STUDIES ON THE INCIDENCE OF SALMONELLA AMONG THE PORCINE POPULATION IN AND AROUND BANGALORE CITY

B. Y. SHASHIDHAR
(I. C. A. R. Junior Fellow)

Department of Veterinary Microbiology & Public Health
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

1974

STUDIES ON THE INCIDENCE OF SALMONELLA AMONG THE PORCINE POPULATION IN AND AROUND BANGALORE CITY

By

B.Y. SHASHIDHAR
(I.C.A.R. Junior Fellow)

Thesis submitted to the University of Agricultural Sciences, Bangalore, in partial fulfilment of the requirements for the degree of
MASTER OF VETERINARY SCIENCE

Bangalore

August 1974

Department of Veterinary Microbiology & Public Health.
UNIVERSITY OF AGRICULTURAL SCIENCES.
Bangalore

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON THE INCIDENCE OF SALMONELLA AMONG THE PORCINE POPULATION IN AND AROUND BANGALORE CITY" submitted by Mr. B.Y. Shashidhar, B.V.Sc., for the degree of Master of Veterinary Science (Microbiology - Main) of the University of Agricultural Sciences, Bangalore, is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

(R.D. NANJIAH)
Chairman,
Advisory Committee

August 1974

APPROVED BY

Chairman

Members 1

2

3

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to Dr. R.D. Nanjiah, B.Sc., G.M.V.C., B.V.Sc., Ph.D., Director of Animal Husbandry and Veterinary Services, Government of Karnataka, Bangalore and Ex-Director of Instruction (Vety), Professor and Head of the Department of Veterinary Microbiology and Public Health, University of Agricultural Sciences, Bangalore, for his valuable guidance, constant support and encouragement throughout this work as Chairman of my Advisory Committee.

My thanks are due to Dr. C.K. Abdulla Khan, Professor of Gynaecology and Obstetrics, and Dr. S.N. Shetty, Associate Professor of Pharmacology, Veterinary College, Bangalore, for having kindly served as members of my Advisory Committee and for making useful suggestions during the course of this work.

I am grateful to Dr. B.S. Keshava Murthy, Associate Professor of Veterinary Microbiology and Public Health, for his guidance and constant support during the course of this work.

I sincerely acknowledge the valuable suggestions from the staff and from my colleagues for their kind co-operation.

I will be failing in my duty, if I do not put on record the inspirations given by the members of my family throughout this work.

I am thankful to Mr. Mohamed Ayaz Ahamed for typing this thesis.

In conclusion, I wish to express deep appreciation to the Indian Council of Agricultural Research for their generous financial support.

August 1974

B.Y. SHASHIDHAR

INTRODUCTION

CHAPTER I

INTRODUCTION

Salmonellosis in animals is a world-wide problem. Surveys conducted on the incidence of salmonella infection in various species of animals in different countries reveal that salmonellae are widely spread and many reservoirs of infection exist. Pigs along with poultry, frequently form the important animal reservoirs for these organisms.

Pigs are easily exposed to salmonella infection owing to their scavenging habit and very often, they are the source of human salmonellae in many parts of the world. It is believed that clinical salmonellosis as a disease in fully grown hogs is rare, but the condition is frequently encountered in young pigs. While primarily a disease of immature animals, it frequently appears in debilitating conditions of young and adult animals. However, in adult domesticated animals, salmonellae usually play a secondary role. They become active only when the resistance of animals is lowered by viral infection, starvation or exposure to other stresses.

Salmonellae are often isolated from the mesenteric lymph glands, liver and intestinal contents of pigs. These animals frequently harbour the same salmonella types as does man in the same area.

Animal adopted salmonellae, often change hosts and become more firmly established in species other than in

those from which they originated. Salmonella derby for instance was first prevalent in pigs, but was later found to be the causal agent of poultry salmonellosis.

It is well known that salmonellosis in animals, besides causing various disease conditions in them, constitutes the chief source of human infection. Several salmonella serotypes other than S. tyohi, which are also potent human pathogens, have been isolated from pigs in this country.

Pigs, inspite of various hygienic measures undertaken in farms, have been found to act as carriers of salmonellae even in developed countries where foods of swine origin are increasingly used for human consumption. At the abattoir too, several workers have recorded the presence of different salmonella serotypes in pigs.

The most striking feature of the genus Salmonella, is the growing number of serotypes. Serology combined with refined bacteriological techniques have been useful in understanding this complex group of organisms. In recent years, salmonella problem has been receiving due attention in this country. Several reports have been made of the isolations of various members of Salmonella from many species of domestic and other animals. There are only a few reports of such isolations from pigs in India.

Considering the importance of the problem, a survey on the incidence of Salmonella among the pig population,

in and around Bangalore city was undertaken to know the different species of Salmonella which have potentialities of becoming public health hazards, besides endangering the health of other domestic livestock.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Salmonella organisms were first isolated in 1885 by Smith and Salmon from pigs, which had died of hog cholera and the name Bacillus cholerae-suis was given to it.

During the last two decades enormous amount of work has been done to understand the epidemiology of the disease. Many surveys have been conducted to find out the various serotypes, present in particular area which are of importance to public and animal health.

ISOLATION OF SALMONELLA SEROTYPES OF ANIMAL IMPORTANCE

Ganguli (1958) reported 32 salmonella serotypes in India, including 15 serotypes from human infections, besides S. typhi and S. paratyphi A, and seven serotypes from animals.

According to Gitter (1959), the recovery rate of S. cholerae-suis from the intestinal contents of pigs was better after 24 hr of incubation at 37°C on brilliant -green-lactose-neutral red agar than on MacConkey agar and also the number of salmonella isolations from the gall bladder of pigs was more on the former medium.

Hoag and Rogers (1961) were of the opinion that S. typhimurium could be isolated more frequently on tetrathionate enriched brilliant green agar than on brilliant green agar alone. They used this method to isolate S. typhimurium from necropsy material of both carrier mice and clinically-ill animals.

The salmonella serotypes from animals recorded in India till 1962 were 83 in number which included S. aberdeen, S. chester, S. poona and S. stanley from pigs (Agarwal, 1962).

S. dublin and S. typhimurium were associated with pneumonia in sheep and goats and no outbreak of illness attributable to salmonella infection was recorded in cattle (Khera, 1962).

Between 1959-1961, S. cholerae-suis was isolated from two, out of 450 slaughtered pigs, by Gitter and Bonson (1963) at Guildford.

Mann (1963) while investigating into the various Salmonella types, found that the mesenteric lymph nodes of swine and cattle harbour many types of salmonellae.

S. cholerae-suis infection was not only concomitent with hog cholera, but also could be responsible singly for outbreaks resembling hog cholera. It was observed by Krishnamurthy and Kaushik (1964) that in most cases in a hog cholera outbreak only S. cholerae-suis was isolated from young unweaned piglets and weaners of one to six months of age. The disease was acute in younger piglets, but was chronic and progressive with marked diarrhoea in the older animals.

Datta and Singh (1965) examined bacteriologically 403 faecal samples of which 308 were from pigs and the

remaining from fowl, duck and pigeon. In their study, 52 salmonella isolates of 11 serotypes, including a new serotype S. gokul from an apparently healthy pig were isolated. They also pointed out that animals and birds were recorded as new hosts for S. senftenberg in India.

Dennis (1965) did isolation studies in western Australia, employing tetrathionate, selenite and brilliant green broths as enrichment media and MacConkey agar and S-S agar as differential media. Thirty-one salmonella isolates belonging to eight serotypes were obtained from pigs. These included S. cholerae-suis and its variant kunzendorf S. typhimurium, S. give etc.

An interesting observation on the increased rate of S. typhimurium infection in a herd of hysterectomised pigs was made by Heard et al. (1965). Rectal swabs from these pigs yielded, besides S. typhimurium, four other salmonella serotypes wherein the morbidity rate was as high as 30%.

An outbreak of S. cholerae-suis infection was described with a 15% mortality in a large pig fattening enterprise in Lancashire by O'Brien (1966). He reported that this outbreak almost resembled swine fever in its symptoms and lesions, thereby the initial diagnosis was difficult. Later the isolation of S. cholerae-suis from three typically affected pigs confirmed the diagnosis and he pointed out the

increasing importance of the organism in such large enterprises.

Gitter and Kidd (1967) isolated S. typhimurium from carrier pigs. The pigs were specific pathogen free, but were accidentally infected with S. typhimurium. The organism was excreted sporadically in the faeces of survivors upto six months after the outbreak ceased. In this study, tonsils and gall bladder were the sites from where the organism could be isolated most consistently.

S. typhi-suis appeared to be a primary pathogen producing progressive infection in pigs in one outbreak in which Barness and Bergeland (1968) observed emaciation, intermittent diarrhoea and in some cases swelling of the throat.

During 1967-68, eight European laboratories were invited to isolate Salmonella from either artificially or naturally contaminated samples of pig faeces and minced meat. In addition, some of these samples had been artificially contaminated with Proteus, Citrobacter, Providencia, Aerobacter and Pseudomonas. All these laboratories isolated Salmonella, when there were approximately 10 cells per sample and when few competitive organisms were present. The efficiency of isolation declined when competitive organisms were in large numbers (Edel and Kampelmacher, 1968).

Heard et al. (1968) in a three-year study with a closed pig herd, isolated S. typhimurium, S. orion, S. durban, S. bradney and S. heidelberg from hysterectomised animals.

Edel and Kampelmacher (1969) stated that a new standard method for the isolation of Salmonella was better than the usual methods used when artificially contaminated pig faecal and minced meat samples were tested. In this method, they emphasised the importance of a second subculture, especially when a large number of competitive bacteria were present in the samples.

In an Hawaiian piggery, the prevalence of salmonellosis was determined by bacteriological examination of rectal swab samples collected from young pigs. An unexpectedly high rate of infection (9 out of 10) was found in one-day and two-day old pigs and a lower rate in pigs of two and three months of age (Gooch and Haddock, 1969).

Heard et al. (1969) examined the faecal samples from a large number of pigs to determine the rate of excretion of Salmonella. Pigs from 344 farms were included in this survey. Pooled faecal samples numbering 3127 were examined, representing 13,355 pigs. The isolation rate during a period of two years' study was 1.3%.

During a 10-month study, Jayaraman and John (1969) made 29 isolates of Salmonella from domestic animals, poultry and from abattoirs. The isolates comprised of 10 serotypes including S. bournemouth, S. lanka and S. sladun which were

the first reports in India.

The potentiality of S. dublin to cause meningitis in piglets was recorded by McErlean (1969). An interesting observations made in this study was that the organisms isolated from the brain and colon of the same animal showed difference in their biochemical properties.

Reynolds (1969) diagnosed S. typhi-suis as the apparent cause of infection in the nine swine herds of Massachusetts. Intestinal and lung lesions were prominent at necropsy. Cultural responses of this organism was not typical of the genus.

Salmonella, a major cause of bacterial gastro-enteritis in the United States was responsible for more than half the number of cases of food borne illness (Aserkoff et al. 1970). The author stressed on the improvement to be done in the poultry and egg processing industries and particularly in the animal rendering industries. A total of 10 serotypes were recorded from animals during this study.

Bhattacharya and Sinha (1970) were of the opinion that in West Bengal, S. choleraesuis was comparatively more common than other types involved in the salmonella outbreaks in pigs. These workers have reported for the first time in this country of isolations of S. enteritidis and S. paratyphi B from pigs.

Besides causing meningitis in piglets, S. dublin

causes other conditions. Dullness, pyrexia, inappetence, dyspnoea and blue discolouration of the ears were very characteristic and in all such cases, Buckley and Donnelly (1970) observed that death occurred within 24 hr of the appearance of clinical condition.

Haddock (1970b) working on the efficiency of rectal swab to detect swine Salmonella carriers, was of the opinion that rectal swabs alone were inadequate for the detection of long term carriers of Salmonella. Of the 256 swabs examined, 40 (15.6%) only yielded salmonellae. Six carriers were identified after three tests and six more after 16 tests. At slaughter, 15 of the 16 pigs yielded salmonellae from ceacal swabs and nine of 10 pooled faecal samples collected from the piggery floor yielded four serotypes which were not detected in the rectal swabs.

According to Linton et al.(1970), an increase in the number of pigs per pen, correspondingly increased the incidence of salmonella infection. According to them the most susceptible age of pigs was between three and seven months.

On examination of the intestines and mesenteric lymph nodes of 200 slaughtered pigs, 54 (27%) yielded salmonellae (Riley, 1970). S. cambridge was reported for the first time from Australia in this study.

Sojka and Field (1970) reported that S. cholerae-suis

was the most common cause of clinical salmonellosis in pigs in Britain. They pointed out that infection of meat and its products may originate from infected animals, but meat from uninfected carcasses can also become contaminated with faeces or bile. In animal populations, the most important factor was the contact and environmental spread, as the animals were herded in groups with young and susceptible ones in close contact.

S. ochi from pigs and S. paratyphi B from tinned pork were reported for the first time in India by Bhatia and Pathak (1971). They also isolated S. cholerae-suis var. kunzendorf, S. colombo, S. paratyphi B and S. hvitting-foss from pigs showing diarrhoea and fever and S. ochi from apparently healthy pigs. They used modified tetrathionate broth for the primary enrichment of the rectal swabs and faecal samples.

Goel and Malik (1971) recorded 13 isolates of Salmonella, 11 out of 252 rectal swabs, one out of 42 mesenteric lymph nodes and one out of 25 specimens of small intestine from pigs. Among the 13 isolates six were S. stanley, five S. cholerae-suis var. kunzendorf and two S. enteritidis. For the primary enrichment of the materials collected Kauffmann modified tetrathionate broth was used by these workers.

The occurrence of salmonella infection in market swine in south western Ontario was studied by Groves et al. (1971). Salmonellae were detected in the mesenteric lymph

nodes of 19.5% of 303 pigs examined. S. muenchen was isolated with the greatest frequency from lymph node samples and S. typhimurium and S. heidelberg were the serotypes from carcass swabs.

Harrington et al. (1971) isolated Salmonella from swine suspected of ailing from hog cholera. Out of 831 salmonella isolates of 2,774 samples examined, 42 serotypes were identified. S. cholerae-suis var. kunzendorf accounted for 62.5% of all the isolates.

Persistence and spread of salmonella excretion in pigs in a breeding farm was studied by Ghosh (1972). He observed that the breeding stock was responsible for the salmonella excretion and perpetuation of infection in the farm.

No serotype was repeatedly isolated from any source by Lee et al. (1972) and they concluded that the salmonella serotypes were not able to establish themselves in the pigs.

S. derby, S. anatum, S. typhimurium and S. indiana were isolated more frequently than S. saintpaul, S. heidelberg, S. london, S. jamaica, S. montevideo and S. schwarzengrund from pig carcasses by Carpenter et al. (1973).

Salmonella organisms were not isolated from gastric and rectal lymph nodes, although ten strains were isolated from digestive tract of pigs. The caecum and the ileal lymph nodes were the most common sites from where salmonellae

were recovered according to Katsube *et al.* (1973).

Salmonellae were isolated from 22 out of 300 bacon pigs sampled at slaughter. McCaughey *et al.* (1973) showed that multiple infection of salmonella serotypes was possible on the farm. Rappaport's medium demonstrated greater overall selectivity in the isolation of salmonella organisms to selenite F broth and brilliant green broth.

ISOLATION OF SALMONELLA SEROTYPES OF PUBLIC HEALTH IMPORTANCE

In an extensive review, Steel (1953) pointed out the marked invasive power of *S. cholerae-suis*, frequently producing fatal septicaemia in man. The prevalence of Salmonella in swine and cattle, the major source of meat for man was discussed.

Sharma and Singh (1961) conducted a Salmonella survey of 2,970 domestic animals, out of which 247 were pigs. Bacteriological examination was mainly confined to faecal samples. However, small pieces of liver, spleen, gall bladder, lungs and intestines with their contents were also examined. In this study several human types like *S. anatum*, *S. stanley* and *S. newport* were encountered.

Sojka and Gitter (1961) pointed out that complete co-operation among the workers on the farm, in the abattoir and in the food factory etc., was essential in the control of salmonellosis. They also stressed the importance of pig as a food animal in causing salmonella food poisoning

in man.

Nath et al. (1966) were of the opinion that in man, apart from host specific salmonellae, many other serotypes which were usually animal pathogens, were also found. The occurrence of S. cholerae-suis, a swine pathogen, producing various clinical manifestations in man was significant.

Khera (1968) stressed the zoonotic importance of salmonellae from animals. From pigs alone among the 17 strains, S. cholerae-suis, S. dublin and S. stanley were recorded by him in India.

Chung and Frost (1969) when examining 1,000 pigs at slaughter for salmonellae, isolated 87 strains belonging to 26 serotypes. S. anatum, S. muenchen, S. give and S. typhimurium constituted the majority of the serotypes. Faeces and mesenteric lymph nodes were the best sources for isolation of the organisms. These workers emphasised the importance of pigs in the incidence of human salmonellosis. Gibson (1969) pointed out the importance of S. cholerae-suis, for which the pig is the natural host although infection occurred due to other salmonellae. He also made a mention of S. cholerae-suis occurring independently of swine fever in pigs. Referring to public health aspects of this disease, he endorsed the view of Sojka and Gitter (loc. cit).

Haddock (1970a) was of the opinion that pork and its products were important potential sources of human salmonellosis. He considered that the continuing emphasis

on the mass production and mass distribution of food stuffs, food borne disease problems would continue to grow. Studies in an Hawaiian piggery supported this conclusion. Asymptomatic salmonellosis, wherein 8 serotypes were involved, contributed to the spread of salmonellosis in the piggery, as did the commercial food.

Prakash and Ghosh Ray (1970) reported for the first time a case of purulent meningitis in a three-year old child due to S. cholerae-suis var. kunsendorf. The infection proved fatal within five days of illness. The causal organism was isolated from cerebrospinal fluid shortly before death.

Ray and Mallick (1970) have emphasised the enteropathogenic potentialities of S. enteritidis and S. typhimurium and the public health importance of these organisms.

Timoney (1970) reported 16% of lairage samples, 16% of caecal and 5% carcass swabs yielded Salmonella. Serotypes which occurred in the lairage were found also in the caeca and on the carcasses of slaughtered pigs. In this study, 8 serotypes, including S. senftenberg an important cause of human salmonellosis in the Dublin area were recorded.

Skovgaard and Nielsen (1972) reported that S. virchow which was isolated from man was also found to be associated with poultry salmonellosis.

MATERIAL AND METHODS

CHAPTER III

MATERIAL AND METHODS

The samples from pigs were collected from the following places in and around Bangalore city.

(1) Border Security Force, Yelahanka, Bangalore North.

(2) Nandidurg Farm, Bangalore.

(3) Veterinary College Hospital.

(4) A.S.C. Centre South, Bangalore.

(5) Post-mortems conducted at the Veterinary College.

(6) Combined Livestock Farms and Research Station, Hesserghatta.

(7) Pig Slaughter House

(8) From Hebbal and other locales.

The samples were divided into two categories, viz., (i) those collected from live pigs, and (ii) those collected from dead/slaughtered pigs.

The pigs examined were subdivided into three groups according to their age and sex as follows:

(a) 0 - 3 months

(b) 3 - 6 months

(c) 6 months and above

From the live pigs, faecal samples were collected. From the dead and slaughtered pigs, along with faecal samples, the following samples were collected with sterile precautions:

(i) Heart blood

(ii) Visceral organs - liver, spleen and mesenteric lymph glands

(iii) Portion of contents from small intestine (Table 1).

Soon after collection, the materials were brought to the laboratory in sterile containers for further processing.

CULTURE MEDIA

The following media were employed for isolating salmonella organisms from the materials collected from both live and dead or slaughtered pigs.

(i) Kauffmann modified tetrathionate broth

(ii) Teepol lactose agar (TLA) and

(iii) Salmonella-Shigella agar (S-S agar)

The various media used were prepared according to the procedures described in the Medical Microbiology (Cruickshank, 1968). Each of the material collected was inoculated into modified tetrathionate broth and incubated for 16 to 18 hr at 37°C. Following incubation a loopful or two of the material from tetrathionate broth was streaked on TLA and S-S agar plates which were incubated for 48 hr at 37°C.

Different colonies suggestive of Salmonella from either of the plates were subcultured into stock culture agar slants for further processing. The two differential media were used in conjuncture to make sure of maximum number of Salmonella isolations.

IDENTIFICATION AND CHARACTERISATION OF THE ISOLATES

Identification of the cultures suggestive of Salmonella was made by subjecting to a systematic study by cultural, biochemical and serological tests. Their classification was done according to Bergey's Manual of Determinative Bacteriology (Breed et al. 1957).

The salmonella isolates were sent to Biological Standardisation Division, Indian Veterinary Research Institute, Izatnagar and Department of Microbiology, Veterinary College, Haryana Agricultural University, Hissar for serological typing.

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

A total of 1,023 pigs were screened to study the incidence of Salmonella among the porcine population in and around Bangalore city. This consisted of 810 live pigs and 213 dead or slaughtered pigs from which 1,336 different samples were collected and examined for the presence of Salmonella (Table 1).

In this study, a total of 1,336 samples from 1,023 pigs yielded 17 isolates of Salmonella. Of these, 13 strains were recovered from faecal samples, two were isolated from mesenteric lymph node samples and one each from liver and heart blood samples.

A higher percentage (8.7%) of incidence was observed in the age group of three to six months than in the other groups of pigs. The incidence of Salmonella was observed to be more in females than in males (Table 2).

The overall incidence of Salmonella numbering 17 among 1,023 pigs examined was 1.66%.

The results of serological typing are shown in Table 3.

Table 1: DETAILS OF THE NUMBER OF PIGS SCREENED AND THE TYPE OF MATERIALS COLLECTED

	0 to 3 months (304)		3 to 6 months (207)		6 months and above (512)		Total (1023)
	Live	Dead	Live	Dead	Live	Dead or slaughtered	
Faecal samples	303	1	207	-	300	212	1023
Small intestine	-	1	-	-	-	78	79
Mesenteric lymph glands	-	1	-	-	-	78	79
Heart blood	-	1	-	-	-	58	59
Liver	-	1	-	-	-	64	65
Spleen	-	1	-	-	-	30	31
Total number of samples	303	6	207	-	300	520	1336

Note: The numbers in the parenthesis indicate the number of animals screened.

TABLE 3: RESULTS OF THE SEROLOGICAL TYPING OF SALMONELLA ISOLATES

Isolate No.	Age of the animal (months)	Sex of the animal	Health status of the animal	Antigenic structure	Serotypes
1	2	3	4	5	6
16	2	Female	Clinical case	9, 12 : g, m : -	S. enteritidis
48	4½	Male	Healthy pig	9, 12 : rough	Salmonella D_1 group with rough flagellar antigens
63	4	Male	"	1, 4, 12, i : 1, 2	S. typhimurium var. copenhagen
97	2	Female	"	9, 12 : rough	Salmonella D_1 group with rough flagellar antigens
124	12	Male	Slaughtered pig	1, 9, 12 : g, m : -	S. enteritidis
171	5	Female	Healthy pig	1, 9, 12 : g, m : -	"
188	6	Female	"	6, 7 : c : 1, 5	S. cholerae-suis

DISCUSSION

CHAPTER V

DISCUSSION

The present study was conducted to know the incidence of Salmonellaamong the porcine population in and around Bangalore city. During the study, a total of 1,336 samples from 1,023 pigs were examined for the presence of Salmonella organisms.

OVERALL INCIDENCE

The samples from 1023 pigs yielded 17 strains of Salmonella accounting for an overall incidence of 1.66%.

The incidence of salmonella organisms which has been recorded in the present study was mostly from apparently healthy pigs and from slaughter house samples rather than from clinical cases. In no instance, two different serotypes were recorded in the same animal during the study, although the possibility of occurrence of multiple infection has been recorded by McCaughey et al. (1973).

The scavenging habits of the pigs are likely to influence the incidence of Salmonella in them primarily and their role in the spread of the disease secondarily. The underlying importance of this habit in the incidence of salmonella serotypes cannot, therefore, be overlooked.

In general, the common salmonella serotypes found in man are those commonly found in domestic animals, excluding the host specific ones. Bhattacharya and Sinha (1970)

reported that S. cholerae-suis was the most common serotype than other types involved in salmonellosis in pigs in Bengal. In the present study, it was found that the nine serotypes were almost equally distributed among the pigs and no one serotype predominated over others.

In other countries, salmonella infection was reported to be fairly common in pigs by Buxton (1957). Heard et al. (1965) reported that 71% of 62 pigs in a hysterectomy produced herd had salmonella infection (Gibson, 1969). Chung and Frost (1969) examined 1,000 pigs at slaughter house for salmonellae and reported an 8.4% incidence among them with the occurrence of 26 serotypes.

During a 10 year period, Sojka and Field (1970) recorded incidence of 83.2% of S. cholerae-suis, 8.2% of S. typhimurium and 3.9% of S. dublin among 895 pigs examined for clinical salmonellosis. Riley (1970) reported an incidence of 27% out of 200 slaughter pigs and he also recorded the incidence of S. cambridge for the first time in Australia.

Pooled faecal samples numbering 3,127 from 13,355 pigs were examined for the presence of salmonellae from 344 farms by Heard et al. (1969). They recorded an 1.3% incidence of Salmonella. A comparable incidence of 1.27% was recorded in the faecal samples examined in the present study.

Linton (1970) observed an increase in the percentage incidence of Salmonella with an increase in the number of pigs per pen. During the present study, the samples were collected from various piggeries, but in only one piggery the animals were stocked in pens and hence a comparison of the percentage incidence and pigs per pen was not possible.

ISOLATION TECHNIQUE

In the present study, the material collected from pigs was put on Kauffmann modified tetrathionate broth for primary enrichment and after 16-18 hr incubation, was plated on two selective media simultaneously. This method resulted in the recovery of 17 isolates of Salmonella, 13 of which were recovered on both teepol lactose agar and Salmonella-Shigella agar, three on teepol lactose agar only and one on Salmonella-Shigella agar alone.

Edel and Kampelmacher (1968) pointed out that the efficiency of isolation of Salmonella declined when competitive organisms were in large numbers.

Although no attempt was made in this study to use different types of enrichment media, the one employed in the present study was considered to be one of the better enrichment media. Hoag and Rogers (1961) reported that S. typhimurium was isolated more frequently on tetrathionate enriched medium. Bhatia and Pathak (1971) used modified tetrathionate broth for the isolation of Salmonella from

both healthy and sickpigs. Goel and Malik (1971) also used the same medium for the primary enrichment of samples collected from pigs suffering from enteritis.

McCaughey et al. (1973) reported that S. cholerae-suis isolations were 80% on Rappaports medium, only 8% on selenite F broth and none on brilliant green broth, when used for enrichment although Gitter (1959) stated that the recovery rate of Salmonella from gall bladder of pigs was high when selenite F broth was used for primary enrichment.

It was obvious that observations made by different workers on the use of enrichment media vary in different situations and each one useful for particular serotype.

Several workers have expressed the usefulness of employing more than one type of selective medium with higher rates of recovery of salmonellae. Gitter (1959) recovered S. cholerae-suis more frequently on brilliant green-neutral red-lactose agar medium than on MacConkey agar. MacConkey agar and S-S agar were used to isolate S. cholerae-suis and its variant kunzendorf, S. typhimurium, S. give etc., by Dennis (1965). But Gitter and Kidd (1967) used 5% sheep blood agar, MacConkey agar and desoxycholate citrate agar for the isolation of S. typhimurium from carrier pigs, although they did not compare the efficiency of the different media. Bhatia and Pathak (1971) used brilliant green agar, MacConkey lactose agar and desoxycholate citrate agar for the isolation of different serotypes from both healthy and

sick pigs. Goel and Malik (1971) also used the same media as selective media and recovered 13 isolates of Salmonella. However, except Gitter (loc. cit) others did not point out the merits of one differential medium over the other.

INCIDENCE AMONG NORMAL AND DISEASE PIGS

In the present work, out of 17 salmonella strains recorded, nine were isolated from apparently normal animals, four from slaughtered pigs while four more from clinically ill pigs. The faecal samples numbering 1023 yielded 13 strains, while two were isolated from among 79 samples of mesenteric lymph nodes and one each from 65 liver and 59 heart blood samples. The percentage recovery from 63 clinically ill pigs was 6.35% only.

Gooch and Haddock (1969) isolated salmonellae in 9 out of 10, one and two day old pigs from a closed herd with a previous history of salmonellosis. Heard et al. (1969) recorded 1.3% incidence after examining 3,127 pooled faecal samples, representing 13,355 pigs. Haddock (1970b) recovered Salmonella from caecum of 15 out of 16 carrier pigs although only 15.6% of the faecal samples taken from them when alive were positive. Riley (1970) reported a 27% incidence of Salmonella when mesenteric lymph nodes and intestines were examined from 200 slaughtered pigs. Goel and Malik (1971) recorded 11 salmonella strains from 252 rectal swabs, one from 48 mesenteric lymph node samples and another strain from 25 specimens of small intestine. Harrington et al. (1971) isolated 831 salmonellae belonging to 42 serotypes from

2,774 samples from swine suspected of having hog cholera. Salmonellae were isolated from 22 out of 300 bacon pigs at slaughter by McCaughey et al. (1973).

These observations indicate that the incidence of Salmonella could be very high in diseased herds, more so if they are closed ones and may vary considerably in others. In the present study, no intensive investigation was possible but the samples covered a cross section of the population which included a small number of clinically ill animals although they were mostly available for casual examination.

AGE AND SEX-WISE INCIDENCE

In animal populations, contact and environmental spread of infection plays and important role, especially in the young ones (Sojka and Field, 1970). In the present study the animals were divided into three groups based on their age and for recording the percentage incidence (see table 2). In the age group of 0-3 months, the incidence was 2.6%, in the group of 3-6 months 8.7% and in the group above 6 months 1.5%. It was obvious that 3-6 months group showed the highest incidence and there was a significant difference between this group and the other two groups ($P > 0.01$).

There appears to be a relationship between the age of the animals and the incidence of salmonella infection in them.

Other workers have also made similar observations. Gooch and Haddock (1969) pointed out that there seemed to be a possible role of colostrum in preventing the occurrence of Salmonella in very young pigs by reducing their susceptibility to infection. Linton (1970) also observed that pigs in the age group of 3 to 6 months were more susceptible to salmonella infection. The high incidence of Salmonella in this age group is still considered important since these are occasionally slaughtered for food and thereby contamination of other carcasses is possible to occur.

In the present study, though 10 strains of Salmonella were isolated from female pigs and seven from the males, the difference in the percentage isolation was not significant ($P < 0.05$). Sex of the pig did not significantly influence the incidence of Salmonella. But, Ghosh (1972) pointed out that salmonella excretor boars and sows in breeding establishments could be responsible for the spread and perpetuation of infection. He also mentioned that the female pigs were more susceptible to the infection than the male ones.

INCIDENCE OF SEROTYPES

Transmission of salmonellae directly from animal reservoirs and indirectly through food products to man is widespread. In recent years the infection of man with animal types and infection of animals with human types have been

observed. The animal types are attaining great public health importance as these hosts are being identified as the source of human infection and in this regard the role of pig has been established (Steel, 1953).

The 17 isolates of Salmonella encountered in the present study belonged to eight serotypes.

Out of the 17 strains, nine strains belonging to six serotypes, viz., S. cholerae-suis, S. virchow, S. enteritidis, S. typhimurium var. copenhagen, Salmonella D_1 group with rough flagellar antigens and a rough strain of Salmonella were isolated from the faecal samples of apparently healthy animals. Four strains were from slaughtered pigs which consisted of S. enteritidis, S. cholerae-suis var. kunzendorf and S. dublin, while four more strains viz., S. enteritidis, S. cholerae-suis, S. virchow and S. typhimurium var. copenhagen were isolated from the clinically ill pigs.

out of the eight serotypes encountered, S. cholerae-suis, S. enteritidis and S. virchow were found to predominate. The isolation of S. enteritidis and S. virchow from slaughtered pigs and apparently healthy animals with their scavenging habits, closely suggested the possibility of human infection.

Sharma and Singh (1961) reported the occurrence of S. anatum, S. newport and S. stanley from pigs in India. Agarwal (1962) reported S. aberdeen, S. chester, S. poona

and S. stanley from pigs.

Gitter and Bonson (1963) isolated S. cholerae-suis from two out of 480 pigs at slaughter. Krishnamurthy and Kaushik (1964) recorded the incidence of S. cholerae-suis var. kunzendorf in a hog cholera disease outbreak.

Datta and Singh (1965) reported the occurrence of S. gokul, a new serotype from an apparently healthy pig. Chung and Frost (1969) reported the occurrence of S. anatum S. derby, S. javana, S. muenchen, S. give and S. typhimurium in pigs.

S. cholerae-suis var. kunzendorf is assuming importance in recent years as a pathogen of other animals and man besides the pig. It is reported that it causes meningitis in man (Prakash and Ghosh Ray, 1970).

S. typhimurium var. copenhagen encountered in the present study has been isolated from apparently healthy pigs and also from a clinical case. This serotype has not been reported earlier from pigs and ~~x~~is probably the first record of its incidence in this animal.

In the present study, S. virchow has been isolated from a pig showing characteristic lesions such as button ulcers in the small intestine. The animal had suffered from acute diarrhoea for three days and then died. The organism was isolated from the intestinal contents collected at the post-mortem examination of the animal. The organism

has also been isolated from two apparently healthy animals in this study. Earlier this organism has been found mostly in man and bird (Skovgaard et al. 1972). The present record of this serotype in pigs is probably the first of its kind in India. This idea further emphasised the fact that human types might be important in pig salmonellosis and was all the more significant, since it was isolated from a clinical case, which probably succumbed to the infection.

A few members of the genus Salmonella demonstrate strict species specificity, although a few others are less so. An understanding of the close relationship between human and non-human salmonellosis and the relative specificity of some of these serotypes are useful in recognising their zoonotic importance. Emphasising this aspect of the disease Khera (1968) was of the opinion that the pig served as an important carrier animal. He recorded 17 strains including S. cholerae-suis, S. dublin, and S. stanley from pigs. McErlean (1969) reported an outbreak of S. dublin meningitis in young pigs. But in the present study, this serotype was recorded from a slaughtered pig. The isolation of S. cholerae-suis and S. dublin in the present work is thus significant.

Three of the 17 isolates, were serologically typed as Salmonella D_1 group with rough flagellar antigens. These two serotypes could not be fitted into any particular aspect of the disease, although they may be of public health importance.

With the continuing emphasis on mass production of animal protein, the food borne disease problems are also multiplying. S. enteritidis, S. cholerae-suis and S. virchow have been isolated from clinical cases, and S. cholerae-suis var. kunzendorf has been isolated from the liver of a slaughtered pig in this study (see table 3). Their incidence in pigs is considered significant since these salmonella serotypes have been reported to be human pathogens (Nath et al. 1966; Prakash and Ghosh Ray, 1970; Skovgaard et al. 1972). Maddock (1970a) also recorded human types such as S. anatum, S. derby and S. typhimurium from an asymptomatic disease in pigs.

A progressive disease manifestation characterised by emaciation, intermittent diarrhoea and occasional swelling of the throat were observed by Barness and Bergeland (1968) in S. typhi-suis infection of pigs. During the course of the present work also, in one clinically ill animal, progressive diarrhoea was seen and the animal succumbed to the infection. On post-mortem, characteristic intestinal lesions were observed and S. virchow was isolated from the intestinal contents.

Four of the 17 isolates in the present study were typed as S. enteritidis. This was the most commonly encountered serotype. It was isolated from apparently normal animals as well as a clinical case.

Pig as a food animal in causing Salmonella food

poisoning in man was rightly pointed out by Sojka and Gitter (1961). According to these workers, S. enteritidis was the most common serotype encountered in man between 1958 and 1966 in United Kingdom. In animals it accounted for 1% of the total incidence report. Ray and Mallick (1970) also emphasised the public health importance of this organism.

ANIMAL AND PUBLIC HEALTH IMPORTANCE

S. typhimurium var. copenhagen isolated from pigs has been encountered in clinical condition in buffalo calves in this state. The calves showed severe diarrhoea and died subsequently (Nagaraja et al. 1973). This emphasises the importance of the occurrence of this serotype in apparently normal pigs that may form a source of infection to other domestic animals, particularly in mixed farming.

It is noteworthy that S. virchow and S. enteritidis, the serotypes commonly considered as non-pathogenic to pig have presently been recorded from clinical cases. The serotypes recorded in the present study such as S. choleraesuis var. kunzendorf, S. virchow, S. enteritidis and S. dublin have been claimed to cause clinical conditions in man and animals besides the pig (Nath et al. 1966; Prakash and Ghosh Ray, 1970; Ray and Mallick, 1970; Grooves et al. 1971).

From the foregoing study, it will be seen that though there is a clear difference in the frequency of

incidence and the serotypes of Salmonella isolated from pigs by different workers, there is considerable unanimity of observation that the presence of increasing number of salmonella serotypes in not only sick animals but also apparently healthy ones, does act as a public health hazard besides being an animal health problem. This investigation of Salmonella in pigs in this city establishes that this animal on the farm and in the abattoir, harboured various salmonella serotypes, many of which are potential pathogens of man and other animals.

SUMMARY

CHAPTER VI

SUMMARY

1. A bacteriological study was conducted to know the incidence of salmonella serotypes among the porcine population in and around Bangalore city.

2. A total of 1,336 different samples were collected from 810 live and 213 slaughtered or dead pigs. Faecal samples from 1,023 pigs and samples of mesenteric lymph nodes, heart blood, liver and spleen were collected from dead or slaughtered animals.

3. Of the two selective media employed in the studies, teepol lactose agar was useful in isolating 16 strains and Salmonella-Shigella agar in isolating 14 strains of Salmonella. Of these 13 appeared on both the media, while three did so on teepol lactose agar alone and one on Salmonella-Shigella agar alone.

4. The overall incidence of Salmonella was 1.66% among the 1,023 pigs examined during the study.

5. In all 17 isolates, representing eight serotypes of Salmonella were isolated.

6. The serotypes encountered were, S. cholerae-suis (2), S. typhimurium var. copenhagen (3), S. virchow (3), S. cholerae-suis var. kunzendorf (1), S. dublin (1), S. enteritidis (4) and Salmonella D_1 group (3) with rough flagellar antigens.

BIBLIOGRAPHY

BIBLIOGRAPHY

Agarwal, S.C., 1962, Salmonella serotypes identified at National Salmonella and Escherichia Centre, Kasauli (1958-60). Indian J. Med. Res., 50:567-568.

Aserkoff, B., Schroeder, S.A., Brachman, P.S., 1970, Salmonellosis in the United States - a five year review. Am. J. Epidem., 92:13-24.

Barness, D.M. and Bergeland, M.E., 1968, Salmonella infection in Minnesota swine. J. Am. Vet. Med. Ass., 152:1766-1770.

Bhatia, A.K. and Pathak, R.C., 1971, Salmonella serotypes from pigs. Indian J. Microbiol., 11:508.

Bhattacharya, H.M. and Sinha, P.K., 1970, Incidence of Salmonella species in pigs. Indian J. Ani. Hlth., 9:131-133.

Breed, R.S., Murray, E.C.D. and Smith, N.A., 1957, Bergey's Manual of Determinative Bacteriology. Edn. 7, Williams and Wilkins Co., Baltimore.

Buckley, H.G. and Donnelly, W.J.C., 1970, Salmonella dublin infection in piglets. Irish Vet. J., 24:74-78. (cited in Vet. Bull., 40:Abstr. No. 4710)

Buxton, A., 1957, Salmonellosis in animals. Farnam Royal, Bucks, C.A.B.

Buxton, A., 1958, Salmonellosis in animals. Vet. Rec., 70:1044.

Carpenter, J., Elliot, J.G. and Reynolds, A.E., 1973, Isolation of Salmonella from pork carcasses. Appl. Microbiol., 25:731-734.

Chung, G.T. and Frost, A.J., 1969, Occurrence of salmonellae in slaughtered pigs. Aust. Vet. J., 45:350-353.

Cruickshank, R., 1968, Medical Microbiology, Edn. 12, E & S Livingstone Ltd., London.

Datta, S.K. and Singh, C.M., 1965, Salmonella serotypes in domestic animals, birds and bonemeal. Indian J. Vet. Sci., 35:121-125.

Dennis, S.M., 1965, Salmonellosis in animals in Western Australia. Aust. Vet. J., 41:315-320.

Edel, W. and Kampelmacher, E.H., 1968, Salmonella isolation in eight European laboratories (a comparative study). Bull. Wld. Hlth. Org., 39:487-493.

Edel, W. and Kampelmacher, E.H., 1969, Salmonella isolation in nine European laboratories using a standardised technique. Bull. Wld. Hlth. Org., 41:297-306.

Ganguli, S., 1958, Salmonella serotypes in India. Indian J. Med. Res., 46:637.

Ghosh, A.C., 1972, An epidemiological study of the incidence of Salmonella in pigs. J. Hyg., 70:151-160.

Gibson, R.A., 1969, Salmonella infection in pigs. Brit. Vet. J., 125:431.

Gitter, M., 1959, Isolation of Salmonella cholerae-suis from post-mortem specimens. Vet. Rec., 7:237.

Gitter, M. and Kidd, A.R.M., 1967, Isolation of Salmonella typhimurium from carrier pigs. Vet. Rec., 81:358-359.

Goel, Y.P. and Malik, B.S., 1971, Isolation of Salmonella organisms from enteritis cases in piglets. Indian Vet. J., 48:1193-1196.

Gooch, J.M. and Haddock, R.L., 1969, Swine salmonellosis in Hawaiian piggery. J. Am. Vet. Med. Ass., 154:1051-1054.

Grooves, B.I., Fish, N.A. and Mitchel, W.R., 1971. The occurrence of Salmonella infection in marketed swine in south Western Ontorio. Can. Vet. J., 12:11-15. (cited in Vet. Bull., 41:Abstr. No.4496).

Harrington, R. (Jr), Hulse, D.C. and Blackburn, B.O., 1971. Salmonella isolated from swine suspected of having hog cholera. Am. J. Vet. Res., 32:1297-1299.

Haddock, R.L., 1970a, Asymptomatic salmonellosis in a swine herd. Am. J. Pub. Hlth., 60:2345-2353.

Haddock, R.L., 1970b, Efficiency of examining rectal swabs to detect swine Salmonella carriers. Am. J. Vet. Res., 31:1509-1512.

Heard, T.W., Linton, A.H., Penny, R.H.C. and Wilson, M.R., 1965. Salmonella typhimurium infection in a hysterectomy produced herd of pigs. Vet. Rec., 77:1276-1280.

Heard, T.W., Jennette, N.E. and Linton, A.H., 1968. The control and eradication of salmonellosis in a closed pig herd. Vet. Rec., 82:92-99.

Heard, T.W., Jennette, N.E. and Linton, A.H., 1969. The incidence of Salmonella excretion in various pig populations from 1966-1968. Brit. Vet. J., 125:635.

Hoag, W.G. and Rogers, J., 1961, Techniques for the isolation of Salmonella typhimurium from laboratory mice. J. Bact., 82:213-218.

Jayaraman, M.S. and John, M.A., 1969, Isolation of salmonellae from domestic animals poultry and abattoirs in Madras State and its public health significance. Indian J. Anim. Sci., 39:213-218.

Katsube, Y., Tanaka, Y., Imiazumi, K. and Masuda, K., 1973. Salmonella carriers in swine. Jap. J. Vet. Sci., 35:25-31 (cited in Vet. Bull., 43:Abstr. No.4808).

Khera, S.S., 1962, Animal salmonellosis in India. Indian J. Med. Res., 50:569-579.

RFT- 6120

Khera, S.S., 1968, Zoonoses in India. Indian Vet. J., 45:811.

Krishnamurthy, D. and Kaushik, R.K., 1964, Occurrence of Salmonella cholerae-suis var. kunzendorf in pigs in India. Indian Vet. J., 41:561-576.

Lee, J.A., Ghosh, A.C., Mann, P.G. and Tee, G.H., 1972, Salmonella on pig farms and in abattoirs. J. Hyg., 70:141-150.

Linton, A.H. and Heard, T.W., 1970, Computer based analysis of epidemiological data arising from salmonellosis in pigs. Res. Vet. Sci., 11:523-532.

McCaughey, W.J., McClelland, T.G. and Reddy, R.M., 1973, Salmonella isolation in pigs. Vet. Rec., 92:191-194.

McErlean, B.A., 1969, A further outbreak of Salmonella dublin meningitis in piglets. Irish Vet. J., 23:10-11. (Cited in Vet. Bull., 39:Abstr. No. 2338).

Mann, P.H., 1963, Salmonella types found in the mesenteric lymph nodes of various domestic animals. Cornell Vet., 53:392-395.

Nagaraja, K.V., Gopal, T. and Keshava Murthy, B.S., 1973, Isolation of Salmonella typhimurium var. copenhagen from calves - Report on the A.I.C.R.P. on investigation of calf mortality in India, Bangalore Centre, (I.C.A.R).

Nath, M.L., Shrivastav, J.B., Sethi, S.K. and Singh, J., 1966, Salmonella pattern in India. Indian J. Med. Res., 54:512.

O'Brien, J.D.P., 1966, An outbreak of Salmonella cholerae-suis infection in a large pig fattening enterprise in Lancashire. Vet. Rec., 79:558-566.

Prakash, R. and Ghosh, Ray, B., 1970, Pyogenic meningitis Caused by Salmonella cholerae-suis var. kunzendorf. Indian J. Exp. Med., 58:155.

Ray, J.P. and Mallick, B.B., 1970. Public health significance of Salmonella infection in laboratory animals. Indian Vet. J., 47:1033-1037.

Reynolds, I.M., Smith, R.E., Smyser, C.F. and Miner, P.N., 1969. Salmonella typhi-suis from Massachusetts swine. J. Am. Vet. Med. Ass., 155:1600-1602.

Riley, M.G.I., 1970. The incidence of Salmonella in normal slaughter pigs. Aust. Vet. J., 46:40-43.

Sharma, V.K. and Singh, C.M., 1961. Occurrence of Salmonella in domestic animals and poultry in India. Nature (Lond), 191:622-623.

Skovgaard, N. and Nielsen, B.B., 1972. Salmonellosis in pigs and animal feeding stuffs in England and Wales and in Denmark. J. Hyg. 70:127.

Sojka, W.H. and Gitter, M., 1961. Salmonellosis in pigs with reference to its public health significance. Vet. Revs. Annot., 7:11-28.

Sojka, W.J. and Field, H.I., 1970. Salmonellosis in England and Wales 1958-1967. Vet. Bull., 40:515.

Steel, J.H., 1953. Veterinary public health. Adv. Vet. Sci., 1:329-377.

Timoney, J., 1970. Salmonellae in Irish pigs at slaughter. Irish Vet. J., 24:141-145. (Cited in Vet. Bull., 41: Abstr. No. 2188).