

## REVERSE TRANSCRIPTION-PCR FOR DETECTION OF PORCINE DIARRHEA ASSOCIATED GROUP A ROTAVIRUS IN FIELD SAMPLES FROM PORCINES FARMED IN NORTH-EASTERN ROMANIA

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### Abstract

*Group A rotaviruses (GARVs) cause acute diarrhea and malabsorption in new-born and young piglets, resulting in high mortality and morbidity. Evidence of this infection has been reported in various European countries. However, there is little evidence of porcine GARV infections in Romania. The aim of this study was the detection of the GARV in an outbreak of diarrhea in piglets and sows farmed in North-Eastern Romania. We examined 25 fecal samples: 20 diarrheic fecal specimens collected from piglets and 5 normal fecal samples collected from healthy sows raised in a closed-circuit farm. The extracted ARN underwent a reverse transcription step, followed by a classical polymerase chain reaction assay, using primers able to amplify a fragment of 317 bp from NSP5 gene, the most conservative gene among GARV strains and isolates. RT-PCR using specific primer for the GARV NSP5 gene detected GARV-positive reactions in 15 (60%) fecal samples. Of these, 12 out of 20 diarrheic fecal samples (60%) and 3 out of 5 fecal samples (60%) tested positive for porcine GARVs. The data showed that GARV was identified in the vast majority of both diarrheic and normal fecal samples, suggesting that the GARV may represent a major pathogen with an important role in this diarrhea outbreak. Thus, this RT-PCR assay proved to be a rapid and precise diagnosis assay for detection of porcine GARV. Furthermore, the primers annealing temperature (60 °C) is able to confer to this assay an increased specificity and sensitivity. In order to prevent the economical losses, the use of a reliable diagnosis method allowing the detection of rotavirus could contribute in achieving this goal, together with the identification and removal of the asymptomatic carriers.*

**Key words:** reverse-transcription, piglet, diarrhea, North-Eastern.

### INTRODUCTION

Rotaviruses are members of the family *Reoviridae*, genus *Rotavirus*, classified into seven antigenically distinct groups (A to G). Groups A, B and C are associated with acute gastroenteritis in humans and animals, while groups D, E, F and G have been detected only in animals (Kapikian et al. 2001). Group A rotaviruses (GARVs) cause acute diarrhea and malabsorption in new-born and young piglets, resulting in high mortality and morbidity. However, the GARV infections are difficult to clinically differentiate from other enteritis such as transmissible gastroenteritis and porcine epidemic diarrhea (Saif and Wesley, 1999). All these entities cause enteritis in swine of all ages, while the clinical signs seen in piglets are including watery diarrhea,

dehydration and high mortality, resulting in serious economic loss (Collins et al. 2010). Furthermore, pigs represent a potential reservoir for zoonotic transmission of RVA to humans (Monini et al. 2014). Noteworthy, the sows, as asymptomatic carriers, may transmit the virus either via transplacental or via milk, thus they contribute in maintaining active outbreaks (Amimo et al. 2015). Since the main route of the infection is represented by the orofecal route, the GARV was associated to up to 89% of all rotavirus diarrhea in commercial piglets. Moreover, GARV was also detected in non-diarrheic piglets (Atii et al., 1989). Evidence of this infection has been reported in various European countries (Collins et al. 2010; Theuns et al. 2014). Since the absence of surveillance programs have resulted in a lack of data on viral

associated diarrhea in pigs, there is little evidence of porcine GARV infections or their genetic diversity in Romania. The aim of this study was the detection of the GARV in an outbreak of diarrhea in piglets and sows farmed in North-Eastern Romania.

## MATERIALS AND METHODS

A total of 25 samples consisting of diarrheic and normal feces were obtained from an outbreak of diarrhea. In this farm, an enteric viral infection was suspected to evolve because of the high mortality; the piglets with diarrhea showed typical signs (vomiting and no response to antibiotics). The cases were selected on the basis of clinical signs, therefore 20 diarrheic fecal specimens from piglets aged 1 to 20 days and 5 normal fecal samples from healthy sows were collected; all the animals were raised in a closed-circuit farm in North-Eastern Romania. All the samples were conditioned in ARN later (Qiagen). The ARN was extracted using a commercial kit (Life Technologies). The extracted ARN samples were stored at  $-20^{\circ}\text{C}$  until use. The reverse transcription reaction was performed using iScript cDNA synthesis kit (BioRad), according to the manufacturer's protocol. The reaction was carried out in a 20  $\mu\text{L}$  reaction volume, consisting of 4  $\mu\text{L}$  5x iScript reaction mix, 1  $\mu\text{L}$  iScript reverse transcriptase, 2  $\mu\text{L}$  RNA template and 13  $\mu\text{L}$  nuclease-free water. The incubation of the reaction mix was carried out at  $25^{\circ}\text{C}$  for 5 minutes, at  $42^{\circ}\text{C}$  for 30 minutes and  $85^{\circ}\text{C}$  for 5 minutes. The products were stored at  $-20^{\circ}\text{C}$  until use. For the classical polymerase chain reaction assay, 2  $\mu\text{L}$  of cDNA was mixed with a reaction mixture containing 2.5  $\mu\text{L}$  of 10 x Gold buffer, 3 mM of  $\text{MgCl}_2$ , 200  $\mu\text{M}$  of each dNTPs, 20  $\mu\text{M}$  of each primers, 0.25  $\mu\text{L}$  of Taq DNA polymerase; water was added to make up a volume of 25  $\mu\text{L}$  for each reaction. The primers used are able to amplify a fragment of 317 bp from NSP5 gene, the most conservative gene among GARV strains and isolates (NSP5 gene of GARV OSU strain, accession no. X15519) (Salem et al., 2010). The nucleotide sequence of the primers used is the following: P1 Fw GGTTTAAAGCGCTACAG

TGATGTCTCT (1-29 bp) and P2 Rev GGTCGTGATTGTGTTGATGAATCCATA GA (289-317 bp). The amplification was carried out using the following thermal cycles: the initial denaturation at  $94^{\circ}\text{C}$  for 4 minutes was followed by 40 cycles of denaturation at  $94^{\circ}\text{C}$  for 1 minute, annealing at  $60^{\circ}\text{C}$  for 1 minute and extension at  $72^{\circ}\text{C}$  for 1 minute, with a final extension step at  $72^{\circ}\text{C}$  for 5 minutes. The PCR products were analyzed by electrophoresis in 2% agarose gel containing ethidium bromide and visualized using a UV transilluminator (BioRad).

## RESULTS AND DISCUSSIONS

The RT PCR assay performed on the diarrheic and normal fecal samples enabled identification of group A rotavirus. Using primers specific for the GARV NSP5 gene, there were detected 15 (60%) out of 25 GARV-positive fecal samples. A fragment of 317 bp was obtained and visualized in 2% agarose gel and UV light (Figure 1).

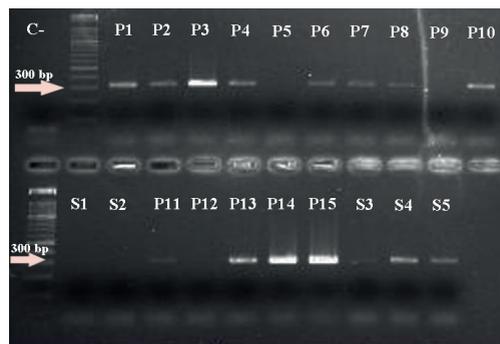


Figure 1. Detection of GARV by RT-PCR assay in fecal samples. A representative image of 2% agarose gel stained with ethidium bromide. C-: negative control; MW: DNA ladder of 100 bp; P1-P15 piglet samples; S1-S5 sow samples.

Out of 20 diarrheic sample, 12 (60%) specimens were identified as positive, while out of 5 normal fecal samples collected from sows, 3 (60%) samples were GARV positive (Table 1).

Table 1. Number of GARV positive samples (out of 25) detected by RT-PCR

Virus	Samples	No. of positive animals			
		Piglets		Sows	
GARV	Normal feces	-	-	3/5	60%
	Diarrheic feces	12/20	60%	-	-
<b>Total</b>	25	12/25	48%	3/25	12%

Similar findings were reported by different authors. Song and the co-workers reported a GARV infection rate of 13.2% in piglets farmed in Korea. Moreover, the same authors were reporting different enteropathogens alone or in combination in same specimens. Furthermore, the concurrent infections with porcine epidemic diarrhea virus and GARV was 43.2%, suggesting that GARV is a major enteropathogen in porcine livestock (Song et al. 2006). Beside the diarrhea-affected piglets, GARV infection may occur as asymptomatic. Indeed, a recent study revealed a prevalence of 26.2% of GARV in asymptomatic young pigs (Amimo et al. 2015). Moreover, it was shown that the farm size may influence the rate of infection; thus, the authors of this study concluded that the age, the management system and the pig density influenced the incidence of GARV infection (Amimo et al. 2015). In several countries, the importance of rotavirus group A in the etiology of diarrhea in suckling and recently weaned pigs is well characterized (Martella et al. 2011, Linares et al. 2009, Alfieri et al. 1991). In this study, we showed that 60% of the sows were asymptomatic carriers, as the antigen was detected in normal fecal samples. These interesting data show the importance of the sows in maintaining the virus circulation among the new born piglets. Interestingly, it was reported that in the week prior to farrow, 35% of the tested sows were excreting the virus by feces, while, during nursing the percent of sow excreting the antigen was higher.

PCR-based assays are becoming more and more the diagnostic tool of first choice in order to detect various pathogens in field fecal samples. In this study, we aimed to detect the presence of GARV in diarrheic piglets since this disorder was suspected for the high mortality of the suckling piglets. Therefore,

this RT-PCR assay proved to be a rapid and precise diagnosis assay for detection of porcine GARV. Furthermore, the primers annealing temperature (60<sup>0</sup> C) is able to confer to this assay an increased specificity and sensitivity. In order to prevent the economical loses, the using of a reliable diagnosis method allowing the rotavirus detection could contribute in achieving this goal, together with the identification and removal of the asymptomatic carriers.

## CONCLUSIONS

Finally, these data bring new insights into the enteropathogens circulating among the pig livestock in North-Eastern Romania and further studies are needed in order to better characterize the rotavirus genotypes detected in piglet fecal samples.

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