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Authors: Thomas, A. D., Forbes-Faulkner, J. C., Speare, R., and Murray, C.

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A. D. Thomas,[1] **J. C. Forbes-Faulkner,**[1] **R. Speare,**[2] **and C. Murray**[3]

[1] Queensland Department of Primary Industries, Animal and Plant Health Service, Oonoonba Veterinary Laboratory, P.O. Box 1085, Townsville, Queensland, Australia, 4810

[2] School of Public Health and Tropical Medicine, James Cook University, Douglas, Townsville, Queensland, Australia, 4811

[3] Australian *Salmonella* Reference Centre, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia, Australia, 5000

ABSTRACT: During a 20 yr period (1978 to 1998), 233 isolates of *Salmonella* spp. were cultured from 179 wildlife animals (representing 25 species), 32 crocodile (*Crocodylus porosus*) eggs and six crocodile nesting sites, and represented 59 different serotypes. *Salmonella* serotype Virchow, the major serotype infecting humans in north Queensland, (Australia) was common in macropodids, but was not found in reptiles and was isolated only once from cane toads (*Bufo marinus*). Investigations of human cases of salmonellosis should include simultaneous studies on wild and domestic animals in contact with the case.

Key words: Reservoirs, *Salmonella* spp., salmonellosis, survey, wildlife.

INTRODUCTION

The Oonoonba Veterinary Laboratory (OVL) is a regional laboratory situated in northern Queensland (Australia) that performs diagnostic and research work for the major industries of cattle, sheep, goats, pigs and aquaculture. Samples from wildlife species, including farmed crocodiles (*Crocodylus porosus*), are also submitted for disease diagnosis and bacterial examination. Submissions are received from within an area from the Torres Strait in the north to Mackay in the south and from the coastline to the Northern Territory border (10° to 21°S; 138° to 149°E). The majority of the area covered is situated in a tropical environment. During the period 1978 to 1998, 233 isolates of *Salmonella* spp. were obtained from 25 species of wildlife ranging from macropodids to reptiles. Part of the data presented in this paper dealing with selected wildlife species has previously been published in other journals. The purpose of this paper is to expand the knowledge of the serotypes of *Salmonella* spp. present in the wildlife population of northern Queensland, especially in relation to the presence of *Salmonella* serotype Virchow, the major serotype infecting humans in this region.

MATERIALS AND METHODS

The names given for *Salmonella* serotypes do not follow the usual rules of nomenclature. Se-rotypes belonging to *Salmonella enterica* subsp. *enterica* (*Salmonella* subspecies I) will be designated *Salmonella* serotype X where X is a specific epithet relating to a disease type, to the host animal, or more recently, to a geographical location (eg. *Salmonella* serotype Dublin). Serotypes belonging to the other subspecies (II, IIIa, IIIb, IV, VI) will be designated by their antigenic formulae (eg. *Salmonella* serotype IIIb 50:k:z). This conforms to the method used by the WHO Collaborating Centre for Reference and Research on *Salmonella* in France (Bopp et al., 1999). The genus 'Arizona' was incorporated into the genus *Salmonella* as subspecies IIIa (monophasic strains) or subspecies IIIb (diphasic strains) (Bopp et al., 1999).

Samples were received for bacterial examination from a variety of sources. These included (1) a survey of the bacterial flora of the intestine of cane toads (*Bufo marinus*) and macropodids, with samples mainly received from the intestinal tract or cloacae; (2) a survey of the bacterial contamination on crocodile carcases during processing at an abattoir, with up to six swabs collected per animal from the skin and meat of 72 crocodiles (23 in 1991 and 49 in 1992); (3) a survey of the natural flora on the shells and/or from the yolks of 689 crocodile eggs (both fertile and infertile) during the hatching process, with these eggs collected during four consecutive breeding seasons (1995–98) from two female crocodile breeders in 1995 to six breeders in 1998; (4) sick animals (brought into OVL with all due care by licensed carers) for bacterial diagnosis of infection, in which necropsy of these animals was performed and the appropriate organs removed for culture; and (5) suspected diseased tissues submitted for bacterial evaluation. Samples

from sources (4) and (5) also were examined for parasitology and histopathology when recommended by the duty pathologist.

The Oonoonba Veterinary Laboratory (Townsville, Queensland) is a NATA (National Association of Testing Authorities)-accredited Laboratory and has permits from a recognised Regional Animal Ethics Committee for the necropsy of all animals.

Samples for salmonellae isolation were macerated in buffered peptone water (BPW) (Oxoid Australia Pty Limited, West Heidelberg, Victoria, Australia) using a Stomacher Blender (Stomacher Lab-Blender 80, Seward Medical, 131 Great Suffolk St, London UK). Eggs were washed whole in 20mL BPW in a Stomacher bag (Sarstedt of Australia, Technology Park, South Australia) and the washes kept for microbiological analysis. The eggs were then wiped with 70% iso-propyl alcohol and aseptically cut open with sterile scissors. The yolks were sampled. Two different procedures were used over the years. Prior to 1993, samples were transferred onto Brilliant Green Agar (BGA) (Oxoid Australia Pty Limited), and into BPW for 24 hr at 37 C. Then a few drops of the BPW were transferred to two bottles of Tetrathionate broth (Oxoid Australia Pty Limited), with added iodine and brilliant green, one bottle incubated at 37 C and the other at 42 C for 24 hr before plating further onto BGA. After 1993, Bismuth Sulphite Agar (BSA) (Oxoid Australia Pty Limited) and Lysine-Mannitol-Glycerol Agar (LMG) (Cox, 1993) replaced the BGA, and Rappaport-Vassiladis broth (Oxoid Australia Pty Limited) replaced the Tetrathionate broth. The procedure remained the same.

Suspect salmonellae colonies were removed for further identification, initially by conventional tests and then progressively by use of the API 20E (bioMerieux, Marcy, l'Etoile, France) and Microbact 24E (Medvet Science Pty. Ltd., Adelaide, South Australia) kit systems. More than one presumptive colony was removed from each selective agar for identification and serotyping if colony variation was obvious. Presumptive salmonellae isolates were sent to the Australian *Salmonella* Reference Centre (Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia), for serotyping. Multiple isolations of the same serotype made from a single host were recorded as only one for this study.

RESULTS

Serotypes of *Salmonella* spp. that we isolated from macropodids are listed in Table 1. The species represented were the agile wallaby (*Macropus agilis*), black-striped wallaby (*Macropus dorsalis*), bridled nailtail wallaby (*Onychogalea fraenata*), eastern grey kangaroo (*Macropus giganteus*), common or eastern wallaroo (*Macropus robustus*), red kangaroo (*Macropus rufus*), allied rock wallaby (*Petrogale assimilis*), spectacled hare wallaby (*Lagorchestes conspicillatus*), Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*), the western grey kangaroo (*Macropus fuliginosus*) and the whiptail wallaby (*Macropus parryi*).

Of the 57 macropodids sampled, 62 isolates were cultured of which 35 were isolated from fecal material only. Isolates were not always associated with diarrhea as this work was part of an ongoing survey into the bacterial flora of macropodid feces. However, three cases that were associated with diarrhea came from situations where the macropodid was a pet living in close proximity to humans and other pets. Of these three cases, two were associated with children and dogs (*Salmonella* serotype Wandsworth, *Salmonella* serotype Litchfield, and *Salmonella* serotype Lansing), and one was associated with a cat (*Salmonella* serotype Newington). Fourteen of the isolates (representing nine serotypes) were collected from lymph nodes (mainly ileocaecal or mesenteric) while the remaining 13 isolates (representing 10 serotypes) were cultured from urine, liver, lung, kidney, and spleen. Multiple serotypes of salmonellae from individual macropodids occurred five times during this study. *Salmonella* serotype Anatum and *Salmonella* serotype Derby and *Salmonella* serotype Bilthoven and *Salmonella* serotype Lansing were isolated from the feces from two eastern grey kangaroos, *Salmonella* serotype Lansing and *Salmonella* serotype Saintpaul were isolated from the feces of a red kangaroo, *Salmonella* serotype Litchfield was isolated from the feces and *Salmonella* serotype Muenchen from the mesenteric lymph node of a spectacled hare wallaby and *Salmonella* serotype IIIb 50:k:z35 was isolated from the heart and abdomen and *Salmonella* serotype Chester

TABLE 1.   Sixty-two isolates of *Salmonella* spp. (belonging to 24 serotypes) cultured from 57 macropodids during the period 1978 to 1998 in Queensland. Most of these animals were sampled during the course of a survey.

Number of isolates	Serotype	Macropodid[a]	Site of isolation[b]
8	Virchow	AW(3); EG(3); WH(1)	E
		RK(1)	S
7	Lansing	EG(3); EW(1); RK(2)	E
		RK(1)	S
6	Anatum	AW(1); EG(2); K?(1)	E
		BL(1); RW(1)	S
6	Litchfield	AW(1); K?(1); SP(1);	E
		TK(1); W?(1)	E
		K?(1)	S
5	Muenchen	AW(1); SP(2)	E
		EG(1); BR(1)	S
5	Wandsworth	AW(1); RK(4)	E
3	II Bilthoven	EG(2)	E
		EG(1)	S
2	Chester	EW(1)	E
		EG(1)	S
2	Infantis	K?(1)	E
		RW(1)	S
2	Newington	EG(1); EW(1)	E
2	Orientalis	EG(2)	E
2	Saintpaul	AW(1); RK(1)	E
1	Aberdeen	K?(1)	E
1	Derby	EG(1)	E
1	Give	RK(1)	E
1	Heidelberg	K?(1)	S
1	Meleagridis	EG(1)	E
1	Orion	EW(1)	E
1	Rubislaw	BL(1)	E
1	Thompson	AW(1)	E
1	Typhimurium	TK(1)	S
1	Zanzibar	K?(1)	E
1	I (rough:1,v:1,2)	W?(1)	E
1	IIIb (50:k:z35)	EG(1)	S

[a] AW = Agile Wallaby; BL = Black-Striped Wallaby; BR = Bridled Nailtail Wallaby; EG = Eastern Grey Kangaroo; EW = Eastern (common) Wallaroo; K? = Kangaroo; RK = Red Kangaroo; RW = Allied Rock Wallaby; SP = Spectacled Hare Wallaby; TK = Lumholtz's Tree Kangaroo; W? = Wallaby; WH = Whiptail Wallaby.

[b] E = samples collected from enteric or related origins—colon, feces, ileocecal lymph node, intestinal contents, mesenteric lymph node; S = samples collected from systemic origins—one or more isolations from lung, liver, spleen, bladder, urine, heart blood, peritoneal fluid, abdomen and often in conjunction with feces or associated lymph nodes.

isolated from the abdomen, stomach and large intestine of a grey kangaroo.

The *Salmonella* serotypes isolated from the cane toad (*Bufo marinus*) we show in Table 2. These animals were collected from the wild as part of a survey into the bacterial flora of their intestinal tract. With one exception (*Salmonella* serotype Saintpaul from a liver sample) all isolates were cultured from the cloacae, intestinal contents or feces. *Salmonella* serotype Mgulani was the most prevalent isolate, occurring in 46% of 22 toads positive for salmonellae. No members of *Salmonella* subspecies III were isolated.

We have indicated serotypes of *Salmonella* isolated from reptiles, excluding crocodiles, in Table 3. These 14 reptiles included eight snakes (python (species unknown), scrub python (*Morelia amethistina*) and western taipan (*Parademansia microlepidota*)), four lizards (including two

TABLE 2.   Twenty-four isolates of *Salmonella* spp. (belonging to 11 serotypes) cultured from 24 cane toads (*Bufo marinus*) during the period 1978–98 in Queensland. These animals were sampled during a survey and appeared healthy at collection.

Number of isolates	Serotype	Site of isolation[a]
11	Mgulani	E
3	Anatum	E
2	Aberdeen	E
1	Chester	E
1	Enteritidis	E
1	Hvittingfoss	E
1	Lansing	E
1	Newington	E
1	Oranienburg	E
1	Saintpaul	S
1	Virchow	E

[a] E = samples collected from cloacae, intestinal contents or feces; S = sample collected from the liver.

blue-tongue lizards (*Tiliqua scincoides*)) and two turtles (species unknown). All cases were associated with disease and were presented by permit owners or from a nearby wildlife sanctuary.

The serotypes of salmonellae isolated from salt-water crocodiles (*Crocodylus po-rosus*) we list in Table 4. Samples from the crocodiles were either from diseased animals on crocodile farms, an abattoir survey of potential pathogens during slaughtering or from eggs and nesting material during the hatching process. *Salmonella* serotype Lansing and *Salmonella* serotype Litchfield were the most common isolates from the farms, while serotypes of *Salmonella* subspecies IIIb were prominent during the abattoir survey. *Salmonella* serotype Welikade was the most common isolate from the eggs and bedding material.

Salmonella serotypes that are isolated from a variety of other wildlife species, including a cassowary (*Casuarius casuarius*), a duck (species unknown), a dugong (*Dugong dugon*), an echidna (*Tachyglossus aculeatus*), six ostriches (*Struthio camelus*), a galah (*Cacatua roseicapilla*), and three common brushtail possums (*Trichosurus vulpecula*) are listed in Table 5. All of these isolates were associated with disease.

DISCUSSION

Salmonella spp. are opportunistic pathogens and can infect a wide range of host

TABLE 3.   Fourteen isolates of *Salmonella* spp. (belonging to 11 serotypes) cultured from 14 reptiles (including 4 lizards, 8 snakes and 2 turtles) during the period of 1978–98 in Queensland. All animals were submitted for bacterial diagnosis of infectious disease.

Number of isolates	Serotype	Reptile	Site of isolation[a]
Snakes			
2	IV Houten	Python	S
		Western Taipan	S
2	Paratyphi B var.	Snake (?)[b]	S
	Java	Scrub Python	E
1	Muenchen	Snake (?)	S
1	Seftenberg	Python	S
1	IIIb (50:r:z35)	Snake (?)	E
1	IIIb (61:1,v:z35)	Python	E
Lizards			
2	II Wandsbek	Blue-tongue lizard	E
		Blue-tongue lizard	E
1	II Freemantle	Lizard (?)	S/E
1	Adelaide	Lizard (?)	S
Turtle			
1	Anatum	Turtle (?)	E
1	Enteritidis	Turtle (?)	S

[a] E = samples collected from enteric origin; S = samples collected from systemic origin.
[b] (?) = species unknown.

TABLE 4.   One hundred and eleven isolates of *Salmonella* spp. (belonging to 38 serotypes) cultured from 72 saltwater crocodiles (*Crocodylus porosus*), 32 eggs and six nesting sites during the period 1978–98 in Queensland.

Number of isolates	Serotype	Animal status		
		Diseased animals[a]	Abattoir survey[b]	Eggs[c]
Crocodiles				
13	Welikade	0	0	13
11	Litchfield	11(7S,4E)	0	0
8	IIIb (50:r:z35)	3(3S)	3	2
7	Lansing	6(4S,2E)	0	1
7	IIIb (61:r:z53)	0	0	7
4	Enteritidis	3(3S)	0	1
4	IIIb (38:1,v:z53[z54]	0	4	0
4	IIIb (60:r:z)	4(2S,2E)	0	0
4	IIIb (61:z52:z53)	0	4	0
3	Anatum	0	0	3
3	Singapore	3(3S)	0	0
3	Typhimurium	0	3	0
3	IIIb (48:k:1,5,[7])	1(1S)	2	0
3	IIIb (61:l,v:z35)	3(2S,1E)	0	0
3	Potsdam	0	0	3
2	Adelaide	1(1S)	1	0
2	Bahrenfeld	0	2	0
2	Cerro	2(2S)	0	0
2	Onderstepoort	2(1S,1E)	0	0
2	Urbana	2(1S,1E)	0	0
1	Ball	1(1S)	0	0
1	Chester	1(1E)	0	0
1	Eastbourne	1(1S)	0	0
1	Havana	1(1S)	0	0
1	IV Houten	1(1S)	0	0
1	Hvittingfoss	0	0	1
1	Jangwani	1(1S)	0	0
1	Muenchen	0	0	1
1	Poona	0	1	0
1	IIIb (61:l,v:1,5,7[z57])	1(1S)	0	0
1	IIIb (50:k:z)	0	1	0
1	IIIb (48:z52:z)	1(1S)	0	0
Nests				
1	Aberdeen			
1	Anatum			
1	Hvittingfoss			
1	Odozi			
1	Rubislaw			
1	Virchow			
1	Welikade			
1	Zanzibar			
1	IIIb (50:r:z35)			

[a] Site of isolation: E = samples collected from enteric origin; S = samples collected from systemic origin.
[b] Samples collected from the skin before skinning and from the meat after skinning.
[c] Samples collected from the outer egg shell or from the yolk.

animals, including man (Morse and Duncan, 1974; Murray, 1991). *Salmonella* subspecies I are normally isolated from warm-blooded animals while those of the other subspecies (II, III, IV, VI) are usually isolated from cold-blooded animals and the environment and rarely from humans (Bopp et al., 1999).

TABLE 5.   Fifteen isolates of *Salmonella* spp. (belonging to nine serotypes) cultured from 14 animals representing seven different species during the period 1978–98 in Queensland. All the animals were submitted for bacterial diagnosis of infectious disease.

Number of isolates	Serotype	Animal species	Site of isolation[a]
Cassowary(1)			
1	Saintpaul	*Casuarius casuarius*	E
Duck(1)			
1	Litchfield	(?)[b]	E
Dugong(1)			
1	IV Lohbruegge	*Dugong dugon*	S
Echidna(1)			
1	Adelaide	*Tachyglossus aculeatus*	E
Galah(1)			
1	Typhimurium	*Cacatua roseicapilla*	S
Ostrich(6)			
5	Saintpaul	*Struthio camelus*	E
1	Hvittingfoss	*Struthio camelus*	E
Possum(3)			
3	Zanzibar	*Trichosurus vulpecula*	S
2	Havana	*Trichosurus vulpecula*	S
1	Mgulani	*Trichosurus vulpecula*	E

[a] E—Samples collected from fecal or intestinal origin; S—samples collected from systemic origin.
[b] (?) = species unknown.

Salmonella serotypes, especially *Salmonella* subspecies III, are frequently isolated from healthy reptiles (including crocodiles) as part of the normal flora of the intestine of these reptiles (Chiodini and Sundberg, 1981; Obwolo and Zwart 1993). These serotypes are rarely incriminated as a cause of gastro-enteritis in man (Chiodini and Sundberg, 1981). *Salmonella* spp. are ubiquitous and can persist very well in the environment (eg. dust, manure, soil) if well protected from direct sunlight (Morse and Duncan, 1974). Spread of the organism can occur via the water system (Murray, 1991; Polo et al., 1999).

Salmonellae-associated problems in animals, as recorded through OVL submissions, do not occur in large numbers in northern Queensland. In all, only 454 *Salmonella* isolates were cultured during the 20 yr period. Of these, 233 were isolated from wildlife. The remainder were cultured from dogs (66), cattle (63), horses (33), birds (23), pigs (19), goats (10), sheep (4), cats (2), and a mouse.

This paper does not specifically deal with the prevalence of salmonellae in north Queensland even though routine survey results have been included. With the exception of the toad and part of the macropodid survey, the samples are biased by animals having been held in captive situations. This increases the risk of salmonellae carriage and disease (Munday, 1988). Alternatively, the hosts have been diagnosed with suspected bacterial infections.

Salmonella spp. are a significant cause of disease in captive macropodids although it can be difficult to interpret the results because of the high prevalence of carrier animals in these species (Munday, 1988). Of the 57 macropodids that tested positive for salmonellae during this period, 37 were orphaned joeys and represented 27% of joeys submitted for survey purposes (Speare and Thomas, 1988). Stress is a major factor in the increased load and excretion of salmonellae in macropodids (Speare et al., 1989). The orphaned joeys were under such stress after removal from their dead mothers and the handling and feeding changes that were subsequently made. Of the 13 macropodids with bac-

terial septicaemia due to *Salmonella* spp., seven were joeys.

Salmonella spp. were isolated from the ileocaecal or mesenteric lymph nodes of the macropodids on thirteen occasions. This is not uncommon in carrier animals. In a study of 100 normal cattle held 4 days before slaughtering, salmonellae were isolated from 76 of the animals and included 54 isolations from the mesenteric lymph nodes (Samuel et al., 1979).

It was interesting to note that a serotype of *Salmonella* subspecies IIIb was only isolated once from the 57 animals. The serotypes isolated from the remaining animals were not dissimilar to those isolated in other studies of Australian mammals (How et al., 1983) and free-ranging macropodids (Speare et al., 1989). One isolation of *Salmonella* serotype Typhimurium, a serotype commonly isolated from infections in humans (National *Salmonella* Surveillance Scheme Reports, 1988–1997) was made as were some unusual serotypes in *Salmonella* serotype Bilthoven and *Salmonella* serotype Zanzibar. The presence of *Salmonella* serotype Virchow is not unexpected. It is a common human pathogen especially in northern Queensland (Ashdown and Ryan, 1990).

Salmonellosis in amphibians was investigated during a survey of the cane toad (*Bufo marinus*). These apparently healthy free-ranging toads yielded 24 isolates of *Salmonella* spp. The common isolate during this study was *Salmonella* serotype Mgulani, a serotype often found in tropical areas (Murray, 1991), and represented 46% of the serotypes recovered. No serotypes of *Salmonella* subspecies IIIb were isolated. Serotypes of *Salmonella* subspecies IIIb are not commonly found in toads or frogs (Roggendorf and Muller, 1976), however Kourany et al. (1970) found 8% of 185 *Bufo marinus* carried *Salmonella* spp., 25% of which were serotypes from *Salmonella* subspecies IIIb. A prevalence of 13% for *Salmonella* spp. (O'Shea et al., 1990) has been recorded in Australia. Most of the serotypes isolated from the toads were different to those found in toads overseas (Bool and Kampelmacher, 1958; Kourany et al., 1970; Everard et al., 1979) and also those found in northern Australia in reptiles and amphibians (Lee and Mackerras, 1955; How et al., 1983). *Bufo* spp. can have up to 1×10^{10} salmonellae/g of feces which is 10 to 100 times the level carried by most other vertebrates (Sharma, 1979) and well above the minimum infectious dose for humans. Toads are therefore a potential vector for the spread of salmonellosis as they move through northern Queensland into the north of the Northern Territory.

The reptile isolates (other than those isolated from crocodiles) were the most diverse in serotypes. All these animals were submitted with disease problems including diarrhea and necrosis of the tail. Seven of the fourteen isolates (50%) were typed as belonging to *Salmonella* subspecies II, IIIb or IV. Snakes, lizards and turtles are renowned for their carriage of salmonellae as part of the normal flora of the intestinal tract (Cooper, 1981; Austin and Wilkins, 1998). The zoonotic potential of these animals has been recognized when they are kept as pets (D'Aoust et al., 1990; Buck and Nicolls, 1997; Mermin et al., 1997).

Stress in lizards can induce disease by the commensal salmonellae in the intestinal tract (Kalvig et al., 1991). A study of lizards in Panama (Kourany et al., 1970) showed 35% of 177 animals cultured carried *Salmonella* spp. and 39% of these were serotypes of *Salmonella* subspecies III. Roggendorf and Muller (1976) found that 62% of 39 lizards studied carried salmonellae and 21% of these were serotypes of *Salmonella* subspecies III.

Although serotypes of *Salmonella* subspecies III can be found in a wide variety of animals, snakes are considered to be their main reservoir (Ikeda et al., 1986; Kraus et al., 1991; van der Walt et al., 1997). Roggendorf and Muller (1976) isolated *Salmonella* spp. from 33% of 24 snakes and half of these isolates were serotypes of *Salmonella* subspecies III.

Salmonella spp. isolated from saltwater crocodiles, their eggs, and nesting material during this period also showed a broad range of serotypes. In all, there were 111 isolates covering 38 serotypes. Although 49 isolates were recovered from 44 crocodiles sent in for disease diagnosis, it is often difficult to interpret the results as *Salmonella* spp. are regarded as commensals of the intestinal tract of these animals (Cooper, 1981; Debyser and Zwart, 1991). Salmonellae are more prevalent in farmed (14%) than wild-caught (3%) alligators (*Alligator mississippiensis*) in the USA (Scott and Foster, 1997) because of the stress factors involved with crowding and handling during the early growth period of the farmed reptile. The prevalence of salmonellae isolated from healthy, farmed juvenile crocodiles ranged from 8% (van der Walt et al., 1997) in the Republic of South Africa to 88% (Obwolo and Zwart, 1993) in Zimbabwe. The major serotypes isolated from our study of diseased crocodiles were *Salmonella* serotype Litchfield and *Salmonella* serotype Lansing. Eight of the isolates (20%) were serotypes of *Salmonella* subspecies III.

The abattoir study covered surveys on *C. porosus* during two slaughtering procedures 12 mo apart. During the first survey, 11 of 23 crocodiles carried *Salmonella* spp. and 65% of the isolates were serotypes of *Salmonella* subspecies IIIb (Rickard et al., 1995). During the second survey of 49 crocodiles, and after a change in the slaughtering routine, only three salmonellae were isolated of which two were serotypes of *Salmonella* subspecies IIIb (Rickard et al., 1995). During the slaughter of *C. porosus* and *C. johnstoni* in the Northern Territory, 16% of 287 meat swab samples yielded salmonellae of which 11% were serotypes of *Salmonella* subspecies III (Manolis et al., 1991). Madsen (1996) isolated salmonellae from 30% of fresh and 20% of frozen meat of the Nile crocodile (*C. niloticus*). Serotypes of *Salmonella* subspecies III accounted for 41% of these isolates.

Over the last four years, this laboratory has sampled 689 *C. porosus* eggs (including both fertile and infertile eggs), 5% of which were contaminated by *Salmonella* spp. representing nine serotypes. The predominant serotype was *Salmonella* serotype Welikade. From six nesting sites, nine serotypes of *Salmonella* were also isolated but only four of these were the same as those found on the eggs. Two of the egg isolates and one of the nest isolates were serotypes of *Salmonella* subspecies IIIb indicating that the majority of the salmonellae were possibly of fecal origin. As all the female breeder crocodiles were captive animals, it is likely that these serotypes were associated with the raw animal material (such as poultry heads and kangaroo meat) used as feed. The breeder crocodiles were not tested for a carrier state.

Of the 17 isolates recovered from a small range of other wildlife species, none were serotypes of *Salmonella* subspecies IIIb. *Salmonella* serotype Saintpaul was isolated from a cassowary and also from five ostriches from a single flock. The details of the case in the dugong have already been reported (Elliott et al., 1981). There are few reports available on monotremes, however, one study found *Salmonella* serotype Bovis-morbificans, *Salmonella* serotype Dublin, *Salmonella* serotype Saintpaul and *Salmonella* serotype Typhimurium involved in the death of six of 73 captive echidnas surveyed (McOrist and Smales, 1986). Other workers have indicated the presence of *Salmonella* serotype Dublin, *Salmonella* serotype Orion, *Salmonella* serotype Typhimurium and serotypes of *Salmonella* subspecies III in routine fecal or intestinal culture of captive echidnas (Whittington, 1988). Rodriguez et al. (1992) found healthy opossums (*Didelphidae*) to be heavily colonised by salmonellae with 40% of the 20 animals surveyed being culture positive. The possums (*Phalangeridae*) in our study were all suffering from disease. One of the isolates was *Salmonella* serotype Mgulani, a sero-

type found commonly in dogs and toads in northern Queensland.

During the period under examination, serotypes of *Salmonella* subspecies IIIb were prominent in snakes and crocodiles, rare in the mammals and not found in the toads. This again indicates that reptiles are the major harbourers of serotypes of *Salmonella* subspecies IIIb in wildlife. Eleven serotypes of this group were isolated.

In north Queensland, *Salmonella* serotype Virchow is the predominant serotype isolated from human clinical cases while in temperate Australia, *Salmonella* serotype Typhimurium predominates and *Salmonella* serotype Virchow is less common (Streeton and Hanna, 1994). *Salmonella* serotype Virchow tends to produce invasive salmonellosis in infants and children in north Queensland, but the epidemiology is poorly understood (Ashdown and Ryan, 1990). Reptiles, including house geckoes (Tan et al., 1978), have been suspected as potential sources, however, no geckoes were examined in this study. No *Salmonella* serotype Virchow were isolated from reptiles and only one isolate was obtained from cane toads. These classes of host appear to be unlikely sources of human infection. Since *Salmonella* serotype Virchow was a common isolate from the macropodids in this survey, mammals including macropodids should be suspected as a possible source of *Salmonella* serotype Virchow for humans in north Queensland.

The potential of captive and pet wildlife to transmit salmonellae to their owners and carers should not be underestimated, and epidemiological studies on sources for human salmonellosis should simultaneously investigate both the human cases and the wild and domestic animals in contact with them.

LITERATURE CITED

ASHDOWN, L. R., AND P. J. RYAN. 1990. Invasive disease due to *Salmonella* Virchow: A north Queensland problem. Medical Journal of Australia 153: 330–335.

AUSTIN, C. C., AND M. J. WILKINS. 1998. Reptiles— associated salmonellosis. Journal of the American Veterinary Medical Association 212: 866–867.

BOOL, P. H., AND E. H. KAMPELMACHER. 1958. Some data on the occurrence of *Salmonella* in animals in Surinam. Antonie van Leeuwenhoek 24: 76–80.

BOPP, C. A., F. W. BRENNER, J. G. WELLS, AND N. A. STROCKBINE. 1999. *Escherichia, Shigella,* and *Salmonella. In* Manual of clinical microbiology, 7th Edition, P. R. Murray (ed.). American Society for Microbiology, Washington, DC, pp. 459–474.

BUCK, J. J., AND S. W. NICOLLS. 1997. *Salmonella arizonae* enterocolitis acquired by an infant from a pet snake. Journal of Pediatrics, Gastroenterology and Nutrition 25: 248–249.

CHIODINI, R. J., AND J. P. SUNDBERG. 1981. Salmonellosis in reptiles: a review. American Journal of Epidemiology 113: 494–499.

COOPER, J. E. 1981. Pathogenic infection by *Salmonella* spp. rare among reptilians. *In* Diseases of reptiles, Vols. 1, 2. J. E. Cooper (ed.). Academic Press, London, UK, pp. 105–191.

COX, J. M. 1993. Lysine-mannitol-glycerol agar, a medium for the isolation of *Salmonella* spp., including *Salmonella typhi* and atypical strains. Applied and Environmental Microbiology 59: 2602–2606.

D'AOUST, J. Y., E. DALEY, M. CROZIER, AND A. M. SEWELL. 1990. Pet turtles: A continuing international threat to public health. American Journal of Epidemiology 132: 233–238.

DEBYSER, I. W. J., AND P. ZWART. 1991. Review of the most important diseases in crocodylia which possibly interfere with human health. Vlaams Diergeneeskd Tijdschrift 60: 164–169.

ELLIOTT, H., A. THOMAS, P. W. LADDS, AND G. E. HEINSOHN. 1981. A fatal case of salmonellosis in a dugong. Journal of Wildlife Diseases 17: 203–208.

EVERARD, C. O. R., B. TOTA, D. BASSETT, AND C. ALI. 1979. Salmonella in wildlife from Trinidad and Grenada. Journal of Wildlife Diseases 15: 213–219.

HOW, R. A., A. J. BRADLEY, J. B. IVESON, C. M. KEMPER, D. J. KITCHENER, AND W. F. HUMPHREYS. 1983. The natural history of salmonellae in mammals of the tropical Kimberley Region, Western Australia. Ecology of Disease 2: 9–32.

IKEDA, J. S., D. C. HIRSH, S. S. JANG, AND E. L. BIBERSTEIN. 1986. Characteristics of *Salmonella* isolated from animals at a veterinary medical teaching hospital. American Journal of Veterinary Research 47: 232–235.

KALVIG, B. A., L. MAGGIO-PRICE, J. TSUJI, AND W. E. GIDDENS. 1991. Salmonellosis in laboratory-housed iguanid lizards (*Sceloporus* spp.). Journal of Wildlife Diseases 27: 551–556.

KOURAN, M., C. W. MYERS, AND C. R. SCHNEIDER. 1970. Panamanian amphibians and reptiles as

carriers of *Salmonella.* American Journal of Tropical Medicine and Hygiene 19: 632–638.

———, L. BOWDRE, AND A. HERRER. 1976. Panamanian forest mammals as carriers of *Salmonella.* American Journal of Tropical Medicine and Hygiene 25: 449–455.

KRAUS, A., G. GUERRA-BAUTISTA, AND D. ALARCON-SEGOVIA. 1991. *Salmonella arizonae* arthritis and septicaemia associated with rattlesnake ingestion by patients with connective tissue diseases. A dangerous complication of folk medicine. Journal of Rheumatology 18: 1328–1331.

LEE, P. E., AND I. M. MACKERRAS. 1955. *Salmonella* infections of Australian native animals. Australian Journal of Experimental Biology 33: 117–126.

MADSEN, M. 1996. Prevalence and serotype distribution of *Salmonella* in fresh and frozen meat from captive Nile crocodiles (*Crocodylus niloticus*). International Journal of Food Microbiology 29: 111–118.

MANOLIS, S. C., G. J. W. WEBB, D. PINCH, L. MELVILLE, AND G. HOLLIS. 1991. *Salmonella* in captive crocodiles (*Crocodylus johnstoni* and *C. porosus*). Australian Veterinary Journal 68: 102–105.

MCORIST, S., AND L. SMALES. 1986. Morbidity and mortality of free-living and captive echidnas, *Tachyglossus aculeatus* (Shaw) in Australia. Journal of Wildlife Diseases 22: 375–380.

MERMIN, J., B. HOAR, AND F. J. ANGULO. 1997. Iguanas and *Salmonella marina* infection in children; a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics 99: 399–402.

MORSE, E. V., AND M. A. DUNCAN. 1974. Salmonellosis—an environmental problem affecting man and animals. Journal of the American Veterinary Medical Association 165: 288–299.

MUNDAY, B. L. 1988. Marsupial diseases. *In* Australian wildlife, Proceedings of the Post-Graduate Committee for Veterinary Science. University of Sydney, Australia pp. 299–365.

MURRAY, C. J. 1991. Salmonellae in the environment. Revue Scientifique et Technique de l'Office Internationale des Epizooties 10: 765–785.

NATIONAL SALMONELLA SURVEILLANCE SCHEME. 1988–1997. Annual reports, Microbiological Diagnostic Unit, University of Melbourne, Victoria, Australia.

OBWOLO, M. J., AND P. ZWART. 1993. Prevalence of *Salmonella* in the intestinal tracts of farm-reared crocodiles (*Crocodylus niloticus*) in Zimbabwe. Journal of Zoo and Wildlife Medicine 24: 175–176.

O'SHEA, P., R. SPEARE, AND A. D. THOMAS. 1990. Salmonellas from the cane toad, *Bufo marinus.* Australian Veterinary Journal 67: 310.

POLO, F., M. J. FIGUERAS, I. INZA, J. SALA, J. M. FLEISHER, AND J. GUARRO. 1999. Prevalence of *Salmonella* serotypes in environmental waters and their relationship with indicator organisms. Antonie van Leeuwenhoek 75: 285–292.

RICKARD, M. W., A. D. THOMAS, S. BRADLEY, J. FORBES-FAULKNER, AND R. J. MAYER. 1995. Microbiological evaluation of dressing procedures for crocodile carcases in Queensland. Australian Veterinary Journal 72: 172–176.

RODRIGUEZ, L., R. CALABUIG, M. LAROCCO, F. G. MOODY, AND J. A. MILLER. 1992. Bacterial flora of the gastrointestinal tract of opossums. Veterinary Microbiology 30: 289–295.

ROGGENDORF, M., AND H. E. MULLER. 1976. Enterobacteria of reptiles. Zentralblatt fur Bakteriology und Hygiene, I Abteilung Original A 236: 22–35.

SAMUEL, J. L., D. A. O'BOYLE, W. J. MATHERS, AND A. J. FROST. 1979. Isolation of salmonellae from mesenteric lymph nodes of healthy cattle at slaughter. Research in Veterinary Science 28: 238–241.

SCOTT, T., AND B. G. FOSTER. 1997. *Salmonella* spp. in free-ranging and farmed alligators (*Alligator mississippiensis*) from Texas and Louisiana, USA. Aquaculture 156: 179–181.

SHARMA, V. K. 1979. Enterobacteriaceae infection in man and animals and detection of their natural reservoirs in India. Zentralblatt für Bakteriologie und Parasitenkunde 1. Abteilung Originale 243: 381–391.

SPEARE, R., AND A. D. THOMAS. 1988. Orphaned kangaroo and wallaby joeys as a potential zoonotic source of *Salmonella* spp. Medical Journal of Australia 148: 619–623.

———, J. A. DONOVAN, A. D. THOMAS, AND P. J. SPEARE. 1989. Diseases of free-ranging Macropodoidea. *In* Kangaroos, wallabies and rat-kangaroos, G. Grigg, P. Jarman, and I. Hume (eds.). Surrey Beatty and Sons Pty Limited, New South Wales, Australia, pp. 705–734.

STREETON, C., AND J. HANNA. 1994. Salmonellosis in far North Queensland, 1993. Communicable Diseases Intelligence 18: 187–188.

TAN, R. J. S., L. EK-WANG, AND B. ISHAK. 1978. Intestinal bacterial flora of the household lizard, *Gecko gecko.* Research in Veterinary Science 24: 262–263.

VAN DER WALT, M. L., F. W. HUCHZERMEYER, AND H. C. STEYN. 1997. *Salmonella* isolated from crocodiles and other reptiles during the period 1985–1994 in South Africa. Onderstepoort Journal of Veterinary Research 64: 277–283.

WHITTINGTON, R. J. 1988. Monotremes in health and disease. *In* Australian wildlife, Proceedings of the Post-Graduate Committee for Veterinary Science. University of Sydney, Australia, pp. 727–787.