Fritz Huchzermeyer Veterinary Science Student Research Assistance Scheme

FINAL REPORT

Analysis of antibiotic sensitivity profiles in *Escherichia coli* isolates, obtained from American Crocodile (*Crocodylus acutus*) cloaca of animals captured in tilapia production ponds in Cañas Guanacaste, Costa Rica

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Goals and objectives

The research has as a general objective: To analyze the profiles of antibiotic sensitivity in isolates of *Escherichia coli* obtained from the cloaca of the American Crocodile (*Crocodylus acutus*) for the identification of the degree of resistance to antibiotics used in the treatment of Gram negative bacteria in human and aquaculture

Specific:

- Determine the antibiotic sensitivity profile of *E. coli* isolates against the main classes of antibiotics used to treat Gram negative bacteria.
- Evaluate the degree of resistance of the main antibiotics used in aquaculture.
- Determine the association between antibiotic sensitivity profiles with sex, size, residence time and place of origin of crocodiles in tilapia production farms in Cañas, Guanacaste.

Goals:

Obtained the sensitivity profiles of the *E.coli* isolates from the cloaca of each sampled crocodile and verified if there is a common pattern. Also comparison with each of variables and significance.

Project summary

In recent years, bacteria have acquired resistance genes causing a new problem "antimicrobial resistance." The agricultural, animal and aquaculture industries are one of the main causes, due to the excessive use of antibiotics that are released in aquatic environments generating changes. The American crocodile found in this type of ecosystem becomes a key and bioindicator. The objective of the research is to analyze the profiles of antibiotic sensitivity in isolates of *Escherichia coli* obtained from the American crocodile cloaca to identify the degree of resistance to antibiotics used in the treatment of gram-negative bacteria and aquaculture. The study was carried out in the AquaCorporación Company in Cañas Guanacaste Costa Rica. Samples of captured crocodiles were taken in tilapia ponds. Subsequently, a swab was made from the cloaca of each animal and transported to the laboratory. The swabs were inoculated into two selective agar media and incubated at 35°C. Then an antibiotic sensitivity profile was performed with the Vitek 2 equipment to the isolates. Those for veterinary use were evaluated by the Kirby Bauer disk diffusion technique and the minimum inhibitory concentration (MIC). The most relevant results were a high prevalence of tetracycline

(75%), with a MIC 50 of 32 µg/ml and MIC 90 of 128 µg / ml. This work concludes that the information obtained in this research is important because resistance was detected in wild animals that are associated with a production system and that in the future it can become a risk to public health and the environment. In addition, it is important to evaluate in the future the role of the dispersion of the resistance mechanisms detected and the possible changes that may occur in the natural structure of aquatic ecosystems.

Introduction

The American Crocodile is a species that has suffered a loss of its habitat due to pressures from urban development, tourism, agricultural activity and aquaculture industries (Bolaños, 2012) and has led it to overlap with human activities (Jiménez, 2005; Sandoval, Durán & Quirós, 2017), being this reptile a wild species can become a reservoir of important pathogens that can be shared with the human being through a "zoonosis", which is an important aspect to consider (Magaña, 2005).

This dynamic can vary depending on different factors however, in recent years zoonoses are not the only alarming problem but is currently "antimicrobial resistance" (Karesh et al., 2012). The extensive use of these components in the aquaculture, agricultural and livestock industry has promoted this situation. Likewise, the uncontrolled use of antibiotics causes these drugs to be released and remain on the surface of the soil or, failing that they are directed towards aquatic environments, a horizontal transfer of genes occurs in the bacteria of the environment generating the emergence of resistant bacteria (Cabello, 2006; Segura, François, Gagnon & Sauvé, 2009).

In this sense, it is mentioned that the severity of bacterial resistance infections is greater causing strong problems in human health as an animal (Silva et al., 2011). Research on bacterial resistance in the country has been developed mainly in animal production domestic animals and wildlife species (pigeons and felines) (Blanco-Peña et al., 2017) but little has been found in reptiles according to the bases of consulted data.

In this way studying antibiotic resistance profiles in crocodiles would allow us to begin to understand if these populations are being directly or indirectly affected by this problem leading them to be bio indicating species of environmental health and public health.

Materials and methods

The research approach is quantitative and with the purpose of knowing more about the species in topics of biological importance and conservation.

The study will be carried out in the town of Cañas, Costa Rica. Samples will be taken from 50 specimens of crocodile (*Crocodylus acutus*), from the Great Tempisque Wetland, which arrives and is captured in the tilapia production ponds and the maintenance site. Each animal's cloaca was sampled using the swab technique and transported in a cold temperature container to the laboratory. Subsequently, they were inoculated in Levine and MacConkey Agar plates without antibiotics and with antibiotics, to obtain isolates of *E.coli* and incubated at 37°C for 24 h. In case of growth of typical colonies of *E. coli* (pink colonies), they were grown in soy trypticase agar at 37°C for 24 h (Cushing, Pinborough & Stanford, 2011; Foti et al., 2013) and identified with the GN card in the VITEK 2 device. Biochemically confirmed isolates will be suspended in sterile skim milk (10%) with sterile glycerol (20%) and cryopreserved at -80 ° C. All of them had an antibiotic sensitivity profile with the Vitek 2 equipment and the AST-279 card, also allowing the confirmation of a BLEE type phenotype (Extended Spectrum β-beta-lactamases) that are of essential health value public For the execution of the tests and the interpretation of the results, the CLSI interpretation guides (Clinical and Laboratory Standards Institute, 2016) will be used.

Additionally, the sensitivity profile to florfenicol and tetracycline (antibiotics for veterinary use) will be analyzed using the Kirby Bauer disk diffusion technique. To assess the degree of resistance, the minimum inhibitory concentration (MIC) will be determined. By means of the profiles, the differences between the two

places of origin and an exploratory analysis of main components to determine correlations between the variables (the length of stay) in the enclosure, place of origin, the size of the animal and the sex).

Results obtained during the investigation

Regarding the results obtained during the investigation, different advances achieved can be mentioned according to the objectives set:

- Obtaining 63 samples of American crocodile cloaca which were able to isolate a total of 53 *E.coli* isolates, already processed to determine the resistance profile in antibiotics for human use
- Resistance to four types of antibiotics for human use was obtained with a prevalence rate of 4 to 17%
- Obtaining 27 isolates of *E.coli*, already processed to assess the degree of resistance in antibiotics for use in aquaculture
- A 75% prevalence was obtained for tetracycline, an antibiotic for use in aquaculture, relevant and warning data since practically all isolates presented it.
- The degree of minimum inhibitory concentration (MIC) was determined for tetracycline positive isolates, where MIC 50 was 32 µg/ml and MIC 90 was 128 µg/ml.
- In determining the association between antibiotic sensitivity profiles with sex, size, time of residence and place of origin of crocodiles, no significant association was found between the characteristics evaluated.

Obstacles encountered during the project

- Among the obstacles was the delay with sampling due to climatic changes that occurred in the sampling area. Which complicated comply with the established dates.
- At the beginning the methodology used to obtain *E.coli* isolates did not work as we expected, which along we had to make some changes at the time of sampling. This situation also delayed us some time.
- The buy of reagents (antibiotics), took a long time for delivery, this also made us wait some time to start processing the samples.

Compliance with the project criteria

Research on "Analysis of antibiotic sensitivity profiles in isolates of *Escherichia coli*, obtained from the cloaca of American Crocodile (*Crocodylus acutus*) of animals captured in tilapia production ponds in Cañas Guanacaste, Costa Rica." The objectives and goals of the project were fully completed. Also, the project provides important information on the detection of resistance in wild animals that are associated with a production system and that in the future may become a risk to public health and the environment.

However, it is important to evaluate in the future the role of the dispersion of the resistance mechanisms detected and the possible changes that may occur in the natural structure of aquatic ecosystems. In addition to observing the important role of crocodiles as environmental bioindicators, this also contributes to future conservation projects of this species.

The product obtained at the end of the support period

Concerning the final product of the project, we are in the drafting part of the last details of the work so that it can be evaluated by the University staff. Therefore we hope to finish in the first months of this next year with 100% of the work.

Assessment how the fund contributed to the TFG

The support obtained by the Crocodile Specialist Group (CSG), contributed in a very positive way in the process of my TGF, since with this help achievement to do part of sampling and offset the expenses of food, transportation of field visits, and the purchase of different laboratory and office materials that are necessary during this research process.

In this way I appreciate the unconditional support of the grants provided by the scholarships of the group of specialists to pre-graduate students, which facilitates the development of our research for the medicine and conservation of crocodiles. Where we know the hard work that is to find subsidies for their development and this type of fund reduces such work.

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