SURVEY ON THE INCIDENCE OF SALMONELLAE IN MEAT ANIMALS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "SURVEY ON THE INCIDENCE OF SALMONELLAE IN MEAT ANIMALS" is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma. associateship, fellowship, or other similar title of any other University or Society.

P.C. James.

Mannuthy,

9 -7-1977.

CERTIFICATE

Certified that this thesis entitled "SURVEY ON THE INCIDENCE OF SALMONELLAE IN MEAT ANIMALS" is a record of research work done independently by Sri. P.C. James under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

INTRODUCTION

Salmonellosis is the most important of the meat borne infections. It is estimated that in certain areas 50 per cent of the slaughter animals are carriers of Salmonella (Schwabe, 1969). Since the first discovery of Salmonella typhi by Eberth in 1880, the genus has swelled in number every year and presently over 1300 serotypes are known to exist, with possibly many more to be detected. This great multitude of serotypes all possess pathogenic potential and can precipitate disease in man and animals under conditions congenial for infection. Since particularly they are ubiquitous in distribution, Salmonella infection is a major zoonotic problem, causing considerable morbidity and mortality and constitutes a public health hazard besides accounting for much economic loss due to reduced production of meat, milk and eggs.

Though much work covering various aspects of Salmonellosis has been carried out all over the world, the knowledge about the serotypes prevalent in the divergent species of livestock in Kerala and the magnitude of their occurrence associated with disease conditions is scanty, except for the studies in pigs reported by Sulochana et al. (1973) and Balakrishna Pillai (1975). Therefore, a project, "Survey on the Incidence of Salmonellae in Meat Animals" was taken up with the objective of gathering information on the extent and magnitude of

prevalence of Salmonellosis in Kerala which would facilitate further investigations on the problem of Salmonellosis in man and animals in the State.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

History

Salmon and Smith isolated and described the Salmonella cholera-suis in 1885 which was in those days, erroneously considered to be the aetiological agent of hog chlera, the causative agent of which has subsequently been identified as virus. Though Salmonella typhi had been recovered prior to the isolation of S. cholerae-suis, the latter became the prototype of the genus Salmonella, because at the time of isolation of typhoid bacillus it was classified under a separate genus Eberthella. Later on, related organisms causing human enteric fevers were identified and designated as paratyphoid A and B. In 1892 Loeffler identified the cause of mouse typhoid as a member of this group and soon occurrence of multitude of organisms evidently related, but deviating in certain cultural characteristics and immunological properties was established (Weil, [illegible] and Saphra, [illegible] 1953).

Description of Salmonella

In Bergey's Manual (Breed et al. 1957) the genus Salmonella is described as possessing the following characteristics: Rods which are usually motile by means of peritrichous flagella, although non-motile forms may occur. Gram-negative, Gelatin not liquefied, indol[illegible] not produced. Hydrogen

sulphide production is variable. Acid is produced from glucose, mannitol, maltose and sorbitol. Gas production is usually observed. Lactose, sucrose, salicin and adonitol are not attacked. The fermentation of other carbohydrates is variable. Acetylmethylcarbinol is not produced. Methyl red test is positive. Nitrites are produced from nitrates. Ammonium citrate is usually utilised. Urea not hydrolysed. Growth does not occur in KCN medium.

Biochemical reactions of the three genera - Salmonella, Arizona and Citrobacter - under the tribe Salmonelleae are furnished below in a tabular form.

Test or substrate	Salmonella	Arizona	Citrobacter
Indol	-	-	-
Methyl red	+	+	+
Voges-proskauer	-	-	-
Simmon's citrate	d	+	+
Hydrogen sulphide (TSI)	+	+	+ or -
Urease	-	-	dw
KCN	-	-	+
Motility	+	+	+
Gelatin (22C)	-	(+)	-
Lysine decarboxylase	+	+	-
Arginine dehydrolase	(+) or +	+ or (+)	d
Ornithine decarboxylase	+	+	d
Phenylalanine deaminase	-	-	-
Malonate	-	+	d
Gas from glucose	+	+	+
Lactose	-	d	d
Sucrose	-	-	d
Mannitol	+	+	+
Dulcitol	d(2)	-	d
Salicin	-	-	d
Adonitol	-	-	-
Inositol	d	-	-
Sorbitol	+	+	+
Arabinose	+(2)	+	+
Raffinose	-	-	d
Rhamnose	+	+	+

+, 90 per cent or more positive in 1 or 2 days. -, 90 per cent or more negative. d, different biochemical types (+), (+). (+), delayed positive. + or -, majority of cultures positive. - or +, majority negative. w, weakly positive reaction.
(Adopted from "Identification of Enterobacteriaceae" - Edwards and Ewing, 1972).

Deviations from the standard cultural characteristics of Salmonella do occur. Hence one has to be cognizant of the possibility of occurrence of strains of Salmonellae which reveal deviation of cultural properties in order to avoid serious diagnostic errors. The deviations that may be encountered are:

1. Lack of gas formation: This is the outstanding peculiarity of Salmonella typhi. This inability to break sugars all the way to carbondioxide and water is seen in S. gallinarum too. Non-gas forming variants occur rarely in other Salmonella serotypes such as S. paratyphi A and B, S. typhimurium, S. montevideo, S. enteritidis, S. sendai and S. anatum (Seligmann et al. 1943).

2. Lack of motility: S. gallinarum and S. pullorum are non-motile. Non-motile variants of S. typhi also do exist. Cultures with poor motility may be mistaken for non-motile strains especially when cultures are grown at 37°C. S. pullorum and non-motile S. typhi can be differentiated by fermentation of rhamnose (Kauffmann, 1941). The former ferments rhamnose while the latter does not.

3. Indol formation: Indol positive strains of S. eastbourne, S. panama, S. muenchen and S. enteritidis have been met with (Seligmann et al. 1943).

4. Liquefaction of gelatin: Slow liquefaction is found in the case of S. abortus ovis, S. gaminara, S. georgia and several others (Watt et al. 1950).

5. Lactose fermentation: Fermentation of lactose has been observed in a variant of S. anatum (Kauffmann, 1941) and in variants of S. newington (Saphra and Seligmann, 1947), in a strain of S. typhimurium and in two cultures of S. tennessi (Edward and Ball, 1966).

6. Failure to produce hydrogen sulphide: Serotypes S. abortus equi, S. pullorum, S. berta, S. seftenberg and some strains of paratyphi A fail to produce hydrogen sulphide (Ewing and Ball, 1966).

7. Non-utilization of citrate: Certain cultures of S. typhi, S. paratyphi A and S. pullorum have been found to fail to grow on Simmon's citrate agar (Ewing and Ball, 1966).

8. Growth in KCN medium: Two cultures of Salmonella have been found to grow in KCN medium (Ewing and Ball, 1966).

9. Failure to decarboxylate aminoacid: One strain of S. cholerae-suis, two cultures of S. pullorum and 16 of paratyphi A were found unable to decarboxylate lysine. One strain of S. paratyphi A failed to decarboxylate the ornithine as well. Twentysix other cultures of S. typhi and bioser gallinarum did not decarboxylate ornithine (Edward and Ball, 1966).

10. Utilization of sodium malonate: Sodium malonate was utilized by two cultures - S. bovis morbificans and S. albony (Edward and Ball, 1966).

11. Acid formation from sucrose: Two cultures of S. tennesse which were lactose fermenters produced acid from sucrose as well (Edward and Ball, 1966).

12. Fermentation of salicin: Three strains which belonged to different serotypes have been found to ferment salicin (Edward and Ball, 1966).

All the members of the genus Salmonella have an antigenic structure by which they can be recognized. They have been classified into types essentially based on the somatic and flagellar antigenic components. Kauffmann-White Schema is the one internationally recognized and adopted for subclassification and typing of Salmonella. In K-W Schema all antigenic relationship in detail has not been elucidated but the antigens of diagnostic importance have been catalogued.

Incidence

Salmonella genus comprises of more than 1300 serotypes and all of them are found to be potential pathogens to man and animals. Certain serotypes are specially pathogenic to certain species of hosts and are host specific, for example, S. typhi

to man; S. abortus equi to horse; S. abortus ovis to sheep and S. gallinarum to poultry. Host specific Salmonellae cause severe septicaemic form of illness. Non-host specific serotypes may be:

1. Commonly occurring and widely distributed among divergent species of animals causing mild disease.

2. Rarely occurring serotypes.

S. typhimurium is an exception and being ubiquitous in distribution produces severe illness in a variety of animal species.

Cattle.

Pigs and fowls were considered as major sources of Salmonellae for human infections than cattle (Wilson and Miles, 1967). But recent works have revealed the significant role of cattle in spreading Salmonella infection. Meat and egg products play a major role in food borne Salmonellosis. But not much effort to adopt standard hygienic measures has been made in the case of meat animals with regard to transport to slaughter houses, methods of slaughter and handling of meat and other products within abattoir and outside. The seriousness of the problem has been brought to light by several investigators through their work on isolation of Salmonellae in abattoirs, butcher shops and it's environments.

Salmonella dublin is found to be the major serotype responsible for paratyphoid infection in cattle. This host-adapted Salmonella has got wide distribution and poses a major problem to cattle owners in many parts of the world. S. typhimurium stands second in the incidence of Salmonellosis in cattle. In places where S. dublin is not prevalent, S. typhimurium is the prominent serotype concerned with bovine Salmonellosis. A wide variety of other Salmonellae may also be incriminated with low incidence (Gibson, 1965). Rothenbacher (1965) recorded 23.6 per cent mortality caused by Salmonellosis among 663 calves during a period of 20 months on 26 farms in Michigan. The average age of calves at death was 13.7 days. Forty-eight isolates were recovered. Of these, 44 were S. typhimurium and 4 S. newport. Jayaraman and John (1969) recovered 29 strains of Salmonellae from 391 samples originated from different species of livestock in Tamil Nadu. Of these 29 strains, one was from a calf and two from bovine abattoir drain sample. Randawa and Kalra (1970) isolated S. anatum (4 strains), S. dublin (1), S. weltevreden (1), S. virginia (1) and non-motile strain (1) belonging to E group from 8 of 100 carcasses examined in abattoirs at Amristar, Hissar and Patiala. Sojka and Field (1970) observed that 70.9 per cent of a total of 8,968 incidence of Salmonellosis in cattle in U.K. were due to S. dublin and 26.7 per cent due to S. typhimurium. For the remaining 2.4 per cent, 55 other

serotypes were met with. In Europe, S. dublin was found to be predominantly associated with abortions in cattle (Hinton, 1971). Thirteen other serotypes were also encountered in association with abortions in bovines. McCaughey et al. (1971) made a survey on Salmonellosis in cattle and found 87 (24%) of 359 carcasses were positive for Salmonellae. S. dublin was isolated from 83 cases. Nagaratnam and Rathantungu (1971) examined 815 rectal swabs from cattle and recorded isolation of 82 strains of Salmonellae. The serotypes recovered were S. typhimurium (65 strains), S. enteritidis (7), S. stanley (3), S. barielly (4) and S. waycross (3). As revealed by the results of investigation in cattle, sheep and goat abattoirs in Tamil Nadu under Salmonellosis Scheme (Jayaraman, 1973) 17 (14%) of 126 drain samples and 6 (0.8%) of 715 tissue samples from cattle abattoir were found positive for Salmonella. The serotypes isolated were S. seftenberg, S. typhimurium, S. chester, S. weltevreden, S. ceyco, S. lanka, S. slaudum, S. loredney and S. dublin. Four of 105 beef samples from butcher shops were found positive to Salmonella, the strains recovered being S. bredney (3) and S. typhimurium (1). Incidence of S. dublin, S. typhimurium and S. agona in 4.3 per cent of carcasses of 720 veal calves has been reported by Nazer and Osbourne (1976).

Buffalo.

A few reports are also available on the incidence of

Salmonellosis in buffaloes in India. Priestly and Artioli (1946) reported 72.1 per cent mortality in 282 buffalo calves in military farms in India. The mortality was within 14 days of birth. Seventy of 124 calves yielded S. typhimurium. S. dublin could be recovered from six calves above one month of age. Khera and Dhanda (1958) recovered S. dublin, S. weltevreden, S. enteritidis, S. richmond, S. newport, S. chester, S. butantan and S. hvittingfoss from buffalo calves which were suffering from gastro-enteritis and dying within three months of age. Mortality was more in young calves. During the last 10 to 15 years several workers have reported numerous serotypes from apparently healthy and diseased buffalo calves and zebu calves in India (Sharma and Singh, 1961; Sharma and Singh, 1963; Prasad and Ahmed, 1965; Gupta and Mataney, 1968 and Sodhi and Singh, 1970).

Pig.

Pigs are very susceptible to Salmonella infection. S. cholerae-suis and S. typhimurium are mainly responsible for clinical Salmonellosis in pigs. However, many apparently healthy pigs harbour Salmonella and excrete through faeces without evincing any clinical manifestation. Localization of the organism occurs in lymphnodes of young animals. There is no dearth of information to prove that apparently healthy pigs are the biggest reservoir of Salmonella infection. A

wide variety of Salmonellae have been isolated. Mesenteric lymphnodes have been found as their prediliction site. These subclinical infections are dangerous because such animals act as carriers promoting perpetuation of infection to other healthy animals and the man alike.

S. typhisuis was described by Glasser (1909). This host adapted serotype which possesses greater potential for primary pathogenicity produces chronic syndrome in pigs (Branes and Bergeland, 1968). S. typhimurium has been frequently isolated from healthy slaughtered pigs (Galton et al. 1954; Vanhoof, 1966; and Chung and Froster, 1969) and also from ailing animals (Heard et al. 1965 and Bruner, 1973). This non-host specific serotype produces either acute or chronic disease (Heard and Linton 1965; Hoorens and Thoonen, 1968). Pateraki et al. (1968) recorded the prevalence of S. paratyphi B, S. typhimurium, S. gatuni, S. saintpaul, S. anatum and S. westerstade in Athens and Greece. Mathisen (1968) noted that S. typhimurium was the most common isolate in Norway during the period from 1964 to 1967. Forty-six per cent of Salmonella cultures typed during 1950 to 1971 in Cornell University were S. typhimurium (Bruner, 1973). Report of losses of pigs because of infection by S. dublin, S. paratyphi B and S. paratyphi C has also been recorded (Field, 1959; Joubert and Francoy, 1963 and Micozzi, 1963).

Khera (1962) consolidating the reports of Salmonellosis in divergent species of animals and birds in India, observed incidence of 52 serotypes of which S. dublin and S. typhimurium were more prevalent. S. brindaban, a new serotype was isolated from apparently healthy pigs (Makholia and Singh, 1963). Dutta and Singh (1964) reported the isolation of S. gokul from pigs. S. cholerae-suis var. kunzendorf was isolated from pigs by Krishnamurthy and Kausik (1964). Nath et al. (1970) detailed the Salmonella serotypes isolated from pigs and identified during the period from 1965 to 1969 at National Salmonella and Escherichia Centre, Kasauli. The common serotypes were found to be S. anatum, S. cholerae-suis, S. dublin, S. enteritidis, S. kentucky, S. newport, S. paratyphi B, S. stanley, S. virginia and S. weltevreden.

Salmonella stanley, S. cholerae-suis var. kunzendorf and S. enteritidis were isolated from pigs in Uttar Pradesh by Goel and Malik (1971). Bhatia and Patak (1971) succeeded in isolating S. cholerae-suis var. kunzendorf, S. colombo, S. paratyphi B and S. hvittingfoss from pigs with enteritis and hyperpyrexia and S. ohio from clinically healthy pigs. Sulochana et al. (1973) isolated S. weltevreden from ailing pigs in Pig Breeding Farm, Mannuthy, Kerala. Sashidhar (1974) recovered S. typhimurium var. copenhagen, S. virchow, S. cholerae-suis var. kunzendorf, S. dublin and S. enteritidis from pigs in Bangalore.

Investigation by Balakrishna Pillai (1975) on bacterial aetiology of gastro-enteritis in pigs culminated in the recovery of S. typhimurium and S. weltevreden from pigs suffering from necrotic enteritis. Singh and Kaura (1976) isolated Salmonella from 35 (8.75%) of 400 rectal swabs of healthy pigs around Hissar. The serotypes isolated were S. anatum, S. kentucky, S. virginia and S. weltevreden. Of these, S. kentucky and S. virginia were recovered for the first time from pigs in India.

Sheep and goat.

Salmonella abortus ovis is host specific serotype causing abortions and gastro-enteritis in sheep in many parts of the world. S. typhimurium, S. dublin, S. reading have also been found associated with abortions in sheep. S. dublin may cause abortion and enteritis in both sheep and goat (Gibson, 1957 and Watson, 1960). S. typhimurium, S. dublin, S. orienburg and S. java have been found responsible to cause Salmonellosis in lambs. Jayaraman and John (1969) isolated four strains of Salmonella from sheep in Tamil Nadu. The serotypes were S. newport, S. barielly and S. virchow. One of the sheep harboured both S. newport and S. barielly indicating the possibility of multiple serotype infection. Fourteen (1.4%) of 1010 ovine tissue samples yielded Salmonellae. The serotypes isolated were S. typhimurium (6), S. seftenberg (4), S. newport (2), S. barielly (1) and S. enteritidis (1).

Nagaratnam and Rathantungu (1971) isolated Salmonella from 16 of 305 rectal swabs collected from goats. Of the 16 cultures, seven were S. typhimurium, eight S. enteritidis and one S.stanley. The investigation of goat and sheep abattoirs (Jayaraman, 1973) resulted in the recovery of S. uchanga (2), S. virchew (2) and S. reading (2) from 145 drain samples examined. The survey (Jayaraman, 1973) in goats used for Rinderpest goat tissue vaccine at Ranipet culminated in the recovery of 92 (15.1%) strains out of 610 mesenteric lymphnodes examined. Majority (58 out of 92) were S. typhimurium. Of the 520 samples of intestinal contents cultured, 39 (7.5%) yielded Salmonellae. Here again S. typhimurium (24) outnumbered the rest nine serotypes. Five per cent (5 out of 95) of liver samples, 1.8 per cent (7 of 385) spleen samples and 1.7 per cent (1 of 60) of bile samples were positive for Salmonellae on culturing. The infection of hospital staff and the patients in a hospital due to S. weltevreden was traced to goat meat (Chitkara and Gull, 1976).

Contributory factors.

Besides the agent Salmonellae, other factors such as exhaustion and stress due to long journey, malnutrition, unhygienic conditions etc., contribute to the upsurge of Salmonellosis (Gibson, 1965 and Steenkamer, 1966). Field (1948) described the classical symptoms of Salmonellosis in cattle due to S.dublin

infection. Pregnant cows may usually abort. S. dublin can be isolated from faeces and possibly also from milk during the active phase of the disease. Recovered animal invariably become constant and life long excretors of Salmonellae. Similarly subclinical cases may either become intermittant or constant excretors (Gibson, 1961). Most of the outbreaks of Salmonellosis in cattle occur due to contact with infected cattle. In cases of abortions, foetal materials and placenta serve as important source of infection. The cattle may acquire infection on their house premises, in transit, in markets, in collecting centres or lorries (Gibson, 1965). Richardson and Watson (1971) investigated factors facilitating flare up of S. dublin infection in 223 affected farms in England and reported that abortions or dysentery was found more prevalent in farms where loose housing system was practiced. Probably, cattle in such herds are more constantly exposed to infection than those in herds housed in byers. Moreover, rectal swabbing will not detect permanent from transient excretors in loose housed herds.

Human sewage has been reported to cause contamination of drinking water and pasture (Mortan, 1962). Jack and Hepper (1969) found that cattle became infected with S. typhimurium from grazing pastures irrigated with cattle slurry.

Ghosh (1972) noted that distribution of symptomless

carriers from pig breeding centres resulted with transmission of infection to rearing units. Symptomless excretors mainly sows and boars were responsible for the spread of disease.

Starvation, dehydration, over-crowding, change of weather, transportation and other stress factors possibly changed the infected but non-excretors into excretors (Williams and Newell, 1967).

Calaprice (1959) demonstrated S. abortus ovis in semen of two rams and observed abortion in ewes mated by these infected rams. He concluded that male is the main source of infection and active transmission occurs during coitus. Robinson (1967) demonstrated that sheep picked up Salmonella infection within 24 hours from contaminated yards and Salmonellae were present in nasal and oral swabs. Mortality due to Salmonellosis has occurred following routine dipping for ectoparasite control. Robinson and Royal (1971) are of opinion that post-dipping-pneumonia may well represent undiagnosed Salmonellosis.

Feeding stuffs and organic manures have been shown to contain a wide range of serotypes. Harvey and Price (1962) reported that 56 of 57 samples of crushed bones imported from India contained Salmonellae and upto 17 serotypes were isolated from them. Lee et al. (1972) carried out studies on Salmonella infection in pig farms and in abattoirs and concluded that infection detected at the time of slaughter originated in the farm

where fish meal introduced and maintained the infection. In a second farm where feedwas given in the form of ready made pellets, there was lower isolation rate at slaughter. Ghosh (1972) also reported that heat treated pellets fed to pigs prevented the introduction of Salmonellae.

Rats have been reported to carry Salmonella (Kerrin, 1928; Bartram et al. 1940; Ghosal, 1941 and Brown and Parker, 1957) and they have been held responsible for the transmission of infection to man and animals. Pigeons, house sparrows, crows and several other birds have been described to be carriers of Salmonella (Kaura and Singh, 1968 and Sambyal and Sharma, 1972). But their role in the transmission of Salmonella infection particularly S. dublin to domestic animals is not clearly known. In general, the role of birds in the transmission of infection seemed to be unimportant since the infection rate depended on infected environment and these organisms did not represent normal flora of the gut of birds (Kaura and Singh, 1968).

Natural sources of Salmonella are furnished in table 1.

MATERIALS AND METHODS

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Source of materials

The various biosamples employed for this work originated from divergent species of livestock belonging to Livestock Farm, Pig Breeding Farm, Goat Farm and Equine Unit under the Kerala Agricultural University, Mannuthy, besides the specimens collected from Municipal Slaughter House, Trichur and butcher shops at Mannuthy. The specimens from rodents (Bandicoots) abounding in and around the Pig Breeding Farm were also collected for cultural screening. Drainage samples collected by way of swabs kept immersed in the drains for one to two hours were also subjected to cultural screening. The specimens from animals included from those suffering from enteritis and also from apparently healthy stock.

Nature of materials

The different types of specimens subjected to cultural screening against Salmonellosis comprised of rectal swabs, intestinal contents, pieces of intestine taken from different locations, mesenteric lymphnodes, liver, bile and lung. These materials were available from ailing animals, died or slaughtered and apparently healthy animals, as the case may be. A total of 823 biomaterials (cattle 221; buffaloe 65; goat 242; sheep 75; pig 210; horse 4 and rodents 6) from 453 animals (cattle 109; buffaloe 45; goat 117; sheep 45; pig 127; horse 4 and rodents 6), besides 50 drain samples were collected and

examined for the presence of Salmonellae. The details of number of animals screened and nature and number of specimens processed under different categories have been furnished in tables 2 and 3 respectively.

Media employed

Brilliant green agar (Kristensen et al. 1925).
Carbohydrate fermentation media.
Citrate medium (Simmon, 1926).
Composite media I and II (Chitin et al. 1972).
Decarboxylase media (Falkow, 1958).
McConkey's lactose bilesalt agar (McConkey, 1905).
McConkey medium - modified (Sharma, 1961).
Malonate medium (Leifson, 1933).
Nitrate reduction test medium.
Nutrient agar.
Nutrient broth.
Selenite broth (Leifson, 1936).
Tetrathionate broth (Muller, 1923).
Triple sugar iron agar (Sulkin and Willett, 1940).
Urease medium (Christensen, 1944).

Isolation technique

Attempts for the isolation of Salmonella were made mainly following the lines advocated by Edwards and Ewing (1972).

Various tissue samples collected with aseptic precautions, to the extent possible, were given one or two momentary dippings in methyl alcohol and flammed in order to minimise excessive extraneous surface contamination, if occurred per chance and subsequently triturated in sterile normal saline to get an approximately 10 per cent homogenate. These tissue suspensions, intestinal contents and rectal swabs were directly and separately streaked on to McConkey's agar medium and Brilliant green agar medium to which 1 per cent sodium citrate had been incorporated to suppress swarming Proteus. These plates were incubated for 24-48 hours at 37°C for the development of colonies.

Duplicate samples of all the materials were seeded in enrichment media, Tetrathionate broth and Selenite broth in the proportion of approximately two in 10 parts of media to facilitate the growth of Salmonella. The seeded Tetrathionate medium was incubated at 37°C and Selenite broth at 41°C for 24 hours with occasional shaking of the tube. From these media after 24 hours of incubation one loopful was used for plating out in McConkey's agar and Brilliant green agar and these plates were incubated for 24-48 hours and examined for the development of non-lactose fermenter colonies which could be Salmonellae appearing in McConkey's agar as colourless round colonies with a raised centre and in Brilliant green agar as pink colonies. From these plates, colonies suspected

to be Salmonellae by the colony characters were first stabbed into the Triple sugar iron agar medium to the bottom of the butt and then streaked the slant. The tubes of Triple sugar iron agar medium which had been inoculated with suspected colonies from primary plates were incubated over night at 37°C. Next day the reactions were observed. The tubes which revealed yellow butt due to acid formation and pink slant due to the development of alkalinity with or without production of hydrogen sulphide and gas were retained discarding other tubes exhibiting non-characteristic reactions. In order to eliminate the possible presence of Proteus which will exhibit typical reactions as of Salmonella in Triple sugar iron agar giving false positivity, modified McConkey's agar (Sharma, 1961) which contained mannitol instead of lactose was streaked with suspected colonies. In this medium Salmonella develop as pink colonies due to the fermentation of mannitol producing acid whereas Proteus fails to do so.

Composite media I and II (Chitin[15] et al. 1972) were also employed to study the glucose and lactose fermentation, hydrogen sulphide production and phenyl pyruvic acid formation in part I and fermentation of mannitol and sucrose, motility and indol production in part II. Identification of the isolates was made based on cultural and other biochemical reactions as described by Edwards and Ewing (1972). The results are furnished in table 4.

Ten of the cultures which had been identified as Salmonella on the basis of biochemical reactions were referred to the National Centre for Salmonella and Escherichia, Kasauli and six to Dr. V.K. Sharma, Haryana Agricultural University to elucidate their serotype identity (Table 5).

Pathogenicity Studies

One strain isolated from a pig which had suffered from necrotic enteritis and identified as S. typhimurium was employed to study its pathogenicity in heterologous and homologous species of animals which included mice, guinea pigs, rabbits, calves and piglets.

Mice.

Four Swiss albino mice of one month age were utilised for this experiment. Saline suspension of Salmonella from 18 hour old growth on nutrient agar was used for infection. The density of culture suspension was adjusted to five to six of Brown's opacity tube so as to contain 10^3 Salmonellae per ml.

The mice were grouped into two, each group comprising of two animals. The first group received 0.2 ml culture suspension intraperitoneally and the second 0.2 ml subcutaneously. The animals were observed for the evidence of illness. The mice which succumbed at various intervals were examined in

detail for pathological changes. The divergent tissue samples were cultured for the recovery of the organisms. The details of experiment have been furnished in table 6.

Guinea pigs.

Four clinically healthy guinea pigs which were under observation for illness were chosen for the experiment. They were divided into two groups, each comprising of two animals. One ml. of saline suspension of Salmonella typhimurium was injected intraperitoneally into the animals of first group. The second group was inoculated subcutaneously with one ml. of the same culture suspension. The animals were watched for the development of disease symptoms. The animals died at various intervals were examined in detail. The recovery of the organism from tissue materials was attempted. The details are furnished in table 6.

Rabbits.

Four adult rabbits were divided into two groups. The first group received intraperitoneally one ml. of culture suspension prepared as described above. The animals of other group were inoculated subcutaneously one ml. of the same culture. These were under observation for the evidence of illness. The animals died or slaughtered at various intervals of observation were examined for pathological changes in the internal organs.

The tissues collected at post-mortem were cultured in appropriate media for the recovery of Salmonella. The details are tabulated in table 6.

Piglets.

Four healthy piglets of one to one and half months which were found negative for Salmonella by culturing their rectal swabs were chosen for the experiment. The animals were kept under close observation for one week prior to the administration of Salmonella culture. Rectal temperature was recorded daily and rectal swabs screened to confirm that they were not Salmonella excretors. At the end of observation period the animals were fed one-third of normal quantity of ration daily to induce stress due to undernourishment. After the observation period, three animals were administered per os 30 ml of 18 hour broth culture of S. typhimurium whose concentration in culture had been adjusted to contain approximately 10^6 - 10^8 organisms per ml. A plastic bottle with a nozzle 15 cm long was used for oral dosing to ensure the deposition of the culture at the pharyngeal region. The fourth animal was kept as control and housed separately.

The animals were closely observed for the development of clinical syndrome. Rectal temperature was recorded in the morning and evening till they were sacrificed. Rectal swabs were taken daily and examined for Salmonella. The blood was harvested

for five consecutive days after the administration of the culture from anterior venacava and cultured in dextrose broth, besides simultaneously subjecting it to wet mount examination under phase contrast microscopy for the presence of organisms in the blood in order to detect septicaemic phase of infection. The animals were sacrificed on 5th, 10th and 15th day post-infection. The intestinal contents, pieces of colon and caecum, mesenteric lymphnodes (pooled and ground), liver, bile, spleen and lung were all cultured for the recovery of Salmonella. The pathological changes and lesions were recorded. Histopathological studies on liver, kidney and spleen were also made. The details of the experiment are presented in table 7.

Calves.

Four calves aged two to three months were employed for the experiment. The general procedure was the same as adopted in the case of piglets excepting that the dose of culture was 50 ml of 18 hour old broth culture. The blood was collected from jugular vein for culturing and wet mount examination. The details have been furnished in table 7.

R E S U L T S

RESULTS

Cattle.

A total number of 221 biosamples collected from 109 animals of different age groups - 35 young animals and 74 adults - were processed for the isolation of Salmonellae. The adults were without any clinical syndrome of Salmonellosis while nine of 35 calves screened had enteritis and diarrhoea. Rectal swabs/faecal samples from 74 adult cattle which appeared apparently healthy were culturally screened. Two of 74 adults yielded Salmonella cultures. Attempt to isolate Salmonella from calves was found to be more productive especially from those suffering from enteritis and diarrhoea. Salmonella could be recovered from rectal swabs taken from two of nine calves having enteritis and from two of 26 apparently healthy calves. Fifty-two mesenteric lymphnodes, 20 liver samples, 15 bile samples and 25 beef samples were employed for cultural isolation of Salmonella. This venture culminated in the recovery of two strains from mesenteric lymphnodes while the beef, liver and bile samples were found negative.

Six drainage samples from milch cow shed and 14 samples from drains of the bovine abattoir were also subjected to cultural screening. None of the former and three of the latter revealed growth of Salmonella.

Buffaloes.

Cultural examination of rectal swabs from 15 healthy animals did not reveal any evidence of Salmonella infection. Mesenteric lymphnodes, 20 in number and 30 samples of intestinal contents collected from 30 animals slaughtered at Municipal Abattoir, Trichur were culturally processed. Of these, intestinal content and mesenteric lymphnode of one and the same animal yielded Salmonella in culture.

Goats.

A total of 262 biosamples originating from 117 animals were subjected to cultural screening for Salmonella. The rectal swabs and faecal pellets from 10 adult goats and 26 apparently healthy kids and 14 samples of diarrhoeic faecal material from kids suffering from enteritis were cultured. Isolation of Salmonella was not successful in the case of adults while one of the healthy kids yielded positive result. Culturing of faecal materials from kids with enteritis resulted in isolation of Salmonella in three instances. Mesenteric lymphnodes, liver and lung samples from 10 kids whose rectal swabs had been screened previously and which died of enteritis were also used for cultural isolation. Two kids whose rectal swabs had been found positive for Salmonella yielded cultures from mesenteric lymphnodes also. The liver and lung were found negative.

Twelve goats employed for the experimental studies on pathogenicity of bacterial isolates from pneumonic cases of goats, on sacrifice at the termination of experiment in the Department of Microbiology were also screened for Salmonella. Both the intestinal contents and the mesenteric lymphnodes were used for cultural processing. Of the 12, two goats yielded Salmonella from intestinal contents and another goat from mesenteric lymphnode alone.

Fifty-five samples of intestinal contents and 33 mesenteric lymphnodes from 55 adults collected from Slaughter House, Trichur were subjected to cultural screening. Of these, mesenteric lymphnodes of one animal was found positive yielding Salmonella. Thirty liver samples and ten lung samples which were cultured failed to yield any organism. Detailed bacteriological examination of 20 mutton samples also proved to be negative. Two of the 20 drain samples collected from abattoir also yielded Salmonella in culture.

Sheep.

Forty-five sheep, all adults and apparently healthy, were screened to detect the prevalence of Salmonella. Although 45 samples of intestinal contents and 30 samples of mesenteric lymphnodes were cultured, none of them revealed the presence of Salmonella.

Pigs.

A total of 220 specimens comprising of 10 drain samples and 210 biomaterials from 127 animals were subjected to cultural screening. The rectal swabs from apparently healthy animals of different age groups and ailing animals which were having enteritis and diarrhoea at the time of collection were used for screening. A total of 20 strains of Salmonella could be recovered from 127 rectal swabs. This included five from 54 apparently healthy piglets, one from 21 healthy adults, seven from 24 piglets which were clinically ill at the time of collection, five from 14 piglets died of enteritis and two from 14 adults died of enteric disorders. Twenty-eight samples of mesenteric lymphnodes, 20 liver, 15 lung and 20 bile samples collected from 28 animals died of necrotic enteritis as confirmed on post-mortem examination, besides lung abscess in the case of one animal were subjected to cultural screening. Three animals yielded Salmonella from mesenteric lymphnodes. The animal which had revealed lung abscess and pneumonia yielded Salmonella from liver, bile and lung abscess as well. Besides the above materials, 10 samples collected by way of swabs from gullies of drains were also screened. Of these, three yielded Salmonella cultures.

Horses.

The faecal materials collected from four horses maintained at the Veterinary College were proved to be negative for Salmonella.

Rodents ◦

Six bandicoots abounding in and around pig styes were trapped and their intestinal contents were cultured for Salmonella. Two of them yielded cultures which were identified to be Salmonella on the basis of cultural and biochemical characters.

Six of the 109 cattle (5.5%), one of 45 buffaloes (2.2%), 10 of 177 goats (8.5%), 20 of 127 pigs (15.7%) and two of six bandicoots (33.3%) were found to harbour Salmonella. Forty-five sheep and four horses which were subjected to cultural screening proved to be negative (Table 2).

The work carried out during this investigation resulted in the recovery of 56 Salmonella strains (11 from cattle, 2 from buffalo, 12 from goats, 29 from pigs and 2 from rodents). The details in respect of nature and number of materials processed and the percentages of isolations have been furnished in table 3.

The Salmonella isolates formed pink colonies in modified McConkey's medium which contained mannitol instead of lactose. The composite media I and II devised by Chitin et al. (1972) efficiently exhibited characteristic reactions. Part I was equally good as Triple sugar iron agar with production of acid butt, alkaline slant and hydrogen sulphide production. In part II

fermentation of mannitol formed blue ring at the top and motility could be observed as formation of 'Fan' shaped extension of growths from the stabline.

Details of cultural and biochemical characters of the Salmonella isolates are presented in table 4.

The serotype identity of 10 cultures from pigs have been elucidated (Courtesy: National Salmonella and Escherichia Centre, Kasauli). Of these 10 cultures, six have been identified as S. weltevreden, three as S. typhimurium and one as S. urbana. Six cultures recovered from other species of animals have been serologically typed by Dr. V.K. Sharma, Haryana Agricultural University, Hissar. Five of them belonged to S. typhimurium and one to S. weltevreden.

The particulars in respect of cultures whose serotype identity has been elucidated are furnished in table 5.

Studies on Pathogenicity of Salmonella typhimurium

Mice .

All the infected mice became dull and inactive following infection after a few hours. The mice which were inoculated with S. typhimurium by intra-peritoneal route succumbed to infection within 24 hours. They did not reveal any lesion except for congestive changes in internal organs. One of the animals

which received the culture by subcutaneous route died on the third day and the other on fourth day post-infection. In these animals lungs were found pneumonic and the liver exhibited necrotic foci. Salmonella could be recovered from liver, spleen, lungs and heartblood of all the four animals.

Guinea pigs.

Guinea pigs which had received culture of S. typhimurium intra-peritoneally died next day. No gross lesions in the visceral organs were discernible except for the congestive changes. The ones which were infected by subcutaneous route died one on the 5th day and the other on the 6th day following infection. There was swelling and thickneing at the site of injection surrounded by hyperaemic zone. The lungs revealed patchy congestive areas and the liver showed necrotic foci. Salmonella was isolated from heart blood, liver, spleen and lung.

Rabbits.

One of the rabbits which received the culture by intra-peritoneal route died next day. Salmonella was recovered from liver, spleen, lung and heart blood. The other animal became gravely ill and was about to collapse. But next day onwards it evinced improvement and started to take feed. This particular animal was sacrificed on fourth day post-infection.

Pulmonary oedema and enlargement of spleen were evident. However, cultural findings were negative for Salmonella. The animals which were inoculated by subcutaneous route failed to develop any clinical manifestation except for the fact that they were dull and inactive for the first two days. Later on they returned to normal state of alertness and activity. They were autopsied on 7th day. No gross lesions were discernible except that the gall bladder contained excessive quantity of thick viscid bile. Neverthless, liver and spleen yielded Salmonella cultures.

Piglets -

The first two days were uneventful and after that the infected animals evinced rise of temperature to 104-105°C for two days. They were dull and consumed less quantity of feed. Subsequently temperature came down. Only one animal developed mild diarrhoea for three days following temperature rise. The blood samples collected from anterior venacava were found negative for Salmonella while the faeces was consistently positive on all days after the administration of culture. The animal that developed diarrhoea was sacrificed on 5th day. Internal organs did not reveal any significant gross lesion except mild catarrhal inflammation of the intestine. However, isolation of Salmonella could be made from faeces and mesenteric lymph-node. The other two animals did not develop diarrhoea. Though

the animals were feeding normally, their condition ran down and became emaciated. These animals were sacrificed on 10th and 15th day after the administration of the culture. The body cavities revealed abnormal quantities of fluid. The intestinal wall had numerous greyish white nodular necrotic areas (Fig. 1) predominently and preponderatingly at the region of colon. Histopathological examination of the internal organs did not reveal much significant lesions except that mesenteric lymphnodes and spleen evidenced congestive changes. However, liver, bile and spleen and faeces on culturing were found positive for Salmonella. The control animal did not show any evidence of illness throughout the period of experiment. The examination of tissues also proved to be negative for Salmonella infection.

Calves.

Two days following the administration of culture a temperature rise of 103-104°C was observed. Two calves developed diarrhoea which persisted for two days followed by recovery from diarrhoea. One calf became evidently ill that it was unable to bear its weight. There was no diarrhoea. It died on the 3rd day. On post-mortem examination all the internal organs were found highly congested. There were petichieae and ecchymosis in visceral organs. The lungs were highly oedematous, the alveoli and bronchi contained frothy fluid. The

other two calves which had diarrhoea and had subsequently recovered were autopsied on the 10th day. The pleural and peritoneal cavities and pericardial sac had excessive accummulation of yellowish fluid. The mesenteric lymphnodes appeared enlarged. No other gross lesions could be discerned. Salmonella was recovered from bile, liver, mesenteric lymphnodes and faeces. The control animal did not show any evidence of infection when examined on the 10th day of the experiment.

Histopathological examination of various internal organs of the calves revealed the changes as given below:

Liver	:	Sinusoidal engorgement, interlobular oedema and mononuclear and lymphoid infiltration in portal areas (Fig. 2).
Kidney	:	Focal areas of mild tubular degeneration.
Spleen	:	Did not reveal any change.
Mesenteric lymphnode	:	Engorgement of capillaries in the medulla and depletion of lymphoid nodules (Fig.3).

The details of experimental infection studies have been furnished in tables 6 and 7.

DISCUSSION

DISCUSSION

Scrutiny of the results of this survey suggests that screening of sewer swabs seems to be more productive in yielding Salmonella. The percentages of isolation from sewer swabs collected from drains of pig styes and abattoir for Capridae and Bovidae tends to be always higher than the percentages of isolation from tissues. As can be seen in table 3 the percentages of isolation from drain samples are 15, 10 and 30 from bovine abattoir, caprine abattoir and pig styes respectively. The rate of isolation from tissue samples under all categories are found less. Jayaraman (1973) also recorded higher percentage of isolation from drain samples when compared to tissue samples. The spectrum of serotype of Salmonella recovered from drains can be expected to be wider than from tissue specimens collected from abattoirs. Greater the rate of isolation, greater the spectrum of serotypes. This wider spectrum makes it possible to get serotypes which otherwise would have been over looked by culturing tissue materials alone. Hence for epidemiological and ecological studies, screening of sewer, floor and environment of abattoirs, butcher shops and other establishments carrying out food processing appears to be more important and rewarding.

Among various tissue materials employed for screening, mesenteric lymphnodes are more preferable to examine for the presence of Salmonella in deeper tissues (Smith, 1959; Guinee et al. 1964). Liver, spleen and bile are less productive in yielding Salmonella.

Jayaraman (1973) reported that lymphnodes had yielded higher isolation rates than liver and spleen in the case of goats. The isolation rates from mesenteric lymphnodes from all species screened in this survey are found higher than from liver, lung and other tissues. But mesenteric lymphnodes are inferior to rectal swabs/faecal materials in yielding Salmonella, because the rate of isolation is found greater in the case of the latter (Table 3). Hence it can be inferred that the animal whose rectal swab/faecal material is positive for Salmonella need not necessarily harbour the organisms in mesenteric lymph-nodes.

Stress factors like prolonged journey, over crowding, inter-current infections etc., help in the build up of infection. (Gibson, 1965 and Steenkamer, 1966). The isolation rate of Salmonella from mesenteric lymphnodes of goats used for Rinder-pest goat tissue vaccine was found to be 15.1 per cent (Jaya-raman, 1973). In the present study attempt to isolate Salmonella from goats which had been employed for studies on pathogenicity of bacterial isolates from pneumonic cases was successful in recovering organism from three of 12 goats making the isolation rate of 25 per cent. This higher percentage of isolation can be attributed to stress induced on them due to other infections intentionally inflicted because other pen mates were found negative when their faecal materials were cultured. The dormant or quiescent infection which would have been confined to deeper

tissues like the mesenteric lymphnodes, the predilliction site when carrying chronic infection, might have been triggered by the stress factor induced by other infecticns, thereby making the animals excretors or shedders of the organisms and giving higher percentage of isolation when such animals are subjected to screening. Moreover, in this group of animals Salmonella was recoverable from intestinal contents of two animals while the third one yielded the growth only from mesenteric lymph-nodes. This fact points to the possibility of an animal being declared negative for Salmonella eventhough actually it harbours the same organism in deep seated tissues like the mesenteric lymphnodes.

Salmonella weltevreden, S. typhimurium and S. urbana have been isolated from pigs during the course of this survey. First two of these serotypes have been incriminated in many outbreaks of Salmonellosis in India and abroad. S. typhimurium has been reported associated with clinical disease in pigs (Heard and Linton, 1965; Hooran and Thoonan, 1968 and Balakrishna Pillai, 1975). This serotype has been isolated by Sasidhar (1974) from pigs in and around Bangalore. Jayaraman and John (1969) have reported that S. weltevreden has a wide range of hosts. Both these serotypes are very important and potent pathogens afflicting human beings next to S. typhi and S. paratyphi A (Nath et al. 1970). S. weltevreden has been found to cause heavy

mortality in laboratory animals like guinea pigs (Jayaraman et al. 1964). S. typhimurium which is a non-host specific and ubiquitous serotype attacks a wide variety of animal hosts and man causing much morbidity and mortality in infected population. In pigs it is the most important pathogen next to S. cholerae-suis.

The two serotypes isolated - S. weltevreden and S. typhimurium - have been reported from various sources by several workers (Dutta and Singh, 1961; Jayaraman et al. 1964; Sadidhar, 1968; Jayaraman and John, 1969; Nath et al. 1970; Randhawa and Kalra, 1970; Chitkara and Gull, 1976 and Singh and Kaura, 1976). A few reports on isolation of S. typhimurium and S. weltevreden from pigs in Kerala are also available (Sulochana et al. 1973 and Balakrishna Pillai, 1975). These serotypes occur in other species of animals too. In cattle, next to S. dublin, S. typhimurium is most important serotype causing paratyphoid infection. Goat and sheep are no exception to infection by S. typhimurium and S. weltevreden both of which enjoy wide host range.

Among the divergent species of animals subjected to screening during the course of present study, swine as a species stands first in yielding greater percentage (15.7) of Salmonella followed by Caprine (8.5), Bovine (5.5) and Bubaline (2.2). The Ovines have failed to yield Salmonella probably due to the combined

effect of two factors - lesser number of materials processed and the lower prevalence rate in sheep. It is worthwhile to note that in a more detailed study conducted in Tamil Nadu only 14 of 1010 (1.4%) ovine tissues specimens were found to be positive (Jayaraman, 1973).

Isolation from piglets which had enteritis and diarrhoea was found to be higher (35.3%). This probably points to the possible association of Salmonella in clinical outbreaks of enteritis either singly or in association with other entero-pathogens. The prevalence rate has been found higher in young population and that too among animals in which enteritis and diarrhoea were major clinical symptoms. This fact suggests that young ones are more prone to infection by Salmonella.

Isolation of Salmonella from apparently healthy animals though prevalence rate is much lower than in ailing animals explains how animals can act as carriers facilitating perpetuation of infection to other non-infected animals and man alike and cross contamination of wholesome meat and meat products during different processing procedures starting from raw meat at slaughtering centre to the finished ready-to-sell products at the other end of processing plants and food packing units. The significance of the presence of this organism in meat and meat products needs no over-emphasis. They provide sources for extensive contamination of other clean healthy

products either directly or indirectly. This explains the explosive and extensive outbreaks of food poisoning in man under conditions which favour the multiplication of the organisms in the animal products. Reports of food poisoning due to consumption of meat and meat products are numerous. Taylor (1967) has reported food poisoning cases due to contaminated meat dishes containing beef and pork. S. weltevreden has been incriminated in the outbreak of Salmonellosis in the staff and patients admitted to a hospital, the source of infection being traced to infective goat meat and chicken meat (Chitkara and Gull, 1976).

Salmonella urbana isolated from pig during the course of this investigation appears to be a rare serotype prevalent in India. Frequency of its isolation is very low as per the available literature. It has no place in the list of "Salmonella Serotypes in India" (July 1965 to December 1969) as given by Nath et al. (1970).

Rodents have got a major role in the epidemiology of Salmonellosis. These animals have close association with and accessibility to animal sheds and human dwellings. They, being natural reservoirs and excretors of Salmonella, can carry infection from population to population and place to place. Their role in the causation and spread of Salmonellosis has been studied by several workers (Goshal, 1941; Kaura and Singh, 1968

and Guinee et al. 1973). During this survey bandicoots have been found as carriers of Salmonella as evidenced by isolation from two of six bandicoots screened giving a recovery percentage of 33.3. The significance of this high percentage is questionable since the number processed is very small. But the fact that they can act as carriers has been proved irrevocably.

The use of modified McConkey medium (Sharma, 1961) which contains mannitol instead of lactose has been found advantageous to differentiate Proteus from Salmonella thereby avoiding spurious results and false positivity. Composite media developed by Chitin[15] et al. (1972) was found superior to T.S.I. because it has got the additional advantage that it could be used for testing deamination of phenylalanine to phenylpyruvic acid. Hence it facilitates elimination of Proteus cultures which simulate Salmonella in reactions in T.S.I. The composite medium II also was found to yield good result to study the motility and the fermentation of mannitol. The motile organisms produced 'Fan' shaped growth in semisolid medium. The motility testing by hanging drop method may lead to false positive result (Edward and Ewing, 1972). The results of biochemical reactions obtained are in conformity with the reactions of Salmonella described by Edward and Ewing (1972).

Studies on pathogenicity of S. typhimurium in laboratory

animals such as mice, guinea pigs and rabbits showed that all the three species of hosts were susceptible to infection by S. typhimurium. Mice were found most susceptible. All of them died in one to four days post-infection. The ones which were infected intraperitoneally did not survive more than 24 hours while those which received the culture subcutaneously could survive for three to four days. The latter group exhibited pneumonic changes in lungs and necrotic foci in liver while the former group revealed high degree of congestion of internal organs without showing any other visible lesions probably due to too short a period to develop lesions. Hence the route of infection has a bearing on the time of onset of disease syndrome and the course of disease. The response of guinea pigs also to experimental infection was similar to what had been in the case of mice except that the survival time was more by one day or two. But rabbits were found a little bit refractory and failed to develop clinical symptoms when the culture was given subcutaneously. The rabbits which received the culture intraperitoneally developed the clinical symptoms and pathological changes in internal organs as confirmed on post-mortem examination. The animal died within a short span of time did not reveal any gross lesions in internal organs while those which were sacrificed at later periods had lesions in internal organs. Similar findings have been reported by Ghosh and Anina Chatterjee (1960). Salmonella was recoverable from different

internal organs. The findings on experimental infection studies in laboratory animals suggest that S. typhimurium has got definite invasive power for the tissues of the experimental hosts. Wilson and Miles (1967) had also observed that S. typhimurium possessed very definite invasive power for tissues of mice and other laboratory animals when infected parenterally.

Experimental infection in piglets produced transient hyperpyrexia and accompanied dullness and off-feed. But they came to apparently normal state of health within three days. However, pathological changes were observed in tissues on post-mortem examination and culture could be recovered from liver, bile and faeces. Hence it is reasonable to speculate that the infected pig need not always evince diarrhoea eventhough it harbours infection. This finding probably explains the fact that animals may act as symptomless carriers eventhough they habour silent infection. Attempts to recover Salmonella from the blood of infected calves during the febrile period had been unsuccessful. Hence it is inferred that infection by S. typhimurium will not always result in septicaemic form of the disease unlike S.dublin infection which cause acute septicaemic form of the disease in calves. W.H.O. report (1967) stated that infection by S. typhimurium was not so acute as in the case of S. dublin infection in calves and that it caused mostly a subclinical form of infection with lesser gravity.

S U M M A R Y

SUMMARY

Survey on the Incidence of Salmonellosis in Meat Animals was conducted.

A total of 873 samples comprising of rectal swabs, faeces, mesenteric lymphnodes, liver, bile, spleen and lung collected from divergent species of animals either apparently healthy or clinically ill or died and drainage samples were culturally screened for the prevalence of Salmonellae.

Enrichment media employed included Selenite broth and Tetrathionate broth with addition of brilliant green. Besides the common media used for routine isolation of Salmonella, modified McConkey's medium (Sharma, 1961) which contained mannitol instead of lactose was made use of. Composite media I and II (Chitin et al. 1972) were advantageously utilized to study many major characteristics.

A total of 56 strains of Salmonella were isolated from different sources. Elucidation of serotype identity of 16 cultures were made. (Courtesy - Dr. V.K. Sharma, Haryana Agricultural University, Hissar and National Centre for Salmonella, Kasauli). The serologically typed strains belonged to Salmonella typhimurium (7), S. weltevreden (8) and S. urbana (1).

The results of this study have proved the prevalence of Salmonella infection in livestock in Kerala. The preponderating

serotypes prevalent were found to be S. typhimurium and S. weltevreden. S. urbana has also been found to occur in pigs though not to the same extent as of the other two serotypes.

Better recovery rate was observed in the case of drainage samples. Among deeper tissues, mesenteric lymphnodes were found to be the material of choice for the recovery of Salmonella.

Isolation of S. typhimurium from the bandicoots points to their possible role in the epidemiology of Salmonella infection.

Pathogenicity studies have revealed that S. typhimurium is pathogenic to laboratory animals like mice, guinea pigs and rabbits. Oral feeding of S. typhimurium culture to calves and piglets resulted in mild clinical manifestation.

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Table 1. Natural sources of *Salmonella*.

Mollusks	Oysters, claws, snails, slugs.
Arthropods	Flies, fleas, cockroaches, ticks, mites, crayfish, lobsters, crabs, other crustacea.
Fish	Carp and other scavenger fish.
Reptiles	Snakes+, lizards+, tortoise.
Birds	Sparrows+, starlings, doves+, ducks, geese, swan, pheasant, turkey, peafowl, guineafowl, crow+, pigeon+.
Mammals	Rodents+, mice, squirrel+, grophers, hedgehogs, carnivores especially foxes, shunks, bears, deer, elk, moose, monkeys, man.
Pets	Sunfish, snakes, alligators, turtles, canaries, parakeets, parrots, chicks+, ducklings, pigeons+, guineapigs+, hamsters, rabbits+, cats, dogs.
Domestic animals	Swine+, cattle,+ sheep+, goats+, equine+, camel+.
Food products	Water+, ice, milk+, and its products, poultry and its products, egg+ and egg products, bakery products especially cakes and pastries containing egg, dessicated coconut, fish, meat and its products, fruits and vegetables, goat meat+
Animal feeds	Meat meal+, poultry feed+.
Others	Sewage+, abattoir drains+, etc.

+ Reported from India.

Adapted from Ayeres (1969)

Table 2. Details of number of animals under different species screened and number positive to Salmonellae.

Species	Young animals						Adult animals						Total no. screened	Total positive	% total positive
	Ailing/died			Apparently healthy			Ailing/died			Apparently healthy					
	No. screened	No. positive	% positive	No. screened	No. positive	% positive	No. screened	No. positive	% positive	No. screened	No. positive	% positive			
Cattle	9	2	22.2	26	2	7.7	0	0	0	74	2	2.7	109	6	5.5
Buffaloe	0	0	0	15	0	0	5	0	0	25	1	4	45	1	2.2
Goat	24	5	20.8	26	1	3.8	12	3	25	55	1	1.9	117	10	8.5
Sheep	0	0	0	0	0	0	0	0	0	45	0	0	45	0	0
Pig	38	12	35.3	54	5	9.2	14	2	14.3	21	1	4.7	127	20	15.7
Horse	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0
Rodent (Bandicoot)	0	0	0	0	0	0	0	0	0	6	2	33.3	6	2	33.3

Table 3. Details of nature and number of materials processed and percentage positive to Salmonellae.

Species	Nature and number of materials processed																					Total no. processed	Total no. positive	% of total positive
	Rectal swabs/ faeces			Mesenteric lymphnodes			Liver			Bile			Lung			Meat sample			Drain sample					
	Number processed	No. positive	% positive	Number processed	No. positive	% positive	Number processed	No. positive	% positive	Number processed	No. positive	% positive	Number processed	No. positive	% positive	Number processed	No. positive	% positive	Number processed	No. positive	% positive			
Cattle	109	6	5.5	52	2	3.8	20	0	0	15	0	0	0	0	0	25	0	0	20	3	15	241	11	4.6
Buffaloe	45	1	2.2	20	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	2	3
Goat	107	6	5.6	55	4	7.3	40	0	0	0	0	0	20	0	0	20	0	0	20	2	10	262	12	5.3
Sheep	45	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0	0
Pig	127	20	15.7	28	3	10.7	20	1	5	20	1	5	15	1	6.6	0	0	0	10	3	30	220	29	13.2
Horse	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Rodent (Bandicoot)	6	2	33.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	2	33.3

Table 4a. Biochemical reactions of Salmonella isolated from cattle and buffaloes.

Test/Substrate	Cultures from cattle											Cultures from buffaloe	
No.	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	B1	B2
Indol	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-
Simmon's citrate	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulphide	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenyl alanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+	+	+	-	+	+	+	-	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+

-, Negative.
+, Positive

Table 4b. Biochemical reaction of *Salmonella* isolated from goats.

Test/Substrate	Culture numbers											
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Indol	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-
Simmon's citrate	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulphide	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+	+
Phenyl alanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	-	+	+	-	+	+	+	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	+	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+

-, Negative.
+, Positive.

Table 4c. Biochemical reaction of *Salmonella* isolated from pigs.

Test/Substrate	Culture numbers																													
	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14	P 15	P 16	P 17	P 18	P 19	P 20	P 21	P 22	P 23	P 24	P 25	P 26	P 27	P 28	P 29
Indol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simmon's citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulphide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenyl alanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+	+	-	+	-	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	+	-	+	+	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

-, Negative.

Table 4d. Biochemical reaction of *Salmonella* isolated from bandicoots.

Test/Substrate	Culture numbers	
	R1	R2
Indol	-	-
Methyl red	+	+
Voges-Proskauer	-	-
Simmon's citrate	+	+
Hydrogen sulphide	+	+
Urease	-	-
Motility	+	+
Lysine decarboxylase	+	+
Phenyl alanine deaminase	-	-
Malonate	-	-
Gas from glucose	+	+
Lactose	-	-
Sucrose	-	-
Mannitol	+	+
Dulcitol	+	+
Salicin	-	-
Adonitol	-	-
Inositol	-	-
Sorbitol	-	-
Arabinose	+	+
Raffinose	-	-
Rhamnose	+	+
Nitrate	+	+

-, Negative.
+, Positive.

Table 5. Particulars of *Salmonella* isolates whose serotype identity has been elucidated.

Culture number	Source of isolation	Species of the host	Age	Symptoms/lesions	Serotype	Antigenic formula
P1	Faeces	Pig	Young	Apparently healthy	*S. typhimurium*	4,5,12:i:1,2
P3	Faeces	Pig	Young	Enteritis	*S. weltevreden*	3,10,:r:z6
P4	Faeces	Pig	Young	Enteritis	*S. weltevreden*	3,10,:r:z6
P5	Faeces	Pig	Young	Enteritis	*S. weltevreden*	3,10,:r:z6
P6	Faeces	Pig	Young	Enteritis	*S. weltevreden*	3,10,:r:z6
P7	Faeces	Pig	Young	Enteritis	*S. weltevreden*	3,10,:r:z6
P9	Mesenteric lymphnode	Pig	Young	Weak	*S. urbana*	30:b:e,n,x
P16	Mesenteric lymphnode	Pig	Adult	Enteritis	*S. weltevreden*	3,10,:r:z6
P20	Faeces	Pig	Adult	Enteritis	*S. weltevreden*	3,10,:r:z6
P24	Faeces	Pig	Young	Enteritis	*S. typhimurium*	4,5,12:i:1,2
G1	Faeces	Goat	Adult	Pneumonia	*S. typhimurium*	4,5,12:i:1,2
G2	Mesenteric lymphnode	Goat	Adult	Pneumonia	*S. typhimurium*	4,5,12:i:1,2
G9	Mesenteric lymphnode	Goat	Young	Enteritis	*S. typhimurium*	4,5,12:i:1,2
G10	Abattoir drain	-	-	-	*S. weltevreden*	3,10,:r:z6
C5	Faeces	Cattle	Young	Apparently healthy	*S. typhimurium*	4,5,12:i:1,2
R1	Faeces	Bandicoot	Adult	Healthy	*S. typhimurium*	4,5,12:i:1,2

Table 6. Details of experimental infection studies in laboratory animals.

Culture used	Type of animal and number	Route of infection	Dose	Period elapsed till death/ sacrifice	Gross lesion	Culture recovered from
Saline suspension of Salmonella typhimurium	Mice					
	1	Intraperitoneal	0.2 ml	Less than 24 hours D	Only congestive changes in internal organs	Liver, spleen, lung and heart blood
	2	,,	,,	,,	,,	,,
	3	Subcutaneous	,,	3 days D	Pneumonic areas in lungs and necrotic foci in liver	,,
	4	,,	,,	4 days D		,,
	Guinea pigs					
	1	Intraperitoneal	1 ml	1 day D	Congestion of internal organs	,,
	2	,,	,,	,,	,,	,,
	3	Subcutaneous	,,	5 days D	Patchy congested areas in lung and necrotic foci in liver	,,
	4	,,	,,	6 days D	,,	,,
	Rabbits					
	1	Intraperitoneal	,,	1 day D	Congestion of internal organs	,,
	2	,,	,,	4 days K	Pulmonary oedema and enlargement of spleen.	Negative culturally
	3	Subcutaneous	,,	7 days K	Gall bladder contained excessive amount of thick viscid bile	Liver and spleen
	4	,,	,,	,,	,,	,,

D = died; K = killed.

Table 7. Details of experimental infection studies in piglets and calves.

Culture used	Type of animal and number	Route of infection	Dose	Period elapsed till death/ sacrifice		Symptoms/lesions	Culture recovered from
18 hour old broth culture of *S. typhimurium*	Piglets						
	1	Per os	30 ml	5 days	K	Dull and inactive, temperature rise 104°C for 3 days followed by mild diarrhoea congestion of intestine.	Faeces and mesenteric lymphnodes
	2	"	30 ml	10 days	K	Temperature rise 105°C for 3 days, nodular necrotic foci in intestinal wall.	Liver, bile, spleen and faeces.
	3	"	30 ml	15 days	K	"	"
	4	Control	Control	15 days	K	Clinically healthy	Negative
	Calves						
	1	"	50 ml	3 days	D	Temperature rise 104°C, off-feed petechieae and ecchymosis in internal organs, lungs oedematous and contained frothy fluid, mesenteric lymphnodes congested.	Bile, liver, mesenteric lymphnodes and faeces.
	2	"	50 ml	10 days	K	Temperature 103°C, diarrhoea, accummulation of fluid in body cavities, enlargement of mesenteric lymphnodes.	"
	3	"	50 ml	10 days	K	"	"
	4	Control	Control	10 days	K	Clinically healthy	Negative

K = Killed; D = Died.

Fig. 1 White nodular necrotic foci on intestinal wall.

Fig. 2 Sinusoidal engorgement and mononuclear and lymphoid infiltration in portal areas.

Fig. 3 Engorgement of capillaries in the medulla and depletion of lymphoid nodules.

Fig. 1

Fig. 2

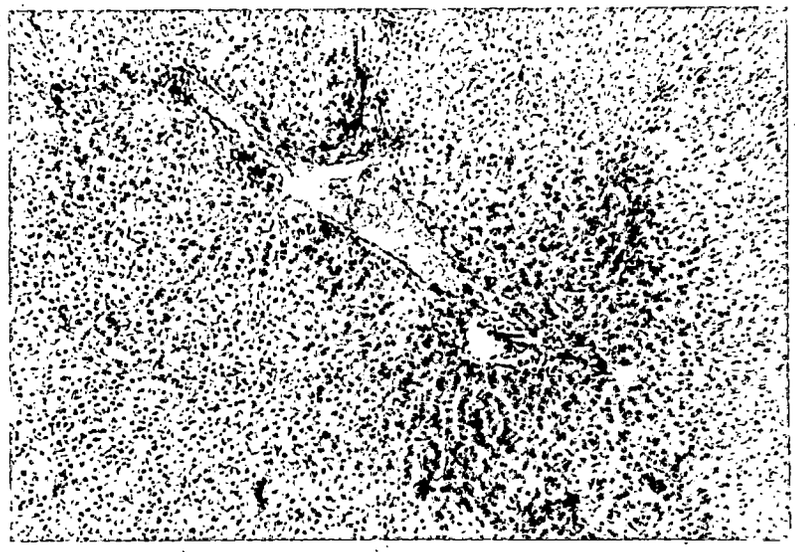

Fig. 3

SURVEY ON THE INCIDENCE OF SALMONELLAE IN MEAT ANIMALS

By

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ABSTRACT OF A THESIS

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A B S T R A C T

ABSTRACT

Prior to this "Survey on the Incidence of Salmonellae in Meat Animals" the information on the serotypes of *Salmonella* prevalent and the magnitude of their occurrence in livestock in Kerala had been meagre except for the reports by Sulochana *et al.* (1973) and Balakrishna Pillai (1975). The work carried out during the present investigation has gathered more information on the prevalence of *Salmonella* in livestock in Kerala.

In this study the prevalence of *Salmonella* serotypes in the different species of animals was probed. A total of 823 biomaterials, besides 50 drain samples were subjected to cultural screening. This venture resulted in the recovery of 56 strains of *Salmonella*. Serological identification of many of these strains proved the prevalence of *S. typhimurium*, *S. weltevreden* and *S. urbana*. The preponderatingly prevalent serotypes were found to be the former two.

Pathogenicity studies employing *S. typhimurium* culture in mice, guinea pigs, rabbits, calves and piglets were conducted. All these animals were found to be susceptible to infection by *S. typhimurium* evincing varying degrees of clinical manifestations.

The advantages of employing modified McConkey's medium

(Sharma, 1961) containing mannitol instead of lactose and composite medium I and II developed by Chitin[is] et al. (1972) to differentiate Salmonella at primary screening level have been discussed.