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Salmonella isolation from reptilian faeces: A discussion of appropriate cultural techniques

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SUMMARY

A short study of salmonella isolation from reptile faeces is described. The samples came from the reptile house at the Bristol Zoological Gardens. The wide range of salmonella serotypes present in the material, including representatives of all four subgenera, is noted. The important factors in the technique of isolation are discussed. These are choice of inoculum, enrichment medium, and plating medium, use of multiple subculture and serological isolation. If the number of samples available is scanty, an extended technique will probably yield more information than a simple method.

INTRODUCTION

Salmonellosis in reptiles has recently aroused interest as a potential source of gastro-enteritis in man (Chiodini & Sundberg, 1981; Anon, 1981). Of the estimated two million salmonella infections in humans in the United States between 1970 and 1971, 280000 (14%) were linked to contact with pet turtles (Anon, 1975). Lizards and snakes have also been associated with zoonotic salmonellosis (Hinshaw & McNeil, 1944; Kaura, Sharma & Singh, 1970; McInnes, 1971; Kaura *et al.* 1972).

Over 1300 serotypes have been identified in reptiles. Relatively few are isolated from man, or are associated with reptilian zoonosis (Chiodini & Sundberg, 1981). Members of all four salmonella subgenera are represented in these reptile isolations.

Our own interest in salmonella serotypes other than those belonging to subgenus I began with a study of contamination of sun dried bones imported from India for the manufacture of gelatin (Harvey & Price, 1962) and continued with investigations on a series of environmental samples from which both subgenus II and subgenus III strains were isolated (Harvey, 1971; Harvey, Price & Hall, 1973). The main habitat of subgenus II salmonellas is the faeces of cold blooded animals. Many are of African origin.

In 1972 and 1973, through the courtesy of the Bristol Public Health Laboratory, we were able to obtain reptile faecal specimens. These came from the reptile house at the Bristol Zoological Gardens. The samples were examined for salmonellas and the results of this study are presented here with a discussion of suitable isolation techniques.

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Table 1. *Biochemical differentiation of the four sub-genera of salmonella*

	Sub-genus			
	I	II	III	IV
Acid from dulcitol	+	+	–	–
Acid from lactose	–	–	+†	–
Acid from salicin	–	–	–	+
Liquefaction of gelatin	–*	+	+	+
Growth of KCN medium	–	–	–	+

* A few exceptions. † Late or irregular result.

MATERIALS AND METHODS

Faecal specimens were received by post. About 1 g of faecal material was cultured. At first, selenite F (Leifson, 1936) alone was employed for enrichment. Later Muller–Kauffmann tetrathionate (Muller, 1923; Kauffmann, 1930, 1935) was used in parallel with selenite F. The enrichment media were prepared from individual ingredients. In the final technique, 1 g of faeces was inoculated into 10 ml of selenite F and the same quantity was seeded into 10 ml of Muller–Kauffmann tetrathionate. The enrichment broths were incubated at 43 °C in an incubator for 24 h and were subcultured to brilliant green MacConkey agar (Harvey, 1956), desoxycholate citrate agar and de Loureiro's (1942) three stock solution modification of bismuth sulphite agar used unripened. Preparation of all media is described elsewhere (Harvey & Price, 1982*a*). Selective agars were incubated at 37 °C for 24 h and suspicious colonies were picked for further examination serologically and biochemically.

RESULTS

Table 1 records selected biochemical reactions for differentiation of the four subgenera of *Salmonella*. Although subgenus III salmonellas are shown as mainly lactose fermenting, some strains present on brilliant green MacConkey agar as non-lactose fermenters. These usually split the sugar fairly rapidly in fluid media.

Table 2 gives the total isolations from each animal species and Table 3 presents the recovery of different serotypes from the two enrichment media where they were used in parallel.

DISCUSSION

The test samples, in this study were potentially contaminated with serotypes belonging to four salmonella subgenera. Some specimens contained more than one serotype. The material was, therefore, unusual and isolation methods developed for samples containing subgenus I strains only were not entirely suitable. The technique actually used in the study was basic and in view of the small numbers involved, a more elaborate and prolonged method of selective culture might have afforded additional information on salmonella content. This we say with hindsight.

The investigation affords an opportunity, however, to discuss techniques appropriate to successful salmonella isolation from such complex material.

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Table 2. *Salmonella serotypes and subgenera in reptile faeces*

Reptile species	No. of samples	Salmonella subgenus and number of isolations: I		II		III		IV	
Python reticulatus	1	*S. typhimurium*	1	—		—		—	
		S. thompson	1	—		—		—	
Boa constrictor constrictor	7	*S. typhimurium*	7	S. 30:b:z_6	5	—		—	
Lizard cage	2	*S. tennessee*	1	*S. nachshonim*	1	Ar:26:32:21	1	*S. lohbruegge*	1
		S. kentucky	1	S. 30:b:z_6	2	—		—	
Cross *python molurus* and *sebae*	5	*S. typhimurium*	4	—		—		*S. lohbruegge*	2
Testudo denticulata	1	*S. typhimurium*	1	S. 16:b:z_{39}	1	—		—	
Python regius	2	*S. typhimurium*	2	—		—		—	
Tiliqua scincoides	1	*S. kentucky*	1	S. 30:b:z_6	1	—		*S. lohbruegge*	1
Varanus niloticus	1			S. 3,10:b:z_{39}	1	—		—	
Ptyas mucosus	2	*S. typhimurium*	2	—		Ar:28:32:28	1	—	
Two banded monitor	1	*S. typhimurium*	1	—		—		—	
Crocodylus palustris	1	*S. thompson*	1	—		—		—	
Mabuya sp.	1	*S. typhimurium*	1	S. 16:b:z_{39}	1	—		—	
Varanus salvator	1	*S. typhimurium*	1	—		—		—	
Testudo graeca	1	*S. albany*	1	—		—		—	
Uromastix acanthinurus	1	*S. lindern*	1	—		—		—	
		S. anatum	1	—		—		—	
Tupinambis nigropunctatus	1	*S. infantis*	1	S. 30:b:z_6	1	—		—	
Cordylus giganteus	1	*S. orion*	1	—		—		—	
		S. anatum	1	—		—		—	
Gerrhosaurus sp.	1	*S. nyborg*	1	S. 30:b:z_6	1	—		—	
Natrix piscator	1	*S. typhimurium*	1	—		—		—	
		S. give	1	—		—		—	
		S. oranienburg	1	—		—		—	
Alligator mississipiensis	1	—		—		—		—	
Osteolaemus tetraspis	1	—		—		—		—	

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Table 3. *Distribution of serotypes isolated from comparable samples cultured in selenite F and Muller–Kauffmann tetrathionate*

Serotype	Isolated in: Selenite F	Isolated in: Muller–Kauffmann tetrathionate
S. albany	+	−
S. anatum	+	+
Ar 28:32:28	+	−
S. give	+	−
S. infantis	+	+
S. kentucky	+	+
S. lindern	+	−
S. lohbruegge	+	+
S. oranienburg	−	+
S. orion	+	−
S. nachshonim	−	+
S. thompson	+	+
S. typhimurium	+	+
S. unnamed 16:b:z_{39}	+	−
S. unnamed 30:b:z_6	+	+
S. unnamed 3,10:b:z_{39}	−	+

+, Isolation of serotype. −, No isolation of serotype.

The factors relevant to efficient salmonella recovery are: the inoculum of the test sample, the fluid media used, the plating media, the timing of subculture from the fluid media and the use of serological isolation methods.

An adequate inoculum of contaminated material is essential for optimum results. In a study of abattoir pigs, 10 serotypes were isolated from 0·5 g inoculum in contrast to 19 from a 80 g quantity of caecal faeces (Harvey, Price & Morgan, 1977). Inocula have varied in different reptile studies. Kaura *et al.* (1972) killed the snakes they examined and cultured post-mortem samples. The inoculum size is not stated, but in quantitative estimates of Arizona species in cobras and in a rattle snake, a 1 g quantity of faeces was used. Habermalz & Pietzsch (1973) cultured 0·1–50 g of material depending on the animal type, while Koopman & Janssen (1973) examined 0·1–1 g of faeces. Wells, Clark & Morris (1974) and Siebeling, Neal & Granberry (1975) kept baby turtles in beakers of water and used 1 ml of container water as inoculum. This procedure may well have given a more representative sample of salmonellas harboured by the animals. The last two studies also examined whole animal blended specimens and from these obtained information not provided by excreta. There was evidence that the blending method was more sensitive than the excretion method. Subgenus III salmonellas (Arizonas) were more often isolated from the turtle homogenate than the container water. This suggests that subgenus III salmonellas have a greater tendency to systemic involvement than other salmonellas.

Fluid media associated with salmonella recovery include both selective and unselective categories. Siebeling *et al.* (1975) recorded that pre-enrichment of homogenates of antibiotic treated turtles prior to enrichment provided less information than direct culture in tetrathionate brilliant green broth. The

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pre-enrichment medium was lactose broth. In theory, a pre-enrichment stage to recover antibiotic damaged salmonellas could have been of value, but the reverse was the case – an interesting finding. Enrichment media useful for salmonella isolation from reptile material are selenite F and tetrathionate (Kaura *et al.* 1972), Rappaport's medium and strontium chloride B medium (Iveson, 1971). None of these is faultless. Selenite F is toxic to *Salm. cholerae suis* (Smith, 1952) and tetrathionate broth can inhibit multiplication of some strains of salmonellas if the inoculum is small (van Schothorst *et al.* 1977; Vassiliadis, Pateraki & Trichopoulos, 1974). Only three out of 11 strains of subgenus III salmonellas were able to multiply in Rappaport's medium (Vassiliadis, 1968) and strontium chloride B was significantly less efficient than modified Rappaport for salmonella isolation from sewage polluted natural water (Harvey & Price, 1982*b*). With reptile samples it is probably best to use a battery of enrichment broths to compensate for the imperfections of any one of them (Harvey & Price, 1974). There is also the theoretical possibility that certain serotypes may be recovered more easily from one of the enrichment media rather than from another. We have demonstrated this with sewage polluted water (Harvey & Price, 1976). Koopman & Janssen (1973), however, in a study of reptile faeces found no definite preference for an enrichment method by certain serotypes. The enrichment broths used in this paper were tetrathionate incubated at 37 and 43 °C and selenite incubated at 37 °C only. With the use of two methods, the yield of serotypes increased by about 38%. The combination of the three methods increased the serotype yield by approximately 64% compared with a single technique. The number of enrichment methods used would depend on the time available for the investigation. Our own preference, in a future study, would be to use three enrichment broths, selenite F, Muller–Kauffmann tetrathionate and modified Rappaport. The preparation of these media is given elsewhere (Harvey & Price, 1982*a*). We would incubate selenite and tetrathionate at 43 °C and Rappaport at 37 °C.

In our view, the best selective agars for reptile salmonella studies are brilliant green MacConkey agar (Harvey, 1956) and a modified bismuth sulphite agar (de Loureiro, 1942). Brilliant green MacConkey agar is less inhibitory than brilliant green agar owing to the incorporation of sodium taurocholate in the formula and permits a wide range of serotypes to form colonies on it (Chau & Leung, 1978). Leaving plates at room temperature, after 37 °C incubation, inproves colonial differentiation (Jameson, 1966; Harvey & Price, 1974). We have used de Loureiro's (1942) modification of bismuth sulphite agar for many years with extremely satisfactory results. Between 1959 and 1971, we isolated 16 different subgenus III serotypes on this medium from various materials. All strains produced colonies surrounded by a typical sheen (Harvey, Price & Hall, 1973). The performance of this selective agar is consistent and it is only stock solution C (containing ferrous sulphate) that requires frequent renewal. In our hands, commercial bismuth sulphite agars have been disappointing. Erdman (1974) has had similar experience with Difco bismuth sulphite agar in an International collaborative assay of salmonella in raw meat. In contrast to our own experience, Iveson (1971) has recorded that subgenus III strains do not always produce typical colonies on bismuth sulphite agar.

Reptile samples often contain multiple salmonella serotypes. In a study of

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multiple serotype contamination, we recorded that when selenite F broth is inoculated with material containing several *salmonella* species, the ratio of one serotype to another is a function of time of subculture. It follows that at least two times of subculture from enrichment media may increase chances of recovery of additional serotypes. This technique was found valuable in sewage and crushed bone studies (Harvey, 1957; Harvey & Price, 1962). Subcultures should be at 24 h and 48 h. In any event, a 48 h subculture is useful to obtain optimum results with Muller–Kauffmann tetrathionate (Edel & Kampelmacher, 1969) and similar spacing of subculture timing is also valuable with selenite F (Harvey, 1965).

Lastly, multiple serotype contamination may also be studied by serological isolation techniques (Loeffler, 1906; Wassén, 1930; Bailey & Laidley, 1955; Juenker, 1957; Harvey, 1957). The method used in Cardiff is described elsewhere (Harvey & Price, 1982*a*). The serological technique is valuable, but time consuming. It requires H agglutinating sera and not all laboratories will have sera corresponding to some of the unusual H antigens possessed by reptile salmonellas.

The complexity of examination used depends on the availability of specimens. When only a few samples are involved, a prolonged and detailed technique may be worth considering to obtain the best return for effort.

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