بسم اللہ الرحمٰن الرحیم

اللّٰھُمَّ اِنِّیْ اَسْئَلُکَ عِلْمًا نَّافِعًا

وَّعَمَلًا مُّتَقَبَّلًا وَّرِزْقًا طَیِّبًا

(حدیثِ نبوی ﷺ)

اے اللہ! میں آپ سے فائدہ مند علم اور مقبول ہونے والا عمل اور پاک روزی کی درخواست کرتا ہوں

Dedicated to

My Father-in-Law

Late. **RANA KHADIM HUSSAIN**

Dedicated to

My Supervisor

DR. ABDUL HAMEED

REVALENCE AND PATHOLOGICAL STUDIES I SALMONELLOSIS IN BROILER BREEDERS

BY

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CONTENTS

CONTENTS

CONTENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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(TARIQ JAVED)

ABSTRACT

The present project was designed to investigate the sources of *Salmonella* infection in broiler breeders, isolate and identity *Salm. serotypes* from broiler breeders, and to improve methods for *Salmonella* isolation and accurate diagnosis of salmonellosis. Other studies included pathogenicity of *Salmonella* isolates and characterization of specific pathological lesions in infected birds. Among the control measures antibiography, feed supplementation, competitive exclusion, elimination of carriers and evaluation of the disinfectants.

During the years 1988-1990, 150 chicken broiler breeder flocks (262454) around Islamabad, Rawalpindi and Abbottabad were screened for *Salmonella* by the rapid hemagglutination test. Most of the flocks were tested between the age of 21-40 weeks of age, a few at a later age. Rapid hemagglutination testing revealed 112 flocks (12159 birds) positive for *Salmonella*. The prevalence of *Salmonella* carriers varied in birds reared on various commercial feeds (69.2-82.2) in chickens of different breeds (100-60.01) in different age group (88.23 - 77.7) those maintained on the varying standards of management (41.66-78.57) and in different sex. Possible source of *Salmonella* infections and necessary control measures against avian salmonellosis are discussed.

Analysis of *Salmonella* serotypes isolation over the years indicated a relation between our flock pattern and isolation in different months of the year during 1988-1990. Significantly isolations were made in 1988 as compared to isolation in 1989 and 1990. An overall increasing trends in motile salmonellae was observed over the prevalence of non-motile serotypes. Motile *Salmonella* serotypes isolated from 18 various sources were 390 (4.73 %), while, non-motile were 325 (3.94 %). Isolation from 8241 samples from 18 different sources including broiler breeders, *Salmonella* was isolated from 715 (8.70 %) samples. The average isolation prevalence at random in chicken broiler breeders was 5.11 per cent, while in day-old broiler breeder chicks it was 4.18 per cent. Isolation prevalence in avifauna birds was 17.83 per cent and in indigenous chickens it was 5.71 per cent.

Isolation and pathological studies were conducted in 753 enlarged liver, spleen and intestines of indigenous chickens. Salmonellae were for the most pant isolated from the intestines (9.96 %), then from the liver (5.97 %) and the spleen (1.19 %). An overall isolation incidence was 5.71 per cent. *Salm. gallinarum, Salm. pullorum and Salm. typhimurium* were isolated.

The occurrence of *Salmonella* in a variety of zoological garden birds was investigated. Of 370 rectal swabs examined, 66 yielded different *Salmonella* serotypes, which includes *Salm. typhimurium* (30), *Salm. gallinarum-pullorum* (23), *Salm. saint-paul* (5), *Salm. butantan* (5) and *Salm. eastbourne* (3). Parrots, Pigeons, Java sparrows, quails, peacocks, doves and pheasants were the common birds positive for *Salmonella*.

In most of the birds, salmonellae were isolated from intestines (37.08 %), liver (24.07 %), spleen (10.18) and ovaries (11.11 %) isolates were also recovered from ceca (5.55 %), lungs (3.70 %), kidneys (1.85%), heart (1.85 %), brain (2.77 %) and bursa of Fabricius (1.85 %). Isolations were also undertaken from dead in shell (9.39 %), embryos (11.52 %), egg shells (4.33 %), egg contents (14.78 %), hatchery fluff (16.19 %), fecal material (8.16 %), cloacal swabs (5.31 %), litter samples (14.42 %), poultry house dust (4.50 %), drinking water (21.08%), poultry feeds (11.89 %), fish meal (21.65 %), meat meal (27.65 %) and rodent feces (9.77 %). Higher prevalence of *Salmonella* in the sources is a serious threat for the development of our poultry industry.

Various antimicrobials, ampicillin, chloramphenicol, erythromycin, flumequine, furazolidone, gentamicin, kanamycin,

lincomycin, neomycin, streptomycin, Terramycin, Tribirssen and vibramycin were antibiographed against isolated 715 isolates. Seventeen (2.37 %) isolates were resistant to all the anti bacterials while 427 (59.72 %) were highly sensitive to all antibacterial and 103 (14.43 %) were intermediately sensitive. Flunequine proved to be drug of choice, as 668 (93.43 %) isolates were sensitive, 30 (4.19 %) intermedially susceptible and only 2.37 per cent were resistant. Vibramycin stood at number two to which only 4.33 per cent resistance was observed. According to the spectrum of susceptibility, maximum resistance (40.27 %) was observed against kanamycin, followed by tribrissen (38.74 %), furazolidone (37.20 %), Terramycin (32.16 %), erythromycin (26.01 %) and neomycin (22.23 %). Antibiography of each serotype was developed.

Pathological studies in infected birds was conducted, the salient pathologic changes were observed in liver, intestines, spleen, lungs, kidneys, ovaries, heart, ceca, brain and bursa of Fabricius. Regardless of the serotypes involved gross, histo and ultrastructural pathological lesions were almost same but variable in intensity. Most of the liver had bronze discoloration, fragility, enlargement, hemorrhage, congestion, degeneration, necrosis, cellular infiltration and hyperplasia of kupffer cells. Single cell necrosis, fatty degeneration and

hyperplasia of biliary epithelium were the salient changes. Almost identical changes were observed in other organs. In spleen dark red discoloration, hypertrophy, necrosis, friability, congestion, hemorrhages and cellular infiltration in all the organs was observed, except capsule thickening was salient feature of spleen. In bursa of Fabricius degeneration, necrotic changes along with connective tissue proliferation was observed.

Ultrastructurally breakage of nuclear membrane, alteration of organellae, exvagination of chromatin, fragmentation of chromatin material, necrotic lesions, dystrophic alteration, cytoplasmic modification, mitochondrial elongation and endoplasmic reticular changes were the salient features.

CHAPTER 1

INTRODUCTION

Salmonellosis caused by *Salmonella* species has been recognized as a worldwide problem in both man and animals. *Salmonella* infections occur in many kinds of birds and mammals (Garg and Sharma, 1979); frequently recorded in poultry. It also occurs in rats, mice and other rodents, in many reptiles and some insects (Sing *et al.*, 1980). Both domestic and wild poultry are vulnerable to *Salmonella* infections (Javed *et al.*, 1990). More than 2300 known *Salmonella* serotypes have so far been reported in the world, which suggests a ubiquitous nature of *Salmonella* (Edwards & Ewing, 1989). Poultry and poultry products constitute one of the major reservoirs of *Salmonella* infections since more than 50 per cent of the serotypes have been isolated from these sources alone (Kohler *et al.*, 1979). Two serotypes i.e. *Salm. pullorum* and *Salm. gallinarum*, causative agents of pullorum disease and fowl typhoid respectively, are of great economic importance due to high mortality, lowered egg production and reduced hatchability (Javed *et al.*, 1992). Pullorum disease is characterized by white diarrhoea and high mortality in young birds (Javed & Hameed, 1989).

Salmonellosis in young adult chicken produces fewer clinical signs, but localization of *Salmonella* goes upto 74 per

cent in non intestinal tissues among which ovary (13 %) is the predilection site. Transovarian transmission in poultry is probably the primary means of its spread to the offspring. The infected hens mostly laid eggs with contaminated shell and/or contents. However, there is still some contovers over whether, in the field most eggs are infected by contamination with intestinal contents during passages through the cloaca and/or by infection in the ovary (Barrow, 1991). Chicks hatched contaminated or infected soon after hatching excrete more *Salmonella* organisms and for a longer periods than do adult birds. This is attributed to the inhibitory activities of the complex microflora of the adult ceca (Barnes and Impey, 1980).

The principal reservoir of salmonellae are animal species which may infect human being *via* ingestion of contaminated food or direct exposure (Drapeau and Jankovic, 1977). Practically all animals (Welchman, 1987), domestic aviary (Javed *et al.*, 1990), wild birds (Javed *et al.*, 1992), rodents (Siddique *et al.*, 1985a), and insects can host salmonellae (Williams *et al.*, 1980). Refuse from hospitals (Leclerec and Oger, 1974) and slaughter houses can contaminate water (Leclerec and Oger, 1975) which may support bacterial multiplication (Wright, 1989). Salmonellae remain viable in sludges, which could be a potential contaminant for streams and other water reservoirs.

Consumption of *Salmonella* contaminated meats and poultry products, resulted in health care cost of $1000 million in the United States in 1987 and 9,00,000 £ in the United Kingdom (Yule *et al.*, 1988). The incidence of human *salm. enteritidis, Salm. virchow* and *Salm. stanley*, has risen significantly between 1981 and 1986. Again poultry remains a major vehicle of disease transmission, however, bovines also contribute in cross-species infections (Humphrey and Lanning, 1988). Poultry-borne salmonellosis is the most common form of food borne infection in Scotland (Yule *et al.*, 1988).

Salm. typhimurium has been described as a facultative intracellular parasite that resides within the macrophages. However, there is controversy over the major location of *Salm. typhimurium* multiplication *in vivo*. Blood clearance occurs within the first few hours after intravenous injection of *Salm. typhimurium*. The surviving *Salm. typhimurium*, which accounted for approximately 5 per cent of the injected bacteria became localized in liver and spleen (Swanson and O'Brien, 1983). The exact localization of *Salm. typhimurium* within these organs is not known, but the rate of multiplication of the surviving *Salm. typhimurium* is controlled by the locus *ity*. Once the salmonellae are in the reticulo endothelial system (RES), host killing of *Salmonella* almost ceases, at least after the first 48 hours

(Benjamin *et al.*, 1990). The ability of *Salmonella* within the spleen and liver to grow in relative essence of killing suggests that the surviving *Salmonella* have reached a safe-site which is probably instrumental in their ability to cause disease. However, direct evidence for a cellular location of *Salmonella* during the early stages of enteric fever is missing. It is reported that the *Salmonella* resides in the spleen and liver either extracellular or in the non-professional phagocytes (Nakoneczna and Hsu, 1980). Vast majority of *Salmonella* are found in the liver and spleen cells within 24 hours post ingestion (Dunlap *et al.*, 1991).

A key pathogenic mechanism of *Salmonella* is their ability to invade the cells of the intestinal epithelium. Electron microscopic studies of the *Salmonella* infected animal tissues (Takeuchi, 1967) and cultured cells (Kohbata *et al.*, 1986) have shown that these organisms enter epithelial cells after transient disruption of their surface microvilli. Bacteria are later seen within the endocytes. Instead, it appears that *Salmonella* strains translocate through the epithelial cell in membrane bound vesicles to later exit at the basolateral surface of epithelium.

Accurate diagnosis is an essential prerequisite in the assessment of the true extent of this recently introduced infection in the poultry. The detection of *Salmonella* in flocks of laying hens has thus become a public health priority and a matter of great concern to egg producers. Testing for the presence of specific serum antibodies in an important aspect of proposed progression of identifying *Salmonella*-positive flocks. The National Poultry Improvement Plan authorizes the use of a variety of macro and microagglutination techniques, for the detection of *Salm. pullorum* antibodies in chicken. Paratyphoid *Salmonella* serotypes, such as *Salm. enteritidis*, generally elicit weaker antibodies responses (Williams and Whittemore, 1975). Conventional agglutination tests have not been much effective for detecting paratyphoid infections in chickens (Olesiuk and Carlson, 1969 and Williams, 1975), perhaps because many such infections in mature birds are limited to colonization of the alimentary tract. Very young chickens are far more susceptible to paratyphoid salmonellae, but the antibody response by chicks has been observed to be insufficient for serological detection of infection. Serological methods have been reported to vary in sensitivity and reliability, but all were found to be more sensitive than cloacal swab cultures for the detection of paratyphoid *Salmonella* infections in chickens. Evidence of systemic infection with *Salm. enteritidis* suggests

that infected hens are likely to have antibody titers high enough to permit efficient serological detection (Gast and Beard, 1990a).

Current practices in poultry production are aimed at breaking infection cycles, such as by producing *Salmonella*-free animal feeds, more stringent control of farm hygiene, treatment of processed carcasses and increasing public awareness of food poisoning hazards (Humphrey *et al.*, 1988). However, *Salmonella* contamination is still a major problem in poultry production. One solution proposed to reduce the incidence of *Salmonella* is through competitive exclusion. However, there are important differences between field and laboratory trials, and these have frustrated the application of this treatment. Reports on the effect of antibiotic additions is limited (Khoshoo *et al.*, 1989 and Wray *et al.*, 1991). Control measures must not only prevent infection in the poultry themselves but must also take into account the extensive cross contamination that occurs during carcasses processing. Some microbiological techniques for reducing infection are currently available. However, these have only been used in UK and USA in a fragmentary fashion and most recently in Pakistan. Some control measures such as chemotherapy have to some extent, been shown to be useful under laboratory conditions but have yet to prove themselves in the field.

Controlling bacterial infection in the animals by the use of bacteriophages needed to be tested in poultry. In view of the renewed interest in salmonellosis, generated by the recent epidemic of egg-associated *Salmonella* infection in the world, it is opportune to consider the prospects for control (Kuhl, 1989 and Mandl *et al.*, 1987).

Previously a few limited reports on the salmonellosis in poultry are available in which extensive studies were lacking on the chicken breeders in Pakistan. A little work has been done on the isolation and identification of *Salmonella* in Pakistan. Bashir and Barya (1969) isolated *Salmonella* from 7.75 per cent of the fecal samples of poultry in and around Faisalabad. Athar (1982) studied the incidence of *Salmonella* in poultry and poultry feed during 1976 to 1982 in and around Karachi. *Salm. pullorum* among broiler breeder in Pakistan was extensively studied on large scale for the first time (Javed and Hameed, 1989 and Siddique *et al.*, 1989). Prevalence of *Salm. typhimurium* in pigeons (Siddique *et al.*, 1985b) and zoological garden birds and avifauna for the first time reported in Pakistan (Javed *et al.*, 1992). Although there seems high incidence of salmonellosis in our breeder flocks but only few limited reports are available (Nafees, 1984, Sajid *et al.*, 1986 and Anjum, 1983). These studies were so small scaled that you cannot estimate the total

population of the chicken broiler breeders. Furthermore, these studies utilized small number of birds which may not be true indicator of *Salmonella* problem at total population level in broiler breeders.

The present project was designed to investigate the prevalence of salmonellosis in chicken broiler breeder flocks. Specific objectives of this study were to:

1) determine the sources of *Salmonella* infection in broiler breeders.
2) isolate and identify *Salmonella* serotypes from broiler breeders.
3) improve methods for isolation of *Salmonella* and accurate diagnosis of salmonellosis.
4) test pathogenicity of *Salmonella* isolates
5) characterize *Salmonella*-specific pathology in infected birds
6) to suggest measures to eliminate this insidious problem of our poultry industry.
7) study the antibiography of the isolate.
8) evaluate the different control measures such as vaccination, Na EDTA supplementation, competitive exclusion, carriers elimination, evaluation of the disinfectants.

Salm. typhimurium is one of the predominant bacteria affecting poultry. Transmission through the hatchery egg may produce either clinical or subclinical infections in chicken younger than 1 week of age (Humphrey *et al.*, 1989b). It has been shown that penetration of *Salm. typhimurium* through the cuticle, shell, and shed membranes occurs very rapidly and that bacterial penetration is greatly influenced by the presence of moisture on the egg shell, either as liquid or as water vapor (Kim *et al.*, 1989). Much more attention has been given to contamination of hatching eggs with moist feces contaminated with salmonellae as an important link in the epizootiology of avian salmonellosis (Williams *et al.*, 1980). Most of the bacterial penetration studies have been performed using eggs several hours after they had been laid (Mario, 1990b).

Epidemiological investigations have implicated raw or uncooked eggs as the vehicle of transmission of *Salm. enteritidis* to consumers in a high percentage of outbreaks. Contaminated eggs, in some instances, have been traced back to cloaca, of laying hens that were culturally or serologically positive for *Salm. enteritidis* (Gast and Beard, 1990c). In United Kingdom, human salmonellosis has been associated with the consumption of foods containing improperly cooked shell eggs contaminated with *Salm. enteritidis.* Isolates from the ovaries

of British laying hens which produces eggs implicated in human outbreaks and has also been responsible for significant mortality in broiler chicks (Lister, 1988). Epidemiologically significant isolates from poultry and humans in the United States cause vertically transmissible systemic diseases in chickens (Gast and Beard, 1990b and Shivaprasad *et al.*, 1990).

CHAPTER 2

REVIEW OF LITERATURE

2.1. HISTORY AND DISTRIBUTION

2.1.1. HISTORY

The name *Salmonella* resulted from the willingness of Dr. Daniel E. Salmon, Director of the Bureau of Animal industry in Washington in the late 1800's to allowed his name to be associated with the bacterium that Theobold Smith, his otherwise brilliant assistant, had mistakenly identified as the cause of "hog cholera" (Grady and Keusch, 1971). It must have been beyond their imagination that despite 102 years of study the pathogenesis of *Salmonella* would still remain an enigma to the scientists. *Salm. typhimurium* and related non-typhoid salmonellae are among the most common causes of food-borne infectious diseases and the economic impacts of *Salmonella* outbreaks are also very large. Salmonellae are subdivided by biochemical characters into so called subgenera of Kauffmann *et al.* (1960). The subdivisions correspond more closely to species or subspecies in other groups of bacteria which were later confirmed by Rohde in 1965 (Baloda, 1987). Kelterborne (1979) revised the kauffmann-white scheme in 1983. He listed 2,104 species of *Salmonella*, compiled from 17 published sources and author's own records for 1.5 million European strains. Bacterium sanguinarum *(Salm. gallinarum)* was first to be isolated from heart blood of baby chicks in different flocks (Beaudette and Brunswick, 1925). Later Cooper and Niak (1931) reported *Salm. gallinarum* isolated from chicks in India. Abdus-Salam and Haq (1950) isolated gram negative bacilli (*Salmonella*) from the heart blood of chickens died or undiagnosed disease in Pakistan. Quddus (1962) reported an outbreak of salmonellosis in 16 weeks old birds in Sudan.

2.1.2. INCIDENCE

2.1.2.1. INCIDENCE OF SALMONELLLOSIS

Salmonella infections have been reported in all species of domestic poultry and many species of wild birds (Javed & Hameed, 1989). Infections have also been recorded in pigeons (Siddique *et al.*, 1985b), parrots, sparrows, canaries and many species from zoological gardens (Javed *et al.*, 1990, 1992; Sojka and Field, 1970 and Bouzouba and Nagaraja, 1984).

Status of *Salmonella* infection varied from country to country. *Salmonella* was isolated from birds which died or became morbid within two weeks of importation into Japan from the Republic of Indonesia, the Netherland and India. During 1980 birds, finches, lorries and parakeets revealed *Salm. typhimurium*. A high infection rate was found in the birds imported from Indonesia. The most prevalent primary biotype isolated was one which was also isolated from food poisoning outbreaks in man (Sawa *et al.*, 1981). Athar (1982) studied the incidence of salmonellosis in poultry, poultry feeds and feed ingredients during 1976-82 in and around Karachi, Pakistan. Occurrence of salmonellae after transportation from the hatcheries to the farms is a common problem, Tests were conducted over a year on 50 batches of chicks. *Salmonella* were recovered from the cardboard containers (Escober 1982). Three thousands cloacal swabs from breeding flocks during 1980-81, 10 yielded *Salm. typhimurium*, 10 *Salm. enteritidis*, 3 *Salm. paratyphi* C and 3 *Salm. thompson* (Shahata *et al.*, 1983).

Occurrence of *Salmonella* among wild birds caught in Italy, *Salm. typhimurium* was recovered from cloacal swabs, intestine, liver or spleen of 3 birds. Out of 229 birds belonging to 30 species, *Salm. bovis-morbificans* was isolated in a Turdus

morula. In addition, 8 of 28 gulls harbored five *salmonella* serotypes (Modugno *et al.*, 1986). *Salmonella* serotypes were prevalent in Romania during the year 1971-1980. In Romania a total of 103, 803 *Salmonella strains* identified upto 1980. Among them 82.88 per cent were of human origin 8.02 per cent were isolated from animals, 6.73 per cent from food and food stuffs and 2.37 per cent from various environmental sources (Negut *et al.*, 1983). The five most frequently isolated serotypes were *Salm. typhimurium, Salm. cholesresuis, Salm. typhimurium* var. copenhagen, *Salm. anatum,* and *Salm. heidelberg.* The most common species of origin, in decreasing order of frequency, were cattle, swine, turkeys and fowls. *Salmonella* carriers in ducklings in Egypt were detected frequently (Shahata *et al.*, 1983 and El-Din *et al.*, 1987).

In Sweden a total of 1266 outbreaks of salmonellosis were recorded between 1978 and 1982, including 687 in cattle, 37 in swine, 220 in poultry and 190 in wild animals. Among 78 different serotypes were identified, the commonest being *Salm. typhimurium* (38.5 %) and *Salm. dublin* (37 %). Twenty five serotypes had never been isolated previously in Sweden, 524 strains were recovered from home-produced feed (Martensson *et al.*, 1984). Moitra and Sexena (1984) recovered 18 strains of 7 serotypes from 460 cloacal swabs from apparently healthy fowls.

Incidence of *Salmonella* carriers in broiler flocks in Australia remained a big problem. In 20 broiler flocks at time of slaughter during a year, the incidence of *Salmonella* carriers was (30 %) in 4 flocks, and a low proportion (1-10 %) in 2 flocks. No *Salmonella* was isolated from 8 flocks. Five *Salmonella* serotypes were isolated, with *Salm. typhimurium* the commonest. *Salmonella* was much less frequently isolated from flocks reared on the old litter than on new littre, (Soerjadi *et*

al., 1981). In Finland and Denmark between 1970 and 1980 *Salm. infantis* accounted for 2918 of the 3539 Finnish isolates while *Salm. typhimurium* accounted for 296 isolates; 16 other serotypes were isolated in small number. A single hatchery was the source of *Salm. infantis* infection. Contaminated feed did not seem to have been the source (Vasa, 1984). Between 1980-1984 over 150 strains of *Salm. typhimurium*
var. copenhagen were recovered from outbreaks of infection in France (Vaissaire *et al.*, 1984).

Serological survey of *Salm. pullorum* in Zaria and Nigeria, 480 apparently healthy chickens (160 of local and 320 of exotic breeds) from free range, semi intensive and intensive management flocks were screened for antibodies to *Salm. pullorum* (Adesiyun *et al.*, 1988). Presence of *Salmonella* is not out of reach on traditional poultry farms in Southern Italy. In a total of 224 small traditional poultry flocks, *Salm. pullorum-gallinarum* was isolated from fecal samples in nine (4 %) and *Salm. typhimurium* in one flock (0.4 %) (Ianieri, 1984). In Germany *Salmonella* isolation was undertaken in cattle faeces, feed meals of animal origin, poultry wild and aviary birds, domestic and wild animals each year from 1977 to 1983. Among 2603 isolates belonging to 50 serotypes, 53 per cent were *Salm. typhimurium*, 4.5 per cent *Salm. typhimurium* var copenhagen, 18.5 per cent *Salm. dublin* and 7.9 per cent *Salm. tennessee* (Schellner, 1985). Incidence of *Salmonella* serotypes varied considerably in different broiler flocks and decreased with the advancement of age. Serotypes originating from the hatchery were less important in the final stages than those introduced in poultry house by number of contaminated vectors during rearing period (Lahellec and Colin, 1986). During 1976-1984 in England 26219 incidents of salmonellosis among which poultry involved in 91 per cent cases. Among the poultry birds, *Salm. typhimurium* was the commonest

serotype in broilers. Bone meal was most frequently contaminated with *Salm. montevideo*, the common serotype (Kirby, 1985).

In Assam, India, 28 *Salmonella* strains were recovered from 16 (10.66 %) of 150 dead poultry birds of which 12 were *Salm. chester*, 11 *Salm. 4, 12: eh* and 5 *Salm. 4, 5, 12: eh.* Most of the strains were from liver (11) and intestine (9), followed by spleen (5) and heart (3) (Saikia and Patgiri, 1986). In New Caledonia, 20 outbreaks of salmonellosis in poultry have been observed since 1981. *Salm. typhimurium* was isolated in 50 per cent of cases, *Salm. london* in 20 per cent, *Salm. muenchen* in 20 per cent *Salm. arizona* in 10 per cent whereas, *Salm. gallinarum* or *Salm. pullorum* have never been isolated, *Salm. typhimurium* and *Salm. virchow*, were the most common serotypes prevalent (Desoutter, 1986).

Occurrence of *Salm. virchow* in chicks is panic in Nagaland (India). A progressive mortality in white leghorn and Rhode Island chicks 2-4 weeks of age occurred in an organized poultry form in January-March, 1986 (Ghosh, 1987). *Salm. gallinarum* was reported in broiler parent stock for the first time in Tamil Nadu. During 1987, the mortality rate was 5 to 10 per cent in chicks produced at a hatchery. Fifteen per cent of birds in 3 breeder flocks (3500 birds) had infective agglutinating titres of *Salm. gallinarum* (Palanisami *et al.*, 1987). Al-Obaidi *et al.* (1987) surveyed a flock of native chickens in Iraq. During 18 months, *Salmonella* was isolated from 15 birds and one litter samples. Investigation of a broiler breeder flock which had been identified as infected with *Salm. enteritidis*, as a result of tracing from diseased infected progeny (Lister, 1988). *Salmonella* is prevalent in animals and animal feeds. Over a four-year period (1981-1985), 146342 samples of organs from birds, cattle, sheep and pigs, or of feed, were tested for

salmonellae. Among forty seven serotypes that were identified, the most frequent were *Salm. typhimurim* (18 %), *Salm. gallianrum-pullorum* (16 %), *Salm. virchow* (13 %), *Salm. choleraesuis* (11 %) *Salm. enteritidis* (8.5 %)and *Salm. heidelberg* (7 %). More than three times as many salmonellae (27 strains) were isolated from birds as from cattle or pigs. *Salm. heidelberg* was the most common finding in feed samples (Topolko *et al.*, 1988).

In Pakistan, rapid hemagglutination testing of 150 chicken broiler breeder flocks having 2,62,454 birds revealed a high prevalence of *Salmonella* seropositive birds. Among these, 12,159 (4.63 %) birds were screened as carriers from 112 positive flocks. The prevalence of *Salmonella* carriers varied in birds reared on various commercial feeds in chicken of different breeds and those maintained on the varying standards of management (Javed and Hameed, 1989). The persistence of *Salmonella* in a variety of zoological garden birds was investigated. Among 370 rectal swabs examined, 66 yielded different *Salmonella* serotypes which includes 30 strains of *Salm. typhimurium,* 23 *Salm. gallinarum-pullorum.* 5 *Salm. saint-paul,* 5 *Salm. butantan* and 3 *Salm. eastbourne.* Parrots, pigeons, Java sparrows, quails, peacocks, doves and pheasants were the common birds positive for *Salmonella* (Javed *et al.*, 1992a). In Pakistan, isolation and pathological studies were conducted in 753 enlarged livers, spleens and intestines of indigenous chickens, salmonellae were isolated mostly from intestines (9.96 %), followed by liver (5.97 %) and minimum (1.19 %) in spleen. An overall isolation incidence was 5.71 per cent *Salm. gallinarum-pullorum* and *Salmonella* of group E were the most common isolates (Javed, *et al.*, 1990). Incidence of salmonellosis have been summarized in Table 1a,b,c,d,e.

Table 1a: Prevalence of Salmonella in Asian Countries

Geographical area & reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
PAKISTAN			
Abdus Salam & Haq,1950	chicken	Salmonella	1 isolate
Qureshi et al., 1981	chicken	pullorum, gallinarum	
Athar, 1982	feed & feed ingredients	gallinarum	13.85 %
		pullorum	2.99 %
Anjum, 1983	broiler breeder	gallinarum	4.50 %
		pullorum	0.9 %
Nafees, 1984	broiler breeders	pullorum-gallinarum	5.64 %
Tariq & Anjum, 1984	fish meal	Salmonella	5.99 %
	meat meal	"	28.57 %
	tankage meal	"	52.00 %
	blood meal	"	5.88 %
	bone meal	"	38.48 %
Chishti et al., 1985	poultry livers	pullorum	22.00 %
		gallinarum	11.00 %
Siddique et al., 1985	pigeons	typhimurium	5.94 %
Sajid et al., 1986	broiler breeder	gallinarum	0.14 %
		pullorum	0.21 %
Rehman et al., 1987	buffaloes	typhimurium	2.00 %
		heidelberg	2.2 %
Anjum et al., 1989	cattle feces	saint-paul	0.5 %
		typhimurium	0.33 %
		reading	0.16 %
		chester	0.16 %
		butantan	0.16 %
Javed & Hameed, 1989	broiler breeder	Salmonella	4.63 %
Javed et al., 1990	indigenous chicken	gallinarum	1.32 %
		pullorum	2.78 %
		typhimurium	1.59 %
Javed et al., 1992	parrots	typhimurium	0.27 %
		gallinarum	2.7 %
		saint-paul	0.81 %
		butantan	0.27 %
		eastbourne	0.27 %
	Java sparrows	gallinarum	0.27 %
		butantan	0.27 %
		eastbourne	0.27 %
	quails	typhimurium	0.54 %
		gallinarum-pullorum	0.81 %
	peacocks	gallinarum-pullorum	0.81 %
		saint-paul	0.27 %
	doves	typhimurium	0.27 %
		gallinarum-pullorum	0.27 %
	Pheasants	gallinarum-pullorum	0.81 %

Geographical area & reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
	Others species of birds	typhimurium, gallinarum, pullorum, saint-paul, butantan	2.97 %
INDIA			
Cooper & Niak, 1931	chicks	gallinarum	-
Garg & Sharma, 1979	calves	Salmonella	15.52 %
Singh et al., 1980	rodents	bareilly	6.2 %
	shrew	newport, welteverden	10.60 %
	cockroackes	enteritidis, typhimurium	1.11 %
	ants	hivitting foss, anatum	70.00 %
	mice	matopeni, waycross paratyphi B.	10.09 %
Sharma et al., 1980	mynahs, parrots,	saint-paul	4.00 %
	house sparrows,	bareilly	3.00 %
	swallow, crow,	welteverden	2.00 %
	grey partridge	typhimurium,	2.00 %
		E_1 group	1.00 %
Moitra and Sexena, 1984	poultry	alachus, saint-paul, newport, anatum	3.91 %
Nag and Koley, 1986	poultry carcasses	anatum	4.3 %
		welteverden	1.3 %
		binza	1.00 %
		orion	0.33 %
		paratyphi B	0.33 %
		matopeni	0.33 %
Saikia and Patgiri,1986	poultry birds	chester	24.00 %
		4,12, eh	22.00 %
		4,5,12, eh	10.00 %
Rao et al., 1986	poultry	indiana	-
Ghosh, 1987	layer chicks	virchow	26.42 %
IRAQ			
Al-Obaidi et al., 1987	chicken flocks	Salmonella	15 birds
	litter	Salmonella	1 sample
	chicks	neuikerk	26.6 %
		montevideo	20.00 %
	litter	java	1 isolate

Geographical area & reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
JAPAN			
Sawa et al., 1981	chicks	typhimurium	14.5 %
	finches	typhimurium	18.00 %
	lories	typhimurium	27.00 %
	parakeets	typhimurium	1.6 %
Venkateswaran et al.,1988	chicken meat	hadar, typhimurium paratyphi B	58.33 %
	beef, pork,	-	0.00 %
	shell fish	-	0.00 %
SRILANKA			
Palanisami et al., 1987	chicks	gallinarum	15.00 %
USSR (RUSSIA)			
Kotova et al., 1988	chicken	Salmonella	16.00 %
	ducks	Salmonella	12.00 %
	meal processing	typhimurium	50.00 %
		newport	14.1 %
		enteritidis	7.6 %
	sheep	enteritidis	7.6 %
	shepherds	typhimurium, enteritidis, Java	-
	farm employees	enteritidis, typhimurium newport, dublin	-

Table 1b: Prevalence of *Salmonella* in Europian Countries

Geographical area and reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
BULGARIA			
Stefanov et al., 1987	poultry	typhimurium oranienberg gallinarum	200 isolates
ENGLAND (UK)			
Sojka and Field, 1970	chicken	gallinarum pullorum	80.50 % 12.80 %
Kirby, 1985	poutry as a vector of infection	typhimurium, montevideo	91.00 %
Smyth and Watson, 1987	hatchery, day-old, chicks, egg shell, floor	infantis, typhimurium enteritidis	0.5 %
	hatchery attendents	typhimurium enteritidis	52.00 %
Cooper et al., 1989	chicks	enteritidis typhimurium	- -
Harwood, 1989	rabbit	typhimurium	26.7
Hosie and Grant, 1990	Pheasants	enteritidis	18.18 %
FINLAND & DENMARK			
Vasa, 1984	chickens	infantis typhimurium	82.4 % 8.4 %
FRANCE			
Vaissairie, 1984	poultry	typhimurium	150 isolates
GERMANY			
Schellner, 1985	cattle feces, feed, poultry, aviary, wild animals.	typhimurium typhimurium var copenhagen dublin tennessee	53.00 % 4.5 % 18.5 % 7.9 %

Geographical area & reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
GREECE			
Iliadis, 1987	chicken, turkey	pullorum, orion	-
		typhimurium	-
HUNGARY			
Jayarao et al., 1989	pig	typhimurium	36.8 %
		derby	4.3 %
		bredney	2.16 %
		agona	1.08 %
		infantis	4.8 %
		london	1.60 %
		panama	1.08 %
ITALY			
Modugno et al., 1986	wild birds	typhimurium	1.31 %
	turdus merula	bovis-morbificans	0.43 %
	gulls	Salmonella	3.49 %
Lodetti and Zavanella, 1990	eggs	typhimurium	1.6 %
NETHERLAND			
Netherlands, 1989	poultry egg	enteritidis	6 flocks
ROMANIA			
Negut et al., 1983	animal	gallinarum	21.76 %
		cholerae suis	16.59 %
		enteritidis	16.47 %
		typhimurium	9.99 %
		abortus ovis	8.83 %
		london	3.09 %
		heidelberg	2.39 %
		anatum	1.99 %
		infantis	1.99 %
		agona	1.67 %
		other 31 serotypes	15.23 %
Siddique et al., 1985a	chicken	typhimurium	6.01 %
		heidelberg	
		blockley, anatum, agona, remo, newport, bredeney, infantis	

Geographical area & reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
SWEDEN			
Martensson et al., 1984	poultry	typhimurium	38.5 %
		dublin	37.0 %
	feed	typhimurium, dublin	524 cases
SWITZERLAND			
Breer, 1985	untreated sludge	Salmonella	97.00 %
	sweage disposal	Salmonella	207 cases
	slurry of cattle	Salmonella	1.3 %
	fecal sample of poultry	Salmonella	24.5 %
YUGOSLAVIA			
Simko, 1985	broiler farms	typhimurium	28.00 %
		agona	23.00 %
		enteritidis	10.00 %
Girao et al., 1985	chicks	pullorum	2.85 %
		gallinarum	0.79 %
		saint paul	0.38 %
		jaffina	0.26 %
		typhimurium	0.12 %
		berta	0.12 %
	meat meal	Salmonella	19.6 %
	feather meal	Salmonella	9.67 %
	feed	saint paul	7.69 %
Mrden et al., 1987	liver of chicks	typhimurium	5.15 %
		virchow	4.21 %
		enteritidis	2.5 %
		heidelberg	1.4 %
		infantis	0.56 %
		bredeney	0.37 %
Topolko et al., 1988	broiler breeder,	enteritidis, typhimurium	8.5 %
	cattle, sheep, pigs	typhimurium	18.0 %
		gallinarum pullorum	16.00 %
		virchow	13.00 %
		choleraesuis	11.00 %
	feed	heidelberg	7.00 %

Geographical area and reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
Mrden and Glavicic, 1988	eggs	virchow	7.1 %
	embryos	virchow	7.8 %
Novak, 1990	rendering material	typhimurium enteritidis,	0.5 %
	fish meal	anatum, agona, harona, senftenberg	1.9 %
	broiler breeder	enteritidis, virchow, typhimurium	3.3 %
	breeder hens	typhimurium	10.6 %
	commercial layer	typhimurium	8.0 %
	turkeys	typhimurium	20.8 %
	ducks	typhimurium,agona	13.07 %
	partridges	virchow	-
	pheasant	pullorum	-
	indigenous chicken	typhimurium, pullorum	7.6 %
	hatching eggs	virchow, enteritidis	4.0 %
	table eggs	orion, virchow, enteritidis	2.5 %
	poultry liver	virchow, typhimurium, anatum, enteritidis, agona, heidelberg, munchen, livingstone,	8.5 %
	chicken meat	virchow, typhimurium, living stone, senftenberg, enteritidis	5.20 %
	Minced meat	typhimurium	1.5 %
	salami	typhimurium	0.7 %

Table 1c: Prevalence of Salmonella in African Countries

Geographical area and reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
EGYPT			
Shahata et al., 1983	duck breeders	typhimurium	0.33 %
		enteritidis	0.33 %
		paratyphi C	0.1 %
		thompson	0.1 %
	ducklings	typhimurium, enteritidis	1.8-8.4 %
Safwat et al., 1986	ducklings	typhimurium	-
Hamed et al., 1987	eggs	gallinarum	-
		pullorum	-
		virchow	-
NIGERIA			
Adesiyun et al., 1988	sheep	colindale, rubislan	4.0 %
	goat	poona, kintambo, sanktgeorg var i, stanbyville, hull	9.5 %
	local and exotic chicken	pullorum	37.1 %
SUDAN			
Quddus, 1962	chicken	gallinarum	2.88 %
Yagoub et al., 1987	chicken	mons amek	-
		uganda	3.89 %
UGANDA			
McAnulty, 1958	animals	Salmonella	2.0 %

Table 1d: Prevalence of Salmonella in North and South American Countries

Geographical area and reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
CANADA			
Swan et al., 1968	poultry	pullorum, typhimurium , heidelberg	0.58 %
Mutalib and Hanson, 1989	pigeons	typhimurium	3 outbreaks
	broiler chicks	typhimurium	1 outbreaks
	mouse	mrandaka	-
		thompson	-
MEXICO			
Mario, 1990a	broiler breeder	gallinarum-pullorum	2.8 %
		typhimurium	2.8 %
USA			
Bivini, 1948	poultry	pullorum	202 isolates
Tablante and Lane, 1989	dairy herd	dublin	16.8 %
	mice	dublin	103 cases
Tay, 1989	Sows (lymph node)	agona, Java	84.0 %
Opengart, 1991	turkey breeder flocks	arizona	33.3 %
		saintpaul, heidelberg, berta, hadar	86.36 %
Waltman, 1991	day-old chicks	Salmonella	5.15 %
	carcasses	Salmonella	14.2 %
	reactor (breeder)	Salmonella	13.93 %
	hatchery fluff	Salmonella	12.8 %
	hatching eggs	Salmonella	12.53 %
	drag swab	Salmonella	16.77 %
	litter, nest, soil	Salmonella	14.92 %
	dead-in-shell	Salmonella	35.48 %
	water	Salmonella	0.71 %
	feeds	Salmonella	0.71

Table 1e: Prevalence of Salmonella in Australian Countries.

Geographical area and reference	Source of infection	Serotypes of Salmonella isolated	Prevalence
AUSTRALIA			
Soerjadi et al., 1981	chicken broiler flocks	typhimurium	0.30 %
Murray, 1986	chicken	sofia	10217 cases
	man, animal	typhimurium	-
NEW CALEDONIA			
Desoutter, 1986	poultry	typhimurium	50 % isolates
		london	20 %
		muenchew	20 %
		arizona	10 %

2.1.2.2. INCIDENCE OF SALMONELLA SEROTYPES

Salmonella serotypes can be categorized according to the frequency of isolation. Kelterborn (1979) analyzed the incidence of *Salmonella* serotypes isolated from 109 countries during 1934-1978 and divided the various serotypes into quite frequent, frequent, rare and quite rare categories. *Salm. paratyphi, Salm. typhimurium, Salm. heidelberg, Salm. infantis, Salm. typhi, Salm. enteritidis, Salm. dublin, Salm. panama* and *Salm. anatum* were categorized as quite common serotypes. Barros and Martins (1984) isolated 23 of *Salm. typhimurium*, 19 of *Salm. typhimurium* var copenhagen, 26 of *Salm. enteritidis*, 10 of *Salm. berta* and 7 of *Salm. havana*. Among 124 total isolates 101 were from poultry, *Salm. pullorum* have several serological variants. Bivini (1984) isolated 202 strains from birds. Persistence of *Salmonella* strains most frequently isolated from animals was recorded in the years 1976-1978 *Salm. typhimurium* was isolated from 222 cases, *Salm. dublin* 250, *Salm. choleraesuis* 188, *Salm. enteritidis* 61 and *Salm. gallinarum-pullorum* in 73 cases, (Haszowski and Truszynski, 1980). Among *Salmonella* species most frequently found in poultry farm employees was *Salm. typhimurium* while *Salm. newport, Salm. enteritidis* and *Salm. dublin* were also isolated (Kotova *et al.*, 1988). Girao *et al.* (1985) isolated salmonellae from meat meal, feather meal, hatchery meal and finished feed. *Salm. saint-paul Salm. senftenberg, Salm. anatum, Salm. dublin, Salm. infantis, Salm. gallinarum-pullorum, Salm. jaffina, Salm. typhimurium and Salm. berta* were the common feed contaminant (Yaziz and Awang, 1985).

Occurrence of 451 strains of salmonellae belonging to 17 serovars were isolated from 61 farms in Slovakia between 1971 and 1981. The commonest was *Salm. typhimurium*, followed by *Salm. agona* and *Salm. enteritidis* (Simko, 1985). *Salm. worthington* was

isolated from the meat meal component of the grower ration. *Salm. infantis, Salm. typhimurium, Salm. heidelberg, Salm. schwarzengrund and Salm. albany* were other contaminants (Rigby, *et al.*, 1980). *Salm. enteritidis* was isolated from 13 broiler breeder birds (Lister, 1988). *Salm. virchow* was isolated from eggs and embryos. Cultural examination of 350 eggs 385 dead embryos from poultry farms in the vojvodina autonomous province resulted in the isolation of *Salm. virchow* from 5 (7.1 %) of the 70 egg pools and 6 (7.8 %) of the embryo pools (Mrden *et al.*, 1988).

In samples taken at 7 chickens and 2 turkey farms during 14-58 months, *Salm. gallinarum* was the most frequently isolated of *Salmonella* species *Salm. pullorum, Salm. orion* and *Salm. typhimurium* were occasionally isolated (Iliadis, 1987). Stefanov *et al.* (1987) isolated 200 strains of *Salmonella* from Stara Zagora region of Bulgaria between 1982-1985, 40 belong to group B (mainly *Salm. typhimurium*), 61 to group C (including 38 of *Salm. oranienberg*), 89 to group D (including 57 of *Salm. gallinarum*) and 8 to *Salmonella* group E. 89 per cent of strains were sensitive to the bacteriophage, including groups B and E, 59 of group C and 69 of group D strains. Multi-resistant strains of *Salmonella* from eggs and embryos were isolated. Cultural examination of 660 eggs yielded 14 *Salmonella* isolates 5 *Salm. typhimurium,* 4 *Salm. pullorum,* 3 *Salm. enteritidis* and 2 *Salm. montevideo.* Presence of the R. factor in multi-resistant *Salmonella* strains isolated from eggs and their significance for human health (Becirevic and Popovic, 1987).

Among day-old chicks delivered from hatcheries salmonellosis is quite prevalent. Fifty deliveries of day-old chicks, were examined for *Salmonella* on arrival as part of routine microbiological monitoring. Paper floor inserts and

faeces from the transport boxes were immersed in peptone water and then cultured in two different enrichment media. *Salmonella* were isolated from six of the 50 samples, one isolated was identified as *Salm. muenchen* and the other five as *Salm. serovar. sieburg* (Nicklas, 1987). Twenty three *Salmonella* strains of six serotypes were isolated from 300 samples from 200 poultry carcasses (intestines and contents, bile and gall bladder). In order of prevalence, the serotypes were *Salm. anatum* (13), *Salm. welteverden* (4), *Salm. binza* (3), *Salm. orion, Salm. paratyphi B* and *Salm. matopeni* (1 each) Nag and Koley (1986). Contaminated eggs with *Salmonella* after I/V or oral inoculation of laying hens. *Salm. typhimurium* was not isolated from ovaries or eggs of 10 hens infected I/V or orally (Becirevic *et al.*, 1986).

Salmonella strains are well spread in nature and infections are practically possible in all the species of domestic and wild poultry. Kelterborn (1979) analyzed the incidence of *Salmonella* serotypes isolated from 109 countries. Intestines carries the maximum number of *Salmonella.* Among 790 intestinal content, 20 yielded different *Salmonella serotypes,* which included 10 strains of *Salm. saint-paul,* 4 of *Salm. bareilly,* 3 of *Salm. weltevreden,* 2 of *Salm. typhimurium* and one of *Salmonella* E_1 group. Nine mynahs, seven house sparrows, a swallow, a grey partridge, a parrot and a crow were positive for *Salmonella* (Sharma *et al.* 1980). Rodents and insects play vital role in the transmission of *Salmonella.* In a survey the 767 rodent, shrew, cockroaches and ants were examined 767 samples examined, 43 yielded different *Salmonella* serotypes. Salmonellae were isolated from 16 of 254 rats, 11 of 109 house mice, 11 of 104 shrews, 3 of 270 cockroaches and 21 of 30 ants. the serotypes isolated included *Salm. saint-paul, Salm. bareilly, Salm. newport, Salm. weltevreden, Salm. enteritidis,*

Salm. typhimurium, Salm. hvittingfoss, Salm. anatum, Salm. matopeni, Salm. waycross, Salm. paratyphi, while dual infection was detected in three shrews and a rat (Singh *et al.* 1980). Prevalence of *Salmonella* serotypes have been tabulated country wise in Table 1a,b,c,d,e.

2.2. ISOLATION AND IDENTIFICATION

2.2.1. ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF *SALMONELLA*

Salmonellosis is usually diagnosed by using different serological tests. However, confirmatory diagnosis can only be made by isolating the disease causing organism. *Salmonella* organisms are aerobic or facultative anaerobic and grow well on ordinary laboratory culture media. However, enrichment media is necessary for material containing few bacteria and sublethally injured organisms (Zecha *et al.*, 1977). Media commonly used for the growth of *Salmonella* include Eosin Methylene Blue (EMB). *Salmonella-Shigella* (SS), Mac-Conkey's (MC) and De-soxycholate (DSA) Agar. These media Posses inhibiting factors for the growth of Gram positive and the Gram negative organisms other than the members of the enterobacteriaceae. In addition, these media also contain indicators which help to distinguish lactose fermenting from non-lactose fermenting bacteria (Harvey and Price, 1982). Mac-Conkey's agar is considered a better medium for the isolation of salmonellae (Siddique *el al.*, 1983). However, it is recommended that for isolation studies at least two media should be employed. Cox and Williams (1976) proposed the use of Triple Sugar Iron (TSI) agar and lysine iron agar (LIA) media alongwith six fermentation sugars to screen *Salmonella* isolates for primary biochemical characterization.

Salm. pullorum is also aerobic and facultatively anaerobic and the optimum growth takes place at 37°C. On meat extract agar, the discrete colonies appear as smooth, glistening,

homogeneous, transparent and round to angular in shape. The organism produces acid with or without gas from different sugars, salmonellae destroyed in old litter due to higher concentration of ammonia leading to high Ph (Turnbull and Snoeyenbos, 1973). Hatchery dust and chick downs are good sources for the isolation of *Salmonella* in hatchery enterprises (Sari and Thain, 1984). *Salm. pullorum* produces acid with gas by fermenting dextrose and mannitol but does not ferment sucrose, dulcitol and maltose, no H_2S gas is produced in TSI agar medium. For routine diagnosis from commercial as well as breeder flocks, culled birds are the best source for isolation. According to other workers the chicks with evident lesions on liver, spleen or ovaries which have not been treated with any antibacterial during the last few days are the best sources (Bercea *et al.*, 1981 and Kim *et al.*, 1989).

Salm. gallinarum is aerobic and facultatively anaerobic and produces small, grayish, circular and transparent to colorless colonies having entire margins. In broth, these organism gave a uniform turbidity and a flocculent sediment. The other properties of this organisms include indole negative and variable production of H_2S gas. It also produces acid but no gas from different sugars (Breed *et al.*, 1974). *Salm. gallinarum* occurs in short rods of 0.3 to 0.6 µm length and 0.8-2.6 µm width with round ends. Gram negative motile and non-motile, non spore forming and non capsulated (Edwards and Ewing, 1989). *Salm. gallinarum* produces acid but no gas by fermenting dextrose, mannitol, maltose and dulcitol. It may or may not produces H_2S gas on TSI agar medium.

Salm. typhimurium is viable in different environmental conditions. *Salm. typhimurium* survived for up to 6 weeks in poultry feed and 2 weeks in litter when kept at 37°C. At room

temperature (23°C) survival was 18-19 months and at 7°C it was 20 months for both materials (Nashed 1986).

In a comparison of various enrichment media for the isolation of salmonellae from seagull cloacal swabs, the relative efficiency of three selective enrichment broths (Muller Kauffmann tetrathionate, Rappaport's and Selenite F) were investigated. Rappaport's broth as modified by vassiliadis for incubation at 43°C yielded the highest number of positive swabs and the widest range of serotypes. It was significantly more efficient than other broths (Fricker, 1984). Use of brilliant green agar containing sulphamandelate supplement detected *Salmonella* in each of 96 positive samples and was the most efficient medium, Brilliant green agar without supplement was the least effective medium. Desoxycholate citrate agar was considerably less inhibitory to *Salmonella* after ageing for four days. Ageing of other media had no effect on their ability to support the growth of *Salmonella* (Fricker, 1984). Original Rapport's medium (R medium) and Rappaport-vassiliadis medium (RV medium) for the isolation of salmonellae from meat products were compared. *Salmonella* was isolated from with at least one enrichment medium. RV medium (10 ml) inoculated with buffered peptone water pre-enrichment medium (0.1 ml) and incubated at 43°C was the most efficient (Vassiliadis, 1983 and Nashed, 1986).

Among the broths used for *Salmonella* enrichments, Rappaport's broth plus magnesium chloride was preferred to Muller-Kauffmann broth and broth media containing sodium selenite with or without brilliant green or strontium chloride (Rambach, 1990). *Salm. heidelberg* and *Salm. montevideo* artificially inoculated and stored for 7 weeks in dry feed, were recovered significantly more samples after pre-enrichment with

M-9 or buffered peptone than with the other procedures such as lactose broth with and without Tergitol and indirect enrichment in selenite cystine or modified tetrathionate broths (Perales and Ana, 1989).

2.2.2. SEROLOGICAL IDENTIFICATION OF *SALMONELLA*

A revised simplified scheme for the identification of *Salmonella* organisms was introduced in 1949 by Kauffmann. According to this scheme, the *Salmonella* antigens were divided into somatic 'O' and flagellar "H" type of antigens. All the members of the genus have an antigenic structure by which they can be recognized. On this basis those organism which were found positive serologically were considered to belong to genus *Salmonella* even if they differed biochemically (Wilson and Miles, 1964). Two modified methods for the identification of *Salmonella* somatic and flagellar antigens has been in practice recently (Rambach, 1990). Over a period of 2 years, both modified methods were found to be approximately three times less labor intensive. Furthermore, 43 "O" antisera reacted exclusively with organisms possessing homologous O antigens when the modified and two standard methods were used. As the antiserum dilutions used H antigen identification, H antisera did not react with O antigen or heterologous H antigens by either the modified or the standard method. Modified method was 20 times more accurate. In modified method antisera is 100 fold more diluted than standard method used for storage (Naguib *et al.*, 1989 and Spears *et al.*, 1990).

2.3. CLINICAL PICTURE

Salmonellosis is one among the most frequent bacterial infections of poultry. The clinical forms of infection are more prevalent during first few weeks after hatching (Barnes and Impey, 1979). Non-motile salmonellae may produce high morbidity

and mortalities at all stages of birds life span, while the infections with motile salmonellae usually results in mortalities during first few weeks of age and in adults infection is mostly non clinical. Experimental infection in day old chicks with non motile or motile *Salmonella* serotypes lead to variable high mortality (Siddique, 1985c). Maximum susceptibility in ducklings have been reported uptil 10th day and in case of goslings upto 3 weeks of age. The frequency of infection decreases with the advancement of age (Volinter, 1975). In *Salmonella* contaminated fertile eggs embryo may die in the eggs or may hatch normally, after which the disease is expressed by anorexia, polydipsia, anemia and immobility. Diarrhoea of pale, greenish, discoloration and some times hemorrhagic. Some birds show conjunctivitis, dyspnoea serous or purulent rhinitis, arthritis and even nervous manifestations (Bercea, 1981). Presence of other infections, especially infectious bursitis, coccidiosis and blue comb virus have been reported to increase the susceptibility (Stephens *et al.*, 1964 and Wyeth, 1975). Mycotoxins have also been reported to increase susceptibility to salmonellosis in chickens (Boonchuvit and Hamilton, 1975).

In *Salm. paratyphi* infections, mortality has been reported uncommon after the age of one month, but pigeons, canaries and parrots were exceptions, in which infections are mostly reported in adults (Javed *et al.*, 1992). Turkey poults are more susceptible during the first 48 days of life (Hofstad *et al.*, 1984).

2.3.1. YOUNG BIRDS

The influence of age and ambient temperature on response to *Salm. typhimurium* endotoxins was studied in chickens. Broiler cockerels 1 to 8 weeks of age received a single I/V injection of

Salm. typhimurium endotoxins. Rectal temperatures were taken over 30 min, for 108 min. In one week-old chicks, a significant increase in rectal temperature was observed (Jones, 1983).

Salm. pullorum infected growing chicks became emaciated, anaemic, and unthrifty. Retardation of growth, distension of the abdomen with fluid and occasionally diarrhoea were the common symptoms. Outbreak in growing chicks was attributed to a low temperature in the hatching house before and after hatching (Gao *et al.*, 1987). Mortality among day-old chicks following oral administration of *Salm. typhimurium* varied considerably with strain and with breed depending upon the invasiveness of the organism. Death probably resulted from anorexia and dehydration (Barrow *et al.*, 1987). Feed containing 6 million cells of *Salm. gallinarum* strain 296 (Bulgarian field strain), offered to 100 day-old-chicks, resulted in 53 per cent death due to septicemia within 11 days. A lower concentration (1.2 million cells per chick) resulted in 32 per cent mortality (Gyurov and Vodas, 1987).

The principal signs of fowl typhoid include somnolence, poor growth, loss of appetite, sudden drop in feed consumption and adherence of whitish material to the vent (Pomeroy, 1978). During subacute and chronic salmonellosis progressive emaciation, diarrhoea, arthritis and in some cases respiratory and nervous symptoms were recorded (Sojka *et al.*, 1977).

2.3.2 ADULT BIRDS

Adult birds usually do not show any symptom, but remain carriers for a long time (Bercea, 1981 and Hofstad *et al.*, 1984). Infections are favored by many factors such as low or high ambient temperatures, high humidity, deficiency of vitamins and minerals as well as intestinal parasites. Low protein

rations have been reported to increase the resistance or birds against *Salmonella* infections (Ganovska, 1978, Palanisami *et al.*, 1987). Presence of other infections, especially infectious bursitis, coccidial infection and blue comb virus have been reported to increase the susceptibility (Stephens *et al.*, 1964 and Wyeth, 1975). Mycotoxins have also been reported to lower the resistance power (Boonchuvit and Hamilton, 1975). A high percentage of young birds which survive of an outbreak become carriers. The organism is localized usually in the ovary and a high percentage of eggs laid by such carriers is infected and produce the disease in their progeny (Gordon and Jordan, 1982). Principal signs of fowl typhoid include somnolence, poor growth, loss of appetite, sudden drop in feed consumption and adherence of whitish material to the vent (Pomeroy 1978 and Siddique *et al.*, 1985b). In subacute and chronic salmonellosis observed progressive emaciation, diarrhoea, arthritis and in some cases respiratory and nervous symptoms (Sojka, *et al.*, 1977).

2.4. DIAGNOSIS

2.4.1 SEROLOGICAL DIAGNOSIS

The detection of *Salmonella* in flocks of laying hens has thus become a public health priority and a matter of great concern to egg producers. Testing for the presence of specific serum antibodies in an important aspect of proposed progression of identifying *Salmonella*-positive flocks. The National Poultry Improvement Plan authorizes the use of a variety of macro and microagglutination technique, for the detection of *Salm. pullorum* antibodies in chicken. Paratyphoid *Salmonella* serotypes, such as *Salm. enteritidis*, generally elicit weaker antibodies responses (Williams and Whittemore, 1975). Conventional agglutination tests have not been effective for detecting paratyphoid infections in chickens (Olesiuk *et al.*, 1969 and Williams, 1975), perhaps because many such infections

in mature birds are limited to colonization of the alimentary tract. Very young chickens are far more susceptible to paratyphoid salmonellae, but the antibody response by chicks has been observed to be insufficient for serological detection of infection. Serological methods have been reported to vary in sensitivity and reliability, but all were found to be more sensitive than cloacal swab cultures for the detection of paratyphoid *Salmonella* infections in chickens. Evidence of systemic infection with *Salm. enteritidis* suggests that infected hens are likely to have antibody titers high enough to permit efficient serological detection (Gast and Beard, 1990a).

On the basis of similarities in somatic antigens, *Salm. enteritidis*, *Salm. pullorum* and *Salm. gallinarum* are all assigned to *Salmonella* sero group D. Cross reactivity with antibodies to shared antigenic determinants, therefore, might enable *Salm. pullorum* antigen preparations to identify birds infected with *Salm. enteritidis* (Gast and Beard, 1990b). The tube agglutination test with the SP antigen have efficiency to detect 81 per cent of the samples as positive while the same sample were 84 per cent positive with micro agglutination test and 86 per cent with whole blood test. Serological testing for specific antibodies is an effective and widely used method of screening poultry flocks for evidence of infections with many common pathogens. Large numbers of samples can be collected and processed with great efficiency sensitivity and accuracy of this approach compare favorably with other testing optics (Gast and Beard, 1990c).

Serological techniques have been used to diagnose *Salmonella* infections. In *Salmonella* infections antibodies appear in the blood after fourth month of life and persist with little fluctuations during the whole life span of the birds.

Higher values are however, recorded during the period of production and molting. Agglutination of *Salmonella* antigen with antibodies is based of commonly analyzed serological diagnosis of salmonellosis. Schaffer (1931) for the first time developed the pullorum stained antigen whole blood test for diagnosis of pullorum disease in poultry and claimed it to be rapid and accurate. This test was modified using the polyvalent antigen called as "K" which contained both the standard and variant type strains of *Salm. pullorum* (Williams and Mac-Donald, 1955). Slide agglutination test was performed as a reliable diagnostic tool for *Salmonella* organisms (Silberstein, 1935). In 1951 Williams used spot agglutination test for the diagnosis of pullorum disease. Salmonellosis in subacute and chronic stages of infection can be detected by rapid plate hemagglutination test and slow tube agglutination test.

Serological testing of motile salmonellae polyvalent antigens are used which consist of specific group antigen of particular serotype. For the tube agglutination test a titer of 1:40 or more is considered as positive. Some therapeutic agents have effect on the antibody titer and allergic response in pullorum disease in fowls. Whole blood and tube serum agglutination tests together with the allergic skin test using 0.2 ml of lipopolysaccharide allergen were carried out in 30 one-year-old hens with naturally acquired high titre of *Salm. gallinarum-pullorum* antibodies (Dimitrov, 1978).

A new method for diagnosing pullorum disease in breeding hens was developed. In gel precipitation test on 827 eggs from 808 hens, a precipitin line was clearly visible between the yolk of infected hens and the antigen. The results of this test agreed with those of rapid whole blood stained antigen agglutination test, whole blood gel precipitation test and

bacteriological culture (Zhao *et al.*, 1981). A test for differential serodiagnosis of *Salmonella* by detection of IgG and IgM antibodies in ELISA was developed by. In a comparative investigation with 192 pigeon sera, 14.5 per cent were positive in the (H+L) chain specific IgG-ELISA, 12 per cent in the tube agglutination test and 6.3 per cent in slide agglutination test.

The peroxidase-antiperoxidase immunoassay was developed by using *Salm. choleraesuis* var Kunzendrof, *Salm. dublin* and *Salm. typhimurium* as test organisms. Strong specific staining with corresponding antiserum was achieved with smears of each *Salmonella* serotype on microscope slides from formalized cell suspension, culture of liver clinical isolates and tissue suspensions from the livers and spleens of experimentally infected mice. In addition *Salm. choleraesuis* var kunzendrof was detected in formalin-fixed and fresh frozen tissues from experimentally infected pigs. Their results indicate that the peroxidase antiperoxidase assay is well suited for the rapid identification of *Salmonella* from pure cultures and that the technique can be useful for detecting *Salmonella* in histological sections (McRill *et al.*, 1984). Enzyme Linked Immune Sorbent Assay (ELISA) is a new and most reliable serological method used for the diagnosis of typhoid fever in human beings (Vior, 1984). Siddique *et al.* (1984) used this method for the differential diagnosis of *Salmonella* infections from other confusing diseases. Excellent results were obtained for the accurate diagnosis as well as for the differentiation of motile and non motile salmonellae.

A direct enzyme immunoassay (EIA) with polyclonal antibodies was developed for *Salmonella* in foods and feeds. *Salmonella* cells were attached firmly to the wells of polystyrene microtitration plates with the capture-antibody

technique. The direct EIA was more sensitive than the indirect EIA of pure culture technique. The direct EIA was sensitive, rapid and could be automated (Anderson and Hartman, 1985). A comparison was made between a commercially available enzyme immunoassay (ELISA) and various culture procedures for detecting *Salmonella* in minced meat contaminated with a standard inoculum. To detect *Salmonella* by ELISA. It was necessary to modify the recommended base line for spectrophotometric measurement to avoid false positive results. The incidence of false negatives was no greater than that with a standard isolation procedure. Both methods were affected by competing microflora (Beckers *et al.*, 1988). Bourhy *et al.* (1988) conducted serological diagnosis of *Salm. gallinarum pullorum* infection and compared the efficacy of rapid plate agglutination and slow microagglutination tests in fowls of different ages. Rapid serum plate and micro agglutination tests were only suitable for adult laying hens. the rapid serum plate test was more accurate. A positive response with a serum dilution of 1: 4 should be considered indicative of *Salm. gallinarum pullorum* infection. Specific monoclonal antibodies was produced against *Salm. typhi* flagellin and possibly applied to immunodiagnosis of typhoid fever. Four murine monoclonal antibodies to *Salm. typhi* flagellin were produced. These monoclonal antibodies did not react with eight other enterobacterial strains tested (Sadallah *et al.*, 1990). Detection of *Salmonella* with fluorescent antibody test is possible (Wray and Callow, 1989).

2.4.2. BACTERIOLOGICAL DIAGNOSIS

Diagnosis of *Salmonella* by conventional methods of culture isolation and identification usually require 2-4 days. To obtain earlier diagnosis of salmonellosis, a coagglutation test was used for rapid detection of *Salm. oranienburg* antigen in enrichment broth cultures of feces specimens from infants. The

overnight enrichment broth cultures of specimens were also examined by coagglutination slide test with stabilized protein A-containing staphylococci sensitized with antisera for *Salmonella* antigens C_1, E and Vi. *Salmonella* was specifically detected in cultures within 20 hours by coagglutination technique.

Development of simplified identification of bacteria belonging to the genus *Salmonella* has remained a problem. A new simplified procedure for identification of bacteria belonging to the genus *Salmonella* has been reported. By this procedure which is a new combination of well known tests. *Salmonella* species are easily and reproducibly differentiated from non *Salmonella* species after 6 hours of incubation at 37°C. Salmonellae were detected in 15 of 167 samples of feed by using the three culturing method including culture technique recommended in ISO. There was no advantage in hydrogen sulphide treatment before applying the ISO technique or in using the membrane filter disk immobilization technique (Mulder *et al.*, 1989).

It is noteworthy that the number of reactors among birds infected orally with various isolates of *Salm. enteritidis* was low in all experiments and some birds did not become positive until 30 days post infection. This idea may reflect the low sensitivity of the rapid plate test, which is known to be less sensitive and less reliable than the tube agglutination test. However, it is clear from the work that *Salm. enteritidis* antigen is more sensitive and specific than commercially available pullorum antigen for the detection of serum antibody to *Salm. enteritidis* in laying hens (Barrow and Lovell, 1991).

Monitoring programs for *Salmonella* have been used in the industry for many years for the purpose of reducing the level of

Salmonella contamination. It appears that the levels of *Salmonella* contamination in poultry will have to be reduced in order to satisfy public concerns. To ensure the effectiveness or identify and controlling infected flocks, isolation procedures must be sufficiently sensitive to detect *Salmonella*. Conventional isolation media such as tetrathionate or selenite enrichment and the use of selective plating media such as brilliant green agar or xylose-lysine-deoxycholate agar are being modified to increase their sensitivity (Millier and Tate, 1990). Other methods are being introduced to increase the sensitivity and speed of detecting *Salmonella* such as various latex agglutination kits and enzyme-linked immunosorbent assay (ELISA) techniques. Recently use of a very sensitive antigen-capture ELISA for detecting *Salmonella* (Mallinson *et al.*, 1989). To approach the sensitivity of the ELISA, they modified conventional *Salmonella* isolation procedures by incorporating novobiocin into plating media and using delayed secondary enrichment (DSE). Because of their reported increase in the isolation rate of *Salmonella* by these two procedures (Waltman *et al.*, 1991). The use of novobiocin-supplemented enrichment and plating media has been advocated for many years (Bailey *et al.*, 1988). The use of reducing the level of other bacteria in the culture. This antimicrobial is particularly effective at preventing the over growth of *Proteus* spp. (Waltman *et al.*, 1991).

Drag-swab (DS) sampling and *Salmonella* antigen-capture immunoassay (SAC) is a current monitoring approach in poultry farms for *Salmonella* (Mallinson *et al.*, 1989). DS and SAC techniques are efficient enough in detecting *Salmonella* to be useful in extensive epizootiological surveys for *Salmonella* control in poultry operations and possibly other livestock production operations. Use of this technology in such surveys

accommodate the evaluation of large numbers of flocks or birds. DS-SAC presented several practical advantages. They are a) time savings gained from collecting the four unpolled DS samples (0.5 to 0.7 man hours/flock VS 2.75 man hours/flock for collecting the subsequently pooled 40 feather samples and 80 fecal samples); b) laboratory samples processing advantages of DS samples over litter and dust samples; and c) the shortened test turn-around time and resulting higher sampling/testing capacity available with SAC (Kingston, 1981).

Cloacal swabs have been used in the past to isolate and monitor *Salm. arizona* prevalence. The fact that infected adult breeders are often intermittent shedders made recovery of the organism difficult. Consequently, studies using this technique have reported poor results (Opengart *et al.*, 1991). Multiple swabbings, moreover, may be physically and economically impractical (Kenneth *et al.*, 1991). Serological tests also have been employed as monitoring tools but they have lacked sensitivity and specificity (Nagaraja *et al.*, 1984). Direct litter sampling has been suggested as a practical method of *Salmonella* detection in poultry flocks (Snoeynbos *et al.*, 1969). Other researchers have found drag swab to be as sensitive as direct litter sampling (Kingston, 1981). Others have reported that *Salm. arizona* can survive in poultry house litter for as long as 19 weeks (Geissler and Youssef, 1981). Drag swab technique may be a sensitive and efficient method of detecting and monitoring flocks that are shedding or have shed *Salm. arizona* in the past.

2.5. ANTIGENIC PROPERTIES

In *Salm. typhimurium*, nearly 50 genes are involved in flagellar formation and function and constitute at least 13 different operons. Flagellar operons can be divided into three

classes, class I, *feh D*, class II *flg A, flg B, flh B, fli A, fli E fli F*, and *fli L*, class III *flg K, fli D, fli C, mot A* (Kutsukake *et al.*, 1990).

Studies of 87 strains of *Salm. typhimurium* var copenhagen mostly from clinically affected pigeons and 7 laboratory or vaccine strains of *Salm. typhimurium* and *Salm. typhi* revealed many different combinations of lysotype, biotype antibiotic resistance and plasmid content. However, all except one laboratory strain possessed fimbriae. The fimbriae were mostly 7 nm in diameter and helicle super structure was demonstrated. All fimbriated strains possessed mannose-sensitive hemagglutination (Grund *et al.*, 1988). Lipopolysaccharide alteration was mediated by the virulence plasmid of *Salmonella*. All wild-type strains had smooth type LPS i.e. LPS with long O specific polysaccharide. The virulence plasmid-cured strain of *Salm. dublin* exhibited a shorter O specific chain than its parent strain (Kawahara *et al.*, 1989)

Salmonellae were screened for mannose sensitivity and mannose-resistance binding properties. Type I fimbriae positive strain adhered significantly better than Type 2 fimbriae-negative strains. Adherence was significantly inhibited by D-mannose, methyl-a-D-manoside, arabinose and galactose. Adherence was both time and temperature dependent. The function of the receptors is dependent on a mannose moiety. Bacteria adhered better to fresh intestine cells than to cells held overnight at 4°C. Thus, adherence was dependent upon a metabolically active host cell (Oyofo *et al.*, 1989). The synthesis of a versatile trisaccharide synthon is combination of protecting groups suitable for preparing higher oligosaccharides of the sequence Man-Rha-Gal and for introducing side-chain subsitituents. This synthon was used for the synthesis of protected trisaccharides

and haexasaccharide fragments of *Salmonella* polysaccharides. (Chernyak *et al.*, 1989).

The study of possibility of detecting the specific antigenic features of *Salm. typhi* L forms has revealed that out of three destructive methods under study (Osmoticlysis, freezing-thawing, sonication) only ultrasonic disintegration has proved to be effective for *Salm. typhi* L forms. Three specific fractions capable of interacting only with specific antibodies to *Salm. typhi* L forms have been revealed in the course of chromatographic separation of the soluble antigenic complex of *Salmonella* stable L forms and the subsequent analysis of the fractions thus obtained in the enzyme immunoassay (Prozorovskii *et al.*, 1989). A procedure was developed for smooth conversion of polyprenyl pyrophosphates into the monophosphates through hydrolysis in the presence of 4-dimethyl aminopyridine. The polyprenyl phosphate prepared were studied as substrate for the enzymes of *Salmonella anatum* O. specific polysaccharide biosynthesis. It is concluded that some changes in the position of the phosphate group may be permissible without any significant loss of substrate properties. Termini of *Salmonella* flagellin are disordered and became organized upon polymenization into flagellar filament. The terminal region of *Salmonella* flagellin is essential for polymerization to form the flagellar filament (Danilov *et al.*, 1989). Cultured mouse kupffer cells were incubated the presence of biologically tritiated *Salm. abortus equi* lipopolysaccharide. Uptake of lipopolysaccharide increased rapidly during the first 2 hours of incubation and then levelled off. Within the first hour of incubation 10^6 Kupffer cells were able to ingest upto 18 µg lipopolysaccharide. Kupffer cells metabolized lipopolysaccharide and released lipopolysaccharide-related substances, but neither the cell-associated lipopolysaccharide nor the released

lipopolysaccharide products were detoxified, as measured by the mouse lethality test (Van *et al.*, 1989). Lipopolysaccharide or defined lipopolysaccharide structures induce tumor necrosis factor-alpha (Feist *et al.*, 1989). Large scale fractionation of S-form lipopolysaccharide from *Salm. abortus* equi have been reported (Galanos et al, 1988).

Limited proteolysis of flagellin from *Salm. typhimurium* by subtilisin, trypsin and thermolysin results in homologous degradation patterns. The terminal regions of flagellin are very sensitive to proteolysis. These parts are degraded into small oligopeptides at the very early stage of a milk acidic digestion that yields a relatively, stable fragment with a molecular weight of 40,000 (Vonderviszt *et al.*, 1989).

Salm. typhi have unique sequence in region VI of the flagellin gene. The H1 (now renamed fli C) alleles specifying antigenically different *Salmonella* flagellins are identical at their ends but differ greatly towards the middle, where there are two hyper variable segments (region IV and VI). The flagellar antigen d, of *Salm. typhi*, is found also as phase-I antigen in many other Salmonella species. Four scattered amino acid differences and ten adjacent amino acids in the inferred *Salm. typhi* sequence, all of which differ from the corresponding nine amino acids in the other salmonellae. The difference in amino acid sequence in segment VI may be responsible for the minor serological differences between antigens d of *Salm. typhi* and antigen *d* of other salmonellae. *Flg B, Flg C, Flg F* and *Flg G* are structurally related proteins in the flagellar basal body of *Salm. typhimurium*. the flagellar basal body of *Salm. typhimurium* consists of four rings surrounding a rod. the rod which is believed to transmit motor rotation to the filament, is not well characterized in terms of its structure and

composition. *Flg G* is known to lie within the distal portion of the rod, in the region where it is surrounded by the L and P rings, just before the rod-hook junction.

The flagellar filaments of *Salmonella* are polymers of a single protein, flagellin and the large number of flagellar antigens (Edward and Ewing, 1989) is a reflection of the wide variety of flagellins produced by the group. These proteins consist of extremely conserved terminal regions with variable control areas (Wei and Joys, 1985). Eight regions of different variabilities were recognized, with two regions (*IV* and *VI*) being hypervariable. It has been suggested (Newton *et al.*, 1989), that parts of these hypervariable regions could be substituted with known important epitopes so that flagellin would act as carrier in a possible vaccine. Attempts at this substitution have used a unique restriction site in the structural gene for the *i* antigen flagellin of *Salm. typhimurium* (Newton *et al.*, 1990) and substitution of a 48-6p region between two EcoRV restriction sites in the gene of the *d* flagellin of *Salmonella* muenchen. A range of known epitopes has been introduced into these flagellins, but results indicating potential vaccine usefulness have been limited (Newton *et al.*, 1990). The next logical step would be to define the natural surface epitopes of one flagellin to identify regions likely to be immunopotent if substituted with foreign epitopes. On the basis of the previously published amino acid sequence for the *d* flagellin of *Salm. muenchen* (Wei and Joys, 1985) progressive octameric peptides had been synthesized or polyethylene pins (Geysen *et al.*, 1987) contiguous exposed antibody-binding regions of the antigen *d* flagellin of *Salm. muenchen* were identified by using octameric peptides synthesized on polyethylene pins. Identification was confirmed by the serological activity of immunolobulins recovered from specified

pin peptides. Peptides equivalent to four region of the *d* flagellin reacted with three different sera tested (Joys and Schodel, 1991 and Sanderson and Roth, 1988).

2.6. VIRULENCY

Bacteria are exposed to a wide range of environments, in which the motility and chemotaxis towards favorable environments may be important factors to increase their chance of survival. Whether motility and chemotaxis constitute one of the virulence factor has been examined in several pathogenic bacteria. Flagella and motility are important for the invasive virulence. A different result was obtained with *Salm. typhimurium*. Using mutants defective in flagellar synthesis, motility or chemotaxis. Carsiotis *et al.*, (1984) have demonstrated that flagella but not motility are important for the virulence of *Salm. typhimurium*. Furthermore, they showed that flagella improved the survival of the pathogen with murine macrophage (Weinstein *et al.*, 1984 and Tsang and Wong, 1989). However they reported that the attenuated virulence of non-flagellate mutants was associated with the loss of a virulence gene *mvi S* adjacent to the *flg* gene and not with the non-flagellate phenotype. Defects in the motility reduces the ability of *Salm. typhimurium* to invade Henle cells *in vitro* but does not affect its virulence for mice (Lockman and Curtiss, 1990 and Peng and Chang, 1989).

The efficiency of invasion of wild-type *Salm. typhimurium* is much higher than that of the isogeneic non-motile mutants. A comparison of invasion between the *mot* and non-flagellated mutants demonstrated that motility *per se* is important for efficient invasion of *Salm. typhimurium* into He La cells. Physical impact caused by bacterial motility enhanced the phagocytic activity of macrophage. While the simple

sedimentation of non-motile bacteria is enough to trigger the internalization of the bacteria (Falsafi *et al.*, 1990 and Lockman and Curtiss, 1990).

Salm. typhimurium is not only motile but also chemotactic. In the wild type strain, the cells swim by rotating there flagella with an occasional change in their swimming direction by reversing the flagellar rotation. A chemotactic response is manifested by modulating the frequency of reversal of the swimming direction. If the cells swim towards a favorable stimulus, the reversal is repressed; if they swim towards a non-favorable stimulus, the reversal is increased. In chemotactic mutants, regulation of the reversal is defective. Therefore, these mutants show aberrant swimming behavior. Mutants defective in *che A, che W, cheY* and *che R* swim ahead without any reversal of the swimming direction. Mutants defective in *che B* and *che Z* change continuously their swimming direction except when they receive strong chemotactic stimuli which causes smooth swimming behavior. The rates of invasion of the smooth swimming chemotactic mutants (*che A, che W, che Y,* and *che R*) is higher than the wild-type in the conventional assay, but approximately equal to the wild-type in the vertical assay. Chemotactic ability of *Salmonella* is not necessary for efficient invasion into He La cells but rather interfered with invasion under some experimental condition (Falsafi *et al.*, 1990).

The *cys PTWA* operons of *E. coli* and *Salm. typhimurium* encode components of periplasmic transport system for sulfate and thiosulfate and are regulated as part of the cysteine regulons. *In vitro* transcription initiation from the *cys P* promoter was shown to require both *cys B* protein and either O-acetyl-L-serine or N-acetyl-L-serine, which act as inducers, and was inhibited by the anti-inducer sulfide. Thiosulfate was found

to be even more potent than sulfide as an anti-inducer. Dnase I protection experiments showed two discrete binding site for *cys B* protein in the presence of N-acetyl-L-serine. CBS-P1 is located between positions -85 and -41 relative to the major transcription start site and CBS-P2 is located between positions -19 and +25. Without N-acetyl-L-serine, the *cys B* protein protected the region between positions -63 and -11, which was designate *CBS-P3*. In gel mobility shift assays, the mobility of *cys B* protein *cys P* promoter complexes was increased by O-acetyl-L-serine. N-acetyl-L-serine had no effect in gel shift experiments, presumably because is anionic charge results in its rapid removal from the complex during electrophoresis. Comparison of DNA fragments differing with respect to binding site position indicated that complexes with *cys B* protein contain DNA that is bent some where between *CBS-P1* and *CBS-P2* and that O'acetyl-L-serin decreases DNA bending. Binding studies with fragments containing either *CBS-P2* along, *CBS-P1* alone, or the entire *cys P* promoter region suggest a model in which the complex of bent DNA observed in the absence of O-acetyl-L-serine contains a single *cys B* protein molecules bound to *CBS-P3*. At relatively low *cys B* protein concentrations, O-acetyl-L-serine would cause a single *cys B* protein molecule to bind tightly to *CBS-P1*, rather than to *CBS-P3*, thereby decreasing DNA bending and increasing complex electrophoretic mobility. At higher *cys B* protein concentrations, O-acetyl-L-serine would cause a second molecule to bind at *CBS-P2*, giving a more slowly migrating complex (Hryniewicz and Kredich, 1991).

Assimilatory sulfate reduction is *Salm. typhimurium* commences with the uptake of extra cellular sulfate, a process requiring a periplasmic transport system termed the sulfate permease system (Dreyfuss, 1964). All but one of the components of the sulfate permease system are encoded by contiguous genes

located at 52 min. on the *E. coli* (Karbonowska *et al.*, 1977) and at 49 min on the *Salm. typhimurium* map (Dreyfuss and Monty, 1963) which in *Salm. typhimurium* were originally designated *cys Aa*, *cys Ab* and *cys Ac*. This genetic region has recently been cloned and sequenced in *E. coli* and found to contain five open reading frames, which beginning furthest upstream, were designated *cys P*, *cys T*, *cys W*, *cys A* and *cys M* (Sirko *et al.*, 1990). *cys M* encodes O-acetyl-serine (thiol)-lyase B, which catalyzes the synthesis of L-cystine from O-acetyl-L-serine and sulfide and also the synthesis of S-sulfocystine from O-acetyl-L-serine and thiosulfate (Nakamura *et al.*, 1984). Sulfate and thiosulfate transport activities vary according to the availability of L-cysteine and reduced sulfide and are presumed to be regulated, together with other activities required for L-cysteine biosynthesis, at the gene level as part of the cysteine regulon (Hryniewicz and Kredich, 1991 and Gulig and Curtiss, 1987).

Most stains of *Salmonella* serovar *typhimurium* harbor a 90 Kb virulence plasmid, whose involvement in the virulence of the host had been well established (Gulig and Curtiss, 1988), further, some virulence genes on the plasmid have been mapped and elucidation of their functions and products has begun (Gulig and Curtiss, 1989). In each of these experiments, however, only one or two strains or plasmids have generally been used. Therefore, we asked whether or not there are strain differences in respect of the virulence of the plasmid or its effect on the virulence of the bacterial host. It appears that whereas the virulence function of the plasmid is intact in all strains, there are differences in the virulence of the bacterial host. Among six strains of *Salm. typhimurium* tested for their mouse virulence, four were highly virulent with an LD_{50} of ten other 50 bacteria. The high virulence of these four required the

presence of the 90 Kb plasmid since when the plasmid was removed, the virulence decreased, and when the plasmid was regained, the virulence level was restored. In contrast, the remaining two were not affected by the presence or absence of the 90 Kb plasmid in their expression of virulence, for the curing or the reintroduction of the plasmid failed to influence their level of virulence. The plasmids retained their virulence function no matter how many passages the plasmids made. Thus, the 90 Kb plasmid requires an appropriate host to support a high level of virulence (Ou and Baron, 1991). Transfer of phospholipid and protein into the envelop of *Salmonella* is possible (Stephen *et al.*, 1989).

There are a number of chromosomal determinants related to the expression of virulence of *Salm. typhimurium* (Sanderson and Roth, 1988). A virulence determinant, termed *mvi A*, located between *trp D* and *sup D* on the *Salm. typhimurium* chromosomal map. There is also a report that showed a virulence gene linked to the *flg* gene at about 23.5 min. on the chromosomal map. It is not known whether these genes require the plasmid for the virulence expression (Ou and Baron, 1991). The psonizing ligand on *Salm. typhimurium* influences incorporation of specific but not azurophil, granule constituents into neutrophil phagosomes (Jiner, 1989).

2.7. PATHOGENESIS

Salmonellae those cause invasive disease in man and animals do so by virtue of their ability to multiply extensively in the tissues, particularly in the reticuloendothelial system (RES). None of the conventional laboratory rodents is naturally susceptible to *Salm. typhi*; the cause of human typhoid fever, and extensive use has been made of the laboratory mouse in which organisms such as *Salm. typhimurium* and *Salm. enteritidis* cause

invasive disease believed to have many points in common with the human infection. Salmonellae are generally accepted to be facultative intracellular pathogens which can proliferate inside macrophage (Collins, 1974) and this has been shown clearly *in vitro* models using cultured mouse macrophage. Infection of macrophage *in vitro* has also been used to isolate non virulent mutants for genetic analysis of virulence mechanism in salmonellae. Natural killer cells play a vital role in salmonellosis (Smith *et al.*, 1989).

However, the intracellular location of salmonellae *in vivo* has been less easy to demonstrate because of the low numbers of organisms present in the tissues during the early stages of the infection, so that investigators have had to inoculate large numbers of salmonellae in order to be able to visualize them by light and electron microscopy (Hsu, 1989). These studies suggest that salmonellae may multiply extensively extracellularly rather than intracellularly. Administration of a large number of dead bacteria therefore caused an accelerated net bacterial growth rate in the RES in very early stages of infection (Hormaeche, 1990). Acceleration of early net growth rate caused by dead bacteria may be partly due to the endotoxin content of the dead salmonellae, rather than by the sheer number of the organisms administered.

Endotoxin causes as multiplicity of biological effects, many of them through the release of mediators (Morrison, 1983). The mechanism by which endotoxin can accelerate a *Salmonella* infection is not clear. It has long been known that the administration of dead salmonellae or their endotoxin will produce a biphasic effect on the phagocytic capacity of the reticuloendothelial system as measure by the clearance of colloidal carbon from the circulation, causing a rapid (24

hours) depression in clearance followed by an increase of 4-5 days. This endotoxin-medicated depression of phagocytosis could be expected to modify the course of an infection with an intracellular parasite. The acceleration of in vivo salmonellae net growth rate caused by endotoxins similar to that caused by the administration of silica (O'Brien *et al.*, 1990), which interferes with macrophage function causing a marked reduction in their phagocytic capacity and also a marked acceleration of early *in vivo Salmonella* net growth rates in the RES (Hormaeche, 1990 and Forrest, 1988).

Whatever the precise nature of the mechanism by which a large bolus of dead organism or endotoxin exert their effect, it is clear that they cause marked acceleration in the cause of a *Salmonella* infection in mice. The possibility that these large doses may be hindering macrophage function suggests that speculations on whether salmonellae are or are not facultative intracellular parasites, derived from observations on their intracellular or extracellular location to the tissues following inoculation of large numbers of organisms may need to be considered with some caution (Hsu, 1989 and Hormaeche, 1990).

The progression of salmonellosis can be divided into different phases, and the types of host defense that control the infection vary between the phases. Blood clearance occurs with in the first few hours after intravenous injection of *Salm. typhimurium*. The surviving *Salmonella*, which account for approximately 5 per cent of injected bacteria, become localized to the liver and spleen (Benjamin *et al.*, 1986). The exact location of *Salmonella* within these organs is not known, but the rate of multiplication of the surviving *Salmonella* is controlled by the *ity* locus (Benjamin *et al.*, 1990). Once in the reticuloendothelial system (RES), host killing of salmonellae

almost ceases, at least during the first 48 hours. The ability of *Salmonella* within the spleen and liver to grow in the relative essence of killing suggests that the surviving *Salmonella* have reached a "safe-site" which is probably instrumental in their ability to cause disease (Dunlap *et al.*, 1991).

Various substances may interact *in vivo* and *in vitro* with either microorganism or animal cell surfaces and thereby influence the attachment of the microorganism to susceptible cells and their subsequent internalization. Both small molecules and biological macromolecule can interfere with the binding of bacteria and viruses by competing for cellular receptors, whereas polyelectrolytes can act on charged molecules on the cell surface and modify early events in the interaction between microorganisms and animal cells (Christensen *et al.*, 1985). Bacteria and most eukaryotic cells have a net negative charge (Wicken, 1985). Lipopolysaccharides on the surface of Gram-negative bacteria contains several ionic groups, including acidic phosphate and carboxyl, moieties, all of which are sources of electrostatic negative charge (Luderitz *et al.*, 1982). The presence of polyions in the environment can modify bacterial surfaces. Polycations such as polylysine and protamine can act as outer membrane perturbing agents of enteric bacteria and polycationic antibiotics are known to bind to lipopolysaccharide (Freudenberg *et al.*, 1991).

Within the enterobacteriaceae family, many species are capable of entering human epithelial cells. Bacteria belonging to *Yersinia* species cross the intestinal epithelium and replicate within the underlying tissues. A chromosomal region from *Yersinia pseudotuberculosis*, containing the *inv* gene has been cloned in *E. coli* HB 101 (Isberg and Falkow, 1985). This

genetic determinant allows the normally non-invasive *E. coli* HB 101 to invade cultured human epithelial cells, but is unable to induce intracellular replication. The peculiar behavior of this bacterial strain has made it possible to study the entry mechanism of enteroinvasive bacteria in a suitable cell model. Various polycations (DEAE-dextran, histone, poly-L-Lysine, protamine and protamine sulphate) and anions (mucin, heparan sulphate, trypsin inhibitor and dextran sulphate) were either added during the early phases of infection or preincubated with the bacteria. It was observed that invasion was only slightly influenced by polyanions whereas all polycations strongly enhanced bacteria entry; this activity was exerted during the attachment step. *Salm. typhimurium* induce fluid secretion by making a complex with leukocytes (Wallis, 1990).

In order to establish which phase of infection by *E. coli* HB 101 (Pr 1203) was affected by the poly cationic agents, the infection was synchronized by a temperature shift. He La cell monolayers were preincubated at 4°C for 30 min. Bacteria were then added to monolayers and allowed to bind at 4°C for 30 minutes. After removal of the unbound bacteria by washing, the bound bacteria were allowed to penetrate by raising the temperature to 37°C for 30 min. In these assays, polycations were used at the minimal doses that in the previous experiments had given the highest values of invasion, and added under for alternative schedules (Conte *et al.*, 1990) to the cell monolayers for 30 minutes prior to infection by *E. coli*; the tissue cultures were washed before the infection; during the bacterial attachment step at 4°C; they were then removed by washing together with the bacterial inoculum; during the penetration step (for 30 minutes at 37°C (Pedersen, 1980) preincubated with the bacteria at 4°C for 30 minutes; the bacteria were then washed and resuspended in MEM before addition

to the *He La* cell monolayers (Conte *et al.*, 1990). The invasion of the host tissues by enteropathogenic bacteria is a complex process which involves many factors.

2.8. PATHOLOGICAL STUDIES

2.8.1. GROSS PATHOLOGICAL STUDIES

Among postmortem lesions in pullorum disease, yellow color of liver, congestion and caseous nodules in the lungs were the salient features. The organs usually affected include ovaries, spleen, liver, gall bladder and kidneys (Doyle, 1925). Mottling and enlargement of liver and kidneys, distension of gallbladder, congestion of the intestinal mucosa were common gross alterations observed (Athar, 1982). Enlargement of liver and spleen, petechiation and whitish foci on their surfaces were common macroscopic lesions in experimental infection with *Salm. gallinarum*. The characteristic greenish discoloration of the liver frequently observable (Khan, 1982). Pathological study of pullorum disease is characterized by synovitis in replacement breeder flocks, along with swelling of the hock joint and/or sternal synovial bursae, exudative synovitis, with mononuclear infiltration of the synovial and fibrous layers (Xu *et al.*, 1987).

Liver and spleen showed the most constant lesions in young birds affected by fowl typhoid. Liver usually enlarged 2 to 3 times, however, typical bronze discoloration, splenomegaly accompanied by mottling in most of the cases was observed. Mild to severe enteritis, pericarditis and enlargement of the heart were also recorded in many infected birds. In laying birds ovarian follicles appeared pedunculated, misshapen and in many instances discolored (Siddique *et al.*, 1987).

Salm. typhimurium produced typical pathological changes in experimentally infected chicken embryos and day-old chicks. SPF chick embryos were infected on 6th day and on 14th day of incubation with *Salm. typhimurium*. Salmonellae primarily damaged chorio-allantoic membrane (CAM), resulted in 10-40 per cent embryonic mortality. Birds died in acute stage showed atrophy of lymphoid organs, bursa of Fabricius and thymus. Depletion of lymphocytes and necrosis are the characteristic features, while the lymphoid organs of chicks held in the subacute stage developed hyperplastic changes. Two weeks-old chicks affected with *Salm. enteritidis* showed a toxic indurated yolk-sac remanent, rarely pericarditis at the time of slaughter (O'Brien, 1988).

In broiler breeder flocks infected with *Salm. enteritidis* birds in full lay had no gross abnormalities. Birds with inactive ovaries had misshapen, shrunken discolored and congested ova. Yolk profusion was abundant in the abdominal cavity (Lister, 1988). Birds died on the farm with *Salm. enteritidis* had ovarian infection. The ovules were congested and misshapen. Egg peritonitis was common in these cases (Hopper and Mawer, 1988). Pathological changes in indigenous chickens showed enlargement of liver with necrotic foci and hemorrhage on the surface. Intestines were commonly affected while spleen had least tendency of alterations. Livers were bronze metallic sheen color and fragile in consistency (Javed *et al.*, 1990). Gross pathologic studies in Australian parrots, pigeons and peacocks were observed frequently. Liver, lungs, spleen and kidneys were invariably enlarged, congested and friable with necrotic foci. Lungs appeared hepatized with exudate (Javed *et al.*, 1992).

2.8.2. HISTOPATHOLOGICAL STUDIES

Histopathologic changes of salmonellosis in chicken and adult hens comprised of focal endothelial proliferation of liver, accompanied with focal necrosis of myocardium, catarrhal bronchitis and enteritis (Suganuma, 1960 and Pomeroy, 1978) observed swelling of liver, spleen and kidneys. Other changes included grayish white miliary foci in myocardium but rarely in the lungs and gizzard of young chicks. Pericarditis and enteritis were also recorded. The heart showed sub-pericardial hemorrhages, the liver showed maximum dilation of the veins and sinusoidal capillaries, on renal section presence of numerous granulocyte (Javed *et al.*, 1991). In the spleen apart from the hyperemic blood vessels, there was marked proliferation of the endothelial cells of the sinus, and an increased number of granulocyte at germination centers (Ahmad, 1982 and Khan, 1982). Purulent meningoencephalitis caused by *Salm. pullorum* in chicks, pullorum disease affected 160.000 chicks from the total of 400,000. Meningoencephalitis developed in some 1000 of the affected chicks (Coelho *et al.*, 1983).

Pathological changes of liver and spleen in avian salmonellosis due to *Salm. pullorum* in the liver showed hemorrhages (16 %) and necrotic foci (11 %), while in the spleen, hemorrhages (13 %) and necrotic foci (25 %) were also found. Histopathological alterations in the liver showed thickening of capsule, congestion, degenerative and necrotic changes, while the spleen showed similar changes in addition to hemorrhages. Birds infected with *Salm. gallinarum* showed liver with bronze discoloration, mottling and hemorrhages. In spleen there was mottling, hemorrhages and necrotic foci were the common lesions. The histopathological alterations in the liver and spleen were similar to those in *Salm. pullorum* infections,

but fatty change was also observed (Chishti *et al.*, 1985; Javed *et al.*, 1990 and Javed *et al.*, 1992).

Histopathological alterations induced by different motile and non-motile salmonellae in SPF chicks were thickened hepatic capsule with a fibrinous exudate containing a few leukocytes and fat droplets. The blood vessels of the capsules were congested, liver cells in the center of the lobules were necrotic but those around the edges were in advanced stages along with parenchymatous degeneration. The capillaries contained large number of leukocytes and red blood corpuscles. A number of small veins were surrounded by dense masses of polymorph leukocytes. Congestion of the large arteries was marked. Some hemorrhages in the tissue elements was present. However, a few differences regarding the species differences were observed (Siddique *et al.*, 1985c). Histopathological examination of the thymus and cloacal bursa of Fabricius from vaccinated chickens showed an expansion of the medullary zone and a narrowing of the cortical zone, with migration of thymocytes to the medullary zone. These changes were more pronounced in vaccinated birds (Purdnikov, 1987 and Gao *et al.*, 1987).

2.8.3. ELECTRON MICROSCOPIC STUDIES

Analysis of the transmission electron-microscopic images (TEM) showed, severe nucleocytoplasmic modifications, irrespective of the organ (tissue) and motile *Salmonella* serotypes involved. In nucleus fragmented nuclear chromatin with frequent exvagatination towards the cytoplasm and breakage of the nuclear membrane were the main features. All the cytoplasmic organellae were altered. Scanning electron-microscopy (SEM) performed on tissue from all the organs of the chicks experimentally infected with both motile salmonellae and *Salm. gallinarum*, showed that appreciable ultrastructural lesions were

generally induced only by *Salm. typhimurium*. Except for the bursa of Fabricius, there were severe inflammatory lesions in all the investigated organs, i.e., liver, spleen, kidney, heart and intestinal wall. The presence of necrotic and necrobiotic zones in the parenchyma, dystrophic alterations. An appreciable reduction in the number of reticular cells and chose of the macrophagic system of the "B" and "T" lymphocytes in lymphoid organs was observed (Siddique *et al.*, 1985c).

Penetration of *Salm. Thompson* and *Salm. enteritidis* through chick ileocecal mucosa was observed. Entry of bacteria into the epithelial cells was associated with a series of pathological changes. Beginning with the appearance of active Golgi apparatus and the production of variety of lysosomal vesicles. No lesion was observed in lamina propria until transported macrophage. Scanning electron microscopy revealed adhering bacteria increased with the age of the chick, that the morphological and quantitative changes of the caecal microflora are completed by about 15 days of age and that a complete bacterial colonization of the caecal wall of a newly hatched chick occur only 24 hours after treatment with an adult caecal microflora (Humbert *et al.*, 1989).

2.9. ANTIBIOGRAPHY

The increasing use of antibacterials for prophylactic, therapeutic and nutritive purposes in agriculture and medicine creates a potentially powerful selective pressure for the spread of antibiotic resistance in bacteria (Duck *et al.*, 1978 and Lofont *et al.*, 1981). As many authors pointed out, the spreading of multidrug resistance strains determined peculiar aspect of gravity in the outbreak evolution, serious economic involvement including loss of work, cost of therapy, expensive laboratory investigations and antiepizootic measures (Barbour

and Nabbut, 1982; McGarr *et al.*, 1980 and Hirsh *et al.*, 1983 and Kawara, 1990).

Antibiotic sensitivity of *Salmonella* strains revealed that ampicillin, gentamicin, kanamycin, neomycin and streptomycin were the most effective against motile as well as non motile salmonellae. A remarkable resistance to tetracycline, tylosin, biseptol and furazolidone was observed. Some susceptibility differences to polymyxin, neomycin and streptomycin were noticed in motile salmonellae (Siddique *et al.*, 1985). *Salm. gallinarum* isolated from outbreaks of infection in poultry in Greece (15) Amman (3) Kenya (2)Lebanon (1) and Yemen (1). Among them 20 were more resistant to furazolidone in vitro than 6 strains that had been isolated in the UK. The minimum inhibitory concentration of furazolidone was approximately 0.3 µg/ml for the sensitive strains and 1.3 or 2.5 µg/ml for the more resistant strains. Chloramphenicol, trimethoprim and sulfadiazine or mixtures of the latter two were the best antibacterial for treating these infections (Smith *et al.*, 1981). Antimicrobial drug susceptibility of *Salmonella* from ducks, rabbits, fowls, mice and guinea pig were tested. Four strains were resistant to ampicillin, cephalothin, chloramphenicol, sulfonamides, 6 per cent were resistant to neomycin and about 5 per cent to streptomycin and tetracycline. Most strains were resistant to kanamycin and penicillin (Yoon *et al.*, 1985).

Ampicillin at 0.6 µg/lit, chloramphenicol, gentamicin and streptomycin at 2 µg/lit and colistin at 6 µg/ml were bacteriostatic against *Salmonella* strains isolated from turkey embryos. Neomycin, oxytetracycline and tylosin were less effective. The minimum bactericidal concentration of ampicillin was 2 µg/ml. The strains were resistant to penicillin, erythromycin, sulfamerazine and sulfachlorpyrazine.

Antimicrobial drugs resistance in 261 *Salmonella* strains revealed resistance to tetracycline (44 %) at 8 µg/ml, ampicillin (3.8 %) at 8 µg/ml and chloramphenicol (9.2 %) at 12 µg. Bacitracin followed by chlortetracycline, tetracycline, polymyxin B, oxytetracycline, erythromycin, streptomycin and neomycin have maximum resistance. Sensitivity to chloramphenicol and nitrofuraltadone was detected in 99.78 per cent and 97.6 % of the Salmonella strains, respectively. the strains were isolated from horses, poultry, guinea pig, frogs and ground lizards (Gupta and Mallick, 1976).

Salm. typhimurium, Salm. saint-paul, Salm. eimsbuettel and *Salm. arizona* of chicken breeder origin were tested against 10 antibacterial drugs. Gentamicin, ampicillin, kanamycin and polymyxin was sensitive streptomycin, chloramphenicol, nalidixic acid oxytetracycline and sulfadiazine were resistant at concentrations of 2 or 5 µg/ml, and to nitrofurazone at 5 or 10 µg/ml. One strain of *Salm. typhimurium* was resistant to nitrofurazone at 20 µg/ml (Silva *et al.*, 1981). Sensitivity to eight antibacterial drugs was tested in 12,903 Salmonella strains isolated from animals and environment during the three year period of 1979 to 1981. A total of 2.834 (21.9 %) strains were sensitive to all eight antibacterial drugs (Sojka *et al.*, 1984). *Salm. gallinarum*, Salm pullorum and motile salmonellae, isolated during 1975-1982 from several species of birds were sensitive to the nitrofurans, chlortetracycline, neomycin and streptomycin. *Salm. pullorum* was very sensitive to chloramphenicol while the motile salmonellae were quite sensitive to ampicillin (Gitthkopoulos, 1984).

Sulfalene and sulfachloramphene at respective concentration of 6.25-12.5 µg/ml and 6.25-25 µg/ml showed in vitro bacteriostatic activity against six strains of *Salm. gallinarum-*

pullorum (Bondarenko *et al.*, 1984). Neomycin sulphate 200 g/ton and or oxytetracycline 200 g/ton for 16 days reduce the infection. The incidence of salmonellosis was lowest in chicken fed or the combined antibiotics and there were fewer viable *Salm. typhimurium* in feces than in chicken receiving only one antibiotic (Williams *et al.*, 1984). Nine serotypes from poultry in Ghana were isolated for the first time. All serotypes tested were sensitive to furazolidone, all but one to chloramphenicol and neomycin but half were resistant to oxytetracycline, chlortetracycline and streptomycin (Boachie, 1985).

Among *Salm. typhimurium* strains antibiographed 250 were *Salm. dublin*, 1988 *Salm. choleraesuis*, 61 *Salm. enteritidis* and 73 *gallinarum-pullorum* was determined to streptomycin, chloramphenicol, colistin, neomycin, ampicillin, nitrofurantoin and sulfathiazole. Comprising with earlier work, an increasingly high percentage of strains showed resistance to several agents suggesting the probability of new determinants of resistance being acquired. Most of the organisms were sensitive to chloramphenicol. *Salm. choleraesuis* was susceptible to streptomycin and *Salm. enteritidis* and *Salm. gallinarum-pullorum* to colistin and neomycin. Many strains were sensitive to streptomycin but a few to ampicillin (Hoszowski and Truszynski, 1980). The sensitivity of apramycin treatment against fowl typhoid and *Salm. gallinarum* was of value (Demirozu, 1982). Field trials with furazolidone at 100 g/ton and chloramphenicol at 100 g/ton gave good results against *Salmonellae* All strains were highly sensitive in vitro to nitrofuran, chloramphenicol and gentamicin, but completely resistant to benzylpenicillin and neomycin erythromycin, ampicillin and sulfamethoxazole (Shahata *et al.*, 1983).

In 1287 strain of *Salmonella* resistance was found to streptomycin in 286 isolates and to tetracycline in 282 isolates. Resistance to other antimicrobial drugs was low and was unrelated to the source. 173 isolates showed multiple resistance to two or more antimicrobial agents with resistance to streptomycin and tetracycline being the most common (Murray *et al.*, 1986). Minimum inhibitory concentration 16 µg/10 ml of cefoxitin sodium against avian *Salmonella* was sensitive. In an outbreak of *Salm. indiana*, was sensitive to oxytetracycline pathogen was resistant to penicillin, chloramphenicol, ampicillin and sulfonamides (Rao *et al.*, 1986). *Salm. typhimurium, Salm. pullorum, Salm. enteritidis* and *Salm. montevideo* were resistant to oxytetracycline, ampicillin, sulfonamide, streptomycin chloramphenicol (Becirevic and Popovic, 1987). Kanamycin administration was associated with a significant increase in the frequency of isolation of drug-resistant transconjungant *Salm. typhimurium* from the livers of poults inoculated with multiple drug-resistant *E. coli* and drug sensitive *Salm. typhimurium.* Kanamycin administration reduced the spread of drug-sensitive *Salm. typhimurium* (Gast, 1991c).

Salm. gallinarum-pullorum, Salm. virchow and *Salm. newport* against eleven antibiotics revealed that the isolates were sensitive to flumequine and chloramphenicol (70-80 %), moderately sensitive to nitrofuration, ampicillin and neomycin (38-57 %) and weakly sensitive to lincomycin and streptomycin (15-18 %), but completely resistant to erythromycin, penicillin, tetracycline and trimethoprim. In vivo flumeguine and chloramphenicol decreased mortality and carrier rates. Efficacy of sulphadiazine-trimethoprim combination against *Salm. gallinarum* is effective (Reddy *et al.*, 1987) Apramycin is of value against *Salm. pullorum* in chickens. Apramycin given at 225

mg/littre for 5 days in drinking water prevents deaths and reduces bacterial excretions in *Salmonella* infected chicks (Tacconi *et al.*, 1987). The feed given to young broiler chickens was contaminated artificially with *Salm. kedougou*. Avilamycin, added to the feed at either 2.5 or 10 ppm (mg/kg), favored the colonization of the intestinal tract of the birds with *Salm. kedougou* when they were challenged with this organism in the feed (Hinton, 1988). There are set requirement to evaluate the activity of antibacterials in field (Lewin and Amyes, 1989).

2.10. CONTROL MEASURES

Dissemination of salmonellosis in chicken operations cannot be controlled without knowing the sources and spread of the organism at the hatchery, breeding and commercial farms as well as processing plants (Javed and Hameed, 1989). A better understanding of this process would help in development of monitoring system for in formulating effective control programs. (Bhatia and Nabb, 1980). In spite of a high prevalence of salmonellosis in breeder flocks, only a few limited steps have been taken to eradicate this insidious problem of our poultry industry. Import of *Salmonella* free day-old parent chicks, feeding *Salmonella* free feed and uncontaminated drinking water are necessary control measures. Restriction on visitors, improving sanitary and managemental conditions are of great value. Competitive exclusion, addition of antibacterial drugs and vaccination is recent intervention to break the cycle (Javed and Hameed, 1989). Attempt with oral immunobiotherapy and immunochemotherapy is of value for the elimination of *Salmonella* in carriers (Munnich and Dalmi, 1989).

2.10.1. PARENT STOCK AND HATCHERY

Bacteria, including *Salmonella*, can penetrate an egg shell is as soon as 30 minutes after laying. Bacterial penetration

through shell pores is facilitated in the first few minutes after lay due to the cooling of a warm and moist egg. Most breeder farms in Georgia do not have an on-farm program for the disinfecting of hatching eggs. Conditions during incubation (temperature and humidity) are favorable for the rapid growth of bacterial population. Invading bacteria usually do not cause extensive decomposition of the egg and, as a result, chicks are often hatched from contaminated eggs. There are numerous factors that can affect the susceptibility of chickens to *Salmonella*, one of the most important is the age of the animal. Milner and Shaffer (1952) found that day-old chicks could be infected with less than five *Salmonella* organisms. Later, when chicks were older, much higher doses of *Salmonella* were required, even to achieve irregular infection. Newly hatched chicks could be infected by a single *Salmonella* organisms. Therefore, the presence, persistence and abundance of *Salmonella* contamination in the commercial hatchery suggest that the vulnerable day-of-hatch chick may be at a greater rich of colonization during the hatching process than during grow-out. A chick that becomes colonized in the hatchery can subsequently spread *Salmonella* contamination to other chicks in the hatchery and to flock mates during grow-out. When such a flock reaches the processing plant, *Salmonella* contamination both interior and exterior can be released into the processing facility and contaminate the final product from this and subsequently processed flocks. Contamination and penetration of the shell of fresh and incubating hatching eggs constitute an early important critical control point in the transmission of *Salmonella* to young birds and perhaps, eventually to the consumer (Mauldin, 1990).

Ozone and formaldehyde were compared as poultry hatchery disinfectants in poultry and evaluated for their effectiveness. Ozone (1.41 to 1.68 % w/w) resulted in significant bacterial

reductions of > 4 log_{10}. In the event that formaldehyde can no longer be used in the hatchery, an effective alternative may be ozone (Whistler and Sheldon, 1989). *Salm. enteritidis* was transmitted vertically to clinically affected progeny flocks. The infected breeder flocks were slaughtered and the infection throughout the organization was controlled and subsequently eradicated. Prevention of *Salmonella* by vertical or reintroduction of infection is only possibly checked by avoiding the entry of infected personnel, other animal species, fomites or transmission through feed. All feed was heated to minimum 70°C for 12 minutes immediately before it was pelleted and subsequently transported to the flocks through a dedicated system of conveyor belts, bins and lorries (McIlroy *et al.*, 1989).

2.10.2. CHEMICAL DISINFECTION

Meat samples were treated with various levels of chlorine dioxide (ClO_2) in large spin type chiller in poultry processing plants. ClO_2 from 0 to 1.39 mg/littre resulted in reducing the bacterial count to the point where salmonellae could not be isolated from the chilled water or the chilled broiler carcasses. Sensory panelists reported no bad flavors for any ClO_2 concentration but rated broiler skin as being slightly lighter in color compared to control carcasses at all concentrations of ClO_2 treatment. Dip disinfection of *Salmonella* infected hatching eggs concerned with hatchability. Dipping eggs in 56°C hot water for 2 minutes or 60°C for 1 minute had no adverse effect on hatchability, nor did dipping in H_2O, or disinfectants affect hatchibility. Hatchability was significantly reduced by dipping in an iodophorphosphoric acid preparation or by combined 60°C water and 1 per cent formalin treatment. Contamination of eggs with *Salm. senftenberg* led to high reisolation rates on the 7th, 9th, 13th, 15th and 19th days

of incubation. No adverse effect was detected on hatchibility with laysovet and water of 60°C at room temperature for 5 minutes (Mandl, 1985).

Lysozyme and ethylene diamine tetra acetic acid have effect on *Salmonella* on broiler parts. A dip system, consisting of lysozyme (1 mg/ml) and acetic acid (ETDA 5 mg/ml), gave a significant reduction in the number of viable *Salmonella*. In a trypticase-soy broth solution, *Salmonella* growth was inhibited by EDTA, while lysozyme had little effect (Samuelson *et al.*, 1985). Total plate counts of *Salmonella* for washed duck eggs were less than 30/shell during the winter, 1982. Clean unwashed eggs had counts less than 9×10^4/shell whereas dirty unwashed eggs had counts as high as 9×10^5/shell. Washing with chlorine disinfectant was highly effective in reducing bacterial counts on egg shells, prolonged storage reduced bacterial counts on clean eggs. Bacterial loads on washed and clean duck eggs from six different breeder farms were low where as dirty eggs had heavily contaminated with *Salmonella*. *Salm. enteritidis* was detected on dirty egg shells in an many farms. Bacterial loads on washed and nest clean eggs from the same breeder farms (nonconfined) was less. *Salm. enteritidis* and *Salm. hadar* were recovered from washed, nest clean and dirty eggs. Proper egg washing and confinement of breeder ducks should minimize the problem of salmonellosis in ducklings (Baker and Goff, 1982). The thermal inactivation of *Salm. thompson* was accelerated by the addition of sodium isoascorbate (1 Mm) to phosphate buffer. The lethal effect of isoascorbate was nullified by heating under anaerobic conditions or by the addition of catalase. The mannitol and formate were not protective whereas histidine was. Histidine may have protected by slowing the rate of isoascorbate auto-oxidation, a property common to the other amino acids tested. The bactericidal effect of mild heat plus isoascorbate

or dehydroascorbic acid both apparently depend on oxidative process related to their respective rate of oxygen consumption or peroxide production (Mackey and Seymour, 1989).

Prevention of *Salm. typhimurium* colonization of broilers with D-mannose is a recent approach. D-mannose blocks *Salm. typhimurium* adherence to chicken intestine in vitro. Certain carbohydrates may provide a mean to reduce *Salm. typhimurium* contamination in broilers (Oyofo *et al.*, 1989).

2.10.3. COMPETITIVE EXCLUSION

Competitive exclusion in preventing *Salm. typhimurium* infection of broiler chickens has been observed with a lyophilized extract of breeder flock litter, an anaerobic culture of adult fowl faeces. Old litter extract and anaerobic culture in combination were placed on litter, exposed at 3 days of age to *Salm. typhimurium* in drinking water. Intestines of all chicks indicated a lower incidence of infection at 708 weeks in treated chicks (Rigby and Pettit, 1980). Chicks treated with avian strain of *Lacto-bacilli* were subsequently infected with either *Salm. typhimurium* or *Salm. enteritidis*. The *Lacto-bacilli* reduced the number of *Salmonella* adhering to the crop mucosa but not on the caecal mucosa, nor did it reduce shedding of *Salmonella* (Soerjadi *et al.*, 1981). A defined mixture of bacterial isolates from the caecal microflora of an adult birds showed competitive exclusion against salmonellae. Colonization of the caeca of newly hatched chicks by *Salm. typhimurium* was prevented by the oral administration of a mixture of cultures comprising 48 different bacterial strains except *Salmonella* from an adult bird (Impey *et al.*, 1984 and Popiel and Turnbull, 1985).

Axenic chickens were given diluted suspension of adult birds feces and exposed to the salmonellae. Exclusion was dose dependent, a large inoculum of the salmonellae (10^7 viable organism per bird) leading to colonization in treated chicks (Lofont *et al.*, 1981). A defined mixture of 48 bacteria isolated from the chicken cecum and undefined anaerobic cultures of the contents of the caecum of both chicken and turkey were compared for their ability to prevent intestinal colonization of newly hatched chicks and turkey poults of *Salm. kedougou* or *Salm. typhimurium*. The defined bacteria mixture protected chicks but not poults even when a further 17 strains of different caecal bacteria were incorporated in the mixture. The undefined culture from chicken was less protective for poults than the corresponding turkey culture (Impey *et al.*, 1984 and Hinton *et al.*, 1990). A further passage culture of adult chicken caecal contents protected against challenge with *Salm. typhimurium* reared on wood-shavings poultry littre. Competitive exclusion of *Salm. typhimurium* by *lactobacilli* in chickens showed prompt effect Gleeson *et al.*, 1989). During a 5-years period, a bacterial flora from caecum of adult birds was given to broiler chickens in order to control *Salmonella* infection. Culture has a *Salmonella* contamination-preventing effect under field conditions (Wierup *et al.*, 1988 and Impey *et al.*, 1987). Poultry feed was contaminated artificially with either *Salm. kedougou* or *Salm. livingstone*. *Salm. kedougou* was the most efficient colonizer although for both serotypes infection rates varied in different groups of birds those were given feed containing similar number of salmonellae (Hinton, 1988c and Schneitz *et al.*, 1989).

2.10.4. FEED TREATMENTS

Salmonella is a common component of the commensal flora of the intestinal tracts of animals. However, some species of the

microorganism can be quite pathogenic. The disease they cause in poultry can have severe adverse effects on the economy of the poultry industry (Williams *et al.*, 1984). A combination of techniques has been utilized to achieve significant control of *Salmonella* infection of chickens (Bryan *et al.*, 1979) and to produce a raise to maturity, for a limited period of time, turkeys that were free of *Salmonella* (Zecha *et al.*, 1977). Failure to eliminate *Salmonella*, or to maintain a permanent *Salmonella*-free status in these projects, was ascribed largely to an inability to eliminate *Salmonella*, or to maintain a permanent *Salmonella*-free status in these projects, was ascribed largely to an inability to eliminate the organism from feed. Contaminated feed is a major source of infection for poultry (Gangarosa, 1978).

2.10.4.1. ADDITION OF ANTIMICROBIALS

Resistance is more serious threat in animal origin *Salmonella* which is less likely to be in human origin *Salmonella* (Pontello *et al.*, 1982). The inclusion of penicillin in the diet was associated with an increase in *Salmonella* shedding, particularly in the first half of the rearing period, but did not influence the lactobacillary count in the crop or the Ph of the contents of the crop, gizzard and caecum. Furazolidone medication (150 mg/kg feed) for the first 10 days had no effect on *Salmonella* carriage at the time of slaughter (Hinton *et al.*, 1986 and Ekperigin *et al.*, 1983). Administration of nosiheptide (20 g/ton) for 33 days against *Salm. typhimurium* var copenhagen has been found quite effective. The effect of feeding halofuginone at 3 and 6 mg/kg of feed on the excretion of *Salm. typhimurium* by experimentally infected chickens was studied. Halofuginone at 3 mg/kg showed no significant increase in excretion rate. The group fed 6 mg/kg showed a slight increase in excretion which was statistically significant (Barrow *et al.*,

1988a and Zabel *et al.*, 1989). It is well documented that prior antimicrobial exposure decreases resistance to infection by antimicrobial sensitive *Salmonella* (Pavia *et al.*, 1990).

2.10.4.2. CHEMICAL TREATMENT

Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed is an efficient method. In three experiments a solution of formic acid was added to feed "naturally" contaminated with salmonellae. In two of them no *Salmonella* infections were demonstrated in broiler chickens given feed containing 0.6 % (w/w) of the formic acid solution for seven weeks and in the third the infection rate was reduced considerably. The treatment of the feed with formic acid plus propionic acid mixture one week before the addition of the salmonellae prevented the establishment of infection in chicks given the treated feed (Hinton and Linton, 1988b). Feed given to laying hens with 0.5 per cent formic acid reduced significantly the isolation rate of salmonellae and was associated with a reduction in the incidence of infection in newly hatched chicks. Formic acid treatment of chicken feed could have important benefits for the public health (Humphrey and Lanning, 1988a). Chemical treatment of poultry feed reduced the chances of survival of *Salmonella*. After treatment with a chemical preservative (Myco-Curb) at 0.25, 0.5, 0.75 or 1 per cent, decontaminate commercial poultry feed. The number of faecal and intestinal samples positive for *Salmonella* was reduced, demonstrating elimination of *Salmonella* in the feed by the use of the feed preservative (Rouse *et al.*, 1988).

2.10.4.3. ADDITION OF SUGARS

Drug resistant *Salmonella* are in abundance in USA where drugs are not free in market, chemical treatment is good approach (Cohen and Tauxe, 1989). Acidification tolerance

response of *Salm. typhimurium* have been detected (Foster and Hall, 1990). The susceptibility of broiler chicks to *Salmonella* colonization is greatest during the first few days of life, after which resistance increases due to growth of normal intestinal flora (Barnes, 1979). Resistance to colonization provided by normal flora has been reported to be dependent on the level of *Salmonella* challenge and may be overcome by continuous or severe rechallenged (Pivnick and Nurmi, 1982). It has been reported that lactose added to the drinking water inhibited *Salm. typhimurium* colonization in 10-day-old broiler chicks (Oyofo *et al.*, 1989).

Proposed mechanisms by which normal intestinal flora prevent colonization by invading enteropathogens include: competition for limited nutrients (Freter, 1962); competition for attachment sites on the intestinal mucosa (Loyd *et al.*, 1977); and the production of short-chain, bacteriostatic VFAs, particularly acetic, propionic, and butyric acids, by anaerobic bacteria present in the ceca and colon (Rolfe, 1984). VFAs produced by anaerobic bacteria were reported to inhibit salmonellae growth and colonization in mice and in poultry. The bacteriostatic action of VFAs is Ph dependent and is exerted only when the acids are present in the undissociated lipophilic state. The concentrations of acetic, propionic and butyric acids present in the undissociated bacteriostatic state progressively increase as the Ph of the environment decreases and approaches the specific dissociation constant (pKa) of each fatty acid (Corrier *et al.*, 1990b).

Salmonella colonization in broiler chickens has affected by certain carbohydrates in the diets of chickens (Oyofo *et al.*, 1989). Mannose (2.5 % in water) reduced the over all cecal concentration of *Salm. typhimurium* by at least two orders of

magnitude. Based on *in vitro* studies with mannose, indicate that it interferes with the adherence of *Salm. typhimurium* to chicken intestinal epithelium cells (Mc Han *et al.*, 1989). Another carbohydrate lactose, was shown to reduce the *Salm. typhimurium* concentration in chicken ceca by two to three orders of magnitude. However, the mechanism of action of lactose and mannose are brought to be different. Lactose probably acts through promoting growth of lactose-fermenting bacteria that produce substances toxic to *Salmonella* (De Loach *et al.*, 1990).

Provision of normal intestinal flora to newly hatched chicks to prevent enteropathogen cecal colonization is known as the Nurmi concept of competitive exclusion and has been shown to effectively decrease cecal colonization by a number of *Salmonella* species. Effect of anaerobic cecal microflora and dietary lactose on colonization resistance of layer chicks to invasive *Salm. enteritidis* has proven of value (Richard *et al.*, 1990).

Salm. enteritidis has been reported to penetrate the cecal epithelium and pass into the lamina properia of newly hatched layer chicks enclosed within membrane bound vesicles and by capture and transport within host macrophage. Differences in invasive activity between virulent and avirulent strains of *Salmonella* were not observed by some researchers, and it was suggested that differences in invasiveness may be associated with a variety of factors, including the ability of *Salmonella* strains to multiply and colonize the ileocecal region of the gut. The normal intestinal flora increase colonization resistance against a variety of *Salmonella* serotypes. The normal intestinal flora and lactose in the feed decrease the *Salmonella* colonization (Richard *et al.*,1990).The Eh and volatile fatty acids in the normal gut flora play antimicrobial role (Meynell,

1963).

2.10.4.4. HEAT TREATMENT

As long as salmonellae are constantly introduced into farms in the feed, nothing substantial can be done about the situation. The only way to tackle *Salmonella* is at the source; feed. If cereals and other feed raw materials are involved as well as animal proteins, they will have to be included. Heat treatment is simple, cheap and effective (Grisedale, 1990).

2.10.4.5. PELLETING

Attempts have been made to eliminate *Salmonella* from poultry feed either by pelleting (Bryan *et al.*, 1979) or by sterilizing feed components of animal origin before their incorporation into feed (Marthedal, 1977). At best, such attempts have resulted only in significant reductions in levels of contamination. Sterilizing only certain components of feed ignored the probability of contamination from one or more of the other components (Marthedal, 1977). Pelleting should have been more effective, since it involved treating the whole feed. However, the pelleting process is weighted heavily in favor of production of good quality pellets, and the conditions favoring the production of good quality pellets are unfortunately not always the same as those who make difficult to the survival of *Salmonella*. Mash is pelleted by forcing it through die openings, the producers being facilitated by treatment of mash with steam (conditioning) for about 20 seconds before compression. Under current pellet-production practices, steam generated in a broiler is the only medium through which the heat energy of fuel can be transferred to mash. This heat transference is accompanied by condensation of the steam and a corresponding increase in moisture content of mash being heated. It has been estimated that the moisture level increases by 1.0 per cent for

every 11.1°C rise in temperature. For the relatively colder feed generally available during winter in cold climates, this means that in order to raise mash temperature to a level high enough to kill *Salmonella,* the moisture content of the mash would have to be allowed to increase beyond the choke point of the pellet mill. In other words, the mash would become too moist for pelleting (McCapes *et al.*, 1989).

Recently, an equipment configuration called the anaerobic pasteurizing conditioning system is introduced to the feed industry (Beaumont, 1986). It is claimed to be capable of permitting the attainment of high mash temperatures without causing mash to become too moist. Fuel is ignited and combined directly with water the vaporator resulting in the production of steam, nitrogen, and other hot gases (CO_2, CO), which are channeled into the conditioner to heat mash. The direct utilization of all the hot products of this combustion is said to make it possible to control temperature independent of moisture levels.

The susceptibility of microorganisms to the lethal effects of heat is influenced by genetic and environmental factors. For instance *Salm. senftenberg* is less susceptible to heat than most other salmonellae. Naked strains of microorganisms, on the other hand, are generally more susceptible than encapsulated or sporulated forms. Susceptibility is also influenced by size of population of the microorganism as well as by changes in temperature, heating time, moisture, acidity, and composition of the medium in which the organism is being heated (Bryan *et al.*, 1979). Optimum conditions of temperature, heating time, and moisture (optimum TTM) that will kill *Salmonella* is 87.8°C for 1.5 minutes, 89.4°C for 0.5 minutes at 15 per cent moisture (Liu *et al.*, 1969). Conventional pelleting is the most effective

anti-*Salmonella* feed processing technique currently available. However, it only reduces the level of *Salmonella* in feed (Marthedal, 1977). More reductions in the level of *Salmonella* contamination of feed cannot be useful in a *Salmonella* elimination program, because only as few as one colony forming unit of *Salmonella* per gram of feed is required to initiate infection (Gangarosa, 1978). If the observed elimination of *Salmonella* from feed in the present study is real, the new pelleting process could be regarded as providing the missing link in the chain of technology needed to eliminate the organism from poultry (McCapes *et al.*, 1989).

2.10.5. MICROBICIDAL EFFECTS OF VARIOUS DISINFECTANTS

The membrane disruptive and antimicrobial activities of cationic surfactants are well recognized. These agents are often active against a broad range of bacteria and other cells and can also inactive certain viruses (Hugo and Russel, 1982). Because of their high affinity for biological membranes, these agents show a low selectivity and can be damaging to a variety of mammalian cells (Pinnaduwage *et al.*, 1989). Since the time needed to kill microorganisms with cationic surfactants is usually short, it could be expected that side effects in the host might be decreased by the use of substances that are subject to hydrolytic degradation. However, the life-time of the compounds must be sufficiently long to allow proper inactivation of the undesired microorganisms. The products obtained in the degradation steps should also be significantly less toxic than the original compounds and should ideally constitute normal metabolites of the host (Lindstedt *et al.* 1990). To explore the possible use of degradable cationic surfactants, a series of amphophilic betaine esters have been studied. The interaction between cationic surfactants and microbial cells is not understood in detail. It seems generally accepted, however, that

lipid bilayer structures of cell membranes are principal targets for this class of compounds. In the process of bindings, the hydrocarbon tail of the cationic amphophilic substance becomes intercalated into hydrophobic interior of the microbial membrane, and the cationic polar head group participates in charge interactions with neighboring surface structures (Jawetz *et al.* 1989).

As for stable quaternary ammonium compounds, the initial site of interaction of the betaine esters is probably the lipid bilayer of the outer membrane. Furthermore, these substances cause leakage of cytoplasmic compounds, indicating that the plasma membrane is also affected (Hugo, 1982). The phospholipids of both types of membranes contain fatty acids, mainly C_{16} and C_{18} (Cronan and Rock, 1987), and there is a rapid exchange between the phospholipids of the outer and inner membranes. In the lipopolysaccharide of *Salmonella* strains, the 3-hydroxy-tetradecanoic acid residues, which are amide linked to the glucosamine moieties of lipid A, are 3-O acylated by C_{12} and $C16$ saturated fatty acids, allowing a hydrocarbon chain length in the outer cell membrane of at least 18 carbon atoms. Thus, the high bactericidal activity of the C_{16} and C_{18} betaine esters may be due to the facts that both the outer and the plasma membrane lipid bilayers may accommodate the entire hydrocarbon chain length of these betaine esters and that longer hydrophobic chains have a greater hydrophobic effect (Tanford, 1980). Change interactions between quaternary nitrogen groups in betaine esters and phosphate groups in phospholipids and lipopolysaccharide may contribute to complex formation. The higher antibacterial activity of octadecyl quaternary ammonium compounds was shown the earlier comparative tests of series of substances with different hydrocarbon chain lengths and other chemical structures adjacent to the quaternary nitrogen

(Linfield, 1970). Because the time needed for microbial killing is short, a reduction of the lifetime of the esters by hydrolysis should allow effective disinfection and antisepsis with reduced toxic effects.

2.10.6. WATER TREATMENT

The drinking water provided to poultry can be a potential source of disease causing organisms. However, this fact is sometimes overlooked by poultry farmers and a loophole is left in their hygiene programs. There are three main point which have to he considered. First, attention to the entire system as part of the terminal cleaning program. Secondly, the quality of the actual supply and regular sanitizing of it, when necessary. Finally, the prevention of transmission of disease causing organisms *via* the drinking water at times of high risk.

To gain the full potential from water sanitizing, as with any other aspect of disinfection, it is vital to follow the correct procedures and to select the most suitable and cost effective product. Traditionally, terminal disinfection of the water system and water sanitization, when carried out, was done with one of a number of chemicals. All have some limitations in activity and, occasionally, there is risk of toxicity to stock if care is not taken. Examples are the quaternary ammonia compounds, the iodine based products and hypochlorite. A recently developed synergised acid peroxide disinfectant, virkon S, provides a safe and effective answer to all aspects of water sanitization. The product is a solid formulation that is readily soluble in water. It is a balanced sterilized blend of peroxygen compounds, surfactants, organic acids and an inorganic buffer system (Woodger, 1990).

2.10.7. VACCINATION

Trial of fowl typhoid live vaccine prepared from *Salm. gallinarum* strain 9R suggested that this vaccine was unsuitable for disease control or eradication programs because of the spread of infection among birds in contact with vaccinated birds, and because of the positive serological reactions that it induced (Kirsche, 1986 and Lee, 1986). *Salm. gallinarum* 9R vaccine was more effective than *Salm. enteritidis* E20 live vaccine. Both vaccines more effective with Freund's complete adjuvant vaccine (Padmanaban *et al.*, 1981). Antibodies to whole bacterial cells and lipopolysaccharide were detected in the serum and bile of all chicks from day 24. Cell mediated immunity to Salmonella antigens was also present in chicks that had acquired the infection naturally (Lee, 1989). Polymer flagellin prepared by acid hydrolysis was given by mouth as aqueous suspension or by intramuscular injection as water oil emulsion. Only parental immunization conferred protection against oral challenge against *Salmonella* (Matsui, 1989). Purified lipopolysaccharides (LPS) and -O-antigenic polysaccharides (O-PS) from *Salm. typhimurium* type 0, 1, 4, 5, 12 incorporated into negatively-charged multiamellar liposomes showed a high affinity for macrophage and other fixed reticulo-endothelial cells *in vivo*. Immunization of mice with liposome associated O-PS induced a specific cellular response to the 0, 1, 4, 5, 12 antigen as determined by delayed type hyper-sensitivity reaction. this vaccine was ineffective in generating a specific cellular response (Desidero and Campbell, 1985).

Salm. gallinarum 9R strain was used as immunogenic support against fowl typhoid. To each of four live 9R vaccines containing 1 x 10^9 micro-organism/ml were added one of the adjutants 25 per cent Al $(OH)_3$, levamisole at 25 mg/ml, 5 per cent saponin, or 50 per cent incomplete Freund adjuvant. Groups

of 4-week old chicks were vaccinated S/C with 0.5 ml of a vaccine, then half of each group was revaccinated when 8 weeks old. The birds were challenged at 4, 12 or 20 weeks after the second vaccination. Protection at 28 weeks of age was only found in birds vaccinated twice with the vaccines containing AL $(OH)_3$ or incomplete Freund adjuvant (Kahraman and Ozcan, 1985). Immunization of pigeons was performed with subunit vaccines against *Salm. typhimurium*. Extracts of *Salm. gallinarum* prepared by acid hydrolysis protected pigeons from *Salm. typhimurium* when given by mouth, but not when inoculated parenterally. Outer membrane protein was isolated from *Salm. gallinarum* by urea extraction. Chickens were vaccinated at 8 and 12 weeks of age with the protein extract at levels of 50, 100, 200, 400 µg/100 gm live body weight. Protein extracts were in aqueous suspension or with a mineral oil adjuvant, formalin-killed whole cell bacterin of the same organism. Chickens were challenged at 15 weeks of age with live *Salm. gallinarum*. Chickens in all groups showed a positive seroconversion with an anamnestic response after the second injection but titres among the groups did not differ significantly (Bouzouba *et al.*, 1987). Multi doses of TY21 a live oral typhoid vaccine proven good with enteric coated capsules (Ferreccio *et al.*, 1989).

Vaccination with a rough mutant strain referred to as 9R for the control of Fowl typhoid has been reported. However, the protection provided by 9R vaccine in highly susceptible chickens exposed to virulent field strains of *Salm. gallinarum* has been minimal and has not limited the egg transmission of FT (Silva *et al.*, 1981). One limitation of both 9R and protein extracts for the control of fowl typhoid is that many vaccinated birds develop antibodies that produce reactions in serological tests for pullorum-typhoid. However, these reactions resulted from antibodies to contaminating lipopolysaccharides (LPS), because

the accurrence of low levels of LPS cannot be prevented in protein preparations from the bacterial cell wall (Bouzoubaa, *et al.*, 1989 and Udhayakumar and Muthukkaruppan, 1987). Oral killed typhoid vaccine in field proven ineffective (Chuttani *et al.*, 1973)

Day-old chicks treated with defined mixtures containing 50, 40, 25 or 10 bacterial isolates, administered orally and challenged 2 days later with 10^4, 10^5 or 10^6 cfu of nalidixic acid resistant *Salm. typhimurium*. The mixture of 50 bacterial isolates gave protection against salmonellosis comparable to that obtained with fecal or cecal cultures of unknown bacterial composition. Less protection was given by mixtures containing fewer cultures. The initial vaccination programs involved the use of killed (Aitken *et al.*, 1982) or rough mutants (Wray *et al.*, 1977) and there have been conflicting reports on the efficacy of these vaccines (Smith *et al.*, 1980). Gal-E mutants of *Salm. typhimurium* (rough mutants) have been used in vaccination program to protect chickens against salmonellosis. Oral administration of these mutants was reported to reduce the shed of virulent *Salm. typhimurium* in feces of chicken challenged with the virulent organisms. However, the lack of detectable serological responses in these investigations and the potential pathogenicity of Gal-E mutants ((Wray *et al.*, 1977) calls into question their usefulness as vaccines. Hoiseth and Stocker (1981) reported on the use of aromatic vitamin-dependent (Aro-A^-) avirulent *Salm. typhimurium*. These Aro-A^-strains have been further improved and shown to protect the animals against challenge with virulent *Salm. typhimurium*. More recently protection against experimental salmonellosis after immunization with aromatic dependent *Salm. typhimurium*. Vitamin dependent *Salmonella* spp. have been preferred in vaccine programs because they are invasive when provided with a source of the vitamin(s)

that they depend on for growth. However, following invasion, the organism quickly exhausts its supply of the vitamin(s) and is eliminated from the host. These features mean the organism is in contact with the host's immune system long enough to enable to strong immune response to be mounted before the organism is eliminated, without detrimental effects to the host. However, much work is still needed to determine the extent and type of immune response elicited by these Aro$^-$ strains when they are introduced into selected animals. Recent work on salmonellosis in chickens has not involved vaccination using an avirulent *Salm. typhimurium*. The work has concentrated an other aspects of salmonellosis control, such as the effects of drug treatment (Barrow *et al.*, 1988) and pre-colonization of the alimentary tract with avirulent *Salm. typhimurium* mutants, and on their effect on the excretion and colonization of virulent *Salm. typhimurium* (Alderton *et al.*, 1991).

Live attenuated salmonellae are believed to give greater protection than killed vaccines due to a sustained antigenic stimulus and the development of cell-mediated immunity. Immunization of adult hens with live *Salm. gallinarum* 9R vaccine caused a significant reduction of ovarian infection with wild type *Salm. enteritidis* PT4 (Mestecky, 1987).

Recently use of peripheral blood antibody secreting cell (ASC) assay as a measure of the immunogenicity of an oral vaccine. Circulatory ASC have been demonstrated in humans after oral vaccination with both live and killed microorganisms. Circulating ASC are also found after parenteral vaccination (Kantele *et al.*, 1991). Comparison of the ASC responses after oral and parenteral immunization suggests qualitative differences between the routes. However, these studies have been carried out using different antigens and some what different

methods of investigations. Protective immunity can be induced by outer membrane proteins of Salm. typhimurium (Udhayakumar and Muthukkaruppan, 1987).

2.11. PUBLIC HEALTH SIGNIFICANCE

Salmonellosis, a common human intestinal disorder primarily caused by *Salmonella* contaminated meats and poultry was estimated to cost Americans around $1000 million in 1987. Control of salmonellosis in animals, is a key objective of regulatory program in reducing human salmonellosis (Roberts, 1988 and Ward and Palmer, 1989)). *Salm. typhimurium* is persistent in dairy animals. Milk filters were positive (35.62 %) for *Salmonella* associated with three human disease incidents. Cows may have been excreting the organism intermittently from the udder for 2-5 years (Giles *et al.*, 1989). An outbreak of *Salm. typhimurium* infection affecting 101 people was reported in England. German salami stick as the vehicle of infection and the product was withdrawn from sale. the epidemiological investigation highlighted the occurrence of a long incubation period, bloody diarrhea (Klonin *et al.*, 1989 and Lam *et al.*, 1985).

Salm. paratyphi B infection in the U. K. was associated with the fish and chips shops. The source of infection for the first handler who was infected overseas 6 years earlier. A second household contact of the proprietor also became a faecal excreter 2 months later. Food handlers living in house holds or in intimate contact with cases or carriers of *Salm. paratyphi* B. (Francis *et al.*, 1989). Food poisoning in a worker's camp in Saudi Arabia was caused by *Salm. minnesota*. Acute gastroenteritis with diarrhea, vomiting, abdominal pain, and low grade fever were the common complaints. the outbreak was confined to those who ate from a single kitchen. One of the 27

cooks was positive for the organism (Al-Ghamdi *et al.*, 1989). *Salm. typhimurium* were the highest isolates in blood, stools and cerebrospinal fluid the. The infection was nosocomial in nature hence there is a need for coordination between the laboratory and clinical staff to prevent the spill-over of infection (Mirza and Wamola, 1985).

The vaccination route, infectivity, thioglycolate broth administration and effect of live vaccine efficacy of *Salm. choleraesuis.* were evaluated. An aromatic-dependent, non-virulent, derivative of mouse virulent strain of *Salm. choleraesuis* previously showed not to be effective as live vaccine when given intraperitoneally. Two doses given intraperitoneally or by feeding did not protect against intraperitoneal or oral challenge (Dragunsky *et al.*, 1989 and Nnalue *et al.*, 1989). Three unusual presentation of *Salmonella* infection in Asian infants in Nepal have been reported. *Salmonella* infections should be considered in the differential diagnosis of acute illness of obscure etiology, especially in *Salmonella*-endemic areas (Klonin, 1989). *Salm. typhimurium* is always associated with severe diarrhea in infants and young children. The organism showed multiple drug resistance. All patients received antibiotics for 10-14 days which was followed by rapid improvement in clinical signs. Antibiotic therapy may benefit children with *Salm. typhimurium* associated severe protracted diarrhea and rapid progress in weight loss (Khoshoo *et al.*, 1990). Outbreaks of *Salm. enteritidis* associated eggs from over seas are still a havoc to human beings (Stevens *et al.*, 1989).

CHAPTER 3

MATERIALS AND METHODS

One hundred and fifty chicken broiler breeder flocks around Rawalpindi, Islamabad and Abbottabad (90 % of the total breeder population of Pakistan) were screened for pullorum. Laying chicken broiler breeder found seropositive were confirmed by yolk agar precipitin, and tube agglutination tests. Isolation studies were carried out from the seropositive and seronegative birds, eggs, dead in shell embryos, litter, water, faeces, hatchery fluff, feed as well as from feed ingredients and poultry houses allied sources. Salmonellae were confirmed by biochemical and serological tests. Experimental studies on virulency factors, competitive exclusion, chemical inactivation of *Salmonella* in feed, drug susceptibility, and gross as well as histopathological studies were also undertaken in naturally and experimentally challenged birds. Ultrastructural alterations were studied to determine any changes in visceral organs of birds infected with *Salmonella*. Various steps for these investigations are described as under.

3.1. SEROLOGICAL STUDIES

3.1.1. RAPID BLOOD AGGLUTINATION TEST

Pullorum stained antigen K polyvalent (Salsbury Inc. Charles city, Iowa, USA) was used to identify seropositive birds

from 150 chicken broiler breeder flocks (2,62,454 birds). Spot agglutination test was performed on an illuminated wooden square box with glass toping at 70 to 80°F temperature. Blood samples in 0.02 ml volume were mixed with 0.05 ml of antigen in one cm area. Assay was read after 30 seconds. The development of blue colored clusters surrounded by clear spaces was considered as positive test for *Salmonella*.

3.1.2. TUBE AGGLUTINATION TEST

Tube agglutination test was performed to monitor the anti-pullorum antibodies in those birds which were delayed or slow reactors in spot agglutination test. Ten fold serial dilutions (0.5 ml) of the test serum were mixed with 0.5 ml volume of 1:25 diluted unstained antigen in normal saline at Ph 8.2. After 20 hours of incubation at 37°C in 65 per cent humidity, the tubes were examined against a dark background. A button formation by the unused antigen at the bottom of the tube was considered as negative and the tubes with agglutination of antigen was considered as positive for *Salmonella* antibodies.

3.1.3. YOLK AGAR PRECIPITIN TEST

Yolks from seropositive laying birds were tested for the presence of anti-*Salmonella* antibodies following the method of Zhao *et al.* (1981). This test was used as a tool to detect *Salmonella* carriers in breeder flock. Fifteen ml of melted noble agar was poured into clear sterile Petri dishes (9 cm) and

stored at 4°C. Four wells of 6.5 mm in diameter each were cut in a circle for sample and fifth in the center for antigen. The inner edges of the outer wells were 5 mm aparted from the circumference of the central well. The central well was filled with 2-3 drops of washed polyvalent unstained antigen of *Salmonella* and 3 surrounding wells were filled with diluted yolk (1:10 with distilled, demineralized sterile water) and forth with phosphate buffer saline (PBS) of Ph 7.2 as negative control. Plates were kept in a humidified container, at room temperature and observed for the development of the precipitin bands after 24, 48 and 72 hours.

3.2. BACTERIOLOGICAL STUDIES

3.2.1. ISOLATION

Liver, lungs, heart, spleen, intestines, caeca, ovary, testis, kidneys, bursa of Fabricius and brain 2,114 of each were randomly collected from broiler breeders. Eggs (1029), cloacal swabs (527), litter samples (215), water (147), house dust (111), dead in shell embryos (1007), hatchery fluff (105), feed (185), feed ingredients (144) and rodent faeces (215) were also collected from seropositive flocks and houses. The samples were subjected to bacteriological analysis to determine the prevalence of *Salmonella* species. Isolation was also undertaken from intestines, liver and spleen 251 of each organ from indigenous chickens. Three hundred and seventy cloacal swab from

various species of avifauna was also attempted.

3.2.1.1. ISOLATION FROM VISCERAL ORGANS OF BIRDS

The surface of the visceral organs was seared with red hot spatula. Material was sucked with sterile pasture pipette and inoculated in selenite, Mac-Conkey's and tetrathionate broths. The inoculated broths were incubated at 37°C for 24 hours and subsequently streaked on Mac-Conkey's (MC), *Salmonella-Shigella* (SS) and Eosin Brilliant Green (EBG) agars. The inoculated plates were incubated at 37°C for 24 hours had examined for the presence of smooth transparent dew drop like colonies (Edward & Ewing, 1989).

3.2.1.2. ISOLATION FROM CLOACAL SWABS

Since young birds lack sufficient circulating anti-*Salmonella* antibodies to allow rapid detection by serum, cloacal isolation for *Salmonella* was performed. Cloacal swabs (527) were obtained from broiler breeder upto 18 weeks of age and 370 cloacal swabs were taken from various birds of avifauna. A sterile glycerine moistened cotton plug was inserted by gentle rotation into cloaca. The swab was then partially inserted into a tube containing 10 ml of selenite broth and incubated at 37°C for 24 hours. Afterwards, the bacterial growth in broth was streaked on MC, SS and EBG media.

3.2.1.3. ISOLATION FROM EGG SHELL AND MEMBRANES

Eggs (508) of seropositive birds were monitored for the interior and/or exterior contamination with *Salmonella*. Intact eggs were dipped in sterile plastic bags containing 40 ml of selenite broth to isolate the shell contaminants. The bags were incubated in the rotary shaker for 20 minutes and the broths were poured in the sterile test tubes for incubation. Isolation was conducted by streaking the broths on the above mentioned solid media.

3.2.1.4. ISOLATION FROM EGG YOLKS

In carrier birds ovary is the predilection site of the *Salmonella*. To evaluate the true transovarian transmission and excluding the possibility of false contamination of eggs with faecal material at the cloaca, isolation from yolk were carried out. Intact (521) eggs were dipped in 2 per cent tincture of iodine and then fumigated in close chamber with formaldehyde gas to exclude the exterior contaminants. Yolks were removed by cutting the shell of the eggs and whole yolk was poured into the flask containing selenite broth and sterile glass beads to homogenize the yolk in the broth. Flasks were incubated and isolations were conducted as described earlier.

3.2.1.5. ISOLATION FROM DEAD IN SHELL EMBRYOS

Eggs of seropositive flocks were monitored in the hatcheries to trace the transovarian transmitted *Salmonella*. These isolations were carried out to evaluate the presence of specific *Salmonella* serotypes in embryos as a source of infection to the hatched chicks. Eggs were dissected and a composite sample of visceral organs and yolk contents was incubated for isolation studies.

3.2.1.6. ISOLATION FROM LITTER

Random litter samples from seropositive flocks were collected in sterile plastic bags. The composite samples were mixed with selenite broth in the rotary shaker for 24 hours. The broth was then transferred to a test tube and incubated for the isolation on the solid media.

3.2.1.7. ISOLATION FROM WATER

To evaluate the possible role of the contaminated drinking water in the spread of infection in the broiler breeders, the water samples from the discharge point of water source, drinkers and from the main storage tanks were also collected. Ten ml of each water sample was dispensed in selenite broth and incubated for isolation of *Salmonella* serotypes.

3.2.1.8. ISOLATION FROM FECES

Sterile drag swabs were drawn through fresh dropping under roosters, near water troughs, and on the nest tops. The swabs were put into 15-20 ml of selenite broth and stirred to maximize the *Salmonella* isolation in feces. The broths were incubated for isolation of salmonellae shedded in the feces.

3.2.1.9. ISOLATION FROM HATCHERY FLUFF

Hatchery fluff samples were collected at the hatching time. Test tubes were selenite broth were filled half the way with the fluff and dust from the separate hatchers. The samples were incubated for isolation on solid media.

3.2.1.10. ISOLATION FROM FEED AND FEED INGREDIENTS

Feed ingredients particularly animal origin protein sources and finished feed samples (upto 100 gm/sample) were collected from different seropositive farms and feed mills. Duplicate Samples (50 gm) were inoculated in the selenite broth and set in the shaking incubators at 37°C for 6 hours before shifting to bacteriological incubator. Samples exhibiting bacterial growth were streaked on solid media for further characterization.

3.2.1.11. ISOLATION FROM RODENT FECES

Rodents play a vital role in the spread of *Salmonella* by contaminating the feed under poor storage conditions as well as

water. Rodent feces from the seropositive flocks allied feed stores were collected in plastic bags. The feces were inoculated in selenite broth and later streaked on solid media, as described above.

3.2.2. IDENTIFICATION

3.2.2.1. BIOCHEMICAL TESTS

Typical lactose negative colonies were cultured on the Triple Sugar Iron (TSI) agar to confirm *Salmonella* strains biochemically (Edwards & Ewing, 1989). The medium composed of three sugars, lactose, glucose, sucrose and ferrous sulphate to determine H_2S production and phenol red as an indicator. The isolates with positive glucose and negative lactose and sucrose fermentation along with +/- H_2S and/or gas production, were suspected as *Salmonella*. Other biochemical tests were performed with self developed kit like API-20E system, modified in the 96 well plate for the biochemical and sugar fermentation tests. Sugar fermentation tests included adonitol, dulcitol, sorbitol, arabinose, xylose, rhamnose, maltose, inositol, lactose, sucrose, mannitol, glucose and galactose. Biochemical tests included indole, H_2S, NH_4 glucose, NH_4 citrate, KNO_3,Voges-Proskauer, methyl red, urea, citrate, mucate, tartrate and KCN tests.

3.2.2.2. SEROLOGICAL IDENTIFICATION

Isolates giving typical biochemical reactions of *Salmonella* were confirmed and typed serologically with standard *Salmonella* somatic (O) polyvalent and group antisera, obtained from *Salmonella* reference laboratories Cantaczino institute, Bucharest, Romania. These antisera, PO (1:5), AO (1:8), BO (1:14), CO (1:8), DO (1:14): etc were diluted by 0.1 % phenolized normal saline (0.85 %) as recommended by the reference laboratory. Colonies of organisms were mixed with a drop of polyvalent "O" antisera and agglutinating isolates were considered to be *Salmonella*. Further serotyping was done by mixing a loopful of culture with a drop of group antisera of AO, BO, CO, DO, EO separately (Edward and Ewing, 1989, Javed *et al.*, 1990 and Javed *et al.*, 1992a).

The isolates, confirmed upto group level by slide agglutination, were further confirmed by tube agglutination with flagellar antisera (Ha, Hb, Hc, Hd, Hr, Hi, Hgst, Ht, Hlv, Hy, Hz etc) respective to the somatic groups. Five ml of 18th hours old motile *Salmonella* cultures in nutrient broth were mixed with ≈ 300 µl of formalin (37 % v/v in saline) to prepare the flagellar antigen of the bacteria for agglutination and denaturing the formalin sensitive O and K antigens. Half ml of the formalized culture was added in 0.5 ml of the diluted

respective flagellar antiserum fraction. The tubes were incubated at 56°C for 2 hours in water bath and cooled to room temperature without disturbing. Agglutination was read at the bottom of the tube with gentle shaking towards light source. The intensity of positive agglutination in term of surface area was scored as +, ++,+++, ++++. This criterion allowed appropriate *Salmonella* strain identification based on flagellar fraction. In case of similar flagellar agglutination, intensities with one antiserum for two *Salmonella* variants, the serotype distinction was obtained by sugar and biochemical reaction results based on Kauffmann scheme (Kauffmann, 1960 and Edward and Ewing, 1989).

3.3 EXPERIMENTAL STUDIES

3.3.1. EGG SHELL PENETRATION

To investigate the extent of *Salmonella* penetration through the egg shell, egg were dipped in red and green aqueous bland food color solution for the detection of positive penetration test areas. Eggs were soaked in the red solution for 3 minutes, and in green solution for 6 minutes before being washed with sterile water. After candling, an area of 1 cm in diameter was marked on the egg shell. Sterilized steel cylinders (1 cm in diameter and height) were attached on the egg shell around the marked points by sealing their outer boundaries with melted paraffin under aseptic conditions. The cylinders were filled with the test strain of salmonellae and upper side of the

cylinder was sealed with the parafilm and eggs were incubated for 18 hours. Eggs were opened under aseptic conditions and isolation of penetrated *Salmonella* was attempted from the stained spots on the shell membrane to confirm the positive penetration.

3.3.2. CHICK EMBRYO INOCULATION

Groups of 10 embryos were exposed by injecting 0.2 ml of 10^{-7} dilutions of 18 to 20 hours broth cultures of selected *Salmonella* strains on day 2 of incubation into the albumen from the small end of the egg (Snoeyenbos *et al.*, 1969). These low infecting dose (100 cells) usually allowed a few infected embryos to survive and hatch. One isolate from each group of salmonellae was studied by this method. Gross and histopathological studies of the embryos were undertaken to characterize the specific lesions at tissue and cellular level in visceral organs.

3.3.3. CHICK INOCULATION

For each selected serotypes of *Salmonella* isolate, 0.5 ml bacterial culture ($\tilde{}$ cfu/0.5 ml) was injected i.p. into 10 day-old chicks obtained from seronegative *Salmonella* parent flock. Chicks were monitored for clinical symptoms. Dead birds were necropsied for gross lesions and tissues from visceral organs were subjected to histopathology.

3.3.4. COMPETITIVE EXCLUSION

The crop and the cecum are the major sites of *Salmonella* colonization. The role of *lactobacilli* in competitive exclusion of *Salmonella* was investigated. Three replicate groups of 15 chicks per replicate were administered for 3 days with *lactobacilli* in drinking water, three days later, birds in three replicates were challenged with 2 x 10^5 cfu with *Salm. gallinarum*, *Salm. pullorum* and *Salm. typhimurium*, respectively. A fourth group of chicks served as control which received no *lactobacilli* but *Salmonella* alone. Five chicks from each group were euthanized at 4, 8, and 12 days of age. Crop and ceca were cultured for *Salmonella*.

3.3.5. FEED SUPPLEMENTATION

To check the influence of feed additive on *Salmonella* carriage in chicken, sodium ethylene diamine tetra acetic acid (Na EDTA) was used in this trial. Five groups of 10 chicks per treatment were fed EDTA at a dosage level of 5 and 10 gm per 50 kg of feed for 7 and 14 days to the respective treatments. The five treatments were designated as, T_1 (control, on normal diet), T_2 (5 g Na EDTA/50 kg of feed for 7 days), T_3 (5 g Na EDTA/50 kg of feed for 14 days), T_4 (10 g Na EDTA/50 kg of feed for 7 days) and T_5 (10 g Na EDTA/50 kg of feed for 14 days). During the feeding trial, birds were given *Salm. typhimurium* in drinking water at a rate of 2000 cfu/ml. Intestines of the birds

were monitored at the end of the experiment for the presence of localized salmonellae. Shedding of *Salmonella* in the faecal material was used as an indicator of the effects of Na EDTA supplementation on the incidence of *Salmonella*.

3.3.6. VACCINATION TRIAL

Salm. gallinarum 9R strain was used as a vaccine strain for fowl typhoid. Twenty chicks in each group were vaccinated with 1 x 10^9 cfu/ml, sub cutaneously, orally and intramuscularly at 8 week of age. Blood samples were collected at 7, 14, 28 and 70 days to check anti-*Salmonella* antibody levels in each group.

3.4. DRUG SENSITIVITY STUDIES

3.4.1. ANTIBIOGRAM

Antibiogram of the various isolated *Salmonella* serotypes was performed. Strength of ampicillin (A), chloramphenicol (C), erythromycin (E), flumequine (AR), furazolidone (F), tetracycline (T), Kanamycin (K), lincomycin (L), neomycin (N) was 30 µg/disk, streptomycin (S), 50 µg/disk while, vibramycin (V) and gentamicin (G) were 10 µg/disk. Tribrissen (TS) 25 µg/disk was a combination of trimethoprim and sulpha methoxazole. Penicillin was 50 IU/disk. Sensitivity was conducted on the Iso- sensitivity media as previously described (Siddique *et al.*, 1985).

3.4.2. SENSITIVITY OF DIFFERENT DISINFECTANTS

Sensitivity of various isolates of *Salmonella* to various disinfectants was determined. Two commercial disinfectants included Belaron (Ciba Giegy), Virkon (Antec Internation). Microbicidal effects (ME) of both disinfectant was established at 20°C for 5, 15 and 30 minutes exposure basis.

M.E. = °C/T - log NC - log ND

Where

NC = The number of cfu/ml without the disinfectant.

ND = The number of cfu/ml evaluation after the disinfectant has taken effect.

°C = Temperature in °C during evaluation.

T = Time of exposure of disinfectant in minutes

Twelve ml of sterile 5 per cent yeast suspension was added to 4 ml of sterile water and 2 ml of the disinfectant in 10 times concentration recommended for routine use. This suspension was shaken after every 10 minutes for 45 minutes. Two ml of 24 hours broth culture of the test organism of each group were added to the disinfectant suspension. After 5, 15 and 30 minutes of addition of culture to the disinfectant suspension, 0.2 ml mixture was pipetted out into 1.8 ml of sterile neutralizer solution to inactivate the disinfectant. Neutralized disinfectant mixture (obtained from TNO -CIVO institutes, Holland) representing 5, 15 and 30 minutes treatments pipetted

out 0.02 ml and streaked on the SS agar plates for incubation. Colony forming units were counted to evaluate the efficacy of disinfectant.

3.5. STUDIES ON THE VIRULENCY FACTORS

3.5.1. ENTEROPATHOGENICITY AND ENTEROTOXIGENICITY

Rabbit intestinal loop assay was performed for the detection of *Salmonella* enterotoxins in concentrated *Salmonella* cell-free culture supernatants. Eight to ten cm long isolated intestinal loops were made by ligating the ileum starting from the distal end. Each loop was 4 cm aparted. Individual loops were injected with 1 ml of culture filtrate. After 18 hours the loops were removed and amount for fluid accumulated per unit length (cm) of ileal loop was calculated. A ratio of > 0.7 was considered as positive test (Gonzalez *et al.*, 1989)

Positive samples were injected intradermally in rabbit skin to perform skin permeability test. Three-cm^2 areas were marked on the belly region and injected with 0.1 ml trial filtrate. After 18 hours, Evan's blue 1.25 per cent was injected iv and 2 hours later rabbits were euthanized with overdosing anesthesia (Barbitol sodium). Results were recorded as induration, blueing, necrosis and blanching of skin in positive cases.

3.6. PATHOLOGICAL STUDIES

3.6.1. GROSS PATHOLOGICAL STUDIES

Different visceral organs from *Salmonella* positive birds, embryos and chicks were examined for the presence of gross abnormalities in term of size, consistency, changes in color, hemorrhages, congestion and necrotic changes.

3.6.2. HISTOPATHOLOGICAL STUDIES

Selected pieces of 5-6 mm size from morbid organs showing gross abnormalities were removed for histopathological studies. These tissues were fixed in 10 per cent formalin solution. The fixed tissue pieces were dehydrated through ascending grades of ethyl alcohol and xylol for clearing. Samples were infiltrated in melted paraffin at 58°C for 6-12 hours depending upon the tissue. The tissues were then embedded in paraffin and tissue blocks were prepared. Sections of < 5 µm thickness were cut with the rotary microtome as previously described Sabri *et al.*, 1986.

The slides were deparaffinized in two successive steps in xylol for two minutes each and then brought to ascending grades of ethyl alcohol. Staining was done with routine hematoxylin and eosin stain. Selected tissue sections were also stained with oil-red-O, Congo red, trichrome and periodic acid schiff to detect the presence of fatty change, amyloid, smooth muscle proliferation, hyaline, fibrin, colloid and integrity of

cacodylate buffer (Ph 7.2) for 15 minutes each time. Tissues were placed in 1 per cent osmium tetraoxide until they turned black in about 1 hour. Osmicated tissues were again washed 3 times with the phosphate buffer. Washed tissues were passed through ascending concentrations of ethanol (50, 75, 95 % and twice in 100 %). these tissues were infiltrated 2 times in 1:1 and 1:3 propylene oxide:epoxy plastic, for 30 minutes each time and kept in pure epoxy at least 2 hours. the capsulized blocks were polymerized at 90°C overnight in a histodryer. thick sections (0.5 µm) were stained with toluene blue and basic fuschin to target the areas with major changes, then thin sections (60-90 nm) were prepared from the target area and stained with uranyl acetate and lead citrate to enhance their ultrastructural visibility under JEOL 100-CX transmission EM (Javed *et al.*, 1992b)

3.7. STATISTICAL ANALYSIS

Data was analyzed for intratreatment and intertreatment differences within each study. Significant of differences were analyzed at the level of $P< 0.01$ through $P< 0.05$ level using the chi square, ANOVA and Dunnet double sided tests among the univariates and multivariates (Javed *et al.*, 1992b).

CHAPTER 4

RESULTS

During the years 1988-1990 chicken broiler breeder flocks around Islamabad, Rawalpindi and Abbottabad were screened for *Salmonella* by the rapid hemagglutination test using Salsbury's stained pullorum antigen. Most of the flocks were tested between the age of 21 to 40 weeks, a few at a later age, while on some farms the birds were tested and the carriers were removed. A complete history of the flocks, including the number of birds, the breed of the parent flock, the type of feed on which the birds were reared and the age of the birds was recorded. Details about the management of the farms were noted and categorized as excellent, good, satisfactory, and poor. The data thus obtained were analyzed statistically to see the effect of the feed, breed, age and management on the prevalence of *Salmonella* seropositive.

4.1. PREVALENCE OF *SALMONELLA* IN BROILER BREEDERS

Rapid hemagglutination testing of 150 chicken broiler breeder flocks showed that 112 (74.7 %) flocks were positive for *Salmonella* and only 38 flocks were negative. On these breeder farms 2,62,454 birds (2,28,583 females and 33,871 males) 12,159 were recorded to be carriers thus indicating a prevalence of 4.63 per cent. Data on the prevalence of *Salmonella* seropositive were analyzed according to various feeds to rule out the possible role of the feed in the spread of the pathogen. Birds in these 150 flocks were reared on eight different feeds and form the commercial point of view

they were designated F_1 to F_8 (Table 2).

4.1.1. FEED WISE PREVALENCE

Prevalence of *Salmonella* carriers varied greatly among birds fed on various commercial feeds. Regarding the flocks on most of the feeds, the prevalence varied from 69.2 to 82.2 per cent, ranging from 50 to 100 per cent on flock basis (Table 2). On one of the feeds, birds showed the prevalence as high as 11.33 per cent in one flock on bird basis.

4.1.2. BREED WISE PREVALENCE

The broiler breeders included in the present studies belonged to live various breeds and from the commercial point of view they were designated from B_1 to B_5 (Table 3). Breed B_4 showed the maximum prevalence (5.87 %) of salmonellosis followed by B_1 (5.13 %) and B2 (4.27 %). Significant low level of prevalence was noted in B3 (0.073 %) and B5 0.45 per cent (Fig. 1). In Pakistan parent flocks are imported from technically advanced countries and there seems to be less chance of getting *Salmonella* from grand parents, we were astonished to see the *Salm. gallinarum-pullorum* organisms were sometimes isolated from one-day old parent flock chicks. Strict measures for the import of *Salmonella*-free parent flocks would be a basic step for the elimination of salmonellosis and for the real development of our poultry industry.

4.1.3. AGE WISE PREVALENCE

Most of the flocks were tested between the age of 21 to 40 weeks but many flocks also at later stages and some of the birds were retested. The prevalence of *Salmonella* carriers

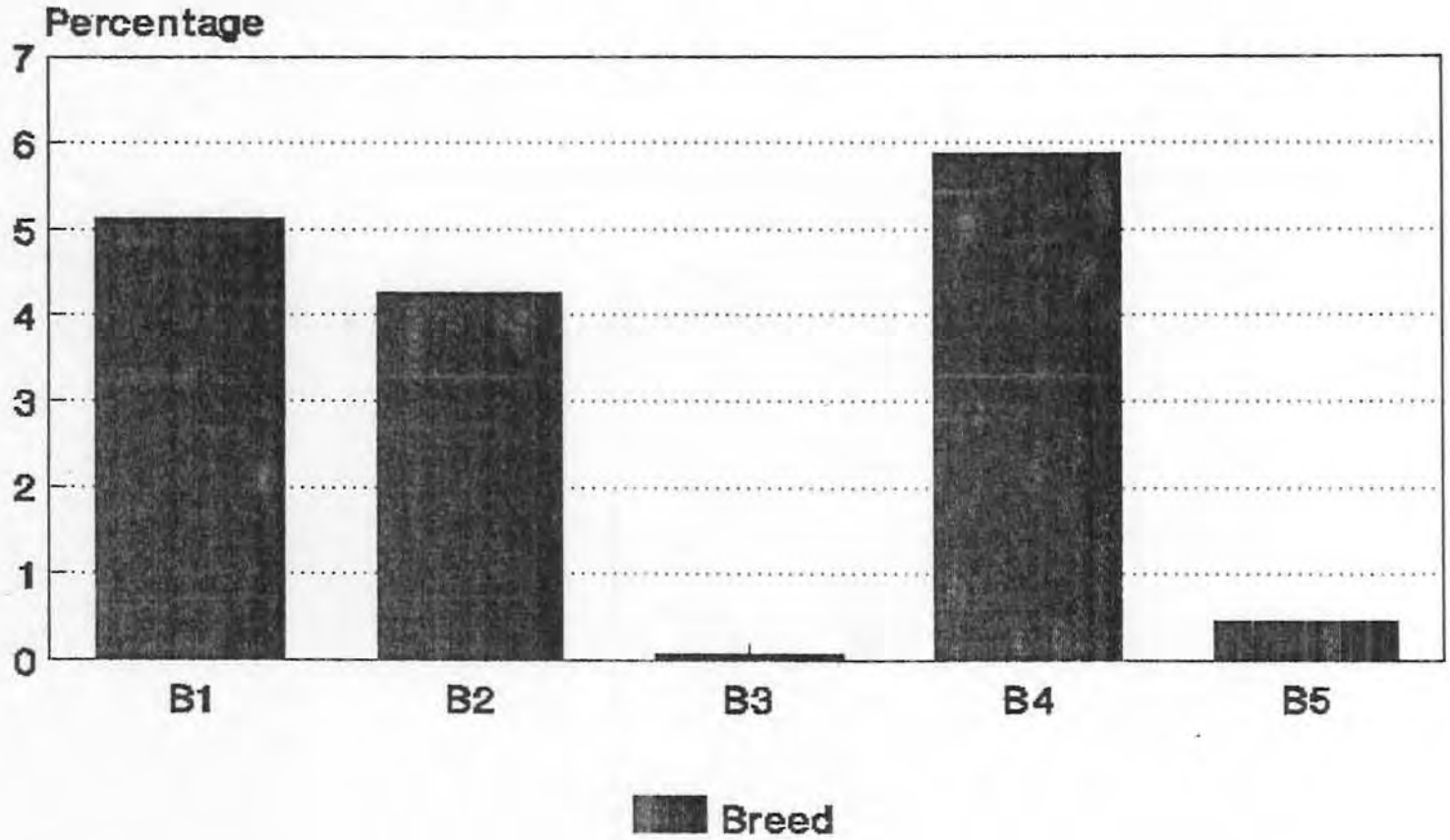
Table 2. Prevalence of Salmonella seropositives among broiler breeders fed different commercial feeds

Feed	No. of flocks tested	No. of birds tested			Flocks positive		Birds positive					
							Female		Male		Total	
		Female	Male	Total	No.	%	No.	%	No.	%	No.	%
F_1	90	112 636	17 261	129 897	74	82.2	4 965	4.4	375	2.17	5 340	4.11
F_2	21	39 697	5 325	45 022	10	47.6	4 982	12.5	122	2.29	5 104	11.33
F_3	13	29 540	4 264	33 804	9	69.2	972	3.3	81	1.9	1 053	3.11
F_4	8	10 800	2 363	13 163	5	62.5	135	1.3	3	0.13	138	1.05
F_5	6	13 260	1 952	15 212	5	83.3	229	1.7	33	1.69	262	1.72
F_6	6	12 650	962	13 612	4	66.67	71	0.56	22	2.27	93	4.63
F_7	4	5 000	1 070	6 070	4	100.0	160	3.2	5	0.46	165	2.72
F_8	2	5 000	674	5 674	1	50.0	3	0.6	1	0.15	4	0.07
Total	150	228 583	33 871	262 454	112	74.7	11 517	5.04	642	1.89	12 159	4.63

Table 3. Prevalence of Salmonella seropositives among broiler breeders of various breeds

Breed	No. of flocks tested	No. of birds tested			Flocks positive		Birds positive					
							Females		Males		Total	
		Females	Males	Total	No.	%	No.	%	No.	%	No.	%
B_1	82	133 552	18 791	152 343	55	67.07	7 576	5.67	244	1.29	7 820	5.13
B_2	58	72 001	12 705	84 706	50	86.21	3 282	4.55	338	2.66	3 620	4.27
B_3	5	10 900	1 381	12 281	3	60.0	4	0.04	5	0.36	9	0.073
B_4	4	11 130	873	12 003	3	75.0	651	5.84	54	6.18	705	5.87
B_5	1	1 000	121	1 121	1	100.0	4	0.4	1	0.83	5	0.45
Total	150	228 583	33 871	262 454	112	74.7	11 517	5.04	642	1.89	12 159	4.63

Fig. 1. Prevalence of Salmonella seropositives among broiler breeders of various breeds.



varied widely in birds of different ages (Table 4). The maximum number of flocks (84.82 %) were affected during 51 to 50 weeks of age (88.23 %). The highest number of carriers (16.75 %) were detected in birds tested during 41-50 weeks followed by 31-40 weeks (82.35 %), 41-50 weeks (81.81%). During 21-30 weeks and over 60 weeks of age increasing and decreasing trends were comparable 68.23 per cent (21-30 weeks) and 66.7 per cent (over 60 weeks) (Fig. 2). There was an exception that no male positive over 60 weeks of age. The reason being that in our poultry industry usually blood tested young males were introduced in old flocks to improve the hatchability.

4.1.4. MANAGEMENT WISE PREVALENCE

The management of a farm has a direct bearing on the spread of infections. A similar pattern was also observed during the present investigations of *Salmonella* carriers (Table 5). Regarding the prevalence of *Salmonella* carriers, a significant difference were recorded in flocks as well as in birds maintained under various managemental conditions. Maximum prevalence (78.57 %) was observed in flocks under poor management, followed by flocks under a satisfactory management (77.47 %) and the minimum prevalence was found in birds kept in excellent farming conditions (41.66 %). Similarly, maximum number (9.47 %) of carriers were detected in birds kept under the poor management, followed by breeders under satisfactory conditions and the minimum in birds with excellent farming practices. A strong correlation has been observed between the degree of contamination of the floor litter and the spread of

Table 4. Prevalence of Salmonella seropositives among broiler breeders of various age groups

Age group (weeks)	No. of flocks tested	No. of birds tested			Flocks positive		Birds positive					
							Females		Males		Total	
		Females	Males	Total	No.	%	No.	%	No.	%	No.	%
21—30	85	127 391	21 618	149 009	58	68.23	7 190	5.64	282	1.31	7 474	5.01
31—40	34	57 057	6 749	63 806	28	82.35	2 301	4.03	209	3.09	2 510	3.93
41—50	11	21 028	2 545	23 573	9	81.81	1 462	6.95	131	5.15	1 593	6.75
51—60	17	18 050	2 348	20 398	15	88.23	415	2.3	18	0.77	433	2.12
Over 60	3	5 057	611	5 668	2	66.7	149	2.95	—	—	149	2.63
Total:	150	228 583	33 871	262 454	112	74.7	11 517	5.04	642	1.89	12 159	4.63

Table 5. Prevalence of Salmonella seropositives among broiler breeders maintained under various managements

Management	No. of flocks tested	No. of birds tested			Flocks positive		Birds positive					
							Females		Males		Total	
		Females	Males	Total	No.	%	No.	%	No.	%	No.	%
Poor	14	18 320	3 672	21 992	11	78.57	1 914	10.45	169	4.60	2 083	9.47
Satis-factory	61	97 186	14 789	111 975	47	77.04	747	7.97	316	2.14	8 063	7.20
Good	63	89 967	11 262	101 229	49	77.77	1 677	1.86	147	1.31	1 824	1.80
Excellent	12	23 110	4 148	27 258	5	41.66	179	0.77	10	0.24	189	0.69
Total	150	228 583	33 871	262 454	112	74.7	11 517	5.04	642	1.89	12 159	4.63

salmonellae through a flock. It is the usual practice that after blood testing the reactors are removed and the non-reactors are left in the same infected litter. In this way the potential source of infection remains and there is every chance of reinfection of the healthy birds. It is recommended that the negative birds should be left in clean premises with a fresh uncontaminated litter.

4.1.5. SEX WISE PREVALENCE

Regarding the sex the prevalence varied in different breeds, feeds and managemental conditions. Although overall incidence was 5.15 per cent in B_1 birds while only 1.29 per cent males were positive as compared to the B_4 where overall prevalence in total flocks was 5.87 per cent, while only 6.18 per cent males were involved. In B_2 overall prevalence was 4.27 per cent with 2.66 per cent of male and 4.55 per cent of females. In B_3 (0.36 %) and B_5 (0.83 %) the higher percentage of males were positive and compared to females B3 (0.04 %) and B5 (0.4 %). Analysis of the data on bird basis gave a strong correlation of higher percentage of females directly proportional to the birds positivity. In case of exception higher percentage of male indicate introduction of new males or in a stage to spread the infection to females.

4.2. COMPARATIVE EFFICACY OF DIFFERENT DIAGNOSTIC TESTS

A number of tests were compare to check the reliability of these diagnostic and carrier birds monitoring tests, Rapid blood agglutination test (RBAT), Tube agglutination test (TAT), Yolk agar precipitin test (YAPT) and cloacal swab (CS)

isolation test were compared in the known 200 *Salmonella* carrier female birds. The 200 (100 %) birds positive in RBAT were also comparatively confirmed by TAT in 98.50 per cent cases. YAPT was effective in 97.50 per cent followed by cloacal swab method where 87.00 per cent were confirmed positive for *Salmonella.*

RBAT was reliable with some field problems as the E. coli shares some of the antigen with *Salmonella* so cross reaction indicate pseudo carriers, which can be detected by TAT. The effectiveness of cloacal swab was of value in birds under 18 weeks. The efficiency of this test is influenced by the intermittent sheders of salmonellae. The yolk agar ppt test (YAPT) was good tool to monitor the birds for primary indications of the carrier state through eggs without disturbing the flock or in those condition where flock and hatcheries are well aparted.

4.3. ISOLATION

4.3.1. MORPHOLOGICAL OBSERVATIONS

Microscopic examination of discrete colonies with Gram's staining showed that bacteria were Gram negative (G-), short rods or coccobacilli of different sizes. The sizes varied with the advancement in the age of culture. However, the average dimensions were 0.4 to 0.6 x 1 to 3 µm. Single or short chains were the common forms observed. Non of the *Salmonella* showed capsule with staining, while motile salmonellae had flagella (Fig.3). Ultrastructurally motile salmonellae had flagella while non-motile were flagella free. The morphology of motile and non motile salmonellae however, was identical (Fig.4).

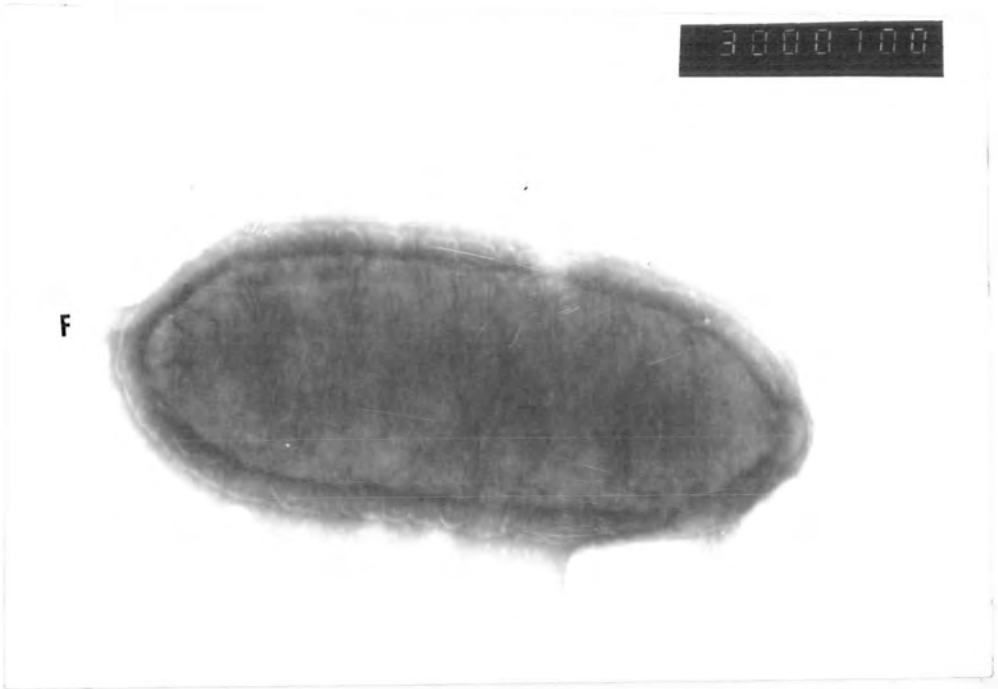


Fig. 3. A motile Salmonella with flagella (F) 8000X

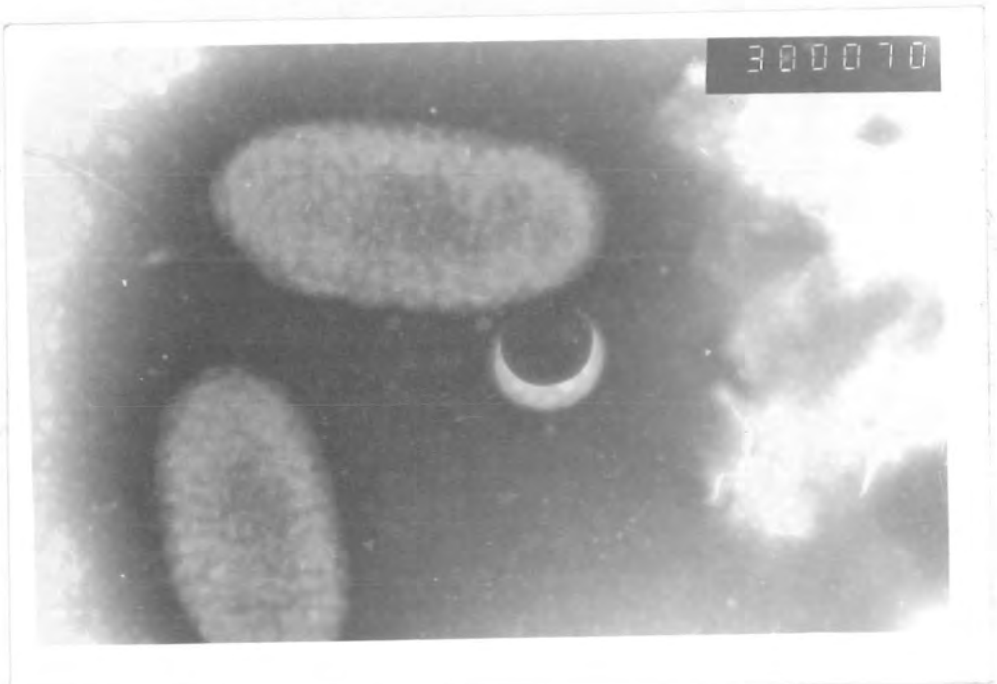


Fig. 4. Group of salmonellae showing specific coccobacilli shape 7000X

4.3.2. EFFICACY OF DIFFERENT CULTURE MEDIA

Among the 715 serotypes of *Salmonella* isolated from various sources selenite broth was proven 100 per cent effective followed by tetrathionate broth (95.8 %) and Mac-Conkey's broth (90.06 %). Among the solid laboratory isolation media Mac-Conkey's agar (MC) was the most effective as all the stains were isolated on Mac-Conkey's (97.20 %) followed by *Salmonella-shigella* agar (88.40 %) and Eosin Brilliant Green (EBG) agar (86.01 %). An overall isolation regimen was best through selenite broth enrichment and isolation on Mac-Conkey's media give almost all the isolates from various sources.

4.3.3. BIOCHEMICAL REACTIONS

The biochemical characteristics of *Salm. pullorum* and *Salm. gallinarum* were found to be almost similar in many biochemical pathways but some of the critical differences regarding their biochemical fermentation are presented.

Salm. pullorum isolates produced acid by fermentation of glucose at the butt of Triple Sugar Iron (TSI) agar and produced alkalinity at the slant surface. In contrast all the *Salm. pullorum* isolates did not ferment lactose and sucrose nor produce black coloration of H_2S on TSI. All the isolates of *Salm. pullorum* produced acid with gas by fermenting dextrose, mannitol and dulcitol. *Salm. gallinarum* produced acid without gas by fermenting dextrose, mannitol, dulcitol but non of the strains fermented lactose and sucrose. Biochemical reactions of all the 19 serotypes isolated.

4.3.4. SEROLOGICAL IDENTIFICATION

Isolates giving typical biochemical reactions of *Salmonella* were confirmed and typed serologically with standard *Salmonella* somatic (O) polyvalent and group antisera. The isolates were confirmed upto group level with slide agglutination. Each group was further processed for flagellar antigens through tube agglutination. All the isolates showed agglutination with polyvalent antisera and their respective group and flagellar antisera.

4.3.5. YEAR WISE ISOLATION PREVALENCE

Analysis of *Salmonella* serotypes isolation over the years indicated a relation between our flock pattern and isolation in different months of the year (Table 9). As it is evident in Pakistan we have seasonal flock system, so the flock raised/reared in December-January-March season come to production in July-August. Hence incidence increases in this period. In 1988 there were more non-motile (192) and motile (211) isolates (Table 9). Highly significant ($P< 0.01$) isolations were made in 1988 as compared to isolation 1989 and 1990. In 1989, isolation of non-motile serotypes was significantly ($P<0.05$) higher than isolation of non-motile salmonellae in 1990, while isolation prevalence of motile salmonellae in 1989 and 1990 was non-significant (Table 9).

4.4. ISOLATION PREVALENCE OF *SALMONELLA*

Among 8241 samples from 18 different sources including broiler breeder birds, *Salmonella* was isolated from 715 (8.7 %) samples. The average isolation prevalence at random in

chicken broiler breeder was 5.11 per cent. While in day-old-broiler breeder chicks it was 4.18 per cent. Isolation prevalence in avifauna birds was 17.83 per cent and in indigenous chicken it was 5.71 per cent (Table 6). Maximum isolation prevalence (27.65 %) was noted in meat meal samples, followed by fish meal (21.65 %), drinking water (21.08 %), hatchery fluff (16.19 %), eggs from carrier birds (14.78 %) and litter (14.42 %). A range of *Salmonella* isolation was 4.50 to 27.65 per cent in various allied sources of positive flocks.

4.4.1 MOTILE AND NON-MOTILE *SALMONELLA*

An overall increasing trends in motile salmonellae was observed over the prevalence of non-motile serotypes. Motile *Salmonella* serotypes isolated from 18 various sources were 390 (4.73 %), while, non-motile were 325 (3.94 %). Among non motile (325), *Salm. gallinarum* was isolated from 181 samples, followed by 144 (44.31 %) of *Salm. pullorum*. Among motile (390) *Salmonella* group, *Salm. typhimurium* was the most prevalent contaminant (19.23 %) followed by *Salm. agona* (10 %) *Salm. saint-paul* (7.95 %) and *Salm. butantan* 7.95 per cent (Table 7).

4.4.2 BROILER BREEDERS

Regarding the isolation of various *Salmonella* serotypes in broiler breeders at random, a relatively higher prevalence of non-motile salmonellae were recorded. *Salm. gallinarum* was recorded in 23.14 per cent, followed by *Salm. pullorum* (14.81 %). Among the motile salmonellae isolation in broiler

Table 6: Isolation of salmonellae from different sources.

Source	No. of Samples Tested	Salmonella isolated	
		No	(%)
Broiler breeders	2114	108	5.11
Day-old B.breeders	502	21	4.18
Indegnious chicken	753	43	5.71
Avifauna birds	370	66	17.83
Dead in shell	511	48	9.39
Embryos	1007	116	11.52
Egg (shells)	508	22	4.33
Egg (Contents)	521	77	14.78
Hatchery fluff	105	17	16.19
Fecal Materials	306	25	8.16
Cloacal swabs	527	28	5.31
Litter samples	215	31	14.42
Poultry house dust	111	5	4.50
Drinking water	147	31	21.08
Poultry feeds	185	22	11.89
Fish meal	97	21	21.65
Meat meal	47	13	27.65
Rodent feces	215	21	9.77
Total	8241	715	8.70

Table 7: Isolation frequency of motile and non-motile salmonellae from broiler breeders and allied sources.

Source tested	Type of *Salmonella* Isolated					
	Non-motile		Motile		Total isolates	
	No.	%	No.	%	No.	%
Broiler breeders	41	1.93	67	3.16	108	5.11 a
Day-old B.breeders	17	3.38	4	0.79	21	4.18 e
Indegnious chicken	31	4.11	12	1.59	43	5.71a
Avifauna birds	23	6.21	43	11.62	66	17.83 f
Dead in shell	29	5.67	19	3.72	48	9.39 b
Embryos	52	5.16	64	6.35	116	11.52 c
Egg (Shells)	12	2.36	10	1.97	22	4.33 e
Egg (Contents)	33	6.33	44	8.44	77	14.78 g
Hatchery fluff	12	11.42	5	4.76	17	16.19 h
Fecal material	12	3.92	13	4.25	25	8.16 i
Cloacal swabs	15	2.85	13	2.47	28	5.31 a
Litter samples	11	5.11	20	9.30	31	14.42 g
P.house dust	5	4.50	-	0.00	5	4.50e
Drinking water	10	6.80	21	14.28	31	21.08 d
Poultry feeds	5	2.70	17	9.19	22	11.89 c
Fish meal	10	10.34	11	11.34	21	21.65d
Meat meal	1	2.13	12	25.53	13	27.65d
Rodents feces	6	2.79	15	6.98	21	9.77 b
Total	325	3.94	390	4.73	715	8.70

a-h = Values with similar superscripts do not differ statistically, while values with different superscripts differ significantly (P< 0.01 to 0.05)

breeders, *Salm. typhimurium* was isolated in 9.26 per cent, followed by *Salm. heidelberg* (6.48 %) and *Salm. butantan* (5.55 %). *Salm. eastbourne, Salm. saint-paul, Salm. remo* and *Salm. agona* have 4.63 per cent isolation prevalence each. Isolation prevalence of other isolates is given in Table 8. Considering the incidence of *Salm. gallinarum* and *Salm. pullorum* in adult broiler breeders the *Salm. gallinarum* is increasing over *Salm. pullorum.*

4.4.3 VISCERAL ORGANS OF BROILER BREEDERS

In most of the birds, salmonellae were isolated from intestines, liver, spleen and ovary. Isolates were also obtained from caeca, lungs, kidney, heart, brain and bursa of Fabricius (Table 10). A higher number of isolates were obtained from intestines (37.08 %) than the liver (24.07 %) and spleen (10.18 %).

Maximum isolation of non-motile salmonellae were observed in intestines (10.18 %), liver (9.26 %), ovary (4.63 %), caeca (2.78 %), spleen (2.78 %) and brain (2.78 %). Isolation of the motile salmonellae in intestines (26.85 %), liver (14.81 %), spleen (7.40 %), ovary (6.48 %) and lungs (1.85 %) were the common organs. Among 108 isolates from various organs, *Salm. typhimurium* was isolated from intestines (3.70 %), liver (1.85 %), ovary (1.85 %), spleen 0.92 %) and lungs (0.92 %). *Salm. eastbourne* was isolated from intestines, liver, spleen and ovary. All the serotypes were isolated from intestines except *Salm. paratyphi A*, which was only isolated from liver. In ceca, *Salm. gallinarum, Salm.*

Table 8: Intensity (no) of isolation of various serotypes of Salmonella from chicken broiler breeders and allied sources

Source	gallinarum	pullorum	typhimurium	east-bourne	saint-paul	butantan	java	reading	chester	remo	heidel-berg	anatum	hadar	orion	ridge	agona	mission	give	p.typhi-A	Total No.	(%)
Broiler breeders	25	16	10	5	5	6	4	3	4	5	7	4	1	2	1	5	3	1	1	108	5.11
Day old B. breeders	10	7	1	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	21	4.18
Indegnious chicken	10	21	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	5.71
Avifauna birds	13	10	30	3	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	66	17.83
Dead in shell	17	12	2	1	2	3	1	1	-	1	2	-	-	-	-	3	-	1	2	48	9.39
Embryos	30	22	-	6	5	4	5	3	7	4	3	2	4	1	1	13	2	3	1	116	11.52
Egg (shells)	5	7	2	-	-	-	-	1	-	1	-	1	-	-	-	-	-	5	-	22	4.33
Egg (contents)	18	15	1	5	4	5	1	2	5	4	1	2	5	2	-	2	-	5	-	77	14.78
Hatchery fluff	7	5	2	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	17	16.19
Fecal materials	8	4	3	2	1	2	2	-	1	-	-	-	-	-	-	2	-	-	-	25	8.16
Clocal swabs	7	8	2	1	-	-	-	1	-	1	1	1	1	1	1	1	-	1	1	28	5.31
Litter samples	7	4	1	2	2	2	1	1	-	2	1	-	1	-	-	3	-	4	-	31	14.42
Poultry house dust	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	4.50
Drinking water	5	5	3	2	1	1	1	-	1	2	-	1	1	3	1	2	-	2	-	31	21.08
Poultry feeds	3	2	2	1	1	1	-	-	-	1	1	-	-	1	1	3	1	2	2	22	11.89
Fish meal	7	3	2	-	-	1	2	1	-	1	-	-	-	1	-	-	1	1	1	21	21.65
Meat meal	1	-	1	-	2	1	-	1	-	1	1	-	1	-	1	1	-	-	2	13	27.65
Rodent feces	4	2	1	-	3	-	1	1	-	-	-	-	-	-	1	4	1	2	1	21	9.77
	(25.31)	(20.13)	(10.49)	(3.91)	(4.33)	(4.33)	(2.52)	(2.10)	(2.52)	(3.35)	(2.52)	(1.95)	(1.95)	(1.54)	(0.98)	(5.45)	(1.12)	3.78	1.68	100.00	
Total (%)	181	144	75	28	31	31	18	15	18	24	18	14	14	11	7	39	8	27	12	715	8.70

Table 9: Isolation (No.) of salmonellae in different months of the year during 1988 through 1990.

Months of the Year	1988		1989		1990		Total No.	(%)
	Non-motile	Motile	Non-motile	Motile	Non-Motile	Motile		
January	17	-	7	1	-	3	28	3.91
February	13	3	3	12	-	5	36	5.03
March	8	5	2	17	3	11	46	6.43
April	11	21	-	12	11	21	76	10.62
May	1	23	-	2	21	13	60	8.39
June	2	31	-	1	3	24	61	8.53
July	32	34	1	3	5	11	86	12.02
August	51	37	23	1	4	3	119	16.64
September	43	51	21	4	2	2	123	17.20
October	3	2	15	11	-	1	32	4.47
November	5	1	7	21	-	-	34	4.75
December	6	3	5	-	-	-	14	1.95
Total No. (%)	192	211	84	85	49	94	715	(8.7)

Table 10: Organ wise isolation of various *Salmonella* serotypes from chicken broiler breeders.

Salmonella serotypes	ORGANS POSITIVE										
	Intes-tines	Liver	Spleen	Ceca	Lungs	Kidneys	Ovar-ies	Heart	Brain	Bursa	Total (%)
pullorum	5	3	1	1	1	1	2	1	1	-	16 (14.81)
gallinarum	6	7	2	2	1	1	3	1	2	-	25 (23.14)
typhimurium	4	2	1	-	1	-	2	-	-	-	10 (9.25)
eastbourne	2	1	1	-	-	-	1	-	-	-	5 (4.62)
saint-paul	1	1	1	1	1	-	-	-	-	-	5 (4.62)
butantan	2	2	1	1	-	-	-	-	-	-	6 (5.55)
java	2	1	1	-	-	-	-	-	-	-	4 (3.70)
reading	1	1	1	-	-	-	-	-	-	-	3 (2.77)
chester	2	2	-	-	-	-	-	-	-	-	4 (3.70)
remo	2	1	-	-	-	-	-	-	1	1	5 (4.62)
heidelberg	4	1	-	-	-	-	2	-	-	-	7 (6.48)
anatum	2	1	1	-	-	-	-	-	-	-	4 (3.70)
hadar	1	-	-	-	-	-	-	-	-	-	1 (0.92)
orion	1	1	-	-	-	-	-	-	-	-	2 (1.85)
ridge	1	-	-	-	-	-	-	-	-	-	1 (0.92)
agona	2	-	-	1	-	-	1	-	-	1	5 (4.62)
mission	1	1	1	-	-	-	-	-	-	-	3 (2.77)
give	1	-	-	-	-	-	-	-	-	-	1 (0.92)
para typhi. A.	-	1	-	-	-	-	-	-	-	-	1 (0.92)
Total (%)	40 (37.04)	26 (24.07)	11 (10.18)	6 (5.55)	4 (3.70)	2 (1.85)	12 (11.11)	2 (1.85)	3 (2.77)	2 (1.85)	108 (100)

pullorum, Salm. saint-paul, Salm. butantan and *Salm. agona* were the common contaminants. Lungs were infected only with *Salm. pullorum, Salm. gallinarum, Salm. typhimurium* and *Salm. saint-paul*. Only *Salm. gallinarum* and *Salm. pullorum* were isolated from kidneys. Ovary was predilection site for *Salm. gallinarum, Salm. pullorum, Salm. typhimurium, Salm. eastbourne, Salm. remo, Salm. heidelberg* and *Salm. agona*. Heart and brain had only positive on isolation with *Salm. gallinarum* and *Salm. pullorum*. *Salm. remo* and *Salm. agona* were isolates from bursa of Fabricius (Table 10).

4.4.4. DAY-OLD BROILER BREEDERS

Among the day-old broiler breeder chicks, a total of 21 (4.18 %) salmonellae were isolated. *Salm. gallinarum* was isolated from 10 (47.61 %), followed by *Salm. pullorum*, 7 (33.33 %), *Salm. typhimurium* 1 (4.76 %) and *Salm. paratyphi A*, 4.76 per cent (Table 7,8). These isolations were attempted from the composite samples of yolks, liver, intestine, lungs and spleen of birds died during transportation from abroad.

4.4.5. INDIGENOUS CHICKEN

Seven hundred and fifty-three enlarged livers, spleens and intestines (251 each) of apparently healthy desi poultry birds were collected from the local market. After searing their surface with a red hot spatula, a loopful of the tissue material was inoculated in the broths for enrichment. The growth in the selenite, Mac-Conkey's and Tetrathionate broths was transferred to S. S., EMB and Mac-Conkey's agar to study the cultural characteristics of the suspected *Salmonella*. Out

of 753 samples of livers, intestines of spleens (251 each), 43 were positive for *Salmonella*. Isolation of *Salmonella* was successfully attempted from 25 (9.96 %) intestines, 15 (5.97 %) livers and 3 (1.19 %) spleens. Out of 43 isolates, 22 isolates were isolated on S.S agar and 21 on Mac-Conkey's agar. Organwise isolation of *Salmonella* on different media is given in Table 11.

Among the 43 positive organs belonging to 31 birds. *Salmonella* could be isolated from 2 birds from the liver, spleens and intestines, in 8 birds from the livers and intestines, while 21 birds proved to be intestinal carriers only. the maximum localization was in the intestine (9.96 %) followed by the liver (5.97 %) and the spleen (1.19 %). A higher percentage of intestinal carriers is also a serious threat to our commercial poultry. Out of 43 isolates, 10 (23.25 %) were of *Salm. gallinarum* and 21 (48.84 %) *Salm. Pullorum* while 12 (27.91 %) were *Salm. typhimurium*.

4.4.6. ISOLATION OF *SALMONELLA* IN AVIFAUNA

Three hundred and seventy rectal swabs were collected from various species of avifauna from zoological garden birds, mainly love, birds, Tena parrots, Australian parrots, peacock, Java sparrows, pigeons, cockatoos, doves, canary, silky, quails, pheasants, partridges, nightingale and wood-pecker. Among 370 birds of various species from different household units, *Salmonella* was isolated from 66 birds, indicating an overall prevalence of 17.83 per cent. *Salmonella* serotypes were isolated from 15 (14.85 %) Australian parrots, 22 (22 %)

11. Organwise isolation of Salmonella on different media

Organs	No. of organs	Media used		Total	
		S.S.	MC	No.	%
Intestines	251	12	13	25	9.96
Livers	251	7	8	15	5.97
Spleens	251	3	—	3	1.19
Total/Average*	753	22	21	43	5.71*

pigeons, 3 (6.67 %) Java sparrows, 5 (12.50 %) quails, 4 (26.67 %) peacocks, 3 (17.65 %) doves, 3 (30.00 %) pheasants and 11 species of other birds (26.19 %). Isolation of *Salmonella* serotypes like *Salm. typhimurium, Salm. saint-Paul, Salm. butantan* and *Salm. east-bourne* (Table 12), which are known for their association with disease condition in man and animals is of animal industry as well as public health significance. *Salm. typhimurium* was isolated from 30 (45.45 %) rectal swabs, *Salm. gallinarum-pullorum* was confirmed in 23 (34.84 %) cases etc. (Table 13).

4.4.7. DEAD IN SHELL

Composite samples of the dead in shell embryos and egg residual contents were taken by tracing back the history record of the egg contents with sero-positive flocks. From a total of 511 composite samples cultured, 48 (9.39 %) gave isolates of *Salmonella*. Among 48 isolates, 29 (60.41 %) were *Salm. gallinarum* and *Salm. pullorum. Salm. butantan* (6.25 %), *Salm. agona* (6.25 %), *Salm. typhimurium* (4.16 %), *Salm. saint-paul* (4.16 %), *Salm. heidelberg* (4.16 %) and *Salm. paratyphi A* (4.16 %) were other motile contaminents. *Salm. eastbourne, Salm. Java, Salm. reading* and *Salm. remo* were isolated one in each case (Table 6,7,8).

4.4.8. EMBRYOS

Isolation was undertaken from 1007 full termed embryos by separating the embryos from egg contents and internal organs were composite aseptically by washing the abdominal area with iodine solution. Among 1007 embryos, 116 (11.52 %)

Table I2: Prevalence of *Salmonella* in various species of birds.

S. No.	Species of birds	No. of birds tested	Birds positive No.	Birds positive %
1.	Australian parrots *(Psittacidae, budgereegah)*	101	15	14.85
2.	Pigeons *(Columba livia)*	100	22	22.00
3.	Java sparrows *(Ploceidae)*	45	3	6.67
4.	Quails *(Cyrtonyx mountezumae)*	40	5	12.50
5.	Peacocks *(Pavo cristatus)*	15	4	26.67
6.	Doves *(Zenaidura macroura)*	17	3	17.65
7.	Pheasants *(Phasianidae)*	10	3	30.00
8.	Other species	42	11	26.19
Total/Average*		370	66	17.83*

Table I3. *Salmonella* strains isolated from various species of zoological garden birds.

Species	Total Isolates	SEROTYPES OF *SALMONELLA* ISOLATED				
		typhi-murium	galli. Pullorum	saint Paul	but-antan	east-bourne
Australian Parrots	15	1	10	3	1	1
Pigeons	22	20	-	-	1	1
Java Sparrows	3	-	1	-	1	1
Quails	5	2	3	-	-	-
Peacocks	4	-	3	1	-	-
Doves	3	1	1	-	-	-
Pheasants	3	-	3	-	-	-
Other Spps.	11	6	2	1	2	-
Total/Average	66	30 45.45 %	23 34.84%	5 7.57%	5 7.57%	3 4.54 %

were positive for *Salmonella*. Fifty two (5.16 %) embryos were positive for non-motile *Salmonella* and 64 (6.35 %) had motile *Salmonella*. Among non-motile salmonellae, *Salm. gallinarum* (25.86 %) and *Salm. pullorum* (18.96 %) were isolated. *Salm. agona* was the major (11.20 %) contaminant in motile group. Most of the *Salmonella* were isolated except *Salm. ridge, Salm. mission,* and *Salm. paratyphi A* (Table 6,7,8).

4.4.9. HATCHING EGGS

External or false contaminations with litter in floor laying or vent contamination were assessed in 22 (4.33 %) cases among 508 total hatching eggs. Intact egg washing was contaminated with 22 isolates of *Salmonella,* among which 12 (2.36 %) were non-motile salmonellae and 10 (1.97 %) were motile salmonellae. Major external contaminants of hatching eggs were *Salm. pullorum* (31.8 %), *Salm. give* (22.72 %), *Salm. gallinarum* (22.72 %) and *Salm. typhimurium* (9.09 %) while *Salm. reading, Salm. remo* and *Salm. anatum* had identical isolation prevalence of 4.55 per cent (Table 6,7,8). *Salm. give* among the motile salmonellae was the common contaminant which was also isolated from litter samples with almost same frequency (12.90 %) as that of external egg contamination.

In 521 internal egg contents, salmonellae were isolated in 77 (14.78 %) hatching egg contents. Thirty three (6. 33 %) isolates were non-motile salmonellae and 44 (8.44 %) were motile salmonellae. In comparison with the external contamination where non-motile isolation was maximum in case of internal contents higher percentage (8.44 %) was isolated.

Ovary was contaminated with 6.48 per cent motile salmonellae as compared to non-motile (4.63 %). Major contaminants of internally hatching eggs are given in Table 6,7,8.

4.4.10. HATCHERY FLUFF

Hatchery fluff is a good monitoring material to check the chick contamination upto hatchery. Among 105 composite samples from individual hatchers of different hatcheries, 17 (16.19 %) were positive for different serotypes of salmonellae. Non-motile salmonellae were confirmed in 12 (11.42 %), followed by 5 (4.76 %) of motile group. *Salm. gallinarum* (41.17 %), followed by 5 (4.76 %) of motile group. *Salm. gallinarum,* (41.17 %), *Salm. pullorum* (29.41 %) and *Salm. typhimurium* (11.76 %) were the frequent contaminants while *Salm. remo, Salm. heidelberg* and *Salm. anatum* were also isolated one of each (Table 6,7,8).

4.4.11. FECAL MATERIAL

As a result of biochemical and serological reactions it was revealed that 25 (8.16 %) fecal samples were positive for *Salmonella.* Among 306 fecal samples, *Salmonella* shedding was confirmed in 8.16 per cent cases. *Salm. gallinarum* (32.00 %), *Salm. pullorum* (16.00 %), *Salm. typhimurium* (12.00 %) were the most prevalent salmonellae. Among motile salmonella, the main serotypes isolated were *Salm. eastbourne, Salm. saint-paul, Salm. butantan, Salm. Java, Salm. Chester* and *Salm. agona* (Table 6,7,8).

4.4.12. CLOACAL SWABS

All (527) the cloacal swabs samples were taken from the serologically unknown birds and 18 weeks of age revealed 28 (5.31 %) isolates of *Salmonella*. Higher percentage (2.85 %) of non-motile salmonellae were isolated followed by motile salmonellae (2.47 %). *Salm. gallinarum* (28.00 %) and *Salm. pullorum* (28.57 %) were the major contaminants while *Salm. typhimurium* (7.14 %), *Salm. eastbourne, Salm. reading, Salm. orion, Salm. ridge, Salm. agona, Salm. paratyphi A* and *Salm. give* each 3.57 per cent was isolated (Table 6,7,8).

4.4.13. LITTER SAMPLES

Salmonella was recovered from 31 (14.42 %) samples by composite method. *Salm. gallinarum* was isolated from 7 (22.58 %) and *Salm. pullorum* in 4 (12.90 %) samples of litter. Other 11 serotypes isolated were motile salmonellae. Among motile salmonellae, higher isolation was revealed of *Salm. give* (12.90 %), followed by *Salm. agona* (9.67 %). *Salmonella eastbourne, Salm. saint-paul, Salm. butantan* and *Salm. remo* had similar isolation percentage (6.45 %). In 215 litter samples taken from seropositive flocks give *Salm. typhimurium, Salm. Java, Salm. reading, Salm. heidelberg* and *Salm. hadar* one isolate of each (Table 6,7,8).

4.4.14. POULTRY HOUSE DUST

Salmonella could be an airborne infection as the dried fecal material spread in the air in form of dust. The contaminated house dust is one of the vehicle to transport infection to the penmate and poultry houses in the vicinity of

the farm. A total of 111 house dust samples were collected and only 5 (4.5 %) samples were positive for salmonellae. All of the isolates were non-motile group. *Salm. gallinarum* was isolated 4 (80.00 %) and *Salm. pullorum* in 1 (20.00 %) dust samples (Table 6,7,8).

4.4.15. DRINKING WATER

Drinking water is an important vector of pathogen's transmission by contamination in house or outside the house. In poultry houses, 147 water samples were collected among these 31 (21.08 %) were positive for *Salmonella*. Water had heavy contamination of motile salmonellae as 21 (14.28 %) were motile salmonellae and only 10 (6.80 %) were non-motile salmonellae. *Salm. gallinarum* and *Salm. pullorum* had identical isolation prevalence (16.12 %) as it was identical in case of *Salm. typhimurium* and *Salm. orion* (9.67 %). *Salm. eastbourne, Salm. remo, Salm. agona* and *Salm. give* had similar isolation number 2 (6.45 %) in each case. *Salm. saint-paul, Salm. butantan, Salm. Java, Salm. chester, Salm. anatum, Salm. hadar* and *Salm. ridge* only 1 (3.22 %) isolate for each (Table 6,7,8).

4.4.16. POULTRY FEEDS

Feed samples were collected from the flocks with history of salmonellosis. Among a total of 185 feed samples, 22 (11.89 %) were positive for *Salmonella*. Among total, 22 isolates from feed 5 (2.70 %) were non-motile salmonellae and 17 (9.19 %) were motile salmonellae. *Salm. gallinarum* (13.64 %), *Salm. pullorum* (9.09 %) were the non-motile isolates while *Salm.*

agona (13.64 %). *Salm. typhimurium, Salm. paratyphi A* and *Salm. give* have identical isolation prevalence (9.09 %). *Salm. eastbourne, Salm. saint-paul, Salm. butantan, Salm. remo, Salm. heidelberg, Salm. orion, Salm. ridge* and *Salm. mission* was isolated 1 (4.54 %) of each (Table 6,7,8).

4.4.17. FISH MEAL AND MEAT MEAL

The average recovery rate from the total 97 fish meal samples was 21.65 per cent. Among the 21 isolates of salmonellae, 10 (10.30 %) were non-motile and 11 (11.34 %) were motile salmonellae (Table 6,7,8). *Salm. gallinarum* was isolated from 7 (33.33 %), followed by *Salm. pullorum* 3 (14.28 %), *Salm. typhimurium* 2 (9.52 %) and *Salm. Java* 2 (9.52 %). *Salm. butantan, Salm. reading, Salm. remo, Salm. orion, Salm. mission, Salm. paratyphi A* and *Salm. give* were isolated 1 (4.76 %) of each (Table 8).

Among 47 meat meal samples, 13 (27.65 %) were positive for *Salmonella*. Among 13 isolates, only 1 (7.69 %) was non-motile *Salm. gallinarum*. Other contaminants of motile group were *Salm. typhimurium* (1), *Salm. saint-paul* (2), *Salm. butantan* (1), *Salm. reading* (1), *Salm. remo* (1), *Salm. heidelberg* (1), *Salm. hadar* (1), *salm. ridge* (1), *Salm. agona* (1) and *Salm. paratyphi A* (2).

4.4.18. RODENT FECES

Rodents are good vector of transmitting the disease organism to the feed store or spreading to the other farms. The contaminated rodent feces are mixed in the feed and

ultimately the insidious material reached to the birds. A total of 215 composite rodent fecal samples were collected and *Salmonella* isolation was confirmed in 21 (9.77 %) samples. Among 2.79 per cent non-motile *Salmonella*, 19.04 per cent was *Salm. gallinarum* followed by *Salm. pullorum* (9.52 %). Among motile group *Salm. agona* (19.04 %), *Salm. saint-paul* (14.28 %) and *Salm. give* (9.52 %) were isolated, while *Salm. typhimurium, Salm. Java, Salm. reading, Salm. ridge, Salm. mission* and *Salm. paratyphi A* each of the isolate was attempted at same frequency of 4.76 per cent (Table 6,7,8).

4.5. ANTIBIOGRAPHY OF *SALMONELLA* ISOLATES

Various antimicrobial agents more commonly used against salmonellosis were evaluated by disc method against the isolated *Salmonella* strains. The antimicrobials used were ampicillin, chloramphenicol, erythromycin, flumequine, furazolidone, gentamicin, kanamycin, lincomycin, neomycin, streptomycin, Terramycin, Tribrissen and vibramycin. On the average, 66.33 per cent of the isolates were highly susceptible to various antimicrobials, 14.43 per cent intermediately susceptible, while 19.26 per cent of the isolates were resistant (Table 14).

Flumequine proved to be the drug of choice, as 668 (93.43 %) isolates were sensitive, 30 (4.19 %) intermediately susceptible and only 17 (2.37 %) were resistant. Vibramycin stood at number two, to which 568 (79.44 %) isolates were sensitive and 116 (16.22 %) were intermediately susceptible, while 31 (4.33 %) isolates were resistent. According to the

Table 14: Susceptibility of 715 isolates of *Salmonella* serotypes to different antimicrobials.

Antimicrobials tested		Susceptibility						
		High		Intermediate		Resistant		
		No.	%	No.	%	No.	%	
Ampicillin	(A)	544	76.08	68	9.51	103	14.40	a
Chloramphenicol	(C)	605	84.61	45	6.29	65	9.09	b
Erythromycin	(E)	368	51.47	161	22.52	186	26.01	c
Flumequine	(AR)	668	93.43	30	4.19	17	2.37	d
Furazolidone	(F)	335	46.85	114	15.94	266	37.20	e
Gentamicin	(G)	625	87.41	54	7.55	36	5.03	f
Kanamycin	(K)	351	49.09	76	10.62	288	40.27	g
Lincomycin	(L)	499	69.79	130	18.18	86	12.02	h
Neomycin	(N)	412	57.62	144	20.14	159	22.23	c
Streptomycin	(S)	537	75.10	132	18.46	46	6.43	i
Terramycin	(T)	319	44.61	166	23.21	230	32.16	e
Tribrissen	(TS)	332	46.43	106	14.82	277	38.74	e
Vibramycin	(V)	568	79.44	116	16.22	31	4.33	f
Total/Average		474	66.33	103	14.43	138	19.28	

$a - i$ = Values with similar superscripts do not differ statistically, while values with different superscripts differ significantly ($P < 0.01$ to 0.05)

Terramycin = Tetracycline
Tribrissen = Sulphadiazine + Trimethoprim
Vibramycin = Doxycycline

spectrum of susceptibility, maximum resistance (40.27 %) was observed against kanamycin, followed by Tribrissen (38.74 %), furazolidone (37.20 %), Terramycin (32.16 %), erythromycin (26.01 %) and neomycin (22.23 %). Seventeen (2.37 %) isolates were resistant to all the antibacterials, while 427 (59.72 %) were sensitive to all the antibacterials, while 103 (14.43 %) were intermediately sensitive.

4.5.1. SUSCEPTIBILITY IN MOTILE VS NON MOTILE

Among total of 715 isolates of salmonella, 390 (54.55 %) were motile and 325 (45.45 %) non-motile. The distribution of resistance in both the groups was statistically ($P<0.05$) non significant in most of the cases. An overall trend of sensitivity was more in motile group in case of almost all the antibacterials as compared to non-motile group where the isolates were facing more resistance to antibacterials (Table 15).

4.5.2. TEMPORAL SUSCEPTIBILITY

Among non-motile group of *Salmonella*, a variable trend in antimicrobials were noted. Ampicillin resistance was more during 1988 and 1989, while resistance increased from 11.98 to 14.28 per cent in 1990. Increase in resistance from 10.41 to 20.41 per cent was noted in chloramphenicol, 24.47 to 28.57 per cent in erythromycin, 30.20 to 48.97 per cent in kanamycin, 16.14 to 26.53 per cent in neomycin 24.47 to 32.65 per cent in Tribrissen. (Table 16). Rest of the antibacterials had fluctuating trend particularly resistance against Terramycin risen from 20.31 per cent to 34.52 per cent in 1989

Table 15: Susceptibility (%) of 715 *Salmonella* serotypes to different antimicrobials.

	Non-motile			Motile		
Antimicrobials	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)	Sensitive No. (%)	Intermediated No. (%)	Resistant No. (%)
Ampicillin	251 (35.10)	31 (4.33)	43 (6.01)	292 (40.97)	37 (5.17)	60 (8.39)
Chloram-phenicol	282 (39.44)	2 (0.28)	41 (5.73)	323 (45.17)	43 (6.01)	24 (3.35)
Erythromycin	168 (23.49)	73 (10.20)	84 (11.74)	200 (27.97)	88 (12.30)	102 (14.26)
Flumequine	308 (43.07)	14 (1.95)	3 (0.42)	360 (50.34)	16 (2.23)	14 (1.96)
Furazolidone	151 (21.11)	52 (7.27)	122 (17.06)	184 (25.73)	62 (8.67)	144 (20.13)
Gentamicin	287 (40.13)	27 (3.77)	11 (1.53)	338 (47.27)	27 (3.77)	25 (3.49)
Kanamycin	175 (24.47)	33 (4.61)	117 (16.36)	176 (24.61)	43 (6.01)	171 (23.91)
Lincomycin	239 (33.42)	57 (7.97)	29 (4.05)	260 (36.36)	73 (10.20)	57 (7.97)
Neomycin	202 (28.25)	62 (8.67)	61 (8.53)	210 (29.37)	82 (11.47)	98 (13.70)
Streptomycin	261 (36.50)	51 (7.13)	13 (1.81)	276 (38.60)	81 (11.32)	33 (4.61)
Terramycin	170 (23.77)	74 (10.35)	81 (11.32)	149 (20.83)	92 (12.86)	149 (20.83)
Tribrissen	183 (25.59)	48 (6.71)	94 (13.14)	149 (20.83)	58 (8.11)	183 (25.59)
Vibramycin	261 (36.50)	55 (7.69)	9 (1.26)	307 (42.93)	61 (8.53)	22 (3.07)

le 16: Susceptibilites (%) of different serotypes of Salmonella to various antimicrobials

monella otypes	Total isolates tested	ANTIBACTERIALS TESTED												
		A	C	E	AR	F	G	K	L	N	S	T	TS	V
tbourne	28	100.00	100.00	71.42	100.00	71.42	100.00	71.42	100.00	42.85	100.00	85.71	42.85	100.00
nt-Paul	31	87.09	100.00	64.51	100.00	80.64	100.00	80.64	100.00	38.70	87.09	80.64	64.51	87.09
antan	31	93.54	100.00	83.87	100.00	67.74	96.74	38.70	83.87	83.87	100.00	90.32	48.38	100.00
a	18	88.86	94.44	61.11	100.00	61.11	94.44	55.55	83.33	88.88	94.44	27.77	44.44	94.44
ding	15	66.66	86.66	66.66	100.00	86.66	86.66	86.66	100.00	40.00	100.00	93.33	66.66	100.00
ster	18	33.33	94.44	88.88	94.44	27.77	94.44	22.22	77.77	33.33	33.33	5.55	27.77	88.88
o	24	83.33	91.66	54.16	87.50	45.83	79.16	29.16	79.16	83.33	95.83	50.00	41.66	91.66
delberg	18	94.44	88.88	83.33	94.44	61.11	94.44	55.55	83.33	88.88	94.44	27.77	44.44	94.44
tum	14	78.57	92.85	64.28	92.85	64.28	78.57	64.88	78.57	85.71	78.57	35.71	64.28	78.57
ar	14	78.57	85.71	42.85	92.85	50.00	100.00	42.85	92.85	85.71	92.85	14.28	42.85	92.85
on	11	90.90	90.90	81.81	100.00	18.18	100.00	54.54	63.63	72.72	81.81	27.27	36.36	90.90
ge	7	71.42	71.42	28.57	85.71	28.57	85.71	28.57	57.14	71.42	88.71	42.85	28.57	71.42
na	39	89.74	94.87	66.66	94.87	53.84	92.36	30.76	82.05	87.71	94.87	89.74	38.41	97.43
sion	8	100.00	100.00	62.50	100.00	37.50	100.00	50.00	87.50	50.00	100.00	50.00	50.00	87.50
yphi.A	12	100.00	100.00	91.66	100.00	83.33	100.00	50.00	91.66	100.00	100.00	41.66	25.00	100.00
e	27	81.48	92.59	81.48	100.00	37.03	92.59	55.55	81.48	85.18	92.59	40.74	48.14	96.29
himurium	75	84.00	92.00	89.33	94.66	86.66	93.33	77.33	84.00	90.66	96.00	78.66	84.00	97.33
al iles	390	330 (84.61)	366 (93.84)	288 (73.84)	376 (96.41)	246 (63.07)	365 (93.58)	219 (56.15)	333 (85.38)	292 (74.87)	357 (91.53)	241 (61.79)	207 (53.07)	368 (94.35)
linarum	181	94.47	95.02	79.00	98.89	58.01	96.13	66.85	96.13	80.11	97.79	81.21	81.21	96.68
lorum	144	77.08	77.77	68.05	99.30	68.05	97.22	60.41	84.72	82.63	93.75	67.36	58.33	97.91
al -motiles	325	(282)	(284)	(241)	(322)	(203)	(314)	(208)	(296)	(264)	(312)	(244)	(231)	(316)
tal)	715 100	612 85.59	650 90.90	529 73.90	698 97.62	449 62.80	679 94.96	427 59.72	629 87.97	556 77.76	669 93.56	485 67.83	438 61.25	684 95.66

and decreased to 18.36 per cent in 1990.

In motile *Salmonella*, trend in resistance was relatively faster as compared to the non-motile salmonellae (Table 17). Significant rise in resistance against Tribrissen was noted where the resistance increased from 39.33 per cent (1988) to 69.14 per cent (1990), followed by kanamycin from 40.28 to 55.32 per cent, erythromycin 16.58 to 47.87 per cent, Terramycin 36.96 to 45.74 per cent and neomycin from 22.27 per cent in 1988 to 31.91 per cent in 1990. A temporal relationship in resistance against traditional antibacterials were noted (Table 18).

4.5.3. GLOBAL ANTIBIOGRAMS OF *SALMONELLAE*

In toto 715 isolates were screened for antimicrobial sensitivity. Sensitivity to all the antibacterial under trial was observed only in 427 (59.72 %) isolates of *Salmonella*. Of which 11 (1.54 %) were sensitive to all except kanamycin to which resistance was created by *Salmonella* serotypes against, while another 11 (1.54 %) created resistance to Tribrissen in addition to kanamycin. Seventeen (2.37 %) isolates were only sensitive to chloramphenicol, fluruequine, gentamicin, lincomycin, streptomycin and vibramycin. Five (0.70 %) isolates were sensitive only to flumequine and vibramycin, while 14 (1.95 %) were sensitive to flumequine. Seventeen (2.37 %) isolates were resistant to all the antibacterials tested (Fig. 5).

Table 17: Sensitivity of 325 non-motile *Salmonella* serotypes (isolated during 1988-1990) to different antimicrobials.

Antimicrobials tested	1988 Susceptibility (%)			1989 Susceptibility (%)			1990 Susceptibility (%)			Sensitive Total No.(%)
	S	I	R	S	I	R	S	I	R	
Ampicillin	79.16	8.85	11.98	73.80	10.71	15.47	75.51	10.20	14.28	282 (86.76) a
Chloram-phenicol	89.06	0.52	10.41	85.71	1.19	13.09	65.30	0.00	20.41	284 (87.38) a
Erythromycin	57.81	17.70	24.47	41.66	30.95	27.38	44.89	26.53	28.57	241 (74.15) a
Flumequine	95.31	3.64	1.04	92.85	5.95	1.19	95.91	4.08	0.00	322 (99.07) b
Furazolidone	47.39	15.10	37.50	44.04	17.85	38.09	46.93	16.32	36.73	203 (62.46) c
Gentamicin	87.49	8.85	3.64	89.28	8.33	2.38	89.79	6.12	4.08	314 (96.61) b
Kanamycin	58.85	10.93	30.20	48.80	9.52	41.66	42.85	8.16	48.97	208 (64.00) c
Lincomycin	77.08	15.10	7.81	67.85	21.42	10.71	69.38	20.41	10.20	296 (91.07) b
Neomycin	65.62	18.23	16.14	58.33	21.42	20.23	55.10	18.36	26.53	264 (81.23) a
Streptomycin	81.77	14.58	3.64	79.76	15.47	4.76	75.51	20.41	4.08	312 (96.00) b
Terramycin	57.29	22.39	20.31	40.47	24.99	34.52	53.06	28.57	18.36	244 (75.07) a
Tribrissen	62.49	13.02	24.47	46.42	16.66	36.90	48.97	18.36	32.65	231 (71.07) a
Vibramycin	82.29	15.10	2.60	76.19	20.23	3.57	79.59	18.36	2.04	316 b

a-C = Values with similar superscripts do not differ statistically, while values with different superscripts differ significantly ($P < 0.01$ to 0.05)

S = Sensitive

I = Intermediately sensitive

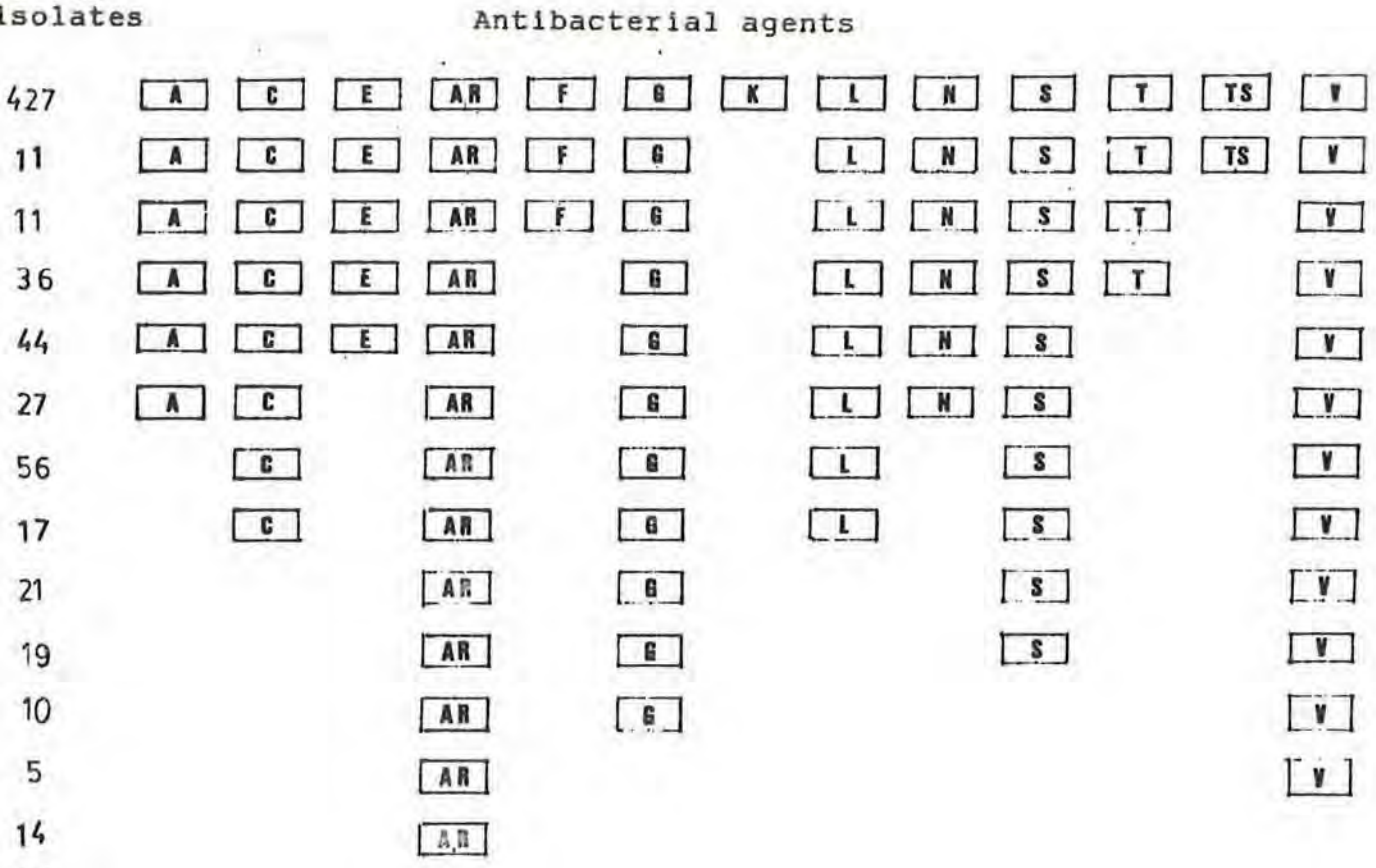
R = Resistant

Table 18: Sensitivity of 390 motile *Salmonella* serotypes (Isolated during 1988-1990) to different antimicrobials.

Antimicrobials tested	1988 Susceptibility (%)			1989 Susceptibility (%)			1990 Susceptibility (%)			Sensitive Total No.(%)
	S	I	R	S	I	R	S	I	R	
Ampicillin	75.82	9.47	14.69	69.41	9.41	21.17	78.72	8.51	12.76	330 (84.61)
Chloram-phenicol	89.09	6.16	4.73	83.52	7.05	9.41	68.08	25.53	6.38	366 (93.84)
Erythromycin	60.66	22.74	16.58	50.58	23.52	25.88	30.85	21.27	47.87	288 (73.84)
Flumequine	90.99	3.79	5.21	91.76	4.70	3.53	95.74	3.19	1.06	376 (96.41)
Furazolidone	46.44	16.11	37.44	44.70	17.64	37.64	51.06	13.82	35.10	246 (63.07)
Gentamicin	87.20	7.58	5.21	82.35	9.41	8.23	89.36	3.19	7.45	365 (93.58)
Kanamycin	49.28	10.42	40.28	45.88	14.11	40.00	35.10	9.57	55.32	219 (56.15)
Lincomycin	69.66	18.01	12.32	67.05	18.82	14.11	59.57	20.21	20.21	333 (85.38)
Neomycin	57.34	20.37	22.27	54.11	21.17	24.70	45.74	22.34	31.91	292 (91.53)
Streptomycin	74.88	18.48	6.63	71.76	18.82	9.41	60.63	27.65	11.70	357 (91.53)
Terramycin	39.81	23.22	36.96	42.31	24.70	32.94	30.85	23.40	45.74	241 (61.79)
Tribrissen	45.97	14.69	39.33	44.70	14.11	41.17	14.89	15.95	69.14	207 (53.07)
Vibramycin	79.15	16.11	4.73	76.47	16.47	7.05	79.78	13.82	6.38	368 (94.35)

S = Sensitive
I = Intermediately sensitive
R = Resistant

Fig.5 : Antibiogram of 715 Salmonella



4.5.3.1. *SALMONELLA GALLINARUM*

Salm. gallinarum was observed more over the *Salm. pullorum* in the previous few years. Among non-motile group the higher percentage was isolated by *Salm. gallinarum.* In a total of 181 *Salm. gallinarum,* 105 (58.01 %) isolates were sensitive to all the antibacterials in trial, another 16 (8.83 %) were sensitive to all except furazolidone to which resistance was observed (Fig. 6). Twenty two (12.15 %) isolates were sensitive to all except furazolidone and kanamycin. Ampicillin, chloramphenicol, flumequine, gentamicin, lincomycin, streptomycin and vibramycin were only sensitive in 24 (13.26 %) isolates. Two (1.10 %) isolates were only sensitive to flumequine, while another 2 (1.10 %) isolates were resistant to all the isolates.

4.5.3.2. *SALMONELLA PULLORUM*

Among non-motile a total of 144 *Salm. pullorum* isolates were attempted for antibiography. Resistance against non-motile salmonellae was more serious as the non-motile group was more prevalent in our broiler breeders. Eighty four (58.33 %) isolates were sensitive to all the antimicrobials tested. Resistance against Tribrissen was the first resistance indicator in 3 (2.08 %) isolates, while these isolates were sensitive to all the other antibacterials. Generally higher problem of resistance was critically with erythromycin, furazolidone, kanamycin, neomycin, Terramycin and Tribrissen. Twelve (8.33 %) isolates were only sensitive to flumequine, gentamicin, streptomycin and vibramycin, while another 6 (4.16 %) lost sensitivity to streptomycin in addition. Two (1.38 %)

isolates were only sensitive to flumequine, while 1 (0.69 %) isolate was resistant to all the antibacterials (Fig. 7).

4.5.3.3. *SALMONELLA TYPHIMURIUM*

Among the motile salmonellae group *Salm. typhimurium* was isolated with the highest prevalence. Among total 75 isolates 50 (66.66 %) were sensitive to all the antibacterial included in sensitivity test. Five (6.66 %) isolates were resistant only to kanamycin. Resistance against furazolidone and kanamycin was observed in 4 (5.33 %) isolates, while another 4 (5.33 %) lost sensitivity to Terramycin in addition. Mostly, furazolidone, kanamycin and Terramycin have fairly high resistance. One (1.33 %) isolate was only sensitive to streptomycin and vibramycin, while another 1 (1.33 %) was sensitive to vibramycin only. Two (2.66 %) isolates were resistant to all the antibacterials (Fig. 8).

4.5.3.4. *SALMONELLA EASTBOURNE*

Among the *Salm. eastbourne* tested against various antibacterials 12 (42.86 %) were sensitive to all the antibacterials used. Eight (28.57 %) serotypes were resistant to neomycin and Tribrissen, while another 4 (14.28 %) serotypes were resistant to erythromycin, furazolidone, kanamycin, neomycin and Tribrissen. Among the antibiography 4 (4.28 %). *Salm. eastbourne* isolates were only sensitive to ampicillin, chloramphenicol, flumequin, gentamicin, lincomycin, streptomycin and vibramycin. Generally *Salm. eastbourne* was resistant to erythromycin, furazolidone, kanamycin, neomycin and Tribrissen at most (Fig. 9).

Fig.6 : Antibiogram of 161 Salmonella gallinarum

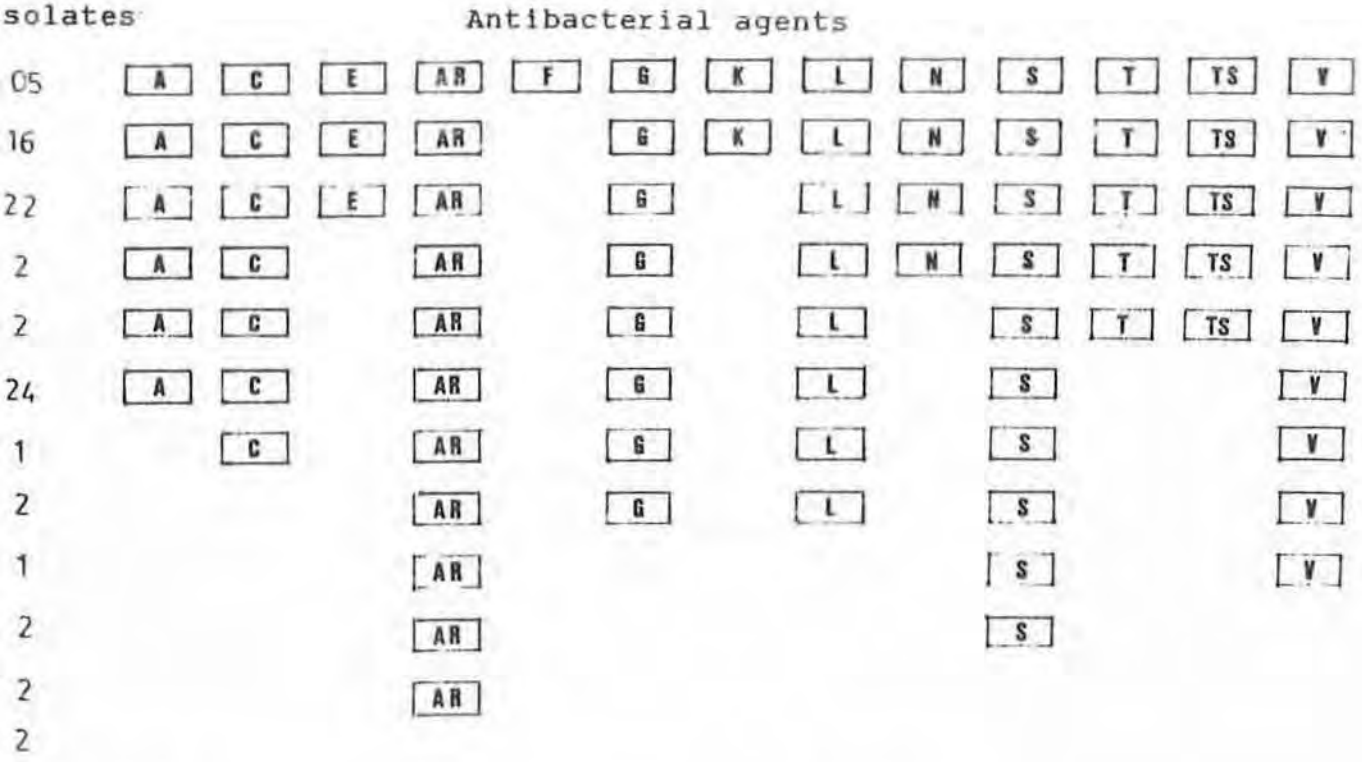


Fig.7 : Antibiogram of 144 Salmonella pullorum

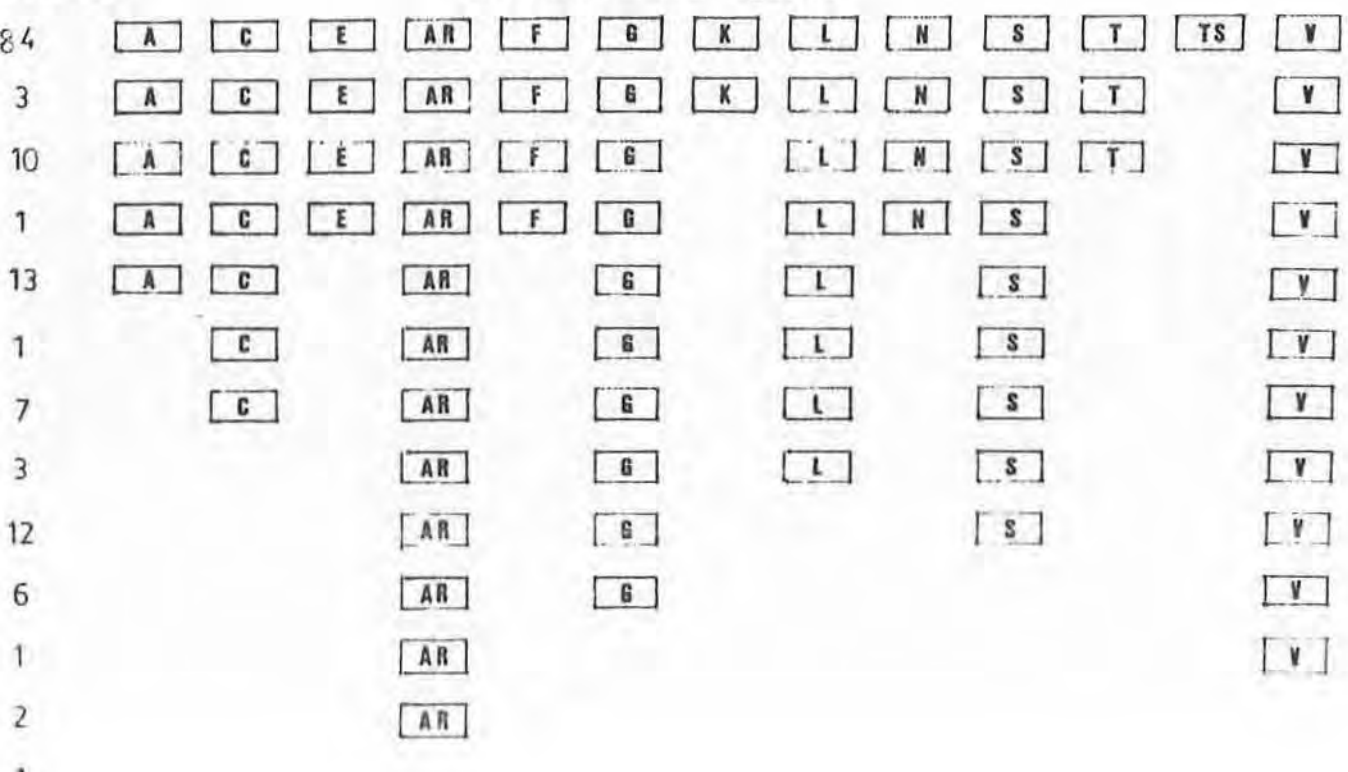


Fig.8 : Antibiogram of 75 Salmonella typhimurium

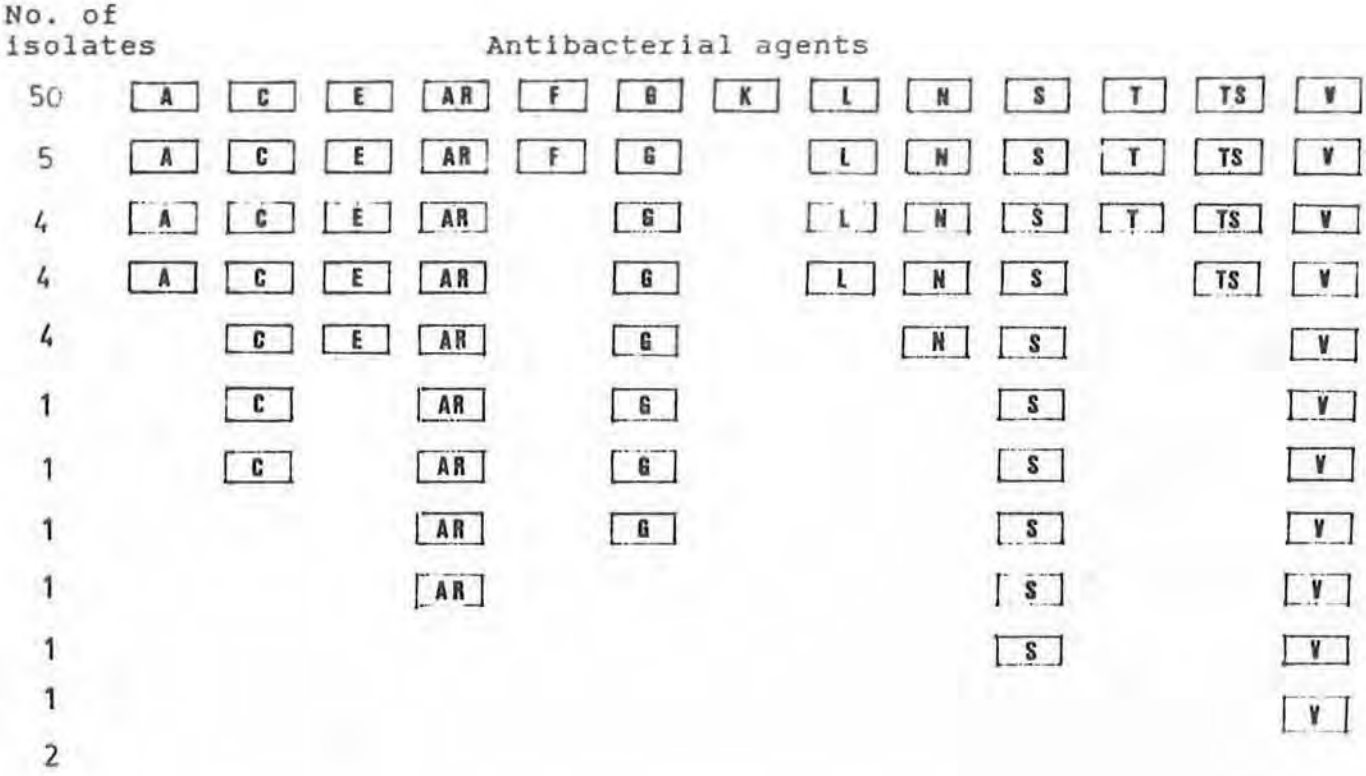
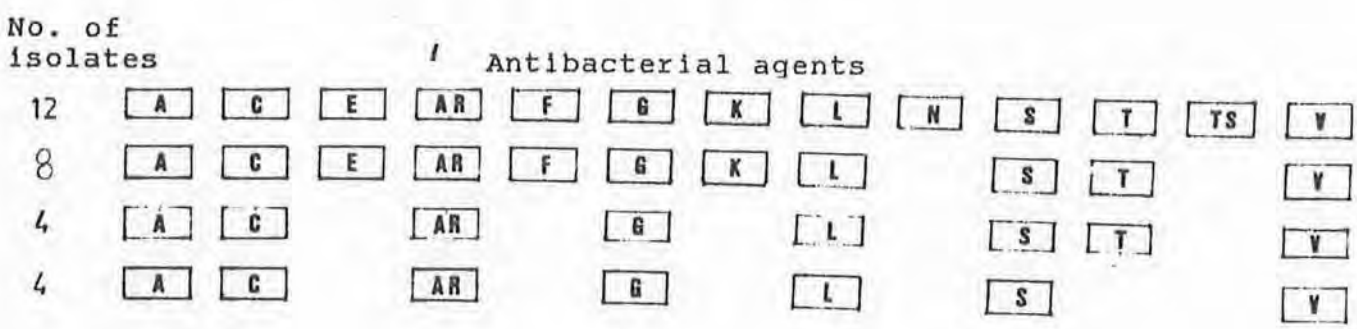


Fig.9 : Antibiogram of 28 Salmonella eastbourne



4.5.3.5. *SALMONELLA SAINT-PAUL*

Among the total 31 isolates of *Salm. saint-paul* tested 12 (38.70 %) were sensitive to all the antibacterials in trials. Only 8 (25.81 %) showed resistance against neomycin, while another 5 (16.12 %) have tendency to survive in the presence of erythromycin, neomycin and Tribrissen. Two (6.45 %) salm. saint-paul were sensitive to ampicillin, chloramphenicol, flumequine, gentamicin, lincomycin, streptomycin and vibramycin. Sensitivity against 4 isolates (12.96 %) of *Salm. saint-paul* diminishes to the extent of chloramphenicol, flumequine, gentamicin and lincomycin (Fig. 10).

4.5.3.6. *SALMONELLA BUTANTAN*

A total of 31 isolates were tested against various antibacterials under trial. *Salm. butantan* 12 (38.70 %) serotypes showed sensitivity to all the tested antimicrobials. Three (9.67 %) isolates were only resistant to kanamycin, while another 6 (19.35 %) showed resistance to Tribrissen in addition. Resistance against furazolidone, kanamycin and Tribrissen was observed in 5 (16.12 %) isolates of *Salm. butantan*. None of the isolate was resistant to all the antibacterials, while some have the sensitivity only to chloramphenicol, flumequine, gentamicin, streptomycin and vibramycin (Fig. 11).

4.5.3.7. *SALMONELLA JAVA*

Salm. java was isolated on 18 occasions from different sources. *Salm. java* was sensitive to all the tested

Fig.10 : Antibiogram of 31 Salmonella saint-paul

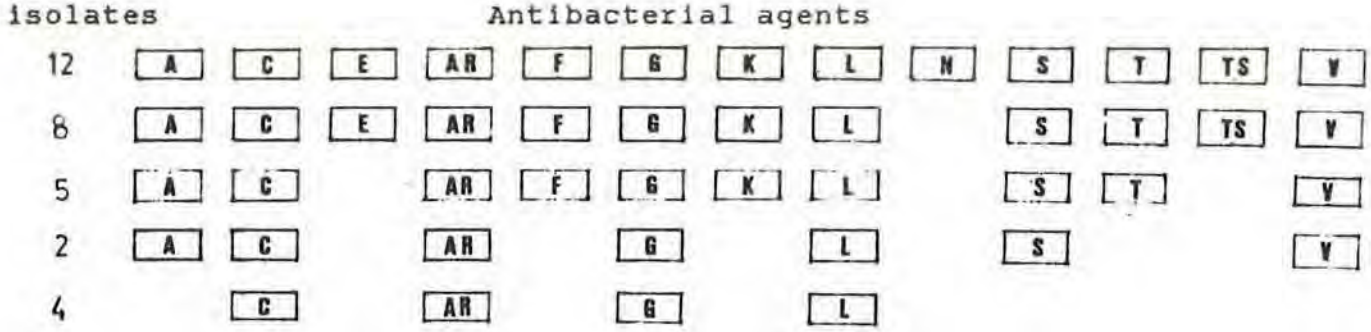
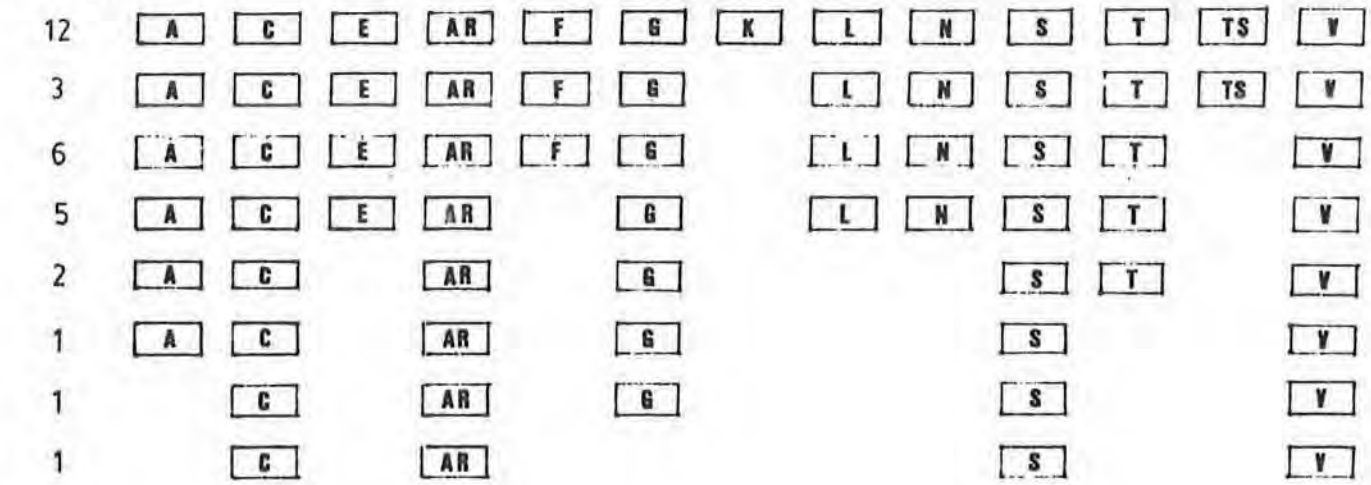


Fig.11 : Antibiogram of 31 Salmonella butantan



antimicrobials for 5 (27.77 %) isolates. Three (16.66 %) isolates were resistant only to Terramycin, while another 2 (11.11 %) were resistent to Tribrissen in addition. Only one (5.55 %) isolate of *Salm. java* was sensitive to only flumequine, while another was sensitive to only chloramphenicol, flumequine, gentamicin, streptomycin and vibramycin (Fig. 12).

4.5.3.8. *SALMONELLA READING*

Salm. reading was mostly sensitive to all the antimicrobials tested. Among total 15 isolates 6 (40.0 %) were sensitive to all the antibacterial tested and another 4 (26.66 %) were sensitive to all antibacterials except neomycin where there showed resistance. As the resistance spectra extended to other antibacterials 3 (20.0 %) isolates showed resistance to ampicillin, erythromycin, neomycin and Tribrissen, only one (6.66 %) isolate was resistant to ampicillin, chloramphenicol, erythromycin, furazolidone, gentamicin, kanamycin, neomycin and Tribrissen (Fig. 13). One (6.66 %) serotype of *Salm. reading* was only sensitive to flumequine, lincomycin, streptomycin and vibramycin.

4.5.3.9. *SALMONELLA CHESTER*

Salm. chester responded only 22.22 per cent to the all antibacterial under trial except Terramycin. One (5.55 %) *Salm. chester* was resistant to kanamycin and Terramycin, another one (5.55 %) showed the resistant against furazolidone and Tribrissen alongwith the previous antibacterials, Resistance was developed in 9 (50.0 %) against ampicillin,

Fig.12 : Antibiogram of 18 Salmonella java

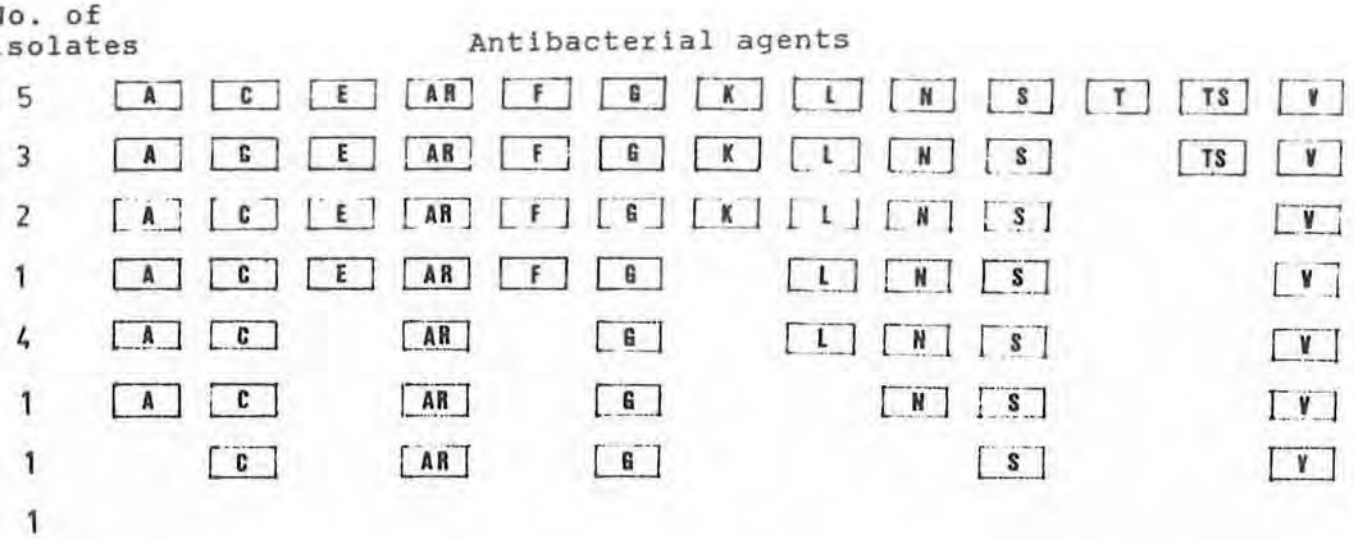
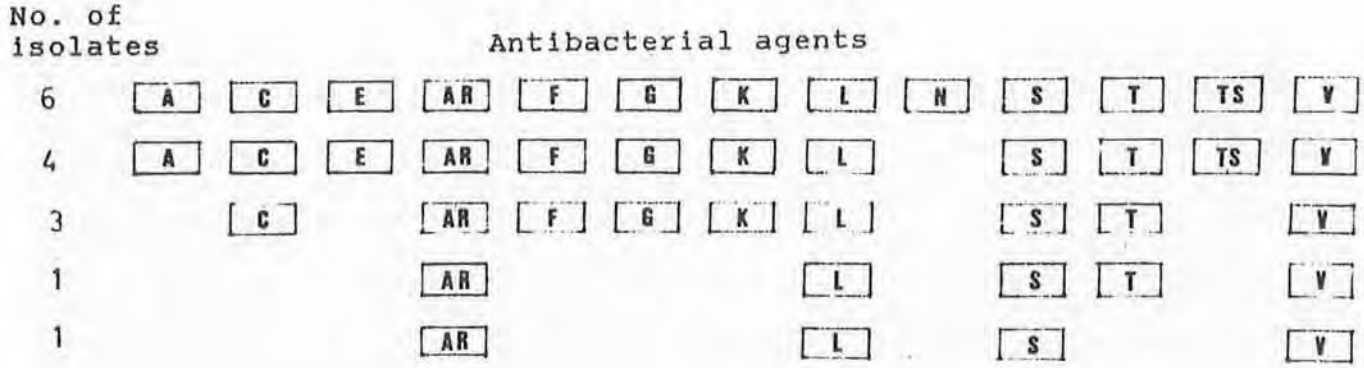


Fig.13 : Antibiogram of 15 Salmonella reading



furazolidone, kanamycin, neomycin, Terramycin and Tribrissen. One (5.55 %) isolate of *Salm. chester* was resistant to all the spectrum of antibacterials under trial (Fig. 14).

4.5.3.10. *SALMONELLA REMO*

Among the total 24 isolates of *Salm. remo* only 7 (29.16 %) were sensitive to all the tested antimicrobials. Three (12.50 %) isolates were resistant to kanamycin only the variable resistance was noted in another 3 isolates. Six (25.0 %) isolates of *Salm. remo* were sensitive to all except erythromycin, furazolidone, kanamycin, Terramycin and Tribrissen. Most of the isolates were sensitive to chloramphenicol, flumequine, vibramycin, streptomycin (Fig. 15). One (4.16 %) isolates was resistant to all the antibacterial tested.

4.5.3.11. *SALMONELLA HEIDELBERG*

A total of 18 isolates of *Salm. heidelberg* were detected among these 5 were sensitive to all the antibacterials. Three (16.66 %) isolates were sensitive to all the antimicrobials except Terramycin against which resistance observed another 2 (11.11 %) isolate lost sensitivity against Tribrissen in addition. Kanamycin, Terramycin and Tribrissen resistance was observed in only one (5.55 %) isolate. Generally the resistance was observed against erythromycin, furazolidone, kanamycin, Terramycin and Tribrissen. One (5.55 %) isolates was resistant to all the antibacterials tested (Fig. 16).

Fig.14 : Antibiogram of 18 Salmonella chester

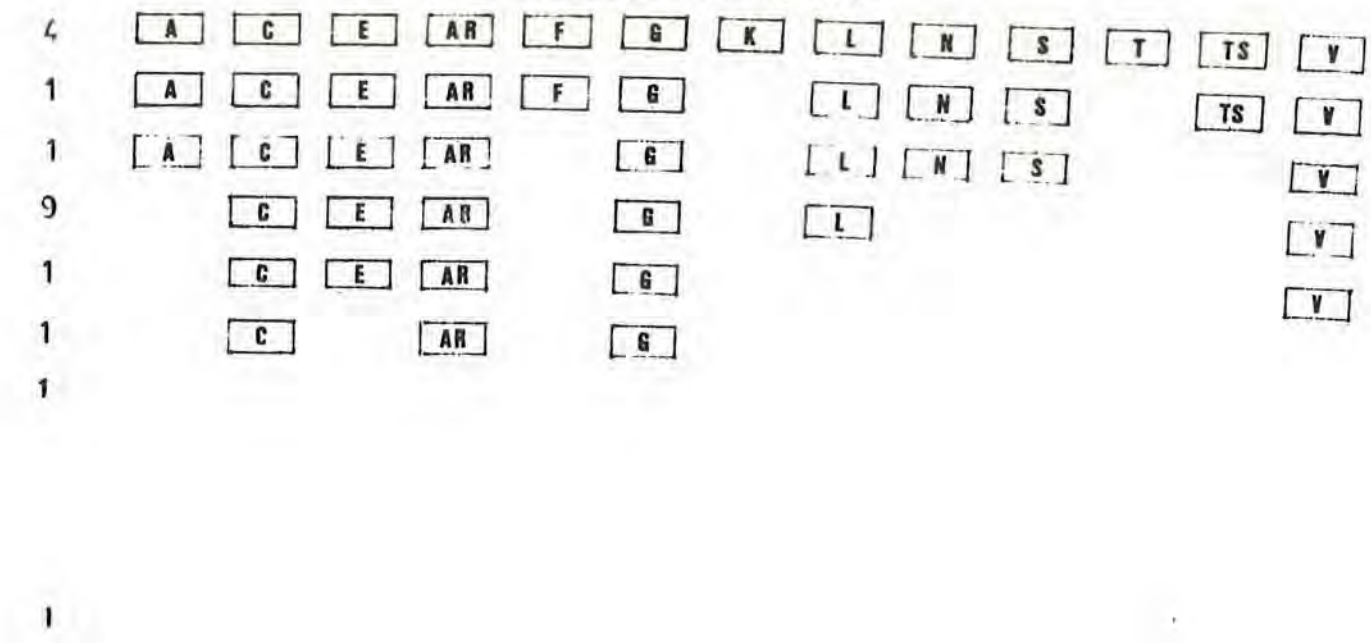
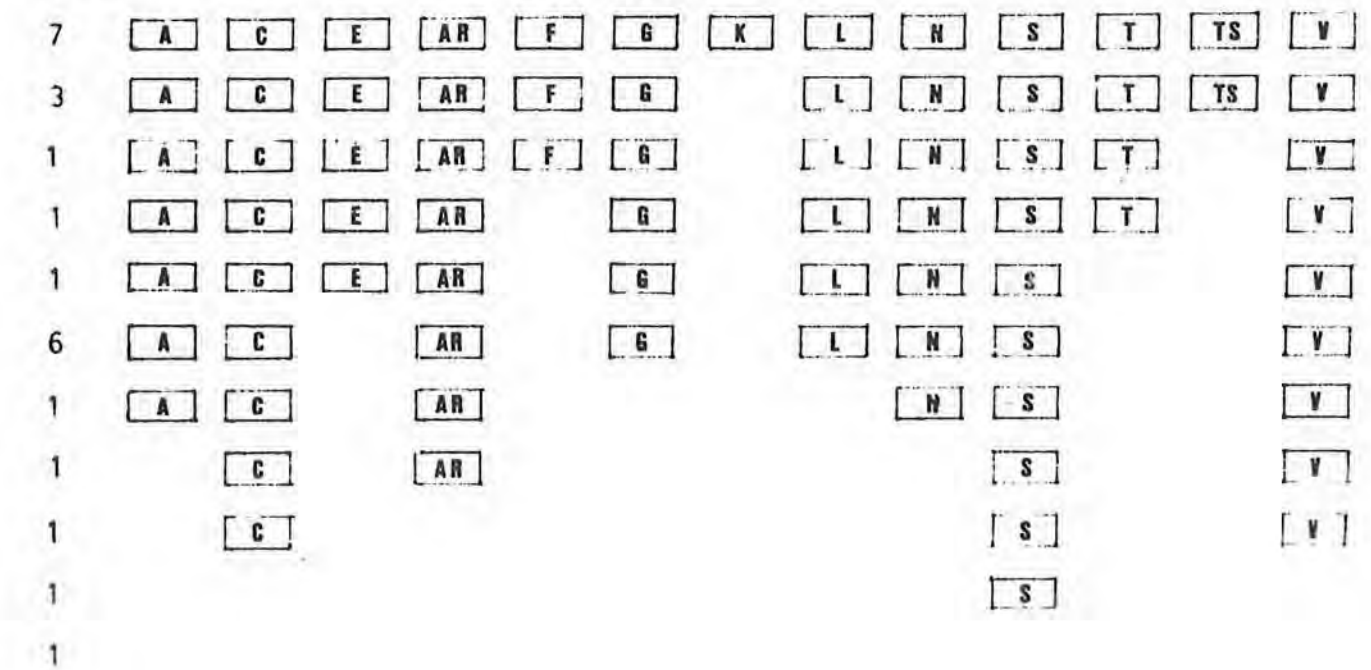


Fig.15 : Antibiogram of 24 Salmonella remo



4.5.3.12. *SALMONELLA ANATUM*

Five (35.71 %) of the total (14) isolates of *Salm. anatum* were sensitive to all the tested antibacterials. Resistance was observed against Terramycin in 4 (28.57 %) cases. Further addition of resistance was observed in 2 (14.28 %) isolates against erythromycin, furazolidone, kanamycin and Terramycin, only one (7.14 %) *Salm. anatum* isolate was sensitive to chloramphenicol, flumequine and neomycin, while another lost sensitivity to neomycin in addition (Fig. 17).

4.5.3.13. *SALMONELLA HADAR*

To the all antibacterials tested only 2 (14.28 %) serotypes of *Salm. hadar* were sensitive, while only 1 (7.14) was sensitive only to gentamicin. Four (28.57 %) isolates were resistant to erythromycin, furazolidone, kanamycin, Terramycin and Tribrissen. One (7.14 %) was only sensitive to chloramphenicol, flumequine, gentamicin, lincomycin, neomycin, streptomycin and vibramycin, while another one (7.14 %) have the same sensitivity except it created resistance against chloramphenicol and neomycin (Fig. 18).

4.5.3.14. *SALMONELLA ORION*

Eleven isolates of *Salm. orion* were tested to check their response to the antibacterials in use, 2 (18.18 %) isolates were sensitive to all the spectra. Only one (9.09 %) isolate was sensitive to flumequine and gentamicin. Resistance was observed against flumequine, kanamycin, lincomycin, neomycin, Terramycin and Tribrissen (Fig. 19).

Fig.16 : Antibiogram of 18 Salmonella heidelberg

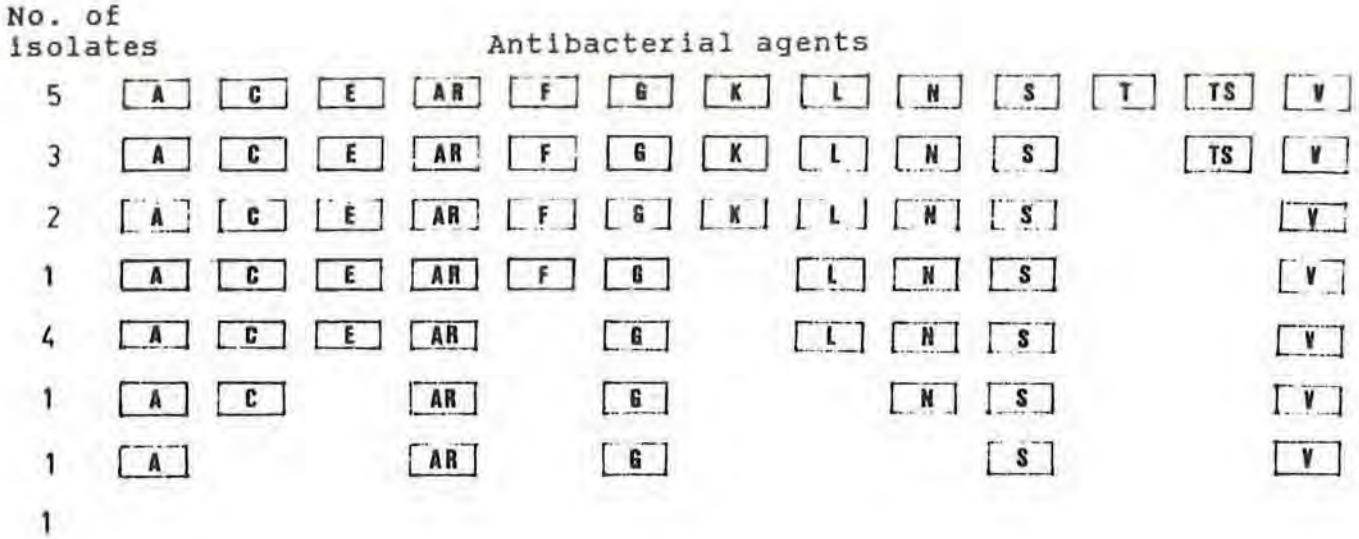


Fig.17 : Antibiogram of 14 Salmonella anatum

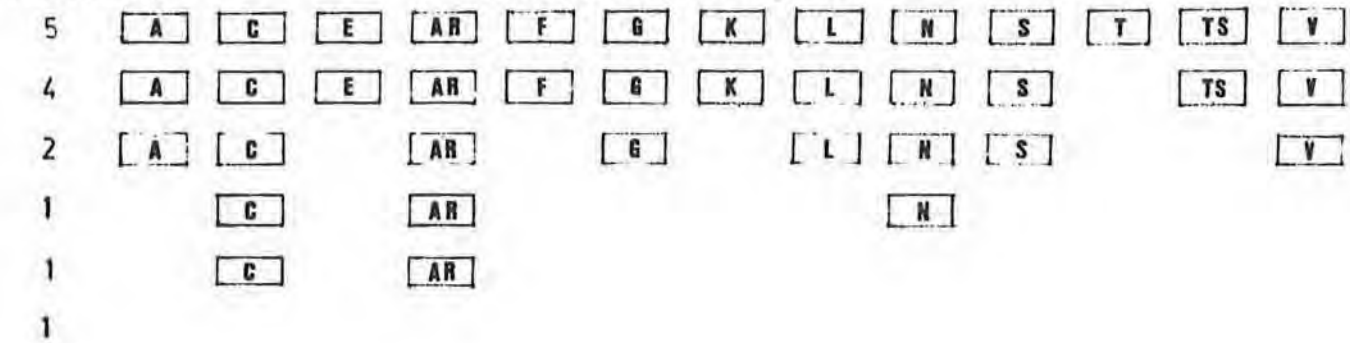


Fig.18 : Antiblogram of 14 Salmonella hadar

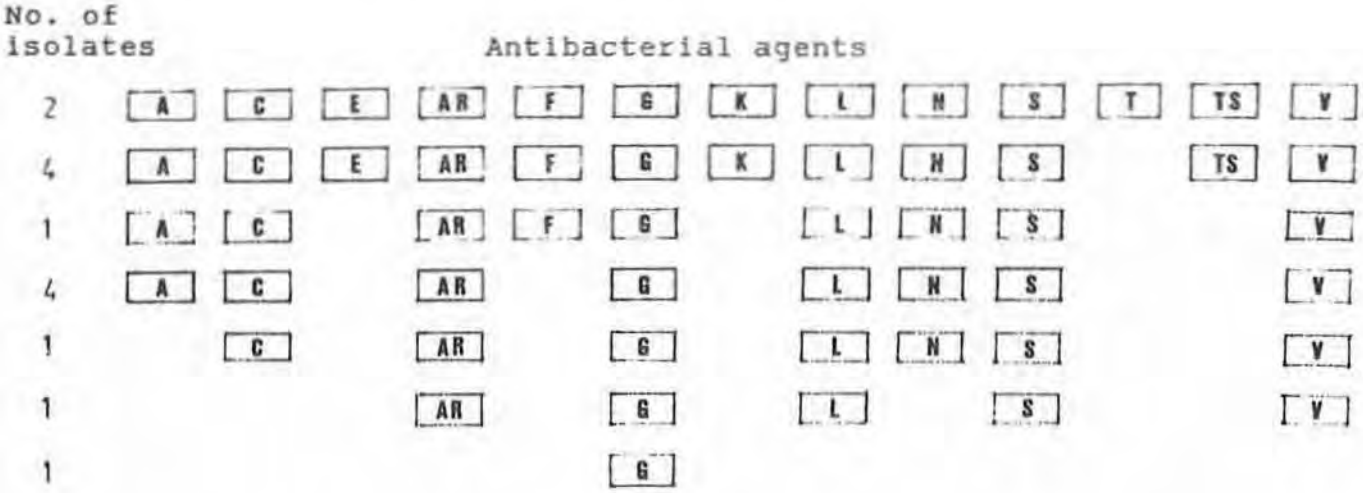


Fig. 19 : Antiblogram of 11 Salmonella orion

4.5.3.15. *SALMONELLA RIDGE*

Salm. ridge was isolated from 7 samples among these isolates 2 (28.57 %) were sensitive to all the antibacterials. One (14.28 %) isolate was resistant to all the spectrum. Other 4 serotypes lost their sensitivity gradually and develop resistent to erythromycin, furazolidone, kanamycin, lincomycin, Terramycin and Tribrissen in general (Fig. 20).

4.5.3.16. *SALMONELLA AGONA*

Thirty nine *Salmonella* isolates were of *Salm. agona*, whereas, only 12 (30.76 %) were sensitive to the antimicrobials under trial. Three (7.69 %) isolates lost sensitivity to kanamycin. Tribrissen was the next antibacterial against which resistant was observed alongwith kanamycin in 6 (15.38 %) isolates of *Salm. agona*. Resistance against furazolidone, kanamycin and Tribrissen was observed in 5 (12.82 %) isolates. Six (15.38 %) were resistant to furazolidone, erythromycin, kanamycin and Tribrissen. One (2.56 %) isolate was only sensitive to vibramycin, while one (2.56 %) isolate was resistant to all the antimicrobial under trial (Fig. 21)

4.5.3.17. *SALMONELLA MISSION*

Among the total of 8 isolates of *Salm. mission* 3 (37.5 %) were sensitive to all the drugs tested in these studies. One (12.50 %) isolate lost its sensitivity against furazolidone, while another one lost its sensitivity to kanamycin, neomycin, Terramycin and Tribrissen in addition. One (12.50 %) isolate was only sensitive to ampicillin,

Fig.20 : Antibiogram of 7 Salmonella ridge

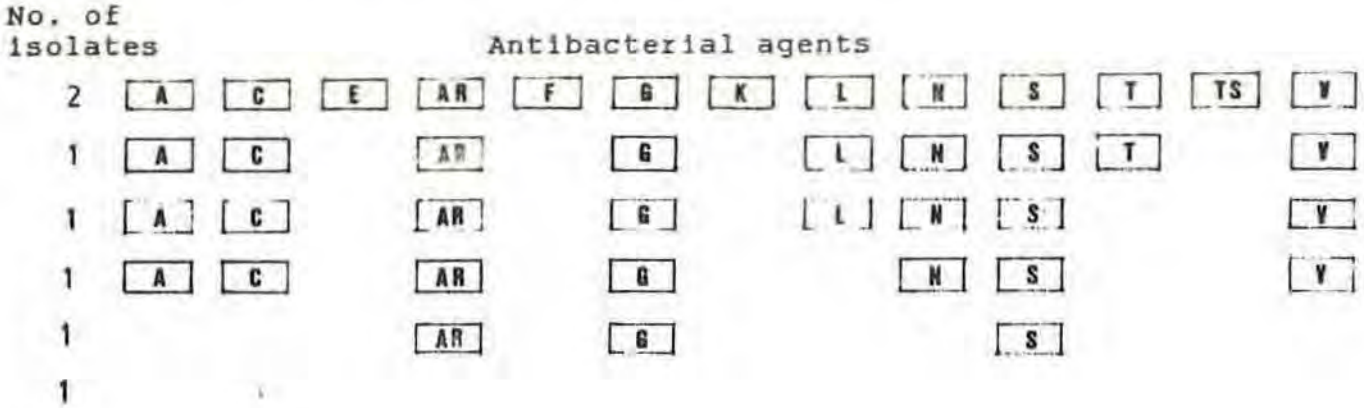
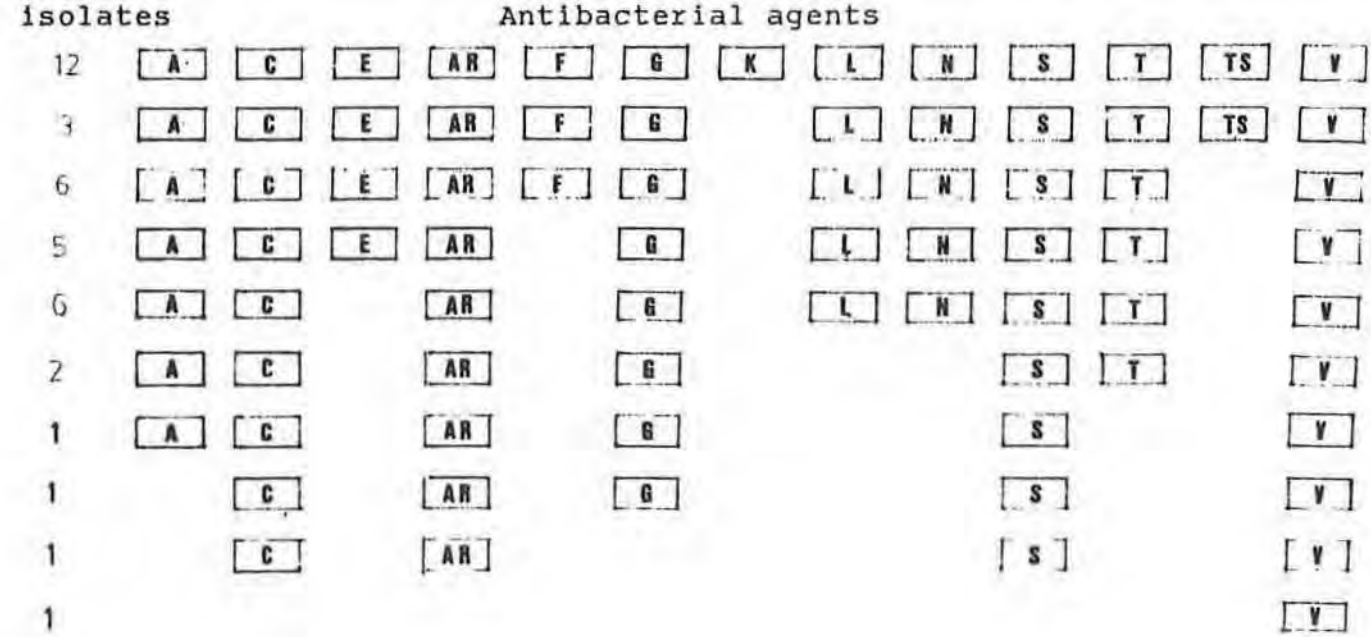


Fig.21 : Antibiogram of 39 Salmonella agona



chloramphenicol, flumequine, gentamicin and streptomycin. Two isolates (25.00 %) developed resistance against erythromycin, furazolidone, kanamycin, neomycin, Terramycin and Tribrissen (Fig.22).

4.5.3.18. *SALMONELLA GIVE*

Salmonella give was isolated on 27 occasions from different sources. *Salm. give* was sensitive to all the antimicrobials under trial in only 10 (37.03 %) cases. One (3.70 %) isolate was resistant only to the furazolidone, while another 2 (7.40 %) lost sensitivity against Terramycin in addition. Generally Terramycin, Tribrissen and furazolidone lost the effectiveness due to resistant bacteria. One (3.70 %) isolate was only sensitive to flumequine and vibramycin, while another one (3.70 %) create resistance against vibramycin (Fig. 23).

4.5.3.19. *SALMONELLA PARATYPHI-A*

Antibiogram of *Salm. paratyphi A* indicated that among the 12 isolates tested 3 (25.00 %) were sensitive to all the antimicrobials. Resistance against only Tribrissen was observed in 2 (16.66 %) isolates, while resistance against Terramycin in addition was observed in 1 (8.33 %) isolate. Four (33.33 %) isolates showed resistance to kanamycin, Terramycin and Tribrissen, while one (8.33) isolate showed resistance to furazolidone in addition (Fig. 24).

Fig.22 : Antibiogram of 8 Salmonella mission

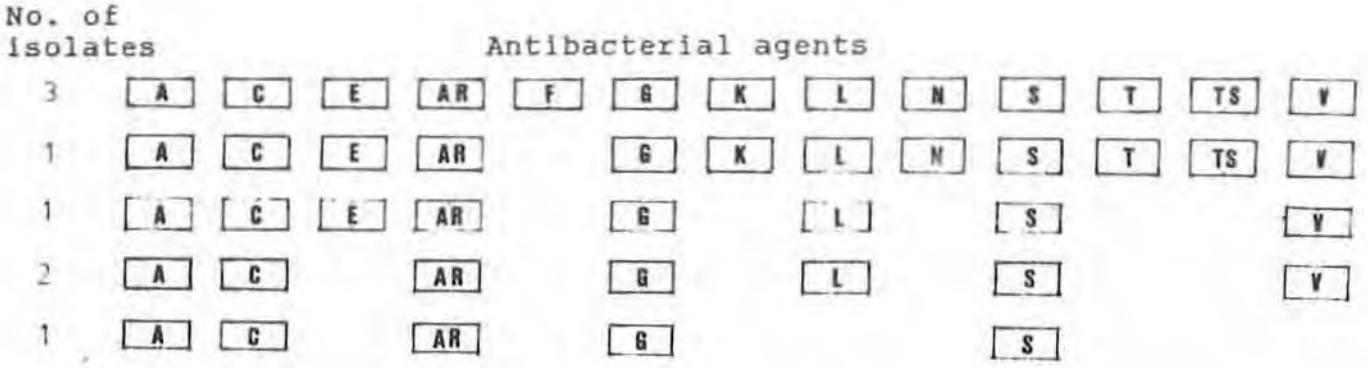


Fig.23 : Antibiogram of 27 Salmonella give

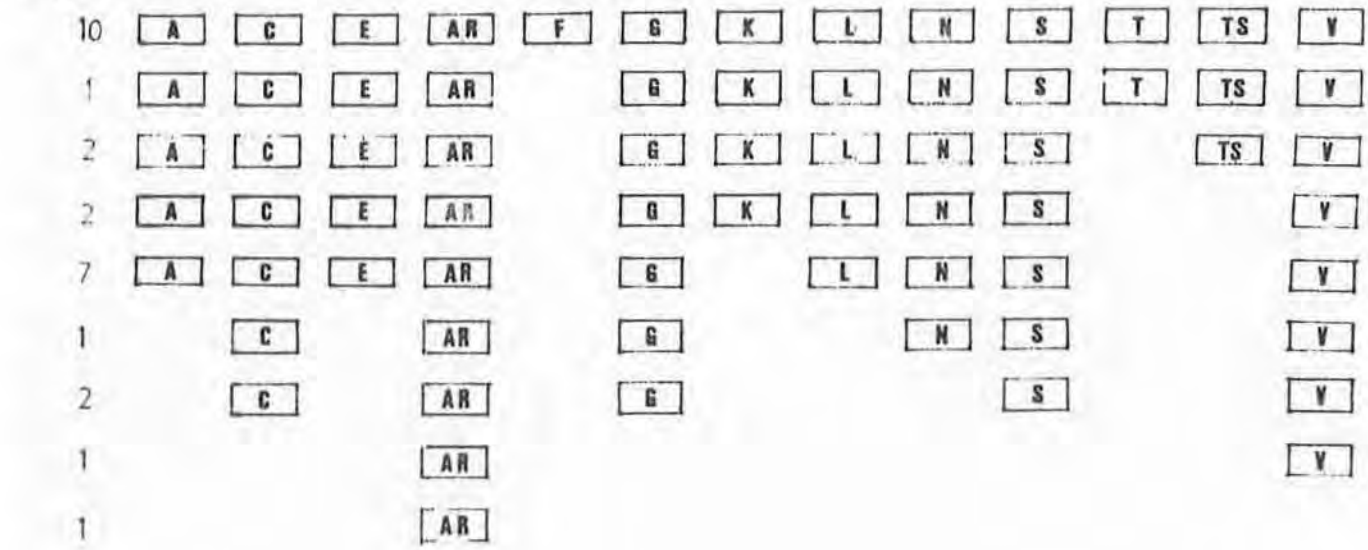
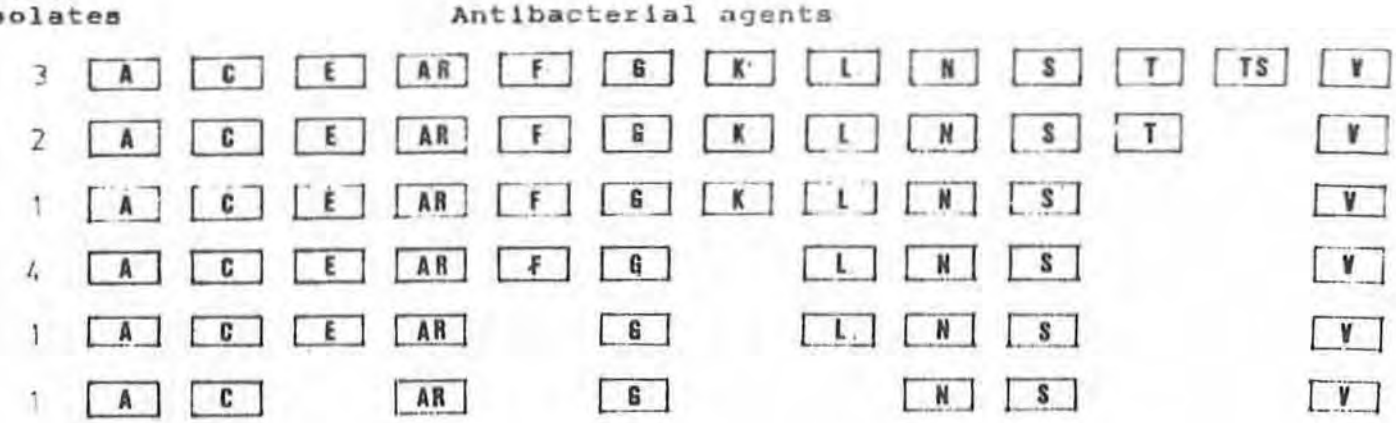


Fig. 24 : Antibiogram of 12 Salmonella para typhi A.



4.6. MACRO-MICRO AND ULTRASTRUCTURAL PATHOLOGICAL STUDIES

Pathological lesions were almost identical in all the affected birds at organ and cellular level but intensity was variable with different serotypes of *Salmonella* involved (Table 19,20).

4.6.1. LIVER

Liver was the main target organ of *Salmonella* serotypes in 83 per cent cases among 108 bacteriological positive birds. Liver affected with remarked enlargement along with soft consistency, hemorrhages and congestion were the primary gross abnormalities leading to severe metallic sheen and bronze discoloration. Toxic induration, necrotic foci, petechial or ecchymotic hemorrhage and severe atrophic changes were the more prominent lesions in *Salm. gallinarum, Salm. pullorum, Salm. typhimurium and Salm. heidelberg*. While other serovars affected the liver milk to moderately (Table 21). The capsule was thick but tissues were friable and crisper in almost all the cases, however, the intensity varied with the serotypes incriminated. These changes were more pronounced at the margins than central areas of the lobes. The size of necrotic foci scattered all over the surface of the liver varied from millet seed to that of a pea.

Histopathologically the most salient lesions found in the liver were congestion, fatty degeneration, thickening of capsule, cloudy swelling along with pyknotic nucleus and degenerative changes were seen in 65 per cent cases (Fig. 25). Diffuse cirrhosis and amyloid deposition in degenerated areas

Table 19: Frequency (%) of involvement of various organs in different *Salmonella* serotypes.

Salmonella serotypes	Total positive	Liver	Intestines	Spleen	Lungs	Kidneys	Ovaries	Heart	Ceca	Brain	Mesa
pullorum	16	83	76	67	54	64	93	25	82	13	58
gallinarum	25	80	73	61	44	45	98	23	84	15	61
typhimurium	10	92	81	72	64	61	93	33	91	21	76
eastbourne	5	54	44	38	41	38	72	13	47	10	13
saint-paul	5	58	41	39	42	37	58	12	42	9	11
butantan	6	61	43	41	45	39	53	13	43	10	13
java	4	57	41	38	42	37	51	12	40	9	11
reading	3	73	65	69	54	41	83	21	77	12	43
chester	4	74	63	68	53	39	78	18	73	10	41
remo	5	71	68	63	51	38	71	15	68	9	42
heidelberg	7	72	67	63	51	42	81	21	75	11	42
anatum	4	67	58	59	50	41	72	18	70	10	38
hadar	1	73	64	58	53	38	77	20	67	11	39
orion	2	67	58	43	51	38	51	13	67	10	28
ridge	1	63	52	47	50	37	53	12	65	9	21
agona	5	68	62	49	51	38	72	18	71	12	42
mission	3	61	48	53	54	41	70	13	70	12	40
give	1	57	49	58	61	38	73	15	73	11	41
para typhi A.	1	76	65	48	62	39	74	19	68	13	43

: Ultrastructure changes of various visceral tissues in different Salmonella serotypes

la s	Breakage of nuclear membrane	Alterat- ion of organellae	Exvagin- ation of chromatin	Fragmen- tation of chromatin	Necrotic lesions	Dystraphic alterations	Reticular cell destruction	Cytoplasmic modific- ation	Mitochondral elongation	Endoplasm relictum changes	Neerobiosis
n	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
rum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
rium	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
rne	+	+	+	+	+	+	+	+	+	+	+
aul	+	+	+	+	+	+	+	+	+	+	+
n	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
	++	++	++	++	++	++	++	++	++	++	++
	++	++	++	++	++	++	++	++	++	++	++
	++	++	++	++	++	++	++	++	++	++	++
erg	++	++	+	+	++	+	++	+	+	+	+
	++	+	+	++	+	++	+	++	++	++	++
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
	++	++	++	+	+	++	+	++	++	+	+
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
-A	++	++	++	++	++	++	++	++	++	++	++

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1: Frequency of involvement of liver with different serotypes of Salmonella

lla es	Total iso-lates	GROSS PATHOLOGY					HISTOPATHOLOGY				
		Hemo-rrhages	Necrotic-foci	Bronze colouration	Fragility	Enlarg-ment	Conges-tion	Degenra-tion	Necrosis	Cellular infiltration	Hyperplasia of Kupffer cells
n	16	+++(12) ++(4)	+++(14) ++(2)	++(16)	+++(15) ++(1)	+++(16)	+++(16)	+++(16)	+++(14) ++(2)	+++(14) ++(2)	+++(13) ++(3)
um	25	+++(21) ++(4)	+++(22) ++(3)	+++(14) ++(11)	+++(15) +++(10)	+++(22) ++(3)	+++(25)	+++(25)	+++(22) ++(3)	+++(25)	+++(13) ++(12)
ium	10	+++(10)	+++(8) ++(2)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)
ne	5	+(5)	++(4) +(1)	+(5)	++(5)	+(5)	++(5)	++(4) +(1)	++(5)	++(5)	++(5)
ul	5	++(5)	++(5)	+(5)	++(5)	+(5)	++(5)	++(5)	++(5)	++(5)	++(5)
	6	++(5) +(1)	++(5) +(1)	+(6)	++(4) +(2)	+(6)	++(4) +(2)	++(5) +(1)	+(6)	++(6)	++(4) +(2)
	4	++(4)	+(4)	+(4)	+(4)	+(4)	+(4)	++(3) +(1)	+(4)	++(2) +(2)	+(4)
	3	+(3)	++(3)	+(3)	++(3)	+(3)	++(3)	+++(2) ++(1)	++(3)	+++(1) ++(2)	+(3)
	4	+(4)	++(4)	+(4)	++(4)	+(4)	++(4)	+++(2) ++(2)	++(2) +(2)	+++(4)	+(4)
	5	++(5)	++(5)	++(5)	++(5)	++(5)	+++(3) ++(2)	+++(4) +(1)	++(4) +(1)	+++(2) ++(3)	+(5)
rg	7	++(7)	+++(4) ++(3)	++(7)	++(3) +++(4)	++(5) +(2)	+++(3) ++(4)	+++(5) ++(2)	++(3) +(4)	+++(3) ++(4)	+(7)
	4	++(3) +(1)	++(4)	+(4)	++(4)	++(4)	+++(4)	++(4)	+++(3) +(1)	++(3)	++(4)
	1	++(1)	+(1)	+(1)	++(1)	+(1)	++(1)	++(1)	++(1)	+(1)	++(1)
	2	+(2)	+(2)	+(2)	++(2)	+(2)	+(2)	+(2)	+(2)	+(1)	+(1)
	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)
	5	++(4) +(1)	++(3) +(2)	++(5)	++(5)	++(5)	++(5)	++(5)	+(5)	+(5)	++(5)
	3	+(3)	+(3)	+(3)	+(3)	+(3)	+(3)	+(3)	++(3)	++(2) +(1)	+(3)
	1	++(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)
	1	+(1)	+(1)	++(1)	++(1)	+(1)	+(1)	++(1)	++(1)	++(1)	++(1)

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were in abundance. Hemorrhagic or hemorrhagic-necrotic hepatitis irrespective of the age of birds and serotype involved was seen with variable intensities. Mono nuclear cellular infiltration was seen replacing the degenerated or necrotic foci in 32 per cent cases (Fig. 26). Perivascular areas showed hemorrhages and lymphocytic hyperplasia of pseudoacini, were the frequent lesions. All the affected sinusoidal spaces and blood vessels were dilated and filled with blood elements. Biliary spaces and ducts have desquamated epithelium along with distension of disse spaces (Fig. 27).

Ultrastructural changes in hepatocytes included cytoplasmic and nuclear enlargement. There were thickened membranes of the smooth endoplasmic reticulum (SER) and dilation of the rough endoplasmic reticulum (RER) with loss of ribosomes (Fig. 28). Some hepatocytes contained vacuolated or deformed mitochondria with a tendency to aggregate near the cell membrane. Dilated bile canaliculi had reduced numbers of microvilli and many were shortened and flattened. There was granular and/or fibrillar degeneration of nucleoli. In severe cases hepatocytes had more degenerative changes with increased numbers of polysomes, lysosomes and bile deposits. Thy cytosol of hepatocytes was electron lucent, edematous and contained increased numbers of fat vacuoles (Fig. 29). Desmonsomes were detached, thickened and irregular at the cell boundaries. Cytoplasmic hydropic degeneration caused scattering of organellae and enlargement of affected cells, causing compression and atrophy of neighboring cells (Fig. 30, 31).

Fig. 27. Liver from Salmonella infected bird having several bile ducts lined by epithelial cells with loss of cell polarity, variation in shape and size with indistinct cell borders ( ); note small indistinct poorly-delineated lumena. H&E 400 X.

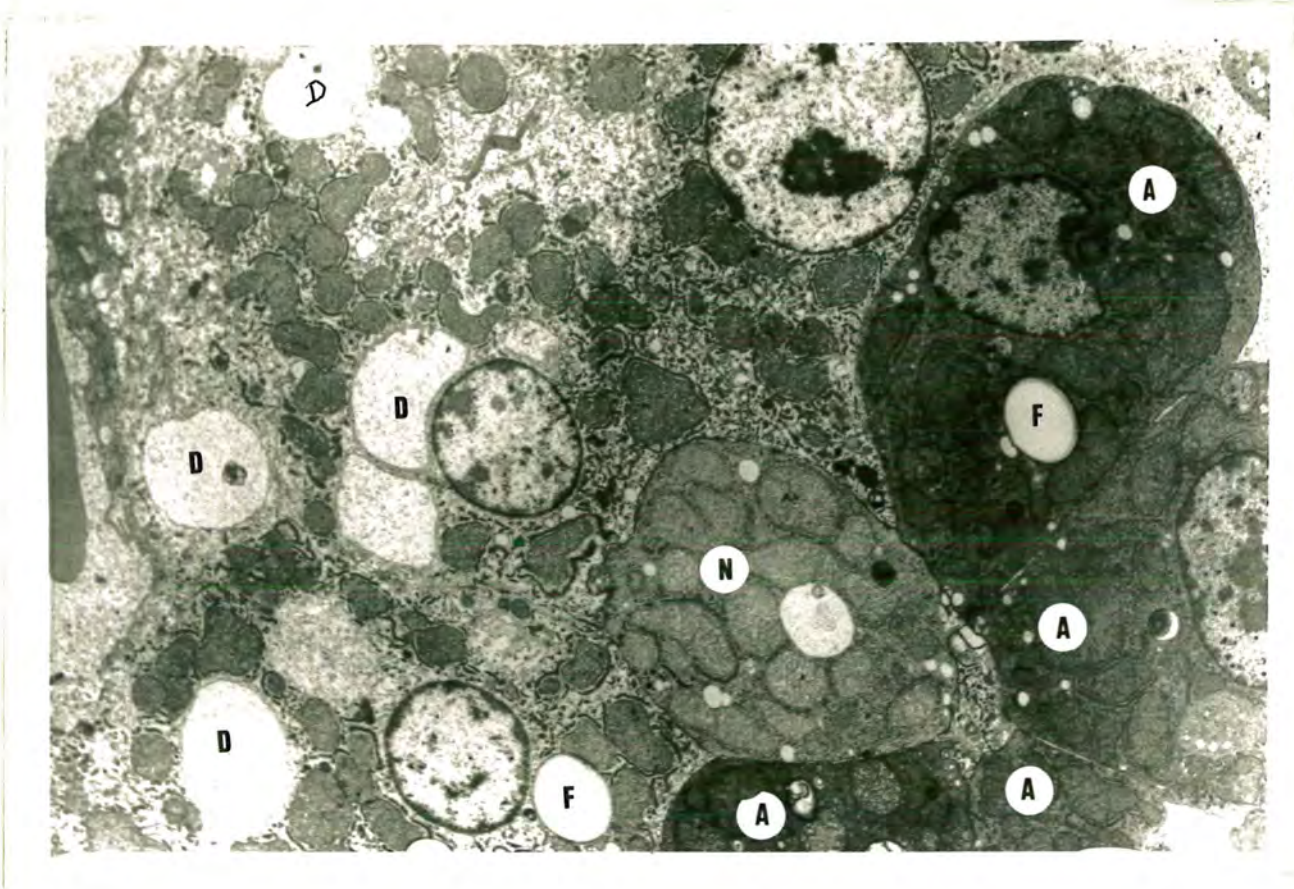


Fig. 29. Normal hepatocytes (A) adjacent to a shrunken necrotic cell (N) and other hepatocytes with indistinct cell borders arrows indicate visible portions of cell membrane, dispersed organellae with intracellular hydropic degeneration (D) and increased fat vacuoles (F) 6270 X.

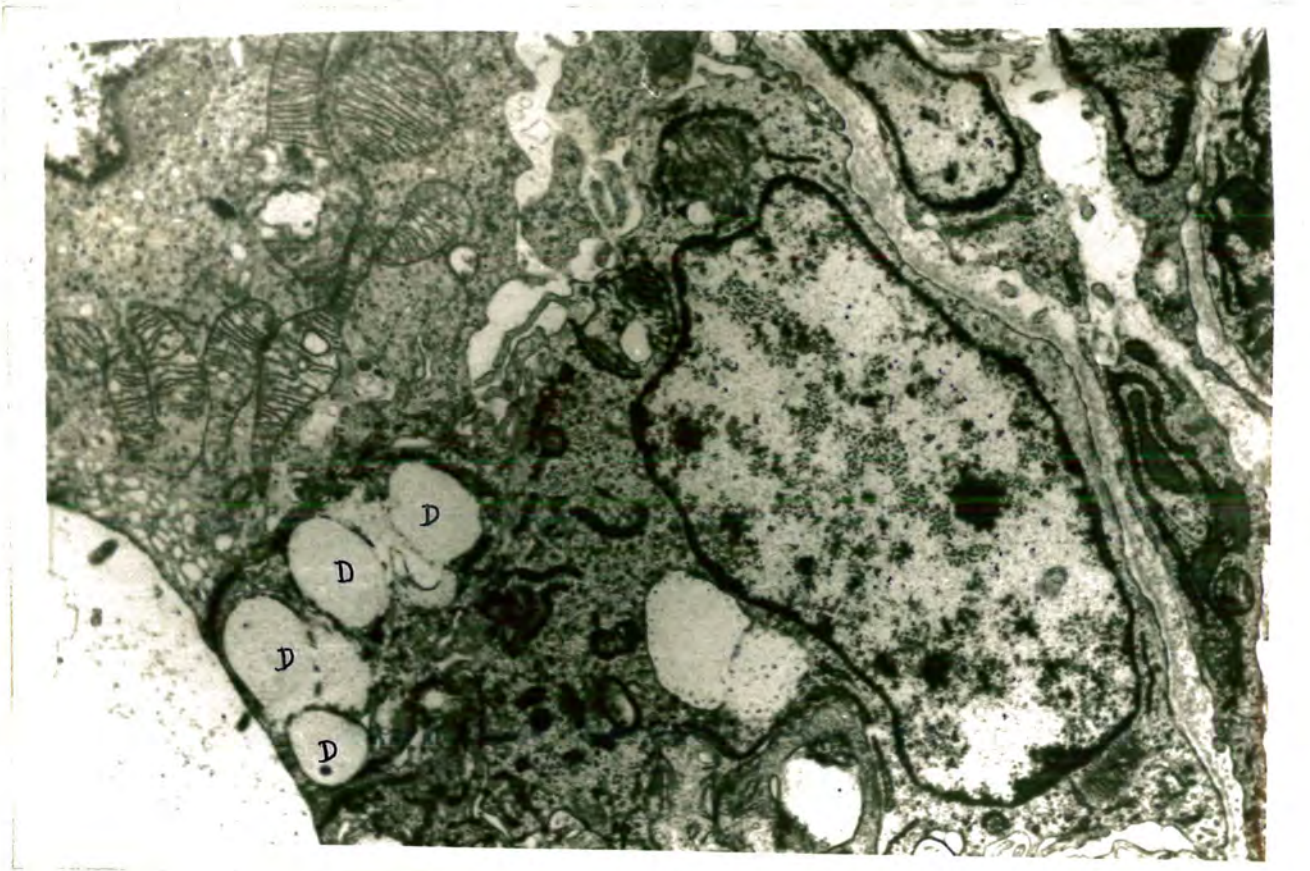


Fig. 30. Single cell necrosis with higher magnification of hepatocytes, dispersed organellae with intracellular hydropic degeneration (D) and increased fat vacuoles (F) 6270 X.

4.6.2. INTESTINES

Among 108 randomly selected bacteriological positive intestines 76 per cent were affected with variable damages with different serotypes grossly. Most of the intestines particularly from chicks contained cheesy core of caseative material and in some cases tinged with blood in young chicks. Intestinal walls was thickened and had hemorrhages pin head size to brush types in almost all the cases along with catarrhal inflammation (20 %). *Salm. typhimurium, Salm. gallinarum* and *Salm. pullorum* affected adversely, while other serotypes ranged from mild to moderate in lesion production (Table 22).

The most salient histopathological lesions in the intestines were thickening of the muscular mucosa along with lymphocytic infiltration. Hemorrhagic exudate was also seen in sections from intestines of 72 per cent cases. There were broken villi, degeneration of intestinal glands and thickening of muscularis mucosae were the lesions in common. Severe hemorrhages and leukocytic infiltration were more frequently in the lamina properia. Hypertrophy of glandular cells, cloudy swelling of degenerated glandular cells and occluded lumen of glands (Fig. 32,33).

Ultrastructurally necrobiosis in some intestinal cells at the nuclear and cytoplasmic level was observed. Cytoplasmic fragmentation with cytovacuolation blabbing of the cell membrane were the salient features in intestinal cells. Necrotic cells in the lumenal mucosae with sloughing of the

Table 22: Frequency of involvement of intestines in different Salmonella serotypes

Salmonella serotypes		GROSS PATHOLOGY		HISTO PATHOLOGY			
		Thickening of intestinal wall	Hemorrhages	Cattarrhal entritis	Hemorrhagic entritis	Degenration of M.Mucosae	Degenration of glands
pullorum	16	+++(16)	+++(16)	+++(10) ++(6)	+++(6) ++(10)	+++(10) ++(6)	+++(10) ++(6)
gallinarum	25	+++(22) +(3)	+++(25)	+++(2) ++(23)	+++(5) ++(20)	+++(20) ++(5)	+++(25)
typhimurium	10	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)
eastbourne	5	++(5)	+++(1) ++(4)	++(4) +(1)	+++(3) ++(2)	+++(5)	++(5)
saint-paul	5	++(5)	++(3) +(2)	++(4) +(1)	++(4) +(1)	++(5)	++(5)
butantan	6	++(6)	++(6)	++(6)	+++(1) ++(5)	+++(4) ++(2)	++(6)
java	4	+(4)	+(4)	+(4)	++(4)	++(3) +(1)	++(4)
reading	3	+++(2) ++(1)	++(3)	++(3)	++(3)	++(3)	+(3)
chester	4	+++(2) ++(2)	+++(2) ++(2)	++(4)	++(4)	++(4)	+(4)
remo	5	+++(2) ++(3)	+++(2) ++(3)	++(5)	++(5)	+(5)	++(5)
heidelberg	7	+++(4) ++(3)	++(7)	++(7)	+++(3) ++(4)	++(7)	++(7)
anatum	4	++(4)	++(4)	+(4)	+(4)	++(4)	+++(2) ++(2)
hadar	1	++(1)	++(1)	++(1)	++(1)	++(1)	+++(1)
orion	2	+(2)	+(2)	+(2)	+(2)	+(2)	++(2)
ridge	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)
agona	5	++(5)	+++(1) ++(4)	+(5)	+(5)	+(5)	+(5)
mission	3	+(3)	+(3)	+(3)	+(3)	+(3)	+(3)
give	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)
P.typhi-A	1	+++(1)	++(1)	++(1)	++(1)	+++(1)	+++(1)

+++ = Severe
++ = Moderate
+ = Mild

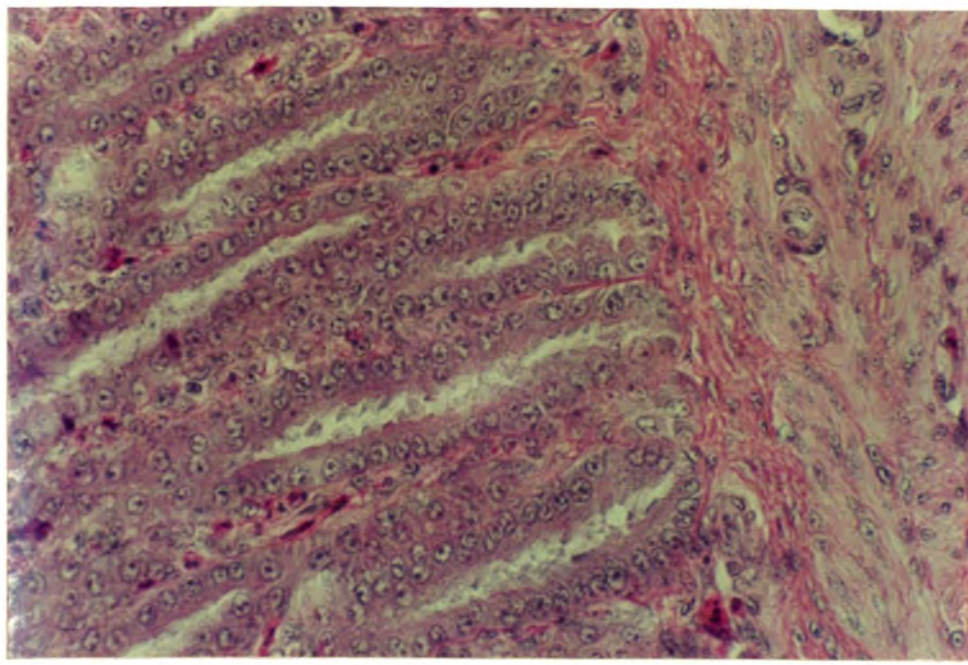


Fig. 32. Intestine with thickened mucosa and degeneration of the glands H&E 400 X.

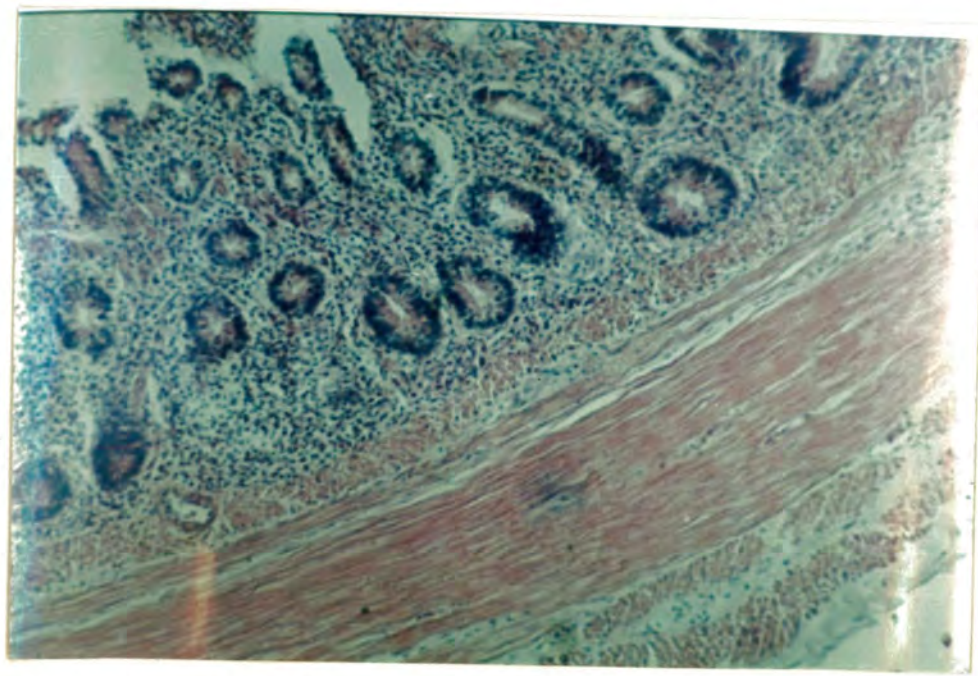


Fig. 33. Intestine showing hypertrophy of glandular cells H&E 400 X.

cells while integrated cells lost microvilli and cilia. The shapes and sizes of the microvilli were inconsistent. The missing microvilli give place to the adjacent microvilli to reduce the length and becomes rounded in abundance. In goblet cells numerous secretory droplets were crowded together where they have been deposited from the surrounding cytoplasm. The goblets have variable degeneration.

4.6.3. SPLEEN

Spleen was involved in 77 per cent cases with variable intensities related to the serotypes involved, spleens were friable in consistency (75 %) and the normal mahogany color was changed to dark plum color (25 %). The capsules were over stretched and the consistency of the affected spleens were friable. Pinpoint hemorrhages, necrotic foci scattered all over the surface of the spleen (12 %). These changes were more pronounced in *Salm. pullorum, Salm. gallinarum, Salm. typhimurium Salm. remo* and *Salm. heidelberg*, while the other serotypes affected the spleen mild to moderately (Table 23). Mottling of spleens having a mixture of hemorrhages and necrotic foci were observed in almost all the cases.

Microscopically splenic capsules along with its trabeculae was thickened, edematous and detached at various sites (74 %). Splenic nodules lost their normal architect (53 %). Congestion, hemorrhages and thickened vascular endothelium were also seen in 42 per cent organs (Fig. 34). Marked proliferation of the endothelial cells of the sinus and increase in number of granulocytes at germination centers were

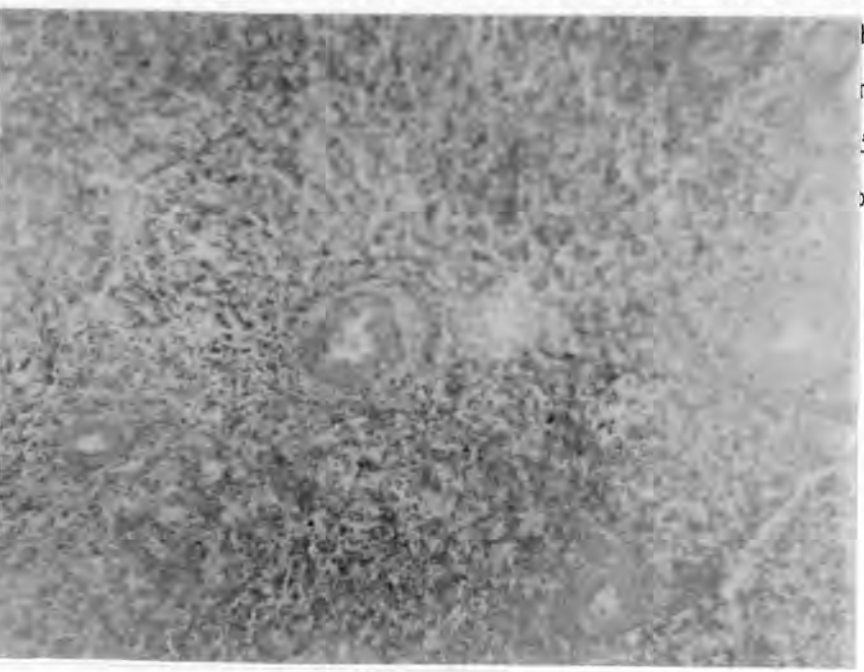
ble 23: Frequency of involvement of spleen in various Salmonella serotypes

Salmonella serotypes		GROSS PATHOLOGY				HISTOPATHOLOGY					
		dark red discolouration	hyper trophy	necrosis	friability	congestion	hemorrhages	thickening of capsule	Detachment of capsule	Thickening of endothelium	Cellular infiltration
llorum	16	+++(4) ++(12)	+++(4) ++(2)	+++(10) ++(6)	+++(5) ++(11)	+++(11) ++(5)	+++(16)	+++(16)	+++(16)	+++(16)	+++(16)
llinarum	25	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)
phimurium	10	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)
stbourne	5	++(5)	+(5)	++(5)	+(5)	++(3) +(2)	++(5)	++(3) +(2)	+(5)	++(5)	++(5)
int-paul	5	++(5)	++(5)	+(5)	++(3) +(2)	++(4) +(1)	++(5)	++(5)	++(5)	++(5)	++(5)
tantan	6	++(5) +(1)	++(6)	++(6)	++(6)	+(6)	++(6)	++(5) +(1)	+(6)	+(6)	++(6)
va	4	++(4)	+(4)	+(4)	++(4)	++(4)	++(3) +(1)	++(4)	+(4)	++(4)	++(4)
ading	3	++(2) +(1)	++(3)	++(3)	++(3)	++(3)	++(3)	+++(3) +(1)	++(3)	++(3)	+++(3)
ester	4	++(4)	+++(4)	++(4)	++(3) +(1)	+++(4)	++(3) +(1)	+++(3) +(1)	++(4)	+++(4)	+++(4)
mo	5	+++(5)	+++(1) ++(4)	++(5)	+++(5)	++(5)	++(5)	++(5)	+++(5)	+++(4) +(1)	++(5)
idelberg	7	+++(7)	+++(5) ++(2)	+++(6) ++(1)	+++(5) ++(2)	+++(5) ++(2)	++(5) +(2)	+++(5) ++(2)	+++(6) +(1)	+++(7)	+++(7)
atum	4	++(4)	++(4)	++(4)	++(4)	++(4)	++(4)	++(4)	+++(3) +(1)	++(4)	+++(3) +(1)
dar	1	++(1)	++(1)	++(1)	+(1)	+(1)	++(1)	++(1)	++(1)	++(1)	++(1)
ion	2	++(2)	+(2)	+(2)	+(2)	+(2)	++(2)	+(2)	++(2)	++(2)	++(2)
dge	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	++(1)	++(1)
gona	5	++(5)	++(5)	++(5)	++(5)	++(4)	++(5)	+(5)	++(4) +(1)	++(5)	+++(5)
ssion	3	++(3)	+(3)	+(3)	+(3)	+(3)	+(3)	+(3)	+(3)	+(3)	++(2) +(1)
ve	1	++(1)	+(1)	++(1)	+(1)	+(1)	+(1)	++(1)	+(1)	+(1)	++(1)
ra.typhi A.	1	++(1)	++(1)	++(1)	++(1)	++(1)	++(1)	++(1)	+(1)	++(1)	+++(1)

++ = Severe
++ = Moderate
+ = Mild

the common lesions. Hyperplastic splenitis irrespective of the age and serotypes involved was observed. Degeneration or deorganization of primary follicles, severe hemorrhages in the medullary region were also seen. Infiltration of lymphocytes along with abnormal number of mononuclear cells were observed infiltrating in the splenic tissues. Blood vessels of splenic nodules were engorged with blood in 48 per cent cases and thickening of the walls in 37 per cent cases (Fig. 35).

On cellular level spleen showed proliferation of reticular cells along with acentric nuclei with necrobiotic changes. Cytoplasm increased translucent tendency. Proliferating reticular cells have cell membrane irregularities. Fragmentation of nuclear material along with degenerated organellae were the common features in almost all the cases with variable intensity. Lymphocytic invasion was in abundant especially lymphocyte T. Splenic cells showed variable degree of degeneration along with necrotic cells. On majority cases regions of cell degeneration which varied from nuclear vacuolation and margination of the heterolucent to karyorrhexis. In addition, there was a high incidence of



he numerous heterophils
nality. The cytoplasmic
iable size. Cytoplasmic
bserved.

4.6.4. LUNGS

Macroscopically in many cases, the lungs appeared normal in size, shape, color and consistency. However, in 53 per cent affected birds the lungs were congested and pneumonic changes along with necrotic foci were recorded. Among the affected lungs, caseous nodules and catarrhal bronchitis were the most common changes in *Salm. pullorum, Salm. gallinarum Salm. typhimurium, Salm. reading, Salm. chester* and *Salm. heidelberg* infection. Miliary foci were rare in lungs with other serotypes, while pullorum nodules were after on margin with *Salm. pullorum* in young chicks (Table 24).

Hemorrhages and/or lympho histiocytic bronchopneumonia accompanied by hyperplastic nodular foci in chicken infected with different serovars of salmonellae was observed. There were areas of hemorrhages in 72 per cent lungs. Small capillaries and blood vessels were engorged with blood and RBCs were also seen in the intra-alveolar areas (Fig. 36). Focal areas of fibrosis along with extensive connective tissue proliferation and pneumonic changes were also seen in 54 per cent lungs. In some zones, the alveoli were collapsed showing atelectasis, whereas alveoli in other zones were dilated showing compensatory emphysema. These were atelectasis of the alveolar septa with hemorrhages and infiltration of inflammatory cells. Excessive extravascular congestion in the form of hemorrhagic areas was observed in 51 per cent cases. Mono nuclear cell infiltration was also seen in 68 per cent lungs. Bronchiole had epithelial necrosis surrounded by hemorrhages, catarrhal bronchitis and nodular lymphocytic

Table 24: Frequency of involvement of lungs in various Salmonella serotypes

Salmonella serotypes		GROSS PATHOLOGY				HISTO-PATHOLOGY				
		pneumonia	hepati-zation	congestion	enlargement	fibrosis	atelectasis	emphysema	nodules formation	necrosis
pullorum	16	+++(10) ++(6)	+++(10) ++(6)	+++(16)	+(16)	+++(6) ++(10)	+++(6) ++(10)	++(16)	+++(2) ++(4)	+++(10) ++(6)
gallinarum	25	+++(20) ++(5)	+++(10) ++(15)	+++(25)	+(25)	++(15) +++(10)	++(25)	++(25)	-	++(25)
typhimurium	10	+++(10)	+++(10)	+++(10)	+(10)	++(5) +++(5)	++(5) +(5)	++(5) +(5)	-	++(10)
eastbourne	5	++(5)	++(5)	++(5)	+(5)	++(5)	++(5)	++(5)	-	++(5)
saint-paul	5	++(5)	++(5)	++(4) +(1)	+(5)	++(5)	++(5)	++(5)	-	+(1) ++(4)
butantan	6	++(6)	++(6)	++(6)	+(6)	++(6)	++(6)	++(6)	-	++(6)
java	4	+(4)	+(4)	++(4)	+(4)	+(4)	+(4)	+(4)	-	+(4)
reading	3	++(1) +++(2)	++(3)	++(3)	+(3)	++(3)	++(3)	++(3)	-	++(3)
chester	4	++(2) +++(2)	++(4)	++(4)	+(4)	++(4)	++(4)	++(4)	-	++(4)
remo	5	++(5)	++(5)	++(5)	+(5)	++(5)	++(5)	++(5)	-	++(5)
heidelberg	7	+++(4) ++(3)	++(4) +++(3)	++(7)	+(7)	++(3) +++(4)	++(7)	++(7)	-	++(3) +++(4)
anatum	4	++(4)	++(4)	++(4)	+(4)	++(2) +++(2)	++(4)	++(4)	-	++(4)
hadar	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	-	++(1)
orion	2	++(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	-	+(2)
ridge	1	+(1)	++(1)	+(1)	+(1)	+(1)	+(1)	+(1)	-	+(1)
agona	5	++(5)	++(5)	++(5)	+(5)	++(5)	++(5)	++(5)	-	++(5)
mission	3	+(3)	+(3)	+(3)	+(3)	+(3)	++(3)	+(3)	-	+(3)
give	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	-	+(1)
para-typhi-A	1	++(1)	++(1)	++(1)	+(1)	+(1)	+(1)	+(1)	-	+(1)

+++ = Severe.
++ = Moderate
+ = Mild

Table 25: Frequency of involvement of kidneys in various Salmonella serotypes.

Salmonella serotypes	Total Positive	GROSS PATHOLOGY				HISTOPATHOLOGY					
		Dis-colouration	Enlargment	Friability	Hemorrhages	congestion	Hypremia	Cloudy swelling	Tubular degenration	Necrosis	Cellular infiltration
pullorum	16	+++(16)	+++(16)	+++(14) ++(2)	+++(16)	+++(14) ++(2)	+++(14) +(2)	+++(16)	+++(16)	+++(16)	+++(66)
gallinarum	25	+++(25)	+++(20) ++(5)	+++(18) ++(7)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)
typhimurium	10	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)
eastbourne	5	++(5)	++(5)	+++(5)	+++(5)	+++(5)	+++(5)	++(5)	++(5)	++(5)	++(5)
saint-paul	5	++(5)	++(5)	++(5)	+++(3) ++(2)	++(5)	++(5)	++(5)	++(5)	++(5)	+++(3) ++(2)
butantan	6	++(6)	++(6)	++(6)	+++(4) ++(2)	+++(4) ++(2)	+++(2) ++(4)	++(6)	++(6)	++(6)	+++(2) ++(4)
java	4	+(4)	++(4)	+(4)	+(4)	+(4)	+(4)	+(4)	+(4)	++(2) +(2)	+++(1) +(3)
reading	3	++(3)	+++(2) ++(1)	++(2) +(1)	+++(1) ++(2)	+++(1) ++(2)	++(2) +(1)	+++(3)	+++(3)	+++(3)	+++(3)
chester	4	++(4)	+++(2) ++(2)	++(2) +(2)	+++(3) +(1)	++(3) +(1)	+++(3) +(1)	+++(2) +(2)	+++(2) ++(2)	+++(2) ++(2)	+++(3) +(1)
remo	5	+++(3) ++(2)	+++(3) ++(2)	+++(4) ++(1)	+++(3) +(2)	++(5)	+++(2) +(3)	++(5)	+++(3) ++(2)	++(5)	+++(3) ++(2)
heidelberg	7	+++(5) ++(2)	+++(3) ++(4)	++(7)	++(7)	+++(3) ++(4)	+++(4) ++(3)	+++(5) ++(2)	++(7)	+++(5) ++(2)	+++(2) ++(5)
anatum	4	+++(4)	++(4)	++(4)	++(4)	++(4)	++(4)	++(4)	++(2)	++(4)	+++(1)
hadar	1	++(1)	+(1)	+(1)	+(1)	+(1)	++(1)	++(1)	++(1)	++(1)	+++(1)
orior	2	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	++(2)
ridge	1	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	++(2)	++(2)
agona	5	++(5)	++(5)	++(5)	++(5)	++(5)	++(5)	+++(2) ++(3)	+++(3) ++(2)	++(5)	+++(4) ++(1)
mission	3	++(3)	+(3)	+(3)	++(3)	++(3)	++(3)	++(3)	+(3)	+(3)	+++(1) ++(2)
give	1	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	++(1)
P.typhi-A	1	++(1)	++(1)	++(1)	++(1)	++(1)	++(1)	++(1)	+++(1)	++(1)	+++(1)

+++ = Sever
++ = moderate
+ = Mild

Fig. 36. Lung from Salmonella infected bird having hemorrhage into tertiary bronchus, atria and air capillaries (H) and atrial air capillaries containing mononuclear inflammatory cells (a) and edema fluid (F); note that some inflammatory cells are shrunken and have pyknotic nuclei (N). H&E 400 X.

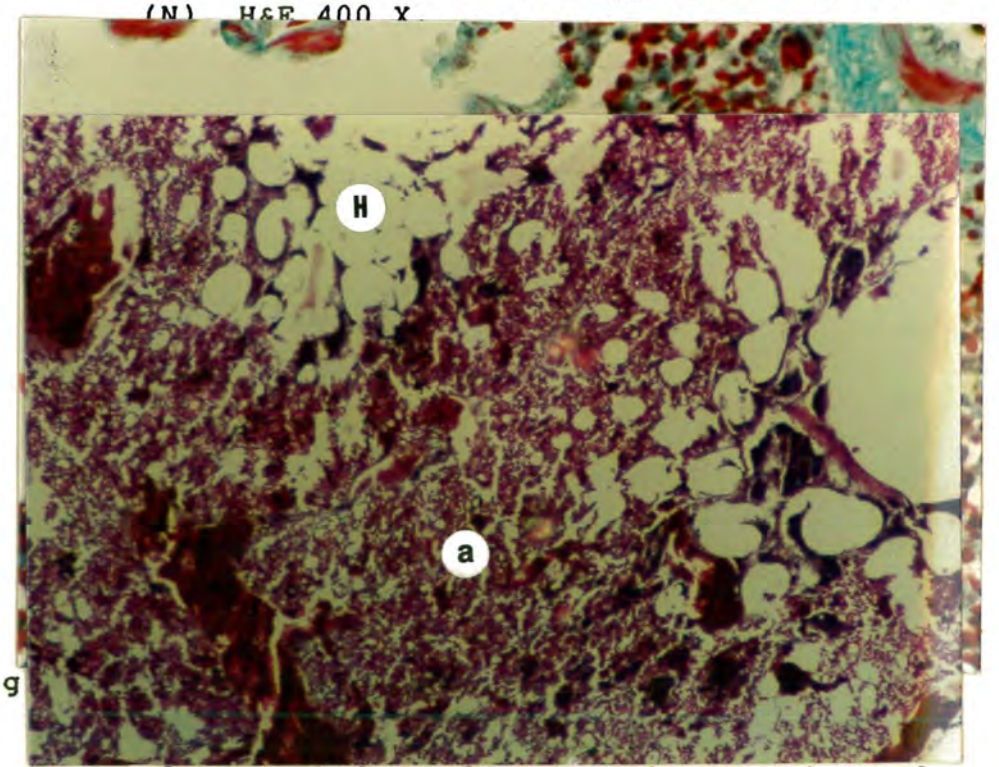


Fig
degenerated and necrotic (▲) and congestion H&E 400 X.

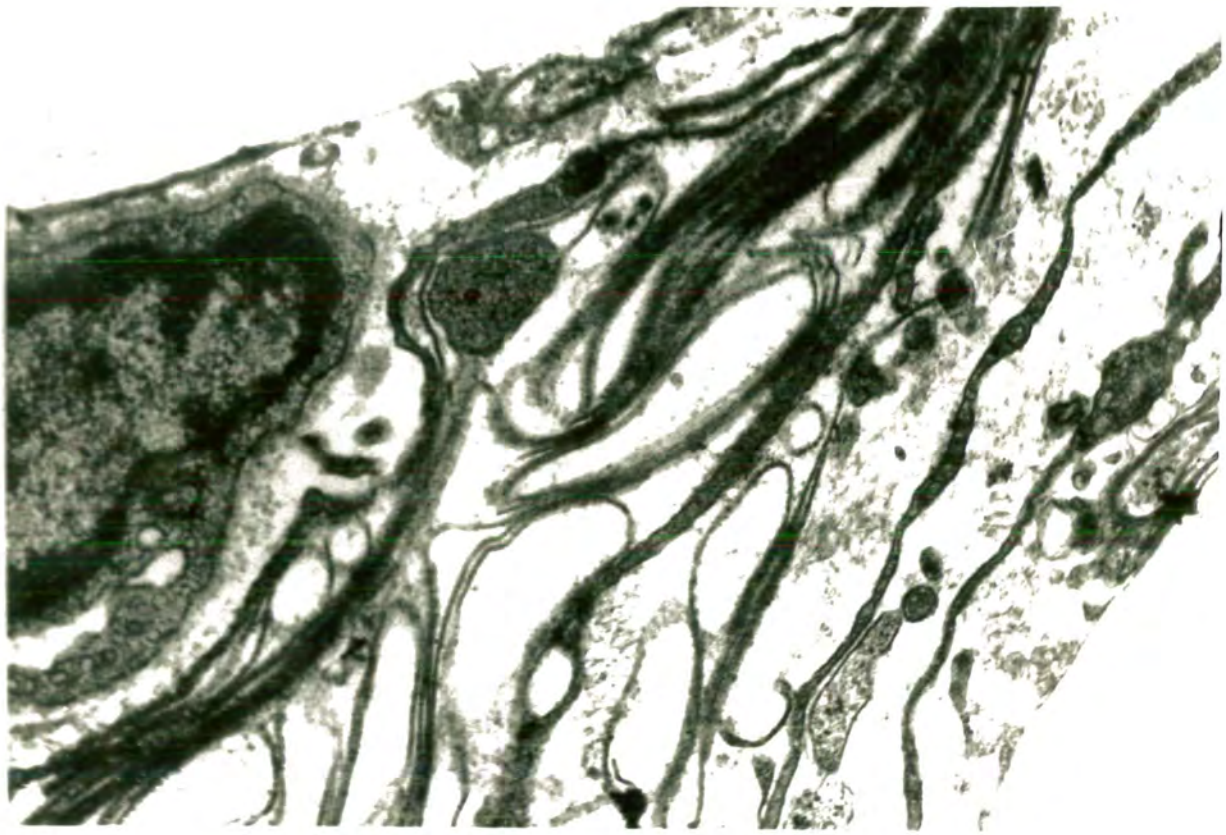


Fig. 39. Lung showing connective tissue proliferation with intercellular edema X 23000.

well as tubules showed very marked cloudy swelling. In many (65 %) cases tubular epithelium showed parenchymatous degeneration leading to necrosis. Blood vessels were engorged with blood in some areas along with cellular infiltration. Among the total kidneys affected with salmonellae, tubules were swollen along with cellular infiltration were seen in 57 per cent cases (Fig. 42). Congestion, fatty degeneration and edematous fluid was also seen in 54 per cent organ (Fig. 43).

Ultrastructurally, tubular epithelial cells were necrotic or in various stages of degeneration. The mitochondria were misshapen, their cristae were widely separated initially, and in later stages degenerated into a uniform homogeneous mass (Fig. 44,45). RER appeared to be a primary target of degeneration. Those RER still intact had scanty ribosomes, but many ribosomes were aggregated in the cytoplasm. Tubular lumens were narrowed but the brush borders were intact. Some of the tubular cells were so enlarged by hydropic degeneration that their membranes were hardly discernable and several cells appeared as a single cell. Glomerular basement membrane podocyte foot processes were detached, thickened, misshapen and elongated. Bowman's spaces were widened (Fig. 46,47).

4.6.6. OVARIES

Ovaries were affected in 81 per cent carrier laying birds. The most characteristic changes seen in the positive ovaries were flaccid, discolored and misshapen ova. Many of the misshapen ova when cut showed cheesy material with

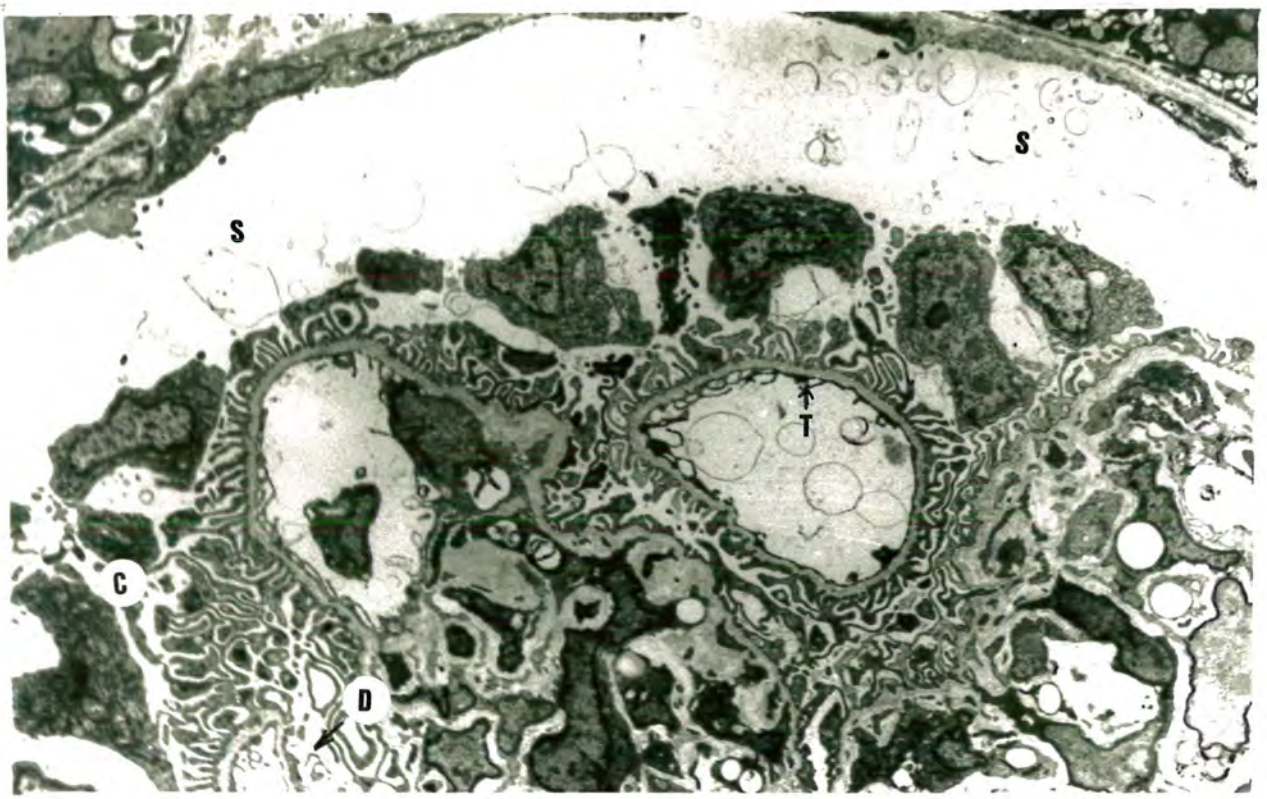


Fig. 45. Glomerular tuft from Salmonella infected bird having widened Bowman's space (S) and detached (D), thickened (T), elongated (C) and misshapen podocyte foot processes. 4940 X.

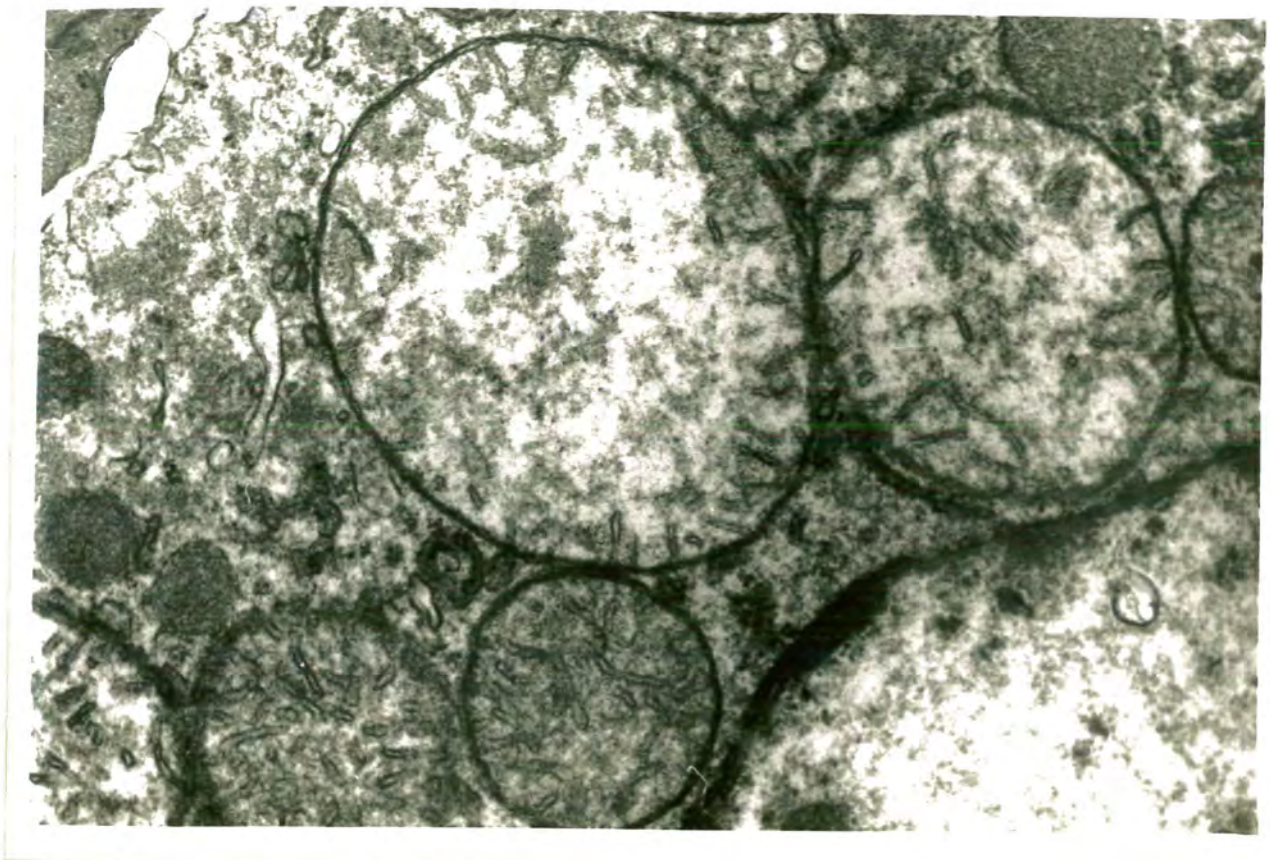


Fig. 47. Renocytes showing hydropic degeneration and mitochondrial destruction 28000 X.

blackish tinge to tar color. Ovarian follicles were attached to the ovary with pedunculated stalk. These all changes were common with all the serotypes with variable intensity except *Salm. Java, Salm. ridge, Salm. mission* and *Salm. give* which produced slight congestion and hemorrhages in the yolks of ova attached to the ovary. However, changes and pathological conditions were not age dependent (Table 26).

Microscopically capsules of ovaries were thickened due to infiltration of chronic inflammatory cells. The cortex contained some nor mal and atretic follicles at different places and were also infiltrated with lymphocytes. The cortical tissue showed evidence of congestion, the follicles were distorted and blood vessels were thickened due to proliferation of tunica media and tunica adventitia. The medullary areas showed excessive proliferation of connective tissue and cellular infiltration, particularly with lymphocytes.

4.6.7. HEART

Grossly heart was the target organ in only 33 per cent cases. Affected hearts were congested and slightly enlarged. Excessive amount of fat was observed in between epicardium and myocardium extending from the coronary grove towards the apex. A tiny areas of ecchymosis were seen in the auricles, while coronary arteries appeared to be normal. Grayish white foci in myocardium were observed less frequently. In few cases, serous fluid in the pericardial sac and hemorrhages in the subpericardium were seen. Necrotic nodules in myocardium were

Table 26: Frequency of involvement of ovaries in various *Salmonella* serotypes.

Salmonella Serotypes	Total isolates	Gross Pathology			Histopathology		
		Mis-shapen ova	Discolou-ration of ova	Pedunculation of ova	Ateratic follicle	Fibro-blastic proliferation	Cellular infiltration
pullorum	16	+++(16)	+++(16)	+++(16)	+++(16)	+++(16)	+++(16)
gallinarum	25	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)	+++(25)
typhimurium	10	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)	+++(10)
eastbourne	5	++(5)	++(5)	++(5)	++(5)	++(5)	+++(5)
saint-paul	5	++(5)	++(5)	++(5)	++(5)	++(5)	+++(5)
butantan	6	++(6)	++(6)	++(6)	++(6)	++(6)	+++(6)
java	4	+(4)	+(4)	+(4)	+(4)	+(4)	+++(2) ++ (2)
reading	3	+++(1) ++(2)	++(3)	++(2) +(1)	+++(1) ++(2)	++(1) +(2)	+++(2) ++(2)
chester	4	+++(3) ++(1)	++(3) +(1)	++(3) +(1)	++(4)	++(4)	+++(4)
remo	5	+++(1) +(4)	++(4) +(1)	++(4) +(1)	+++(1) +(4)	++(2) +(3)	+++(1) ++(4)
heidelberg	7	+++(2) ++(5)	++(7)	++(7)	+++(1) ++(6)	+++(1) ++(6)	+++(1) +(6)
anatum	4	++(4)	++(4)	++(2) +(2)	++(2) +(2)	++(2) +(2)	+++(1) +(3)
hadar	1	++(1)	+(1)	+(1)	+(1)	+(1)	++(1)
orion	2	++(2)	+(2)	+(2)	+(2)	+(2)	++(2)
ridge	1	+(1)	+(1)	+(1)	+(1)	+(1)	++(1)
agona	5	++(3) + (2)	++(5)	++(5)	++(5)	++(5)	+++(1) ++(4)
mission	3	+ (3)	+(3)	+(3)	+(3)	+(3)	+++(1) ++(2)
give	1	+(1)	+(1)	+(1)	+(1)	+(1)	++(1)
para typhi A.	1	++(1)	++(1)	++(1)	++(1)	++(1)	+++(1)

+++ = Severe
++ = Moderate
+ = Mild

also seen in *Salm. typhimurium, Salm. gallinarum* and *Salm. pullorum* infection.

Histopathologically myocardial fibers showed kinky pattern along with severe hemorrhages throughout the heart muscles (Fig. 48). Subpericardial hemorrhages were observed frequently. Focal areas of necrosis leading to large areas of coagulative necrosis. Grayish white miliary foci in myocardium along with pericarditis. Acidophilic necrosis of Zenker's type were mostly encountered (Fig. 49). Some cases showed edema and infiltration of mononuclear leukocytes in between the cardiac muscle fibers. There was also thickening of the blood vessels (Fig. 50,51).

Ultrastructural changes included extensive fragmentation of muscle fibers, destruction of the Z, H and I bands along with a haphazard mixture of degenerated mitochondria and myofibril (Fig. 52). The Z bands were changed to a wavy pattern. There was detachment and dissolution of myofibril. RER and mitochondrial changes were the same as described above. Intercellular junctions had a wavy pattern and frequent loss of desmosomal attachments (Fig. 53).

4.6.8. CECA

Grossly typhlitis along with lumen filled with brownish oily feces were the common findings in all the affected ceca.

Microscopically ceca among 108 bacteriological positive birds were affected in 91 per cent cases, irrespective of the

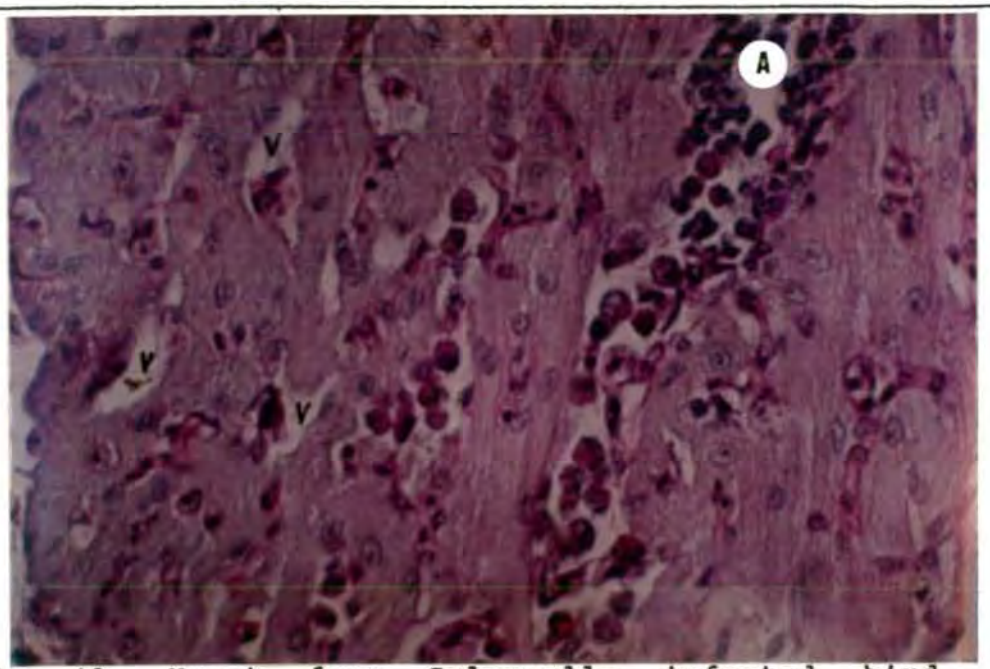


Fig. 48. Heart from Salmonella infected bird having myofiber vacuolation (V) disorientation, fragmentation and infiltrate of heterophils, macrophages and lymphocytes (A) H&E 400 X.

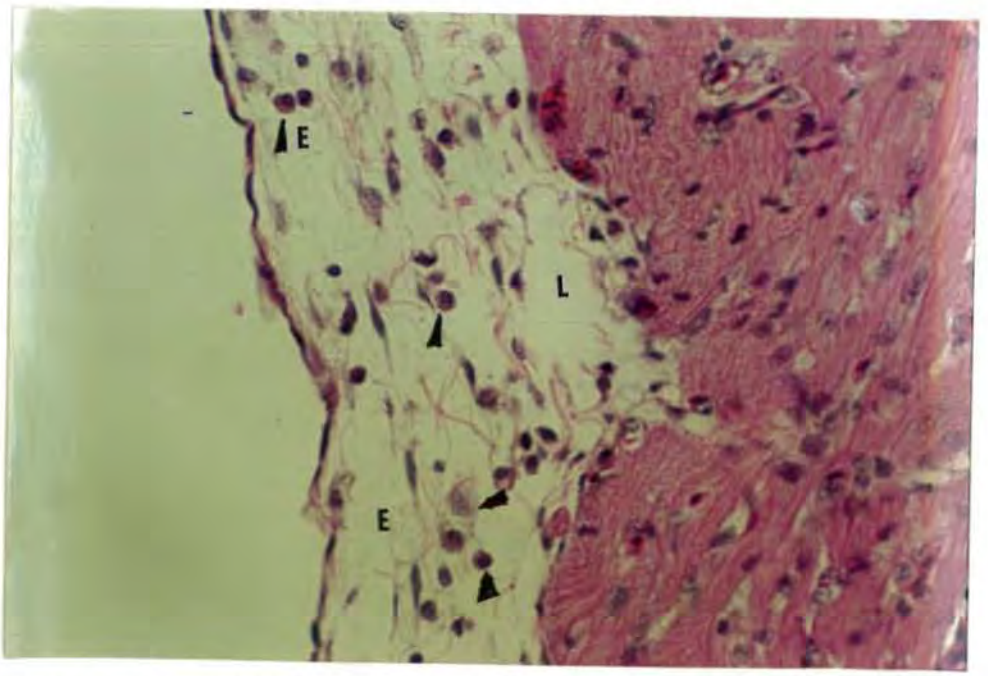


Fig. 49. Heart from Salmonella infected birds having epicardium thickened by edema fluid (E) and containing dilated lymphatic channels (L), macrophages and lymphocytes; the myocardium contains foci of heterophils and macrophages (▲) H&E 400 X.

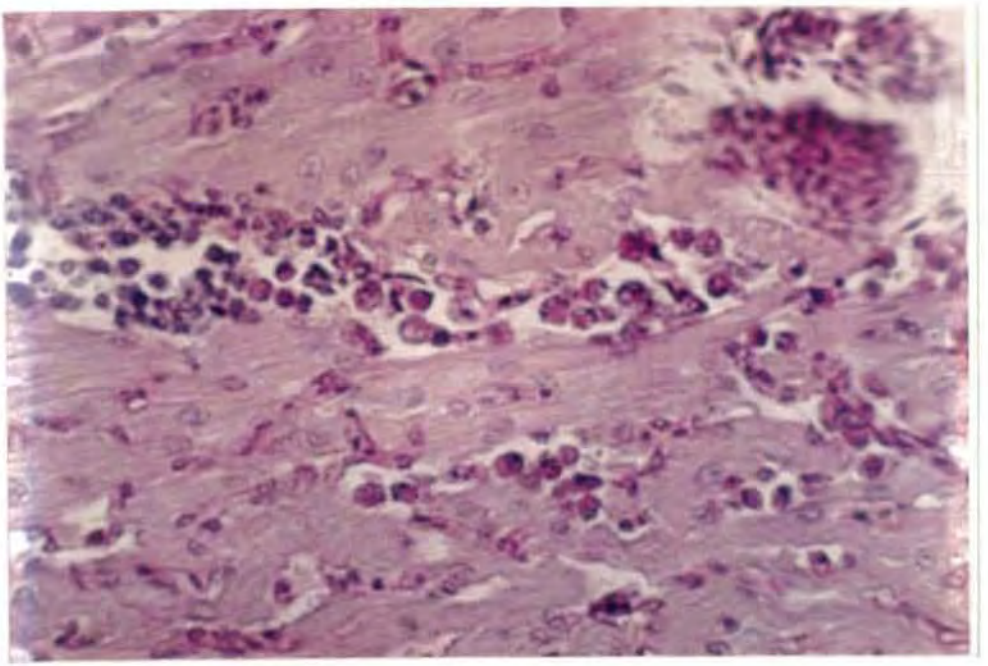


Fig. 50 Heart muscles showing heavy degenerative changes with leukocytic infiltration. H&E 400 X.

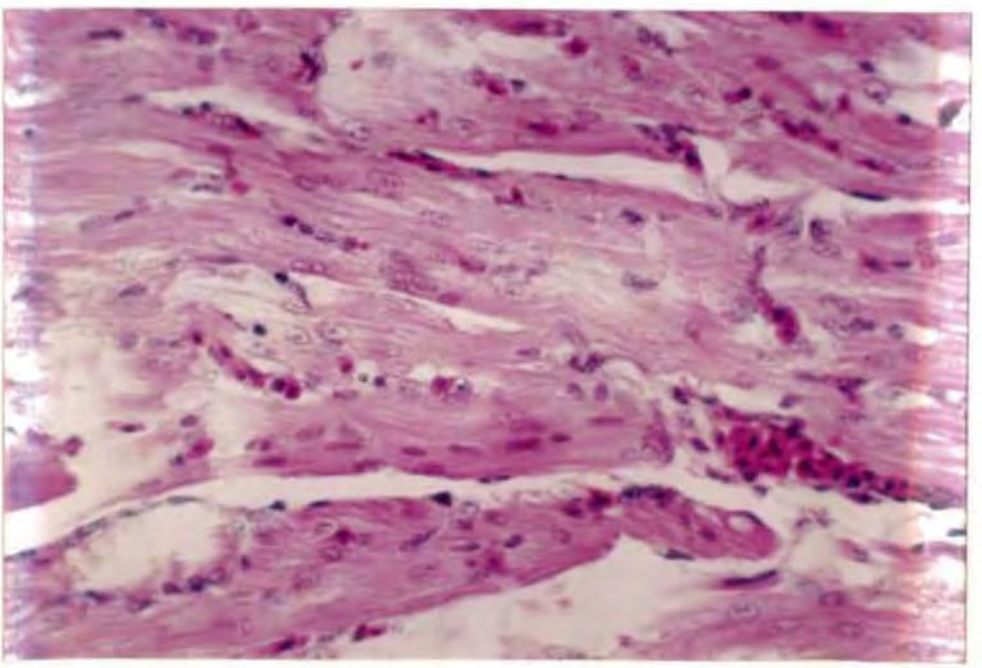


Fig. 51. Necrosed area alongwith leucocytic infilatration H&E 400 X.

Fig. 52. Heart muscles showing fragmentation alongwith leukocytic infiltration 12500 X.

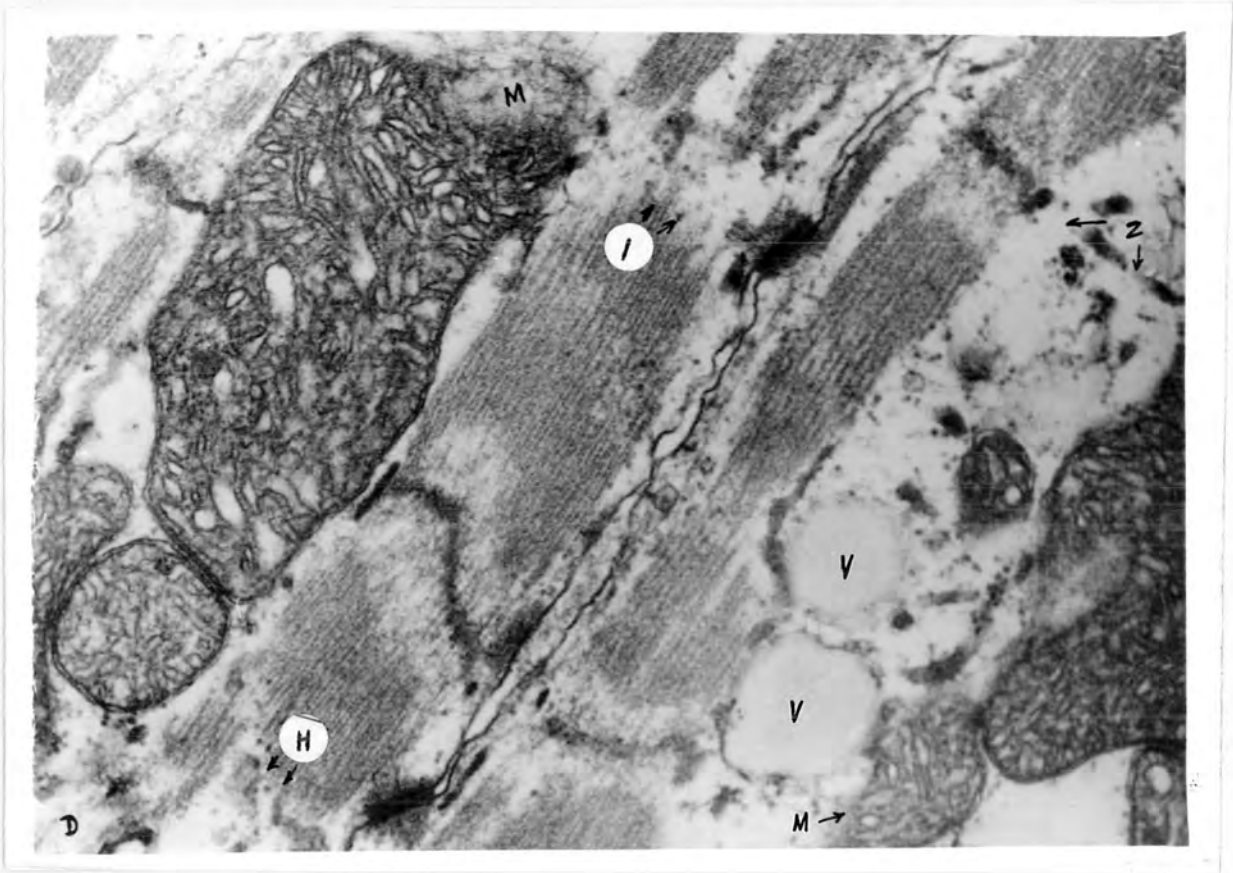


Fig. 53. Heart from Salmonella infected bird having detachment and dissolution of myofibrils (D) with vacuolation of sarcoplasm (V), destruction of Z, I and H bands; and mitochondrial swelling and membrane dissolution (M). 38000 X.

strain involved. Lesions were recorded in almost all the cases. *Salm. gallinarum, Salm. pullorum, Salm. typhimurium* and *Salm. heidelberg* affected most adversely. Eroded surface of cecal lumen, necrotic foci, zonal necrosis and hemorrhages were the salient gross pathological lesions.

4.6.9. BRAIN

Grossly the brain invariably showed changes in color and consistency. The cerebrum had extensive edema, hemorrhages and an increased presence of astrocytes and oligodendroglia cells around neuronal cell bodies (satellitosis). The cerebellum contained increased numbers of microglial and gitter cells in the granular layer. There was also multifocal purkinje cell degeneration (Fig. 54,55).

Ultrastructurally, there was cytovacuolation and intracellular hydropic degeneration. Mitochondrial and RER changes were prominent in neuronal cells (Fig. 56,57).

4.6.10. BURSA OF FIBRICIUS

Bursa of Fabricius in *Salmonella* effected birds contained thick white, grumous material along with hard consistency. There was severe depletion and necrosis of lymphocytes in the medulla. The cortical mantles were thickened by connective tissue, resulting in the compaction of parenchymal cells, and lymphoid follicles were small and variable in size (Fig. 58).

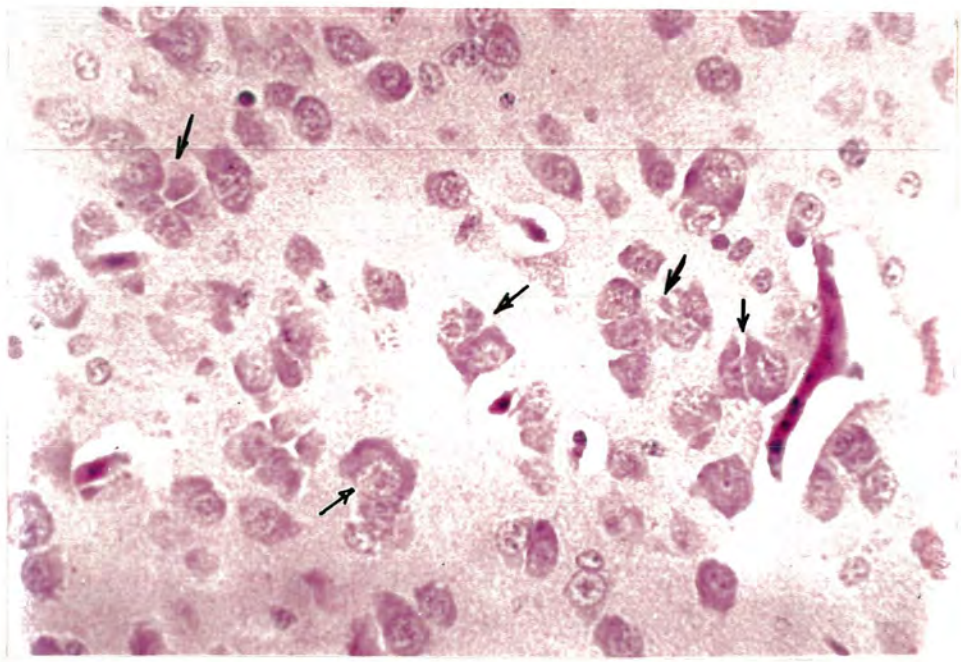


Fig. 54. Brain from Salmonella infected bird having satellitosis (↑). H&E 400 X.

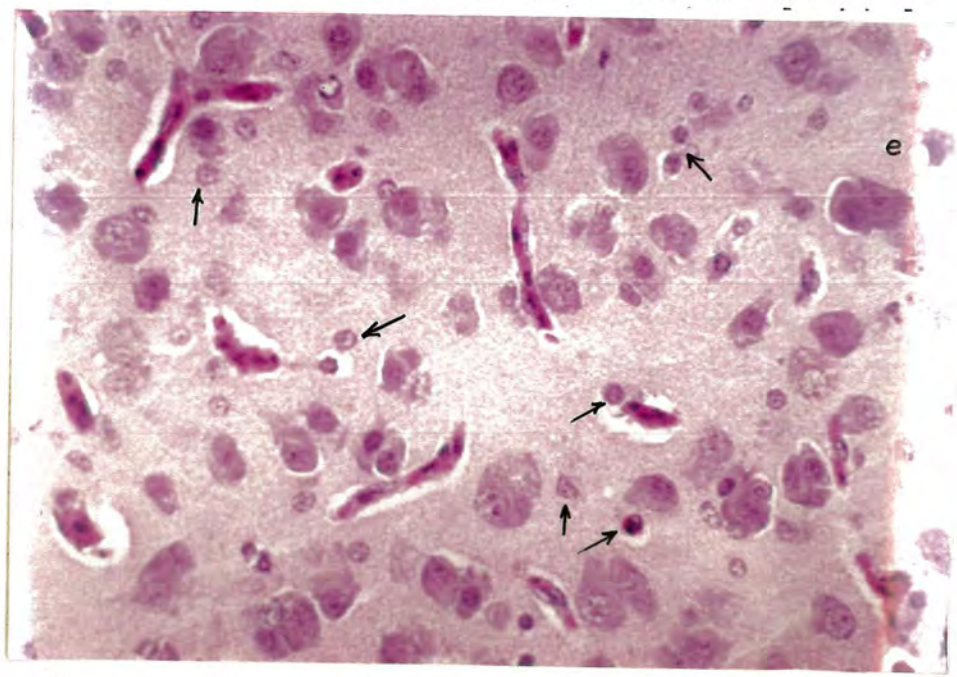


Fig. 55. Brain from Salmonella infected bird having neuronal degeneration, reduced cellularity, astrocyte proliferation (↑) and cytovacuolation (e). H&E 400 X.

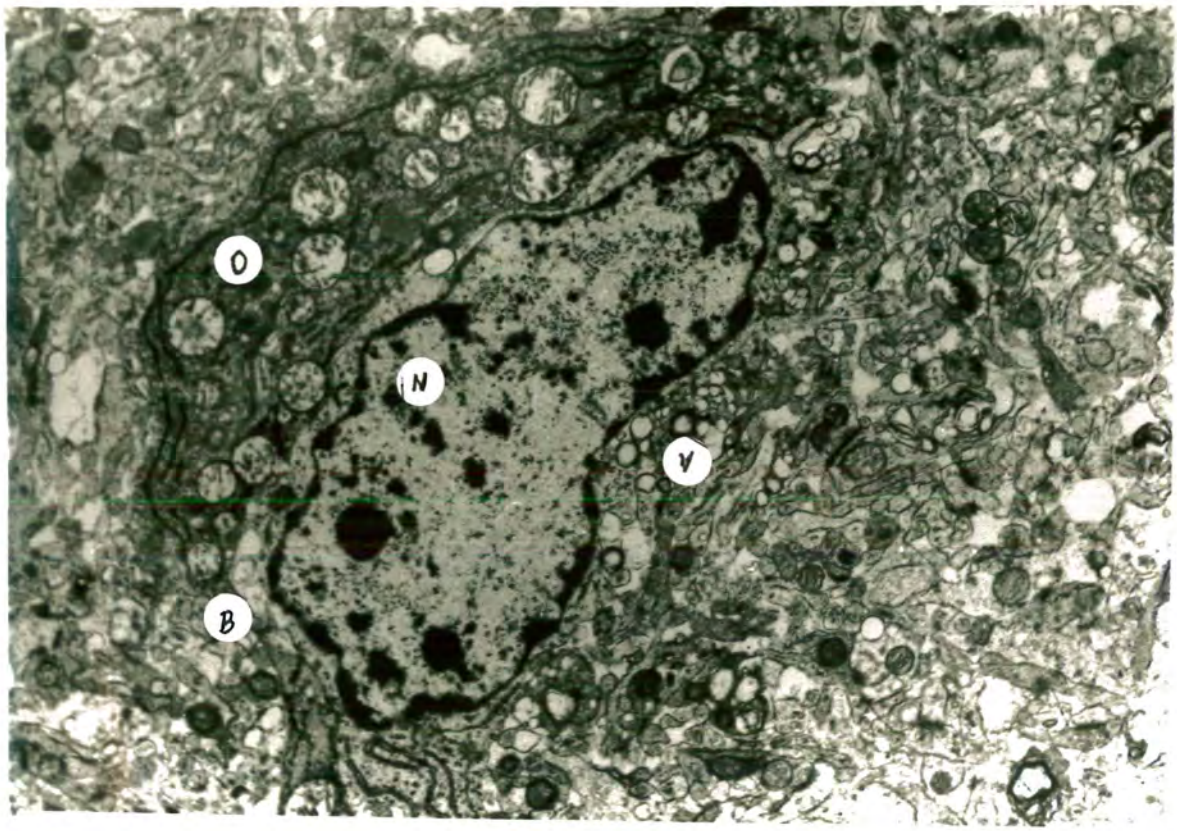


Fig. 56. Brain from Salmonella infected bird having satellitosis (oligodendrocyte (O) adjacent to neuronal cell, cytoplasm (B) and nucleus (N): note cytovacuolation (V), and mytochrondrial degeneration. 9500 X.

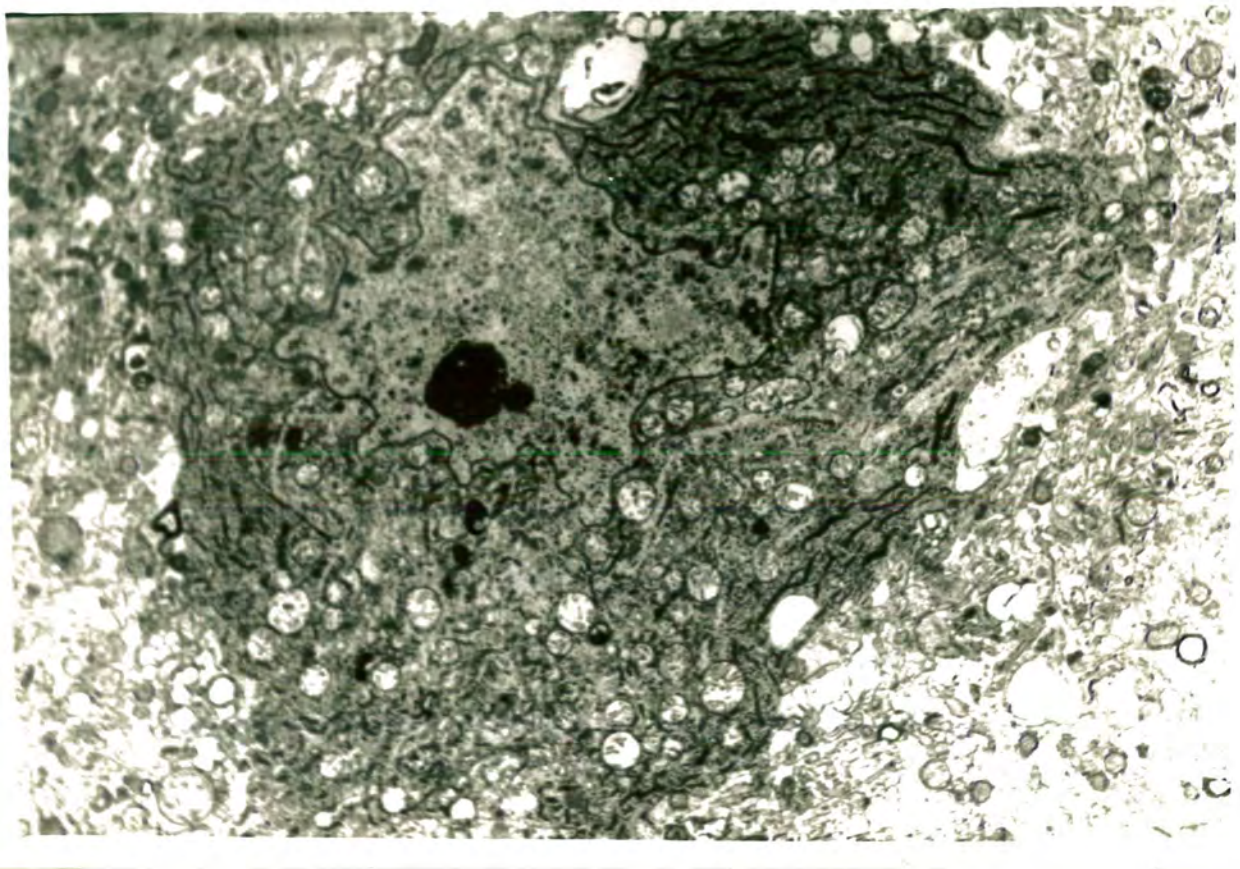


Fig. 57. Brain cells showing satellitosis with diffusing neucleous 9500 X.

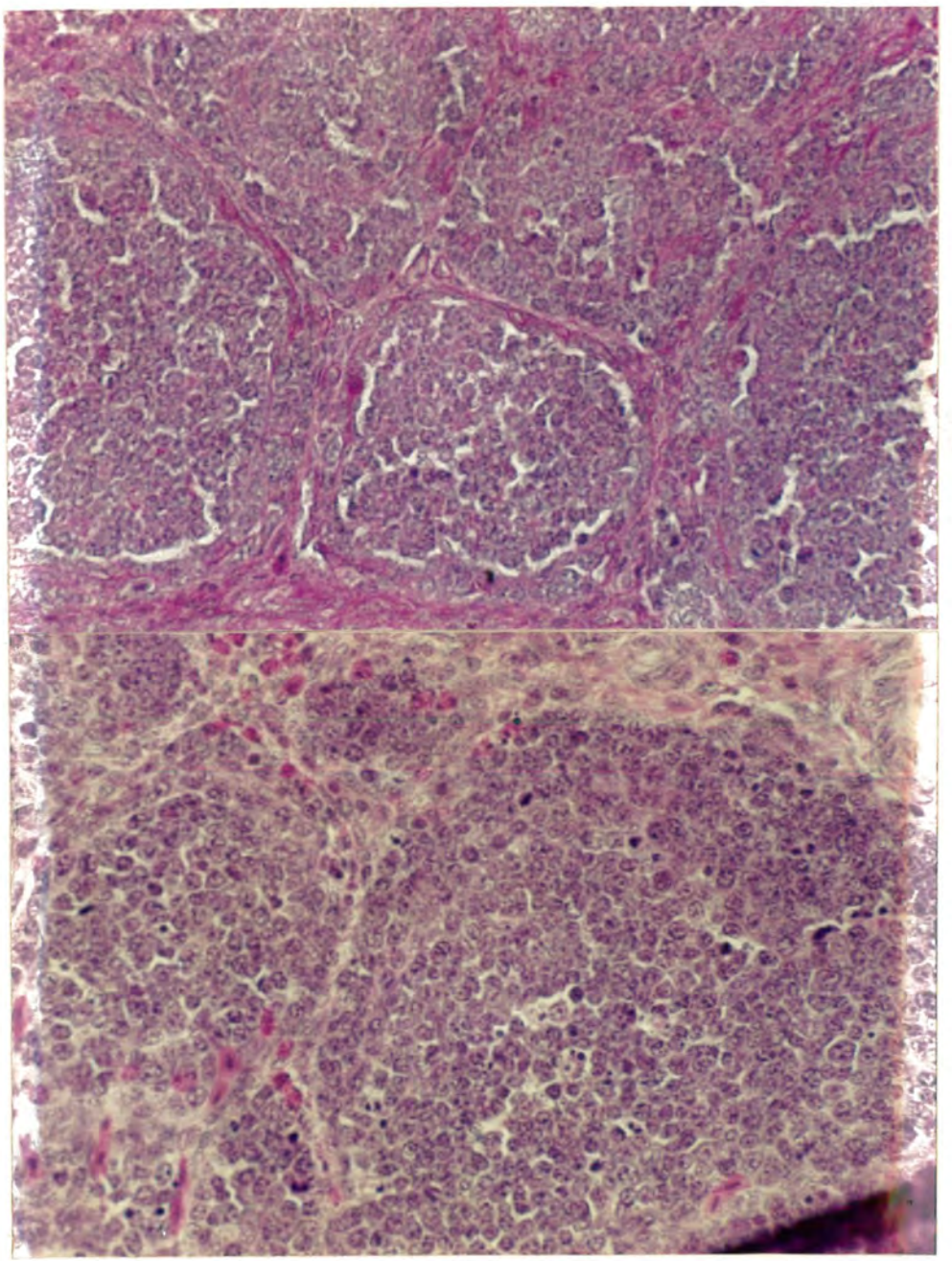


Fig. 58. Bursa of Fabricus showing connective tissue proliferation and necrosis of lymphocytes H&E 400 X.

Ultrastructurally, lymphocytes had cytoplasmic and nuclear enlargement. There were thickened and connective tissue proliferated in the cortical mantles resulting in the compaction of the neighboring cells to change their morphology. Cytoplasmic vacuolation along with nuclear fragmentation was in abundance. Variable sizes of lymphocytes were noted. Cytoplasmic membrane morphology was inconsistent with variable fragmentation and foldings. Cellular intensity decreased while connective tissue proliferation was in abundant.

4.7. EXPERIMENTAL STUDIES

4.7.1. ENTEROPATHOGENICITY AND ENTEROTOXIGENICITY

Salmonella serotypes were proven to be invariably enteropathogenic and enterotoxigenic (Table 27). Congo red binding and rabbit ileal loop system showed 100 per cent serotypes positive for enteropathogenicity, while ileal loop system and skin toxigenicity was variable ranging from 20-100 per cent (Table 27,28).

The occurrence of gross and microscopic lesions in the rabbit ileal loops exposed to enteropathogenic and enterotoxigenic *Salmonella* serotypes were studied. The most severe lesions were produced after 24 hours, the loops were distended and filled with an abundant fluid, containing mucus and blood (Table 28). Varying degree of histopathological alterations were demonstrated in the intestine were congestion and edematous fluid was the consistent findings. The enterotoxigenic serotypes produce degenerative and

Table 27: Comparison of Pathogenicity and Enterotoxigenicity of Salmonella serotypes.

Salmonella serotypes	Tested	PATHOGENICITY		Toxigenicity on skin
		Chemically*	Biologically**	
pullorum	10	10 +	10 +	5 +
gallinarum	10	10 +	10 +	10 +
typhimurium	10	10 +	10 +	10 +
eastbourne	5	5 +	5 +	3 +
saint paul	5	5 +	5 +	5 +
butantan	5	5 +	5 +	4 +
jave	5	5 +	5 +	2 +
reading	5	5 +	5 +	3 +
chester	5	5 +	5 +	2 +
remo	5	5 +	5 +	3 +
heidelberg	5	5 +	5 +	1 +
anatum	5	5 +	5 +	3 +
hadar	5	5 +	5 +	4 +
orion	5	5 +	5 +	2 +
ridge	5	5 +	5 +	1 +
agona	5	5 +	5 +	4 +
mission	5	5 +	5 +	2 +
give	5	5 +	5 +	1 +
para typhi. A	5	5 +	5 +	3 +

* = Positive (+) or negative (-) for congo red binding

** = Positive (+) in lesion production in rabbit ileal loop

Table 28: Comparative Pathogenicity of Salmonella serotypes by Chemical and Rabbit Ileal Loop Inoculation.

Salmonella serotypes	Tested	Congo red binding*	Ileal Loop Pathogenicity(a)
pullorum	10	+	++++
gallinarum	10	+	++++
typhimurium	10	+	++++
eastbourne	5	+	+++
saint paul	5	+	+++
butantan	5	+	++++
jave	5	+	++
reading	5	+	++
chester	5	+	++
remo	5	+	++
heidelberg	5	+	++++
anatum	5	+	+++
hadar	5	+	++
orion	5	+	+++
ridge	5	+	+++
agona	5	+	++
mission	5	+	++++
give	5	+	++
para typhi. A	5	+	++++

* = Positive (+) or negative (-) for cong red binding.

a = Intensity of pathology, mild (+), moderate (++), severe (+++) and extremely severe (++++)

inflammatory changes but no hemorrhages was observed in all the cases of enterotoxigenic serotypes inoculated in rabbit ileal loops.

4.7.2. EGG SHELL PENETRATION

To investigate the extend of *Salmonella* penetration through the egg shell membrane, eggs were dipped in red(group A) and green (group B) aqueous bland food color solutions for the detection of positive penetration areas. In group A (20) eggs were soaked in the red solution for 3 minutes where as same number of eggs in group B were dipped in green color for 6 minutes. Highly significant ($P< 0.01$) green spots were detected with candler as compared to red spots in group A after 3 minutes exposure. The long exposure of the colored solutions have more tendency to expose the positive penetration point. The higher spot number have direct strong correlation with the bacterial penetration. Those points developed after 3 minutes exposure had more potential points of easy penetration as compared to all the points developed after 6 minutes exposure. Food color penetration through the shells detect open areas in the shell surface of chicken eggs. The surface of the eggs give a true reflection of penetration points. This method is good non-microbiological marker for the confirmation of its potential to microbial invasion.

Penetration patterns of tested salmonellae through the outer structures of the eggs. Among total 190 eggs (950 points) maximum (30.00 %) penetration was in area III, where salmonellae invaded through cuticle, shell, inner and outer

shell membranes followed by area II (14.77 %) and area I (4.60 %). It was very well evident that penetration of salmonellae to the contents of eggs was maximum, while in area II the penetration was upto outer shell membrane and in least cases through the cuticle and shell. Penetration in area I is not significant and to some extent in area II as well, while invasion in the are III is highly significant as the high (30.00 %) number of isolation was undertaken in area III. Most recently egg architecture needs lot consideration to prevent the pseudocontamination of the pathogens.

Motile salmonellae have quite bit higher tendency of penetration deep into egg contents through shell as *Salm. typhimurium* and *Salm. hadar* penetrated in 28 per cent points in area III. Among other motile serotypes Salm. reading penetrated through 22 per cent shells to area III followed by *Salm. anatum* (20 %), *Salm. remo* and *Salm. agona* (18 %), *Salm. chester* and *Salm. mission* (16 %) and *Salm. heidelberg* (14 %). Non-motile salmonellae were poorly penetrated through area I, II, and III as *Salm. gallinarum* and *Salm. pullorum* have only 2 per cent penetration intensity (Table 29).

4.7.3. EMBRYO INOCULATION

One hundred and ninety fertile eggs (Hubbard) were assigned to 19 groups of 10 eggs each inoculated on day 2nd of incubation with one serotype of each selected from 19 serotypes isolated. Gross pathological changes were identical in almost all the embryos exposed to various serotypes of salmonellae. Hemorrhages were noted on the surface of feed,

Table 29: Percentage of *Salmonella* penetration in various areas of the egg.

Salmonellae tested	No. of isolates/egg	Mean per cent of penetration into Area I (Cuticle & Shell)	Area II (Cuticle, shell & membrane)	Area III (Area II & inner shell membrane)
pullorum	10*	0.00	2.00	2.00
gallinarum	10	0.00	2.00	2.00
typhimurium	10	2.00	12.00	28.00
eastbourne	10	2.00	8.00	16.00
saint-paul	10	4.00	6.00	12.00
butantan	10	4.00	8.00	8.00
java	10	2.00	6.00	10.00
reading	10	4.00	10.00	22.00
chester	10	4.00	6.00	16.00
remo	10	4.00	8.00	18.00
heidelberg	10	0.00	14.00	14.00
anatum	10	2.00	4.00	20.00
hadar	10	0.00	14.00	28.00
orion	10	0.00	1.00	4.00
ridge	5	4.00	8.00	16.00
agona	10	4.00	8.00	18.00
mission	5	4.00	6.00	16.00
give	10	0.00	6.00	8.00
para typhi A.	10	2.00	4.00	12.00
Total/Average	180 900	42 (4.6%)	133 (14.77%)	278 (30.00%)

* = On each egg 5 cylinders were attached (No. of egg x No. of cylinders) were consider for percentage calculation.

legs, breast, neck and skull. Enlargement of the posterior part of the skull was also noted in all the embryos. The intensity was more pronounced in embryos exposed to *Salm. gallinarum, Salm. pullorum* and *Salm. typhimurium*. In the later stages of development embryos had incomplete closure of the umbilicus, hemorrhages in the egg membranes and yolk sacs, and increase viscosity of yolks. The yolk become a faded yellow in case of *Salm. gallinarum* and *Salm. pullorum* while blackish in case of motile salmonellae especially with *Salm. typhimurium*. The yolk become solid coagulum while the albumin lost its viscosity. The surviving chicks were weak, under developed and unable to break the shell. The toe nails and beaks of the salmonellae exposed chicks were softer than those of normal chicks. The surviving embryos that hatched had metallic or rust colored features, as compared to the normal pale or sulfur yellow color.

Gross lesions in internal organs were more pronounced with *Salm. gallinarum, Salm. pullorum, Salm. typhimurium* and *Salm. saint paul*. Liver were yellow, friable and crumbly. Kidneys were pale with focal hemorrhages. Hearts had myocardial petechiae with opaque and thickened pericardial sac. Lungs were congested, firm and plum colored. Intestines had thickened walls and severe hemorrhages in the lamina propria and muscularis mucosae. Breast muscles had whitish coloration.

Microscopically hepatocytes were in various stages of necrosis with disrupted hepatic cord pattern. Myocardial

fibrils were of variable sizes nuclei, including pyknotic forms. The renal tubular epithelium cells were intact and forms tubules along with dissociated, individualized and sloughed into lumen. These cells were recorded, irregular in shape and usually had irregular fimbriated cell margins.

4.7.4. CHICK INOCULATION

Among the various serotypes selected at random from the total isolated serotypes were inoculated intra peritoneally in day-old chicks. Acute mortalities were observed in case of *Salm. pullorum* (100 %), *Salm. gallinarum* (100 %), *Salm. typhimurium* (100 %). In case of *Salm. hadar, Salm. orion* and *Salm. agona* mortality was 100 per cent but pattern was not acute. Death time range was 4 to 5 days in case of *Salm. gallinarum, Salm. pullorum, Salm. typhimurium Salm. orion, Salm. agona, Salm. give, Salm. ridge, Salm. anatum, Salm. reading* and *Salm. Java.* While other salmonellae inoculated chickens showed mortality range up to day 8. Survived chicks remained alive upto day 14 at the end of experiment. In chicks inoculated with different serotypes of *Salmonella* highly significant ($P< 0.01$) mortalities were observed as compared to control, where no mortality was observed. Mortality pattern varied with different serotypes. The differences between *Salm. gallinarum, Salm. pullorum, Salm. typhimurium Salm. saint paul, Salm. reading, Salm. chester, Salm. anatum, Salm. hadar, Salm. orion, Salm. ridge, Salm. agona* and *Salm. paratyphi A,* where mortality ranged from 90-100 per cent. Mortality with these serotypes was significantly ($P< 0.01$) less where chicks were exposed to *Salm. eastbourne, Salm. butantan, Salm. java,*

Salm. remo, Salm. heidelberg, Salm. mission and *Salm. give* (Table 30).

4.7.5. COMPETITIVE EXCLUSION

It has been reported that the normal anaerobic flora of the ceca of adult chickens produces short-chained VFAs that inhibit the growth of *Salmonella*. The antibacterial activity of VFAs increases as the pH and oxidation reduction (redox) potential (Eh) decreases. The Ph effects the antibacterial activity of the VFAs by altering the amount of dissociation of the acid.

Competitive exclusion is a physical and chemical overcome of non-pathogenic *lactobacilli* over the pathogenic salmonellae by competitive exclusion. *Lactobacilli* compete with the salmonellae on receptors to discourage the *Salmonella* colonization. *Salmonella* isolation were undertaken on day 4, 8 and 12 post *lactobacilli* treatment from crop and ceca in all the treatment groups *Salm. gallinarum, Salm. pullorum* and *Salm. typhimurium* colonization was discouraged significantly ($P< 0.05$) by *lactobacilli* in T2, T3 and T4. Five chicks from each treatment groups were slaughtered on day 4, 8 and 12 and homogenate as well as washing of crop and ceca were cultured for the mean log *Salmonella* count. The mean log *Salmonella* count in control (T1) group increased in crop on day 4, 8 and 12 in homogenate samples while there was decreasing trend in washing samples from crop on day 8 and 12 as compared to day 4. The *Salmonella* count increased in cecum on day 8 and 12 as compared to day 4 in control group (T1) among homogenate samples, while there was marked decrease on day 8 and 12 in washing samples. As compared with typical trend in control

Table 30: Mortality pattern of day-old chicks inoculated with different serotypes of *Salmonella*.

Salmonella serotypes	Mortality (%) days post inoculation											Mortality	
	1	2	3	4	5	6	7	8	9	10 // 14		No.	(%)
pullorum	2	4	3	1	-	-	-	-	-	-	-	10	(100)
gallinarum	1	3	4	2	-	-	-	-	-	-	-	10	(100)
typhimurium	-	2	3	4	1	-	-	-	-	-	-	10	(100)
eastbourne	-	1	1	3	1	1	-	-	-	-	-	7	(70)
saint-paul	-	-	1	2	3	1	1	1	-	-	-	9	(90)
butantan	-	-	1	2	4	-	1	-	-	-	-	8	(80)
java	1	1	1	3	2	-	-	-	-	-	-	8	(80)
reading	-	2	1	3	3	-	-	-	-	-	-	9	(90)
chester	1	1	3	2	1	1	1	-	-	-	-	10	(100)
remo	-	1	1	2	1	1	1	1	-	-	-	8	(80)
heidelberg	-	1	1	2	1	-	1	-	-	-	-	6	(60)
anatum	1	2	3	2	1	-	-	-	-	-	-	9	(90)
hadar	-	1	2	4	1	1	1	-	-	-	-	10	(100)
orion	-	1	3	4	2	-	-	-	-	-	-	10	(100)
ridge	1	1	2	2	3	-	-	-	-	-	-	9	(90)
agona	1	2	3	4	-	-	-	-	-	-	-	10	(100)
mission	1	1	2	1	1	1	1	-	-	-	-	8	(80)
give	1	2	2	1	1	-	-	-	-	-	-	7	(70)
para typhi A	-	1	3	2	1	1	1	-	-	-	-	9	(90)
Total No. (%)	10	27	40	46	27	7	8	2	-	-	-	167	(87.89)
	5.26	14.21	21.05	24.21	14.21	3.68	4.21	1.05	-	-	-	87.89	

group salmonellae count in T2 where *Salm. gallinarum* was challenged the count in homogenate samples were increased upto day 8 and then decreased on day 12. Almost identical trend was noted in the T3 and T4 groups where *Salm. pullorum* and *Salm. typhimurium* were challenged respectively. In treatment groups *lactobacilli* reduced the colonization rate that was the reason of reduced mean log *Salmonella* count in homogenate samples and washing on day 12. The increased *Salmonella* count in control is due to colonization and shedding of salmonellae in the organ's lumen. A significant reduction was noted in cecum and crop where *lactobacilli* were administered (Table 31).

4.7.6. FEED SUPPLEMENTATION

Compared with controls, the mean log number of *Salmonella* shedding decreased significantly ($P < 0.05$) with the addition of Na EDTA in all the treatment groups. In treatment groups T2 and T4 where the Ma EDTA were amended in the diet @ rate of 5 g and 10 g/50 kg of feed for 7 days respectively reduced intestinal colonization and fecal shedding significantly ($P < 0.05$) as compared to control treatment group (Table 32). The reduction in the shedding and colonization was significantly less in T4 vs T2. The groups

Table 31: Mean log number of various *Salmonella* serotypes in cecum and crop of birds given *Lactobacilli* in drinking water.

Treat-ments	Birds chall-enged with	Samples Description	Culture results (Days-Post-Treatment) in Mean log number Days-Post-Inoculation 4 Crop	4 Cecum	8 Crop	8 Cecum	12 Crop	12 Cecum
T1	-	Homogenate	1.86±0.52	6.21±1.04	6.53±1.14	7.18±0.93	7.71±0.50 a	7.15±0.93 a
Untreated		Washing	6.10±1.30	4.86±0.98	3.79±0.63	2.19±0.71	0.96±0.56 b	1.54±1.01 b
T2	SG	Homogenate	1.54±0.75	3.51±0.46	2.50±0.57	1.90±0.70	1.70±1.01 c	1.50±1.01 b
Lactobacilli		Washing	0.95±0.31	4.28±0.36	4.10±1.51	2.38±0.27	1.03±1.34 c	0.82±0.31 c
T3	SP	Homogenate	1.45±0.65	3.60±0.54	2.40±0.67	1.93±0.65	1.80±0.88 c	1.45±0.95 b
Lactobacilli		Washing	0.88±0.28	4.32±0.31	4.13±0.65	2.23±0.31	1.01±1.25 c	0.77±0.22 c
T4	ST	Homogenate	1.40±0.66	3.42±0.26	2.20±0.82	1.70±0.65	1.80±0.87 c	1.47±1.22 b
Lactobacilli		Washing	0.93±0.42	4.50±0.47	4.18±0.88	2.40±0.31	1.22±1.35 c	0.87±0.31 c

a – c = Values with similar superscripts do not differ statistically, while values with different superscripts differ significantly ($P < 0.01$ to 0.05)

SG = *Salm. gallinarum*
SP = *Salm. pullorum*
ST = *Salm. typhimurium*

Table 32: Effect of EDTA on colonization of *Salmonella*.

Treatment Groups	No. of chicks	Na EDTA (g/50 kg feed)	Days of Exposure	Mean log No. of Salmonella (CFU) per gm on 14th day	
				Fecal material*	Intestine*
T1	10	Control diet	1-14 days	7.31 ± 0.62a	2.21 ± 0.29a
T2	10	5	1-7 days	3.07 ± 0.51b	1.02 ± 0.17b
T3	10	5	1-14 days	1.88 ± 0.62c	0.62 ± 0.20c
T4	10	10	1-7 days	2.21 ± 0.77c	0.87 ± 0.27c
T5	10	10	1-14 days	1.51 ± 0.65c	0.53 ± 0.23c

* = 24 hours composite sample on weight basis on day 14.

a - c = Values with similar superscripts do not differ statistically, while values with different superscripts differ significantly ($P < 0.01$ to 0.05)

offered Na EDTA for 14 days in T3 and T5 at a dosage level of 5 and 10 g/50 kg of feed significantly ($P< 0.01$) reduced the salmonella colonization in intestine and fecal shedding as compared to the control group (T1). The use of Na EDTA at a dosage level of 5 g/50 kg feed for 7 days in treatment T2 reduced significantly colonization and shedding, however, the same level (T4) for 14 days reduced the colonization and shedding as compared to control and T2. The treatment groups where the dosage level was 10 g/50 kg feed for 7 days (T3) and 14 days (T5) were ideally depressed the colonization in intestine and shedding of salmonellae in feces. However, the differences between 7 days (T3) and 14 days (T5) medication were non significant between these two groups as well as with T3. There was a good temporal correlation was observed with different dosage level of Na EDTA medication. Either the high dosage or the prolong use, significantly reduce the colonization of *Salmonella* and shedding in the feces.

4.7.7. VACCINATION TRIAL

Vaccination through different routes was tried to attempt the suitable route of administration. There was non-significant differences were observed on the 0,7,14,28 and 70 days post vaccination. Routes of administration were of no

value in case of vaccination with *Salm. gallinarum* 9R strain vaccine. The humoral immune response was of moderate type (Table 33).

4.7.8. MICROBICIDAL EFFECT OF DISINFECTANTS

The most desirable means of disease prevention is to be in an area where certain diseases do not exist. This is accomplished by excluding diseases from theses areas through a system of strict isolation, sanitation and regulation. Virkon and Beloran two disinfectants were tried to chick their killkinetic *in vitro*. Virkon and Beloran proven to be the best choice of disinfectants against salmonellae. Virkon have a little more effective than Beloran but the differences were nonsignificant ($P< 0.05$). There was good temporal response among both the disinfectant. There was a strong correlation between time and microbicidal effect of Virkon and Beloran. Standard microbicidal effect index is > 6.0 according to this standardization at 20°C with one per cent concentration for 5 minutes exposure. Virkon and Beloran was effective 100 per cent against all the isolates of salmonellae except *Salm. gallinarum* where it was not effective 100 per cent for 5 minutes exposure. Beloran was effective to all the isolates tested whereas it was not effective 100 per cent against *Salm. gallinarum* and *Salm. typhimurium* at 5 minutes exposure. Beloran kill 100 per cent *Salm. gallinarum* at 30 minutes

Table 33: Humoral immune response in broilers vaccinated through various routes.

Treatment Groups (Route of vaccination)	Days Post Vacci-nation	HI Titres								GMT
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
V1	0	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-
	28	-	-	-	-	-	-	-	-	-
Control	70	-	-	-	-	-	-	-	-	-
V2	0	-	-	-	-	-	-	-	-	-
	7	-	5	7	8	-	-	-	-	0.9424 a
	14	-	2	6	5	7	-	-	-	1.1589 a
	28	-	2	4	4	8	2	-	-	1.0837 a
(Sub/cut)	70	-	1	5	4	7	2	1	-	1.3094 a
V3	0	-	-	-	-	-	-	-	-	-
	7	2	5	7	6	-	-	-	-	0.8558 a
	14	1	6	5	7	1	-	-	-	0.9178 a
	28	-	4	7	8	1	-	-	-	0.9933 a
(Orally)	70	-	2	4	7	7	-	-	-	1.1890 a
V4	0	-	-	-	-	-	-	-	-	-
	7	-	4	8	7	1	-	-	-	0.9783 a
	14	-	3	5	4	6	2	-	-	1.1890 a
	28	-	1	4	7	7	1	-	-	1.2492 a
(I/m)	70	-	-	3	8	5	3	1	-	1.3696 a

a = Values with similar superscripts do not differ statistically, while values with different superscripts differ significantly (P< 0.01 to 0.05)

exposure and *Salm. typhimurium* at 15 minutes exposure (Table 34).

Virkon was more efficient as compared to Beloran, while the both disinfectants were ideal for in farm or poultry house-ware disinfectants. Both the disinfectant were 100 per cent effective to kill the isolates *in vitro* at 20°C in 5 minutes except *Salm. gallinarum* where it needs 15-30 minutes exposure. As a control measure one per cent solution of disinfectants is 10 time more as recommended level so farm spray will be effective at 0.1 per cent level.

Table 34: Mean microbicidal effect of disinfectants (at 20°C with 1 % concentration) at different time exposure intervals (in minutes).

Salmonellae tested	No. of isolates tested	VIRKON 5	VIRKON 15	VIRKON 30	BELORAN 5	BELORAN 15	BELORAN 30	Standard M. E.
pullorum	5	6.02	7.23	7.34	5.98	6.58	7.21	>6.0
gallinarum	5	5.96	6.05	6.58	5.91	5.96	6.45	>6.0
typhimurium	4	6.05	6.95	7.25	5.98	6.21	7.13	>6.0
eastbourne	2	7.21	7.53	8.12	7.15	7.22	7.59	>6.0
saint-paul	2	6.51	6.93	7.25	6.21	6.54	7.11	>6.0
butantan	3	7.39	7.81	8.15	6.89	7.52	8.12	>6.0
java	4	7.12	7.53	8.11	7.11	7.42	7.95	>6.0
reading	5	6.95	7.54	8.52	6.73	7.43	8.19	>6.0
chester	6	6.88	7.12	7.93	6.49	7.11	7.88	>6.0
remo	2	7.93	8.11	8.53	7.51	7.81	8.22	>6.0
heidelberg	2	6.57	7.31	8.11	6.27	7.11	7.98	>6.0
anatum	5	7.13	7.51	8.10	6.98	7.43	7.95	>6.0
hadar	5	6.93	7.22	7.35	6.21	6.98	7.31	>6.0
orion	5	7.13	7.18	7.21	7.11	7.15	7.35	>6.0
ridge	5	7.91	7.93	8.21	7.73	7.89	8.11	>6.0
agona	5	6.95	7.15	7.54	6.81	7.11	7.43	>6.0
mission	5	7.91	7.99	8.11	7.88	7.93	8.05	>6.0
give	5	6.93	7.12	7.58	6.81	6.98	7.34	>6.0
para typhi A	3	6.53	7.11	7.53	6.41	6.98	7.11	>6.0

CHAPTER 5

DISCUSSION

Salmonellosis caused by *Salmonella* species has been recognized as a worldwide problem in both man and animals. *Salmonella* infections occur in many kinds of birds and mammals; frequently recorded in poultry. It also occurs in rats, mice and other rodents, in many reptiles and some insects (Barnes and Impey, 1980). Both domestic and wild poultry are vulnerable to *Salmonella* infections (Javed *et al.*, 1990). More than 2300 known *Salmonella* serotypes have so far been reported in the world which suggests a ubiquitous nature of *Salmonella* (Edwards & Ewing, 1989). Poultry and poultry products constitute one of the major reservoirs of *Salmonella* infections since more than 50 per cent of the serotypes have been isolated from these sources alone (Kohler *et al.*, 1979). Two serotypes i.e. *Salm. pullorum* and *Salm. gallinarum*, causative agents of pullorum disease and fowl typhoid respectively, are of great economic importance due to high mortality, lowered egg production and reduced hatchability (Javed *et al.*, 1992). Pullorum disease is characterized by white diarrhea and high mortality in young birds (Javed & Hameed, 1989).

The principal reservoir of salmonellae are animal species which may infect humanbeing *via* ingestion of contaminated food or direct exposure (Drapeau and Jankovic, 1977). Practically all animals (Welchman, 1987), domestic aviary (Javed *et al.*, 1990), wild birds (Javed *et al.*, 1992), rodents (Siddique *et al.*, 1985), and insects can host salmonellae (Williams *et al.*, 1980).

Refuse from hospitals (Leclerec and Oger, 1974) and slaughter houses can contaminate water (Leclerec and Oger, 1975) which may support bacterial multiplication (Wright, 1989). Salmonellae remain viable in sludge, which could be a potential contaminant for streams and other water reservoirs.

Consumption of *Salmonella* contaminated meat and poultry products, resulted in health care cost of $1000 million in the United States in 1987 and 9,00,000 £ in the United Kingdom (Yule *et al.*, 1987). The incidence of human *Salm. enteritidis, Salm. virchow* and *Salm. stanley*, has risen significantly between 1981 and 1986. Again poultry remains a major vehicle of disease transmission, however, bovines also contribute in cross-species infections (Humphrey and Lanning, 1988). Poultry-borne salmonellosis is the most common form of food borne infection in Scotland (Yule *et al.*, 1988).

Rapid hemagglutination testing of 150 chicken broiler breeder flocks showed that 112 (74.7 %) flocks were positive for *Salmonella* and only 38 flocks were negative. On these breeder farms 2,62,454 birds (2,28,583 females and 33,871 males) 12,159 were recorded to be carriers thus indicating a prevalence of 4.63 per cent. Data on the prevalence of *Salmonella* seropositive were analyzed according to various feeds to rule out the possible role of the feed in the spread of the pathogen. The prevalence of *Salmonella* carriers varied greatly among birds fed on various commercial feeds. Regarding the flocks on most of the feeds, the prevalence varied from 69.2 to 82.2 per cent, ranging from 50 to 100 per cent. On one of the feeds, birds showed the

prevalence as high as 11.33 per cent. Feed as an important source of *Salmonella* infections has been indicated by a number of research workers. (Zecha *et al.*, 1977). Many times feed ingredients of animals origin are the potential sources. Proper sanitary measures during the procurement and processing of feed ingredients can greatly help to avoid feed contaminations. Although many of the organism are killed during the pelleting of feed, care should always be taken during transportation and particularly during storage, as the presence of rodents, such as mice and lizards which are usually carriers and active spreaders of *Salmonella*, may reinfect the feed (Lahellec *et al.*, 1986).

The five breeds of broiler breeders were included in the present studies from the commercial point of view these were designated from B1 to B5. Breed No.4 showed the maximum prevalence of salmonellosis and it varied significantly from B2 and B3. Transovarian transmission is the most important route of *Salmonella* infections. Although in Pakistan parent flocks are imported from technically advanced countries and there seems to be less chance of getting *Salmonella* from grand parents, we were astonished to see that *Salmonella-free* parent flocks would be a basic step for the elimination of salmonellosis and for the real development of our poultry industry.

Most of the flocks were tested between the age of 21 to 40 weeks but many flocks also at later stages and some of the birds were retested. The prevalence of *Salmonella* carriers varied widely in birds of different ages. The maximum number of flocks were affected during 51 to 60 weeks of age (88.23 %), followed

by birds of 31 to 50 weeks of age and other groups. The highest number of carriers (6.75 %) were detected in birds tested during 41-50 weeks, followed by 21-30 weeks (5.01 %) and at other stages. The antibody titre in *Salmonella* carrier birds starts at an early age, rises with the advancement of age and is usually detectable by the hemagglutination test after the age of 20 weeks. This titre has also a direct correlation with the production of the birds and the maximum titre is recorded during the peak production periods. Considering these factors, the rapid hemagglutination test is recommended after the age of 22 weeks when the egg production rises up to 5-10 per cent (Hofstad *et al.*, 1984).

A direct correlation was recorded between the age of the breeders and incidence of salmonellosis. Maximum (6.75 %) incidence was recorded in adult birds during 41 to 50 weeks while, in the youngest flocks it was maximum (5.01 %) between 21 to 30 weeks. This may be due to fact that infection mostly remain localized in the carrier birds, but occasionally they secrete the organism in their secretions and excretion contaminating the environment, feed, water, egg nest, litter etc., and remain a permanent source of infection for their penmates. This may be a probable factor for higher incidence in older age group (Mario, 1991).

The management of a farm has a direct bearing on the spread of infections. A similar pattern was also observed during the present investigations of *Salmonella* carriers. Regarding the prevalence of *Salmonella* carriers, a highly significant

difference was recorded in flocks as well as in birds maintained under various conditions of management. The maximum prevalence (78.57 %) was observed in flocks under the poor management, followed by flocks under a satisfactory management (77.04 %) and the minimum prevalence was found in birds kept in excellent farming conditions (41.66 %). Similarly, the maximum number (9.47 %) of carriers were detected in birds kept under the poor management, followed by breeders under satisfactory conditions and the minimum in birds with excellent farming practices.

There are numerous sources of salmonellae in a contaminated poultry flock, once a flock becomes infected, the organisms may be transmitted from chicken to chicken through several pathways and the floor litter can harbor salmonellae for long periods. However, most of the organisms are destroyed in the deep litter system due to a high concentration of ammonia (Bhatia and Nabb, 1980). Oral infection is generally considered a likely pathway for a natural infection but percloacal infection is also possible in chickens reared on litter (Moitra and Saxena, 1984). A strong correlation has been observed between the degree of contamination of the floor litter and the spread of salmonellae through a flock. It is the usual practice that after blood testing the reactors are removed and the non-reactors are left in the same infected litter. In this way the potential source of infection remains and there is every chance of reinfection of the healthy birds. It is recommended that the negative birds should be left in clean premises with a fresh uncontaminated litter (Javed and Hameed, 1989).

Poor managemental conditions on the breeder farm may therefore help in the spread of organisms. Maximum incidence was recorded in the flock maintained under poor managemental conditions such as inadequate space, dirty drinkers, dumpy litter, improper ventilation and unhygienic drinking water. The incidence was lowest in a flock kept under good managemental conditions and where regular screening was conducted. This underlines the importance of managemental practices for the control of *Salmonella* infections. However, the possibilities of reinfection from infected feed and water cannot be eliminated.

Regarding the sex the prevalence varied in different breeds, feeds and managemental conditions. Although overall incidence was 5.15 per cent in B_1 birds while only 1.29 per cent males were positive as compared to the B_4 where overall prevalence in total flocks was 5.87 per cent, while only 6.18 per cent males were involved. In B_2 overall prevalence was 4.27 per cent with 2.66 per cent of male and 4.55 per cent of females. In B_3 (0.36 %) and B_5 (0.83 %) the higher percentage of males were positive and compared to females B3 (0.04 %) and B5 (0.4 %). Analysis of the data on bird basis gave a strong correlation of higher percentage of females directly proportional to the birds positivity. In case of exception higher percentage of male indicate introduction of new males or in a stage to spread the infection to females.

A number of tests were compare to check the reliability of these diagnostic and carrier birds monitoring tests. Rapid blood agglutination test (RBAT), Tube agglutination test (TAT), Yolk

agar precipitin test (YAPT) and cloacal swab (CS) isolation test were compared in the known 200 *Salmonella* carrier female birds. The 200 (100 %) birds positive in RBAT were also comparatively confirmed by TAT in 98.50 per cent cases. YAPT was effective in 97.50 per cent followed by cloacal swab method where 87.00 per cent were confirmed positive for *Salmonella*.

RBAT was reliable with some field problems as the E. coli shares some of the antigen with *Salmonella* so cross reaction indicate pseudocarriers, which can be detected by TAT. The effectiveness of cloacal swab was of value in birds under 18 weeks. The efficiency of this test is influenced by the intermittent sheders of salmonellae. The yolk agar ppt test (YAPT) was good tool to monitor the birds for primary indications of the carrier state through eggs without disturbing the flock or in those condition where flock and hatcheries are well aparted (Siddique *et al.*, 1989). Spot agglutination test is not considered to be 100 per cent accurate as many false negatives as well as false positives have been reported (Sajid *et al.*, 1986).

The detection of *Salmonella* in flocks of laying hens has thus become a public health priority and a matter of great concern to egg producers. Testing for the presence of specific serum antibodies in an important aspect of proposed progression of identifying *Salmonella*-positive flocks. The National Poultry Improvement Plan authorizes the use of a variety of macro and microagglutination technique, for the detection of *Salm. pullorum* antibodies in chicken. Paratyphoid *Salmonella*

serotypes, such as *Salm. enteritidis*, generally elicit weaker antibodies responses (Williams and Whittemore, 1975). Conventional agglutination tests have not been effective for detecting paratyphoid infections in chickens (Olesiuk *et al.*, 1969 and Williams, 1975), perhaps because many such infections in mature birds are limited to colonization of the alimentary tract. Very young chickens are far more susceptible to paratyphoid salmonellae, but the antibody response by chicks has been observed to be insufficient for serological detection of infection. Serological methods have been reported to vary in sensitivity and reliability, but all were found to be more sensitive than cloacal swab cultures for the detection of paratyphoid *Salmonella* infections in chickens. Evidence of systemic infection with *Salm. enteritidis* suggests that infected hens are likely to have antibody titers high enough to permit efficient serological detection (Gast and Beard, 1990).

A new method for diagnosing pullorum disease in breeding hens was developed. The results of this test agreed with those of rapid whole blood stained antigen agglutination test, whole blood gel precipitation test and bacteriological culture (Zhao *et al.*, 1981). A test for differential serodiagnosis of *Salmonella* by detection of IgG and IgM antibodies in ELISA was developed. In a comparative investigation with 192 pigeon sera, 14.5 per cent were positive in the (H+L) chain specific IgG-ELISA, 12 per cent in the tube agglutination test and 6.3 per cent in slide agglutination test.

The peroxidase-antiperoxidase immunoassay was developed by using *Salm. choleraesuis* var Kunzendrof, *Salm. dublin* and *Salm. typhimurium* as test organisms. Strong specific staining with corresponding antiserum was achieved with smears of each *Salmonella* serotype on microscope slides from formalized cell suspension, culture of liver clinical isolates and tissue suspensions from the livers and spleens of experimentally infected mice. In addition *Salm. choleraesuis* var kunzendrof was detected in formalin-fixed and fresh frozen tissues from experimentally infected pigs. Their results indicate that the peroxidase antiperoxidase assay is well suited for the rapid identification of *Salmonella* from pure cultures and that the technique can be useful for detecting *Salmonella* in histological sections (McRill *et al.*, 1984). Enzyme Linked Immune Sorbent Assay (ELISA) is a new and most reliable serological method used for the diagnosis of typhoid fever in human beings (Vior, 1984). Siddique (1985a) used this method for the differential diagnosis of *Salmonella* infections from other confusing diseases.

In Pakistan, rapid hemagglutination testing of 150 chicken broiler breeder flocks having 2,62,454 birds revealed a high prevalence of *Salmonella* seropositive birds. Among these, 12,159 (4.63 %) birds were screened as carriers from 112 positive flocks. The prevalence of *Salmonella* carriers varied in birds reared on various commercial feeds in chickens of different breeds and those maintained on the varying standards of management (Javed and Hameed, 1989). The persistence of *Salmonella* in a variety of zoological garden birds was investigated. Of 370 rectal swabs examined, 66 yielded different

Salmonella serotypes which includes 30 strains of *Salm. typhimurium*, 23 *Salm. gallinarum-pullorum*. 5 *Salm. saint-paul*, 5 *Salm. butantan* and 3 *Salm. eastbourne*. Parrots, pigeons, Java sparrows, quails, peacocks, doves and pheasants were the common birds positive for *Salmonella* (Javed *et al.*, 1992). In Pakistan, isolation and pathological studies were conducted in 753 enlarged livers, spleens and intestines of indigenous chickens, salmonellae were isolated mostly from intestines (9.96 %), followed by liver (5.97 %) and minimum (1.19 %) in spleen. An overall isolation incidence was 5.71 per cent *Salm. gallinarum-pullorum* and *Salmonella* of group E were the most common isolates (Javed, *et al.*, 1991). This variation might have been due to the difference in the number of the birds studied, the types of cases recorded and the husbandry and managemental conditions prevailing at the farm. Other reasons which could be advocated were the breeds involved, the genetic resistance of the birds, geographical and seasonal variation and use of preventive medicine for the control of diseases particularly in breeding flocks.

Among the 715 serotypes of *Salmonella* isolated from various sources selenite broth was proven 100 per cent of effective followed by tetrathionate broth (95.8 %) and Mac-Conkey's broth (90.06 %). Among the solid laboratory isolation media Mac-Conkey's agar (MC) was the most effective as all the stains were isolated on Mac-Conkey's (97.20 %) followed by *Salmonella-shigella* agar (88.40 %) and Eosin Brilliant Green (EBG) agar (86.01 %). An overall isolation regimen was best through selenite broth enrichment and isolation on Mac-Conkey's media

give almost all the isolates from various sources. The biochemical characteristics of *Salm. pullorum* and *Salm. gallinarum* were found to be almost similar in many biochemical pathways but some of the critical differences regarding their biochemical fermentation were noted.

Analysis of *Salmonella* serotypes isolation over the years indicated a relation between our flock pattern and isolation in different months of the year. As it is evident in Pakistan we have seasonal flock system, so the flock raised/reared in December-January-March season come to production in July-August. Hence incidence increases in this period. In 1988 there were more non-motile (192) and motile (211) isolates. Highly significant isolations were made in 1988 as compared to isolation 1989 and 1990. In 1989, isolation of non-motile serotypes was significantly higher than isolation of non-motile salmonellae in 1990, while isolation prevalence of motile salmonellae in 1989 and 1990 was non-significant.

Regarding the isolation of various *Salmonella* serotypes in broiler breeders at random, a relatively higher prevalence of non-motile salmonellae were recorded. *Salm. gallinarum* was recorded in 23.14 per cent, followed by *Salm. pullorum* (14.81 %). Among the motile salmonellae isolation in broiler breeders, *Salm. typhimurium* was isolated in 9.26 per cent, followed by *Salm. heidelberg* (6.48 %) and *Salm. butantan* (5.55 %). *Salm. eastbourne, Salm. saint-paul, Salm. remo* and *Salm. agona* have 4.63 per cent isolation prevalence each. Isolation prevalence of other isolates is given in Table . Considering the incidence of

Salm. gallinarum and *Salm. pullorum* in adult broiler breeders the *Salm. gallinarum* is increasing over *Salm. pullorum.*

In most of the birds, salmonellae were isolated from intestines, liver, spleen and ovary. Isolates were also obtained from caeca, lungs, kidney, heart, brain and bursa of Fabricius. A higher number of isolates were obtained from intestines (37.08 %) than the liver (24.07 %) and spleen (10.18 %).

Incidence of *Salmonella* serotypes isolated from 109 countries during 1934-1978 and divided the various serotypes into quite frequent, frequent, rare and quite rare categories. *Salm. paratyphi, Salm. typhimurium, Salm. heidelberg, Salm. infantis, Salm. typhi, Salm. enteritidis, Salm. dublin, Salm. panama* and *Salm. anatum* were categorized by Kelterborn (1979) as quite common serotypes. Barrow et al. (1988b) isolated 23 of *Salm. typhimurium,* 19 of *Salm. typhimurium* var copenhagen, 26 of *Salm. enteritidis,* 10 of *Salm. berta* and 7 of *Salm. havana.* Among 124 total isolates 101 were from poultry, *Salm. pullorum* have several serological variants. Bivini (1984) isolated 202 strains from birds. Persistence of *Salmonella* strains most frequently isolated from animals was recorded in the years 1976-1978 *Salm. typhimurium* was isolated from 222 cases, *Salm. dublin* 250, *Salm. choleraesuis* 188, *Salm. enteritidis* 61 and *Salm. gallinarum-pullorum* in 73 cases, (Haszowski and Truszynski, 1980). Among *Salmonella* species most frequently found in poultry farm employees was *Salm. typhimurium* while *Salm. newport, Salm enteritidis* and *Salm. dublin* were also isolated (Kotova *et al.*, 1988). Girao *et al.* (1985) isolated salmonellae from meat meal,

feather meal, hatchery meal and finished feed. *Salm. saint-paul Senftenberg, anatum, dublin, infantis, gallinarum-pullorum, jaffina, typhimurium and berta* were the common feed contaminant (Yaziz and Awang, 1985).

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Among the day-old broiler breeder chicks, a total of 21 (4.18 %) salmonellae were isolated. *Salm. gallinarum* was isolated from 10 (47.61 %), followed by *Salm. pullorum*, 7 (33.33 %), *Salm. typhimurium* 1 (4.76 %) and *Salm. paratyphi A*, 4.76 per cent. These isolations were attempted from the composite samples of yolks, liver, intestine, lungs and spleen of birds died during transportation from abroad.

Salmonellosis is one of the most important zoonotic problem throughout the world. Domestic chickens are close inhabitants of our human population, particularly in rural areas. There are

therefore fair chances of transmission of *Salmonella* infection from chickens to human beings and vice versa (Siddique *et al.*, 1985). The present studies were designed to examine the presence of *Salmonella* among rural chickens. The incidence of *Salmonella* would help understand the role of desi (indigenous) birds as a potential health hazard for the human population and also for commercial poultry (Adesiyun *et al.*, 1988).

Domestic poultry are reported to be the largest single reservoir of *Salmonella* and the principal source of *Salmonella* infection in man. Eggs infected by direct ovarian transmission and tissues from the affected poultry are of importance. The foremost role in the epidemiology of the disease in man is played by egg shells contaminated during laying and by poultry carcasses contaminated during slaughter. Inapparent intestinal carriers are common in poultry flocks, and both the egg-shells and carcasses may be contaminated by feces from these inapparent carriers. Programs aimed at eliminating this source of infection will therefore depend on information on the factors influencing both the initiation of inapparent intestinal infection and shedding of the organism in the feces (Sadler *et al.*, 1969). The objectives of the present studies were to know the status of *Salmonella* infection in desi chickens.

There have been numerous reports on the factors that influence the shed pattern of *Salmonella* and its localization in different visceral organs. As our desi birds roam about in dirty places and sewages and always get their feed from contaminated sources, this leads them towards carriers of pathogens. The

localization of *Salmonella* in the intestines, liver and spleen has also been reported by Siddique et al. (1985c). A higher percentage of intestinal carriers is also a serious threat to our commercial poultry. Out of 43 isolates, 31 (72.09 %) were of *Salmonella* gallinarum-pullorum and 12 (27.91 %) were motile Salmonellae. All the paratyphoid organisms belong to group E. Sharma et al. (1980) isolates 20 different *Salmonella* serotypes out of 790 intestinal contents, which included 10 strains of *Salmonella* saint paul, 4 *Salmonella* bareilly, and 6 *Salmonella* of group E_1. This work is in line with our findings.

This higher prevalence of salmonellosis in these domesticated birds posses a serious threat to human health, as these birds live quite close to human, particularly the children (Sharma *et al.*, 1980). *Salmonella* carrier birds commonly excrete these organisms through faeces (Impey *et al.*, 1984) and ultimately may infect human mostly through dust borne infections (Breer, 1985). For the control of human salmonellosis apart from other hygienic measures, elimination of important reservoirs in domestic and wild birds would be of paramount importance.

Isolation of *Salmonella* serotypes like *Salm. typhimurium, Salm. saint-Paul, Salm. butantan* and *Salm. east-bourne,* which are known for their association with disease conditions in man and animals is of animal industry as well as public health significance. *Salm. typhimurium* and *Salm. saint -paul* were recovered from goats having diarrhoea (Singh *et al.*, 1981). Isolation of *Salm. typhimurium* from pigeons has earlier been reported by Siddique *et al.*, (1985). *Salm. typhimurium* was

isolated from 30 (45.45 %) rectal swabs, *Salm. gallinarum - pullorum* was confirmed in 23 (34.84 %) cases etc.

Salmonella could be an airborne infection as the dried fecal material spread in the air in form of dust. The contaminated house dust is one of the vehicle to transport infection to the penmate and sheds in the vicinity of the farm. A total of 111 house dust samples were collected and only in 5 (4.5 %) cases salmonellae were isolated. All of the isolates were non-motile group. *Salm. gallinarum* was isolated 4 (80.00 %) and *Salm. pullorum* in 1 (20.00 %) dust samples.

Drinking water is an important vector of pathogen's transmission by contamination in house or outside the house. In poultry houses, 147 water samples were collected among these 31 (21.08 %) were positive for *Salmonella*. Water had heavy contamination of motile salmonellae as 21 (14.28 %) were motile *Salmonella* and only 10 (6.80 %) were non-motile salmonellae. *Salm. gallinarum* and *Salm. pullorum* had identical isolation prevalence (16.12 %) as it was identical in case of *Salm. typhimurium* and *Salm. orion* (9.67 %). *Salm. eastbourne, Salm. remo, Salm. agona* and *Salm. give* had similar isolation number 2 (6.45 %) in each case. *Salm. saint-paul, Salm. butantan, Salm. Java, Salm. chester, Salm. anatum, Salm. hadar* and *Salm. ridge* only 1 (3.22 %) isolate for each.

Rodents are good vector of transmitting the disease organism to the feed store or spreading to the other farms. The contaminated rodent feces are mixed in the feed and ultimately

the insidious material reached to the birds. A total of 215 composite rodent fecal samples were collected and *Salmonella* isolation was confirmed in 21 (9.77 %) samples. Among 2.79 per cent non-motile *Salmonella*, 19.04 per cent was *Salm. gallinarum* followed by *Salm. pullorum* (9.52 %). Among motile group *Salm. agona* (19.04 %), *Salm. saint-paul* (14.28 %) and *Salm. give* (9.52 %) were isolated, while *Salm. typhimurium, Salm. Java, Salm. reading, Salm. ridge, Salm. mission and Salm. paratyphi A* 1 (4.76 %) each of the isolate was attempted.

Various antimicrobial agents more commonly used against salmonellosis were evaluated by disc method against the isolated *Salmonella* strains. The antimicrobials used were ampicillin, chloramphenicol, erythromycin, flumequine, furazolidone, gentamicin, kanamycin, lincomycin, neomycin, streptomycin, Terramycin, Tribrissen and vibramycin. On the average, 66.33 per cent of the isolates were highly susceptible to various antimicrobials, 14.43 per cent intermediately susceptible, while 19.26 per cent of the isolates were resistant.

Flumequine proved to be the drug of choice, as 668 (93.43 %) isolates were sensitive, 30 (4.19 %) intermediately susceptible and only 17 (2.37 %) were resistant. Vibramycin stood at number two, to which 568 (79.44 %) isolates were sensitive and 116 (16.22 %) were intermediately susceptible, while 31 (4.33 %) isolates were resistent. According to the spectrum of susceptibility, maximum resistance (40.27 %) was observed against kanamycin, followed by Tribrissen (38.74 %), furazolidone (37.20 %), Terramycin (32.16 %), erythromycin

(26.01 %) and neomycin (22.23 %). Seventeen (2.37 %) isolates were resistant to all the antibacterials, while 427 (59.72 %) were sensitive to all the antibacterials, while 427 (59.72 %) were sensitive to all the antibacterials tested.

The increasing use of antibacterials for prophylactic, therapeutic and nutritive purposes in agriculture and medicine creates a potentially powerful selective pressure for the spread of antibiotic resistance in bacteria (Duck *et al.*, 1978 and Lofont *et al.*, 1981). As many authors pointed out, the spreading of multidrug resistance strains determined peculiar aspect of gravity in the outbreak evolution, serious economic involvement including loss of work, cost of therapy, expensive laboratory investigations and antiepizootic measures (Barbour and Nabbut, 1982; McGarr *et al.*, 1980 and Hirsh *et al.*, 1983).

Antibiotic sensitivity of *Salmonella* strains revealed that ampicillin, gentamicin, kanamycin, neomycin and streptomycin were the most effective against motile as well as non motile salmonellae. A remarkable resistance to tetracycline, tylosin, biseptol and furazolidone was observed. Some susceptibility differences to polymyxin, neomycin and streptomycin were noticed in motile salmonellae (Siddique *et al.*, 1985).

Although there are so many anti-*Salmonella* products are available in the market but these products are constantly loosing their efficacy by resistance development in the pathogen. For few years product remains highly sensitive and than fall to intermediately sensitive and finally fully

resistent. This fashion of resistance against chemotherapeutic have been documented every where, in Romania, Greece, Amman, Kenya, India, Lebanon, Yemen USA, UK, Egypt (Yoon, *et al.*, 1981, Gupta and Mallick, 1976, Boachie, 1985, Hinton, 1988a). Resistance against tetracycline, tylosin, biseptol, furazolidone, ampicillin, chloramphenicol, gentamicin, bacitracin, polymyxin B, erythromycin, Kanamycin, nalidixic acid, sulpha, and streptomycin has already been documented in the world (Siddique, *et al.*, 1985, Silva 1985). Still we are using these products in abundance. The reason being the free availability of antimicrobials in market without prescription. Antibiotic prescription permission to any body incontrast with other countries permitted to only licensed veterinarians. Free availability and medication should be checked to reduce the insidious problem of resistance. Use of antibiotic by the veterinarian without getting the information of antibiography with blind prescription is another technical handicap against this problem. As it is evident that resistance against antimicrobials varied strain to strain and country to country in temporal fashion. The data of one country will not be helpful to the other country for the use of the new products. Many companies in Pakistan discourage the antibiotic sensitivity to mask their insufficiencies. Wisely use of antibiotic need due consideration because the drug development need at least 10-20 years where as on circumstances resistance becoming matter of months.

Carriers breeders showed little evidence of gross pathological changes in the different organs. However, in some

cases ovarian follicles appeared misshapen, discolored containing cheesy material. Live and spleen showed congestion and at places necrotic foci. In some cases heart showed enlargement and accumulation of fat with ecchymotic hemorrhages on the auricles. It may be mentioned that *Salm. pullorum and Salm. gallinarum* infections were indistinguishable from the gross lesions as were also observed by pomeroy, 1978, Athar, 1982, Javed *et al.*, 1990;1992. Congestion of the liver and spleen, pedunculated ova and accumulation of fat on the myocardium was also observed by Sajid, et al. (1986).

The organs which was studied histopathologically showed no difference in the histopathological lesions of broiler breeder affected with *Salm. pullorum and Salm. gallinarum* except fatty degeneration of the liver in *Salm. gallinarum* and not in *Salm. pullorum.* Our results are in agreement with Hofstad *et al.*, 1978 who also reported fatty degeneration is *Salm. gallinarum* affected birds. Liver in many cases showed congestion, focal areas of coagulative necrosis and cellular infiltration in the carrier breeder had already reported.

The spleen showed congestion and thickening of the trabeculae in most of cases. Diffuse areas of hemorrhages were also seen in a few spleens. Similar histopathological changes were also seen in *Salmonella* affected spleens by other workers Pomeroy, 1978, Siddique *et al.*, 1985c and Javed *et al.*, 1991;1992.

In the lungs, there was congestion and areas of hemorrhages in many cases. Interalveolar septa were frequently infiltrated by R.B.C.'s and lymphocytes. These findings closely resemble with those reported by Hofstad *et al.*, 1978 and Siddique *et al.*, 1984.

The kidneys showed congestion, cloudy swellings leading to necrotic changes in the tubular epithelium. Blood vessels were engorged with blood in some cases, while in many cases areas of hemorrhages were also seen. Similar lesions have been mentioned by Hofstad *et al.*, 1984 and Bercea *et al.*, 1981.

In the heart, there were areas of coagulative necrosis in the myocardium and infiltration of mononuclear cells in surrounding area. Zenker's necrosis has been mentioned in the myocardium of day old SPF chicks affected with *Salm. gallinarum* by Siddique *et al.*, 1984. The difference in the type of necrosis may be due to age factor and the type of birds (SPF and conventional).

Salmonella is one of the predominant bacteria affecting poultry. Transmission through the hatching egg may produce either clinical or sub clinical infectious in chicken younger than 1 week of age. It has been shown that penetration of *Salm. typhimurium* through the cuticle, shell and shell membranes occurs very rapidly and that bacterial penetration is greatly influenced by the presence of moisture on the egg shell, either as liquid or as water vapor. Much more attention has been given to contamination of hatching eggs with moist feces contaminated

with salmonellae as an important link in the epizootiology of avian salmonellosis. Most of the bacterial penetration studies have been performed using eggs several hours after they had been laid. Studies demonstrate the penetration of *Salm. typhimurium* through shell of newly laid broiler hatching eggs under two methods of exposure. Eggs were challenged either by lightly spraying the bacteria over the blunt end of the egg or by contact with contaminated dry nest litter. Isolation from sprayed groups are positive upto 100 per cent while in litter contact group upto 50 per cent (Mario, 1990b).

Dissemination of salmonellosis in chicken operations cannot be controlled without knowing the sources and spread of the organism at the hatchery, breeding and commercial farms as well as processing plants (Javed and Hameed, 1989). A better understanding of this process would help in development of monitoring system for in formulating effective control programs. (Bhatia and Nabb, 1980). In spite of a high prevalence of salmonellosis in breeder flocks, only a few limited steps have been taken to eradicate this insidious problem of our poultry industry. Import of *Salmonella* free day-old parent chicks, feeding *Salmonella* free feed and uncontaminated drinking water are necessary control measures. Restriction on visitors, improving sanitary and managemental conditions are of great value. Competitive exclusion, addition of antibacterial drugs and vaccination is recent intervention to break the cycle (Javed and Hameed, 1989).

Control measures must not only prevent infection in the poultry themselves but must also take into account the extensive cross contamination that occurs during carcass processing. Some microbiological techniques for reducing infection are currently available. However, these have only been used in the UK and USA in a fragmentary fashion. Some control measures such as chemotherapy have, to some extent, been discredited. Others, such as competitive exclusion, chemical supplementation and immunization, have been shown to be useful under laboratory conditions but have yet to prove themselves in the field. Controlling bacterial infections in animals by the use of bacteriophages has yet to be tested in poultry. In view of the renewed interest in salmonellosis, generated by the recent epidemic of egg-associated *Salmonella* infections in the world, it is opportune to consider the prospects for control.

Chickens which are infected soon after hatching excrete more *Salmonella* organisms and for longer periods than do adult birds (Barrow *et al.*, 1988). This is attributed to the inhibitory activities of the complex microflora of the adult caeca (Barnes and Impey, 1979). Young chicks inoculated orally with a suspension or crude culture of faeces or caecal contents obtained from adult birds (Nurmi and Rantala, 1973) acquire the full resistance to infection possessed by the adult. Such undefined cultures are effective after extensive in vitro passage or after lyophilization and can be administered via the drinking water or by spray.

The mechanism of protection is poorly understood (Mead and Impey, 1987). It has been suggested that the normal flora competes with *Salmonella* organisms for sites of attachment within the caeca. Other explanations include the inhibitory effect of bacterial metabolites such as hydrogen sulphide and volatile fatty acids and the low redox potential generated by these organisms.

Salmonella is a common component of the commensal flora of the intestinal tracts of animals. However, some species of the microorganism can be quite pathogenic. The disease they cause in poultry can have severe adverse effects on the economy of the poultry industry (Williams *et al.*, 1984). A combination of techniques has been utilized to achieve significant control of *Salmonella* infection of chickens (Bryan *et al.*, 1979) and to produce a raise to maturity, for a limited period of time, turkeys that were free of *Salmonella* (Zecha *et al.*, 1977). Failure to eliminate *Salmonella*, or to maintain a permanent *Salmonella*-free status in these projects, was ascribed largely to an inability to eliminate *Salmonella*, or to maintain a permanent *Salmonella*-free status in these projects, was ascribed largely to an inability to eliminate the organism from feed. Contaminated feed is a major source of infection for poultry (Gangarosa, 1978).

The inclusion of penicillin in the diet was associated with an increase in *Salmonella* shedding, particularly in the first half of the rearing period, but did not influence the lactobacillary count in the crop or the Ph of the contents of

the crop, gizzard and caecum. Furazolidone medication (150 mg/kg feed) for the first 10 days had no effect on *Salmonella* carriage at the time of slaughter (Hinton *et al.*, 1986). Administration of nosiheptide (20 g/ton) for 33 days against *Salm. typhimurium* var copenhagen has been found quite effective. The effect of feeding halofuginone at 3 and 6 mg/kg of feed on the excretion of *Salm. typhimurium* by experimentally infected chickens was studied. Halofuginone at 3 mg/kg showed no significant increase in excretion rate. the group fed 6 mg/kg showed a slight increase in excretion which was statistically significant (Barrow *et al.*, 1988a).

Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed is an efficient method. In three experiments a solution of formic acid was added to feed "naturally" contaminated with salmonellae. In two of them no *Salmonella* infections were demonstrated in broiler chickens given feed containing 0.6 % (w/w) of the formic acid solution for seven weeks and in the third the infection rate was reduced considerably. The treatment of the feed with formic acid plus propionic acid mixture one week before the addition of the salmonellae prevented the establishment of infection in chicks given the treated feed (Hinton and Linton, 1988b). Feed given to laying hens with 0.5 per cent formic acid reduced significantly the isolation rate of salmonellae and was associated with a reduction in the incidence of infection in newly hatched chicks. Formic acid treatment of chicken feed could have important benefits for the public health (Humphrey and Lanning, 1988b). Chemical treatment of poultry feed reduced the chances of

survival of *Salmonella*. After treatment with a chemical preservative (Myco-Curb) at 0.25, 0.5, 0.75 or 1 per cent, decontaminate commercial poultry feed. The number of faecal and intestinal samples positive for *Salmonella* was reduced, demonstrating elimination of *Salmonella* in the feed by the use of the feed preservative (Rouse *et al.*, 1988).

The susceptibility of broiler chicks to *Salmonella* colonization is greatest during the first few days of life, after which resistance increases due to growth of normal intestinal flora (Barnes, 1979). Resistance to colonization provided by normal flora has been reported to be dependent on the level of *Salmonella* challenge and may be overcome by continuous or severe rechallenged (Pivnick and Nurmi, 1982). It has been reported that lactose added to the drinking water inhibited *Salm. typhimurium* colonization in 10-day-old broiler chicks (Oyofo *et al.*, 1989).

Proposed mechanisms by which normal intestinal flora prevent colonization by invading enteropathogens include: competition for limited nutrients (Fretor, 1956); competition for attachment sites on the intestinal mucosa (Loyd *et al.*, 1977); and the production of short-chain, bacteriostatic VFAS, particularly acetic, propionic, and butyric acids, by anaerobic bacteria present in the ceca and colon (Rolfe, 1984). VFAs produced by anaerobic bacteria were reported to inhibit salmonellae growth and colonization in mice and in poultry. The bacteriostatic action of VFAs is Ph dependent and is exerted only when the acids are present in the undissociated lipophilic

state. The concentrations of acetic, propionic and butyric acids present in the undissociated bacteriostatic state progressively increase as the Ph of the environment decreases and approaches the specific dissociation constant (Pka) of each fatty acid (Corrier *et al.*, 1990a and 1991).

Attempts have been made to eliminate *Salmonella* from poultry feed either by pelleting (Bryan *et al.*, 1979 and 1981) or by sterilizing feed components of animal origin before their incorporation into feed (Marthedal, 1977). At best, such attempts have resulted only in significant reductions in levels of contamination. Sterilizing only certain components of feed ignored the probability of contamination from one or more of the other components (Marthedal, 1977). Pelleting should have been more effective, since it involved treating the whole feed. However, the pelleting process is weighted heavily in favor of production of good quality pellets, and the conditions favoring the production of good quality pellets are unfortunately not always the same as those inimical to the survival of Salmonella. Mash is pelleted by forcing it through die openings, the producers being facilitated by treatment of mash with steam (conditioning) for about 20 seconds before compression. Under current pellet-production practices, steam generated in a broiler is the only medium through which the heat energy of fuel can be transferred to mash. This heat transference is accompanied by condensation of the steam and a corresponding increase in moisture content of mash being heated. It has been estimated that the moisture level increases by 1.0 per cent for every 11.1°C rise in temperature. For the relatively colder feed

generally available during winter in cold climates, this means that in order to raise mash temperature to a level high enough to kill *Salmonella*, the moisture content of the mash would have to be allowed to increase beyond the choke point of the pellet mill. In other words, the mash would become too moist for pelleting (McCapes *et al.*, 1989).

Recently, an equipment configuration called the anaerobic pasteurizing conditioning system is introduced to the feed industry (Beaumont, 1986). It is claimed to be capable of permitting the attainment of high mash temperatures without causing mash to become too moist. Fuel is ignited and combined directly with water the vaporator resulting in the production of steam, nitrogen, and other hot gases (CO_2, CO), which are channeled into the conditioner to heat mash. The direct utilization of all the hot products of this combustion is said to make it possible to control temperature independent of moisture levels.

The susceptibility of microorganisms to the lethal effects of heat is influenced by genetic and environmental factors. For instance *Salm. senftenberg* is less susceptible to heat than most other salmonellae. Naked strains of microorganisms, on the other hand, are generally more susceptible than encapsulated or sporulated forms. Susceptibility is also influenced by size of population of the microorganism as well as by changes in temperature, heating time, moisture, acidity, and composition of the medium in which the organism is being heated (Bryan *et al.*, 1979). Optimum conditions of temperature, heating time, and

moisture (optimum TTM) that will kill *Salmonella* is 87.8°C for 1.5 minutes, 89.4°C for 0.5 minutes at 15 per cent moisture (Liu *et al.*, 1969). Conventional pelleting is the most effective anti-*Salmonella* feed processing technique currently available. However, it only reduces the level of *Salmonella* in feed (Marthedal, 1977). More reductions in the level of *Salmonella* contamination of feed cannot be useful in a *Salmonella* elimination program, because only as few as one colony forming unit of *Salmonella* per gram of feed is required to initiate infection (Gangarosa, 1978). If the observed elimination of *Salmonella* from feed in the present study is real, the new pelleting process could be regarded as providing the missing link in the chain of technology needed to eliminate the organism from poultry (McCapes *et al.*, 1989).

Increased interest in methods to improve immunity to bacterial infections has arisen largely out of the fear that multiple antibiotic resistance might develop as a result of extensive chemoprophylaxis and chemotherapy. In poultry, development of such resistance in *Salm. typhimurium* and Escherichia coli has been demonstrated experimentally (Smith and Tucker, 1975, 1978). It has been suggested that antibiotics might be used in conjunction with competitive exclusion, the latter being used to re-establish a resistant flora after *Salmonella* organisms had been reduced by antibiotic treatment. The effect of such treatment on the development of antibiotic resistance, however, has not yet been studies.

Recently it has been shown that virulent bacteriophages are

more effective at controlling murine experimental E. coli infections than are antibiotics (Smith *et al.*, 1980). Phages have been used to treat and prevent neonatal E. coli diarrhoea in calves and pigs (Smith *et al.*, 1981). One of the many advantages of this approach over using antibiotics is that the mutants that arise following the development of phage resistance are frequently rough and because of this are of reduced virulence. It would be interesting to known whether suitable broad host range virulent phages could be found for *Salmonella*. Further studies on the use of phage have been advocated.

Competitive exclusion is a physical and chemical overcome of non-pathogenic *lactobacilli* over the pathogenic salmonellae by competitive exclusion. *Lactobacilli* compete with the salmonellae on receptors to discourage the *Salmonella* colonization. *Salmonella* isolation were undertaken on day 4, 8 and 12 post *lactobacilli* treatment from crop and ceca in all the treatment groups *Salm. gallinarum, Salm. pullorum* and *Salm. typhimurium* colonization was discouraged significantly by *lactobacilli* in T2, T3 and T4. Five chicks from each treatment groups were slaughtered on day 4, 8 and 12 and homogenate as well as washing of crop and ceca were cultured for the mean log *Salmonella* count. The mean log *Salmonella* count in control (T1) group increased in crop on day 4, 8 and 12 in homogenate samples while there was decreasing trend in washing samples from crop on day 8 and 12 as compared to day 4. The *Salmonella* count increased in cecum on day 8 and 12 as compared to day 4 in control group (T1) among homogenate samples, while there was marked decrease on day 8 and 12 in washing samples. As compared

with typical trend in control group salmonellae count in T2 where *Salm. gallinarum* was challenged the count in homogenate samples were increased upto day 8 and then decreased on day 12. Almost identical trend was noted in the T3 and T4 groups where *Salm. pullorum* and *Salm. typhimurium* were challenged respectively. In treatment groups *Lactobacilli* reduced the colonization rate that was the reason of reduced mean log *Salmonella* count in homogenate samples and washing on day 12. The increased *Salmonella* count in control is due to colonization and shedding of salmonellae in the organ's lumen. A significant reduction was noted in cecum and crop where *lactobacilli* were administered.

The most desirable means of disease prevention is to be in an area where certain diseases do not exist. This is accomplished by excluding diseases from theses areas through a system of strict isolation, sanitation and regulation. Virkon and Beloran two disinfectants were tried to chick their killkinetic *in vitro*. Virkon and Beloran proven to be the best choice of disinfectants against salmonellae. Virkon have a little more effective than Beloran but the differences were nonsignificant. There was good temporal response among both the disinfectant. There was a strong correlation between time and microbicidal effect of Virkon and Beloran. Standard microbicidal effect index is > 6.0 according to this standardization at 20°C with one per cent concentration for 5 minutes exposure. Virkon and Beloran was effective 100 per cent against all the isolates of salmonellae except *Salm. gallinarum* where it was not effective 100 per cent for 5 minutes exposure. Beloran was

effective to all the isolates tested whereas it was not effective 100 per cent against *Salm. gallinarum* and *Salm. typhimurium* at 5 minutes exposure. Beloran kill 100 per cent *Salm. gallinarum* at 30 minutes exposure and *Salm. typhimurium* at 15 minutes exposure.

Virkon was more efficient as compared to Beloran, while the both disinfectants were ideal for in farm or poultry house-ware disinfectants. Both the disinfectant were 100 per cent effective to kill the isolates *in vitro* at 20°C in 5 minutes except *Salm. gallinarum* where it needs 15-30 minutes exposure. As a control measure one per cent solution of disinfectants is 10 time more as recommended level so farm spray will be effective at 0.1 per cent level.

The membrane disruptive and antimicrobial activities of cationic surfactants are well recognized. These agents are often active against a broad range of bacteria and other cells and can also inactive certain viruses (Hugo and Russel, 1982). Because of their high affinity for biological membranes, these agents show a low selectivity and can be damaging to a variety of mammalian cells (Pinnaduwage *et al.*, 1989). Since the time needed to kill microorganisms with cationic surfactants is usually short, it could be expected that side effects in the host might be decreased by the use of substances that are subject to hydrolytic degradation. However, the life-time of the compounds must be sufficiently long to allow proper inactivation of the undesired microorganisms. The products obtained in the degradation steps should also be significantly less toxic than

the original compounds and should ideally constitute normal metabolites of the host (Lindstedt *et al.* 1990). To explore the possible use of degradable cationic surfactants, a series of amphophilic betaine esters have been studied. The interaction between cationic surfactants and microbial cells is not understood in detail. It seems generally accepted, however, that lipid bilayer structures of cell membranes are principal targets for this class of compounds. In the process of bindings, the hydrocarbon tail of the cationic amphophilic substance becomes intercalated into hydrophobic interior of the microbial membrane, and the cationic folar head group participates in charge interactions with neighboring surface structures (Jawetz *et al.* 1989).

As for stable quaternary ammonium compounds, the initial site of interaction of the betaine esters is probably the lipid bilayer of the outer membrane. Furthermore, these substances cause leakage of cytoplasmic compounds, indicating that the plasma membrane is also affected (Hugo, 1982). The phospholipids of both types of membranes contain fatty acids, mainly C_{16} and C_{18} (Cronan and Rock, 1987), and there is a rapid exchange between the phospholipids of the outer and inner membranes. In the lipopolysaccharide of *Salmonella* strains, the 3-hydroxy-tetradecanoic acid residues, which are amide linked to the glucosamine moieties of lipid A, are 3-O acylated by C_{12} and $C16$ saturated fatty acids, allowing a hydrocarbon chain length in the outer cell membrane of at least 18 carbon atoms. Thus, the high bactericidal activity of the C_{16} and C_{18} betaine esters may be due to the facts that both the outer and the plasma

membrane lipid bilayers may accommodate the entire hydrocarbon chain length of these betaine esters and that longer hydrophobic chains have a greater hydrophobic effect (Tanford, 1980). Change interactions between quaternary nitrogen groups in betaine esters and phosphate groups in phospho lipids and lipopolysaccharide may contribute to complex formation. The higher antibacterial activity of octadecyl quaternary ammonium compounds was shown the earlier comparative tests of series of substances with different hydrocarbon chain lengths and other chemical structures adjacent to the quaternary nitrogen (Linfield, 1970). Because the time needed for microbial killing is short, a reduction of the life time of the esters by hydrolysis should allow effective disinfection and antisepsis with reduced toxic effects.

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