

## Descripción fenotípica y microscópica de bacterias aisladas del contenido cecal de conejos con enteropatía epizootica en granjas del Valle de Tulancingo

Phenotypic and microscopic description of isolated bacteria of cecal content of rabbits with epizootic enteropathy in farms of Tulancingo Valley

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### Abstract:

Epizootic Rabbit Enteropathy (ERE) is one of the health problems that cause the major economic losses to rabbit farmers, it is a condition that is potentially fatal for infected animals and continues to threaten the rabbit production industry nationally and internationally. The first report of the condition dates back 100 years, which is based on a description of symptoms like ERE; however, the first definitive description of ERE dates to France, at the end of 1996 and to Galicia, Spain in September 1996. Etiologic of the disease and the candidate organisms that seem to be associated with it, are not fully defined, it has been There has been much discussion about its viral or bacterial origin, however, food and the environment play a decisive role in keeping the disease under control; sanitary control of drinking water and food, hygiene and monitoring of comfort factors, are decisive in preventing this disease.

### Keywords:

Rabbit farming, Bacterial diseases, Rabbit diseases

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### Resumen:

La enteropatía epizootica del conejo (EEC) es uno de los problemas de salud que causan las principales pérdidas económicas para los criadores de conejos, es una condición potencialmente mortal para los animales infectados y continúa amenazando a la industria de producción de conejos a nivel nacional e internacional. El primer informe de la condición se remonta a 100 años, se basa en una descripción de síntomas similares a EEC; sin embargo, la primera descripción definitiva de EEC data de Francia, a finales de 1996 y de Galicia, España, en septiembre de 1996. La etiología de la enfermedad y los organismos candidatos que parecen estar asociados con ella no están completamente definidos. Se ha debatido mucho sobre su origen viral o bacteriano, sin embargo, los alimentos y el medio ambiente juegan un papel decisivo para mantener la enfermedad bajo control; el control sanitario del agua potable y los alimentos, la higiene y el control de los factores de confort son decisivos para prevenir esta enfermedad.

### Palabras Clave:

Cunicultura, Enfermedades bacterianas, Enfermedades en conejos

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### Introduction

Rabbit farming is a livestock activity with great development potential for small and medium producers, because the rabbit is a species of easy handling and very prolific; furthermore, its meat is considered one of the most nutritious for its significant content of polyunsaturated fatty

acids, low in sodium and its content of vitamins and minerals 1.

Although in Mexico, rabbit breeding has not made significant progress, compared to productions of poultry, pigs and cattle, rabbit meat production has increased, mainly in the central states of the country, its progress has

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been limited due to the lack of interest in public policies that promote the development of this species of production, as well as the scarcity of health policies that help prevent and minimize diseases, mainly enteropathies, which most affect this species 2. Rabbit is predisposed to suffer digestive disturbances, especially in weaned animals, due to the changes that the gastrointestinal tract undergoes to adapt to solid food, at peculiarities of the cecal microbiota and its sensitivity to stress; in the presence of a digestive problem, it is difficult to know its origin, mainly due to the participation of various etiological agents and the similarity of clinical signs and injuries with other disease 3. Epizootic Rabbit Enteropathy (ERE) is one of the main health problems that generate most economic losses in productions, it is a condition that is potentially fatal for infected animals and continues to threaten rabbit productions national and international 2. Although it is a disorder of the digestive tract, the impact of the disease can extend beyond digestive problems, mainly affecting rabbits between 3 and 7 weeks of age, sick rabbits show a 50% reduction in their daily food consumption by going from 110 g per day to 55 g per day for about 7 days; although ERE is not always fatal, rabbits that survive the disease are lighter compared to healthy rabbits, these conditions cause a decrease in productivity, due to growth retardation and the low rate of daily weight gain; in turn, this leads to a decrease in the amount of meat produced, and affects profit margins 4. ERE is transmitted horizontally by the oral-faecal route (caecotrophy) and oral-oral contact (socialization), this is a reflection of the close contact that exists between animals in the productions 5. It is initially characterized by a moderate and slight increase in food and water consumption, then comes an abdominal dilation, after this phase, food consumption is drastically reduced until nothing is consumed, so that animals only ingest water and overtime the consumption is reduced, as the disease progresses, rabbits are located in areas far from the cage, with decreased reflexes and mobility 6, abdominal dilation remains and may progress with formation of a long, hard mass in the abdomen, in some cases a gnashing of teeth may be heard; diarrhea does not occur in all animals, sometimes diarrhea is preceded by the emergence of a translucent gelatinous mass after a period of three to five days, then of the first signs, the animal dies; survival rate is usually very low 7.

Currently the etiology of ERE has not been well defined; however, the first description given for this disease dates back to 1996 in the countries of France and Spain, where it was suggested that the main microorganisms that provoked disease were *Escherichia coli*, *Clostridium perfringens*, *Salmonella tiphymurium* and the parasite *Eimeria sp.*, caused anorexia, abdominal distention due to the accumulation of gas and liquid in the stomach,

constipation, diarrhea and the presence of clear mucus in the stool, despite the fact that the signs of the disease is well defined, its etiology and the possible microorganisms that seem to be associated with the incidence of the disease are not very clear 4, it has been suggested that it exists a relationship between rabbit gastrointestinal microbiota with the presence of ERE 8. Traditional bacterial phenotypic identification schemes are based on the observable characteristics of bacteria such as: morphology, development, biochemical and metabolic properties; bacterial cultures continues to be the diagnostic major method; allowing the isolation of the microorganism involved, for the culture the correct choice of the growth medium and the incubation conditions followed by a morphological, biochemical and microbiological analysis of the isolated bacteria is essential 9; for the above, the aim of this research was to describe phenotypically and microscopically the bacteria isolated from the cecal content of rabbits that presented signs of ERE in rabbit farms in the Tulancingo Valley, Hidalgo.

#### Materials and methods

A survey was conducted of 11 rabbit producers located in the Valle de Tulancingo: "Mi Pequeña Esperanza" of Mr. Daniel Villalpa Romero located in Santiago Tulantepec de Lugo Guerrero municipality (20°2'23" N, 98°21'27" W); "Campis" of Mr. Sergio Hernández Maldonado, "Roberto" of Mr. Roberto Rondal, "Jofaya" of Mr. Jorge Enrique González García and "Karla" of Mr. Carlos Muñoz Hernández located in Cuauhtepic de Hinojosa municipality (20°09'00" N, 98°26'00" W); "Mi granjita" of Mrs. Nancy Natali Rosal Fernández, "Jaltepec" of Miss Laura Nataly Hernández Terrazas, "Gayoso" of Mrs. Rosa María Pérez Ramírez and "Espuela de oro" of Mr. Jaime Ibáñez located in Tulancingo de Bravo municipality (20°05'09" N, 98°21'48" W); "El Encinal" of Mrs. Karla Álvarez Otero and "Tolteca" of Mr. Edgar Ricardo López Vargas located in Singuilucan municipality (19°58'03" N, 98°31'02" W). A monitoring was carried out during the months of February to May 2019 in order to select rabbits with signs of ERE; rabbits with signs were taken to the Anatomy and Necropsies laboratory of Instituto de Ciencias Agropecuarias of Universidad Autónoma del Estado de Hidalgo; were given death according to the NOM-056-SAG-ZOO-2014, to subsequently perform the necropsy and take sample of cecal content.

Cecal content was obtained under aseptic conditions and conserved in a peptone water buffer with a pH of 7.2 for 2 hours at 4°C for subsequent phenotypic and microscopic analysis. 1 ml of cecal content was used to make dilutions  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ , which were plated on standard agar bead, then were incubated at 35°C during 48 hours; after, was chosen dilution with best colonies isolated, which were transferred under sterile conditions

and using a bacteriological loop to soy broth and triple casein with 0.05% yeast extract and incubated at 35°C, during 24 hours; after time, 1 ml of the culture was recovered, it was centrifuged at 3500 rpm for 5 minutes and the bacterial button was stored with triple casein soy broth and 50% v/v glycerol in cryo tubes at -20°C to microscopic analysis and staining Gram.

Sterile conditions, petri dishes were prepared with 15 ml each of violet red bile agar; the strains used were sown by cross striations. It was incubated for 18 to 24 hours at 37°C, after the incubation time, plates with the presence of colonies were checked, were isolated was taken and placed on a slide with a drop of peptone water, once mixed a drop was placed and then was fixed 10. Subsequently, the Gram stain was performed, first all the bacterial smear was soaked with violet crystal as the primary dye for 60 seconds and rinsed with running water, then lugol was added for 60 seconds and it was rinsed with running water, later for the discoloration two drops of alcohol-acetone were used for 10 seconds and rinsed with running water, finally safranin was added for 60 seconds and it was last washed with running water to remove excess stain 10, then they were observed under a microscope. For the catalase test, a roast was taken from an isolated colony from a 24-hour culture. The sample was contacted over a drop of 3% hydrogen peroxide. When bubbles appear, it was reported as catalase positive 11.

### Results

Of the rabbits that presented ERE signology in the farms of the Tulancingo Valley, 128 bacteria were isolated from the cecal content; regarding the colonial morphology, most presented circular shapes, medium size, convex, with rounded edges and the centre darkened on the other hand, only 15 of the 128 bacteria analysed presented small shapes and pointed (Figure 1).



**Figure 1.-** Colonial morphology of bacteria isolated from the cecal content of rabbits with ERE on Red Violet Bile Agar.

Regarding the results of Gram staining, results was observed in Table 1, 84 of the 128 samples analysed were Gram negative bacilli (Figure 2), 33 were Gram positive

bacilli (Figure 3), 10 Gram positive cocci (Figure 4) and only one Gram negative coco (Figure 5).

**Table 1.-** Gram identification of bacteria isolated in the cecal content of rabbits that presented singnology the ERE.

Morphology	Number
Gram negative bacilli	84
Gram positive bacilli	33
Gram positive coco	10
Gram negative coco	1
<b>Total</b>	<b>128</b>



**Figure 2.-** Gram negative bacilli.



**Figure 3.-** Gram positive bacilli.

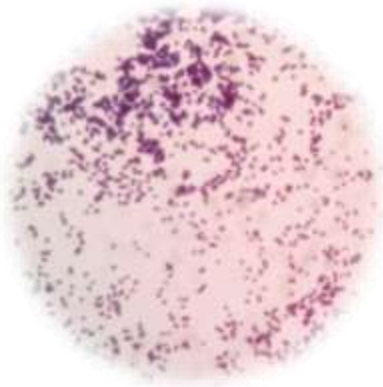


Figure 4.- Gram positive cocci.

The results of the catalase test showed 79 of 128 samples analysed were positive (Figure 6) and 49 were negative (Table 2).

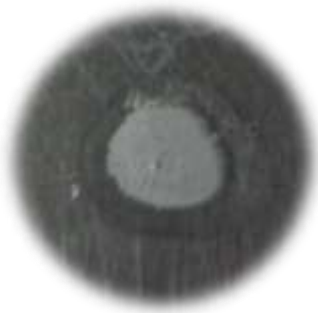


Figure 6.- Catalase test positive result.

Table 2.- Catalase test in bacteria isolated in the cecal content of rabbits that presented the ERE.

Catalase test	Number
Positive	79
Negative	49
<b>Total</b>	<b>128</b>

### Discussion

Epizootic Rabbit Enteropathy is one of the main health problems that generate most economic losses in productions of rabbits 2. Even though the signology of the disease is well defined, its etiology and the possible microorganisms that seem to be associated with the incidence of disease are not very clear 4. The presence of habitual gram-negative aerobic bacteria in the microbiota of the intestines of rabbits such as *E. coli*, *Streptococci*, *Clostridia*, *Salmonella* 6, suggest that there may be a relationship between the gastrointestinal microbiology of the rabbit with the presence of the Epizootic Rabbit Enteropathy 8. The increase in the concentration of

enterobacteria which are characterized by being mainly gram-negative, can unbalance the bacterial symbiosis causing enteropathy in rabbits, affecting production and causing losses in rabbit farms 12

### Conclusion

Gram negative, catalase positive bacteria are more present in the cecal content of rabbits with Epizootic Rabbit Enteropathy in farms in the Tulancingo Valley, these results give similar information with the characteristics of the bacteria that have been related to ERE in the literature cited. Molecular or biochemical testing is necessary to identify the genus and species of each bacterium.

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