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Molecular characterization of a Porcine Group A rotavirus of VP6 gene of by Reverse Transcriptase–Polymerase Chain Reaction

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Abstract
The present study aimed to detect *Rotavirus* in the feces of diarrheic piglets using a VP6 gene-based RT-PCR assay. 15 fecal samples from piglets aged 0-6 months were analyzed for the presence of group A *Rotavirus*. Results showed that 10 out of the 15 samples (66.6%) tested positive for the *Rotavirus* VP6 gene, indicating a significant prevalence of group A *Rotavirus* in this population. It was observed that piglets less than one-month-old exhibited a higher rate of *Rotavirus* detection compared to older piglets. The findings suggest that the RT-PCR assay is a highly sensitive and specific method for the rapid detection of group A *Rotavirus* in fecal samples from piglets.

Keywords: Piglets, *Rotavirus,* VP6 gene, diarrhoea, RT–PCR

Introduction

Gastroenteritis poses a major threat to swine farms globally, leading to significant financial losses (Doerksen *et al*., 2022) [1]. Pigs are vulnerable to infectious and non-infectious gastrointestinal diseases due to their physiology and eating habits. Suckling and weaned piglets lack maternal antibodies, increasing their susceptibility to enteric infections like diarrhea leading to stunting growth, and increasing mortality (Sinha *et al*., 2019) [13]. Diarrhea in piglets can be caused by various pathogens including bacteria, viruses, and parasites.

Porcine *rotavirus* (PRV) is a major cause of diarrhea in newborn piglets, showing symptoms like watery diarrhea, fever, dehydration, vomiting, and nausea. *Rotavirus*, a member of the Reoviridae family within the *Rotavirus* genus, exhibits a tri-laminar viral structure characterized by two double capsid layers enveloping its genomic core, comprised of double-stranded RNA organized into 11 segments. These segments encode a total of six non-structural proteins (NSP1–NSP6) and six structural proteins (VP1–VP4, VP6, and VP7) (Maclachlan and Dubovi, 2010) [7]. The classification of *rotavirus*es into 10 main groups (RVA-RVJ) is based on the antigenicity and genetic attributes of the outer shell protein VP6. *Rotavirus* A(RVA), has been identified as the primary causative agent of diarrhea in piglets aged within 8 weeks (Wang *et al*., 2024) [16]. The binomial nomenclature system for *rotavirus*es relies on the VP7 and VP4 segments, which determine the G (glycoprotein) and P (protease-sensitive protein) genotypes respectively. To date, RVAs have been classified into 42G and 58 P genotypes (Neira *et al*., 2023) [11].

RT-PCR has emerged as the primary diagnostic method for detecting *rotavirus* infections in pigs (Min *et al*., 2006) [9]. Preference for RT-PCR is due to the risk of false negative results associated with alternative techniques like ss-PAGE and ELISA when diagnosing *rotavirus* infections in pigs with diarrhea (Medici *et al*., 2011) [8]. Logeshwaran *et al*., (2020) [6] reported 5(5.74 %) samples positive for group A *Rotavirus* by VP6 gene-based reverse transcriptase–polymerase chain reaction (RT-PCR) assay from diarrheic pigs.

Limited research on porcine *rotavirus* exists in Western Maharashtra, India. Given the economic importance of pig farming and the potential for zoonotic transmission, comprehensive surveillance, and sensitive RNA-based tools are crucial for detecting and

distinguishing local *rotavirus* strains, aiding disease diagnosis, and formulating effective control strategies.

Materials and Methods

One hundred fecal specimens were collected from pigs aged between 0 to 6 months, experiencing diarrhea, during the period from July 2023 to February 2024. The sampling was conducted in the Satara and Pune districts of Maharashtra, India. Fifteen out of one hundred fecal samples were positive upon screening using a rapid *rotavirus* antigen detection kit. Confirmation of group A *Rotavirus* was subsequently carried out via VP6 gene-based RT-PCR assay.

Detection of *Rotavirus*- A 10% fecal suspension was made in 10 mM phosphate buffer saline (PBS; pH 7.2). After thorough vortexing, centrifugation at 13000 rpm for 15 minutes at 4 °C was done to remove coarse debris. The supernatant was collected for RNA extraction using Trizol extraction reagent. The double-stranded RNA (ds-RNA) of the virus was subjected to a One-step reverse transcription-polymerase chain reaction (RT-PCR) to amplify a specific segment of gene 6, responsible for encoding the VP6 protein of *rotavirus* using 309 bp primers optimized by Elschner *et al*., (2002) [2]. The sequences of oligonucleotide primers are shown in Table 1.

A one-step RT-PCR assay was conducted using a 50 µl reaction volume to detect the VP6 gene. The reaction mixture included 25 µl of 2x one-step RT-PCR master mix, 2 µl of each primer (10 pmol), 2 µl of RNA, 2.5 µl of 50 mM Mn $(OAc)_2$, and 16.5 µl of RNAse-free water. Cycling conditions are provided in Table 2. Vaccine-derived RNA served as the positive control. The PCR amplification products were visualized using agarose gel electrophoresis.

Table 1: List of oligonucleotide Primers used for VP6 gene RT-PCR

Sr. No	Gene	Primers Sequence (5´–3´)	Amplicon size (bp)	Reference
1.	VP6-F (Rot3)	AAAGATGCTAGGGACAAAATTG	309	(Elschner *et al.*, 2002) [2]
	VP6-R (Rot5)	TTCAGATTGTGGAGCTATTCCA		

Table 2: Cycling conditions for one-step RT-PCR of VP6 gene

Cycling steps	Time and temperature
1. Polymerase Activation	30 sec at 90 °C
2. Reverse Transcription	60 min at 60 °C
3. Denaturation	1 min at 94 °C
4. Denaturation	30 sec at 94 °C
5. Annealing	30 sec at 50 °C
6. Extension	2 min at 72 °C
Repeat steps 4-6 for 35 cycles	
7. Final Extension	7 min at 60 °C

Results and Discussion

Out of 100 fecal samples,15 fecal samples initially positive for *rotavirus* by rapid antigen detection test, 10 (66.6%) were confirmed positive for *rotavirus* using VP6 gene-based one step RT-PCR assay (Fig.1). These findings are consistent with those of Song *et al*., (2006) [14], Mondal *et al*., (2013) [10], Tumlam *et al*., (2019) [15], and Logeshwaran *et al*., (2020) [6], who utilized VP6 gene-based RT-PCR assay to confirm group A *Rotavirus* presence.

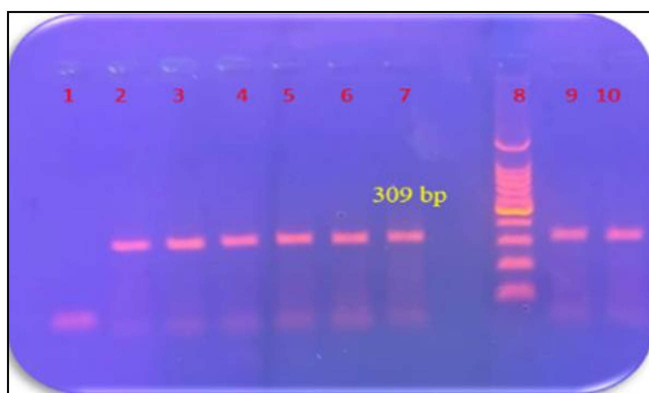

Fig 1: Detection of VP6 gene amplicon of porcine *rotavirus* (309 bp) by PCR

Lane 1: Negative Control, Lane 2: Positive Control, Lane 8: Molecular weight marker (100bp ladder), Lane 2, 3, 4, 5, 6, 7, 9, 10: Porcine *rotavirus*-positive samples.

In the present study, the stratification of age groups of pigs using one-step RT-PCR of VP6 gene analysis indicated a positivity rate of 22.2% (2 out of 9 samples) in the below 3 weeks age group, with a 9.8% (5 out of 51 samples) positivity rate in the 3-10 weeks age group (table-3). These findings are consistent with those of Gachanja *et al*., (2016) [5] reported 80% positivity rate (4 out of 5 samples) in pigs below 3 weeks of age and a 41.3% positivity rate (19 out of 36 samples) in the 3-10 weeks age group from a total of 110 screened fecal samples. According to the investigation conducted by Ferrari *et al*., (2022) [4] nursing piglets within the age category of less than one month demonstrated a heightened incidence of *rotavirus* detection compared to older age groups.

Table 3: Age-wise occurrence of porcine *rotavirus* by RT-PCR

Age group	No of the cases	No of positive cases by RT-PCR	Percentage
<3 Weeks	9	2	22.2
3 - 10 W	51	5	9.8
>10 W	40	3	7.5
total	100	10	10

The RT-PCR is advantages for detecting *Rotavirus* in fecal samples because of its high sensitivity and specificity (Fedorova *et al*., 2005) [3]. It allows early detection of viral nucleic acids. RT-PCR is effective for the specific and sensitive detection of the *Rotavirus* VP6 gene in fecal samples of pigs (Niture *et al*., 2011) [12].

In conclusion, *Rotavirus* stands out as a predominant cause of diarrheal episodes in piglets. RT-PCR based detection of porcine *rotavirus* is most sensitive and specific assay. Nursing piglets are most succeptible to *rotavirus* infection.

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Conflict of Interest: All authors declare no conflict of interest.

Conclusion

Gastroenteritis, particularly caused by porcine rotavirus, presents a significant threat to the health of piglets and the economic viability of swine farming. This study highlights the effectiveness of RT-PCR as a sensitive and specific method

for detecting rotavirus in fecal samples, confirming its status as a leading cause of diarrhea in young pigs. With a notable prevalence of infection among nursing piglets, the findings underscore the importance of implementing rigorous surveillance and diagnostic practices in swine farms. By enhancing detection capabilities and understanding rotavirus dynamics, we can develop more effective control strategies to mitigate its impact on pig health and farm productivity.

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