Pathology of Digestive System in Japanese Quail *(Coturnix coturnix japonica)* With Special Reference to Bacterial Enteritis

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA, (SAMASTIPUR), BIHAR

(FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(VETERINARY PATHOLOGY)

By

Latika

Registration No. - M/Vety. Patho./45/1998-99

Department of Veterinary Pathology

BIHAR VETERINARY COLLEGE

PATNA - 800 014

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Dedicated

To

My Father

Late. Jwala Prasad Ram

Department of Veterinary Pathology
Rajendra Agricultural University, Bihar
Bihar Veterinary College, Patna – 14.

CERTIFICATE – I

This is to Certify that the thesis entitle **"Pathology of Digestive system in Japanese quail (*Coturnix coturnix japonica*) with special reference to bacterial enteritis"** submitted in Partial fulfilment of the requirement for the degree of "Master of Veterinary Science in Pathology" of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar is the record of bonafide research carried out by Dr. Latika under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further Certified that the assistance and help received during the course of investigation have been fully acknowledged.

23/9/2010

L. N. Prasad

Associate Professor,

Department of Veterinary Pathology.

CERTIFICATE – II

We, the undersigned, members of the Advisory Committee of Dr. Latika a candidate for the Degree of Master of Veterinary Science with major in Pathology have gone through the manuscript of the thesis and agree that the thesis entitled **"Pathology of Digestive System in Japanese quail (*Coturnix coturnix japonica*) with special reference to Bacterial enteritis"** may be submitted by Dr. Latika in partial fulfillment of the requirements for the Degree.

23/9/2

L. N. Prasad

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Advisory Committee.

Members of the Advisory Committee :

1. Dr. Binay Kumar Sinha, 23/9/2
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3. Dr. M. K. Roy, 23/9/200
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   Associate Professor (Dept. of Animal Breeding and Genetics)
5. Dr. Mani Mohan,
   Dean cum Principal, Bihar Veterinary College. 23.9.2000
   Nominee, Dean Post-Graduate Studies.

CERTIFICATE – III

This is to Certify that the thesis entitled **"Pathology of Digestive system in Japanese quail (*Coturnix coturnix japonica*) with special reference to bacterial enteritis"** submitted in partial fulfilment of the requirement for the Degree of Master of Veterinary Science (Pathology) of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar was examined and approved on ..17.02.2001..... ~~2000.~~

L. N. Prasad

Chairman, Advisory / Examination Committee

Members of Advisory Committee

1. Dr. Binay Kumar Sinha,
2. Dr. Basant Kumar Sinha,
3. Dr. M. K. Roy,
4. Dr. S. B. Verma.
5. Dr. Mani Mohan,
   Dean cum Principal, Bihar Veterinary College.
   Nominee Dean, Post – Graduate Studies.

ACKNOWLEDGEMENTS

It is my great privilege and honour to express my deep sense of gratitude to my major advisor Dr. L. N. Prasad, Ph. D, Associate Professor, Department of Veterinary Pathology, Bihar Veterinary College, Patna for erudite guidance, close supervision and constant encouragement in consumption of this ardous task and above all a keen interest throughout the period of study.

I accord my thanks to Dr. B. K. Sinha, Associate Professor and Head Department of Veterinary Pathology for his inspiring guidance and valuable suggestions.

I wish to express my sincere thanks and indebtness to Dr. M. K. Roy, Associate Professor and Head, Department of Vet. Anatomy and Histology for his whole hearted cooperation and constant effort extended throughout the course of the study.

My profound gratefulness are due to the members of the advisory committee, Dr. B. K. Sinha, Associate Professor and Head, Department of Veterinary Microbiology. Dr. M. K. Roy, Associate Professor and Head, Department of Veterinary Anatomy and Histology, Dr. S. B. Verma, Associate Professor,

Department of Animal Breeding and Genetics for their generous help and valuable suggestions.

I am highly thankful to Dr. Mani Mohan, Dean-cum-Principal, Bihar Veterinary College, Patna for providing necessary facilities during the research period.

My special thanks to Dr. Ujwal Kumar, Farm Veterinary officer, Central poultry farm and Mr. Ram Janam Prasad for their excellent cooperation during the study.

My thanks are also due to Dr. Rekha T. Kujur, Dr. Vinita Rani, Kusum Khalkho and other friends for their friendly cooperation in many ways during the present investigation.

Grateful thanks is also expressed to all the technical and non-technical staffs of Department of Veterinary Pathology, Department of Veterinary Anatomy and Histology and Department of Veterinary Microbiology for their constant help.

Author is also thankful to campus librarian, Dr. B. K. Mishra for providing all the library facilities.

I wish to express my most sincere and deep sense of reverence to my husband, Dr. Rajesh Kumar Sinha for his understanding, perseverance and constant encouragement shown throughout the tenure of the

study. Above all, I would like to express my love to my beloved daughter 'Shreya' for her unlimited patience.

I am highly thankful to my brother-in-law, Dr. Shashi Kumar Sinha, my dear sisters, Dr. Smita and Sucheta for their spell bound efforts endured throughout the tenure of the study.

I am greatly indebted to my loving mother, Smt. Lajja Singh for her unceasing moral support and encouragement.

Thanks are also due to all staffs, M/S Sanjeevani Art Computers, Patna- 14. for their ever available assistance and final shaping of this thesis.

Last but not the least, I thank 'God' for giving me patience and strength to overcome the difficulties which crossed my way in accomplishment of this endeavour.

Place : Patna

Lalika

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Chapter • 1

INTRODUCTION

INTRODUCTION

Coturnix coturnix japonica had previously a nomadic life (about 50-60 years) in Japanese Islands. Japanese quail a natural habitat of Japanese Islands belong to the **Class**-*Aves*, **Family**-*Phasianidae*, and **Genus**-*Coturnix* and hence the name *Coturnix coturnix japonica.* It is popularly known as bater in Hindi.

Japanese quail were introduced in India by the Central Avian Research Institute (Poultry Research Division of IVRI) in 1974 by procuring hatching eggs from University of California, U.S.A. under ICAR/UNDP project. During 1978 two more quail lines were procured under the UNDP from the University of Cohenheim, Stutgart, West Germany and during 80's another line was added from Democratic Republic of Korea (Agrawal, 1996). The successful propagation of the bird at Central Avian Research Institute has generated greater interest among private breeders. Today quail has become the third largest avian species in number next only to chicken and ducks in the country. (Ahuja, 1990). The number of quails produced for commercial use has also increased considerably during recent years and estimated to be about one lakhs. (Agrawal, loc.cit.).

Japanese quail (synonyms-Coturnix quail, pharoah's quail, stubble quail and eastern quail) is used as pilot animal. Recognizing the multifarious advantage of quail farming, many people are coming forward to take up quail as a major enterprise and it is rapidly becoming an important

component of the Indian Poultry Industry. Broiler quails are marketed at the age of around six weeks.

Inspite of all these, quails are very sensitive to abrupt environmental changes particularly during first two weeks of life. Programmes for disease control should be made to meet the demand of quails in which loss due to enteric disease is very important. They probably suffer from disease similar to those of chicken (Mohanty and Verma, 1982).

The common pathogens affecting health status of quail are bactria, virus, fungi and parasites. Disease affecting the intestine leads to heavy loss which arises mainly due to unhygienic handling, contaminated feed and environmental conditions.

Enterobacteriaceae are commonest pathogen affecting quail which are causative agent for bacterial enteritis. Among the enteric bacterial diseases –

(i) Colibacillosis, Coligranuloma, (ii) Fowl typhoid and paratyphoid

(ii) Bacillary white Diarrhoea (BWD), (iv) *Salmonella gallinarum* infection, (v) Tuberculosis, (vi) Ulcerative enteritis are common.

Between 1979 and 1983, post-mortem examination of 1985 quails (*Coturnix coturnix japonica*) were carried out at Hissar, Haryana. In 292 (i.e. 19.7%) ulcerative enteritis, in 157 (10.6%). Colibacilosis, in 73 (4.9%) hepatitis cases were reported. The cases were recorded only in birds of five weeks or above i.e. adult birds. The maximum mortality was recorded during the winter and lowest during the rainy season (Sharma and Kaushik 1986).

A compilation of five years mortality records from 1989 to 1992 for different ages of Japanese quail was undertaken at Poultry Research Station,

Madras. Mortality was higher among the age groups of 0-1 and 2 to 6 weeks. (Ravindran *et al;* 1994). Majority of quail chicks used to die due to enteritis during the age of 2-3 three weeks and it was found that enteritis was a major problem in breeding stocks. In an investigation that carried out at Orissa Veterinary College (Misra *et al;* 1991) 84 samples were analysed bacteriologically, 79 yielded bacteria and the rest were found contaminants. The bacteria isolated were *Staphylococcus aureus, Escherichia coli, Streptococcus sp, Klebsiella sp. Salmonella sp. Proteus sp. Shigella sp.* isolated in pure or mixed forms.

Sarma *et al.* (1988) observed heavy mortality in 1-3 days old Japanese quail during the months of September-December, 1986 followed by on outbreak of low mortality in cockerels). A heavy mortality of 71 percent was observed in the outbreak in Japanese quails particularly baby quails, Reddy *et al.* (1994) in an experimental study at Namakkal reported that the infection of *Escherichia coli* was found to be more acute in nature in seven day old quails chicks with rapid deaths and few lesions.

Enterobacteriaceae group of organisms are the common inhabitants of the intestinal tract of the poultry birds among which *Escherichia coli* plays a vital role in causing enteritis resulting in heavy mortality.

Prevention is better than cure, so it is very necessary to control the diseases of digestive system in the quails otherwise it will adversely affect the profitability and economy of the farmers who are taking quail farming as their profession based on agro-industry. Although, studies have been made on digestive disorder of quail in India, but a little effort have been made

especially in this part of Bihar to ellucidate the pathological condition of digestive system. It is specially warranted to make elaborate study in view of the growing popularity of quail farming and in face of some reported incidence of *Escherichia coli,* Salmonellosis, Fowl cholera in Japanese quail.

Keeping in view of the above facts the present study has been undertaken with the following aim and objectives :-

I. To find out the type, pattern and various morphological changes in digestive system of quail particularly in this part of Bihar.

II. To study the pathology of different conditions of the digestive system in Japanese quail particularly enteritis.

III. Isolation of Bacterial agents, if any will be carried out to know the role of these agents in causation of Enteritis in Japanese quail.

Chapter • 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Seeing the importance and utility in the present investigation the literatures have been reviewed on two aspects i.e. the gross and microscopic pathology of digestive system and the isolation of bacteria from the enteritis cases separately.

Pathology of Digestive System in quails :

Parihar and Rao (1969) reported ulcerative enteritis in poultry resembling Quail's disease. The outbreak occurred in a private farm at Izatnagar, India. Caeca and adjacent portions of small intestine were more frequently involved. Lesions in the intestine could be detected from the serous surface as circumscribed, circular or elliptical grey spots. In the intestine lesions varying from mere haemorrhages to well formed ulcers were observed. Tissue adjacent to these ulcers invariably showed degenerative changes.

Chawla and Sharma (1972) reported occurrence of ulcerative enteritis simulating Quail's desease in domestic fowls. Out of total 8335 birds autopsied at Indian Veterinary Research Institute, Izatnagar. Nine birds revealed lesions associated with ulcerative enteritis amongst 2350 birds showing lesions of gastro-intestinal tract. The gross and histopathological lesions had a close similarity to Quails disease.

Cygan and Nowak (1974) reported acute necrotic enteritis in chicks and isolated *Clostridium perfringes* types C.

Bendele, (1976) has made a histopathologic and immuno-fluorescence microscopic study of the pathogenesis of ulcerative enteritis in bob white quail. He found that after experimental infection, *Clostridium*

colinum was seen penetrating the intestinal epithelial cells causing the sub adjacent brush border to be denuded. The lesions progressed as the bacteria continued to invade deeper into the intestinal mucosa until (within 3-4 days) well developed ulcers are produced. In chronic cases lesions were seen in the liver and consisted of focal coagulation necrosis of hepatocytes surrounding bacterial colonies.

Cygan and Nowak (1974) reported acute necrotic enteritis in chicks and isolated *Clostridium perfringes type C.*

Mohanty *et al.* (1979) has presented a record on comparative study on the isolation of *Escherichia coli* serogroups involved in enteritis cases and also in healthy birds in Patna. They reported that infection of *Escherichia coli* was 88.88 percent in apparently healthy birds and 80 percent in diseased birds. Percentage of isolation of *Escherichia coli* from internal organs showing pathological lesion was 62.5 pecent.

Srinivasan *et al.* (1979) studied the colibacillosis in poultry mostly 0-6 weeks of age. Both in natural and experimental cases of colisepticaemia, heart, liver lungs and intestine were found to be involved. On gross and microscopic examination, it was found that the birds died of colisepticaemia (natural or experimental) irrespective of the species involved revealed serofibrinous, pericarditis and perihepatitis. The peritoneal fluid was found invariably to be increased in quantity. The lungs and other visceral organs were congested.

Kapoor *et al.* (1980) conducted an epidemiological and pathological studies in outbreak of *Salmonella bareilly* infection in chicken and quails. Necropsy revealed necrotic foci on the surface of liver, catarrhal enteritis, enlargement as well as congestion of spleen. Histopathologically, the lesion

in the small intestine comprised acute congestion, heterophilic infiltration and haemorrhages followed by infiltration of lymphocytes and few plasma cells in the mucosa. Liver revealed focal necrotic areas. The histopathological picture in quails was almost similar to that of chicken, but the reaction was more severe. Mortality of quails in the outbreak of *Salmonella bareilly* infection was 22 percent against 92.5 per cent in broiler chicks.

Srinivasan *et al.* (1980) observed mortality patttern in quails during the years 1976 and 1977 at Haryana Agricultural Farm, Hissar. The crude mortality rate was 9.8% in the 1976 and 29.54 in the year 1977. Under cause specific mortality rate he reported the mortality caused by different conditions are hepatitis, enteritis, pneumonia, internal haemorrhages, Colisepticaemia and Aspergillosis.

Srinivasan et al. (1981) reported ulcerative enteritis among 110 quails chicks of 2-3 weeks of age which caused 88 deaths.

Panigrahy and Glass (1982) described case report of three outbreaks of fowl cholera in quail in Texas. In first case at necropsy the livers were enlarged, friable and had focal areas of necrosis. The intestinal mucosa was haemorrhagic. In second case at necropsy livers were haemorrhagic and had whitish pin-point areas of necrosis. The intestine contained watery ingesta and the intestinal mucosa were haemorrhagic. In third case, 33 quails from a flock of 300 died. Necropsy revealed severe enteritis. Acute deaths in the three flocks of domestic quails were attributed to fowl cholera.

Sharma *et al.* (1985) described *Escherichia coli* infection (coli bacillosis) in poultry and its control. On post-mortem the liver was enlarged

congested with gelationous exudate over its surface and the spleen was engorged.

Sharma and Kaushik (1986) performed post mortem examination of 1485 quails *(Coturnix coturnix japonica)* between 1979 and 1983 at Hissar, Haryana was carried out. In 292 (i.e. 19.7%) ulcerative enteritis, in 157 (10.6%) colibacillosis, in 73 (4.9%) hepatitis cases were reported. The cases were reported only in birds of five weeks or above i.e. adult birds.

Myint. (1987) reported 65% mortality in quail due to *Proteus mirabilis* infection in Burma.

Kondo *et al.* (1988) reported ulcerative enteritis in broiler chickens caused by *Clostridium colinum* at five poultry farms in southern Japan. This was the first report of the disease in Japan. The mortality rate was estimated at 1-5%.

Kulkarni *et al.* (1988) presented a paper on outbreak of fowl cholera in 6 days old chicks in a organised poultry farm in Parbhani city. On detail post-mortem examination, in majority of dead chicks the lesions were general hyperemia of viscera, enlargment of spleen 5-10 times than normal, enlargment of liver with petechial haemorrhages in a few and coagulative necrosis and necrotic foci on liver with discolouration of whole liver.

Panda and Verma (1988) reported *Escherichia coli* as potent pathogen in poultry. *Escherichia coli* in poultry causes colibacillosis (Hijarre's disease) perihepatitis, peritonitis as well as other diseases. In chronic cases, these may result in coligranulomas which are hard tumor like tissue in liver and intestine.

Sarma *et al.* (1988) reported a natural outbreak due to *Salmonella gallinarum* in Japanese quails and its subsequent spread to chicken has been

reported. A heavy mortality of 71 per cent was observed particularly in baby quails. Lesion found in quails were perihepatitis and necrotic foci on liver, slight enlargement of spleen and acute haemorrhagic enteritis. In cockerels, the lesion consists of congestion of liver and enteritis.

Glisson *et al.* (1989) reported *Pasteurella multocida* infection in Japanese quails. Histopathologically detected multifocal splenic and necrotic necrosis. Experimental studies showed Japanese quails to be highly susceptible to disease cuased by the *P. multocida.*

Silva *et al.* (1989) reported occurrence of coligranulomatosis in Coturnix quails in Uberlandia, Brazil. Lesions were located on the mesentry, intestine, gizzard and liver.

Mathew and Sulochana (1990) reported an outbreak of Salmonnellosis in a government owned poultry farm at Chengannur where chicken and quails were reared simultaneously. Heavy mortality was reported among quails from July to November, 1989. Nearly 95 percent birds died during the outbreak. On post-mortem examination, lesion noticed were spleenomegaly with necrotic areas as in the case with Salmonellosis in chicken.

Ahmed and Sarkar. (1991) reported *Escherichia coli* infection as a major threat to the poultry industry in North Bengal. He studied that the disease incidence in North Bengal is 16.7 percent whereas in India it is around 14 percent. Post-mortem findings were enlargement and darkening of liver which indicates impairment of liver function. The spleen was also damaged.

Das and Som (1992) studied the pathological changes occuring in induced *Escherichia coli* infection, 40 birds were taken into investigation. The gross changes were characterised by enteritis (35 quails) focal necrosis

and congestion in liver (15 quails). The congestion of gizzard, peritonitis were recorded in few cases (approx-7). Histopathology of intestine revealed extensive haemorrhagic enteritis together with linning desquamation and degeneration of linning epithelial cells. The structural details of the villi were lost, submucosa was oedematous and markedly infiltrated with heterophils. The changes in the liver were characterised by congestion, swelling of hepatocytes, extensive fatty changes, focal areas of necrosis and diffusely aggregation of lymphocytes.

Mohapatra (1992) had made an ellaborate study of Salmonella infection of quail. In *Salmonella gallinarum* infection he noticed that spleen was enlarged with the presence of patchy necrotic areas. The liver shows the lesions of perihepatitis with necrotic foci, the lesion of haemorrhagic enteritis in encountered in the intestine.

The lesions in case of other Salmonella infections includes congested, enlarged spleen, distended gall bladder, necrotic foci on liver and enteritis with acute congestion. In the intestine there was heterophilic infiltration, haemorrhages, infiltration of lymphocytes. Reticuloendothelial cell hyperplasia is also observed. In the liver there was perivascular lymphocyte proliferation with focal necrosis.

Patro *et al.* (1992) studied etiopathology of quail disease in Orissa. 650 adult Japanese quails were examined at necropsy. Enteritis was found to be one of the major cause of mortality. Necrotic hepatitis, Salmonellosis and Staphylococcal infection were also found in some cases.

Das and Panda (1993) made an ellaborate study on Salmonellosis in Poultry. Liver was enlarged and yellowish green in colour. Pancreas shows

minute necrotic foci. Mesentric vessels were congested along with fibrinous exudate. Liver showed haemorrhagic streaks.

Moharana *et al.* (1993,a) conducted an experimental trial of *Escherichia coli* infection in poultry. The histopathology of intestine showed catarrhal and necrotic enteritis characterised by hyperplasia of goblet cells, loss of villi, desquamation and degeneration of the linning epithelial cells of mucosa exposing the underlying submucosa which was oedematous and infilterated with heterophils. The section of liver showed generalised venous congestion with engorged central vein and sinusoids. The hepatic cells showed retrogressive change characterised by cloudy swelling and hydropic degeneration.

Mayahi *et al.* (1994) has studied the clinico-pathology condition of experimentally induced *Salmonella Virchow* infection in chicks. The gross pathological lesion comprised of congestion and pin-point greyish necrotic foci in the spleen and liver and petechiae on the intestinal mucosa. Histopathologically, focal parenchymal necrosis and perivascular, lymphocytic infiltration in the liver use observed.

Reddy and Koteeswaran (1994) has conducted an experimental *Escherichia coli* infection in Japanese quail, gross lesion such as enteritis, perihepatitis as well as other lesion were found.

Singh *et al.* (1996) reported some of the emerging discases of quails among which salmonellosis, fowl cholera, *Proteus mirabilis* infections coryza colibacillosis are the main bacterial diseases.

Kokosharov *et al.* (1997) observed clinical bacteriological and pathological conditions on experimental fowl typhoid. Liver was enlarged friable and hyperaemic with distended gall bladder Spleenomegaly and

catarrhal to haemorrhagic enteritis were also noticed. Histopathologically, liver revealed congestion, diffuse vascular degeneration and reactive necrosis.

Singh *et al.* (1997) reported the pathogenecity of different serogroups of *Escherichia coli* from poultry farms in Tarai region of U. P. In liver lesions of perihepatitis and hepatomegaly were recorded. Intestine revealed the changes of congestion, haemorrhages, blood mixed contents in the lumen and necrosis of mucosal epithelium.

Miguel *et al.* (1998) reported a case of subacute to chronic fowl cholera in a flock of pharoah breeder quail. Mortality rate was 13 percent during a 7-day period. The necropsy findings were emaciation, generalised carcas congestion, mild hepatomegaly with green discolouration, congested intestinal mucosae and thickened crop epithelium. Microscopically, there was amyloid deposits in the liver.

Pandey *et al.* (1998) had made an attempt to study the Avian colibacillosis outbreak in Lusaka, Zambia. The disease was diagnosed on the basis of gross post-mortem lesions.

ISOLATION OF BACTERIA FROM CASES OF ENTERITIS

Harris (1961) observed mortality in quail in U. S. A. Ulcerative enteritis was the main lesion in which coliform organisms was present.

Verma and Adalka (1971) isolated Klebsiella from different disease condition in poultry.

Berkhoff *et al.* (1974) isolated the causative anaerobe of ulcerative enteritis (quail disease). The causal bacterium was found to be anaerobe with

subterminal oval spores. Preliminary identification included colony characteristic staining ability, size and shape of vegetative cells and the location of spores.

Further Berkhoff *et al.* (1974) isolated an anaerobic bacterium from cases of ulcerative enteritis in chickens, pheasants and quails. According to biochemical characteristics and fermentation products it appeared to be a new species of pathogenic clostridium tentatively named *Clostridium colinum.*

Mohanty *et al.* (1979) isolated *Escherichia coli* involved in enteritis cases and also from healthy birds. The characteristic colony growth on Mac-Conkey's agar plates suggestive of *Escherichia coli* which were characterised on IMVIC and sugar fermentation reactions.

Srinivasan *et al.* (1979) made an attempt to asses the prevalence of *Escherichia coli* associated with different disease condition in chickens, turkeys, ducks and quails with a view to conform their identify, Gram-negative bacilli were subjected to detail bacteriological examinations including biochemical tests.

Bergmann *et al.* (1980) reported *Staplylococcus aureus* infection of fowls. He reported that the incidence of infection increased from 5.49% in 1975 to 28.95% in 1978.

Gupta *et al.* (1981). found out the effect of *Salmonella typimurium* on viscera of poultry birds when injected intravenously. The lesions observed were petechiae on serous surfaces, jaundiced liver, petechial haemorrhages in Peyer's patches, catarrhal enterites, congestion and enlargement of liver mainly. Changes in other organs were also noticed.

Sah *et al.* (1983) isolated *Proteus mirabilis* from heart, blood, liver and lungs of affected quail at IVRI, Izatnagar. India.

Sinha *et al.* (1985) isolated *Escherichia coli* 41.7 percent from intestine, heart blood, liver, lungs and spleen of birds.

Pranjape and Das (1985) investigated prevalence of bacterial infections in poultry farms of Bombay. Materials comprised of heart blood, spleen and lung. Prevalence of Colibacillosis was highest 37.6%. Salmonellosis 15.59%, Pasteurellosis 9.4%, Proteus infection 6.43%, Pseudomonas 3.96%, Klebsiella 1.98%, Shigella 0.24%, Staphylococcus 2.72%, Bacillus 0.99% and Streptococcus 0.74%.

Dwivedi and Sodhi (1987) isolated *Pasteurella multocida* strains from various poultry farms of the Punjab from healthy and apparently disease birds.

Myint (1987) reported *Proteus mirabilis* infection in quail in Burma.

Sandhu *et al.* (1987) dealt with eight outbreaks of fowl typhoid in different poultry farms of Punjab during the year 1986-1987. Direct culture on MLA yielded lactose negative colonies after 18-24 hours which was subsequently confirmed as *Salmonella gallinarum*.

Shimizu *et al.* (1987) isolated 100% coagulase negative *Staphylococcus epidermidis* from quail in Japan.

Minakshi *et al.* (1988) isolated *Campylobacter jejuni* from quails for the first time. From 23 domestic quails four isolates (17.39%) of Campylobacter were recovered.

Panda & Verma (1988) isolated coliform organisms from lesion area in chicken. Confirmatory diagnosis was done by isolation of *Escherichia coli* from heart blood of sick birds with special media.

Sharma *et al.* (1988) reported heavy mortality in Japanese quail due to *Salmonella gallinarum* infection.

Gupta and Verma (1989) made an effort to provide an overall picture about the frequency of various Salmonella serotypes in avian hosts on the basis of National Salmonella centre at I. V. R. I. Total of 170 salmonella strains were identified during the period (1977-86) from avian sources from different parts from India and Nepal.

Saini *et al.* (1989) isolated *Salmonella gallinarum* from 11 sample out of 20 samples suspected for fowl typhoid was received from different poultry farms.

Silva *et al.* (1989) isolated *Escherichia coli*, the causative agent of Coligramulomatosis in a Brazilian flock of 1500, 8-12 months old *Coturnix quail.*

Mathew and Sulochana (loc.cit.) reported an outbreak of Salmonellosis in a government owned poultry farm where chicken and quails were reared simultaneously.

Ahmed and Sarkar. (1991) studied that the incidence of *Escherichia coli* infection among poultry in North Bengal was 16.7 percent whereas in India it was arround 14 percent.

Katoch *et al.* (1991) isolated Salmonella and characterised Salmonella serotypes prevalence among poultry in Himachal Pradesh. He found out that in Himachal Pradesh *Salmonella gallinarum* is chiefly involved in causation of Salmonellosis in poultry.

Misra *et al.* (1991) analysed 84 samples from enteritis cases in quail in Bhubaneshwar, Orissa. On bacteriological examination 79 yielded bacteria and rest were found to be contaminants. The bacteria isolated were

Staphylococcus aureus (39.28%), *Escherichia coli* (28.57%) *Streptococcus sp* (39.9%) *Klebsidla sp* (21.42%) *Salmonella sp* (10.71%) *Proteus sp* (8.33%) and *Shigella sp* (16.66%) in pure or mixed form.

Patro *et al.* (1992) reported some cases of Staphylococcal infection in quail in Orissa. 650 adult Japanese quails were examined at necropsy. Enteritis was found to be one of the major cause of mortality. Necrotic hepatitis, Salmonellosis and Staphylococcal infection were also found in some cases.

Raja Rajeshwari *et al.* (1992) conducted bacteriological examination of 65 samples collected from respiratory, intestinal tracts and oviducts of 30 healthy Japanese quails and isolated 305 bacterial organisms. Micrococcus (81.53%) Staphylococcus (27.69%), Streptococcus (33.84%), Bacillus (86.15%), Corynebacterium (27.69%), Listeria (4.16%), Erysipelothrix (1.53%), Escherichia (52.3%), Shigella (18.46%), Citrobacter (12.3%), Klebsiella (23.06%), Enterobacter (27.69%), Proteus (38.46%) Providencia (12.3%), Pseudomonas (4.61%), Pasteurella (6.15%) and Alealigens (10.76%). There was predominance of Gram-positive organisms 171 (56.06%) over the Gram – negative 134 (43.94%). *Escherichia coli* was found to be highest 34 (11.15%) among Gram-negative organism.

Das and Panda (1993) made an ellaborate study on Salmonellosis in Poultry. Liver was enlarged and yellowish green in colour. Pancreas shows minute necrotic foci. Mesentric vessels were congested along with fibrinous exudate. Liver showed haemorrhagic streaks.

Moharana *et al.* (1993,b) reported *Proteus sp., Shigella sp.* in poultry from enteritis cases.

Reddy and Koteeswaran (1994) isolated the relative susceptibility of Japanese quails to serotypes of pathogenic *Escherichia coli* isolated from chicken from Namakkal area.

Reddy *et al.* (1994) isolated and identified *Escherichia coli* from poultry from different pathological conditions such as Colisepticaemia, enteritis, omphalitis and necrotic hepatitis.

Singh *et al.* (1996) reported *Proteus sp* infection as one of the emerging bacterial diseases in quail.

Itoh *et al.* (1997) examined intestinal microflora of the quail. The total number of facultatively anaerobic are aerobic bacteria in the intestine were $10^{5.9}$ to 10^{9}/g being smaller in the upper intestine *streptococcus* including *Enterococcus; Lactobacillus* and *Peptococaceae* were the main bacteria found in the intestine.

Shoba *et al.* (1997) detected staphylococcus infection in Japanese quail in a breeder farm at Namakkal, India.

Miguel *et al.* (1998) isolated *Pasteurella multocida* from flock of 1300 of breeder pharaoh quail.

Pandey *et al.* (1998) isolated *Escherichia coli* from poultry in the outbreaks from 1993-95 in Lusaka, Zambia. He concluded that November to March was the peak period of Colibacillosis outbreaks among broilers in Zambia.

Jones *et al.* (2000) studied the extent of Escherichia coli infection in poultry in Mahaboob nagar district of Andra Pradesh. Isolation and identification of *Escherichia coli* was undertaken in 250 field cases of Colisepticaemia, omphalitis, peritonitis, enteritis and coligranuloma.

Chapter • 3

MATERIALS AND METHODS

MATERIALS AND METHODS

Source of Materials :

A total number of 120 naturally dead quails were taken in this investigation. The samples were collected from Central poultry farm, Patna and local meat shops.

For pathological study and bacterial isolation the different parts of the digestive tract of quails was examined carefully for any gross lessions. The tissue pieces were collected aseptically and kept separately in sterilized petridishes for bacterilogical examination Another pieces of tissue were collected separately in glass container and presevered in 10% formal saline solution for histopathological studies.

Materials for histopathology :

For histopathological examination 0.5 cm. thick tissue pieces from the collected samples were preserved in 10 percent formal saline solution.

Preparation of histopathological sections of tissue :

After proper fixation tissues were subjected for washing in running tap water. After proper washing the tissues were dehydrated by using ascending grades of alcohol and acetone and cleared in benzene. The dehydrated tissue were passed through three sets of paraffin for embedding. After proper infiltration of paraffin into the tissues, paraffin blocks were prepared. The tissue sections were cut at 5-6 microns in thickness with the help of rotary microtome and slides were stained with haematoxylin and eosin stain (Luna,

1968). Special staining for showing the different constituents as well as bacteria were as follows :-

1. Periodic-Acid schiff (PAS) staining for demonstrating neutral mucopolysaccharides (Culling, 1974).
2. Ziehl-Nelsen staining method for demonstating acid fast bacteria (Luna, 1968).
3. Mucicarmine for demonstrating epithelial mucin (Luna, 1968).
4. Brown and Brenn method for demonstrating Gram-positive and Gram-negative bacteria (Luna, 1968).

Isolation of Bacteria:

Small pieces of intestines (different parts) were inoculated in a test tube containing nutrient broth and tetrathionate broth for *Salmonella sp.* separately which acted as enrichment medium. Nutrient broth tubes were incubated at 37^0C for 24 hours where as tetrathionate broth was incubated for 8-12 hours Sub culture from nutrient broth was done on blood agar, nutrient agar and Mac-Conkey's agar plates and the tertrathionate broth on Mac-Conkey's agar or Desoxycholate citrate agar plates. These were incubated at 37^0C for another 24 hours.

After incubation, the plates were examined for types of growth and also cultural characters e.g. colony characters etc. Different types of representative colonies were obtained in pure form on nutrient agar slants by conventional methods and this process was repeated till a single similar type of colonies were obtained and kept at 4^0C for further study.

Identification of the Isolates :

The identification of the isolates obtained in pure form was done as described by Cruick shank et al. (1975). Briefly the identification was based on the staining characters, motility, different biochemical tests and sugar fermentation tests of the isolates.

Morphology :

Smears from all representative colonies were made on clean, dry microscopic slide and stained with Gram's stains.These stained smears were examined under oil immersion lens of microscope for staining characters, their shape and arrangements.

Motility :

The motility of the organisms was examined by means of hanging drop method in 12-15 hours broth cultures as described by Cruickshank *et al.* (1975).

Biochemical reactions :

The suggestive colonies were subjected to various biochemical and sugar fermentation tests. Biochemical test used to characterized the different enteric bacteria are IMViC test.

1. ***Sugar fermentation :*** Break down of sugar is tested in various sugar media-glucose, lactose etc. Acid production is indicated by development of pink colour of the medium and gas produced accumulates in Durham's tube.

old culture in glucose phosphate broth (buffered glucose broth), 4 to 5 drops of 0.04% methyl red solution is added, mixed well and read immediately. Positive reactions are bright red (indicating persistent acidity) and negative are yellow (significantly new or transient acidity).

3. ***Voges Proskauer (VP) test- (Barrits method)*** – In a 48 hours growth in 2.5 ml glucose phosphate broth 0.5 ml of 40% KOH and 1.5 ml of 5% alphanaphtal in absolute alcohol are added. In a positive test, deep pink colour appeared in 2 to 5 minutes which deepens into magenta or crimson colour in 30 minutes. The media remained colourless in negative test. For maximum aeration the tube was to be shaken at intervals.
4. ***Citrate utilization :*** Simmon's citrate medium was inoculated with fresh nutrient broth culture and incubated for 48 hours at 37^0C. Results were noticed every 24 hours. Streaks of growth and blue colour (due to change of pH) denote positive reaction.
5. ***Indole Production :*** Bacteria are grown in tryptophan rich peptone for 48 to 96 hours in incubator. To the bacterial growth in peptone water, 0.5 ml. Kovac's reagent (Paradimethyl aminobenzaldehyde – 10g, amyl or isoamyl alcohol-150 ml, Conc. HCL-50 ml) is added and gently shaken. A red colour near the surface indicates positives reaction.
6. ***Hydrogen sulphide production :*** Organisms were grown in peptone water and a lead acetate strips (filter paper soaked in 10% lead acetate solution and dried) is inserted between the cotton plug and culture tube suspending it above the culture. The lead acetate strip turns black which indicates H_2S production.
7. ***Phenyl alanine deaminase test (PPA)*** – Nutrient agar slope containing DL – phenylalanine is inoculated with a fairly heavy inoculum of culture

and incubated at 37^0C for 24 hours. A few drops of 10% ferric chloride solution is added, positive reaction is indicated by development of green colour in the medium and in the fluid. Certain bacteria (Proteus group) produce green colour.

8. ***Urease :*** Christensen's urea medium (peptone water, urea, phenol red) slants were inoculated heavily with the proper culture. A 4 to 24 hours growth of an organism acquired pink colony by urease producing organisms which changed colour of indicator. Urease production is not to be considered negative till a 4-day old tested as some of the members of certain groups other than proteus can give delayed reaction.

9. ***Oxidase reaction :*** The organisms were cultured on a solid medium like nutrient agar and then a freshly prepared 1 to 1.5 per cent solution of tetra-methyl-p—phenylene diamine hydrochloride was poured on the surface of colonies and the excess amount was thrown out. In positive reaction, the colonies become maroon, purple and black in 10-30 minutes.

10. ***Catalase production :*** With the help of platinum loop, the H_2O_2 was inoculated on colonies on nutrient agar. Prompt effervescence indicated production of catalase. Positive catalase reaction shows production of gas bubbles almost immediately.

11. ***Nitrate reduction test :*** This test was used to detect presence of enzyme nitrate reductase present in almost all enteric bacteria which reduce nitrate to nitrite. In a 5 days growth in broth containing KNO_3, 0.1 ml of test reagent was added. In positive, red colour developed with in a few minutes.

12. ***Coagulase test :*** For differentiation of *Staplylococcus species* coagulase test was done. A small amount of solid culture was emulsified in a drop

of saline on a clean slide to form a smooth suspension. A loopful of rabbit plasma was added into the bacterial suspension on the slide and was stirred well Coarse clumping becoming visible to the naked eye within 9-10 seconds and was taken as positive results. Coagulase positive were identified as *Staphylococcus aureus* where coagulase negative as *Staphylococcus epidermidis*.

Chapter • 4

RESULTS

RESULTS

Recently, the Japanese quail has been introduced in India. Seeing its importance to meet the meat requirement of the over growing population of India. Nowadays, Japanese quail are used as commercial line as its meat it comparatively tender having better taste and fat in comparision to chicken and as such many people are coming forward to take quail as major commercial industry. Hence the present study has been made to know the pathological changes occuring in different parts of digestive tract particularly bacterial enteritis.

In the present investigation altogether 120 dead quails were brought to the laboratory for detail pathological observation and isolation purposes. Out of 120 birds only 90 showed some gross abnomalities in their digestive tract which were preserved and processed for histo-pathological examinations. Out of 90 specimens only 72 samples were showing different types of lesions. The different pathological changes encountered on microscopic examination are shown in Table :1.

TABLE – 1

Showing various types of intestinal affection observed in 72 quails :

Sl. No.	Types of intestinal affection	Total no.	Percentage	χ^2_6df
1.	Mucoid enteritis	12	16.66	61.04**
2.	Catarrhal enteritis	22	30.35	
3.	Haemorrhagic enteritis	16	22.22	
4.	Ulcerative enteritis	10	13.88	
5.	Suppurative enteritis	8	11.11	
6.	Chronic Proliferative enteritis	1	1.38	
7.	Necrotic enteritis	3	4.16	

**Significant at P<0.01.

(1) **Mucoid Enteritis :** Twelve out of seventy two (16.66%) specimens of intestine showed the changes of mucoid enteritis. The changes varied from mild to subtle form.

Grossly, the affected intestine mostly, the small intestine showed mild congestion of the blood vessel in their serosal layer (Fig. 1). The intestine also appeared slighly swollen. On opening the lumen, there was presence of faecal mass mixed with mucous material which was slimy to touch. The mucous membrane also appeared to be studded with some mucin-like flakes. On removal of those mucous mixed faeces the lining epithelium appeared reddened.

Types of intestinal affection

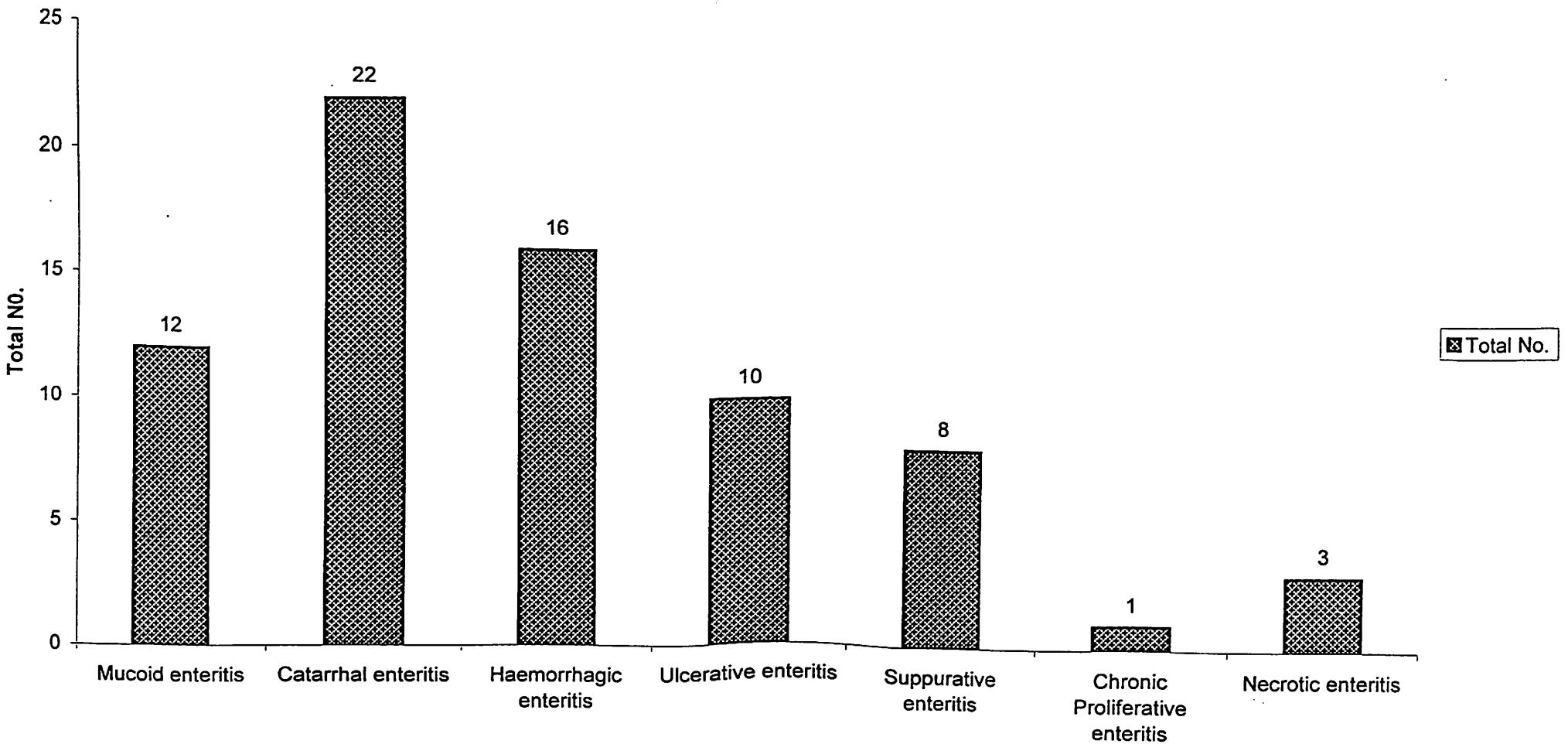
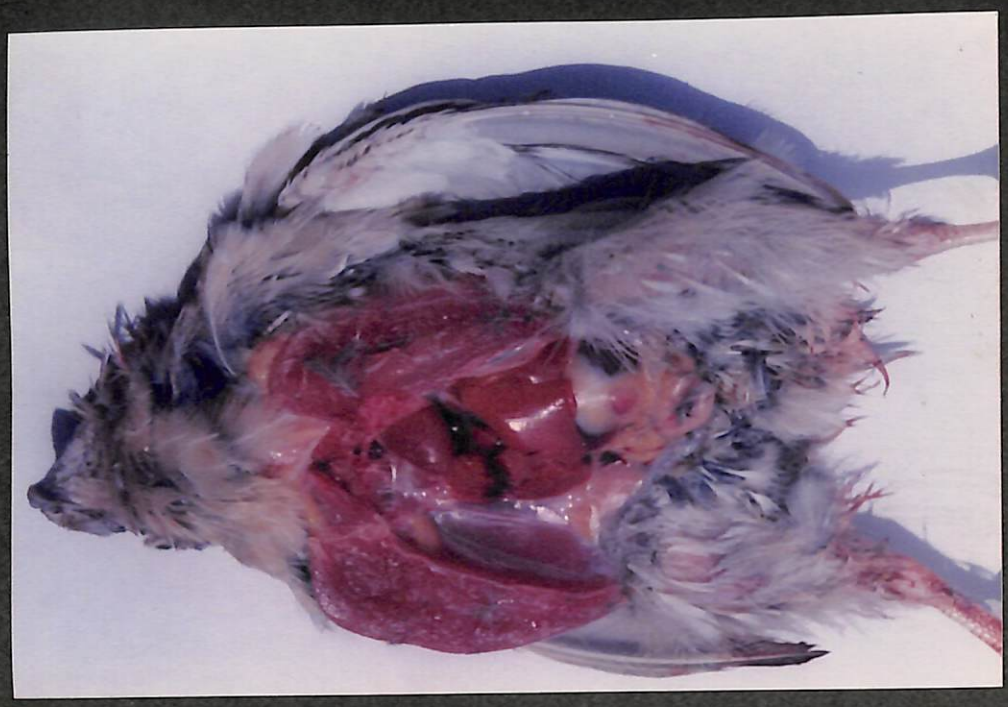
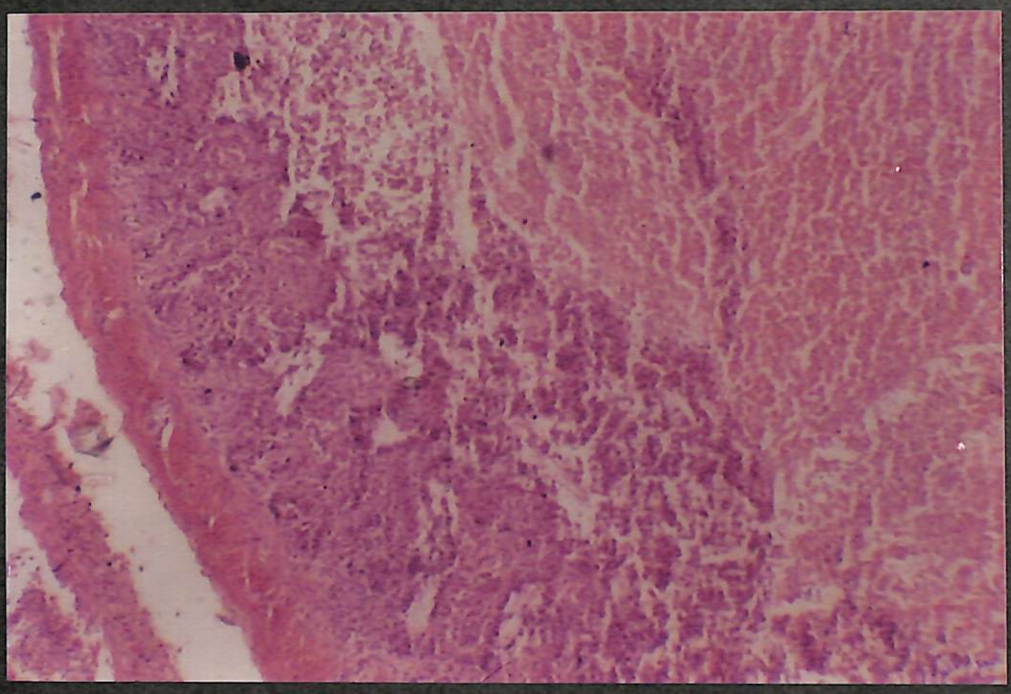


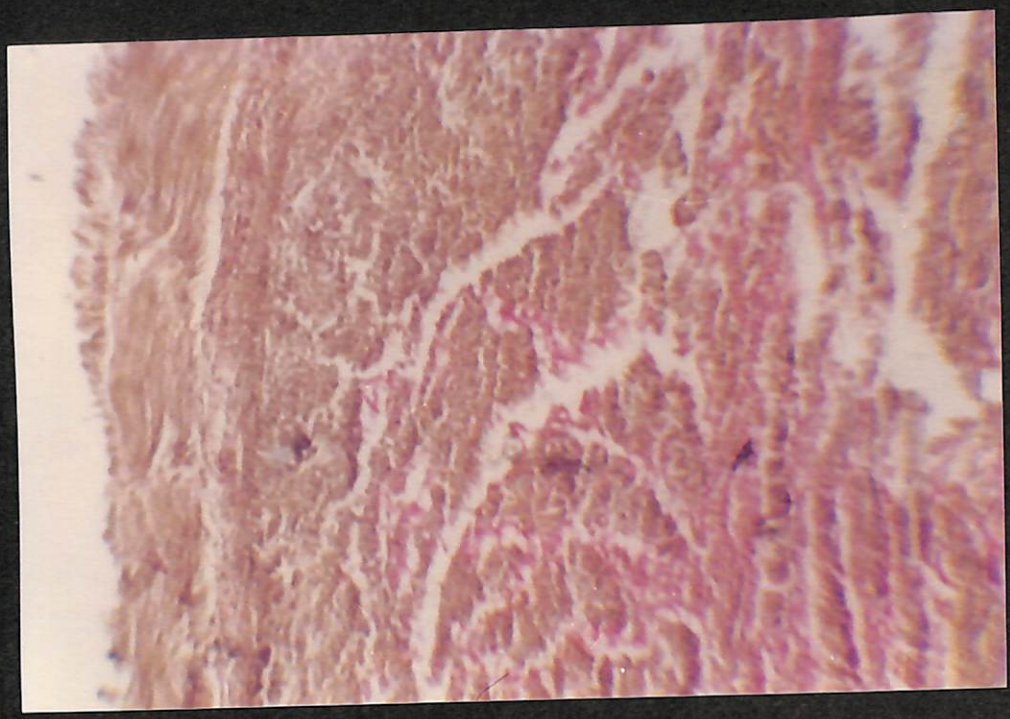
DIAGRAM-1

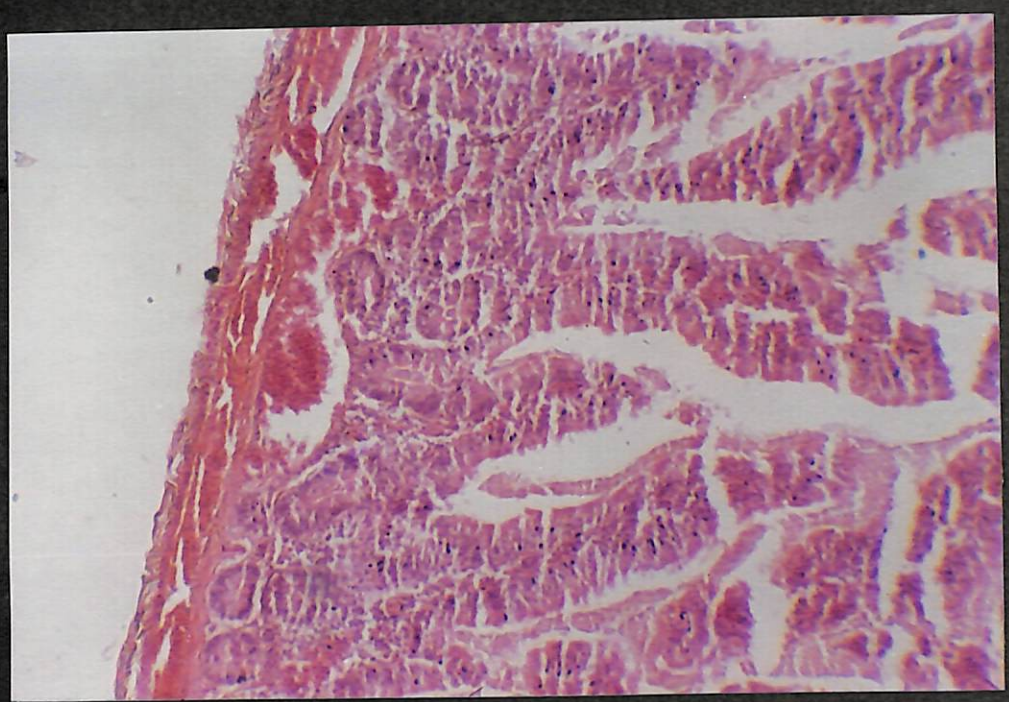




Microscopically, the mucoid enteritis was characterised by presence of few mucous in the goblet cell as well as in the Brunner's glands. (submucosal glands of the duodenal region). There were few heterophils and mononuclear cell infiltration in the lamina propria of the affected intestine (fig. 2).

(2) Catarrhal Enteritis :- Twenty two (30.55%) of intestine showed changes of catarrhal enteritis which varied from acute to chronic form. This is very common form of enteric inflammation producing a variety of morbid appearances. Acute catarrhal enteritis is the severe form of inflammation which cause excessive secretion of mucinous materials. Catarrhal enteritis was found to be occuring in a diffuse manner which affected throughout the intestine. This type of enteritis however, was more severe at one end of the small intestine or the other. Grossly, the mucosal layer was reddish in colour and was covered with a mucinous exudate. The mucinous exudate appeared clear which was slimy and may described as mucous. The wall of the intestine was thickened. The mucosa was found to be smooth and thickned. In few cases the intestinal content were found to be mixed with some desquamated cellular debris. In acute fatal cases of Catarrhal enteritis, there was copious fluid in the lumen consisting of a serous or watery exudate which were yellowish or white in colour. These flocules of materials were mucin with desquamated epithelial lining. Acute catarrh with a longer course tended to become purulent and the lumen of the small intestime contained a thin creamy exudate which was copious in quantity. In more prolonged course the volume of the mucin produced was greater and it was





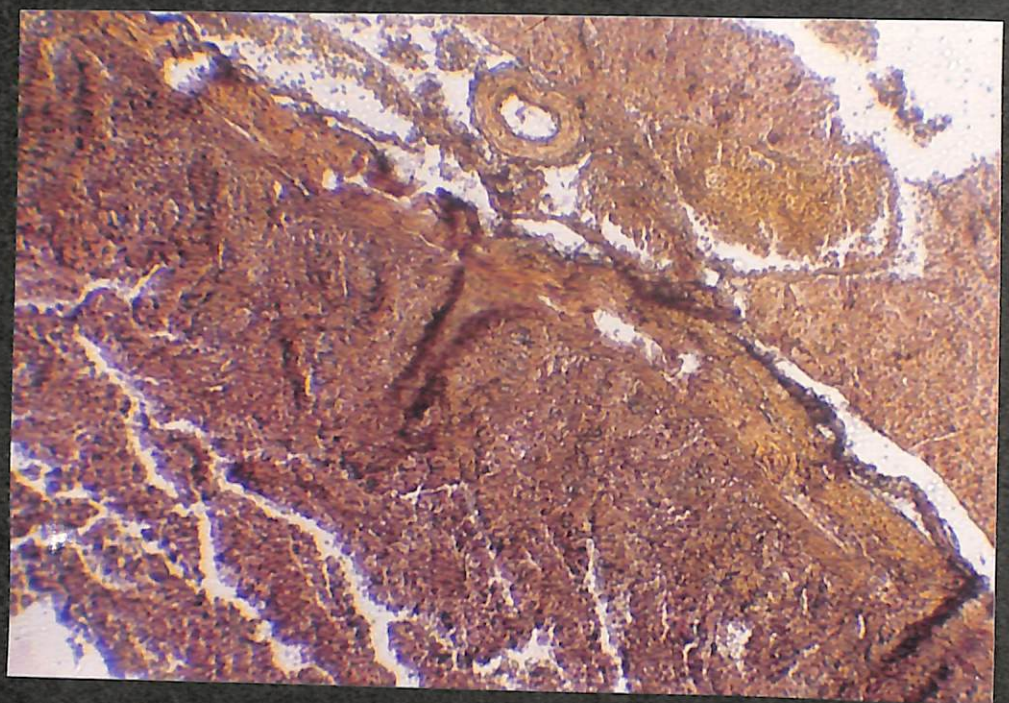
characterised by a tenacious semitransparent mucus which clings tightly to the mucosal surface.

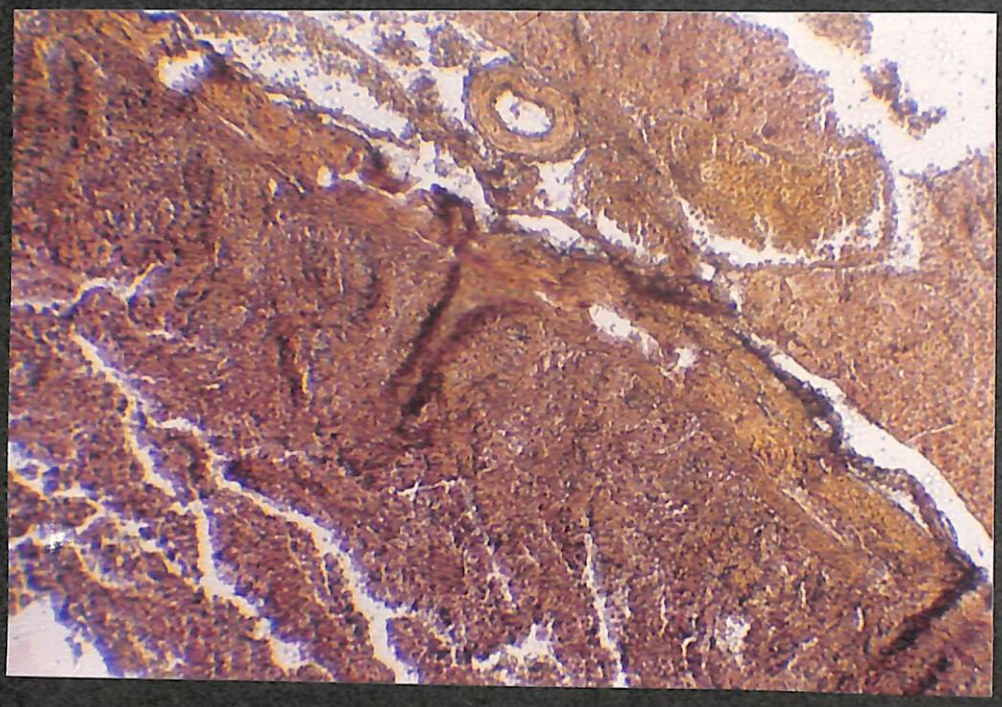
Microscopically, the oedema of the intestine was observed. There were infiltration of few heterophils in the lamina propria and to a little extent in the sub-mucosa. Epithelial desquamation and the infiltration of leucocytes were usual. Plasma cells were the most frequently noticed.

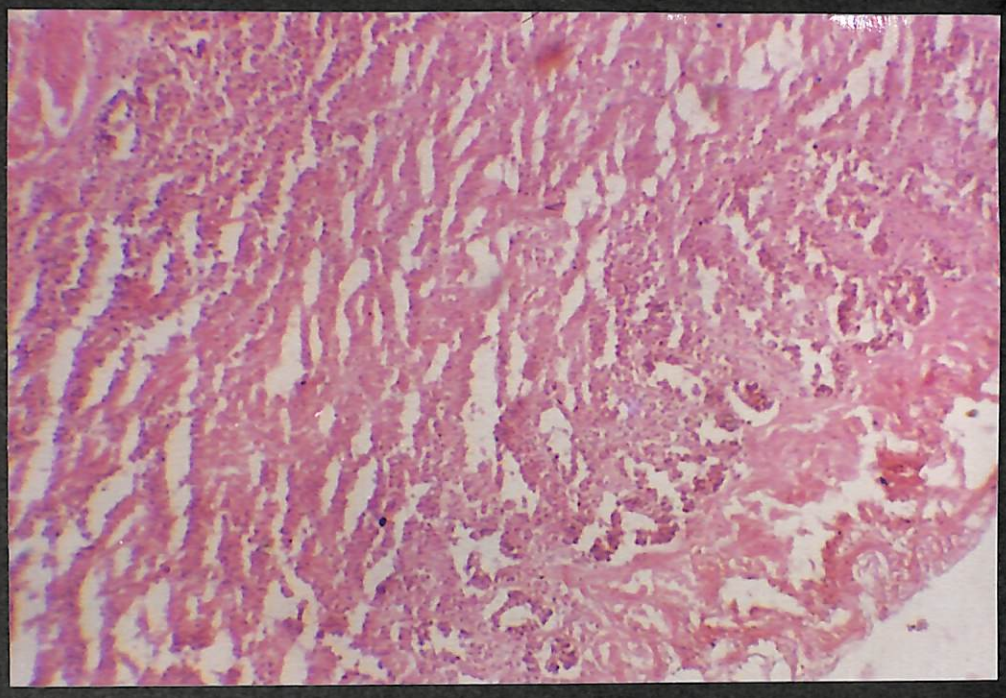
In few section, mononuclear cells chiefly lymphocytes were noticed. Commonly the excessive mucus was readily visible as blue strands of mucin. Goblet cells were numerous in number and were found to produce large amount of mucinous materials. In many cases however, the important feature was noticed to excess of mucinous cells which appeared pink in colour when stained with mucicarmine method (fig. 3). Rather frequently this became the predominant features of catarrhal enteritis leaving the affected mucous membrane denuded from its epithelial covering. There was hypereamia, oedema and cellular infiltration in the lamina propria. The tips of the villi were found to be reddened and swollen. (Fig. 4).

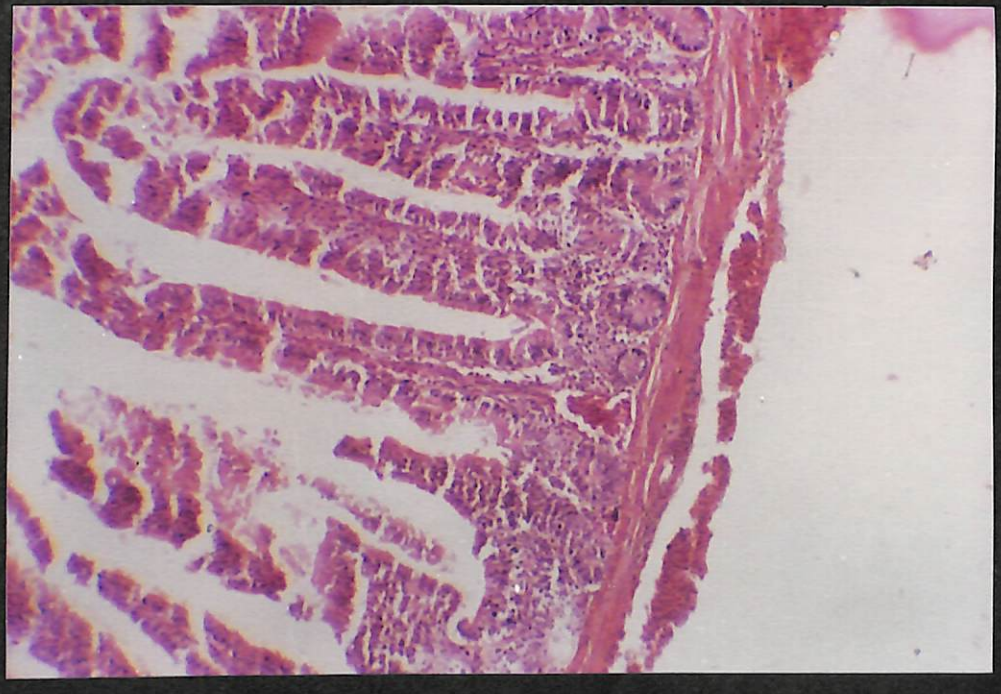
3. Haemorrhagic enteritis : This is the more severe form of enteritis characterised by the presence of red blood cells in the different layers of affected intestine. Sixteen (22.22%) intestine pieces showed the pathological changes of haemorrhagic enteritis.

Grossly, they were characterised by superficial extravasation and staining of the ingesta with blood. The mucosa of the small intestine and large intestine was severely congested and haemorrhagic. There was oedematous thickening of the wall of the bowel which extended into the mesenteric attachment (Fig. 5). In some cases the peritonium was swollen









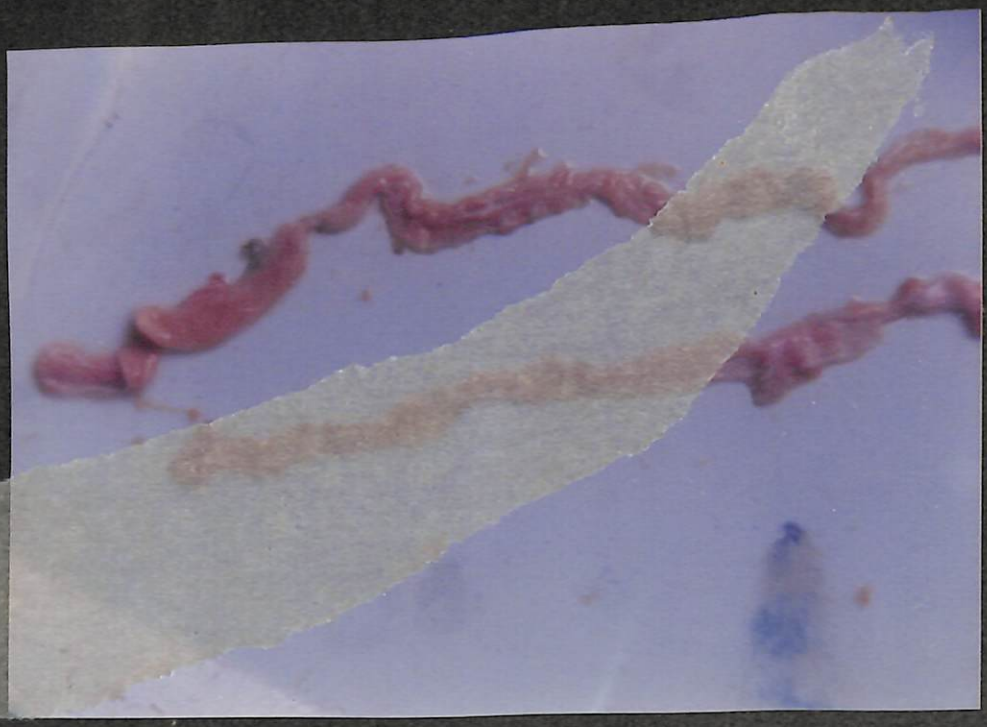
which were diffusely reddended. The anterior protion of the affected intestine was brown coloured, showed some whitish materials where as posterior of the intestine appeared slightly brigther red in colour. When the intestine was opened there was presence of blood tinged dirty material in the lumen. The mucus membrane was highly reddened.

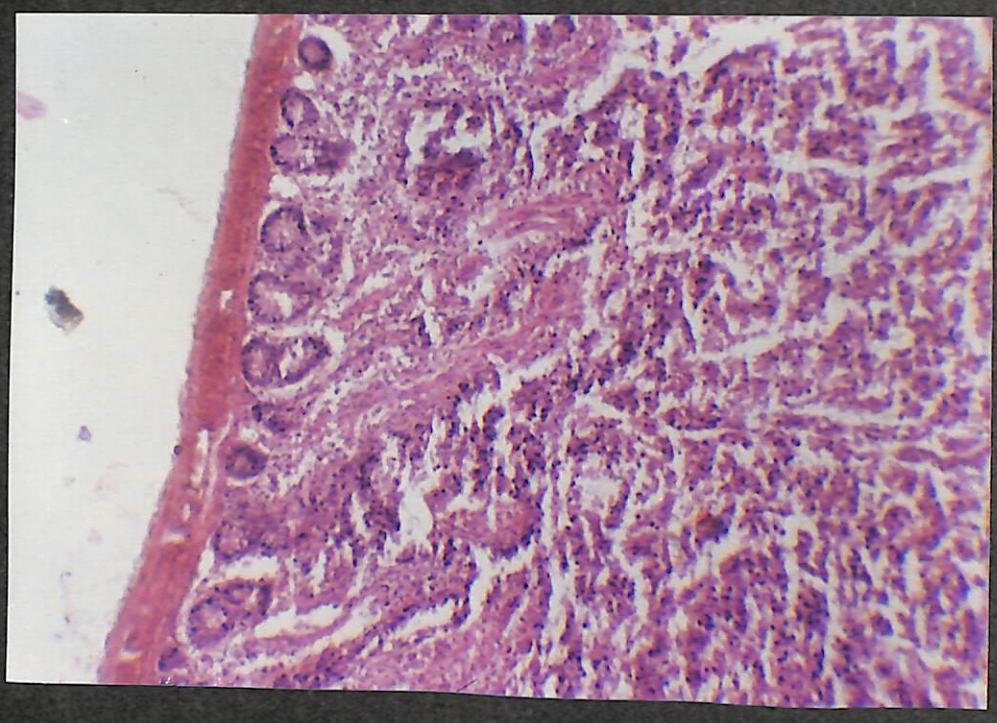
Microscopically, this type of enteritis in the present study was characterised by focal infiltration of red cells in the submucosal and mucosal layer of the intestine.

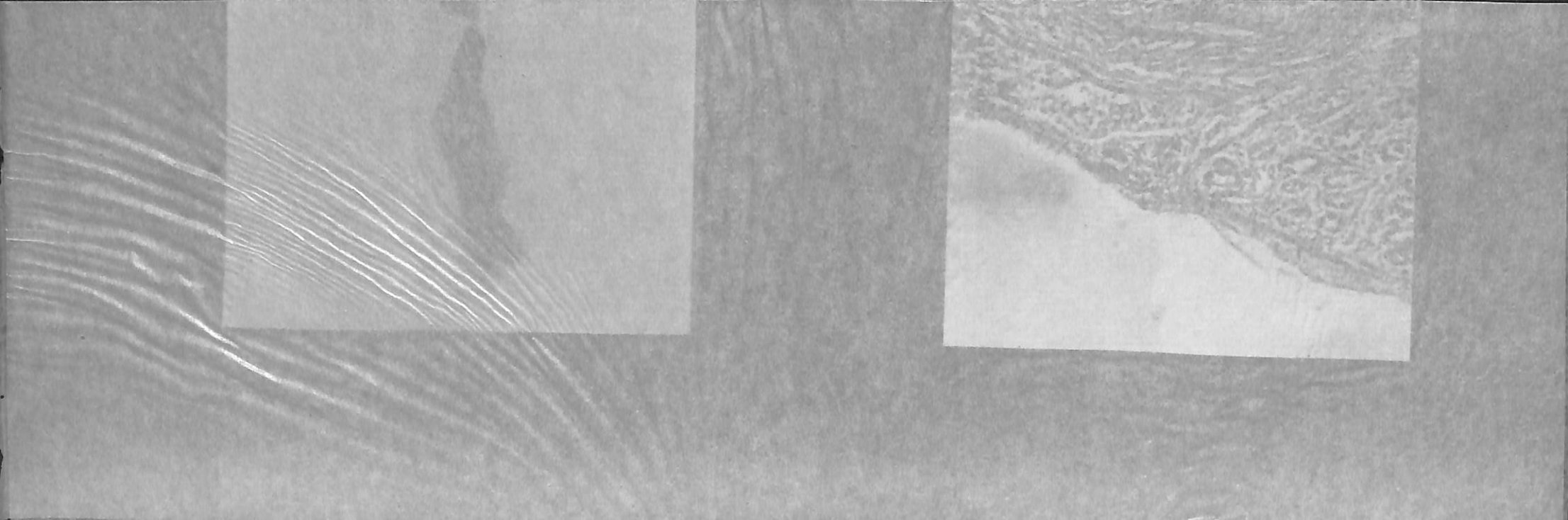
Such changes was mostly observed in the jejunum and ileum. The blood vessels were found to be ruptured at places and few showed thickening of the middle layer of the blood vessels (Fig. 6). The lining epithelium was almost erroded from their basement and the tip of the villi appeared blunt and rounded. They were marked pitting of the villi in some of the intestinal section (Fig. 7-8). This type of enteritis was is the severe from the catarrhal type and such too mucous cells were also noticed in the submucosal glands which showed positive reaction when stained by mucicarmine method. In one section of the intestine the blood vessels was found to be highly distended and engorged with the blood.

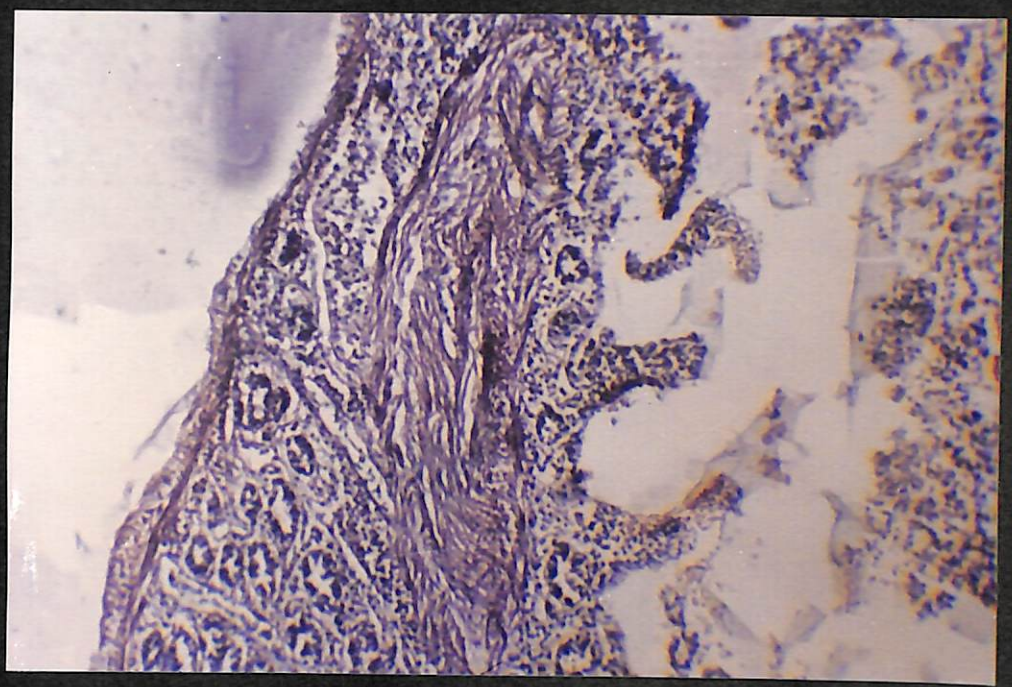
4. Ulcerative enteritis : Ten out of 72 samples (13.38%) showed the pathological changes of Ulcerative enteritis.

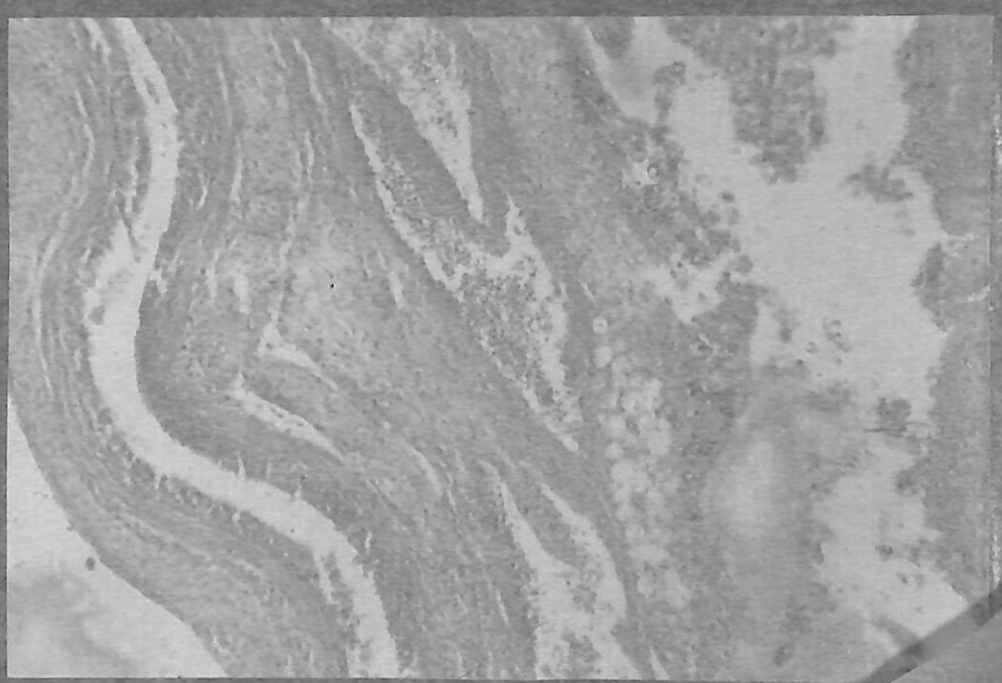
Grossly, the lesions include the ulceration of the small intestine, as well as the caeca and upper portion of the large intestine. Acute lesions were characterised by marked haemorrhagic changes mostly in the duodenum. Small penetrated haemorrhages were seen in the intestinal wall in some cases which was followed by necrosis and ulceration. Intestinal mucosal











FI... – 15

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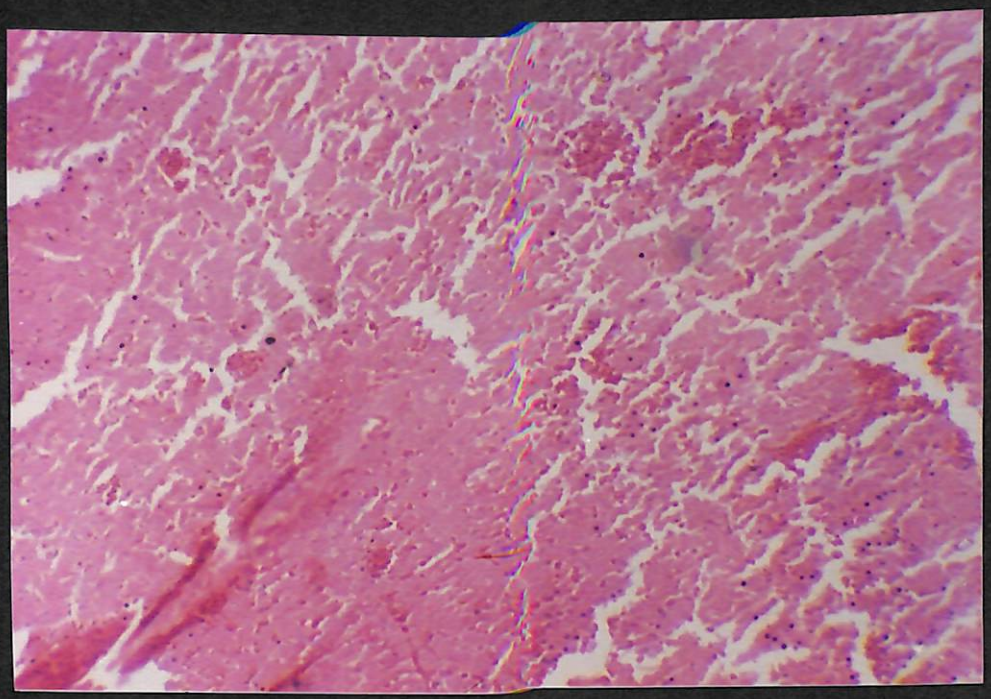

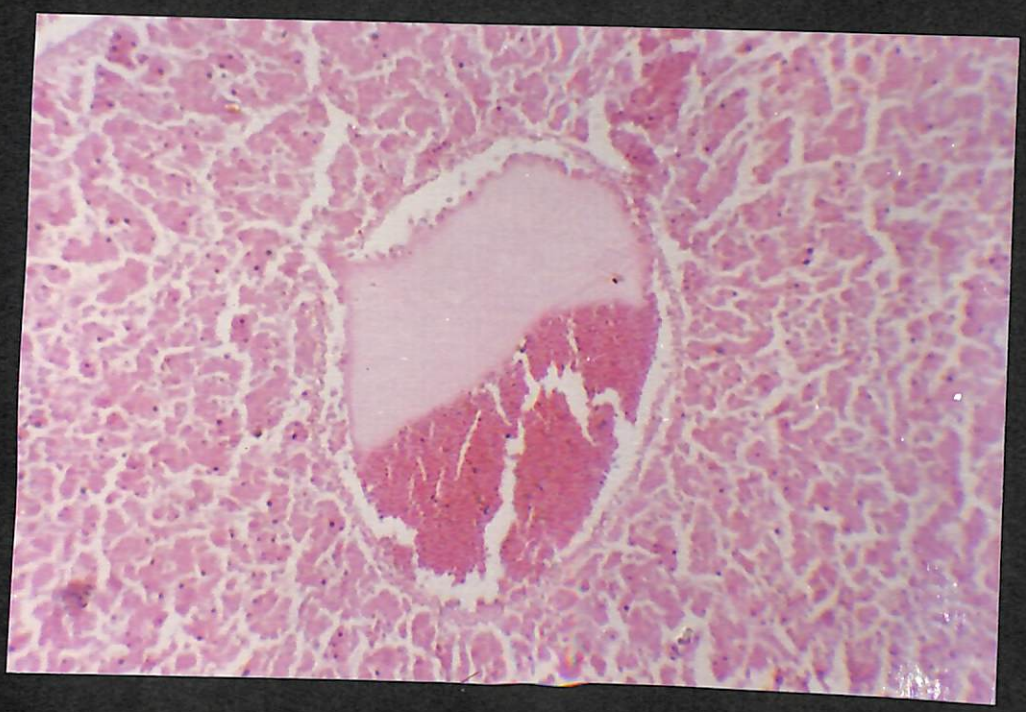

necrosis of hepatocytes has been seen at places. Perivascular lymphocytic infiltration in portal areas. Focal neerosis was also noticed (Fig. 18).

Proventriculus – In four cases congestion of proventriculus was noticed..

On microscopic examination necrotic lesions were noticed. There was desquamation and degeneration of subrugrosal glands. The proventriculuar subrugrosal glands appeared to be separated with proliferation of connective tissue in the interglandular spaces in some cases (Fig. 19,20).

Microbiological study :

Bacteriological isolation from enteritis cases :

Intestinal tissues of 72 quails grossly suspected for enteritis, were processed for bacterial isolation. For the isolation purposes the different types of culture media were used viz Nutrient broth, Nutrient agar, Mac-conkey's agar, blood agar media and tetrathionate broth. The nutrient broth appeared hazy suggesting the bacterial growth. The broth cultured was subcultured on the Mac-Conkey's agar plates gave pink colour colony after 24 hrs. of incubation (Fig. 21 & 22) Subculture of tetrathionate broth was done on Mac-Conkey's agar plates or Desoxycholate citrate agar.

Different types of organisms were isolated from 50 cases only. The details of the isolates with associated intestinal tissues changes is summarised in Table 2.

Escherichia coli was isolated from 19 (38%) of the intestinal tissue examined. Next in order of prevalence were *Staphylococcus species* which

was isolated from 9 (18%), *Klebsiella sp.* 6 (12%), *Proteus sp.* 5 (10%), *Shigella sp.* 4 (8%), *Streptococcus sp.* 2 (4%) and Gram-positive as well as Gram-negative *Bacillus sp.* were isolated from 5 (10%) cases.

Table – 2

Bacteria isolated from enteritis cases of Japanese quails.

Sl. No.	Type of Bacteria	No.	Percentage	Types of enteritis observed histopathologically	χ^2_6df
1.	*Escherichia coli*	19	(38%)	Haemorrhagic enteritis, Catarrhal enteritis Necrotic enteritis.	
2.	*Staphylococcus spp.*	9	(18%)	Suppurative enteritis, Catarrhal enteritis Ulcerative enteritis	
3.	*Klebsiella sp.*	6	(12%)	Mucoid enteritis, Catarrhal enteritis, Chronic proliferative enteritis.	26.728**
4.	*Proteus sp.*	5	(10%)	Catarrhal enteritis, Suppurative enteritis.	
5.	*Shigella sp.*	4	(8%)	Suppurative enteritis, Catarrhal enteritis.	
6.	*Streptococcus sp.*	2	(4%)	Haemorrhagic enteritis.	
7.	*Bacillus sp.*	5	(10%)	Necrotic enteritis, Suppurative enteritis.	

**Significant at P<0.01

Bacteria isolated from enteritis cases of Japanese quails.

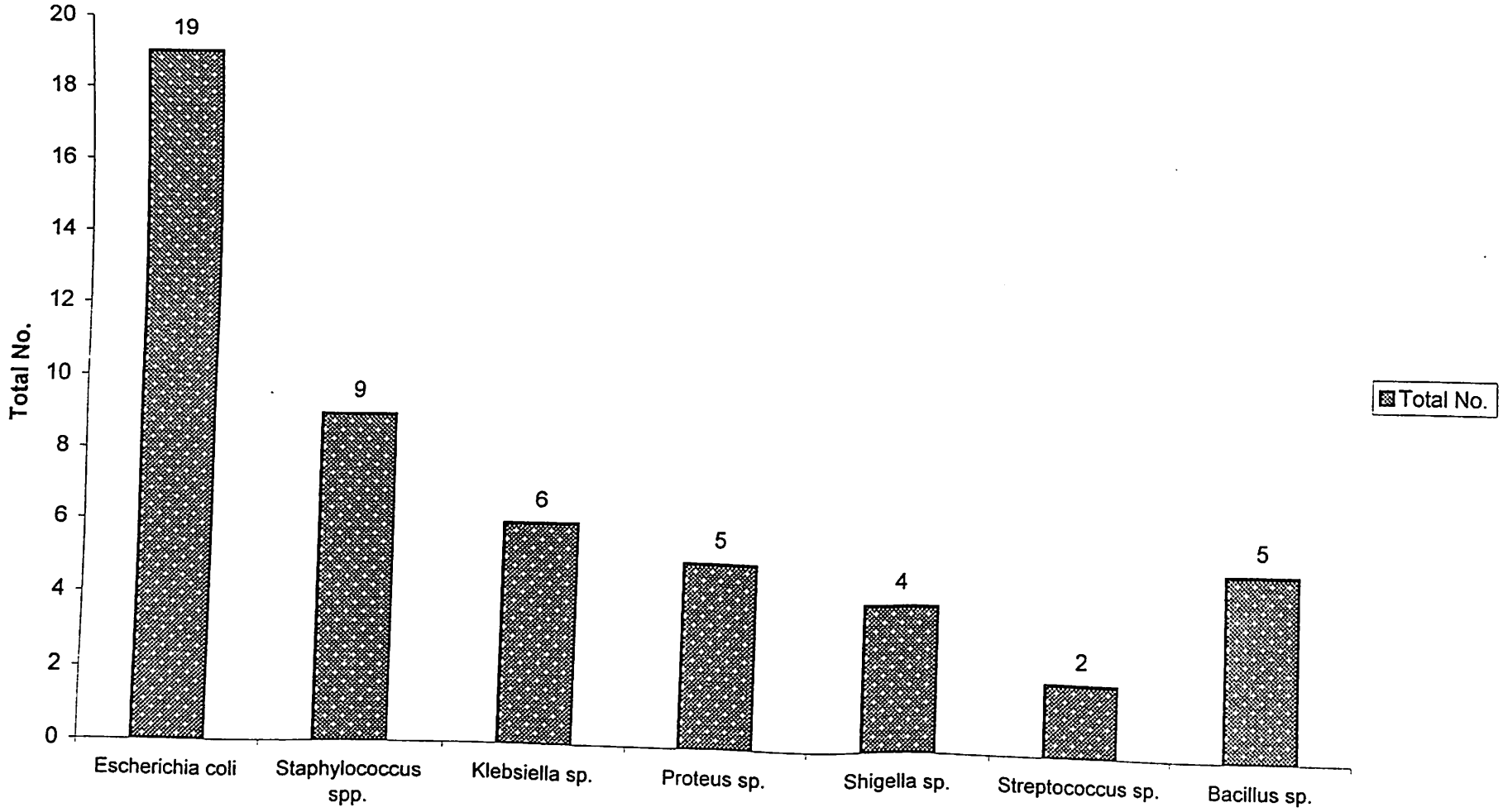

DIAGRAM-2

Chapter • 5

DISCUSSION

DISCUSSION

Quails are considered to be more resistant than chickens However, they are susceptible to the various bacterial diseases such as infection of *Escherichia coli, Ulcerative enteritis*, Salmonellosis and Fowl cholera. Perusal of the literatura revealed that though much works has been done with regard to the husbandry aspects of quail, the information on disease condition in quails are not. There are some reported incidence of bacterial isolation from quail but still there is scanty work done on the pathology of digestive system of quail in India and specially in this part of Bihar. In the present study an attempt has been made to study the pathological changes affecting the digestive system particularly in case of bacterial enteritis.

Intestine was the most affected part, therefore, extensive study has been made on this very part of the digestive system.

PATHOLOGY OF DIGESTIVE SYSTEM IN QUAIL

1. MUCOID ENTERITIS

In the present study twelve out of seventy-two (16.66%) of the intestinal specimen revealed the changes of mucoid enteritis which was characterised by presence of mucinous materials in the intestinal lumen. Microscopically, there was presence of mucous in the goblet cell in the affected intestine. The submucosal glands also appeared to be very active with presence of mucous cells. There was mild infiltration of heterophils along with few mononuclear cells in the lamina propria. However, similar type has not been described by earlier worker as per literature available.

2. *CATARRHAL ENTERITIS*

Twenty two (30.55%) of intestinal pieces showed changes of catarrhal enteritis. In acute fatal cases there was copious fluid in the lumen and the mucosal layer was reddened due to hyperaemic changes. Similar observation was made by Srinivasan *et al.* (1979) who reported that the peritoneal fluid was invariably increased in quautity and the visceral organs were congested. However, no peritonial fluid was found during the present investigation in such cases. Singh *et al.* (1997) also reported congestion of intestine in chickens.

Acute catarrhal with a longer duration, becomes purulent and the lumen of the small intestine was found to contain a thin creamy exudate in copious quantity. Such changes were noticed by Singh *et al.* (loc.cit.) in some chicks in which there was deposition of creamy exudate in peritoneal cavity. There was infiltration of the heterophils in the lamina propria and to a little extent in the submucosa. Epithelial desquamation and infiltration of leucocytes was usual. This observation corroborate the observation made by Das and Som (1992) who reported the similar changes in quails whereas Moharana *et al.* (1993, a) reported the similar histopathological changes in poultry. Nayak *et al.* (1980) reported that there was marked thickening of all the serous membrane in ducks. They also found infiltration of lymphocyte in cases of colisepticaemia in ducklings. Goblet cells were found to be numerous in number. Moharana *et al.* (loc cit.) noticed the similar change in poultry. Gupta *et al.* (1981) noticed catarrhal enteritis in experimentaly produced Salmonellosis in poultry.

3. *HAEMORRHAGIC ENTERITIS*

Sixteen (22.22%) intestinal samples showed the pathological changes of haemorrhagic enteritis. It was characterised by superficial extravasation and staining of the ingesta with blood. The mucosa of the small intestine and large intestine was severely congested and haemorrhagic. Singh *et al.* (loc. cit.) also noticed the similar changes in chickens in Tarai region. Extensive haemorrhagic enteritis was recorded in quails by Das and Som (loc. cit). Sharma and Joshi (1987) characterised haemorrhagic enteritis in chicken by bloody diarrhoea. There was focal infiltration of heterophils in the submucosal and mucosal layer of the intestine which is in agreement with the findinges of Das and Som (loc. cit) who also noticed marked infiltration of heterophils in submucosa. Gupta *et al.* (loc. cit.) observed petechial haemorrhages in Peyer's patches and enlarged caecal tonsils. in case of experimental salmonellosis in poultry. But these changes were not observed in the present study except the highly congested area of mesentery. Kapoor *et al.* (1980) noticed acute congestion, heterophilic infiltration and haemorrhages in the small intestine followed by infiltration of lymphocytes and few plasma cells in the mucosa in *Salmonella bareilly* infection similar to haemorrhagic enteritis but in the present investigation Salmonella could not be isolated from any case. Panigrahy and Glass (1982) noted intestinal mucosa to be haemorrhagic in some cases of severe enteritis in an outbreak of Fowl cholera. in quail. But in the present study the casuative agent of Fowl cholera i.e. *Pasteurella multocida* could not be isolated from any cases of enteritis which were subjected for isolation. Sarma *et al.* (1988) noticed acute

haemorrhagic enteritis in quail in case of *Salmonella gallinarum* outbreak.

4. *ULCERATIVE ENTERITIS*

Ten out of seventy two samples (13.88%) showed the pathological changes of Ulcerative enteritis. The lesions include the ulceration of the small intestine as well as the caeca and upper portion of the large intestine. Small penetrated haemorrhages were seen in the intestinal wall in some cases which was followed by necrosis and ulceration. They sometimes penetrated deeply into the serosa. Early ulcers resembled small haemorrhagic necrotising areas involving villi of the intestine.

Bendele (1976) observed similar changes in ulcerative enteritis in bob white quail in *Clostridium colinum* infection. Srinivasan *et al.* (1981) reported an outbreak of ulcerative enteritis which caused 88 deaths out of 110 quails. Ulcerative enteritis in the present study was found in less cases compared to the observation made by Bendele (loc.cit.) and Srinivasan *et al.* (1981) which might be due to the fact the collection of material was made from the organised farm. However, the findings of the author is in agreement with the findings of Chawla and Sharma (1972)., Parihar and Rao (1967) in domestic fowls.

5. *SUPPURATIVE ENTERITIS*

Eight (11.11%) of the specimen showed the changes of suppurative enteritis, Grossly, the affected intestine appeared swollen and the lumen contained viscid slimy materials.

Microscopically, there was infiltration of heterophils in the submucosal and tunica mucosa muscularis. The blood vessels were also

found to be engorged. Desquamation of the epithileal linning of the villi were the prominent changes noticed. However, similar description has not been reported as per persual of the literature though many workers have found the similar infiltration of heterophils and mononuclear cells in the submucosal layer (Kapoor *et al.*, 1980; Srinivasan., 1981).

6. *CHRONIC PROLIFERATIVE ENTERITIS*

Intestine of one quails revealed the changes of chronic proliferative enteritis. Grossly, the tiny nodular structure was present on the serosal layer of the intestine which microscopically showed excessive proliferation of connective tissue in the lamina propria. The lining epithelium also appeared desquamated. Almost similar observation have been reported in poultry in Hjarre's disease.

7. *NECROTIC ENTERITIS*

Three (4.16%) of the intestinal specimen showed changes of necrotic enteritis during the present study. In small intestine the wall was markedly thickened. These was also extensive necrosis of the epithelial lining of the intestine. Similarly Das & Som (loc.cit.) and Moharana (1993, a) reported necrotic enteritis in quail and poultry respectively. The changes encountered were desquamation and degeneration of lining epithelial cells of mucosa exposing the underlying submucosa which was oedematous and infiltrated with heterophils. Singh *et al.* (1997) also found mucosal necrosis in chickens. Das and Som (loc. cit.) observed similar necrotic lesions in caeca as well as other parts of digestive tract. Mazurkiewiez *et al.* (1968) and Silva *et al.* (1989) reported similar

necrotic changes in quails. The lumen contained dirty faecal material which were mixed with cellular debris which appeared like the flakes Sharma *et al.* (1985) reported similar changes in young chicks in which caeca was found to be filled with caseous material.

Cygan and Nowak (1974) found lesions of acute necrotic enteritis in quail. They reported the changes were similar to those found in naturally infected chickens in *Clostridium perfringes* type C infection.

LIVER

In ten cases liver showed some gross pathological changes. Liver was found to be enlarged, congested and pale in colour. There was discolouration with petechial haemorrhages and darkeing of liver in some cases. Das and Som (loc.cit) reported congestion of liver in quails while Sharma *et al.* (1985) and Ahmed *et al.* (1991) in poultry in case of *Escherichia coli* infection. Kulkarni *et al.* (1988) reported enlargement of liver with petechial haemorrhages and necrotic lesion on liver.

On microscopic examination liver showed generalised venous congestion with engorged vein and sinusoids. The degeneration and necrosis of hepatocytes was seen in some cases. Perivascular lymphocytic infiltration in portal areas and focal necrosis was also noticed. Similar observations were made by Das and Som (loc. cit.) Kapoor *et al.* (1980) and Silva *et. al.* (1989) in quails. Moharana *et al.* (1993, a); Mayahi *et al.* (1994) and Singh *et al.* (1997) reported similar changes in liver in poultry.

Nayak *et al.* (1980) reported the similar changes in ducklings in case of colisepticaemia.

Proventriculus

In four cases congestion of proventriculus was noticed. The lining epthelium and the follicles were also congested.

The microscopic examination revealed necrotic lesions. There was desquamation and degeneration of subrugrosal glands There was proliferation of connective tissue. Similar obsevation has not been reported as per persual of the available literature.

ISOLATION OF BACTERIA

Escherichia coli, besides being a normal inhabitant of the intestinal tract is also associated with a variety of pathological problems under conditions of stress, inadequate ventilation and overcrowding (Sojka, 1965). The disease incidence in India in poultry is around 14 per cent.

In the present study the prevalence of *Escherichia coli* was highest in occurrence. It was found to be associated with 19 (38%) enteritis case out of 50 samples subjected for bacterial isolation from the case of enteritis. The present finding is in agreement with the finding of Misra *et al.* (1991) and Srinivasan *et al.* (1979). Misra *et al.* (loc. cit.) isolated *Escherichia coli* from cases of enteritis in quail in pure form 21.5% and in mixed form 9%. Over all prevalence was 33.6%. Srinivasan *et al.* (1979) reported 36.88% from quails suspected for colibacilosis. Sinha *et al.* (1985) and Singh *et al.* (1997) reported *Escherichia coli* from poultry in 41.17% and 36.32% respectively.

Paranjape and Das (1985) during their study on the prevalence of bacterial infections in poultry farms at Bombay isolated colibacillosis to the extent of 37.62 percent from different organs of poulry which is

similar to the observation made in the present study. However, they have also isolated Salmonella (15.59%), Pasteurella (9.4%), Proteus (6.43%), Pseudomonas (3.96%), Klebsiella (1.98%), Shigella (0.24%), Staphylococcus (2.72%), Bacillus (0.99%), Streptococcus (0.74%). These organisms have also been isolated from the quail during this study though Salmonella, Pasteurella could not be isolated from any case.

These findings establishes that the prevalence of enteritis due to *Escherichia coli* infection in quail is similar to that of chickens. Reddy and Koteeswaran (1994) concluded that quail chicks are susceptible to *Escherichia coli* as chicken. However, Srinivasan *et al.* (loc. cit.) isolated 23.77% from chicken suspected for colibacillosis.

Raja Rajeshwari *et al.* (1992) isolated *Escherichia coli* 52.3% from healthy quails from different parts as well as intestine. Jones *et al.* (2000) found out the extent of *Escherichia coli* infection to be 66% in Mahaboobnagar district of Andra Pradesh. Srinivasan *et al.* (1980) studied that mortality rate due to colisepticaemia in quail in 0-6 weeks of age was 0.63-0.98 whereas in adult it was 0.69. Mohanty *et al.* (1979) isolated *Escherichia coli* from intestinal contents of dead quails and reported a marked high incidence 100%. These findings greatly vary from the present findings. This may be due to locality variation, the managemental condition and prevaling climatic conditions in this part of Bihar.

Sarma *et al.* (1978) isolated 12% *Escherichia coli* from enteric microflora of healthy duck. Bowman and Jocobson (1980) found that *Escherichia coli* was the most commonly species among Gram-negative in cloacal flora of normal Psittacine birds.

Staphylococcus Sp

Staphylococcus are ubiquitous, normal inhabitants of skin and mucous membrane and are common organisms environment where poultry are hatched, reared or processed.

In the present investigation the prevalence of *Staphylococcus sp.* was 18% next to *Escherichia coli.* Raja Rajeshwari *et al.* (1992) isolated 27.69% from various parts of quail. Misra *et al.* (1991) isolated 39.28% *Staphylococcus aureus* from enteritis case of quail. Shoba *et al.* (1997) detected Staphylococcus infection in Japanese quail in a breeder farm at Namakkal. Patro *et al.* (1992) reported mortality in quail due to staphylococcal infection. Naqui *et al.* (1970) isolated *Staphylococcus Sp* from turkey as normal microflora . Bergmann *et al.* (1980) reported *Staphylococcus aureus* injection of fowls. He noticed that the incidence of infection increased from 5.49% in 1975 to 28.95% in 1978. Thompson *et al.* (1980) isolated *Staphylococcus aureus* from chicks, pullets and hens. Bhatia *et al.* (1980) reported 25% mortality in an outbreak of *Staphylococcus aureus* infection among turkey poults Shimizu *et al.* (1987) isolated 100% coagulase negative *Staphylococcus epidermidis* from quail in Japan. Sarma *et al.* (1978) isolated 23% from enteric flora of healthy ducks.

Klebsiella Sp

Klebsiella sp are widely distributed in nature, occupying both as commensals in human and animal intestines as well as saprophytes in soil, water and vegetation.

In the present investigation *Klebsiella sp* was isolated from 12% cases of enteritis. Misra *et al.* (loc. cil) found it in 21.42% cases of enteritis in quail in mixed form. Raja Rajeshwari *et al.* (loc. cil) from 23.06% from various parts of healthy quails. Verma and Adalka (1971) isolated a very low 3.34% from different disease condition in poultry.

Sarma *et al.* (1978) isolated 3% *Klebsiella sp* from enteric microbial flora of healthy ducks.

Proteus Sp

In the present investigation, *Proteus sp* was isolated from 10% intestinal samples screened for bacteriological examination. This finding is in agreement with the finding of Misra *et al.* (loc.cil) who isolated 8.33% from enteritis cases of quail in mixed form.

However, Raja Rajeshwari *et al.* (1992) isolated 38.46% *Proteus sp* from various parts of healthy quails. Singh *et al.* (1996) reported *Proteus sp* infection as one of the emerging bacterial diseases of quail. Myint (1987) reported *Proteus mirabilis* infection in quail in Burma which caused 65% mortality. The findings which differs from the present study might be due to the fact that isolation was make only from the enteritis cases of the quails. Sah (1983) isolated *Proteus sp* from blood, liver and lungs of affected quail. While Mall and Shah (1979) reported the association of *Proteus sp* from Japanese quail with Pneumonia. Moharana *et al.* (1993, b) reported Proteus sp with the cases of enteritis in poultry.

Sarma *et al.* (1978) found 1.4% *Proteus sp* from enteric microflora of healthy ducks.

Shigella

In the present study *Shigella sp* was isolated from 8% cases of enteritis. Misra *et al.* (loc.cit) isolated 16.66% *Shigella sp* from enteritis in quail in Orissa. Raja Rajeshwari *et al.* (1992) reported 18.46% from healthy quails. Moharana *et al.* (1993, b) reported *Shigella sp* from enteritis cases in poultry.

Streptococcus

Streptococcus are found commonly in environment like staphylococcus. *S. faecalis* is normally found in the intestine of healthy birds. In acute outbreaks mortality may go up to 50%.

In the present investigation *Streptococcus sp* was isolated in 4% cases of enteritis. Itoh *et al.* (1997) found Streptococcus as the main bacteria found in the intestine of the normal quail. Misra *et al.* (loc.cit.) found a high percentage of 36.90% in mixed form. Raja Rajeshwari *et al.* (loc.cit.) also found a high percentage 33.84% in healthy quail.

However, Sarma *et al.* reported *Streptococcus sp.* 5.1% in enteric microbial flora of 36 healthy ducks in Tirupati.

Bacillus sp

In the present investigation *Bacillus sp* was isolated from 10% enteritis cases in quail.

Raja Rajeshwari *et al.* (loc.cit.) found a very high (86.15%) prevalence of *Bacillus sp.* from different part as well as intestine of healthy quails. This variation may be due to difference in specimen taken during the study.

Venkanagouda *et al.* (1996) reported *Bacillus cereus* infection in the cases of early chick mortality.

Sarma *et al.* (1978) found 23.2% *Bacillus sp* from enteric microbial flora of healthy ducks. Mazurkiewiez *et al.* (1982) reported *Bacillus subtilis* infection in ducks. Bowman and Jacobson (1980) noted the high prevalence of *Bacillus sp.* in cloacal swabs of Psittacine birds.

Chapter • 6

SUMMARY

SUMMARY

The present study was undertaken with the objective to find out the type, pattern and various morphological changes in digestive system of quail particularly Bacterial enteritis including isolation of bacterial agents.

120 naturally dead quails were collected from Central poultry farm and local meat shops. On necropsy, the gross lesions were noticed in different parts of digestive system of quails mainly intestine. Out of which 90 quails showed some gross changes in their intestine mainly and few in liver and proventriculous also. Gross changes noticed in intestine were mainly congestion, swelling of intestine and hyperaemic changes. These samples were further processed for histopathological studies.

For histopathological examination 0.5 cm thick tissue pieces of intestine, were collected from 90 samples and were preserved in 10.0 percent formal saline solution. Ten (10) samples of liver and 4 samples of proventriculus showing some gross changes were also processed, same as the samples of intestine for histopathological study. After proper fixation, paraffin blocks tissue sections and slides were prepared by standard technique. Slides were stained with Haematoxylin and eosin stain for routine examination. Some special stains such as Mucicarmine, Brown and Brenn were also used and the changes were carefully noticed.

On the basis of histological findings the different types of pathological changes were found in 72 (60%) specimens of intestine collected. They were as follows :-

Mucoid enteritis, Catarrhal enteritis, Haemorrhagic enteritis, Ulcerative enteritis, Suppurative enteritis, Chronic proliferative enteritis, Necrotic enteritis.

Characteristic features of different types of enteritis observed during study :-

1. ***Mucoid enteritis*** :- Twelve out of seventy two (16.66%) intestinal samples showed changes of mucoid enteritis. There was mild congestion, intestine was slightly swollen, Faecal mass was found to be mixed with mucous material. Presence of few heterophils and mononuclear cell infiltration in the lamina propria of the affected intestine were noticed.
2. ***Catarrhal enteritis*** :- Twenty – two (30.55%) of intestinal specimen showed the changes of Catarrhal enteritis. This was found to be the most frequently occuring enteritis. Excessive secretion of mucinous materials was noticed. Mucosal layer was reddish in colour and was covered with mucous. The odema of the intestine was observed. Mucous membrane was found to be denuded from its epithelial covering and there was infiltration of few heterophils in the lamina propria.
3. ***Haemorrhagic enteritis*** :- Sixteen (22.22%) cases of enteritis were noticed. Ingesta was found to be stained with blood. The mucosa of the small and large intestine was severely congested and haemorrhagic. There was infiltration of heterophils in the submucosal and mucosal layer of the intestine. The lining epithelium was almost erroded from their basement and the tip of the villi appeared blunt and rounded.
4. ***Ulcerative enteritis*** :- Ten (13.88%) showed the pathological changes of Ulcerative enteritis. It was characterised by ulceration of the small intestine as well as the caeca and upper portion of the large intestine.

Ulcers were found to be numerous in some caecum and large intestine. Desquamation of mucosal epithelium, lymphocytic infiltration were noticed.

5. ***Suppurative enteritis*** :- Eight (11.11%) samples showed the changes of suppurative enteritis. The affected part of the intestine appeared slightly swollen, the serosal layer was thickened and the lumen was full of viscid, slimy, yellowish materials mixed with intestinal content. The blood vessels appeared congested and full of heterophils. Gram-negative thin rods were noticed in the submucosal layer when stained by Brown and Brenn method.

6. ***Chronic Proliferative enteritis*** :- One (1.38%) intestinal specimen showed the changes of chronic proliferative enteritis. Several tiny nodules were present in the wall of the affected intestine. Tunica lamina was highly thickened with the excessive proliferation of the connective tissue. Some of the villi also appeared elongated. There was infiltration of mononuclear cells chiefly lymphocytes.

7. *Necrotic enteritis* :- Three (4.16%) of the intestinal samples showed the changes of necrotic enteritis. The whole of the mucosa was affected leaving only samll patchy necrotic areas. Fibrin like deposits were noticed on the mucosal surface in the area of necrosis. Beside hyperaemia, exudate and cellular infiltration, necrosis of the epithelium of the mucosa was seen. Necrosis of the villi was almost complete in duodenal regions.

Liver

Ten liver showed gross pathological changes. Liver was found to be enlarged and pale in colour. There was discolouration of liver, petechial haemorhages with degenerative changes and congestion. Ten samples showing some gross changes were processed for histopathological study. The changes noticed were congestion, swelling of hepatocytes, focal areas of necrosis and diffusely scattered aggregation of lymphoctyes while degeneration and necrosis of hepatocytes has been seen at places.

Proventriculus

Four samples of proventriculus showing some pathological lesion with congestion were processed for histopathological study. Necrotic lesions in proventriculus was mainly desquamation and degeneration of subrugrosal glands. The subrugrosal glands were also found to be separated with proliferation of connective tissues in the interglandular spaces in some cases.

For isolation of bacteria, intestinal tissues of seventy two quails, suspected for enteritis were processed for bacterial isolation. For the isolation purpose the different types of culture media were used viz. Nutrient broth, Nutrient agar, Mac-Conkey's agar, blood agar media and tetrathionate broth.

Different types of organism were isolated from 50 cases only.

Prevalence of *Escherichia coli* in the present study was highest in 19 (38%) cases of enteritis. *Staphylococcus sp* 9 (18%), *Klebsiella sp* 6 (12%), *Proteus sp* 5 (10%), *Bacillus sp* 5 (10%), *Shigella sp* 4 (8%), *Streptococcus sp* 2 (4 %) were isolated from cases of bacterial enteritis.

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