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## RETROSPECTIVE AND LONGITUDINAL STUDY OF SALMONELLOSIS IN CAPTIVE WILDLIFE IN TRINIDAD

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**ABSTRACT:** Morbidity and mortality of captive wildlife at the Emperor Valley Zoo, Trinidad from 1993 to 1996 were analysed to determine involvement of *Salmonella* spp. A 6 mo longitudinal study was conducted to determine the frequency of isolation of *Salmonella* spp. from apparently healthy, sick and dead wild mammals, birds, and reptiles. The antibiograms of *Salmonella* isolates were determined using the disc diffusion method. Fecal samples randomly selected from animal enclosures and cloacal swabs of snakes were cultured for *Salmonella* spp. following enrichment in tetrathionate and selenite cystine broths. For the 1993–96 period, *Salmonella* spp. was implicated in 17 (12%) of 141 sick or dead animals and the predominant serotype was *S. typhimurium*. During the 6 mo prospective study in a mean animal population of 1,186, there were 20 (2%) and 14 (1%) animals that were sick and died respectively; *Salmonella* spp. was implicated in only one mortality. Overall, of 1,012 samples from apparently healthy wildlife cultured, 66 (7%) yielded 24 serotypes of *Salmonella*. The predominant serotype were *S. seigburg* (16 isolates), *S. gaminara* (6 isolates), and *S. thompson* (6 isolates). None of the samples yielded *S. typhimurium*. The frequency of isolation of *Salmonella* spp. in reptiles (14%) was significantly higher than found in either mammals (7%) or birds (3%). Sixty-five (99%) of 66 *Salmonella* spp. isolates exhibited resistance to one or more of the nine antimicrobial agents tested. Resistance was high to cephalothin (92%), moderate to streptomycin (35%) and tetracycline (29%), but significantly low to gentamicin (2%), chloramphenicol (0%), and sulphamethoxazole/trimethoprim (0%). The prevalence of asymptomatic infections by *Salmonella* spp. in zoo animals was high and the very high prevalence of antimicrobial resistance could be a problem when treating salmonellosis.

**Key words:** Captive wildlife, salmonellosis, *Salmonella* spp., survey, zoo.

### INTRODUCTION

Diseases caused by *Salmonella* spp. are well recognized in humans, livestock, companion and zoo animals which may result in morbidity and mortality and significant economic losses (Okoh and Onazi, 1980; Hull et al., 1982; Minga et al., 1985; Stone et al., 1993). *Salmonella* spp. have assumed increased significance due to their ubiquitous distribution, the growing number of serotypes, wide host range, complex pathogenesis, and complicated epizootiology involving humans, animals, and environment (Morse and Duncan, 1974; Moustafa, 1989; D'Aouost, 1989).

*Salmonella* spp. inhabit the intestinal tract of vertebrate and invertebrate animals worldwide and their excretion results in contamination of water, food and the environment (Wray and Sojka, 1977; Turnbull, 1979). The microorganism has been isolated from wild mammals, birds and reptiles (Mohan et al., 1973; Williams et

al., 1977; Harper and Price, 1983; Minette, 1984). Although salmonellae may survive for long periods in the environment, it is the carrier state that provides the major source of infection for animals and humans (Minette, 1984; Moustafa, 1989) and various carrier states are recognized (Wray and Sojka, 1977).

Salmonellae may be involved in morbidity and mortality of zoo animals (Cambre et al., 1980; Okoh and Onazi, 1980). Probable source of *Salmonella* spp. for zoo animals are fruits and foods indiscriminately provided by zoo visitors and native rodents and wild small birds which gain access to the enclosures (Okoh and Onazi, 1980). The zoonotic nature of salmonellosis may also result in the interchange of *Salmonella* spp. between zoo attendants and captive wildlife (Sedgwick et al., 1975; D'Aouost, 1989; Pelzer, 1990).

Salmonellosis occurs in the three major forms of enteritis, septicemia, and abor-

tion; however during an epizootic or even in a single animal, a combination of the three may be observed (Pelzer, 1990). The clinical manifestation of salmonellosis depends upon virulence of the serotype, nature and chronicity of the lesion, and innate immunity of the host (Hoff and Hoff, 1984). Resistance to salmonellosis generally increases as the animal host ages (Wray and Sojka, 1977; Turnbull, 1979).

An epizootic of salmonellosis may have occurred in snakes at the Emperor Valley Zoo, (Port of Spain, Trinidad) in 1995–96. Adesiyun et al. (1998a) reported the prevalence of *Salmonella* spp. and *Campylobacter* spp. in animals at the zoo during the epidemic. No information was available on the antibiograms of the *Salmonella* spp. isolates.

This retrospective and longitudinal study of captive mammals, birds and reptiles at the zoo was conducted to determine the prevalence of *Salmonella* spp. in apparently healthy, sick and dead wildlife and to determine the antibiograms of *Salmonella* spp. isolates.

#### MATERIALS AND METHODS

Morbidity and mortality of animals housed at the Emperor Valley Zoo from 1993–96 were obtained from zoo records. Between September 1997 and February 1998, all cases of illness and death were recorded and *Salmonella* spp. isolation was conducted.

Over a 6 mo period (September 1997–February 1998), we sampled enclosures of every class of species of all mammals, birds, and reptiles every 3 wk but individual animals were not sampled. During each visit to animal enclosures, fecal samples in each enclosure were randomly selected. From enclosures housing <5 animals, one sample was randomly collected on each sampling day while for enclosures containing  $\geq 5$  animals, two randomly selected fecal samples were collected. Freshly voided feces by animal species were collected from the cages or enclosures with the exception of snakes where cloacal swabs were directly obtained from randomly selected animals. As a routine, the first freshly voided droppings or feces encountered in the enclosures were sampled. In cases where two samples were obtained, feces farthest away from the first were sampled. All samples were transported to the

laboratory ice-cooled in sterile plastic containers, within 2 hr of collection.

Feces (approximately 1 g) or cloacal swabs were enriched each in 10 ml of tetrathionate broth (Difco, Detroit, Michigan, USA) and 10 ml of selenite cystine broth (Difco) and incubated overnight at 37 C and 42 C, respectively. Growths were plated for isolation on xylose lysine desoxycholate (XLD) agar (Difco) and incubated at 37 C overnight. Colonies with black centers were subcultured onto blood agar and using standard methods (Macfaddin, 1980. Food and Agricultural Organization, 1992) suspect colonies were biochemically identified as *Salmonella* spp. The polyvalent antisera (A-1 $\times$ Vi) (Difco) for *Salmonella* spp. was used to serologically identify the isolates. Confirmation of *Salmonella* spp. isolates and serological typing using standard methods were done by the Caribbean Epidemiology Centre (CAREC; Port of Spain, Trinidad) the regional center for salmonellosis affiliated with the Pan American Health Organization (World Health Organization, Washington, D.C., USA).

The disc diffusion method (Bauer et al., 1966) was used to determine the antibiograms of all *Salmonella* spp. isolates. Antimicrobial agents (Difco) and their concentrations that were used included ampicillin (30 mcg), cephalothin (30 mcg), chloramphenicol (30 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg), neomycin (10 mcg), streptomycin (10 mcg), sulphamethoxazole/trimethoprim (23.75/1.25 mcg), and tetracycline (30 mcg). Isolation rates of *Salmonella* spp. from various mammals, birds, and reptiles, antibiograms and serotypes were compared using the chi-square test for independence with Epi Info (Center for Disease Control and Prevention, Atlanta, Georgia, USA; Version 6.02)

#### RESULTS

Table 1 shows the serotypes of *Salmonella* spp. isolated from the zoo from 1993–96. Of 141 animals documented to be either sick or to have died, 17 (12%) were documented in the records to have been due to salmonellosis based on clinical manifestation and isolation of *Salmonella* spp. Of 14 isolates *Salmonella* spp. which were serotyped, eight distinct serotypes were isolated with the prevalent serotypes being *S. typhimurium* (four isolates) and *S. brandenburg* (three isolates). Thirteen (76%) of the 17 zoo animals with salmonellosis were reptiles, mainly snakes, based on zoo records.

TABLE 1. Serotypes of *Salmonella* spp. isolated from sick or dead animals from the Emperor Valley Zoo between 1993 and 1996.

Year	Common name	Scientific name	<i>Salmonella</i> spp. serotypes isolated
1993	Porcupine	<i>Coendou prehensilis</i>	<i>S. typhimurium</i>
1994	Tiger	<i>Panthera onca</i>	<i>Salmonella</i> spp. <sup>a</sup>
1995	Local brocket deer	<i>Mazama americana trinitalis</i>	<i>S. enteritidis</i>
	Rainbow boa	<i>Epicrates cenchria maurus</i>	<i>S. brandenburg</i>
	Macajuel	<i>Boas constrictor</i>	<i>S. denver</i> , <i>S. mbandaka</i>
	Regal python	<i>Python</i> sp.	<i>S. brandenburg</i>
	Cook's tree boa	<i>Corallus enydris cookii</i>	<i>S. miami</i> , <i>S. brandenburg</i>
1996	Rabbit	<i>Dryctolagus cuniculus</i>	<i>Salmonella</i> spp. <sup>a</sup>
	Cook's tree boa	<i>Corallus enydris cookii</i>	<i>S. miami</i>
	Anaconda	<i>Eunectes murinus gigas</i>	<i>S. typhimurium</i>
	Rainbow boa	<i>Epicrates cenchria maurus</i>	<i>S. typhimurium</i>
	Macajuel	<i>Boas constrictor</i>	<i>Salmonella</i> spp. <sup>a</sup> , <i>S. typhimurium</i>
	Laura "Lora"	<i>Leptodeira annulata</i>	<i>S. panama</i>
	Mapepire balsain	<i>Imantodes cenchoa</i>	<i>S. mundonobo</i>

<sup>a</sup> *Salmonella* spp. isolates were not serotyped.

Of a total of 1,186 animals housed at the zoo from September 1997–February, 1998, 20 (2%) and 14 (1%) were sick and died, respectively. *Salmonella* spp. was responsible for death of a capybara (*Hydrochoerus hydrochoerus*). Morbidity experience in mammals (6%) (10/176) was statistically significantly ( $P < 0.01$ ;  $\chi^2$  test) higher than found in birds (1%) and reptiles (1%), (7/493). Similarly, the mortality rate was higher in mammals, 3% (6/176) compared to birds (1%) (3/157) and reptiles (1%) (5/493).

Table 2 shows the frequency of isolation of various *Salmonella* spp. serotypes. Overall, of 1,012 samples 66 (7%) yielded *Salmonella* spp. with 7% (29/404) from mammals, 3% (12/435) from birds and 14% (25/173) from reptiles. The isolation rate of *Salmonella* spp. from reptiles was significantly higher than from mammals and birds ( $P < 0.001$ ;  $\chi^2$  test). Twenty-four serotypes were isolated from all three classes of animals studied. The prevalent serotype was *S. siegburg* which constituted 24% (16/66) of isolates.

Of the total of 404 samples tested from mammals, 29 (7%) yielded *Salmonella* spp. Four (57%) of the seven mammalian orders including Carnivora, Primates, Rodentia, and Artiodactyla yielded *Salmonel-*

*la* spp. The highest frequency (17%) was detected in Carnivora while none of the samples from Perissodactyla, Chiroptera, and Lagomorpha yielded the microorganism. Overall, the ocelot (*Felis pardalis*) and kinkajou (*Potos flavus*) had prevalences of *Salmonella* spp. at 28% (8/29) and 25% (4/16), respectively.

Twelve (3%) of 435 bird samples tested from September 1997–February 1988 were positive for *Salmonella* spp. The highest frequency (7%) (2/30) was in Piciformes but *Salmonella* spp. also were recovered from 6% (6/106) of waterbirds, 6% (3/53) of raptor and from < 1% (1/140) of psittacines.

Of 173 reptilian samples collected from September 1997–February 1998, 25 (14%) were positive for *Salmonella* spp. Three (60%) of five samples from the family Teiidae yielded *Salmonella* spp. while none of the samples from the Pelomedusidae (4), Kinosternidae (3), Leptodactylidae (1), Iguanidae (1), and Elapidae (1) was positive for the microorganism. Six (55%) of the 11 families yielded *Salmonella* spp.

Resistance to antimicrobial agents by *Salmonella* isolates is shown in Table 3. Sixty-five (98%) of 66 isolates exhibited resistance to one or more of the antimicrobial agents tested. There was no significant

TABLE 2. Frequency of isolation of *Salmonella* spp. serotypes from mammals, birds, and reptiles from September 1997–February 1998 at the Emperor Valley Zoo.

Animal group	Animal source (family)	Number tested	Number (%) positive	Serotype of <i>Salmonella</i> [ ] <sup>a</sup>	
MAMMALS		404	29 (7)		
Primates	<i>Ateles belzebuth</i> (Cebidae)	9	1 (11)	<i>S. alachua</i>	
	Other Cebidae	51	0 (0)	—	
	Callithricidae	7	0 (0)	—	
	Cercopithecidae	41	0 (0)	—	
	Pongidae	8	0 (0)	—	
	Subtotal	116	1 (<1)	—	
Carnivora	<i>Felis pardalis</i> (Felidae)	29	8 (28)	<i>S. seigburg</i> [4] <i>S. isangi</i> <i>S. newport</i> <i>S. alachua</i> <i>S. kentucky</i>	
	<i>Pantera tigris sumatrae</i> (Felidae)	7	1 (14)	<i>S. seigburg</i>	
	<i>Pantera tigris altaica</i> (Felidae)	7	2 (29)	<i>S. aberdeen</i> [2]	
	<i>Pantera onca</i> (Felidae)	9	1 (11)	<i>S. uganda</i>	
	Other Felidae	9	0 (0)	—	
	<i>Potos flavus</i> (Procyonidae)	16	4 (25)	<i>S. seigburg</i> [2] <i>S. javiana</i> <i>S. oldenburg</i> <i>S. kentucky</i> <i>S. seigburg</i>	
	<i>Procyon cancrivorus</i> (Procyonidae)	15	2 (13)	<i>S. kentucky</i> <i>S. seigburg</i>	
	Other Procyonidae	8	0 (0)	—	
	<i>Eira barbara trinitatis</i> (Mustelidae)	24	3 (13)	<i>S. seigburg</i> <i>S. alachua</i> <i>Salmonella</i> Group G	
	<i>Pteronura brasiliensis</i> (Mustelidae)	3	1 (33)	<i>S. aberdeen</i>	
	<i>Lutra longicaudis</i> (Mustelidae)	8	1 (13)	<i>S. seigburg</i>	
		Subtotal	135	23 (17)	—
	Rodentia	<i>Herpestes auropunctatus</i> (Veverridae)	8	2 (25)	<i>S. thompson</i> <i>S. seigburg</i>
		<i>Hydrochoerus hydrochoerus</i> (Veverridae)	16	1 (6)	<i>S. thompson</i>
		<i>Coendou prehensilis</i> (Erathizontidae)	8	1 (13)	<i>S. thompson</i>
Dasyproctidae (Rodentia)		26	0 (0)	—	
Sciuridae (Rodentia)		8	0 (0)	—	
Caviidae (Rodentia)		4	0 (0)	—	
Rat (Rodentia)		7	0 (0)	—	
Mice (Rodentia)		7	0 (0)	—	
		Subtotal	84	4 (5)	—
Artiodactyla	<i>Tayassu tajacu</i> (Tayassuidae)	16	1 (6)	<i>S. gaminara</i>	
	Cervidae	34	0 (0)	—	
	Subtotal	50	1 (2)	—	
Other mammals	Phyllostomidae	7	0 (0)	—	
	Tapiridae	8	0 (0)	—	
	Leporidae	4	0 (0)	—	
		Subtotal	19	0 (0)	—
BIRDS		435	12 (3)	—	
Psittacidae	<i>Piuous mentrurus</i>	8	1 (13)	<i>Salmonella</i> Group C	
	Other Psittacidae	132	0 (0)	—	
		Subtotal	140	1 (<1)	—

TABLE 2. Continued.

Animal group	Animal source (family)	Number tested	Number (%) positive	Serotype of <i>Salmonella</i> [ ] <sup>a</sup>	
Waterbirds	Anatidae	35	3 (9)	<i>S. gaminara</i> <i>S. javiana</i> <i>S. heidelberg</i>	
	<i>Endocimus ruber</i> (Threskiornithidae)	28	2 (7)	<i>S. thompson</i> <i>S. javiana</i>	
	Other Threskiornithidae	9	0 (0)	—	
	<i>Pelicanus accidentalis</i>	18	1 (6)	<i>S. aberdeen</i>	
	Ardeidae	13	0 (0)	—	
	Phoenicopteridae	2	0 (0)	—	
	Rallidae	1	0 (0)	—	
	Subtotal	106	6 (6)		
	Raptor	<i>Pandion haliactus</i> (Pandionidae)	8	1 (13)	<i>S. thompson</i>
		<i>Milvago chimachima</i> (Falconidae)	4	1 (25)	<i>S. seigburg</i>
<i>Ciccaba virgata</i> (Strigidae)		8	1 (13)	<i>S. thompson</i>	
Other Strigidae		16	0 (0)	—	
Catharidae		8	0 (0)	—	
Accipitridae		9	0 (0)	—	
Subtotal		53	3 (6)		
Ramphastidae	<i>Ramphastos vitellinus</i>	22	2 (9)	<i>S. javiana</i> <i>S. rubislaw</i>	
	Other Ramphastidae	8	0 (0)	—	
	Subtotal	30	2 (7)		
Others	Phasianidae	71	0 (0)	—	
	Cracidae	19	0 (0)	—	
	Fringillidae	15	0 (0)	—	
	Trichillidae	1	0 (0)	—	
	Subtotal	106	0 (0)		
REPTILES		173	25 (14)	—	
Boidae	<i>Boa constrictor</i>	13	1 (8)	<i>S. livingston</i>	
	<i>Corallus enydris cookii</i>	2	1 (50)	<i>S. miami</i>	
	<i>Eunectes murinus gigas</i>	4	2 (50)	<i>S. uganda</i> [2]	
	<i>Epicrates cenchrus cenchrus</i>	3	2 (67)	<i>S. mbandaka</i> <i>S. heidelberg</i>	
	<i>Corallus canina</i>	1	1 (100)	<i>S. miami</i>	
	Other Boidae	22	0 (0)	—	
	Subtotal	45	7 (16)		
	Testudinidae	<i>Rhinodemmus punctularis</i>	7	1 (14)	<i>S. braenderup</i>
<i>Geocholone denticulata</i> and <i>Geocholone carbonaria</i>		24	3 (13)	<i>S. gaminara</i> [2] <i>S. rubislaw</i>	
Other Testudinidae		9	0 (0)	—	
Subtotal		40	4 (10)		
Columbridae	<i>Mastigodryas bordatia</i>	7	4 (57)	<i>S. seigburg</i> [4]	
	<i>Mastigodryas boddaesti</i>	3	1 (33)	<i>S. paratyphi</i> B	
	<i>Leptophis ahaetulla</i>	1	1 (100)	<i>S. reading</i>	
	Other Columbridae	21	0 (0)	—	
	Subtotal	32	6 (19)		
Viperidae	<i>Bothrops lanceolatus</i>	5	1 (20)	<i>S. uganda</i>	
	<i>Bothrops asper/atrox</i>	18	2 (11)	<i>S. mondonobo</i> [2]	
	<i>Crotalus durissus</i>	12	1 (8)	<i>S. mondonobo</i>	
	Subtotal	35	4 (12)		

TABLE 2. Continued.

Animal group	Animal source (family)	Number tested	Number (%) positive	Serotype of <i>Salmonella</i> [ ] <sup>a</sup>
Crocodylidae	<i>Caiman crocodilus</i>	3	1 (33)	<i>S. gaminara</i>
	Other Crocodylidae	3	0 (0)	—
	Subtotal	6	1 (17)	
Teiidae	<i>Tupinambis teguixin</i>	5	3 (60)	<i>S. gaminara</i> <i>S. gallinarum/pullo-</i> <i>rum</i> <i>S. oranienburg</i>
	Subtotal	5	3 (60)	
	Others			
Others	Pelomedusidae	4	0 (0)	—
	Kinosternidae	3	0 (0)	—
	Leptodacylidae	1	0 (0)	—
	Iguanidae	1	0 (0)	—
	Elapidae	1	0 (0)	—
	Subtotal	10	0 (0)	
TOTAL		1,012	66 (7)	

<sup>a</sup> Number of *Salmonella* spp. isolated.

difference ( $P > 0.05$ ;  $\chi^2$  test) in resistance amongst *Salmonella* spp. isolates from mammals, birds, and reptiles. Fourteen resistance patterns were observed in *Salmonella* spp. isolates; 10 patterns were detected in isolates from mammals, seven from birds and eight from reptiles.

### DISCUSSION

The finding that salmonellosis was indicated, based on clinical, pathological, and microbiological data, to have been possibly responsible for 12% of morbidity and mortality experiences amongst animals (mammals, birds and reptiles) kept at the Emperor Valley Zoo from 1993 to 1996

emphasizes the clinical importance of salmonellosis in captive wildlife. However, it was significant that during the 6 mo longitudinal study (September 1997 to February 1998) *Salmonella* spp. was isolated from only 1 (5%) of 20 sick animals at the Emperor Valley Zoo. Salmonellosis has been reported to be responsible for morbidity and mortality in zoo animals in other countries (Cambre et al., 1980; Okoh and Onazi, 1980). Although no epidemiological studies were performed, the possible sources of *Salmonella* spp. for captive animals at the Emperor Valley Zoo may have been food, particularly raw meat and live mice or improper quarantine measures for

TABLE 3. Prevalence of resistance to antimicrobial agents of *Salmonella* spp. isolates from animals at Emperor Valley Zoo.

Animal class	Num-ber of isolates tested	Number of isolates resistant <sup>a</sup> (%)	Number of <i>Salmonella</i> spp. isolates resistant (%):								
			KF <sup>b</sup>	TE	NA	N	S	AMP	SXT	C	GN
Mammals	29	28 (97)	26 (90)	6 (21)	1 (3)	5 (17)	10 (34)	1 (3)	0 (0)	0 (0)	1 (3)
Birds	12	12 (100)	12 (100)	3 (25)	1 (8)	1 (8)	5 (42)	1 (8)	0 (0)	0 (0)	0 (0)
Reptiles	25	25 (100)	23 (92)	10 (40)	2 (8)	0 (0)	8 (32)	2 (8)	0 (0)	0 (0)	0 (0)
Total	66	65 (98)	61 (92)	19 (29)	4 (6)	6 (9)	23 (35)	4 (6)	0 (0)	0 (0)	1 (2)

<sup>a</sup> Resistant to one or more of the antimicrobial agent(s).

<sup>b</sup> KF = cephalothin; TE = tetracycline; NA = nalidixic acid; N = neomycin; S = streptomycin; AMP = ampicillin; SXT = sulphamethoxazole-trimethoprim; C = chloramphenicol; GN = gentamicin.

wild animals, mostly reptiles, which are brought to the facility by the public. Other sources of infection include native rodents at the zoo and birds which gain access to animal enclosures (Okoh and Onazi, 1980).

Reptiles were demonstrated to be highly susceptible to salmonellosis (Harper and Prince, 1983; Minette, 1984). Over 80% of the animals which experienced *Salmonella* spp.-induced morbidity and mortality during the 1993–96 period were, in fact, squamates. The epidemic salmonellosis experienced during this period may have been responsible for the rather high frequency of isolation of *Salmonella* spp. Among apparently healthy turtles, snakes, and lizards, frequency of *Salmonella* spp. isolation was 9%, 14% and 50% respectively, which is lower than published reports. Turtles may have infection rates varying from 12% to 85% (Jackson and Jackson, 1971; Keymer, 1972), snakes may have 16% to 92% infection rates (Iveson et al., 1969; Roggendorf and Muller, 1976) and lizards may have infection rates from 40% to 77% (Iveson et al., 1969; Koopman and Janssen, 1973). The higher frequency of isolation of *Salmonella* spp. from apparently healthy reptiles (14%) in this study compared to mammals (7%) and birds (3%) further confirms the importance of reptiles as carriers of the microorganism. Adesiyun et al. (1998b) reported a prevalence of 21% for *Salmonella* spp. in captive reptiles on individual farms across Trinidad. Stress and immunosuppression have been reported to predispose to clinical salmonellosis in reptiles (Wray and Sojka, 1977).

A wide variety of *Salmonella* spp. serotypes were found in the present study. Adesiyun et al. (1998a) reported 12 serotypes among 24 isolates of *Salmonella* spp. from apparently healthy captive wildlife at the Emperor Valley Zoo during the salmonellosis epidemic. Multiple infections by *Salmonella* spp. serotypes may occur in asymptomatic and clinical salmonellosis

and some serotypes are more pathogenic than others (Borland, 1995; Pelzer, 1990).

Of epizootiological relevance was finding a predominant infection by some *Salmonella* spp. serotypes during different times at the Emperor Valley Zoo. During the 1993–96 period of epidemic salmonellosis, *S. typhimurium* and *S. brandenburg* were the predominant serotypes isolated from cases of clinical salmonellosis. During the 1995–96 period, *S. miami* was the predominant serotype isolated, although *S. typhimurium* also was isolated (Adesiyun et al., 1998a). However, in the present longitudinal study on apparently healthy mammals, birds, and reptiles, *S. seigburg* was the most prevalent serotype isolated. Some serotypes, specifically *S. miami*, *S. mbandaka* and *S. mundonobo*, were commonly isolated during all the periods earlier mentioned. The finding of varying predominant serotypes may represent a changing pattern of *Salmonella* spp. infections at the Emperor Valley Zoo.

It was of particular interest to isolate *S. pullorum/gallinarum*, an avian-adapted serotype from a tegu (*Tupinambis teguixin*). Poultry is fed to tegus at the Zoo which may explain, in part, the finding. The food of animals in captivity is known to act as vehicles for *Salmonella* spp. infections of animals and humans (Okoh and Onazi, 1980; D'Aoust, 1989).

Of zoonotic significance was isolation of *S. paratyphi* B, a human-adapted serotype from a snake (*Mastigodryas boddaesti*). Consumption of contaminated foods may have been possible sources of exposure of the snake to *S. paratyphi* B. Human handlers have been documented as sources of infection of *Salmonella* spp. to animals (Okoh and Onazi, 1980; Pelzer, 1990; Sedgwick et al., 1975). Sedgwick et al. (1975) found that transmission of pathogens from humans to zoo animals occurred more frequently than from zoo animals to humans. However, reptiles have been implicated as sources of *Salmonella* spp. for humans (Minette, 1984; Pelzer, 1990).

The significantly higher frequency of



isolation of *Salmonella* spp. from Carnivora compared to other mammalian classes can be explained, in part, by feeding these animals raw meat which could be contaminated by *Salmonella* spp. *Salmonella* spp. infections have been documented in livestock and slaughter animals in Trinidad (Adesiyun, 1993; Adesiyun and Kaminjolo, 1994). The frequency of isolation of *Salmonella* spp. from primates was relatively low; it is considered rare for free-living wild primates to be infected at the time of capture but they frequently become infected in captivity (Chiodini and Sundberg, 1981). The frequency of isolation of *Salmonella* spp. from captive mammals (7%) in the present study is similar to the prevalence of 8% found in confined mammalian wildlife on individual farms across Trinidad (Adesiyun et al., 1998b).

The low frequency of isolation of *Salmonella* spp. from avian species (3%) was not unexpected as similar results have been reported by other investigators (Kapperud and Rosef, 1983). In a study of captive avian wildlife in individual households in Trinidad, none of the birds were carriers of *Salmonella* spp. but a 5% infection rate detected in racing pigeons was attributed to observed poor sanitary practices at the two lofts which yielded *Salmonella* spp. positive racing pigeons (Adesiyun et al., 1998b). In our studies, waterbirds had the highest frequency (6%) of detection of *Salmonella* spp. *Salmonella* spp. was isolated from digestive tracts of flamingos and a waterbird, and mortality due to the microorganism was reported (Aguirre et al., 1991).

During the longitudinal aspect of the investigation, the isolation frequency of *Salmonella* spp. from feces in enclosures or cages of various animal classes varied among the 3 wk sampling periods. It is however pertinent to mention that sampling of feces in animal enclosures rather than individual animals every 3 wk, may be a limitation of the study. However, these findings support reports of intermittent nature of shedding *Salmonella* spp. by ap-

parently healthy animals, thus making identification of carriers difficult (Chiodini and Sunberg, 1981). This may be responsible for the observed wide variation of salmonellosis rates in wildlife (Chiodini and Sunberg, 1981; Roggendorf and Muller, 1976).

A high percentage of *Salmonella* spp. isolates exhibited resistance to one or more antimicrobial agents tested and the prevalence of multiple resistance was high. White and Forrester (1979) reported that antimicrobial resistance in bacteria isolated from wild animal species is rare. Captive wildlife kept in zoos may be exposed to human strains of bacteria through contact with human handlers who may be carriers of various strains of microbes and foods they consume which may also be contaminated with human strains of bacteria. Bacteria isolated from wildlife with close human contact have been documented to exhibit resistance to antibiotics (Roland et al., 1985) and the multi-resistance detected among *Salmonella* spp. isolates agrees with a published report (Hoff and Hoff, 1984). Misuse of antimicrobial agents at the zoo, may also have been responsible for the high prevalence of resistant *Salmonella* spp. isolates in the present study. Large scale indiscriminate use of antimicrobial agents in prophylaxis and therapy have been reportedly responsible for an increase in incidence of drug resistance to salmonellae in animals and humans (Cohen and Tauxe, 1986; Heffernan, 1991; Wray et al., 1991).

The antimicrobial agents to which many isolates of *Salmonella* spp. were resistant are commonly used in veterinary practice in Trinidad. These isolates may have been acquired from raw meat or milk fed to zoo animals. The regulations governing the presence of antibiotics in meat and milk in Trinidad and Tobago is not presently enforced (Adesiyun et al., 1998c). Transfer of resistance factor among enterobacteriaceae is well documented (Marsik et al., 1975; Tsubokura et al., 1995).

Sensitivities of *Salmonella* spp. isolates

to chloramphenicol, gentamicin, and sulphamethoxazole/trimethoprim might be due to their uncommon use in veterinary practice in the country either because of restriction (chloramphenicol) or cost and impractical routes, i.e., by injection only, of administration for wildlife (gentamicin). Sulphamethoxazole/trimethoprim is very effective against *Salmonella* spp. (Lawrence, 1983).

Intermittent shedding of diverse serotypes of *Salmonella* spp. which were resistant to many antimicrobial agents by apparently healthy captive wildlife at the Emperor Valley Zoo may have clinical, therapeutic, and public health significance.

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