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ISOLATION OF *FUSOBACTERIUM NECROPHORUM* AND *PASTEURELLA MULTOCIDA* IN RABBITS FROM MÉXICO

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ABSTRACT

In the following study, a total of 51 samples were collected from individual cases of rabbits that presented respiratory signs, of dead rabbits and of apparently healthy rabbits, originating from breeding farms located in the state of Mexico. Samples were taken for bacteriological analysis, obtaining 19 isolations (37.2%) of P. *multocida*. Of the samples collected, a 3 year-old male rabbit, of New Zealand breed, presented consistent lesions during the necropsy in purulent abscesses in lungs, from which samples were taken for bacterial cultivation, isolating F*usobacterium necrophorum* and P*asteurella multocida*. The isolation of P. *multocida*, was tested by PCR assays to confirm identification and to determine how many of these belong to capsular serotype A. The 19 isolates showed positive results, therefore, all were type A capsules, obtaining associations with macrolide resistant strains.

Key words: Rabbit, Bacteria, F*usobacterium necrophorum*, P*asteurella multocida*.

INTRODUCTION

Farm rabbits are extremely sensitive animals to stress, pathogens and any factor that alters their environment, which translates into the development of digestive and respiratory processes that compromise the health and productivity of the farm. (Villa et al., 2001). Respiratory tract infections occupy second place in terms of the degree of importance, occupying the first place is infections of the digestive system, due to the percentage of farms affected, and the number of animals that get sick (Rosell, *et al*., 2000b).

Pasteurellosis is one of the non-digestive infectious diseases, more frequently found in cunicultural farms (Coudert 1999). P. multocida is a prominent pathogen in farm rabbits; however, pathological characteristics have already been reproduced with P.*haemolytica* (now known as M*annheimia haemolytica*) (Ramirez-Romero, *et al*., 1997).

As well as Pasteurellosis, F*usobacterium necrophorum* is an important animal pathogen (Nagaraja *et al.*, 2005). Because infections caused by F*usobacterium spp*. are characterized for causing severe sepsis known as necrobacillosis or Lemierre's syndrome, and if it is not treated properly, septic embolus formation occurs more commonly at the pulmonary level (Grasa *et al*, 2018), thus causing death.

Objective: Identification of bacteria present in rabbits with respiratory symptoms.

MATERIALS AND METHODS

Sample collection:
In the period from January to August 2019, 51 samples of rabbits with respiratory clinical signs were collected (nasal and ocular secretion, rhinitis, dyspnea, sneezing), apparently healthy rabbits and dead rabbits from different farms of cunicultural production in the State of Mexico. Of the samples

obtained, the C50 stands out, corresponding to a male rabbit, 3 year-old, of New Zealand breed, intended for breeding in the rural area in the town of Texcoco, Mexico, it also presented the characteristic signs of a respiratory infection, an evident hemorrhage in the ear canal.

Necropsy

Of the 51 rabbits sampled in the C50, mucopurulent secretion was observed in nasal passages and evident bleeding in the ear canals (Figure 1 and Figure 2).

In the necropsy, all the organs were inspected, from cranial to caudal, in this procedure significant lesions were found such as purulent abscesses in the lungs, congestion in the heart, and areas with necrosis in the small intestine (Figure 3).

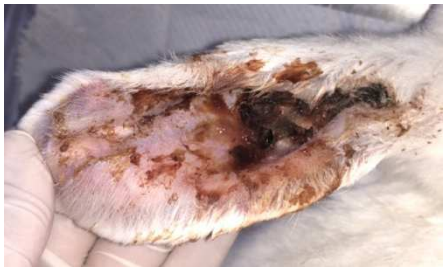

Figure 1. Auditory cavity with apparent hemorrhage and secretion

Figure 2. Mucopurulent secretion in nostrils

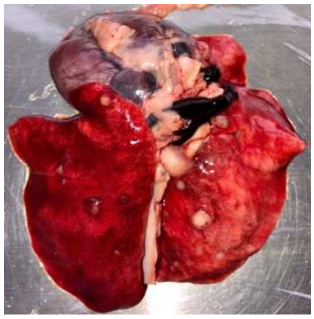

Figure 3. Purulent abscesses in the lungs

Sampling for bacteriology

The collection of the samples was conducted in the microbiology laboratory of the UAEM Amecameca University Center, located in the State of Mexico. Samples of purulent exudate from lung abscesses and intestinal contents of the aforementioned lesions of the rabbit were collected by sterile swabs for immediate sowing in blood agar.

Obtaining pure samples

The isolation and identification of the colonies were done by sowing in blood agar boxes from the swab, they were incubated for 24 h at 37℃. Subsequently, Gram staining was performed. The morphological characteristics of the developed colonies were observed. A new sowing was performed selecting those that matched the specific morphology of P. *multocida* (round, gray, mucoid and without the presence of hemolysis) and F. *necrophorum* (round, whitish and mucoid) in order to obtain pure samples (Figure 4), observed previously under the microscope and finally the biochemical identification was conducted.

Figure 4. Second sowing of previously selected colonies.
B.-P*asteurella multocida*
C.-F*usobacterium necruphorum*

Biochemical tests

The biochemical identification of the P. *multocida* and F. *necrophorum* strains was performed, through the identification tests of LIA, MIO and TSI. The tests were performed on colonies incubated for 18 and up to 24 h in blood agar.

RESULTS AND DISCUSSION

Results

The blood agar, for its high nutritional value, allows the growth of a large variety of microorganisms, including those nutritionally demanding, therefore, when sowing in this medium, we were allowed to grow different colonies, nevertheless; colonies were selected with a very specific morphology such as

the P. *multocida* and F. *necruphorum* strains. For the observation of the isolated colonies, Gram staining was used and subsequently observed under a microscope (Figure 5 and Figure 6).

The identification by biochemical tests was performed with TSI, LIA and MIO (Table 1).

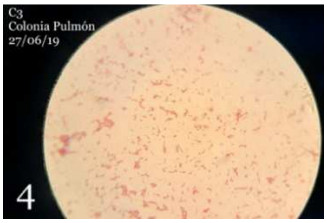

Figura 5. Coccobacillus gram negativos, P*asteurella multocida*

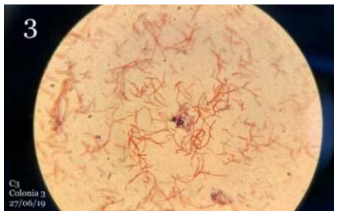

Figure 6. Elongated folate bacillus, Fusobacterium necrophorum.

Table 1. Results of identification by biochemical tests.

N°	ID	TSI				MIO			Ox	Ca	Ur	MacConkey	
		Gl	La/Sa	Gas	H_2S	M	I	O				C	F.L.
1	C-2	+	+	-	+	-	+	+	+	+	-	-	-
2	C-3	+	+	-	+	-	-	-	+	+	-	-	-
3	C-4	+	+	-	+	-	+	+	+	+	-	-	-
4	C-7	+	+	-	+	-	+	+	+	+	-	-	-
5	C-9	+	+	+	+	-	+	+	+	+	-	-	-
6	C-11	+	+	-	+	-	-	+	+	+	-	-	-
7	C-16	+	+	-	+	-	-	+	+	+		-	-
8	C-17	+	+	-	+	-	+	+	+	+	-	-	-
9	C-20	+	+	-	+	-	+	+	+	+	-	-	-
10	C-21	+	+	-	+	-	+	+	+	+	+	-	-
11	C-27	+	+	-	+	-	+	+	+	+	-	-	-
12	C-28	+	+	-	+	-	+	+	+	+	-	-	-
13	C-33	+	+	-	+	-	+	+	+	+	-	-	-
14	C-35	+	+	-	+	-	+	+	+	+	-	-	-
15	C-39	+	+	+	+	-	+	-	+	+	-	-	-
16	C-40	+	+	-	+	-	+	-	+	+	-	-	-
17	C-43	+	+	-	+	-	+	+	+	+	-	-	-
18	C-44	+	+	-	+	-	+	-	+	+	-	-	-
20	C-50	+	+	-	+	-	+	+	+	+	-	-	-
	C.50b	+	+	+	-	-	+	-				F*usobacterium necrophorum*	

TSI: Gl (fermentación de glucosa), La (fermentación de lactosa), Sa (fermentación de sacarosa), Gas (presencia de gas), H2S (producción de ácido sulfhídrico).

MIO: M (motilidad), I (indol), O (ornitina decarboxilasa). Ox (oxidasa). Ca (catalasa). Ur (ureasa). MacConkey: C (crecimiento), F.L. (fermentación de lactosa).

DISCUSSION

Pasteurellosis has been recognized as a disease of great economic impact in rabbit farms. This pathogen is generally endemic in cunicultural populations, whose prevalence has been estimated from 7% to almost 100%, both in rabbits housed in laboratory conditions and in meat producing farms (DiGiacomo et al. 1983). Its confirmation is difficult due to the variety in clinical signs and because the procedures for its diagnosis can take a long time. Bacterial agents are the most commonly involved pathogens, there is currently no effective and simple test to determine the etiology of the disease. In this study, it was identified the presence of F*usobacterium necrophorum* which is not common in rabbits, nevertheless, this bacterium is part of the oropharynx Flora and the digestive system of humans, all this makes it an anthropozoonotic disease, it means it may be misdiagnosed and unreported; P. *multocida* was identified in 37.2% of the total of samples analyzed, a result superior to that reported in the United States, (7%) Brazil (21%) (Ferreira et al. 2012) and in Egypt, (27%) and 9.4% (Asran et al. 2016).This prevalence may be due to various factors, such as geographical locations, environmental conditions , and mainly due to differences in the types of rabbit production.

In this study the sampled farms were backyard, type of production that represents more than 95% of the rabbit production in Mexico (SAGARPA, 2015).

CONCLUSIONS

Since there is a great disinformation on the part of the producers regarding the knowledge of the signs caused by Fusobacterium, there is a high probability that several cases have been misdiagnosed, being confused with diseases that present similar clinical characteristic such as Pasteurellosis, allowing the bacteria to remain on farms constantly and thus, causing significant economic losses. The use of microbiological laboratory tests allows to reach the final diagnosis.

REFERENCES

Asran, E.A., Khalil, S.A., Abd El-Halim M. 2016. Identification and Molecular Analysis of Pasteurella Multocida Isolated from Rabbits, *Alexandria Journal of Veterinary Sciences, 48, 34–41*.

DiGiacomo, R.F., Garlinghouse, L.E., Van Hoosier, G.L. Jr., 1983. Natural history of infection with Pasteurella multocida in rabbits, *Journal of the American Veterinary Medical Association, 183, 1172–1175.*

Ferreira, T.S., Felizardo, M.R., Sena de Gobbi, D.D., Gomes, C.R., Nogueira Filsner, P.H., Moreno, M., Paixao, R., Pereira, JdeJ., Micke Moreno, A., 2012. Virulence genes and antimicrobial resistance profiles of Pasteurella multocida strains isolated from rabbits in Brazil, *ScientificWorld Journal,2–6.*

Grasa, F., Palomo, D., Bollici, L., Antúnez, S., Serratos, F., Gómez, A., Pradas, M.. , 2018. Necrobacilosis abdominal como causa inusual de shock séptico . *Cirugía Andaluza, 29, 11-15.*

Nagaraja, T. G., Narayanan, S. K., Stewart, G. C. & Chengappa, M. M. , 2005. Fusobacterium necrophorum infections in animals: pathogenesis and pathogenic mechanisms. *Anaerobe11,239-246.*

Ramirez-Romero, M., Bustos,P., Girard,L., Rodriguez, O., Davila,G.. ,1997. Sequence, localization and characteristics of the replicator region of the symbiotic plasmid of Rhizobiurn etli. Universidad *Nacional Autonoma de Mexico, 143, 2825-2831.*

Scalan, Ch.. , 1991. Familia enterobacteriaceae, bacilos gramnegativos aerobios. En Introducción a la bacteriología veterinaria. *España: Acribia, S.A..(87-179).*

SAGARPA. 2015. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. Manual de Buenas Prácticas de Producción de Carne de Conejo. *Disponible en http://sistemaproductocunicola.org.mx/descargas/ManualdeBuenasPracticasdeProduccíondeCarnedeConejo.pdf [Última consulta: 14 de julio de 2019].*

Selva, L.; Viana, D.; Ortega, J. y Corpa, J.M. , 2007. Pasteurelosis: principal patología respiratoria en cunicultura industrial. *Boletín de Cunicultura, ISSN 1696-6074, Nº. 150, 2007, pags. 14-24.*

Percy, D., Barthold, S.. 2007. Rabbit. *En Pathology of laboratory rodents and rabbits. (264-285). Malaysia: Blackwell Publishing.*

Villa, A, E. Garcia, A. Fernandez, I. Albizu, R. Baselga. ,2001. The detection of mycoplasmas in the lung of rabbits with respiratory disease. *Veterinary Record, 23, 148, 788-789*