

An Arizona serotype isolated from a case of gastro-enteritis in Britain

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Human infection with one of the Arizona group of organisms has not previously been reported in Britain, but Edwards, Kauffman & Stucki, (1960) have reported one case in Europe, of gastro-enteritis in a man recently returned from holiday in the Balearic Islands. The organism was a new Arizona serotype, biochemically atypical.

In 1962 Harvey & Price reported the presence of Arizona strains in Indian crushed bone. More recently Harvey, Price & Dixon (1966) gave an account of Arizona strains isolated from abattoirs in England and Wales, and Brookes & Fife (1966) reported twenty-two new Arizona serotypes isolated from coconut, bone-meal, dried egg and reptiles. Nevertheless, no members of the Arizona group were included in the recently published list of salmonellas isolated from human, animal or other sources and identified in Great Britain between 1951 and 1963 (Taylor *et al.* 1965) as none were isolated from human or animal, and only rarely from other sources.

It may therefore be of interest to record a recent case of gastro-enteritis in a child from whom an Arizona strain was isolated. A symptomless excretor was also found in the same family. The source of the infection was traced to pet terrapins (a type of small turtle belonging to the genus *Graptemys*) recently purchased by the family from a pet shop in Sheffield.

CASE REPORT

A 3-year-old girl, living with her parents and 5-year-old brother in Sheffield, developed gastro-enteritis on 27 May 1966. She had a fever and was quite ill, her symptoms continuing intermittently for the following 8 days. A specimen of faeces was sent to the Public Health Laboratory on the first of June. A provisional report of 'an organism has been isolated which is probably a salmonella' was made 2 days later. The patient was treated with a proprietary mixture of kaolin and neomycin and quickly responded to treatment. Food poisoning was not suspected as the

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cause of infection and she appeared to be an isolated case within the household. Neither the parents nor brother experienced gastro-enteritis, although on direct questioning the mother admitted that the brother felt sick at the time of his sister's illness.

After the organism had been provisionally identified as an Arizona, the family were asked whether they had any reptiles, tortoises or turtles as pets. They admitted that they had recently purchased two terrapins. It was ascertained that the daughter was fond of playing with them and frequently put them to her mouth. The terrapins were sent for examination to the Public Health Laboratory. Enquiries showed that these reptiles had been purchased from a Sheffield pet shop, but by the time a public health inspector visited the premises all the remaining terrapins from the particular batch (twelve in number) had been sold. This batch originated from Florida, U.S.A., and were imported via Liverpool docks, and a distributor in Knutsford, Cheshire. Other batches had been imported from Ceylon and Singapore. Terrapins do not breed in Britain because the climate is too cold. Water from the tank in which they had been kept was still available. This water together with a sample of dried flies upon which terrapins feed were examined. A week later two of the terrapins sold by the shop were traced and later examined.

LABORATORY INVESTIGATIONS AND FINDINGS

The faeces were cultured by direct inoculation on three solid media, MacConkey agar, brilliant green MacConkey and deoxycholate citrate agar. Three liquid media, tetrathionate broth, selenite F. and peptone water were also inoculated. After 18 hr. incubation at 37° C. the tetrathionate broth was subcultured on brilliant green MacConkey agar, and the selenite F. and peptone water on deoxycholate citrate agar. All the plates were incubated at 37° C. overnight.

Table 1. *Biochemical findings in the preliminary screen*

Test	Result
Fermentations of:	
Triple sugar*	—
Glucose	AG
Mannitol	AG
H ₂ S production	+
Indole production	—
Urease	—

* Triple sugar contains 0.5% lactose, sucrose and salicin in peptone water. The other sugars were 1% solutions in peptone water. AG = acid and gas production.

A few non-lactose fermenting colonies were isolated from the faeces of the daughter on the brilliant green MacConkey agar and deoxycholate citrate agar. The colonies were indistinguishable from salmonella colonies morphologically. A heavy, almost pure, growth of non-lactose fermenting colonies was obtained on each of the plates subcultured from liquid media.

The results from a small routine set of biochemical tests were consistent with the

biochemical reactions of a salmonella (Table 1). However, when further biochemical tests were performed the results were no longer typical (Table 2). Serologically the organism was not agglutinated by polyvalent salmonella 'O' antisera but was strongly agglutinated by polyvalent salmonella 'H' and by single factor salmonella 'H' c antisera.

The organism was sent to the Salmonella Reference Laboratory at Northallerton, where it was identified as a possible Arizona, and the identification was confirmed by the reference laboratory at Colindale. The organism was serotyped and had the following antigenic structure, Ar 26:32-21, the equivalent salmonella antigenic structure being S 61:c-z₃₅. Serum obtained from the daughter 5 weeks after the onset of her symptoms was titrated against Arizona O antigen 26 and was positive at a dilution of 1/16, but against Arizona H antigens 32 and 21 it was negative at 1/10 and upwards.

An Arizona of identical serotype was isolated from the water in which the terrapins belonging to the family were kept. No Arizona was isolated from the intestines of these terrapins at postmortem, but from one of them four salmonellas were isolated, *S. oranienburg* (6,7:m,t-), *S. saint paul* (1,4,5,12:e,h,-1,2), *S. bredeney* (1,4,5,12,28:1,v-1,7) and *S. carrau* (6,14,24:y-1,7). The last strain produced indole.

From one of two other terrapins obtained from the same pet shop an Arizona of identical serotype was also isolated. No isolations, Arizona or salmonella, were obtained from the tank water or dried fly food from the pet shop. No isolations were made either from the girl's mother or father, but an Arizona of similar serotype was isolated from the faeces of her brother. Four weeks after the first isolation Arizona was still present in the faeces of both children. Later specimens, however, proved negative.

DISCUSSION

The Arizona group of organisms is closely related serologically to the salmonella group but is distinguishable by biochemical methods (Table 2). An occasional strain of salmonella may also fail to ferment dulcitol and a few liquify gelatin. The malonate and the O.N.P.G. tests, however, provide a firm differentiation between Arizona and salmonella strains. The first description of an Arizona was by Caldwell & Ryerson (1939), who isolated this strain from diseased chuckwallas, horned lizards and Gila monsters. This organism was designated *Salmonella* sp (Dar-es-Salaam type, var from Arizona) because it liquified gelatin, as does the true Dar-es-Salaam type. Later Kauffman (1941) studied a culture which, although it fermented lactose and liquified gelatin, he placed in the genus *Salmonella* because of the close relationship of its H antigens to those of known salmonella types. The organism was called *S. arizona*. Soon afterwards other serologically related organisms were isolated with similar biochemical properties and it was decided that they belonged to a group of enterobacteria not previously described. Edwards & West (1945) were the first to describe phase variation in a member of the Arizona group. Previously only monophasic members had been described. The first comprehensive study was by Edwards, West & Bruner (1947), who studied 382

cultures. Later Edwards, Fife & Ramsey (1959) published another comprehensive study with a review of published work to date. The final classification of the Arizona group is still undecided, but it seems probable that they will be placed on their own as a separate subgenus (subgenus III) within the genus *Salmonella*.

Table 2. *Comparison of the properties of a typical Salmonella and a typical Arizona with the organism isolated from the case*

	Salmonella	Arizona	Organism isolated from the case
Fermentations of:*			
Lactose	—	+ or ×	+2
Maltose	+	+	+
Mannitol	+	+	+
Sucrose	—	—	—
Dulcitol	+	—	—
Salicin	—	—	—
Gas from glucose	+	+	+
Indole production	—	—	—
Methyl red test	+	+	+
Voges-Proskauer test	—	—	—
Growth in Simmon's citrate	+	+	+
H ₂ S production	+	+	+
Gelatin liquefaction	—	(+)	+10
Phenylalanine deamination	—	—	—
Malonate	—	+	+
O.N.P.G.† test	—	+	+

+ = Prompt positive. +2 = positive on 2nd day. × = late and irregularly positive. (+) = delayed positive.

* 1% sugars in peptone water.

† O.N.P.G. = O nitrophenyl-β-D-galactopyranoside (Lapage & Jayaraman, 1964).

There have been many previous isolations of Arizona from human faeces from countries other than Britain (Seligmann, Saphra & Wassermann, 1944; Edwards, 1945; Ferris, Hertzberg & Atkinson, 1945; Buttiaux & Kesteloot, 1948; Murphy & Morris, 1950; Seligmann & Saphra, 1951; Edwards, McWhorter & Fife, 1956*a, b*). The patients in these series suffered from diarrhoea, vomiting and fever. Arizonas have also been isolated from other organs of the body, the blood (Seligman & Saphra, 1951), pus in a case of otitis media (Butt & Morris, 1952), liver abscess, arthritic joints and osteomyelitic bone (Krag & Shean, 1959). This organism can spread to the blood stream and from there to all parts of the body, producing clinical pictures indistinguishable from salmonella infection.

The majority of Arizonas isolated have been from snakes, lizards and other reptiles, poult and turkeys, eggs and spray-dried egg powder. There are several reports (from countries other than Britain) where human salmonella infections were traced to turtles and tortoises (Alin, 1956; Williams & Helsdon, 1965; Rosenstein, Russo & Hinchliffe, 1965; United States Department of Health, 1964; Reardon & Wilder, 1964; Fleming & Williams, 1964; Ager 1963; United States Public Health Service, 1963; Barr & Williams, 1964). In Britain Boycott, Taylor & Douglas (1953) and Boycott (1962) have published reports of salmonellas

isolated from tortoises and turtles and found evidence of human infection from this source. Thomas (1957) described another outbreak in which the causative organism proved to be a paracolonic bacillus intermediate between the *Salmonella* and Arizona groups. There has been only little mention of Arizona strains isolated from similar sources (Dimow, 1965*a, b*; Dimow & Rohde, 1965). Rosenstein *et al.* (1965) isolated an Arizona from the turtle water taken from the retail store during their investigation.

In the Report on the Health of the City of Liverpool (Semple, 1965) there is an interesting account of a family outbreak of *S. paratyphi B.* infection traced to a pet boxer bitch. She also infected her eight pups, some of which died. The bitch recently dug up the remains of a tortoise which had died 2 years previously. This was thought to be a possible source of the salmonella, but bacteriologically the remains proved negative. The family also had a bowl of terrapins, the water of which the bitch and her pups frequently drank. The terrapins were later found to be excreting *S. paratyphi B.* They were therefore supposed to have been infected by the bitch or her pups, but it is possible that it was the terrapins that infected the dogs.

In the recently published list of Sources of *Salmonella* 1951–63 (Taylor *et al.* 1965) 443 salmonella strains were isolated from tortoises in Britain. Among these strains there were 54 different serotypes, the commonest being *S. bleedon* (45 strains), *S. kottbus* (36 strains), *S. uphill* (29 strains) and *S. taunton* (27 strains). However, tortoises and turtles seem to be an uncommon source of the four salmonella serotypes reported in this paper. Neither *S. saint-paul*, *S. bredeney* nor *S. carrau* are present in the above list and only six strains of *S. oranienburg* were isolated from this source. As mentioned previously there were no isolations of Arizona from either human or animal sources.

CONCLUSIONS

Terrapins, like tortoises and turtles, are excretors of salmonellas and Arizonas. If the habit of keeping terrapins as pets increases then these small innocuous animals may well become an important source of human *Salmonella* infection. This is especially so in young children. Terrapins are more likely to act as a source of human infection than tortoises for the following reasons. Terrapins are smaller and therefore easier and more tempting for young children to handle. They are usually kept indoors and in a tank of water. It has been observed that children do play with them and are in the habit of transferring them in and out of the tank at frequent intervals. If the terrapins are excretors then the water in the tank will become infected, so increasing the possibility of spread to humans or to other pets.

Members of the Arizona group of organisms are well known causes of salmonella-like infections, both of gastro-enteritis and of a more generalized infection. An Arizona has not previously been isolated from a human source in Britain: but this may well be because it has not been recognized, as this one might not have been if lactose had been fermented earlier. Arizonas are less likely to be missed on media such as Wilson and Blair, which do not rely on lactose fermentation for preliminary

differentiation. Their colonies are identical with those of other salmonellas, after overnight incubation about 1 mm. in diameter with a black centre surrounded by a clear translucent edge. The medium surrounding the colony has a metallic sheen.

The importance of knowing all possible sources of infection must be stressed. Serotyping as a method of pinpointing the source of infection, as well as in the tracing of contacts, must also be stressed.

SUMMARY

Two isolations of Arizona from humans were made in Sheffield in 1966. The first was from a 3-year-old girl with a history of gastro-enteritis; the second was from her 5-year-old brother who was a symptomless excretor. Infection was traced to pet terrapins recently purchased from a local pet shop. The relative epidemiological significance of Arizona infection and the potential hazard of pet terrapins has been reviewed.

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