Studies on
EXPERIMENTAL INFECTION AND TREATMENT OF ESCHERICHIA - COLI IN CHICKS

A Thesis

Submitted to the

RAJENDRA AGRICULTURAL UNIVERSITY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

IN

VETERINARY MEDICINE

By

Ram Prasad Gupta, B. V. Sc. & A. H.

BIHAR VETERINARY COLLEGE

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Dr. S.S. Mishra,
Ph. D. (U.S.S.R.)
Professor and Chairman
Post Graduate Department of Medicine
Bihar Veterinary College, Patna
Rajendra Agricultural University
BIHAR.

PATNA,

Dated, the 12th December, 1977.

This is to certify that the work embodied in this Thesis entitled "STUDIES ON EXPERIMENTAL INFECTION AND TREATMENT OF ESCHERICHIA COLI IN CHICKS" is the bonafide work of Shree Ram Prasad Gupta and was carried out under my guidance and supervision.

S.S. Mishra

(S.S. MISHRA)

C E R T I F I C A T E

Certified that the research work encorporated in this Thesis has not been published in part or in full in any of the journal.

(Ram Prasad Gupta)

MOST FILIALLY DEDICATED

TO MY

LATE MOTHER

ACKNOWLEDGEMENT

A C K N O W L E D G E M E N T S

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(R. P. GUPTA).

C O N T E N T S

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INTRODUCTION

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The present decade has seen a breakthrough in the poultry industry in this country. With the phenomenal exploitation in the field of poultry economy, more modern and scientific methods of poultry keeping has become popular. At the same time, however, such methods of intensive poultry keeping has exposed the bird to various bacterial and viral infections which spread through contact.

In India, about 40 % of total bird mortality are attributed to bacterial and viral infection (Naidu, 1959). Among the bacterial infections affecting the poultry health, the role of Esch. coli can not be overemphasised. A number of poultry health problems may be attributed to such tiny-ubiquitous organism, as coli-granuloma, haemorrhagic-enteritis, coli-septicaemia, egg-peritonitis, salpingitis, oophoritis, and infectious arthritis. Coli-septicaemia is alone responsible for the 50 % death among the broilers (Gordon, 1961). Besides affecting a high percentage of death, this organism is reported to cause great economic loss to the poultry industry by affecting the weight gains and poor carcass quality of infected birds.

Several workers interested in poultry health, have

investigated and reported on the Esch. coli infection in poultry (Hjarre and Wramby, 1945; Cole and Hutt, 1953; Edward and Ewing, 1954; Barr and Carman, 1957; Quareshi, 1957; Gross, 1958; Gross and Siegel, 1959; Sojka and Carnaghan, 1961; Savov, and Pavlov, 1965; Nagi and Khanna, 1967; Prasad et al., 1967; Gupta and Singh, 1969; Yadav and Malik, 1971).

Resistance of Esch. coli to various antibiotics and nitrofurans have also been reported by Savov, 1967 and Butura and Sahleanu (1972).

It is needless to emphasize here that the effective control of patnogens depend on the effective specificity of chemotherapeutic agents. Keeping this inview, for the fruitful control of bacterial infection, sensitivity test of drugs were conducted so as to spotout the most effective drug.

As poultry farming in the Bihar State has gained much momentum and the Esch. coli infection has spread, the present problem of coliform infection in Bihar needs to be tackled in a scientific manner.

Comprehensive effort hence has been made to study the behaviour of Esch. coli in poultry and in causation of various changes after infection in terms of loss of body weight, appearance of symptoms, gross and histopathological changes.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

Escherich (1885) for the first time isolated Esch. coli from a faecal sample of a breast fed infant and was of opinion that it was an exclusively saprophytic organism.However, this concept changed and thereafter this organism, besides being considered a normal inhabitant of intestinal tract of different species of animals and man (Wilson and Miles, 1961) has been implicated as the etiological agent for certain well-defined diseases which included white scour of calves, cases of cystitis, pyelonephritis, naval-ill, and joint-ill, some cases of mastitis in bovines, uterine infection of bitches and oedema diseases of swine (Merchant and Packer, 1967; and Lovell,1959).

The list of diseases being caused by Esch. coli in various species of animals has progressively been increasing and during last decade or so, many workers have indicated about its role in the causation of several diseases of poultry which include "coli-septicaemia" (Edward and Eweing, 1954; Gross, 1958; Sojka and Carnaghan, 1961, and Gupta, 1963); Haemorrhagic enteritis (Quareshi, 1957; Barr and Carman, 1957); Hjarre and Wramby diseases (Hjarre and Wramby, 1945; Hamilton and Conard, 1958), Chronic respiratory diseases (CRD) (Wassermann et al., 1954, Gross, 1956, 1958; and Glantz et al., 1962), and in

cases of egg-peritonitis, salpingitis, and oophoritis (Gross and Siegel, 1959; Sharma and Singh, 1968; Gupta and Singh, 1969; Kulkarni et al., 1970 and Pradhan et al., 1973).

ISOLATION AND PATHOGENICITY.

Hjarre and Wramby (1945) reported for the first time a condition characterised by tuberculosis like granulomatous lesions in the liver and caeca of fowls. A pure culture of mucoid Esch. coli was isolated from the lesions and disease was produced experimentally by inoculating intravenously.

Wramby (1948) isolated 50 mucoid Esch. coli strains from cases of "coli-granuloma" in fowls.

Kohler's (1951) recorded 95 cases of "coli-granuloma' out of 2,583 post mortem cases. This condition was frequently seen in liver and intestine and other organs were less affected. The reproduction of the disease was possible by inoculating the culture, however, oral administration had no effect.

Edward and Ewing (1954) reported that 30 Esch. coli cultures were recorded from internal organs of fowls died in widely separated areas in the U.S.A., which belonged to single serological variety O2 : K_1 : K_5. This type of culture was neither found among 200 cultures from the intestines of fowls and other domestic animals, nor it could be found in the stools of the food handlers or persons suffering from diarrhoea.

Quareshi (1957) reported that Esch. coli caused fatal enteritis in two flocks of birds in West Pakistan following New-Castle disease vaccination. The disease was reproduced by inoculating the suspension of the organism from the suffering birds, the organism was recovered from 207 out of 378 chicks with enteritis.

Barr and Carman (1957) reported an outbreak of fatal-diarrhoea in a flock of 3,000 ten week old chicks in which causal organism was Esch. coli.

Gross (1957) recorded pan-opthalmitis in chicks associated with severe Esch. coli bacteraemia and subsequently this condition was experimentally produced.

Hamilton and Conard (1958) reported an outbreak of Hjarre disease in two flocks of 2000 and 1000 pullets in which 75 % of the birds were affected. Generalised T.B. like lesions were produced and a mucoid, encapsulated Esch. coli was isolated.

Gross (1958) reported that certain types of Esch. coli were important complicating factors in chronic respiratory disease and other respiratory diseases. Infections through aerosals route were easiest and probably the natural one.

Gross and Siegel (1959) described egg-peritonitis in fowls caused by Esch. coli.

Pathak et al. (1960) reported frequent isolation

of Esch. coli in course of examination of yolk-flora of 100 dead chicks.

Sojka and Carnaghan (1961) reported that Esch. coli was constantly isolated from the internal organs, from the cases of coli-septicaemia and a remarkable degree of serological uniformity was found among those isolates. The same conditions were produced by inoculating the isolates of Esch. coli experimentally.

Gurumurthi and Panduranga Rao (1962) reported that non-haemolytic strain of Esch. coli were isolated from the affected birds. The strains were pathogenic for Swiss-albino mice and had a variable pathogenicity for rabbits.

Kulkarni (1964) isolated Esch. coli belonging to different serotypes from coli-granuloma.

Iyre et al. (1965) reported a granulomatous condition in fowls caused by Esch. coli in mucoid phase. This strain affected internal organs of birds, particularly caeca and liver. They could reproduce the nodules in the liver.

Narulah and Kuppuswamy (1966) reported a case of coli-granuloma from Bihar. They found lesions similar to tuberculosis over surface of the liver.

Sarkar (1966) reported Esch. coli isolated from chicks in one of the farms in Bihar which had high mortality.

Nayak et al. (1967) recorded a case in a Rhode Island

Red Cockerel involving the proventriculus, small intestine, caeca, liver, pancreas, kidney, bone marrow and eyelids. Esch. coli was isolated in pure culture from affected organs and was also demonstrated in the tissue sections.

Heller and Perek (1969) described the pathogenic Esch. coli strains prevalent in poultry flocks in Israel. Out of 308 birds, 203 were examined which had characteristic lesions of coli-septicaemia. The 267 pathogenic strains of Esch. coli were isolated and identified by biochemical and serological methods. The pathogenicity of strains was ascertained by injecting the organism intraperitoneally to several groups of chicks.

Gupta and Singh (1969) isolated seventeen strains of Esch. coli from 21 cases of egg-peritonitis in hens, of these, fifteen proved pathogenic for mice on experimental inoculation by intraperitoneal route.

Butura and Cernea (1969) examined 378 strains of Esch. coli and found that half of these strains came from fowls, chicks, and embryos with specific pathological conditions.

Betitskii and Panikar (1969) isolated Esch. coli from broiler with septicaemia. Coli-septicaemia caused 10 % death in 42 to 44 day old chicks but death was less common in younger groups in comparison to the older ones.

Kulkarni et al. (1970) isolated 52 strains of Esch. coli from 105 specimen of suspected cases of chronic respiratory disease. 32 of these strains proved pathogenic for mice.

Grosheva (1971) isolated 30 strains of Esch. coli from organs of chickens with coli-septicaemia and classified them into five biotypes. The majority of the strains were haemolytically active and produced exotoxins and endotoxins. Tney were pathogenic and killed 28 to 42 per cent of chickens infected intranasally or intratracheally, 75 per cent by aerosal inhalation, 96 and 92 per cent by airsac and intraperitoneal routes, 31 per cent by contact and 16 per cent when given orally.

Butura and Sahleanu (1972) isolated 198 strains of E. coli from fowls which died of septicaemic condition.

Yadav and Malick (1972) isolated 52 strains of Esch. coli from Egg-yolk, embryos and various organs of the dead chicks.

Pradhan et al. (1973) isolated 16 strains of Esch. coli from 25 natural cases of oophoritis, salpingitis and egg-peritonitis. Out of these, 6 strains were pathogenic for albino mice.

Mauel (1973) isolated 138 strains of Esch. coli, out of these 103 isolates were pathogenic for chicks by producing intraperitoneal injection.

Sharma et al. (1977) isolated 32 strains of Esch. coli from various organs of poultry. Some of the isolated Esch. coli strains were pathogenic for chicks.

SYMPTOMATOLOGY.

Quareshi (1957) observed that Esch. coli caused fatal enteritis in two flocks of birds in West Pakistan when some birds were vaccinated against New-Castle disease. Barr and Carman (1957) reported an outbreak of fatal-diarrhoea in a flock of 3000 ten week old chicks in which causal organism was Esch. coli.

Sojka and Carnaghan (1961) observed acute depression, severe green diarrhoea, and lameness in experimentally infected chicks. According to Gordon (1961) coli-septicaemia proved major concern to the broiler industry and economic losses were not only due to mortality but also due to carcass condemnation, inferior meat quality and loss in weight gains.

Gurumurthi and Pandurenga Rao (1962) observed coli-bacillosis causing heavy mortality in brooder chicks under one to four weeks of age. Death occurred within a few hours after the onset of symptoms. White-pasty diarrhoea, blocking of vent with dried faeces and wetting of surrounding area were noticed in about 30 per cent of the affected chicks.

Savov (1965) recorded 14 - 25 per cent less body

weight, than the control, in infected chicks which survived after showing some or no clinical signs.

Nagi and Khanna (1967) reported a cholera like disease in chicks due to haemolytic Esch. coli strain. They found 95 per cent death in a flock of 1000 chicks, the age group of, between six to eight weeks showing the symptoms of weakness, inappetance, diarrhoea, cynosis of Wattle and Comb.

Stipkovits and Solyom (1968) reported a condition characterised by weakness, inappetance, diarrhoea, lameness and 20 - 30 per cent loss in body weight, mostly amongst the chicks of 3 to 10 days age group in three salmonella and mycoplasma free flocks.

Sharma et al. (1977) reported two outbreaks in Pantnagar and Haldvani. The first outbreak was among 3 to 4 days old chicks with symptoms of dullness, depression, and slightly blood tinged diarrhoea. The second outbreak was observed among laying, white leghorn flock of 1500 birds with the symptoms of offed, bluish-comb, watery-diarrhoea with mucous flakes, subnormal temperature and sudden drop in egg production (95.4 per cent).

LESIONS AND HISTOPATHOLOGY.

Gross (1957) reported that Esch. coli, in experimentally infected poultry and turkeys, produced aerosacculitis,

fibrinous pericarditis, perihepatitis, salpingitis and panophthalmitis. In histopathology, cellular infiltration showed heterophilic and mononuclear phagocytes. Necrotic areas were at first lined with mononuclear phagocytes and later with giant cells.

Sojka and Carnaghan (1961) reported that in experimentally infected birds the most common lesions were fibrinous pericarditis, hepatitis and inflammation of the airsacs. The liver was markedly enlarged, dark in colour and covered by gelatinous material and the airsacs were thickened and covered by yellow caseous materials. The other lesions observed in a proportion of cases were nephritis, congestion of internal organs, pin-point haemorrhages on the myocardium, abscess formation (Particularly on the site of inoculation) and panophthalmitis.

Savov and Pavlov (1965) observed pericarditis, pneumonia, perihepatitis, aerosacculitis and leucocyte predominated in the inflammatory exudate. Hyperaemia with extravasates and fatty dystrophy were also found in the liver.

Nagi and Khanna (1967) observed petechiation of the intestines, heart, lungs, kidney, and enlargement of the liver with small necrotic foci on post mortem examination.

Stipkovits and Solyom (1968) reported catarrhal enteritis with congestion of the yolksacs, serofibrinous inflamation of the serous membranes hepato and splenomegaly and

arthritic changes.

Micevski and Naletoski (1972) observed granulomata lesions most commonly in caecum and also in intestine, liver, mesentry, heart, lung, and musculature.

Truscolt et al. (1974) reported that the common histopathological findings were subepicardial oedema and congestion, focal necrosis in the spleen, focal necrosis, congestion, oedema, and accumulation of fibrin in the liver.

DRUGS SENSITIVITY.

Antibiotics are being used as feed supplement in poultry for prevention of diseases and for better growth. This has given rise to many drug resistant strains of Esch. coli.

Starr and Reynolds (1951) reported that the use of streptomycin as a growth promoting supplement in the feed of turkeys poults resulted in the appearance of streptomycin resistant Esch. coli strain within three days. Resistance was of the level of 60 mg/ml in 60 per cent of streptomycin.

Barr and Carman (1957) described an outbreak of diarrhoea in a ten week old broiler flocks on feeding a ration containing tetracycline. This outbreak was associated with tetracycline resistant Esch. coli strains which were sensitive both in in-vitro and in-vivo to neomycin and polymyxin. They came to a conclusion that tetracycline and oxytetracycline

resistant Esch. coli strains were increasing day by day.

Sojka and Carnaghan (1961) tested 797 Esch. coli strains isolated from septicaemic condition with antibiotics and found that all strains were sensitive to furazolidone.

Gross (1961) studied the effect of chlortetracycline, erythromycin and nitrofurans for the treatments of experimentally produced airsacs disease. He found that airsac disease could be controlled by furaltadone given in feed or in water. Chlortetracycline and erythromycin could not control the disease due to Esch. coli alone but could reduce the severity of lesions in pleuro-pneumonia like organism.

Glantz (1962) studied antibiotics sensitivity to 287 strains of Esch. coli isolated from animals and poultry. He performed the sensitivity test to different antibiotics and nitrofurans. The most effective ones were colistin, chloramphenicol, furazolidone, N-1-amino-2 Pyrrolidone, thiofuradane, and polymycin. Dihydrostreptomycin, chlortetracycline, tetracycline and oxytetracycline were intermediate in effects. Penicillin, oleandomycin, furaltadone and nidroxyzone were least effective.

Malik (1963) tested eight antibiotics against Esch. coli and found that neomycin, tetracycline, oxytetracycline, and chlorampnenicol were most effective whereas penicillin and erythromycin were not effective.

Savov (1963) worked with 64 strains of which 45 were

resistant to chlortetracycline, 40 to tetracycline, 39 to oxytetracycline in-vitro, and 62 were sensitive to neomycin.

Takahasi (1966) studied 251 strains of Esch. coli isolated from diseased fowls on one farm for antibiotics sensitivity and found that all strains were sensitive to chloramphenicol, 64 were resistant to tetracycline, one to streptomycin, and 12 for both tetracycline and streptomycin.

Andreani et al. (1969) studied the sensitivity of 14 antibiotics to 215 strains of Esch. coli isolated from various species of animals and found that nitrofurazone, cephaloridin, gentamycin, and ampicillin were most effective. Many of these strains were resistant to streptomycin, chlor-tetracycline and chloramphenicol and multiple resistance was found in 52 per cent of the isolates.

Butura and Sahleanu (1972) prepared antibiogram for 198 strains of Esch. coli isolated from fowls, which died due to septicaemia and found that 95.5, 96, 83, 81, 37 per cent were sensitive to chloramphenicol, polymyxin B, neomycin, furazolidone and tetracycline respectively. However, they were resistant to erythromycin, penicillin G, Penicillin V, and streptomycin.

CHEMOTHERAPY.

Mecarty (1953) got the encouraging result with

neomycin at the dose rate of 50 mg each at an interval of eleven days in an outbreak of Paratyphoid (S. derby) in Goslings.

Sojka and Carnaghan (1961) found that furazolidone was most useful in small experimental trials. 0.04 per cent level of furazolidone for a period of a week to ten days was recommended for treatment.

Matvienko and Rudenko (1964) proved the effectiveness of colistin, mycerin, furazolidone and monomycin at the dose rate of 75,000 unit, 20,0000 units and 0.2 to 0.3 gm orally and 50,000 units intramascular respectively in Esch. coli infection.

Butura and Sahleanu (1972) reported that groups of fowls experimentally infected subcutaneously with a lethal dose of Esch. coli were protected by the addition of chloramphenicol to the diet from the three days after the infection, either 0.005 gm per head every day or 0.010 gm per head for three days, however, furazolidone in same conditions was 80 - 87 per cent effective.

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MATERIALS AND METHODS

MATERIALS AND METHODS

Sources of materials.

In the present study, for the isolation of Esch. coli, different organs of poultry such as lung, liver, kidney, spleen, and heart were collected. The details about the collected materials have been shown in Table I.

TABLE - I.

S.No.	Place of collection	No. of birds	Condition of birds
1.	Dressing plant of the Central Poultry Farm, Patna.	20	Apparently healthy birds dressed for human consumption.
2.	Disease investigation wing of the Institute of Animal Health and Production, Patna.	10	Dead birds brought for ascertaining the cause of death.
3.	Military Farm, Dinapur, Patna.	10	Dead birds having history of diarrhoea and respiratory trouble.

Isolation and identification of Esch. coli.

The materials collected from these birds aseptically

were plated directly on MacConkey's Lactose Agar (MLA) and Eosin Methylene Blue (EMB) plates taking due care to avoid contamination. The inoculated plates were incubated at 37°C for 24 hours. Typical lactose fermenting, round, translucent, convex colony of about 2 mm in diameter on MacConkey's plates and colonies having moderate size with dark (almost black) centre and showing metallic sheen in reflected light were selected for preservation.

The isolates were stored and maintained on nutrient agar slants, and identification of Esch. coli were made as per technique advocated by Edward and Ewing (1972).

Pathogenicity test.

Pathogenicity test of the strains of Esch. coli isolated during the present study were carried out in albino mice (Italian). Five isolates from each group were used for studying the pathogenicity.

Albino mice of 1 to 2 months age group irrespective of their sex, were used for this test. For each strain of Esch. coli, six mice were used. Three mice were inoculated with the isolated culture while the rest three kept as control. 0.5 ml of 18 hours broth culture were inoculated intraperitoneally in each mice taking aseptic precautions and similarly 0.5 ml nutrient broth inoculated in each mice of control group.

The inoculated mice were kept under observation for 72 hours. The post mortem examination of the mice which died during this period of observation were carried out. The cultures from heart blood, lungs, liver, spleen and kidney of the dead animals were prepared to recover the inoculated organism.

The strains which killed the mice and were recovered subsequently after post mortem examination were considered as pathogenic strains.

Drug sensitivity test.

The following commonly used antimicrobial drugs were used in the present work to study the sensitivity of Esch. coli strains isolated from the birds. The concentration of disc used for each drug is indicated against their respective names (Table II).

TABLE - II.

S.No.	Antimicrobial disc used in sensitivity	Concentration.
1.	Ampicillin	10 mcg.
2.	Chloramphenicol	30 mcg.
3.	Furadantoin	100 mcg.
4.	Kanamycin	30 mcg.
5.	Neomycin	30 mcg.
6.	Gramoneg	30 mcg.
7.	Streptomycin	10 mcg.
8.	Septran	100 mcg.
9.	Sulfuno	250 mcg.
10.	Furoxone.	100 mcg.

The antimicrobial discs of first seven drugs were obtained from M/s Bharat Laboratories, Thana, BOMBAY. The anitimicrobial discs of the rest three drugs were prepared as per the method described by Cruickshank (1965) and Mishra(1977). 5 ml of eighteen hours old broth culture of the isolated strains of Esch. coli were poured over the nutrient agar plates and evenly spread. Plates containing broth culture were allowed to dry for 1 to 2 hours in incubator at 37°C.

The antimicrobial discs were carefully placed on the surface of the plates. Then, the plates containing antimicrobial discs were incubated at 37°C for 24 hours and the zone of inhibtion was measured. The zone of inhibition included the diameter of discs as well as surrounding zone of inhibition. Zone of inhibition (15 mm or more) was considered as sensitivity of the organisms to the particular antimicrobial drugs.

Experimental infection.

The strains which killed the mice within the shortest period was selected for experimental infection. The experimental infection with pathogenic strain of Esch. coli was studied on two groups of birds. The procedure adopted has been depicted in the schematic diagram shown on page 23. The chicks of both groups were reared from day old chick for one month under the possible hygienic condition. The chicks were kept on chick ration procured from Central Poultry Farm, Patna. In addition

vitablend WM Fort (Glaxo) (at the dose rate of 2 ml in a liter of water) were supplemented in drinking water. The chicks were also supplied coccidiostat (Aprolium Hydrochloride at the dose rate of 0.6 gm per liter of water) as prophylaxis measure during rearing.

In the first group, 26 apparently healthy chicks were to study the effect of infection on body weight and to observe the symptoms produced after experimental infection. The macroscopic and microscopic changes in extra intestinal organs of the infected chicks were also studied. In this group 10 chicks were infected by intraperitoneal route and 10 chicks were given oral infection while the rest six were kept as control. Nutrient broth culture of 18 hours incubation of the selected Esch. coli strain was used for infection.

0.5 ml of this culture was infected intraperitoneally and same dose was instilled in the mouth for experimental infection through oral route. The chicks used as control were infected with nutrient broth, three, intraperitoneally and three, through the oral route respectively. The infection in chicks were considered by culturing the blood on MacConkey's lactose agar plate and examination of the blood smear.

The second group consisted of 46 apparently healthy chicks upto one month age. The chicks were divided in five groups of eight and one group of six chicks only. The two groups consisting of eight chicks each were infected intraperitoneally and the

rest two groups were given oral infection with 18 hours broth culture of the selected strain of Esch. coli. The next two groups consisting of eight and six chicks respectively, were used as control. In the first group of control birds, four were given oral infection and rest four were infected intraperitoneally. Similarly second group of control chicks were divided into two sub-groups and simple nutrient broth was given through intraperitoneally and oral route respectively.

The infected chicks were treated with the antimicrobial durgs. The drugs were administered on appearance of visible symptoms viz. off-fed, depression, enteritis (pasty-diarrhoea with blood tinged), respiratory trouble etc. coupled with presence of organisms in blood. Four drug, namely (1) septran paediatric suspension (2) chloromycetin capsule (3) neomycin capsule (4) furoxone were used for treatment. The dose and make of the drugs used have been detailed in the Table III.

The drugs found efficacious in order of preference towards this strain, on the basis of sensitivity test were selected to study their therapeutic value. One drug particularly was used for the treatment of one group only of the infected chicks on appearance of symptoms.

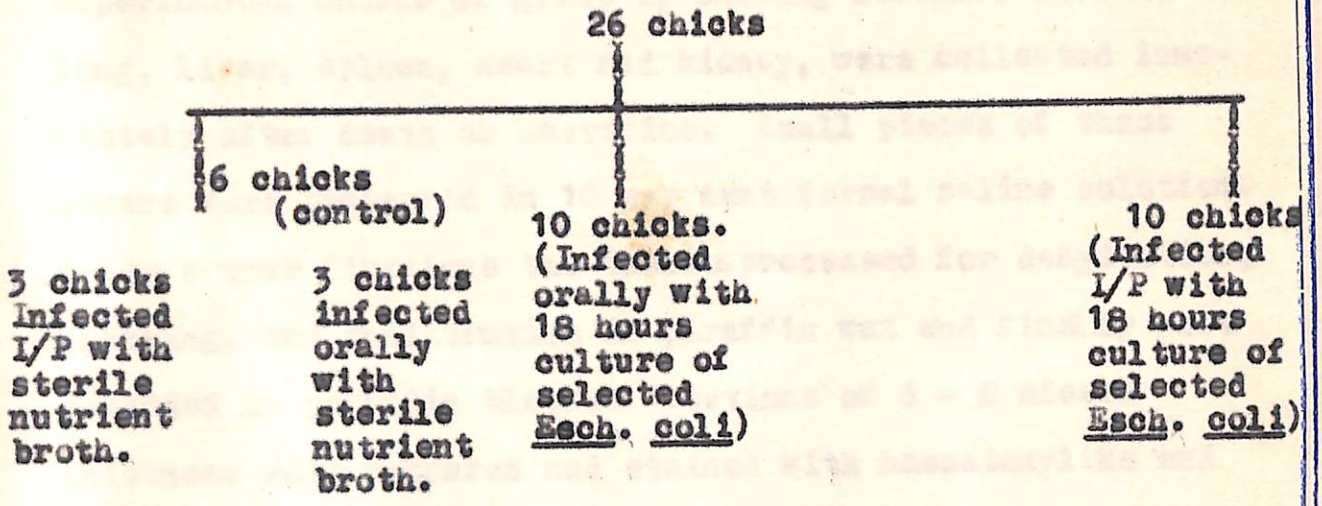
TABLE - III.

Chemotherapeutic agents used for the treatment of experimental Eschi. coli infection in chicks.

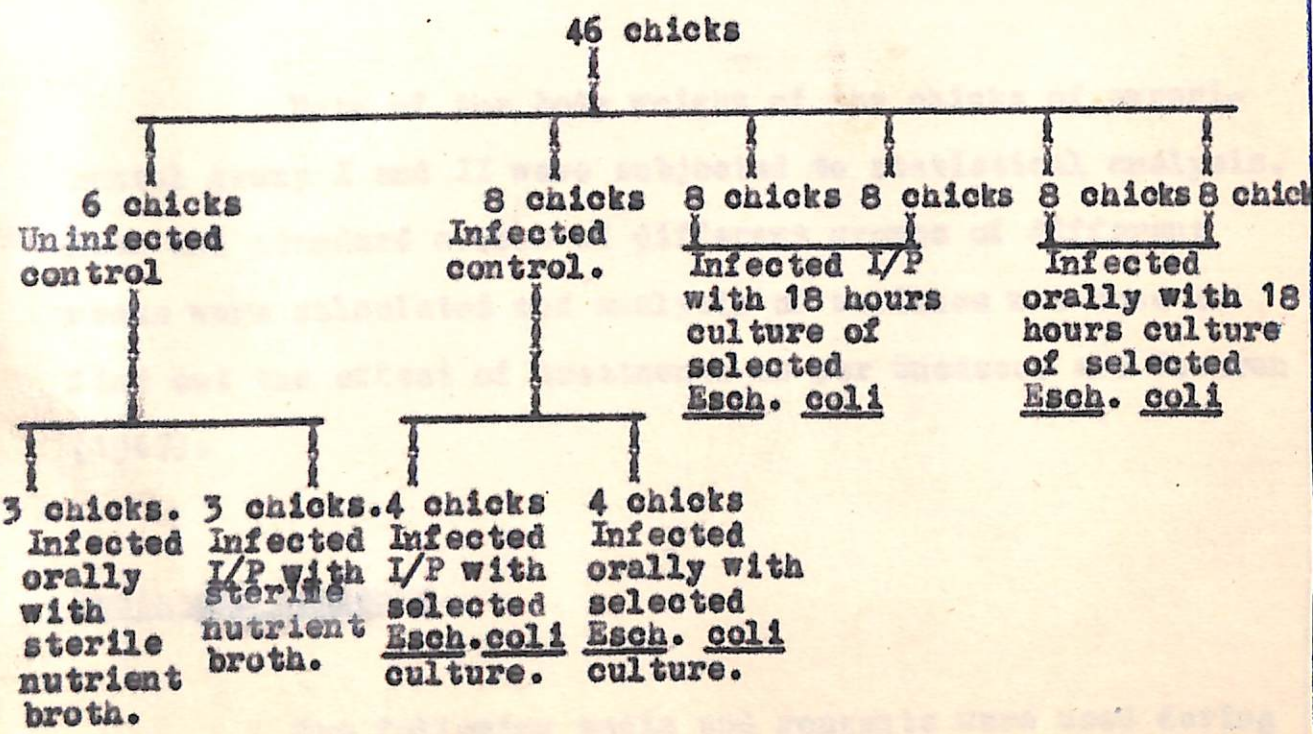
S. No.	Trade name and firm	Composition and presentation.	Dose used	Route of administration.
1.	Septran paediatric suspension (Burrough Wellcome & Co. (India) Pvt. Ltd., Bombay).	Each 5 ml contains Trimethoprim BP 40 mg Sulphamethoxazole 200 mg.	0.04%	Drinking water.
2.	Chloromycetin capsule (Parke-Davis India Ltd., Bombay).	Chloramphenicol 250 mg capsule.	10 mg	Oral
3.	Neomycin capsule (Unichem Laboratories Ltd., Bombay).	Neomycin sulphate 350 mg capsule.	30 mg.	Oral
4.	Furaxone tablets (Smith Kline & French (India) Ltd., Banglore.	Furazolidone	0.04%	In feed.

SCHEMATIC DIAGRAM

Group I : To study the effects of experimental infection of Esch. coli in chicks.



Group II : To study the efficacy of four chemotherapeutic agents against Esch. coli infection in chicks.



Histopathology.

For microscopic examination, the organs of the experimental chicks of group I, showing lesions, such as lung, liver, spleen, heart and kidney, were collected immediately after death or sacrifice. Small pieces of these organs were preserved in 10 per cent formal saline solution. After proper fixations the tissues processed for dehydration, clearing, and infiltration in paraffin wax and finally were embedded in paraffin blocks. Sections of 6 - 8 micron thickness were prepared and stained with haematoxyline and eosin method for the routine examination (Luna, 1958).

Statistical analysis.

Data of the body weight of the chicks of experimental group I and II were subjected to statistical analysis. Mean and standard errors of different groups of different weeks were calculated and analysis of variance was done to find out the effect of treatments as per Snedecor and Cochran (1967).

Media and Reagents.

The following media and reagents were used during

the present study :

1. Nutrient broth.
2. Nutrient agar media.
3. MacConkey's lactose agar media (MLA).
4. Eosin methylene blue (EMB) agar.
5. Peptone water.
6. One per cent sugar solution of lactose, glucose, sucrose, mannitol and maltose.
7. Glucose phosphate peptone water.
8. Koser's citrate media.
9. Methyl red.
10. V.P. reagent.
11. Andrades reagent.
12. 10 per cent lead acetate paper.
13. Hydrogen peroxide 3 per cent.
14. Gram's stain.

***
*

RESULTS

RESULTS

During present investigation altogether 200 samples (viz. lung, liver, spleen, heart, and kidney) from forty birds were examined for the isolation of Esch. coli as detailed in Table I. A total of 72 strains of Esch. coli were isolated in pure form based on biochemical and other tests. The number and percentage of organism isolated from different internal organs have been shown in Table IV.

The strains isolated were Gram negative rods measuring about 2 to 4 u x 0.5 u. All strains reduced nitrate into nitrite, produced indol and were positive for methyle red (M.R.) test. They were found negative for Voges-Proskauer (V.P.) reaction and did not utilize Koser's citrate. Only five strains out of 72 isolates produced hydrogen sulphide (H_2S).

Fermentation of lactose, glucose, mannitol, and maltose with production of acid and gas was observed in all isolates. However, the ability of the isolates to ferment sucrose was variable. Only 62 strains were able to ferment sucrose while the rest failed to attack it.

The percentage of Esch. coli isolated from lungs,

liver, spleen, heart, and kidney of apparently healthy birds were 35, 30, 25, 10 and 15 respectively and in diseased birds the percentage were 80, 55, 20, 40 and 50 respectively (Table IV).

TABLE - IV.

Name of the organs	Apparently healthy birds		Diseased birds	
	Number of Esch.coli isolated on examination of 20 samples each.	Percentage	Number of Esch.coli isolated on examination of 20 samples each.	Percentage.
Lung	7	35	16	80
Liver	6	30	11	55
Spleen	5	25	8	40
Heart	2	10	4	20
Kidney	3	15	10	50
Total.	23		49	

Pathogenicity test.

The pathogenicity test of the 15 randomly selected strains of isolated Esch. coli on albino mice revealed that only seven strains to be pathogenic. The result has been depicted in Table V.

TABLE - V.

Showing results of pathogenicity test of Esch. coli isolated from internal organs of birds.

Strain no. isolated from	Number of mice inoculated*	Mortality within 18 hours	Mortality within 19-30 hours	Mortality within 31-72 hours	Total death in inoculated mice.	No. of death/No. of inoculated.
PLi 32	3	0	0	0	0	0/3
PLu 36	3	0	0	0	0	0/3
PLu 66	3	0	0	0	0	0/3
PLi 67	3	0	0	0	0	0/3
PK 75	3	0	0	0	0	0/3
PK 105	3	0	0	0	0	0/3
PLu 106	3	0	0	1	1	1/3
PK 110	3	0	0	1	1	1/3
PLu 121	3	0	1	0	1	1/3
PLi 132	3	0	0	0	0	0/3
PLu 146	3	2	0	0	2	2/3
PK 150	3	0	2	0	2	2/3
PH 169	3	0	0	0	0	0/3
PS 178	3	0	2	0	2	2/3
PLi 192	3	0	2	0	2	2/3

PLi = Poultry liver; PLu = Poultry lung; PK = Poultry kidney.
PH = Poultry heart; PS = Poultry spleen.
* 0.5 ml 18 hours nutrient broth culture I/P.

Drug sensitivity test.

The isolated strains of Esch. coli from healthy and diseased birds were subjected to sensitivity test against ten commonly used antimicrobial drugs. The results of drug sensitivity of the isolated strains have been outlined in Table VI.

In terms of percentage, 62.5, 90.2, 41.6, 55.5, 86.1, 50.1, 12.5, 100.0, and 80.5 per cent strains were found sensitive to ampicillin, chloramphenicol, furadantoin, kanamycin, neomycin, gramoneg, streptomycin, septran, sulfuno and furazolidone respectively.

TABLE - VI.

Showing result of drug sensitivity test of Esch. coli strains isolated from internal organs of birds.

Antimicrobial drugs used	No. of strains of Esch. coli examined	Strains sensitive		Strains resistance	
		Number	Percentage	Number	Percentage
Ampicillin	72	45	62.5	27	37.5
Chloramphenicol	72	65	90.2	7	9.8
Furadantoin	72	30	41.6	42	58.4
Kanamycin	72	40	55.5	32	44.5
Neomycin	72	62	86.1	10	23.9
Gramoneg	72	36	50.0	36	50.0
Streptomycin	72	10	12.5	62	87.5
Septran	72	72	100.0	-	-
Sulfuno	72	-	-	72	100.0
Furazolidone	72	58	80.5	14	19.5

Experimental infection.

The chicks infected with pathogenic strain of isolated Esch. coli produced the symptoms as detailed below. Within 24 hours after infection the chicks were found depressed, off-fed, and developed respiratory trouble like gasping inhalation and had tendency to sit together. Syndrome of blood tinged pasty-diarrhoea developed in surviving chicks after 2 days which continued upto 30th day except in few cases, where this symptoms disappeared gradually within a fort-night.

The symptoms in intraperitoneally infected chicks appeared rather quickly in comparison to orally infected chicks. Two chicks infected intraperitoneally collapsed within 24 hours. Among the orally infected chicks one died within 30 hours and the second on 8th day after infection. The post mortem examination of dead chicks revealed congestion of lung, liver, spleen, heart, and kidney and the infective organisms were recovered from the internal organs as well.

Body weight.

In the first groups of experiment, infected lot showed decrease in body weight in comparison to the control group. The difference in body weight between the control group and infected groups were found to be highly significant ($P<0.01$) (Table VIII).

Between the oral and intraperitoneally infected groups no significant difference in body weight could be found. The mean body weight along with standard error (S.E.) (in gm) of chicks under different groups treatments in different weeks are appended in Table VII.

Similarly for the second group of experiment, the mean body weight of chicks of infected control was found to differ significantly from those of treated and uninfected groups. But mean body weight difference between different treatment group showed no significant difference (Table IX and X).

TABLE - VII.

Showing mean body weight along with S.E. (in gm) of different treatment groups in different weeks.

Groups / weeks	1st group Mean ± S.E.	2nd group Mean ± S.E.	3rd group Mean ± S.E.	Overall Mean ± S.E. week
1	149.83±1.44	149.16±1.62	149.5±1.33	149.5±0.8
2	179.00±3.36	165.16±3.68	162.0±1.36	168.72±2.4
3	205.83±4.54	177.14±3.47	173.5±1.38	185.5 ±3.9
4	229.1 ±4.19	189.66±2.77	185.5±1.30	201.44±7.6
5	258.66±3.87	202.16±1.85	198.16±0.83	219.66±4.8
Overall groups Mean ± S.E.	204.5±7.19	176.66±3.64	173.73±3.22	184.62±5.95

TABLE - VIII.

Analysis of variance table showing effects of treatments and weeks on body weight of experimental chicks.

Sources of variation	df	M. S.	
Between treatment	2	8649.45	
Between weeks	4	13489.47	
Interaction	8	1018.78	22.41**
Error	75	45.46	
Total	89		

** denotes significant at 1% level.

C.D. value at 1% = 4.59

C.D. value at 5% = 3.46

Group I (Control)	Group II (Infected orally)	Group III (Infected I/P)
204.5	176.66	173.33

TABLE - IX.

Showing mean body weight along with S.E. (in gm) of different treatment groups in different days.

Groups / days	Group I Mean ±S.E.	Group II Mean ± S.E.	Group III Mean ± S.E.	Group IV Mean ± S.E.	Group V Mean ± S.E.	Group VI Mean ± S.E.	Overall Mean±S.E./ days
0 day	128.16 ± 1.22	127.83 ± 0.91	126.66 ± 1.05	128.5 ± 0.57	126.83 ± 1.13	127.83 ± 0.94	127.63 ± 0.41
2nd day	130.83 ± 1.3	128.83 ± 1.01	129.00 ± 0.73	129.6 ± 0.92	127.83 ± 0.94	128.83 ± 1.30	129.16 ± 0.43
4th day	134.33 ± 1.50	130.33 ± 0.88	130.16 ± 1.25	131.5 ± 1.50	129.33 ± 0.84	131.16 ± 7.5	131.13 ± 1.2
6th day	139.5 ± 1.8	132.00 ± 0.68	134.5 ± 1.11	131.8 ± 1.01	133.00 ± 0.61	134.5 ± 1.28	134.27 ± 0.6
8th day	141.0 ± 1.52	133.33 ± 1.05	137.33 ± 1.50	136.00 ± 0.88	135.83 ± 0.71	135.16 ± 1.74	136.44 ± 0.63
10th day	144.83 ± 1.51	135.16 ± 1.19	141.61 ± 1.17	138.83 ± 0.98	138.66 ± 0.72	138.66 ± 1.28	139.63 ± 0.67
12th day	148.33 ± 1.96	136.16 ± 1.04	146.83 ± 0.94	140.83 ± 0.77	142.66 ± 0.72	140.83 ± 1.01	142.61 ± 0.8
Overall groups Mean±S.E.	138.41 ± 1.19	131.95 ± 0.57	134.07 ± 2.91	133.88 ± 0.75	133.5 ± 0.92	133.85 ± 1.28	134.4 ± 0.91

Group I = Uninfected control; Group II = Infected control; Group III = Infected I/P and treated with septran paediatric suspension; Group IV = Infected I/P and treated with chloromycetin; Group V = Infected orally and treated with neomycin; Group VI = Infected orally and treated with furazolidone.

TABLE - X.

Analysis of variance table showing effects of treatments and days on body weight of experimental chicks.

Sources of variation	df	M. S.	
Between treatment	5	179.06	13.05**
Between days	6	3156.43	230.06**
Error	240	13.72	
Total	251		

** denotes significant at 1% level.

C.D. value at 1% = 2.08

C.D. value at 5% = 3.46

Groups					
I	II	III	IV	V	VI
138.14	131.95	134.07	133.88	133.50	133.85

Lesions and histopathology.

The gross and microscopic examination of the internal organs (viz. lung, liver, spleen, heart and kidney) were done in experimentally infected chicks after sacrificing them on 10th, 15th and 30th day of post infection. The gross lesions were observed in pericardial sac, heart, liver, and kidney.

A small amount of serous fluid were present in the pericardium of almost all infected chicks. The pericardium appeared thickened and was at places adherent to the epicardium. The heart, liver, kidney, spleen, and lungs were congested. In some chicks petechial haemorrhage was noticed on epicardium, liver, spleen, kidney, and even on skeletal muscles. Liver and spleen were invariably enlarged in size (Fig. 1 and 2). The liver of chicks which were infected with Esch. coli by per os showed rounding of their edges and bulging of cut surfaces. The culture of infective Esch. coli could not be isolated from the sacrificed chicks.

The microscopic examinations of the internal organs of intraperitoneally infected chicks revealed the following changes.

Heart.

Muscle fibers were separated from each other due

to oedematus fluid in some places. There were also necrotic foci and infiltration of mononuclear cell between muscle fibers. The myocardium of chicks which died in early stage of infection revealed blood vessels packed with erythrocytes and there were also focal haemorrhagic spots (Fig. 3).

Lung.

In this groups of chicks, the blood vessels of inter-alveolar space, peribronchiolar and subepithelial region were packed with erythrocytes and at some places there was also erythrodiapedesis. The epithelial cells of bronchi and bronchiole were beraft from there basement membrane.

In the subepithelial region there was also formation of lymphoid nodule. In one case the epithelial cells of the bronchiole were hyperplastic and even the peribronchiolar lymphoid nodules were hyperplastic in which haemorrhagic spots were also seen (Fig. 4). The walls of the alveolar sacs were thickened due to infiltration of mononuclear cells. The cells lining of alveolar sacs also appear hyperplastic.

Liver.

The change in liver of this group of chicks were very mild in nature. The only alteration was the infiltration of a few mononuclear cells in the portal tracts. The

hepatocytes, central veins, synosoides and blood vessels of the portal tracts and bile duct revealed no significant change on microscopic examination.

Spleen.

In early stage of infection (10 days PIP) the lightly stained cells of lymphoid series located centrally in the lymphoid follicles showed hyperplasia (Fig. 5). The number of germinal centres also appeared to have increased. However, the cells of paracortical regions showed least alteration due to infection of Esch. coli. The changes in germinal centres were not so pronounced in late stage of infection i.e. 30 days PIP.

Kidney.

In early stage of infection there was severe congestion of intertubular blood-vessels, glomurular and major vessels of pelvic region. There were also haemorrhage in the intertubular areas. Some of the tubules showed rounding of the tubular epithelial cells and epithelial cells in general were deeply stained with eosin. In the late stages (i.e. 30 days PIP) there was also formation of granulomatous lesions which consisted of macrophages and lymphocytes. There was also destruction of tubular structure within the granulomatous lesions (Fig. 6 and 7).

The microscopical examination of the internal organs of orally infected chicks revealed the following changes.

Heart.

Most prominent change in heart on microscopic examination was found to be increased in sarcolemmal space (Fig. 8). The heart muscle fibers were separated from each other probably due to the presence of oedematous fluid. Frank necrosis and formation of granuloma were not observed in any part of the organ.

Lung.

The histological alteration in lungs varied with the time of sacrifice. The chicks which were sacrificed on 10th day post infection showed severe congestion of blood vessels, haemorrhage in between two alveolar sacs and thickening of the wall of air sacs due to wide spread haemorrhage. The congestion of blood vessels continued even after 15th day post infection. The wall of air sacs were still loaded with a large number of erythrocytes but thereafter appearance of epitheloid cells and a few lymphocytes in the wall of air sac's were evident.

The chicks sacrificed/died 30th day PIP showed

reduction in the severity of congestion and haemorrhage in lung parenchyma. At this stage the wall of the air sacs were mostly infiltrated by mononuclear cells, consisting of macrophages and lymphocytes. The most drastic change was seen at the peribronchiolar region. The lymph nodules were hyperplastic and tremendously enlarged in size (Fig. 9). However, the epithelial cells lining the bronchioles showed least involvement in the reaction to Esch. coli. In addition, at some places there was formation of nodular lesions consisting of mainly macrophages, lymphocytes and erythrocytes. Giants cells of foreign body type were also seen (Fig. 10).

Liver.

On microscopic examination of the liver it was observed that per unit area the sinusoidal space had increased. The most striking changes were observed around the blood vessels. There was congestion of blood vessels and infiltration of plasma cells, macrophages and lymphocytes in the periportal tract or around some of the central veins. At some location there was a focal necrosis of hepatocytes and formation of granulomatous lesions. In fact, the periphery of these lesions showed proliferation of connective tissue which surrounded the granulomatous lesions incompletely (Fig. 11). In some cases there was a definite sign of proliferation of connective tissue in the periportal tract.

The Van-Kupffer cell in general showed hypertrophy and hyperplasia. Proliferation of bile duct was not a feature in any of the chicks examined. The histopathological alteration in the liver of chicks which were sacrificed/died at 10th days or 15th days post infection showed tissue alteration similar to the above, except that the necrosis and cellular infiltration were less pronounced.

Spleen.

The white pulp were depleted of lymphocytes but the large lymphoid cells in the germinal centres were increased in number. The red pulp showed large number of nucleated erythrocytes and presence of macrophages and lymphocytes. However, the formation of nodular lesions with giant cells were not observed in any part of splenic parenchyma.

Kidney.

The blood vessles of kidney parenchyma were packed with nucleated erythrocytes. In the cortex there was haemorrhage. In the chicks which were sacrificed on 10th day of post infection, the proliferation of glomerular endothelial cells were also evident. The glomerular space was reduced due to cellular proliferation. Some of the tubules showed necrosis of epithelial cells and in some, the lining epithelial cells of tubules were desquamated. There was also individualization

of the epithelial cells of tubules. Cellular infiltration was not observed in any part of the kidney.

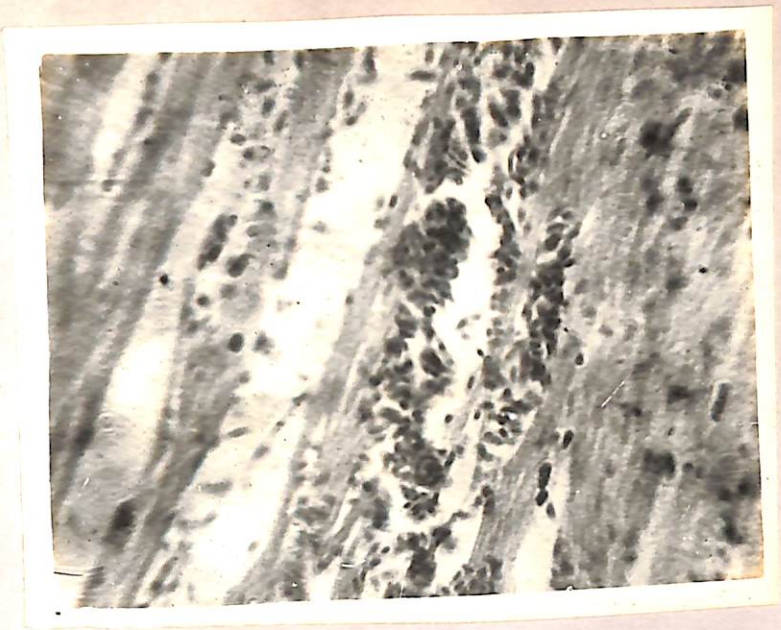
The drug trial was conducted in the second group of the infected chicks after the appearance of symptoms. Four chemotherapeutic agents were used for the drug trial in infected chicks.

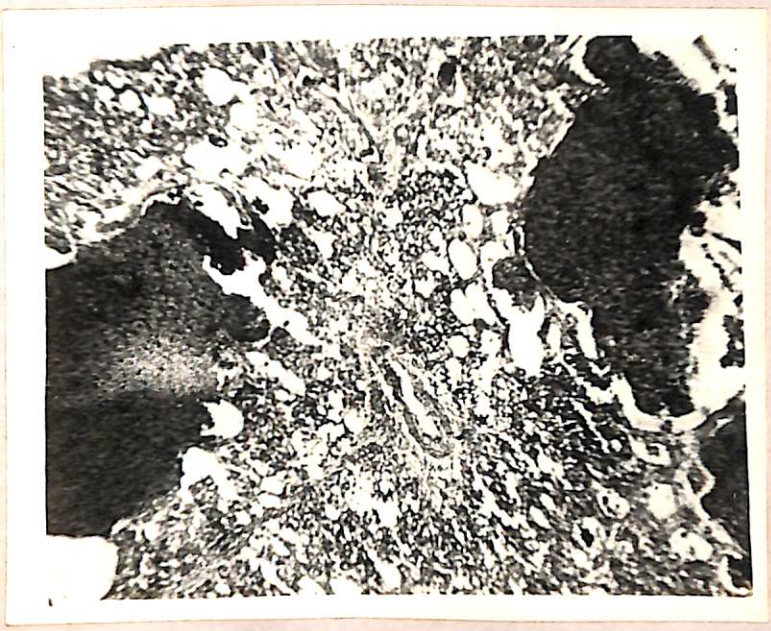
1. Septran paediatric suspension.

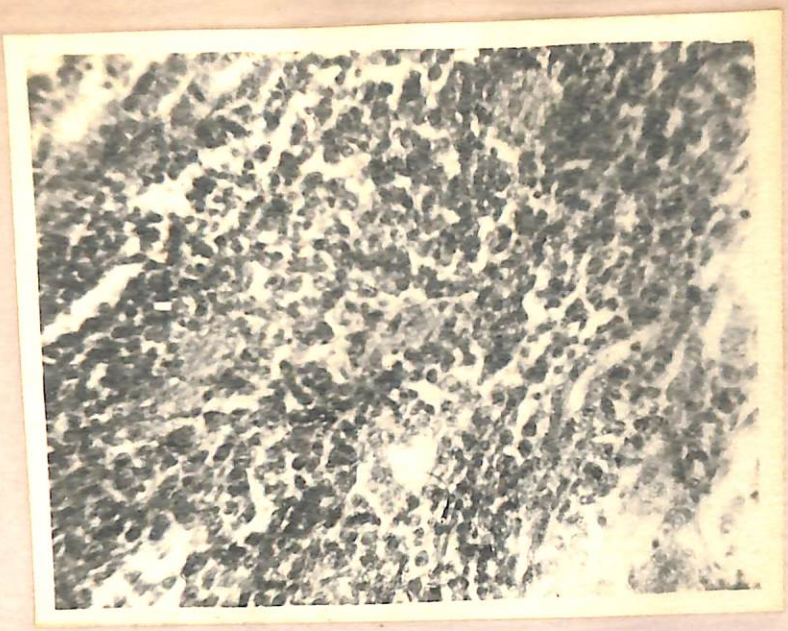
The group of eight infected chicks was treated with "septran paediatric suspension". The drug was administered through drinking water in the concentration of 0.04 per cent. After two days of treatment the diarrhoea in chicks was checked and other symptoms also disappeared. The treatment was continued for four days. All birds of this group were treated successfully.

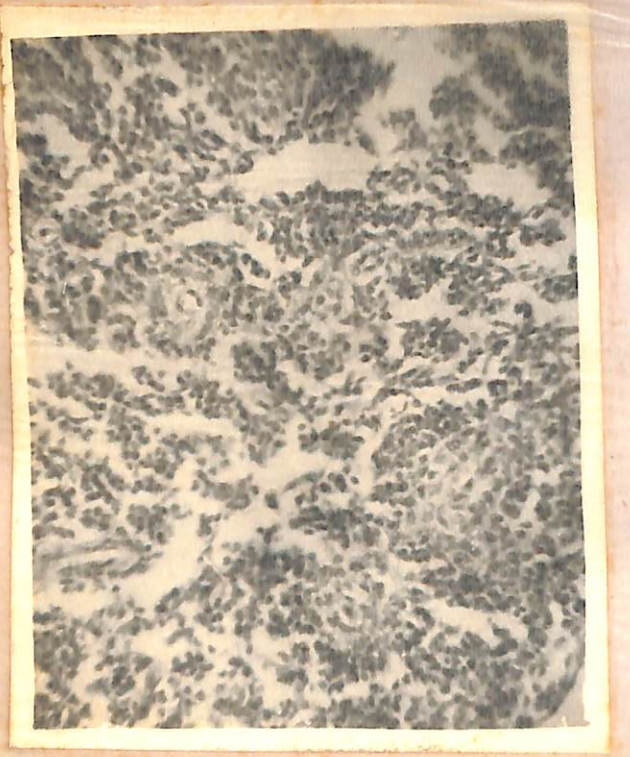
2. Chloromycetin.

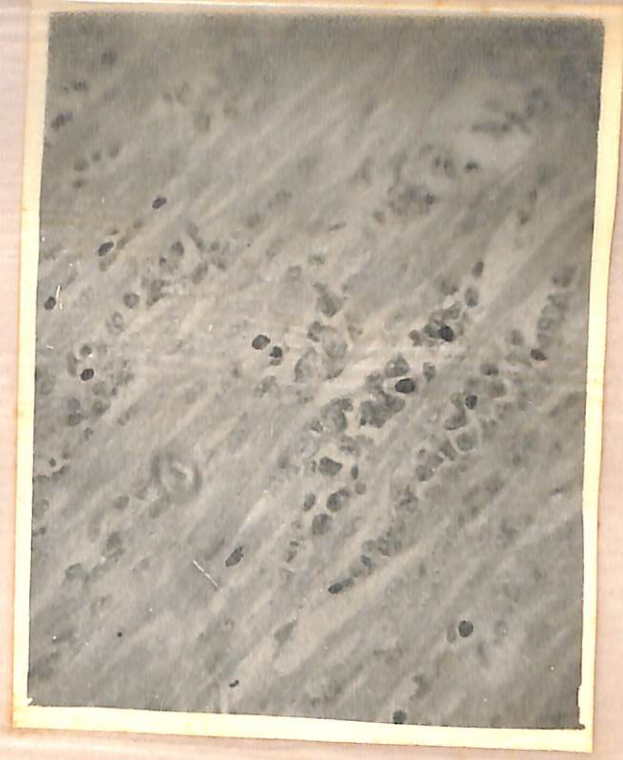
The second group of eight chicks was treated with chloromycetin capsule at the dose rate of 10 mg per chick orally. After three days of treatment the diarrhoea in chicks were cnekced and all visible symptoms disappeared. The treatment was continued for five days. Once the treatment started no loss of life was observed in this group.

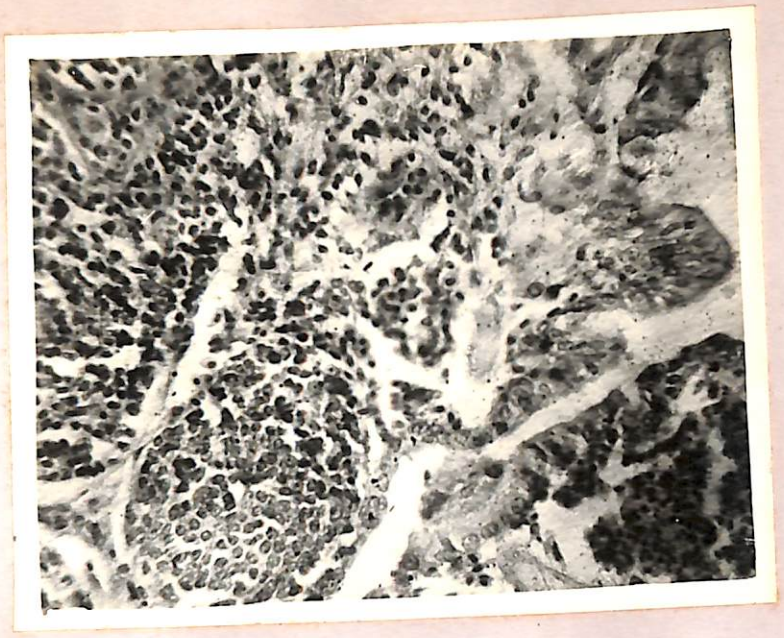


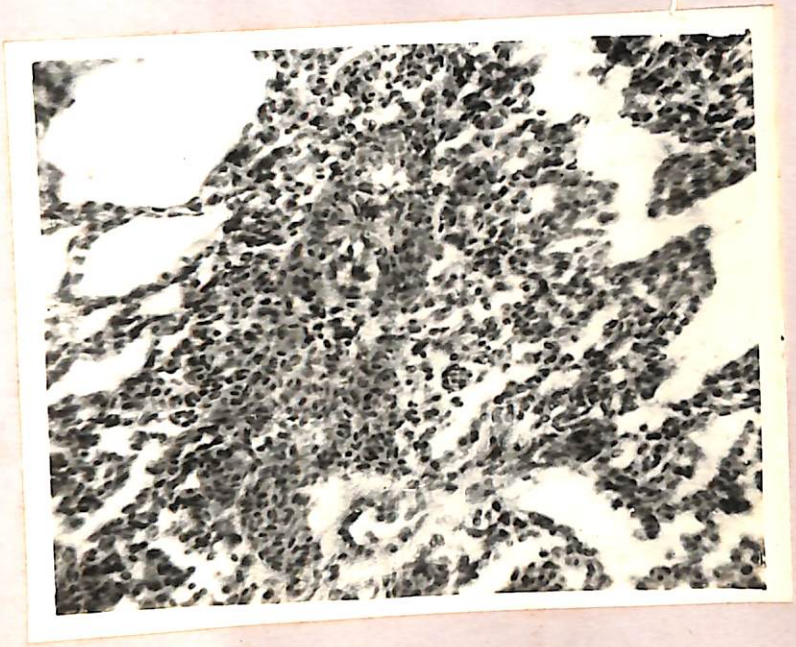












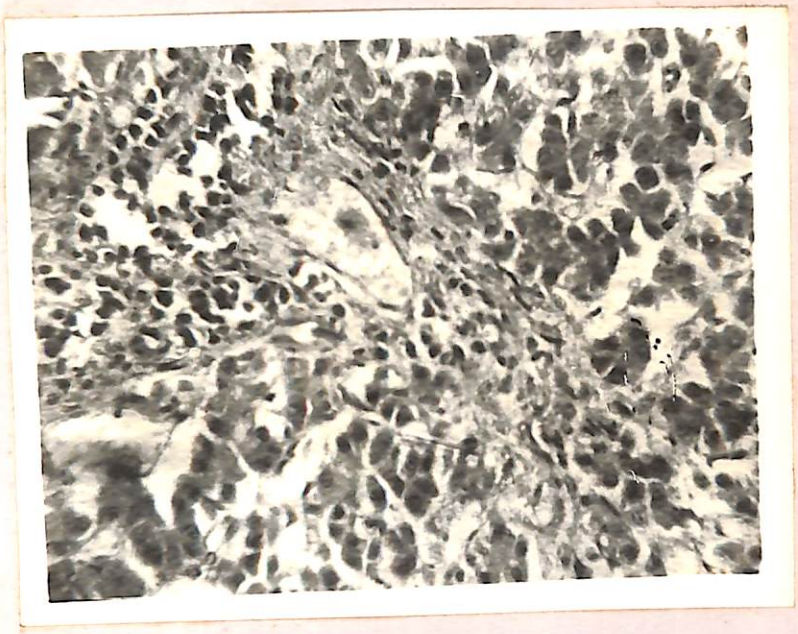



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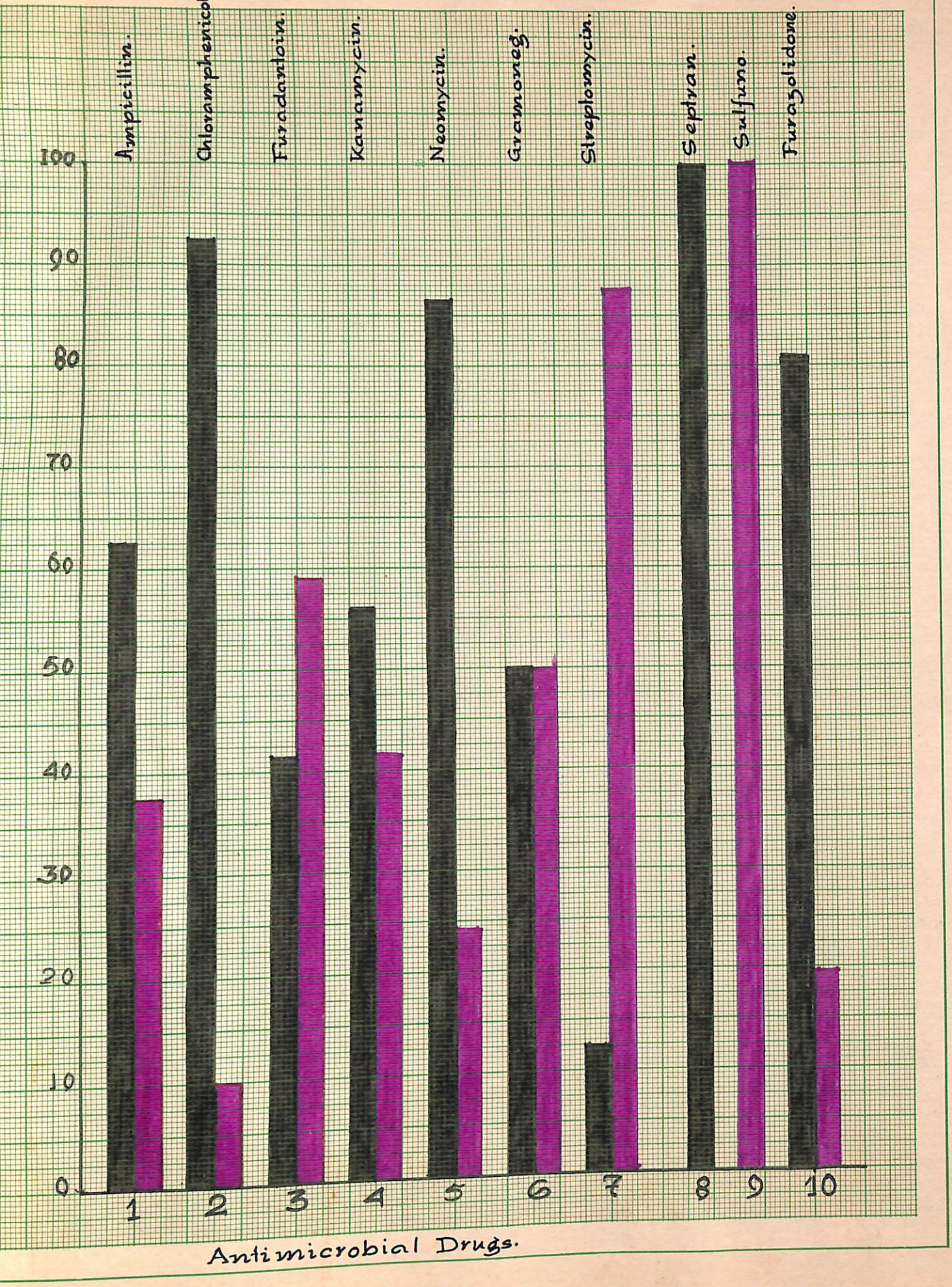
FIGURE 13.


Ampicillin.
Chloramphenicol.
Furadantoin.
Kanamycin.
Neomycin.
Gramoneg.
Streptomycin.
Septran.
Sulfuno.
Furazolidone.
100
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70
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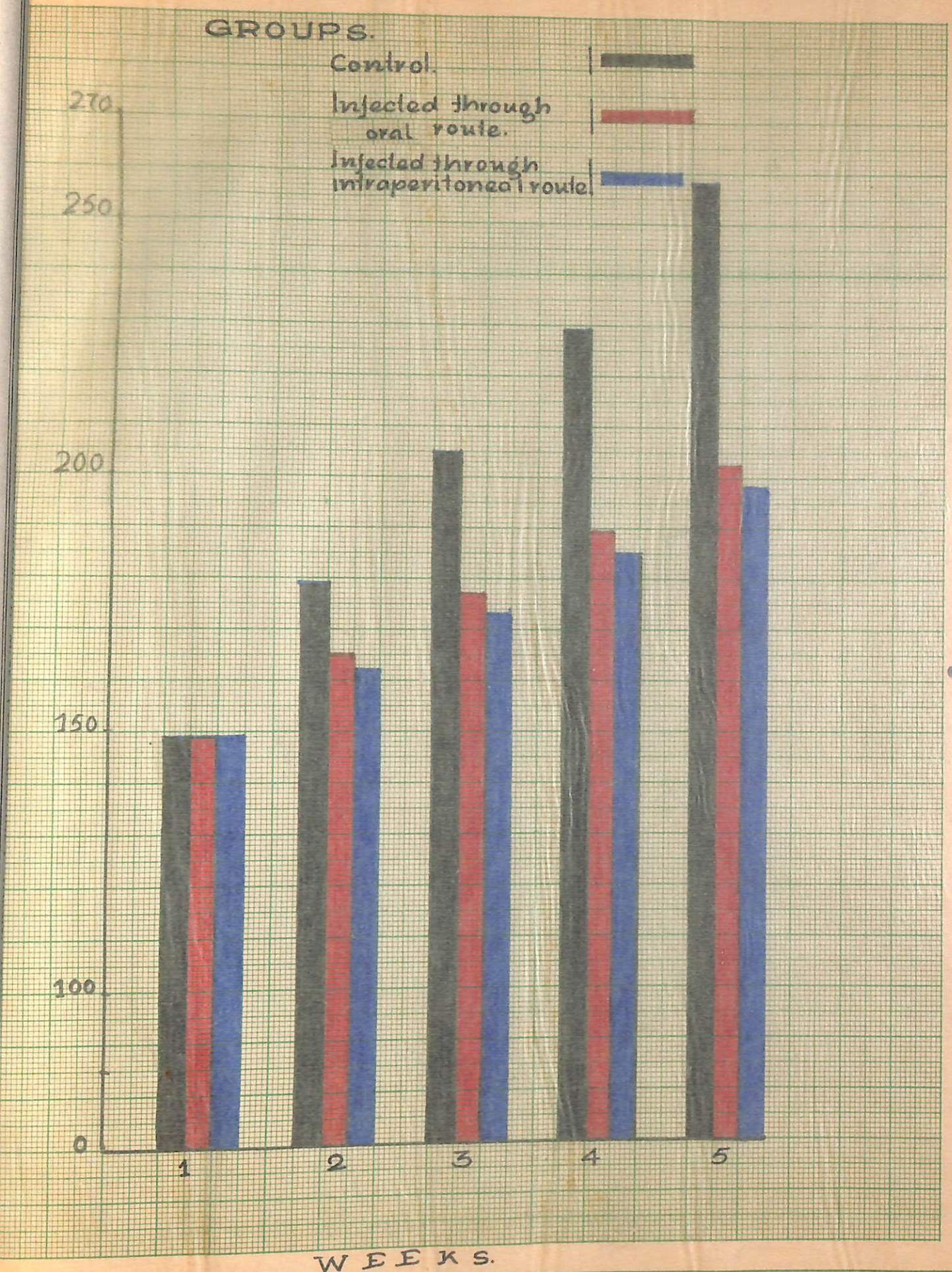
Antimicrobial Drugs.

FIGURE-14.


GROUPS.
Control.
Injected through oral route.
Injected through intraperitoneal route.
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250
200
150
100
0
1
2
3
4
5
WEEKS.

DISCUSSION

D I S C U S S I O N

Esch. coli has been found associated with a number of disease conditions of poultry resulting into heavy mortality, such as, coli-granuloma or Hjarre's diseases (Hjarre and Wramby, 1945), C.R.D. complex (Gross, 1956, 1957), Egg-peritonitis (Gross and Siegel, 1959), coli-septicaemia (Sojka and Carnaghan, 1961) and haemorrhagic-enteritis (Quareshi,1957; Barr and Carman, 1957).

Altogether 200 samples (viz. lung, liver, spleen, heart and kidney) from forty birds were examined for the isolation of Esch. coli as detailed in Table I. Based on biochemical and other tests, out of the 72 strains isolated, 49 were from diseased birds and 23 were from apparently healthy birds. The percentage of incidence of this organism in diseased group from lung, liver, spleen, heart and kidney were 80, 55, 40, 20, 50 respectively and in apparently healthy group its incidence were 35, 30, 25, 10, 15 from lung, liver, spleen, heart, and kidney respectively (Table IV).

As evident from Table IV, that the percentage incidence of Esch. coli isolated from diseased birds was higher as compared to that of healthy ones.

The isolation of Esch. coli from different organs of diseased birds was not surprising as perusal of the literature revealed that this organism might produce various pathological conditions in the above mentioned organs. Edward and Ewing (1954) isolated Esch. coli of O2 : K_1 : K_5 serotypes from the different internal organs of fowls died in U.S.A. Several workers have isolated Esch. coli from different internal organs of poultry producing different clinical conditions (Sojka and Carnaghan, 1961; Gurumurthi and Panduranga Rao, 1962; Gupta and Singh, 1969; Prasad et al., 1967; Yadava and Malick, 1972; Sharma et al., 1977).

The protean manifestation of the organisms affecting several organs i.e. proventriculus, small intestine, caeca, liver, pancreas, kidney, bone marrow and even eye lids was reported by Nayak et al. (1967).

The isolation of Esch. coli from different organs of apparently healthy birds was also carried out to study their pathogenic property if present. It has been seen that apparently healthy birds also harbour some pathogenic strains of Esch. coli. These potential pathogens may prove havoc to the flock by being disseminated by carrier birds under poor managerial condition (Savov, 1966; Sojka and Carnaghan, 1965; Andrey et al., 1968). These pathogens may prove hazardous even to the carrier birds under various stress conditions as Quareshi (1957) reported fatal enteritis in two flock of birds following "New Castle Disease Vaccination".

Pathogenicity.

The association of Esch. coli with certain avian disease condition has been recognised since long. But at the same time it is known that all strains of Esch. coli do not have capacity to produce the diseases. It has been found by several workers that there are certain serotype of Esch. coli which oftenly produce diseases. Sojka and Carnaghan (1961), have shown over 60 per cent of strains from coli septicaemia belong to the serological "O" group i.e. 02, 078, 011. Keeping in view the above factors the pathogenicity of Esch. coli isolated in present investigation was carried out. Possible pathogens were searched among the randomly selected 10 strains of Esch. coli from diseased group and 5 strains from healthy groups.

The pathogenicity test was done in albino mice. The test was designed as depicted in Table V. From the Table V, it is evident that the seven strains out of 10 isolated from diseased birds were proved to be pathogenic for albino mice. The two albino mice died in 18 hours, seven mice in 19-30 hours and only two mice survived upto 72 hours.

In almost all mice septicaemic condition was observed on post mortem examination and Esch. coli were isolated in pure from heart, lung, liver, spleen and kidney.

It has been observed by various workers also that

generally the Esch. coli isolated from different pathological conditions of poultry have proved pathogenic to one or other experimental laboratory animals. Sojka and Carnaghan (1961) reported that Esch. coli isolated from coli-septicaemia produced the same condition in the fowl experimentally. The pathogenicity of Esch. coli in albino mice has been reported by Gurumurthi and Panduranga Rao (1962), Nagi and Khanna (1967), Gupta and Singh (1969), Kulkarni (1970) and Sinha (1975).

However, Gurumurthi and Panduranga Rao (1962) have opined that their strains had variable pathogenicity for rabbits. This difference may be attributed to the species variations. Similar results have been reported by other workers on pathogenicity test of the Esch. coli isolated from the diseased birds in chicks (Iyre et al., 1965; Nagi and Khanna, 1967; Grosheva, 1971; Blauel, 1973; Sharma et al., 1977).

The five strains of Esch. coli isolated from apparently healthy birds could not produce disease in any of the albino mice even after 72 hours of observation. However, two mice showed dullness within 12 hours after inoculation but again they became as normal showing their nonpathogenic character.

Drug sensitivity test.

Antimicrobial sensitivity tests with the isolates of Esch. coli were carried out during this study to find out

suitable antimicrobial agents to be utilised as therapeutic agents in the control of Esch. coli infection. The results of in-vitro antimicrobial sensitivity tests against Esch. coli given in Table VI; septran, chloramphenicol, neomycin, and furazolidone were found to be 100, 90.2, 86.1 and 80.5 per cent sensitive respectively.

The septran has been observed by other worker (Mishra, 1977) of the same value as observed in the present study. Chloramphenicol was found to be the next effective drug against Esch. coli, on the basis of antibiotic sensitivity tests. This finding is in agreement with that of Glantz(1962), Takahsi (1966), Butura and Sanleanu (1972), Butura et al. (1973), Heller et al. (1973).

The neomycin and furazolidone has also been found effective against Esch. coli by different workers (Sojka and Carnaghan, 1961; Glantz, 1962; Butura and Sanleanu, 1972). The present finding is also inaccordance with the above workers.

It is evident from Table VI that the ampicillin, furadantoin, kanamycin, gramoneg, and streptomycin, were found to be sensitive in the percentage of 62.5, 41.6, 55.5, 50 and 12.5 respectively. Sinha (1973) and Sinha (1975) reported 57.1 and 46.1 per cent strains sensitive to ampicillin respectively, whereas in the present study 62.5 per cent strains were found to be sensitive to ampicillin which is almost atpar with the finding of above workers. But Mishra (1977) found 100 per cent strains resistant to ampicillin. Ampicillin

resistance of Esch. coli may be due to the fact, because this strains were of milk derived from the respective animals might have been kept on antibiotic therapy for longer period resulting into the resistance strain.

Sinha (1975) and Mishra (1977) reported 76 and 79.1 per cent strains sensitive to nitrofuradantoin but Sinha (1973) found high percentage of resistance. However, in present study only 41.6 per cent strains were sensitive to nitrofuradantoin.

As shown in Table VI, Kanamycin was only 55.5 per cent sensitive to Esch. coli. As regards to kanacin quite dissimilar result has been reported by Farrag and Oof (1967), Verma (1973) and Sinha (1975) who observed more than 90 per cent strains of Esch. coli sensitive to kanamycin. Variations in sensitivity of Esch. coli to kanamycin as compared to the findings of above workers with the present one might be due to the strain variations as Farrag and Oof (1967) and Verma (1973) used the Esch. coli strain of mastitis origin whereas Sinha (1975) used the strains of dog origin having urinary tract infection for antibiotic sensitive test. In the present study 50 per cent strains were sensitive to gramoneg, whereas Heller et al. (1973) observed that none of the Esch. coli, which resulted into the fatal infection of poultry, were resistant to gramoneg. Such wide difference might be due to the variable pattern of antibiotic sensitive tests followed in different laboratories, as till now there is no uniform pattem chalked out to be followed in all the laboratories for having

fruitful comparison of the antibiotics sensitive result.

During the present study only 12.5 per cent strains were sensitive to streptomycin. Butura and Sanleanu (1972) while studying the antibiogram pattern of Escn. coli isolated from septicaemia of fowl showed that all the 198 strains were resistant to streptomycin. During the present study 100 per cent strains were resistant to sulfuno. However, Mishra (1977) observed only 4.00 per cent strains were sensitive to sulfuno.

Some critical observation, with regard to variability of efficacy of some antibiotics in the light of observation of other workers could not be explained as there are many limitations and fallacies of in-vitro sensitivity tests of the antimicrobial drugs (association of Clinical Pathologists, 1965). The interpretations of sensitivity result is also not free from controversies, as it depends on the types of methods used (Ericsson and Sherris, 1971), choice of drug with their concentration for sensitivity and size of inoculum used for sensitivity (Castle and Elstab, 1971) and zone of inhibition (Wolf et al., 1975). That is way an expert committee of W.H.O. (1961) has stressed the use for a standardized method for conducting microbial sensitivity, so that it could provide a reliable guide to the research worker and clinicians. Recently Garrod and Wateruth (1971) have also suggested that there should be an international standards for disc content to obtain uniformity of the result.

Experimental infection.

In the present study the total number of 15 strains used for pathogenic test, the most pathogenic one which killed the mice in shortest period has been selected to establish the infection in experimental chicks. The common and the characterestic symptoms observed in both the routes of infection viz. intraperitoneally and orally, were found to be severe depression, off-fed, respiratory trouble accompanied by blood tinged diarrhoea in most of the cases.

Quareshi (1957) observed that Escn. coli caused fatal enteritis in two flocks of birds in West Pakistan when some birds were vaccinated against "New Castle Disease". The infection was reproduced from the organ suspension.

Sojka and Carnaghan (1961) observed the symptoms of acute depression, severe green diarrhoea, and lameness in experimentally infected chicks.

Gurumurthi and Panduranga Rao (1962) observed colibacillosis condition with the symptoms of severe enteritis, white pasty diarrhoea, blocking of vent with dried faeces in cases of field outbreak. The authors were also able to reestablish the infection in chicks. The similar manifestation have also been observed by Nagi and Khanna (1967), Stipkovits and Solyom (1968), Sharma et al. (1977).

However, the lameness could not be observed

markedly during the present study but other clinical manifestation were almost the same as reported by other workers.

The symptoms in intraperitoneally infected chicks in comparison to orally infected ones appeared much earlier, which is evident on the basis of death which took place i.e. 24 hours in intraperitoneally infected chicks, whereas the chicks infected orally died 30 hours after infection. It has also been recorded that one orally infected chicks died on 8th day after infection. In later case the cause of death seems to be due to exhaustion and dehydration being caused by diarrhoea, debility followed by lowintake of feed. Thus, it appeared that death in intraperitoneally and orally infected birds was 20 and 20 per cent respectively.

Betitskii and Panikar (1969) have reported 10 per cent death in 42 to 44 days old chicks but death was less common in younger chicks in comparison to older chicks in broiler with septicaemia. Sojka and Carnaghan (1961) has also reported variation in mortality from 33 to 100 per cent. The death occurring in 24 to 72 hours following inoculation of pathogenic strains profuse and pure culture of the same serotype of Esch. coli were obtained on primary isolation of the internal organs. The death occurring later, inspite of presence of lesions the number of Esch. coli colony obtained on primary culture from viscera was relatively small, and in some instances bacteriological examination of internal organs was negative. The report of the authors are in similarity

with the findings of the present work, where culture of the infective Esch. coli could not be isolated from the birds sacrificed on 10th, 15th and 30th day of infection.

Sharma et al. (1977) have also reported 15 to 20 per cent death in two groups of intraperitoneally infected white leghorn chicks.

In the present study both the groups of experimentally infected chicks showed decrease in body weight in comparison to control group. The different in body weight between control and infected groups have been found to be highly significant (P 0.01). Here the difference in body weight in infected and control groups appears to be due to the stress produced by the different pathological condition being caused by infective organism. Such loss of body weight due to infection caused by Esch. coli has also been reported by Gordon (1961) and Stipkovits and Solyom (1968). It is not always possible that loss in body weight should occur only after appearance of clinical manifestation as Savov (1965) observed 14 - 25 per cent less body weight than the control, in infected chicks which survived after showing some or no clinical signs. It is interesting to note that the difference in body weight was not statistically significant so far the route of inoculation (I/P, orally) was concerned whereas the early characteristic symptoms have been recorded in I/P infected birds as comparison to orally infected chicks.

As expected, in the second group of experiments,

marked difference in the mean body weight of birds were observed with the infected control as compared to that of treated and uninfected groups. This finding is not surprising, if the infected birds left untreated, decrease in body weight will occur due to progressive pathological condition. Subsequent loss in body weight due to infection has also been observed by Gordon (1961).

Histopathology.

There has been conflicting reports in the literature regarding the production of lesions in experimentally infected birds (Hamilton and Conard, 1958). This might be due to the difference in the route of infection. Hence, in the present study oral and intraperitoneal routes were chosen to set up infection in chicks. Critical perusal of the findings indicate that liver was damaged only when the infection was given through per os. It is a well known fact that any organism travelling through oesophagus, proventriculus and duodenum is likely to reach the liver via portal vein.

The organism inoculated intraperitoneally would also reach the liver but only after a long tortuous route via heart and during this journey their main strings are likely to be made blunt. Thus, it is possible that the same organism by oral route has caused much more appreciable tissue alterations in liver than through intraperitoneal route.

During early stages of the infection hyperaemia was very much pronounced in both the group of chicks in heart, lung, and kidney. A similar observation was reported earlier by Sojka and Carnaghan (1961) and Nagi and Khanna (1967) in this condition.

Another important histological change was noted in the spleen in early stages of the disease. There was a definite evidence of proliferation and differentiation of large lymphoblast cells in the germinal centres in the spleen. In recent years ample evidence has been put forward to show that the lightly stained cells of the germinal centres were responsible for the production of antibodies against the antigen. It is also known that Esch. coli is disposed off in the body by humoral mechanism and as such proliferation of the cells of the germination centres of the spleen in Esch. coli infection in the present study is not surprising.

In the lungs both bronchioles and alveolar sacs were affected in the present experiment. The subepithelial tissue was infiltrated with mononuclear cells and the walls of the alveolar sacs were thickened due to infiltration by mononuclear cells. The lesions were suggestive of granulomatous inflammation.

The changes in kidney and heart were also suggestive of granulomatous lesions. The formation of granulomatous lesions in lung, heart, and kidney, was also observed by several other workers (Nayak et al., 1967; Micevski and

Naletoski, 1972; Truscolt et al., 1974).

It may be concluded that early stages of the infection due to Esch. coli is marked by vascular changes while focal necrotic and the granulomatous lesions are formed only in later stages of the infection.

To combat the economical loss being caused by Esch. coli infection, a number of chemotherapeutic agents have been tried at different dose rate by various workers (Sojka and Carnaghan, 1961; Matvienko and Rudenko, 1964; Butura and Sahleanu, 1972; Butura et al., 1973).

Esch. coli infection causes marked decreased in body weight, low egg production and poor carcass quality. It thereby leads to heavy loss to poultry industry (Gordon, 1961; Savov, 1965; Stipkovits and Solyom, 1968; Sharma et al.,1977).

In the present study four drugs such as (i) septran paediatric suspension, (ii) chloromycetin capsule (iii) neomycin capsule (iv) furazolidone have been selected to treat the experimentally infected chicks and also to assess the comparative efficacy of the drugs.

Septran, a newly introduced broad spectrum sulfa drug has been put to trial in a group of eight experimentally infected chicks at the dose rate of 0.04 per cent in drinking water. It is interesting to note that in this group of chicks the diarrhoea was checked and other symptoms disappeared only within two days of treatment. The drug has been found to be

100 per cent efficacious as the chicks were fully recovered and no chick died during the course of treatment and thereafter. It is evident from percentage of recovery, that this can successfully be used for treating the Esch. coli infected chicks. The cost of drug is comparatively higher as compared to the other drugs used in the present experiments but recovery period is very short. However, it has not been substantiated by the other workers.

So far, only in-vitro test has been conducted and it has been reported to be 94.8 per cent effective to Esch. coli isolated from milk sample (Mishra, 1977). Similarly in the present study septran has been found to be 100 per cent effective in in-vitro test.

Butura and Sahleanu (1972) were able to protect the experimentally infected birds by adding chloramphenicol to the diet, from three days after infection, either 0.005 gm per head every day or 0.010 gm per head for three days. Similarly in the present study the second group of eight chicks was treated with 10 mg per chicks orally, after three days of treatment the diarrhoea was checked and all visible symptoms disappeared. However, treatment was continued for five days to establish the effective cure.

The effectiveness of neomycin at the dose rate of 30 mg per chick orally has also been assessed in 3rd group of experimentally infected chicks, where diarrhoea was checked

and other symptoms disappeared on 7th day after commencement of treatment. It was also noticed that one chick died during the course of treatment. In this case dose rate was higher as compared to other drugs of this experiment and recovery period was also more. Though the drug is effective neverthless it can not be considered much efficacious and drug of choice in relation to high cost, due to its higher dose rate along with longer period of recovery in treating the ailing chicks keeping in view loss in weight, and poor carcass quality which were the subsequent losses in Esch. coli infection.

Sojka and Carnaghan (1961) has reported furazolidone to be most useful in experimental trials and 0.04 per cent level of furazolidone for a period of a week and ten days has been recommended for treatment. In the present study the chicks of fourth group has been treated with furazolidone at the dose rate of 0.04 per cent in feed. This drug was found efficacious on the basis that the diarrhoea was checked after four days of treatment in four chicks whereas the symptoms were further controlled in rest four chicks on sixth day. The course of treatment was ten days. Thus the present finding is in agreement with the findings of the above authors, were the efficacy of furazolidone is same both on level of dose as well period of treatment.

Butura and Sahleanu (1972) have claimed the furazolidone to be 80 - 87 per cent effective in experimentally infected birds. The effectiveness of furazolidone in present

study can also be claimed to be 100 per cent which is evident on the basis that all the infected chicks recovered and symptoms disappeared after completion of treatments.

In the present study, the comparative efficacy of four drugs can be explained by analysing level of doses, course of treatment and period of recovery. In the present trials revealed that septran and chloromycetin have proved much effective as compared to furazolidone and neomycin. In the first two the dose level is low, course of treatment short, and recovery period is early and in the rest two the dose level is bit higher but course of treatment and recovery period is longer.

Thus, the above two drugs viz. (i) septran paediatric suspension (ii) chloromycetin capsule can be considered the drug of choice whereas rest two viz. (i) neomycin (ii) furazolidone are, though effective, but take longer course and recovery period is not early as compared to the medication by former drug.

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SUMMARY AND CONCLUSION

S U M M A R Y

1. Seventy two strains of Esch. coli were isolated from 200 samples (viz. lung, liver, spleen, heart, and kidney) collected from forty birds.

2. Esch. coli was isolated both from healthy and diseased birds. They were positive for indol and M.R. tests, negative for Voges-Proskauer reaction and did not utilize koser's citrate. All strains reduced nitrate into nitrite.

3. Only five strains out of 72 isolates produced hydrogen sulphide (H_2S). All isolates fermented lactose, glucose, mannitol, and maltose within 24 hours. 62 strains were able to ferment sucrose within 24 hours, while the rest failed to attack it.

4. 15 strains, randomly selected, were tested for their pathogenicity in albino (Italian) mice. Only seven strains were found pathogenic whereas eight were non-pathogenic.

5. The sensitivity of Esch. coli strains was tested against 10 antimicrobial agents viz. ampicillin, chloramphenicol, furadantoin, kanamycin, neomycin, gramoneg, streptomycin, septran, sulfuno, and furazolidone. Septran, chloramphenicol, neomycin, and furazolidone were found to be the most effective; whereas all the isolates were 100 per cent resistant to sulfuno.

BIBLIOGRAPHY

BIBLIOGRAPHY

Andreani, E., Agrimi, P., Cardini, G. and Dimitra, V.	1969	Study of 215 strains of Esch. coli isolated from various species of animals. Biochemical characteristic, haemolytic activity and sensitivity to antibiotics. Annali Fac. Med. vet. Pisa, 21: 308-328. (Vet. Bull., 40: 166).
Andrey, W.B., Peterson, C.F. and Hagart, M.	1968	Experimental coli-bacillosis and the development of carriers in laying hens. Avian Dis., 12: 505-511.
Association of clinical Pathologists.	1965	Antibiotics sensitivity test trial. J.Clin.Path., 18: 1-5.
Barr, F. S. and Carman, P.E.	1957	South. East. Vet., 9: 11. (Cited by Sojka, 1965).
Betitskii, B.I. and Panikar, I.I.	1969	Pathogenicity of Esch. coli isolated from broilers with septicaemia. Veterinariya Moscow 1969 No. 12: 25-26. (Vet.Bull., 40: 760).
Blauel, G.B.	1973	Determination of the pathogenic properties of field strains of Esch. coli from poultry. Inaugural Dissertation, Ticrar-Ztliche Fakutat, Munchen (1973) pp. 58. (Vet.Bull., 44: 285).
Butura, I. and Cernea, J.	1969	Incidence and pathogenicity of Esch. coli serotypes on chicken farms. Incr. Inst.Cerc.Vet. Bioprep.Pasteur, 6(1967):115-125. (Vet.Bull., 40:688).

Butura, I. and Sahleanu, C.M.	1972	Research into the prophylaxis and treatment of Esch. coli infection in fowls. Lucrarile Inst. Cerc.Vet.Bioprep. Pasteur, (1972) 8: 223-232. (Vet. Bull., 42: 75).
Butura, I., Cernea, I. and Sahleanu, C.M.	1973	Epidemiological and experimental studies of Esch. coli infection of fowls. Incr. Inst. Cerc. Vet. Bioprep. Pasteur, (1973) 9: 155-166. (Vet.Bull., 43: 667).
Castle, A.R.S. and Elstab, J.	1971	Antibiotics sensitivity testing. A survey under taken in Sept. 1970 in-vitro. J.Clin.Path., 24: 775-778.
Cole, R.K. and Hutt, F.B.	1973	Normal ovulation in non-laying hens. Poult. Sci., 32: 481-492.
Cruickshank, R.	1965	Medical microbiology. 11th Ed. E.and S. Livingtone Ltd. Great Britain.
Edward, P.R. and Ewing, W.H.	1954	Studies on a coliform type isolated from organ of fowls. Cornell.Vet., 44: 50-53.
Edward, P.R. and Ewing, W.H.	1972	Identification of Enterobacteriaceae. 3rd edn., pp. 7-108, 337-358. Burges Publishing Company Minn.
Ericsson, H. and Sherris, J.C.	1971	Antibiotic testing report of an international collaboration study. Act.Path.Microbial.Scand. Set.B., Suppl. No. 217.
Farrag, H. and Oof, F.	1967	"Sensitivity of organisms isolated from cases of bovine and goat mastitis to various antibiotics". Ind. Vet.J.,44: 640-646.

Garrod, L.P. and Waterutn, P.N.	1971	A study of antibiotic sensitivity testing with proposals for simple uniform method. J.Clin.Patn., 24: 779-784.
Glantz, P.J.	1962	In-vitro sensitivity of Esch. coli to antibiotic and nitrofurans. Cornell Vet., 52: 552-562.
Glantz, P.J., Narotsky, S. and Bubash, G.	1962	Esch. coli serotypes isolated from salpingitis and chronic respiratory disease of poultry. Avian Dis., 6: 322-328.
Gordon, R.F.	1961	Broiler's, 20: 66. (Cited by Sojka, 1965).
Grosneva, G.A.	1971	Properties of Esch. coli strains isolated from birds with septicaemia. Trudy Vse-Soyuznogo Instituta Eksperimental'noi Veterinarii (1971) 39: 166-171. (Vet.Bull., 42: 758).
Gross, W.B.	1956	Escherichia coli as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of Turkeys. Poult.Sci.,35: 765-771.
Gross, W.B.	1957	Pathological changes of an Escherichia coli infection in chickens and turkeys. Amer.J. Vet. Res., 18: 724-730.
Gross, W.B.	1958	Symposium on chronic respiratory diseases of poultry. II. The role of Esch. coli in the cause of chronic respiratory disease and certain other respiratory diseases. Amer.J.Vet.Res., 19: 448-452.

Gross, W.B.	1961	A synovitis caused by strains of Esch. coli. Avian Dis.,5: 218.
Gross, W.B. and Siegel, P.B.	1959	Coliform peritonitis of chicks. Avian Dis., 3: 370-373.
Gupta, R.N.	1963	Studies on phagetyping of Escherichia coli strains from domestic animals and poultry. M.V.Sc.Thesis, Agra University.
Gupta, R.N. and Singh, C.M.	1969	Studied on Esch. coli from Egg-peritonitis in poultry in India. Indian J.Anim.Hlth., 8: 1-10.
Gurumurthi, V. and Panduranga Rao.	1962	Colibacillosis in brooder chicks. Ind.Vet.J., 39: 66-69.
Hamilton, C.M. and Conard, R.D.	1968	Extreme mortality in Hjarre's disease (coli-granuloma) in chickens. Jour.Amer.Vet.Med. Ass., 132: 84-85.
Heller, E.D. and Parek, M.	1969	Pathogenic Esch. coli strains prevalent in poultry flocks in Israel. Br. Vet. J., 124:509-583. (Vet.Bull., 39: 317).
Heller,E.D. and Smith, H. Williams.	1973	The incidence of antibiotic resistance and other characteristic amongst Esch. coli strains causing fatal infection in chickens. J. Hygiene, 71 No. 4: 771-78.
Hjarre and Wramby,G.	1945	Skand.Vet.Tidsskr., 35: 449. (Cited by Sojka, 1965).
Iyre,P.K.R., Srinivasan, U.V., and Joshi, T.P.	1965	Ind.Vet.J., 42: 237.

Kohler, H.	1951	Exp.Vet.Med., 75: 54. (Cited by Sinha, 1973).
Kulkarni, M.N.	1964	Studies on serology and phage-typing of Esch. coli from poultry. M.V.Sc. Thesis, Agra University.
Kulkarni, M.N., Gupta, R.N. and Singh, C.M.	1970	Studies of Esch. coli strain isolated from chronic respiratory disease of poultry in India. M.V.Sc. Thesis, Agra University.
Lovell, R.	1959	Coliform diseases. In diseases due to Bacteria Vol. I (Edtd. by Stableforth, A.W. and Galloway, I.A.) pp. 229-238. Butter Worths Scientific Publications, London.
Luna, LEE G.HT (Ascp)	1968	Mannual of histological strains methods of the Armed Forces Institute of Pathology. 3rd Edn. McGraw Hill Book Company, New York.
Malik, K.	1963	Med. Vet., 19: 511-514. (Cited by Tiwary, 1969).
Matvienko, B.A. and Rudenko, T.P.	1964	Veterinariya Mascow, 41: 22. (Cited by Sinha, 1973).
McCarty, R.T.	1953	J.Amer.Vet.Med.Ass., 122: 386. (Cited by Daykin, 1966).
Merchant, T.A. and Packer, R.A.	1967	Veterinary bacteriology and virology, 7th edn., pp. 273-285. The Iowa State University Press, Iowa, U.S.A.
Micevski,C. and Naletoski.	1972	Coligranulomatosis of fowls. Makedonski Veterinaren Pregled (1972) 1 No. 1/2: 57-65. (Vet.Bull., 44: 638).

Mishra, S.K.	1977	Studies on incidence of coliform bacteria at raw and pasteurised market milk of Patna with special reference to Esch. coli. M.Sc. (Vet.) Thesis, Rajendra Agricultural University.
Nagi, M.S. and Khanna, P.N.	1967	A cholera like disease in chick due to haemolytic Esch. coli. Ind. Vet. J., 44: 629- 633.
Naidu, P.M.N.	1959	Poultry keeping in India. I.C.A.R., New Delhi.
Narula, A.S.	1966	M.V.Sc. Thesis, Magadh University.
Nayak, B.C., Mishra, B. and Biswal, G.	1967	Avian coligranulomatosis - Hjarre's disease. Ind. Vet. J., 44: 355-389.
Pathak, R.C., Singh, C.M. and Tangari, R.P.	1960	Chick mortality and contamination of yolk's by means of enterobacteriaceae. Brit. Vet. J., 2: 81-84.
Pradhan, H.K., Dutta, N.K., Panda, S.N. and Nayak, B.C.	1973	Studies on the pathology of the female reproductive tract of domestic fowls. IV. Bacteriological studies with reference to Escherichia coli organisms from cases of Egg-peritonitis. Indian J.Poult. Sci., 8: 218-223.
Quaresni, S.H.	1957	The incidence of infectious enteritis (coli-bacillosis) in chickens in West Pakistan. Agric. Pakist., 8: 48-52. (Vet.Bull., 28: 287).
Sarkar, R.N.	1966	Personal communication. (Cited by Narula, 1966).

Savov, D.	1963	Studies on colisepticaemia in chicks. Izv. Vet.Inst. Zaroz. Parasit. Bolesti. Sofia, 9: 97-111. (Vet.Bull., 34: 385).
Savov, D.	1965	Studies on strains of Escherichia coli isolated from fowls. Vet. Med. Nauki. Sof., 2: 825-832. (Vet.Bull., 36: 402).
Savov, D.	1966	Pathogenic Esch. coli carrier in fowl. Vet.Med. Nauki.Sof., 3: 519-525. (Vet.Bull., 37: 343).
Savov, D. and Pavlov, D.	1965	Experimental Esch. coli infection in chicks. Vet.Med.Nauki.Sof., 2: 695-700. (Vet.Bull.,36:276).
Sharma, D.N. and Singh, C.M.	1968	Studies on pathology of female genital tract of poultry with special reference to egg-peritonitis, incidence, Patho-anatomy and experimental study. Indian J. Vet. Sc. Anim. Husb., 38: 737-746.
Sharma,V.D.,Sethi, M.S., Prasad,A.K. and Yadav, M.P.	1977	Uncommon Escherichia coli (Migula) Lehmann and Neumann 1896, serogroups associated with disease outbreaks in poultry. Patnagar J.Res. Vol.2 No. 1, 77-79.
Sinha, S.N.P.	1973	Detailed studies of certain serotypes of Esch. coli especially associated with colibacillosis in poultry. M.Sc.(Vet) Thesis, Rajendra Agricultural University.
Sinha, V.K.	1975	Studies on the incidence,clinical symptoms clinical pathological changes, histopathology and therapy of clinical and experimental urinary tract infection in dogs. M.Sc.(Vet.) Thesis,Rajendra Agricultural University.

Snedecor, George W. and Cochran, William G.	1967	Statistical Methods. 6th edn. Oxford & IBH Publishing Co.
Sojka, W.J.	1965	Escherichia coli in domestic animals and poultry. Commonwealth Agricultural Bureaux Publication 1965.
Sojka, W.J. and Carnaghan, R.B.A.	1961	Escherichia coli infection in poultry. Res. Vet. Sci., 2: 340-352.
Starr, M.P. and Reynolds, D.M.	1951	Amer.Jour.Pub.Hlth., 41: 1375. (Cited by Sojka, 1965).
Stipkovits, L. and Solyom, F.	1968	Esch. coli induced disease in day old chicks. Magy. Allatorv. Lap. 23: 605-608. (Vet. Bull., 39: 467).
Takahsi, K.	1966	Studies on Esch. coli isolated from diseased chickens with special reference to "O" group of isolates. Jap.J.Vet.Res., 14: 134. (Vet.Bull., 37: 524).
Truscolt, R.B., Lopez Alvarez, J. and Pettit, I.R.	1974	Studies on Esch. coli infection in chickens. Canadian Journal of Comparative Medicine (1974) 38 No. 2: 160-167.
Verma, T.N.	1973	"Bacteriological study of milk from clinical and subclinical cases of bovine mastitis and its Public Health significance". M.Sc.(Vet.) Thesis, Rajendra Agricultural University.
Wasserman, B, Yates, V.J. and Fry, D.E.	1954	On so-called airsac infection. Poultry Sci., 33: 622.

Wilson, G.S. and Miles, A.A.	1961	Topley and Wilson's principles of Bacteriology and Immunity. 4th edn. (Vol.I), pp. 751-783. Edward Arnold (Publishers) Ltd., London.
Wolf, P.L., Russel, B. and Shimoda, A.	1975	"Practical clinical Microbiology and Mycology" Techniques and interpretations. John Wiley & Sons, New York.
Wramby, G.	1948	Investigation into antigenic structure of B. coli isolated from calves with special reference to colisepticaemia (white scours). Acta.Path.Microbial. Scand.Supp., 96. (Cited by Sojka, 1965).
Yadav, M.P. and Malick, B.S.	1972	Isolation and serotyping of Esch. coli from chicken and eggs in India. Ind. Vet. J., 48: 879-884.

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