2 (2%) calves, Rotavirus + Clostridium perfringens in 1 (1%) calf, Rotavirus + Cryptosporidium spp. in 1 (1%) calf, and Escherichia coli - Clostridium perfringens in 1 (1%) calf. As a result, data on the presence and distribution of enterogenous pathogens that cause diarrhea in neonatal calves in the Eşme district of Uşak were presented, and it was concluded that it would shed light on future studies on diarrheal calves in the Eşme.

*Keywords:* Immunochromatographic test kit, Neonatal calf, Prevalence

## INTRODUCTION

The neonatal period is the period between 0 and 28 days following the birth and the most critical period of calf rearing (Mickelsen and Evermann, 1994). Calf diarrhea, which causes significant economic losses in Turkey and abroad, is one of the important health problems in the cattle

breeding business. The economic loss is mainly due to calf loss, loss of genetic material with death, treatment costs, performance retardation in the later stages of life despite recovery (Eskiizmirliler *et al.*, 2001). The regular number of calves obtained by the enterprises during the year, keeping these calves healthy, and increasing productivity will ensure the growth of the enterprises and increase the profit rate. Problems such as calf mortality due to various factors, increased treatment costs as a result of diseases, and growth deficiency and productivity slowdown in treated calves can reduce profitability and cause businesses to experience significant financial losses

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(Mellor and Stafford, 2004). Calf diarrhea is attributed to both infectious and non-infectious factors. The majority of neonatal calf diarrhea is caused by infectious agents. Infectious diarrhea is one of the most important health problems observed in neonatal calves until the weaning age and is responsible for approximately half of the calf mortality up to this age (Izzo *et al*., 2011). Infectious diarrhea in calves is most commonly associated with Escherichia coli, Salmonella spp., Clostridium perfringens, Cryptosporidium parvum, Eimeria spp., Giardia spp., Rotavirus, Coronavirus, and Bovine Viral Diarrhea Virus (BVDV) agents; the presence of factors such as Torovirus and Calicivirus, which play a minor role in the development of diarrhea, has also been demonstrated recently (Foster and Smith, 2009; Cho and Yoon, 2014).

Various variables can cause diarrhea in newborn calves. In general, factors that predispose to diarrhea include colostrum administration to calves on time and in sufficient quantities or not at all (Wells *et al*., 1996), nutrition, the immunization status of mother and calf (Wind*eyer et al*., 2014), lack of cleaning and disinfection of tools used in barns or exposing the calf to intense microbial load before consuming colostrum, genetic and environmental factors (Gulliksen *et al*., 2009), lack of postpartum umbilical cord disinfection and managerial factors (Khan and Khan, 1991; Lundborg *et al.,* 2005; Larson Tyler, 2005; Radostits *et al.,* 2007).

It has been determined that immunochromatographic tests in calf feces show high specificity for Coronavirus, Rotavirus, Escherichia coli K99, and Cryptosporidium parvum, and the sensitivity is very high. With the use of these kits, the etiological factors in calves with acute diarrhea will be determined in the shortest time possible, specific treatments can be applied for the agent, and the survival chances of the calves will increase (Klein *et al.,* 2009).

Diagnosing the causative agent in neonatal diarrhea in a short time is significant for the diagnosis and treatment of the disease, especially for veterinarians working in the field. This study aims to contribute to the treatment and prevention methods by detecting the prevalence of the agents causing diarrhea in neonatal calves by using immunochromatographic test kits in the Eşme district of Uşak.

## MATERIALS AND METHODS

### Animals
The animal material of the study consisted of 100 1-28 days old neonatal calves in the Eşme district of Uşak. Clinical examinations were made and calves with diarrhea were included in the study. Stool samples from calves with diarrhea were taken by rectal stimulation and placed in sterile stool containers. Systemic clinical examinations of calves with diarrhea were performed. Stool consistency, stool content, mucous membranes, and conjunctival color were checked in clinical examination. In the examination of calves with diarrhea, skin elasticity, the position of the eyeball in the orbit, status parezens, and sucking reflex were taken into consideration. Stool analyzes were performed using a commercial immunochromatographic ready-made diagnostic kit containing enteropathogens of Rotavirus, Coronavirus, Cryptosporidium, Clostridium perfringens, and Escherichia coli (F5-K99).

### Test procedure
Stool samples were taken from the middle part of calf feces using a cotton swab. The cotton sampling swab was mixed in the tube containing the assay diluent until the sample was dissolved. It was kept for 30 seconds for sedimentation. Supernatants were taken with a disposable vacuum pipette; the test cartridge was placed on a flat surface; four drops were dropped into each test chamber; after a waiting period of 5 to 10 minutes, the test was evaluated according to the positive

and negative values indicated in the application brochure.

## RESULTS

As a result of the test performed with the immunochromatographic ready diagnostic kit from the stool of neonatal calves with diarrhea, none of the disease factors sought in the study were found in 10 (10%) of 100 calves, while one or more disease factors were detected in 90 (90%) calves. Rotavirus was detected in 27 (27%) calves, Escherichia coli in 14 (14%) calves, Coronavirus in 8 (8%) calves, Clostridium perfringens in 19 (19%) calves, Cryptosporidium spp. in 17 (17%) calves, Rotavirus + Coronavirus in 2 (2%) calves, Rotavirus + Clostridium Perfringens in 1 (1%) calf, Rotavirus + Cryptosporidium spp. in 1 (1%) calf, and Escherichia coli - Clostridium perfringens in 1 (1%) calf (Table 1).

**Table 1:** As a result of stool analysis, the total number of disease agents and their ratios according to the total number of calves (n=100)

|  | Ratios |
| --- | --- |
| Total number of calves | 100 (%100) |
| Negative | 10 (%10) |
| Positive | 90 (%90) |
| Rotavirus | 27 (%27) |
| Escherichia coli | 14 (%14) |
| Coronavirus | 8 (%8) |
| Clostridium perfringens | 19 (%19) |
| Cryptosporidium spp. | 17 (%17) |
| Rotavirus + Coronavirus | 2 (%2) |
| Rotavirus + Clostridium perfringens | 1 (%1) |
| Rotavirus + Cryptosporidium spp. | 1 (%1) |
| Escherichia coli - Clostridium perfringens | 1 (%1) |

## DISCUSSION

It is reported that the incidence of diarrhea in neonatal calves is higher than 50%, and the calf mortality rate due to diarrhea varies between 1.5% and 8% (Frank and Kaneene 1993). Calf diarrhea, which is characterized by high morbidity and mortality, continues to be a current problem in Turkey and causes significant economic losses due to high treatment costs, poor performance, and death (Kozat and Tuncay, 2017). Due to the high mortality rate of calf diarrhea and the very complex etiological factors, the difficulty of performing an effective treatment depends on performing adequate, regular, and correct fluid-electrolyte treatment (Kozat 2017).

In the present study, it was determined that, in the Eşme district of Uşak, the Rotavirus (27%) was detected most, and the Coronavirus (8%) was detected least. Also, the mixed infection rates were found very low. It was determined that Rotavirus, which was detected the most in our study, also contributed to mixed infections (Rotavirus + Coronavirus (2%), Rotavirus + Clostridium Perfringens (1%), Rotavirus + Cryptosporidium spp. (1%)). Many studies have been conducted on the prevalence of the causative agents of neonatal calf diarrhea at different times and in different regions of Turkey. Küliğ and Coşkun (2019) conducted a study on 138 calves with diarrhea in and around Sivas and found Escherichia coli 23%, Rotavirus in 7%, Coronavirus in 4%, Clostridium perfringens in 22%, and Cryptosporidium spp. in 4% as the sole factor in the etiology of the study. In their study with 100 animals with diarrhea and 10

healthy animals in Siirt province, Kozat and Tuncay (2017) found 4% Rotavirus, 5% Rotavirus + Escherichia coli, 6% Escherichia coli, 12% Rotavirus + Cryptosporidium, 10% Cryptosporidium, 4% Rotavirus + Giardia, and 7% Escherichia coli + Coronavirus enterogenous pathogens in calves with diarrhea and the ratios of other factors were found to be 52%. The findings of the present study were higher. It can be stated that the reason for this is related to the regional difference.

Contrary to the current study, Cho and Yoon (2014) found that at least one infectious agent is responsible for diarrhea in 80% of calves, and the rate of mixed infection in which two or more agents are detected can exceed 50%.

Ekici et al. (2011) determined the prevalence of Cryptosporidiosis as 39.4% in their study. In the presented study, Cryptosporidium spp. has been detected in 17%, a percentage below the Turkey average. This result can be attributed to the fact that the animals included in the present study were better cared for and fed in the first month. Because this infection is more common in calves younger than one-month-old, and the most significant factors in the emergence of the disease are age and whether the immune system is sufficiently developed.

Rotavirus and coronavirus have a significant role in newborn diarrhea. Many other viruses (parvovirus, astrovirus, and BVDV) also cause neonatal calf diarrhea. Coronavirus and rotavirus infections are often encountered in young animals (Alkan, 1998). Rotavirus infection is usually seen in calves aged between two days and three weeks (Gökçe, 1995). Data are indicating that rotavirus infections are frequent in calves with diarrhea. Abraham et al. (1992) found rotavirus infection in calves with diarrhea in the first eight weeks of their postnatal life in Ethiopia as 16.7%. In studies on rotavirus infection in calves with neonatal diarrhea in Turkey, Yazıcı (1992)

found rotavirus rates as 17%, Alkan et al. (1998), 26.8%, and Burgu (1995), 34%. In the present study, rotavirus was detected with a rate of 26%, highest, in the Eşme district of Uşak.

Escherichia coli serotype (F5) with K99 pilus was isolated from 30-35% of calves with diarrhea in Turkey. About 30% of calves with severe diarrhea and depression have a bacterial infection associated with Escherichia coli Infections were observed more commonly in calves less than two weeks old and with inadequate colostral IgG concentration (Akyüz et al., 2017). Contrary to previous study, Escherichia coli was detected at a lower rate, 14%, in the present study.

As in the rest of the world, calf mortality maintains its significance in Turkey, and these losses in growing enterprises are reaching very critical levels. The calf mortality rate in Turkey can reach 10% in state enterprises and up to 50% in individual enterprises. The main causes of calf mortality are known as diarrhea, pneumonia, and sepsis (Klein et al., 2009). Therefore, in the present study, the factors of diarrhea, which is the main cause of calf mortality, were investigated.

## CONCLUSION

Traditional diagnostic methods used to diagnose enteropathogens of calf diarrhea have some disadvantages. Instead of traditional diagnostic methods, with rapid immunochromatographic test kits, which are more advantageous in field conditions, different disease agents can be detected from calf feces within 10-15 minutes, and it is possible to plan prophylactic and treatment methods quickly. In the present study, rapid immunochromatographic test kits were used in the field to detect disease agents and we believe that the prevalence of rapid immunochromatographic test kits should increase. As a result, this study had concluded that in the Eşme district of Uşak,

the Rotavirus was detected at the highest rate (27%), and this factor should be considered in the treatment and prevention of neonatal calf diarrhea in this region.

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