MOLECULAR DIAGNOSTICS OF ROTAVIRUS IN RABBITS, IN THE SOUTH-EAST OF MEXICO STATE

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ABSTRACT

In our country, the Mexico State is the major rabbit production entity, has an 45,000 bellies of inventory and here is produced about 2340 tons. Enteric diseases have an important role in rabbit production, because they cause severe economic losses due to mortality, growth depression and worsening of conversion index. Rotaviruses (RV) are members of the *Reoviridae* family, are non-enveloped viruses with a segmented double-stranded RNA (dsRNA) genome, is considered the main cause of acute viral gastroenteritis in different animals including rabbits. This agent is also a zoonotic disease. Among all enteric pathogens in humans, Rotavirus is the leading cause of severe acute gastroenteritis in infants and young children worldwide affect 95% of children under 5 years of age and cause 453,000 infant deaths annually. Group A rotavirus, Lapine strain (infecting rabbits), has been isolated by investigators in Europe, Japan, and the United States, however haven´t studies that identified molecularly Lapine Rotavirus strain in our country, for this reason, in this study we develop a molecular diagnostic of Rotavirus through the Reverse Polymerase Chain Reaction (RT-PCR), which will allow genotyping of strains that infect rabbits meat producers in the South-East of the State of Mexico. The molecular diagnosis was carried out through the use of the VP6 primers (VP6-F [sense] 5' GACGGVGCRACTACATGGT 3' and VP6-R [antisense] 5' GTCCAATTCATNCCTGGTGG 3') reported by Gómara., *et al* (2002).

Viral RNA was extracted using the GeneJET Viral DNA and RNA Purification kit of Thermo Scientific according to the manufacturer's instructions. As a positive control was used the RotaTeq vaccine. Has been performed the standardization and has been achieved the amplification of a 379pb region of VP6. The results show the presence of rotavirus in rabbits in the study region. We provide the first data on rotavirus in rabbits in Mexico. Data collected may contribute to avoiding economic loss, development of a vaccine and will assist in the resolution of public health problems.

Keywords: Rotavirus, gastroenteritis, rabbits, zoonotic, molecular diagnostic, genotyping**.**

Introduction

Rotaviruses (RV) are members of the *Reoviridae* family, *Sedoreovirinae* subfamily, are non-enveloped viruses with a segmented double-stranded RNA (dsRNA) genome, cause severe diarrheal disease in various species of birds and mammals, including humans.

Among all enteric pathogens in humans, Rotavirus is the leading cause of severe acute gastroenteritis in infants and young children worldwide, affect 95% of children under 5 years of age, although the main impact of rotavirus disease occurs in developing countries, where it causes over 453.000 deaths annually. Different Rotavirus strains infect particular species, however occasionally happens, for genetic reassortment, interspecies transmission, evidence of this is exposed in a study (Matthijnssens *et al.,* 2006), demonstrating transmission Lapine Rotavirus (which affects rabbits) to the human species, which gives Rotavirus infection, classification of zoonotic disease. Moreover, enteric diseases have an important role in rabbit production, because they cause severe economic losses due to mortality, growth depression and worsening of conversion index. Group A rotavirus, is considered the main cause of acute viral gastroenteritis in different animals including rabbits.

The Lapine strain (LRV) is considered slightly pathogenic, however it can primarily cause enteric disease in post-weaning rabbits, in addition it could also be involved in the etiology of severe enteritis outbreaks in association with bacteria, parasites and other viruses. Rabbits become infected by the oro-fecal route and the extension and the severity of the lesions (microvillus degeneration, malabsorption and diarrhoea) are dose dependent.

The Rotavirus infection is characterised by a high rate of morbidity and not specific clinical signs such as diarrhea, anorexia, depression, etc. Diarrhoea appears at the beginning of viral excretion that lasts for 6-8 days, and is generally followed by constipation. Lesions observed at necropsy are not constant: catarrhal, haemorrhagic or necrotic entero-tiflitis and caecal impaction. Rabbits patients may die due to dehydration and secondary infections while those that recover from the infection commonly show a decrease in productivity due to reduced absorption capacity.

Materials and methods

For the molecular identification of Rotavirus, Polymerase Chain Reaction Inverse technique was performed, wherein were used the *primers* designed and reported by Gómara., *et al* (2002), for amplification of a 379 bp fragment of the structural protein VP6 (VP6-F [sense] 5' GACGGVGCRACTACATGGT 3' and VP6-R [antisense] 5' GTCCAATTCATNCCTGGTGG 3'). Standardization was carried out using the pentavalent vaccine RotaTeq (live attenuated Rotavirus), which contains the human-bovine rotavirus strains variants: G1, G2, G3, G4 and P1A (genotype P1 [8]), same that was used as a positive control in all experimentation.

Viral RNA was extracted using the GeneJET Viral DNA and RNA Purification kit of Thermo Scientific according to the manufacturer's instructions.

After obtaining the viral RNA, RT (reverse transcription) was performed using the ImProm-II Reverse Transcription System, of Promega, whose final solution was 20 μl, the details are shown in Table 1. The PCR was performed in a final volume of 25 μl, the conditions and the result of standardization process are shown in Table 2.

Mixture 1	RNA	3 μl
	Oligo DT	2 μl
Mixture 2	Improm II 5x	4 μl
	Cl Mg	4.6 μl
	dNTP´s	2 μl
	RNA sin	0.5 μl
	RT	1 μl
	H2O	2.9 μl
Final volume		20 μl

Table 1. Components and volumes of the mixture to RT.

Element	Volume	Program features of PCR		
H2O	7.7 μl	Temperature	Time	Cycles
Buffer	5 μl	96°	10m	-
Cl Mg	4 μl	96°	45s	45
dNTP´s	1 μl	61°	45s	
VP6 Forward	2 μl	72°	45s	
VP6 Reverse	2 μl	72°	5m	-
Taq	0.3 μl	4°	10m	-
cDNA	3 μl			
Final volume	25 μl			

Table 2. Final features of standardization process of PCR for amplification of VP6.

Once obtained the reaction, it was placed in an agarose gel 3% and the electrophoresis was performed, with a time of 30 minutes at 100 ° C.

Finished the standardization for amplification of the desired fragment, 7 samples of intestines of rabbits from the zootechnical place from Centro Universitario UAEM Amecameca were processed, aged approximately 45 days, 3 of which were positive for Rotavirus.

Results and discussion

Thanks to standardization process, we have achieved amplify a fragment of 379 bp of VP6 from RotaTeq vaccine (Figure 1). Same way, amplification was obtained in 3 of 7 processed samples (Figure 2).

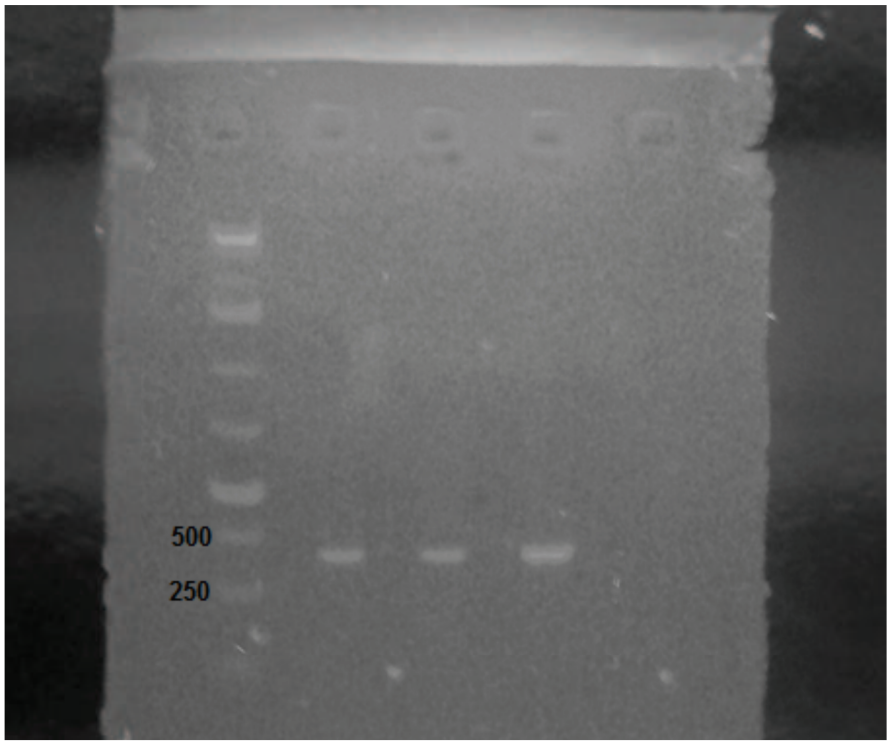

Figure 1. Amplified fragment of 379pb Rotavirus VP6 from RotaTeq vaccine

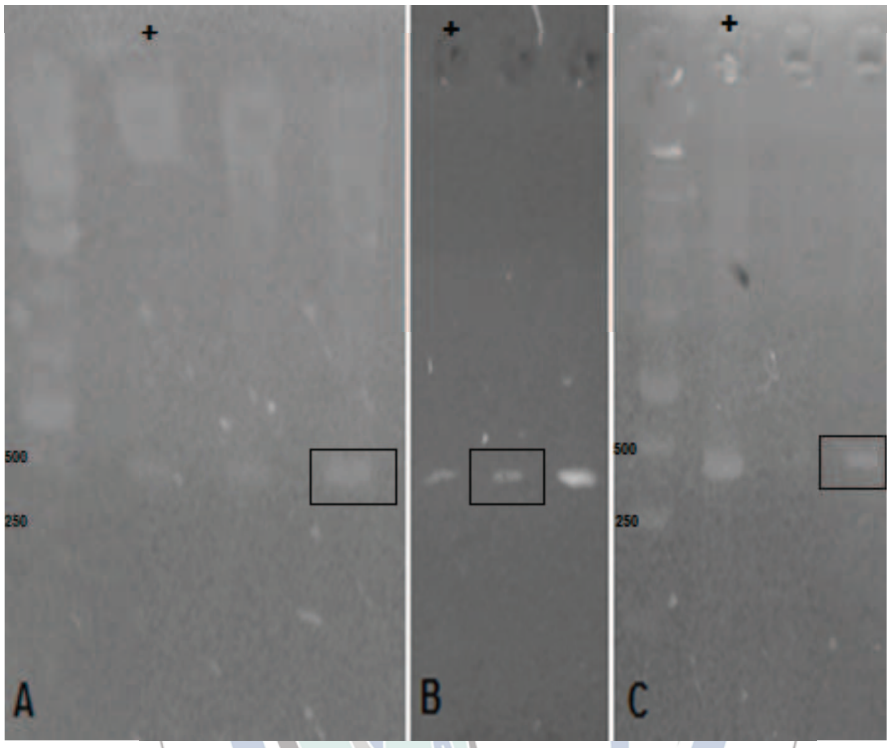

Figure 2. Amplified fragments of VP6 of 379pb: A and C; samples from asymptomatic rabbits. B; sample from rabbit with gastroenteric signs.

By the characteristics of the rabbits from which the samples were taken, the results show that rabbits can be asymptomatic carriers of Rotavirus, or their presence is determined by the dose and may be a transient infection with short periods of excretion. The pathogenic role and importance of rotavirus as primary aetiological agent rabbit enteritis is questioned.

Conclusions

Starting from multiple modifications in the protocols for the molecular identification of Rotavirus, we have achieved standardize the technique for the amplification of a fragment of 379pb of VP6. Rotavirus has been identified in asymptomatic rabbits and in one rabbit with gastroenteric signs, this being the first report of the presence of rotavirus in rabbits in Mexico.

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