Salmonella isolated from crocodiles and other reptiles during the period 1985–1994 in South Africa

MARTHA L. VAN DER WALT[1]*, F.W. HUCHZERMEYER[2] and HELENA C. STEYN[1]

ABSTRACT

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Over a 10-year period, 173 isolates of *Salmonella* were obtained during routine isolation from reptiles. Of the 173 isolates, 92 different *Salmonella* serovars were identified. Of them, 61 (66 %) belonged to subspecies I, nine to subspecies II and 21 to subspecies III (IIIa and IIIb), and one to subspecies IV. The majority of isolates were from farmed Nile crocodiles (145), three from wild-caught African dwarf crocodiles, 11 from captive snakes, 13 from lizards and one from a tortoise. The isolates from the tortoise and lizards were subspecies I isolates (Zaire and Tsevie, respectively). Of the snakes, nine isolates were S.III. The serovars isolated most often from the crocodiles were of subspecies I (32 serovars). Eight were from subspecies II, seven from subspecies III and one from subspecies IV. The most frequently identified serovars were Typhimurium (seven), Tsevie (six), Duval (six), Schwerin (six), Tinda (six), and Tallahassee (six). On two commercial crocodile breeding farms that had experienced ongoing problems for about two years, many isolates of *Salmonella* were made. Some of these serovars were isolated more than once, and also months apart. No single *Salmonella* serovar predominated, nor did a single pathological condition. These salmonellas were predominantly of subspecies I.

Keywords: Crocodiles, isolate, reptiles, *Salmonella*, serovars

INTRODUCTION

Members of the *Salmonella* genus are well-known pathogens of humans, livestock and companion animals, and they have also been implicated to play a major role in reptile diseases (Ross & Marzek 1984). However, salmonellae in reptiles have been reported mainly with reference to their zoonotic significance, whereas infections had been associated mostly after contact with lizards and snakes (Onderka & Finlayson 1985; Greenberg & Sechter 1992).

* Author to whom correspondence is to be directed

[1] Salmonella Laboratory, Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

[2] Pathology Section, Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

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Reptiles are often asymptomatic carriers of *Salmonella* (Zwart, Poelma & Strik 1970; Koopman & Janssen1973) while cases of clinical salmonellosis are documented less frequently (Huchzermeyer 1991; Manolis, Webb, Pinch, Melville & Hollis 1991). Most of the identified serovars of *Salmonella* have been isolated from reptiles, but relatively few have been isolated from man, or have they been associated with reptilian zoonoses (Chiodini & Sundberg 1981; Harvey & Price 1983; Greenberg & Sechter 1992). These studies showed that reptiles represent a large natural reservoir for *Salmonella*, a large percentage of animals are infected, and that a large variety of serovars of the subspieces I–IV are represented.

There has been much development in crocodile farming world-wide over the past ten years, and with this has come an interest in the major diseases associated with farmed crocodiles. Bacterial infections are probably the most common cause of death in farmed crocodiles, and severe ourbreaks of disease with massive mortality can occur (Foggin 1992; Huchzermeyer 1991). Many potentially pathogenic bacteria, especially enterobacteria, are harboured by clinically normal crocodiles, and these bacteria are probably the most frequent cause of disease. This report documents the occurrence of *Salmonella* mostly from post mortem cases of salmonellosis in captive reptiles. The data presented were collated from Annual Reports of the Bacteriology Section, Onderstepoort Veterinary Institute (OVI), during 1985–1994.

MATERIALS AND METHODS

Samples

Over a ten-year period (1985–1994) 173 *Salmonella* isolates were made. Of these, 148 isolates originated from farmed Nile crocodiles (*Crocodylus niloticus*) and free-living African dwarf crocodiles *Osteolaemus teraspis*, 11 from captive snakes [Indian python (*Python molurus*), Brown snake (*Storeria* sp.), California kingsnake (*Lampropeltis getulus californiae*), Rufus beaked snake (*Rhamphiophis oxyrhynchus*) and Mozambique spitting cobra (*Naja mossambica*)], 13 from clinically normal, free-living lizards and one sample from a tortoise. Most of the crocodile specimens derived from post mortem examinations carried out at the Pathology Section of the OVI. These included clinical samples from a number of crocodile-breeding farms, where problems were encountered over extended periods, an exception to this being the three specimens from wild-caught African dwarf crocodiles that were collected at markets in Brazzaville (Huchzermeyer & Agnagna 1994). The samples from the snakes were from cloacal swabs from clinically normal animals, or from animals that had died of various conditions. The samples from the lizards, all caught at the same time in one locality in the Western Cape, were taken from the intestines. The sample from the tortoise was from a rectal swab of an imported, clinically normal animal. In many instances histories were not available.

Isolation and identification procedures

For the isolation of *Salmonella*, specimens were directly plated out onto blood tryptose agar and MacConkey's agar, and also inoculated into selenite F broth for enrichment of *Salmonella*, which were then subcultured onto the agar media. Presumptive *Salmonella* colonies were biochemically identified to determine to which *Salmonella* subspecies they belong (Brenner 1984; Ewing 1986; Le Minor & Popoff 1987). Serovars were identified according to the latest available editions of the Kauffmann and White Diagnostic Scheme. Standard agglutination procedures were used as described by Ewing (1986). Commercial typing antisera [Diagnostics Pasteur, Sanofi Southern Africa (Pty) Ltd, P.O. Box 2884, Parklands, 2121 South Africa] were employed.

RESULTS

The identified serovars are listed in Table 1. Of the 173 samples, 92 different *Salmonella* serovars were identified. Most of the isolates (139) (80 %) belonged to subspecies I, nine to subspecies II, 24 to subspecies III (IIIa and IIIb) and one to subspecies IV (serovar 50;z4z23;–). Three rough salmonellas belonging to subspecies I–III were identified; there were also three untypable *Salmonella* isolates, and a number of isolates with antigenic formulae not in the Kauffmann-White Scheme, and a number of subspecies III isolates of which no H-antigens could be determined. From the intestines of the 13 wild-caught, clinically normal lizards, serovar Tsevie was identified, and from the rectal swab from the tortoise, serovar Zaire. From the 11 snakes, an untypable *Salmonella* I, and serovar Lindenburg were identified; the *Salmonella* III isolates (nine) from snakes were either rough, untypable, or belonging to serovars 48;–;–, 11;z42z23;–, 50;–;– and 58;z52;z. No subspecies II serovars were identified from snakes.

Most of the samples (148) were from crocodiles. The organisms were isolated from diseased animals (young animals and adults), but also from the intestines of clinically normal, slaughtered, farmed Nile crocodiles as well as from free-living African dwarf crocodiles, to determine whether salmonellas were present in healthy animals. The serovars identified were mostly of subspecies I (32 different serovars), eight of subspecies II, seven from the two subspecies III and one of subspecies IV. The most frequently identified serovars were Typhimurium (seven), Tsevie (six), Duval (six), Schwerin (six), Tinda (six), Tallahassee (six), Aarhus (six), Agama (six) and Blockley (four). Most of the other serovars were identified only once. A rough *Salmonella* I and two untypable *Salmonella* III isolates were also found. In some of the outbreaks of *Salmonella* on a farm, more than one serovar were identified, and sometimes more than one serovar from a single animal were identified. In one instance where a specific commercial breeder had had an ongoing problem for 4 months, eight different serovars were identified, more than one serovar was isolated from individual affected animals at a given stage and only one serovar (Schwerin) was identified more than once, but 2 months apart. For an extended period all crocodiles that had died on farms 1 and 2 were submitted for examination, as they had experienced ongoing fatalities. The isolates from two different crocodile farms are detailed in Tables 2 and 3.

From commercial breeding farm 2, which had had an ongoing problem with mortalities over a period of 24 months, 16 isolates were made. Most of the isolates (five) were serovar Antarctica, and they were made on two occasions, nine months apart. The *Salmonella* III isolates (two) were made 12 months apart, and the three S.IIIb isolates, eight and 13 months apart. Most isolates (11) were made from the intestines, and six from the liver. Enteritis was the clinical sign most often encountered. The three isolates encountered more than once were isolated either from the intestines or the liver.

From farm 1, which had had a problem for 2 years and 3 months, 79 isolates were made, and 41 different salmonellas isolated. Of them, 16 were made more than once [i.e. Tinda (six), Tallahasse (seven), Arechavalata (four), Blockley (five), Agoueve (two), Aarhus (four), Israel (two), Schwerin (three), Kisangani (three), Typhimurium (two), Dabou (two) and Brancaster (two)]. The pathological conditions observed most often were enteritis and conjunctivitis. Some of these serovars were isolated at various intervals, and no serovar was associated with a single pathological condition, except Blockley, which was isolated four out of five times from conjunctivitis.

DISCUSSION

A wide range of serovars were found in this study, including representatives of four of the six *Salmonella* subspecies. This was in accordance with data from other authors (Zwart *et al.* 1970; Harvey & Price 1983; Greenberg & Sechter 1992), but in contrast with the findings of Obwolo & Zwart (1993) who isolated almost exclusively serovars of the S.IIIa or S.IIIb subspecies from slaughtered crocodiles on one farm in Zimbabwe. Subspecies I contributed the largest proportion of serovars and although most of the animals in this study were clinically affected by *Salmonella* infections, similar results have been reported from other studies of healthy captive reptiles (Koopman & Janssen 1973; Harvey & Price 1983; Manolis *et al.* 1991). It would appear that reptiles are healthy carriers of *Salmonella*, and that a single animal can carry more than one serovar (Koopman & Janssen 1973; Chiodini & Sundberg 1981; Harvey & Price 1983). The general belief is that cold-blooded animals are the major reservoir for subspecies III salmonellae, but this study, as well as the others referred to, indicates that these animals also harbour a wide variety of subspecies I serovars.

Under intensive farming conditions as well as in other captive situations, the salmonellas harboured by clinically normal animals may be a source of infection to other animals as well as for man, i.e. from the handling of exotic pets. In this study, infection of farmed crocodiles with more than one serovar occurred, as was also seen by others (Onderka & Finlayson 1985). In reptiles other than crocodiles, infections with more than one serovar have been described as well (Koopman & Janssen 1973; Harvey & Price 1983;

TABLE 1 *Salmonella* serovars isolated from reptiles during the period 1985–1992

Serovar	No. of times isolated	Animal species
Salmonella I [a]		
Untypable	6	Snake, crocodile
Aarhus	5	Crocodile
Blockley	4	Crocodile
Duval	6	Crocodile
Lindenburg	1	California king snake
Schwerin	6	Crocodile
Tallahassee	7	Crocodile
Tinda	7	Crocodile
Tsevie	16	Crocodile, lizards
Typhimurium	8	Crocodile
Zaire	1	Tortoise
Salmonella II		
Rough	1	Crocodile
6,8; eh; enz$_{15}$	1	Crocodile
9,12; gz$_{62}$; –	1	Crocodile
16; gt; z$_{42}$	1	Crocodile
16; z; enx	1	Crocodile
39; mt; enx	1	Crocodile
40; b; –	1	Crocodile
48; k; enxz$_{15}$	1	Crocodile
55; –; –	1	Crocodile
Salmonella III		
Untypable	3	Snake, crocodile
Rough	1	Mocambique spitting cobra
48; –; –	1	Brown house snake
50; –; –	2	Crocodile
Salmonella IIIa		
11; z$_{42}$z$_{23}$; –	1	Snake
43; z$_{4}$z$_{23}$; –	1	Indian python
48; k; z$_{53}$	1	Crocodile
Salmonella IIIb		
Untypable	1	Rufous beaked snake
28;–;–	1	Crocodile
28; z10; z	1	Crocodile
30; k; enx	1	Crocodile [b]
38; k; z$_{35}$	1	Crocodile
48; k; z53	1	Crocodile
48; r; enxz$_{15}$	1	Crocodile
50; untypable	1	Gaboon adder
50; r; Z$_{35}$	1	Crocodile
50; r; z$_{53}$	1	Crocodile
50; z$_{52}$; z$_{35}$	1	Crocodile
58; z$_{52}$;z	1	House snake
59; lv; z53	1	Shield-nose snake
60/65; k; z	1	Crocodile
Salmonella IV		
50; z$_{4}$z$_{23}$	1	Crocodile
Total: 92	173	

[a] The following serovars were found one to three times, and only from crocodiles: Rough, 17;Z$_{4}$Z$_{23}$Z$_{32}$, Aberdeen, Adelaide, Agama, Agodi, Agoueve, Alamo, Albany, Anatum, Antartica, Arechavaleta, Bangui, Braenderup, Brancaster, Brazos, Bron, Brisbane, Budapest, California, Dabou, Diguel, Duesseldorf, Farsta, Good, Haardt, Herston, Israel, Javiana, Kingston, Kisangani, Naestved, Ndolo, Newlands, Os, Oslo, Othmarschen, Phaliron, Ried, Sandiego, Schwarzengrund, Simi, Somone, Tange, Tshiongwe, Wagenia, Wangata, Westhampton, Yaba, Yoruba

[b] Wild-caught African dwarf crocodile

TABLE 2 *Salmonella* isolates from crocodiles of farm 1 over the period 1991–1993

Date mm yy	Isolate	Isolated from			Pathology
		Intestine	Liver	Other	
02 91	Typhimurium	+			Wounds
03 91	III		+		Abscesses
05 91	Bangui	+	+		Enteritis
05 91	Kisangani		+		Hepatitis
05 91	Tinda	+	+		Splenomegaly
07 91	S I		+		Gastritis
07 91	S III	+			Gastritis
07 91	Tshiongwe	+	+		Gastritis
08 91	Yaba		+		None
08 91	Schwerin		+		Gastritis
08 91	S II rough		+		Gastritis
	S I rough	+	+		
08 91	S II	+			Gastritis
09 91	S II			Kidney	Gout
10 91	Blockley		+		None
11 91	Tinda	+			Enteritis
	Israel			Foot pad	Foot abscess
11 91	S IIIb		+		Enteritis
	Israel	+			
01 92	S II	+			Gastritis
03 92	Schwerin	+			Gastritis
03 92	Schwerin		+		Runt
03 92	Somone	+			Runt
03 92	Arechavalata	+	+		Wounds
03 92	Arechavalata	+	+		Wounds
03 92	Arechavalata	+	+		Wounds
03 92	Tsevie	+	+		Runt
03 92	Tallahassee		+		Runt
03 92	Aarhus	+			Runt
04 92	Kisangani		+	Stomach	Pneumonia
04 92	Agodi		+		Runt
04 92	Kisangani		+		Gastritis
04 92	Braenderup	+			Gastritis
05 92	Adelaide	+			Colitis
06 92	Tsevie	+			Enteritis
06 92	Tinda		+		Enteritis
06 92	Tinda		+		Enteritis
06 92	Tinda	+			Enteritis
07 92	Wagenia	+	+		Sudden death
07 92	Duval		+		Splenomegaly
07 92	Duval	+	+	Lung	Enteritis, lung oedema
08 92	Brancaster	+			Enteritis
09 92	Aarhus	+			Enteritis
10 92	Phaliron	+			Runt
11 92	S II	+			Runt
12 92	Agoueve	+			Gout
01 93	Dabou			Yolk sac	Y/s infection
01 93	Diquel	+			Gastritis
01 93	Bron		+		Hepatitis
01 93	Dabou	+			Malformation
01 93	Tallahassee		+		Gastritis
	Brancaster		+		
01 93	Ried		+		Gastritis
	California		+		
01 93	Herston	+			Gastritis
01 93	Tinda	+	+		Gastritis
02 93	Othmarschen		+	Yolk sac	Y/s infection
02 93	Haardt	+		Stomach	Runt
02 93	Westhampton	+	+		No lesions
02 93	Agoueve	+			Hepatitis
02 93	Arechavalata			Stomach	Gastritis
03 93	Aarhus	+			Conjunctivitis
03 93	Albany	+	+		Splenomegaly
03 93	Tallahassee		+		Splenomegaly
	Tallahassee	+			Splenomegaly
	Tallahassee	+			Splenomegaly
	Tallahassee	+			Splenomegaly
03 93	Tanger	+			Injury
03 93	Duval	+			No lesions
03 93	Duesseldorf		+		Runt
03 93	Tallahassee		+		Conjunctivitis
03 93	Blockley		+		Conjunctivitis
	Blockley		+		Conjunctivitis
03 93	Good	+			Conjunctivitis
	Good			Lung	Conjunctivitis
04 93	Blockley		+		Conjunctivitis
04 93	Typhimurium			Lung	Conjunctivitis
04 93	Blockley		+		Conjunctivitis
	Aarhus		+		Conjunctivitis
05 93	Kissi	+			Splenomegaly

TABLE 3 *Salmonella* isolated from crocodile farm 2 during 1989–1991

Date mm yy	Isolate	From organ: Intestine	Liver	Other	Pathology
05 89	S III	+			Enteritis
07 89	Antarctica	+			Enteritis
	Antarctica		+		Enteritis
	S IIIb		+		Enteritis
03 90	Budapest			Eye	Conjunctivitis
	Alamo	+	+		Enteritis
	S IIIb	+	+		Enteritis
03 90	Antarctica	+			Enteritis
	Antarctica	+			Enteritis
	Antarctica	+			Enteritis
03 90	S IV		+		Enteritis
06 90	Ndolo	+			Hepatitis
	S III	+			Double infection
06 90	Anatum	+			Runt
07 90	Wagenia	+		Abscess	Foot abscess
04 91	S IIIb		+		Enteritis

TABLE 4 *Salmonella* isolates from other crocodile farms

Date mm yy	Farm	Isolate	From organ: Intestine	Liver	Other	Pathology
03 89	3	Aarhus		+		Dermatitis
05 90		S I		+		Hepatitis
07 90	4	Agama		+		Hepatitis
07 92		Duval			Nose	Rhinitis
02 91	5	Tsevie	+	+		Enteritis
04 91	6	Israel	+			Peritonitis
07 91	7	S III		+		Foot abscess
12 91	8	S II	+			Enteritis
05 92	9	Yaba	+			Enteritis
08 92	10	Sandiego	+			Enteritis
08 92	11	Simi	+			Enteritis
02 93	12	Aberdeen		+	Yolk sac	Retained yolk sac
07 93	13	Schwerin	+			Enteritis
05 94	14	S I		+		Runt
		S III	+			Runt

Onderka & Finlayson 1985; Greenberg & Sechter 1992). This indicates that in cases of reptiles in which salmonellosis is suspected, several colonies per animal specimen should be examined to get the complete range of organisms involved. In this study, only 11 samples from snakes were analysed and only one serovar per case was identified. As multiple infection with *Salmonella* is common in reptiles, it is necessary to examine several *Salmonella* colonies from the agar media to obtain a complete picture of the infection in an individual animal or in an outbreak.

Serovars which were also found in other studies from reptiles were Adelaide, Anatum, Blockley, Oslo, Typhimurium, Wagenia and Wangata (Zwart *et al.* 1970; Koopman & Janssen 1973; Onderka & Finlayson 1985; Manolis *et al.* 1991; Foggin 1992; Greenberg & Sechter 1992). In this and other studies, Typhimurium was the serovar most frequently isolated from reptiles, and a number of untypable isolates were found (Zwart *et al.* 1970; Harvey & Price 1983; Huchzermeyer 1991; Manolis *et al.* 1991; Foggin 1992). This may well indicate that reptiles could harbour a large number of yet unrecognized serovars. It was surprising that so few subspecies II serovars were found, as the faeces of cold-blooded animals, many of African origin, are believed to be the main reservoir of this subspecies (Harvey & Price 1983).

Most of the samples from snakes (nine out of 11) belonged to the two subspecies III, while the majority of the isolates from crocodiles were from subspecies I. The high prevalence of subspecies III isolates in snakes and the low incidence of these isolates in farmed crocodiles were also observed by other authors (Zwart *et al.* 1970; Greenberg & Sechter 1992).

Many of the subspecies I serovars found among the crocodiles in this study are also found in poultry and cattle in South Africa (Annual Reports, Bacteriology Section, OVI).

In South Africa, farmed crocodiles are kept indoors in heated rooms, have a daily change of water and are mostly fed raw meat from farm fatalities. This appears to constitute an important source of infection under the intensive farming conditions. Flies and rodents, which are common sources of infection in other farming situations, do not occur in crocodile-rearing houses. Heat sterilization of the minced meat has been recommended to overcome the problem of introducing salmonellae into the crocodile-rearing houses with the feed (Huchzermeyer 1991). Another approach would be the exclusive feeding of compounded rations, which are being developed for crocodilians as well (Staton, McNease, Theriot & Joanen 1990), and which are gaining popularity with crocodile farmers in South Africa. In the case of commercial crocodile farms, where the animals are kept under captivity, it would appear that the S.I serovars are far more pathogenic than the S.II or S.III serovars. It would also appear that these animals are susceptible to infection from any *Salmonella* (at least S.I) serovar, and that the clinical signs of infection may manifest as enteritis, septicaemia, conjunctivitis or localized abscesses.

Incidence and distribution of *Salmonella* infections in wild crocodiles are mostly unknown, with the exception of the three *Salmonella* isolates from African dwarf crocodiles reported above, which were obtained from 21 faeces samples (Huchzermeyer & Agnagna 1994). The inaccessibility of wild-crocodile populations and the consequent unavailability of such specimens for isolation generally, prevent studies of this nature being carried out on wild crocodiles. The difference in subspecies distribution between snakes and crocodiles may also be due to differences of feed sources in captivity.

From each of the 13 lizards, serovar Tsevie was isolated. Lizards, like other reptiles, also have a very high carrier rate of *Salmonella* (Zwart *et al.* 1970; Kalvig, Maggio-Price, Tsuji & Giddens 1991). In another study, it has also been found that a particular serotype may be present at a high frequency in a given reptile species when it is captured in the same locality or possibly if the animals are exposed to the same contaminated environment prior to examination (Chiodini & Sundberg 1981).

All the animals in this study, that died of salmonellosis were kept in captivity. Although experimental infection of cold-blooded animals with *Salmonella* has not caused disease (Chiodini & Sundberg 1981), cases of fatal septicaemic salmonellosis have been reported in this study as in others, as well as cases of abscesses involving salmonellae (Boam, Sanger, Cowan & Vaughan 1970; Onderka & Finlayson 1984). Gross histological lesions have not been described and these cases are probably a result of the maladaptation syndrome and do not necessarily represent the manifestations of a primary pathogen.

The culture media used in this study to select for *Salmonella* were MacConkey's agar and selenite F broth. Other authors (Koopman & Janssen 1973; Harvey & Price 1983) made a comparison of culture media for the isolation of *Salmonella* from reptiles and found that selenite F broth gave reliable results. They noted, however, that if the organisms in a sample were scanty, extended enrichment of the selenite F broth would yield more information than direct plating out on agar media.

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