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Salmonella and Arizona in reptiles and man in Western Australia

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INTRODUCTION

During the course of investigations into the prevalence of *Salmonella* in foodstuffs produced in Western Australia, and as a result of findings in routine medical diagnostic bacteriology, it was evident that a wide variety of serotypes existed in the State. During a 5-year period seventy distinct serotypes were isolated from human infections in the community. Further, it was remarked that a geographical distribution of serotypes existed. A relative prominence of salmonellas with numerically high somatic antigens, the isolation of new serotypes, the incidence of multiple infections, and the finding of strains in *Salmonella* subgenera II, III and IV were associated with specimens which originated in the remoter and more recently developed areas of the north. The converse was found in the metropolitan district of Perth and the more populous areas of the south-west.

A possible explanation for this geographical distribution of serotypes was the ecological relationship of *Salmonella* species and the fauna of the country. It appeared that in the remoter regions the strains were originating in a reservoir of infection that was unlikely to be human owing to the sparseness of the population and the diversity of types isolated from human cases of salmonellosis. Since the geological history of the Australian subcontinent is one of evolutionary development in relative isolation from the land masses of the world, a fact illustrated in the unique fauna of reptiles, marsupials and the last surviving monotremes, and, since isolations of salmonellas were made from reptiles caught in areas remote from human habitation, it was reasoned that these creatures might serve as the reservoir of types that had evolved independently of strains in other parts of the world.

A limited investigation was undertaken to assess the importance of reptiles in Australia as carriers of *Salmonella* and Arizona groups of organisms and this report records the findings of our study.

MATERIALS AND METHODS

Specimens

One hundred and sixteen reptiles, comprising 70 lizards, 40 snakes, 4 tortoises and 2 crocodiles, originating in Australia were examined. At least five of the snake species examined were venomous. The size of the reptiles ranged from a 14 ft. python to legless lizards a few inches long. Specimens were obtained from captive creatures and from those in their natural environment either remote from or near

to human habitation. Rectal swabs from a number of native birds and rodents, from a domestic cat and a captive kangaroo, all of which were living in close association with some of the reptiles, were examined. In addition, sixteen samples of litter from captive reptiles were tested; this litter was contaminated by rodent litter from live animals fed to snakes. Some of the larger lizards were infested with ticks which were removed, macerated and cultured separately.

The majority of specimens were taken on a single examination, but repeat specimens were taken from two wild lizards after 6 weeks in captivity, and on several occasions from two captive pythons; the last occasion being 9 months after first sampling and following a period of hibernation. Samples of contaminated soil and reptile litter, stored in sealed jars, were examined after an interval of 12 weeks from first examination. This repeat sampling was a part of survival experiments to be reported elsewhere.

Cloacal contents on swabs were placed in 1–5 ml. volumes of Sachs's (1939) buffered glycerol saline transport medium. Organs from reptiles submitted dead or diseased were removed with aseptic precations. All samples were homogenized before processing.

Culture procedure

All samples were first inoculated direct on SS agar (Oxoid or Difco) and modified bismuth sulphite agar (BSA) (Hobbs, 1943). Approximately 0·5 ml. of each sample, delivered by pasteur pipette, was sown into 10 ml. volumes of enrichment media of Leifson (1936), and of Rappaport, Konforti & Navon (1956) (as modified by Iveson & Kovacs, 1967), and of selenite F. broth. Also included were two new enrichment media (to be published separately) designed to recover a wider range of salmonella serotypes from human and animal sources and from food. Subcultures were made from all enrichment media after 18–24 hr. incubation, and occasionally after 48 hr, at 37° C. on SS and modified BSA media.

The BSA medium was prepared by reconstituting dehydrated bismuth sulphite agar (Oxoid or Difco) as recommended by the manufacturer, but, immediately before pouring into plastic Petri plates, 10 ml. of 1 % ferrous sulphate and 3·0 ml. 10 % ferric citrate were added to 1 litre of the molten medium. Plates were dried for 30–45 min. with the lids removed, and were stored at 4° C. overnight or up to 4 days before use. The additive solutions were sterilized by heating at 60° C. for 1 hr., and remained stable for several weeks at 4° C.

Non-lactose fermenting colonies on SS agar, or colonies resembling *Salmonella* or Arizona species on the modified BSA medium were examined biochemically by inoculation on a composite medium slope, to differentiate subgenus I and III strains and related Enterobacteriaceae. The differential composite medium detected fermentation of glucose, lactose, sucrose, sorbose, mannitol, dulcitol, production of hydrogen sulphide and splitting of urea after 16–24 hr. incubation. Growth from the slope was used for serological testing and further biochemical reactions. Dulcitol-negative cultures were further screened biochemically for reactions with dulcitol and sorbose peptone water, lysine, malonate, gelatin, and O.N.P.G. tests. Serological confirmation of subgenus III strains was made where

appropriate. The use of sorbose, as reported by Kauffmann (1956) and Stenzel (1960), coupled with lysine reaction served to differentiate *Citrobacter* spp. Colony selection was limited to a maximum of five colonies per plate, or an average of fifteen for each specimen. During the investigation up to fifty colonies were serologically screened from selected specimens as a rough check on routine recoveries.

RESULTS

From 116 reptiles examined 97 yielded *Salmonella* or Arizona species (Table 1). Of 70 lizards in the investigation 54 (77 %) were positive and among 40 snakes tested 37 (92 %) proved positive. The 4 tortoises and 2 crocodiles examined were all positive for *Salmonella* but negative for Arizona. Forty-four (63 %) lizards yielded *Salmonella* only (5 infected with more than one strain) and 4 (6 %) were positive for Arizona only; whereas 4 (10 %) snakes were positive for *Salmonella* only, while 15 (37 %) were positive for Arizona only. The distribution of serotypes was: 29 salmonella and 7 Arizona serotypes in lizards, compared with 14 salmonella and 10 Arizona serotypes in snakes.

Table 1. *Isolations of salmonella and Arizona strains from* 116 *reptiles*

Reptile	No. tested	No. positive	No. positive for: Salmonella only	No. positive for: Arizona only	No. of: Salmonella serotypes	No. of: Arizona serotypes
Lizard	70	54 (77)	44 (63)	4 (6)	29	7
Snake	40	37 (92)	4 (10)	15 (37)	14	10
Tortoise	4	4	4	0	4	0
Crocodile	2	2	2	0	2	0
Totals	116	97 (84)	54 (47)	19 (16)	49	17

Figures in parentheses indicate percentages

The distribution of salmonella and Arizona serotypes in the ninety-seven reptilian genera and species from which positive isolations were made is shown in Table 2. In general, the reptiles imported from Australian states, and those in the southern region of West Australia (including metropolitan Perth), were captive creatures or were caught in proximity to human habitation. Those reptilia caught in the northern region of the state (which has been demarcated for convenience from the southern region at 28° latitude) were free-ranging. There appeared to be no peculiar distribution of serotypes as between captive and wild reptiles, although *S. typhimurium* was absent from specimens taken in geographically remote regions, whether from wild or captive reptiles; neither was *S. typhimurium* isolated from the birds, domestic cat or rodents which were sampled in the north of the state although five isolations from the birds and the domestic cat were made. However, *S. typhimurium* was occasionally found in reptiles, including snakes, living in captivity in the metropolitan area. *S. typhimurium* was isolated from rodents fed to these snakes and from the mixed rodent and reptile litter.

From ten litter samples associated with the reptiles imported from the Eastern

Table 2(*a*). *Salmonella and Arizona isolations from fifty-four Australian lizards*

Lizards			*Salmonella*			*Arizona*			
	State of origin	No.	Serotype	No.	Total no.	Serotype	No.	Total no.	Total isolations
Amphibolurus barbatus	NSW	1	*ohlstedt*	1	1	.	.	.	1
	Vic	2	42:Z; –	1	1	5:29 -21	1	1	2
	WA 1	1	*bullbay*	1	.	.	.	.	.
			nashua	1	2	.	.	.	2
	WA 2	8	*adelaide*	2	.	.	.	.	.
			boecker	1	.	.	.	.	.
			chester	1	.	.	.	.	.
			emmastad	1	.	.	.	.	.
			kisarawe	6	.	.	.	.	.
			*mowanjum**	1	.	.	.	.	.
			muenchen	1	.	.	.	.	.
			orion	2	.	.	.	.	.
			rubislaw	1	.	.	.	.	.
			senftenberg	4	.	.	.	.	.
			tenessee	1	.	.	.	.	.
			wandsworth	1	22	.	.	.	22
A. ornatus	WA 1	11	*kisarawe*	4	.	.	.	.	.
			nashua	9	13	.	.	.	13
Diporiphora bilineata	WA 2	1	47:k:–	1	1	.	.	.	1
Lialis burtoni	WA 1	2	*hvittingfoss*	1	.	.	.	.	.
			newport	1	2	.	.	.	2
Moloch horridus	WA 1	1	*chester*	1	1	.	.	.	1
Omolepida branchiale	WA 1	1	*muenchen*	1	1	.	.	.	1
Physignathus leseurii	Q	4	*chester*	2	.	1,33:23–21	1	.	.
			adelaide	1	.	28:23–25	1	.	.
			rubislaw	1	.	29:24–31	1	.	.
			wandsbek	2	6	.	.	3	9
Tiliqua occipitalis	WA 1	2	*alsterdorf*	1	.	.	.	.	.
			lindern	1	2	.	.	.	2
T. occipitalis multifasciata	WA 2	1	47:k; –	1	1	.	.	.	1
T. rugosa	Vic	1	*charity*	1	.	.	.	.	.
			give	1	2	.	.	.	2
T. scincoides	Vic	2	*chester*	1	.	.	.	.	.
			saintpaul	1	2	5:29–30	1	1	3
	WA 2	1	*adelaide*	1	.	.	.	.	.
			muenchen	1	.	.	.	.	.
			orion	1	.	.	.	.	.
			senftenberg	1	4	.	.	.	4
Trachysaurus rugosus	WA 1	3	*give*	1	.	.	.	.	.
			ohlstedt	1	.	.	.	.	.
			orientalis	1	.	.	.	.	.
			singapore	1	4	.	.	.	4
Varanus tristis	WA 1	7	*alsterdorf*	1	.	26:26–25	1	.	.
			champaign	5	.	.	.	.	.
			give	2	.	.	.	.	.
			wandsbek	1	9	.	.	1	10
V. varius	NSW	1	.	.	.	1,33:23–21	1	1	1
	Q	1	.	.	.	16:23–25	1	1	1
	SA	1	*saintpaul*	1	.	species	1	.	.
			chester	1	2	.	.	1	3
	Vic	1	.	.	.	26:24–25	1	1	1
Unidentified	WA 2	1	*kisarawe*	1	1	.	.	.	1
Total lizards		54	.	.	77	.	.	10	87

* Indicates new serotypes

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Table 2(*b*). *Salmonella and Arizona isolations from thirty-seven Australian snakes*

Snakes			*Salmonella*			*Arizona*			
	State of origin	No.	Serotype	No.	Total no.	Serotype	No.	Total no.	Total isolations
Ahaetulla punctulata	Q	7	*adelaide*	1	.	1,33:23–21	1	.	.
			muenchen	2	.	15:24–31	1	.	.
				.	.	9a9b:26–21	4	.	.
				.	.	24:26–21*	1	.	.
				.	.	25:23–25*	2	.	.
				.	3	28:32–28	1	10	13
Aspidites melanocephalus	WA 2	1	*chester*	1	1	5:29–30	1	1	2
Demansia affinis	WA 1	2	*potsdam*	1	1	16:23–25	1	.	.
						Species	1	2	3
D. nuchalis	WA 1	1	.	.	.	28:32–35	1	1	1
D. olivacea	Vic	1	*newington*	1	1	1,33:23–21	1	1	2
Denisonia superba	Vic	1	*hvittingfoss*	1	.	5:29–31	1	.	.
			orientalis	1	2	.	.	1	3
Liasis amethystinus	Q	1	.	.	.	20:29–25	1	.	.
						30:22–31	1	2	2
L. childreni	Q	1	*typhimurium*	1	1	25:23–25*	1	1	2
Morelia spilotes spilotes	Q	4	*saintpaul*	2	.	16:23–25	1	.	.
			typhimurium	1	.	16:23–37	1	.	.
				.	3	30:26–21	1	3	6
M. spilotes variegata	Q	5	*chester*	1	.	16:23–25	1	.	.
			enteritidis	1	.	30:26–21*	4	.	.
			typhimurium	3	.	29:29–25*	1	.	.
				.	5	30:22–31	1	7	12
M. variegata	WA 1	1	.	.	.	29:29–25*	1	1	1
Notechis scutatus	Vic	4	*onderstepoort*	1	.	26:33–31	3	.	.
			orientalis	1	.	5:29–21	1	.	.
			typhimurium	1	.	16:27–25	1	.	.
			wandsbek	1	4	.	.	5	9
	WA 1	3	*kibusi*	1	.	16:23–25	2	.	.
			newport	1	.	.	.	.	.
			typhimurium	1	3	.	.	2	5
Pseudechis	NSW	2	.	.	.	16:23–25	1	.	.
			.	.	.	26:33–31	1	2	2
	Q	1	.	.	.	20:29–25	1	1	1
Unidentified	WA 1	2	*muenchen*	2	2	26:23–21	1	1	3
Total snakes		37	.	.	26	.	.	41	67

* Indicates new serotypes.

Table 2(*c*). *Salmonella and Arizona isolations from four Australian tortoises and two Australian crocodiles*

Tortoises and crocodiles			*Salmonella*			*Arizona*			
	State of origin	No.	Serotype	No.	Total No.	Serotype	No.	Total no.	Total isolations
Chelodina rugosa	WA 2	3	*champaign*	1	.	.	1	1	1
			orion	1	.	.	.	.	.
			emmastad	1	.	.	.	.	.
			wandsbek	1	4	.	.	.	4
Emydura australis	WA 2	1	*champaign*	1	.	.	.	.	.
			orion	1	.	.	.	.	.
			wandsbek	1	3	.	.	.	3
Crocodylus porosus	WA 2	2	*adelaide*	1	.	.	.	.	.
			O group G	1	2	.	.	.	2
Total tortoises and crocodiles		6	.	.	9	.	.	.	9

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States of Australia detailed in Table 3, twenty-two isolations of salmonella and Arizona serotypes were made; while from six litter samples of reptiles and rodents in metropolitan Western Australia twelve isolations of salmonella and Arizona strains were achieved. *S. typhimurium* was isolated from both sources.

Infections by more than one serotype were frequent. Twelve multiple infections, involving three serotypes, were detected in individual lizards and snakes. One tortoise yielded three salmonella serotypes.

Table 3. Salmonella *and Arizona serotypes isolated from reptilian and associated rodent litter of captive reptiles*

Source of litter	Salmonella Serotypes	No.	Arizona Serotypes	No.	Total
Imported reptiles from eastern states (10 samples)	*adelaide* (2) *alsterdorf* (1) *blukwa* (1) *charity* (1) *chester* (3) *saintpaul* (1) *typhimurium* (2) *uzuramo* (1) *wandsbek* (6)	18	5:29–21 (2) 26:27–25 (1) 26:33–31 (1)	4	22
West Australian (6 samples)	*chester* (2) *enteritidis* (4) *saintpaul* (2) *typhimurium* (2)	10	16:23–25 (1) 16:23–27 (1)	2	12
Totals		28		6	34

The distribution of serotype recoveries in the various organs from seven dissected reptiles are shown in Table 4. Up to five serotypes were recovered from various sites in lizards, while both salmonella and Arizona types were found widely distributed in the organs of snakes, including the ovaries, stomach and gall bladder.

Of the nineteen identified serotypes of Arizona isolated from snakes only two (1,33:23–21 and 5:29–21) were also found among the seven identified strains from lizards.

DISCUSSION

Reptiles were first investigated bacteriologically during coliform studies by Bettencourt & Borges (1908), and Konrich (1910). Salmonella serotypes from lizards, snakes, and Galapagos turtles were reported by McNeil & Hinshaw (1944, 1946) and Hinshaw & McNeil (1945, 1947), who speculated that lizard infection was food-borne from human carriers and that lizards and snakes were reservoirs of infection for turkeys and chickens. Also in America, Parker & Steinhaus (1943) reported *Salmonella* infection in ticks.

Rewell, Taylor & Douglas (1948) isolated a salmonella strain from a captive West African python in England, and Boycott, Taylor & Douglas (1953), linked possible human infection with isolations from imported Moroccan tortoises describing the finding as 'both novel and alarming'. Recent examination of

Table 4. Salmonella *and Arizona serotype colony distribution in seven reptiles*

Reptile	Origin	Serotype organ distribution: Stomach	Other organs	Cloaca and intestine	Total colonies sero-typed	Sero-types
Notechis scutatus	Southern West Australia	Arizona 16:23–25	*S. kibusi* Arizona 16:23–25	*S. kibusi* Arizona 16:23–25	> 20	2
Amphilbolurus barbatus (3 reptiles)	Northern West Australia	*S. senftenberg* (2) *S. kisarawe* (1) *S. rubislaw* (1) *S. chester* (9) *S. adelaide* (2)	*S. senftenberg* (5) *S. wandsworth* (9) (liver)	*S. senftenberg* (9) *S. kisarawe* (13) *S. rubislaw* (3) *S. chester* (8) *S. adelaide* (5) *S. tennessee* (1) *S. orion* (1)	69	8
Tiliqua scincoides	Northern West Australia	*S. senftenberg* (9) *S. orion* (3)		*S. senftenberg* (6) *S. adelaide* (12) *S. muenchen* (9) *S. orion* (2)	41	4
Tiliqua scincoides multi-fasciata	—	*Salmonella* 47 k:– (8)	.	*Salmonella* 47 k:– (27)	35	1
Notechis scutatus	Victoria	*S. onderstepoort* *S. typhimurium* Arizona 16:27–25	Arizona 16:27–25 Arizona 5:29–21 *S. onderstepoort*	Arizona 16:27–25 . .	> 50	4

reptiles in the zoological gardens of Basle, Berne and Zurich by Rudat *et al.* (1966) provided fifteen serotypes, including three occurring in Western Australia. The authors comment that infected reptiles were of little consequence in infection trends in the attendants. The frequent presence of *Salmonella* in African reptiles has been recorded by Mackey (1955) and Collard & Sen (1960). Mackey considered the numerous house lizards observed might be primary reservoirs of human infection. He showed that among thirty-three different serotypes found in lizard droppings, twenty-one of the types were also isolated from human cases in the same area, but only one of these isolations was *S. typhimurium*. Fulton, Szafran & Lesko (1961) isolated *Salmonella* from reptiles in the Congo in an area where infection from man and rodents was unlikely and suggested that the strains obtained were present in reptiles as intestinal commensals.

In Australia, salmonella isolations from native animals were described by Lee & Mackerras (1955), who considered that the carrier state was the more usual condition in reptiles, but that adverse environmental factors might lead to disease in the host. These workers also noted the relative absence of *S. typhimurium*.

The first reported isolations of Arizona organisms from lizards were made in America by Caldwell & Ryerson (1939). Further isolations were described by Edwards, Cherry & Bruner (1943) from reptiles, fowls, mammals and man. Le Minor, Fife & Edwards (1958) isolated strains of Arizona from 134 venomous snakes obtained in France for the garnering of venom. Brookes & Fife Asbury (1966), investigating cultures from South Africa and the London Zoo, found fourteen new Arizona serotypes from snakes, a tortoise, a lizard, and from a sample of human faeces. In a total of 229 infections reported by Edwards, Fife & Ramsey (1959) 205 were accompanied by symptoms; and Krag & Shean (1959) have reported two fatal infections. Caldwell & Ryerson (1939) considered the aetiological role of Arizona in disease difficult owing to a lack of specific knowledge of reptilian disease.

Outbreaks of infection associated with subgenus III strains have been reported in France by Buttiaux & Kesteloot (1948), and in America by Murphy & Morris (1950).

The first Arizona isolation in Western Australia was made in 1962 from an infected pig gland, and 2 years later a second strain was isolated from the faeces of an 8-year old boy with acute gastro-enteritis. A further eight human isolations have been obtained from the faeces of patients between the age of 3 months and 65 years during 1967 to May 1968. All patients had gastro-intestinal symptoms; at least one continued to excrete the organism for several weeks, during which time the symptoms persisted. All the human and domestic animal Arizona isolations, including those from kangaroo and rabbit meat, were made from specimens received from remote areas, mainly in the northern regions of the State. A further subgenus IV strain was isolated from a sewage sample collected in the extreme northern Kimberley region of the State.

The absence of *S. typhimurium*, together with increased isolations of new serotypes, subgenus II, III and IV strains, as well as multiple serotype infection and re-infection in the less developed regions of the State is illustrated in Table 5, which

shows the distribution of Salmonella serotypes in human infections by the geographical regions of Western Australia. The table also shows the *S. typhimurium* distribution. Edwardsiella species, as reported by Ewing *et al.* (1965), have not yet been isolated, although we were aware of their possible presence in the later stages of the investigation.

Table 5. *Geographic distribution of* Salmonella *serotypes isolated from human faeces*

Region	Total persons	Salmonella serotypes						Persons with multiple serotype isolations
		Subgenus			O groups			
		I	II	III	A–G	Other	*typhi-murium*	
Northern	*642*	*616*	*21*	*5*	*446*	*191*	*33*	*68*
Southern	776	773	1	2	723	51	434	15
Totals	1418	1389	22	7	1169	242	467	83

Our investigation has shown no distribution differences in the serotypes isolated from captive and free-ranging reptiles with the exception of *S. typhimurium*, which was not isolated from wild reptiles but only from captive snakes and the rodents provided for their food and their combined litter. There was a higher incidence of *Salmonella* infection in lizards than in snakes, while the reverse was found for Arizona infection.

The significance of salmonella and Arizona organisms in reptiles is complicated by our lack of knowledge of other host–parasite relationships that may be present. The ectoparasite *Amblyoma triguttatum* from lizards, included in this report, frequently contained *Salmonella* species. One lizard captured in a metropolitan cemetery yielded both *S. give* and *S. ohlstedt* from the cloaca, *S. ohlstedt* from ticks deep inside the left ear cavity, and *S. houten* from ticks in the right ear. Arizona isolations were also recorded from ticks on the larger monitor lizards (*Varanus varius*). Endoparasites and ectoparasites including intestinal flagellates, ciliates, amoebae, and haematozoa in Australian reptiles, monotremes, and marsupials have been recorded by Johnston (1932) and Mackerras (1961, 1962), the latter commenting that in the ancient group of reptiles it could be expected that their parasites would be widely distributed. However, some lizards have restricted ranges, which could lead to a relatively isolated development not only of particular species, or even races of parasite, but of particular strains of *Salmonella* or Arizona. Ten strains of *S. kisarawe*, for instance, were isolated from twenty *Amphibolura* but from only one other lizard (which was not identified).

Lizards and snakes occur throughout Australia. In Western Australia their distribution is widespread and varies from remote uninhabited desert to areas close to human habitation. This report has shown that both groups of reptiles are frequently colonized or infected with salmonella and Arizona groups of organisms. Furthermore, the evidence here presented suggests that these organisms were established in Australian reptiles before the invasion of the subcontinent by European man and his introduced fauna. Reptiles thus provide a reservoir of strains from which man

and his domestic animals can be infected or from which his food can become contaminated. The implication of this ecological relationship may extend beyond the shores of the Commonwealth to any country where reptiles abound and foods favouring the survival of enteropathogenic bacteria are produced for home or foreign consumption.

SUMMARY

1. Ninety-seven (83·6%) of 116 reptiles, comprising 70 lizards, 40 snakes, 4 tortoises and 2 crocodiles, yielded isolations of organisms in the Salmonella and/or Arizona groups.

2. The reptiles were captive or free-ranging; the former were drawn from all states of mainland Australia, while the latter were from West Australia only.

3. The relative prominence of *Salmonella* serotypes containing numerically high somatic antigens, the finding of new serotypes, of multiple infections, and of strains in subgenera II and III was remarked.

4. The lack of evidence of differences in the serotypes isolated from captive or wild reptiles (except for the isolation of *S. typhimurium* in creatures closely associated with man and his domestic fauna), and the apparent absence of a specific geographical distribution of serotypes in reptiles, lent support to the conclusion that reptiles provide a natural reservoir for *Salmonella* and Arizona strains in Australia. The possible spill-over to man, his domestic animals and his foodstuffs is discussed.

It is a pleasure to record our indebtedness to Dr Joan Taylor for her continuous interest and support in providing confirmation and identification of many *Salmonella* serotypes; to Dr W. H. Ewing and later Dr R. Rhode for serotyping the Arizona strains; to Dr G. M. Storr, Curator of Reptiles in the Museum of Western Australia, for identifying the reptiles and to Dr W. S. Davidson, Commissioner of Public Health, Western Australia, for permission to publish.

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