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PREVALENCE AND MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* AND *SALMONELLA SPP*. IN GUINEA FOWLS (*NUMIDA MELEAGRIS*) IN TWO RANCHES OF CHIAPAS, MEXICO

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— Abstract—

The objective of the study was to evaluate the prevalence and molecularly characterize the isolates of *Escherichia coli* and *Salmonella spp*. in Guinea fowl (*Numida meleagris*) in two ranches in the municipalities of Villaflores and La Trinitaria, Chiapas, Mexico. A total of 23 guineas were randomly sampled, from which cloacal samples were taken for bacterial identification using selective media, while the *rfb*O157 gene (producer of *Shiga* toxin) was analyzed for molecular characterization. Data analysis was carried out using point prevalence. The results showed that there was no presence of *Salmonella spp*. nor of *Escherichia coli* in the guineas of the La Trinitaria ranch. Regarding the guineas sampled in the Villaflores ranch, no presence of *Salmonella spp*. was found, however, a 95% prevalence of *Escherichia coli* was found. The molecular characterization of 10% of the *Escherichia coli* samples allowed the identification of the serotype O157: H7 (producer of *Shiga* toxin). There was no prevalence of *Salmonella spp*. in guineas; however, the study shows that guinea fowl are carriers of *Escherichia coli* O157: H7, so it is necessary to continue with studies of the impact of the serotype on animal health, as well as on public health.

Keywords
Enterohemorrhagic strain; Public health; Animal health.

Poultry represents one of the main sources of animal protein and for this reason, it is common to find different domestic birds under backyard conditions, where chickens (*Gallus gallus domesticus*) and turkeys (*Meleagris gallopavo*) are mainly observed, as well as ducks (*Anas platyrhynchos domesticus*), geese (*Anser anser*) and guinea fowl (*Numida meleagris*) (Zaldívar-Pedroso, 2007).

In Mexico, guineas as an exotic species occupy an area of approximately 5,648 ha. (SEMARNAT, 2000), being reported mainly in wildlife conservation management units (UMAS) in Coahuila, Hidalgo, and Nuevo León (Goméz-de Silva, et al., 2005).

Currently, the guinea fowl has become popular among people for breeding, either as an ornamental bird or as meat and/or egg producer, due to its hardiness and low management. This bird has its origins in Central and East Africa, and although it was already known by the Greeks and Romans, it has only recently been used for meat production (Zaldívar-Pedroso, 2007). Among the advantages of guinea fowl production is its hardiness and good adaptation to adverse conditions, qualities that make it an effective and novel poultry species for backyard producers (Gómez-de Silva, et al., 2005; Salgado, et al., 2011), although information about this species in terms of production is scarce, it is known that it is kept in some communities in the country (Camas-Robles, et al., 2017).

However, some diseases originated by bacteria directly affect birds, causing a considerable drop in production. The *Enterobacteriaceae* family is the largest and most heterogeneous family of Gram-negative bacilli with importance in human and animal health (Araujo-Guerra, et al., 2019). Among the most common bacterial diseases are *Salmonella spp*. and *Escherichia coli*, which affect the intestinal tract of the poultry, causing its death; additionally, some bacterial serotypes can reach the consumer through meat or eggs, generating a negative impact on food safety and security (Leotta, et al., 2005; Rincón-Acero, et al., 2011; Araujo-Guerra, et al., 2019).

Regarding *Escherichia coli*, many serotypes have been associated with hemolytic uremic syndrome in humans (Ochoa, et al., 2004), with serotype O157:H7 being its main cause. On the other hand, *Salmonella spp*. infections are related to diseases transmitted by contaminated food (Rincón-Acero, et al., 2011) such as meat, which are dangerous when kept under circumstances that favor their multiplication, a situation that represents a serious public health problem (Bello-Pérez, et al., 1990; Mussaret, et al., 2006).

Although guinea fowl farming represents an efficient alternative for small-scale food production, it is important to carry out studies to identify the microorganisms that endanger the health of both the animal and the consumer. Therefore, the objective of this study was to evaluate the prevalence and molecular characterization of *Escherichia coli* and *Salmonella spp*.

isolates in guinea fowl (*Numida meleagris*) on two farms in the municipalities of Villaflores and La Trinitaria, Chiapas, Mexico.

MATERIALS AND METHODS

The present study was carried out in the ranches of "Las Amazonas" in the municipality of Villaflores, Chiapas, located at the geographical coordinates 16° 13' 57''N, 93° 15' 57''W and "Monte Calvario" in the municipality of La Trinitaria, Chiapas, located at the geographical coordinates 16° 7' 13''N, 92° 2' 27''W. The identification of bacteria, as well as their molecular characterization, was carried out in the Molecular Biology Laboratory of the Faculty of Veterinary Medicine and Husbandry Campus (FMVZ) of the Universidad Autónoma de Chiapas (UNACH).

A random sampling without replacement was used, selecting 30% of the total population of birds in both ranches. Samples were taken by cloacal swabbing of the guineas with the support of Stuart medium, then placed under refrigeration at a temperature of 4°C until processing. The samples were inoculated in Petri dishes with *Salmonella Shigella* agar media (MCD Lab®) and *Methylene Blue Eosinia* (MCD Lab®) for the identification of *Salmonella spp*. and *Escherichia coli*, respectively. Subsequently, they were incubated at 37°C for 24 h; after this period, the bacteria were identified, being positive for *Escherichia coli* when the colonies had a phosphorescent green coloration and for *Salmonella spp*. when the colonies had dark coloration centers. Subsequently, a bacterial reseeding was performed, incubating the samples at a temperature of 37°C for 24 h, to isolate the positive colonies, then they were treated with a *Brain Heart Infusion* broth (MCD Lab®), to avoid the death of the colonies inside the agar medium, then they were homogenized with a Vortex type shaker and incubated at 37°C for 24 h after the time elapsed, a cloudy coloration was observed in the samples, indicating that the colonies grew in the broth; Subsequently, the samples were inoculated on *Muller Hinton* agar (MCD Lab®) and incubated at 37°C for 24 hours. Subsequently, the samples were preserved with glycerol and brought to a temperature of -26°C.

Then, the DNA extraction process was performed following the methodology used by Leotta, et al. (2005), where a small bacterial colony was placed in sterile distilled water. The samples were homogenized and transferred to a dry bath incubator (Thermo Shaker® TSI-100, USA) at a temperature of 100°C for 15 min with 600 rpm; subsequently, the samples were centrifuged with a centrifuge (VelaQuin® VE-4000, Mexico) at 1500 rpm for 5 min. Later, the supernatant was extracted and the amount of DNA was analyzed in a nanodrop spectrophotometer (Thermo Scientific®, ND-ONE-W, USA).

For the identification of the *Escherichia coli rfb*O157 gene, the polymerase chain reaction (PCR endpoint) technique was used, placing in microcentrifuge tubes, 6.5 µL of sterile water, 2.5 µL of forwarding primer, 2.5 µL of reverse primer (using the nucleotide sequences described by Leotta, et al, 2005 shown in Table 1), 12.5 µL of premixed solution with Taq DNA polymerase, dNTP, MgCl2 and reaction buffers (GoTaq® Master Mix) and 1 µL of DNA per sample; samples were placed in a thermal cycler (Bio Rad® C-1000, USA) under the following cycles: 94°C for 5 min, 30 cycles at 94°C for 30 s, 52°C for 30 s, 72°C for 1 min, with a total volume of 25 µL at 105°C.

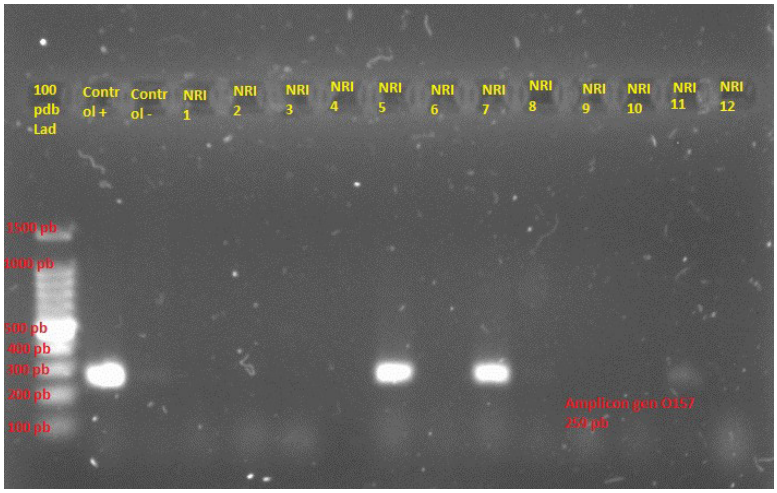
Table 1
Characteristics of the rfbO157 gene used in the study (Leotta, et al., 2005)

Gen	Sequence (5'-3')	Size (Pb)
O157a	CGGACATCC ATGTGATATGG	259
O157b	TTGCCTATGTACAGCTAATCC	25.62%

*Pb: Base pairs

Source: Own elaboration

The bands were detected using a 2% agarose gel stained with ethidium bromide, placing 1 µL of bromophenol blue and 4 µL of DNA in each well, in addition to placing the positive and negative reference of the gene in the first and second wells, respectively; the electrophoresis chamber was programmed at 60 V for 45 min. Visualization of the electrophoresis results (Image 1) was performed with UV light in a transilluminator (Bio Rad® Chemi-Doc XRS+, USA).



*pb/pdb: base pairs; NRI: *Numida*-related isolate.

Source: Own elaboration

Data were analyzed using point prevalence formula (Rodriguez-Hernandez, 2015), as shown below:

$$\text{Point prevalence} = Ct / Nt$$

Where:

Ct: Number of existing cases (prevalent) at a given time or age.

Nt: Total number of individuals in the population at that given time or age.

RESULTS AND DISCUSSION

No prevalence of *Salmonella spp*. was found in any of the farms studied, results similar to those of García, et al. (2009), who carried out a study in Spain with laying hens, finding a prevalence of 1.3% of *Salmonella spp*. in feces collected by cloacal swabbing; this shows that the prevalence of *Salmonella* is low in this type of sampling method; however, these results differ from those obtained by Castañeda-Salazar, et al. (2018), who found a prevalence of 29.2% in chickens for human consumption in Colombia.

In the case of *Escherichia coli*, no prevalence was found in the "Monte Calvario" ranch in La Trinitaria, while in the "Las Amazonas" ranch in Villaflores, 95% of the samples were positive (Image 2), this is attributed to the type of management employed with the guineas and the variation of temperatures between municipalities. Findings of Hernández-Fillor, et al. (2017), who conducted a study in Cuba with laying hens and found a prevalence of 45.9%, differs from this study; on the other hand, Gibert-Perelló, in 2010, found a prevalence of 72.7% in samples of different organs from laying hens in Spain. However, for the identification of serotype O157:H7 (*Shiga* toxin producer), 10% prevalence was found (Image 3), this differs from the findings of Zotta, et al. (2016), who did not report the presence of the gene in chicken viscera in Argentina. A study by Rípodas-Navarro, et al. (2017), found 12.12% presence of the gene in meats and their derivatives from domestic slaughter species in Madrid, Spain.

Studies have demonstrated the ability of *Escherichia coli* O157:H7 to colonize the intestinal mucosa of chickens and spread to the environment (Best, et al., 2005; La Ragione, et al., 2005), which may explain that chickens and guineas can act as carriers of *Escherichia coli* O157:H7, being a possible threat to public health.

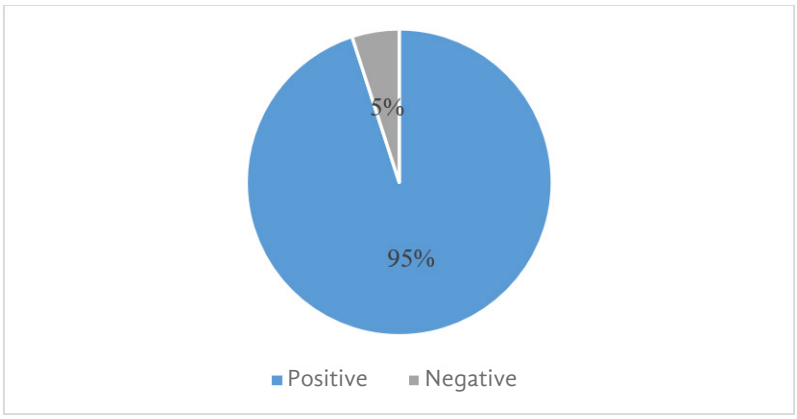


Image 2. Prevalence of *Escherichia coli* in guinea fowl at "Las Amazonas" ranch, Villaflores, Chiapas. Source: Own elaboration

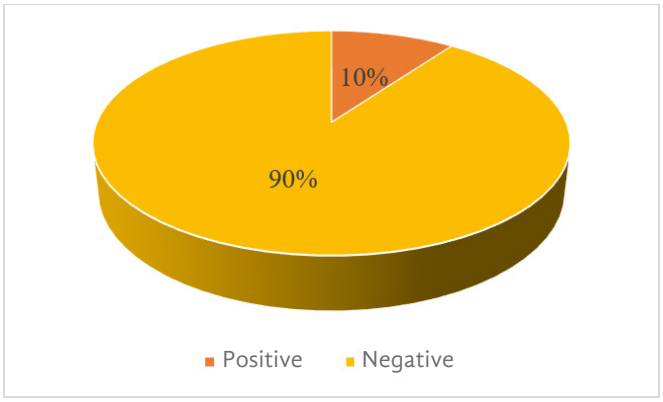


Image 3. Prevalence of *Escherichia coli* serotype O157:H7 (*Shiga* toxin producer) in guinea fowl from "Las Amazonas" ranch, Villaflores, Chiapas. Source: Own elaboration

CONCLUSION

The breeding of guinea fowl represents an alternative for backyard producers due to their easy adaptation to the environment, as well as the low initial investment, in addition to presenting a source of animal protein.

Although no prevalence of *Salmonella spp*. was found in guinea fowl, the study demonstrates that guinea fowl are carriers of *Escherichia coli* O157:H7, so it is necessary to continue with studies on the impact of the serotype on animal health, as well as on public health.

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