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Rapid diagnosis of the viral causative agents in diarrheal calves

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Abstract
Numerous infectious agents induce diarrhea in calves, which may be present either singly or in combination. *Rotavirus, coronavirus, astrovirus* and *calicivirus* are the common viral agents that can lead to diarrhea in calves. This study was designed to detect the viral agents of calves diarrhea in Baghdad governorate using rapid diagnostic Cards test. Hundred fecal samples were collected from calves from different area in Baghdad governorate. Combo Cards test (Certest) was used for the rapid field diagnosis of viral diarrhea. The results of this study revealed that the viral agents were constituted to 96% percentage of calves suffering from diarrhea, while the rested 4% were non-viral agents. The percentages of viruses infection were; 75%, 6.25% and 2.08% for rotavirus, adenovirus and norovirus respectively. Moreover, the percentages of mixed viral infections with *astrovirus* and rotavirus or rotavirus and adenovirus or *astrovirus*, rotavirus and norovirus were 8.33%, 5.21% and 3.13% respectively. In conclusion, this study revealed that rapid diagnostic Cards test were able to approve different viral agents in diarrheal calves. The author suggests to implement the rapid diagnostic card test on large numbers of calves suffering from diarrhea in order to approve the dominant causative agents and employ the preventive program to reduce the economic implication on the production of the calves.

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Introduction

Diarrhea in calves occurs as a results of the complex interactions of three sets of factors including: the calf, the dam and the calf's environment, which are including management, and infectious agents. Diarrhea in new-born calves is caused by many infectious or non-infectious agents. Many infectious agents, e.g., rotavirus, coronavirus, astrovirus, calicivirus are incriminated and transmission occurs via the faecal-oral route. Rotavirus, Norovirus and Astrovirus are RNA viruses with either single or double stranded nucleic acid belong to Reoviridae, Caliciviridae and Astroviridae families respectively (Gunther and Otto, 1987; Prasad and Chiu 1994;

Pesavento *et al.,* 2006; Green 2007, Brown *et al.,* 2008). On the other hand, Adenovirus is a double stranded DNA virus belong to Adenoviridae. All these viruses are icosahedral and non-enveloped. Rotavirus contains structural and nonstructural viral proteins.

In Iraq, Hasso (1982) detected the presences of rotavirus in fecal specimens of calves in Baghdad by using agar gel precipitation test. Moreover, the virus was isolated from the diarrheic calves in Mosul by Saeed (1992). Several studies were also recorded numerous isolation of rotavirus from buffalo or dairy cattle at (16.8%), (19.4%), (26.4%) and (8.5%) from Southern Italy (Pisanelli *et al.,* 2005), Brazil (Alfieri et *al* 2006), Quebec (Hussein *et al* 1995) and Turkey (Duman and Aycan, 2010) respectively. Norovirus are one of the causative agents of diarrhea in calves. It can genetically 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). Newbury agent (calici-like virus) was caused xylose malabsorption, anorexia and diarrhea in gnotobiotic calves at aged (17 – 60) days (Bridger *et al.,* 1984). In contrast, other surveys were reported the following rates :(1.6%), (72%) and (4%) by RT-PCR in Canada (Matsui *et al.,* 2001), US veal calves (Smiley *et al.,* 2003) and Dutch dairy cattle specimens (Van der Poel *et al.,* 2003) respectively.

Several studies recognized the astrovirus from some species of mammalian animal. Bovine astrovirus was first recognized in England in 1978. Later on, this virus was isolated from feces of a neonatal calf with diarrhea and experimentally transmitted to two gnotobiotic calves (GC) (Woode and Bridger, 1978). The authors considered this isolate to be non-pathogenic in calves, as no clinical effect was observed. When alone astrovirus infected calves, no clinical signs of the disease were observed. However, the mixed infection with astrovirus and rotavirus or Breda virus 2, led to develop severe diarrhea in the infected animals. Meanwhile, the astrovirus-infected calves remained clinically normal, although the feces became yellow in color and slightly soft. This change coincided with excretion of astrovirus. In addition, it was approved that the calf infected with rotavirus plus astrovirus (GC52) developed profuse yellow diarrhea and excreted both viruses (Woode *et al.,* 1984).

Adenovirus contains 10 serotypes recognized in Africa and Central America. It was approved that this virus causes disease of the respiratory or gastrointestinal tract in cattle (Benkö *et al.,* 2000). Also, infection with virus may not result in disease but can be isolated from healthy cattle. These cattle were remained the virus shedding for about (10 days) in the feces or respiratory secretions. Moreover, some cattle may become persistently infected, resulting in excretion of the virus for much longer. Clinical signs are more appearance in younger animals due to the levels of maternal antibodies, which begin to wane 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). However, 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). So far as the author aware, studies regarding viral causes in diarrheal calves in Iraq are scarce, so this study was designed to detect the viral agents of calves diarrhea in Baghdad governorate using rapid diagnostic Cards test.

Materials and Methods

One hundred fecal samples were collected from calves suffering from diarrhea from different areas in Baghdad governorate. The calves were one month old. Directly after collection, each fecal sample were kept in disposable suitable container. These samples treated with buffered and tested by combo card test from Certest biotec. Combo card test, Rota+ Adeno+ Noro is a one-step a coloured chromatographic immunoassay for the simultaneous qualitative detection of Rotavirus and Norovirus in fecal samples. This test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of Rotavirus, adenovirus and Norovirus infection. This test remains as the simplest method for diagnosis of these viruses. The Biotec Certest is devoted to product diagnosis of clinical field in veterinary, human and Agri-food.

Results and Discussions

The results of this study showed that the viral agents were detected by rapid Combo card test. Out of the 100 examined samples, 96 were infected with viruses. The rested four fecal samples were revealed non- viral causative agents (Table.1). In addition, the results of this study revealed the mixed viral infection, which was less abundant than single causes. However, the Rotavirus, as single or mixed infection, appeared as a dominated among all the isolates.

The result of this study revealed that 75 % percentage of the examined calves were infected with rotavirus. This percentage consider as the highest rate in compare to the other causative agents that recognized in the calves suffered from watery whitish diarrhea for 4-5 days. This result is in agreement with previous studies (Pisanelli *et al.*, 2005, Badiei *et al.*, 2010, Duman and Aycan, 2010). However, the result of this study also revealed that adenovirus infection was reported in 6.25% percentage of calves that showed diarrhea and intestinal colic, and this result is in agreement with previous study (Woods *et al.*, 2008). The results of this study also approved that the norovirus occurred in 2% percentage of diarrheal calves. These calves were suffered from greenish diarrhea at (4) days. Meanwhile, other study, showed that (100%) of new borne cattle were infected experimentally with bovine norovirus (Otto and Ian *et al.*, 2011).

In this study, a mixed infection with rotavirus and astrovirus was detected in 8.33% percentage of calves suffering from diarrhea for 12 days duration. This result is compatible with Woode *et al.*, (1984), who detected the Astrovirus and Breda virus infections of dome cell epithelium of bovine ileum.

Table (1): .,

Infection	No. of samples	Percentage %	Duration of diarrhea (day)	Clinical sings
Rotavirus	72	75	4-5	Watery whitish diarrhea
Adenovirus	6	6.25	5	Mild diarrhea & intestinal colic
Norovirus	2	2	4	Watery and Sandy greenish diarrhea
Rotavirus + Astrovirus	8	8.33	12	Yellowish watery diarrhea
Rotavirus + Adenovirus	5	5.21	5	Died after healing from diarrhea
Rotavirus + Norovirus + Astrovirus	3	3.13	24*	Watery greenish diarrhea
Sum	96	100		

***Intermittent**

However, astrovirus was considered to be nonpathogenic in calves, as no clinical effect was observed (Woode and Beidger, 1978). Woode *et al* (1982) was also approved that the infected calves with bovine astrovirus alone showed no clinical signs of diarrhea, however the infection with rotavirus plus astrovirus (GC52) developed profuse yellow diarrhea and excreted both viruses. The results of this study revealed also that the 5.21% percentage of examined calves were showed mixed infection with rotavirus and adenovirus. These calves were suffered from diarrhea at 5 days and were died after healing of diarrhea and this result is compatible with previous studies (Smith, 2008; Anderson and Rings, 2009). Moreover, the results of this study revealed that 3.13% percentage of examined calves were infected with rotavirus, norovirus and astrovirus. These calves were suffered from long watery greenish intermittent diarrhea at 24 days and this result is compatible with previous study (Bridger *et al.,* 1984), who characterized the Calici-like virus (Newbury agent) that found in association with astrovirus in bovine diarrhea.

In conclusion, this study revealed that rapid diagnostic Cards test were able to approve different viral agents in diarrheal calves. The author suggests to implement the rapid diagnostic card test on large numbers of calves suffering from diarrhea in order to approve the dominant causative agents and employ the preventive program to reduce the economic implication on the production of the calves.

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