Short Communication

Group A rotavirus and *Mycobacterium avium subspecies paratuberculosis* associated with diarrhea in dromedary camels in Eastern province, Saudi Arabia

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Abstract

Diarrhea in newborn calves as well as in adult dromedary camels is considered the most serious constrains of camel production. It is recorded in the Eastern Province of Saudi Arabia to be very high, therefore, it is necessary to investigate the role of certain pathogenic agents. In the present study, group A rotavirus was detected in 5/50 (10%) and 6/50 (12%) diarrheic samples collected from one- to three-month-old camel calves using immune chromatographic and ELISA techniques, respectively. The characteristic wheel-like morphology of rotavirus particles was identified in the positive samples by electron microscopy. Also, *Mycobacterium avium subspecies paratuberculosis* was detected in 5/50 (10%) and 30/50 (60%) three- to five-year-old diarrheic dromedary camels, respectively, using direct Ziehl-Neelsen staining of fecal smears and PCR assays.

Keywords: group A rotavirus, *Mycobacterium avium subspecies paratuberculosis*, diarrhea, dromedary camels, Saudi Arabia

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Introduction

Dromedary camel (*Camelus dromedaries*), which is estimated at 850,000 heads of different breeds (Aljumaah et al., 2012a), is considered an iconic animal in the Saudi Arabian heritage. It is considered as an important source of milk, meat and wool. The meat and milk of camel comprise proteins for the wide range of population (Breulmann et al., 2007). In Saudi Arabia, camel meat constitutes 30% of the meat production (Hamam, 1993), whereas the total annual milk production measures 2500-4900 liter/head (Aljumaah et al., 2012b). Regarding camel diseases, camels are considered resistant to most of the pathogens affecting livestock animals. Recently, camels have been found to be susceptible to several numbers of pathogens as a result of more carried out research. Neonatal camel calf diarrhea causes great losses of camel calves worldwide (Mohammed et al., 2003). Calf diarrhea is regarded the major cause of calf mortality in camels less than six months of age. In Sudan, the mortality rate due to camel calf diarrhea reached 39.9% (Ali et al., 2005). Group A rotavirus was detected in 20% of diarrheic camel calves aged between 0-3 months in Sudan (Khalafalla and Ali, 2007). *Mycobacterium avium subspecies paratuberculosis* (MAP) (Johne's disease or paratuberculosis) affects camels by causing severe diarrhea. The disease is usually more severe in camels aged 3-5 years (Behr and Collins, 2010).

Rotaviruses are common causes of severe gastroenteritis and mortality in neonates of all animals and human (Estes and Cohen, 1989). They were first isolated from calves with diarrhea by Mebus et al. (1969) and have been identified in most animal species of veterinary importance (Estes and Cohen, 1989). Rotaviruses constitute a genus of the family Reoviridae. The virus is composed of 11 segments of double-stranded RNA (dsRNA) in a 70 nm, double-shelled, icosahedral capsid. Rotaviruses are composed of three layers of the structural proteins: core, inner capsid and outer capsid. The core comprises viral proteins VP1, VP2 and VP3 and the inner capsid layer is composed of the most abundant protein VP6, to which a majority of the group-specific antibodies is directed. The outer capsid layer contains the major surface glycoprotein VP7 (glycoprotein, G) and hemagglutinin spike VP4 (Protease sensitive, P), both of which contain neutralization antigens (Estes and Cohen, 1989). Rotaviruses are classified into three important antigenic categories: group, subgroup and serotypes. On the basis of group-specific protein (VP6), which is the predominant group antigen, there are at least 7 serogroups coded (from A to G) and there are similar RNA electropherotypes within each serogroups (Saif and Jiang, 1994). Group A bovine rotavirus is the major cause of severe diarrheal syndrome in calves throughout the world. A dual classification scheme defines group A rotavirus strains into G and P serotypes. G serotypes are based on the type of VP7 because VP7 is a glycoprotein and P serotypes are based on the type of VP4 because VP4 is protease sensitive. Rotavirus G serotypes have been identified using fluorescent focus and plaque reduction virus neutralization assays (Estes and Cohen, 1989; Fukai et al., 2004).

MAP is an intracellular slow growing bacilli of 0.5-1.5 µm diameter. The Ziehl-Neelsen staining method shows it as short rods, red in color and arranged in clumps (Cocito et al., 1994). Using restriction fragment length polymorphism (RFLP) assay combined with hybridization to the insertion sequence (IS900), MAP can be classified into three groups (cattle, sheep and intermediate types) (Pavlik et al., 1995). IS900 is an unusual DNA insertion element (15 to 20 copies) in the genome of MAP. IS900 has been used extensively as a specific and sensitive DNA marker for identification and characterization of MAP from other mycobacterial species by PCR (Miller et al., 1996; Alhebabi and Alluwaimi, 2010; Haghkhah et al., 2015).

The present study was conducted to determine rotavirus, coronavirus and MAP infections in dromedary camels in Al-Hassa region of the Eastern Province of Saudi Arabia

Materials and Methods

Clinical samples: A total of 100 fecal samples, 50 samples from diarrheic camel calves (1-3 months old) and 50 from diarrheic adult camels (3-5 years old), were collected in sterile plastic bags, rapidly transferred to central Biotechnology Laboratory, College of Veterinary Medicine, King Faisal University and stored at -80^{o}C until tested.

Detection of group A rotavirus and coronavirus using ELISA: An antigenic ELISA kit (Bio-X Diagnostics, Belgium) for detection of rotavirus and coronavirus was used. The test procedures were performed according to the manufacturer's recommendations. Briefly, the fecal samples were diluted volume per volume into dilution buffer, then 100 µl of each diluted samples were added to wells of microplates sensitized by specific antibodies for coronavirus and rotavirus, and incubated at room temperature for 1 hour. The plates were rinsed 3 times with washing buffer. One hundred microliters of conjugated solution was added: anti-rotavirus in rows A, B, E and F and anti-coronavirus in rows C, D, G and H, and incubated at room temperature for 1 hour before thoroughly washing 3 times with washing buffer. Color was developed by addition of 100 µl chromogen solution and incubation at room temperature for 10 minutes. Further color development was stopped by adding 50 µl stop solution, then the plates were read at 450 nm wavelength.

Detection of group A rotavirus using immunochromatography technique (ICT): The Rota-Adeno test device kit [VIKIA®Rota-Adeno (BIOMERIEUX)] was used for dual detection of rotaviruses and adenoviruses in a single fecal sample based on the association of monoclonal antibodies specific to rotavirus and adenovirus. ICT was carried out according to the manufacturer's instructions.

Electron microscopy (EM): A total of 6 ELISA positive samples were examined by EM after staining of ultracentrifuged viral pellets by 3% phosphotungistic acid (Alain et al., 1987).

Ziehl-Neelsen acid fast staining: Fecal smears were stained with carbol fuchsin Ziehl-Neelsen acid-fast stain for 3-5 minutes. The smears were washed for 2 minutes in water, then decolorized with acid alcohol for 20-30 seconds in two brief changes. The smears were washed for 2 minutes in water and briefly counterstained with methylene blue for 2 minutes, then washed for 2 minutes in water and allowed to dry. All smears were examined by specialists.

Oligonucleotide primers: Primers used in PCR and RT-PCR assays were selected according to sequence database, analyzed by OligoAnalyzer 3.1 Integrated DNA Technologies, USA and synthesized by Metabion International AG, Germany. The complete data of primers are shown in Table 1.

Table 1 Details of oligonucleotide primers

Primer name	Primer Sequence (5´- 3´)	PCR Size	Reference
ROT1F	CTCTGGCAAARCTGGTGTCA		
ROT2R	CATTCGACGCTGATGACATY	492	Asano et al., 2010
ROT3R	ARCAATCRACCAACCASTCCTGTA	228	
VP6F	GGCTTTTAAACGAAGTCTTCAACATGG	1356	El-Sabagh, 2006
VP6R	GGTCACATCCTCTCACTACGC		
VP7F	GCGGTTAGCTCCTTTTAATGTATGG	1030	El-Sabagh, 2006
VP7R	GGTCACATCATATACAACCTCTAATCTAACATG		
$P90^{+}$	GAAGGGTGTTCGGGGCCGTCGCTTAGG	413	Millar et al., 1996
$P91^{+}$	GGCGTTGAGGTCGATCGCCCACGTGAC		

RT-PCR for detection of group A rotavirus: Five microliters of extracted dsRNA was mixed with 3.5 µl of dimethylsulfoxide in a microcentrifuge tube, denaturated by heating at 95ºC for 5 minutes and immediately cooled on ice. cDNA was carried out with SuperScript™ III Reverse Transcriptase (Invitogen). Seven microliters of denatured RNA was mixed with 4 µl 5X buffer, 2 µl dNTPs of 10 µm, 2 µl DTT, 1 µl SuperScript™ III Reverse Transcriptase, 1 µl Random hexamer and 3 µl RNase free water. The RT reaction was performed at 37ºC for 60 minutes, 70ºC for 15 minutes and 4ºC until sample collection. Different protocols for amplification of rotavirus genes were carried out. Semi-nested PCR for amplification of VP1 gene according to Asano et al. (2010) and VP6 and VP7 PCR according to El-Sabagh (2006) was performed. Five microliters of each cDNA was amplified in 20 µl of the final volume of a 2X HotStartTaq Plus Master Mix (QIAGEN, USA) containing 1.5 mM $MgCl_2$, 200 µM of each dNTP and 1 unit HotStartTaq Plus DNA polymerase and 10 µM of each primers. Thermocycling conditions were enzyme activation and initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds and 72ºC for 90 seconds, and a final extension step at 72ºC for 10 minutes. The amplified PCR products were electrophoresed in 1.2% agarose gel containing 0.5 µg/ml ethidium bromide and documented using ultraviolet gel documentation system (BIORAD).

DNA extraction: Total DNA was isolated from the fecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN, USA) according to the manufacturer's recommendations. Briefly, 180-220 mg of the fecal samples were diluted by adding 1.4 ml of ASL buffer, mixed by pulse vortexing and incubated at 70ºC for 5 minutes. One inhibited tablet was added to 1.2 ml of the supernatant samples and vortexed, then centrifuged at full speed for 3 minutes. Fifteen microliters of proteinase K was added to 200 µl of the sampled supernatant, then 200 µl buffer AL was added and vortexed for 15 seconds and incubated at 70ºC for 10 minutes. Two hundred microliters of absolute ethanol was added and mixed for 15 seconds by pulse vortexing. Complete lysate was transferred to a QIAamp spin column followed by centrifugation at full speed for 1 minute. The binding DNA was washed by the addition of 500 µl AW1 buffer and centrifuged at full speed for 1 minute, followed by the addition of 500 µl AW2 buffer and centrifuged at full speed for 3 minutes. The DNA was eluted from a column by adding 200 µl of buffer AVE, incubation at room temperature for 1 minute and centrifugation.

RNA extraction: Total RNA was extracted with QIAamp Viral RNA Mini Kit (QIAGEN, USA) from rotavirus positive fecal samples according to the manufacturer's recommendations. Briefly, 140 µl of the fecal samples were lysed by adding 560 µl of AVL buffer containing carrier RNA. After complete lysis, 500 µl of absolute ethanol was added and mixed for 15 seconds by pulse vortexing. Aliquots of 630 µl were transferred sequentially to a QIAamp spin column followed by centrifugation at 8,000 rpm for 1 minute after each addition. Binding RNAs were washed by the addition of 500 µl AW1 buffer and centrifuged at 8,000 rpm for 1 minute, followed by the addition of 500 µl AW2 buffer and centrifugation at 14,000 rpm for 3 minutes. The RNAs were eluted in 60 µl of buffer AVE and stored at -85ºC until used.

PCR for Mycobacterium avium subspecies paratuberculosis: Amplification MAP was performed in a total volume of 20 µl of HotStartTaq® Plus Master Mix Kit (2x) (QIAGEN, USA). The PCR cycling profile consisted of one cycle for initial heat activation of 95ºC for 5 minutes, 35 cycles of denaturation at 94ºC for 1 minute, annealing at 55ºC for 30 seconds and extension at 72ºC for 30 seconds, and final cycle at 72ºC for 10 minutes. Ten microliters of each amplified product was analyzed by agarose gel electrophoresis on 1.5% agarose containing 0.5 µg/ml ethidium bromide and visualized using gel documentation system.

Results and Discussion

Diarrhea is considered the most serious constrains of camel production. The morbidity and mortality rates due to camel calf diarrhea could reach

up to 33% and 100%, respectively (Schwartz and Dioli, 1992). Group A rotaviruses are one of the major causes of diarrhea in camel calves. In Sudan, group A rotavirus was firstly reported in 11 out of 117 (9.4%) samples of 1- to 3-month-old diarrheic calves using ELISA (Mohamed et al., 2003), whereas Ali et al. (2005) reported 13.9% of rotavirus infection in camel calf diarrhea. In Egypt, Abo Hatab et al. (2009) reported 8 out of 85 (9.4%) samples of 2-week-old to 4-month-old diarrheic calves with rotavirus infection. In the North Province of Saudi Arabia, Group A rotavirus was recorded in 34/255 (13.3%), 41/278 (14.7%) and 48/256 (18.7%) samples from one-month-old to one-year-old diarrheic and healthy camel calves using immune chromatographic, ELISA and latex agglutination assays, respectively (Al-Ruwaili et al., 2012). In our study, group A rotavirus was detected in 5/50 (10%) and 6/50 (12%) samples from one- to three-month-old diarrheic camel calves using immune chromatographic and ELISA techniques, respectively. De Verdier and Esfandiari (1996) compared immune chromatographic and ELISA techniques for group A rotavirus antigen detection in 161 fecal samples collected from bovine, porcine and equine and recorded 89% and 99% sensitivity and specificity of the immune chromatographic assay. Comparison of our findings with the results obtained by Al-Ruwaili et al. (2012) on samples collected from the North Province of Saudi Arabia revealed certain differences in detection percentage of group A rotavirus using immune chromatographic and ELISA techniques. This difference could be due to the number of tested samples and/or age of diarrheic animals. However, group A rotavirus was detected in the fecal samples by the immune chromatographic and ELISA assays and confirmed by the observation of the characteristic wheel-like morphology of rotavirus particles using electron microscopy (Fig. 1). All trials for the amplification of VP1, VP6 and VP7 genes were negative, which could be due to empty viral capsid (natural lack of viral RNA) (Sheridan et al., 1984; Brüssow et al., 1990). These empty viral capsids can be detected by serological assays and observed with electron microscopy, but produce negative RT-PCR amplification. Regarding coronavirus, all tested samples revealed negative result using ELISA.

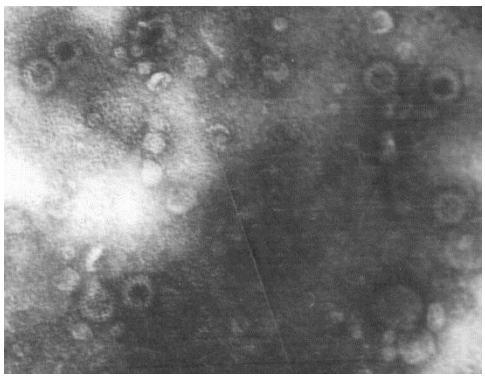

Figure 1 Characteristic wheel-like morphology of camel rotavirus particles by electron microscopy

Mycobacterium avium subspecies paratuberculosis, also called paratuberculosis, causes Johne's disease, which is characterized in camels by intermittent diarrhea, reduced milk production, dehydration and emaciation (Hereba et al., 2014). The control program of Johne's disease is based on test and culling strategy that require sensitive and specific diagnostic assay to reduce infection rate in the herd. Culture of fecal samples is the gold standard for the diagnosis of Johne's disease, but it is considered time-consuming, requiring 12-16 weeks, and can detect only 38-50% of infected animals (Stabel et al., 2004). In our study, the direct Ziehl-Neelsen staining of the fecal smears revealed that 5/50 (10%) samples of the 3- to 5-year-old dromedary camels were suspected to be positive to MAP. In contrast, 30/50 (60%) of the dromedary camel samples were positive in the fecal samples by PCR targeting the IS900 gene (Fig. 2). Alhebabi and Alluwaimi (2010) reported 97/310 (31.29%) dromedary camels positive by PCR, whereas in Iran Haghkhah et al. (2015) reported 4/50 (8%) positive in fecal samples by PCR. The results of our study compared with those of previous studies revealed that the optimization of PCR targeting the IS900 gene could be used as a potential diagnostic assay for rapid and effective diagnosis of Johne's disease in camel.

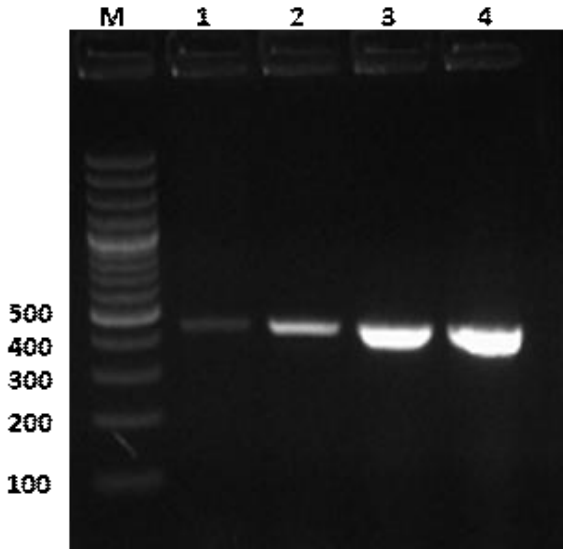

Figure 2 Ethidium bromide-stained agarose gel showing 413 bp *Mycobacterium avium subspecies paratuberculosis* band of PCR products. Lane M: 100 bp molecular weight PCR markers; Lanes 1-4: four representative positive samples.

In conclusion, this study highlights the widespread of group A rotavirus and MAP infection in camel population in Saudi Arabia. Further research is urgently needed to understand the molecular epidemiology of these pathogens, which will be helpful in the protection and control strategy.

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References

Abo Hatab E, Hussein HA, El-sabagh IM and Saber MS 2009. Isolation and antigenic and molecular characterization of G10 of group A rotavirus in camel. IJV 5(1): 18-27.

Alain R, Nadon F, Séguin C, Payment P and Trudel M 1987. Rapid virus subunit Visualization by direct sedimentation of samples on electron microscope gride. J Virol Methods, 16:209-216.

Alhebabi MA and Alluwaimi MA 2010. Paratuberculosis in camel (*Camelus dromedarius*): The diagnostic efficiency of ELISA and PCR. Open Vet Sci J; 4: 41-44.

Ali YH, khalafalla AI and Gaffar ME 2005. Group A rotavirusassociated camel calf diarrhea in Sudan. J. Anim. Vet. Adv. 4 (3), 401-406.

Aljumaah RS, Almutairi FF, Ismail E, Alshaikh MA, Sami A and Ayadi M 2012b. Effects of Production System, Breed, Parity and Stage of Lactation on Milk Composition of Dromedary Camels in Saudi Arabia. J Anim Vet Adv 11(1): 141-147.

Aljumaah RS, Samara EM and Ayadi M 2012a. Influence of Introducing Machine Milking on Biothermal Parameters of Lactating Camels (*Camelus dromedarius*). Ital J Anim Sci 11: e73.

Al-Ruwaili MA, Khalil OM and Selim SA 2012. Viral and bacterial infections associated with camel (Camrlus dromedarius) calf diarrhea in North Province, Saudi Arabia. Saudi Journal of biological sciences 19(1): 35-41.

Asano KM, Souza SP, Barros IN, Ayres GR, Silva SO, Richtzenhain LJ and Brandão PE 2010. Multiplex semi-nested RT-PCR with exogenous internal control for simultaneous detection of bovine coronavirus and group A rotavirus. J. Virol. Methods 169(2): 375-379.

Behr AM and Collins MD 2010. Paratuberculosis, organism, disease, control. Oxfordshire, UK: CABI; p. 179-183.

Breulmann M, Böer B, Wernery U, Wernery R, El Shaer H, Alhadrami G, Gallacher D, Peacock J, Chaudhary A and Brown G 2007. The Camel from Tradition to Modern Times. The United Nations Educational, Scientific and Cultural Organization Office, Doha.

Brüssow H, Bruttin A and Marc-Martin S 1990. Polypeptide Composition of Rotavirus Empty Capsids and Their Possible Use as a Subunit Vaccine. journal of virology, 64(8): 3635-3642.

Cocito C, Gilot P, Coene M, de Kesel M, Poupart P and Vannuffel P 1994. Paratuberculosis. *Clinical Microbiology Reviews*, 7(3): 328-345.

De Verdier KK and Esfandiari J 1996. Evaluation of a one-step test for rapid, in practice detection of rotavirus in farm animals. Vet. Rec. 138(16): 393-395.

El-Sabagh I 2006. Coloning and expression of bovine rotavirus genes thesis. Ph.D. thesis, Depatment of virology, Faculty of Veterinary Medicine, Cairo University, Egypt.

Estes MK and Cohen J 1989. Rotavirus gene structure and function. Microbiol. Rev. 53(4): 410-449.

Fukai K, Onoda H, Iton T, Sato M, Miura Y and Sakai T 2004. Genetic and serological characterization of novel serotype G8 bovine group A rotavirus strains isolated in Japan. J. Vet. Med. Sci. 66(11): 1413-1416.

Haghkhah M, Derakhshandeh A, Jamshidi R, Moghiseh A, Karimaghaei N, Ayaseh M and Mostafaei M 2015. Detection of Mycobacterium avium subspecies paratuberculosis infection in two different camel species by conventional and molecular techniques. Veterinary Research Forum. 6(4): 337-341.

Hamam EAM 1993. Effect of Roughages on Growth Rate and Feed Conversion in Young Camels. M.Sc. Thesis, Animal Production Dept., Fac. Agric., King Saud Univ., Saudi Arabia.

Hereba A, Hamouda M and Al-hizab F 2014. Johne's Disease in Dromedary Camel: Gross Findings, Histpathology and PCR. JCPR 21: 83-88.

Khalafalla AI and Ali YH 2007. Observations on risk factors associated with some camel viral diseases. In: Proceedings of the 12th International Conference of the Association of Institutions for Tropical Veterinary Medicine AITVM, Montpellier, France.

Mebus CA, Underdahl NR, Rhodes MB and Twiehaus MJ 1969. Calf diarrhea (Scours): reproduced by the virus from a field outbreak. Univ. Nebraska Res. Bull. 233: 1-16.

Millar D, Ford J, Sanderson J, Withy S, Tizard M, Doran T and Hermon-Taylor J 1996. IS900 PCR to detect Mycobacterium paratuberculosis in retail supplies of whole pasteurized cows' milk in England and Wales. Appl Environ Microbiol 62(9): 3446-3452.

Mohammed M., Hart CA and kadden OR 2003. Viruses and bacteria associated with neonated camel calf diarrhea in eastern Sudan. Emir. J. Agric. Sci. 15 (1): 56-62.

Pavlik I, Bejčková L, Pavlas M, Rozsypalova Z and Koskova S 1995. Characterization by Restriction Endonuclease Analysis and DNA Hybridization Using IS900 of Bovine, Ovine, Caprine and Human Dependent Strains of Mycobacterium paratuberculosis Isolated in Various Localities. Veterinary Microbiology, 45(4): 311-318.

Saif LJ and Jiang B 1994. Non-group A rotaviruses in humans and animals. In: Raming, R., ed. Rotaviruses, Berlin: Springer-Verlag 185:339-371.

Schwartz HJ and Dioli M 1992. The one-humped camel in Eastern Africa. A Pictorial Guide to Diseases, Health Care and Management. Weikersheim, Germany, Verlag Josef Margraf Publishers, p. 282.

Sheridan JF, Smith CC, Manak MM and Aurelian L 1984. Prevention of rotavirus-induced diarrhea in neonatal mice born to dams immunized with empty capsids of simian rotavirus SAl1. J. Infect. Dis. 149(3): 434-438.

Stabel JR, Bosworth TL, Kirkbride TA, Forde RL and Whitlock RH 2004. A simple, rapid, and effective method for the extraction of *Mycobacterium paratuberculosis* DNA from fecal samples for polymerase chain reaction. J Vet Diag Invest 16(1): 22-30.

บทคัดย่อ

โรต้าไวรัสกลุ่ม A และ *Mycobacterium avium subspecies paratuberculosis* และโรคท้องเสียในอูฐหนอกเดียวในจังหวัดทางตะวันออก, ซาอุดีอาระเบีย

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โรคท้องเสียในอูฐหนอกเดียว ทั้งในลูกอูฐแรกเกิดและอูฐโต มีผลกระทบที่สำคัญในการผลิตสัตว์ โดยเฉพาะอย่างยิ่งในเขตจังหวัดทางตะวันออกของประเทศซาอุดีอาระเบีย ในการศึกษาครั้งนี้ พบว่าในลูกอูฐอายุ 1-3 เดือน ตรวจพบเชื้อไวรัสโรต้ากลุ่ม A ด้วยวิธี immune chromatographic คิดเป็น 10% (5/50) และด้วยวิธี ELISA คิดเป็น 12% (6/50) และเมื่อตรวจด้วยกล้องจุลทรรศน์อิเล็กตรอน พบลักษณะทางสัณฐานวิทยาของเชื้อโรต้าไวรัสเป็นรูปวงล้อ นอกจากนี้ในอูฐโตอายุ 3-5 ปี ตรวจพบเชื้อ *Mycobacterium avium subspecies paratuberculosis* ด้วยวิธีการย้อมสี Ziehl-Neelsen คิดเป็น 10% (5/50) และด้วยวิธี PCR คิดเป็น 60% (30/50)

คำสำคัญ: ไวรัสโรต้ากลุ่ม A *Mycobacterium avium subspecies paratuberculosis* ท้องเสีย อูฐหนอกเดียว ซาอุดีอาระเบีย

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