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Isolations of subgenus III salmonellas (arizonas) in Cardiff, 1959–1971

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SUMMARY

Isolations of subgenus III salmonellas (arizonas) made in the regional Public Health Laboratory, Cardiff, between 1959 and 1971 are reviewed. The techniques of isolation are listed for the various materials examined. The necessity of using bismuth sulphite agar as a plating medium is stressed, as some strains might appear on brilliant green MacConkey agar as rapid lactose fermenters and be missed. The serotypes isolated in Cardiff are discussed with reference to isolations by other authors. The culture of a subgenus III salmonella from pig faeces is described. It is thought that this is the first record of such an isolation from a pig in the United Kingdom.

INTRODUCTION

Arizona species have not aroused much interest in Britain. This may be because they are rarely encountered except as a manifestation of imported infection (Edwards, Kauffmann & Stucki, 1960; Harvey & Price, 1962; Timms, 1971; Hughes *et al.* 1971). In this paper we record a series of isolations of strains of this subgenus made in the Regional Public Health Laboratory, Cardiff, in the period 1959–71.

MATERIALS

The samples examined for salmonellas were: (1) crushed bones imported from India and Pakistan, (2) crushed bone imported for fertilizers from Argentina, (3) abattoir drain swabs, (4) retail meat market drain swabs, (5) swabs of a tank in which terrapins were kept, (6) samples of caecal faeces and mesenteric glands from pigs slaughtered at Cardiff abattoir.

METHODS

Isolation methods varied with the materials examined and it will be simplest to present them in tabular form. This information is recorded in Table 1. These isolation techniques have been published in full elsewhere (Harvey, 1956; Harvey, Price & Dixon, 1966; Harvey & Price, 1967*a*, *b*; Harvey & Price, 1970; Kampel-

Table 1. *Methods of isolation of subgenus III salmonellas (arizonas)*

Material	Method			
	Pre-enrichment	Enrichment	Secondary enrichment	Plating media used
Crushed bones from India and Pakistan	Nutrient broth 24 hr. at 37° C.	Selenite F broth 24–72 hr. at 43° C.	Migration through soft agar at 37° C. with and without agglutinating sera	Wilson & Blair bismuth sulphite agar and brilliant green MacConkey incubated at 37° C.
Abbattoir and meat market drain swabs. Swabs from terrapin tank	Nil	Selenite F broth 24 hr. at 43° C.	Migration through soft agar at 37° C. with and without agglutinating sera	Wilson & Blair bismuth sulphite agar; brilliant green MacConkey agar; deoxycholate citrate agar
Samples from pigs slaughtered at Cardiff abbattoir	Nil	Selenite F broth 24 hr. at 43° C.; Kauffmann-Muller tetrathionate broth 24 hr at 43 °C.	Migration through soft agar at 37° C. without agglutinating sera	Brilliant green MacConkey agar; deoxycholate citrate agar
Crushed bone for fertilizer from Argentina	Nutrient broth 24 hr. at 37° C.	Selenite F broth 24 hr. at 43° C.	Migration through soft agar at 37° C. with and without agglutinating sera	Brilliant green MacConkey agar; deoxycholate citrate agar

Selenite F broth: Hobbs & Allison (1945). Kauffmann-Muller tetrathionate broth: Kampelmacher (1967), Heard, Jennet & Linton (1969). Wilson & Blair bismuth sulphite agar: de Loureiro (1942). Brilliant green MacConkey: Harvey (1956). Deoxycholate citrate agar: Leifson (1935), Hynes (1942). Secondary enrichment technique: Harvey & Price (1967*a*, *b*).

macher, 1967; Heard, Jennett & Linton, 1969). It will be noted that Kauffmann-Muller tetrathionate broth was used in the pig investigation in parallel with selenite F broth. This medium uses 0·018 M tetrathionate (Knox, Gell & Pollock, 1943) in comparison with 0·039 M tetrathionate employed in Rolfe's B formula (J. Morgan, personal communication, Rolfe, 1946). The medium with the lower concentration of the selective agent functions very well at 43° C. while that incorporating the 0·039 M concentration does not.

RESULTS

The results are shown in Table 2. The period of each separate investigation is recorded.

Some strains when first isolated from bone products were rapid lactose fermenters on brilliant green MacConkey agar, although they presented as typical salmonella-like colonies with surrounding metalic sheen on bismuth sulphite agar.

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Table 2. *Subgenus III salmonellas isolated from different materials*

Material	No. of samples	Serotypes isolated
Crushed bone from India and Pakistan: 1959–1964	82	Ar 26:23:30 Ar 26:23:21 Ar 26:26:25 Ar 9a, 9c:29:31 Ar 16:22:31 Ar 20:24:28 Ar 29:24:31 Ar 29:33:21 Ar 30:23:31 Ar 30:27:28 Ar 9a, 9c:26:25
Abbatoir drain swabs: 1961–65	1641	Ar 26:29:30 Ar 26: – :30
Meat market drain swabs: 1967	446	Ar 26:32:21
Water from terrapin tank: 1967	1	Ar 26:23:30 Ar 24:24:28
Crushed bone from Argentina: 1968–9	170	Ar 30:22:31
Pig samples: 1968–71		
(*a*) Faeces	1796	Ar 26:29:30
(*b*) Mesenteric gland pools	132	Nil

On storage in the laboratory several of these strains failed to ferment lactose promptly on brilliant green MacConkey agar on re-examination and appeared as large green salmonella-like colonies after 24 hr. incubation at 37° C. The colonies produced by the strains found in abattoir and market drain swabs were indistinguishable from subgenus I salmonellas on both plating media. The two strains found in the terrapin tank sample differed in that serotype Ar 26:23:30 was a rapid lactose fermenter, both in lactose peptone water and on brilliant green MacConkey agar. In contrast, serotype Ar 24:24:28 presented as a salmonella-like colony on the neutral red indicator medium. Both strains were ONPG positive. The serotype isolated from the pig, Ar 26:29:30 had the same antigenic formula as some of the abattoir drain swab isolations and, like them, appeared on brilliant green MacConkey agar as colonies identical with subgenus I salmonellas. As this appears to be the first recorded finding of a subgenus III salmonella in a pig in the United Kingdom, the biochemical reactions of the strain are given in greater detail in Table 3.

The reactions recorded in Table 3 identified the strain as belonging to salmonella subgenus III, also known as the *Arizona* group. The organism, when cultured on a moist agar slope, readily agglutinated with *Salmonella* polyvalent H phase 1 and 2 serum, *Salmonella* polyvalent H phase 2 serum and *Salmonella* H phase 2 serum, factor 5. It did not agglutinate with *Salmonella* polyvalent O serum. Sera were provided by the Public Health Laboratory Service Standards Laboratory.

Cultures isolated up to 1962 were sent to Dr P. R. Edwards, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia. Cultures isolated

Table 3. *Biochemical reactions of the* Arizona *strain isolated from pig faeces*

Dextrose	Acid and gas (1)	Citrate (Simmons)	+
Lactose, 1%	Acid and gas (2)	Potassium cyanide	−
Salicin	− (14)	Lysine decarboxylase	+
ONPG	+	Ornithine decarboxylase	+
Dulcitol	− (14)	Arginine dihydrolase	+
Gelatin liquefied		H_2S	+
Malonate utilized			

Figures in parentheses indicate days of incubation of tests.

subsequently were identified serologically at the Salmonella Reference Laboratory, London.

The pig serotype was identified as Ar 26:29:30 (Sa 61:k:1,5,7).

DISCUSSION

The serotype Ar 26:29:30 isolated from swabs placed in drains receiving material from slaughtered sheep and cattle and currently isolated from pig faeces has a close association with sheep. It has been found in sheep in several parts of the United States of America and also in Europe. There is a record of a previous isolation from a pig (Edwards, Fife & Ramsey, 1959). It has also been recovered from members of American red indian tribes in close contact with sheep (P. R. Edwards, personal communication).

Two of the eleven serotypes isolated from Indian and Pakistani crushed bone (Harvey & Price, 1962) had not been described before (Ar 20:24:28; Ar 26:26:25). Four of the serotypes (9a, 9c:29:31; 26:23:21; 26:23:30; 29:33:21) have since been found in Indian snakes (Kaura *et al.* 1972; I. P. Singh, personal communication). The serotype Ar 26:23:30, which we found in the terrapin tank, has also appeared repeatedly in monkeys affected with diarrhoea in which no shigellas or salmonellas were found (Edwards *et al.* 1959).

The strain 26:32:21, isolated in 1967 from the meat market drain swab, had been found in 1966 in a girl in Sheffield. This was the first isolation of a subgenus III salmonella from man in the United Kingdom. The source of infection was traced to a terrapin imported from Florida, U.S.A. (Plows, Fretwell & Parry, 1968). It is interesting that terrapins were on sale in the gallery of the Cardiff meat market.

From our own observations between the years 1959 and 1972, many of the strains isolated have been slow lactose fermenters. This may give a false picture of the situation. Some authors maintain that when cultures are selected without regard to lactose fermentation, the majority of *Arizona* strains recognized ferment lactose promptly (Solowey, 1947; Le Minor, Fife & Edwards, 1958). It is essential, if one is searching for arizonas to use a selective agar not dependent on lactose fermentation. In our experience, Wilson and Blair's bismuth sulphite agar, as modified by de Loureiro (1942), is very efficient in this connexion. Several strains found in Indian and Pakistani bones would have been missed had we relied entirely on brilliant green MacConkey agar. This brilliant green medium is, however, in our hands the plating medium of choice when subgenus I salmonallas

only are being considered (Harvey, 1956). For economy, it is undesirable to use multiple plating media. We do not employ bismuth sulphite agar in many of our routine investigations. Our examination of Indian crushed bone required the use of this medium and was somewhat in the nature of an academic exercise in the isolation of multiple serotypes. This prolonged study increased the chances of finding arizonas (Harvey & Price, 1967*b*).

We have little evidence that arizonas are of much importance in the United Kingdom. The recent introduction, however, of Ar 7a,7b:1,7,8:– in day-old turkey poults from California, U.S.A. (Joan Taylor, personal communication; Timms, 1971) and the record of serious disease in a Negro woman infected with a serotype of similar antigenic formula 7:1,7,8:– (Guckian, Byers & Perry, 1967) suggest that some attention should be paid to these rare invaders of Britain.

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