

STUDIES ON COLIBACILLOSIS IN CHICKS WITH PREVENTION AND CONTROL

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in
VETERINARY MEDICINE**

**BY
BAIDYA NATH MUKHOPADHYAY**

**DEPARTMENT OF VETERINARY MEDICINE AND PUBLIC HEALTH
FACULTY OF VETERINARY AND ANIMAL SCIENCES
BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
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*Dedicated in memory
to my beloved parents*

BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
FACULTY OF VETERINARY AND ANIMAL SCIENCES
MOHRNPUR, NADIA, WEST BENGAL.

Dr. S. K. Misra,
C.M.V.C., B.V.Sc., P.G.(Edin),
F.R.V.A.C. (Copenhagen), Ph.D.
Professor,
Veterinary Clinical Medicine,
Department of Veterinary Medicine
and Public Health

C E R T I F I C A T E

This is to certify that the work recorded in the thesis entitled "STUDIES ON COLIBACILLOSIS IN CHICKS WITH PREVENTION AND CONTROL", submitted by Shri Baidya Nath Mukhopadhyay for the award of the Degree of Doctor of Philosophy in Veterinary Medicine of the Bidhan Chandra Krishi Viswavidyalaya, is the faithful and bonafide research work, carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

Dated: Mohanpur

The 25.11.1989.


(S. K. MISRA)
ADVISOR

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CHAPTER I

INTRODUCTION

Poultry farming in India has for a long time remained largely a rural cottage enterprise but today it has become one of the fastest growing industry in the country. It has made spectacular progress during the last two decades from a non-commercial backyard farming to a fulfilled commercial and profitable agro-industrial enterprise helping in the development of national socio-economic growth of the country. This has been achieved through improved breeding, management and health cover. Poultry industry has a potential source of employment to the rural mass. In addition, this is a significant tool for providing self employment, supplementary income and eradication of rural poverty and at the same time combat malnutrition by providing cheap source of protein of high biological value. Poultry farming has all the year round business and the farmers get a continuous flow of income through egg and meat productions (Sidhu and Rangl, 1980).

To-day, India ranks amongst the world's fifteen leading countries in egg production. The poultry population in India is 182 millions including 95 million broiler and 87 million layers. The total egg production per year is 15,225 millions (Panda, 1987). The present availability of eggs in India is only 20 per head per year as against 352 in U.S.A., 341 in Belgium, ^{and} 305 in U.K. (Taneja, 1988). The annual per capita consumption of poultry meat in this country is about 160 g

(Supekar, 1983). We have yet a long way to go from the current per capita consumption of 20 eggs to 180 eggs and 160 g of poultry meat to 10 kg per annum. The broiler production is gaining momentum attaining a level of 50 million in 1983, 70 million in 1985 compared to 4 millions in 1971 (Taneja, 1988). In next 5 years the broiler production in the country is expected to double to 150 million on a moderate estimate.

In West Bengal, according to 1984 Census of the Government of West Bengal there are about 28 million poultry including ducks etc. There has been a noticeable development in the poultry industry in the State due to much awareness among the farmers particularly for broiler production (Report, 1984).

A major threat to the development of poultry industry in the country is due to a number of devastating infectious diseases which cause substantial economic loss every year. Intensification has improved production but disturbed the ecosystem of the birds which put them under stress leading to complications in disease problems (Panisup and Verma, 1986). Mortality can be attributed to many factors but Colibacillosis in chicks is one of the diseases causing heavy loss to poultry industry. Most cases occur in chickens of 6-10 weeks age but there is often an early smaller peak of incidence in chicks upto the age of 2 weeks. Colisepticaemia affects ducks and turkeys also. The peak incidence is usually earlier in ducks

and later in turkeys. This is a major disease problem in chicks. According to Hofstad (1975) Escherichia coli infection includes colibacillosis, colisepticaemia (Hjarres diseases) coligranuloma, peritonitis, pericarditis, salpingitis, synovitis, omphalitis, airsac disease and all other disease conditions caused entirely or partly by E. coli. The common diseases caused by E. coli in chicks are yolk sac infection (omphalitis, naval infection, Mushy chick disease), colibacillosis, pericarditis, perihepatitis, air sacculitis, salpingitis, peritonitis, synovitis, cophoritis and in chronic infections coligranuloma in liver, airsac and intestines are seen (Panda and Verma, 1988). Collectively, they are responsible for a major economic loss to the poultry industry (Savov, 1973). In India, omphalitis (Pathak et al., 1960), colisepticaemia (Verma and Adlakha, 1971) and egg peritonitis (Gupta and Singh, 1969 and Kulkarni et al., 1970) in poultry have been reported. The poultry industry suffers heavily due to E. coli infection since it causes early chick mortality and lower meat/egg production. Though it was recognised since long, yet it was over looked or was considered as a secondary infection. In the recent past, E. coli has posed a serious threat to poultry industry and it has become one of the major problem^h of poultry farmers (Shukla et al., 1987). In recent years more attention has been paid to the role of E. coli as a potential pathogen of domestic fowl (Pradhan et al., 1973, and Srinivasan et al., 1979). There are a good number of reports available implicating E. coli in causing severe economic losses to poultry industry.

Infection caused by E. coli ^{is} are becoming more and more prevalent among all poultry. This microorganism is widely spread in nature and is a normal permanent inhabitant of the lower intestines of all animals including man. However, when sufficiently large numbers are present, infectious occur that result in 2% to 10% among young birds frequently at an age of 7-9 weeks.

Conditions favourable to the rapid multiplication of this organism must be avoided. An outbreak of E. coli indicates poor sanitary conditions exist, so a great care should be exercised to maintain high level of sanitation. Stressful conditions frequently result in outbreak where marginal level of microorganisms are present.

Escherichia coli is a typical coliform organism commonly seen in the intestinal tract and is present in large numbers in the faecal samples of animals and birds. In healthy birds, the coliform content of the intestine is less than one percent. The organism is a non-capsulated, motile short rod in size but short coccobacillary and long filamentous forms are occasionally seen. On solid media E. coli produces large colonies which are smooth, ^{and} glistening. On blood agar media it is non-haemolytic but haemolytic forms are sometimes seen. Majority of them ferment lactose with production of acid and gas. Does not liquify gelatin.

Approximately about 100 serotypes of E. coli are reported. All over the world more than 50 different 'O' groups are mainly responsible for some of the disease conditions like colisepticaemia (Sojka and Carnagham, 1961). Serotypes 01, 02 and 078 are common pathogen to avian species. Serogroups 08, 09, 018 and 011 were isolated by Gupta and Singh (1969b) as pathogen to avian species. Colibacillosis is a major disease problem in chicken. E. coli organisms, though normal inhabitants of the intestinal tract, are potential pathogens to the chicks exposed to various stress factors. The stress of deficiency of vitamin A and E, general debility, cold exposure, transportation, faulty incubation, fowl pox and Ranikhet disease vaccination, presence of other diseases or poor quality of feed ingredients precipitate this infection (Shukla et al., 1987). The primary infection with pathogenic E. coli can occur but is rare. Many other stress factors bring about weakness in ~~body~~ defences caused by other infectious agents like Mycoplasma gallisepticum infection (CRD), Adenovirus infection, immunosuppression due to IBD and aflatoxicosis, non-fatal Ranikhet disease, infectious Bronchitis, excess ammonia, high environmental temperature etc. may induce colibacillosis (Panda and Verma, 1988). There may be other stress-factor(s) present which need further investigation.

Colibacillosis is transmitted by shell penetration, artificial insemination, transovarian infection and horizontal

transmission by contamination of feed and water, infected litter, dust particles, contaminated instruments, equipment in the farm and mechanical carriers. The clinical manifestations of the disease are diarrhoea and death in acute forms in very young chicks. There is poor growth and loss of production. The mortality and morbidity rate is very high. Chronic cases are seen as coligranulomatous changes in liver, airsac and intestines. Post-mortem changes include pericarditis, perihepatitis and air sacculitis. Sharma and Joshi (1983) reported that in cases of oophoritis, salpingitis and egg peritonitis 6 serotypes of E. coli are associated. Bandyopadhyaya and Dhawedkar (1984) isolated pathogenic serotypes 017, 019, 05 and 060 from cases of salpingoperitonitis. Post-mortem lesions are swollen, dark red ovaries, misshapen and congested ova and inflamed oviduct. In case of broilers, synovitis of hock joint is commonly seen due to E. coli infection. Yolksac infection (Mushy chick disease), omphalitis or naval infection due to E. coli is the commonest of all diseases causing early mortality. The clinical signs are that the chicks so affected are usually normal to start with but die mostly from 24-72 hours after placing them in the brooder. Dead chicks show blowup abdomen, wet or scabby naval, ^{and} offensive smell which ^{are} ~~is~~ diagnostic. Post-mortem changes show unabsorbed yolk filled with discoloured brown liquid and the whole abdominal cavity may be infected. This is usually due to mismanagement

of the disease flock or bad hygiene, poor hatchery management etc.

Colibacillosis in chicks can be diagnosed from clinical symptoms, post-mortem lesions of affected birds, isolation of coliform organisms from the lesion sites. Confirmatory diagnosis can be made by isolation of E. coli from heart blood of sick birds in special media. Pathogenicity tests in laboratory animals like mice, rabbits and chicks can be done.

Various antibiotics and sulphonamides have been widely used for treatment and control of the disease but found unsuccessful in the control of E. coli infections due to drug resistance. Due to indiscriminate use of several antimicrobial agents emergence of drug resistance among various bacteria has developed (Kapoor et al., 1978). The emergence of multiple drug resistant strains of E. coli recovered from poultry have been reported by Kulshrestha and Kumar (1976). The gut strains of E. coli are resistant to chloramphenicol and Furazone (Mehrotra et al., 1984). Higher rate of tetracycline resistance is recorded by Walton (1984). Transfer of resistance has been reported by Ramaswamy et al. (1982) in 38 strains of E. coli isolated from diseased poultry to the extent of 64.1%, 62.5%, 55.0% and 35.0% with streptomycin, kanamycin, chloramphenicol and Trimexazole respectively. Sambyal et al. (1981) also reported the following drug sensitivity given in parenthesis : Nalidixic acid (83%), Nitrofurazone

5 or 8

(76%), Streptomycin (69%), Kanamycin (93%), Oxytetracycline (31%), Chlortetracycline (23%), Tetracycline (39%), Chloramphenicol (86%) and Tripple sulphonamide (19%). However Doxycycline is reported to possess high activity. The drug is given @ 50 ppm in drinking water. Sulphadimethoxine or Furazolidone also ^{has} have similar effect. Hence, many of the commonly available drugs are not responsive to the infection in many cases leading to hazzards in poultry farms. Hence, sensitivity against the pathogenic isolates is essential for therapeutic and control measures in an outbreak.

Adequate ventillation without dry dusty condition avoidance of overcrowding and clean, regularly disinfected waters are important factors to avoid outbreak of colibacillosis.

In view of the above facts, the present study was undertaken with the following objectives :

1. To study the incidence of colibacillosis in chicks in poultry farms.
2. To isolate and identify the strains of Escherichia coli from suspected cases of colibacillosis in chicks.
3. Serological typing of E. coli in collaboration with the National Typing Centres for E. coli.
4. To study the pathogenicity of the isolates in laboratory animals like mice and rabbits.

5. Experimental production of colibacillosis in young chicks with most virulent strain(s) and to study the clinical manifestations and post-mortem changes.
6. Antibiotic sensitivity tests on the most pathogenic isolates.
7. Experimental production of colibacillosis in chicks with the most virulent isolate with and without latent infection of coccidiosis and therapy.
8. Control

CHAPTER II

REVIEW OF LITERATURE

2.1 Incidence of Colibacillosis in Chicks

2.1.1 Incidence of colibacillosis in chicks in poultry farms

Harry (1964) reported that outbreak^b of Colisepticaemia were particularly frequent in chickens aged 7 to 9 weeks. He also recorded the disease in 1 to 2 week old chicks. Mortality in most cases was less than 2.5% and morbidity above 10%.

Sandulescu et al. (1977) examined 445 chicken carcasses from 6 broiler units which yielded 121 strains of E. coli.

Oskolkov and Saltykov (1977) isolated 869 strains of E. coli from dead fowls of 7 poultry farms in U.S.S.R.

Bozorgmehri-Fard and Gilani (1980) made a survey of colibacillosis in chicks in 9 month period around Teheran and reported that from SISO chicks in 500 flocks, 18% birds were positive for colibacillosis.

Sambyal et al. (1981) carried a survey of large poultry farms around Ludhiana and isolated E. coli from Cloacal samples of healthy fowls, feed, water and litter samples.

Watanbe et al. (1983) reported colibacillosis in chicks from Japan. Out of 12,000 broiler chicks of 7 to 9 weeks age groups 738 (6.1%) died of colisepticaemia.

Dho and Lafont (1984) observed virulant strains of E. coli isolates from poultry.

Barbour et al. (1985) isolated E. coli from post-mortem materials of chicks with symptoms and lesions of typical colisepticaemia in Saudi Arabian broiler farms.

Cloud et al. (1985) isolated 177 E. coli serogroups from 145 broiler farms of peninsula area over a period of two and half years and 20 from normal 1 day old hatchery chicks.

Dominick (1985) observed that virulant (high and low) strains of E. coli were present in intestinal villi and lungs of 3 week old probiotic turkeys.

Sinha et al. (1985) reported the prevalence of E. coli in intestine and extra - intestinal sites of poultry. Out of 195 samples received from various poultry farms, 85 strains of E. coli were isolated.

Nakamura et al. (1986) observed that out of 134 broiler chicks screened for colibacillosis, 48 were microbiologically diagnosed to have colibacillosis.

Prukner (1986) reported that 81.46% E. coli strains were isolated in bacterial diseases of poultry from Yugoslavia.

Sporenberg et al. (1986) observed field outbreak of colibacillosis associated with haemorrhagic enteritis infections in 6 to 12 week old turkey birds.

Verma (1987) studied 205 samples (90 sick or dead birds, 65 litters and 50 dead-in-shell embryos from 11 poultry farms. In all 41 (45.5%) E. coli isolates were obtained from the sick/dead birds, 15 (30%) from dead embryos and 53 (81.5%) from litter samples.

Phukan (1988) during the course of survey isolated 103 E. coli strains from dead birds. He estimated the overall infection rate of colibacillosis as 50.84%. The infection in younger chicks was 63.20%.

2.1.2 Sources of materials, and isolation of Escherichia coli

Glantz et al. (1962) isolated 122 strains of Escherichia coli from fowls with chronic respiratory disease and 72 from cases of salpingitis.

Bakajle and Prost (1963) collected materials from sero-fibrinous pericarditis caused by E. coli in 211 cases out of 30,230 table poultry birds.

Nayak et al. (1967) isolated strains of E. coli from proventriculus, small intestine, caeca, liver, pancreas, kidney, bone marrow and eyelid.

Prasad et al. (1967) isolated strains of E. coli from organs of an adult white leghorn hen with granulomatous lesions.

Stipkovits and Solyom (1968) recovered strains of E. coli from the yolk sac and liver of 222 out of 267 dead and 51 out of 78 clinically ill chicks.

Gupta and Singh (1969a) isolated 17 strains of E. coli from 21 cases of egg peritonitis in hens.

A total of 40 E. coli strains isolated from the heart blood of chicks with fresh septicaemic infections by Gupta and Singh (1969b).

Kulkarni et al. (1970a) isolated 33 E. coli strains from 231 samples of caseous exudate in the peritoneal cavity, inflamed peritoneum, oviduct and ovary in cases of egg peritonitis.

Kulkarni et al. (1970b) examined 105 specimens culturally from cases of chronic respiratory diseases in poultry and yielded 52 strains of E. coli.

A total of 64 E. coli strains were isolated from 82 dead birds and 10 apparently healthy birds by Garg and Sethi (1971).

Jayaraman and John (1971) isolated 51 strains of E. coli from 251 specimens of poultry examined.

Verma and Adlakha (1971) isolated 108 E. coli strains from 359 cases of pneumonia, septicaemia, egg peritonitis, enteritis, persistent yolk sac and chronic respiratory diseases of poultry.

Yadav and Malik (1971) isolated 42 strains of E. coli from egg yolk, embryos and various organs of the dead chickens.

Damodaran and Thanikachalam (1972) isolated 2 strains of E. coli from one case of coligranuloma.

Venugopalan et al. (1974) isolated 78 strains of E. coli from fowl with enteritis or colisepticaemia or dead in shell embryos.

Rao et al. (1976) recovered a total of 347 E. coli strains from five different regions of the intestinal tract of poultry.

Kulshrestha and Kumar (1977) recovered 1215 E. coli isolates from poultry to various biochemical tests. They

reported that majority of the isolates were positive for Methyl Red and Indol tests. Few non-motile and H_2S producing variants were also reported.

Sandulescu et al. (1977) examined 445 chicken carcasses from 6 broiler units which yielded 121 strains of E. coli.

Farid et al. (1977) recovered 295 E. coli isolates from faeces of apparently healthy chickens (150) and from the internal organs of dead chickens with typical post-mortem picture of colisepticaemia.

Oskolkov and Saltykov (1977) isolated 869 strains of E. coli from dead fowls of 7 poultry farms of U.S.S.R.

Escherichia coli was isolated from 40 of 110 cases of oophoritis and from 35 of 85 cases of salpingitis by Sahota et al. (1978).

Farias (1979) examined 414 specimens (poultry and eggs) and isolated 166 strains of E. coli.

From 5150 fowls examined within a period of 9 months Bozorgmehri - Fard and Gilani (1980) isolated 90 strains of E. coli.

In 75 cases of reproductive disorders like oophoritis, salpingitis, egg peritonitis and other conditions (34, 13, 23

and 5 respectively), a bacteriological aetiology was established in 69 cases of which E. coli was isolated from 45 cases (Sharma et al., 1980).

Farid et al. (1981) isolated E. coli from 138 heart blood samples of 1450 dead chickens.

Ramaswamy et al. (1982) observed that out of 117 E. coli strains isolated from 247 chickens with various diseases, 10 different serotypes were recorded.

Kin and Tak (1983) from Korea studied pathogenic E. coli isolated from chicks with colibacillosis. They isolated 488 E. coli strains from pericarditis, hepatitis, airsac infections, salpingitis, septicaemia and various other conditions.

Bandyopadhyay and Dhawedkar (1984) examined 711 hens and salpingitis was detected in 80 cases. E. coli strains were isolated from 27.5% of these cases.

Cloud et al. (1985) obtained 177 E. coli strains from 145 field reared broiler flocks and 20 from clinically normal one day-old chicks.

Meliota et al. (1985) isolated 246 strains of E. coli from the brain, spleen and pericardium of fowl and turkeys.

Rosenberger et al. (1985) studied factors associated with pathogenicity of E. coli strains isolated from commercial broiler chicks. Most isolates had similar metabolic activity determined by amoniacid decarboxylation, and carbohydrate fermentation. It was also observed that in pathogenic strains adonitol was frequently fermented.

Sinha et al. (1985) by selective isolation on 195 samples from birds received from different poultry farms, recovered 85 E. coli strains of which 35 were from intestines, 15 from hearts, 13 from lungs, 14 from livers and 8 from spleen.

Wang et al. (1985) from China studied E. coli infections in the reproductive organs of geese and isolated 5 strains of E. coli.

Sarma et al. (1987) isolated 100 E. coli strains from different organs of 57 diseases in domestic fowls.

Verma (1987) isolated E. coli strains from materials collected from sick/dead birds, litter samples and dead embryos.

2.1.3 Serological typing of Escherichia coli

Glantz et al. (1962) isolated 122 strains of Escherichia coli from cases of chronic respiratory disease and salpingitis of fowls, of which 31 belonged to group 2a, 19 to 078,

14 to 0109, 6 to 082, 5 to 0103, 4 each to 01ab and 013 and 3 each to 0 groups 15, 17, 111ac and 119. Fifteen strains did not react with standard '0' group antisera.

Bekajle and Prost (1963) could identify serotypes 071, 08 and 03 from cases of serofibrinous pericarditis of poultry.

Stipkovits and Bereznai (1967) could isolate number of strains from carcasses with mild lesions having most frequent '0' groups 78, 87, 3, 35, 15, 95, 74, 109, 20 and 141. Some were found to be untypable.

Out of 21 strains of E. coli isolated from chickens aged 42 - 44 days by Belitskii and Panikar (1969), 17 belonged to group 078, while four belonged to 111.

• Serotyping of 15 strains of E. coli isolated from 21 cases of egg peritonitis in hens revealed that four belonged to serogroup 08 and one each to 09, 017, 018, 023, 051, 088, 0101 and 0111, while the rest three were untypable (Gupta and Singh, 1969a).

Gupta and Singh (1969b) obtained 40 strains of E. coli from the heart blood of 351 chicks of which nine belonged to serogroup 078, k80, three to 01:k1, five to 02, four each to 08 and 023, two each to 010. 04, 026 and 0111 and one each

to 039, 07, 0143, 021 and 054, one strain was untypable and another was a rough variant.

Kulkarni et al. (1970b) observed that out of 52 strains of E. coli isolated from poultry, six belonged to 'O' group 111, four to 8, two each to 78, 1 and 6, and one each to 0136, 03, 0115, 023, 0103, 024. The remaining strains could not be typed.

Yadav (1970) isolated ten 'O' groups from infections of poultry septicaemia. They are 01, 02, 086, 091, 0101, 0103, 0108, 0119, 0128 and 0143, out of them the most common were 086, 0119 and 0128.

Jayaraman and John (1971) isolated 51 strains of E. coli belonging to groups 01, 02, 09 and 027 from poultry.

Verma and Adlakha (1971) recovered E. coli from 108 cases of pneumonia, septicaemia, egg peritonitis, enteritis, persistent yolk sac and chronic respiratory disease of poultry. About 30 percent of them could be serotyped as group 08 being predominant followed by groups 01, 078, 018, 02 and 0111.

Yadav and Malik (1971) isolated 42 E. coli strains from egg yolk, embryos and various organs of dead chickens. Out of them only 29 could be serogrouped. The strains associated with colisepticaemia were grouped as 08, 078, 028, 086, 02,

051 and 0143, 'O' groups encountered in other conditions were 8, 83 and 105 from oophoritis, 14, 83 and 89 from enteritis and 147 from chronic respiratory disease.

Arunachalam et al. (1974) reported isolation of sero-groups 078, 02, 08, 09 and 029 of Escherichia coli from septicaemia and enteritis in poultry birds.

Venugopalan et al. (1974) isolated 78 strains of E.coli from fowls with enteritis or colisepticaemia or dead in shell embryos which belonged to 'O' groups 69, 131, 15, 8, 53, 61, 51 and 23.

Sandulescu et al. (1977) isolated 121 E. coli strains from 445 chickens and were subjected to serotyping of the strains. The commonest 'O' groups were found to be groups 2, 5, 8, 1 and 78.

Farid et al. (1977) isolated 295 E. coli strains. The serological typing revealed that the faecal isolates taken from 9 different breeds belonged to 'O' groups 1, 2, 11, 42, 53, 71 and 78 while those from the internal organs of dead chickens belonged to 'O' groups 1, 2, 8, 22, 42, 53, 71 and 78.

Oskolkov and Saltykov (1977) recovered 869 strains of E. coli from fowls. The most prevalent groups were 0111, 02, 01, 078, 0103 and 08.

Sahota et al. (1978) reported the isolation of 18 strains of E. coli from cases of oophoritis and salpingitis which belonged to 'O' groups 8, 17, 1, 15, 20, 29, 23, 26, 69, and 79.

Farias (1979) reported isolation of E. coli strains from poultry. From 414 samples (birds and eggs) 166 were positive to E. coli and out of them only 22 could be typed. Most common was 04. The other serogroups rarely found were 08 and 078.

Srinivasan et al. (1979) reported that the most common serogroups prevalent in chicks are 029 and 01; 03 and 01 in turkeys; 01, 010 and 02 in quails and 042 and 01 in ducks.

Sharma et al. (1980) conducted serological typing of 45 strains of E. coli from cases of reproductive disorders which revealed that the most predominant serotype was 01 followed by 08 and 078.

Farid et al. (1981) from Egypt reported isolation of E. coli in pure cultures from 183 heart blood samples of 1450 dead chickens, 131 strains were serotyped into 24 different 'O' groups. They were 08, 02, 0128, 078, 0126, 0114, 0127, 0119 and 0157.

Ramaswamy et al. (1982) observed that out of 117 E.coli strains isolated from 247 chickens with various diseases, 10 different serotypes were recorded of which 01, 05 and 022 were the most common.

Kim and Tak (1983) isolated 488 strains of E. coli from various disease conditions of birds of which 224 were classified into 18 - O groups, the most prevalent 'O' group being 0128.

Sarma and Boro (1983) made isolation of 23 'O' sero groups from 202 diseased and apparently healthy chicks 183 diseased and 53 apparently healthy.

Saxena et al. (1983) reported that the serogroups frequently isolated from poultry were 01, 02, 017, 022, 025, 029, 034, 060, 069 and 078.

Sechter et al. (1983) recovered 3117 E. coli strains from diseased fowls which belonged to 49 'O' groups. Groups 78, 2 and 88 represented together 55.55 percent of all the isolates. Other frequent groups were 111, 18, 1 and 8 (7%, 5.1%, 3.2% and 2.6% respectively).

Watanbe et al. (1983) isolated 'O' serogroup of E.coli from colibacillosis in chicks from Japan. Serogrouping of 137 strains showed 89.8% belonged to eight 'O' groups, '01' was

detected in 43.1%, 0.78 in 26.3%, 02 in 14.6% and 08 was not detected.

Bandyopadhyay and Dhawedkar (1984) reported that the most prevalent E. coli serotype was 017 (43-45%) in salpingitis cases of poultry. The other serogroups were 05, 019 and 060.

Cloud et al. (1985) obtained 177 E. coli strains from broiler flocks. Serotyping of these isolates revealed a predominance of 02, 035 and 078 serogroups and a large number of untypable isolates.

Mellota et al. (1985) studied Escherichia infection in poultry and 'O' group of the strain isolated group 'O' antigens were identified by slow agglutination test in 246 strains of E. coli isolated from brain, spleen and pericardium of the fowls and turkeys. The common group identified was 078, followed by 02, 0141 and 0139.

Sinha et al. (1985) isolated 85 E. coli strains from different organs of diseased poultry of which 38 belonged to 10 serogroups : 01 (4 isolates), 02 (3), 05 (19), 07 (1), 017 (3), 025 (3), 034 (1), 035 (1), 060 (2) and 066 (1).

Wang et al. (1985) from China studied the E. coli infection in the reproductive organs of geese. Five

strains were isolated from affected ova and peritoneum which belonged to 02, k89, 039 and two unknown groups.

Orajaka and Mohan (1986) randomly selected dead in shell chicken embryos from commercial hatcheries in Nigeria and examined for E. coli. Bacterial cultures were made from 79 pooled samples containing 632 dead in shell embryos. Twenty three strains of E. coli among which serogroups 01, 02, 055, 08, 086, and 0111 were known to be associated with pathological changes in poultry were isolated.

Prukner (1986) observed that E. coli strains were isolated from poultry and 078 serotype was most often isolated.

Murray (1986) isolated and serotyped 425 E. coli isolates from various sources, out of which 115 were from chicken sources and typed. They belonged to '0' groups.

Ghosh (1987) isolated 50 strains of E. coli from 437 dead embryonated eggs which were serotyped. The dominant serotype was 061 (22.44%) followed by 062, 0126 and 0147.

Ghosh (1987) reported mortality of 37% in an organised poultry farm between July and September 1985 in Nagaland. On post-mortem examination the cause of death was found to be E. coli infection on the farm. Of the 25 serotypes isolated, the predominant serotypes were 061, 0143, 0147, 091 and 0119.

Sarma et al. (1987) recovered 100 E. coli isolates from different organs of 57 diseased domestic fowls of which 99 were classified into 56 serotypes. Serotypes 02, K1:H5, 078:H, 08:H19, 09:H8, 023:H, 01:H7, 023:H16, 01:H1 and 0143,H4 represented 45, 4% of the total typed strains.

Verma (1987) examined 205 samples (90 sick or dead birds, 65 litter samples and 50 embryos (dead in shell) from poultry farms of isolated E. coli from 41 of 90 (45.5%) sick/dead birds, belonged to 14 serotypes of which the most frequent were 087, 022, 0132 and 088, 53 (81.5%) litter samples yielded E. coli while 10 serotypes were identified from 15 of 50 (30%) dead embryos. Isolates from pathological conditions were similar to those from other poultry sources.

Phukan (1988) while conducting a survey in Guwahati area of Assam, isolated E. coli serotypes as 02, followed by 020, 088, 0158, 078, 0137, 03, 07, 085 and 0154.

2.2 Pathogenicity Test of Escherichia coli Isolates

2.2.1 Pathogenicity test in mice

Bekajle and Prost (1963) conducted pathogenicity test of E. coli serotypes 071 and 08 on mice. By injecting 0.1 ml of 24 hour culture intravenously, the mice were killed.

Gupta and Singh (1969b) studied pathogenicity of E. coli strains in white swiss mice of about $1\frac{1}{2}$ - $2\frac{1}{2}$ months of age. Out of 54 strains of E. coli, 40 strains were found to be pathogenic for mice on experimental inoculation through the intraperitoneal route. Out of these strains, 9 belonged to 078, 3 to 01, 5 to 02, 4 each to 08 and 023, 2 each to 018, 04, 026 and 0111 and 1 each to 039, 07, 0143, 021 and 054. One was untypable and one was rough.

Ellis et al. (1974) used suckling mice for detection of enteropathogenicity of E. coli isolated from calves. Bacteria-free supernatant fluids from cultures of E. coli tested by oral administration to unweaned mice showed pathogenic to them.

Gupta (1975) observed pathogenicity of E. coli serogroups in adult white mice weighing 18 - 25 g after intraperitoneal injection. Four serogroups (05, 09, 011 and 015, were highly pathogenic and one serogroup (03) of doubtful pathogenicity. Fifteen serogroups (06, 07, 010, 013, 015, 016, 023, 034, 037, 041, 042, 054, 057 and 065) were nonpathogenic.

Dey et al. (1977) reported that 016 and 036 serogroups from diarrhoeic calves were pathogenic for mice. Virulent and avirulent strains occurred with same 'O' group due to difference in 'H' antigen type.

• Srivastava and Arya (1979) reported isolation of E.coli serogroups from 13 calves died of enteric colibacillosis. They tested the pathogenicity of the isolates in mice by intraperitoneal injection, serogroup 022 was reported pathogenic for mice.

Sharma et al. (1981) studied the pathogenicity of E. coli of poultry origin. They observed that out of 24 E.coli strains belonging to 'O' group, 23 were pathogenic to chicks and 21 to mice. E. coli strains of groupⁿ 025, 049, 060, 078, 090 and 0160 were equally pathogenic to mice and chicks producing 100% mortality of the two E. coli strains belonging to group 017, one was nonpathogenic while the other was pathogenic to chicks, E. coli strains in groups 015, 018, and 048 were pathogenic to mice. The macroscopic lesions observed in chicks were peritoneal haemorrhages while in mice, congestion of liver, spleen and haemorrhages in spleen were observed.

2.2.2 Pathogenicity test on rabbits

Smith and Hall (1967) studied the enterotoxic activity of pathogenic E. coli serotypes collected from pigs, calves, lambs and rabbits in the ligated intestine loops of rabbit. They also studied the characters of enterotoxigenicity, enteropathogenicity and enteroinvasiveness from histopathological changes in tissue sections. In all cases, haemorrhages and necrosis of mucosa and submucosa were seen.

Olson and Soderland (1980) reported the enterotoxic activity of untypable E. coli serotypes isolated from porks and inoculated into ligated gut loops of rabbit. The enterotoxic fluid was heat stable.

Saxena and Yadav (1982) recorded that the enterotoxic activity of the pathogenic E. coli serotypes can be studied by inoculating the supernatant of the culture filtrate of the pathogenic isolates into the ligated intestinal loops of rabbit. Adult healthy male rabbit was used for the experiment. The test organisms were grown in nutrient broth and incubated at 37°C for 8 hours. The culture supernatant was prepared by centrifuging at 5000 rpm for 15 minutes.

Yadav (1982) reported that the E. coli serotypes 02 and 011 which caused septic infection in man on parenteral infection, caused nonenterotoxic activity in ligated loops of rabbit. He concluded that this might be due to strain variation, type of enterotoxin production (Thermostable and thermolabile). The nonenterotoxic fluid collected was seen thick, viscous and haemorrhagic.

Borah et al. (1988) reported that the fluid accumulation in the ligated ileal loops of rabbit (adult healthy male) be collected separately from each loop and the volume, colour, consistency and pH of the fluid studied. The culture

was enterotoxic if the ratio of the fluid volume (ml) to the length (cm) of each loop was 0.4 or more.

2.3 Antibiotic Sensitivity Test

Savov (1963) reported that out of 64 strains of E.coli isolated from chicks, 45 were resistant to chlortetracycline in vitro, 40 to tetracycline, 39 to tetracycline while 62 were sensitive to neomycin.

Hemsley et al. (1967) tested 213 avian strains of E. coli against 24 drugs, out of which polymyxin-B and nitrofurazone exhibited the greatest inhibitory activity.

Aller Gancedo et al. (1969) recorded the highest sensitivity of E. coli isolates (82) to kanamycin, neomycin and chloramphenicol while 70 percent of the strains were found to be resistant to tetracycline.

Among 60 strains of E. coli from colibacillosis in poultry tested by Ezhov et al. (1970), the level of resistance recorded against tetracycline, neomycin and ampicillin was 30-40%, 56-58%,_A and 23% respectively.

Butura and Sahleanu (1972) isolated 198 strains of E. coli from fowls with septicaemia. Drug sensitivity test showed that they were highly sensitive to chloramphenicol,

polymyxin-B, neomycin, furazolidone and tetracycline (97.5%, 96%, 83%, 81% and 37% respectively) and resistant to erythromycin and streptomycin.

Drug sensitivity patterns of 25 strains of E. coli from birds were recorded by Bessarabov et al. (1973) revealed that 40% of the strains were sensitive to neomycin, 52% to polymyxin and 36% to furazolidone.

Gupta (1975) recorded no resistance strain against furadentin. He detected resistance against chloramphenicol, ampicillin, neomycin, streptomycin and tetracycline at 34.20%, 25.70%, 56.80% and 90.20% respectively.

Kapoor et al. (1978) reported that the antibiogram of 220 E. coli isolates from poultry showed a high percentage of resistance to ampicillin, chlortetracycline and streptomycin (94.1%, 76.9% and 69.1% respectively). Fewer strains were resistant to nitrofurazolidone, polymyxin and chloramphenicol (25%, 9.1% and 26.4% respectively).

Sahota et al. (1978) reported that most of the strains of E. coli isolated from cases of oophoritis and salpingitis were sensitive to streptomycin and nitrofurantoin and resistant to tetracycline.

Farias (1979) from Brazil isolated 166 E. coli sero - types from 414 samples, of which 22 were typed. In antibiotic sensitivity 95% were positive to chloramphenicol and gentamycin, 91% to kenamycin and tetracycline, 82% to ampicillin and 68% to streptomycin.

Out of 126 E. coli isolates tested by Srinivasan et al. (1979) 26 were sensitive to ampicillin, chloramphenicol, kanamycin and furadentin while most of them were resistant to erythromycin, polymyxin and tetracycline.

Bozorgmehri-Fard and Gilani (1980) from Teheran isolated 90 strains of E. coli (18%) from 5150 fowls in a period of 9 months. The E. coli isolates were 92% sensitive to trimethoprim, 64% to chloramphenicol, 62% to streptomycin, 55% to neomycin, 38% to streptomycin, 35% to sulphachlorpyridazine and 13% to tetracycline.

Gyurov et al. (1981) reported multiple drug resistance among 143 E. coli strains isolated from internal organs and bone marrow of poultry with septicaemia, enteritis, pericarditis and perihepatitis. The isolates recovered belonged to serotype 078, 01, 04, 0111 and 12 were not typed. All the strains were resistant to coxacillin, obondomycin and tylosin. Only one strain was resistant to gentamycin and carbanicillin, 77% of the strains were sensitive to ampicillin, 74.1% to

furazolidone, 67.2% to Borgal (Sulphadoxin and trimethoprim), 39.2% to kanamycin and neomycin, 37.7% to streptomycin, 34.3% to chloramphenicol, 4.9% to sulphathiazole and 1.4% to tetracycline. Most strains had 1-4 resistant markers.

Kumar et al. (1981) reported drug resistance of 125 E. coli strains isolated from chicks and hens. The isolates were resistant to bacitracin, tetracycline and sulphonamide.

Sambyal et al. (1981) made a survey of large poultry farms around Ludhiana, collected 235 cloacal swabs, 18 feed samples, 23 water samples and 30 litter samples, 161 faecal swabs, 4 feed samples, 15 water samples and 27 litter samples and 49 tissue samples were positive for E. coli. The isolates were sensitive to the following drugs, nalidixic acid (83%), nitrofurantoin (76%), Streptomycin (69%), Kanamycin (93%), Oxytetracycline (31%), Chlortetracycline (23%), Tetracycline (86%) and Triple sulphonamides (19%).

Goren et al. (1982) studied 223 gram negative bacteria (E. coli, Salmonella and Pasteurella multocida) in poultry in Netherlands. Among them, many multiresistant strains were susceptible in vitro to the bactericidal activity of flumequin. No cross resistance with tetracycline, chloramphenicol, neomycin, ampicillin, sulphanamides, trimethoprim or furazolidone was found.

Kim and Tak (1983) studied on pathogenic E. coli isolated from chicks with colibacillosis. Almost all of the 391 strains isolated were resistant to tetracycline and streptomycin and 30 - 80% were resistant to sulphonamides nitrofurantoin, carbenicillin and ampicillin.

Nakamura et al. (1983) studied the drug resistant E. coli isolates in chicks. They administered faeces containing dominant E. coli sensitive isolates to newly hatched chicks and were successful in reducing the drug sensitivity.

Mehrotra et al. (1984) studied the transfer of drug resistance to chloramphenicol and furazone in E. coli strains isolated from the guts of four month old pullets, fed with feed containing chloramphenicol and furaxone. They compared the drug sensitivity with strains from untreated pullets. Resistance to chloramphenicol developed quickly and more than half of the isolates developed resistance to furazolidone by the tenth day.

Smith and Lovel (1984) reported antibiotic resistance in E. coli causing generalised infection in chicks in U.K. in 1982. They also studied the relationship between the results of in vitro and in vivo furazolidone sensitivity tests. Forbidding the routine use of antibiotics in feeds, incidence of resistance to tetracycline, furazolidone and sulphonamides to E. coli strains decreased.

Vidotto et al. (1984) reported drug resistance of 29 E. coli isolates collected from post-mortem materials of poultry. Out of them 79% were resistant to tetracycline, 68% to sulpho-namide, 59% to streptomycin and 24% to chloramphenicol, Nine of them transferred resistance to recipient strains. However, they were sensitive to polymyxin-B, colistin, gentamicin and nitrofurantoin.

Barbour et al. (1985) reported drug resistance of 11 E. coli isolates, recovered from post-mortem materials of Saudi Arabian broiler farms. They found that the isolates were resistant to streptomycin, sulphathiazole and tetracycline.

Gloud et al. (1985) isolated 177 E. coli serotypes over a period of two years and a half from 145 field reared broiler flocks and 20 day old hatchery chicks from peninsula. Most of the isolates were sensitive to chloramphenicol, gentamicin, nalidixic acid, ormetoprim - sulphadimethexin, streptomycin, neomycin and ampicillin. About half the isolates were sensitive to nitrofurantoin and sulphacompounds. Less than 25% were sensitive to streptomycin, erythromycin, tetracyclines, novobiocin, penicillin, bacitracin and lincomycin.

Sinha et al. (1985) isolated 195 E. coli serotypes from different poultry farms. The isolates were from the intestines, heart, lungs, liver and from spleen. All the

Strains were not sensitive to bacitracin and nalidixic acid (96%) and most resistant to ampicillin, tetracycline (96% each), doxycycline (92%) and sulphafurazole (60%).

Menna and Fioretti (1986) recorded 11 serotypes of E. coli sensitive to cefotaxime at a MIC of 20 strains of E. coli was between 16-32 μ g/10 ml.

Tagawa (1986) studied 184 isolates of E. coli from faecal swabs of broiler chicks. He found that they were resistant to at least one antibiotic. The pattern of antibiotic resistance differed with host species. R-plasmids were detected in 79% of E. coli isolates from fowls.

Ghosh (1987) isolated 50 strains of E. coli from 437 dead embryonated eggs. He serotyped the isolates and found the serotypes 061, 068, 0126 and 0147. All isolates were sensitive to gentamycin and 80% to Chloramphenicol. Resistance levels to the other drugs were high.

Prukner et al. (1987) conducted sensitivity of 160 strains of Escherichia coli and tested against 8 common therapeutic agents - a furan preparation, flumequin, trimethoprim with sulfafurazole, ampicillin, chloramphenicol, streptomycin, oxytetracycline and lincomycin with spectinomycin. The latter combination was the most effective being active against 94% of the strains. When this combination was given prophylactically

to groups of broiler chickens at 2-4 or 24-25 days of age, mortality rate was lower and average weight and daily weight gain and feed consumption was higher than in chickens given trimethoprim with sulfafurazole or tylosin tartarate.

Sarma et al. (1987) reported drug susceptibility of Escherichia coli isolated from domestic fowls. Out of 202 E. coli isolates collected from diseased and apparently healthy chicks, most of them (95 - 100% were sensitive to chloramphenicol, nalidixic acid and neomycin. 94-100% of the isolates from healthy chicks were resistant to chlortetracycline, tetracycline oxytetracycline and tripple sulphonamides, compared with 77 - 80% from diseased chicks, 90% showed multiple resistance to chlortetracycline, tetracycline, oxytetracycline, erythromycin, streptomycin, tripple sulphonamides and nitrofurantion. Rough strains were relatively more susceptible to all antibacterial agents than the 'O' serotypes.

2.4 Experimental Production of Colibacillosis

2.4.1 Experimental production of colibacillosis in young chicks

Stipkovits and Solyom (1968) reported that subcutaneous infection of 0.1 ml undiluted or tenfold diluted broth cultures of two strains of E. coli from chicken killed all of 14 (3 day old) chicks injected.

A total of 15 strains proved to be pathogenic for day old chicks and all except three for 15 day old chicks (Belitskii and Panikar, 1969).

Closek (1973) injected bacterial strains isolated from cases of colibacillosis, lipopolysaccharide and aqueous extracts from these strains intraperitoneally into chickens and mice. It was observed that live bacteria rather than their toxins were pathogenic for 3-4 day old chicks but mice reacted to both injections.

Farid et al. (1977) reported that the 'O' group 2, 11, 42 and 78 of E. coli isolated from internal organs of dead chickens were highly pathogenic to one day old chicks.

Kulshrestha and Kumar (1977) reported the pathogenicity of 1215 E. coli isolates from the respiratory tract infections and heart blood of poultry. They inoculated 2-3 day-old healthy chicks intra-peritoneally with 0.1 ml six hours broth culture of E. coli and found that majority of the isolates had lethal index between 4-7.

Rakhmanian and Shubin (1978) exposed experimental groups of chicks of various ages, either once or twice, to aerosol infection with cultures of one of the same three serotypes (01, 02 and 078). It revealed that serotypes 02 and 078 caused characteristic symptoms and lesions of

colisepticaemia upto the age of 40 days. Very severe symptoms were produced in chicks infected at 2 days of age.

Sharma et al. (1981) studied the pathogenicity of E. coli of poultry origin. They observed that out of 24 E. coli strains belonging to 'O' group, 23 were pathogenic to chicks and 21 to mice, E. coli strains of group 025, 060, 078, 090 and 0160 were equally pathogenic to mice and chicks producing 100% mortality. Of the two E. coli strains belonging to group 017, one was nonpathogenic while the other was pathogenic to chicks. E. coli strains in groups 015, 018 and 048 were pathogenic to chicks but nonpathogenic to mice.

Pages and Costa (1985) detected some factors which increased the pathogenicity of Escherichia coli in broiler chicks. Experimental infection with 023 or 08 were done in chickens. The factors are ammonia, air pollution, H-120 spray vaccination, fluctuating air temperature, infectious bursal disease virus, H-120 oral vaccination, mycoplasma gallisepticum infection and lasota oral vaccination.

Prukner (1986) observed that E. coli strains were isolated in bacterial diseases of poultry from Yugoslavia. 078 serotype was most often isolated and its pathogenicity was confirmed by subcutaneous or intravenous infection in 1 - 40 day-old chicks which died within 24 - 48 hours. The other isolates were 02, 01, 055 and 06.

2.4.2 Clinical manifestations and gross lesions in young chicks

Hamilton and Conrad (1958) reported that experimental chickens inoculated with mucoid encapsulated E. coli isolated from Hjarre's disease did not develop any pathological lesions.

Reid et al. (1961) and Siccardi (1966) reported that omphalitis and other yolk sac infection had been produced in chicks by exposing eggs to E. coli broth cultures.

It was observed by Siccardi (1966) that as few as 10 organisms of serotype 01 could cause 100% mortality in day-old chicks when inoculated into yolk sac. In many cases there was also omphalitis. Chicks living more than 4 days often had pericarditis as well as infected yolks. There was no chick mortality, the only manifestation being retained infected yolk and reduced weight gain.

Stipkovite and Solyom (1968) reported that subcutaneous injection of 0.1 ml undiluted or tenfold diluted broth cultures of two strains of E. coli from chicken killed all 14 (3 day-old) chicks.

A total of 15 strains proved to be pathogenic for day old chicks and all except three for 15 days old chicks (Belitski and Panikar, 1969).

Seneviratna (1969) reported chronic granulomatous condition in domestic fowls due to E. coli. Vague clinical signs like emaciation and unthriftiness were seen. Lesions like TB found in caeca, liver and small intestinal necrosis but the incidence is low and has less economic loss.

Ciosek (1973) injected bacterial strains isolated from cases of colibacillosis, lipopolysaccharide and aqueous extracts from these strains intraperitoneally into chickens and mice. It was observed that live bacterial rather than their toxins were pathogenic in chicks 3-4 days old but mice reacted to both injections.

Hofstad (1975) described the lesions of uncomplicated coliform "air sac disease" which could be reproduced easily by inoculating pathogenic serotypes of E. coli into the air sac. Air sacculitis occurred within an hour. Bacteremia and pericarditis developed by 6 hours. Lesions could be developed 48 hours after inoculation. Most mortality could occur during the first 5 days. Recovery might be rapid if birds survive initial infection although few birds with persistent anorexia became emaciated and died. He further stated that E. coli could be isolated from acute infectious diseases resembling fowl typhoid and fowl cholera. Affected birds were in good flesh and had full crops which suggested acute nature of infection. The most characteristic lesions were green liver, ^{and} congested

pectoral muscles. In some cases, there were small white focal areas in the liver. As in the other coliform septicaemias, there was a tendency towards pericarditis and peritonitis. The disease continued to cause acute losses in mature and growing chickens and turkeys. There was no reports of the experimental production of the condition even though some serotypes were also isolated from other diseases in poultry.

Rakhmanian and Shubin (1978) exposed experimental groups of chicks of various ages, either once or twice to aerosol infection with cultures of one of the same three serotypes (01, 02 and 078). It revealed that serotypes 02 and 078 caused characteristic symptoms and lesions of colisepticaemia upto the age of 40 days. Very severe symptoms were produced in chicks infected at 2 days of age.

Srinivasan et al. (1979) reported that E. coli normally live in intestinal tract of poultry and ducks producing sometimes colisepticaemia and omphalitis, peritonitis, salpingitis and air-sac disease in young chicks.

Kim and Tak (1983) recorded that out of 488 strains from fowl with pericarditis, hepatitis, air sac infection, salpingitis, septicaemia and various other conditions, 224 were classified upto 18 '0' groups. The most prevalent of which was 0128.

Sharma and Joshi (1983) reported that six strains of oophoritis, salpingitis and egg peritonitis were used for experimental production of disease in laying hens. All six strains were pathogenic for laying hens. Killed birds had swollen, dark red ovaries, misshapen and congested ova and inflamed oviducts. Microscopic lesions in the ovary were congestion, mild haemorrhages, fibrinous exudate and foci of lymphoid and plasma cell. Microscopic lesions in oviduct were formation of lymphoid follicles and degenerative changes in the glandular epithelium.

Bandopadhyaya and Dhawedkar (1984) while screening 711 post-mortem cases from AICRPP farm, 11.2% showed salpingo-peritonitis. The 80 cases of salpingoperitonitis when studied bacteriologically revealed 7.5% positive for E. coli type was 017. The other serotypes were 019, 05, and 060.

Morley and Thomson (1984) reported the occurrence of swollen-head syndrome in broiler chickens of 4-6 weeks of age in S. Africa. It appeared to be caused by mixed corona virus and E. coli infection.

Berry et al. (1985) observed the ability of an E. coli strain associated with human haemorrhagic colitis (Serotype 057 : H7) to colonize chicken caeca for 90 days. Afterwards when challenged at 1 day-old chick confined to the proximal

caeca of healthy normal appearing chickens principally at 14-28 days after inoculation. Attachment and penetration of caecal surface epithelium by E. coli 0.157 : H7 were observed with exception of splenic hepatic and caecal tonsil immune related changes and caecal damages and colonisation. No other organs, systems or portions of gastrointestinal tract were affected by bacteria. The ability of this organism to colonize chicken caeca indicated that chicken might serve as host and possibly as reservoir for E. coli 0157 :H7 .

Phukan (1988) conducted experimental infection of chicks with isolated E. coli strains and observed that two serotypes of E. coli (0137 and 020) which were used for experimental infection produced 100% mortality in 3 day-old chicks within 5 days post-inoculation by intra-peritoneal and subcutaneous routes. On the other hand, the same strains could not produce disease in chicks by oral administration. Maximum mortality in chicks inoculated intra-peritoneally was observed on the first day post-inoculation.

On post-mortem, gross lesions observed in the internal organs were congestion of liver, lung, heart and kidneys, enteritis, accumulation of fluid in the peritoneal cavity, thickened airsacs, accumulation of fluid in the pericardial sac, white necrotic areas on the liver. The inoculated E. coli organisms were reisolated from heart blood, lung, liver intestinal

content, pericardial and peritoneal fluid of dead experimental chicks.

2.4.3 Histopathological changes in the internal organs in experimental young chicks

Siccardi (1966) observed that as few as 10 organisms of serotype 01a caused 100% mortality of day old chicks when inoculated into the yolk sac. In many cases, there was omphalitis. Those chicks or poults who lived more than 4 days often had pericarditis as well as infected yolks. The microscopic changes in the wall of the infected yolk sacs appeared to be mild. The wall was oedematous. There was an outer connective tissue layer followed by a layer of inflammatory cells containing heterophils and macrophages, a layer of giant cells, a zone of necrotic heterophils and masses of bacteria and then the inner infected yolk contents. In some yolk sacs, there were a few plasma cells.

2.4.4 Experimental production of colibacillosis in older chicks (3 weeks and above) with and without latent infections or stress factors

Bakajle and Prost (1963) recorded that E. coli serotypes 071 and 08 killed mice when injected intravenously with 0.1 ml of a 24 hours culture but 1 ml doses given orally to 2 month old chicks proved harmless.

savov (1963) stated that out of 48 E. coli strains from chicks 21 belonged to group 01, 9 to 02 and 8 to 078. The disease was reproduced in 3 to 21 day-old chicks by administering 6 hours broth cultures containing 10^9 organisms/ml at a dosage of 0.2 ml S/c, 0.2 ml I/P, or 0.8 ml on 3 consecutive days by mouth. There was a much higher mortality when there was also a coccidial infection. Immersion of eggs in 24 hour broth cultures for 1-2 min caused death of most embryos. Chicks hatching from such eggs were carriers of the disease.

Doroshko et al. (1965) could experimentally reproduce the disease (colibacillosis) in fowls, pigeons, turkeys and mice by intraperitoneal inoculation of cultures of E. coli isolated from birds affected with granulomatosis.

Kulkarni et al. (1970a) could induce egg peritonitis and salpingitis experimentally in laying hens by intraperitoneal injection of E. coli types 078 : K80 and 08 alone or with sterile egg yolk.

savov (1973) reported that between 0.5 and 6% of eggs from normal hens were found to contain E. coli. He experimentally inoculated hens which shed E. coli upto 26.5% of their eggs.

Sandulescu et al. (1977) and Sharma et al. (1977) demonstrated the enteropathic and enterotoxic effect in chicks and in ligated intestinal loops of chickens by inoculating the E. coli '0' groups 2, 5, 8, 1 and 78. It was observed that strains belonging to '0' group 1, 2 and 78 had the most severe enterotoxic effect. Out of 10 isolates tested for pathogenicity two were mildly pathogenic for 3 week old chicks.

Harry (1980) stated that the resistance of chicks to colisepticaemia following injection of approximately 10 cells of E. coli 078 was increased in terms of mortality by the supplementation of their diet with 360 mg/kg or more of iron (Ferrous sulphate). This was related to an increase in plasma iron. The results suggested that increased resistance to infection could be partly achieved by correcting the hypoferraemia in chicken produced by injection of E. coli endotoxin lipopolysaccharide (LPS). Haematological disturbances can persist for upto at least 30 days after infection in survivors of E. coli septicaemia.

Ibragimov et al. (1983) reported that ten groups of 30 month old chickens were infected with mycoplasma gallisepticum, infectious bronchitis and E. coli single or various combinations. They were killed 2 to 45 days after infection. It was concluded that the mycoplasma played primary or infectious bronchitis virus sensitised the bird to E. coli septicaemia.

There was little difference in the pathology of pure and mixed E. coli infection.

Sharma and Joshi (1983) used six E. coli strains for experimental production of disease in laying hens. All six strains were found to be pathogenic for laying hens.

Egizhev (1985) stated that in Kazakstan U.S.S.R. chicks with natural coccidial and nematode (capillaria) infections and ducklings with experimental gastrotaenia infection were investigated for the presence and virulence of E. coli. Pathogenicity of E. coli was directly related to the presence of parasites, their localisation in the intestines and species and age of the host. In duckling, E. coli was much less pathogenic than the chicks probably because of the localisation of gastrotaenia in gizzard.

Bolin (1986) studied the effect of E. coli inoculation on serum iron concentrations of Turkeys and the effect of oxogenous iron as ferric ammonium citrate on E. coli septicaemia in turkeys. Administration of iron with E. coli increased mortality, frequency and degree of bacteraemia and severity of lesions in inoculated turkeys compared to with those in turkeys given E. coli but not given iron.

Dhillon (1986) recorded that 10 strains of adenovirus representing 10 serotypes were administered intratracheally to 3 week old SPF chickens which also received 2.5×10^5 colony forming units of pathogenic E. coli intranasally. The birds had been inoculated by eye drop at 1 day of age with a virulent infectious bursal disease virus (IBDV). Controls consisted of Groups of chickens inoculated with IBDV and E. coli, IBDV only, E. coli only and no virus or E. coli. Gross pulmonary alterations at 5 days after inoculations (PI) congestion and consolidation of one or both lungs of a chick inoculated with two IBDV - infected groups which exhibited bursal change. Other groups except control showed varying degree of tracheitis and pneumonia. Lesions were slight to pronounced in severity. None of the control groups showed gross or histological lesions.

Golubrichii et al. (1986) conducted experiments on 2 month old chicks with simultaneous dual infection with field strains of influenza virus from ducks and E. coli of avian origin of group 02. In general, the bacteria inhibited infection with a low dose of virus but seemed to promote infection with a higher dose of virus. Dual infection of chicks led to respiratory disease.

Larsen et al. (1986) observed experimentally that E. coli (1.02×10^8) when injected intravenously or into the airsac 7 days after virulent haemorrhagic virus inoculation to chicks,

80% died out of 40 ~~after~~ 8-15 days after of virus inoculation whereas 2.5% only with E. coli inoculation.

Prukner (1986) isolated E. coli in 81.46% of bacterial disease of poultry in 1985 in Yugoslavia. Serotype 078 was found most often (9.20%) and its pathogenicity was confirmed by S/c and I/v infection of chickens aged one and 40 days which died within 24 and 48 hours respectively. Other types seen were 02, and 01 neither of which was pathogenic, and 055 and 06. A fast procedure for determination of virulence of E. coli is by S/C or I/V injections of a mixture of types. Virulent and avirulent strains did not differ in sensitivity to medication.

Zahdeh (1987) dipped ten 18 day embryonated chicken eggs in a 24 hour broth culture of E. coli 078; K80 isolated from a field out break of omphalitis. Examination of two at 21 days revealed severe oedematous swelling around the naval orifice. The remaining 8 hatched but 6 had severe omphalitis and 2 had incomplete withdrawal of yolk sac. Naval yolk infection has been suggested as a better term than omphalitis.

Panda and Verma (1988) reported that many stress factors exploit weakness in birds defences due to other infectious agents. They ascribed the stress factors like Mycoplasma gallisepticum (Chronic Respiratory Diseases) adeno virus infections, immunosuppression due to infectious Pursal disease and

aflatoxicosis, nonfatal Ranikhet Disease (RD), Infectious Bronchitis (IB) and many live virus vaccines, excess of ammonia, extreme temperature and induced colibacillosis in chicks.

2.4.5 Clinical manifestations and gross lesions

Seneviratna (1969) observed that in chronic granulomatous conditions in domestic fowl and Turkey due to E. coli, the vague clinical signs like emaciation, unthriftiness, lesions like T.B. found in caeca, liver and small intestine necrosis. The incidence of the disease was low.

Hofstad (1975) described the sources of infection of colibacillosis in chicks and the gross lesions observed. He recorded that E. coli was present in the yolk sacs of 70% of the chicks with "Mushy chick disease" which was characterised by oedema and infected yolk. Faecal contamination of eggs was considered to be the most important source of infection or salpingitis. The yolk sac of the embryo is the focus of infection. Many embryos die before hatching with losses continuing upto 3 weeks later. In many cases there was also omphalitis. Chicks or poults living more than 4 days often had pericarditis as well as infected yolk. There might not be embryo or chick mortality and only symptom seen was reduced weight gain. E. coli often infect respiratory tract, pericardium, joints, and oviduct. Acute septicaemia due to E. coli was also recorded.

Srinivasan et al. (1979) reported that in chickens serotypes 01, 02 and 078 were pathogenic. Ducks were susceptible to 042 and 01 serotypes and quails to 01, 010 and 02 serotypes of E. coli. On gross microscopical lesion, it was found that the birds died of colibacillosis (colisepticaemia) natural and experimental revealed pericarditis and perihepatitis and little in quails, ducklings and turkeys.

Dholakia et al. (1983) stated that 25% of the E. coli organisms isolated from heart blood indicating responsible for septicaemia and producing pericarditis, peritonitis, congestion of lung, kidney and liver.

Kim and Tak (1983) studied 488 strains of E. coli from fowls and observed 224 cases were showing lesions like pericarditis, hepatitis, airsac infection, salpingitis, septicaemia and various other conditions. The most prevalent isolate was 0128.

Sharma and Joshi (1983) reported that six strains of E. coli isolated from natural cases of oophoritis, salpingitis and egg peritonitis were used for experimental production of disease in laying hens. All six strains were pathogenic for layer hens. Killed birds had swollen dark red ovaries, misshapen congested ova and inflamed oviduct.

Bandopadhyaya and Dhawedkar (1984) while screening 711 post-mortem cases from AICRPP farm 11.2% showed salpingoperitonitis and 7.5% were positive for E. coli bacteriologically. The most common sero type of E. coli was 017. The other serotypes were 019, 05 and 060.

Morley and Thomson (1985) reported swollen head syndrome in broiler chicks of 4-6 weeks of age in South Africa supposed to be due to mixed infection of coronavirus and E. coli.

Berry et al. (1985) reported that E. coli strain associated with human haemorrhagic colitis (Serotype 0157 : H7) to colonise chicken caeca for 90 days after oral challenge at 1 day old confined to the proximal caeca of healthy normal appearing chicks principally at 14-28 days after inoculation. Caecal leakage and portions of gastro-intestinal tract were affected. The ability of the organism (E. coli) to colonise chicken caeca indicated that the chicken might serve as host and possibly as reservoirs for E. coli (0157 : H7).

*well
noted* Dunnington and Siegel (1985) observed changes in body weight, surface and cloacal temperature, lesions on heart and airsacs and mortality in 3 and 30 day old chicks due to colibacillosis.

Ling et al. (1987) carried out comparative studies on the pathology of septicaemic colibacillosis in 30 naturally

infected and 24 experimentally infected birds. Macroscopic lesions consisted mainly of petichial haemorrhages in the internal parenchymatous organs and a fibrinous serositis and granulomatoid changes in the serous coverings of the pericardium, air sacs and liver capsule.

2.5 Therapy

Gross (1961) reported that Escherichia coli infection associated with airsac disease can be controlled by furaltadone in the feed or water.

Herberts and Chang (1969) tested nine drugs (furazolidone, oxytetracycline, chlortetracycline, bacitracin, methylene disalicylate, zinc bacitracin, erythromycin, procaine penicillin, penicillin plus streptomycin and tylosin) for the treatment of colibacillosis in poultry and found furazolidone to be the most effective drug in controlling experimental infection.

Hamdy and Blanchard (1969) tried combination of lincomycin and spectinomycin at the dose rate of 3 g daily for 7 days increased the survival rate from 33 to 80 percent in E. coli infection of birds.

Groups of fowls experimentally infected subcutaneously with a lethal dose of E. coli were all protected by addition of chloramphenicol to the diet, from three days after the

infection, either 0.005 g per head every day, or 0.010 g per head for three days (Eutura and Sahleanu, 1972). The efficacy of furazolidone and oxytetracycline in the same condition was found to be 80.87 and 7 to 13 percent respectively.

Artem'Eva et al. (1977) studied the efficacy of three chemotherapeutic agents against experimentally induced colibacillosis in chicks and it was recorded to be 81 percent for clioquinol, 74 percent for Kanamycin and 50 percent for chloramphenicol.

Kapoor et al. (1978) reported that a majority of E. coli isolates of poultry were sensitive to polymyxin, nitrofurazolidone, chloramphenicol. The results obtained in the study indicated that an alarming number of E. coli isolates of poultry were resistant to chlortetracycline and streptomycin.

Maestrone et al. (1979) reported that Rofenaid (113.5 g sulphadimethoxine plus 68.1 g ormethoprim/lb feed) at a feed concentration of 0.02% was effective prophylactically and therapeutically against experimentally induced E. coli air sac infection in chickens.

Srinivasan et al. (1979) tested 26 isolates from various species for sensitivity/resistance to penicillin, chloramphenicol, erythromycin, furadentine, kanamycin, polymyxin and tetracycline.

Apramycin in the drinking water at 0.5 g/litre for five days was found to be effective against E. coli septicaemia in broilers (Donos, 1982).

Dholakia et al. (1983) observed high degree of sensitivity of E. coli cultures to gentamycin and varying degree to furadentine, tetracycline, penicillin and furazolidone.

Goren et al. (1984) demonstrated good therapeutic effects of sulphaquinoxaline (200 mg/litre), sulfadimidine sodium (2 g/litre), sulfachloropyridazine 30% (1 g/litre) and to a lesser degree of sulfadiazine sodium (250 mg/litre) in broilers infected with a sulfonamide susceptible E. coli strain.

Cracknell et al. (1986) conducted trials in Greece, Italy and Jordan to test the efficacy of apramycin against natural E. coli infection, given at the dose rate of 125, 250 and 500 mg per litre drinking water for five consecutive days. Treatment was given only after the infection had been confirmed by the laboratory examination of dead birds. Not all treatments were tested in every house. Each house contained a group of untreated controls. The remaining birds were allocated to one or more apramycin treatment groups. There was a reduction in mortality and an improvement in the final weight at economic feed conversion ratio in all three treatment groups. The results supported the use of apramycin sulphate in drinking water for the treatment of E. coli infections in broilers.

Karmy et al. (1987) isolated E. coli 078:K80 from broilers and ducks with respiratory symptoms and air sacculitis caused respiratory symptoms and mortality in intratracheally infected broilers four days after they had been stressed by vaccination with Lasota spray at fluctuating air temperatures. Flumequine was the most active (microbial) agent in vitro and was highly effective when given prophylactically in the drinking water. It was recommended the use of flumequine in drinking water at a dose rate of 6-12 mg/kg body weight at the time of aerosol vaccination.

Goren et al. (1987) stated that in contrast to the recommended dose of 2 g salphadimidine sodium per litre of drinking water for treatment of broilers against colibacillosis, it appeared that under the experimental conditions treatment at 50 mg/litre (60 mg/kg body weight) gave the best therapeutic effects. At higher concentrations, the drinking water consumption and body weight gain were reduced. During treatment high blood plasma concentrations were measured.

Prukner et al. (1987) conducted sensitivity of 160 strains of E. coli and tested against eight common therapeutic agents - a furan preparation, flumequine, trimethoprim with sulfafurazole, ampicillin, chloramphenicol, streptomycin, oxytetracycline and lincomycin with spectinomycin. The latter combination was the most effective being active against 94% of the

strains. When this combination was given prophylactically to groups of broiler chickens at 2-4 or 24-25 days of age, mortality rate was lower and average weight and daily weight gain and food consumption was higher than in chickens given trimethoprim with sulfafurazole or tylosin tartrate.

Goren et al. (1988) treated experimentally induced colibacillosis in broilers with doxycycline hydrate in drinking water and found effective at a dose rate of 200 mg/kg body weight. The achieved therapeutic effects were similar to those of tetracycline at the same dose and of flumequine at a dose of 19 mg/kg body weight.

2.6 Prevention and Control

Hofstad (1975) described the methods for the prevention and control of colibacillosis in chicken. Methods which could reduce the introduction of pathogenic serotypes into poultry flocks were essential to the control of colibacillosis. E.coli infection of the respiratory tract of birds could be reduced by raising mycoplasma free birds and by reducing the exposure of birds to viruses causing respiratory diseases. Reducing the level of E. coli contaminated dust in the air of poultry houses by means of increased ventilation would reduce respiratory tract exposure.

Methods for reducing the level of pathogenic E. coli in the intestinal tract and faeces were wellknown, also consideration that pelleted feed has fewer E. coli than mash and that rodent droppings a source of pathogenic E. coli should not be overlooked.

The most important source for the transmission of pathogenic E. coli between flocks has been faecal contamination of hatching eggs. Transmission could be reduced by fumigating or disinfecting eggs $1\frac{1}{2}$ - 2 hours after they were laid and by discarding cracked eggs or eggs with faecal contamination. Infected eggs if broken during incubation, their contents were a serious source of infection to other eggs, especially when personnel and egg handling equipment were soiled. Contaminated chicks could survive if they could be kept warm and not starved.

Tengerdy and Nockels (1975) advocated supplementation of diet with either vitamin E (300 mg/kg) or vitamin A (60,000 i.u./kg) of feed and found to protect chicks against E. coli infections.

Morley and Thomson (1984) stated that swollen-head syndrome was a disease seen in broiler chickens 4-6 weeks of age in South Africa. It appeared that the disease was caused by mixed coronavirus and Escherichia coli infection. The coronavirus appeared to be a new serotype. The disease was controlled

with an attenuated live virus vaccine and antibacterial therapy.

Rosenberger et al. (1985) reported that broiler breeder hens were vaccinated once at 20 weeks or twice at 20 weeks or 25 weeks of age with a formalin inactivated oil immersion E. coli bacterin composed of serogroups 02, 078 and 035. Serological responses as assessed by microglutination revealed an increase in serotype-specific antibody in vaccinated birds. Challenge of progeny from vaccinated to nonvaccinated with homologous E. coli demonstrated that maternally derived antibody could protect against mortality and/or lesions for as long as 2 weeks after hatching.

CHAPTER III

MATERIALS AND METHODS

3.1 Incidence of Colibacillosis in Chicks in Poultry Farms

Studies on the incidence of colibacillosis in chicks were undertaken during the period from March 1986 to February 1987 in small, medium and large poultry farms selected at random in and around Bidhan Chandra Krishi Viswavidyalaya, Mohanpur of Nadia district, 24-Parganas (North and South) districts and subdivisional ^{area} of Hooghly district of West Bengal. Poultry farms of the area were arbitrarily classified as small (birds strength below two thousand), medium (birds strength between two to five thousands) and large farms (above five thousand birds). Majority of the poultry farms were having small number of birds because of small holdings.

3.1.1 Sources of materials, collection of samples and isolation and identification of E. coli

Sources of material :

In the present investigation dead birds were collected from the above sources suspected to have died of colibacillosis. The materials to be tested were collected from dead birds at necropsy from suspected cases of colibacillosis (Septicaemia, enteritis, omphalitis, pericarditis and respiratory tract infections). While conducting post-mortem, typical lesions in internal organs were taken into consideration.

Collection of samples :

Bacteriological samples were collected from heart blood, pericardial fluid, peritoneal fluid, intestinal content, liver, lungs, trachea and unabsorbed yolk materials. Heart, blood, pericardial fluid and peritoneal fluid were collected aseptically with the help of sterile pasteur pipettes. Clinical samples from liver, lung, unabsorbed yolk materials and intestinal content for bacteriological study were collected by sterile swabs. The collected samples were inoculated on to MacConkey's Lactose Agar (MLA) plates immediately after collection.

Isolation and identification of E. coli

Media used: The following media were used for isolation, purification and subculture of Escherichia coli.

- i) MacConkey's Lactose Agar (MLA)
- ii) Nutrient Agar (NA)
- iii) Eosin-Methyleneblue Agar (EMB)

The media were prepared according to Edward and Ewing (1972) and Cruickshank et al. (1975).

Inoculation : Primary inoculation of the collected materials was done on MLA by streak plate method as described by Collins and Lyne (1976). The plates were incubated at 37°C

for 24 hours aerobically and the suspected pinkish colonies were subcultured on EMB plates. The plates were incubated at 37°C for 24 hours aerobically.

Maintenance of culture : After purification, the suspected Escherichia coli colonies were picked up and streaked on to Nutrient Agar (NA) slants and incubated at 37°C for 24 hours aerobically. The slants were preserved in a refrigerator with serial number. Viability was maintained by subculturing the organisms at every six weeks interval in NA slants.

Characterisation and identification of the organisms

Characterisation and identification of the organisms were made as per Edwards and Ewings (1972) on the basis of

- i) Morphology,
- ii) Colony characteristics and
- iii) Biochemical tests.

Morphology :

The morphological characteristics of the isolated organisms were studied by staining the culture smears with Gram's stain. Morphological characters like size, shape and arrangement along with Gram's staining reaction were recorded.

Colony characteristics :

The colony characteristics like size, shape and physical appearance on the culture media were observed and recorded.

Biochemical tests :

For the identification of Escherichia coli organisms, the biochemical tests required were performed as per the methods described by Edwards and Ewing (1972) and Cruickshank et al. (1975). The biochemical tests conducted were :

- i) Tests for fermentation of carbohydrates e.g., glucose, lactose, sucrose, salicin, dulcitol, maltose, inositol and adonitol.
- ii) Test for H₂S production.
- iii) Methyl Red (MR) and Voges Proskauer (VP) tests.
- iv) Citrate utilisation test.
- v) Indol test.
- vi) Test for urease production.

3.1.2 Serotyping of Escherichia coli

The E. coli isolates thus obtained on the basis of the above criteria were despatched to the National Salmonella and Escherichia centre, Central Research Institute, Kasauli, H.P. (India) for serotyping.

3.2 Pathogenicity Tests of E. coli

Pathogenicity test of the isolates were performed in Swiss Albino mice and in rabbits.

3.2.1 Pathogenicity test in mice

Source of mice :

White albino-mice weighing 18-20 gms were employed for the test. From each serogroup, one E. coli isolate was tested for pathogenicity in adult white mice as per the method described by Gupta and Singh (1969). Adult healthy Swiss albino mice of the same age group obtained from M/s. S.K. Chose, Lab-animal supplier of the Deptt. of 6/C Motilal Basak Garden Lane, Calcutta-54 were reared in the laboratory for a period of one month before inoculation. During this period, the animals were neither treated nor supplemented with any antibiotic or sulpha.

Preparation of the inoculum :

A representative E. coli serotype from each serogroups was incubated at 37°C for 24 hours. A single colony from this growth was inoculated into 5 ml of nutrient broth from young agar slants and incubated at 37°C for 24 hours. Directly stained smears from the broth cultures were examined to ensure purity of the cultures. A serial tenfold dilution of each broth culture was made in sterilised normal saline solution to determine the viable count of the cultures as per Cruickshank

et al. (1975). The broth culture was centrifused and the sediment was resuspended in nutrient broth so as to contain about 800 million organisms in 0.5 ml suspension.

Inoculation of mice :

Swiss albino mice were randomly divided into groups comprising of 6 animals in each group. For each serotype of E. coli selected for pathogenicity test, one group of mice was injected intraperitoneally (I/P) with 0.5 ml of the inoculum containing about 800 million organisms per dose. The control group consisting of six mice was inoculated with 0.5 ml of sterile nutrient broth by I/P route and all groups were kept for observation.

Post-inoculation observation :

Inoculated mice were observed at every 6 hours interval during the first 24 hours post-inoculation and subsequently at 12 hours interval for 4 days. Mortality was recorded at each observation. Mice died due to probable infection were immediately necropsied under strict aseptic condition and the gross pathological changes in the internal organs were recorded. Reisolation of E. coli was attempted from heart blood, pericardial fluid, peritoneal fluid, liver, lungs and kidneys. All the survivors were sacrificed on the fifth day post-inoculation and the gross pathological lesions if any were recorded.

Reisolation of the E. coli organisms were attempted from heart blood pericardial fluid, peritoneal fluid, liver, lungs intestinal contents. If E. coli was isolated specially from the heart blood of the dead mice, it was considered pathogenic. Pathogenicity of E. coli was graded according to the following table.

<u>No. of dead mice</u>	<u>Hours after inoculation</u>	<u>Pathogenicity</u>
3-4	18	+++
3-4	19-30	++
2-3	31-96	+
1	96	doubtful
Nil	96	non-pathogenic

The isolate thus obtained was again checked up by microscopical, cultural and biochemical observations. The lethal index was calculated as per Stipkovite et al. (1969).

3.2.2 Pathogenicity test in rabbits

Test for the enterotixic activity of Escherichia coli

Some isolated E. coli serotypes were tested for their enterotoxic activity in rabbit ligated ileal loop using the culture supernatant according to the method described by Saxena and Yadava (1982). Three representative highly pathogenic isolates as per pathogenicity test on white albino-mice

were selected for the experiment (01, 02 and 078). One adult healthy male rabbit reared in the laboratory were taken and fasted over-night before the experiment.

Preparation of the inoculum :

The test-organisms were grown in 5 ml of brain heart infusion broth and incubated at 37°C for 8 hours. The culture supernatant was prepared by centrifusing the whole culture at 5000 rpm for 15 minutes.

Preparation of ileal loops :

The surgical technique described by De and Chatterjee (1953) was followed for preparation of ligated ileal loops. The rabbits used for the experiment were anaesthetised with chloroform vapour. The area over the abdomen was depilated and sterilised with 70 percent alcohol. An incision measuring about 10 cm was made parallel to the linea alba. The underlying muscles and the peritoneum were incised and the abdominal cavity was opened. About 50 to 60 cm of the small intestine, particularly the ileum part was taken out and the intestinal content was flushed out injecting 10 ml of warmed normal saline solution. The exposed intestine was divided into 5 loops of 10 cm length by ligation with sterile threads. Further, each loop was separated from the other by ligating 2 cm apart which was kept as a buffer zone.

Inoculation :

Each loop was inoculated with 1 ml of the culture supernatant except the control one which was inoculated with 1 ml of plain brain heart infusion broth. After inoculation, the intestinal loops were reinserted into the abdominal cavity. The abdominal musculature and the skin were sutured.

Post-inoculation observation :

The rabbit was sacrificed after 18 hours post-inoculation giving over dose of chloroform vapour. The abdominal cavity was opened by removing the sutures. The intestinal loops were taken out and observed for accumulation of fluid. Fluid from each loop was collected separately and the volume, colour, consistency and pH of the fluid were recorded.

Interpretation :

A culture was considered as enterotoxic when the dilatation index was 0.4 or more. Dilatation indices were worked out applying the following formula :

$$\text{Dilatation Index} = \frac{\text{Volume of fluid in the intestinal loop (ml)}}{\text{Length of the intestinal loop (cm)}}$$

3.3 Antibiotic sensitivity tests

Escherichia coli isolates were tested in vitro for their sensitivity pattern against various antimicrobial agents by using the disc diffusion technique (Cruickshank et al., 1975). The antimicrobial agents and their concentration per disc used in the test were as follows :

<u>Sl. No.</u>	<u>Antimicrobial agents (abbreviations used)</u>	<u>Concentration per disc</u>
1.	Ampicillin (A)	10 mcg
2.	Carbenicillin (CA)	100 mcg
3.	Cephalexin (CE)	30 mcg
4.	Chloramphenicol (CH)	30 mcg
5.	Contrimoxazole (CT)	25 mcg
6.	Gentamycin (G)	10 mcg
7.	Amikacin (AN)	10 mcg
8.	Sulphadiazine (SN)	150 mcg
9.	Cefotaxime (CF)	30 mcg
10.	Neomycin (N)	30 mcg
11.	Nalidixic Acid (NA)	30 mcg
12.	Furazolidone (Fx)	300 mcg

The discs were obtained from M/s. DYNAMICRC Thane, Bombay, India.

Test procedure :

For the test, E.coli colony was picked up from E.N.E. media (Eosin-methylene blue) and inoculated into nutrient broth tubes (pH 7.2) and incubated at 37°C for 18 hours aerobically. The purity of the organism was determined by microscopic examination. The turbidity of the inocula was matched with that of Brown's opacity tube No. 3. Eighteen hour old pure cultures under test were swabbed with Sterile swab and spread evenly on the nutrient agar surface and the antimicrobial discs were gently fixed at equal distances with sterilised forceps on the agar surface maintaining adequate distances among them. Each disc was gently pressed down with a pair of sterile forceps to ensure complete contact with the medium. The plates were then incubated at 37°C for 18 hours to 24 hours in an inverted position aerobically.

Reading and interpretation :

The diameter of the zone of inhibition around the disc was measured to the nearest millimeter (mm) which included 6mm diameter of the disc. The results were interpreted by using the "zone size interpretative chart" provided by the manufactures of the discs as follows :

A clear inhibiting zone of 15 mm or more was taken as highly sensitive (+++), at 12 - 15 mm as moderately sensitive

to that particular drug. Ten to twelve mm inhibitory zone was taken as weakly sensitive (+) and less than 10 mm inhibitory zone were considered resistant. Depending on the drug sensitivity results the treatment was formulated.

3.4 Experimental Production of Colibacillosis

For production of experimental infection with most pathogenic strains of E. coli, 3 and 21 day old chicks were taken and inoculation was done according to the method described by Savov (1963) with slight modification.

3.4.1 Experimental production of colibacillosis in young chicks (3 day-old chicks).

For producing experimental infection two representative pathogenic strains (01 and 02) were inoculated into chicks as per the above method. Each of the strains was inoculated by different routes (S/C, I/P and oral) into groups of chicks having 10 chicks in each group. On the basis of morbidity and mortality one most pathogenic strains ~~was~~ selected for experimental infection in older chicks (3 week old).

Source of the chicks :

- One hundred healthy day-old chicks were obtained from Government poultry farm, Haringhata, Deptt. of Animal Husbandry, West Bengal for the experimental study.

Observation of chicks before infection :

The experimental day old broiler chicks were kept under strict observation during the period before infection. During this period, the chicks were neither treated nor supplemented with any antimicrobial (antibiotic or sulpha) and coccidiostat. The chicks were routinely examined for health before exposure to infection and revealed no apparent abnormalities. Random faecal samples were examined by culturing on MacConkey's lactose Agar plate for presence of E. coli.

Grouping of chicks :

The experimental chicks were randomly divided into 10 groups having 10 chicks in each group as follows.

Gr 1 Noninfected Control for I/P route with sterile broth culture

Gr 2 Noninfected Control for S/C route with sterile broth culture

Gr 3 Noninfected Control for oral route with sterile broth culture

Gr 4 Infected with E. coli I/P route (01 strain)

Gr 5 Infected with E. coli S/C route (01 strain)

Gr 6 Infected with E. coli oral route (01 strain)

Gr 7 Infected with E. coli I/P route (02 strain)

Gr 8 Infected with E. coli S/C route (02 strain)

Gr 9 Infected with E. coli oral route (02 strain)

The chicks were reared in battery brooders with special broiler chick mash water adlib free from infection.

Preparation of the inoculum :

The cultures of serotype 01 and 02 selected for experimental infection were inoculated into nutrient broth from young agar slants and incubated at 37°C for 12 hours. From the broth cultures, direct smears were taken, stained and examined to ensure purity of the cultures. The broth culture tubes were centrifuged and the sediment was suspended in sterile normal saline and the turbidity was matched with Brown's opacity tube no.3.

Dose of the inoculum and route of administration :

For each strain of E. coli (01 and 02) selected for experimental infection, one group of chicks was infected intraperitoneally (I/P) with 0.2 ml, one group was injected subcutaneously (S/C) with 0.2 ml and one group orally with 0.9 ml of the inoculum containing about 10^9 organisms per ml. Three groups of chicks were kept as control. One of these groups was injected intraperitoneally (I/P) with 0.2 ml, one group subcutaneously (S/C) with 0.2 ml and the other group fed

orally with 0.9 ml of sterile nutrient broth. The inoculation was done when the chicks were three day old.

Post-inoculation observation :

Inoculated chicks were observed at every 6 hours interval during the first 24 hours post-inoculation and subsequently at 12 hours interval for 7 days. Mortality and morbidity were recorded at each observation. Clinical manifestations if any were also recorded. Chicks died due to probable infection were at once necropsied and the gross pathological changes in the internal organs were recorded. For histopathological studies, sections of the heart, lungs, liver, kidney and intestine were collected and processed in the laboratory. Attempts were made for reisolation of E. coli from heart blood, liver, lungs, intestinal contents, pericardial fluid and peritoneal fluid by inoculating on to MacConkey's Lactose Agar plates.

3.4.2 Clinical manifestations and gross lesions in young chicks

As described above, the inoculated chicks were observed at every 6 hours interval during the first 24 hours post-inoculation and subsequently at 12 hours interval for 7 days. Clinical manifestations, morbidity and mortality were recorded at each observation. Chicks died due to probable infection were

immediately necropsied and the gross pathological changes in the internal organs were recorded.

3.4.3 Histopathological changes in young chicks

The young 3 day-old chicks infected with virulent E.coli serotypes (01 and 02) by intraperitoneal, subcutaneous and oral route were immediately necropsied after death. After noting the gross pathological changes in the internal organs, the tissues of organs showing gross pathological lesions/changes were collected in small pieces in 10% formal saline.

After fixation, the tissues were cut into small pieces and processed according to routine procedure described by Luna (1968). The paraffin embedded tissues were cut into sections of 4-5 μ thickness and stained with haematoxylin and eosin (H & E) stain (Collins and Lyne, 1976). The stained tissue sections were examined under the microscope and the histopathological changes recorded.

3.4.4 Experimental production of colibacillosis in older chicks (3 weeks old) with or without latent infection of coccidiosis

For producing experimental infection one most virulent strain (02) was inoculated into 3 week old broiler chicks as per Savov (1963).

Source of chicks :

One hundred day-old healthy chicks were obtained from Government Poultry Farm, Haringhata, Department of Animal Husbandry, West Bengal for the study.

Observation of chicks before infection :

The chicks were kept under constant observation during the period before the infection. During this period, the chicks were neither treated nor supplemented with any antimicrobial and coccidiostat. The chicks were routinely examined for health before exposure to infection and revealed no apparent abnormalities. Random faecal samples were examined by culturing on MacConkey's Lactose Agar plate for presence of E. coli. Faeces were also examined for the presence of any oocysts. The chicks were maintained on specially prepared broiler chick mash and ad-lib drinking water free from infection without any antimicrobial or coccidiostat.

Grouping of chicks :

The experimental chicks were randomly divided into 10 groups comprising 10 chicks in each group as follows :

- Gr I Control for I/P route with sterile broth culture.
- Gr II Control for S/C route with sterile broth culture.
- Gr III Control for oral route with sterile broth culture.

- Gr IV Infected with E. coli by I/P route (02 strain)
- Gr V Infected with E. coli by S/C route (02 strain)
- Gr VI Infected with E. coli by oral route (02 strain).
- Gr VII Latent infected with coccidiosis and infected with E. coli by I/P route (02 strain)
- Gr VIII Latent infected with coccidiosis and infected with E. coli by S/C route (02 strain)
- Gr IX Latent infected with coccidiosis and infected with E. coli by oral route (02 strain)

The experimental chicks were reared carefully till 3 weeks when the experimental infection was given. The chicks were reared in battery brooders separately for each group.

Preparation of the inoculum :

The culture of the most virulent serotype (02) was selected for the experimental infection to 3 week old chicks with and without latent infection with coccidiosis. The culture for the experimental infection was inoculated into nutrient broth from young agar slants, and incubated at 37°C for 12 hours. From the broth culture direct smears were taken, stained and examined to ensure purity of the culture. The broth culture tube was centrifuged and the sediment was suspended in sterile normal saline and the turbidity was matched

with Brown's opacity tube No.3. Gr VII, Gr VIII and Gr IX were fed 1000 infective oocysts of E. tenela at age 2 weeks to produce latent infection of coccidiosis. Faecal samples were examined for all chicks regularly to detect the discharge of infective oocysts in the faeces from day 21 after E. coli infection till the end of the experiment to confirm the latent infection.

Dose of inoculum and route of administration :

The virulant E. coli strain (02) was selected for the experimental infection. Each chick of group IV and group V was injected intraperitoneally (I/P) and subcutaneously respectively with 0.2 ml of the virulant broth culture containing about 10^9 organisms per ml. Each chick of group VI was also fed orally 0.9 ml of the said culture. The chicks of group I, II & III were kept as control. Each chick of groups I, II were infected with sterile broth culture at the dose rate of 0.2 ml injected to each chick intraperitoneally and subcutaneously respectively. The chicks of group III were fed 0.9 ml of the said broth culture of E. coli. The inoculation was done when the chicks attained the age of 3 weeks.

Post-inoculation observation :

Inoculated chicks were observed at every 6 hours interval during the first 24 hours post-inoculation and

subsequently at 12 hours interval for 7 days. Mortality and morbidity were recorded at each observation. Chicks died due to probable infection were immediately necropsied and the gross changes in internal organs were recorded. Clinical observations if any were also noted. Attempts were made for reisolation of E. coli from heart blood, liver, lungs, intestinal contents, pericardial and peritoneal fluid by inoculating on to MacConkeys' Lactose Agar plates.

3.4.5 Clinical manifestations and gross lesions in 3 week old chicks

As described above, the inoculated chicks were observed at every 6 hours interval during the first 24 hours post-inoculation and subsequently at 12 hours interval for 7 days. Clinical manifestations, morbidity and mortality were recorded at each observation. Chicks died due to probable infection were immediately necropsied and the gross pathological changes in the internal organs were recorded.

3.5 Therapy

On the basis of the in-vitro drug sensitivity test. An experimental in vivo trial of the drug against E. coli infection (serotype 02) on day 3 old broiler chicks post-infection on different routes was done to study the efficacy of the drug.

Source of experimental chicks and rearing :

Ninety healthy day-old broiler chicks were obtained from the Government Poultry Farm, Haringhata, Department of Animal Husbandry, West Bengal and reared in battery brooder, without any antimicrobial feed additive and anticoccidial drugs. They were fed with standard chick mash and water adlib. Vitamin supplement under the trade name, "Vimeral" was administered at the dose rate of 10 ml/100 birds with drinking water.

Observation of chicks before infection :

All the chicks were kept under constant observation during the period before infection. During this period, the chicks were neither treated nor supplemented with any antibiotics, sulpha or coccideostat. Routine health check up of chicks before exposure to infection revealed no apparent abnormality. Random faecal samples were examined by culturing on MacConkey's Lactose Agar plate for presence of E. coli. The chicks were divided into nine groups (GA₁, A₂, A₃, B₁, E₂, E₃ and C₁, C₂ & C₃) having 10 chicks in each group as follows and reared in battery brooders separately.

- Gr A₁ - Control (injected with sterile broth with 0.2 ml I/P)
- Gr A₂ - Control (injected with sterile broth with 0.2 ml S/C)
- Gr A₃ - Control (fed with sterile broth with 0.9 ml orally)

- Gr B₁ - Infected untreated control (injected with broth culture of E. coli serotype 02 by I/P route) with 0.2 ml
- Gr B₂ - Infected untreated control (injected with broth culture of E. coli serotype 02 by S/C route) with 0.2 ml
- Gr B₃ - Infected untreated control (administered with broth culture of E. coli serotype 02 orally with 0.9 ml)
- Gr C₁ - Infected and treated (injected with broth culture of E. coli serotype 02 I/P with 0.2 ml) and treated with sulphadiazine in feed.
- Gr C₂ - Infected and treated (injected with broth culture of E. coli serotype 02 S/C with 0.2 ml) and treated with sulphadiazine in feed.
- Gr C₃ - Infected and treated (fed with broth culture of E. coli serotype 02, orally) and treated with sulphadiazine in feeds.

All the infected and treated groups were C₁, C₂ & C₃.

Preparation of the inoculum :

The culture of serotype 02 E. coli selected for experimental infection was inoculated into nutrient broth from young agar slant and incubated at 37°C for 12 hours. Directly stained smears from broth culture were examined to ensure purity of the culture. The broth culture tube was centrifused and the sediment was suspended in sterile normal saline and

the turbidity was matched with the Brownie opacity tube No. 3.

Dose of the inoculum and route of administration :

The serotype 02 of E. coli was selected for experimental infection for its virulence. Each chick of the infected treated group (Gr C₁) at age day 3 was injected intraperitoneally (I/P) with 0.2 ml, group Gr C₂ was injected subcutaneously (S/C) with 0.2 ml and group Gr C₃ orally with 0.9 ml of the inoculum containing 10⁹ organisms per millilitre (ml). All the three groups C₁, C₂ & C₃ of chicks were administered simultaneously at age day 3 with sulphadiazine at the dose rate of 0.4% i.e. 400 mg in 100 gms of feed mash for 5 days.

Three groups of chicks (Gr E₁, Gr E₂ and Gr E₃) were kept as infected untreated control. At age day 3 each chick of the infected untreated control group Gr E₁ was injected intraperitoneally (I/P) with 0.2 ml. Group Gr E₂ was injected subcutaneously (S/C) with 0.2 ml and Group Gr E₃ orally with 0.9 ml of the above inoculum.

Three groups of chicks (Gr A₁, Gr A₂ and Gr A₃) were kept as uninfected untreated control. At age day 3 each chick of the uninfected untreated control group Gr A₁ was injected intraperitoneally (I/P) with 0.2 ml of the sterile broth; Group (Gr A₂) was injected subcutaneously (S/C) with 0.2 ml

of sterile broth and group (Gr A₃) was fed orally with 0.9 ml of the sterile broth.

Post-inoculation observation :

Inoculated chicks were observed at every 6 hours interval during the first 24 hours post-inoculation and subsequently at 12 hours interval for 10 days. Mortality and morbidity were recorded at each observation. Chicks died due to probable infection were immediately necropsied and the gross pathological changes in the internal organs were recorded. Reisolation of E. coli was attempted from heart, blood, liver, lung, intestinal contents, pericardial fluid and peritoneal fluid by inoculating on to MacConkey's Lactose Agar plates. The efficacy of the drug used was assessed.

3.6 Prevention and Control

For prevention and control of colibacillosis in two problem broiler poultry farms A and B in Hooghli district where incidences of colibacillosis were frequently observed were taken up for controlling and preventing the further occurrence of the disease in these farms without vaccination. The following measures were taken :

- i) Disinfection and changes of litters, utensils etc.
- ii) Improved sanitation in poultry houses.
- iii) Supply of wholesome drinking water and feed.

- iv) Increased ventilation
- v) Preventive measures against respiratory diseases (CRD and Ranikhet, coccidiosis and other stress factors).
- vi) Preventing faecal contamination of hatching eggs.
- vii) Prevention of rodents against contamination of feeds by rodent droppings.
- viii) According to the sensitivity of the serotype of E. coli isolated from affected chicks, regular preventive treatment of the chicks was followed at age 3 and age 21 for 5 days each for the newly introduced chicks.
- ix) E. coli infected chicks/birds were to be disposed off from the poultry premises.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Incidence of Colibacillosis in Chicks in Poultry Farms

4.1.1 Isolation and identification of E. coli

The results of the isolation and identification of E. coli from different sources were presented in Table 1. A total of 1008 samples tested for E. coli from 508 dead chicks comprising 524 from small poultry farms, 364 from medium poultry farms and 120 from large poultry farms, were processed for isolation and identification of E. coli in the present study. Altogether 304 strains of E. coli could be isolated from 508 dead chicks. All the 304 isolates were included from the small poultry farms (160), medium poultry farms (104) and large poultry farms (40). Out of 508 dead chicken examined 304 yielded E. coli with an overall incidence rate of 59.8 per cent (Table 1) whereas according to small, medium and large poultry farms the incidence of cultural positive cases of E. coli in relation to samples examined were 63.2%, 53.3% and 66.6% respectively.

Isolation and identification of E. coli from dead chicks and other sources were carried out by Saltykov (1977), Sandulescu et al. (1977), Eozorgmehri Fard and Gilani (1980), Samiyal et al. (1981), Wantanbe et al. (1983), Cloud et al. (1985), Sinha et al. (1985), Nakamura et al. (1986), Prukner (1986), Verma (1987) and Phukan (1988). Yadav and Malik (1971), Verma (1987) and Phukan (1988) reported 69.04%, 45.5% and

Table No. 1. Isolation of E. coli from different sources.

Sl.No.	Source of dead chicks	No. of chicks examined on P.M.	No. of samples tested for <u>E. coli</u> .	No. of samples +ve for <u>E. coli</u> .
1	Small poultry farms	253	524	160(63.2%)
2	Medium poultry farms	195	364	104(53.3%)
3	Large poultry farms	60	120	40(66.6%)
Total :		508	1008	304(59.8%)

50.84% respectively of infection rate of colibacillosis in poultry farms which were close to the results recorded in the present study.

Seasonal incidence of colibacillosis

The seasonal incidence of colibacillosis among chicken according to different seasons of the year (summer, rainy and winter) was presented in Table 2. In a year, the total number of outbreaks, mortality rate among chicken, dead chicken examined on post-mortem, samples collected and samples positive for E. coli were recorded as 35, 560, 253, 524 and 160 in small poultry farms; 12, 323, 195, 364 and 104 in medium poultry farms; 5, 250, 60, 120 and 40 in large poultry farms respectively. According to summer, rainy and winter seasons, number of outbreaks, mortality rate among chicken, dead chicks examined on post-mortem, samples collected and samples positive for E. coli on cultural basis were 12, 12 and 11; 205, 198 and 157; 82, 96 and 75; 164, 192 and 168; and 42, 58 and 54 in small poultry farms, 5, 4 and 3; 114, 126 and 83; 55, 75 and 65; 105, 170 and 89; and 35, 42 and 27 in medium poultry farms and 1, 2 and 2; 46, 84 and 120; 10, 20 and 30; 30, 35 and 55; and 10, 12 and 18 in large poultry farms respectively.

Study indicated no seasonal variation in the incidence of colibacillosis observed in the area. Work on the seasonal incidence of the disease were scarce in the literature.

Table No. 2. Seasonal incidence of Colibacillosis in chicken

Season	Small poultry farms					Medium poultry farms					Large poultry farms				
	No. of outbreaks	No. of mortalities	No. of dead chicks	No. of samples collected	No. of samples collected for E. coli	No. of outbreaks	No. of mortalities	No. of dead chicks	No. of samples collected	No. of samples collected for E. coli	No. of outbreaks	No. of mortalities	No. of dead chicks	No. of samples collected	No. of samples collected for E. coli
Summer (March to June)	12	205	82	164	48	5	114	55	105	35	1	46	10	30	10
Rainy (July to October)	12	198	96	192	58	4	126	75	170	42	2	84	20	35	12
Winter (November to February)	11	157	75	168	54	3	83	65	89	27	2	120	30	55	18
Total	35	560	253	524	160	12	323	195	364	104	5	250	60	120	40

Out of 508 dead chickens examined 304 yielded E. coli culturally with an overall incidence rate of 59.8% (Table 1). The incidence recorded in the present study was almost nearer to the incidence rate observed by Yadav and Malik (1971), Verma (1987) and Phukan (1988) in other parts of the country.

Incidence of E. coli in different age groups of dead chickens were presented in Table 3. The incidence was 66.5% in the chicks in the age group of 0 - 30 days; 20.9% in the chicks of age group 31 - 60 days and 11.5% in the chicken in the age group of 61 days and above. The incidence of colibacillosis was found higher among younger chicks than older ones which was statistically significant ($P < 0.01$).

The present study also revealed that the incidence of colibacillosis was higher (66.5%) in the younger age group (0 - 30 days) than the older birds. This was in accordance with the findings of Phukan (1988) who similarly reported higher incidence (63.20%) in younger birds. This might probably be due to the under developed body defensive mechanism. Further, the chicks as they came out of the hatcheries face a hostile environment and bad management and hygiene. Bacterial colonisation of the gastro-intestinal tract of animals takes place soon after birth or hatching when the young ones ingest food. During the first 2 to 4 days enterobacteria colonise small intestine and caecum and remain throughout the life of the chicken.

Table No. 3. Incidence of E. coli in different age groups.

Sl.No.	Age group in days	Number of dead birds examined on postmortem	<u>E.coli</u> isolated from numbers of birds	Percentage of Prevalence (%)
1	0 - 30 days	308	205	66.5**
2	31 - 60 days	105	22	20.9
3	Above 60 days	95	11	11.5
Total :		508	238	46.8

** P/0.01

Attempt was made to demonstrate the distribution of E. coli in different sites and pathological conditions of dead chicks and the results were given in Table 4. It was clear that the majority of E. coli strains were isolated from intestinal contents (106) and heart blood (92) followed by yolk materials (67), pericardial fluid (12), liver and peritoneal fluid (10 each), lungs (5) and kidney (2). It was further seen that highest number of E. coli strains were isolated from cases of enteritis (106), enlarged and congested heart (92), unabsorbed and putrified yolk (67), pericarditis and cloudy pericardial fluid (12) necrosis and congestion with white streaks on the liver (10), peritoneal fluid (10), congested lungs (5) and congested and enlarged kidneys (2).

Recovery of E. coli from intestine and heart blood and yolk material in higher frequency was reported by Sinha et al. (1985), Verma (1987) and Phukan (1988). The incidence of isolation of E. coli from intestine was highest because E. coli strains were normally present in the intestine. Presence of E. coli strains in the heart blood indicated that the organisms were capable of invading the blood stream resulting in septicæmic condition. These findings were in agreement with the results obtained by Stipkovits and Solyom (1968), Gupta and Singh (1969a), Kulkarni et al. (1970a), Verma and Adlakha (1971), Yadav and Malik (1971), Rao et al. (1976), Sharma et al. (1980), Kim and Tak (1983) and Sinha et al. (1985).

Table No. 4. Isolation of E. coli from different sites and pathological conditions of dead chicks.

Sl.No.	Samples collected from liver, heart, lungs, intestines etc.	Pathological conditions observed.	No. of samples examined.	No. of samples positive for <u>E.coli</u> .
1	Intestinal contents.	Enterites.	356	106 (29.7%)
2	Heart blood.	Enlarged and congested heart.	356	92 (25.8%)
3	Liver.	Necrosis and congestion with white streaks.	82	10 (12.1%)
4	Pericardial fluid.	Pericarditis, cloudy appearance of Pericardial sac with accumulation of fluid inside the pericardial sac.	53	12 (22.6%)
5	Peritoneal fluid	Fluid accumulated inside the peritoneal cavity.	50	10 (20%)
6	Lungs	Congestion of Lungs.	20	5 (25%)
7	Yolk materials.	Unabsorbed putrified yolk.	82	67 (81.7%)
8	Kidneys.	Congested and enlarged	9	7 (22.2%)
Total :			1008	304 (30.15%)

Biochemical tests for Escherichia coli

Altogether 304 E. coli strains were isolated and identified on the basis of morphology, staining reactions, cultural characteristics and biochemical tests. The results of the biochemical tests were presented in Table 5a and 5b. Out of 304 strains of E. coli 300 strains produced indole, all the 304 strains of E. coli showed positive results to M.R. and negative to V.P. reaction and fermented lactose, glucose, manitol and maltose whereas fermentation of sucrose, dulcitol and Salicin was shown by 130, 222 and 95 respectively. On the other hand, none of the 304 strains of E. coli fermented adonitol and inositol and utilised citrate and produced urease and H_2S . The positive biochemical values obtained for the isolates were in full agreement with the results obtained by Phukan (1988).

4.1.2 Serological typing of Escherichia coli

A total of 304 strains of E. coli were serotyped and the results of which were displayed in Table 6. Altogether, 183 (60.19%) strains belonged to different 'O' types, 42 (13.81%) strains were untypable and the remaining 79 (25.98%) were rough strains. Overall 14 different 'O' serotypes were identified. Twenty six of the strains belonged to 01, 49 to 02, 10 to 036, 8 to 045, 6 to 055, 25 to 078, 12 to 079, 5 to 080, 15 to 088, 7 to 0120, 2 to 0157, 13 to 0158, 4 to 0159 and

Table No. 5a. Biochemical characteristics of E. coli isolated from dead chicks (contd..)

Species	Number of isolates showing positive results to						
	Number of isolates.	N.P. test %	V.P. test %	Indole production %	H ₂ S production %	Citrate utilization %	Urease production %
<u>Escherichia coli.</u>	304	304 100%	0 0%	0 0%	300 98.68%	0 0%	0 0%

Table No. 5b. Biochemical characteristics of E. coli isolated from dead chicks.

Species	Number of Isolates tested.	Number of isolates showing +ve results to									
		Fermentation Test for									
		Glucose %	Lac-tose %	Suc-rose %	Dulci-tol %	Adoni-tol %	Irosi-tol %	vari-tol %	Sali-cir %	Maltose %	
<u>Escherichia coli.</u>	304	304 100%	304 100%	130 42.75%	222 73%	0 0%	0 0%	304 100%	95 31.24%	304 100%	

Table No-6. Serotypes of E. coli strains isolated from dead chicks.

Sl.No.	' O ' group	Total number of strain in each group.
1	01	26
2	02	49
3	036	10
4	045	8
5	055	6
6	078	25
7	079	12
8	080	5
9	082	15
10	0120	7
11	0157	2
12	0158	13
13	0159	4
14	0162	1

No. of Typable strain - 183(60.19%)

No. of Nontypable " - 42(13.81%)

PoUgh " - 79(25.98%)

Total No. of strains isolated - 304.

1 to 0162 isolates. According to Bekajle and Prost (1963), Stipkovits and Bereznai (1967), Oskolkov and Saltykov (1977), Sandulescu et al. (1977), Secliter et al. (1983), Wantanhe et al. (1983), Cloud et al. (1985), Mellota and Nigralli (1985), Prukner (1986) and Murray (1986) 'O' serotypes were most commonly isolated. Among the 'O' serogroup most frequent pathogenic serotypes were 078, followed by 01, 02, 03, 08, 0103 and 0128. In India isolation of serotypes 01, 02, 023, 078, 08, 086, and 023 were commonly reported from cases of colisepticaemia in chicken (Gupta and Singh, 1969b; Yadav, 1970; Jayaraman and John, 1971; Verma and Adlakha, 1971; Yadav and Malik, 1971; Arunachalam et al. 1974; and Farid et al., 1981). Sahota et al. (1978) and Sharma et al. (1980), isolated E.coli serotypes 01, 08, 017 and 078 from cases of Salpingitis and oophoritis in chicken whereas Bandopadhyay and Dhawedkar (1984) reported that the most prevalent E. coli serotype was 017, in salpingitis of poultry. Other serogroups isolated by them were 05, 019 and 060. The predominant serotypes in the present study were found to be 02 followed by 01, and 078. Gupta and Singh (1969b) also obtained similar results of isolation of 078, 02 and 01 E. coli serotypes whereas Phukan (1988) recorded predominant serotype of E. coli as 02 followed by 020. Yadav (1970), Jayaraman and John (1971), Verma and Adlakha (1971), Yadav and Malik (1971), Arunachalam et al. (1974), Sandulescu et al. (1977), Farid et al. (1977),

Oskolkov and Saltykov (1977), Srinivasan et al. (1979), Sharma et al. (1980), Saxena et al. (1983), Cloud et al. (1985), Sinha et al. (1985) also recorded predominant serotypes of E. coli as 01, 02 and 078 in addition to other serogroups in their studies. Variations in the recovery of different serogroups might probably be due to different geographical area, environment and managerial factors.

4.2 Pathogenicity Test of Escherichia coli Isolates

4.2.1 Pathogenicity test of E. coli in white albino mice

The results of the pathogenicity test against different '0' serotypes of E. coli isolates in white albino mice were presented in Table 7a. It was observed that 83.66% mortality in white albino mice was recorded within 96 hours of inoculation of the serotype 02 E. coli isolate. Serotypes 01 and 078 of the isolates recorded 66.66% and 50% mortality in white albino mice respectively. Serotypes 079, 080 and 088 showed 50% mortality and 036, 0120, 0158, 0159 and 0162 recorded 33.33% mortality and 045, 055 and 0157 caused 16.66% mortality in experimental white albino mice. Further, the control group which was injected with sterile nutrient broth did not show any mortality.

Experimental mice which died due to probable infection as per the table were immediately necropsied. Cross

Table No. 7a. Results of the pathogenicity test against E. coli serotypes in white albino mice

Sl. No.	E. coli Serotype	Route of inoculation	Dose (ml)	No. of mice inoculated	No. of mice died within days												Total no. died	Per-centage of mortality (%)	Pathogenicity	Control group
					No. of mice died within days															
					24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	120 hrs.	120 hrs.	120 hrs.	120 hrs.	120 hrs.	120 hrs.	120 hrs.				
1	01	I/P	0.5	6					1	1	1	1	1	4	66.66	+++	6	NIL		
2	02	I/P	0.5	6					1	1	1	1	1	5	83.66	+++	6	-		
3	036	I/P	0.5	6									1	2	33.33	+	6	-		
4	045	I/P	0.5	6									1	1	16.66	?	6	-		
5	055	I/P	0.5	6									1	1	16.66	?	6	-		
6	078	I/P	0.5	6							1	1	1	3	50.00	++	6	-		
7	079	I/P	0.5	6					1		1	1		3	50.00	+	6	-		
8	080	I/P	0.5	6					1	1	1	1		3	50.00	+	6	-		
9	088	I/P	0.5	6					1		1	1	1	3	50.00	+	6	-		
10	0120	I/P	0.5	6					1				1	2	33.33	?	6	-		
11	0157	I/P	0.5	6									1	1	16.66	?	6	-		
12	0158	I/P	0.5	6							1		1	2	33.33	+	6	-		
13	0159	I/P	0.5	6								1	1	2	33.33	?	6	-		
14	0162	I/P	0.5	6								1	1	2	33.33	+	6	-		

I/P = Intraperitoneal; ? = Doubtful

pathological changes observed in the internal organs of dead mice were congestion of heart liver, spleen, lungs and kidneys in all the dead mice (Fig. 1, 2, 3, and 4). Tissue suspensions particularly from heart blood were collected aseptically and inoculated into blood agar plates and reisolation and identification of E. coli was made to confirm the cause of death (Table 7b).

The surviving mice which did not die were sacrificed on day 5 post-inoculation. No gross pathological changes in the internal organs like heart, liver, spleen, lungs, kidneys were observed. Tissue materials from heart blood collected aseptically and inoculated into blood agar plates did not show any growth of E. coli. Similarly, the control experimental mice which did not die were sacrificed and nothing abnormal was noticed in the internal organs, specially heart, liver, spleen, lungs and kidneys (Fig. 5). Inoculation of heart blood in blood agar plates did not reveal any microbial growth, particularly E. coli.

It was evident from Table 7a that the degree of pathogenicity of different serotypes of E. coli used for pathogenicity test were 01 and 02 as '+++', 078 as '++', 036, 080, 088, 049, 0158 and 0162 as '+' and the rest serotypes were having doubtful pathogenicity.

Table No. 7.b. Results of Reisolation of E. coli from different sites of inoculated mice.

Sl. No.	Isolate No.	Route of inoculation	Total No. of white albino mice used	No. of mice died	No. of dead mice showing +ve reisolation.					
					Heart blood	Liver	Lungs	Intestinal contents.	Pericardial fluid	Peritoneal fluid.
1	01	I/P	6	4	4	2	1	1	-	1
2	02	I/P	6	5	5	3	1	3	2	1
3	036	I/P	6	2	1	1	-	1	1	-
4	045	I/P	6	1	1	1	1	-	-	-
5	055	I/P	6	1	1	-	-	-	-	-
6	078	I/P	6	3	3	-	1	-	-	4
7	079	I/P	6	3	2	1	1	-	1	-
8	080	I/P	6	3	1	1	-	-	-	-
9	088	I/P	6	3	1	-	-	-	1	-
10	0120	I/P	6	2	-	1	-	1	-	-
11	0157	I/P	6	1	-	1	-	-	-	1
12	0158	I/P	6	2	1	-	1	-	-	-
13	0159	I/P	6	2	2	-	1	-	1	-
14	0162	I/P	6	2	2	1	1	1	-	-

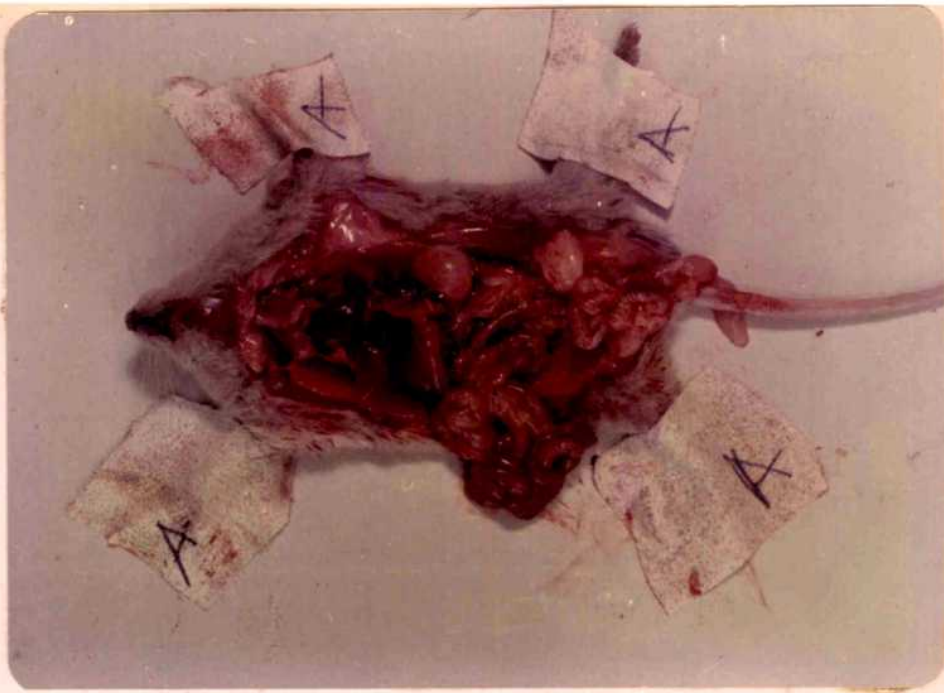


Fig.1 Showing gross pathological changes in the internal organs of white albino mice (dead) suggesting congestion of heart, liver, spleen, lungs and kidneys, due to E. coli. Serotype 01(A) experimental inoculation.

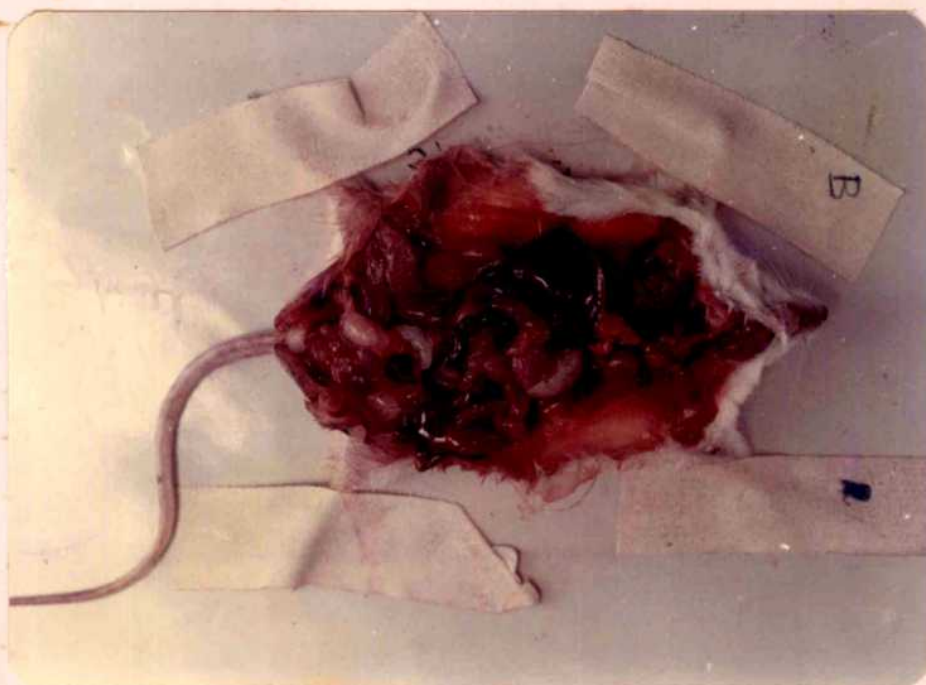


Fig.2 Showing macroscopic changes like congestion of heart, liver, spleen, lungs and kidneys of dead white albino mice with experimental inoculation of E. coli 02 serotype(B)

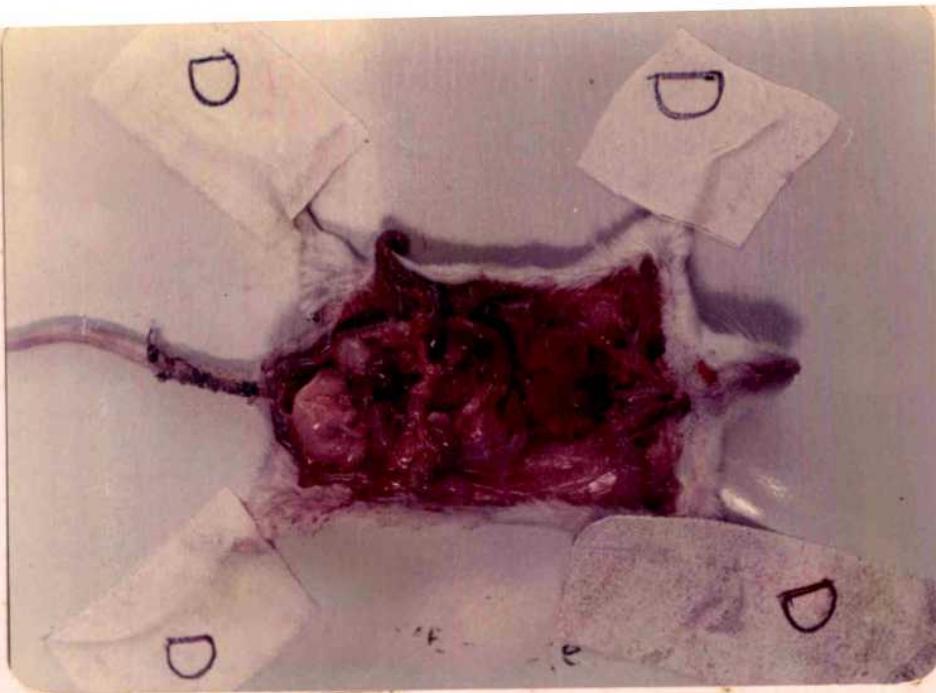


Fig.3 Showing macroscopic lesions in white albino mice(dead), like congestion of heart, liver, spleen, lungs and kidneys due to E.coli 078(D) serotype, experimentally inoculated.

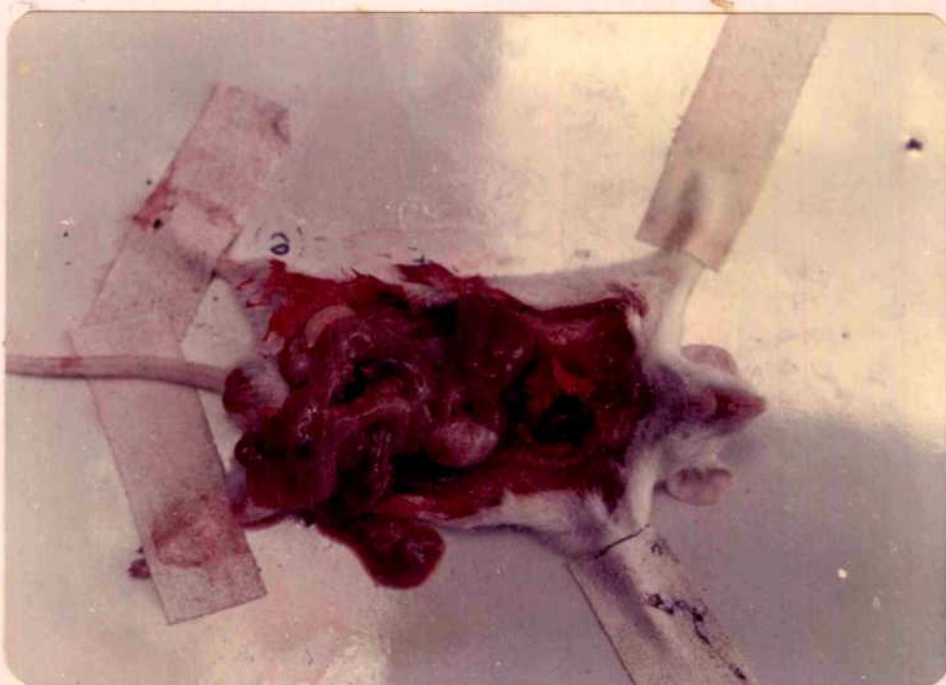


Fig.4 Showing gross lesions in experimentally inoculated white albino mice(dead) with E.coli 0158(C) serotype, suggesting congestion of heart, liver, spleen, lungs and kidneys.

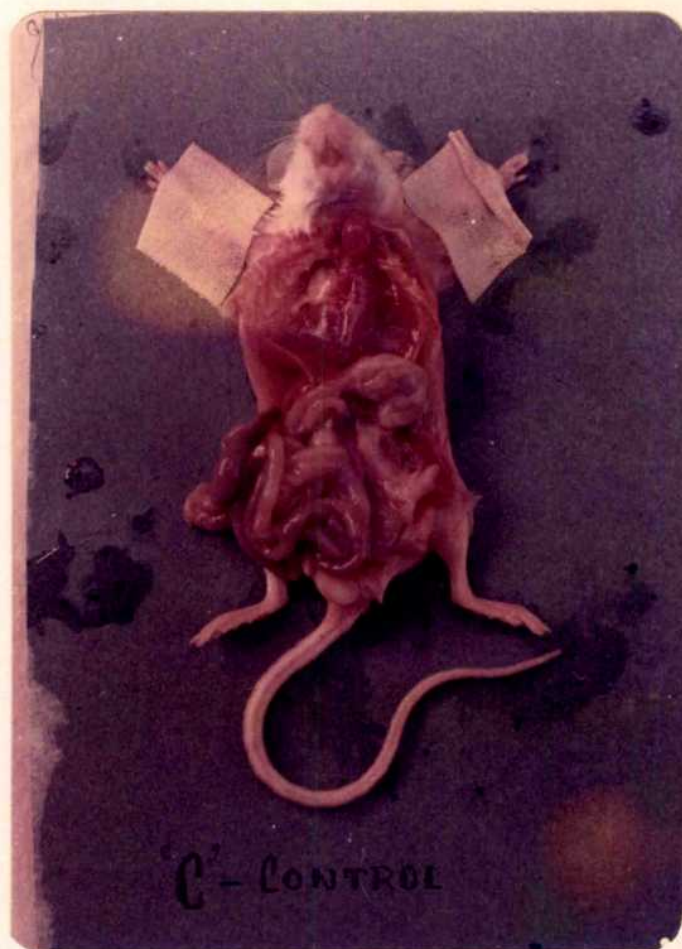


Fig.5 Showing absence of gross pathological lesions in the visceral organs of white albino mice (killed) of control group(C).

Gupta and Singh (1969b) studied the pathogenicity of E. coli strains in white swiss mice and recorded 40 pathogenic strains out of 54 isolates of E. coli. They observed that serotype 01, 02 and 078 were highly pathogenic to mice experimentally which was in agreement with the present findings. In addition, serotypes 07, 08, 018, 021, 023, 026, 039, 054, 0111 and 0143 were also found pathogenic to mice. Besides, Gupta (1975) observed E. coli serogroups (05, 09, 011 and 015) as highly pathogenic to white mice. He further observed that 15 serogroups (06, 07, 010, 013, 015, 016, 023, 034, 037, 041, 042, 054, 057 and 065) were nonpathogenic. Srivastava and Arya (1979) reported serogroup 022 as highly pathogenic to mice whereas Sharma et al. (1981) recorded E. coli strains of groups (025, 049, 060, 078, 090 and 0160) were 100% pathogenic to mice and 015, 018, and 048 were also pathogenic to mice whereas 017 was found nonpathogenic. They further recorded macroscopic lesions like congestion of liver, spleen in dead mice which corroborated with the present findings.

4.2.2 Pathogenicity test in rabbits for the enterotoxic activity of E. coli.

For testing the enterotoxic activity of the most virulent serotypes of E. coli as determined by white albino mice test, serotypes 01, 02, 078, and 0158 were selected for their enterotoxic activity in rabbit ligated ileal loop using their culture supernatant. The results of the experiment on

the enterotoxic activity of the above E. coli serotypes were recorded in Table 8 and Figure 6. The dilatation index in cases of the serotypes 01, 02, 078 and 0158 were calculated as 0.68, 0.68, 0.25, 0.25 respectively. In case of control loop, the dilatation index was 0.16. According to Dorah et al. (1982) the culture of the serotypes 01 and 02 were highly enterotoxic. The ligated illeal loop used in cases of the serotype 01 and 02 were highly swollen with fluid and the mucosa of the inflamed loops were also haemorrhagic and necrosed whereas the degree of swelling of the ligated illeal loop in case of serotype 078 was less marked. The results of the experimental study thus indicated that both serotype 01 and 02 were highly enterotoxic. Work on this respect on the E. coli isolates from chicken were scarce. However, Smith and Hall (1967) studied the enterotoxic activity of pathogenic E. coli serotypes collected from pigs, calves, lambs and rabbits in the ligated intestine loop of rabbit and observed haemorrhages and necrosis of mucosa and submucosa in all cases which was in agreement with the present study.

4.3 Antibiotic Sensitivity Tests

The results (Table 9a) revealed that 97.55%, 96.69% and 80.99% of E. coli strains isolated were sensitive to Furazolidone, Cefotaxime and Ampicillin respectively whereas 78.51% and 61.15% were sensitive to carbanicillin and sulphadiazine

Table No-8. Results of the enterotaxic activity of some pathogenic serotypes of E. coli in rabbit
 located ileal loop.

Sl. No.	Serotype of <u>E. coli</u> .	Dilatation of intestinal loop observed for accumulation of fluid.	Characters of accumulated fluid from intestinal loops. Volume Colour Consistency (ml)	pH.	Volume of fluid in the intestinal loop. (ml)	Length of the intestinal loop.	Dilatation Index.
1	01	+++	5.5 Brownish red.	5.4	5.5	8	0.68
2	02	+++	7.5 Brownish red.	5.6	7.5	11	0.68
3	078	++	2 Straw Coloured.	6.2	2	8	0.25
4	0158	++	2 Straw Coloured.	6.3	2	8	0.25
5	Control	---	0.5 Yellowish grey.	6	0.5	3	0.16

Table No-9 a. Sensitivity pattern of E. coli against different antimicrobial agents.

Species	No. of isolates tested	Sensitivity patterns of <u>Escherichia coli</u> against different antimicrobial agents						
		A	CA	CE	CH	CF	C	
<u>Escherichia coli.</u>	121	98 (80.99%)	95 (78.51%)	39 (32.23%)	43 (35.53%)	36 (29.75%)	43 (35.53%)	(Cortef)

Species	No. of isolates tested	Sensitivity patterns of <u>Escherichia coli</u> against different antimicrobial agents						
		AN	SN	CF	N	NA	FX	
<u>Escherichia coli.</u>	121	43 (35.53%)	74 (61.15%)	117 (96.69%)	43 (35.53%)	56 (46.28%)	118 (97.55%)	

A = Ampicillin 10mcg. C = Gentamycin 10mcg. F = Neomycin 30mcg.
 CA = Carbanicillin 100mcg. AN = Amikasin 10mcg. NA = Nalidixic acid 30mcg.
 CF = Cephaloridine 30mcg. SN = Sulphadiazine 150mcg. FX = Furazolidone 300mcg.
 CH = Chloramphenicol 30mcg. CF = Cefotaxime 30mcg.
 CT = Cotrimoxazole 25mcg.



Fig.6 Showing congestion and dilatation of ligated illeal loops of rabbit, with experimental inoculation of pathogenic E.coli serotypes, 01(A), 02(B), 078(D) and .0158(E) and control (C) with no dilatation of the loop.

respectively. Moderate degree of sensitiveness to cephaloridine (32.23%), Chloramphenicol (35.53%), Cotrimexazole (29.75%), Gertamycin (35.53%), Amikasin (35.53%), Neomycin (35.53%) and Nalidixic acid (46.28%) were recorded. Sensitivity pattern according to different serotypes were presented in Table 9b and Figures 7 to 13. It was seen that serotype 01 ^{was} ~~were~~ sensitive to only carbanicillin, Cephaloridine, Cefotaxime and Furazolidone and resistant to other antimicrobials used in the study. Serotype 02 was sensitive to Ampicillin, Carbanicillin, Sulphadiazine, Cefataxime and furazolidone but resistant to other antimicrobials used in this study. Serotype 078 was found to be sensitive to all antibiotics except Carbanicillin and Cephaloridine. Serotype 0158 was only sensitive to Ampicillin, Carbanicillin, Cephaloridine, Nalidixic acid and Furazolidone but resistant to others. It is evident from the Table 9b that serotype 0120 was resistant to only Cephaloridine, Sulphadiazine and Furazolidone but sensitive to all other antimicrobials used in the experiment. Serotypes 055 and 0162 were resistant to Ampicillin, Carbanicillin, Cephaloridine, Cotrimexazole and Sulphadiazine but sensitive to the rest except furazolidone for serotype 055. Serotype 0159 was resistant to Carbanicillin, Cephaloridine and Sulphadiazine only but susceptible to all other antibiotics. Samkya et al. (1981) reported drug sensitivity in Nalidixic acid (83%), Nitrofurazone (76%), Streptomycin (69%), Kanamycin (93%), Oxytetracycline (31%), Chlorotetracyclin (23%), Chloramphenicol (86%)

Table No. 9b. Sensitivity pattern of E. coli against different antimicrobial agents

Species	No. of sero- type tested	No. of serotypes sensitive to													Remarks
		A (cm)	CA (cm)	CE (cm)	CH (cm)	CT (cm)	C (cm)	AN (cm)	SN (cm)	CF (cm)	N (cm)	NA (cm)	FX (cm)		
<u>E. coli</u>	01		1.2	1.1						1.8			1.2	A = Ampicillin 10mcg	
<u>E. coli</u>	02	1.0	1.0						1.3	0.8			0.9	CA = Caranicillin 100 mcg	
<u>E. coli</u>	078	1.0			2.0	2.2	2.1	1.6	1.5	1.6	1.3	1.8	0.9	CE = Cephaloridine 30 mcg	
<u>E. coli</u>	0158	0.8	0.8	0.7								2.0	1.0	CH = Chloramphenicol 30 mcg	
<u>E. coli</u>	0120	0.7	0.8		2.3	0.6	2.3	2.2		2.0	2.0	2.1		CT = Cotrimexazole 25 mcg	
<u>E. coli</u>	055				1.2		2.0	1.6		1.5	1.3	1.5		G = Gentamycin 10 mcg	
<u>E. coli</u>	0159	1.0			1.8	2.2	1.8	1.7		1.5	1.5	2.0	1.0	AN = Amikasin 10 mcg	
<u>E. coli</u>	0162				2.1		2.3	2.3						ST = Sulphadiazine 150 mcg	
											1.7	1.2	1.3	CF = Cefetaxime 30 mcg	
														F = Neomycin 30 mcg	
														NA = Nalidixic acid 30 mcg	
														FX = Furazolidone 300 mcg	

Cm : Zone of inhibition expressed in centimeter

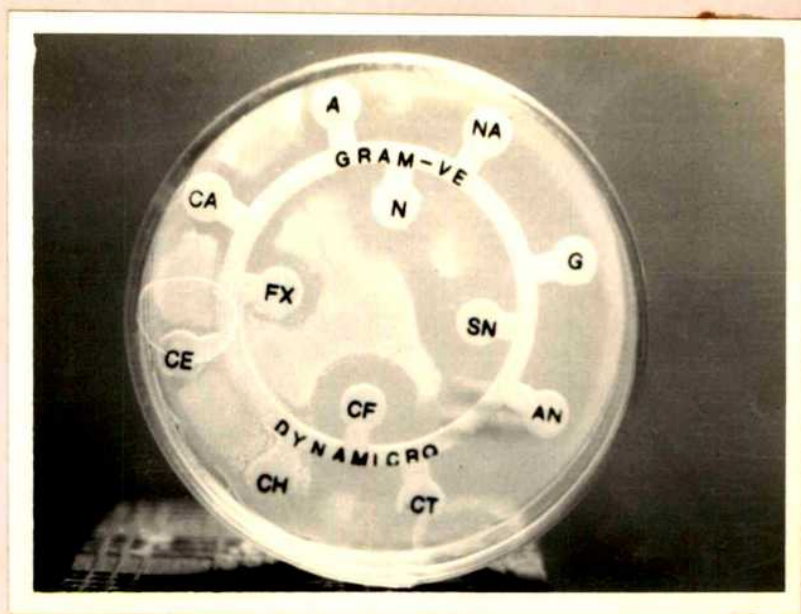


Fig.7 In vitro sensitivity of E.coli 01 sero isolate to different drugs, indicating sensitivity to carbanicillin cephaloridine, cafotaxime and Furazolidone.

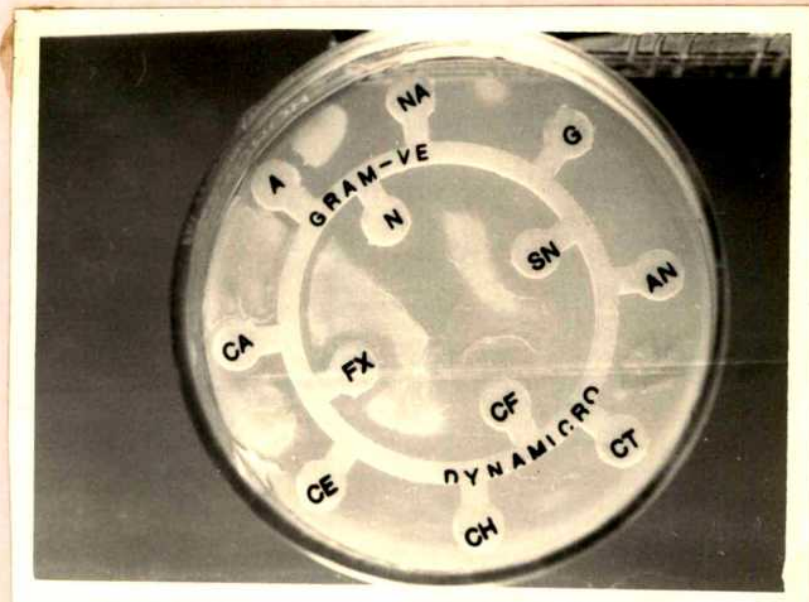


Fig.8 In vitro sensitivity of 02 E.coli sero isolates, indicating sensitive to ampicillin, carbanicillin, Sulphadiazine, cafotaxime and furazolidone.

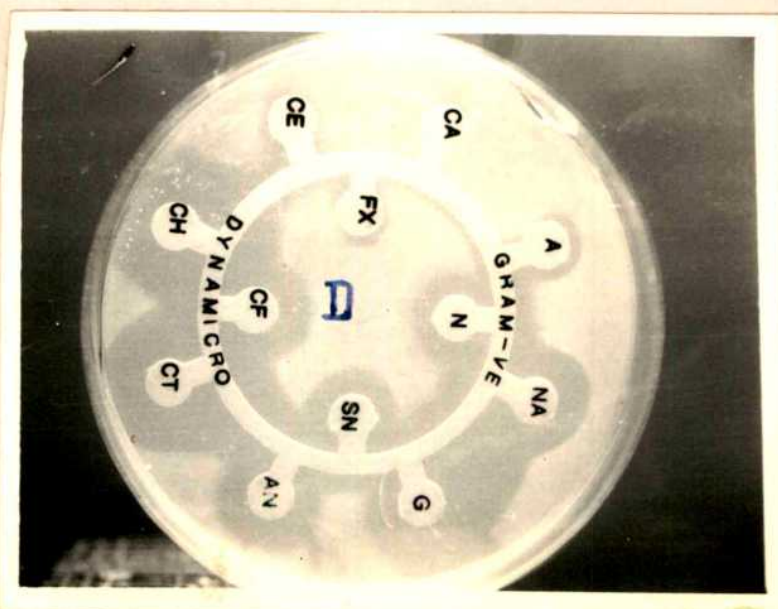


Fig.9 In vitro sensitivity of 078^(D) E.coli sero-isolate indicating sensitivity to all antibiotics used except carbenicillin and cephaloridine.

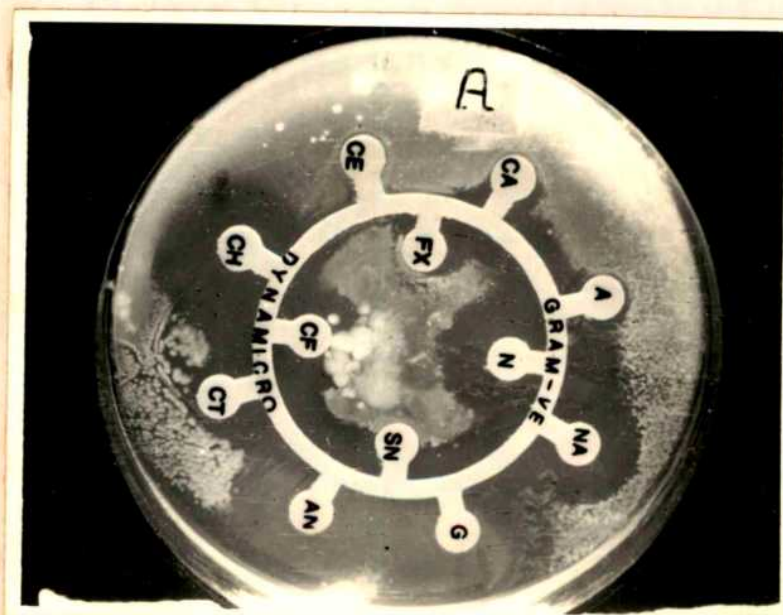


Fig.10 In vitro sensitivity of 0158(A) E.coli isolate to ampicillin, carbenicillin, cephaloridine, nalidixic acid and furazolidone and resistance to other antimicrobials used.

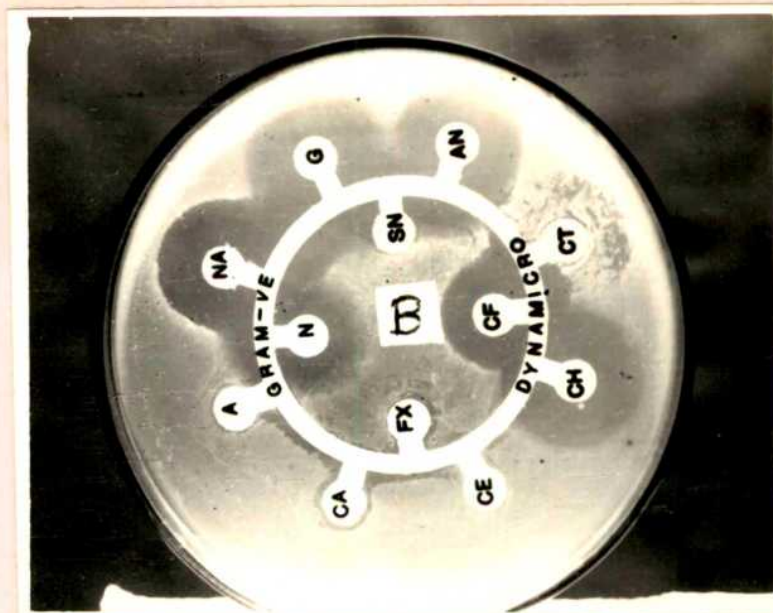


Fig.11 In vitro drug resistance of 0120(B) E.coli seroisolate to cephaloridine, sulphadiazine and furazolidone and sensitivity to other antimicrobials used.

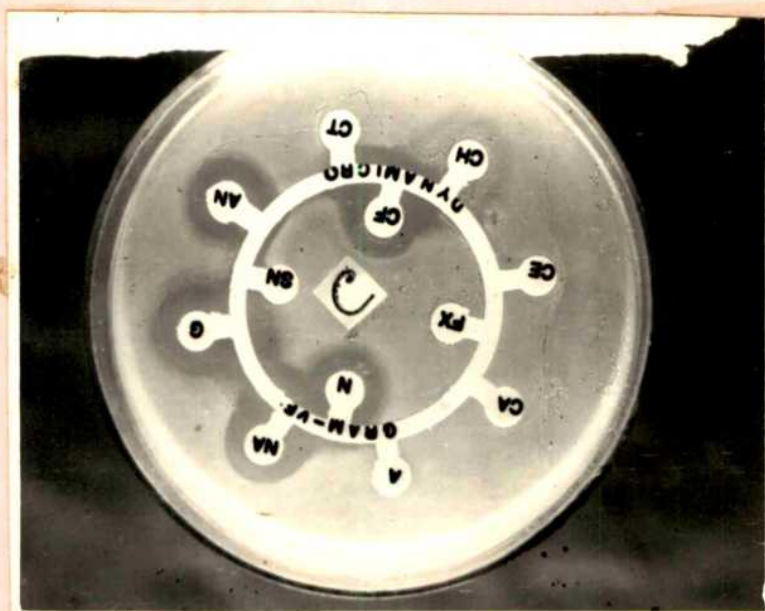


Fig.12 In vitro drug resistance of 055(c) E.coli seroisolate to ampicillin carbanicillin, cephalonidine, cotrimexazole and sulphadiazine and sensitivity to others used.

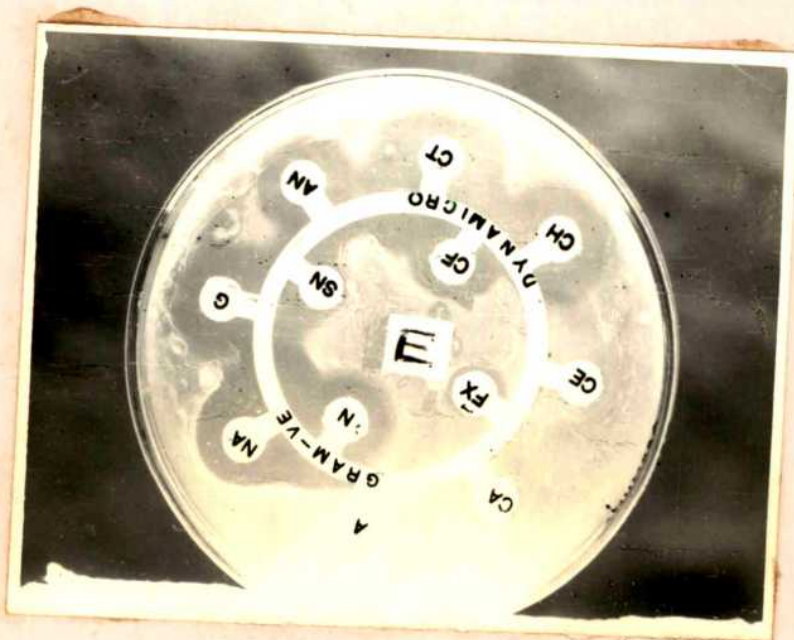


Fig.13 In vitro drug resistance of 0159(B) E.coli isolate to carbonicellin, cephaloridine and sulphadiazine, and sensitive to other antimicrobials, used.

and Tripple Sulphonamide (19%). However, Doxycycline was found to possess high activity against E. coli if given @ 50 ppm in drinking water. Sulphadimethazone or furazolidone also had similar effect. In the present study high level of resistance was observed by most of the E. coli serotypes to ampicillin, carbanicillin, cephaloridine, cotrimexazole, amikasin and sulphadiazine and no single antimicrobial was found sensitive to all the E. coli serotypes. Further, no single antimicrobial was 100% sensitive against the E. coli strains. This might be due to the development of resistance in E. coli due to indiscriminate use of antibiotics. Savov (1963), Aller Gancedo et al. (1969), Ezhov et al. (1970), Butura and Sahleanu (1972), Kapoor et al. (1978), Sahota et al. (1978), Srinivasan et al. (1979), Gyurov et al. (1981), Kumar et al. (1981), Sarma et al. (1981), Kim and Tak (1983), Smith and Lovel (1984), Vidotto et al. (1984), Barbour et al. (1985), Sinha et al. (1985), Tagawa (1986) and Ghosh (1987) reported varied degree of resistance of E. coli strains to different antimicrobials. Transfer of drug resistance has been a present day problem in controlling colibacillosis. Ramaswamy et al. (1982) reported that transfer of resistance was observed in 38 strains of E. coli isolated from diseased poultry ^{which was} 64.1%, 62.5%, 55% and 35% with streptomycin, Kanamycin, Chloramphenicol and Trimexazole respectively. Mehrotra et al. (1984) studied the transfer of drug resistance to Chloramphenicol and Furazone in E. coli.

4.4 Experimental Production of Colibacillosis

4.4.1 Experimental production of colibacillosis in young chicks (3 days old)

• The results of the experimental production of Escherichia coli (serotype 01 and 02) infection in 3 days old broiler were presented in Table 10a. Both the serotypes were highly pathogenic to 3 days old chicks. Serotype 02 produced 100% mortality in chicks within 7 days in all routes of administration whereas serotype 01 produced 100% mortality by intraperitoneal, (I/P) and subcutaneous (S/C) routes and 80% mortality in oral route administered to the chicks. Maximum mortality was observed in chicks which were inoculated intraperitoneally within the first 3 days in both the serotypes. None of the control group of chicks which were inoculated with only sterile broth culture by different routes died.

Stipkovits and Solyom (1968), Belitskii and Panikar (1969), Closek (1973), Farid et al. (1977), Sharma et al. (1981) and Phukan (1988) observed that most of the E. coli strains particularly some 'O' group strains were highly pathogenic to young chicks. Sharma et al. (1981) recorded E. coli strains of group 025, 060, 078, 090 and 0160 as highly pathogenic producing 100% mortality while Farid et al. (1977) reported E. coli serogroup 02, 011, 042 and 078 which were highly pathogenic to chicks. The results obtained in the present study in regard

Table No. 10a. Results of experimental production of E. coli infection in 3 day old chicks

Sl. No.	E. coli Sero-types	Route of inoculation	Dose in (ml)	No. of chicks died within days							Total no. of chicks died	Percent- tage of morta- lity (%)	Control Group	
				No. of chicks inocu- lated	1	2	3	4	5	6	7		No. of chicks used	No. of chick died
1	01	I/P	0.2	10	2	2	1	2	2	1	-	100	10	-
	01	S/C	0.2	10	-	-	-	2	6	2	-	100	10	-
	01	0	0.9	10	-	-	-	2	4	2	-	80	10	-
	02	I/P	0.2	10	8	2	-	-	-	-	-	100	10	-
	02	S/C	0.2	10	-	-	2	2	4	2	-	100	10	-
	02	0	0.9	10	-	-	2	6	-	2	-	100	10	-

I/P : Intraperitoneal

S/C : Subcutaneous

0 : Oral

to the pathogenicity of E. coli serotype 01 and 02 agreed with the findings of Siccardi (1986) and Prukner (1986).

4.4.2 Clinical manifestations and gross lesions in young chicks

Specific clinical manifestations of colibacillosis could not be appreciated. The chicks before death were huddled together, dropped and died showing symptoms of colisepticaemia. Inoculated chicks which died during the experimental observation period were immediately necropsied and the post-mortem examination was done. Gross lesions observed in the internal organs were congestion of the heart, liver, lungs, kidneys and intestines. Pericarditis and perihepatitis were also seen. In many cases, accumulation of fluid in the pericardial sac, peritoneal cavity, thickened airsacs and white necrotic areas on the liver were seen (Figs. 14 to 19). The inoculated E. coli strains of organisms were reisolated from the heart, lungs, liver intestinal contents pericardial and peritoneal fluid of dead chicks (Table 10b). Similar findings were also described by Hofstad (1975), Rakhmanian and Shubin (1979), Srinivasan et al. (1979), Kim et al. (1983) and Phukan (1988) in young chicks due to E. coli strains. On the other hand, Sharma and Joshi (1985) made experimental production of colibacillosis with six strains of E. coli in laying hens and observed that the strains were highly pathogenic for them. Killed birds had

Table No. 10.b. Reisolation of E. coli from different sites of inoculated chicks.

Sl. No.	Isolate No.	Route of inoculation	Age of chicks in days	No. of chicks died	Number of dead chicks showing +ve reisolation				
					Heart blood	Liver lungs	Intestinal contents	Pericardial fluid	Pertoreal fluid
1	01	I/P	3	10	10	3	1	5	3
	01	S/C	3	8	8	4	1	4	2
	01	0	3	6	4	4	-	6	2
2	02	I/P	3	10	10	3	2	2	2
	02	S/C	3	10	6	6	6	1	4
	02	0	3	10	7	6	1	9	4
3	02	I/P	21	32	30	30	10	15	10
	02	S/C	21	27	20	25	2	10	10
	02	0	21	32	22	20	5	30	10



Fig.14 Showing congestion of lungs, kidneys, intestines pericardities and perihepatites in 3 days old experimentally inoculated (oral) chicks with 01(A) E.coli isolate.



Fig.15 Showing congestion of liver, heart, lungs, intestines and kidneys with experimental inoculation of 01(A) E.coli serotype by route.



FIG.16 Showing acute congestion of heart, lungs, liver, intestine and kidneys of 3 day old chick, experimentally inoculated with 01(A) E.coli isolate.



FIG.17 Showing congestion of liver, lungs, intestines heart and kidneys in 3 day old chick, experimentally inoculated (oral) with 02(B) E.coli isolate.



Fig.18 Showing pericardites, perihepatites, and congestion of lungs, intestine, kidney of 3 day old experimentally inoculated chick with 02(B) E.coli isolate by S/C route.

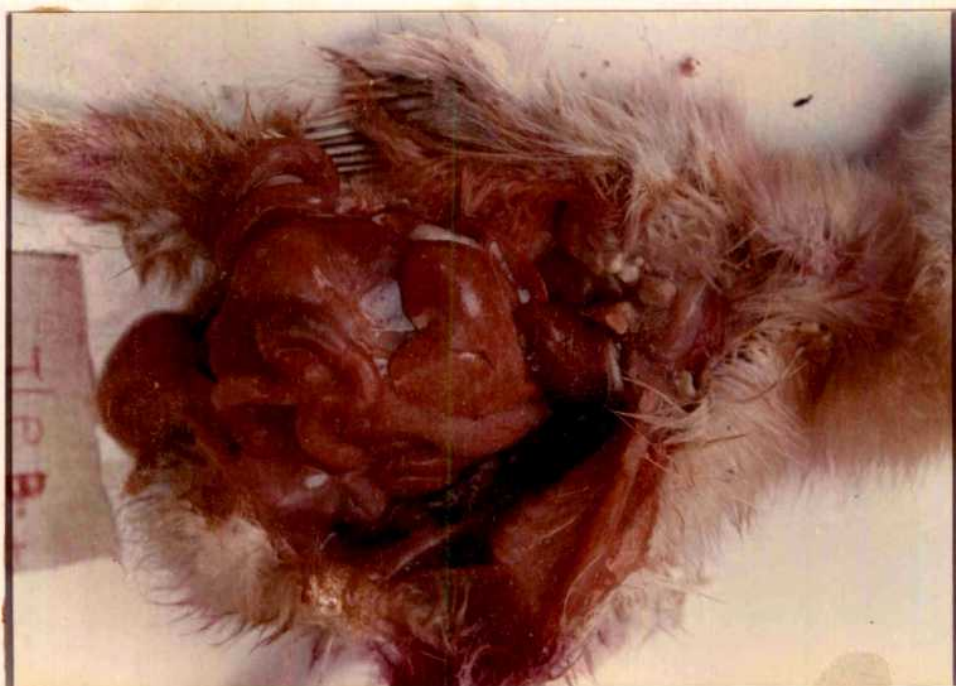


Fig.19 Showing extensive congestion of heart, liver, lungs, intestine, kidney in 3 day old experimentally inoculated with 02(B) E.coli isolate (I/P route).

swollen, dark red ovaries, misshapen and congested ova and inflamed oviducts.

4.4.3 Histopathological changes in the internal organs in experimental young chicks with E. coli 01 and 02 serotypes

(A) E. coli serotype 01 (oral route)

(i) Intestine

Macroscopic changes :

Highly inflamed and congested.

Microscopic changes :

Section of intestine showed extensive, acute haemorrhagic enteritis (Fig. 20) characterised by the presence of bloody exudates, admixed with numerous heterophils. Architecture of the villi was completely lost and there was desquamation and degeneration of the lining epithelial cells. The submucosa was markedly oedematous and infiltrated with number of leucocytes. There was hyper-activity of the submucosal glands. The serosal vessels were also congested (Fig. 21).

(ii) Heart

Macroscopic changes :

Heart congested with less pericardial fluid.

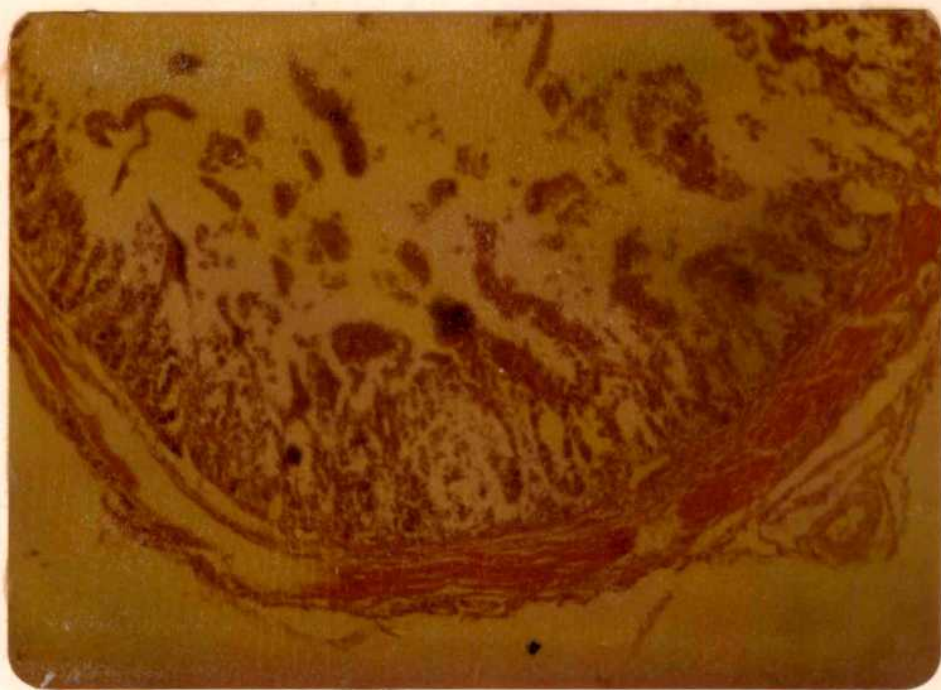


Fig.20 Section of intestine of young chick, experimentally infected (oral) with E.coli serotypes 01, showing extensive haemorrhagic enteritis; architecture of villi completely lost with degeneration and desquamation of lining epithelial cells. H.E X 100

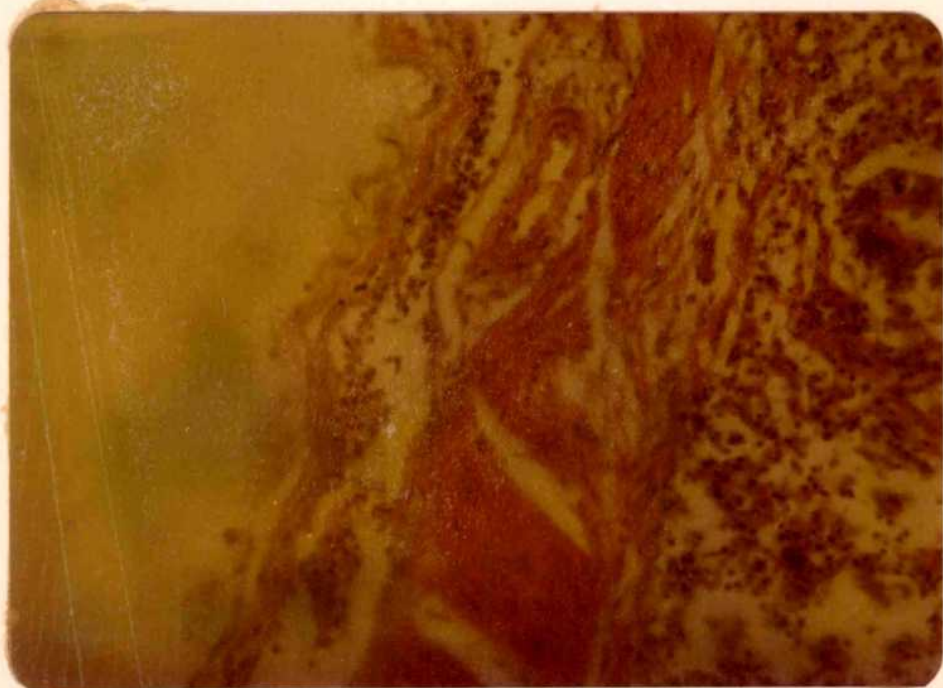


Fig.21 Section of intestine showing marked oedema, leucocytic infiltration, hyperactivity of submucosal glands, and congestion of serosal vessels. H.E. X 450

Microscopic changes :

The intermyseal vessels were markedly congested and the cytoplasm of the cardiac muscles had taken an intense eosinophilic stain (Fig. 22).

(iii) Kidneys :

Macroscopic changes :

Kidneys were much congested and inflamed.

Microscopic changes :

The parenchyma of the kidneys and perirenal tissues were markedly congested and oedematous (Fig. 23). The lining epithelial cells of the tubules showed severe degenerative changes like swelling of the cells which resulted in occlusion of the lumen and increased eosinophilia of the cytoplasm (Fig. 24).

(iv) Liver

Macroscopic changes :

The liver was congested with white streaks at the border.

Microscopic changes :

Liver parenchyma was congested and hepatic cells were swollen due to vacuolar degeneration and some of the hepatic cells showed frank necrosis (Fig. 25).

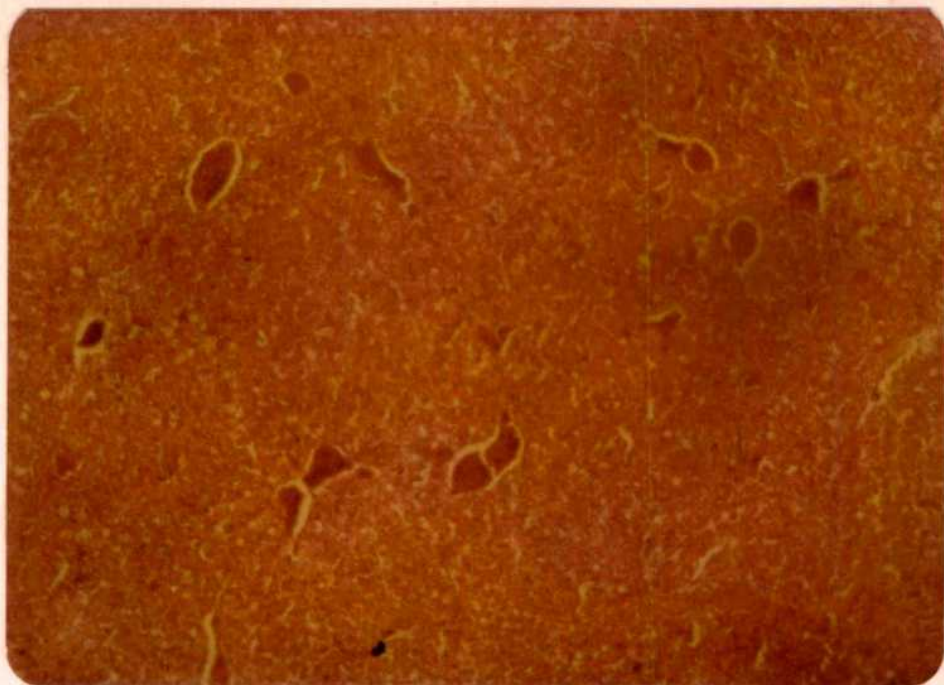


Fig.22 Section of the heart of young chick, experimentally infected(oral) with 01E.coli Serotype, showing marked congestion of the intermyocardial vessels and cytoplasm of cardiac muscle taking intense eosinophilic stain. H.E. X 450

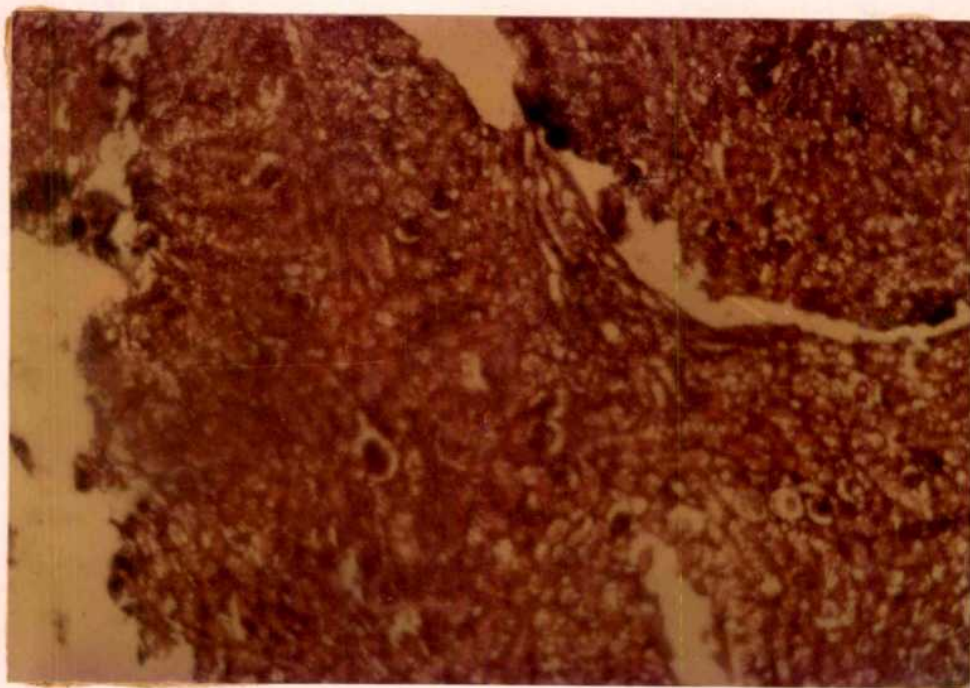


Fig.23 Section of kidney of young chick, experimentally infected (oral) with 01 E.coli serotype showing marked congestion and edema. Tubules showing degenerative changes. H.E. X 100

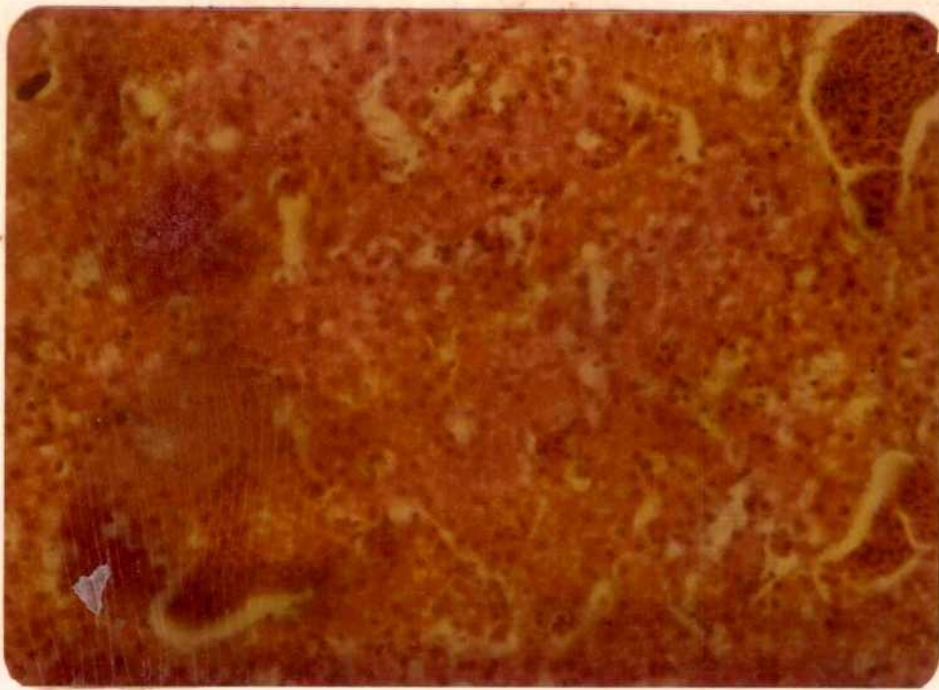


Fig.24 Section of kidney showing swelling of the epithelial cells, lining the tubules,
H.E. X 450

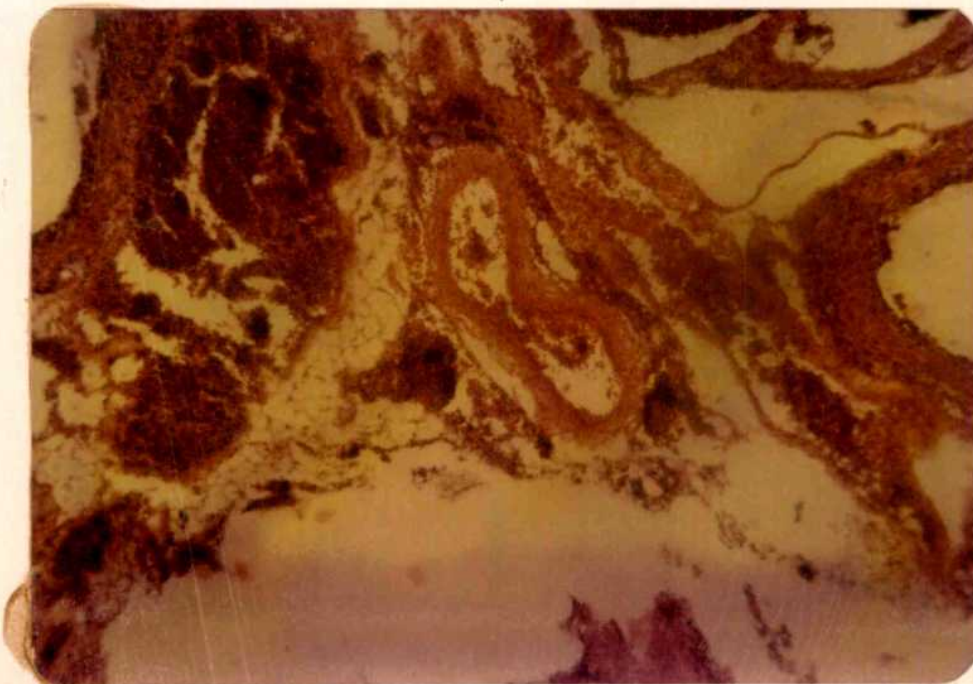


Fig.25 Section of liver of young chick, experimentally infected (oral) with 01 E.coli serotype, showing congestion of liver parenchyma; vacuolar degeneration and necrotic changes.
H.E. X 450

E. coli serotype 01 (subcutaneous route)

(i) Intestines

Macroscopic changes :

Intestine was highly congested as in oral route.

Microscopic changes :

The lesions in the intestine were similar to those as described for oral route.

(ii) Kidneys

Macroscopic changes :

The kidneys were highly congested and oedematous.

Microscopic changes :

Congestion of the kidney parenchyma and degenerative changes of the tubules were more prominent than those seen in the oral route (Fig. 26). Some of the glomeruli were very much swollen with congestion and there was hypertrophy of the visceral layer of the bowman's capsule (Fig. 27).

(iii) Liver

Macroscopic changes :

Gross changes seen in the liver were same as seen in oral route.

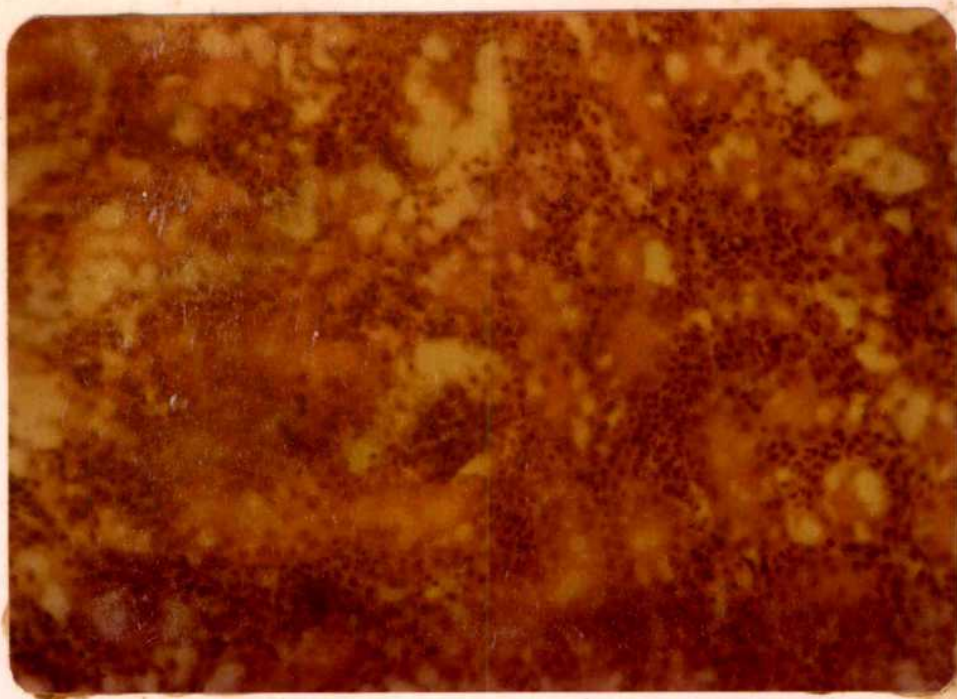


Fig.26 Section of kidney of young chick, experimentally infected (s/c) with 01 E.coli serotype showing marked congestion.
H.E. X 100



Fig.27 Section of kidney showing glomeruli much swollen.
H.E. X 450

Microscopical changes :

Histopathological changes observed in the liver were same as in oral route.

(iv) Heart

Both macroscopic and microscopic changes were similar to those seen in oral route.

E. coli serotype 01 (Intraperitoneal route)

(i) Intestine

Macroscopic changes :

Intestine was highly congested with haemorrhagic patches.

Microscopic changes :

Lesions were same as seen in oral route.

(ii) Kidneys

Macroscopic changes :

Kidneys showed congestion and haemorrhagic patches.

Microscopic changes :

Microscopic lesions were same as seen in oral route.

(iii) Liver

Macroscopic changes :

Liver was congested with white streaks at the parital surface of the organ.

Microscopic changes :

The parenchyma was markedly congested. Hepatic cells were very much swollen due to vacuolar degeneration and in some of the hepatic cells there was frank necrosis (Fig. 28 & 29).

(iv) Heart

Macroscopic changes :

Heart was congested with large amount of pericardial fluid.

Microscopic changes :

Microscopical changes observed were similar to the changes seen in oral route.

Siccardi (1966) observed that as few as 10 organisms of E. coli serotype 01 caused 100% mortality of day-old chicks when inoculated into the yolk sac. In many cases there was omphalitis. Informations are scarce in the literature on the changes (both macro and microscopical) in the intestines,

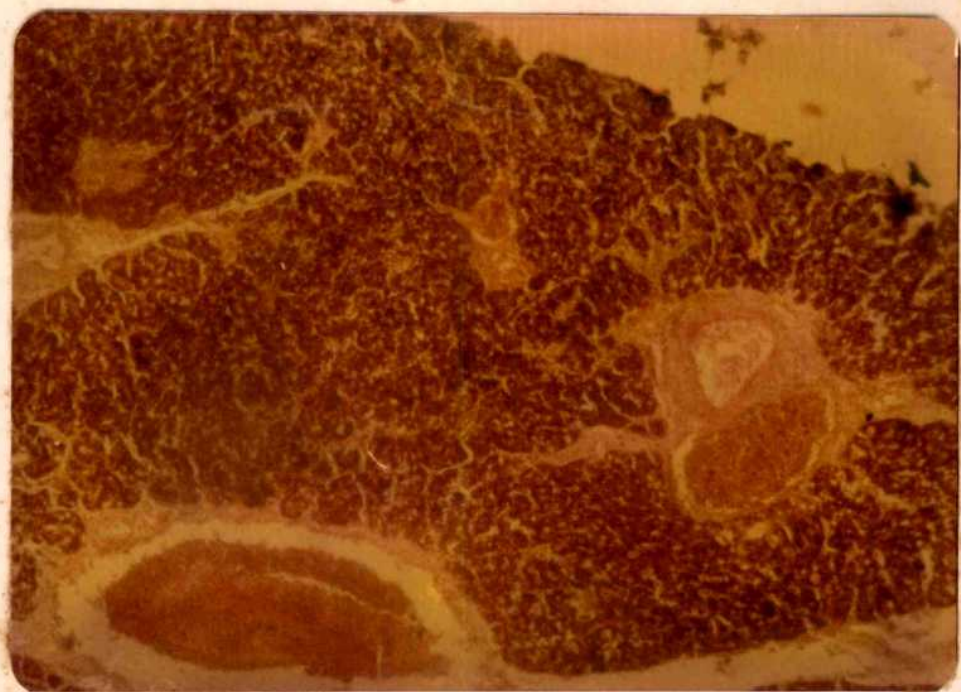


Fig.28 Section of liver of young chick, experimentally infected (I/P) with 01 E.coli serotype showing congestion of central veins, sinusoids and the periportal vessels. H.E. X 100

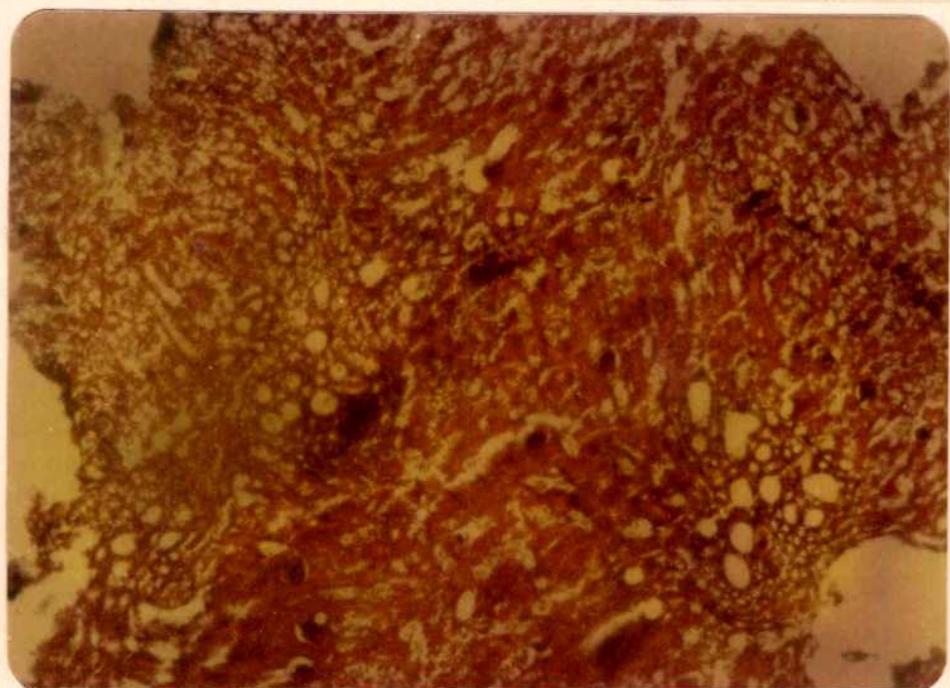


Fig.29 Section of liver showing vacuolar degeneration and necrotic changes. H.E. X 450

liver, kidney, heart of young chicks inoculated with the cultures of E. coli serotype 01 by oral, subcutaneous and intraperitoneal route for comparison.

B. E. coli serotype 02 (intraperitoneal route)

(i) Intestine

Macroscopic changes :

The organ showed severe congestion and haemorrhagic patches here and there over a large area.

Microscopic changes :

- There was complete denudation of the intestinal mucosae and the villi which were completely lost and the necrotic debris was seen in the lumen. The vessels in the pancreas were markedly congested. The serosal vessels were also markedly congested (Figs. 30 to 33).

(ii) Heart

Macroscopic changes :

Pericardial muscles were congested with large amount of pericardial fluid.

Microscopic changes :

Section of heart showed marked oedema and moderately congested (Fig. 34).

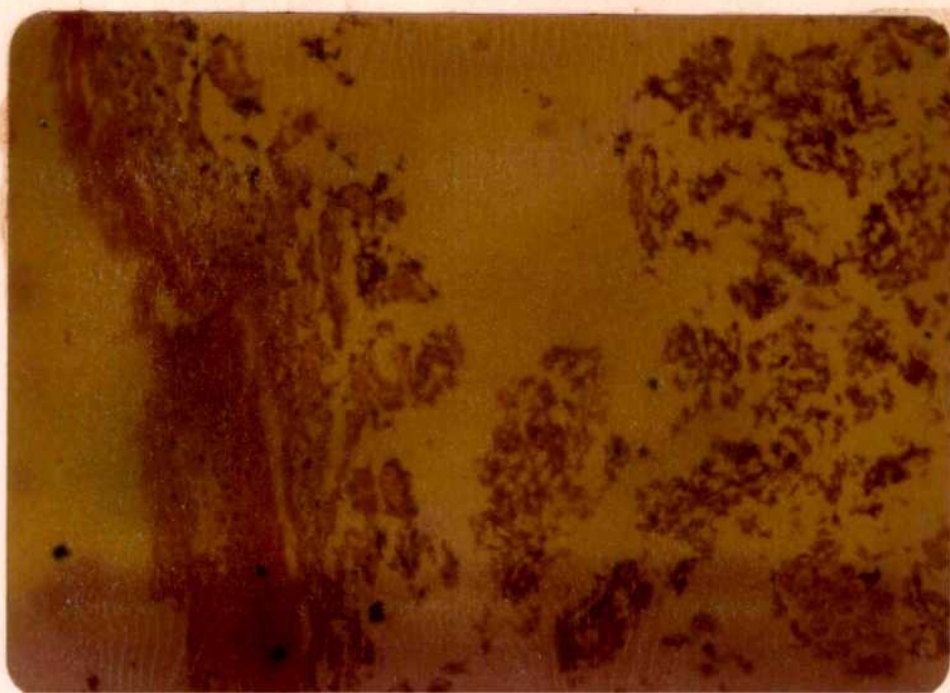


Fig.30 Section of intestine of young chick experimentally infected (I/P) with 02 E.coli serotype showing necrotic debris in the lumen and loss of villi. H.E. X 100



Fig.31 Section of pancreas showing marked congestion. H.E. X 100

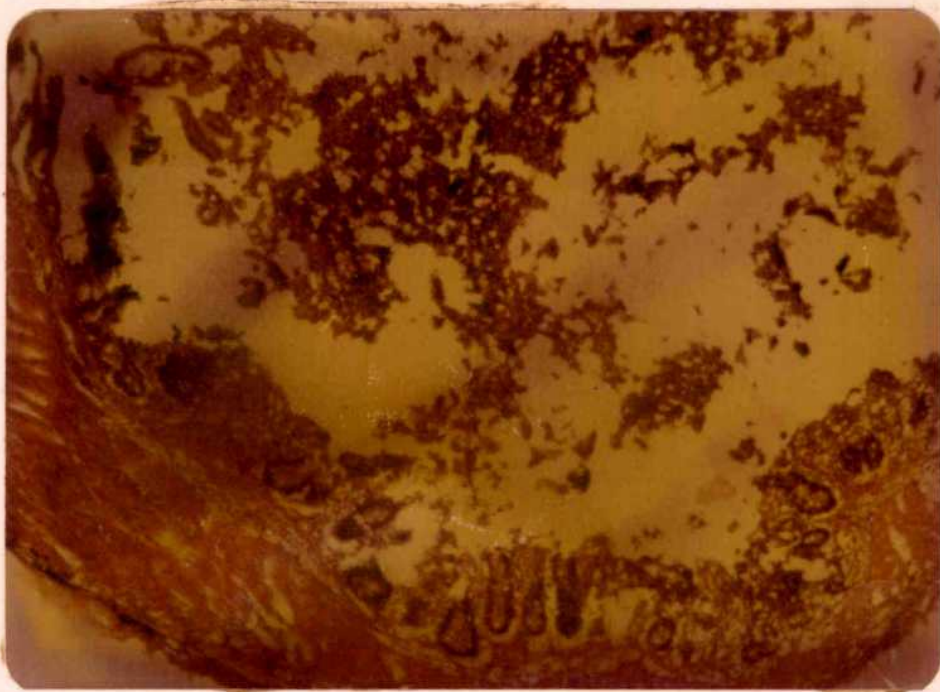


Fig.32 Section of intestine showing necrotic enteritis with hyperactivity of goblet cells. H.E. X 100

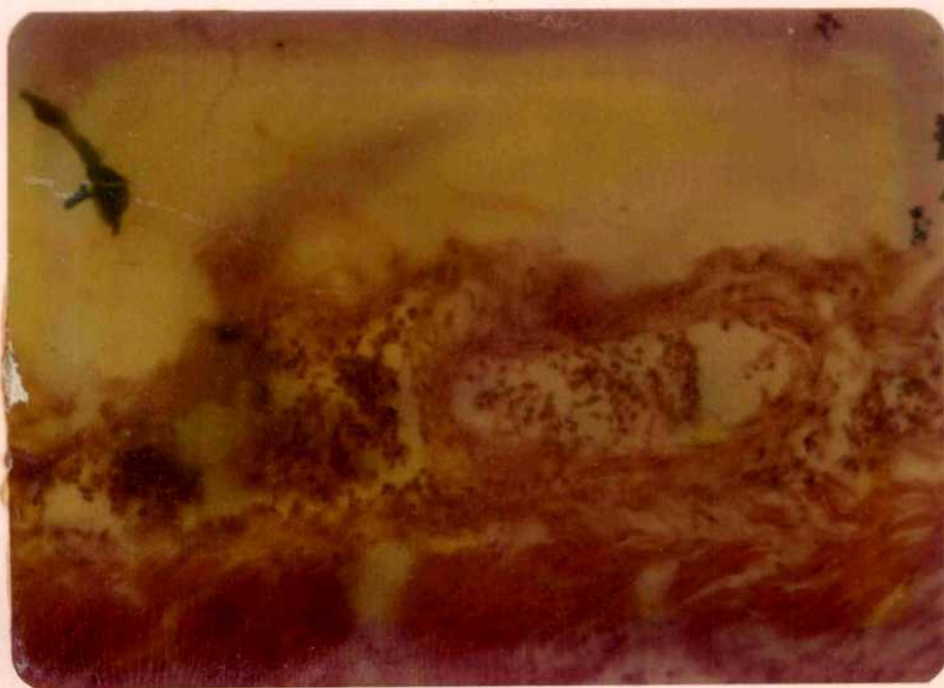


Fig.33 Section of intestine showing congestion of serosal vessels. H.E. X 100

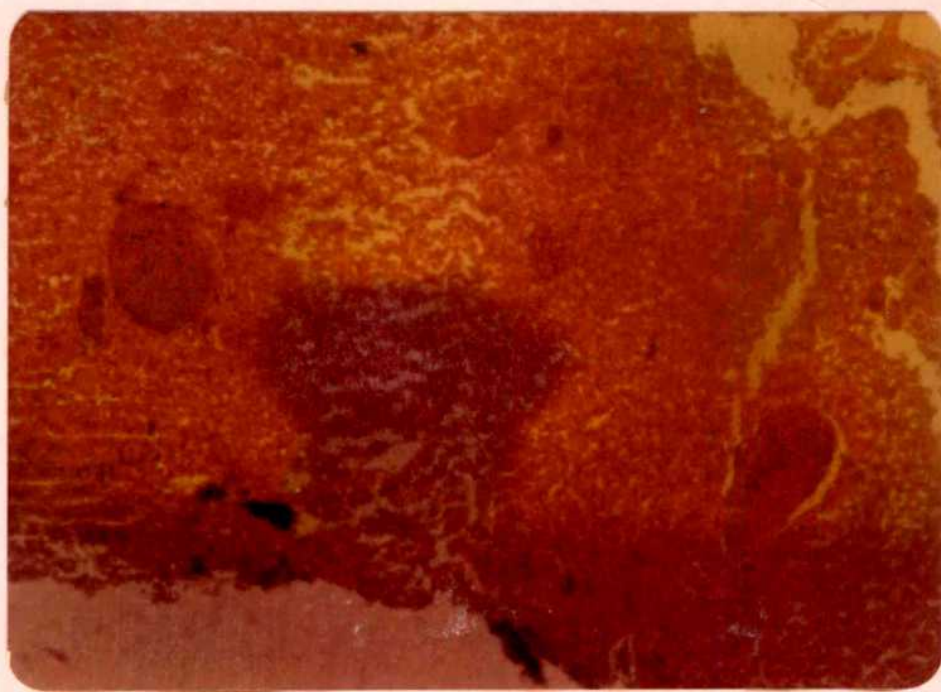


Fig. 34 Section of heart of young chick, experimentally infected (I/P) with 02 E.coli serotype showing marked oedema and moderate congestion. H.E. X 100

(iii) Kidneys

Macroscopic changes :

The kidneys showed severe congestion.

Microscopic changes :

The interlobular vessels of the kidneys were markedly congested and the epithelial cells lining the tubules were markedly swollen due to necrobiotic changes (Fig. 35).

(iv) Liver

Macroscopic changes :

The liver showed congestion and white streaks on the parital surface of the organ.

Microscopic changes :

The organ was very much congested and the hepatic cells showed vacoular degeneration (Fig. 36).

E. coli serotype 02 (subcutaneous route)

(i) Intestines

Macroscopic changes :

The intestine showed congestion and haemorrhagic patches.

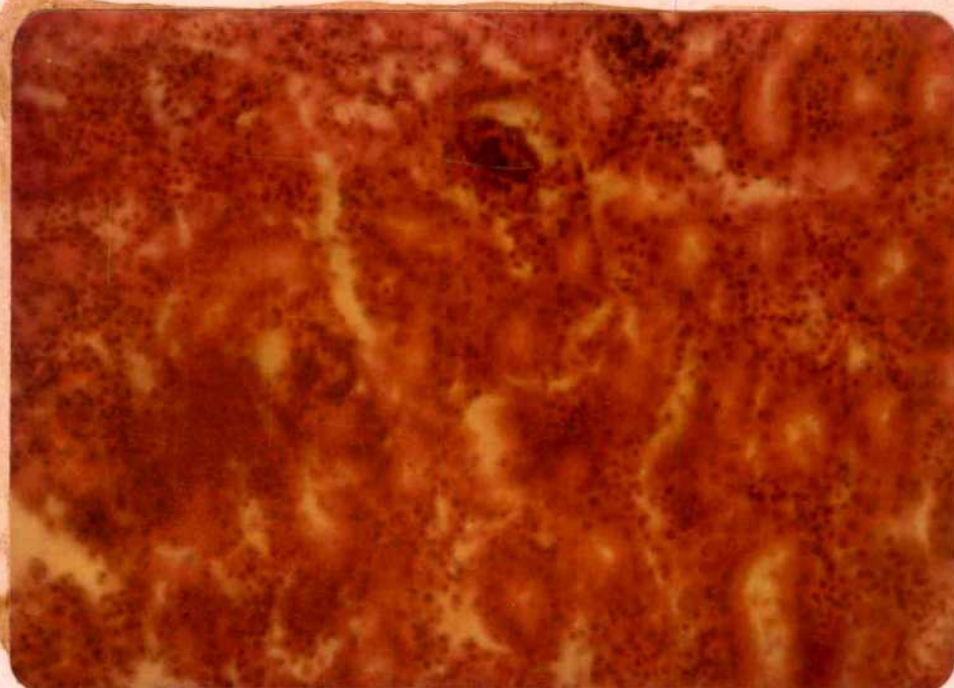


Fig.35 Section of kidney of young chick, experimentally infected (I/P) with 02 E.coli serotype showing congestion.

H.E. X 450

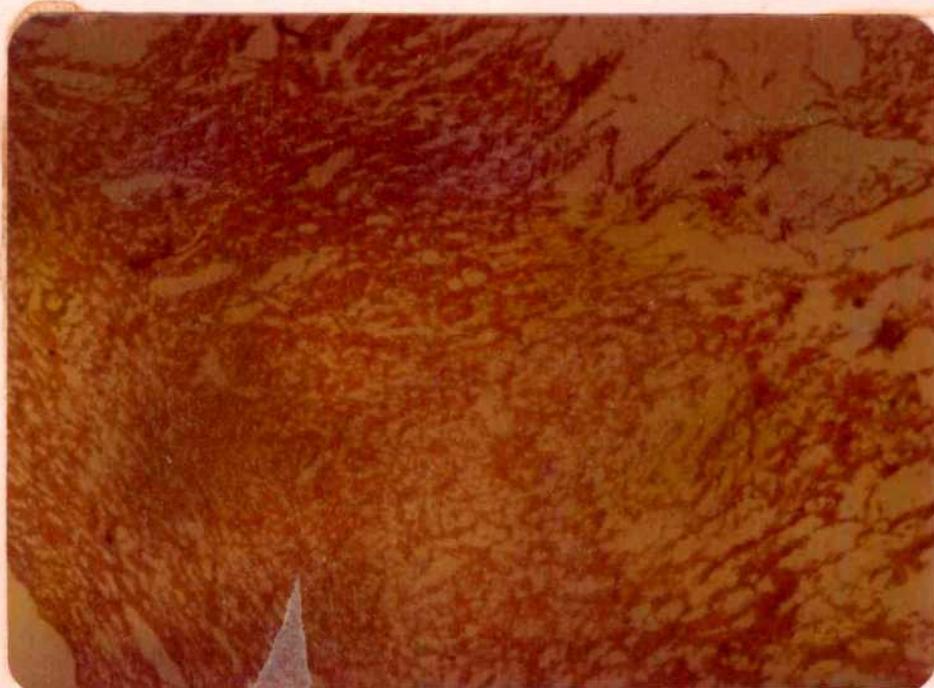


Fig.36 Section of liver of young chick, experimentally infected with E.coli serotype 02 showing congestion.

H.E. X 100

Microscopic changes :

The lesions observed were the same as seen in intraperitoneal route (Fig. 37).

(ii) Kidneys

Macroscopic changes :

Extensive congestion of the kidneys were seen.

Microscopic changes :

Changes were same as seen in intraperitoneal route.

(iii) Liver

Macroscopic changes :

Liver was congested with white streaks on the surface.

Microscopic changes :

Changes were same as seen in intraperitoneal route.

(iv) Heart

Macroscopic changes :

Heart was highly congested with less amount of pericardial fluid.

Microscopic changes :

Lesions were seen same as in intraperitoneal route.

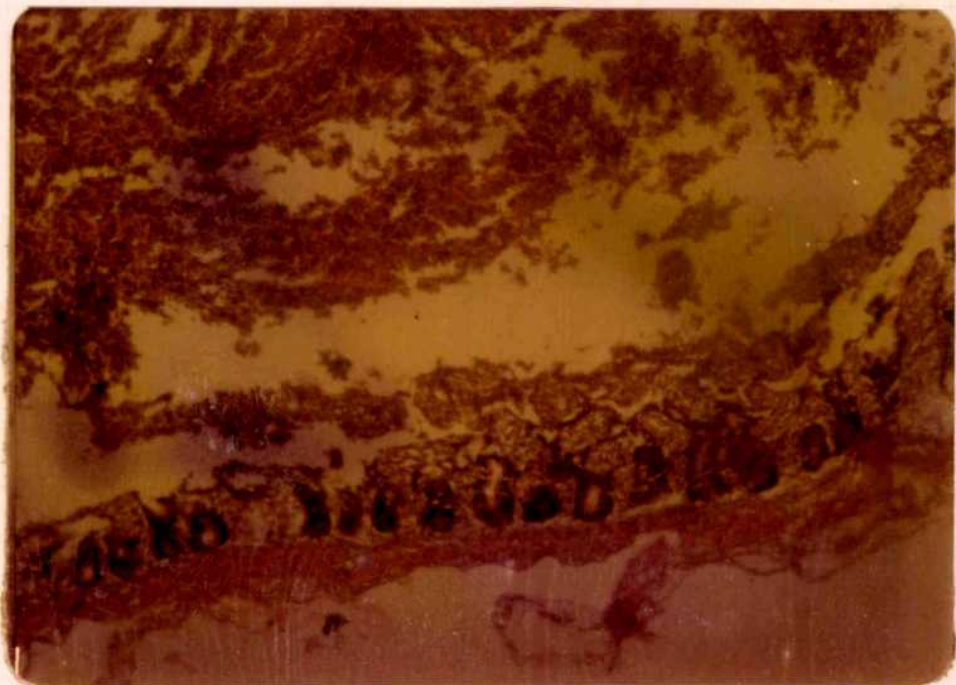


Fig. 37 Section of intestine of experimentally infected(S/C) young chick with 02 C.coli serotype showing necrotic lesions with exudate in lumen. H.E. X 100

E. coli serotype 02 (oral^v route)

(i) Intestines

Both macroscopic and microscopic changes were similar to those seen in intraperitoneal route.

(ii) Kidneys (Fig. 38)

(iii) Liver

(iv) Heart

All the above organs also exhibited similar lesions in both macroscopically and microscopically as seen in intraperitoneal route.

Hofstad (1975) observed gross lesions in chicks like green liver and congested pectoral muscles. In some cases there were small white focal areas in the liver with pericarditis and peritonitis. Phukan (1988) also recorded gross lesions in the internal organs of experimental chicks died of colibacillosis like congestion of liver, lung, heart, kidneys, enteritis, accumulation of fluid in the pericardial cavity and white necrotic areas on the liver. These findings were very close to the results obtained in the present study. As regards the histopathological changes in the internal organs in experimental young chicks, the informations were scarce in the literature. However, Siccardi (1966) recorded mild microscopical changes in the wall of the infected yolk sacs with oedematous

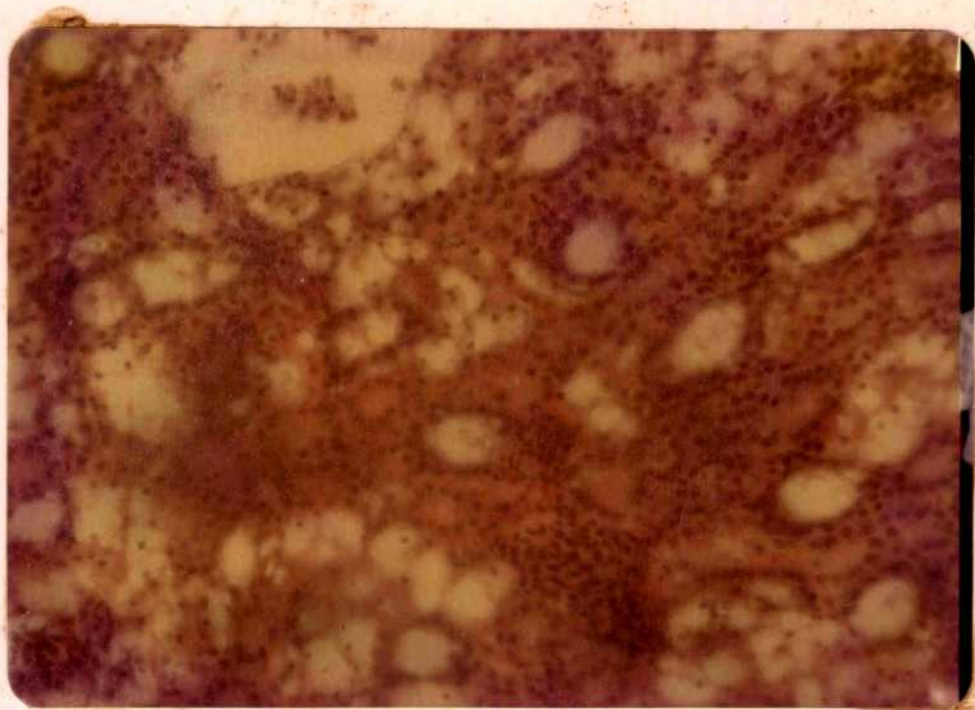


Fig.38 Section of kidney of experimentally
infected(oral) young chick with O2
E.coli serotype showing congestion of
renal parenchyma. H.E. X 450

conditions having inflammatory cells containing heterophils and macrophages, a layer of giant cells, a zone of necrotic heterophils and masses of bacteria with inner infected yolk contents. In some yolk sacs, there were a few plasma cells.

4.4.4 Experimental production of colibacillosis in older chicks (21 days old) with and without latent infections of coccidiosis

The results of the experimental production of colibacillosis (serotype 02) in 21 days old chicks with or without latent infection of coccidiosis were shown in Table 11. It was observed that experimental chicks of Gr. IV, V and VI without latent infection of coccidiosis had mortality rate of 40% in case of intraperitoneal route, 20% for subcutaneous route and 30% for oral route respectively. The mortality in Gr. IV started from day 4 post-inoculation whereas mortality in Gr. V and VI started from day 6 post-inoculations.

In case of Gr. VII, VIII and IX which were subjected to latent infection of coccidiosis had mortality rate of 100% (for intraperitoneal route), 80% (for subcutaneous route) and 70% (for oral route) respectively. The mortality was about double in chicks with latent infection of coccidiosis than the chicks without latent infection and the mortality also started earlier in those groups.

Table No. 11. Results of experimental production of E. coli infection (with serotype 02) in 21 day-old chicks with and without latent infection with coccidiosis

Name of sero-type	<u>E. coli</u>	Route of inoculation	Dose of <u>E. coli</u> culture (ml)	No. of chicks inoculated	No. of chicks died within days							Total % of no. died	Control group I, II & III	
					1	2	3	4	5	6	7		No. used	No. died
Gr. IV	02	I/P	0.2	10	1	1	1	1	1	1	4	40	10	-
Gr. V		S/C	0.2	10				1	1	1	2	20	10	-
Gr. VI		O	0.9	10				1	1	1	2	20	10	-
Gr. VII		I/P	0.2	10 With latent infection of coccidiosis	2	2	2	2	4	-	10	100		
Gr. VIII		S/C	0.2	10 With latent infection of coccidiosis				2	4	2	8	80		
Gr. IX		O	0.9	10 With latent infection of coccidiosis	2	2	2	2	1	1	7	70		

In case of control groups (Gr. I, II & III) which were inoculated by intraperitoneal, subcutaneous and oral routes with sterile broth culture did not have any mortality. During the course of the experimental study, the faecal samples examination of chicks of Gr. VII, VIII and IX revealed latent infection of Coccidiosis. The appetite was normal. No blood streaks in the faeces were also observed during the study. The faecal sample of other groups (Gr. I, II, III, IV, V and VI) were found negative for oocysts of coccidia. The chicks that died were immediately necropsied and the gross lesions in the internal organs were noted. Attempts were made for reisolation of E. coli from heart blood, liver, lungs, peritoneal fluid, pericardial fluid and intestinal contents and the results were positive for reisolation of E. coli.

The results indicated that the mortality due to E. coli infection in chicks suffering from latent Coccidiosis was much higher than chicks without latent Coccidial infection. Savay (1963) also observed much higher mortality in chicks due to colibacillosis when there was a coccidial infection. Ibragimov et al. (1983) concluded that the mycoplasma played primary or infectious bronchitis virus which sensitised the bird to E. coli septicemia. Fgizber (1985) stated that the virulence and pathogenicity of E. coli in chicks and ducks was directly related to the presence of parasites, their localisation in the intestines and the species and age of the host.

Dhillon (1986) and Golubrichii et al. (1986) observed dual infections (E. coli with field strain of influenza virus or with Infectious Bursal Disease Virus (IBDV) promoted the viral infection in chicks. Panda and Verma (1988) reported that many stress factors like infectious agents (Chronic Respiratory Disease, adenovirus infections, immuno suppression due to infectious Bursal disease and aflatoxicosis, nonfetal Ranikhet Disease (RD), Infectious Bronchitis (IB) and many live virus vaccines, excess of ammonia, extreme temperature and induced colibacillosis promote infection in chicks.

4.4.5 Clinical manifestations and gross lesions in 3 week old chicks

The present study revealed that the chicks before death showed symptoms of dullness and huddling at the corners and found lying dead. Gross pathological changes in dead chicks of Gr. IV, V & VI showed congestion of heart, pericarditis, congestion of liver with white streaks and congestion of lungs, kidneys, and intestines. The dead chicks of Gr. VI which were fed orally with virulent culture had enteritis and swollen bowels. The chicks of Gr. VII, Gr. VIII and Gr. IX also exhibited similar changes in the internal organs. In some cases accumulation of fluid in the pericardial and peritoneal sacs was observed.

Hofstad (1975) recorded salpingitis, omphalitis, pericarditis and infected yolk and acute septicaemia in chicks. Dunnington and Siegel (1985) observed loss in body weight, increased body and cloacal temperature, lesions on heart, air sacs and high mortality in chicks. Srinivasan et al. (1979), Dholakia et al. (1983), Kim et al. (1983) and Ling et al. (1987) observed pericarditis, perihepatitis, air sac infection and septicaemia which were very close to the present findings in experimental Colibacillosis in 3 week old chicks. However, Sharma and Joshi (1985) recorded swollen darkred ovaries, misshapen congested ova and inflamed oviduct in layer hens due to colibacillosis.

4.5 Therapy

Treatment was carried out from day 1 post-infection with sulphadiazine (0.4%) in chick mash and continued for 5 days. The results of the treatment with sulphadiazine against E.coli serotype 02 infection in chicks were recorded in Table 12. From the table it was evident that the percentage of efficacy of the drug in groups C₁, C₂ and C₃ infected with intraperitoneal, subcutaneous and oral routes with E. coli serotype 02 was 90%, 100% and 100% respectively. In case of infected untreated chicks of groups B₁, B₂ and B₃, all experimental chicks died off colibacillosis within 6 days. The chicks of healthy control groups A₁, A₂ and A₃ did not show any mortality. The

Table No. 12. Results of therapy in experimentally infected 3 day-old chicks with virulent E. coli serotype (02) and administration of sulphadiazine in feed
(Treatment groups with control.)

Name of groups	E.coli Serotype	Route of inoculation	Dose (ml)	No. of chicks inoculated	No. of chicks died within days										Total no. died	Total no. survived	% of survival	Control group A ₁ , A ₂ & A ₃			Gr. No. used	No. of mortality
					1	2	3	4	5	6	7	8	9	10				A ₁	A ₂	A ₃		
<u>INFECTED TREATED</u>																						
Gr. C ₁	02	I/P	0.2	10	1										1	9	90	A ₁	10	-	-	-
Gr. C ₂	02	S/C	0.2	10												10	100	A ₂	10	-	-	-
Gr. C ₃	02	0	0.9	10											-	10	100	A ₃	10	-	-	-
<u>INFECTED UNTREATED</u>																						
Gr. B ₁	02	I/P	0.2	10	6	3	1								10	-	0					
Gr. B ₂	02	S/C	0.2	10		3	4	2	1						10	-	0					
Gr. B ₃	02	0	0.9	10		2	3	3	2						10	-	0					

chicks which died during the experimental study were necropsied and the gross changes in the internal organs were noted and efforts were made to reisolate E. coli from heart blood, liver, kidneys, lungs, pericardial and peritoneal fluid and intestinal contents to confirm colibacillosis.

Various antibiotics like furaltadone, furazolidone, oxytetracycline, chlortetracycline, tetracycline, bacitracin, erythromycin, procaine penicillin streptomycin, tylosin, lincomycin, spectinomycin, chloramphenicol, kanamycin, polymyxin, doxycycline, nitrofurazolidone, ormetoprim, erythromycin, furadentine, apramycin, ampicillin, lincomycin, trimethoprim etc. were tried by various workers against colibacillosis with encouraging results (Gross, 1961; Herberts and Chang, 1969; Hamdy and Blanchard, 1969; Butura and Sahleanu, 1972; Artem Eva et al., 1977; Kapoor et al., 1978; Maestrone et al., 1979; Srinivasan et al., 1979; Goren et al., 1980; Donos, 1982; Dholakia et al., 1983; and Cracknell et al., 1986). However, drug resistance to number of antibiotics by alarming number of E. coli isolates of poultry were recorded by Kapoor et al. (1978), Srinivasan et al. (1979). Goren et al. (1984), Goren et al. (1987) and Prukner et al. (1987) tried sulpha preparations with good response in cases of colibacillosis in chicken. Resistance of E. coli isolates to different antimicrobials had been encountered due to indiscriminate use of the same.

In the present study sulphadiazine was very effective since the reports in use of this drug were scarce.

4.6 Prevention and Control

Previous outbreak of colibacillosis in the poultry farms (A & B) indicated that E. coli serotypes 078 and 0159 were isolated respectively. The sensitivity pattern of these isolates of E. coli was highest in case of cotrimexazole to both the serotypes. Preventive medication of the chicks were taken up at regular intervals with other sanitation and hygienic measures as detailed in the chapter materials and methods. The measures continued for one year without any incidence of colibacillosis in both the farms. Hofstad (1975) and Tengerdy and Nockels (1975) recommended preventive measures in the farms in controlling colibacillosis. Further informations in this regard were lacking.

CHAPTER V

SUMMARY AND CONCLUSION

The study was undertaken to isolate and identify strains of Escherichia coli from suspected cases of colibacillosis in poultry and to serotype the isolates. The study was limited to small, medium and large poultry farms selected at random in and around Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia district, 24-Parganas (North and South) districts and sub-divisional area of Hooghli district of West Bengal.

A total of 1008 samples were tested for E. coli from 508 dead chicks comprising 524 from small poultry farms, 364 from medium poultry farms and 120 from large poultry farms were processed for isolation and identification of E. coli in the present study. Altogether 304 strains of E. coli could be isolated from 508 dead chicks. All the 304 isolates were included from the small poultry farms (160), medium poultry farms (104) and large poultry farms (40). Out of 508 dead chicken examined 304 yielded E. coli with an overall incidence rate of 59.8% whereas according to small, medium and large poultry farms, the incidence of cultural positive cases of E. coli in relation to sample examined were 63.2%, 53.3% and 66.6% respectively.

The seasonal incidence of colibacillosis among chicken according to different seasons (summer, rainy and winter) were studied. In a year, the total number of outbreaks, mortality

rate among chicken, dead chicken examined on post-mortem, samples collected and samples positive for E. coli were recorded as 35, 560, 253, 524 and 160 in small poultry farms; 12, 323, 195, 364 and 104 in medium poultry farms, 5, 250, 60, 120 and 40 in large poultry farms respectively. According to summer, rainy and winter seasons, number of outbreaks, mortality rate among chicken, dead chicks examined on post-mortem, samples collected and samples positive for E. coli on cultural basis were 12, 12 and 11; 205, 198 and 157; 82, 96 and 75; 164, 192 and 168; 48, 58 and 54 in small poultry farms, 5, 4 and 3; 114, 126 and 83; 55, 75 and 65; 105, 170 and 89; 35, 42 and 27 in medium poultry farms and 1, 2 and 2; 46, 84 and 120; 10, 20 and 30; 30, 35 and 55; 10, 12 and 18 in large poultry farms respectively.

Attempt was made to demonstrate the distribution of E. coli in the different sites and pathological conditions of dead chicks. Majority of E. coli strains were isolated from intestinal contents (106) and heart blood (92), yolk material (67), pericardial fluid (12), liver and peritoneal fluid (10 each), lungs (5) and kidney (2). It was further seen that highest number of E. coli strains were isolated from cases of enteritis (106), enlarged and congested heart (92) unabsorbed and putrified yolk (67), pericarditis and cloudy pericardial fluid (12), necrosis and congestion with white streaks on the liver (10), peritoneal fluid (10), congested lungs (5) and congested and enlarged kidneys (2).

In the present study altogether 304 E. coli strains were isolated and identified on the basis of morphology, staining reaction, cultural characteristics and biochemical tests. Out of 304 strains of E. coli, 300 strains produced indole, all the 304 strains of E. coli showed positive results to M.R. and negative to V.P. reactions and fermented lactose, glucose, manitol and maltose whereas fermentation of sucrose, dulcitol and salicin was shown by 130, 222 and 95 respectively. On the other hand, none of 304 strains of E. coli fermented adonitol and inositol and utilised citrate and produced urease and H_2S .

A total of 304 strains of E. coli were serotyped. 183 (60.19%) strains belonged to different 'O' type, 42 (13.81%) strains were untypable and the remaining 79 (25.98%) were rough strains. Overall 14 different 'O' serotypes were identified. Twenty six of the strains belonged to 01, 49 to 02, 10 to 036, 8 to 045, 6 to 055, 25 to 078, 12 to 079, 5 to 080, 15 to 088, 7 to 0120, 2 to 0157, 13 to 0158, 4 to 0159 and 1 to 0162.

Pathogenicity test against different 'O' serotypes of E. coli isolates was conducted on white albino mice. It was observed that 83.66% was recorded as pathogenic in white albino mice within 96 hours of the inoculation of serotype 02 E. coli isolate. Serotype 01 and 078 recorded 66.66% and 50% mortality in white albino mice respectively. Serotype 079, 080 and 088 showed 50% mortality and 036, 0120, 0158, 0159 and

0162 recorded 33.33% mortality and 045, 055 and 0157 caused 16.66% mortality in experimental white albino mice. There was no mortality seen in control group. The experimental mice which died due to probable infections were immediately necropsied and gross pathological changes were observed in internal organs of dead mice (congestion of heart, liver, spleen, lungs and kidneys). Heart blood from dead mice collected aseptically and reisolation and identifications of E. coli was made to confirm the cause of death. The surviving mice which were sacrificed on days 5 post-inoculation did not show any pathological changes in internal organs like heart, liver, spleen, lungs and kidneys. When heart blood collected aseptically was inoculated in blood agar did not show any growth of E. coli. Similarly, the control experimental mice which did not die were sacrificed and nothing abnormal in internal organs was noticed. Inoculation of heart blood in blood agar plates did not reveal any microbial growth, particularly E. coli.

The most virulent E. coli serotypes (01, 02 and 078) as determined by white albino mice test were tested for their enterotoxic activity in rabbit ligated ileal loop using their culture supernatant. The dilatation index in cases of E. coli serotypes 01, 02 and 078 were calculated as 0.68, 0.68 and 0.25 respectively. In case of control loop the dilatations index was 0.16. Hence, serotype 01 and 02 were found highly pathogenic.

The results on the antibiotic sensitivity tests indicated that 97.55%, 96.69% and 80.99% of E. coli strains isolated were sensitive to Furazolidone, Cefotaxime and Ampicillin respectively whereas 78.51% and 61.15% were sensitive to carbanicillin and sulphadiazine respectively. Moderate degree of sensitivity to cephaloridine (32.23%), chloramphenical (35.53%), cotrimexazole (29.75%), Gentamycin (35.53%), Amikasin (35.53%), Neomycin (35.53%) and Nalidixic acid (46.28%) were recorded. It is seen that serotype 01 and 02 were sensitive to only carbanicillin, Cephaloridine, Cefotaxime and Furazolidone and ampicillin, Carbanicillin, Sulphadiazine, Cefotaxime and Furazolidone respectively but resistant to other antimicrobials used in this study. Serotype 078 was found to be sensitive to all the antibiotics except Carbanicillin and Cephaloridine. Serotype 0158 was only sensitive to Ampicillin, Carbanicillin, Cephaloridine, Nalidixic acid and Furazolidone but resistant to other antimicrobials used. Serotype 0120 was resistant to only Cephaloridine, Sulphadiazine and Furazolidone but sensitive to all other antibiotics used in this study. Serotypes 055 and 0162 were resistant to Ampicillin, Carbanicillin, Cephaloridine, Cotrimexazole and Sulphadiazine but sensitive to the rest except Furazolidone for serotype 055. Serotype 0159 was resistant to Carbanicillin, Cephaloridine, and Sulphadiazine only but susceptible to all other antibiotics. In the present study resistance was observed by most of E. coli serotypes to Carbanicillin, Cephaloridine,

Co-trimexazole, Sulphadiazine and no single antimicrobial was sensitive to all the E. coli serotypes. Further, no single antimicrobial was 100% sensitive against the E. coli strains.

Experimental production of Escherichia coli (Serotype 01 and 02) was done in 3 days old young broiler chicks. Both the strains were found to be highly pathogenic to young chicks. Serotype 02 produced 100% mortality in chicks within 7 days in I/P, S/C and oral routes of administration whereas serotype 01 produced 100% mortality by I/P and S/C routes and 80% mortality in oral route administration to chicks. Maximum mortality was observed in chicks which were inoculated I/P within the first 3 days in both the serogroups. None of the control group of chicks which were inoculated with only sterile broth culture by different routes died.

Clinical symptoms and gross lesions in young chicks due to experimental production of colibacillosis were sudden death, huddling together and death, symptoms of colisepticaemia; gross lesions observed on post-mortem of dead chicks were congestion of the heart, liver, lungs, kidneys and intestines. Pericarditis and perihepatitis were also seen. In many cases accumulation of fluid in the pericardial sac, peritoneal cavity, thickened arisacs and white necrotic areas on the liver were visible.

Histopathological changes (macroscopic and microscopic) observed in varying degrees in internal organs in experimentally produced colibacillosis in young chicks by different routes (I/P, S/C and oral routes) were as follows :

Intestines :

Highly inflamed and congested. Section of intestine showed extensive, acute haemorrhagic enteritis characterised by the presence of bloody exudates admixed with numerous heterophils. Architecture of the villi was completely lost and there was disquamation and degeneration of the lining epithelial cells. The submucous layer of the intestine was markedly oedematous and infiltrated with number of leucocytes. The blood vessels were congested and the submucosal glands were hyperactive.

Heart :

Congested with varying amount of pericardial fluid. The intermyocardial vessels were markedly congested and the cytoplasm of the cardiac muscle has taken as intense eosinophilic stain.

Kidneys :

Kidneys were congested and inflamed. Parenchyma of the kidneys were markedly congested and oedematous. Tubules showed degenerative changes resulting in occlusion of the

lumen of the tubules. Increased eosinophilia was observed in the cytoplasm.

Liver :

The liver was congested with white streaks. Liver parenchyma was congested and hepatic cells were swollen due to vacuolar degeneration and necrosis.

Experimental production of colibacillosis in older chicks (21 day old) with or without latent infection with coccidiosis was done. In this experiment E. coli serotype 02 was used. The results indicated that the experimental chicks of Gr. IV, V and VI without latent infection of coccidiosis had mortality rate of 40% (I/P route), (20% S/C) and 30% (oral route) respectively. The mortality in group IV started from day 4 post-infection whereas mortality in Gr. V and VI started from day 6 post-inoculation. In case of Gr. VII, VIII and IX which were subjected to latent infection of coccidiosis had mortality rate of 100% (I/P route), 80% (S/C) and 70% (oral) respectively. In case of control Gr. I, II and III which were inoculated with sterile broth culture in 3 different routes as above, did not show any mortality. Chicks died of colibacillosis were necropsied and the changes in internal organs were noted. Reisolation of E. coli from the materials from heart blood, liver, lungs, peritoneal fluid, pericardial fluid and intestinal contents was done.

Clinical manifestations of colibacillosis in older chicks (21 days old) were noted. The chicks before death showed symptoms of dullness and huddling at the corners and found lying dead. Gross pathological changes observed in dead chicks of Gr. IV, V and VI were congestion of heart, pericarditis, congestion of liver with white streaks and congestion of lungs, kidneys, intestines. The dead chicks of Gr. VI which were fed orally with virulent E. coli culture had enteritis and swollen bowels. The chicks of Gr. VII, Gr. VIII and Gr. IX also exhibited similar changes in internal organs. In some cases fluid in the pericardial sac and peritoneal cavity was observed.

Therapy was carried out from day 1 post-infection in young chicks with sulphadiazine (0.4%) in chick mash for 5 days. It was calculated that the percentage of efficacy of the drug in Gr. C₁, Gr. C₂ and Gr. C₃ infected with I/P, S/C and oral routes with E. coli serotype 02 was 90%, 100% and 100% respectively. In case of infected untreated chicks of Gr. E₁, Gr. E₂ and Gr. E₃ all experimental chicks died of colibacillosis within 6 days. The chicks of healthy control Gr. A₁, Gr. A₂ and Gr. A₃ did not show any mortality. Dead chicks were necropsied and pathological changes noted in the internal organs. Reisolation of E. coli was also done from heart blood, liver, kidneys, lungs, pericardial and peritoneal fluid and intestinal contents to confirm colibacillosis.

Prevention and control of colibacillosis was tried in two problem poultry farms A & B. E. coli strain 078 and 0159 were isolated respectively from the farms. The sensitivity pattern of these E. coli isolates was highest to cotrimaxazole. Preventive medication of the chicks were taken up at regular intervals along with other sanitation and hygienic measures in these farms for one year without any incidence of colibacillosis.

CONCLUSIONS

1. Incidence of colibacillosis in the area under survey in West Bengal was found to be high.
2. Study indicated no seasonal variation in the incidence of colibacillosis. The incidence of the disease in young chicks was higher than older birds.
3. Majority of E. coli strains were isolated from intestinal contents followed by heart blood and yolk material.
4. Altogether 304 strains of E. coli were isolated having 14 serotypes (01, 02, 036, 045, 055, 078, 079, 080, 088, 0120, 0157, 0158, 0159 and 0162).
5. Pathogenicity test conducted on white albino mice with all the isolated serotypes revealed that E. coli serotype 01, 02 and 078 were highly pathogenic.

6. Enterotoxic activity of the E. coli serotypes 01, 02 and 078 in rabbit ligated ileal loop revealed that the dilatation index was 0.68 in cases of serotype 01 and 02.
7. Antimicrobial sensitivity test conducted on different E. coli isolates indicated varying degree of sensitivity. No drug was found to be 100% effective for all strains. Further, degree of resistance was also high to different antimicrobials used.
8. Experimental production of colibacillosis with E. coli serotypes 01 and 02 in 3 days old broiler chicks revealed that serotype 02 produced 100% mortality in chicks within 7 days in I/P, S/C and oral routes of administration whereas serotype 01 produced 100% mortality by I/P and S/C routes and 80% mortality in oral route. Clinical symptoms revealed colisepticaemia with gross lesions like congestion of heart, liver, intestines, lungs and kidneys. In many cases there were accumulation of pericardial and peritoneal fluid. Severe inflammatory changes were also observed in most of the internal organs in dead chicks.
9. Study revealed that the mortality rate in 21 day old chicks having latent infections of coccidiosis with E. coli serotype 02 was 100% for I/P, 80% for S/C and

70% for oral routes of administration. The chicks without latent infections when subjected to experimental production of colibacillosis with 02 strain revealed mortality rate as 40% by I/P, 20% S/C and 30% for oral routes of administration.

10. Therapy when carried out with Sulphadiazine (0.4%) in chick mash for 5 days to experimental induced colibacillosis with 02 serotype revealed about 100% recovery.

Study also indicated that regular preventive treatment as per sensitivity test along with improved sanitation, hygiene and management could control the diseases (colibacillosis) in two problematic poultry farms.

CHAPTER VI

FUTURE SCOPE OF RESEARCH

Further work on the following aspects of colibacillosis in poultry may be taken up which could not be incorporated in the present investigation.

- i) The incidence study of colibacillosis may further be undertaken on wider area in all poultry pockets of the state to isolate and identify the prevalent serotypes existing.
- ii) Pathogenicity test in white albino mice and enterotoxic activity in ligated ileal loop of rabbit may be done to identify the most prevalent pathogenic strains of E. coli in the state.
- iii) Basing on the isolation of virulent E. coli isolates, efforts may be made to evolve polyvalent vaccine against E. coli.
- iv) Study may be made to assess the economic loss in poultry farms due to high incidence of colibacillosis in the state.
- v) Effective hygiene, sanitation and management systems for prevention of colibacillosis may be chalked out.

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