Detection of Rotaviruses in diarrheic and non- diarrheic fecal samples of captive animals and human workers in Al-Zawra Zoo

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Abstract

The study included detection of rotaviruses in diarrheic and non-diarrheic feces of different species of captive animals and human workers in Al-Zawra Zoo by latex agglutination test (LAT) and negative staining method of electron microscope (EM). A total of 127 fecal samples from different species of captive animals and human workers have been collected from November 2012 to March 2013. The results showed that 10 out of 115 fecal samples from different species of captive animals were positive for rotavirus infection (monkeys, cats, and deer) and 3 positive stool samples from 12 samples of human workers by latex agglutination test. Throughout the study, it have been noticed that 23 captive animal were diarrheic, 8 (34.78%) of them were positive for rotavirus, while the other 15 (65.21%) animals had diarrhea caused by enteropathogens rather than rotavirus. Statistical analysis by using Chi-square test showed that there was significant difference ($P \leq 0.05$) between, diarrheal cases due to rotavirus and diarrhea by other causes. A total of 6 selected positive fecal samples in LAT from 13 positive samples of captive animals and humans were examined by negative staining method EM, the results showed that 4 (66.66%) were positive, 2 (33.33%) fecal samples belonged to captive animals (monkey and wild cat) and 2 (33.33%) other stool samples were from human workers. All samples showed the typical structure of the rotavirus: double-shelled particles, with mostly smooth appearance.

الكشف عن فايروسات الروتا في عينات براز الحيوانات الاسيرة والعاملين في حديقة حيوانات الزوراء لحالات إسهال وغير إسهال

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الخلاصة

تضمنت الدراسة الكشف عن فايروسات الروتا في براز حيوانات حديقة الزوراء والعاملين من حالات إسهال وغير إسهال بواسطة اختبار التلازن الحبيبي اللاتكس وطريقة الصبغة السالبة بواسطة المجهر الالكتروني. جمعت 127 عينة براز من انواع مختلفة من الحيوانات الاسيرة والعاملين خلال فترة الدراسة (من تشرين الثاني 2012 لغاية شهر اذار 2013)، وبينت نتائج الدراسة وجود فايروس الروتا في 10 عينات براز لأنواع مختلفة من الحيوانات (القردة والغزلان والقط البري) من مجموع 115عينة و3 عينات براز للعاملين من مجموع 12 عينة. لوحظ خلال الدراسة وجود 23 حالة اسهال في الحيوانات الاسيرة منها 8 عينات (34.78%) كانت موجبة للروتا فايروس بينما 15 عينة (65.21%) موجبة لمسببات أخرى. اظهر التحليل الاحصائي باختبار مربع كاي وجود فرق معنوي ($P \leq 0.05$) في حالات الإسهال بسبب الروتا والإسهال بمسببات أخرى. كذلك تضمنت الدراسة فحص 6 عينات براز انتخبت من 13 عينة موجبة باختبار اللاتكس من الحيوانات والعاملين بطريقة الصبغة السالبة للمجهر الإلكتروني، بينت النتائج 4 عينات براز

(66.66%) موجبة لفايروسات الروتا، 2 (33.33%) في الحيوانات الاسيرة (القردة، القط البري) و2 (33.33%) بالعاملين. اظهرت جميع العينات التي فحصت بالمجهر الالكتروني التركيب النموذجي لفايروسات الروتا باحتوائها على طبقة مزدوجة الغلاف والمظهر الأملس لفايروسات الروتا.

Introduction

Captivity of animals- that live under human care are in captivity. The captivity can be used as a generalizing term to describe the keeping of either domesticated animals (livestock and pets) or wild animals. wild animals may play an important role in the epidemiology of diseases which has considerable public health significance or potential impact on livestock population. In recent decades, infectious pathogens that originate in wild animals have become important throughout the world due to increasing human contact with domestic and wild animals (1, 2). Diarrheal diseases are a major cause of morbidity and mortality in humans and animals, including non-human primates (3). One of the major causes of diarrheal diseases is rotavirus, which is the prototype of the genus *Rotavirus* in the family *Reoviridea* causes severe diarrheal disease in neonates and adults throughout the world (4, 5). Seven classified rotavirus groups (A-G) are recognized, based on the antigenic variability of their inner capsid protein, VP6 (6). Neonatal diarrhea induced by rotavirus causes significant economic losses due to high morbidity, mortality, treatment cost and reduced growth rate of infected animals. It is estimated that rotaviruses are responsible for more than one-half a million deaths annually among children aged <5 years, with the majority of these deaths occurring in developing countries (7, 8). Several techniques have been developed for rotavirus diagnosis. In the first rotavirus surveys, the viral agent detection was performed by electron microscopy (EM); afterwards, other techniques were developed, such as polyacrylamide gel electrophoresis (PAGE), immunofluorescence (IF), radioimmunoassay (RIA), reverse passive hemagglutination (RPH), immunoenzimatic assay (IEA), latex agglutination test (LAT) and more recently, a reverse transcription polymerase chain reaction (RT-PCR) and immunochromatography (IMC) (9,10, 11). As rotaviruses have a wide host range infecting abroad range of animal species, but little is known about rotavirus infection in captive (exotic) animals through the world so we conducted our study:

1. Detection of rotaviruses in feces of diarrheic and non-diarrheic exotic animals of Al-Zawra Zoo by LAT and EM.
2. Detection of rotaviruses in feces of human workers in Baghdad Zoo by the former tests.

Materials and Methods

- Collection of fecal samples: A total 115 fecal samples of diarrheic and non- diarrheic exotic animals were randomly collected from Al-Zawra Zoo in Baghdad during the period of the study from November 2012- March 2013, in addition to Twelve stool specimens were obtained from human workers who were in contact with captive animals. Each fecal sample of 20 gms were collected in sterile, screw capped containers, stored in a cool box and transported to the laboratory at the same day then were stored at -20 C° until tested.
- LAT examination: Samples prepared were then tested for rotavirus using commercial LAT Kits - Rota-virus latex test kit (plasmatec laboratory products- United Kingdom) in accordance with the manufacture instructions.
- EM examination: Some positive specimens by LAT were examined by negative stain method EM (C10 Philips) in Al-Nahrain University, College of Medicine, as described by (12).

Results and Discussions

- **Rotavirus antigen detection by LAT in captive animals:** In the present study, investigation of rotavirus in feces of diarrheic and non-diarrheic captive animals by LAT. The results revealed that 10 (8.69%) out of 115 fecal samples of different captive animals were positive to rotavirus antigen. The reactive animals included deer 1 (9.09%) positive case out of 11 deer fecal samples; in cats 1 (25%) positive case out of 4 cat fecal samples, and in monkeys 8 (19.51%) positive cases out of 41 monkey fecal samples as shown in table (1) and pictures (1, 2).

Our result was agreed with the findings of other researchers who detected rotavirus in feces of different exotic animals (13, 14,15). It has been found that the percentage of positive cases to rotavirus was lower than the results of (15) who detected rotavirus in feces of some captive animals in a percentage of 13.07% by using LAT and the reactive animals included 1 llama, 2 cats, 3 monkeys, and 4 goats, whereas in other study (14) proved that positive cases to rotavirus were in 1 monkey, 1 fox and 1 dog in a percentage of 2.9% by using agar gel diffusion test. This variation may be due to many factors including management and hygienic situation ranches, nutritional and immunological status of the animals, treatments, overcrowding, environmental condition, vaccination programs applied in the Zoo and finally the method used in detection of rotavirus may contribute to this variation. Throughout the study, it have been noticed that 23 captive animals were diarrheic, 8 (34.78%) of them were positive for rotavirus, the other 15 (65.21%) animals had diarrhea caused by enteropathogens rather than rotavirus as mentioned in table (2). This result was agreed with result of (15) who found that other etiological agents such as bacteria, protozoa, parasites and other viruses may cause diarrhea, this finding was supported by (3, 16).

Table (2) Percentage of positive fecal samples to rotavirus in diarrheic captive animals by LAT

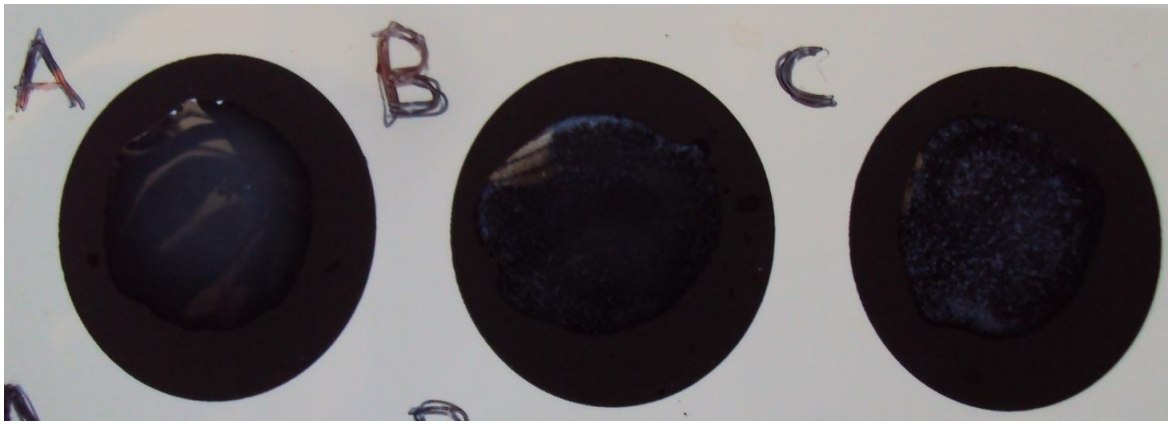
Total number of fecal samples with diarrhea	No. of positive diarrheic fecal samples to rotavirus (%)	Other causes of diarrhea (%)
23	8 (34.78 %)	15 (65.21 %)

Chi-square= 4.26, Degrees of freedom = 1, Probability= 0.039

Statistical analysis using Chi-square test showed that there was significant difference between, diarrheal cases due to rotavirus and diarrhea caused by other agents.

Table (1) Rotavirus detection in fecal samples of captive animals by LAT

Animal species	Scientific names	Samples tested	No. of positive cases (%)	No. of negative cases (%)
Monkeys	*Papio hamadryas* *Pan troglodytes* *Macaca mulatta* *Rhesus macaqye*	41	8 (19.51)	33
Goats	*Capra hircus*	9	0	9
Gray wolf	*Canis Lupus*	10	0	10
Lion	*Panthera leo*	11	0	11
Tiger	*Panthera tigeris*	6	0	6
Dog	*Canis familiaris*	7	0	7
Cat	*Felis Silvestris* *Panthera onca*	4	1 (25)	3
Deer	*Gazella leptoceros* *Gazella gazella*	11	1 (9.09)	10
Camel	*Camelus bactrianus*	6	0	6
Llama	*Lama glama*	5	0	5
Brawn bear	*Ursus arctos*	3	0	3
Fox	*Vulpes Vulpes*	2	0	2
Total		115	10 (8.69)	105 (91.30)



Pic. (1) Positive fecal sample for rotavirus by LAT

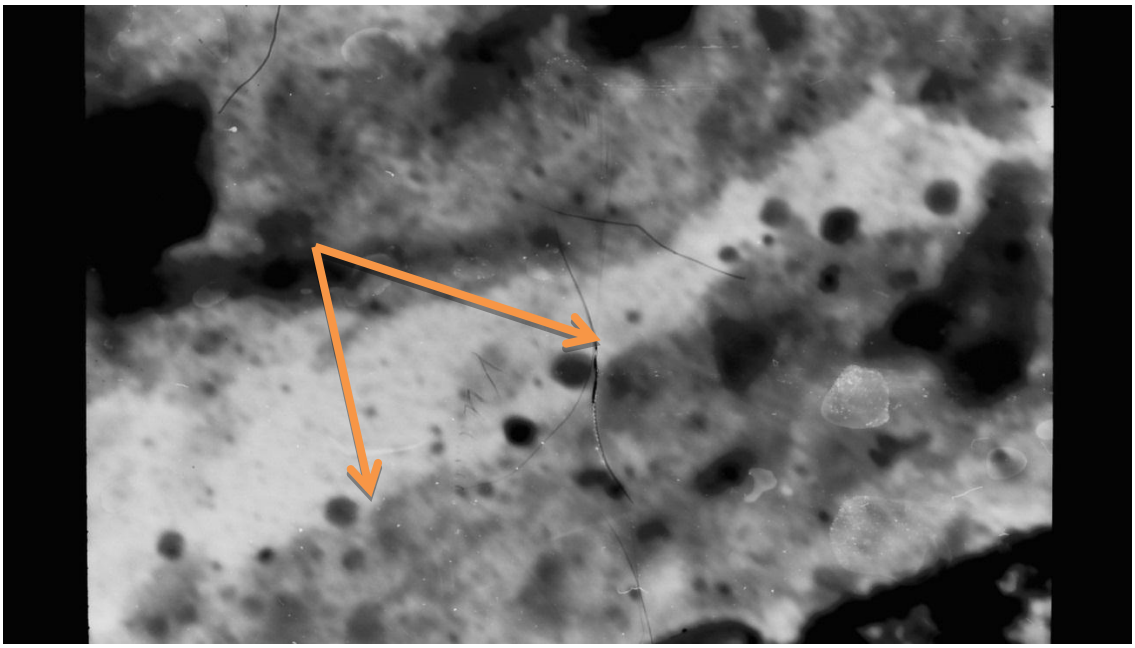
A= Control Negative
B= Positive sample
C= Control Positive

Pic. (2) Negative fecal sample for rotavirus by LAT

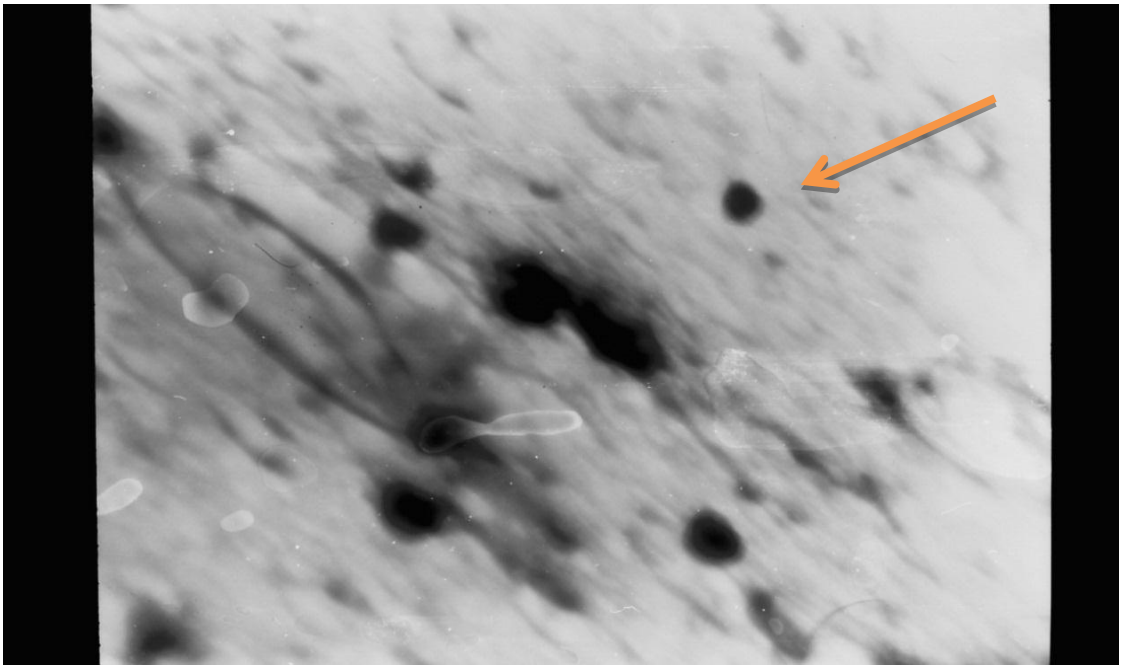
A= Control Negative
B= Negative sample
C= Control Positive

- **Rotavirus antigen detection by LAT in human workers:** Rotavirus was detected in 3 (25%) out of 12 workers, stool samples by Latex Agglutination Test. All collected stool samples were without diarrhea. The finding indicated that a high percent of rotavirus was detected in human workers in Baghdad Zoo. As there is little studies concerning the prevalence of rotavirus in human workers in Zoos, so the present result was discussed with the study of (17) who identified rotavirus in stool samples of two adult patients with diarrhea by nucleotide sequences. One of them had a history of contact with cows and goats in a petting Zoo in the 5 days prior to onset of illness, as well as contact with the family cat and dog. The other patient had reported contact with a dog and parrot. This finding adds further support to the natural interspecies transmission among human and animal, suggesting a putative anthropozoonotic characteristic of the infection (18). In the present study, the detection of rotavirus in 3 stool samples was observed in adult asymptomatic human workers, this probably because they have some degree of protection from clinical disease owing to previous infection with rotavirus. Many reports supported our explanation, that in slightly older children and adults, rotavirus infection can be asymptomatic (19, 20).

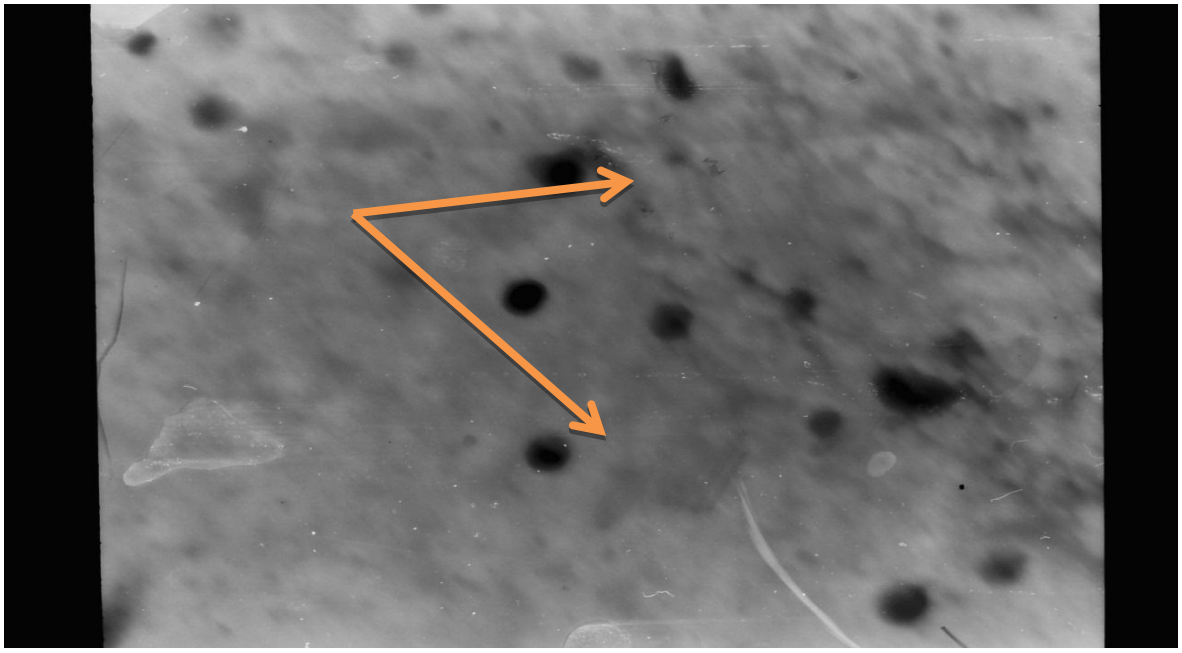
- **Rotavirus antigen detection by EM in captive animals and human workers:** We were unable to test all the specimens by EM in Al-Nahrain University, so only 6 of the positive cases to LAT were examined by negative staining method EM. Results showed that 4 (66.66%) fecal samples were positive to rotavirus infection, 2 positive fecal samples belonged to captive animals, whereas the other 2 positive samples were belonged to human workers (Pic. 3, 4, 5).



Pic. (3). Electron micrograph of a negatively stained rotavirus in non-diarrheic wild cat using a Philips C10 EM at magnification of 130.000



Pic. (4). Electron micrograph of a negatively stained method rotavirus in diarrheic monkey using a Philips C10 EM at magnification of 130.000



Pic. (5). Electron micrograph of a negatively stained rotavirus in non- diarrheic human worker using a Philips C10 EM at magnification of 130.000

As shown in pictures (3, 4) viral particles of similar morphology to rotavirus were observed in the feces of cat and monkey, as well as, in human workers (pic. 5) by the negative staining of EM. The viruses were spherical with smooth appearance, double-shelled layer, and morphologically were identified as rotaviruses. This result was agreed with the results of (13, 21, 22, 23, 24), but disagreed with the observation of (3) who revealed that by EM, rotavirus in fecal specimens of monkeys with diarrhea appeared as empty and severely damaged particles due to unstability and atypically appearance of rotaviruses in the stools of monkeys. The 2 negative fecal samples obtained by EM, which they were formerly positive to LAT, may be due to low concentration of the virus in feces, with variable pH and time used for the staining which were supported by the result of (25).

References

1. Heuschele, W. P. (1981). Viral disease of captive wild animals: The case for increased research effort. J. Zoo Ani. Med., 12 (4): 132-134.
2. Jones, K. E.; Patel, N. G.; Levy, M. A.; Storeygard, A.; Balk, D.; Gittleman, J. L. & DasZak, P. (2008). Global trends in emerging infectious diseases. Nature, 451: 990-993.
3. Wang, Y.; Tu, X.; Humphrey, C.; McClure, H.; Jiang, X.; Qin, C.; Glass, R. I. & Jiang, B. (2007). Detection of viral agents in fecal specimens of monkeys with diarrhea. J. Med. Primatol., 36: 101-107.
4. Kobayashi, N.; Ishino, M.; Wang, Y. H.; Chawla-Sarkar, M.; Krishnan, T. and Naik, T. N. (2007). Diversity of G-type and P-type of human and animal rotaviruses and its genetic background. Communi. Cur. Res. and Educ. Top. and Trends. In Applied Microbiol. Mendez-Vilas A. (Ed.), PP. 847-858.
5. Manuja, B. K.; Prasad, M.; Gulati, B. R.; Manuja, A. & Prasad, G. (2010). Comparative efficacy of immunological, molecular and culture assays for detection of group

A rotavirus from fecal samples of buffalo (*Bubalus bubalis*) calves. Trop. Anim. Hlth. Prod., 42: 1817-1820.

6. Mulherin, E.; Bryan, J.; Beltman, M.; O'Grady, L.; Pidgeon, E.; Garon, L.; Lloyd, A.; Bainbridge, J.; O'Shea, H.; Whyte, P. & Fanning, S. (2008). Molecular characterization of a bovine-like rotavirus detected from a giraffe. BMC. Vet. Res., 4: 46-53.
7. Roger, K. M.; Grooms, D. L.; Wis, A. G.; Han, C.; Ciesicki, V. V.; Han SON, L.; Vickers, M. I.; Kanitzand, C. & Holland, R. (2003). Evaluation of human group A rotavirus assay for on-site detection of bovine rotavirus. J. Clin. Microbiol., 41: 290-294.
8. Mukherjee, A.; Nayak, M. K.; Roy, T.; Ghosh, S.; Naik, T. N.; Kobayashi, N. & Chawia-Sarkar, M. (2012). Detection of human G10 rotavirus strains with similarity to bovine and bovine-like Equine strains from untypable samples. Infec. Gene. and Evol., 12: 467-470.
9. Ibrahim, O. S.; Sunderland, D. & Hart, C. A. (1990). Comparison of four Methods for detection of rotavirus in feces. Tropic. Doctor., 20: 30-32.
10. Marshall, J.; Botes, J.; Corrie, G.; Boardman, C.; Gregory, J.; Griffith, J.; Hogg, G.; Dimitriadis, A.; Catton, M. & Bishop, R. (2003). Rotavirus detection and characterization in outbreak gastroenteritis in aged-care Facilities. J. Clin. Virol., 28:331-340.
11. Ferreira, T. L.; Becho, A. R.; Bernardo, A. R.; Chaves, T. C. B.; Ribeiro, R. S.; Lima, J. S.; Fialho, A. M.; Leite, J. P. G.; Mazur, C. & Danelli, M. G. M. (2006). Performance of a latex agglutination test in the diagnosis of acute gastroenteritis by rotavirus. Brazilian J. Microbiol., 37:587-589.
12. Bozzola, J. J. & Russell, L. D. (1999). Specimen staining and contrast method for transmission electron microscope. In: Jones and Bartlett, Boston, M. A., electron microscope, principles and techniques for biologists. PP. 130-133.
13. Eugster, A. K.; Stropher, J. & Hartfiel, D. A. (1978). Rotavirus (reovirus-like) infection of neonatal ruminants in a Zoo nursery. J. Wildl. Dis.,14: 351-354.
14. Hasso, S. A. (1997). Rotavirus in animals of Baghdad Zoo. Iraqi J. Vet. Sci., 10 (2): 121-128.
15. Al-Mafraji, A. M. (2012). The investigation of some pathogens in feces of animals in Al-Zawra Zoo. M.Sc. Thesis. University of Baghdad, College of Veterinary Medicine. (In Arabic).
16. Sestak, K.; Merritt, C. K.; Borda, J.; Saylor, E.; Schwamberger, S. R.; Cogswell, F.; Didier, E. S.; Didier, P. J.; Plauche, G.; Bohm, R. P.; Age, P. P.; Alexa, P.; Ward, R. L. & Lackner, A. A. (2003). Infectious agents and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infec. Immun., 71: 4079-4086.
17. Midgley, S. E.; Hjulsager, C. K.; Larsen, L. E.; Falkenhorst, G. & Ottiger, B. (2012). Suspected zoonotic transmission of rotavirus group A in Danish adults. Epidemiol. Infect., 140: 1013-1017.
18. Nakagomi, O. & Nakagomi, T. (1993). Interspecies transmission of rotaviruses studied from perspective of genogroup. Microbiol. Immunol., 37: 337-348.
19. Bos, P.; Kirsten, M. & Cronje, R. E. (1995). Monitoring of rotavirus infection in a paediatric hospital by RNA electrophoresis. S. Afr. Med. J., 85: 887-891.

20. Raboni, S. M.; Meri, B. M. D.; Nogueira, M. S. C.; Hakim, V. M.; Torrecilha, V. T. G.; Lerner, H. & Tsuchiya, L. R. V. (2002). Comparison of latex agglutination with enzyme immunoassay for detection of rotavirus in fecal specimens. Am. J. Clin. Pathol., 117: 392-394.
21. Kawamoto, H.; Tanaka, H.; Urasawa, S.; Urasawa, T. & Taniguchi, K. (1990). Serotype analysis of group A human rotavirus related to acute gastroenteritis in winter in Gifo city. Microbiol. Immunol., 34: 675-681.
22. Mohammed, M. E. H.; Hart, C. A. & Kadden, O. R. (2003). Viruses and bacteria associated with neonatal camel calf diarrhea in Eastern Sudan. Emir. J. Agric. Sci., 15 (1): 56-62.
23. Ali, Y. H.; Khalafalla, A. I.; Gaffar, M. E.; Peenze, I. & Steele, A. D. (2008). Detection and isolation of group A rotavirus from camel calves in Sudan. Vet. Arch., 78 (6): 477-485.
24. Santhana Raj, L.; Saraswathy, T. S.; Teh Hamidah, Z.; Nor Asiha, C. P.; Norshahidah, K.; Ravindran, T.; Roslina, I.; Lye, M. S. & Lokman Hakim, S. (2009). Use of Electron microscopy in the laboratory investigation of the acute gastroenteritis outbreak in Cameron Highlands, Pahang. Annals Microscopy, 9: 45-49.
25. Nakata, S.; Petrie, B. L.; Calomeni, E. P. & Estes, M. K. (1987). Electon microscopy procedure influences detection of rotaviruses. J. Clin. Microbiol., 25 (10): 1902-1906.