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MOLECULAR IDENTIFICATION AND PHYLOGENETICS ANALYSIS OF *Pasteurella spp.* IN RABITS FROM THE SOUTH EAST OF THE MEXICO STATE

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ABSTRACT

One factor that affects the efficient development of rabbits in Mexico is the high mortality rate in rabbit farms (SAGARPA, 2012). *Pasteurella multocida* is responsible for the most economically important diseases in animals in both developed and developing countries (Dziva, et al., 2008). the objective of this study is to perform molecular identification and phylogenetic of *Pasteurella multocida* on rabbits that present respiratory symptoms of all ages as well as in apparently healthy individuals who are in the same units of rabbit production where they are presenting respiratory symptoms.

Keywords: *Pasteurella multocida*, molecular diagnostics , genetic diversity.

Introduction

Poverty is one of the great challenges to be met by the State, that 47% of the Mexican population is in some degree of food poverty, capacity or equity, SEDESOL (2008) mentions that more than 86% of the poor are located in rural areas. The rabbit is a livestock activity has been shown to be important in the past four decades, the business has established itself as an alternative to solve food problems and poverty in rural and suburban society. The production of rabbit noted for its productive and reproductive easy handling, quality of derived products, the beneficial characteristics of meat and integration and acceptance in markets (Garcia et al., 2005). One factor that affects the efficient development of rabbits in Mexico is the high mortality rate in rabbit farms (SAGARPA, 2012). *Pasteurella multocida* is responsible for the most economically important diseases in animals in both developed and developing countries (Dziva, et al., 2008). It is the most common pathogen reported in rabbits, lodges in the nasal cavity causing rhinitis and chronic asymptomatic infections, resulting in a variety of clinical manifestations including pneumonia, otitis media, conjunctivitis, abscesses, respiratory tract infections and septicemia (De Long, 2012). As discussed above, the objective of this study is to perform molecular identification and phylogenetic of *Pasteurella multocida* on rabbits that present respiratory symptoms of all ages as well as in apparently healthy individuals who are in the same units of rabbit production where they are presenting respiratory symptoms.

Material and Methods

Bacterial ID

The sampling was a convenience, in rabbits with respiratory clinical symptoms and apparently healthy, who is housed in the same facilities and conditions of different units of rabbit production in the south east of the State of Mexico. A total of 49 samples of rabbits from Tlalmanalco Amecameca, Atlautla and Ozumba municipalities,, were collected from January to September 2013. isolates have been recovered from nasal passages, in live animals, and in the case of dead rabbits, the trachea and lungs was recovered using sterile swabs.

Sampling was performed using swabs, these are placed in Stuart transport medium (Difco-BBL) and maintained under refrigeration until analysis. Each swab is sown in Petri dishes containing blood agar (Difco-BBL) with 5% whole sheep blood and incubated at 37 ° C for 24 hours, then the routine bacteriological procedures for isolation is used, including biochemical tests catalase, oxidase, indole, urease activity, production of ornithine decarboxylase and carbohydrate fermentation.

Molecular identification

A colony from blood agar cultures (Difco-BBL) with 5% sheep whole blood and incubated at 37 ° C for 24 hours. It was processed for extraction of DNA using the Wizard ® Genomic DNA Purification Kit PROMEGA. A fragment of 520pb of *hyaC-hyaD* (access number AF067175) gene was amplified using the primers RGPMA6 and RGPMA5 described by and Gautam, et al., 2004. The amplified fragments were purified from gel and sequenced for phylogenetic analysis. Multiple sequence alignment was performed using the MEGA 6 software(Tamura et al. 2007).

RESULTS

The presence *of Pasteurella multocida* in 37% of the isolates were found. Of the 49 samples collected from rabbits of the Tlalmanalco, Ozumba, Amecameca and Atlautla municipalities, 18 isolates corresponded to *Pasteurella multocida*. Other bacteria identified in the sample were *Manhemia haemolitica*, *Streptococcus, Staphylococcus, Corynebacterium,* and *Bordetella bronchiseptica* .

The program for amplification of *Pasteurella multocida* has been implemented by the PCR technique, which seeks to determine from these 18 isolates of *P. multocida* rabbit, The amplified fragments were purified from gel and sequenced for phylogenetic analysis comparing the sequences obtained with the seven reported worldwide, from the United States, Germany, Czech Republic and Australia, using the 6 MEGA software (Tamura et al., 2007). In Figure 1, shown

the phylogenetic tree constructed with the Neighbor Joining method for the first two isolates sequenced. It can be seen that the strains isolated in this region belong to capsular type A and are genetically closer to those reported for isolates from pigs in the United States than those reported in other countries like Germany, Czech Republic, Australia, however remain in an independent branch of the isolated from other species. Suggesting the relative specificity of the bacteria host and those variants in the State of Mexico, but it is necessary to enrich the tree to get a larger number of sequences *P multocida* strains isolated in this study, order to confirm whether the bacteria found maintain diversity to those reported to worldwide level. It is important to highlight the genetic distance giP7 strain, also isolated from rabbit Czech Republic. Which is of interest to know to implement prevention strategies has considered strains to be included in the development of biological for use in cunícolas units in the region under study.

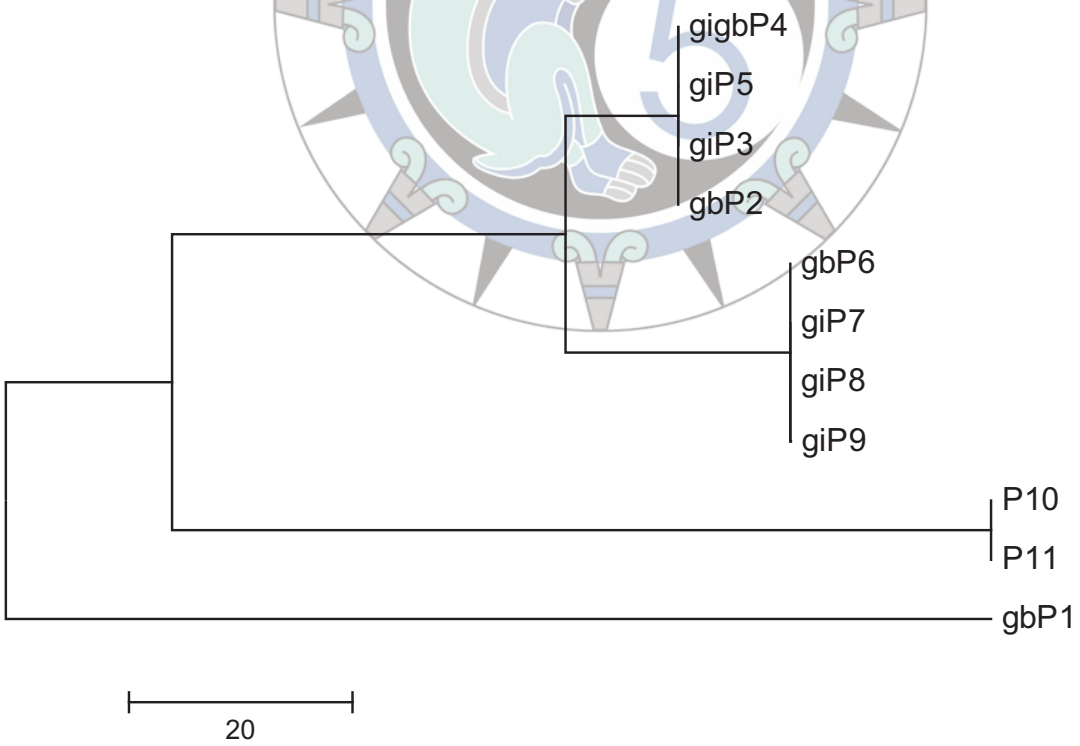

Figure 1. Phylogenetic Three of *Pasteurella multocida*.

GBP1 isolated Pasteurella multocida strain pig US isolate GBP2 bovine Germany, United States giP3b strain without host reported, gigb4 isolate mouse without the country reported, without strain giP5 host country or reported, GBP6 avian strain US giP7 rabbits strain isolated in the Czech Republic, giP8 turkey isolate US giP9 without host strain reported from Australia, P10 strain isolated rabbit Mexico, P11 strain isolated rabbit Mexico.