USE OF VARIOUS METHODS FOR DETECTION OF SERO-GROUP A
ROTAVIRUS IN PORCINE INTESTINAL AND FECAL SPECIMENS

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ABSTRACT

The study was made to find out the etiological agent involved in piglet diarrhoea during 2007-
2009 in and around Bareilly. Group A rotavirus (GARV) was found as the main agent using various
methods applied to intestinal and fecal samples. Out of 199 necropsied intestinal samples, none was
positive in agarose gel electrophoresis, 5 (2.51%) were positive in double antibody sandwich ELISA
(DAS-ELISA), 5 (2.51%) in RT-PCR and 18 (9.04%) in real-time RT-PCR. Out of 96 fecal samples, 8
(8.33%) were positive in agarose gel electrophoresis, 11 (11.45%) in DAS-ELISA, 16 (16.66%) in RT-
PCR and 23 (23.95%) were found positive in real-time RT-PCR. Thus, it was found that real-time RT-
PCR, RT-PCR, ELISA and agarose gel electrophoresis have a descending order of sensitivity for
detection of rotavirus as has already been reported.

Key words: - Feces, Group A rotavirus, Intestine, Methods, Porcine.

Rotavirus is one of the major agents of severe
infantile gastroenteritis affecting humans and
livestock worldwide (Hoshino and Kapikian, 1994).
Diarrhoea in piglets causes considerable economic
loss to the pig industry (Moxley and Duhamel, 1999).
An accurate diagnosis of rotavirus is essential since
it obviates the unnecessary use of antibiotic therapy.
During the 1990s, the importance of rotavirus as a
cause of illness and mortality has been clearly
documented, and substantial progress has been
made towards developing rapid and sensitive
diagnostic methods (Blacklow and Greenberg,
1991). Currently, batteries of diagnostics including
electron microscopy, polyacrylamide
gel electrophoresis (PAGE) of viral nucleic
acid, antibody-based assays such as
enzyme immunoassay, immunofluorescence,
radioimmunoassay, solid-phase aggregation of
coated erythrocytes, latex agglutination test, and
nucleic acid based tests like PCR and real-time PCR
are being used for the detection of rotavirus. In the
present study, an attempt was made to find out the
etiology of piglet diarrhoea prevailed during the period
of study in and around Bareilly using few available
tests in fecal and intestinal specimens of piglets.

A total of 199 intestinal samples from piglets
were collected from necropsy room, Division of
Pathology, IVRI, Izatnagar. A total of 96 fecal samples
were collected from piglets of AICRP pig farm, IVRI
as well as from various local areas of Bareilly during

GARV antigen detection: The DAS-ELISA kit
(1.1.RT.K2.Ingezim Rotavirus DAS.dot)
manufactured in Madrid, Spain by INGENASA was
used for detection of GARV antigen.

RT-Polymerase chain reaction: Total RNA was
extracted from fecal supernatants and necropsied
intestinal samples using TRIZOL-LS and TRIZOL
reagent (Invitrogen) respectively, as per
manufacturer’s recommendation and stored at
-70°C. Oligonucleotide primers (IDT, India Pvt. Ltd)
targeting gene segment 6 i.e. GAR309F: 5’-AAA GAT
GCT AGG GAC AAA ATT G-3’ and GAR309R:
5’-TTC AGA TTG TGG AGC TAT TCC A-3’
used for RT-PCR and thermal cycling conditions were

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similar to those described by Song et al. (2006). Reverse transcription was performed using 4 µg RNA for intestinal sample and 5 µl RNA for fecal samples with random primer (Promega) and SuperScript III Reverse Transcriptase (Invitrogen) in a 20 µl reaction volume following standard protocol and cDNA was stored at -20°C. The confirmation of specific amplicons (309 bp) was done by electrophoresis in 1.5% agarose in TBE buffer with ethidium bromide (0.5 µg/µl) and was visualized in gel documentation system (Innotech, USA).

**Real-time RT PCR:** Oligonucleotide primers (HPLC grade from IDT, India Pvt. Ltd) targeting gene segment 6 i.e. GAR433F: 5’-AAG TAG CTG GAT TTG ATT ATT C-3’ and GAR433R: 5’-GAC TCA CAA ACT GCA GAT TCA A-3’ used for real-time RT PCR and the cycling conditions were similar to those described by Schwarz et al. (2002). It was performed on Stratagene MX-3000P Sequence Detection System using SYBR green mix from Finnzymes DyNAmo HS SYBR Green qPCR kit (Lot No. 322, F-410L) following manufacturer’s instructions.

**Agarose gel electrophoresis:** For electropherotyping/identification of rotavirus RNA in agarose gel, a slight modification of horizontal electrophoresis in a 5 mm thickness of 1.5% agarose was carried out as described by Psikal et al. (1991) and viewed in a gel documentation system.

Out of 199 necropsied intestinal samples, none was found positive in agarose gel electrophoresis, 5 (2.51%) were positive for GARV antigen in DAS-ELISA, 5 (2.51%) in RT-PCR and 18 (9.04%) were found positive in real-time RT PCR. Out of 96 fecal samples, 8 (8.33%) showed positive banding pattern in agarose gel electrophoresis, 11 (11.45%) were positive in DAS-ELISA, 16 (16.66%) in RT-PCR and 23 (23.95%) were positive in real-time RT PCR. Out of 11 segments of rotaviral RNA, only 8 were clearly observed in gel documentation system. Markowska-Daniel et al. (1996) and Barman et al. (1998) used ELISA to detect rotavirus antigen in fecal samples of diarrhoeic piglets. Because of its simplicity and sensitivity, ELISA is the method of choice in many laboratories today (McNeal et al., 2005). RT-PCR targeting VP6 gene was found to be 100 times more sensitive than ELISA (Buesa et al., 1996). Zhao et al. (2005) used VP6 gene for real-time RT-PCR of GARV. Pang et al. (2004) also reported the assay to be 1000 times more sensitive than conventional RT-PCR. Rapid identification of bovine rotavirus by agarose gel electrophoresis was done by Psikal et al. (1991). The RNA banding patterns in this study well correlated with that of Psikal et al. (1991), though in some samples, the RNA segments migrated very fast and were not sharply visible. While Kalica et al. (1978) described 11 segments of rotavirus RNA in PAGE; in our experiment only 8 bands were discernible in agarose gel. Basing on a closer analysis of our electrophoreogrammes and on data published by other investigators, we presume that this reduction of number of bands is due to the positions of the segments 2 and 3, and 7, 8 and 9, which are so close in agarose that the segments become indiscernible. Buesa et al. (1996) compared RT-PCR, ELISA, PAGE and found positivity rates of 30% for RT-PCR, 29% for ELISA, 26.8% for PAGE.

Thus, in this study, it was found that real-time RT PCR, RT-PCR, ELISA and agarose gel electrophoresis have a descending order of sensitivity as has been reported earlier. The study demonstrated that the positivity rates of various tests varied considerably. So, any of the methods depending upon the convenience would be useful as a screening procedure to detect rotavirus in fecal samples of piglets with diarrhoea.

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