International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(10): 89-91

www.veterinarypaper.com
Received: 27-08-2025
Accepted: 29-09-2025

Ibrahim AH Al-Zubaidy
Zoonotic Diseases Researches Unit. College of Veterinary Medicine. University of Baghdad, Baghdad, Iraq

Corresponding Author:
Ibrahim AH Al-Zubaidy
Zoonotic Diseases Researches Unit. College of Veterinary Medicine. University of Baghdad, Baghdad, Iraq

The first identification of rotavirus infection in wild boars in Iraqi marshes using molecular technique

Ibrahim AH Al-Zubaidy

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i10b.2610

Abstract
This study on molecular rotavirus in wild boars in Iraq represents a pioneering effort. The virus is recognized as a significant pathogen due to its link to gastroenteritis characterized by severe diarrhea across various animals and human resulting in a notably high documented morbidity and mortality rates. Twenty -four samples from ileal areas of wild boars living in marshes were taken in the period between December 2020 to April 2021.The results showed 6 positive (16.6%).The highest positive samples appeared in January 2021 and was 4 (8.3%).There was 1 sample positive in each February and in March. There was no infection in December. We used electrophoresis and RT-PCR together to g*et al*l the samples documented.

Keywords: RT-PCR, Iraqi wild boars, Rotavirus diarrhea, First identifing, Iraq

1. Introduction
Rotaviruses recognized as common pathogens affecting animals and birds. The rotavirus belongs to Rioviridae. Rota genome have eleven segments. The item has been unwrapped and classified depending on vp6 into eight categoreis from A to G (Johne *et al*., 2024; Estes and Greenberg, 2013) [11, 6]. Virus target site is GIT particularly the interic cells (He wt al., 2003; Liu *et al*., 2013) [13]. In Turkey they record rotavirus infectivity in Turkish sheep to be infected in ages between 2-3 years. Old aged pigs get higher chances for Rotavirus B infections; however, younger get infection almost due to rotavirus C. High detection of rotavirus B and C indicate the major of this virus as a important etiological factor of diarrhea in swine. Rota virus recorded as sporadic cases in Iraq are in cattle, sheep, some birds, dogs, and rodents specially rat. (Marthaler, 2014; Aljabory *et al*., 2019) [14, 1]. In Iraq rotavirus detected in camels and cattle (Saif, 1999; Amimo *et al*., 2013; Hassan and Kashash, 2017) [18, 2, 7]. Signs include profuse enteritis, gastritis and diarrhea. Atrophied villi also seen so lead to marked decrease of rejection and pumping and losses of fluid and important electrolytes inside bowel cavity leading to sever dehydration and death that results in effective economic losses over all areas in the world (Tazipori *et al*., 1980; Shaw *et al*., 1989; Hassan *et al*., 2014) [21, 19, 8]. Wild boars represent a major factor affect epidemiology of rota virus infection in other mammals due to helping spread between animals in European countries (Massei *et al*., 2015) [15]. Researchers record wild boar to be a significant vector in transmission of this infection. We design our research to investigate possibility of infection in wild boars and the risk that may exist and the role of transmission of rotavirus among cattle and human because wild boars live in marked numbers depending on areas of cattle breeding.

Methods
Collection of samples
Twenty-four intestinal contents samples of wild boars from ileac area of the intestine were collected. Animals were in dissimilar age and sexes following hunted by the hunters for the period of December 2020 to April 2021. Boars were grassed in areas of buffaloes breeding and hunted because they harm buffaloes, cattle, sheep and even human properties. Intestinal contents were located in disinfected cold containers and preserved at -20 ° C till examined.

Primers

Primers used in Molecular examination assay were depending on sequences database, proved by NCBI, rotavirus Avp6 with a length of product 416bp and begin at 699 bp and stopped at 1114bp. Primer extent R= 20 and F= 20, Table. 1

Table 1: Primer sequences used for amplification of the rotavirus *vp6* gene

Primer name		Sequence	length of Product
rotavirus vp6	F	5-CTACCAGACGCGGAAAGGT-3	416 bp
	R	5-CCTGGTGGAAAGACTGGTC-3	

RNA Extraction

Extraction of RNA was done from the supernatant of intestinal content solution prepared in dilution of 1:4 in Diethylpirocarbonate water (DEPC), then centrifuged the solution in cold centrifuge 5000 rpm for 15 minutes. The entire RNA content was extracted from rotavirus-positive stool samples using the QIAamp Viral RNA Collection Kit (QIAGEN, USA), following the manufacturer's protocol.

RT-PCR to detect the rotavirus group

Five microliters of RNA extract were added to 3.5 microliters of dimethyl sulfoxide and mixed in a microcentrifuge tube, followed by heating at 95°C for five minutes and then immediately chilled on ice. Complementary DNA (cDNA) synthesis was performed using SuperScript™ III reverse transcriptase (Invitrogen). Subsequent steps were carried out according to Ibrahim *et al*., 2025. The PCR products were then run on a 1.2% agarose gel containing 0.5 µg/mL ethidium bromide and visualized using the BIORAD UV gel

Results and Discussion

Rotavirus infection considered to be an important viral disease causing gastroenteritis and diarrhea in cattle, buffaloes, sheep and human. It considered as being a common disease (El-Sadek *et al*., 2019) [5]. The infection with rota virus was diagnosed in Brazilian pigs with infectivity (23.6%) by using real time Polymerase Chain Reaction and gel electrophoresis. This outcome were out of 288 animal examined and show (34/288). A percentage of (11, 8%) were positive for rota antigen (29/34) with no diarrhea in pigs with percentage of 85.3% in Southern of Mozambique (Mediei *et al*., 2011; Boene *et al*., 2019) [16, 3]. In Thailand researchers record that ages infected not older than 3 months was 24 out of 132 (18.2%). They infected with type A rotavirus (18). The results gained in Iraq represenes the earliest resting on southren Iraqi wild boars that well thought-out be common over countries in the world. Results in current work concerning molecular diagnosis were obtained by means of electrophoresis of real time-PCR and shown in (Fig.1). The current study agreed with various researches (El-Sadek *et al*., 2019) [5] who recored that in Egypt the infection was (17%) 22 out of 82 of infect cattle with type A. In Thailand about 21.6% (11/51) in pigs infected (Bulgin *et al*., 1988) [4], and in Japan 4 out of 90 wid boars (4.4%) infection were recorded (Tuanthap *et al*., 2018) [20]. The positive outcomes that recorded in our study were high in January, we recorded 4 positive cases, then in February and March at 1 positive in each month as shown in Table 1. The relation with seasons was by studied (Okadera *et al*., 2013; Yu *et al*., 2019) [17, 22]. who indicate winter is the max out spread of the infection, which support our conclusion. We suggest that wild boars in Iraq take part in the epidemiology of infection in calves, calf buffalo and also human because they survive near area of cattle breeding, this was mentioned by several field workers (Tzipori *et al*., 1980; Shaw *et al*., 1989) [21, 19].

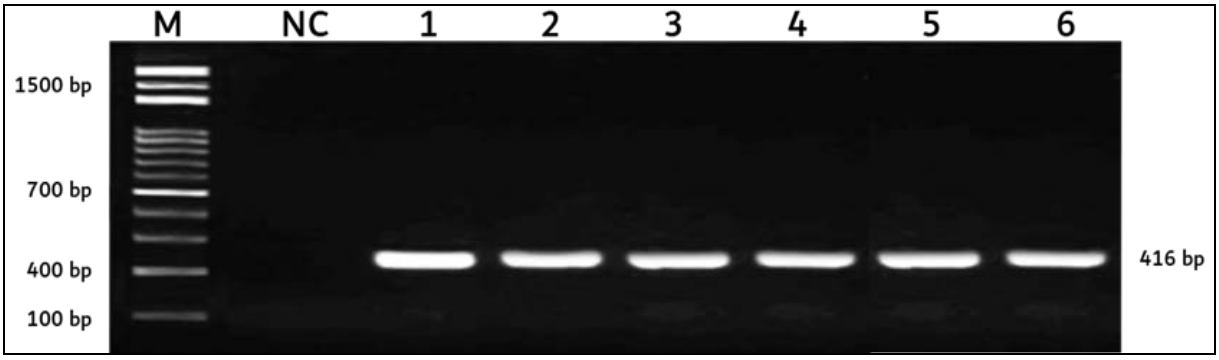

Fig 1: Posative RT_PCR for Rota virus, shows lane 1-4, (416bp) bands

Table 2: Number and percentage of positive samples by month according to months

Samples No	Positive		%
24	December	0	0
	January	4	16.6
	February	1	4.1
	March	1	4.1

Acknowledgements

I would like to express my sincere thanks to the staff members of the Zoonotic Diseases Research Unit at the College of Veterinary Medicine, University of Baghdad, for their valuable support and assistance. I also extend my gratitude to the laboratory technicians who helped with sampling and laboratory analyses.

Conflict of Interest

The authors declare that they have no conflicts of interest

Reference

1. Aljabory HA, Alzubaidy IH, Neamah AJ. Rotavirus A strain AHI1 VP6 gene, partial cds (Accession No. MN234239.1). National Center for Biotechnology Information (NCBI). https://www.ncbi.nlm.nih.gov/nuccore/MN234239.1

2. Amimo JO, Vlasova AN, Saif LJ. Detection and genetic diversity of porcine group A rotaviruses in swine fecal samples in Ohio: Predominance of the *G9P* [13] genotype in nursing piglets. Journal of Clinical Microbiology. 2013;51(4):1142-1151. https://doi.org/10.1128/JCM.03135-12
3. Boene SS, João E, Munlela B, Nabetse E, Cala A, Mapaco L, *et al*. Detection and molecular characterization of *Rotavirus A* antigen in fecal samples from diarrheic and non-diarrheic pigs in Maputo City and Province, southern Mozambique. International Journal of Infectious Diseases. 2019;79:70. https://doi.org/10.1016/j.ijid.2018.11.179
4. Bulgin MS, Ward AC, Barrett DP, Lane VM. Detection of rotavirus and coronavirus shedding in two beef cow herds in Idaho. Canadian Veterinary Journal. 1989;30(3):235-239.
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 Veterinary Medicine Journal (SCVMJ). 2019;24(2):257-272. https://doi.org/10.21608/scvmj.2019.79238
6. Estes MK, Greenberg HB. *Rotaviruses.* In: Knipe DM, Howley PM, *et al*., editors. Fields Virology. 6th ed. Philadelphia (PA): Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013. p. 1347-1401.
7. Hassan HA, Kshash QH. Molecular detection of *Rotavirus* type A in diarrheic calves of Mid-Euphrates governorates, Iraq. Iraqi Journal of Veterinary Medicine. 2017;41(2):48-53.
8. Hassan HA, Kshash QH, Mansur KA. Detection of bovine rotavirus in diarrheic calves by using rapid test in some Mid-Euphrates provinces. Al-Qadisiyah Journal of Veterinary Medicine. 2014.
9. 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. Journal of Virology. 2013;87(22):12357-12366. https://doi.org/10.1128/JVI.02013-13
10. Ibrahim AHA, Noor MM, Al-Rubaiey MGA. Identification of rotavirus in human and calf buffalo using RT-PCR technique and rapid testing. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(8):297-299. https://doi.org/10.22271/veterinary.2025.v10.i8.4315
11. Johne R, Donato C, Bányai K. Rotavirus diversity, evolution, and lineage classification. Bacterial and Viral Bioinformatics Resource Center (BV-BRC). 2024. https://www.bv-brc.org/docs/downloads/subspecies-classification-workshop/040824S2.4-Donato.pdf
12. Kumthip K, Khamrin P, Kongkaew A, Vachirachewin R, Malasao R, Ushijima H, *et al*. Molecular epidemiology and characterization of porcine adenoviruses in pigs with diarrhea in Thailand. Infection, Genetics and Evolution. 2019;67:73-77. https://doi.org/10.1016/j.meegid.2018.11.021
13. 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. Journal of Pediatric Gastroenterology and Nutrition. 2013;57(6):770-778. https://doi.org/10.1097/MPG.0b013e3182a35616
14. Marthaler D. Widespread *Rotavirus H* in commercially raised pigs, United States. Emerging Infectious Diseases. 2014;20(7):1195-1198. https://doi.org/10.3201/eid2007.131680
15. Massei G, Kindberg J, Licoppe A, Gačić D, Šprem N, Kamler J, *et al*. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Management Science. 2015;71(4):492-500. https://doi.org/10.1002/ps.3965
16. Médici KC, Barry AF, Alfieri AF, Alfieri AA. Porcine *Rotavirus* groups A, B, and C identified by polymerase chain reaction in a fecal sample collection with inconclusive results by polyacrylamide gel electrophoresis. Journal of Swine Health and Production. 2011;19(3):146-150.
17. Okadera K, Abe M, Ito N, Morikawa S, Yamasaki A, Masatani T, *et al*. Evidence of natural transmission of group A *Rotavirus* between domestic pigs and wild boars (*Sus scrofa*) in Japan. Infection, Genetics and Evolution. 2013;20:54-60. https://doi.org/10.1016/j.meegid.2013.08.002
18. Saif LJ. Enteric viral infections of pigs and strategies for induction of mucosal immunity. Advances in Veterinary Medicine. 1999;41:429-446.
19. Shaw DP, Morehouse LG, Solorzano RF. *Rotavirus* replication in colostrum-fed and colostrum-deprived pigs. American Journal of Veterinary Research. 1989;50:1966-1970.
20. Tuanthap S, Phupolphan C, Luengyosluechakul S, Duang-in U, Theamboonlers A, Vongpunsawad S, *et al*. Molecular characterization of porcine *Rotavirus C* in pigs with gastroenteritis in Thailand, 2011-2016. PeerJ Preprints. 2018;6:e3515v1. https://doi.org/10.7287/peerj.preprints.3515v1
21. Tzipori SR, Makin TJ, Smith ML. The clinical response of gnotobiotic calves, pigs and lambs to inoculation with human, calf, pig and foal *Rotavirus* isolates. Australian Journal of Experimental Biology and Medical Science. 1980;58:309-318.
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. Journal of Infection. 2019;78(1):66-74. https://doi.org/10.1016/j.jinf.2018.09.002

How to Cite This Article
Al-Zubaidy IAH. The first identification of rotavirus infection in wild boars in Iraqi marshes using molecular technique. International Journal of Veterinary Sciences and Animal Husbandry 2025; 10(10): 89-91.