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PREVALENCE OF ROTAVIRUS INFECTION IN CAMELS AND OTHER ANIMAL SPECIES

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ABSTRACT

Rotaviruses are among the main causes of enteritis in naive humans, animals, and birds. The affected animals or birds showing signs of enteritis in the form of diarrhoea, emaciation, dehydration and finally death in some cases. Little is known about its prevalence and the circulating strains of the virus is not well characterised in the eastern province of Saudi Arabia. The main objective of the current work was to detect and study the morphology of rotavirus particles in faecal suspensions from various animals from the eastern province of Saudi Arabia. To achieve these goals, we collected 140 faecal samples from dromedary camels, chicken, sheep, goat, and turkeys showing signs of diarrhoea. We processed these faecal specimen and prepared tissue suspension per each sample. We tested these faecal suspensions with the commercial available Rotavirus latex agglutination kits. Our results showed 5.17% of sheep samples positive. This represented about 2.14% of the overall tested samples from all species. We further tested these positive samples by the transmission electron microscope technique (TEM). The TEM pictures showed a typical Rotavirus shape in icosahedral in symmetry, and wheel shape. The morphometric analysis of the virus particles revealed the size of the virus ranging from 60-75 nm in diameter. These results suggested the potential roles of sheep in the transmission of rotavirus to other species of animals particularly dromedary camels living in their close proximity. There was a consistency in the results of both the latex agglutination tests and the electron microscope in the detection of rotavirus infection in faecal samples of different animals species studied.

Key words: Diarrhoea, dromedary camels, EM, enteritis, latex agglutination, rotavirus, sheep

The young children, animals and birds are usually suffering from signs of enteritis manifested clinically in the form of diarrhoea. There are many causes of enteritis in young children and animals including viruses, bacteria, parasites, toxins and many other environmental factors. Rotavirus is one of the main leading causes of enteritis in animals and birds (Ghosh and Kobayashi, 2014; Dennehy, 2015). It is well known that all children should have at least one round of rotavirus infection during the first 5 years of their life (Nguyen *et al,* 2004). Despite the presence of human vaccine against human rotaviruses, there are over 2 lacs cases of rotavirus infection reported every year particular from the developing countries (Crawford *et al,* 2017). Rotavirus belongs to the family Reovirdae that include large number of viruses affecting animals, birds as well as humans. The virus particle is non-enveloped and icosahedral in symmetry. The viral capsid composed of several layers of proteins. The virus particle is around 75 nm in diameter (Estes and Cohen, 1989). The rotavirus genome is segmented and consists of 11 segments encodes many important proteins for the virus replication (Estes and Cohen, 1989). Some human and animal strains of rotaviruses are belonging to one sero-group of the virus (Green *et al,* 1987). Detection of rotaviruses was mainly depends on the serological techniques especially the immunofluorescence, enzyme linked immunosorbent assay (ELISA) and the complement fixation test (Estes and Cohen, 1989). Several laboratory techniques were developed to detect rotavirus in clinical specimens particularly stools from human and faecal specimens from animals and birds (Al-Yousif *et al,* 2001; Xiang *et al,* 2020). New generation of the latex agglutination tests against rotavirus were developed and showed high sensitivity in the detection of the virus in faecal specimens (Dusetty *et al,* 2013). Although the electron microscope (EM) discovered long time ago, it have been still of valuable use especially in the diagnosis of the etiology of the viral gastroenteritis (Arcangeletti *et al,* 2005). The EM have great advantages in the field of diagnosing the gastroenteritis. This may be due to its ability to detect the dual infection of some common viral causes of diarrhoea such as rotavirus and Caliciviridae members. It is also useful in the

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diagnosis of some viruses that are difficult to be isolated in cell culture (Arcangeletti *et al,* 2005). Rotavirus was detected in Saudi Arabia and was responsible for gastroenteritis in young children particularly those under two years old (Kheyami and Nigel, 2006). However, there is a scarcity of research on rotaviruses from various species of animals particularly dromedary camels in Saudi Arabia. The main goal of the current study was to conduct a rotavirus surveillance among some various species of animals and birds suffering from diarrhoea in the eastern province of Saudi Arabia. To our knowledge, this is the first surveillance study among various species of animals and birds in Saudi Arabia.

Materials and Methods

All animal experiments were conducted according to the regulations of the King Abdul-Aziz City of Science and Technology, Royal Decree No. M/59, (http://www.kfsh.med.sa/KFSH_WebSite/usersuploadedfiles%5CNCBE%20Regulations%20ENGLISH.pdfAnimal ethics statement. This project was approved by the deanship of scientific reports, King Faisal University (Project No: 150050).

Samples collection and processing

A total of 140 faecal samples were collected from various species of animals and birds including (dromedary camels, sheep, goat, chicken and Turkeys) from Al-Ahsa, during late 2019 and early 2020. Each sample was collected by introducing the swabs into the cloaca of birds or the rectum of animals. The collected swabs were transferred to a sterile tube containing sterile phosphate buffered saline. Each sample was processed by centrifugation at 10000 rpm for 5 minutes. The supernatants were collected and stored at -20°C for further processing.

Latex agglutination test (LAT)

The latex agglutination test (LAT) was conducted on the faecal specimens processed above as per the manufacture's instructions. The test was conducted using the commercial available kits (Simply, Quick Stripe™ Rotavirus, Savyon® Diagnostics Ltd. 3 Habosem St. Ashdod 77610, (Catalog No. 50214)). Each strip was placed in one test tube containing the tested sample. The reaction was kept at room temperature for 10 min. Each strip has two lines, i.e. (C) designated for the control while (T) is designated for the test. Sample considered positive when two lines appear at both (C) and (T) while sample considered negative when only one line appear at (T) (Fig 1).

Processing of collected specimens for the EM testing

Processing of the faecal samples collected from various species of animals and birds for the EM technique was done as previously described (Conner *et al,* 1983). Simply, two subsequent centrifugation steps were carried out. The first cycle was done under 1ow speed 1000 RPM for 5 min then the supernatants were collected. The supernatants were subjected to a second round of centrifugation at high speed (100000 RPM) for 30 min. The supernatant were discarded then the pellets were suspended in 100 µl of distilled water.

Electron microscope (EM)

One drop of the separated faecal suspensions were placed on the Formvar coated grids for 4-5 min until drying. The grids were examined under the EM ((JOEL, JSM-5510LV, Japan) Version 5.04, JOEL Technicon's LTD, Japan) as previously described (Conner *et al,* 1983).

Results

Detection of the rotavirus in specimens from various livestock and birds in Al-Ahsa

We tested 140 faecal samples from various species of animals and birds suffering from diarrhoea. Our surveillance study showed that about 5.17% of the tested sheep samples were positive by the latex agglutination test (Table 1). This was about 2.14% of the total tested samples from all species of animals and birds. Fig 1 is showing the latex agglutination testing for specimens collected from various species of animals and birds in Al-Ahsa, Saudi Arabia in 2019-2020.

Confirmation of the detected rotavirus particles by transmission electron microscope

Fig 2 is showing the results of the transmission electron microscopy of some positive rotavirus specimens. A small virus particles ranged in size from 47-67 nm in diameter. The virus particles resembled wheel shape.

Discussion

Rotaviruses are considered among the main cause of viral diarrhoea in young animals, birds and children all over the world (Dhama *et al,* 2009; Ghosh and Kobayashi, 2014; Crawford *et al,* 2017). The virus infection induce several clinical syndromes particular in young animals, birds and children ranging from mild enteritis to severe diarrhoea, dehydration and

finally death (Nguyen *et al,* 2004; Crawford *et al,* 2017). Enteritis is a multifactorial and complex syndrome in young animals and birds. The etiological agents of diarrhoea includes various types of bacteria, viruses and parasites. The Rotavirus type-A was detected in some young children and some other young domestic animals in Sudan using the antigen detection ELISA test (Ali *et al,* 2005). Rotavirus type-A was reported in camel calves in Sudan (Ali *et al,* 2005). It was also reported in sheep in India in several regions by the commercial ELISA as well as by the RT-PCR for the VP6 genes (Yilmaz *et al,* 2017). All these evidences suggested the potential roles of sheep and goats in the transmission of rotavirus to other species of animals including dromedary camels (Yilmaz *et al,* 2017). High prevalence of diarrhoea in the newborn camels in the northern region of Saudi Arabia have been reported (Al-Ruwaili *et al,* 2012). Same study reported the presence of rotavirus type-A in 14% of the tested camel calves. This is in addition to other bacterial causes of diarrhoea including bacteria such as (*E. coli*, Salmonella species and Enterococcus) (Al-Ruwaili *et al,* 2012). Recent studies showed the zoonotic potential of rotavirus infection among various species of animals and humans in Morocco where several group of animals including camels, sheep, and goat are present in close proximity of each other. It could lead to interspecies transmission of rotaviruses among various species of animals and humans (Alaoui *et al,* 2020). Detection of rotaviruses usually requires rapid, accurate, and sensitive techniques. A comparison study conducted to compare between the latex agglutination test and the EM in the detection of rotavirus in faecal specimens. Although LAT was very rapid and required less labour and time to be conducted, its sensitivity was less when compared to the EM (Moosai *et al,* 1985). This suggests initial screening with the LAT followed by further confirmation by other techniques, i.e. EM. Our results showed only 5.17% of the collected sheep faecal samples positive for rotavirus (Table 1 and Fig 1). The positive animals were young lambs suffering from diarrhoea for several days. This may postulate the high concentration of the virus particles in these collected samples. Taken in consideration that the sheep and goat usually live in close proximity of dromedary camels, they may play some roles in the transmission of rotaviruses and many other enteric pathogens to the dromedary camels. These results may be hampered by the sensitivity of the LAT and EM techniques. Further studies using some molecular based techniques are required for more wide surveillance not only for rotaviruses but also

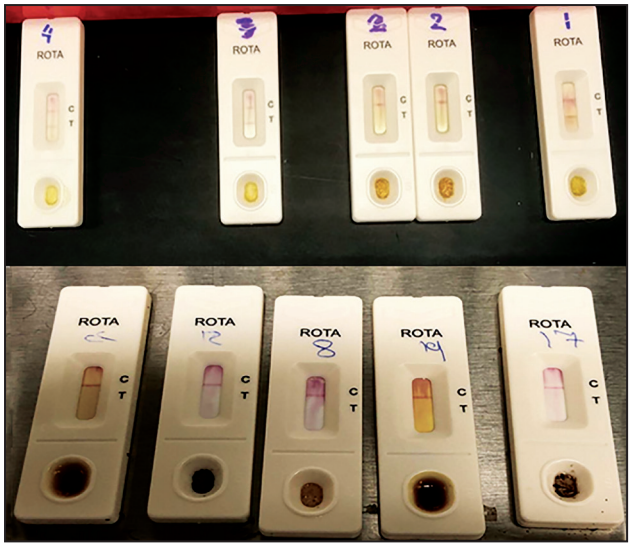

Fig 1. Results of the latex agglutination test on various faecal suspensions of some animals including camel, sheep as well as chickens. The positive results showing two lines while the negative results showing only one line. Positive and negative controls are included.

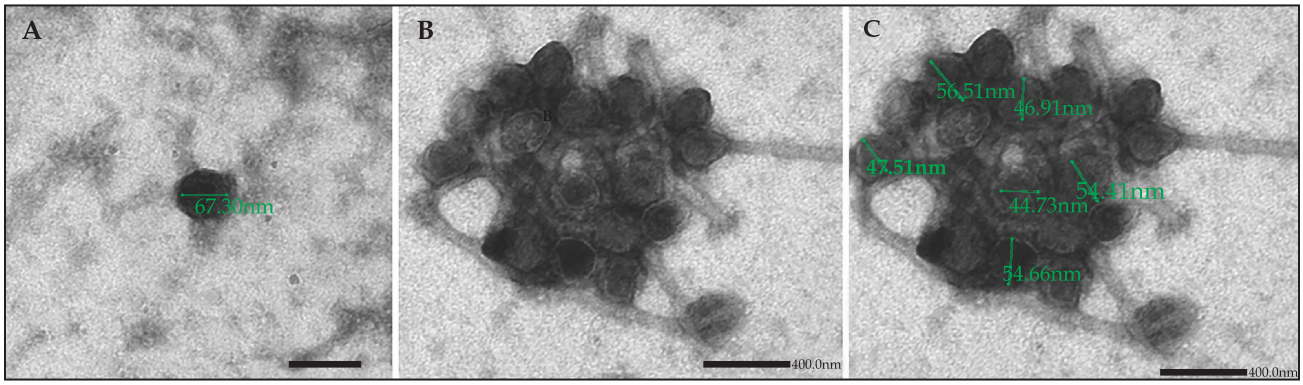

Fig 2. Detection of the rotavirus particles in faecal suspension of sheep by electron microscope. The virus particles are showing typical rotavirus shape (Wheel shape). (A) EM picture of one virus particle about 76.30 nm in diameter. (B) Clusters of rotavirus particles in the faecal suspension from sheep (C) EM picture showing the average diameter of several rotavirus particles in sheep faecal suspension.

for other viral causes of diarrhoea in various species of birds and animals.

Table 1. Summary of the surveillance of rotavirus in domestic animals and birds in Al-Ahsa, Saudi Arabia (2019-2020).

No.	Species	No of tested animals	Positive	Negative
1	Sheep	58	3	55
2	Goat	18	0	18
3	Dromedary camel	25	0	25
4	Chicken	30	0	30
5	Turkey	9	0	9
Total		140	3	137

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