STUDIES ON EXPERIMENTAL INFECTION OF *Escherichia coli* IN ADULT QUAILS *(Coturnix coturnix japonica)*

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Master of Veterinary Science
in
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BY

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Dedicated

to

My beloved Parents

Approval Sheet

Approval of Examiners for the Award of the Degree of Master of Science (Veterinary).

We, the undersigned, having been satisfied with the performance of Miss Sanchita Das, in the viva voce Examination, conducted to-day, the 1991, recommend that the thesis be accepted for the award of the Degree.

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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 experimental infection of Escherichia coli in adult quails (Coturnix coturnix Japonica) submitted by Miss Sanchita Das, in partial fulfilment of the requirements for the Degree of Master of Science in "Veterinary Pathology" of 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 :

The 2nd Sept. 1991

Signature of the Advisor

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Chapter : I

INTRODUCTION

Introduction

It is needless to mention that poultry industry has gained a tremendous momentum during the past few decades in India. Poultry industry covers a huge variety of birds like pea fowl, chicken, guinea fowl, turkey, pigeon, quails, ducks, and geese, etc.

Japanese grey quail (Coturnix coturnix Japonica) was first domesticated in the beginning of nineteenth century by Japanese fanciers.

In India, the Japanese quails were first imported from California (U.S.A.) and maintained at the Indian Veterinary Research Institute, Izatnagar.

Japanese quail (Coturnix coturnix Japonica) is a new avian species which has created much interest of poultry growers on account of its high quality of meat and egg production, less weight and feed consumption rate. The great advantage of quail rearing lies in the fact that it matures early, rate of growth and egg production is very high. The most stricking feature of quail rearing is the high resistance to common intercurrent poultry diseases. There exists other advantages of quail rearing namely, they can be accommodation in relatively smaller spaces, high rate of egg production, small incubation period and no necessity of frequent vaccination. The quails can produce

three to four generations per year and consequently it has been used as a laboratory model for various experiments in the field of medical nutrition and Biochemical research (Padgett and Ivey, 1959).

They have got other advantages of surviving the adverse climatic conditions, especially, the hot and humid climate of the tropics. Therefore, the Japanese quail has opened a new vist in the field of poultry research and egg and meat production.

Various Agricultural Universities and commercial farms are now enthusiastic for quail breeding and management. The quail meat is superior to poultry meat on the grounds that it is having low calories with high protein, rich in vitamins, aminoacids, unsaturated fatty acid, and phospholipids, etc. which are very useful for human health and development.

It has already been stated earlier that quails are adequately resistant to very many maladies to which the poultry are easily susceptible. It is an established fact that quails are refractory to Ranikhet disease virus, resistant to Ascardia galli infection and coccidiosis ~~in chicken~~.

On the contrary, they are suceptible to a few common poultry diseases.

During the recent past, poultry has disturbed the eco-system of the birds which compell them under strees leading to certain disease problems (Panisup and Verma, 1986).

Poultry mortality can be caused by multiple etiological factors but colibacillosis in chicks is a concurrent disease problem causing heavy loss and mortality in poultry industry. Escherichia coli infection causes multiple disease conditions in poultry which includes colibacillosis, coli-septicaemia, coligranuloma, peritonitis, pericarditis salpingitis, omphalitis, synovitis, and air-sac disease, etc. in poultry. Directly or indirectly E. coli is responsible for heavy economic loss in poultry industry (Savov - 1973).

In conjunction with other organisms E. coli is responsible for causing so many pathological conditions in poultry.

E. coli is a typical coliform organism usually found in the intestinal tract and in the fecal samples of animals and birds.

Roughly about 100 serotypes of E. coli have been identified. E. coli infection flares up in chicks when the bird is under stress of certain intercurrent diseases, like fowl pox, R.D. vaccination, etc. and in the deficiency of Vit. A, E and in general debility or poor quality of feed ingredients (Shukla et al., 1987).

Considering the multiple pathogenic conditions caused by E. coli in chicken it has been decided to study the gross and histopathological changes in quails following experimental inoculation of E. coli. The available literature is very scanty on the topics. In view of the great pathogenic role of E. coli in chicks, the present study was undertaken with the experimental inoculation of E. coli through different routes in quails.

E. coli infection in quails can be diagnosed from clinical symptoms, post-mortem lesions of affected birds. Confirmatory diagnosis can be made by reisolation of E. coli from heart blood of experimentally infected quails. 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. Hence, sensitivity against the pathogenic isolates is essential for therapeutic diagnosis and control measures in an outbreak.

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

1) To study the pathological changes in quails following experimental inoculation of E. coli through different routes.

2) To isolate the organisms from the experimental birds.
3) To study the pathogenicity of the isolates in laboratory animals like mice.
4) To conduct antibiotic sensitivity test.

Chapter: II

REVIEW OF LITERATURE

Review of Literature

Though elaborate and comprehensive informations are available in literature on the incidence and mortality of E. coli infections in chickens, the available literature is very scanty on ~~the incidence~~ of E. coli infections in quails. However, the following information would throw some light on the incidence of E. coli infection in quails.

A. Incidence of E. coli infection in quails

Mazurkierueiez, M., et al. (1968) reported E. coli infection in Japanese quail in a experimental farm. The salient symptoms were incoordination of movements, tremors, dullness, loss of appetite and falling over with head thrown back in response to stimuli.

Post-mortem examination revealed severe emaciation, inflammation and swelling of the ear. Granulomas were found in meninges and in outer covering of the hind brain and in the middle and inner ear. Necrosis of caecal mucosa was also noticed.

Srinivasan et al. (1979) reported that ~~in~~ quails were suceptible to serotypes of 01, 010 and 02 ~~E. coli~~. Gross lesions indicated ~~that the birds~~ died of colibacillosis,

revealed pericarditis and perihepatitis in quails following natural and experimental inoculation.

Silva et al. (1989) studied the occurance of coli-granulomatosis in quails and indicated 85% drop in egg production and 15% mortality. The lesions were located on the mesentery, intestine, gizzard, heart, oviducts, ovaries and liver. ~~Bacteriological isolation and pathogenosity assay indicated~~ E. coli. The isolated ~~organism was~~ inoculated I/m or ~~I/v~~into two hens ~~which after four weeks of intravenous inoculation,~~ develop anorexia, loss of weight, difficulty in standing, facial swelling and conjunctivitis with a mucopurulent occular discharge. On the contrary, the hen inoculated with intramuscular route appear healthy.

It has already been mentioned earlier that E. coli infection causes multiple disease conditions in poultry, namely colibacillosis, colisepticaemia, peritonitis, synovitis, air-sac disease in poultry and many other pathological conditions. In the following paragraphs, an attempt has been taken to review the available literatures on the pathological behaviour of experimental E. coli infection in poultry including turkey, ducks, pegions and in mice through different routes.

B. Experimental infection of colibacillosis in poultry.

Bekajie and Prost (1963) reported that E. coli serotypes 071 and 08 killed mice when injected intravenously with 01 ml of a 24 hours culture but 1 ml doses given orally to a month old chicks proved harmless.

Savov (1963) suggested 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. The mortality was higher in conjunction with the coccidial infection.

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

Ramkhmanian 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 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.

Golubrichii et al. (1986) conducted experiments on 2 month old chicks with simultaneous dual infections 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 higher dose of virus. Dual infection of chicks lead to respiratory disease.

Larsen et al. (1986) reported experimentally that E. coli (1.02×10^8) when injected intravenously or into the air-sac 7 days after virulent haemorrhagic virus inoculation to chicks, 80% died out of 40, 8 - 15 days after of virus inoculation whereas 2.5% only with E. coli inoculation.

C. Clinical manifestations and gross lesions in young and older chicks.

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

Stipkovite and Solyom (1968) stated that subcutaneous injection of 01 ml undiluted or tenfold diluted broth

cultures of two strains of E. coli from chicken killed all 14 (3 day old) chicks.

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

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

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.

Sharma and Joshi (1983) studied that six strains of E. coli isolated from natural cases of ~~o~~oporitis, 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, misshapened, congested ova and inflammed oviduct.

Morley and Thomson (1984) reported the occurence of swollen head syndrom in broiler chickens of 4 - 6 weeks of age in S. Africa supposed to be due to mixed infection of corona virus and E. coli.

Dunnington and Siegel (1985) reported the changes in body weight, surface and cloacal temperature, lesions on heart and airsac and mortality in 3 and 30 day-old chicks due to colibacillosis.

Ling et al. (1987) studied the comparative studies on the pathology of septicaemic colibacillosis in 30 naturally infected and 24 experimentally infected birds. Macroscopic lesions consisted mainly of petechial haemorrhages in the internal parenchymatous organs and a fibrinous serositis and granulomatoid changes in the serous covering of the pericardium, air-sacs and liver capsule.

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 intraperitoneal 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 of 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 airsac, accumulation of fluid in the pericardial sac, while 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.

D. Histopathological changes in the internal organs in experimental young and older chicks.

Adopting 10 organisms of serotype 01, Siccardi (1966) recorded 100% mortality of day-old chicks through yolk sac inoculation. Omphalitis was common, while few chicks suffered with peritonitis and infected yolks.

Histopathological changes indicated an outer connective tissue layer followed by a layer of inflammatory cells and masses of bacteria with few plasma cells.

E. Pathogenicity test in mice.

Behajle, R. et al. (1963) reported pathogenicity test of E. coli serotypes 071 and 08 on mice. By injecting 0.1 ml of 24 hrs. cultures intravenously, the mice were killed.

Gupta et al. (1969b) studied pathogenicity of E. coli strains in white mice of about 1.5 - 2.5 months of age. 40 strains of E. coli were found to be pathogenic for mice on experimental inoculation through intraperitoneal routes.

Ellis et al. (1974) inoculated E. coli in suckling mice for detection of eteropathogenicity and demonstrated pathogenic lesions.

Gupta et al. (1975) performed pathogenicity test of E. coli serogroup in adult white mice through intraperitoneal route. Four serogroups (05, 09, 011, 015) were highly pathogenic and one serotype (03) indicated doubtful pathogenicity while the rest 15 serogroups were found to be non-pathogenic.

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

Srivastava et al. (1979) stated isolation of E. coli serogroups from 13 calves died of enteric colibacillosis. They performed 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 and they observed that 23 were pathogenic to chicks and 21 to mice.

E. coli strains of group 025, 049, 060, 078, 090 and 0160 were equally pathogenic to mice and chicks inducing 100% mortality. The microscopic lesion in mice were congestion of liver and spleen and haemorrhages in the spleen.

F. Antibiotic sensitivity test.

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

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

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

Butura et al. (1972) isolated 198 strains of E. coli from fowls with septicemia. Drug sensitivity test indicated that they were highly sensitive to chloramphenicol, polymyxin - B, neomycin, furazolidon and tetracycline, while they are resistant to erythromycin and streptomycin.

Kapoor et al. (1978) reported that out of 220 E. coli isolates from poultry, a high percentage of strains were resistant to ampicillin, chlorotetracyclin and streptomycin. Fewer strains were resistant to nitrofurazolidon polymixin - B and chloramphenicol.

Sahota et al. (1978) reported that most of the E. coli strains isolated from cases of Oophoritis and

salpingitis were sensitive to streptomycin and nitrofurantoin but resistant to tetracyclin.

Srinivasan et al. (1979) reported that out of 126 E. coli isolate from colibacillosis in poultry, 26 were sensitive to ampicillin, chloramphenicol, kanamycin, and furadentin while most of them were resistant to erythromycin, polymyxin and tetracycline.

Farias et al. (1979) isolated 166 E. coli serotypes from poultry of which 95% were positive to chloramphenicol and gentamycin. 91% to kanamycin and tetracycline, 82% to ampicillin and 68% to streptomycin.

Gyurov et al. (1981) recorded 143 E. coli strain isolated from poultry. All of them were resistant to coxacillin, oleondomycin and tylosin.

Kumar et al. (1981) reported drug resistance of 135 E. coli strains isolated from chicks and hens. The isolates were resistant to bacitracin, tetracycline and sulphonamide.

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) suggested 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.

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, sulphathiazol and tetracycline.

One hundred ninety five (195) E. coli serotypes were isolated by Sinha et al. (1985) 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%).

Manna 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 µg/10 ml.

Chapter: III

MATERIALS AND METHODS

Materials and Methods

A. Source of material :

(a) Escherichia coli isolated by Mukhapadhayay (1989) from the Department of Clinical Medicine and Public Health was obtained for the present study. The serotypes (01 and 02) were supplied for the present investigation.

B. Methods

c) Experimental infection of E. coli in quails.

For production of experimental infection with most pathogenic strain of E. coli sixty adult quails were taken and inoculation was done according to the method described by Savov (1963) with modification.

For producing experimental infection two representative pathogenic strain (01 and 02) were inoculated into quails. Each of the strains was inoculated by different routes (I/P and oral) into groups of quails containing 10 quails in each group.

(b) Source of quails :

One hundred healthy day-old quail chicks were obtained from the Department of Animal Genetics and Breeding

for the experimental study. The entire experiment was continued for a period of six months ranging from January, 1991 to June, 1991.

Observation of quail chicks before infection :

The quail chicks were kept under constant observation for ... days before the infection. During this period the quail chicks were not treated or supplimented with any antimicrobial agents. The quail chicks were routinely examined 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. The quail chicks were maintained on specially prepared good quality broiler chick mash and ad-lib drinking water free from infection without any antimicrobial agent.

Grouping of quails : One hundred quails were procured but only 60 were put to experimental trial of period of 40

The experimental quails were randomly divided into 6 groups containing 10 quails in each group as follows :-

Group 1. Non infected control for I/P route with sterile broth culture.

Group 2. Non infected control for oral route with sterile broth culture.

Group 3. Infected with E. coli I/P route (01 strain).

Group 4. Infected with E. coli oral route (01 strain).

Group 5. Infected with E. coli I/P route (02 strain)

Group 6. Infected with E. coli oral route (02 strain)

The quails were reared with battery brooders with special broiler chick mash and ad-lib drinking water 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 stants 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 (Gr 3) of quails was infected intraperitoneally (I/P) with 2 ml and other group (Gr 4) orally with 2.5 ml of the inoculum containing about 10^9 organisms per ml. Two groups (Gr 1&2) of quails were kept as control.

5 and 6 week, respectively.

One of these groups was injected intraperitoneally (I/P) with 2 ml. and the other group fed orally with 2.5 ml. of sterile nutrient broth. The inoculation was done when the quails were 6 weeks of age.

Post-inoculation observation, and Necropsy and Isolation

Inoculated quails 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 manifestation, if any, were also recorded. Quails 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, liver intestines and gizzard were collected and processed in the laboratory. The isolation of E. coli was done from heart blood, liver, intestinal contents, pericardial fluid and peritoneal fluid by inoculating on to MacConkey's Lactose Agar plates following routine bacteriological methods. The media used were:

C. Histopathological changes in quails.

The adult quails infected with virulent E. coli serotypes (01 and 02) by intraperitoneal and oral route were immediately necropsied after death. The gross pathological changes in the internal organs were noted and the tissues of

Repetation of above para.

organs showing gross pathological lesions/changes were collected in small pieces in 10% formalin.

After fixation, the tissues were cut into small pieces and processed according tc routine procedure described by Luna (1968). The paraffin embedded tissues were cut into sections of 4 - 5 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.

D.(a) Collection of samples for reisolation of E. coli from dead quails.

Already mentioned at page → Rejected

Bacteriological samples were collected from heart blood, pericardial fluid, intestinal contents, liver, peritoneal fluid of experimental infected quails by E. coli were collected aseptically with the help of sterile pasteur pipettes. Clinical samples from liver 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.

~~(b) 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 prepared on MLA by streak plate method as described by Collins and Lyne (1976). The MLA plates were incubated at 37°C for 24 hours aerobically and the suspected pinkish colonies were subcultured on E.M.B. plates which 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. Viability was maintained by subculturing the organism at every six weeks interval in NA stants.

(c) Characterisation and identification of the organisms :

Characterisation and identification of the organisms isolated 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 performed 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~~ characteristics of ~~re~~isolated organisms ~~(Escherichia coli)~~

The biochemical tests were performed ~~for the identification~~ of Escherichia coli organisms 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, salicin, dulcitol, maltose, inositol and adonitol.

ii) Test for H_2S production.

iii) Methyl Red (MR) and Voges Proskauer (VP) tests.

iv) Citrate utilisation test.

v) Indol test.

vi) Test for urease production.

E. Pathogenicity Tests of E. coli.

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

Source of mice :

From each serogroup, one reisolated E. coli was used 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. Ghosh, Laboratory animal supplier, of 6/C, Motilal Basak Garden Lane, Calcutta-54, were maintained at the laboratory for a period of one month before inoculation. During this period, the animals were neither treated nor supplimented with any antibiotic or sulpha drug.

Preparation of the inoculation :

The reisolated E. coli were incubated at 37°C for 24 hours. A single colony from this group was inoculated into 5 ml. of nutrient broth from nutrient 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 containing about 800 million organisms in 0.5 ml suspension.

Inoculation of mice :

Swiss albino mice were devided into two groups, each group containing 6 animals. 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 under 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. Dead Mice died due to probable infection were immediately necropsied under strict aseptic

condition and the gross pathological changes in the internal organs were recorded. 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.

No. of dead mice.	Hours after inoculation	Pathogenicity.
3 - 4	18	+++
3 - 4	18	+++
2 - 3	19-30	++
Nil	96	non-pathogen

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).

F. 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 :

Sl.No.	Antimicrobial agents (abbreviations used)	Concentration per disc
1.	Ampicillin (A)	10 meg
2.	Carbenicillin (CA)	100 meg
3.	Cephaloridine (CE)	30 meg
4.	Chloramphenicol (CH)	30 meg
5.	Contrimoxazole (CT)	25 meg
6.	Gentamycin (G)	10 meg
7.	Amikasim (AN)	10 meg
8.	Sulphadiazine (SN)	150 meg
9.	Cepotaxime (CF)	30 meg
10.	Neomycin (N)	30 meg
11.	Nalidixic Acid (NA)	30 meg
12.	Furazolidone (FX)	300 meg

The disces were obtained from M/s. DYNAMICRO Thame, Bombay, India.

Test procedure :

For the test, E. coli colony was picked up from E.M.B. 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 sterilised 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 source 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 innibition around the disc was measured to the nearest millimeter (mm) which included 6 mm diameter of the disc. The results were interpreted by using the "Zone size interpretatine 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.

Chapter: IV

RESULTS AND DISCUSSION

Results and Discussion

A. The results of the experimental production of Escherichia coli (serotype 01 and 02) infection in adult quails have been presented in Table I. Both the serotypes were highly pathogenic to adult quails. Serotypes 02 produced 100% mortality in quails within 7 days by both the routes of administration whereas serotype 01 produced 100% mortality in intraperitoneal (I/P) routes and 30% mortality in oral route. Maximum mortality was observed in quails which were inoculated intraperitoneally within the first 3 days in both the serotypes. However, none of the quail from control groups exhibited any pathological change or mortality when inoculated (I/P) with sterile broth cultures a loopful of 24 hr culture and orally routes.

Stipkovists and Solym (1968), Phukan (1988) observed that most of the E. coli strains were highly pathogenic to young chicks while Srinivasan et al. (1979) recorded E. coli strains of group (01, 02) as highly pathogenic to quails as producing 100% mortality. The results obtained in the present study regarding the pathogenicity of E. coli serotype 01 and 02 were supported the findings of Siccardi (1966).

Table : 1. Result of experimental infection of E. coli in adult quails

Sl. No.	E.coli sero-types.	Route of ino-cula-tion	Dose ~~in~~ (ml)	No.of quails inocu-lated	No.of quails died ~~with~~ at days							Total No.of quails died	Percen-tage of mortali-ty (%)	Control group		
					1	2	3	4	5	6	7			No. of quails used.	No.of quail died.	Percentage of mortali-ty (%)
1	01	I/P	2 ml	10	2	3	2	1	2	-	-	10	100	10	0	0
	01	Oral	2.5 ml	10	-	-	-	2	4	2	-	8	80	10	0	0
2	02	I/P	2 ml	~~01~~ 10	7	2	1	-	-	-	-	10	100	10	0	0
	02	Oral	2.5ml	10	-	-	2	1	5	2	-	10	100	10	0	0

(I/P : Intraperitoneal route)

B. Result of reisolation of E. coli from experimentally infected adult quails by E. coli and the pathological conditions of dead quails.

Representative samples were collected from heart blood, pericardial fluid, intestinal contents, liver and other organs from the experimentally infected adult quails by E. coli at different time intervals immediately after the death of the quails. The hours and days of such death varied from 1 day to 7 days. The isolation, characterisation and identification of E. coli organisms were made as per methods suggested by Edward and Ewing (1972) and Cruickshank et al. (1975). The result of such reisolation of E. coli has been presented in Table - II. The table - II reveals the highest incidence of Enteritis in the intestinal contents in 35 samples. Necrosis and congestion with white streaks/foci on liver were recorded in a number of 15 cases while enlarged and congested heart observed in 30 cases only. The sporadic incidence of congestion of gizzard was found only in one case, while pericarditis and peritonitis were noted in two cases in each category. Congested and enlarged kidneys were found in two cases.

Table : II ~~Re~~Isolation of E. coli from experimentally infected adult quails by E. coli and the pathological conditions of dead quails.

Organs involved

Sl. No.	Samples collected from liver, heart, intestine, etc.	Pathological ~~conditions~~ observed.	No. of quails (%) ~~samples examined.~~
1.	Intestinal contents	Enteritis	35 ( )
2.	Heart blood.	Enlarged and congested heart.	30 ( )
3.	Liver.	Necrosis and congestion with white streaks/foci.	15 ( )
4.	Pericardial fluid.	Pericarditis, cloudy appearance of pericardial sac with accumulation of fluid inside the pericardial sac.	2 ( )
5.	Peritoneal fluid.	Fluid accumulated inside the peritoneal cavity.	2 ( )
6.	Gizzard.	Congestion of gizzard.	1 ( )
7.	Kidney.	Congested and enlarged.	2 ( )
	Total :		87

In how many cases isolation was attempted & in how many the isolation gave +ive result should have been included in this table.

= Only 40 quails were used for expt. other how 87 has come in total.

C. Reisolation of E. coli from different sites of inoculated quails.

The results of isolation studies have been summarised in table - III

Results of the Table - III obtained under the present investigation indicate that the number of dead quails showing positive reisolation of E. coli (01 strain) through intraperitoneal route from heart blood, liver, intestinal contents, pericardial fluid and peritoneal fluids were 9, 4, 6, 4, 2 respectively while through oral route were 5, 5, 7, 2, 3 respectively.

The number of dead quails showing positive reisolation of E. coli (02 strain) through intraperitoneal route from heart blood, liver, intestinal contents, pericardial fluid and peritoneal fluids were 9, 3, 2, 2, 2 respectively while the oral route recorded such positive reisolation 6, 5, 9, 3, 3 respectively.

It is, ~~therefore~~ evident from the above table that in I/P group (01 & 02) highest isolation was recorded from heart blood the number of positive reisolation of E. coli (01) strains ~~from heart blood of dead quails~~ were highest (9 only) through ~~intraperitoneal route.~~ The number of such positive ~~re~~isolation of E. coli (02 strain) were highest from the heart blood and in oral route ~~of dead quails through intra-peritoneal route.~~ ~~It is also interesting to note that the highest number of such positive~~ (01 & 02) the highest isolation was noted from reisolation cases of E. coli (02 strain) were in intestinal contents ~~contents through oral route~~.

Clinical manifestation and gross lesions in young chicks.

Specific clinical manifestation of E. coli infection could not be appreciated. Though the quails before death hurdled together, dropped, showed incoordination of movements, tremors, dullness, loss of appetite and backward twisting of head in response to stimuli. In less severe cases septicemia subsided and the animal recovers but the organisms would have localised causing arthritis with swelling and pain of the joints (Fig 1). Inoculated quails which died during the experimental observation period were immediately necropsied and the post-mortem examinations were conducted. Gross lesions observed in the internal organs were congestion of the heart, liver, gizzard, kidneys and intestines. Pericarditis and perihepatitis were also found (Figs 2 to 7). The inoculated E. coli strains of organisms were reisolated from the heart, liver, intestinal contents, pericardial and peritoneal fluid of dead quails (Table - III).

Similar findings were also described by Muzurkierueiez, M., et al. (1958), Silva et al. (1989) Srinivasan et al. (1979) in quails and Rakhmanian and Shubin (1978), Srinivasan et al. (1979) in young chicks due to E. coli strains. On the other hand, Sharma and Yoshi (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 swollen dark red ovaries, misshapen and congested ova and inflammed oviducts.

Table - III. Reisolation of *E. coli* from different sites of inoculated quails.

Sl. No.	Isolate ~~No.~~ used	Route of inoculation.	Age of quails in weeks.	No. of quails died.	Number of dead quails showing +ve reisolation				
					Heart blood	Liver	Intestinal contents.	Pericardial fluid	Peritoneal fluid.
1.	01	I/P	6	10	9	4	6	4	2
	01	Oral	6	6	5	5	7	2	3
2.	02	I/P	6	9	9	3	2	2	2
	02	Oral	6	8	6	5	9	3	3

Fig. 1 ✓

The adult quails, experimentally inoculated with E. coli (01/ 02) showing symptoms of incoordination of movements, tremors, dullness, loss of appetite, hurdled together, dropped and died.

Histopathology :

Histopathological changes in the internal organs in experimental adult quails with E. coli 01 and 02 serotypes :-

A. E. Coli serotype 01 (Oral route)

i) Intestine

Macroscopic changes :

Highly inflammed and severely congested.

Why the Fig. Nos have not been put serially?

Microscopic changes :

Section of intestine showed the following changes :-

Extensive acute haemorrhagic enteritis (Fig. 8). 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 hyperactivity of the submucosal glands. The serosal vessels were also congested. The lamina propria showed diffuse infiltration with mononuclear cells. Proliferation of the lymphocytic cells in the submucosa. Mucous producing cells are present in the lining epithelial cells of the villi (Fig. 15).

Intestine

The organism is a common inhabitant of the alimentary canal of adults. So long as it is confind

to the intestine it lives as a saprophyte, but if the integrity of the alimentary mucous membrane is impaired in any way it may invade the tissues. Proliferation of certain strains of E. coli in the intestines produce a potent endotoxin, which causes hypotension, vacular collapse and hypothermia.

ii) Heart :

Macroscopic changes :

Heart was congested with ~~less~~ reduced pericardial fluid.

Microscopic changes :

The intermyseal vessels were markedly congested. There were accumulation of the r.b.c. and haemorrhages between the myocardial fibres. In some places there r.b.c. become coagulated (Fig. 10).

This changes were seen ~~more towards the~~ mostly in sub epicardial regions.

iii) Liver :

Macroscopic changes :

The liver was congested with white streaks on the partial surface of the organ.

Microscopic changes :

Congestion were seen in the interhepatic cords and central vein. Extenssive fatty changes and necrosis which were seen more intense towards the central vein. Aggregation of the lymphocytes were showed in the liver parenchyma which had taken the appearance of a lymphatic nodules. Degeneration and thickening of the blood vessels were seen in some places. Liver parenchyma was congested and hepatic cells were swollen due to vacular degeneration (Fig.12,16).

iv) Kidney :

Macroscopic changes :

Kidneys were swelled, congested and inflammed.

Microscopic changes :

Severe haemorrhagic changes were seen throughout the section. Most of the tubules were distorted. The lining epithelial cells of the tubules showed severe degeneration and necrosis. Tubular epithelium were hypertrophied and become columnar in shape and sometimes papillary projection like in appearance in the tubules (Fig.12). Degeneration of the tubules were noticed both in the cortex and medulla.

Glomeruli become congested and hyperimic. The r.b.c. were accumulated more in the interstitial spaces. Some glomeruli were atrophied and some glomeruli were hypertrophied. Congestion in the big blood vessels were present (Fig.21,22).

B. E. coli sero-type 01 (Intraperitoneal route)

i) Intestines

Macroscopic changes :

Intestine was highly congested with haemorrhagic patches.

Microscopic changes :

Lesions were same as seen in oral route (Fig.16).

It is a section of heart & not intestine

ii) Liver :

Macroscopic changes :

Liver was congested with white streaks.

Microscopic changes :

Lesions were same as seen in oral route (Fig.17).

iii) Heart :

Macroscopic changes :

Heart was congested with less amount of pericardial fluid.

Microscopic changes:

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

iv) Kidneys :

Macroscopic changes :

Kidneys showed congestion and haemorrhagic patches.

Microscopic changes :

Microscopic lesions were same as seen in oral route.

C. 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. Proliferation of the lymphocytic cells in the submucosa. There serosal vessels were also markedly congested (Fig. 9, 13).

ii) Heart :

Macroscopic changes :

Pericardial muscles were congested with less amount of pericardial fluid.

Microscopic changes :

Section of the heart revealed moderately congested appearence (Fig. 19).

iii) Liver :

Macroscopic changes :

The liver showed congestion and white streakson the partial surface of the organ.

Microscopic changes :

The organ was very much congested. Extenssive fatty changes and necrosis were noticed (Fig. 25).

iv) Kidney :

Macroscopic changes :

Kidneys showed congestion and haemorrhagic patches.

Microscopic changes :

Microscopic lesions were same as seen in oral route.

D. E. coli serotype 02 (oral route).

i) Intestines :

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

ii) Liver.

iii) Heart.

iv) Kidney :

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

Phukan (1988) also recorded gross lesions in the internal organs of experimental chicks died of colibacillosis like congestion of liver, lung, heart, enteritis, accomulation 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 quails, the information were scarce in the literature.

When infection occurs, the organisms first multiply in the clot of blood and liquefy it. The clot disintegrates and emboli containing bacteria are thus carried by the portal veins or posterior vena cava to the liver and general circulation respectively. Ultimately septicemia is set up.

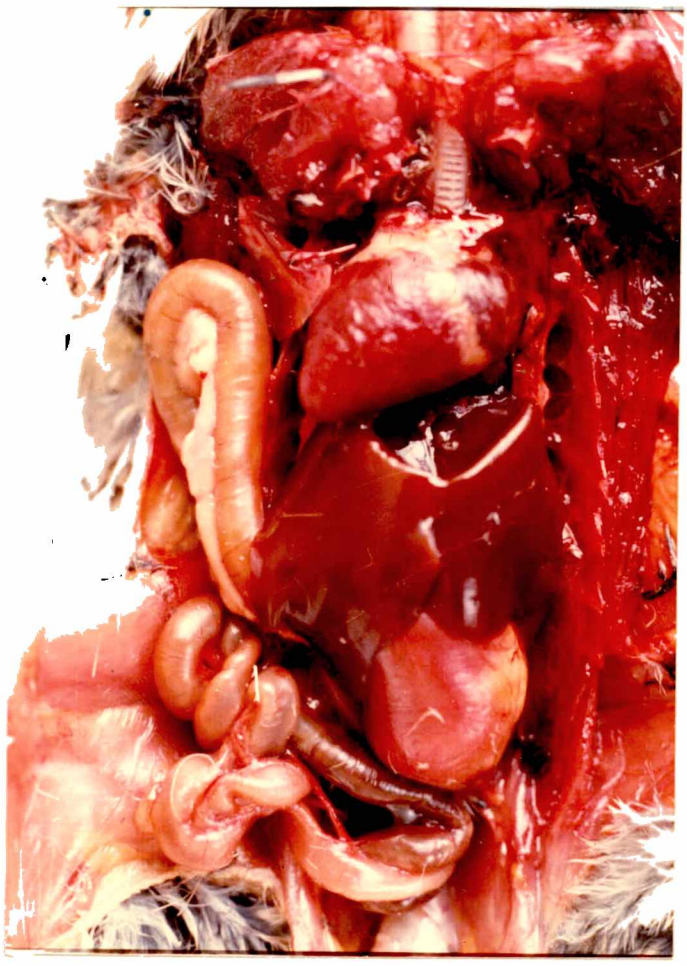

Fig. 2

Showing congestion of heart, intestines, pericarditis and perihepatitis in experimentally inoculated (oral) quails with 01 E. coli isolate.

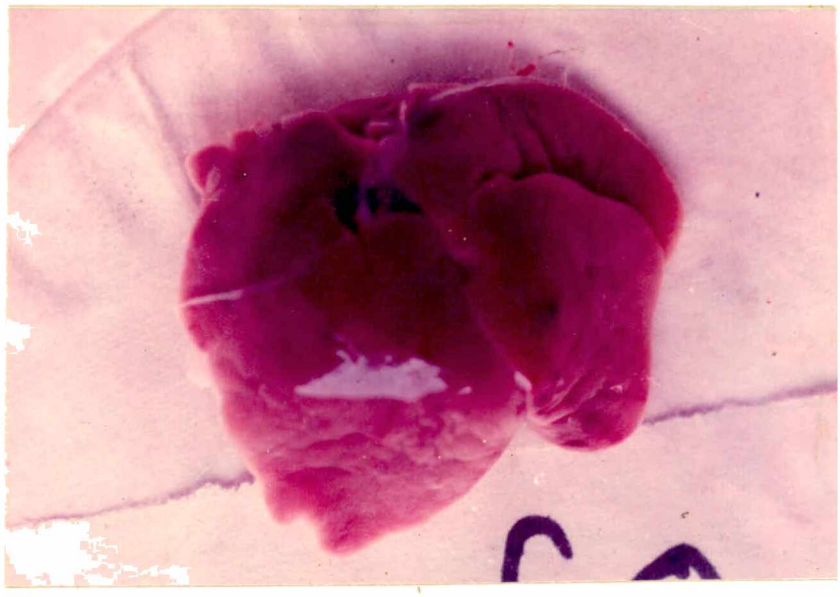

Fig.3 ✓

Showing congestion of liver with white streaks on the surface of the organ in experimentally inoculated (oral) quails with 01 *E. coli* isolate.

Fig. 4 ✓

Showing acute congestion of heart, liver, intestine, and gizzard of adult quails, experimentally inoculated with 01 E. coli isolate (I/P route).

Fig. 5

Showing congestion of liver, intestine, heart and kidneys in adult quails, experimentally inoculated (oral) with 02 E. coli isolate.

Fig. 6

Showing congestion of liver with white streaks on the surface of the organ in experimentally inoculated quails with 02 E. coli isolate, (I/P route).

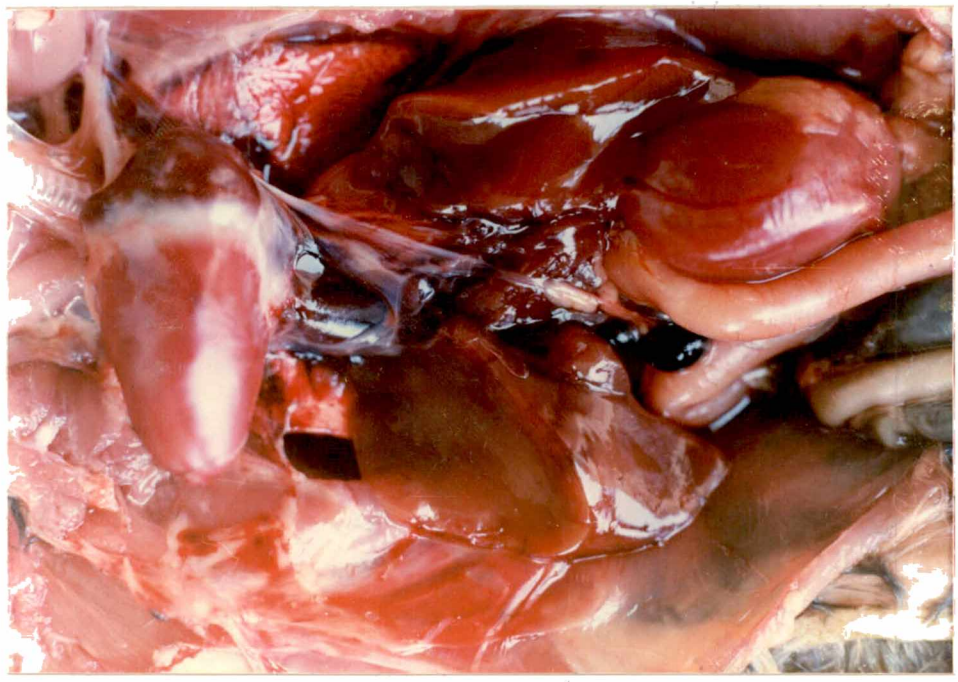

Fig.7

Showing extensive congestion of heart, liver, intestine in adult quails, experimentally inoculated with 02 E. coli isolate (I/P route).

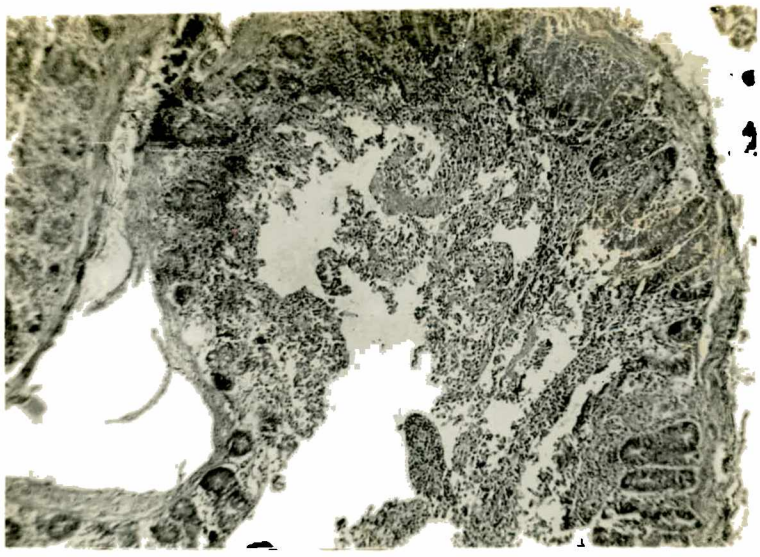

Fig. 8 ✓

Section of intestine of quails 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.

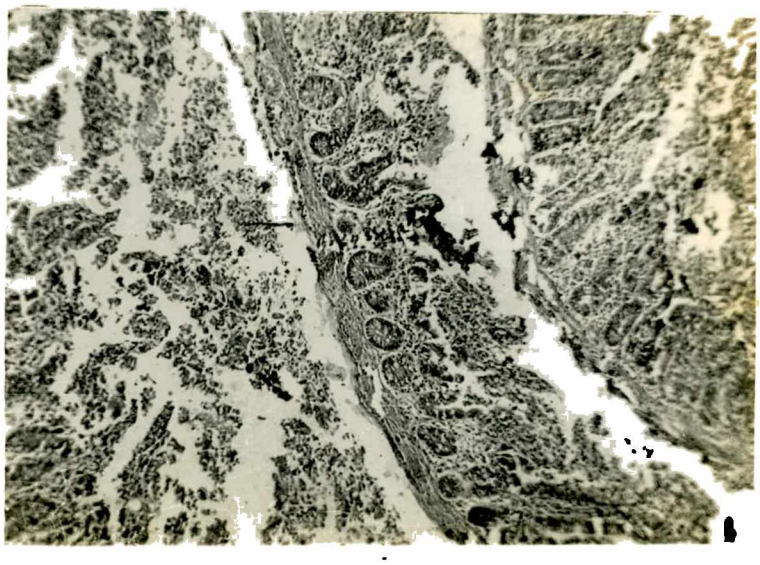

Fig. 9 ✓

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

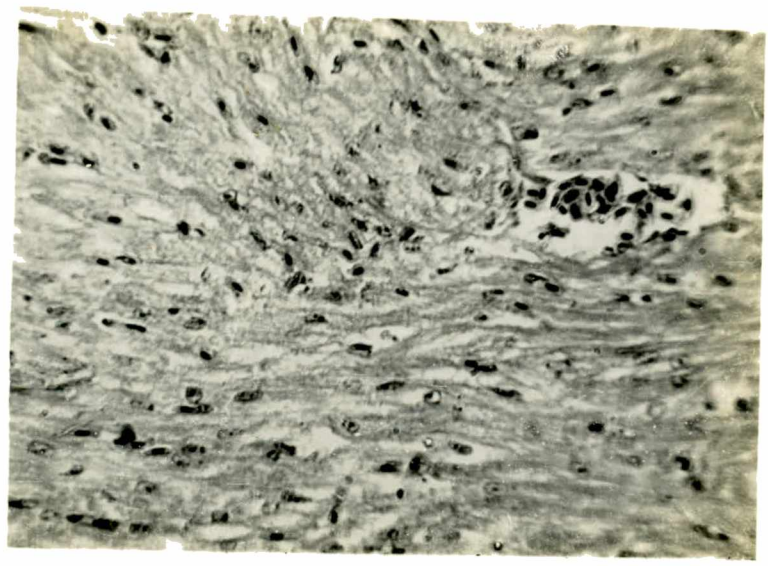

Fig. 10 ✓

Section of the heart of quails experimentally infected (oral) with 01 E.coli serotype, showing marked congestion of the intermyseal vessels. There were accumulation of r.b.c. and haemorrhages between the myocardial fibres.
H. E. X450

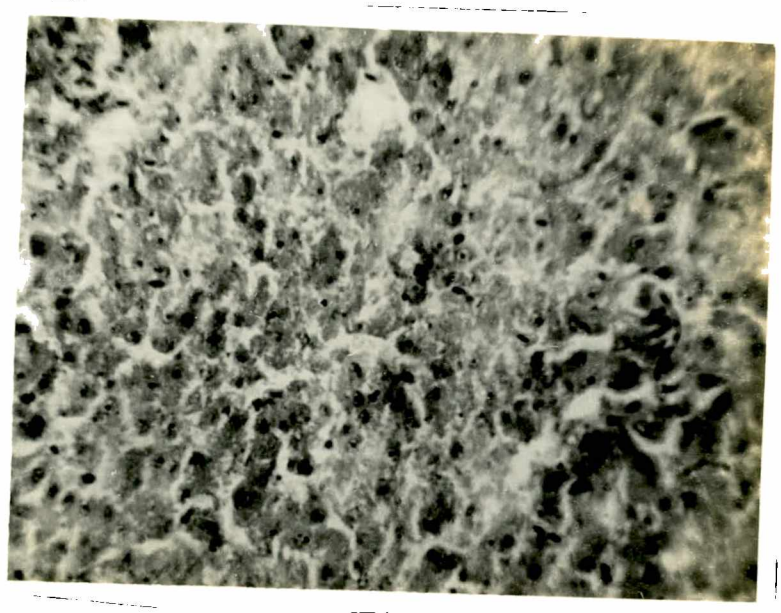

Fig.11 ✓

Section of liver of quails, experimentally infected (oral) with 01 E. coli serotype, showing ~~congestion of liver~~ parenchyma, degeneration and necrosis ~~which were found distributed throughout the field.~~ H. E. X 450.

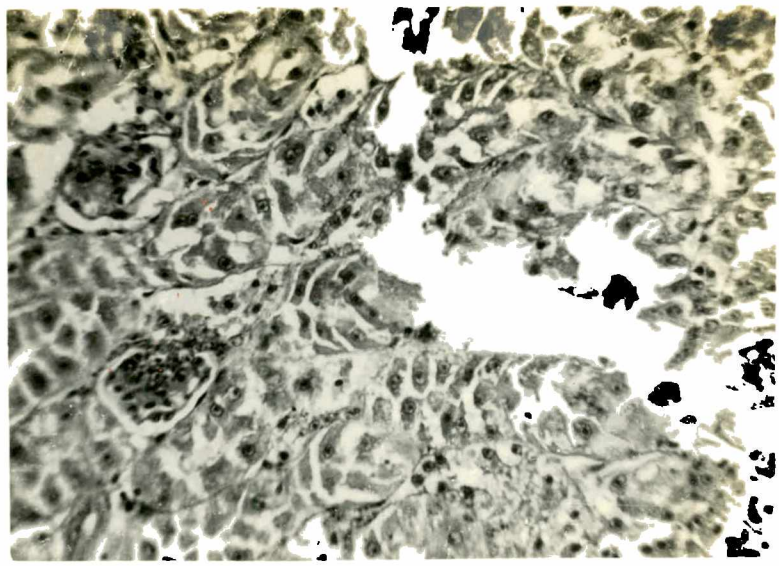

Fig. 12. ✓

Section of kidney of quails, experimentally infected (oral) with 01 E. coli serotype, showing severe degeneration and necrosis of the lining epithelial cells of the tubules.

H.E. X 450

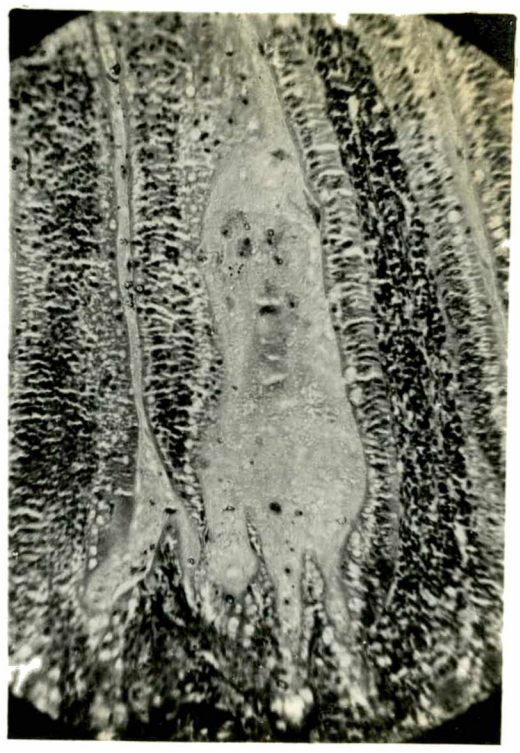

Fig. 13 ✓

Section of intestine of quails, experimentally infected (I/P route) with 02 E. coli serotype, showing diffuse infiltration with mononuclear cells in the lamina propria. H. E. X 450

Fig. 14

Section of intestine of quails, experimentally infected (I/P route) with 01 E. coli serotype, showing mucous producing cells in the lining epithelial cells of the villi.
H. E. X 100

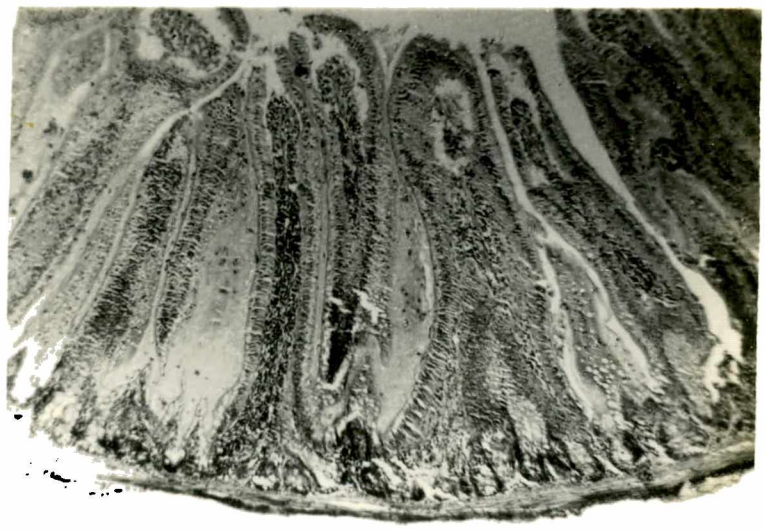

Fig. 15

Section of intestine of quails showing lucocytic infiltration, markedly oedematous of submucosal glands and proliferation of the lymphocytic cells in the submucosa. H. E. X 100.

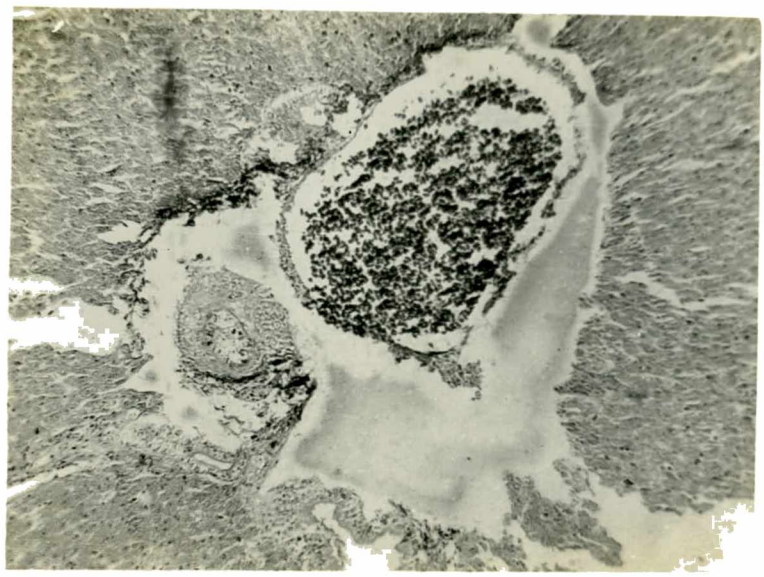

Fig. 16 ✓

Section of liver of quails experimentally infected (I/P route) with 01 E. coli serotype, showing ~~accumulation~~ engorgement of bloody exudate and r.b.c. in the blood vessels. ~~with degeneration and thickening of the blood vessels in some places~~. H. E. X 100.

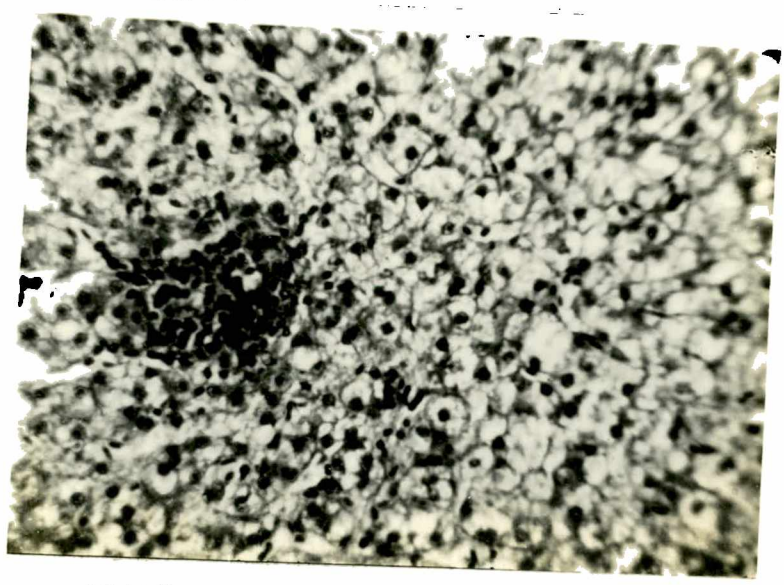

Fig.17 ✓

Section of liver of quails, experimentally infected (I/P route) with 01 E. coli serotype showing aggregation of the lymphocytes in the liver parenchyma and necrosis. H. E. X 450.

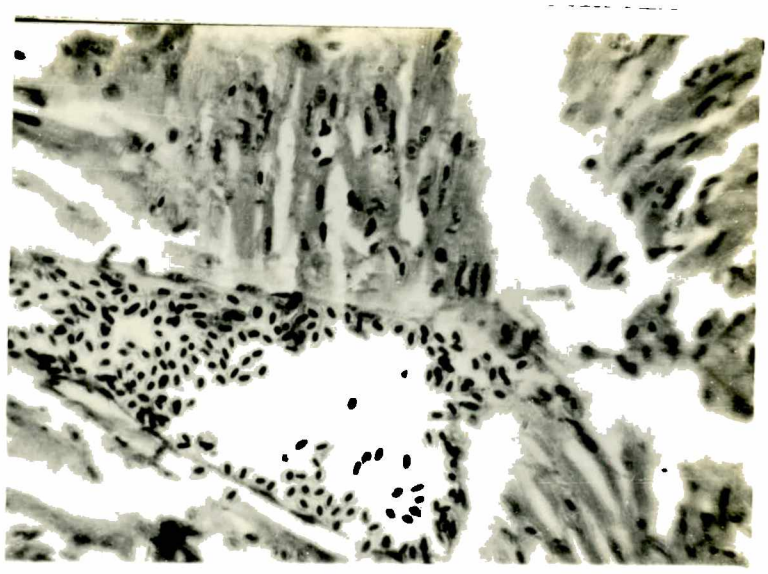

Fig.18

Section of heart of quails, experimentally infected (I/P route) with 01 E. coli serotype showing accumulation of r.b.c. in the blood vessels and fragmentation of the cardiac muscle fibres.
H. E. X 450.

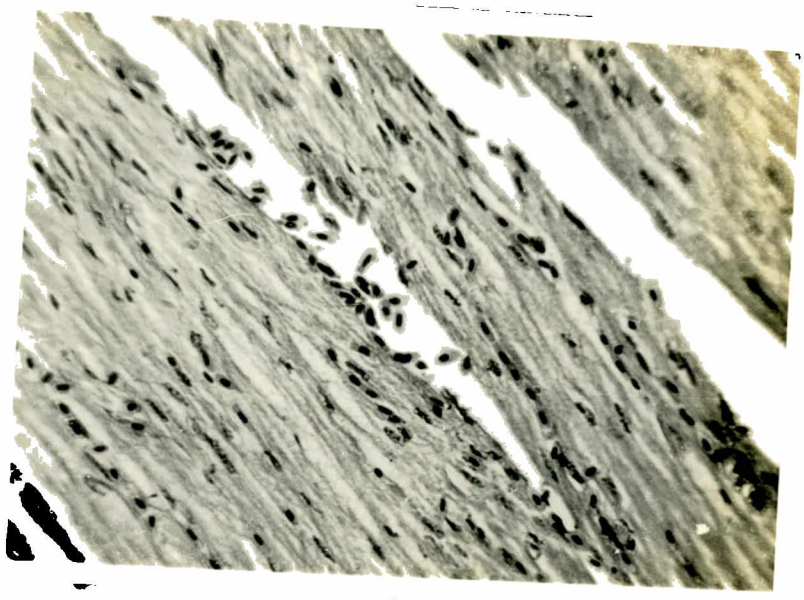

Fig. 19 ✓

Section of heart of quails, experimentally infected (I/P) with 02 E. coli serotype showing moderate congestion.
H. E. X 450.

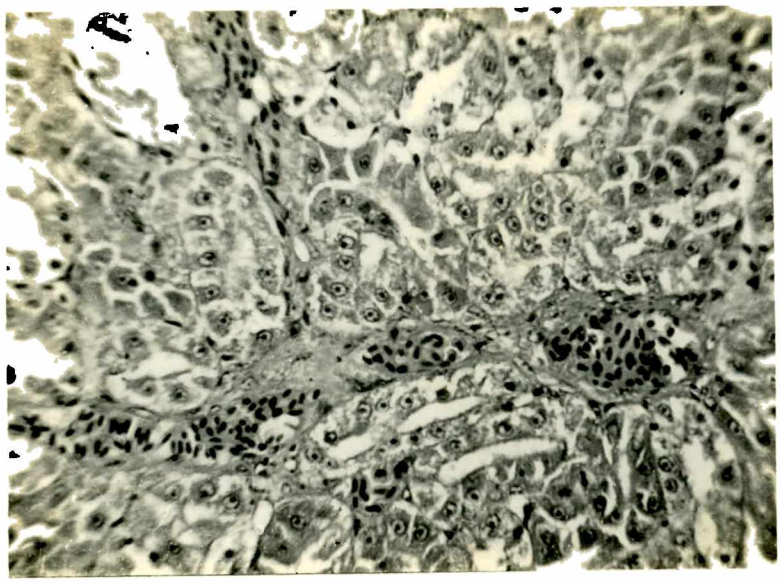

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Fig. 20.

Section of kidney of quails, experimentally infected with E. coli serotype 02 showing severe haemorrhagic changes throughout the section and accumulation of r.b.c. more in the interstitial spaces. H. E. X 450

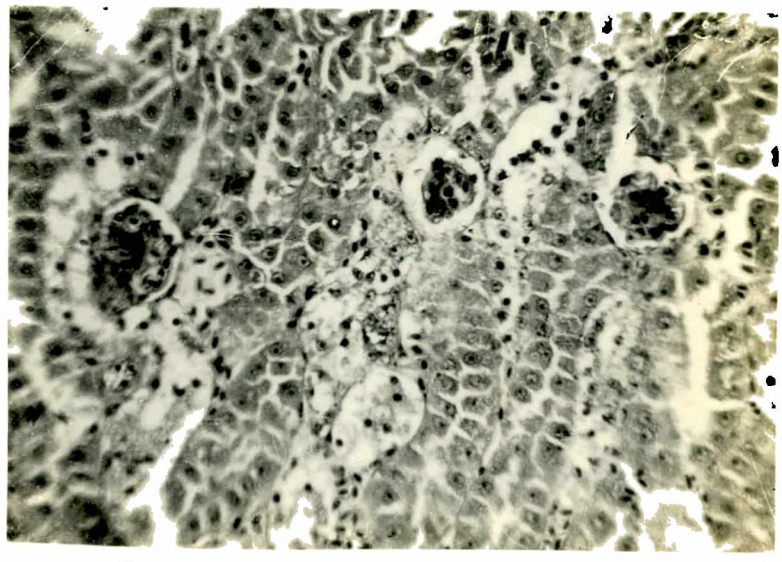

Fig. 21 ✓

Section of kidney of quails infected with E. coli serotype 01 showing congestion and hypermia of glomeruli. H. E. X 450

No room in Fig 2

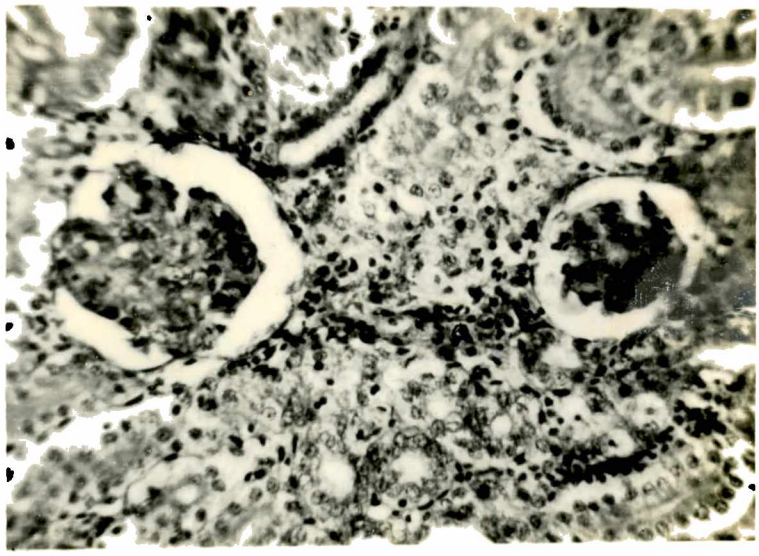

Fig. 22

Section of kidney of quails showing atrophy and hypertrophy of glomeruli.
H. E. X 450.

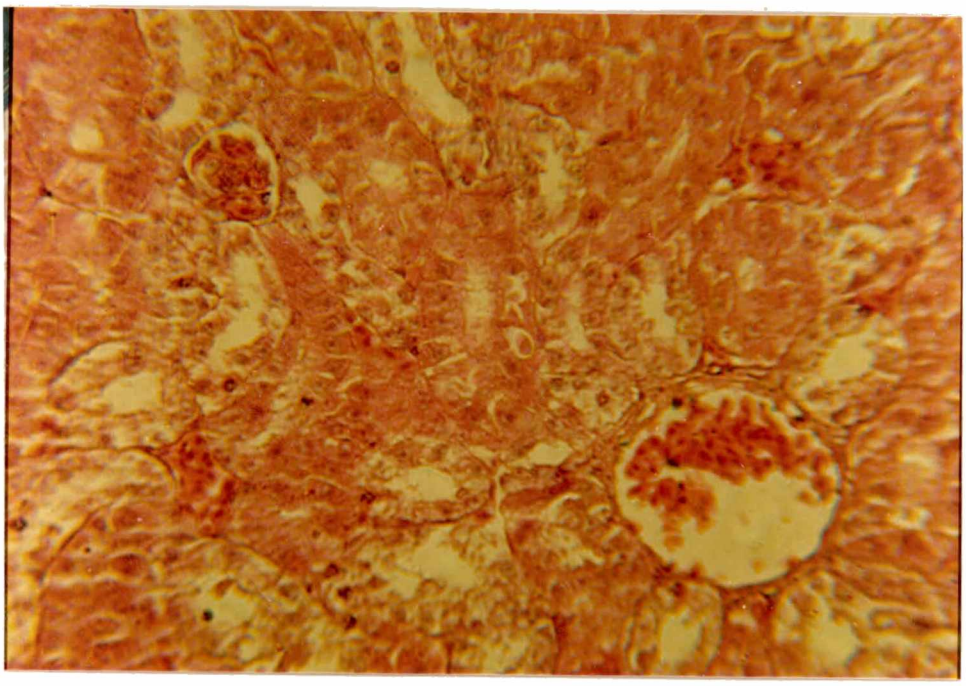

Fig. 23

Section of kidney of quails, experimentally infected (I/P route) with 01 serotype, showing severe haemorrhagic changes throughout the section and accumulation of r.b.c. in glomeruli.
H. E. X 450

No indication in text

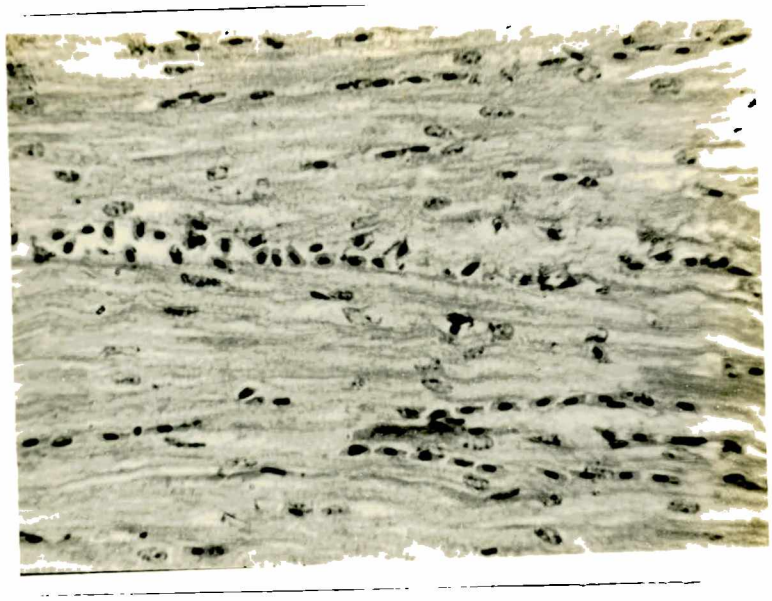

Fig.24 ✓

Section of heart of quails, experimentally infected (oral) with 02 E. coli serotype, showing severe haemorrhagic changes between the myocardial fibres.
H. E. X 450

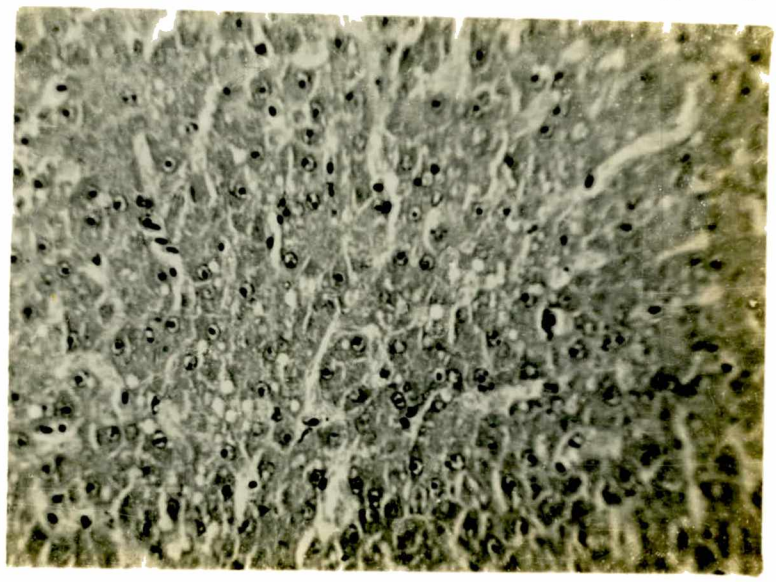

Fig. 25 ✓

Section of liver of quails, experimentally infected with E. coli serotype 02 showing degenerative changes ~~of~~ (fatty degeneration) and necrosis.
H. E.X 450

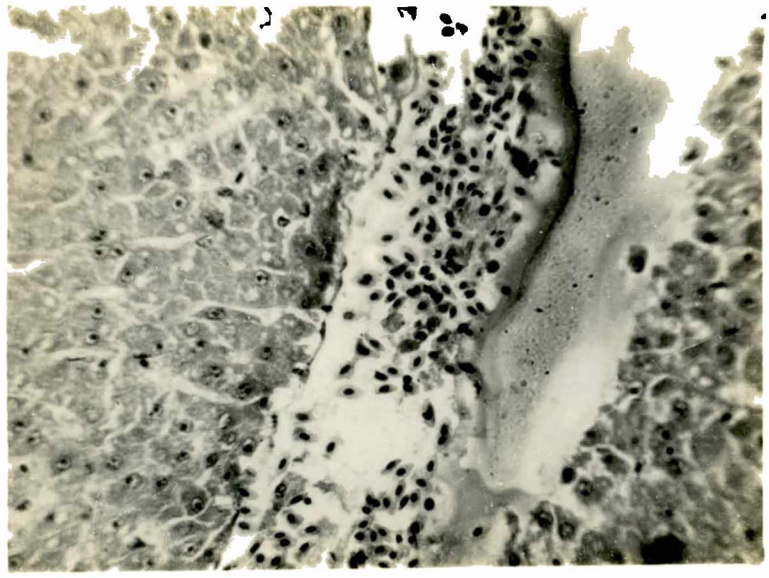

Fig. 26

Section of liver of quails, infected with E. coli serotype 02 showing accumulation of bloody exudate and r.b.c. in the blood vessels.
H. E. X 450

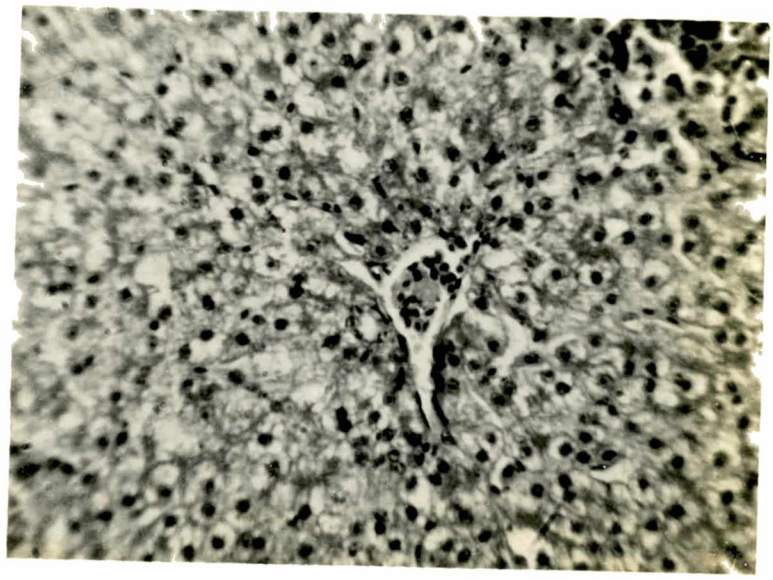

Fig. 9

Section of liver of quails infected with E. coli serotype 01, showing congestion in the interhepatic cord, central vein and necrosis. H. E. X 450

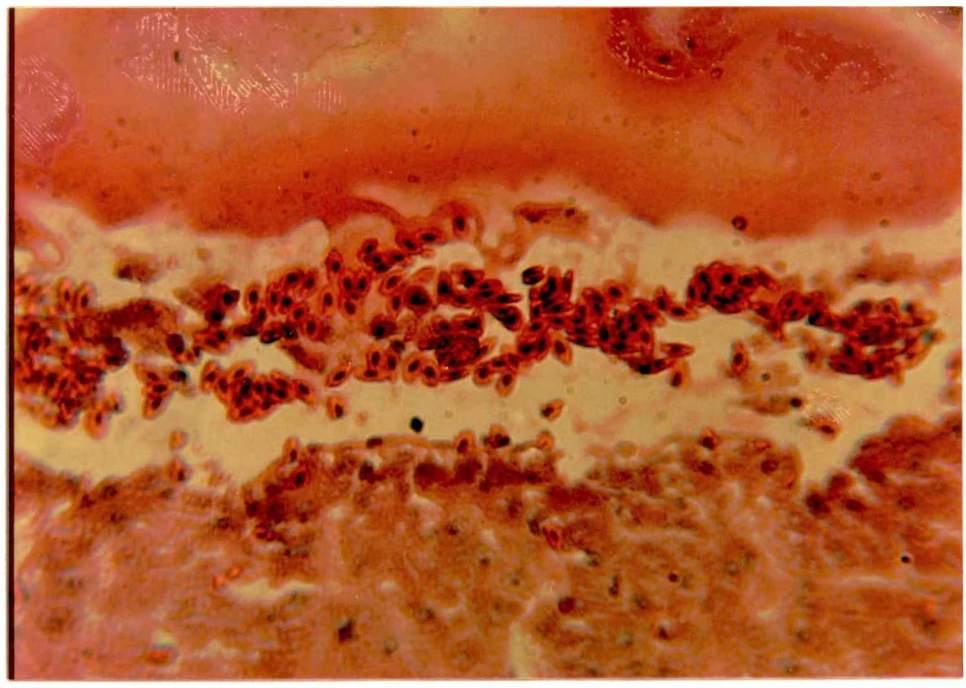

Fig 9.

Section of liver of quails, infedted with E. coli serotype 02, showing accumulation of bloody exudate and r.b.c. in the blood vessels.
H. E. X 450.

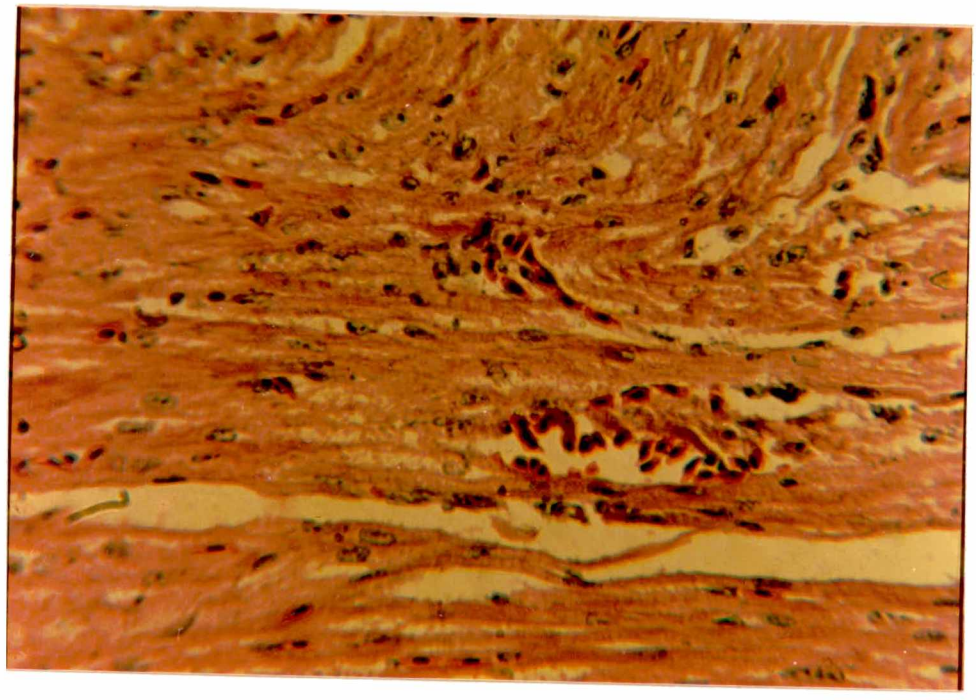

Fig. 9

Section of heart of quails, experimentally infected with E. coli, showing severe haemorrhagic changes between the myocardial fibres. H.E. X 450.

D. Biochemical tests for Reisolated Escherichia coli.

Altogether 40 E. coli (01 and 02) 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 - IV and V. Out of 40 strains of E. coli 30 strains produced Indole, all the 40 strains of E. coli showed positive results to M.R. and negative to V. P. reactions and fermented lactose, glucose, manitol, maltose, dulcitol and salicin. On the other hand, none of the 40 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 confirmity with the results obtained by Phukan (1988).

Table - IV. Biochemical characteristics of E. coli reisolated from experimental infected quails by E. coli.

Species	Number of isolates showing positive results to						
	Number of isolates.	M.R.test %	V.P.test %	Indole production.	H_2S production	Ureas production	Citrate utilisation
Escherichia coli	40	40	0	30	0	0	0
		100%	0%	75%	0%	0%	0%

Table - V. Biochemical characteristics of E. coli reisolated from dead quails.

Species	Number of isolates tested.	Number of isolates showing +ve results to Fermentation test on							
		Glucose %	Lactose %	Dulcitol %	Adonitol %	Inositol %	Manitol %	Maltose %	Salicin %
Escherichia coli		A	A	A	-	-	A	A	A

A = Production of acid on fermentation.

E. Results of the pathogenecity test against reisolated E. coli in white albino mice.

A review of the Table - VI clearly indicates that the percentage of mortality in white albino mice is 66.66% in E. coli (01 serotype) through intraperitoneal route. Similar is the outcome in pathogenecity test in E. coli (02 serotype) through the same route. Further, the control group ~~which was injected with sterile nutrient broth~~ did not show any mortality.

It was found that the white albino mice injected with large doses subcutaneously develop local abscesses, but if injected intravenously or by the intraperitoneal route they may die from septicaemia.

F. Results of Reisolation of E. coli from different sites of inoculated mice.

A survey on the results of positive reisolation of E. coli from the different sites of inoculated mice indicated a highest number in heart blood (4) through intraperitoneal route in 01 strain. Similar is the result of such positive reisolation in heart blood, the number of dead mice being 5, through the same route in 02 strain. Gross pathological changes observed in the internal organs

of dead mice were congestion of heart, spleen, liver, kidneys, lung in all the dead mice (Fig.27,28). 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 VII).

It was evident from (Table - VI) that the degree of pathogenicity test were 01 and 02 as '+++'.

The control experimental mice which ~~were not died~~ did not die were sacrificed ~~and nothing abnormal was noticed in the~~ but no lesion of pathological significance could be noticed internal organs, specially heart, liver, spleen, lungs and kidneys (Fig. 29). Inoculation of heart blood in blood agar plates did not reveal any microbial growth, particularly E. coli.

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.

Besides, Gupta (1975) observed E. coli serogroups (05, 09, 011 and 015) as highly pathogenic to white mice. He further observed that 15 serogroups 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 whereas 017 was found nonpathogenic. They further recorded macroscopic lesions like congestion of liver, spleen in dead mice which coroborated with the present findings.

Table - VI. Results of the pathogenicity test against reisolated *E. coli* in white albino mice.

Sl. No.	*E.coli* sero-type.	Route of ino-cula-tion.	Dose (ml)	No.of mice inocu-lated.	No. of mice died within days.											Total no. died	Per-cen-tage of mor-tali-ty (%)	Pathogeni-city.
					24 hrs.			48 hrs.			72 hrs.			96 hrs.		120 hrs.			
					6	12	18	24	36	48	60	72	84	96	108	120			
1	01	I/P	0.5	6						1		1	1	1			4	66.66	+++
2	02	I/P	0.5	6				1	1	1		1	1				5	83.66	+++

Control group		
No. used	No. died	% of mortality.
6	Nil	Nil
6	-	-

I/P = Intraperitoneal route.

Table - VII. 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	4	2	3	1	1

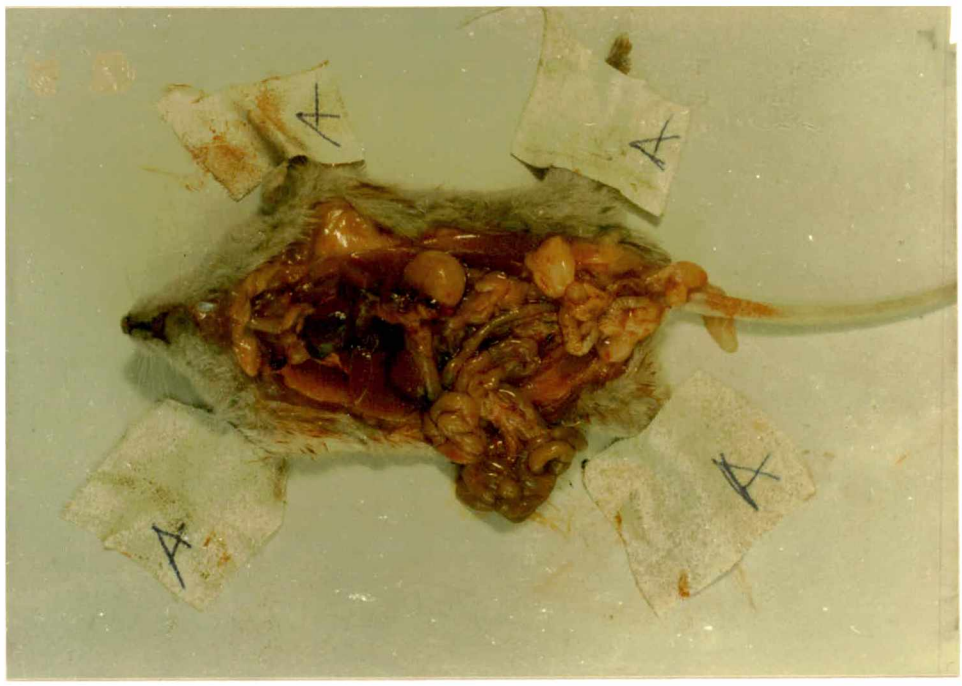

Fig. 27

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.

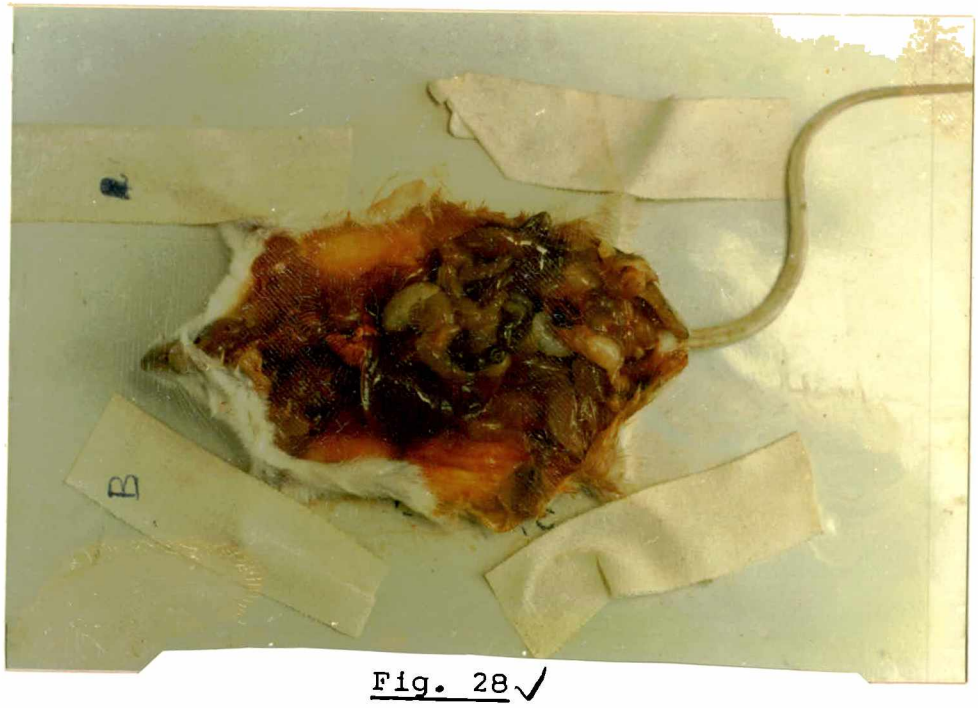

Fig. 28

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. 29

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

Table - VIII. Sensitivity pattern of Reisolated *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)	G (cm)	AN (cm)	SN (cm)	CF (cm)	N (cm)	NA (cm)	FX (cm)	
E. coli	01		1.2	1.1						1.8			1.2	A = Ampicillin 10 mcg
E. coli	02	1.0	1.0						1.3	0.8			0.9	CA = Carbanicillin 100 mcg
														CE = Cephaloridine 30 mcg
														CH = Chloramphenicol 30mcg
														CT = Cotrimexazole 25 mcg
														G = Gentamycin 10 mcg
														AN = Amikasin 10 mcg
														SN = Sulphadiazine 150mcg
														CF = Cefotaxime 30 mcg
														N = Neomycin 30 mcg
														NA = Nalidixic acid 30 mcg
														FX = Furazolidone 300 mcg

Should come after pp 51

G. Sensitivity pattern of Reisolated E. coli against different antimicrobial agents.

A close observation on Table - VIII and (Figures 30,31) vividly indicates that the E. coli (01 stain) is sensitive to carbanicillin, cephaloridine, cefotaxime, Furazolidone, while it is not sensitive to other antimicrobial agents. Further, it is found that E. coli (02 strain) is sensitive to Ampicillin, carbanicillin, Sulphadiazine, Cefotaxime and Furazolidone, but it is not sensitive to other antimicrobial agents.

In the present study no single antimicrobial agent was found sensitive to all the E. coli serotypes. Further, no single antimicrobial agent was 100% sensitive against the E. coli strains.

Savov (1963), Aller Gencedo et al. (1969), Butura and Sahleanu (1972), Kapoor et al. (1978), Sahota et al. (1978), Srinivasan et al. (1979), Gyurov et al. (1981), Kumar et al. (1981), Kim and Tak (1983), Barbour et al. (1985), Sinha et al. (1985), reported varying degree of resistance of E. coli strains to different antimicrobials. Transfer of drug resistance has been a present day problem in controlling colibacillosis. Mehrotra et al. (1984) reported the transfer of drug resistance to chloramphenicol and Furazone in E. coli.

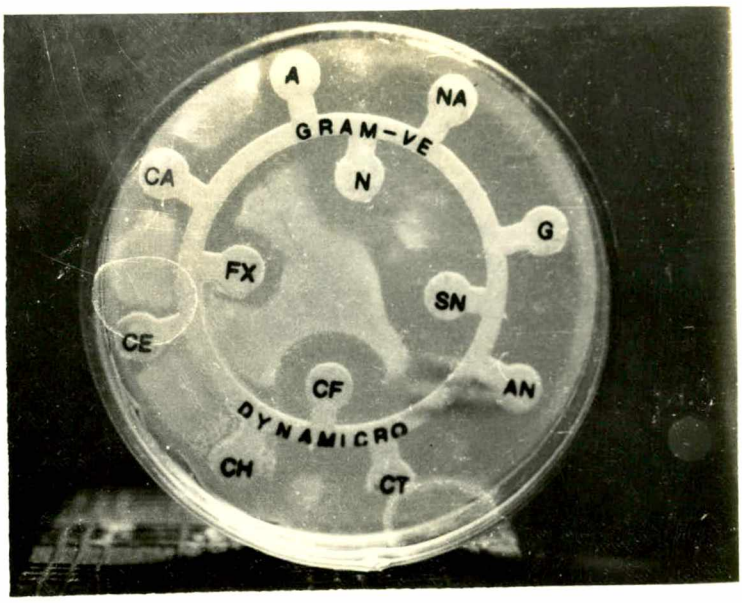

Fig. 30

In vitro sensitivity of E. coli 01 seroisolate to different drugs, indicating sensitivity to carbanicillin, cepharidine, cafotaxime and Furazolidene.

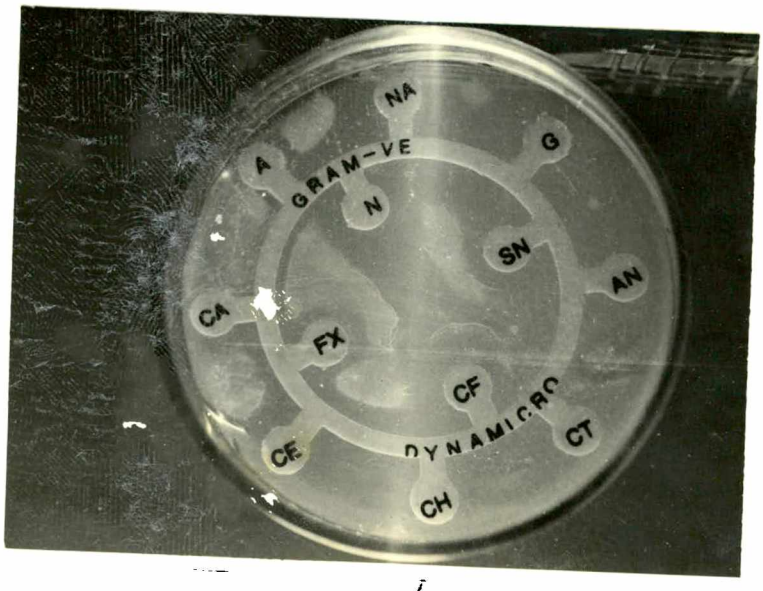

Fig. 31

In vitro sensitivity of 02 E. coli seroisolate indicating sensitive to ampicillin, carbanicillin, sulphadiazine, cafotaxime and furazolidone.

Chapter : V

SUMMARY AND CONCLUSION

Summary and Conclusion

Experimental infection of Escherichia coli (serotype 01 and 02) was done in adult quails. Both the strains were found to be highly pathogenic to quails. Serotype 02 produced 100% mortality in quails within 7 days in I/P, and oral routes of administration whereas serotype 01 produced 100% mortality by I/P routes and 80% mortality in oral route administration to quails. Maximum mortality was observed in quails which were inoculated I/P within the first 3 days in both the serogroups. But none of the control groups of quails exhibited any pathogenic change or mortality when inoculated with sterile broth cultures through I/P and oral routes.

Clinical symptoms and gross lesions in quails due to experimental infection of E. coli were sudden death, huddling together and death, symptoms of incoordination of movements, tremers, dullness, loss of appetite. Gross lesions observed on Post-mortem examination of dead quails were congestion of the heart, liver, intestines, gizzard. Pericarditis and perihepatitis were also seen. In many cases thickened air-sacs and white necrotic areas on the liver were visible.

Varying degree of

Histopathological changes (macroscopic and microscopic) observed ~~in varying degrees~~ in internal organs ~~in experimental infection of E. coli in adult quails by different routes~~ (I/P and oral routes) were as follows :

Intestines :

Highly inflammed and congested. Section of intestine showed extensive, acute haemorrhagic enteritis, characterised by the presence of bloody exudates.

Architecture of the villi was completely lost and there was desquamation 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. Proliferation of the lymphocytic cells in the submucosa.

Heart :

Congested with varying quantity pericardial fluid. The intermyseal vessels were markedly congested. There were accumulation of the r.b.c. and haemorrhages between the myocardial fibres. In some places these r.b.c. become coagulated.

Liver :

The liver was congested with white streaks. Liver parenchyma was congested and hepatic cells were swollen due to vacular degeneration and necrosis. Bloody exudates were present.

Reisolation of E. coli was also done from heart blood, liver, pericardial and peritoneal fluid and intestinal contents to confirm E. coli infection. The isolated organisms were identified on the basis of morphology, staining reaction, cultural characteristics and biochemical tests. Out of 40 strains of E. coli, 30 strains produced indole. All the 40 strains of E. coli showed positive results to M.R. and negative to V.P. reactions and fermented lactose, glucose, manitol and maltose whereas fermented to sucrose, dulcitol and salicin. On the other hand, none of 40 strains of E. coli fermented adonitol and inositol and utilised citrate and produced urease and H_2S.

Pathogenicity test of E. coli was conducted on white albino mice. It was observed that 83.66 was recorded as pathogenic in white albino mice within 96 hours of inoculation of serotype 02 E. coli isolate, serotype 01 recorded 66.66% mortality in white albino mice. There was no mortality in control experimental white albino mice. 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 plates did not reveal andy microbial growth, particularly E. coli.

The results on the antibiotic sensitivity tests indicated that 01 and 02 E. coli strains isolated were sensitive to Ampicillin, Sulphadiazine, Carbanicillin, Cephaloridine, Cefotaxime, Furazolidone respectively, while it is resistant to other antimicrobials used in this study. Further no single antimicrobial was 100% sensitive against the E. coli strains.

Conclusions

1) Majority of *E. coli* strains were isolated from intestinal contents followed by heart blood.

2) Pathogenicity test conducted on white albino mice with the isolated serotypes revealed that *E. coli* serotype 01, 02 were highly pathogenic.

3) Antimicrobial sensitivity test conducted on *E. coli* isolates indicated varying degree of sensitivity. No drugs was found to be 100% effective for all strains. Further, degree of resistance was also high to different antimicrobials used.

Study also indicated that regular preventive treatment as per sensitivity test along with improved sanitation, hygiene and management could control the diseases.

Chapter: VI

FUTURE SCOPE OF RESEARCH

Future scope of Research

A preliminary attempt has been made to study the pathological changes in quails following experimental infection of E. coli. Though extensive and elaborate works have been carried out to study the behaviour of E. coli infection in poultry through different routes, little work has been done in the existing literature.

As quail industry has gained colassal importance in recent part, an arena has been opened to us for such experimental study in quails. Further research work on the experimental behaviour of E. coli infection in quails will be attributed to the following lines of investigation :-

a) The pathogenecity test and the enterotoxic activity may be studied in rabbits and chicks to study the pathogenic strains.

b) Attempts may be taken to evolve a polyvalent vaccine against E. coli basing on the isolation of virulent E. coli strains.

c) Studies may be conducted to combate the incidence of E. coli in quails to prevent the economic loss in quail farms and to enhance the quality of disease-free quail-meat.

d) Proper sanitation, hygiene and other managerial methods may be adopted for control and prevent of E. coli infection in quails.

Chapter: VII

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