# ISOLATION OF *SALMONELLA* SPP. FROM YACARE CAIMAN (*CAIMAN YACARE*) AND BROAD-SNOUTED CAIMAN (*CAIMAN LATIROSTRIS*) FROM THE ARGENTINE CHACO

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ABSTRACT: Presence of Salmonella spp. was evaluated in yacare caiman (Caiman yacare) and broad-snouted caiman (Caiman latirostris) from a ranching facility in the Argentine Chaco. Crocodilian ranching programs are based on captive breeding of wild-harvested eggs and release of excess hatchlings into the wild. Samples for bacterial isolation were collected from 102 captive (35 C. yacare and 67 C. latirostris) and seven free-ranging caiman (four C. yacare and three C. *latirositris*) between 2001 and 2005 and from three artificially incubated C. yacare wild eggs. Two Salmonella spp. of known zoonotic potential, S. infantis and S. nottingham, were isolated from captive caiman in 2001 and 2002, respectively. This is the first report for S. nottingham in reptiles and of S. infantis in caiman. Salmonella spp. prevalence varied significantly between years, with a 77% prevalence peak in 2002. Although the cause of this increase was not confirmed, we found no correlation with the type of enclosure, caiman species, or body weight. Deteriorated physical condition of caiman hatchlings due to dietary changes in 2002 could have influenced Salmonella spp. shedding. However, external sources such as food, water, or enclosures could not be ruled out. Pathogenic Salmonella spp. present a risk for human infection. Inadvertent introduction of Salmonella spp. or other bacteria into the environment when caiman are released could pose a threat to wild caiman populations. Prophylactic measures to detect and decrease Salmonella spp. presence in caiman ranching facilities are recommended to reduce risk to humans and make caiman-ranching a sound conservation strategy for crocodilian species.

Key words: Argentina, Caiman spp., cloacal swabs, crocodilian, ranching, Salmonella spp.

## INTRODUCTION

Both endothermic and ectothermic vertebrates are important reservoirs of *Salmonella* spp. in nature (Chiodini and Sundberg, 1981). Infection usually is subclinical, and most animals act as asymptomatic carriers that shed the bacteria intermittently (Mörner, 2000). Reptiles host diverse serovars of *Salmonella* spp. in their intestinal flora (Onderka and Finlayson, 1985; Thomas et al., 2001). Clinical salmonellosis is infrequent in reptiles, and infected animals most often remain unidentified (Johnson-Delany, 1996). However, stress related to captivity might induce clinical disease (Lane, 1996). Several *Salmonella* spp. serovars have been isolated from sick captive reptiles (Onderka and Finlayson, 1985), and from alligators with septicemic or gastrointestinal disease (Lane, 1996). These generalized infections have caused anorexia, weight loss, and sudden death in crocodilians, with or without clinical signs including lack of coordination or lethargy (Lane, 1996).

The ranching system described in this study generally is associated with species conservation and sustainable-use programs (Onderka and Finlayson, 1985; Huchzermeyer, 1991; Madsen, 1996; Millan et al., 1997; Scott and Foster, 1997). Caiman ranching typically consists of collecting eggs from wild nests and incubating them artificially to obtain higher hatching rates than would be achieved in the wild (Thorbjarnarson, 1992). After raising the hatchlings in captivity for 1-2 yr, some are returned to the wild and others are harvested for hides and meat (Larriera and Imhof, 2006). In Argentina, yacare caiman (*Caiman yacare*) and broad-snouted caiman (Caiman latirostris) have been ranched since 1990 (Larriera, 1994; Prado et al., 2000, 2005; Cardozo et al., 2005; Larriera and Imhof, 2006). The main commercial caiman product is leather, although a few ranching facilities also sell caiman meat to local restaurants (W. Prado pers. comm.).

Human salmonellosis acquired from captive reptiles has been widely described (Millan et al., 1997; Cyriac and Wozniak, 2000; Warwick et al., 2001; Sanchez et al., 2002; Willis et al., 2002) and often is associated with contact with reptile feces, contaminated animal parts, or fomites (Sanchez et al., 2002). Contamination of crocodilian meat with Salmonella spp. has been documented (Manolis et al., 1991; Madsen, 1996). Strict hygiene measures at breeding facilities and during slaughter have been shown to reduce the risk of zoonotic infections (Millan et al., 1997). Similarly, sound health management and prerelease screening have been recommended for population reinforcement (restocking) programs (Viggers et al., 1993; IUCN, 1998). To the best of our knowledge, ranchers in Argentina rarely conduct pathogen screening prior to release of captive raised caiman, nor do they conduct systematic bacterial screening of food, water, enclosures, or animals.

Detection of zoonotic pathogens such as *Salmonella* in reptiles from ranching facilities is important for the reduction of public health risks, and for avoiding inadvertent introduction of disease into wild populations of susceptible species, including birds of prey, waterfowl, wild

mammals, and other reptiles (Onderka and Finlayson, 1985; Battistini et al., 1998; Gopee et al., 2000; Tizard, 2004). Our goal was to determine the presence of *Salmonella* spp. in caiman in areas where ranching is implemented. This information is essential to assess health risks and apply preventive measures in ranching programs.

## MATERIALS AND METHODS

During February and November 2001, December 2002, and February 2005, cloacal swabs were collected from 102 captive individuals (35 C. yacare and 67 C. latirostris) from a caiman ranching facility in Chaco province, Argentina  $(26^\circ53'39''\mathrm{S},~59^\circ01'07''\mathrm{W}).$  Swabs also were collected from seven wild caiman captured in February 2005 in areas where eggs usually are harvested (lagoons Vilches [26°46′58″S, 58°59′17″W], Vilchito [26°46'22"S, 58°59'06"W], Piccili [26°41'48"S, 59°09'36"W], Milón [26°46'58"S, 58°56'20"W], Alvareda [26°43'21"S, 59°06'15"W] and Arroyo Guaycurú: [26°51′32″S, 59°05′54″W]; Table 1). No animals used for this study had clinical signs of illness. Additional sampling in February 2005 included three nonembryonated eggs from one of the yacare caiman clutches harvested. Eggs were collected manually from wild caiman nests, stored at environmental temperature for <24 hr during transport, and incubated for 70 days at 31.5 C and 98% humidity at the ranching facility (Prado et al., 2001).

Cloacal swabs from February 2001 and 2005 were transported in Stuart culture medium (Becton Dickinson and Company, Sparks, Maryland, USA). Swabs collected in November 2001 and December 2002 were transported in enriched culture medium (Selenite Broth and Tetrathionate Broth [TTB]; Merck<sup>®</sup>, Darmstadt, Germany) and refrigerated until processed, 48-96 hr after collection. Cloacal swabs from 2001 (n=44) were cultured on Desoxycholate Citrate Agar (DCA; Difco<sup>®</sup>, Becton Dickinson), incubated at 35-37 C for 72 hr, enriched in TTB (Britania Labs Britania, Buenos Aires, Argentina), incubated at 37-42 C, and again transferred to DCA at 48–72 hr. Cloacal swabs (n=68) and eggs (n=3) collected in 2002 and 2005 (Table 1), were cultured in Peptone broth for 8 hr, transferred to TTB (Merck), and 24-48 hr later cultured on Salmonella-Shigella Agar (Merck; Barrow and Feltham, 1999). Changes

	Number by species			
Time of sampling	Caiman yacare Positive/Total	Caiman latirostris Positive/Total	Source	Isolate
February 2001	0/3	1/13 <sup>a</sup>	captive	Salmonella infantis (6,7: r:1,5)
November 2001	0/14	0/14	captive	
December 2002	$7/7^{\mathrm{a}}$	20/28 <sup>a</sup>	captive	Salmonella nottingham <sup>b</sup> (16:d:e,n,z <sub>15</sub> )
February 2005	0/14	0/12	captive	
February 2005	$0/4^{c}$	0/3°	wild	_
February 2005	eggs 0/3	—	wild	—

TABLE 1. Salmonella spp. isolates from wild and captive yacare caiman (*Caiman yacare*) and broad-snouted caiman (*Caiman latirostris*) from the Chaco region of Argentina.

<sup>a</sup> Positives for Salmonella spp. by standard biochemical methods used to classify enterobacteria (Le Minor and Richard, 1993).

<sup>b</sup> Only one colony was investigated and serotyped. The species of caiman from which the single colony was selected for serotyping was not identified.

<sup>c</sup> From lagoons Vilches, Vilchito, Piccili, Milón, and Alvareda, and Arroyo Guaycurú.

in methodology between years were due to using different diagnostic laboratories.

Colonies resembling Salmonella spp. were biochemically tested by methods used to classify enterobacteria (Le Minor and Richard, 1993). For all years, isolates identified as Salmonella spp. were submitted to the reference laboratory Enterobacteria Service, Instituto Nacional de Enfermedades Infecciosas (I.N.E.I.), Administración Nacional de Laboratorios e Institutos de Salud (A.N.L.I.S.) "Dr. Carlos G. Malbrán" (Buenos Aires, Argentina), where they were typed according to standardized biochemical reactions (Ewing, 1986). Serotyping was conducted following the scheme proposed by the Pasteur Institute, Paris, France (Popoff, 2001), using polyvalent and monovalent antisera and somatic and flagellar factors, supplied by "Servicio Antígenos y Antisueros," Instituto de Producción de Biológicos, A.N.L.I.S. Comparisons between years were done using chi-square tests. Continuous variables were tested using the Mann-Whitney U-test.

#### RESULTS

Two Salmonella serovars were isolated from captive caiman. In February 2001, Salmonella infantis (6.7:r:1.5) was isolated from one C. latirostris (Table 1). In December 2002, Salmonella colonies were cultured from 20 C. latirostris swabs and seven C. yacare swabs. Only one of these 27 colonies was serotyped due to budget and logistic restrictions. This isolate was characterized as Salmonella nottingham (16:d:e, $n,z_{15}$ ; Table 1). The identities of the additional 26 colonies were not determined.

Salmonella isolation prevalence varied significantly between years: 0% in February 2001, 4% in November 2001, 77% in December 2002, and 0% in February  $2005 (X^2 = 66.3, df = 2, P < 0.0001)$ . Analysis of the effects of type of enclosure (mud vs. cement floor) on Salmonella prevalence in captive caiman was not statistically significant ( $X^2 = 0.05$ , df=1, P = 0.822), nor was the weight of sampled animals (U=912,P=0.526). While the proportion of individuals from each species varied significantly between years  $(X^2 = 9.8, \text{ df}=3,$ P=0.020), in 2002 the prevalence between species did not differ  $(X^2 = 2.6, df = 1,$ P = 0.107).

#### DISCUSSION

Prior to this study, *S. nottingham* had not been reported in South America and, to our knowledge, had never been isolated from reptiles. We report *S. infantis* in caiman for the first time, although it had been described in crocodilians from Australia (Manolis et al., 1991). Our findings are important for public health, because both serovars can cause human salmonellosis (Ludlam et al., 1953; Chiodini and Sundberg, 1981).

Although swabs were collected four times over 5 yr, Salmonella was cultured only twice. This sporadic success in isolation could be due to intermittent shedding of the pathogen (Chiodini and Sundberg, 1981; Bradley et al., 1998), suboptimum preservation of samples, or lack of sensitivity of our techniques (Corrente et al., 2004). However, given that significantly more positives were consistently obtained from both species during a single sampling event (December 2002), it is more likely that a factor not related to sample handling enhanced Salmonella detection at that time. When reviewing husbandry practices at the ranching facility, we noted that, due to lack of refrigeration in 2002, caiman feed was switched from minced beef with vitamin and mineral supplements, to plain meat meal. The physical condition of caiman hatchlings appeared to have deteriorated in 2002 and was attributed to this dietary change. In particular, the average weight of yearlings of both species was significantly lower in 2002 than the previous year (U=3,160, P<0.0001). Hematologic parameters such as packed cell volume, total solids, calcium, and phosphorus also were significantly lower than previously recorded (data not shown). This loss of condition might have influenced Salmonella shedding (DuPonte et al., 1978; Beldomenico and Begon, 2010). In addition, starting in November 2001, swabs were collected from caiman housed in both mud- and cement-floor pools. Although evidence of poor hygiene such as the presence of Aeromona hydrophila in the mud-floor pools was found in 2002 and 2005, Salmonella was isolated only in 2002, and from both types of pools, suggesting that the type of enclosure and poor hygiene probably were not the only factors contributing to increased Salmonella presence. Finally, we cannot rule out external sources of Salmonella, because our sample size for free-ranging caiman is low and we did not investigate the source of the pathogen. Investigators in future studies should

contemplate larger sample sizes and additional sampling, especially of wild caiman and eggs, thus allowing prevalence estimates and comparisons with captive-raised individuals, as well as testing of water and food at the ranching facilities.

Humans can become exposed to Salmonella spp. during daily management of captive crocodilians (through direct contact with infected individuals, their feces, or contaminated surfaces) and when the animals are processed for sale (Manolis et al., 1991; Madsen, 1996; Willis et al., 2002). We are unaware of reported cases of human salmonellosis from live caiman or from caiman meat. However, Salmo*nella* spp. have been isolated from crocodile meat (Manolis et al., 1991; Madsen, 1996). Furthermore, the Salmonella isolates in our study were Salmonella enterica, which includes a number of serovars pathogenic to humans, and S. Nottingham, which has been associated with human salmonellosis (Ludlam et al., 1953). Because Salmonella spp. are ubiquitous and often are antibiotic resistant, it is difficult to eliminate the pathogen in a captive population (Marcus, 1971; Bradley et al., 1998; Corrente et al., 2004). Therefore, prophylactic measures that reduce environmental bacteria and host-to-host transmission are considered the most important hygiene efforts in ranching systems (Millan et al., 1997; Warwick et al., 2001; Corrente et al., 2004). Such endeavors should be facility-based, systematic, and aim to reduce the risk of contamination to animals, by-products, and humans.

Although the low number of freeranging caiman in our study precludes conclusions on *Salmonella* spp. exposure in the wild, our results suggest that screening of captive-raised caiman prior to release might help avoid introduction of unusual *Salmonella* spp. or other pathogens into the environment. There are numerous reports of negative effects of stress during captivity and transport on the immune system of reptiles (Zwart, 1986; Jacobson, 1993). Latent infection activated by immunosuppression could lead to morbidity or mortality of released individuals, increased susceptibility to predation, or reduced survival and reproductive capacity (Cunningham, 1996). New pathogens could be introduced inadvertently with asymptomatic carriers, affecting the survival of recipient populations (Jacobson et al., 1991; Karesh, 1995). Over 100,000 caiman are farmed annually in Argentina, 10% of which are released at egg collection sites each season with poor if any prerelease pathogen testing (W. Prado pers. comm.).

Based on our findings, shedding of Salmonella appears to be intermittent. Therefore, targeting instances of high Salmonella prevalences should lead to feasible strategies to reduce infection. However, unless ranching facilities implement regular and systematic surveillance for Salmonella spp. and other bacteria, these peaks in prevalence will remain unnoticed. As a first step to reduce risk to humans and make ranching a sound conservation strategy for crocodilian species, we recommend that ranching facilities regularly screen animals, food, water, and enclosures for potentially pathogenic (to humans or wildlife) bacteria.

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