



Serotyping and antimicrobial resistance profile of *Salmonella* spp. isolated from crocodilians in captivity

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ABSTRACT

Objective. Determine the presence of *Salmonella* spp and the AMR in isolates of cloacal and muscle swabs in specimens of *Caiman crocodilus fuscus* and *Crocodylus acutus* in captivity, in Colombia. **Materials and methods.** A cross-sectional and descriptive sampling was carried out with a non-probabilistic design for convenience, once, during the month of February 2021. 150 *Caiman crocodilus fuscus* and *Crocodylus acutus* animals were included, with a 50/50 distribution. Cloacal and muscle samples were taken with sterile swabs. These were planted on MacConkey agar, then on SS agar. These positive samples were then reconfirmed on TSI agar. Serotyping was performed with specific monovalent and polyvalent antisera. The diffusion test was performed on Mueller-Hinton agar. **Results.** There was no growth of colonies in any muscle sample. The presence of the bacteria in cloacal samples of *Caiman crocodilus fuscus* was 36% and for *Crocodylus acutus* 50.6%. The serotypes with the highest detection percentage in both groups were serogroup C1 and polyvalent serogroup B. Some colonies were resistant to tetracycline, while other colonies had intermediate resistance to ampicillin, cefoxitin, sulfa trimethoprim, and tetracycline. **Conclusions.** This is the first report related to the detection of *Salmonella* spp. in alligator carcasses in the country, without finding the presence of the agent in any sample evaluated, however, the importance of continuing its monitoring is emphasized to guarantee innocuous products

Keywords: Reptiles; antimicrobial resistance; foodborne diseases; meat quality (Source: MeSH).

RESUMEN

Objetivo. Determinar la presencia de *Salmonella* spp y el PRA en aislamientos de hisopados cloacales y musculares en especímenes de *Caiman crocodilus fuscus* y *Crocodylus acutus* en cautiverio en Colombia. **Materiales y métodos.** Se realizó un muestreo de tipo transversal y descriptivo con un diseño no probabilístico por conveniencia una única vez, durante el mes de febrero de 2021. Se incluyeron 150 animales de *Caiman crocodilus fuscus* y *Crocodylus acutus*, con una distribución

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50/50. Se tomaron muestras cloacales y musculares con hisopos estériles. Estas se sembraron en agar MacConkey, posteriormente en agar SS. Luego, se reconfirmaron las muestras positivas en agar TSI. Se realizó la serotipificación con antisueros monovalentes y polivalentes específicos. Se realizó la prueba de difusión en agar Mueller-Hinton. **Resultados.** No existió crecimiento de colonias en ninguna muestra muscular. La presencia de la bacteria en muestras cloacales de *Caiman crocodilus fuscus* fue del 36% y para *Crocodylus acutus* de 50.6%, los dos serotipos con mayor porcentaje de detección en ambos grupos fueron el serogrupo C1 y el serogrupo polivalente B. Ocho colonias fueron resistentes a tetraciclina, mientras que otras colonias tuvieron una resistencia intermedia a ampicilina, cefoxitin, sulfatrimetopim y tetraciclina. **Conclusiones.** Este es el primer reporte relacionado con la detección de *Salmonella* spp. en canales de caimanes en el país, sin encontrar presencia del agente en ninguna muestra evaluada, sin embargo, se recalca la importancia de seguir su monitoreo para garantizar productos inocuos.

Palabras clave: Reptiles; resistencia antimicrobiana; enfermedades transmitidas por alimentos; calidad de carne (*Fuente: MeSH*).

INTRODUCTION

Salmonella spp. is a gram-negative bacterium with pathogenic potential. It can be found in a variety of vertebrate hosts, both endothermic and ectothermic. Within the latter, in reptiles such as caimans, it is present in their gastrointestinal tract in a normal way without causing characteristic clinical symptoms (1,2). However, in stressful situations, mainly related to captivity and management, the clinical disease can be triggered, generally of a gastrointestinal type and even septicemic. Gastrointestinal disease is characterized by generating anorexia, progressive weight loss, and even sudden death without apparent symptoms, depending on the type of serovar involved (1,2).

Salmonella spp. is also a pathogen associated with outbreaks of moderate to severe gastrointestinal diseases in humans, which is important in public health. Humans acquire it due to direct contact with the bacteria on exposed skin surfaces and by ingesting products contaminated with it, mainly meat with an ineffective hygienic control process during slaughter. (3). In Colombia, caiman breeding occurs in around 47 establishments, whose main objectives are: the production of meat and skins, and repopulation in areas where, due to human action, their number has decreased in the ecosystem (4).

The increase in demand for meat and skins derived from these animals has grown gradually in recent decades worldwide, mainly due to nutritional characteristics such as the presence of high

biological value proteins with high digestibility and the low percentage of polyunsaturated fatty acids compared to conventional meats (5,6,7,8). However, with this demand, the requirements of consumers also increased so that these products have sufficient nutritional and microbiological quality, which does not put their health at risk, for which it is necessary to reduce the sources of bacterial contamination that occurs between the carcass, skin, and waste during slaughter to reduce pathogens such as *Salmonella* spp in the final product (7,8,9).

In other countries, human salmonellosis originating in captive reptiles is a reported disease and is mainly associated with contact with feces, animal parts, fomites, and contaminated meat. (2). In Colombia, investigations have been carried out to detect and identify *Salmonella* species present in caimans (10), however, the information in this area is scarce and it is necessary to increase it to have better judgment tools when improving production processes such as slaughtering and even implementing pathogen elimination methodologies to ensure that products intended for human consumption meet the expected sanitary standards (11,12,13,14).

Therefore, the objective of this study was to determine the presence of *Salmonella* spp and the antimicrobial resistance profile (AMP) in isolates from cloacal and muscle swabs in specimens of *Caiman crocodilus fuscus* and *Crocodylus acutus* in captivity in Colombia.

MATERIALS AND METHODS

Site and sampling type. A cross-sectional and descriptive sampling was carried out with a non-probabilistic design for convenience only once. The animals were on the same farm located in the Magdalena Medio region in the department of Tolima. The samples were obtained every eight days during the four weeks of February 2021.

The ponds where the animals were extracted for the sampling process have a closed water system, it is filtered and reused. Zoo breeding has 42 ponds distributed throughout the place, divided according to the stage of growth, separating them into neonates, juveniles, and adults.

Animals included in the study. 150 *Caiman crocodilus fuscus* and *Crocodylus acutus* animals were included, with a 50/50 distribution. The specimens of *Caiman crocodilus fuscus* are destined for slaughter and they had a length between 60-70 centimeters at the time of slaughter. On the other hand, *Crocodylus acutus* specimens are destined for repopulation processes, and their size was also in the range between 60 - 70 centimeters at sampling.

The following samples were taken from this population: two different samples were taken from the specimens destined for slaughter, using sterile swabs; a sample was taken from the cloaca at the time prior to slaughter. After this process, another sterile swab was rubbed on the *Caudofemoralis longus* muscle, located in the ventral and caudal region. Therefore, a total of 150 samples were taken from *Caiman crocodilus fuscus*. On the other hand, the specimens destined for repopulation only had a cloacal swab sample taken, therefore, 75 samples of *Crocodylus acutus* specimens were obtained.

Collection and sample storage. Sampling was performed using gloves and sterile swabs. For the cloacal sample, the specimen was placed in dorsal decubitus, held manually to reduce its movements, and, using the sterile swab, circular movements were made in the cloaca until it was completely covered, lasting about five seconds in each sample. For muscle sampling, the swabs were passed with linear movements for five seconds until covering the entire length of the *Caudofemoralis longus* muscle.

The samples were labeled according to the type of sample (cloacal or muscular) and according to the type of caiman, and with internal serials of the researchers. The swabs were introduced into Stuart medium and kept refrigerated during transport to the Universidad Pedagógica y Tecnológica de Colombia, Tunja - Boyacá, to the veterinary microbiology laboratory.

Laboratory procedure. The samples were kept refrigerated at 4°C for 18 h until processing; they were cultured on MacConkey agar (Merck KGaA, Germany) and incubated for 24 h at a temperature of 37°C. The lactose-negative colonies were cultured on specific SS agar (Salmonella-Shigella Agar, Merck KGaA, Germany) where they were incubated for 48 h. Later, positive samples were reconfirmed by reseeded on TSI agar (Triple Sugar Iron Agar-Merck KGaA, Germany) and incubated for 48 h at a temperature of 35°C, after which time the results were taken. These were considered positive when the colonies took on a reddish-brown coloration throughout the tube, indicating the presence of enterobacteria unable to ferment the lactose and H₂S demonstrated before with SS agar. Each sample was plated in duplicate to validate the results obtained.

Serotyping. Positive samples were serotyped to somatic antigen with specific polyvalent antisera (PB and PC) and group monovalent antisera (B, C1, C2, and D1), they were identified according to the Kauffman-White scheme (10,14).

Antimicrobial resistance profile. The antimicrobial resistance profile of each sample evaluated was performed using the Mueller-Hinton agar diffusion test (Merck KGaA, Germany), the following antibiotics were evaluated: Sulfamethoxazole 25 µg, Norfloxacin 10 µg, Ampicillin 10 µg, Chloramphenicol 20 µg, Cefoxitin 20 µg, and Tetracycline 30 µg (10) (Becton Dickinson, USA). The strains were classified as susceptible, intermediate, or resistant according to the size of the inhibition halo after incubation at 37°C for 24 h and comparing it with the manufacturer's instructions for the sensi-discs.

Statistical analysis. The results obtained for growth and serotyping were recorded in the Excel® program from which the graphs were made to describe the presence or absence of the agent, as well as the antimicrobial susceptibility determined using descriptive statistics.

RESULTS

Detection of *Salmonella* spp. in samples of *Caiman crocodilus fuscus* and *Crocodylus acutus*. The number of positive samples for each type of sample in each caiman species can be seen in Figure 1. It is interesting to note that in no muscle sample was a growth of colonies compatible with *Salmonella* spp., meanwhile, for cloacal swabs, the number of positive samples was 27, which corresponds to 36% of the total cloacal samples of the *Caiman crocodilus fuscus*. On the other hand, the cloacal positive samples of *Crocodylus acutus* specimens correspond to 38, which is 50.6% of the total samples evaluated in this species.

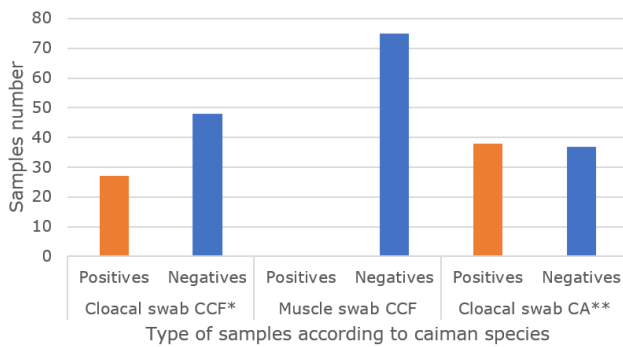


Figure 1. Distribution of samples positive for *Salmonella* spp. in *Caiman crocodilus fuscus* and *Crocodylus acutus* in captivity
*CCF: *Caiman crocodilus fuscus*.
**CA: *Crocodylus acutus*.

Serotyping of bacterial isolates. The total 65 bacterial isolates recovered (27 from CCF and 38 from CA) were serotyped. Figure 2 shows the percentage of detection for each serotype of *Salmonella* spp. with respect to the total number of positive samples for each alligator species evaluated. It is interesting to note that the two serotypes with the highest percentage of detection in both groups were the C1 serogroup (blue) and the polyvalent serogroup B-PB (purple).

Antimicrobial resistance profile of *Salmonella* spp isolates. The results obtained for the susceptibility/resistance profile for the microorganisms used are found in table 1. It is evident that, except for tetracycline, no *Salmonella* spp. was resistant to the antimicrobials evaluated. The study highlights that the microbial isolates in the two groups had similar behavior against the drugs used.

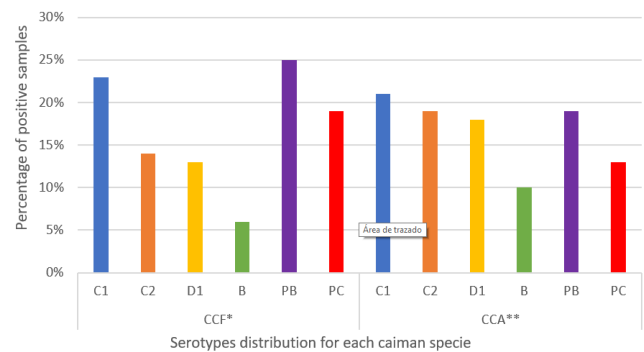


Figure 2. Percentage of serotypes by positive samples according to the caiman species evaluated.
*CCF: *Caiman crocodilus fuscus*.
**CA: *Crocodylus acutus*.

Table 1. Results obtained for the antimicrobial resistance profile of the *Salmonella* spp. isolates, according to the caiman species evaluated.

Medicament	<i>Caiman crocodilus fuscus</i>		
	Susceptible (n)	Intermediate (n)	Resistant (n)
Ampicillin	25	2	
Cefoxitin	23	4	
Chloramphenicol	27		
Norfloxacin	27		
Sulfatrimethopim	26	1	
Tetracycline	20	5	2
	<i>Crocodylus acutus</i>		
	Susceptible (n)	Intermediate (n)	Resistant (n)
Ampicillin	32	6	
Cefoxitin	29	9	
Chloramphenicol	38		
Norfloxacin	38		
Sulfatrimethopim	35	3	
Tetracycline	30	2	6

DISCUSIÓN

In Colombia, studies have detected the presence of various serotypes of *Salmonella* spp. in reptiles, mainly caimans and some species of turtles. In their study, Pachón et al (10) determined a positivity of 40% of the sampled ponds where caiman specimens (*Caiman crocodilus fuscus* and *Crocodylus intermedius*) were in captivity in the eastern plains, a result similar to that reported in this study for both caiman species evaluated (*Caiman crocodilus fuscus* and *Crocodylus acutus*).

The presence of *Salmonella* spp. varies according to the environment where the caimans are found, thus, the bacteria have a greater capacity to survive in the water and their recirculation in the ponds, which favors the constant exposure of caimans to the bacteria, a hypothesis that is supported by the results of Gay et al (15) who determined that despite the fact that caimans are the least frequent type of reptile, infection with *Salmonella* spp. is more frequent in animals that remain in captivity.

Due to *Crocodylus acutus* specimens sampled in this study having repopulation purposes, more than 50% of the specimens had the presence of *Salmonella* spp., could alter the dynamics of exposure to the agent in nature by other caimans and another fauna present (Figure 1).

Regarding serotyping, the serogroups determined in the highest percentage were: C1, PB, and PC, for the two alligator species, however, serogroups D1 and C2 were also present in CA specimens. These results agree with the results of Pachón et al (10) for captive specimens. This could be related to possible factors specific to these serogroups that favor their multiplication, survival, and, therefore, their transmission between animals, this highlights their importance as causes of possible diseases in alligators and, in the case of farms, the possible involvement in the health of workers.

Related to the reports of antimicrobial resistance in bacterial isolates from caimans, (10), they determined that multiple isolates present resistance to drugs such as tetracyclines and cefoxitins, which is a similar result to that reported in this study and to that reported by Merkevičienė et al (16); Evidence that supports the management practices of control of shared diseases that lead to a misuse of drugs allowing the development of these resistances. This could influence the health of workers since *Salmonella* spp has been shown to circulate in multiple spaces in zoos, a situation that manifests cross-contamination of different places and, therefore, the possibility of humans acquiring the bacteria infection and disease development. Regarding the latter, in their study, Bauwens et al (17) determined the circulation of multiple serovars of *Salmonella* spp., with possible pathogenic potential for humans, not only in the reptiles tested but also in areas such as zoo kitchens, closed environments, utility rooms, and public spaces.

Uhart et al (2) determined in Argentina that the prevalence of salmonellosis in caimans both in the wild and in captivity can reach 77%, mainly affecting caimans at the gastrointestinal tract level. In addition to the possible effects on the gastrointestinal tract of *Salmonella* spp. in caimans, its implication in neurological manifestations is reported as the agent responsible for cases of osteomyelitis (18), and also in sudden deaths due to coinfections with other bacterial agents, as reported by Silva et al (19); In addition, studies have shown that *Salmonella* spp. is a pathogen that is involved in gastrointestinal diseases in caimans of the *Crocodylus intermedius* and *Crocodylus acutus* species, mainly related to omphalitis in neonates and pup syndrome in Venezuelan farms (1).

Some mechanisms have been proposed to try to reduce the presence of *Salmonella* spp. in caimans using probiotics in the feeding of Caiman yacare specimens, however, the results obtained by Carvalho et al (20) showed that there is no effect of this technique in the presence of the bacteria.

To the best of the authors' knowledge, this is the first report in the country where muscle samples were analyzed after slaughter to determine the presence of this bacterium, which has an important pathogenic potential for human health. Despite obtaining negative results, which shows the strength of the slaughter method used by the operators, it is necessary to continue investigating the presence of the bacteria in the carcasses and also to identify which practices favor or do not favor contamination in order to obtain standardized procedures that potentiate the national sector. All this is important because there are reports around the world where an important source of human salmonellosis is these wild species (8,15,21,22,23).

In conclusion, this was the first report related to the detection of *Salmonella* spp. in caiman carcasses in the country, demonstrating that they were not positive for the agent, however, the importance of continuing their monitoring is emphasized to guarantee safe products. In addition to this, it was shown that the percentage of positive samples varies between ponds of the caimans evaluated, the serogroups: C1, PB, and PC were the most common for CCF and CA. Reports of antibiotic resistance profiles show that despite the fact that most drugs are still effective, those such as tetracyclines and

cefotixin present resistance in some isolates, which should lead to thinking about correcting management practices and improving measures of pharmacological control that is used in the ponds of the farm.

Conflict of interests

The authors declare that there are no conflicts of interest during the planning, implementation, writing, and presentation of the document to the Journal MVZ Córdoba.

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