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Identification of rotavirus in human and calf buffalo by using RT-PCR technique and rapid testing

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Abstract
The molecular rotavirus, a significant zoonotic disease affecting calf water buffaloes and children within the same geographical region, was examined for the first time in Iraq. This virus is significant due to its activity, which induces gastroenteritis characterized by severe diarrhoea in all species, resulting in elevated mortality rates. Eighty-four fecal samples were collected from calves aged 4 to 6 months in the Abo-Gareeb area of Baghdad from December 2023 to April 2024. PCR identified positive results in 14 samples (16.6%) from a total of 84, with the peak infection rate occurring in January, comprising 8 samples (9.5%). In February and March, there were 4 samples (4.7%) and 2 samples (2.3%), respectively, along with 2 positive samples in December. Nevertheless, these statistics were documented in December. All samples underwent RT-PCR analysis using electrophoresis. Concurrently, 40 fecal bloody diarrhea swabs were obtained from children under the age of 2 belonging to buffalo breeders at Abo Gareb Hospital. The findings indicated that 8 of 40 samples tested positive for rotavirus (20%) using a Rapid Rotavirus test kit.

Keywords: Iraq, RT-PCR, Calf buffalo, gastroenteritis, rotavirus diarrhea

Introduction
Rotavirus is a common infection that impacts avians, animals, and mammals. Rotavirus belongs to the family Rioviridae, characterized by having an 11-segment genome. The material is unwrapped and classified into eight categories, labeled A through G, according per vp6 (Estes and Greenberg, 2013; Li *et al*., 2024) [6, 23]. The virus particularly targets a location within the gastrointestinal tract, resulting in the infection of intestinal cells (Liu *et al*., 2013) [15]. Rotavirus infection was detected in indigenous sheep breeds aged 2-3 years in Turkey. Older pigs exhibit the highest prevalence of RVB infections, while younger pigs may encounter a greater incidence of RVC infections. Elevated levels of RVB and RVC in swine indicate a notable association of these viruses with diarrhoea in this species. On rare occasions, these bacteria have been found in dogs, ferrets, lambs, calves, and rodents (Barua, 2009; Aljabory and Al-Zubaidy, 2020) [3, 2]. Rotavirus has been detected in Camelus dromedarius and calves in Iraq (Matthijnssens *et al*., 2008; Ved, 2014; Hassan and Kshash, 2017) [17, 21, 11]. It leads to acute gastroenteritis and severe diarrhoea by inducing atrophy and impairing absorption, resulting in increased fluid and electrolyte loss into the intestinal lumen, which contributes to significant global economic losses (Ge *et al*., 2013; Hassan *et al*., 2014; Patel *et al*., 2023) [7, 12, 24]. The wild boar has been identified as a vector in the transfer of infections and diseases among animals in Europe. Wild boars serve as significant vectors for rotavirus.

Materials and methods
Ethical approval
Ethical approval for this study obtained by Baghdad university, college of veterinary medicine university, Scientific Affairs Committee, under (Approval Code: P.G:1242, 22\5\2025).

Sample collection and testing

In Abu Ghraib district, west of Baghdad, between December 2020 and April 2021, 84 fecal samples were taken from male and female calves aged between four and six months. All samples were stored in sterile containers covered with ice and kept at -20 °C prior to analysis. Meanwhile, 40 fecal swabs were collected from breeder buffaloes under two years of age, suffering from bloody diarrhea, from Abu Ghraib Hospital. The swabs were transported directly on ice to the Zoonosis Unit laboratory at the College of Veterinary Medicine. All calf samples were immediately tested using a rotavirus test kit.

Primers

The primers utilized in the PCR and RT-PCR assays were selected and evaluated using the sequence database. The National Centre for Biotechnology Information (NCBI). The rotavirus Avp6 sequence consists of 416 base pairs, beginning at position 699 and ending at position 1114. Table 1 presents information on primer identification, nucleotide sequence, and product length for primers of length F=20 and R=20.Table 1.

Table 1: RT-PCR primers targeting the rotavirus VP6 gene

Primer name.		Sequence.	Product length.
Rotavirus vp6	F	5-CTACCAGACGCGGAAAGGTT-3	416 bp
	R	5-CCTGGTGGAAAGACTGGTCC-3	

RNA Extraction

After centrifuging a fecal solution made at a 1:4 dilution in DEPC water for 15 minutes at 4 °C, the supernatant was used to extract RNA. Rotavirus-positive feces samples were subjected to total RNA extraction using the QIAamp Viral RNA collection kit (QIAGEN, USA) in compliance with the manufacturer's instructions.

Real Time PCR to detect the rotavirus group

Super Script™ III reverse transcriptase (Invitrogen) was used to synthesize complementary RNA (cDNA) after heating five microliters of extracted RNA and 3.5 µl of dimethyl sulfoxide to 95°C for five minutes and then cooling them on ice in a microcentrifuge tube. Four microliters of 5x buffer, two microliters of 10 mM dNTPs, two microliters of DTT, one microliter of reverse transcriptase, one microliter of random hexamers, and three microliters of RNase-free water were mixed with seven microliters of denatured DNA. For 60 minutes, the RT reaction took place at 37 °C. After 15 minutes at 70 °C, it was maintained at 4 °C until the sample was collected. Rotavirus genes can be enhanced using a variety of techniques. While the PCR for VP6 and VP7 was performed using the procedure outlined by Pigment (2006), the PCR for VP1 was carried out in accordance with the protocol created by Asano *et al*. (2010). A final volume of 2X Hot Start Taq Plus Master Mix (QIAGEN, USA) containing 1.5 mg of each dNTP per 2,200 titers, 1 Hot Start Taq Plus DNA polymerase, and 10 cycles for both primers was used to amp up five copies of each cDNA in 20 titers. The thermal cycling process included a 5-minute initial heating phase at 95 °C, 35 cycles at 94 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 90 seconds, and a final extension at 72 °C for 10 minutes. A 1.2% agarose gel containing 0.5 g/mL ethidium bromide was used to evaluate the PCR hyperplasia products, and a UV gel documentation system (BIORAD) was used for documentation.

Results and Discussion

Rotavirus is a significant pathogen responsible for gastroenteritis and diarrhoea in humans and animals. Consequently, the disease has become increasingly significant, transitioning from a prevalent condition (El-Sadek *et al*., 2019). PCR identified positive results in 14 samples (16.6%) from a total of 84, with the peak infection rate occurring in January, comprising 8 samples (9.5%). In February and March, there were 4 samples (4.7%) and 2 samples (2.3%), respectively, as indicated in Table (2).

Table 2: RT-PCR positive results for rotavirus in buffalo calves by month

No of samples	Positive samples		Percentage%
84	December	2	2.3
	January	8	9.5
	February	2	2.3
	March	2	2.3
	April	0	0

These strong results indicate the importance of rotavirus in the diarrhea of buffalo calves, which may either lead to dehydration and death or propose to lethal bacterial infections similar to those caused by Enterobacteriaceae members especially *E. coli, Salmonella* or *Shegilla*. The findings from Iraq represented the first study on water buffalo calves as a possible source of human rotavirus infection, which is considered widespread in various regions around the world. The findings obtained through RT-PCR electrophoresis were positive, as demonstrated in (Figure 1). These findings are consistent with the work of multiple experts (Bulgin *et al*., 1989) [4] who reported that in Egypt, 22 samples (17%) out of 82 originated from calves infected with rotavirus. A. Additionally, in India, among diarrheic calves, the prevalence was 11.8% -26.8%. In European countries, the prevalence was higher and reached 47% (Hagbom, 2015) [10].

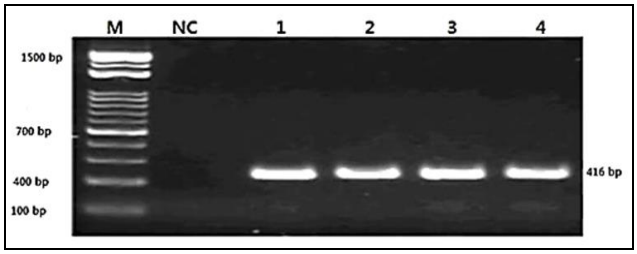

Fig 1: Positive samples of Rotavirus PCR products containing amplicons (416bp) on 1.0% Agarose gel. Lanes 1, 2, 3 and 4

This disagreement with our study may be due to the low temperature. The positive results in children were concentrated on December 1 case, January 3 cases, February and March at 2 cases, as shown in Table 2, identifies winter as the highest season for rotavirus propagation, corroborating our findings. The indicate of rotavirus infection children differs depending on the sociodemographic characteristics of the country. In India reach 39% (Maher *et al*., 2016) [16] and In Iraq in 2016 was 18-57% depending on governorate circumstances (Ahmed *et al*., 2023) [1], these results agree with our study. The relationship between the infection of rotavirus and other viruses in humans and animals was recorded by many researchers (Gorziglia *et al*., 1990; Mohamed *et al*., 2019) [9, 18]. Recent knowledge pertains to the emergence of an atypical rotavirus strain that has persistently disseminated across humans and animals, resulting in a novel

and diversified population of rotavirus progeny (Geletu *et al*., 2021) [8].

Table 3: RT-PCR positive results for rotavirus in children by month

No of samples	Positive samples		Percentage%
	Month	+ve	
40	December	1	*2.5*
	January	3	*7.5*
	February	2	*5*
	March	2	*5*

Conclusion

Rotavirus was identified in calf buffalo firstly in Iraq and it confirmed as a major pathogen causing diarrhea or bloody diarrhea, also its behavior as zoonotic infection was also concluded in this study represented by the high percentage of infection in children in same geographical area.

Conflict of Interest: Not available

Financial Support: Not available

Reference

1. Ahmed S, Khashman BM, Khalaf AK, Areej H. Rotavirus infection in Iraq: A review. Microbiol Sci Arch. 2023;3(1):12-17.
2. Al-jabory HA, Al-Zubaidy IA. Rapid-test and ELISA based identification of Rotavirus in camel calves in middle and south of Iraq. Plant Arch. 2020;20(2).
3. Barua SR. Clinico-pathology and molecular characterization of bovine rotavirus infection in calves in South-Eastern part of Bangladesh [Dissertation]. Chattogram: Chittagong Veterinary and Animal Sciences University; 2019.
4. Bulgin MS, Ward AC, Barrett DP, Lane VM. Detection of rotavirus and coronavirus shedding in two beef cow herds in Idaho. Can Vet J. 1989;30(3):235.
5. El-Sadek E, Shahein M, Abdelwahab S, El Shahidy M. Investigation of Rota and Corona viruses as causative agents for diarrhea in Egyptian calves. Suez Canal Vet Med J. 2019;24(2):257-72.
6. Estes MK, Greenberg HB. Rotaviruses. In: Knipe DM, Howley PM, editors. Fields Virology. 6th Ed., Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2013, p. 1347-401.
7. Ge Y, Mansell A, Ussher JE, Brooks AE, Manning K, Wang CJ, *et al*. Rotavirus NSP4 triggers secretion of proinflammatory cytokines from macrophages via toll-like receptor 2. J Virol. 2013;87(20):11160-11167.
8. Geletu US, Usmael MA, Bari FD. Rotavirus in calves and its zoonotic importance. Vet Med Int. 2021;2021:6639701.
9. Gorziglia M, Larralde G, Kapikian AZ, Chanock RM. Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4. Proc Natl Acad Sci USA. 1990;87(18):7155-7159.
10. Hagbom M. Rotavirus disease mechanisms: diarrhea, vomiting and inflammation-how and why [dissertation]. Linköping: Linköping University; 2015.
11. Hassan HA, Kshash QH. Molecular detection of Rotavirus type A in diarrheic calves of Mid-Euphrates governorates, Iraq. Iraqi J Vet Med. 2017;41(2):48-53.
12. Hassan HA, Kshash QH, Mansour KA. Detection of bovine rotavirus in diarrheic calves by using rapid test in some Mid-Euphrates provinces. Al-Qadisiyah J Vet Med Sci. 2014;13(2):20-26.
13. He B, Yang F, Yang W, Zhang Y, Feng Y, Zhou J, *et al*. Characterization of a novel G3P[3] rotavirus isolated from a lesser horseshoe bat: a distant relative of feline/canine rotaviruses. J Virol. 2013;87(22):12357-12366.
14. Kumar S. Textbook of microbiology. New Delhi: Jaypee Brothers Medical Publishers; 2012.
15. Liu F, Li G, Wen K, Wu S, Zhang Y, Bui T, *et al*. Lactobacillus rhamnosus GG on rotavirus-induced injury of ileal epithelium in gnotobiotic pigs. J Pediatr Gastroenterol Nutr. 2013;57(6):750-758.
16. Maher G, Pradhan G, Shetty S, Ranshing S, Dample A, Chitambar S. Rotavirus infection in children with acute gastroenteritis in Aurangabad, central Maharashtra. Indian Pediatr. 2016;53:631-633.
17. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, *et al*. Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J Virol. 2008;82(7):3204-3219.
18. Mohamed NS, Kandeil A, Al-Zubaidy IA, Kayali G, Ali MA. Genetic and antigenic characterization of avian influenza H9N2 viruses during 2016 in Iraq. Open Vet J. 2019;9(2):164-171.
19. Nakagomi O, Mochizuki M, Aboudy Y, Shif I, Silberstein I, Nakagomi T. Hemagglutination by a human rotavirus isolate as evidence for transmission of animal rotaviruses to humans. J Clin Microbiol. 1992;30(4):1011-1013.
20. Singh S, Singh R, Singh KP, Singh V, Malik YPS, Kamdi B, *et al*. Epidemiological study of naturally occurring bovine rotavirus infection in organized dairy farms, India. Biol Rhythm Res. 2019;52(1):1-9.
21. Ved N. Molecular characterisation and genotyping of human and animal rotaviruses isolates from Northern India [dissertation]. India; 2014.
22. Yu J, Lai S, Geng Q, Ye C, Zhang Z, Zheng Y, *et al*. Prevalence of rotavirus and rapid changes in circulating rotavirus strains among children with acute diarrhea in China, 2009-2015. J Infect. 2019;78(1):66-74.
23. Li X, Liu Y, Zhang J, Wang Y. Advances in rotavirus classification and molecular epidemiology. J Adv Res Virol. 2024;15:102-18.
24. Patel M, Steele AD, Parashar UD. Global review of rotavirus-associated gastroenteritis and its economic impact. Vaccine. 2023;41(5):1275-1284.

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