SALMONELLA INFECTIONS

I. INCIDENCE IN WILD ANIMALS

II. EVALUATION OF CULTURE MEDIA FOR ISOLATION FROM FECES

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Radhey Mohan Sharma

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INTRODUCTION

Salmonellosis has been the subject of many studies since Salmon and Smith, in 1885, isolated the first member of the genus *Salmonella* from swine. The valuable work carried out by White (1925, 1926) and Kauffmann (1930a, 1930b, 1941) established the present method of antigenic analysis of the *Salmonella* group. The investigations on various Salmonella infections in different species of animals conducted in many countries have added several new sero-types. There are at present over 1000 sero-types recognized. Salmonella infections occurring in man and domestic animals and poultry have received considerable attention during the last quarter of a century but no large scale systematic studies seem to have been undertaken in this country to study the occurrence of Salmonella infections in wild animals, though several workers have recorded a number of sero-types occurring in a variety of wild animals. In spite of the fact that there is a general awakening to the serious nature of Salmonella infections in animals and man, and the possible steps taken by the state and private organizations to eliminate these infections, the incidence of Salmonellosis is even today by no means meagre. It may be likely that the Salmonella organisms in wild animals, especially in those living in the proximity of animal and human habitations, may contribute to the perpetuation of infection. The wild animals may contaminate ponds, fields and pastures used for watering and grazing of domestic animals and thus act as reservoirs of the infection. It was with this object in mind that a survey of Salmonella infections in wild animals in Iowa was undertaken as 'Part I' of the study to assess the degree of infection among them.

The isolation of Salmonellae is interesting but involves rather detailed laboratory technology, especially when isolation is attempted from contaminated materials, like feces, sewage, etc. The degree of Salmonella infection in animals and man varies under different conditions of health and disease and the detection of light infection is likely to present difficulty in view of the possibility of the presence of normal bacterial contamination in the feces. A number of enrichment and selective media have been used by various workers for the isolation of Salmonella organisms from organs and feces and varying claims have been made for the superiority of one or the other medium. In view of the diversity of opinion existing in literature on the subject, it was considered desirable to carry out controlled trials as 'Part II' of the study, using commonly employed enrichment broths and selective media, to evaluate their comparative efficiency for the isolation of Salmonellae from feces.

REVIEW OF LITERATURE

Incidence in Wild Animals

Salmonella infections in wild animals have been reported by various workers from different countries. Olson (1940) reported that the most commonly occurring Salmonella types in foxes were *Salmonella typhimurium* and *S. enteritidis* (Gartner) but *S. cholerae-suis* and *S. enteritidis* var. *danysz* were also known to infect them. He mentioned that the cubs were more often attacked and that the infection was rarely encountered in animals over 6 months of age. Benedict *et al*. (1941) isolated *S. anatum*, *S. enteritidis* var. *danysz*, *S. newington*, *S. typhi-murium*, and *S. cholerae-suis* from silver foxes. Momberg-Jorgensen (1942) investigated a number of severe enzootics caused by *S. dublin* amongst foxes during the summer of 1941. Edwards and Bruner (1943) reported frequent occurrence of *S. cholerae-suis* and *S. dublin* in carnivorous animals. *S. dublin* was recorded in 35 per cent of the cultures isolated from carnivorous animals. *S. pullorum* was also recognized in cultures from foxes and mink. Abortion in 16 foxes due to *S. cholerae-suis* was reported by Czarnowski (1958), and later by Malanowska (1963) in 10 out of 46 vixens affected. Rakhmatullin (1962) recorded *S. enteritidis* and *S. cholerae-suis* infection in foxes on a fur farm. Kauffmann and Henningsen (1938) recorded *S. braenderup* in a cat in Denmark. Wickham (1948) isolated *S. cambridge* from the mesenteric lymph nodes of a cat. *S. pullorum* was recovered from a three-month old kitten and the animal died after 4 days of illness (Weidenmuller, 1950). Armstrong (1942) reported *S. typhi-murium*

infection responsible for mortality in muskrats on a fur-animal field station. The infection was believed to have been introduced by wild mice.

Penn (1947) reported that 65 per cent of the Salmonella organisms isolated from mink in U.S.A. were S. typhi-suis, 21 percent S. cholerae-suis, and the rest were S. enteritidis and S. paratyphi. Bacteriological examination of 1602 carcasses received from mink farms throughout Germany revealed Salmonella infection in only 25 (1.6 per cent). The usual agents were S. typhi-murium and S. enteritidis. There were three cases of S. infantis and one of S. london infection (Loliger, 1956). Morkovic and Dordevic (1963) isolated S. berta from six, and S. cholerae-suis from 20 of 100 organ samples from mink which had died or been killed on a mink farm near Belgrade. Zimmermann (1962) recorded S. dublin in 35 and S. typhi-murium in 9 mink which had died of distemper. Salmonella organisms were also isolated from four of 518 fecal samples of apparently healthy stock during an outbreak of distemper. Bigland (1962) reported S. cholerae-suis and S. typhi-murium infections in mink. S. montevideo was recovered from the feces of a monkey suffering from chronic enterocolitis in Uruguay (Hormaechu and Peluffo, 1936); S. enteritidis from intestines of a laboratory monkey (Stasilevich, 1961); S. poona from a colobus monkey (Zwart, 1962); and S. paratyphi B was found in spider monkeys (Galton et al., 1948). Morris and Coburn (1948) isolated S. typhi-murium for the first time from ferrets.

Cruickshank and Smith (1949) examined feces of 500 dogs and 500 cats and found 1 per cent dogs and 1.4 per cent cats positive for Salmonella

organisms. All the types isolated were amongst the commonest "food poisoning" Salmonellae. From one cat a typical S. paratyphi B was isolated. The authors suggested that, in addition to the excretors, there were other animals that were infected but did not excrete the bacilli. They indicated that these findings had an obvious bearing for tracing the source of infection in man. S. new-brunswick was isolated for the first time from feces of dogs and cats from non-urban areas (Gorham and Garner, 1951). S. quiniele was recovered from rectal swab of a normal greyhound (Stucker et al., 1951). Mortelmans et al. (1960) recorded a new serotype, S. ngozi, in a dog during the course of a rabies control survey in the Ngozi province of Ruanda-Urundi. Van der Schaaf (1961) mentioned that he isolated Salmonellae (S. thompson, S. dublin, S. bovis morbificans and S. typhi-murium) from eight sick dogs. S. bareilly and S. paratyphi B were recovered from two other dogs. Wachnik (1963) reported isolation of S. cholerae-suis var. kunzendorf from two sick dogs, aged 3 months; kitchen waste was thought to be the source of the infection. Butler and Herd (1965) recovered Salmonella organisms from 16 dogs out of 98 examined. Six animals yielded two or three Salmonella species. They added that the organisms appeared to be transient carriers, showing no signs of intestinal infection.

S. rowbarton, S. uphill, S. taunton and S. weston were reported from feces of tortoises (Boycott et al., 1953). Bovre and Sandbu (1959) isolated Salmonella organisms from feces of 27 out of 33 tortoises in Oslo. Nineteen serological types were identified including two new ones: S. adamstua and S. lindern. All the strains isolated killed mice within

1-3 days after intraperitoneal injection. Huisman (1961) recovered *S. tel-aviv* from a tortoise and indicated its possible transmission to a girl. Delage *et al.* (1963) examined 1022 small mammals and reptiles and found the highest incidence of Salmonella infections in tortoises, 35 out of 47, and in 84 out of 210 hedgehogs. Veselinov and Feodorov (1965) reported that the incidence of Salmonellosis in captive tortoises varied from 57 to 92 per cent, and in free living tortoises from 12 to 71 per cent. Of the 44 sero-types isolated, three were new: *S. sladun*, *S. halle* var. *vidin* and *S. slatograd*. They were of the opinion that Salmonella formed part of the natural intestinal flora of tortoises.

Rossi and Emanuel (1952) reported an outbreak of *S. typhi-murium* infection which caused 356 deaths on a rabbit breeding farm comprising 1530 animals. Three dogs fed viscera from the dead rabbits contracted severe illness with diarrhoea and fever. *S. typhi-murium* was isolated from their feces. Gusev and Babicheva (1961) recorded a severe outbreak of Salmonellosis that killed 1,100 rabbits. *S. typhi-murium* was isolated from organs and tissues. *S. hessarek* was found in sick and dead rabbits (Minev and Petev, 1964). Cherakasskii and Sorina (1961) described two outbreaks of Salmonella infections in nutria causing abortion and still birth in 200 cases, and death in 500 young stock. The most common organism isolated was *S. typhi-murium*, but two cultures of *S. enteritidis* Gartner and one of *S. typhi* were also recovered.

Cameron *et al.* (1963) reported occurrence of a septicaemic disease due to *S. typhi-murium* amongst blue wildebeest in the Kruger National Park. They thought that the disease was possibly endemic, occasionally

becoming epidemic and seriously affecting the young calves.

Contamination of kangaroo meat with several sero-types of Salmonella has been reported by Mayer and Hang (1962), and Anderson *et al.* (1964). Among the isolates reported by the former, 31 were *S. adelaide*, 9 *S. muenchen*, 5 *S. chester*, 4 *S. anatum*, 3 each of *S. typhi-murium*, *S. kottbus* and *S. onderstepoort*, 2 *S. orion*, and one each of *S. emmastad* and *S. rubislaw*. *S. london* was isolated from a kangaroo (Simmons *et al.*, (1963). *S. teddington* was found in a chimpanzee (Zwart, 1962). *S. typhi-murium* was isolated from 13 of 33 hedgehogs in the Hamilton suburban area in New Zealand (Smith and Robinson, 1964). The hedgehogs might play an important role in the occurrence of this Salmonella in household animals. Watson (1966) reported an outbreak of *S. dublin* infection amongst chinchillas which proved fatal in 18 out of 42 animals.

S. cholerae-suis in a cougar, *S. anatum* in monkeys, and *S. bredney*, *S. javiana*, *S. montevideo*, *S. newington*, *S. newport*, *S. paratyphi B* and *S. typhi-murium* in mink were recorded in Canada. *S. san diego* was recently isolated from a chinchilla. Monkeys were found to harbor *S. seftenburg*, *S. stanley* and *S. typhi-murium* there (Bynoe and Yurack, 1964). The United States Salmonella surveillance report for 1964 revealed that 49 cultures were isolated from zoo and wild animals during the year (Brachman, 1965). The frequency of types recovered was: *S. typhi-murium* (15), *S. anatum* (8), *S. newington* (5), *S. typhi-murium* var. *cop*. (4), *S. chester* (3), *S. newport* (3), *S. derby* (2), *S. pomona* (2), *S. enteritidis* (1), *S. give* (1), *S. heidelberg* (1), *S. manhattan* (1), *S. miami* (1), *S. poona* (1), and *S. saint paul* (1).

It would thus be observed that information in regard to the occurrence of Salmonella infections in wild animals is at present meagre especially in comparison to the amount of knowledge worked out on Salmonella infections of domestic animals and man. The wild animals are likely to play a significant role in the dissemination of infection to animals and man. Keeping this aspect in view the Salmonella Seminar, held at Hyattsville, Maryland, in May, 1964, emphasized the urgent necessity to study the role played by wild life in the overall Salmonella picture.

Evaluation of Culture Media

The isolation of Salmonellae from organs and feces involves the use of enrichment broths and selective culture media. Leifson (1936) developed selenite broth and reported that, used in conjunction with desoxycholate citrate agar, it was very effective for isolation of typhoid bacilli from feces, sewage or water. Edwards and Ewing (1964) mentioned that tetrathionate broth of Mueller (1923) as modified by Kauffmann (1930b) (the combined enrichment medium) was a useful enrichment medium for the isolation of Salmonellae. The efficacy of this medium was attested by the results of Kauffmann (1935) who found that the medium, when used along with phenol red-brilliant green agar of Kristensen, Lester, and Jurgens (1925), the isolations of Salmonella paratyphi B were increased 100 per cent and the isolations of Salmonella from acute gastro-enteritis were increased 500 per cent over methods in which no enrichment was used. Knox, Gell and Pollock (1943) reported that a medium containing balanced tetrathionate provided optimum selectivity. It contained very little excess

sodium thiosulfate and gave consistent results as a routine solid medium for plating of fecal specimens for the isolation of Salmonellae. Preuss (1949) mentioned that better results were obtained with a tetrathionate medium in which a relatively pure preparation of potassium tetrathionate was substituted for the iodine and sodium thiosulfate solutions usually used.

Many persons prefer selenite broth for general use as S. typhi usually is inhibited when tetrathionate broth is used in conjunction with brilliant green agar (Edwards and Ewing, 1964). Hobbs and Allison (1945) found selenite superior to tetrathionate in the isolation of S. typhi and equally as good as tetrathionate in the isolation of S. paratyphi B. These findings were confirmed by Cook, Friesby and Jebb (1951) who reported that selenite broth was better than tetrathionate broth as an enrichment medium. Plating from selenite broth to desoxycholate citrate agar was the most successful method for the isolation of Salmonellae from feces. Smith (1952) studied the sensitivity of different culture media for isolating Salmonellae from the feces of man and various species of animals. He reported that selenite and tetrathionate media were greatly superior to liquid desoxycholate citrate medium, liquid Wilson and Blair's medium, cacotheline broth and brilliant green peptone water, and that it was usually possible to recover Salmonellae from fecal samples to which less than ten Salmonellae had been added. He mentioned that selenite medium was preferable to tetrathionate for examining cow and chicken feces but the reverse was true in the case of dog feces; slight differences only were noted in the other species. Taken as a whole, selenite was

slightly superior to tetrathionate. Banwart and Ayres (1953) carried out studies to determine the effect of commonly used enrichment broths upon the multiplication of several species of *Salmonella*. They found that tetrathionate broth, though it supported the growth of most of the species of *Salmonella* studied by them, it was definitely inhibitory to *S. paratyphi*. Selenite-F broth appeared to be one of the better broths; however, during the initial incubation period, inhibition and actual destruction of *S. anatis* was statistically significant.

Smith (1959) reported that it was necessary to use brilliant green-MacConkey broth for the isolation of *S. cholerae-suis* since the organism failed to develop in selenite broth. Slavin (1943) found brilliant green-neutral red-lactose agar quite useful to isolate 23 out of 28 *Salmonella* strains, mostly *S. suipestifer* on direct culture, from artificially infected pig feces.

Nagel (1950) and Zschuke (1951) advocated the addition of small amounts of streptomycin to selenite broth. Hajna (1955) constituted a gram-negative (GN) broth containing mannitol, glucose, sodium desoxycholate, sodium citrate and phosphate buffers, and reported that increased numbers of Salmonellae were isolated by this method. Bregman (1953) recommended the addition of magnesium chloride to tetrathionate broth, and Rappaport *et al*. (1956a) mentioned that better results were obtained in the recovery of Salmonellae other than *S. typhi* with magnesium chloride-malachite green enrichment broth than with the selenite or tetrathionate medium. Collard and Unwin (1958) supported the superiority of Rappaport's medium (1956b) to selenite and tetrathionate broths. They

mentioned that the addition of one tube of Rappaport's medium to selenite and tetrathionate in routine use in their laboratory increased the number of isolations by enrichment from 16 to 26 in 1,000 consecutive unselected stools. Mackie and McCartney (1953) indicated that best results were obtained by employing two or three different methods simultaneously as it gave a higher percentage of positive results than when one method only was used. They advocated direct plating on desoxycholate citrate medium, and the selenite or tetrathionate enrichment method might be used together, or Wilson and Blair's medium might be substituted for or used in addition to desoxycholate citrate agar. For the isolation of the paratyphoid B bacillus, tetrathionate enrichment gave somewhat better results than selenite.

Brilliant green agar is recommended as a useful medium for the isolation of Salmonellae. Banwart and Ayres (1953) reported that brilliant green agar supported more luxuriant growth of all the species of Salmonella, representative of the various antigenic groups, than did the other media tested. It appeared that Shigella-Salmonella agar and desoxycholate-citrate-lactose-sucrose agar significantly inhibited them. Bismuth sulfite agar was found to be significantly inhibitory to four of the six species of Salmonella used in their study. The use of brilliant green agar is advocated especially in conjunction with the combined enrichment medium of Kauffmann for isolating the largest number of Salmonellae. The use of these two media is the most efficient method yet devised for the isolation of Salmonella types other than S. typhi (Edwards and Ewing, 1964). Galton, Lowery and Hardy (1954) advocated the addition

of 8 to 16 mg. of sodium sulfadiazine per 100 ml. of brilliant green agar to give excellent results to inhibit Pseudomonas. Thomson (1954) mentioned that brilliant green MacConkey agar was the best for the isolation of S. paratyphi B. Harvey (1956) studied the comparative efficacy of brilliant green-MacConkey agar, desoxycholate citrate agar and Tabet's modification of bismuth sulfite agar for the isolation of Salmonellae other than S. typhi. He reported that brilliant green-MacConkey agar was found superior to other media irrespective of the sero-type of Salmonella present, with the exception of S. typhi. The results were independent of the type of material examined, the medium being equally satisfactory for the examination of feces or sewage. Jameson and Emberly (1956) reported that Teepol, an anionic detergent, was a reliable and cheap substitute for bile salts in culture media used for organisms of the colityphoid group. Media containing 0.1% Teepol and an indicator of the sulphonphthalein group were found to possess advantages over MacConkey's medium. Nogrady (1959) developed a medium identical in composition to MacConkey's agar but containing in addition brom-thymol-blue and urea. He claimed that Proteus colonies could be distinguished on this medium by the characteristic strong alkalinization produced in their immediate neighborhood and the isolation of Shigellae and Salmonellae was thus simplified.

Dixon (1961) mentioned that brilliant green MacConkey agar was the most satisfactory solid selective medium for direct plating and a subculture medium giving large characteristic colonies after 24 hours incubation. Selenite F medium inoculated with undiluted feces, incubated at 43°C, and subcultured after 6 hours incubation on to brilliant green

MacConkey agar, was the most successful rapid method of enrichment, though the results were considerably inferior to those obtained after 24 hours incubation. Hoag and Rogers (1961) reported that S. typhimurium in mice could be isolated more frequently by tetrathionate enrichment used in conjunction with brilliant green agar than on brilliant green agar alone. He indicated that the organ tissues might be inoculated separately into tetrathionate enrichment broth for optimal results. Guinee and Kampelmacher (1962) found that Muller-Kauffman's medium and selenite brilliant green broth detected equal numbers of Salmonella infected porcine faeces and skin scrapings; the number of positive findings was increased by using both media simultaneously. With lymph nodes, selenite brilliant green broth detected more infected samples than Muller-Kauffmann's medium. Heidrich (1963) investigated the comparative evaluation of tetrathionate broth and selenite broth for the demonstration of Salmonellae and reported that there was no appreciable difference between them with regard to the isolation of Salmonellae from faecal and organ samples. Guinee, Kampelmacher and Hoejenbos-Spithout (1965) examined feces and mesenteric lymph nodes of pigs and mentioned thattwo jars of Muller-Kauffmann's tetrathionate broth and two jars of Osborn-Stoke's selenite brilliant green medium were inoculated. Plating after 24 hour and 72 hour incubation resulted in an increase in the number of positive results, whereas plating in duplicate after 24 hours failed to do so. Plating after the 24 hour incubation period from two different jars containing the same enrichment medium resulted in an increase in the number of positive results equivalent to that obtained by plating after a 72 hour

incubation period from one jar. With feces, both enrichment media gave approximately an equal number of positive results. With lymph nodes, the selenite-brilliant green medium gave more positive results than Muller-Kauffmann's tetrathionate broth.

Miller and Banwart (1965) conducted a study of the inhibitory effect of 24 different combinations of brilliant green and bile salts concentrations using seven species of microorganisms capable of fermenting mannitol. They found that the inhibitory effect of brilliant green decreased as the concentration of bile salts increased. Staphylococcus aureus and Proteus rettgeri were inhibited by all test media. Escherichia coli was inhibited in all but two combinations of brilliant green and bile salts. Three of the 24 combinations of brilliant green and bile salts showed little or no inhibition of Salmonellae but did inhibit the other organisms studied. Georgala and Boothroyd (1965) reported that Leifson's selenite F broth was more selective for Salmonella when incubated at 43°C instead of the traditional 37°C. Different selective agar media produced different numbers of colonies from similar inocula of Salmonella cells, but Difco brilliant green agar consistently gave the highest recoveries. Combined with selenite broth enrichment at 43°C, it provided a useful system for isolating Salmonellae and compared favorably with the more classical techniques employing enrichment of each sample at 37°C in two different enrichment broths, followed by streaking on two selenite agars. Palyusik (1966) developed a surface inoculation method. Suspension of feces mixed with a solution of sodium tetrathionate and streptomycin was poured on to the surface of the nutrient agar containing lactose and bromthymol blue. The agar was immediately dried and placed in the incubator. He reported that

the method proved to be superior to existing methods for isolating *S. gallinarum* and *S. pullorum* from the feces of fowls, as well as *S. typhi-suis* from pig feces.

PART I. INCIDENCE IN WILD ANIMALS

MATERIALS AND METHODS

Intestinal Samples

The intestinal samples, collected from the wild animals in Iowa, were received from the Head, Veterinary Medical Diagnostic Laboratory, Iowa State University, Ames. The samples had been collected from apparently healthy animals destroyed to reduce the number of rabies susceptible animals under the Rabies Eradication Program. The pieces of intestines were collected from them soon after destruction. The samples were placed in polyethylene bags and were duly labelled with the description of the animal, name of locality, and date of collection. They were either stored in a deep-freeze or in the freezing chamber of a refrigerator.

The samples had been collected during the months from October, 1965 to January, 1966.

Enrichment and Selective Media

The following culture media were used in the study:

Enrichment media:

Selenite F broth (Leifson, 1936)

Bacto-Tryptone	5 gms.
Bacto- Lactose	4 gms.
Sodium selenite	4 gms.
Disodium phosphate	10 gms.
Distilled water	1,000 ml.
(Final pH 7.0 ± 0.1)	

Tetrathionate broth (Mueller, 1923, as modified by Kauffmann, 1935)

Basal medium

Proteose-Peptone, Difco	5 gms.
Bacto-Bile salts	1 gm.
Sodium thiosulfate	30 gms.
Calcium carbonate	10 gms.
Distilled water	1,000 ml.

Iodine solution

Iodine	6 gms.
Potassium iodide	5 gms.
Distilled water	20 ml.

2.0 ml. of iodine solution was added to each 100 ml. of base.

1.0 ml. of 1:1,000 solution of brilliant green was added per 100 ml. of base medium as recommended in Kauffmann's combined enrichment medium (1935).

Selective media

Bismuth Sulfite agar (BSA)

Bacto-Beef extract	5 gms.
Bacto-Peptone	10 gms.
Bacto-Dextrose	5 gms.
Disodium phosphate	4 gms.
Ferrous sulfate	0.3 gm.
Bismuth sulfite indicator	8 gms.
Bacto-Agar	20 gms.
Bacto-Brilliant green	0.025 gms.
Distilled water	1,000 ml.

Desoxycholate citrate agar (DCA)

Pork infusion from	330 gms.
Proteose-Peptone No. 3, Difco	10 gms.
Bacto-Lactose	20 gms.
Sodium citrate	20 gms.
Ferric ammonium citrate	2 gms.
Sodium desoxycholate	5 gms.
Bacto-Agar	13.5 gms.
Bacto-Neutral red	0.02 gm.

MacConkey lactose-bile salt agar

Bacto-Peptone	17 gms.
Peptone (Proteose)	3 gms.
Bacto-Lactose	10 gms.
Bile salts	1.5 gms.
Sodium chloride	5.0 gms.
Neutral red	0.03 gm.
Crystal violet	0.001 gm.
Distilled water	1,000 ml.
(pH 7.1)	

Carbohydrate fermentation media

Fermentation broth base

Peptone	10 gms.
Meat extract	3 gms.
Sodium chloride	5 gms.
Andrade's indicator	10 ml.
Distilled water	1,000 ml.

The pH was adjusted to 7.1 to 7.2. The medium was tubed with an inverted Durham tube and was sterilized at 121°C for 15 minutes.

Glucose, lactose, sucrose, and mannitol were employed in a final concentration of 1 per cent. Other carbohydrates, such as, dulcitol, salicin, etc., were used in a final concentration of 0.5 per cent. Glucose, mannitol, dulcitol, salicin, adonitol, and inositol were added to the basal medium prior to sterilization for 8 minutes at 121°C. Disaccharides, such as, lactose and sucrose (10 per cent solution in distilled water) were sterilized by filtration or at 121°C for 10 minutes and added to previously sterilized basal medium. Xylose and arabinose were also sterilized separately.

Isolation Procedure

Direct plating

The intestine was cut open in a sterile petri dish. An inoculating loopful from the intestinal contents was streaked on plates of MacConkey/desoxycholate citrate agar and bismuth sulfite agar. The inoculated plates were incubated at 37°C for 48 hours. The growth on the culture media was examined at 24 and 48 hours after inoculation.

Enrichment

A piece of intestine was put into Selenite F broth and another piece into tetrathionate broth. They were incubated for 18 hours.

A loopful from the incubated selenite enrichment broth was then inoculated on (1) bismuth sulfite agar and (2) desoxycholate citrate agar. Similarly, a loopful from the incubated tetrathionate broth was streaked on (1) bismuth sulfite agar and (2) desoxycholate citrate agar. The plates were incubated for 48 hours at 37°C.

The growth of organisms was examined on direct streaked plates as well as those inoculated from the enrichment media and the observations were recorded. Colonies suspected to be Salmonella were picked up individually with a slightly curved platinum needle and inoculated on triple sugar iron (TSI) agar by stabbing to the base of the butt and by streaking the slant. The TSl agar tubes were incubated at 37°C overnight. Tubes which exhibited an acid butt and an unchanged or alkaline slant and H_2S production were inoculated on urea agar of Christensen. The medium was inoculated heavily on the surface without stabbing. The tubes were incubated for 6 to 8 hours and were examined; marked alkalinity produced

in 6 to 8 hours is indicative of Proteus culture. Negative tubes were incubated overnight and reaction was read on the following morning. Those which proved negative were further incubated to 72 hours to detect any delayed reaction.

Biochemical Reactions

A colony from TSI agar was inoculated into peptone water. The culture was incubated for approximately 6 hours, and on the same day, the growth in the peptone water was inoculated on the following media. Each medium received two drops of the culture delivered by means of a sterile Pasteur pipette:

Lactose
Maltose
Dulcite
Sucrose
Glucose
Mannitol
Xylose
Salicin
Inosite
Simmons citrate agar
Peptone broth
Tryptose agar slant (usually 5-6 drops of the inoculum)

All the above tubes were incubated overnight and the results recorded on the following morning. The sugar tubes were incubated for further 14 days and were checked daily for any change in their reaction. The peptone water culture was incubated for 24/48 hours to test for indol production.

Indol test

The test for indol production was performed after 24 hours incubation,

using 1 or 2 ml. of the culture, removed aseptically from the tube. It was carried out with Kovac's reagent. About 0.5 ml. of the reagent was added and the tube was shaken gently. A deep red color developed in the presence of indol. In case the test was negative, the remaining portion of the peptone water was further incubated for an additional 24 hours, and the test was repeated.

Methyl red test (MR Test)

A tube of peptone glucose broth was inoculated lightly from a young agar slant culture of the organism. The tube was incubated at 37°C for 48 hours or longer. Five to six drops of the test reagent (Methyl red 0.1 gm., Ethyl alcohol 300 ml.) were added to 5 ml. of the culture. The reaction was read immediately. Bright red color indicated positive reaction and negative reaction was shown by yellow color. An orange red color was indicative of a weakly positive test.

Voges-Proskauer test (V.P. test)

The same medium was used as for MR test. The broth was inoculated from a young agar slant culture and was incubated at 37°C for 48 hours. The test reagent (O'Meara, modified) consisted of Potassium hydroxide 40 gms., creating 0.3 gm., and distilled water 100 ml. The reagent was added to the culture in equal quantity. The tests were left at room temperature and readings were taken after 4 hours.

The cultures which reacted as follows were tentatively identified

as Salmonellae.[1]

Glucose - Fermented, usually with gas (*S. typhi* and *S. gallinarum* are anaerogenic).

Mannitol - Fermented.

Dulcitol - Usually fermented.

Inosite - Fermented or not fermented.

Lactose - Not fermented.

Salicin - Not fermented.

Sucrose - Not fermented.

Indol - Not produced.

Methyl red - Positive.

Voges - Proskauer - Negative

Simmon's citrate - Positive (*S. paratyphi A*, *S. cholerae-suis* 'diphasic', *S. typhi*, *S. sendai*, *S. pullorum*, *S. gallinarum*, and some other types fail to utilize ammonium salts as a sole source of nitrogen).

H_2S-Positive (*S. paratyphi A*, *S. cholerae-suis* 'diphasic', *S. typhi-suis*, *S. sendai*, *S. berta*, some cultures of *S. typhi*, and a few other types, which occur very rarely, fail to produce H_2S.)

Urea - Negative.

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[1]Adapted from Edwards, P. R. and Ewing, W. H. Identification of Enterobacteriaceae. Minneapolis, Minnesota, Burgess Publishing Company. 1964.

The cultures, so isolated, were then tested in lysine decarboxylase broth and potassium cyanide (KCN) medium.

Lysine decarboxylase broth test (Falkow method, 1958)

The lysine decarboxylase broth was inoculated lightly from a young agar slant culture. A control tube was incubated with each culture under investigation. The tubes were incubated at 37°C for 4 days. The medium first turned yellow due to acid production from glucose; later, if decarboxylation occurred, the medium became purple. The control tube remained yellow.

Potassium cyanide (KCN) test

The KCN medium was inoculated with 1 loopful (3 mm. loop) of a 24-hour broth culture. The test was incubated at 37°C and observed daily for two days. Growth of organisms in the tube indicated positive reaction. Absence of growth was indicative of a negative reaction, typical of Salmonella group, to differentiate it from *Citrobacter*, *Klebsiella-Aerobacter-Serratia*, and *Proteus-Providence* group.

Serological Examination

'O' antigens

Two types of 'O' antigens were used: (1) Inactivated Salmonella antigen and (2) live Salmonella saline suspension. The inactivated antigen was prepared by the method recommended by White (1926). One ml. of absolute alcohol was added to the growth of organism in the nutrient agar tube. The growth was emulsified in absolute alcohol with a platinum loop. It

was transferred to a sterilized test tube and heated in a water bath at 60°C for 1 hour. The organisms were sedimented by centrifugation, the alcohol decanted, and the bacilli resuspended in 0.5 ml. of phenolized saline. It was again centrifuged, supernatant discarded, and the sediment resuspended in 0.5 ml. phenolized saline. This method was found useful in the examination of cultures which were slightly rough.

For routine testing, live saline suspension of the organism was used as an antigen. Its preparation was simple and is mentioned under the Plate agglutination test.

'H' antigen

The peptone broth culture of the organism was inactivated by using equal volume of 0.6 per cent formalized physiological saline solution to constitute the antigen.

Plate agglutination test

1. Typical Salmonella cultures growing on triple sugar iron agar (TSI) slants and which produced no alkalinization of Christensen's urea agar were used for serological testing.

2. A dense 1 ml. suspension of the organism to be tested was prepared using 0.85 per cent sodium chloride solution. Care was taken to ensure a uniform suspension.

3. A drop of normal saline was placed in a square of the ruled plate. A loopful of bacterial suspension was transferred to the normal saline drop. The two were mixed thoroughly to check that there was no agglutination in the control test.

4. A drop of 0.05 ml. of Bacto-Salmonella Poly 'O' antiserum was placed in the next square of the glass plate. To it was added 1 loopful

of the bacterial suspension and they were mixed thoroughly to obtain a uniform suspension.

5. The plate was rocked for 1 to 2 minutes to bring the organisms in close proximity to each other. Care was taken to avoid excessive evaporation.

6. Rapid and complete agglutination was recorded as a positive reaction. A delayed or partial agglutination was considered negative.

7. Each culture, which gave a positive agglutination with Poly O antiserum, was tested with Salmonella O antisera, groups A, B, C_1, C_2, D, E_1, E_2, E_4, F, G, H, and I in the manner mentioned above. The organism was placed accordingly in the specific group.

If, however, the culture reacted with Bacto-Salmonella O antisera Poly, but did not react with the specific O antisera groups, it was checked with Bacto-Salmonella Vi antiserum. The culture reacting with Vi antiserum was heated in a boiling water bath for 10 minutes and cooled. The heated culture was retested with the individual Salmonella O antisera groups A to I and the Vi antiserum, and the organism placed in the respective group. In case the heated culture continued to react with the Vi antiserum, the organism was believed to be a member of *Escherichia freundii* group.

8. The somatic antigens having been determined, the culture was tested for H antigens using Salmonella H antisera Spicer-Edwards. The antisera employed in this rapid serological procedure are given below:

Salmonella H antiserum Spicer-Edwards 1

Salmonella H antiserum Spicer-Edwards 2

Salmonella H antiserum Spicer-Edwards 3

Salmonella H antiserum Spicer-Edwards 4

Salmonella H antiserum Spicer-Edwards en complex

Salmonella H antiserum Spicer-Edwards L complex

Salmonella H antiserum Spicer-Edwards I complex.

The first four antisera permit the detection and identification of fourteen H-antigens while the latter three are adjunctive antisera for identifying additional H antigens.

H antigen analysis

The rehydrated Spicer-Edwards H antisera were diluted in the ratio of 0.1 ml. antiserum to 25 ml. 0.85 per cent sodium chloride solution.

One-half ml. of the required groups of diluted Salmonella H antisera were added to serological tubes. To each of the tubes was added 0.5 ml. of the test organism suspension. The tests were incubated in a water bath at 50°C for one hour. The results were read for the presence or absence of agglutination.

The observations recorded were interpreted with the help of Spicer-Edwards H antisera-antigen table.[1]

Isolation of phases

A majority of Salmonella are diphasic and we have to demonstrate both phases to determine the flagellar antigens. However, in the examination of H antigens, it may be assumed that any strain which contains anti-

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[1]Difco. Supplementary literature. Difco Laboratories, Detroit 1, Michigan. 1962.

Table 1. Salmonella H antisera Spicer-Edwards and H antigens with which each reacts

H antigens	Salmonella H antisera Spicer-Edwards			
	1	2	3	4
a	+	+	+	-
b	+	+	-	+
c	+	+	-	-
d	+	-	+	+
eh	+	-	+	-
G complex[a]	+	-	-	+
i	+	-	-	-
k	-	+	+	+
r	-	+	-	+
y	-	+	-	-
z	-	-	+	+
Z_4 Complex[b]	-	-	+	-
Z_{10}	-	-	-	+
Z_{29}	-	+	+	-

H antigens	Salmonella H antisera
enx, en, z 15	en complex
lv, lw, lz_{13}, lz_{28}	L complex
1, 2; 1, 5; 1, 6; 1, 7	I complex

[a]The G complex component of Salmonella H antisera Spicer-Edwards 1 and 4 antisera reacts with antigens fg, fgt, gm, gms, gmt, gp, gpm, gq, qst, ms and mt.

[b]The z4 complex component reacts with z_4, z_{23}, z_4z_{24}, and z_4z_{32}.

gens g, m, p, q, s, t, z_4, z_{27}, z_{36}, or z_{38} is monophasic and it is not necessary to search for a second phase. On the contrary, if a bacillus contains any of the other H antigens of the genus, it is very likely to be diphasic. Both phases of some diphasic cultures are immediately apparent. At times, only one phase can be detected. It is possible to isolate and identify the suppressed phase. A convenient method is the use of semi-solid agar to which agglutinating serum is added - the "Craigie tube" method.

The Craigie tube containing semi-solid agar (broth +0.25 per cent agar) is heated in a water bath. When the agar of the medium is melted to a fluid form, the Craigie tube is cooled by placing it in a water bath at 58°C for 10 to 15 minutes. One-half to 1.0 ml. of diluted anti-flagellar serum (titre 1:250), or a few drops of serum of high titre is added to the melted medium (serum of the same phase, in which the organism was found on agglutination test, is added). After thorough mixing by rolling the tube between the palms of the hands, the agar is allowed to harden and then a small amount of culture from the agar slope is stabbed by means of a straight platinum wire into the agar inside the small glass tube of the "Craigie tube". The culture is incubated at 37°C. On over-night incubation, the culture spreads through the medium inside the small tube and then spreads upwards and appears at the top of the medium of the large Craigie tube. The growth from the top of the medium is subcultured on agar slope and in nutrient broth, and incubated overnight. After incubation, the agar slope culture, ex Craigie, is tested by the rapid agglutination test with (a) the "O" serum of the group in which the organism

was found in the primary examination, (b) with the flagellar serum of the phase in which the organism was found prior to passing it through the Craigie tube, and (c) flagellar serum of suspected phase (judged by information from Kauffmann-White Diagnostic Schema[1]).

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[1] Edwards, P. R. and Ewing, W. H. Identification of Enterobacteriaceae. Minneapolis, Minnesota, Burgess Publishing Company. 1964.

RESULTS

Incidence of Infection

Three hundred and twenty-four samples of intestines, belonging to the following species of wild animals, were examined for Salmonella organisms. Seventeen strains were isolated. The results are tabulated below.

Table 2. Incidence of Salmonella infections in wild animals in Iowa

Species of animal	Number examined	Number positive	Percentage positive	Serotype isolated	
Raccoon	66	2	3.03	*S. derby*	1
				S. curacao	1
Fox	71	3	4.23	*S. cholera-suis* (var. *kunzendorf*)	2
				S. anatum	1
Opossum	56	7	12.50	*S. typhi-murium*	4
				S. anatum	1
				S. derby	1
				S. cholerae-suis (var. *kunzendorf*)	1
Mink	36	3	8.33	*S. typhi-murium*	1
				S. derby	1
				S. thompson	1
Skunk	40	2	5.00	*S. anatum*	1
				S. thompson	1
Squirrel	20	-			
Owl	8	-			
Wolf	5	-			
Civit	8	-			
Badger	3	-			
Hawk	6	-			
Muskrat	5	-			
	324	17	5.24		

Evaluation of Enrichment and Culture Media for the Isolation of Salmonellae

One hundred and ninety-eight intestinal samples were subjected to comparative study to evaluate the efficiency of the different enrichment and selective media. Ten specimens proved positive. The table below shows the number of Salmonella strains isolated by means of different culture media.

Table 3. Isolation of Salmonellae on different culture media

S. no.	Description of animal	Direct culture		Enrichment culture			
		BSA	DCA	Tetrathionate BSA	Tetrathionate DCA	Selenite broth BSA	Selenite broth DCA
1	Lake View opossum 6	X[a]	X	X	X	-[b]	X
2	Lake View skunk	-	-	X	X	X	X
3	Oskaloosa raccoon	-	X	X	X	X	X
4	Lake View opossum 1	X	-	X	-	X	-
5	Oskaloosa skunk	-	-	X	X	X	X
6	Oskaloosa fox	-	-	-	-	-	X
7	Creston mink 2	X	X	-	X	X	X
8	Creston opossum 2	-	-	-	X	-	-
9	Clarion mink 2	-	-	X	-	X	-
10	Creston mink 4	-	X	X	X	-	X
		3	4	7	7	6	7

[a]X = positive.

[b] - = negative.

Evaluation of Direct Plating versus Sub-culture from Enrichment Broths

Direct culture examination of intestinal specimens from 198 wild animals on bismuth sulfite agar and desoxycholate citrate agar revealed Salmonella organisms in 4 specimens against 10 on subculture from enrichment media.

Table 4. Direct culture versus subculture from enrichment broths

No. animals examined	Enrichment culture No. positive on BSA and DCA	Direct culture No. positive on BSA	 No. positive on DCA
198	10	3	4

DISCUSSION

The incidence of Salmonella infections amongst wild animals in Iowa was found to be 5.24 per cent, based on a study of 324 animals. The infection was observed to be highest in opossum, followed by mink, skunk, fox, and raccoon in the order named. Seventeen strains, isolated from them, belonged to six sero-types. *S. typhi-murium* seemed to occur more frequently and was isolated from four opossums and one mink. It has often been reported in mink (Loliger, 1956; Zimmermann, 1962; Bigland, 1962; and Bynoe and Yurack, 1964) but does not appear to have been frequently isolated from opossum. It is known to cause a variety of syndromes in different species of animals. Cherakasskii (1961) reported 200 cases of abortion and stillbirth amongst nutria on a fur animal farm due to *S. typhi-murium*. Rossi and Emanuel (1952) described an outbreak of *S. typhi-murium* infection that caused 356 deaths on a rabbit breeding farm. It causes an endemic disease in blue wildebeest in the Krugar National Park, which occasionally becomes epidemic and seriously affects young calves (Cameron *et al.*, 1963). It has also been reported in foxes (Olson, 1940; and Benedict *et al.*, 1941); in monkeys (Bynoe and Yurack, 1964), dog (Van der Schaaf, 1961); Kangaroo (Mayer and Hang, 1962); hedgehog (Smith and Robinson, 1964); and muskrat (Armstrong, 1942). *S. typhi-murium* is very widely distributed amongst various species of domestic animals, and is the species of *Salmonella* most commonly isolated from man in North and South America, and in Europe.

S. Cholerae-suis was isolated from two foxes and one opossum. This

organism is known to infect foxes (Olson, 1940; Benedict *et al.*, 1941, Edwards and Bruner, 1943; Rakhmatullin, 1962; and Czarnowski, 1958). It has also been reported from other wild animals, such as mink (Penn, 1947; Bigland, 1962; and Markovic and Dordevic, 1963); dog (Wachnik, 1963); and cougar (Bynoe and Yurack, 1964). It gives rise to generalized infections in pigs and affects various species of domestic animals. It is also an important human pathogen, not because it gives rise to many infections, but because of the severity of its effects. Smith (1952) reported that it was difficult to cultivate it from feces by means of the modern enrichment broths and that direct plating was superior to the use of enrichment media. Edwards, Bruner and Moran (1948) mentioned that the inability of *S. cholerae-suis* to grow in the more modern enrichment media was a serious handicap to the study of the epidemiology of disease caused by this organism and might account for some of the observations that healthy fecal excretors were rare. Gitter (1959) also preferred direct culture on selective media for the isolation of *S. cholerae-suis* from feces. The three strains of *S. cholerae-suis* var. *kunzendorf* obtained in the present survey were isolated on MacConkey agar. They could not be isolated by means of selenite broth. This is in conformity with the findings of Smith (1952), and Gitter (1959) as selenite broth does not permit the growth of this organism.

S. derby was isolated from one opossum, one raccoon and one mink. It has been reported from various species of animals and man. *S. anatum* was obtained from one fox, one opossum, and one skunk. It is known to infect fox (Benedict *et al.*, 1941); monkey (Bynoe and Yurack, 1964);

and kangaroo (Mayer and Hang, 1962). It is one of the more frequent causes of human infection in America and western Europe. S. thompson was isolated from a mink and a skunk. It is known to infect dog (Van der Schaaf, 1961). It is uncommon in America but is very common in Great Britain. It has occasionally been isolated from pigs, rats, and cattle. S. curacao was found in one raccoon. It does not appear to have been previously recorded in this species.

Salmonellae may be responsible for various infections in wild animals. The occurrence of gastroenteritis producing Salmonellae among wild animals may be of public health importance through fecal contamination of food and water, arthropod vectors, and direct contact of rodents with domestic animals and man. The wild animals may act as reservoirs of Salmonellae and may cause spread of the infection to domestic animals and man. The fecal contamination of water in ponds and springs by wild animals, especially in under-developed countries, may constitute an important source of infection for man and animals, as water from such water reservoirs is used at some places for drinking by human beings and livestock.

It was observed that out of 10 Salmonella positive cases, 9 were detected on enrichment in tetrathionate broth and an equal number of them proved positive on enrichment in selenite broth. However, one specimen negative in the tetrathionate broth group proved positive in the selenite broth group. Similarly, one specimen that proved negative in the selenite broth group was positive for Salmonella on enrichment in tetrathionate broth.

With regard to the two selective media, bismuth sulfite agar and desoxycholate citrate agar, the former detected 7 positive samples on sub-

culture from tetrathionate broth enriched incubated material and 6 from selenite broth inoculum. The desoxycholate citrate agar detected seven positives from the tetrathionate broth and an equal number from the selenite broth enriched incubated material. Taking the 'sensitivity index' as the ratio between the number of strains of Salmonellae isolated from the intestinal specimens by the individual medium and the total number of strains isolated from the specimens by the various methods, the sensitivity index for each method was: Tetrathionate broth - bismuth sulfite agar 70 percent, tetrathionate broth - desoxycholate citrate agar 70 per cent, selenite broth-bismuth sulfite agar 60 per cent, selenite broth-desoxycholate citrate agar 70 per cent. However, from the practical point of view there appears to be little difference in the efficiency of the two selective media as each one of them, when used in conjunction with the two enrichment broths, detected an equal number of positive samples. The two media, used simultaneously with the two enrichment broths, helped to detect 10 positive cases. These observations are in conformity with those reported by Guinee and Kampelmacher (1962); and Heidrich (1963). It is, therefore, advantageous to use a number of enrichment and selective media to detect the maximum number of positive cases (Knox *et al.*, 1942; Mackie and McCartney, 1953; Guinee and Kampelmacher, 1962; and Edwards and Ewing, 1964).

The direct culture examination of intestinal specimens detected only 4 out of 10 positive samples. Thomson (1954), however, mentioned that *S. typhi* or *S. paratyphi B* were found in very large numbers in the feces

of enteric carriers in human beings. Of the twenty cases found positive, almost all harbored many millions of bacilli. A minute inoculum of feces on a culture plate only rarely failed to reveal all the positives without the use of an enrichment medium. Dixon (1961) reported that out of 279 positive human cases, 76 of them proved negative for Salmonella organisms on direct culture on three selective media used. Slavin (1943) stated that direct culture was inferior to a good enrichment medium because in naturally infected feces, Salmonellae were probably present in most cases in small numbers and it was impracticable, even with the most satisfactory plating medium, to inoculate enough feces to be sure of obtaining a positive result. Smith (1952) mentioned that direct plating could only detect Salmonellae frequently when as many as 2,000 or 20,000 were added to the feces. It appeared that infection in the remaining 6 animals might not have been high enough to be detected by direct culture on the above two selective media.

The above data are not sufficient enough to draw any definite conclusions regarding the efficiency of the different culture media in view of the fact that although 198 samples were examined, only 10 yielded Salmonella organisms, as they were from healthy animals living in the wild state. It was, therefore, considered advisable to carry out the study under controlled conditions by adding known number of Salmonellae to feces and then endeavoring to recover them by means of culture media under test.

SUMMARY

A survey of Salmonella infections carried out amongst 324 wild animals in Iowa revealed 5.24 per cent infection in them. Of the 17 positive animals, 7 were opossums, 3 foxes, 3 mink, 2 skunks, and 2 raccoons.

The strains of *Salmonella* isolated belonged to 6 sero-types. Their distribution was: *S. typhi-murium* 5, *S. cholerae-suis* 3, *S. derby* 3, *S. anatum* 3, *S. thompson* 2, and *S. curacao* 1.

S. typhi-murium was isolated from four opossums and one mink; *S. cholerae-suis* from two foxes and one opossum; *S. derby* from one opossum, one raccoon, and one mink; *S. anatum* from one fox, one opossum, and one skunk; *S. thompson* from one mink and one skunk; and *S. curacao* was found from one raccoon.

The comparative efficiency of direct culture versus enrichment culture for the isolation of Salmonellae was studied on intestinal samples from 198 wild animals. Only 10 specimens proved positive for Salmonella organisms, 4 on direct culture against 10 by means of enrichment media. *S. cholerae-suis* could be isolated on direct culture only. It did not grow in selenite broth.

The size of the sample, 10 isolations only, was too small to evaluate the comparative efficiency of the different culture media for the isolation of Salmonellae from intestinal specimens.

The desirability of carrying out the study under controlled conditions by adding a known number of Salmonellae to feces was indicated.

PART II

EVALUATION OF CULTURE MEDIA FOR ISOLATION OF SALMONELLAE FROM FECES

MATERIALS AND METHODS

Enrichment and Selective Media

Enrichment broths

(i) Selenite F broth (see page 17)

(ii) Tetrathionate broth (Mueller, 1923, as modified by Kauffmann, 1935) (see pages 17-18)

(iii) Brilliant green MacConkey broth, with the lactose replaced with mannitol, and brilliant green 1 in 10,000.

Peptone (Bacto)	4 gms.
Mannitol	2 gms.
Oxgall	1 gm.
Bromcresol purple 1%	2 ml.
Brilliant green 0.1%	20 ml.
Water	200 ml.

Selective media

(i) Bismith sulfite agar (BSA)(see page 18)

(ii) Desoxycholate citrate agar (DCA) (see page 18)

(iii) Brilliant green - neutral red - lactose agar (BGNRLA). Concentration of brilliant green added was 1 in 30,000.

Peptone (Proteose)	10 gms.
Beef extract	3 gms.
Lactose	10 gms.
Sodium chloride	5 gms.
Saccharose	5 gms.

Agar		20 gms.
Neutral red		0.1 gm.
Brilliant green 0.1%		33.3 ml.
Water	q.s.	1,000 ml.

Salmonella cultures

Stock cultures of (1) *Salmonella anatum* (2) *S. typhi-murium* (3) *S. newport* and (4) *S. cholerae-suis* were kindly supplied by the Head, Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames. They had been typed at the Animal Health Division, National Animal Disease Laboratory, Ames, Iowa.

Actively motile 6 hour tryptose broth cultures of the above organisms were used.

Estimation of number of Salmonella

Serial ten-fold dilutions of the broth cultures were made in sterile distilled water containing 0.1 per cent bovine albumin and bacterial counts were made on nutrient agar by the method of Miles and Misra (1938). Bovine albumin was used as it was found by Smith (1952) to be the best for maintaining small numbers of Salmonellae without any apparent decrease in the viable count. Ten fold dilutions were made by adding 0.1 ml. of the culture to 0.9 ml. of the diluent, and a fresh pipette was used for each dilution. Ten dilutions of each culture were usually made. Nutrient agar plates, after the routine 24 hour drying at 37°C with the lids closed, were dried for a further two hours with the lids raised. The plates were dry enough to absorb 0.02 ml. drop in 15-20 minutes. Six plates were used for each culture. The plates were divided into 8 sectors. On such

plates, 0.02 ml. of the diluted culture, dropped from a height of 2.5 cm, spread over an area of 1-5 - 2.0 cm. in diameter. One ml. disposable syringes with 18 gauge needles or dropping pipettes, calibrated to deliver 50 drops to 1 ml, were employed for the purpose. Each of the six plates received one drop of each of the dilutions in numbered sectors. After the absorption of the drops, the plates were incubated in the usual way. The drop areas from the higher concentrations of culture yielded circular patches of confluent growth. Counts were made in drop areas containing the largest number of colonies without signs of confluence or of gross diminution in colony size, due to overcrowding (Figure 1). The number of colonies was estimated from the mean of six counts.

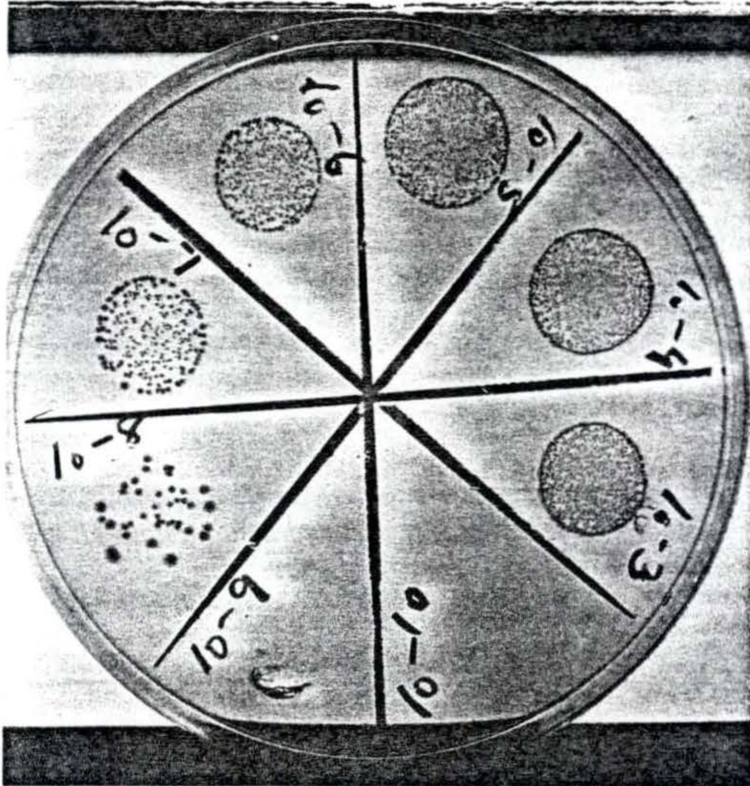
Method of Testing

Specimens of feces were obtained from a healthy cow of the Obstetrics Laboratory, and a healthy pig of the Animal Science Department, of the University. The feces of these animals were examined for Salmonella infection before including them in the study. The fecal samples were collected from the same animals throughout the study to avoid individual differences. The feces were stored in a refrigerator at 4°C and were used within a few days of collection.

The feces were diluted with sufficient sterile distilled water to impart a liquid consistency to them. One ml. volume of feces was added to each of the three enrichment media tubes. Four fecal specimens were examined on each occasion. Each specimen was infected[1] separately with

[1] The term 'infected' has been used to denote contamination with a disease-producing agent.

Figure 1. Estimation of number of Salmonella organisms


10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10

each of the four Salmonella serotypes. Thus there were 16 specimens in each run. Each specimen was added to each of the three enrichment broths. There were, therefore, 12 tubes of enrichment media per sample. Inoculum from each enrichment tube was subcultured on three selective media plates, half of a plate was used for each sample.

A dilution of the broth culture that contained the desired number of Salmonella organisms was added to each tube, and the tube was well shaken. The enrichment broths were incubated for 24 hours at 37°C and then streaked onto plates of selective media, using a 7 mm. platinum loop. The plates were incubated for 24 hours at 37°C. Those which did not show growth of Salmonellae were incubated for a further period of 24 hours. Colonies resembling Salmonellae were tested by slide agglutination with anti O-serum of the particular species of Salmonella with which the enrichment broth had been inoculated.

Approximately 4 Salmonellae were added to each tube of enrichment broth. In case of Salmonella cholerae-suis, the number had to be raised to 16 as no growth occurred with 4 and 8 organisms added to the tubes.

RESULTS

Four Salmonella serotypes were included in the study. Observations recorded in respect of Salmonella cholerae-suis revealed that this organism could not be recovered through tetrathionate broth and selenite medium but was recovered by means of brilliant green MacConkey broth. In view of this variation and the difference in the number of organisms added, it has been considered appropriate to present data in respect to S. anatum, S. typhi-murium, and S. newport separately from S. cholerae-suis to facilitate correct interpretation of results.

The recovery rates of S. anatum, S. typhi-murium, and S. newport from 76 samples of cow and 24 samples of pig feces, each of which had been infected with 4 organisms, by means of tetrathionate broth, selenite broth, and brilliant green MacConkey broth, are given in the following tables:

Table 5. Recovery rate of S. anatum from feces using enrichment broths and selective media

Species of animal	No. samples examined	Number positive by								
		Tetrathionate broth			Selenite broth			Brilliant green MacConkey broth		
		DCA	BSA	BGNRLA	DCA	BSA	BGNRLA	DCA	BSA	BGNRLA
Cow	76	60	57	57	59	50	56	58	51	59
Pig	24	18	16	18	14	14	17	14	14	15
Total	100	78	73	75	73	64	73	72	65	74

Table 6. Recovery rate of S. typhi-murium from feces using enrichment broths and selective media

Species of animal	No. samples examined	Number positive by								
		Tetrathionate broth			Selenite broth			Brilliant green MacConkey broth		
		DCA	BSA	BGNRLA	DCA	BSA	BGNRLA	DCA	BSA	BGNRLA
Cow	76	58	49	55	57	42	56	54	53	57
Pig	24	17	18	19	18	16	15	18	18	18
Total	100	75	67	74	75	58	71	72	71	75

Table 7. Recovery rate of S. newport from feces using enrichment broths and selective media

Species of animal	No. samples examined	Number positive by								
		Tetrathionate broth			Selenite broth			Brilliant green MacConkey broth		
		DCA	BSA	BGNRLA	DCA	BSA	BGNRLA	DCA	BSA	BGNRLA
Cow	76	54	50	51	50	45	55	57	52	56
Pig	24	16	16	16	16	14	18	17	18	18
Total	100	70	66	67	66	59	73	74	70	74

Of the 100 samples inoculated with S. newport, a maximum of 70 were found to be positive by tetrathionate broth, used in conjunction with the three selective media, 73 by selenite broth, and 74 by brilliant green MacConkey broth. The following table shows the results in respect to the three organisms:

Table 8. Comparative efficiency of recovery of three Salmonella serotypes from feces through enrichment broths used in conjunction with three selective media

Serotype	No. samples examined	Maximum number positive by		
		Tetrathionate broth	Selenite broth	Brilliant green MacConkey broth
S. anatum	100	78	73	74
S. typhi-murium	100	75	75	75
S. newport	100	70	73	74

Statistical analysis of the data revealed that the above differences were not significant (Table 11).

Enrichment Broths

With a view to study the efficiency of the three enrichment broths, used in conjunction with the three selective media, the positive results obtained for the three organisms were pooled (Table 9).

Table 9. Total number of positive results when S. anatum, S. typhi-murium, and S. newport were used (summarized from Tables 5, 6 and 7)

Species of animal	No. samples examined	Number positive by								
		Tetrathionate broth			Selenite broth			Brilliant green MacConkey broth		
		DCA	BSA	BGNRLA	DCA	BSA	BGNRLA	DCA	BSA	BGNRLA
Cow	228	172	156	163	166	137	167	169	156	172
Pig	72	51	50	53	48	44	50	49	50	51
Total	300	223	206	216	214	181	217	218	206	223

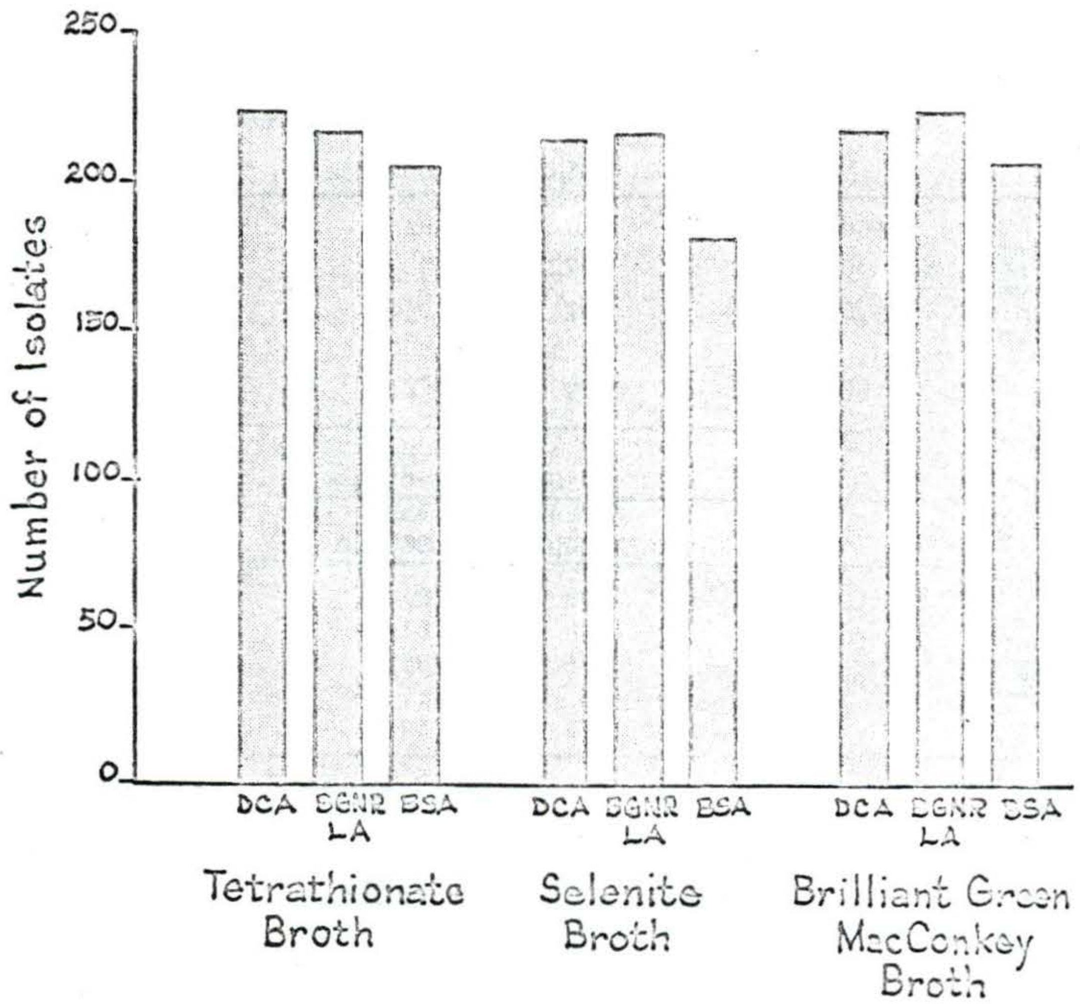
It was observed that the maximum positive results were obtained by means of brilliant green MacConkey broth, 647, against 645 with tetrathionate, and 612 with selenite broth. Using tetrathionate broth with the three selective media, maximum positive results were obtained on desoxycholate citrate agar (DCA), followed by brilliant green - neutral red - lactose agar (BGNRLA), and least with bismuth sulfite agar (BSA), the three figures being 223, 216, and 206 respectively. The corresponding figures for selenite broth were 214, 217, and 181. The combination of selenite broth with BSA gave the least number of isolations. The three figures in respect of brilliant green MacConkey broth were 218, 223, and 206. The combination of brilliant green MacConkey broth with BGNRLA, and tetrathionate broth with DCA gave equal number (223) of isolations (Figure 2). Statistical analysis showed that the difference between broths was significant at the 5 per cent level (Table 11).

Selective Media

The data in Table 9, when analyzed to evaluate the efficiency of the individual selective media in relation to the three enrichment broths, revealed 656 isolations on BGNRLA, 655 on DCA, and 593 on BSA. The factor responsible for decrease in the number of isolations on BSA was the selenite broth - bismuth sulfite agar combination, which isolated 181 cultures against an average of 204.0 of that group.

The differences between selective media, on statistical analysis, were found to be highly significant at the 1 per cent level (Table 11).

Figure 2. Histogram showing efficiency of three enrichment broths used in conjunction with the three selective media


250
200
150
100
50
0
Number of Isolates
DCA
BGNR LA
BSA
DCA
BGNR LA
BSA
DCA
BGNR LA
BSA
Tetrathionate Broth
Selenite Broth
Brilliant Green MacConkey Broth

Selective Media Combinations

The effect of using two or more selective media on the rate of recovery of Salmonellae from artificially infected fecal samples was studied on 300 samples. The following table shows the number of isolations when two or more selective media were used in conjunction with one or more enrichment broths:

Table 10. Rate of recovery of Salmonellae from feces using two or more selective media.

Serotype	No. samples examined	Number positive by Tetrathionate broth			
		DCA-BSA	DCA-BGNRLA	BSA-BGNRLA	All three
S. anatum	100	93	96	92	100
S. typhi-murium	100	93	97	90	99
S. newport	100	88	91	90	98
Total	300	274	284	272	297
		Number positive by Selenite broth			
		DCA-BSA	DCA-BGNRLA	BSA-BGNRLA	All three
S. anatum	100	91	92	87	96
S. typhi-murium	100	91	94	81	98
S. newport	100	82	86	86	88
Total	300	264	272	254	282
		Number positive by Brilliant green MacConkey broth			
		DCA-BSA	DCA-BGNRLA	BSA-BGNRLA	All three
S. anatum	100	92	94	89	96
S. typhi-murium	100	92	93	92	100
S. newport	100	93	92	92	98
Total	300	277	279	273	294

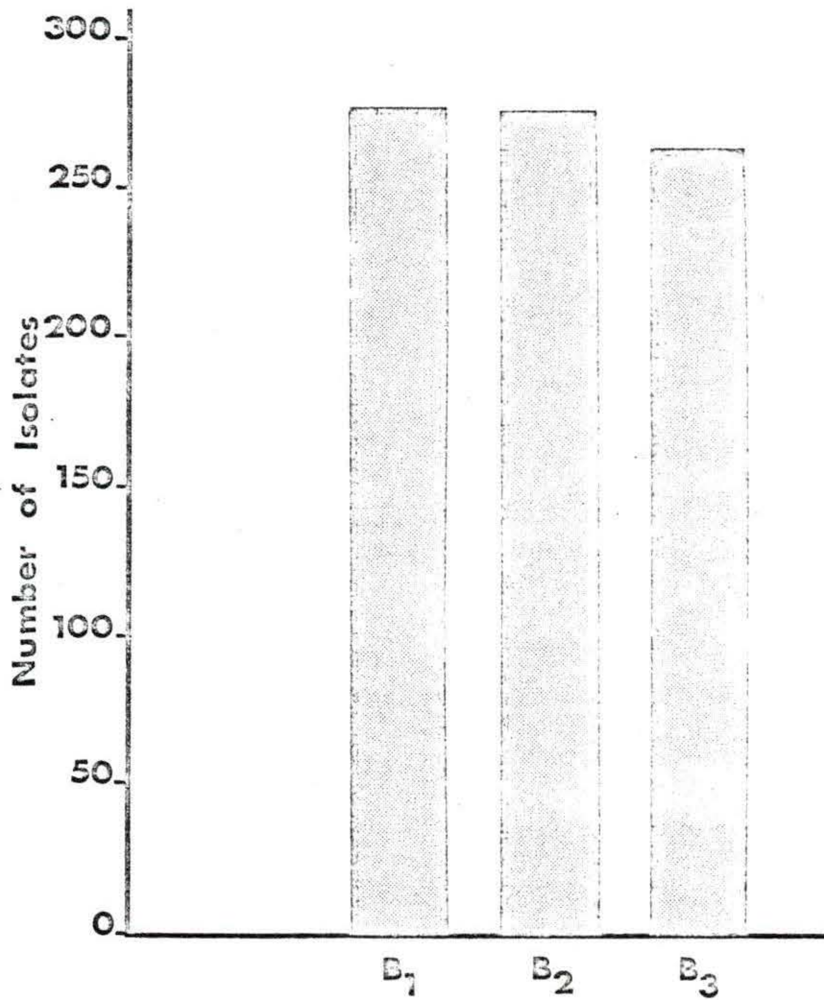
The above results indicated that of the three enrichment broths, used in conjunction with 3 two selective media combinations, tetrathionate broth and brilliant green MacConkey broth gave almost equal number of isolations, the average being 276.67 and 276.33, followed by an average of 263.33 isolations obtained by means of selenite broth. A histogram showing the above results was constructed. It is presented in Figure 3.

The data analyzed in relation to the selective media, used in conjunction with the three enrichment broths, revealed that of the two media combinations DCA-BGNRLA yielded highest number of recoveries, the average being 278.33 against 270.67 for DCA-BSA, and 266.33 for BSA-BGNRLA. The use of three selective media, however, gave still better results, the corresponding figure being 291.00. The inhibitory effect of selenite broth-BSA combination on the growth of Salmonellae adversely affected the results in the related combinations mentioned above. Histogram (Figure 4) shows the results reported above.

Isolation of *S. cholerae-suis* from Feces

The study to evaluate the efficiency of the commonly used enrichment and selective media was initiated with four *Salmonella* serotypes but it was observed that the addition of 4 and 8 *S. cholerae-suis* organisms did not promote its growth in tetrathionate and selenite broths. The number of organisms in the inoculum was increased to 16, 32, 64, 100, 1,000, 2,000, and 3,000 but still no growth could be obtained on any of the three selective media. Using brilliant green MacConkey broth containing a 1 in

Figure 3. Histogram showing efficiency of individual enrichment broths used in conjunction with 3 two selective media combinations



B_1 Tetrathionate broth

B_2 Brilliant green MacConkey broth

B_3 Selenite broth

Figure 4. Histogram showing efficiency of two or more selective media combinations used in conjunction with the three enrichment broths

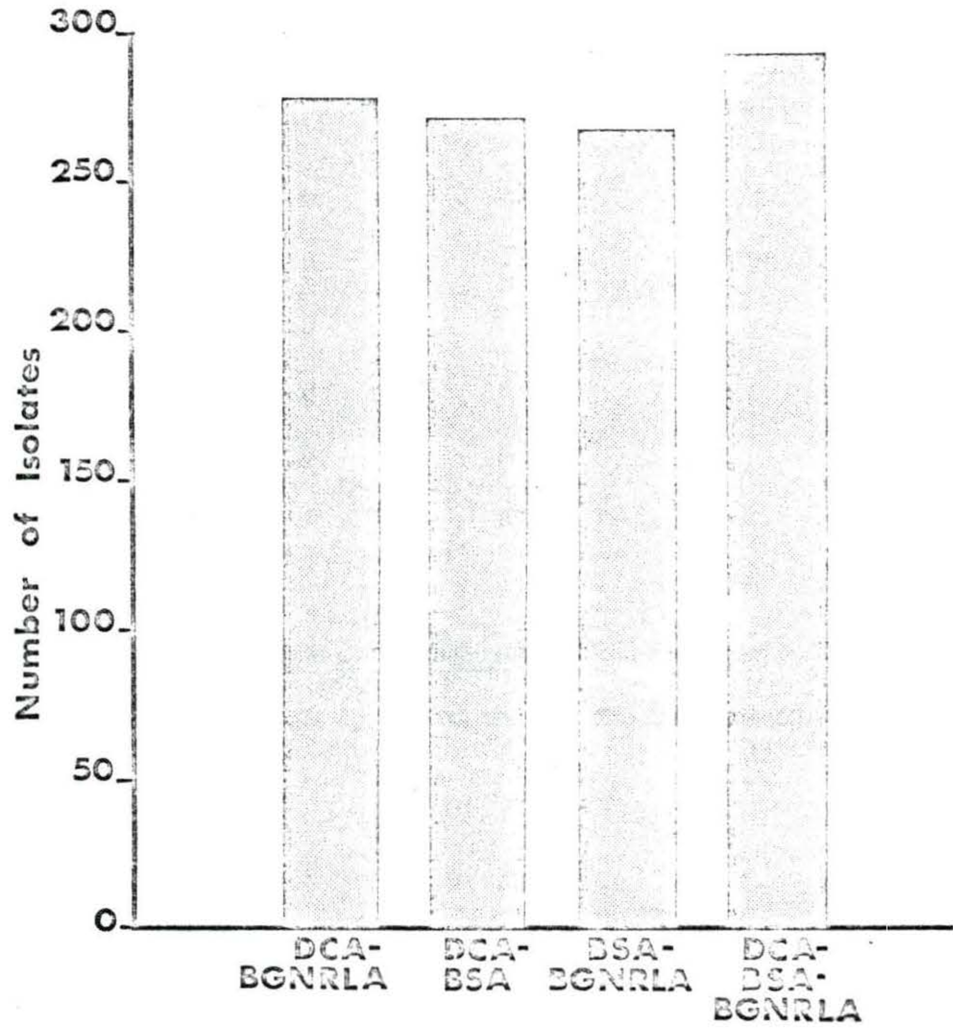

300
250
200
150
100
50
0
Number of Isolates
DCA-
BGNRLA
DCA-
BSA
BSA-
BGNRLA
DCA-
BSA-
BGNRLA

Table 11. Statistical analysis of data

Analysis of variance - variates Due to	DF	Mean square	F
A - Days	24	2.90	
B - organisms	2	1.01	1.99
C - Enrichment broths	2	1.62	3.19*
D - Selective media	2	6.16	12.14**
Interactions			
B X C	4	0.67	1.32
B X D	4	0.14	0.28
C X D	4	0.70	1.38
B X C X D	8	0.30	0.60
Error	624	0.508	
Total	674		

Means

B. *S. anatum* 2.88	*S. typhi-murium*	2.83	*S. newport* 2.75
C. Tetrathionate broth 2.86	Selenite broth	2.72	Brilliant green MacConkey broth 2.87
D. Desoxycholate citrate agar 2.91	Bismuth sulfite agar	2.63	Brilliant green-neutral red-lactose agar 2.91

5,000 concentration of brilliant green, it was found that it inhibited the growth of the organism even when 160 organisms had been added to the broth. The addition of 1,600 organisms to the brilliant green MacConkey broth, however, permitted recovery of S. cholerae-suis. Trials were carried out to find a suitable concentration of brilliant green which could inhibit the growth of contaminants but promote growth of S. cholerae-suis. It was observed that brilliant green, used in a concentration of 1 in 10,000 in MacConkey broth, did not inhibit the growth of S. cholerae-suis when 16 organisms had been added to it. It was, therefore, decided to infect the feces with 16 S. cholerae-suis instead of 4 organisms.

Seventy-two fecal samples (48 cow, and 24 pig), each of which had been infected with 16 organisms, were examined. Tetrathionate and selenite broth media did not promote the growth of the organism. The results obtained with brilliant green MacConkey broth, used in conjunction with the three selective media are, therefore, reported.

Table 12. Rate of recovery of S. cholerae-suis from artificially infected fecal samples through brilliant green MacConkey broth

Species of animal	No. of samples	Number positive by Brilliant green MacConkey broth		
		DCA	BSA	BGNRLA
Cow	48	26	26	42
Pig	24	10	12	15
Total	72	36	38	57

The use of brilliant green-neutral red-lactose agar gave the maximum number of isolations, 57 out of 72.

Effect of Autoclaving the Feces Before Infection on Rate of Recovery

In this study, the feces were autoclaved for 15 minutes at 120°C before artificial infection with S. cholerae-suis with a view to study the rate of recovery of the organism as compared to that obtained when the feces were not autoclaved. Seventy-two fecal samples (48 cow, and 24 pig) were examined. Tetrathionate broth and selenite broth did not promote the growth of the organism. The results obtained with brilliant green MacConkey broth are given below.

Table 13. Rate of recovery of S. cholerae-suis from autoclaved fecal samples, artificially infected

Species of animal	No. of samples	Number positive by Brilliant green MacConkey broth		
		DCA	BSA	BGNRLA
Cow	48	47	44	46
Pig	24	24	23	24
Total	72	71	67	70

It was observed that autoclaving the feces before infection helped to recover S. cholerae-suis from 71 out of 72 samples. Using non-autoclaved feces, the maximum recovery rate was 57 out of 72 specimens.

Effect of Duration of Incubation of Enrichment Broths on the Isolation of Salmonellae from Lightly Infected Feces

With a view to study the effect of duration of incubation of enrichment broths, inoculated with Salmonella infected feces, on the isolation of organisms from them, ten tubes, each of tetrathionate broth, selenite broth, and brilliant green MacConkey broth were taken. One ml. of liquid feces from the cow, under study, was added to each of the thirty tubes of enrichment broths. They were then inoculated with 0.02 ml. of suitably diluted Salmonella culture, containing approximately 15 _S. anatum_. Similarly, a second set of enrichment broth tubes, containing feces were infected, each with approximately 15 _S. typhimurium_, and the third set of broth tubes were inoculated, each with approximately 15 _S. newport_ organisms.

The broths were incubated at $37^{\circ}C$, and after 0, 6, 9, 12, 15, 18, 24, 36 and 48 hours were streaked onto plates of DCA. Salmonellae were not recovered from any of the specimens before 12 hours. The optimum time of incubation in the case of tetrathionate broth was between 24 and 30 hours, when Salmonellae were recovered from twenty-eight of the thirty specimens. The efficiency of this medium then decreased. Twenty-five specimens proved positive at 36 hours and Salmonellae could be recovered from twenty-one only out of 30 specimens at the end of 48 hours incubation. In the case of selenite broth and brilliant green MacConkey broth, the maximum number of twenty-eight positive isolations were obtained at 24 through 48 hours. There was no falling off of efficiency at 36 or 48 hours, as was noticed in respect to tetrathionate broth. The following

table shows the observations recorded:

Table 14. Effect of duration of incubation of enrichment broths on the recovery of Salmonellae from infected feces

No. of fecal samples examined	Duration of incubation in hours	No. of fecal samples positive by		
		Tetrathionate broth	Selenite broth	Brilliant green MacConkey broth
30	0	0	0	0
	6	0	0	0
	9	0	0	0
	12	16	18	18
	15	23	23	23
	18	26	25	26
	24	28	28	28
	30	28	28	28
	36	25	28	28
	48	21	28	28

Effect of Duration of Incubation of Cultures on the Growth of Salmonellae

The selective media plates inoculated with the three serotypes of *Salmonella* were examined after 24 hours and 48 hours incubation at 37°C. It was observed that some specimens negative after 24 hours incubation showed growth of the specific organism on subsequent incubation, when examined at the end of 48 hours. The following table shows the observations recorded:

Table 15. Effect of duration of incubation of cultures on the growth of Salmonellae

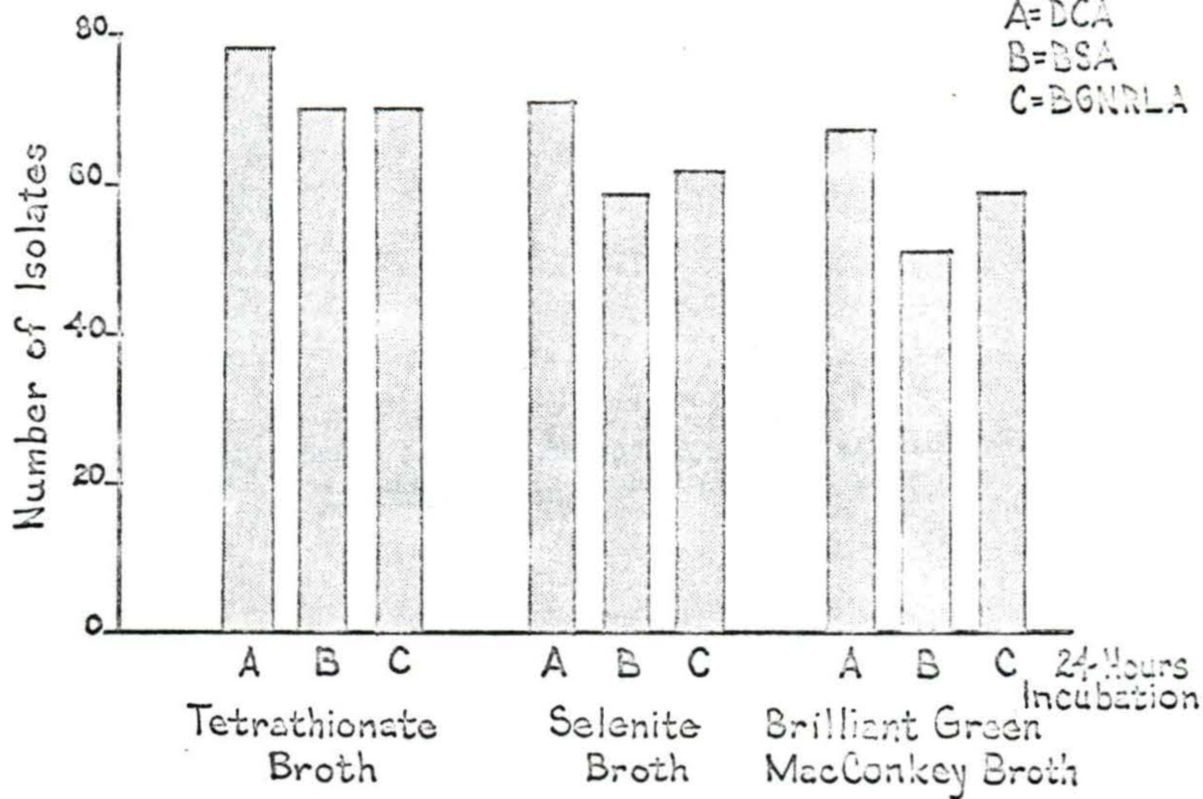
Species of Salmonella	Tetrathionate broth			Selenite broth			Brilliant green MacConkey broth		
	DGA	BSA	BGNRLA	DCA	BSA	BGNRLA	DGA	BSA	BGNRLA
a) S. typhi-murium 24 hours	71[a]	58	64	71	50	63	62	61	57
b) S. typhi-murium 48 hours	75	67	74	75	58	71	72	71	75
a) S. anatum 24 hours	78	70	70	71	59	62	67	51	59
b) S. anatum 48 hours	78	73	75	73	64	73	72	65	74
a) S. newport 24 hours	59	61	58	61	52	67	63	59	60
b) S. newport 48 hours	70	66	67	66	59	73	74	70	73

[a]The figures are numbers of isolations out of 100.

The above results revealed that the number of isolations on each of the three selective media was generally greater after 48 hours as compared to 24 hours incubation. Histograms showing the effect of duration of incubation were constructed. They are presented in Figures 5 through 10. This indicates the desirability of incubating cultures for 48 hours to detect larger number of positives and thus to contribute to the efficiency of diagnostic work.

Figure 5. Histogram showing effect of 24 hours incubation of cultures on the growth of *S. anatum*

Figure 6. Histogram showing effect of 48 hours incubation of cultures on the growth of *S. anatum*


A=DCA
B=BSA
C=BGNRLA
Number of Isolates
80
60
40
20
0
A B C
A B C
A B C
24 Hours Incubation
Tetrathionate Broth
Selenite Broth
Brilliant Green MacConkey Broth

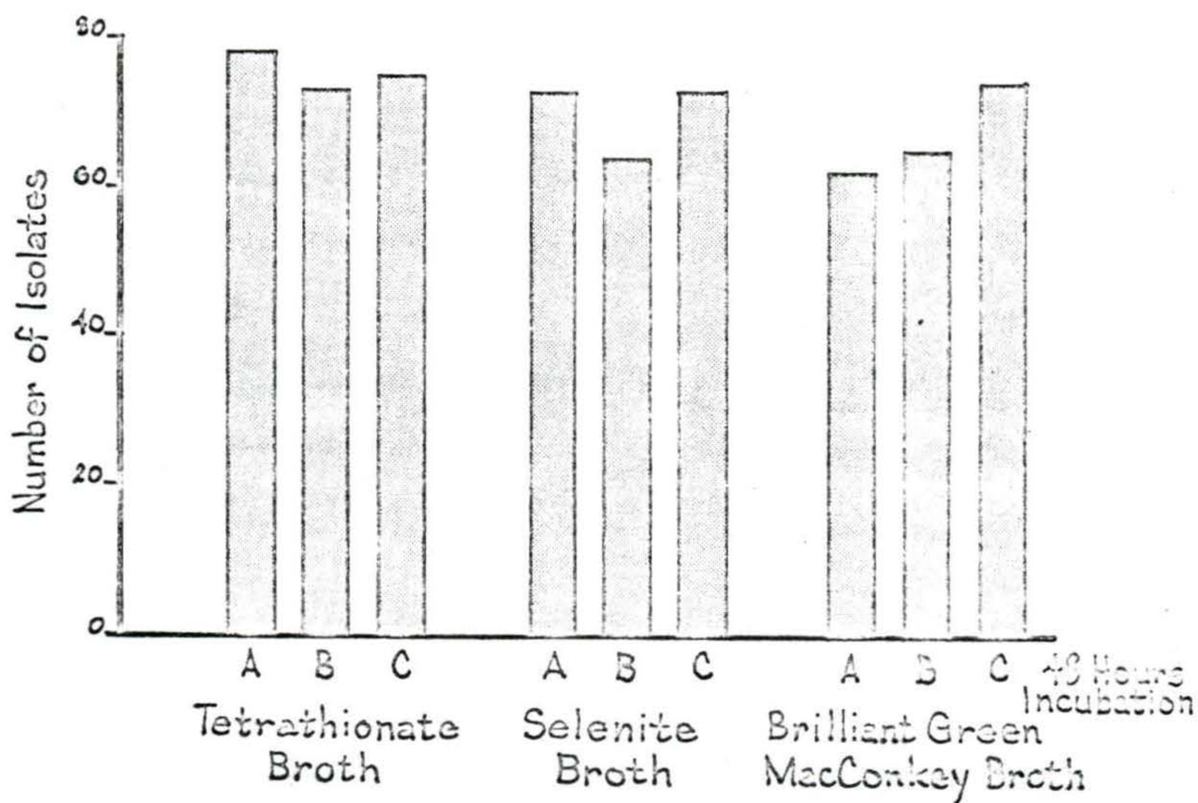
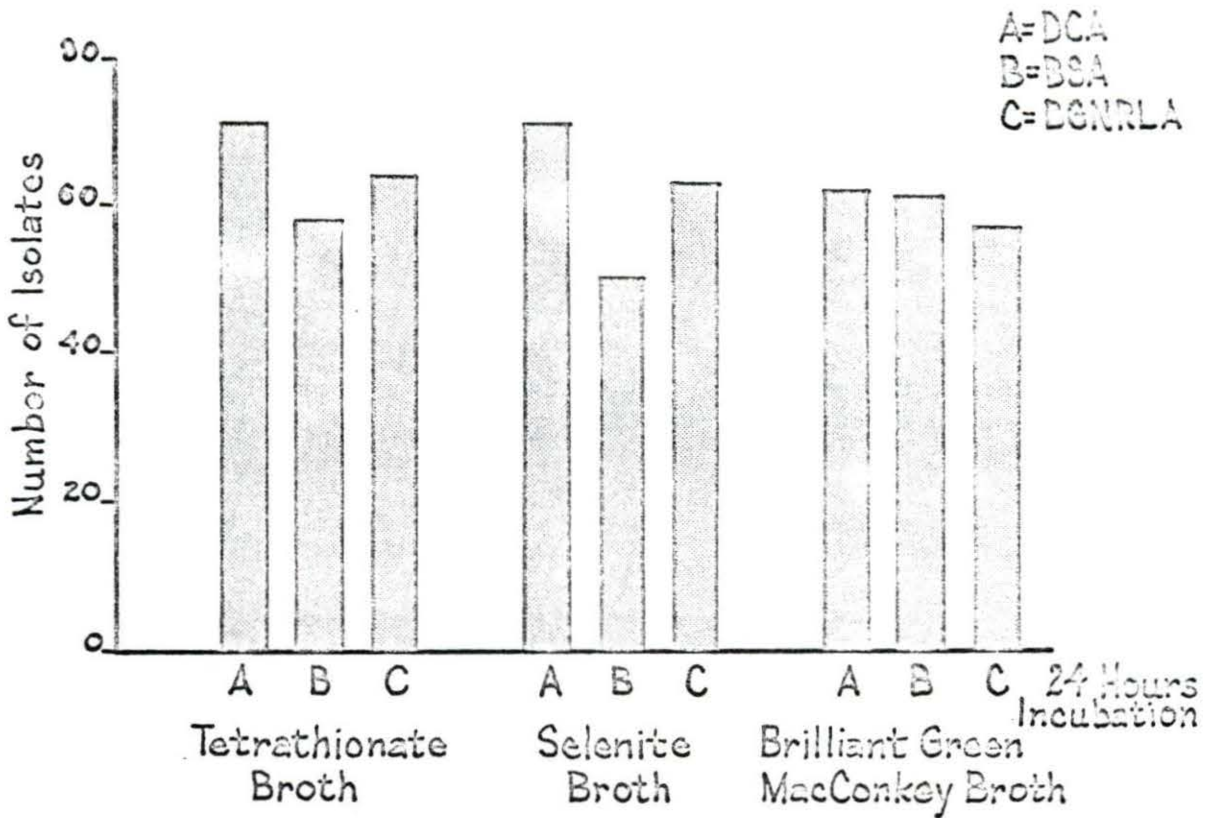

Number of Isolates
80
60
40
20
0
A B C
A B C
A B C
48 Hours Incubation
Tetrathionate Broth
Selenite Broth
Brilliant Green MacConkey Broth

Figure 7. Histogram showing effect of 24 hours incubation of cultures on the growth of _S. typhi-murium_

Figure 8. Histogram showing effect of 48 hours incubation of cultures on the growth of _S. typhi-murium_


A= DCA
B= BSA
C= DGNRLA
80
60
40
20
0
Number of Isolates
A B C
A B C
A B C
24 Hours
Incubation
Tetrathionate
Broth
Selenite
Broth
Brilliant Green
MacConkey Broth

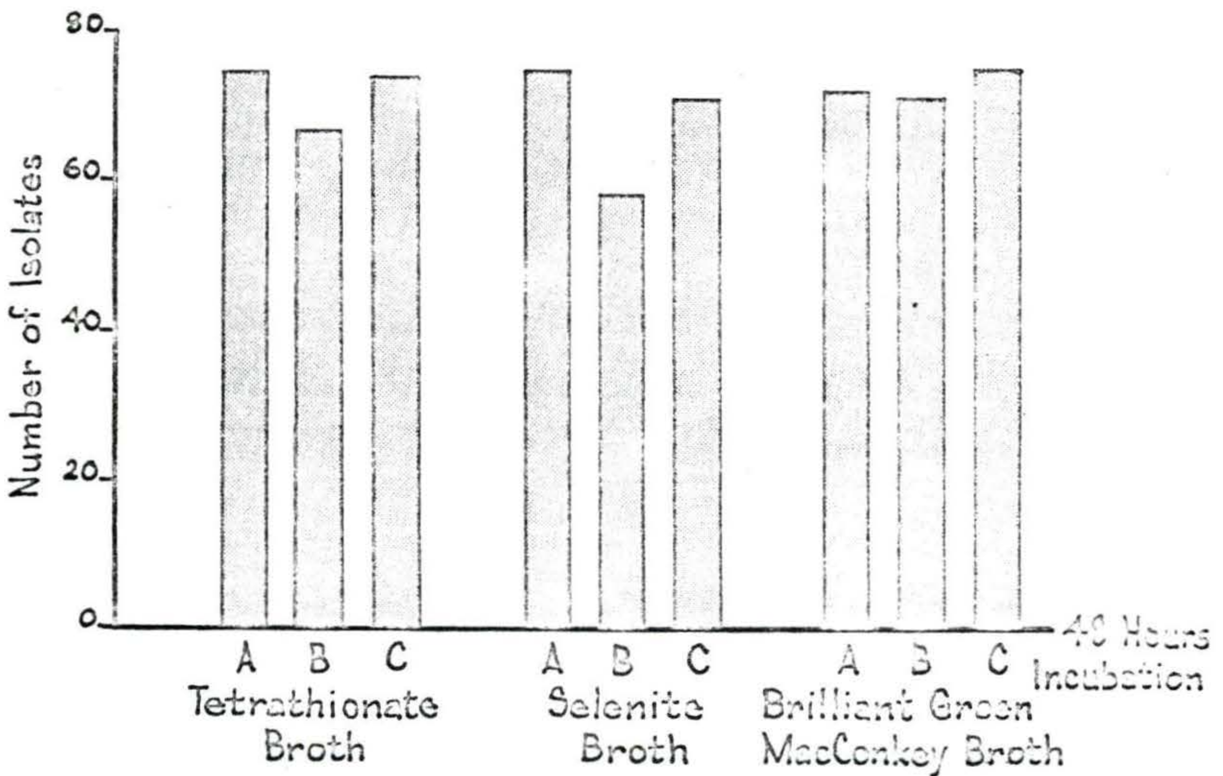
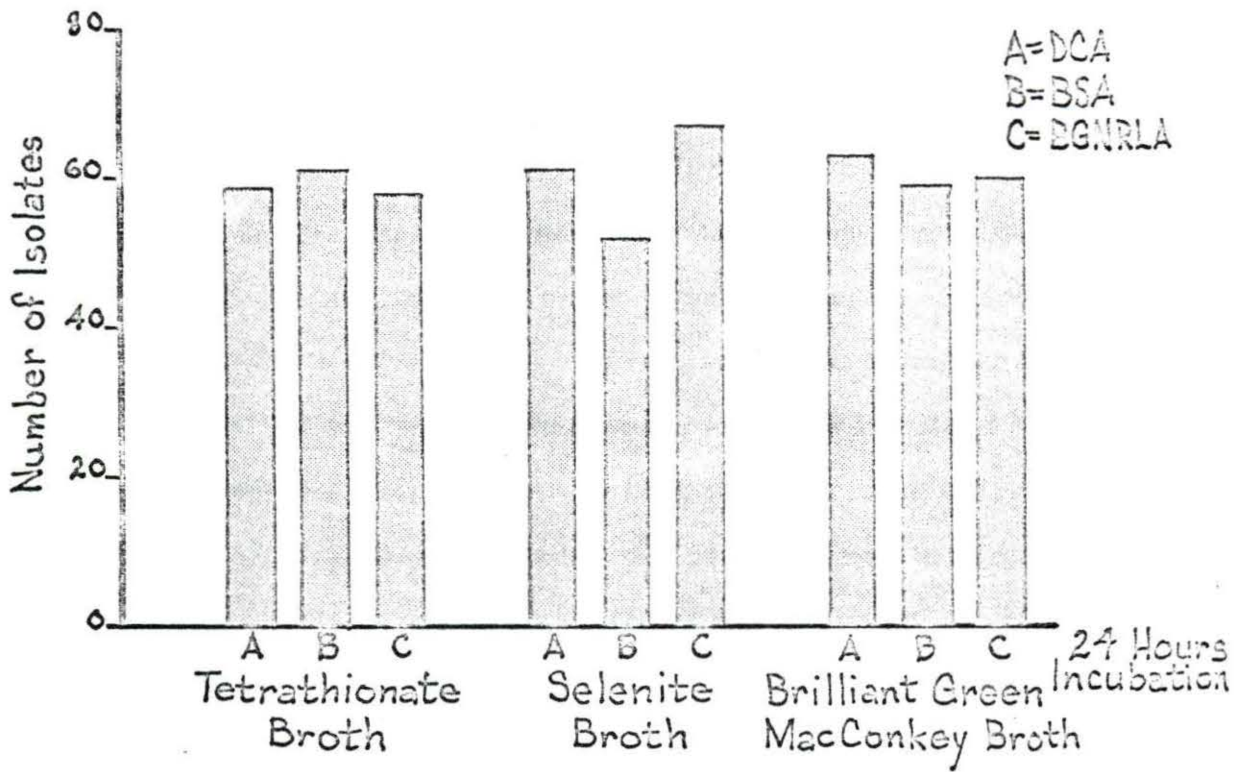
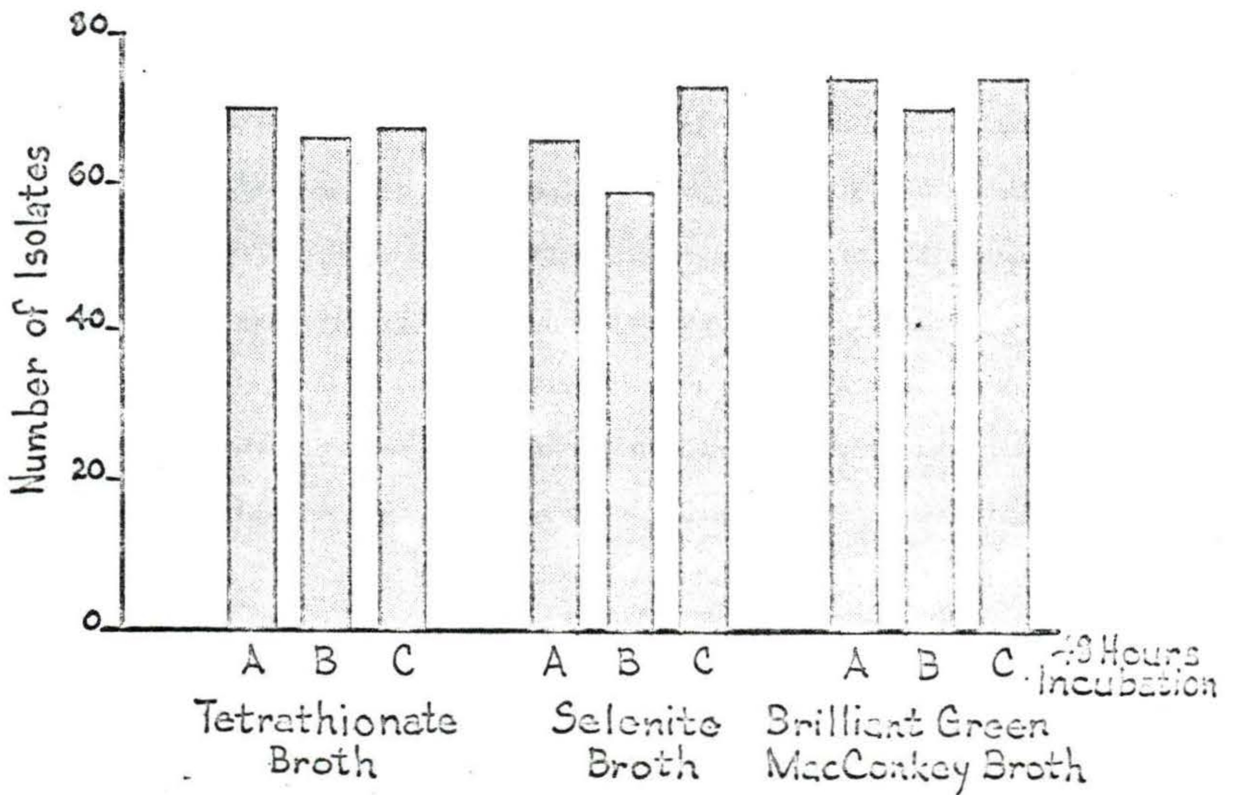

80
60
40
20
0
Number of Isolates
A B C
A B C
A B C
48 Hours
Incubation
Tetrathionate
Broth
Selenite
Broth
Brilliant Green
MacConkey Broth

Figure 9. Histogram showing effect of 24 hours incubation of cultures on the growth of *S. newport*

Figure 10. Histogram showing effect of 48 hours incubation of cultures on the growth of *S. newport*


80
60
40
20
0
Number of Isolates
A=DCA
B=BSA
C=BGNRLA
A B C
Tetrathionate Broth
A B C
Selenite Broth
A B C
Brilliant Green MacConkey Broth
24 Hours Incubation


80
60
40
20
0
Number of Isolates
A B C
Tetrathionate Broth
A B C
Selenite Broth
A B C
Brilliant Green MacConkey Broth
48 Hours Incubation

DISCUSSION

Work carried out under this study indicated that of the three enrichment broths, used in conjunction with the three selective media, brilliant green MacConkey broth gave maximum number of positive results, tetrathionate broth was next in order of efficiency, and the least number of isolations were obtained by means of selenite broth. The analysis of data on the efficiency of the three selective media in relation to the three enrichment broths revealed maximum isolations on BGNRLA, followed by DCA, and least on BSA. The comparatively low results obtained with selenite broth appeared to be due to the selenite broth-BSA combination which picked up only 181 positives against an average of 215.50 isolations obtained by means of selenite broth used in conjunction with the other two selective media, and an average of 215.00 of tetrathionate broth, and 215.67 of brilliant green MacConkey broth, each used in conjunction with the three selective media.

The observation that selenite broth-BSA combination exercised some inhibitory effect on the growth of Salmonellae is in conformity with the findings of Smith (1952), Hobbs and Allison (1945) on _S. typhi_, Cook _et al._ (1951) on _S. paratyphi B_, and Banwart and Ayres (1953). Smith (1952), however, reported that, taken as a whole, selenite broth, used in conjunction with DCA, was slightly superior to tetrathionate but best results were obtained by the use of both media. Banwart and Ayres (1953) mentioned that Selenite F appeared to be one of the better broths; however, during the initial incubation period, inhibition and actual destruction of _S. anatis_ was statistically significant. They added that bismuth

sulfite agar was shown to be significantly inhibitory to four of the six species of Salmonella used in their study. It may be mentioned that the results obtained in this study with approximately 4 Salmonella cells added to the enrichment broths revealed a lesser number of isolations by means of selenite broth on DCA, as compared to tetrathionate broth and brilliant green MacConkey broth on DCA. It resulted in one more isolation on BGNRLA in comparison to tetrathionate. The inoculation of approximately 15 organisms into the three enrichment broths revealed an equal number of isolations, when cultured on DGA, at the end of 24 hours incubation. This seems to indicate that perhaps selenite broth exercises some inhibitory effect when the feces are very lightly infected. Banwart and Ayres (1953) reported that Selenite F broth caused a secrease in numbers of viable cells during the initial period and that the decline was shown to be statistically significant at the 1 per cent level in the case of S. anatis. They added that it was particularly undesirable in the case of samples with low counts of Salmonella since destruction during the lag phase might result in failure to isolate organisms of this genus. The selectively inhibitory action of sodium selenite on S. thompson was reported by Weiss (1964) and it was attributed to be a function of the rate of selenite uptake. It was observed in the present study that selenite broth seemed to score over tetrathionate broth in that there was no falling off of efficiency during incubation at 36 and 48 hours, whereas a decrease in the efficiency of tetrathionate broth was noticed beyond 30 hour incubation. This is consistent with the results obtained by Smith (1952).

The observations recorded in this study in relation to the three

selective media used in conjunction with the three enrichment broths indicated that of the two media combinations, DCA-BGNRLA yielded highest number of recoveries, the average being 278.33 out of 300 known positive samples. The use of all three selective media, however, gave still better results, the corresponding figure being 291.00. This is in conformity with findings of Knox, Gell and Pollock (1942); Cook, Friesby and Jebb (1951); Mackie and McCartney (1953); Guinee and Kampelmacher (1962); and Edwards and Ewing (1964).

The most significant result obtained is the isolation of *S. cholerae-suis* from feces by means of enrichment through brilliant green MacConkey broth. Smith (1952) reported that for the isolation of *S. cholerae-suis*, direct culture on brilliant green agar was superior to the use of enrichment media. He added that it was necessary to use an inoculum of 30,000 *S. cholerae-suis* in tetrathionate broth and liquid desoxycholate citrate medium in order to recover the organisms in 55 to 75 per cent of the specimens. With an inoculum of 3,000 organisms, the recovery rate was markedly reduced. Edwards, Bruner and Moran (1948) mentioned that the inability of *S. cholerae-suis* to grow in the more modern enrichment media was a serious handicap to the study of the epidemiology of disease caused by this organism and that this might account for some of the observations that "healthy" fecal excretors of this organism were rare. Slavin (1943) used BGNRLA to isolate 23 out of 28 *Salmonella* strains, mostly *S. suipestifer*, on direct culture from artificially infected pig feces. He, however, remarked that in spite of the high efficiency of the brilliant green agar, it must be allowed that a direct method was inferior to a

good enrichment method in view of the fact that in naturally infected feces Salmonellae were probably present in most cases in small numbers. He added that it was, therefore, impracticable even with the most satisfactory plating medium to inoculate enough feces to be sure of obtaining a positive result. Gitter (1959) reported that direct culture on BGNRLA was superior to the use of selenite F enrichment medium. He recovered 29 out of 30 strains of _S. cholerae-suis_ on BGNRLA from intestinal contents of pigs heavily infected with undiluted cultures of the organism.

The results obtained in this study, using brilliant green MacConkey broth in conjunction with the three selective media, revealed that it gave maximum number of isolations on BGNRLA, 57 out of 72 specimens, against 36 on DCA, and 38 on BSA. The use of brilliant green in the enrichment and selective media for the isolation of Salmonellae is often advocated (Banwart and Ayres, 1953; Thomson, 1954; Harvey, 1956; Smith, 1959; Dixon, 1961; and Georgala and Boothroyd, 1965). The concentration of brilliant green suitable for the purpose was found to be 1 in 10,000, the higher concentration of 1 in 5,000 was noticed to be inhibitory for the organism in as much as it did not permit its growth in the broth tubes containing 160 organisms but showed the growth in the broth tubes with 1,600 Salmonellae. A concentration of 1 in 30,000 of brilliant green in the BGNRLA, when used in conjunction with brilliant green MacConkey broth (brilliant green 1 in 10,000), was found useful for the isolation of _S. cholerae-suis_ from feces. The higher concentration of 1 in 10,000 brilliant green in the selective medium inhibited the growth of organisms as they had already been subjected to this higher concentration in the enrichment broth and a second exposure to the same concentration seemed

to inhibit their growth. Gitter (1959) recommended the use of a concentration of 1 in 30,000 brilliant green in the selective medium in preference to 1 in 10,000. Slavin (1943) used brilliant green in the selective media in 1 in 10,000 and 1 in 20,000 concentrations as he found that its inhibitory effect varied with the source of the dye. Miller and Banwart (1965) indicated that brilliant green proportionately lost its inhibitive effect on bacteria in the presence of increasing amounts of organic matter.

Smith (1952) mentioned that the reason for failure to recover *S. cholerae-suis* from feces was not primarily that the feces contained bacteria which outgrew Salmonellae but that many of the media used were too toxic to permit the growth of the organism. In the present study, the feces were autoclaved before infecting them with *S. cholerae-suis*. This treatment resulted in a higher rate of isolations as compared with the rate of recovery from non-autoclaved feces. The number of isolations obtained in the case of the former were 70 out of 72 on BGNRLA, and the corresponding figure in the latter was 57 out of 72 on the same selective medium; these observations appear to suggest that contaminant bacteria present in the feces perhaps outgrow and mask *S. cholerae-suis*. It was observed during the course of the study that *S. cholerae-suis* grew at a slower rate as compared to *S. newport*, *S. typhi-murium*, and *S. anatum*, and that the colony size was also comparatively smaller, given the same period of incubation. It may probably be for this reason that before *S. cholerae-suis* could grow in sufficient numbers, the contaminant bacteria multiply in large numbers to mask them and thus render their isolation difficult in naturally infected feces. Smith reported (1959) that *S. cholerae-suis*

could be isolated from mesenteric lymph nodes of pigs by means of brilliant green MacConkey broth, but not from feces. Evidently it does not appear to be the toxicity of the enrichment broth for the organism that contributes towards failure to isolate it from feces but it may be due to the possibility indicated above.

The observation that a 24 to 30 hour was the best incubation time for tetrathionate broth, and that there was no falling off of efficiency in respect of selenite broth from 30 to the end of 48 hours, as happened with tetrathionate broth, were in conformity with the findings reported by Smith (1952). He, however, mentioned that Salmonellae were not recovered from any of the specimens before 15 hours, whereas it was observed in this experiment that the organisms could be isolated at the end of 12 hours incubation of enrichment broths in 53 to 60 per cent of the samples on DCA, used in conjunction with the three enrichment broths. Having used an equal number of organisms for inoculating the enrichment broths as Smith did, the difference may perhaps be due to variation in the nature and amount of the fecal matter added. Smith employed feces from various species of animals in contrast to the use of bovine feces from one animal only used in this experiment. Dixon (1961) isolated Salmonellae on brilliant green MacConkey agar from feces incubated in selenite F medium for 6 hours at 43°C. He recommended that it was the most successful rapid method of enrichment, though the results were considerably inferior to those obtained after 24 hours incubation. This variation appears to be due to the fecal samples, examined by him, to have been heavily infected with Salmonellae, and thus they could multiply to attain an optimal concentration in

the enrichment medium more rapidly as compared to the lightly infected specimens used in this study. The incubation of enrichment medium at $43^{\circ}C$ instead of $37^{\circ}C$ might also be responsible for the difference in results.

SUMMARY

The results of comparative efficiency of the commonly employed enrichment and selective media for the isolation of Salmonellae from feces are reported. The study was based on 300 fecal samples of cow and pig, each infected with approximately 4 organisms, using *S. anatum*, *S. typhimurium*, and *S. newport*.

Of the three enrichment broths used in conjunction with the three selective media, the maximum positive results were obtained by means of brilliant green MacConkey broth, followed by tetrathionate, and the least through selenite broth. The combination of brilliant green MacConkey broth with brilliant green-neutral red-lactose agar, and tetrathionate broth with desoxycholate citrate agar, gave an equal number of isolations, 223 out of 300 infected specimens. The data analyzed to evaluate the efficiency of the individual selective medium in relation to the three enrichment broths revealed maximum number of isolations on brilliant green-neutral red-lactose agar, followed by desoxycholate citrate agar, and least number on bismuth sulfite agar. The differences in the comparative efficiency of the three broths were found to be statistically significant at the 5 per cent level, and those between the selective media were highly significant at the 1 per cent level.

Of the two selective media combinations used in conjunction with the three enrichment broths, desoxycholate citrate agar-brilliant green-neutral red-lactose agar combination yielded highest positive results. The use of all three selective media gave still better results.

Selenite and tetrathionate broths were found unsuitable for isolating

S. cholerae-suis from feces. Brilliant green MacConkey broth, containing 1 in 10,000 brilliant green, proved to be a useful enrichment medium for the isolation of this organism from fecal samples of cow and pig, each infected with approximately 16 organisms.

The number of isolations of *S. cholerae-suis* from fecal samples, which had been autoclaved before infection, was found to be higher as compared to isolations from unautoclaved feces. This seems to suggest that contaminant bacteria present in the feces perhaps outgrow and mask *S. cholerae-suis*.

The combination of selenite broth-bismuth sulfite agar appeared to be somewhat inhibitory for the growth of Salmonella organisms.

An incubation period of 24-30 hours was found optimum for the three enrichment broths when the feces were lightly infected. A longer period was detrimental in the case of tetrathionate but not with selenite and brilliant green MacConkey broths.

Incubation of inoculated selective media plates for 48 hours yielded an increased number of isolations as compared to 24 hour incubation.

BIBLIOGRAPHY

Anderson, K., Crowder, E. F. and Woodruff, P. 1964. The isolation of Salmonellae from kangaroo meat sold as pet food. Medical Journal of Australia 2:668-669. Original not available; abstracted in Veterinary Bulletin 35:1210. 1965.

Armstrong, W. H. 1942. Occurrence of typhi-murium infection in musk-rats. Cornell Veterinarian 32:87-89.

Banwart, G. J. and Ayres, J. C. 1953. Effect of various enrichment broths and selective media upon the growth of several species of Salmonella. Applied Microbiology 1:296-301.

Benedict, R. G., McCoy, E. and Wisnicky, W. 1941. Salmonella types in silver foxes. Journal of Infectious Diseases 69:167-172.

Bigland, C. H. 1962. Salmonella reservoirs in Alberta. Canadian Journal of Public Health 53:97-104.

Bovre, K. and Sandbu, P. 1959. Salmonella excreting tortoises in Oslo. Acta Pathologica et Microbiologica Scandinavica 46:339-342.

Boycott, J. A., Taylor, J. and Douglas, S. H. 1953. Salmonellosis in tortoises. Journal of Pathology and Bacteriology 65:401-411.

Brachman, P. S. 1965. Summary of Salmonella surveillance, 1964. Atlanta, Georgia, Communicable Disease Center, U.S. Public Health Service.

Bregman, E. 1953. Improved diagnostic methods for isolation of pathogenic enteric bacilli. International Congress of Microbiology, 6th, Proceedings 1:391-394.

Butler, C. E. and Herd, B. R. 1965. Human enteric pathogens in dogs in central Alaska. Journal of Infectious Diseases 115:233-236.

Bynoe, E. T. and Yurack, J. A. 1964. Salmonellosis in Canada. In Oye, E., ed. The world problem of Salmonellosis. pp. 397-419. The Hague, Holland, W. Junk.

Cameron, C. M., Tustin, R. C. and Meeser, M. J. N. 1963. Salmonella typhi-murium infection in blue wildebeest calves (Connochaetes taurinus). South African Veterinary Medical Association Journal 34:53-55.

Cherakasskii, E. S. and Sorina, S. E. 1961. Salmonellosis in nutria (translated title). Vestnik sel'skokhozyaist vennol Nauki 4:99-101. Original not available; abstracted in Veterinary Bulletin 31:3161. 1961.

Collard, P. and Unwin, M. 1958. A trial of Rappaport's medium. Journal of Clinical Pathology 11:426-427.

Cook, G. T., Friesby, B. R. and Jebb, W. H. H. 1951. The routine use of selective and enrichment media for the isolation of Salmonellae. Gt. Britain Ministry of Health, Public Laboratory Service Monthly Bulletin 10:89-95. Original not available; abstracted in Veterinary Bulletin 22:39. 1952.

Cruickshank, J. C. and Smith, H. W. 1949. Isolation of Salmonellae from dogs, cats and pigeons. British Medical Journal 1949:1254-1258.

Czarnowski, A. 1958. Abortion in foxes caused by Sal. cholerae-suis (translated title). Medycyna Weterynaryjna 14:459-460. Original available but not translated; abstracted in Veterinary Bulletin 29:1691. 1959.

Delage, B., Chevrier, L., Neel, R. and Ascione, L. 1963. Investigation into Salmonella and Arizona bacteria in animals in Morocco. Marco Medical 42:420-425. Original not available; abstracted in Veterinary Bulletin 33:4420. 1963.

Dixon, J. M. S. 1961. Rapid isolation of Salmonellae from feces. Journal of Clinical Pathology 14:397-399.

Edwards, P. R. and Bruner, D. W. 1943. The occurrence and distribution of Salmonella types in the United States. Journal of Infectious Diseases 72:58-67.

Edwards, P. R., Bruner, D. W. and Moran, A. B. 1948. Further studies on the occurrence and distribution of Salmonella types in the United States. Journal of Infectious Diseases 83:220-231.

Edwards, P. R. and Ewing, W. H. 1964. Identification of Enterobacteriaceae. Minneapolis, Minnesota, Burgess Publishing Company.

Galton, M. M., Lowery, W. D. and Hardy, A. V. 1954. Salmonella in fresh and smoked pork sausages. Journal of Infectious Diseases 95:232-235.

Galton, M. M., Mitchell, R. B., Clark, G. and Riesen, A. H. 1948. Enteric infections in chimpanzees and spider monkeys with special reference to a sulfadiazine resistant Shigella. Journal of Infectious Diseases 83:147-154.

Georgala, D. L. and Boothroyd, M. 1965. A system for detecting Salmonellae in meat and meat products. Journal of Applied Bacteriology 28:205-212.

Gitter, M. 1959. Isolation of Salmonella cholerae-suis from post-mortem specimens. Veterinary Record 71:234-237.

Gorham, J. R. and Garner, F. M. 1951. The incidence of Salmonella infections in dogs and cats in a non-urban area. American Journal of Veterinary Research 12:35-37.

Guinee, P. A. M., Kampelmacher, E. H. and Hoejenbos-Spithout, H. H. M. 1965. Further studies on the influence of variations in the enrichment method for the detection of Salmonellae. Antonie van Leeuwenhoek Journal of Microbiology and Serology 31:1-10.

Guinee, P. A. M. and Kampelmacher, E. H. 1962. Influence of variations of the enrichment method for the detection of Salmonella. Antonie van Leeuwenhoek Journal of Microbiology and Serology 28:417-427.

Gusev, B. A. and Babicheva, A. J. 1961. Salmonella typhi-murium infection in rabbits (translated title). Krolikovodstvo Zverovodstvo 5: 19-20. Original not available; abstracted in Veterinary Bulletin 31: 3162. 1961.

Hajna, A. A. 1955. Public Health Laboratory 13:59. Original not available; cited in Edward, P. R. and Ewing, W. H. Identification of Enterobacteriaceae. Minneapolis, Minnesota, Burgess Publishing Company. 1964.

Harvey, R. W. S. 1956. Choice of a selective medium for the routine isolation of members of the Salmonella group. Gt. Britain Ministry of Health Monthly Bulletin 15:118-124. Original not available; abstracted in Veterinary Bulletin 26:3385. 1956.

Heidrich, D. 1963. Comparative evaluation of tetrathionate broth and selenite broth for the demonstration of Salmonella in bovine feces and in bacterological meat inspection. (English summary). Deutsche Tierärztliche Wochenschrift 70:595-601.

Hoag, W. G. and Rogers, J. 1961. Techniques for the isolation of Salmonella typhi-murium from laboratory mice. Journal of Bacteriology 82:153-154.

Hobbs, B. C. and Allison, V. D. 1945. Studies on the isolation of Bact. typhosum and Bact. paratyphosum B. Monthly Bulletin Ministry of Health (Gt. Britain) 4, No. 12:40-46. Original not available; cited in Edwards, P. R. and Ewing, W. H. Identification of Enterobacteriaceae. 2nd printing. Minneapolis, Minnesota, Burgess Publishing Co. 1964.

Hormaechu, E. and Peluffo, C. A. 1936. Archivos. Uruquayos de Medicina, Cirugia Y Especialidades 9:673. Original not available; cited in Wilson, G. S. and Miles, A. A. Principles of bacteriology and immunity. p. 837. 4th ed. London, England, Edward Arnold, Ltd. 1955.

Huisman, J. 1961. Salmonella tel-aviv transmitted to man by a tortoise (translated title, English summary). Tijdschrift voor Diergeneeskunde 86:899-902.

Jameson, J. E. and Emberly, N. W. 1956. A substitute for the bile salts in culture media. Journal of General Microbiology 15:198-204.

Kauffmann, F. 1930a. Der Antigen-aufban der Typhus-Paratyphus-Gruppe. Zeitschrift fürHygiene und Infektionskrankheiten 111:233-246.

Kauffmann, F. 1930b. Die Technik der Typhenbestimmung in Der Typhus-Paratyphus-Gruppe (English summary). Zentralblatt für Bakteriologie Originale 119:152-160.

Kauffmann, F. 1935. Weitere Erfahrungen mit dem Kombinierten Anreicherungsverfahren für salmonellabacillen. Zeitschrift für Hygiene und Infektionskrankheiten 117:26-32.

Kauffmann, F. 1941. Die Bakterologic der Salmonella Gruppe. Einar Munksgaard, Copenhagen. Original not available; cited in Oye, E., ed. The world problem of Salmonellosis. p. 421. The Hague, Holland, W. Junk. 1964.

Kauffmann, F. and Henningsen, E. J. 1938. Über einen neuen Salmonella-Typhus bei Mensch und Katze. Zeitschrift für Hygiene und Infectionskrankheiten 120:640-641.

Knox, R., Gell, P. G. H. and Pollock, M. R. 1942. Selective Media for organisms of the Salmonella group. Journal of Pathology and Bacteriology 54:469-483.

Knox, R., Gell, P. G. H. and Pollock, M. R. 1943. The selective action of tetrathionate in bacterological media. Journal of Hygiene 43: No. 3:147-158.

Kristensen, M., Lester, V. and Jurgens, A. 1925. On the use of trypsinized casein brom-cresol purple, phenol-red and brilliant green for bacteriological nutrient media. British Journal of Pathology 6:291-299.

Leifson, E. 1936. The effect of sodium selenite on the growth of bacteria and its use as the basis for a new enrichment medium for the isolation of typhoid bacilli from feces, water, milk, etc. Journal of Bacteriology 31:26-27.

Loliger, H. C. 1956. Salmonella infection in mink (translated title). Berliner und Munchener Tierärztliche Wochenschrift 69:31-32.

Mackie, T. J. and McCartney, J. E. 1953. Handbook of Practical Bacteriology. 9th ed. London, England, E. and S. Livingstone, Ltd.

Malanowska, T. 1963. Salmonella cholerae-suis as the cause of infection in foxes. (translated title) Medycyna Weterynaryjni 19:396-397. Original available but not translated; abstracted in Veterinary Bulletin 34:777.

Mayer, H. and Hang, E. 1962. Salmonella in kangaroo meat. Archiv für Lebensmittelhygiene 13:34-36. Original not available; abstracted in Veterinary Bulletin 32:2826. 1962.

Miles, A. A. and Misra, S. S. 1938. The estimation of bactericidal power of the blood. Journal of Hygiene 38:732-749.

Miller, V. R. and Banwart, G. J. 1965. Effect of various concentrations of brilliant green and bile salts on Salmonellae and other organisms. Applied Microbiology 13:77-80.

Minev, M. and Petev, P. 1964. Salmonellosis in ducks caused by S. canastel and in rabbits by S. hessarek in the Kolarovgrad-district (translated title). Veterinaro Meditsinski Nauki 1, No. 9:9-11. Original available but not translated; abstracted in Veterinary Bulletin 35:2057.

Momberg-Jorgensen, H. C. 1942. Paratyphoid in silver foxes (translated title). Maanedsskr Dyrlaeger 54:20-31. Original not available; abstracted in Veterinary Bulletin 17:295. 1947.

Markovic, B. and Dordevic, M. 1963. Salmonellosis in mink. (translated title) Veterinarski Glasnik 17:415-418. Original not available; abstracted in Veterinary Bulletin 34:60. 1948.

Morris, J. A. and Coburn, D. R. 1948. The isolation of Salmonella type (S. Ngozi) isolated separately in its monophasic and biphasic form from a dog and a gecko. Antonie van Leeuwenhoek Journal of Microbiology and Serology 26:255-256.

Mueller, L. 1923. Milieu d'enrichissment pour la recherche du Bacille typhique et des Paratyphiques. Comptes Rendus Societe de Biologie 89: 434.

Nagel, V. 1950. Aerztliches woechenschrift H. Heft. 51. Original not available; cited in Edwards, P. R. and Ewing, W. H. Identification of Enterobacteriaceae. p. 12. Minneapolis, Minnesota, Burgess Publishing Co. 1964.

Nogrady, G. 1959. Primary plating medium for differentiating the colonies of the genus Proteus from certain other enteric bacteria. Applied Microbiology 7:314-317.

Olson, A. B. 1940. Paratyphoid in silver foxes (translated title). Maanedsskr. Drylaeger 52:241-245. Original not available; abstracted in Veterinary Bulletin 19:207.

Palyusik, M. 1966. Surface inoculation technique. A new simple method for isolation of S. gallinarum-pullorum from hen's feces in a liquid medium containing sodium tetrathionate and streptomycin. Acta Veterinaria Academiae Scientiarum Hungaricae 16:33-36.

Penn, K. E. 1947. Salmonella infections in mink. American Fur Breeder 19:38.

Preuss, H. 1949. Über eine neue Tetrathionate-Anreicherung. Zeitschrift für Hygiene und Infektiouskrankheiten 129:187-213.

Rakhmatullin, R. G. 1962. Treatment and prophylaxis of paratyphoid in fur bearing animals (translated title). Krolikovodstvo Zverovodstvo 5:23-24. Original not available; abstracted in Veterinary Bulletin 32:3361. 1962.

Rappaport, F., Konforti, N. and Navon, B. 1956a. A new enrichment medium for certain Salmonellae. Journal of Clinical Pathology 9:261-266.

Rappaport, F., Stark, G. J. and Konforti, N. 1956b. UMAGIS medium: a medium for single tube differentiation of enteric bacteria. Applied Microbiology 4:157-160.

Rossi, C. and Emanuel, C. 1952. Outbreak of _Sal. typhi-murium_ infection in rabbits on a large rabbit farm (translated title, English summary). Atti Della Societa Italiana Delle Scienze Veterinarie 6:599-604.

Salmon, D. E. and Smith, T. 1885. Report on swine plague. United States Department of Agriculture Bureau of Animal Industry. Second Annual Report 2:476-521.

Simmons, G. C., Connole, M. D. and Elder, J. K. 1963. _Salmonella_ species isolated from animals and birds in Queensland during the period, 1951-60. Queensland Journal of Agricultural Science 20:173-179.

Slavin, G. 1943. Brilliant green-neutral red-lactose agar for the isolation of _S. suipestifer_ from pig feces. Journal of Comparative Pathology and Therapeutics 53:315-322.

Smith, H. W. 1952. The evaluation of culture media for the isolation of Salmonellae from feces. Journal of Hygiene 50:21-36.

Smith, H. W. 1959. The isolation of Salmonellae from the mesenteric lymph nodes and feces of pigs, cattle, sheep, dogs, and cats and from other organs of poultry. Journal of Hygiene 57:266-273.

Smith, J. M. B. and Robinson, R. A. 1964. _Salmonella typhi-murium_ in New Zealand hedgehogs. New Zealand Veterinary Journal 12:111-112.

Stasilevich, Z. K. 1961. Experimental salmonellosis in monkeys (translated title). Journal of Microbiology, Moscow, No. 2:70-74. Original not available; abstracted in Veterinary Bulletin 31:2078. 1961.

Stucker, C. L., Galton, M. M., Edwards, P. R. and Fife, M. A. 1951. Five new _Salmonella_ types. _S. quiniela_. U.S. Public Health Service Public Health Report 66:1058.

Thomson, S. 1954. The number of bacilli harbored by enteric carriers. Journal of Hygiene 52:67-70.

Van der Schaaf, A. 1961. Salmonellosis in dogs, cats, and fur animals. (English summary). Tijdschrift voor Diergeneeskunde 86:99-110.

Veselinov, V. and Feodorov, V. 1965. Pathogenic Enterobacteriaceae in reptiles. III. The Salmonella carrier state in tortoises in Bulgaria (translated title). Veterinarno Meditsinski Nauki 2:491-498. Original available but not translated; abstracted in Veterinary Bulletin 36:852. 1966.

Wachnik, Z. 1963. Salmonella cholerae-suis infection in dogs. Medycyna Weterynaryjni 19:260-263. Original available but not translated; abstracted in Veterinary Bulletin 34:776. 1964.

Watson, W. A. and Watson, F. I. 1966. An outbreak of Salmonella dublin infection in chinchillas. Veterinary Record 78:15-17.

Weidenmuller, H. 1950. Salmonella pullorum infection in a cat. (translated title) Berliner und Munchener Tierärztliche Wochenschrift No. 4:71-72. Original available but not translated; abstracted in Veterinary Bulletin 21:951. 1951.

Weiss, K. F. 1964. The inhibitory action of selenite on Escherichia coli, Proteus vulgaris and Salmonella thompson. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

White, P. B. 1925. An investigation of the Salmonella group with special reference to food poisoning. British Medical Research Council Special Report 91.

White, P. B. 1926. Further studies of the Salmonella group. British Medical Research Council Special Report 103.

Wickham, N. 1948. Salmonella cambridge infection in a cat. Australian Veterinary Journal 24:337.

Zimmermann, H. 1962. Salmonella as a secondary invader in mink distemper. (translated title) Monatshefte für Veterinärmedizin 7:306-307. Original available but not translated; abstracted in Veterinary Bulletin 32:2934. 1962.

Zschucke, J. 1951. Die Ausnutzung der elektiven wirkung des Streptomycin auf Saprophyten zur Verbesserung der Kulturellen Typhus-und Paratyphus-diagnose in stuhl und urin. (English summary). Zentralblatt für Bakteriologie Original 157:65-70.

Zwart, D. 1962. Notes on Salmonella infections in animals in Ghana. Research in Veterinary Science 3:460-469.

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APPENDIX

Table 16. Incidence of Salmonella infections in wild animals reported by different workers

Species of animal	S. no.	Salmonella serotype	Reported by	Year
Fox	1	*S. typhi-murium*	Olson	1940
			Benedict *et al.*	1941
	2	*S. enteritidis* (Gartner)	Olson	1940
			Rakhmatullin	1962
	3	*S. enteritidis* var. *danysz*	Olson	1940
			Benedict *et al.*	1941
	4	*S. cholerae-suis*	Olson	1940
			Benedict *et al.*	1941
			Edwards and Bruner	1943
			Czarnowski	1958
			Rakhmatullin	1962
			Malanowska	1963
	5	*S. anatum*	Benedict *et al.*	1941
	6	*S. newington*	Benedict *et al.*	1941
	7	*S. dublin*	Momberg-Jorgensen	1942
			Edwards and Bruner	1943
	8	*S. pullorum*	Edwards and Bruner	1943
Cat	1	*S. braenderup*	Kauffmann and Henningsen	1938
			Wickham	1948
	2	*S. pullorum*	Cruickshank and Smith	1949
			Weidenmuller	1950
	3	*S. new brunswick*	Gorham and Garner	1951
Mink	1	*S. montevideo*	Bynoe and Yurack	1964
	2	*S. typhi-suis*	Penn	1947

Table . (Continued)

Species of animal	S. no.	Salmonella serotype	Reported by	Year
Mink (continued)				
	3	S. cholerae-suis	Penn	1947
			Bigland	1962
			Morkovic and Dordevic	1963
	4	S. enteritidis	Penn	1947
			Loliger	1956
	5.	S. paratyphi B	Penn	1947
			Bynoe and Yurack	1964
	6	S. newington	Bynoe and Yurack	1964
	7.	S. pullorum	Edwards and Bruner	1943
	8.	S. typhi-murium	Loliger	1956
			Zimmermann	1962
			Bigland	1962
			Bynoe and Yurack	1964
	9	S. infantis	Loliger	1956
	10	S. london	Loliger	1956
	11	S. javiana	Bynoe and Yurack	1964
	12	S. berta	Markovic and Dordevic	1963
	13	S. newport	Bynoe and Yurack	1964
	14	S. dublin	Zimmermann	1962
	15	S. bredney	Bynoe and Yurack	1964
Monkey	1	S. typhi-murium	Bynoe and Yurack	1964
	2	S. montevideo	Hormaechu and Peluffo	1936
	3	S. enteritidis	Stasilevich	1961
	4	S. seftenburg	Bynoe and Yurack	1964
	5	S. poona	Zwart	1962
	6	S. stanley	Bynoe and Yurack	1964

Table . (Continued)

Species of animal	S. no.	Salmonella serotype	Reported by	Year
Monkey (Continued)				
	7	S. paratyphi B	Galton et al.	1948
	8	S. anatum	Bynoe and Yurack	1964
Dog	1	S. thompson	Van der Schaaf	1961
	2	S. dublin	Van der Schaaf	1961
	3	S. bovis morbificans	Van der Schaaf	1961
	4	S. typhi-murium	Van der Schaaf	1961
	5	S. paratyphi B	Van der Schaaf	1961
	6	S. cholerae-suis var. kunzendorf	Wachnik	1963
	7	S. quiniele	Stucker et al.	1951
	8	S. bareilly	Van der Schaaf	1961
	9	S. new brunswick	Gorham and Garner	1951
	10	S. ngozi	Mortelmans et al.	1960
Kangaroo	1	S. adelaide	Mayer and Hang	1962
	2	S. muenchen	Mayer and Hang	1962
	3	S. chester	Mayer and Hang	1962
	4	S. anatum	Mayer and Hang	1962
	5	S. typhi-murium	Mayer and Hang	1962
	6	S. kottbus	Mayer and Hang	1962
	7	S. onderstepoort	Mayer and Hang	1962
	8	S. orion	Mayer and Hang	1962
	9	S. emmastad	Mayer and Hang	1962

Table 16. (Continued)

Species of animal	S. no.	Salmonella serotype	Reported by	Year
Kangaroo (Continued)				
	10	S. rubislaw	Mayer and Hang	1962
	11	S. london	Simmons et al.	1963
Tortoises	1	S. rowbarton	Boycott et al.	1953
	2	S. uphill	Boycott et al.	1953
	3	S. taunton	Boycott et al.	1953
	4	S. weston	Boycott et al.	1953
	5	S. adamstua	Bovre and Sandbu	1959
	6	S. lindern	Bovre and Sandbu	1959
	7	S. tel-aviv	Huisman	1961
	8	S. sladum	Veselinov and Feodorov	1965
	9	S. halle var. vidin	Veselinov and Feodorov	1965
	10	S. slatograd	Veselinov and Feodorov	1965
Rabbit	1	S. typhi-murium	Rossi and Emanuel Gusev and Babicheva	1952 1961
	2	S. hessarek	Minev and Petev	1964
Nutria	1	S. typhi-murium	Cherakasskii and Sorina	1961
	2	S. enteritidis (Gartner)	Cherakasskii and Sorina	1961
	3	S. typhi	Cherakasskii and Sorina	1961
Blue wilde-beest	1	S. typhi-murium	Cameron et al.	1963
Chinchilla	1	S. san diego	Bynoe and Yurack	1964
	2	S. dublin	Watson	1966
Chimpanzee	1	S. teddington	Zwart	1962
Hedgehog	1	S. typhi-murium	Smith and Robinson	1964
Ferret	1	S. typhi-murium	Morris and Coburn	1948
Cougar	1	S. cholerae-suis	Bynoe and Yurack	1964
Muskrat	1	S. typhi-murium	Amrstrong	1942