J. Hyg., Camb. (1981), **86**, 369
Printed in Great Britain

Observations on the occurrence of *Salmonella cholerae-suis* and other salmonellas in two herds of feeder pigs

By D. R. WILLIAMS, D. HUNTER, JEANNETTE BINDER and ELAINE HOUGH

Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service, Quarry Dene, Weetwood Lane, Leeds LS16 8HQ

(*Received* 4 *December* 1980)

SUMMARY

Modified brilliant green agar (BGA), Muller-Kauffmann tetrathionate, Rappaport's and selenite F broths were compared for their efficiency in isolating salmonellas from pigs and their excreta. It was concluded that BGA and Rappaport's broth were the media of choice. Where searches were made for *Salmonella cholerae-suis* alone, the use of a trehalose McConkey agar provided a rapid method of differentiating *S. cholerae-suis*, which does not ferment trehalose, from the majority of other salmonellas, which do ferment trehalose.

Casualties were collected from two farms where infection with *S. cholerae-suis* was endemic. The isolation rates of *S. cholerae-suis* from different carcase sites were compared in order to determine the relative importance of the salivary, upper respiratory and faecal routes of excretion. *S. cholerae-suis* was isolated from numerous carcase sites in carriers including the salivary glands, tonsils, trachea and lungs. However, isolations from the nasal passages, mouth, pharynx and gastro-intestinal tract of carriers were either infrequent or absent. When, in a further study, *S. cholerae-suis* was isolated from only 3/414 faeces, 1/170 nasal swabs and not at all from 170 oral swabs taken from live pigs, it was concluded that there must be more significant modes of transmission than from the salivary glands, upper respiratory or gastro-intestinal tracts. Cannibalism was considered to be a possibility.

In contrast to *S. cholerae-suis*, other salmonellas were frequently isolated from randomly collected faeces and from the gastro-intestinal tract as well as other sites in casualties.

INTRODUCTION

Isolations of *Salmonella cholerae-suis* from pigs made by Ministry laboratories in England and Wales fell from 106 in 1965 to only 10 in 1975 (Sojka & Field, 1970; Sojka *et al.* 1977). Reasons for the decline are not known although a number of contributory factors, including the availability of a vaccine and changes within the pig industry, have been suggested (Thomas, 1977). There, nevertheless, remain herds where infection has persisted. The present investigation was carried out in two of these in order to determine why this had occurred. The premise was made

that infection had persisted because of the husbandry conditions. In both herds pigs were reared in sweat boxes, a system under which they live in a warm and extremely humid environment. Opportunities for faecal transmission were considerable but faecal excretion was thought to be a minor source of infection in view of the low isolation rates from faeces and caecal contents reported by other workers (McCaughey, McClelland & Roddy, 1973). Since *S. dublin* had been isolated from the salivary glands of calves (Richardson & Fawcett, 1973) and chronic infection of the nasal passages with *S. typhimurium* and *S. bovismorbificans* had been induced in mice (Tannock & Smith, 1971) it was thought that in pigs, *S. cholerae-suis* might be excreted from the respiratory tract or in the saliva. These possibilities were explored and methods of isolating *S. cholerae-suis* and other salmonellas from pigs and their excreta were compared.

MATERIALS AND METHODS

Surveys

Surveys were undertaken on two farms, A and B, with similar histories. Both reared weaners to a slaughter weight of approximately 100 kg. Salmonellosis due to *S. cholerae-suis* had been diagnosed on farm A in 1969, 1970, 1971 and 1975 and on both farms in 1977. Losses were reduced by immunizing incoming weaners with a live attenuated *S. cholerae-suis* vaccine (Suscovax manufactured by The Wellcome Foundation Limited, Crewe).

Two surveys were carried out, the first from November 1976 to July 1977, the second from September 1977 to April 1978. One to six casualties, and sometimes a number of faeces as well, were collected at weekly intervals. Post mortem examinations were made to determine the causes of death and to collect tissues for culturing. Pigs dying from salmonellosis were arbitrarily defined as those with a septicaemia, pneumonia or necrotic typhlitis and/or colitis from which salmonellas were isolated from at least 75% of the extra-intestinal sites. In the first survey, 48 carcases and 83 faeces from farm A and 47 carcases and 161 faeces from farm B were examined. Cultures were taken from the mediastinal, hepatic and mesenteric lymph nodes, the tonsils, lung, spleen, liver and bile (extra intestinal sites) and from the stomach, jejunum, caecum and rectum (gastro-intestinal sites).

During the second survey in which 17 carcases from farm A and 54 from farm B were examined, cultures were taken from the mouth, sub-lingual, parotid and submaxillary salivary glands, the nasal passages, tonsils, trachea, pharynx, the submaxillary, post-pharyngeal and supra-pharyngeal lymph nodes and from the liver and caecum.

In addition to the above surveys, two extra visits were made to farm B in order to collect 170 random faeces and to take separate nasal and oral swabs from each of 170 pigs, no more than 5 animals being sampled from any one pen.

Collection of samples

Separate cotton wool swabs were rubbed vigorously against the oral, nasal, pharyngeal and tracheal mucosae. Portions of tonsil, lymph nodes and salivary

glands were immersed in boiling water for 15 s to sterilize the outer surfaces. Each portion was then macerated in 10 ml of sterile isotonic saline. The tonsils were too tough to macerate so portions (0·5 cm^3) were excised instead. Aliquots of bile were collected whilst the other sites were sampled by taking loopfuls of contents from the gastro-intestinal sites and loopfuls of tissue from the parenchymatous organs.

Bacteriological examinations

Livers were cultured on sheep blood agar at 37 °C for 24 h. All samples were taken onto modified brilliant green agar (BGA) (Oxoid CM329) supplemented with 0·1 % of Teepol (manufactured by Shell Chemicals Limited, London) (Jameson & Emberley, 1956) and/or into one or more of the following enrichment broths; Rappaport's (Rappaport, Konforti & Navon, 1956), Muller–Kauffmann tetrathionate (TTB) and selenite F broths. The BGA was incubated at 37 °C for 24 h whilst the enrichment broths were incubated at 43 °C for 24 h before sub-cultures were taken onto BGA for a further 24 h at 37 °C. On occasions, sub-cultures were also taken onto trehalose McConkey agar which was prepared by adding trehalose (1 %) to a McConkey agar (Oxoid CM7.).

Searches for *S. cholerae-suis* were carried out by picking non-lactose/trehalose fermenting colonies from the selective media for serological and biochemical identification. Confirmatory serological testing was carried out by the Central Veterinary Laboratory, Weybridge. Thirty-four out of 60 isolates of *S. cholerae-suis* from the first survey and all isolates of *S. cholerae-suis* from the second and subsequent surveys were phage typed and classified as smooth virulent or rough vaccinal strains. The phage lysed the rough strains.

The identification of salmonellas, other than *S. cholerae-suis*, was restricted to non-lactose fermenting colonies isolated during the first survey from 46 casualties and 83 faeces taken from farm A and 8 casualties collected from farm B.

RESULTS

The isolation of S. cholerae-suis

The number of isolations of *S. cholerae-suis* from extra-intestinal sites was significantly greater after enrichment through Rappaport's broth than after direct cultures on BGA ($P < 0·01$). The reverse was true for TTB ($P < 0·01$) (Table 1).

In the case of isolations from the gastro-intestinal tract, statistical comparisons between media were not possible because the numbers of isolations were too small but similar trends were observed. There was one isolation after direct cultures on BGA, none through TTB and four via Rappaport's broth.

The number of isolations of *S. cholerae-suis* on BGA and trehalose McConkey agar, following sub-cultures from enrichment broths, was similar ($\chi^2 = 0·1$ for 1 df). *S. cholerae-suis* was isolated on both selective media on 20 occasions, on BGA alone on six and on trehalose McConkey agar alone on eight.

Table 1. *A comparison of cultural methods for the isolation of* S. cholerae-suis *from extra-intestinal sites*

Methods compared	Isolations*	χ^2	P
BGA:R	12:32	8·2	<0·01
BGA:TTB	15:3	6·7	<0·01
R:TTB	23:2	16·0	<0·01

BGA, direct cultures on modified brilliant green agar supplemented with 0·1 per cent Teepol; R, enrichment through Rappaport's broth with subsequent sub-cultures onto BGA; TTB, enrichment through Muller-Kauffmann tetrathionate broth with subsequent sub-cultures onto BGA.

* The left hand figure shows the number of occasions when *S. cholerae-suis* was isolated by the left hand but not by the right hand method described in the preceding column. The right hand figure shows the reverse, i.e. the number of occasions when *S. cholerae-suis* was isolated by the right hand but not the left hand method. The numbers of concordant isolations (i.e. isolations by both methods) are not shown as they do not contribute to the comparisons.

Table 2. *A comparison of cultural methods for the isolation of salmonellas, other than* S. cholerae-suis, *from porcine carcases and faeces*

Site cultured	Methods compared	Isolations	χ^2	P
Carcase – extra-intestinal	BGA:TTB	5:22	9·5	< 0·01
	BGA:R	4:43	30·7	< 0·001
	TTB:R	2:24	17·0	< 0·001
Carcase – gastro-intestinal	BGA:TTB	6:6	0	n.s.
	BGA:R	1:32	27·3	< 0·001
	TTB:R	1:32	27·3	< 0·001
Faeces	BGA:TTB	3:20	11·1	< 0·001
	BGA:Sel	0:37	35·0	< 0·001
	BGA:R	0:42	40·0	< 0·001
	TTB:Sel	4:24	12·9	< 0·001
	TTB:R	1:26	21·3	< 0·001
	Sel:R	3:8	1·5	n.s.

Sel, enrichment through selenite F broth with subsequent sub-cultures onto BGA and n.s., not significant; otherwise as Table 1.

The isolations of salmonellas other than S. Cholerae-suis

The number of isolations of salmonellas, other than *S. cholerae-suis*, from faeces was significantly greater after enrichment through Rappaport's, selenite F or TTB broths than after direct cultures on BGA (Table 2). The efficiency of enrichment was similar for Rappaport's and selenite F broths, both being better than TTB ($P < 0·001$, Table 2).

A similar picture was observed when carcases were examined, except that selenite F broth was not used. Significantly more salmonellas were isolated after enrichment through Rappaport's broth than by direct cultures on BGA but when TTB was used, the advantage of enrichment was seen only in the case of isolations from the extra-intestinal sites (Table 2). The number of isolations from the gastro-intestinal tract following direct cultures on BGA and after enrichment through TTB was similar (Table 2).

Table 3. *The prevalence of infection with* S. cholerae-suis *on Farms A and B*

Survey	Farm	Number of pigs dying from salmonellosis	Number of carriers	Number of pigs infected with rough isolate	Number of pigs examined
1	A	0	14*	NI	48
	B	8	14*	NI	47
2	A	0	1	0	17
	B	4	14	5	54

NI, Not identified.

* Only 60% of isolates were phage typed so it was not possible to differentiate between carriers and those pigs infected with the rough strain. This figure therefore includes both categories.

Table 4. *Isolation of salmonellas from casualties during survey* 1

	Number of isolations from		
	Pigs dying from salmonellosis due to *S. cholerae-suis*	Carriers* infected with *S. cholerae-suis*	Carriers infected with other salmonellas
Number of pigs in each group	8	28	30
Cultured sites			
Extra-intestinal sites			
Mediastinal lymph node	8	13	5
Hepatic lymph node	7	13	9
Mesenteric lymph node	7	13	18
Tonsil	4	8	9
Lung	8	5	8
Spleen	8	1	1
Liver	8	0	2
Bile	5	0	0
Intestinal sites			
Stomach	1	0	13
Jejunum	0	0	12
Caecum	1	3	12
Rectum	2	0	6

* Includes carriers and pigs infected with the rough strain of *S. cholerae-suis*.

The isolation of S. cholerae-suis *from different sites*

Salmonellosis due to *S. cholerae-suis* was diagnosed in 12 out of 101 casualties from farm B (Table 3). In these *S. cholerae suis* was consistently isolated from most sites except for the digestive tract where the isolation rates varied from 0–50% (Tables 4 and 5).

Where *S. cholerae-suis* was isolated from pigs which had died from other causes, phage typing showed that the majority of isolates were smooth. During the first survey in which 34 out of 60 isolates were typed, 28 smooth and 6 rough strains were found. On farm B in the second survey, the smooth strain was isolated from 12 casualties, the rough strain from three and both from two more.

The distribution of rough and smooth strains in different sites from carriers was

Table 5. *Isolation of* S. cholerae-suis *during Survey* 2 *from pigs infected with the rough strain, carriers and pigs dying from salmonellosis*

	Number of isolations from		
	Pigs dying from salmonellosis	Carriers	Pigs infected with rough strain
Number of pigs in each group	8	15	5
Cultured sites			
Mouth	1	0	0
Sub-lingual salivary gland	7	4	0
Parotid salivary gland	7	5	0
Sub-maxillary salivary gland	5	4	1
Nasal passages	3	1	1
Tonsil	4	3	1
Trachea	4	2	1
Pharynx	4	0	0
Post-pharyngeal lymph node	7	3	2
Sub-maxillary lymph node	7	10	1
Supra-pharyngeal lymph node	7	4	0
Liver	8	3	1
Caecum	2	0	0

similar (Table 5) and subsequent observations refer to both strains. In the first survey, *S. cholerae-suis* was most frequently isolated from the lymph nodes and tonsils and infrequently from the gastro-intestinal tract (Table 4). In the second survey, *S. cholerae-suis* was most frequently isolated from the submaxillary lymph node but also found in the other lymph nodes, the salivary glands, tonsils, nasal passages, trachea and liver. It was not isolated from the mouth or pharynx of carriers (Table 5). Smooth isolates of *S. cholerae-suis* were also cultured from 3/414 faeces, 1/170 nasal swabs but not from the 170 oral swabs.

The prevalence of salmonellas, other than S. cholerae-suis, *in survey* 1

Salmonellas, other than *S. cholerae-suis*, were isolated from 23/46 pigs and 64/83 faeces from farm A and from 7/8 pigs collected from farm B. *S. london*, *S. heidelberg*, *S. takoradi*, serotype 04: 12: d, *S. typhimurium*, *S. takasony*, *S. agona*, *S. livingstone*, *S. derby*, *S. give* and *S. schwartzengrund*, listed in order of prevalence, were isolated from the casualties and faeces from farm A whilst *S. london*, *S. worthington*, *S. bredney* and *S. derby* were isolated from the pigs on farm B.

The number of isolations from different carcase sites is shown in Table 4. Salmonellas were frequently cultured from both intestinal and extra-intestinal sites but none of the pigs was considered to have been suffering from salmonellosis. Eighteen pigs were infected with a single serotype, 11 with two and one with three serotypes. Where pigs were infected with a single serotype, the salmonella was isolated from the intestinal sites alone in five pigs, from the extra-intestinal sites only in seven and from both intestinal and extra-intestinal sites in a further six pigs. The distribution of salmonellas in the 11 casualties with dual infection was similar, the corresponding figures being 4:9:9.

Where there were isolations from two or more of the three enrichment broths used for each faeces, the probability of the isolates being the same or different serotypes was similar (20:28. $\chi^2 = 0{\cdot}04$ ns) but there was only one faeces sample where each of the three broths yielded a different serotype and there were no occasions where more than one serotype was isolated from the same broth.

DISCUSSION

Brilliant green agar, Muller–Kauffmann tetrathionate and Rappaport's broths have each been advocated for the isolation of *S. cholerae-suis* (Smith, 1952; Heard, Jennett & Linton, 1969; McCaughey *et al.* 1973) but the results of the present surveys showed that direct cultures on BGA and enrichment through Rappaport's broth were superior to enrichment through tetrathionate broth (Table 1). Furthermore, although tetrathionate broth was superior to direct cultures on BGA for the isolation of other salmonella serotypes, it was inferior to both Rappaport's and selenite F broths, confirming the observations of Harvey, Price & Morgan (1977) who considered this particular broth (Oxoid CM343) to be unsatisfactory. The isolation rates from faeces via Rappaport's and selenite F broths were similar (Table 2) but the use of selenite F broth is contra-indicated where porcine samples are being examined as it is known to be toxic for *S. cholerae-suis* (Smith, 1952). It was concluded that BGA and Rappaport's broths were the media of choice for the isolation of salmonellas from pigs and their excreta.

Apart from the first survey searches were restricted to *S. cholerae-suis* and the selection and identification of *S. cholerae-suis* isolates from large populations of non-lactose fermenting colonies was laborious. A trehalose McConkey agar was devised to reduce the tedium. Salmonellas, except for *S. cholerae-suis*, *S. abortus ovis* and some strains of *S. typhimurium*, ferment trehalose so its addition to McConkey agar provided a rapid method of differentiating *S. cholerae-suis* from the majority of other salmonella serotypes. Where sub-cultures were taken from enrichment broths, trehalose McConkey agar was as efficient as BGA for the detection of *S. cholerae-suis* but its ability to isolate *S. cholerae-suis* on direct cultures was not explored.

In carriers, *S. cholerae-suis* was most frequently isolated from the sub-maxillary, mediastinal, hepatic and mesenteric lymph nodes (Tables 4 and 5). Few animals appeared to excrete the organisms in the faeces, saliva or from the respiratory tract. *S. cholerae-suis* was rarely isolated from the digestive tract even in animals dying from salmonellosis and was only isolated from 3/414 faeces. Isolations were quite frequently made from the salivary glands and lungs but there was only one isolation from the nasal passages and none from the pharynx or mouth (Tables 4 and 5). Similarly when nasal and oral swabs from 170 live pigs were examined, there was only one isolation of *S. cholerae-suis*. It was concluded that there must be other methods of transmission. Excretion in the urine was rejected because many of the faeces samples, collected from the sweat boxes, were contaminated with urine and therefore urine had, inadvertently, already been sampled. The

most likely mode of transmission, although it was impossible to prove, appeared to be cannibalism. This was frequently observed and in view of the high prevalence of infection in casualties (Table 3) and the considerable annual mortality (8–9% of intake on farm B according to the manager and probably higher on farm A – personal observations), cross infection by this route seemed likely. These factors were also thought to be the reasons why infection had persisted on these premises.

Thomas (1977) has pointed out that the decline in the number of isolations of *S. cholerae-suis* made by Ministry laboratories in England and Wales coincided with the introduction of a live vaccine but commented that it was doubtful if the use of the vaccine had made a significant contribution to the decline of the disease. Our observations support his opinion. Regular vaccination was said to have been practised on farm A since 1971 but smooth isolates of *S. cholerae-suis* were still being found in 1976 and 1977. Vaccination had not eliminated field infection.

The prevalence of infection with salmonellas, other than *S. cholerae-suis*, was high on both farms. The reasons for this were not determined but since both farms bought in weaners from a number of sources and used a wide variety of meat and fish meals in home mixed rations, it would have been surprising if the prevalences had not been high. Statistical comparisions between the number of isolations from different sites was not attempted since numerous serotypes were involved and there were considerable sampling variations. For example, where two isolations were made from the same faeces, the probability of their being the same or different serotypes was similar. However, trends were observed. Isolations were most frequently made from the mesenteric lymph nodes and, in contrast to *S. cholerae-suis*, from the intestinal tract. There were few isolations from the liver and spleen and none from the bile. The latter observation was not unexpected as bile from animals with healthy biliary tracts is usually sterile (Scott, 1971). All infections were subclinical; this too was expected since, apart from *S. cholerae-suis* and occasional outbreaks in young pigs associated with *S. derby*, *S. dublin* or *S. typhimurium*, salmonella infections of pigs in England and Wales are usually sub-clinical.

REFERENCES

HARVEY, R. W. S., PRICE, T. H. & MORGAN, J. (1977). *Salmonella* surveillance with reference to pigs – Cardiff abattoir 1968–1975. *Journal of Hygiene* **78**, 439.

HEARD, T. W., JENNETT, N. E. & LINTON, A. H. (1969). The incidence of *Salmonella* excretion in various pig populations from 1966 to 1968. *British Veterinary Journal* **125**, 635.

JAMESON, J. E. & EMBERLEY, N. W. (1956). A substitute for bile salts in culture media. *Journal of General Microbiology* **15**, 198.

MCCAUGHEY, W. J., MCCLELLAND, T. G. & RODDY, R. M. (1973). *Salmonella* isolations in pigs. *Veterinary Record* **92**, 191.

RAPPAPORT, F., KONFORTI, N. & NAVON, B. (1956). A new enrichment medium for certain *salmonellae*. *Journal of Clinical Pathology* **9**, 261.

RICHARDSON, A. & FAWCETT, A. R. (1973). *Salmonella dublin* infection of calves: the value of rectal swabs in diagnosis and epidemiological studies. *British Veterinary Journal* **129**, 151.

SCOTT, A. J. (1971). Bacteria and disease of the biliary tract. *Gut* **12**, 487.

SMITH, H. W. (1952). The evaluation of culture media for the isolation of *salmonellae* from faeces. *Journal of Hygiene* **50**, 21.

SOJKA, W. J. & FIELD, H. I. (1970). Salmonellosis in England and Wales 1958–1967. *Veterinary Buletin, Weybridge* **40**, 515.

SOJKA, W. J., WRAY, C., SHREEVE, J. & BENSON, A. J. (1977). Incidence of *salmonella* infection in animals in England and Wales 1968–1974. *Journal of Hygiene* **78**, 43.

TANNOCK, G. W. & SMITH, J. M. B. (1971). A *salmonella* carrier state involving the upper respiratory tract of mice. *Journal of Infectious Diseases* **123**, 502.

THOMAS, P. (1977). *Salmonella cholerae-suis* infection in pigs with special reference to England and Wales. *Veterinary Bulletin, Weybridge* **47**, 731.