ORIGINAL ARTICLE Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ

Diagnosis of viral causative agents in neonate small ruminant using rapid card test

Karima Al Salihi
College of Veterinary Medicine / Al Muthanna University/ Department of veterinary medicine

ARTICLE INFO

Received: 13.12.2017
Revised: 25.12. 2017
Accepted: 30.12. 2017
Publish online: 15.02.2018

***Corresponding author:**
Karima A AlSalihi
Email address:
kama-akool18@mu.edu.iq

Abstract

Diarrhea in lambs and goat kids is caused by various infectious agents. It may be occurred alone or in combination with each other's. The more common viral agents are including rotavirus, coronavirus, astrovirus and calicivirus that lead to diarrhea in neonate small ruminant. This study intended to detect the viral agents of lambs and goat kids diarrhea in Al Muthanna province using rapid diagnostic Card test. One hundred fifty fecal samples (100 lambs and 50 kids) were collected from various areas in Al Muthanna province. One-step rapid field diagnostic Card test (Combo /Certest) was used for diagnosis the presence viral agents in fecal samples that collected from diarrheic animals. The viral agents were identified in 140 out of 150 at percentage 93.6 % of examined fecal samples, while 10 out of 150 at percentage of 6.4% revealed negative results in rapid card test. The percentages of viral infection were 123 (87.857 %), 2 (1.428 %) and 4 (2.857%) respectively for rotavirus, adenovirus and norovirus respectively. Besides, the percentages of mixed viral infections with rotavirus & adenovirus and norovirus, rotavirus & adenovirus, and rotavirus & norovirus were 6 (4.385 %), 3 (2.142 %), and 2 (1.428 % respectively. In conclusion, this study approved that one-step rapid diagnostic Cards test was efficient to diagnosis different viral agents in diarrheal lambs and goat kids. The author recommends to use this test to diagnosis viral diarrheal infection in the field to reduce the long diagnostic procedures that used routinely in order to apply the rapid preventive program to decrease the economic implication in the large number herds of sheep and goat.

To cite this article: Karima Al Salihi. (2018). Diagnosis of viral causative agents in neonate small ruminant using rapid card test. MRVSA. 7 (1), 40-46.
DOI: http://dx.doi.org/10.22428/mrvsa-2018-00714

Keywords: Al Muthanna province, Kids, lamb, one step rapid card test, Rotavirus.

Introduction

Newborn farm animals diarrhea under 30 days of age particularly in the first week of life, is one of the most common disease complexes that the large-animal clinician encounters in practice. The causes of lambs and kids diarrhea are complex and usually

involve an interaction between enteropathogenic bacteria, viruses, and protozoa, the colostral immunity of the animal and the effects of the milieu. Accordingly, the term acute undifferentiated diarrhea of newborn lambs and kids is used to describe the acute diarrhea that occurs in newborn small ruminant under 30 days of age, characterized clinically by acute copious watery diarrhea, progressive dehydration and acidosis and death in a few days, or earlier after onset if treatment is not provided. According on the clinical findings alone, it is not usually potential to distinguish between the common known causes of diarrhea in newborn calves and lambs, which include enterotoxigenic *E. coli* (ETEC), verocytotoxic *E. coli* (VTEC), necrotoxigenic *E. coli* (NTEC), rotavirus, coronavirus, bovine torovirus (Breda virus), calicivirus, norovirus (Norwalk-like virus), Cryptosporidium spp., Giardia spp., and Salmonella spp. Rotavirus is a genus of double-stranded RNA viruses in the family Reoviridae (Gunther and Otto, 1987; Prasad and Chiu, 1994; Pesavento et al., 2006; Green, 2007, Brown et al., 2008). Rotavirus contains structural and non-structural viral proteins. The presences of rotavirus in fecal specimens was detected in the calves in Iraq (Hasso, 1982). Besides, the virus was also isolated from the diarrheic calves in Mosul (Saeed, 1992). The rotavirus has been isolated from the feces of diarrheic lamb under 3 weeks of age. However, the disease occurs sporadically and no actual epidemiological characteristics have been recorded. Clinical signs of rotavirus were observed as early as 12-16 h after birth (Thiel *et al.*, 1996) and the morbidity reached to 100% and mortality 10%. The rotavirus experimental infection in lambs is mild and characterized by mild diarrhea, abdominal distress and healing in a few days. However, the mortality in lambs is much higher when both the rotavirus and enteropathogenic *E. coli* are used. Noroviruses, previously known as Norwalk like viruses have been known as the most common pathogens involved in outbreaks of acute non-bacterial gastroenteritis in humans. The Norovirus genus, is one of four genera in the Caliciviridae family. Norovirus-specific DNA has been detected in the fecal samples of diarrheic calves in Michigan and Wisconsin (Wise *et al.,* 2004). Genetically, norovirus can be classified into 5 genogroups (G) (Zheng *et al.,* 2006). GI, GII and GIV in human, GIII of bovine, GII in porcine and GV of murine (Greening and Wolfs, 2010). Bovine GIII contain 2 genotypes (Oliver *et al.,* 2006) and are represented by Newbury agent. The Jena virus (JV) were isolated from cattle in Germany (Günther and Otto, 1987) and was also identified in the fecal specimens of calves with diarrhea in the United Kingdom (Woode and Bridger, 1978). In Africa and Central America, there are 10 serotypes of adenovirus. It was approved that this virus causes disease of the respiratory or gastrointestinal tract in cattle (Benkö *et al.*, 2000). Moreover, the virus was isolated from healthy cattle that acts as virus shedding for about (10 days) in the feces or respiratory secretions. Additionally, some cattle may become persistently infected, resulting in excretion of the virus for much longer. The younger animals reveal the more clinical signs due to the levels of maternal antibodies, which begin to diminish at aged 2 weeks. The Clinical signs of gastrointestinal infection are include abdominal distention, reduced appetite and diarrhea (Benkö *et al.,* 1989). Neonatal cattle have 2 types (3 and 5) of adenovirus. This virus was appeared to be more pathogenic than others and produced disease of the gastrointestinal and respiratory tracts (Lehmkuhl *et al.,* 1975). Other previous study in china, were isolated Adenovirus type 3 in MDBK cells from cattle (Zhu *et al.,*

2011). Moreover, Lehmkuhl *et al.,* (1975) approved that (33.33%) of experimentally infected calves with isolated bovine Adenovirus type 3 were suffered from mild diarrhea. Adenovirus type 5 were also isolated from calves with clinical sings of weak calf syndrome in United Status (Coria *et al.,* 1975). Review of literatures revealed scarce publications regarding the viral causative agents of viral diarrheic lambs and goat kids in Al Muthanna province. Therefore, this study intends to investigate the viral causative agents in neonate lambs and goat kids using rapid diagnostic Card test in Al Muthanna province.

Materials and Methods

One hundred fifty fecal samples were collected from neonatal lambs and goat kids that suffering from diarrhea in different areas in Al Muthanna province. The lambs and goat kids were less than 2 weeks old. Each fecal sample were collected and kept in disposable plastic container. Fecal samples were mixed with buffered and tested by one-step combo card test (Rota+ Adeno+ Noro) from Certest biotec. It is a colored chromatographic immunoassay that based on the simultaneous qualitative detection of Rotavirus, adeno and Norovirus in fecal samples and offers a simple and highly sensitive screening assay to make a presumptive diagnosis of Rotavirus, adenovirus and Norovirus infection.

Results and Discussions

The results of this study showed that the viral agents were detected by one-step rapid card test. The viral agents were identified in 140 out of 150 at percentage 93.6 % of examined fecal samples, while 10 out of 150 at percentage 6.4% revealed negative results in rapid card test (Table. 1).

Table. 1: Shows the percentages of positive and negative samples of tested fecal samples

Total number of samples (Lambs & Kids)	No. of Positive samples/ percentages	No. of Negative samples/ percentages
150	(140) 93.6 %	(10) 6.4%

Rotavirus, Adenovirus and Norovirus were reported in 123 (87.857 %), 2 (1.428 %) and 4 (2.857%) respectively. Moreover, 6 (4.385 %), 3 (2.142 %), and 2 (1.428 %) were revealed mixed infection of Rotavirus+ adenovirus + Norovirus, rotavirus + adenovirus and rotavirus + norovirus respectively. Nonetheless, the dominated viral infection was the Rotavirus (Table. 2). The result of this study revealed that 87.857 % percentage of the examined samples were infected with rotavirus. This percentage appeared as the highest rate in compare to the percentages of other causative agents. This result is in agreement with previous studies (Kaminjolo and Adesiyun, 1994, Pisanelli *et al.,* 2005, Badiei *et al.,* 2010, Duman and Aycan, 2010). Kaminjolo and Adesiyun, (1994) approved that the prevalence of rotavirus infection were 27.7% (73/264) in calves, 27.8% (45/162) in piglets, 48.6% (18/37) in lambs and 28.6% (2/7) in goat kids.

However, rotavirus antigen was not detected in calves and lambs < 1 week old and in piglets < 2 weeks old.

Table. 2: Shows the causative agents identified with one-step rapid test

Virus	Number of samples (Lambs & Kids)	Percentages (Lambs & Kids)	Length of diarrhea / days	Clinical signs
Rotavirus	123	87.857 %	3-5	Whitish watery diarrhea
Adenovirus	2	1.428 %	4	Moderate diarrhea and GIT colic
Norovirus	4	2 . 8 5 7 %	3	Watery, greenish grey diarrhea
Rotavirus+ adenovirus + Norovirus	6	4.385 %	7-10	Yellowish watery diarrhea
rotavirus +adenovirus	3	2.142 %	6	Death after healing from diarrhea due to dehydration
rotavirus + norovirus	2	1.428 %	6	Watery yellowish diarrhea
Total	140	100%		

Moreover, this study found that the highest prevalence of rotavirus was found in calves between the ages 1-6 weeks (72.6%); piglets, 2-8 weeks (91.1%) and in lambs 1-8 weeks (88.9%). The overall prevalence of infection was 39.9% for diarrheic and 13.4% for non-diarrheic animals and the difference was statistically significant (P < or = 0.001; X2). Moreover, the study approved that the differences among husbandry systems in relation to the prevalence of rotavirus infection were not statistically significant (P > or = 0.05; X2). The rotavirus infection in the young tested animals revealed relatively high prevalence coupled with the detected significantly higher infection rates in diarrheic animals. This results indicated that rotavirus may be important in livestock diarrhea in Trinidad (Kaminjolo and Adesiyun, 1994).

Moreover, the result of the current study also revealed that adenovirus infection was reported in 1.428 % of lambs and kids accompanied with diarrhea and gastrointestinal colic, and this result is compatible with previous study (Woods *et al.,* 2008). The results of the current study also found that the norovirus occurred in 2.857% of diarrheal examined animals. These calves were suffered from Watery, greenish grey diarrhea for 3 days. Meanwhile, other study, showed that (100%) of bovine neonate were infected experimentally with bovine norovirus (Otto and Ian *et al.,* 2011).

A mixed infection with rotavirus, adenovirus and Norovirus was detected in 4.385% percentage of lambs and kids suffering from diarrhea for 7-10 days duration. This result is compatible with Woode *et al.,* (1984), who detected mixed infection between Astrovirus and Breda virus infections of dome cell epithelium of bovine ileum. The results of this study also revealed that rotavirus +adenovirus and rotavirus + norovirus occurred in 3 (2.142 %) and 2(1.428 %) fecal samples respectively. These animals were

suffered from diarrhea for 6 days and were died after healing of diarrhea and this result is compatible with previous studies (Smith, 2008; Anderson and Rings, 2009).
In conclusion, this study revealed that rapid diagnostic Cards test were able to diagnosis different viral infectious agents in diarrheal lambs and kids. The author recommends to use this test to diagnosis viral diarrhea in the field in order to reduce the long diagnostic procedures that used routinely. Therefore, decrease in the economic implication due to rapid application of preventive program for sheep and goat herds.

References

Anderson DE and Rings MD. (2009). Curr. Vet. Thera: Food Animal Practice, 5th ed. Saunders S and Louis M. P. 179.

Badiei K, Pourjafar M and Ghane M. (2010). Fecal rotavirus antigen in diarrheic calves of high and average producing Holstein dairy cows. Global Veterinaria. 5(3): 175-197.

Benkö M, Bartha A, Mostl K, Burki F. (1989). Benko M, Bartha A, Mostl K, Bürki F (1989): A heteroploid permanent cell line originating from embryonic calf thyroid supporting the replication of all known bovine adenovirus serotypes. Vet Microbiol 19:317-324.

Benkö M, Harrach B, Russell WC. (2000). Family Adenoviridae. In: Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B. (eds), Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, New York, San Diego. 227-238.

Brown DW, Gunning KB, Henry DM. *et al.* (2008). A DNA oligonucleotide microarray for detecting human astrovirus serotypes. J. Viro. Method.147 (1):86–92.

Coria MF, McClurkin AW, Cutlip RC, Ritchie AE. (1975). Isolation and characterization of bovine adenovirus type 5 associated with weak calf syndrome. Archives of Virology. 47 ;(4):309-317.

Duman, R. and Aycan AE. (2010). Rotavirus prevalence infection of calves with diarrhea in Konya region. J. Anim. Vet. Adv. 9 ;(1):136-138.
Green KY. (2007). *Caliciviridae*: the noroviruses. In Knipe DM. *et al*. (ed.). Fields virology. 5th ed. Lippincott, Wilkins, Williams and Philadelphia. 949–979.

Greening G and Wolf, S. (2010). Calicivirus environmental contamination. In Hansman GS; Jiang XJ & Green KY, Caliciviruses. 25–44.

Günther H, and Otto, P. (1987). Diarrhea in young calves, 7. "Zackenvirus" (Jena agent 117/80) a new diarrhea pathogen calves Arch. Exp. Vet. Med. 41: 934-938. (In German). [PubMed].

Hasso SA. (1982). Rotavirus investigation of neonatal cattle and buffalo calves. Thesis of M.Sc. in Vet. Med. College of Vet. Med. University of Baghdad.

Kaminjolo JS, Adesiyun AA. (1994). Rotavirus infection in calves, piglets, lambs and goat kids in Trinidad. Br Vet J. 1994 May-Jun; 150 (3):293-9.

Lehmkuhl HD, Smith MH, Dierks RE. (1975). A bovine adenovirus type 3: isolation, characterization, and experimental infection in calves. Arch. Viro. 48:39–46.

Oliver SL, Batten CA, Deng Y, Elschner M, Otto P, et al. (2006) Genotype 1 and genotype 2 bovine noroviruses are antigenically distinct but share a cross-reactive epitope with human noroviruses. J Clin Microbiol 44: 992–998.

Otto H Peter, Ian N. Clarke, Paul R. Lambden, Omar Salim, Jochen Reetz and Elisabeth M. Liebler-Tenorio. (2011). Infection of calves with bovine norovirus GIII.1 strain jena virus: an experimental model to study the pathogenesis of norovirus infection. American Society for Microbiology. 85; 22. 12013-12021.

Pesavento JB, Crawford SE, Estes MK, Prasad BV. (2006). Proteins of rotavirus: structure and assembly. Curr. Top. Microbio. and Immuno. (309):189–219.

Pisanelli G, Martella V, Pagnini U, Demartino L, Lorusso E, Iovane G, Buonavogalia C. (2005). Distribution of G VP7 & VP4 genotypes in buffalo group A rotaviruses 21, isolated in Southern Italy. Vet. Microbio. 110:1-6.

Prasad BV and Chiu W. (1994). Rotavirus Structur. Curr. Top. Microbio. and Immuno. 185:9–29.

Saeed AE. (1992). Rotavirus isolation from diarrheic calves. Thesis of M.Sc. in Vet. Med. College of Vet. Med., Mosul University.

Smith BP. (2009). Large Animal Internal Med. 3rd ed. Mosby S and Louis M. 558.

Van der Poel WH, van der Heide R, Verschoor F, Gelderblom H, Vinjé J, Koopmans MP (2003). Epidemiology of Norwalk-like virus infections in cattle in The Netherlands. Vet. Microbiol. 92:297–309.
Thiel KW *e t a1*., (1996). IVet Diag Invest 1996; 8:245.

Wise AG *et al.*, (2004).Vet Res 2004; 100:165.

Woode GN and Bridger JC. (1978). Small viruses isolation resembling caliciviruses and astroviruses from acute enteritis of calves. J. Med. Microbiol. 11:441–452.

Woode GN, Pohlenz JF, Gourley NE, Fagerland JA. (1984). Astrovirus and Breda virus infections of dome cell epithelium of bovine ileum. Jo. Clin. Microbio. 19:623-630.

Woods LW, Lehmkuhl HD, Hobbs LA, Jackie C, Parker MM. (2008). Evaluation of the pathogenic potential of cervid adenovirus in calves. Jo. Vet. Diagnostic. Invest. 20:33–37. doi: 10.1177/104063870802000106

Zheng D-P, Ando T, Fankhauser RL, Beard RS, Glass RI, et al. (2006) Norovirus classification and proposed strain nomenclature. Virology 346: 312–323doi:10.1016/j.virol.2005.11.015 [PubMed]**.**

Zhu Yuan-Mao, Yu Zuo, Cai Hong, Gao Yu-Ran, Xiu-Mei Dong, Zhao-Li Li, Hong-Fei Shi, Qing-Feng Meng, Chuang Lu,1 and Fei Xue. (2011). Isolation, Identification and complete genome sequence of bovine adenoviruse type 3 from cattle in China. Virol. J. (8):557.