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## MOLECULAR IDENTIFICATION OF PATHOGENS IN THE ENTERIC SYNDROME IN RABBITS FROM MEXICO

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### ABSTRACT

In Rabbits the enteropathies are difficult to diagnose; their etiology involves different pathogens that act concomitantly, causing damage to the intestine. The aim of the present study was identification by molecular techniques of the pathogens present in enteric rabbits in Mexico. From May 2014 to December 2019, we screened 228 samples of the intestinal content of rabbits having a clinical history of enteric disease. Out of the samples analyzed, *Eimeria* spp. were found in 63.59%, of this *E. Vejdovski* was identify in the 85% of the one sample screened by specific primers. Followed by Bacteria (49.15%) of this *Escherichia coli* was present in the 33.23%, which were identified as enteropathogenic *E. coli* (EPEC), Viruses was present in the 14.47% of the analyzed samples.

**Key words:** Rabbits, Enteropathies, Pathogens, Mexico.

### INTRODUCTION

Enteric diseases have an important role in breeding farms, as they cause serious economic losses due to mortality, growth depression and decline in the conversion rate [Lavazza et.al. 2008; Papp et. al. 2013]. The observed symptoms include anorexia, polydipsia, weakness, abdominal distension, profuse diarrhea, dehydration, hypothermia and death. Enteropathies are recognized as a problem for domestic rabbits, being their etiology and pathogenesis poorly understood. In commercial rabbit production, enteropathies are hard to diagnose; they can have a multifactorial etiology.

**Objectives :** The aim of this study was to isolate pathogens from enteric disease in rabbits from Mexico.

### MATERIALS AND METHODS

#### Animals and experimental design

Two hundred twenty-eight rabbits, with an enteric clinical profile were obtained from several rabbit meat production from the southeastern part of the State of Mexico, Mexico. The animals exhibited an enteric clinical profile that included depression, anorexia, dehydration, abdominal distension, liquid-to-mucoid diarrhea. The affected animals were young rabbits of 40-60 days old, they were humanely slaughtered according to the Official Mexican Standard NOM-033-SAG/ZOO-2014, and examined *post mortem*. Bacteriological and coproparasitoscopic examination was performed from intestinal contents. This study was authorized by the Bioethics Committee of the CU Amecameca (CBE/06/2013).

## Pathogens Identification

Intestinal content samples were assessed by a coproparasitologic study by means of the McMaster technique to detect the qualitatively and quantitatively *Eimeria* spp. oocysts present. Of the positive samples from the coproparasitoscopic examination, we proceeded to extract total DNA directly from 1 g of faeces, using the ZR faecal Microbe DNA Zymo Research®, following the manufacturer's instructions. The obtained DNA was visualised by electrophoresis on agarose gel 1%. For molecular identification, we used the primers ITS1F (5'GGGAAGTTGCGTAAATAGA 3') and ITS1R (5'CTGCGTCCTTCATCGAT 3') reported by Oliveira et al. (2011) that amplified a fragment of 400–600 bp for the, region of the eleven *Eimeria* species that affect rabbits. The amplification conditions were; Initial denaturalization at 96 °C for 10 minutes, 30 cycles consisting of 96 °C for 45 seconds, 61 °C for 45 seconds at and 72 °C for 1 minute at with a final extension step of 72 °C for 5 minutes. The amplified products are separated by electrophoresis on agarose 3% gels and stained with ethidium bromide.

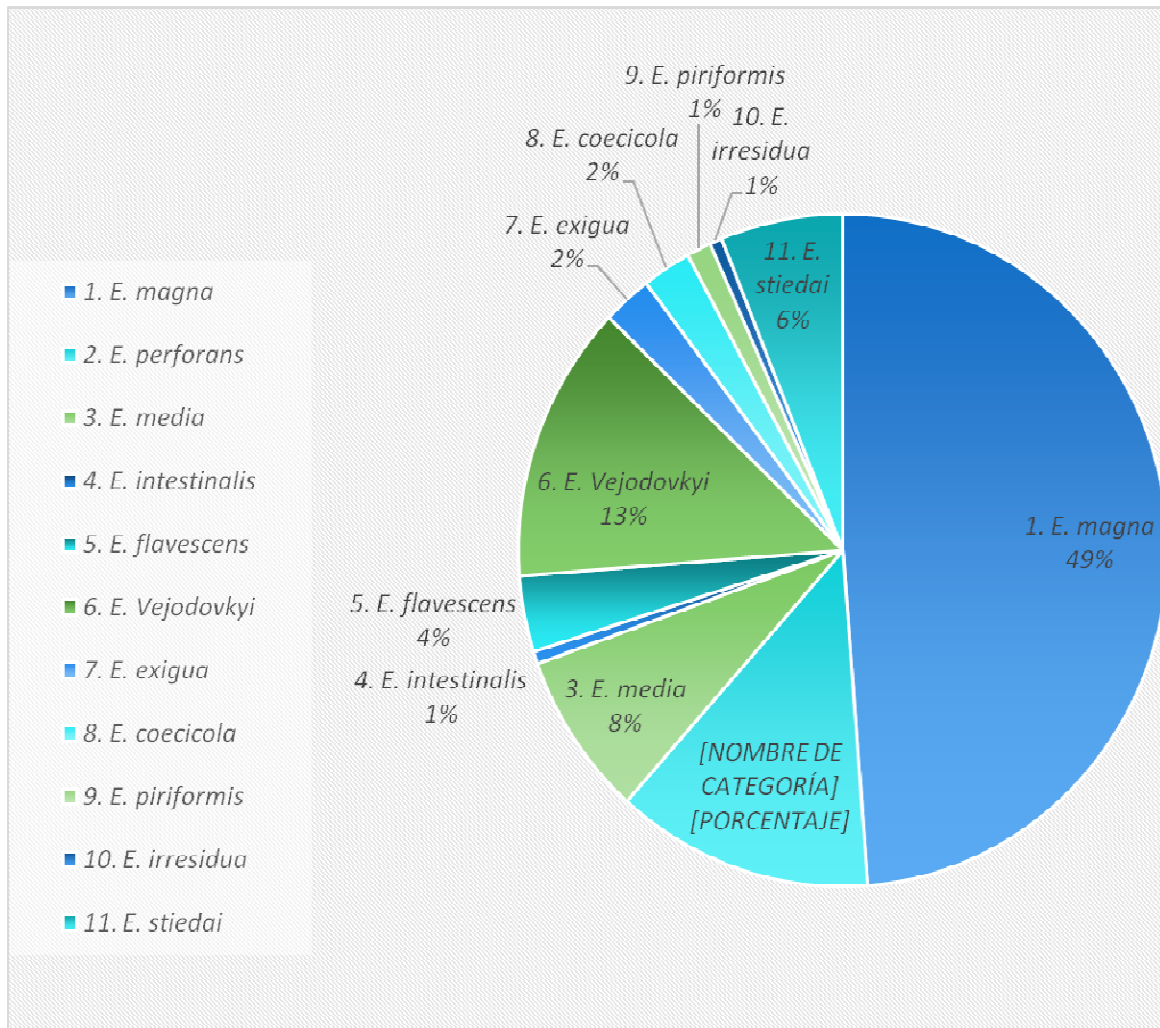
The bacteriological analyses involved primary isolation in blood agar; identification was performed by colonial morphology and Gram staining. Gram negative bacteria were cultured in the following selective media: MacConkey, Salmonella-Shigella and brilliant green agar at 37 °C incubation temperature and aerobiosis for 24-48 h.

The virological examination consisted of the detection of the VP4 and VP7 genes of rotavirus through the reverse polymerase chain reaction (RT-PCR), for norovirus, we amplified a 350 bp fragment of ORF1, that synthesizes a protein RDRP (RNA dependent of RNA polymerase), and for Astrovirus a fragment of 409bp of ORF1b was amplified. From the total RNA extracted from the sample. Primers reported by Gentsch et al., (1992) were used for the amplification of an 876 bp fragment that encodes the VP4 protein (Con 3 [nt 11–32] F 5' TGGCTTCGCCATTTTATAGACA 3' and Con 2 [nt 887–868] R 5' ATTCGGACCATTATAACC 3'). For VP7 amplification, the primers reported by Gouvea et al., (1990) were used, (Beg 9 [nt 1–28] F 5' GGCTTTAAAAGAGAGAATTTCCGTCTGG 3' and End 9 [nt 1062–1036] R 5' GGTCACATCATACAATTCTAATCTAAG 3'). The primers NoVFor TGACAATGTAATCATCACCATA y NoVRev TGACGATTCATCATCACCATA (Caddy, et. Al. 2015), was used for Norovirus and panASTVFor2 5'GARTTYGATTGGRCKAGKTAYGA3' and panASTVRev 3'GGYTTKACCCACATNCCRAA5' (Chu et al., 2008). For Astrovirus. Viral RNA was obtained from both tissue and intestinal contents using the GeneJET Viral DNA and RNA Purification kit (Thermo Scientific™), according to the manufacturer's instructions. Once the viral RNA was obtained, a single-step RT-PCR was performed using the commercial kit SuperScript® III One Step RT-PCR with Platinum® Taq (Invitrogen™). PCR products were visualized on 2% agarose gels stained with ethidium bromide.

## RESULTS AND DISCUSSION

**Table 1.** Concomitant pathogens found in rabbit intestine samples from Mexico

Concurrent identification	Frequency	Percentage
<i>Eimeria</i> spp.	145	63.59
<i>Bacteria</i>	112	49.15
<i>Virus</i>	33	14.47
<i>Eimeria</i> spp. + <i>Bacteria</i>	91	39.91
<i>Eimeria</i> spp. + <i>Virus</i>	12	5.26
<i>Bacteria</i> + <i>Virus</i>	9	3.94
<i>Eimeria</i> spp. + <i>Bacteria</i> + <i>Virus</i>	12	5.26
<i>Astrovirus</i>	12	5.26
<i>Rotavirus</i>	5	2.19
<i>Norovirus</i>	16	7.01



**Figure 1.** Percentage of *Eimerias* by species found in twenty random samples

Out of 228 intestine content samples, (100.00%) were positive for at least one identification and 124 (54.38%) were in concurrence, including: 112 (49.12%) with two identifications and 12 (5.26%) with three identifications. Rotavirus, Norovirus and Astrovirus was identified for the first time in rabbits from Mexico in 33 samples (14.47%), of which 6 samples (2.63%) showed the identification of concomitant infection by Rotavirus, Astrovirus and Norovirus.

Enteric diseases causes 30-80% of deaths in farm animals from 6 to 14 weeks old, across the world, by this have an important role in rabbit production, as they can lead to severe economic losses due to mortality, growth depression and reduced feed conversion rate. Enteric syndrome is one of the most important diseases in rabbits, especially in relation to its productive and economic impact. Among the different pathogens that can be found in rabbits with enteric disease, viruses seem to have an important but not definitive role. (Lavazza and Capucci, 2008). Bacteriological and coproparasitoscopic tests identified the presence of potential pathogens in rabbits (Licois, 2004); *E. coli* in three samples and *Eimeria* spp.,

Studies in other countries have reported the presence of eleven *Eimeria* species that affect rabbits in up to 70% of enteropathies, and, according to their level of pathogenicity, can cause reduced growth rate and feed conversion, and increased mortality.

## CONCLUSIONS

The presence of different pathogens in more than half of the intestinal content samples of rabbits with enteric symptoms indicates the need to carry out a greater number of studies to determine if these pathogens in isolation cause enteric profiles distinct than when they are found concomitantly.

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