STUDIES ON COCCIDIOSIS IN JAPANESE QUAIL

(Coturmix Coturnix Japonica)

And

ITS CONTROL MEASURES

T H E S I S

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR

PUSA (SAMASTIPUR)

(FACULTYOF VETERINARY SCIENCE)

In partial fulfilment of the requirements

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

(PARASITOLOGY)

BY

VIJAY KU MARSINGH

Department of Veterinary Parasitology

BIHAR VETERINARY COLLEGE

PATNA-800014

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DEPARTMENT OF VETERINARY PARASITOLOGY
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C E R T I F I C A T E - I

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It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

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

I feel extremely happy to express my deep appreciation and idebtedness to dr. M. N. Sahay, Associate Professor, Department of Parasitology for his valuable guidance, constant inspiration, constructive criticism and keen interest throughout the present investigation.

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Vijay Kumar Singh.

(VIJAY KUMAR SINGH)

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INTRODUCTION

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M Y P A R E N T S

INTRODUCTION

The quail, commonly known as 'Bater' by the common people is the preferential table delicacy since the ancient days. Quail farming for eggs and meat is common not only in South East Asian Countries like Japan, Hongkong and Korea, but also in European Countries such as Italy, France, Germany and Great Britain. Japanese quail (Coturnix coturnix japonica) as a domesticated species was introduced in this country in mid seventies. It belongs to the wild variety of poultry and covered under " Wild Life Protoction Act, 1972". During the recent period, the quail farming had been attractive alternative for poultry farmers due to great consumer acceptance not only as a table delicacy but its comparative softness, taste and lower fat content. The japanese quail has now attracted the promising poultry farmers, Agricultural Universities, Central and State Animal Husbandry Departments and Research Institutes and Commercial establishements because of the low establishment cost, less rearing space and convenience of management. The medicare like routine vaccinations, deworming and general medication cost are very limited. This species is bestowed in having a shorter reproductive cycle followed by lower feed intake and early marketing age which adds another feather to its cap and utility in quick economic gains as well as assured early

returns. Commercial exploitation of quail farming for their potential of better egg production and quality meat having preferential consumer taste, has compelled investigators to use this species as a research model for conducting laboratory research. This species has also been recognised as an ideal laboratory animal for workers in the fields of avian embryology and Physiology for specific Endocrinolical research (Padgett and Ivey, 1959).

Quail belongs to the order Gallefornes, family Phasianidae, genus Coturnix, species C. coturnix and sub-species Coturnix coturnix japonica. Males and females are readily recognised by their throat feathering which is of deep cinnamon colour in male and light cinnamon in female. The female has long pointed throat feathers which are longer and heavier than the male, while the reverse is true in chicken (Pani, 1978). It is usually monogamus; being polygamous is an exception (Ahuja, 1990). Quail has the unique characteristics of faster juvenile growth rate, early sexual maturity, short incubation period (16 - 17 days). These birds are comparatively less susceptible to diseases as compared to the common chicken. It is highly suited for breeding under high density (six to eight birds per square feet) for rearing (Roay, 1993). Its hatching is very sensitive to the variations of temeratures which is usually lower than thirty five degree centigrade (Reddy, 1991). The

body weight of japanese quail is very low the male and female weighing about 100 and 130 grams, respectively.The egg weighs about 10 gram i.e. eight percent of the body weight (Kumar, 1977). It is entirely ground loving species, the bird never perchas on trees, the male quail tends to limit its activity to an area in which it allows no other male. The females, when they arrive, are not preoccupied with the males but proceed to select a nest site of its own (Prakash Babu et al. 1990).

Japanese quail, a nocturnal habitat of japanese Islands, is a domesticated avian species. It was introduced for the first time in India at the Centre of advanced studies in poultry sciences at Izatnagar, Utter Pradesh, under the UNDP/ICAR collaborative education project. The hatching eggs were obtained from the avian science Department University of California, Davis, USA in 1974 and subsequently in 1976. (Panda et al. 1990). It is probably the smallest avian species and now constitutes the third largest in number, only next to chicken and ducks used for commercial poultry production. Its weekly production varies from 50,000, 1,00,000 birds (Agarwal, 1996).

Poultry rearing is mainly for commercial Production of eggs and meat and almost it is of recent origin of India's most innovative industries. It has evolved from vertically integrated and organised farms. It has achieved

unprecedented growth during the last thirty years. Its progressive development has not been only in size but also in its productivity, sophistication and quality (Mohapatra, 1994). About seventy percent poultry is reared in backyards of India (Kumar, 1997). The term poultry includes all those birds that can be domesticated and serve as an economic means of earning. Consumer preference and agro-climatic conditions favour different kinds of poultry in different places. To create awareness of the advantages of rearing different avian species among the masses, diversification in poultry farming is the need of the recent times.

Acute shortage of proteinous food accompanied with malnutrition due to population explosion is becoming more complex problem of the masses day by day. The diet of average indian is lacking in nutritive food like milk, meat, fish, poultry and poultry products which are most essential for human health. In this respect, poultry is occupying an important position in supplementing the meat and egg requirement in human diet. However, the potentiality of quail farming is yet to be exploited. This particular species is available in both wild and domestic conditions in India and abroad. Quail rearing for meat and eggs will be a good steps to augument this shortfall. To acheive the recommendations of the National Institute of Nutrition (per capita consumption) of 10-8 kg of meat, we are long way to go (Mahapatra, 1992).

Thus, with the additional source of quail meat and eggs, India may increase the poultry production potential to meet its demand and can face the protein deficiency, Malnutrition and hunger of the poor masses.

Among the many deadly diseases of poultry, coccidia may constitute an important group of parasitic protozoa and constitute to be one of the most commonly diagnosed diseases in chicken under modern managemental practices in confinement over a small area. Coccidiosis is thus a major problem of domesticated birds. The increasing scientific knowledge available during the current century has turned the modern poultry breeder wiser than before in assesing the important role of coccidia in the economy of poultry production. The species of coccidia described from japanese quail are Eimeria bateri (Bhatia et al., 1965; Norton and Peirce, 1971), E. uzura (Tsunoda and Muraki, 1971), E. tsunodai (Tsutsumi, 1972) and E. Taldykurganica (Ruff et al. 1984). Tsunoda and Muraki (1971) reported coccidial infection by Eimeria uzura in japanese quail in India which is very similar to Eimeria acervulina of chicken (Tsunoda and Muraki, 1971), Ruff et al., 1984, Rao, 1988). Rao and Sharma (1992) also reported Eimeria uzura infection in japanese quail in India. Coccidiosis induced morbidity due to E. uzura resulting in reduced plasma pigment, diarrhoea, anaemia, and depressed body weigh in quail (Ruff et al.1984,

Rao, 1988). Coccidiosis has also been recognised as a potential for producing a chronic wasting type of diseases in quail. The information on the prevalence of coccidiosis in japanese quail in Bihar is scanty and hence a limited study has been conducted by the past investigators on the occurrance of coccidiosis in japanese quail. The present study is, therefore, undertaken for the fulfilment of the following obsectives:-

1. To study the prevalence of coccidial parasites in Japanese quail in Patna through the examination of faecal samples and intestinal scrappings.

2. To study the haematological examinations such as TLC, DLC, and Hb% of coccidia affected birds .

3. To study the histopathology of some coccidia affected parts of gastointestinal tract of both in light and heavy infection in quails.

4. To study the efficacy of certain coccidiostats which are being used under the field conditions against coccidial parasites in poultry.

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REVIEW OF LITERATURE

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Incidence:

Madsen (1941) reported that Coccidiosis occurs in the majority of free living birds including quail. Coccidia in general are highly host-specific. However, birds belonging to certain closely related genera also seem to be susceptible to other coccidia. For example, Eimeria dispersa (Tyzzer, 1929) a coccidia primarily of turkeys has been recorded from Bobwhite quail (Colinus verginianus virginianus) by Howkins, 1952 and Venard, 1993. Earlier Henri (1931) and Herman et al., (1942) described the presence of coccidiosis in california quail from a natural infection. However, Herman and Jakiewicz (1942) reported the first experimental infection of coccidia (Eimeria species) in California quail (Lophortyx californicus).

The occurrance of E. dispersa in Bobwhite quail has still not been fully elucidated through Edger et al. (1964), observed that E. dispersa was infective to quail. No chicken of Turkey coccidia have, however, been found to develop in these Coturnix species of quail.

Mishra (1944) reported for the first time of quail coccidiosis from India on the incidence of Wenyonella bahli from small intestine in the common grey quail (C. coturnix). Oocyst morphology has been the basis of the identification of species of the coccidia.

Chakravarty and Kar (1947) reported Eimeria coturnicis from the intestine of common grey quail based on the morphological studies on the exogenous developmental stages of the parasite.

Bhatia et al. (1965) described yet another new species from the small intestine of the common grey quail (C. c. cotunix) encountered during routine examination of the birds. The patches of the small intestine revealed distinct lesions characterised by small congested and oedematous area, which on scraping yielded gametogenic and oocystic stages. The information on the various endogenous stages was provided by histological study of the intestinal tissue. The endogenous stages viz; schizonts, macro and micro gametocytes, observed by these workers were mostly confined to lower regions of the small intestine.

Ouchi et al. (1968) reported a disease characterised by haemorrhage and swelling of caecum in Japanese quail reared in a field ranch and observed coccidial oocysts in the faeces. Subsequently, Liburd and Mahrt (1970) described new species Eimeria lophortygis and C. okanaganensis from californian quail in British Columbia. Cross transmission experiments conducted with japanese quail (Coturnix c. japonica) by the above workers were however unsuccessful.

Norton and Peirce (1971) studied the life cycle of E. bateri in the Japanese quail(C.c.japonica) as an alternative host. The first generation schizonts developed in the glands of the duodenum and upper intestine, 2nd, 3rd and 4th generation schizonts and gametocytes occured in the villous epithelium. There was a gradual spread along w the small intestine until the whole organ was affected. The prepatent period of 4 days and the patent period 6 days were recorded. Graded doses of 5000 to 12,80,000 oocysts produced very little pathogenic effect in young quail. The above workers attempted unsuccessfully the cross transmission of this species to young phaesants and gallus chicks.

Shah and Johnson, (1971) reported the outbreak of coccidiosis due to Eimeria bateri attended by high mortality in breeding flock of Hungarian quail (C. c. coturnix) maintained for laboratory use in USA. Lesions were observed in the duodenum and lower intestine including the caecal neck of the dead birds. Attempted cross transmission of this species to chicken, however, did not result in patent infection.

Tsunoda and Muraki (1971) also investigated a new aspecies of coccidium Eimeria uzura from duodenum and upper part of small intestine which resembled E. coturnicis and E. bateri in the morphology of oocysts exept for the presence of microplyle. A pure strain of this species was obtained by the single oocyst infection

method. This coccidial species was not so pathogenic to japanese quail. Twenty common species of coccidia of birds toxonomically similar to Coturnix species of quail were not found to be infective to Japanese quail.

Acedo and Regura (1972) reported an outbreak of coccidiosis involving 10% birds showing 1% mortality due to E. bateri in C. c. coturnix comprising 10,000 birds of 2 months age in spain. Tsutsumi (1972) simultaneously reported a highly pathogenic species of coccidium E. tsunodai from japanese quail, which infected the caeca alone extensively and induced symptoms of caecal enteritis. They failed to infect the Japanese quail with nine species of coccidia isolated from six species of gallinaceous birds or vice versa. Extensive studies were conducted on the pathogenic features of infection with E. tsunodai (Tsutsumi and Tsunoda 1972). These results were contradictory to the reported views of Mazurkiewicz et al. (1967) that the organism detected from quail may be E. tenella . Subsequently, Savanbaev and Utebaeva (1973) reported the occurrance of Eimeria taldykurganica in coturnix quail and Ryley et al.(1975) made an excellent review of the pathological effects of coccidia, particularly in fowls while Anwar (1976) described E. bateri from C. c. coturnix in Iran. Similar, studies were also reported by Bayer et al. (1976) in chicks, Betke et al. (1976) in geese, Mohan and Pande (1976) in Japanese quail, Szalay et al. (1976).

Svanbaev and Utebaeva (1977) recorded W. bahli among coturnix quail from Kazakhastan (USSR) followed by Panda (1978) and Thanikachalam et al. (1979) from India, Reid et al. (1978) from chickens, turkeys geese ducks and pigeons . Other investigators who worked on the prevalence and development of coccidiosis in gallinaceous birds included Srinivasan et al. (1980), Anwar (1981), Sen et al. (1981), Hernandez et al.(1981), Ruff et al. (1984), Dimerdash (1984), Mathis and MC Dougald (1987), Rao et al. (1987), Panda (1989) and Rao & Sharma (1992).

Pathological Studies:

Tsutsumi (1972) reported the detailed clinical symptoms and pathogenesis of coccidosis in experimentally infected quails with graded doeses of infection followed by Arnastauskiene et al. (1977), Ruff et al. (1978) while Kappor (1980) Ganti & Shastri (1983) , Lozanov (1983), Lozanov (1983), Dakshinkar et al. (1985) and Anwar (1981), made detailed haematological & histopathological studies on the coccidiosis in chickens respectively.

Fan et al. (1985) made observation on the pathomorphology of experimental coccidiosis in duckling.

Majhar and Rano (1985) reported histopathology of coccidiosis experimentally infected with Eimeria garnhami in quail. Which caused infarcts in intestinal mucosa 36 hours post infection, superficial errosion and

hypertrophy of villi after 48-72 hours and severe tissue damage after 76-96 hours in mild infection. There was loss of identity in epithelial cells, tunica propria and muscale cells of muscularis mucosa. However, in chronic to heavy infection, infaracts, oedema and atrophy of tissues were the major changes. Later Ruff et al. (1985) differentiated the effects of coccidiosis between game birds and poultry coccidia.

Panda et al. (1989) studied the pathology of coccidiosis in japanese quail with gross lesions of enteritis, balloning and bleaching of intestine and caeca coupled with a few Haemorrhagic spots on the caecal surface. Feldman et al.(1990) stated that stress due to shipment of quails resulted in a wasting syndrome. Aprotozoan isolated from the epithelial cells of the caecum and small intestine was identified as Eimeria brunetti. The diagnosis was avian leucosis and coccidiosis.

Rao et al. (1990) studied on the histopathology caused by Eimeria oocysts pretreated with amprolium and Monensin in Japanese quail in which E. uzura oocysts pretreated in vitro with Amprolium 20 percent (0.025%) or Monensin (100 ppm), successfully.

Gragory et al. (1990) Dlariu et al. (1994) Marshall et al. (1995) and Shukla et al. (1996) were the other investigators who studied the clinicopathological changes in poultry due to coccidiosis.

Chemotherapy :

Mazurkiewicz et al. (1967) reported failure in their attempts to treat an out break of acute caecal coccidiosis in Japanese quail showing signs of blood stained diarrhoea with DOT (Zoalene) and furasol. However, Ouchi et al. (1968) obtained some reprieve from increasing mortality in outbraks of coccidiosis by using a mixture of sulphamezathine, sulphamerazine and Sulphadimehoxine in feed for six consecutive days.

Tsutsumi and Tsunoda (1972) conducted Prophylactic and therapeutic trials against infection of E. tsunodai in Japanese quail using known anticoccidial agents. Comprising Amprolium @ 0.0125 - 0.12%, Methylbenzoauate @ 0.001 - 0.94% and clopidol @ 0.125% in feed for seven consecutivedays resulted in practically complete suppression of oocyst excretion in birds infected with 10^4 E. tsunodai. They also reported that administration of 0.05 - 0.2% of sulphadimethoxine and sulphamonomethoxine was effective in experimental coccidiosis caused by E. tsunodai.

Panda (1978) also used amprosol 20% powder, 1.2 g/l or Bifuran tablets (1 tablet/l water) in an outbreak of E. bateri infection in 10-15 day old birds for a week, followed by preventive medication of lg/3.3 l. in water or 1 tablet/4l water for another 2 week and reported both drugs to be equally effective. Similar

studies were also conducted by Sasmal & Sharma (1981) with monensin .

Singh et al. (1982) made study on effect of medication in inducing immunity against coccidial infection of chicken following use of pancoxin, amprol plus, embazin, nicarbazin, coyden, bifuran, amprolsol, docoxin and saquadil in Eimeria tenella, E. nicatrix and E. acervulina infection in chicks. All the drugs, used prophylactically, fully protectedchicks against E.tenella and E. recatix .

Sasmal and Sinha (1983) made study on efficacy of monensin and lasolacid against amproliumresistant strain of Eimeria tenella in poultry.

Ruff et al. (1985) made study on medication of coccidiosis in game birds and given suitable treatment regimes for pheasants and japanese quail, Norton bobwhite, chukar and partridge are described.

Deghidy et al. (1989) made study on efficacy of baycox (toltrazuril) and sofra-vitaminee for controlling experimentally in duced coccidiosis in chicks, followed by Rao and Sharma (1989a) with amprolium or monensin containing double the concentration of active ingredient used when adding the drugs to feed as prophylactics .

Rao and Sharma (1989b) described the comparative prophylactic efficacy of our anticoccidial agents viz. Arpocox, coban, stenorol and amprolium against experimental E. uzura infection (10^5) in one hundred inetytwo day old chicks of japanese quail.

Srivastava and Sinha (1989) conducted a study on anticoccidial efficacy of salinomycin (Coxistac premix) against drug resistant strains of Eimeria tenella in Broiler chicks. The described anticoccidial efficacy of salinomycin (Coxistac 6% premix) against drug resistant strains of E. tenella in broiler chicks. Salinomycin at 60 ppm recorded highly significant (P/0.001) weight gains over infected, non-treated control, and resulted in highly significant reduction in lesion score. Salinomycin treated group recorded on improvement of 24.3% in feed efficiency over infected non-treated control. Mortality was also checked by addition of salinomycin to feed. In overall assessment, the experiment proves possessing significantly high anti-coccidial efficicacy of salinomycin in broilers.

Rao and Sharma (1990) assessed the effect of pretreatment of E. uzura oocysts with halofuginone or arprinocid on aporulation and infectivity in Japanese quails.

Rao and Sharma (1990) assessed the effect of pretreatment of E. uzura oocysts with halofuginone or arprinocid on aporulation and infectivity in japanese quails.

Vanparijs et al. (1992) reported anticoccidial effic acy of diclazuril in game birds. Diclazuril was tested in phaesants experimentally infected by E. colchisi, E. duodanalis and E. phasiani, in partridge infected with E. legunensis, in quail infected with E. legunensis and in quail infected with E. bateri. Diclazuril was effective at 2 ppm in partridge and quail. At 4 ppm, diclazuril was fully effective in all Eimeria species.

Samad et al. (1993) investigated chemotherapeutic management of dcute outbreaks of caecal coccidiosis in five batches of 2408 broiler chickens in Bangladesh.

Panda and Dwivedi (1994) conducted study on non-coccidiostat drugs such a levamisole, vit. E. septilin for their chemoprophylactic effecacy in experimental coccidiosis in japanese quail chicks devided into 6 groups of 100 chicks. They concluded that these drugs have coccidiostatic activity and it is suggested that they may be used as an adjunct to specific therapy to solve the problem of drug resistance.

Rao et al. (1994) carried out study on the unsporulated oocysts of E. uzura which were treated in vitro for 48 hours with arprinocid and halofuginone at double the concentration recommended for prophylactic use.

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS AND METHODS

Four hundred faecal samples and two hundred intestinal scrapings were examined for the prevalence and incidence of coccidial parasites in Japanese quail (Coturnix coturnix japonica) maintained at Central Poultry Farm, Patna and also with various suppliers of the local market.

These samples were collected from birds of different age groups in different seasons which were kept in individual vials properly labelled. The faecal materials were processed through standard sedimentation and floatation techniques for examination of oocysts of coccidia. Microscopic examination of faecal materials as well as the intestinal scrapings were done in the laboratory. The epidemiological studies included observations on quantitative oocyst production in Japanese quail of different age groups starter(0 to 3 weeks), growers (3 to 6 weeks) and layers (over 6 weeks) to investigate the comperative susceptibility of the birds. Faecal oocyst output was calculated by standard McMaster method and expressed as egg per gram (EPG) of faeces.

Pathological studies

Histopathological examination of the gastrointestinal tract of coccidia infected quails was carried out by the following techniques.

(i) The portion of gastointestinal tract representing the characteristic changes was cut (about 1/2" sq. and not more than 1/5" thick) and fixed in 10% formaline salane (this solution was prepared by adding 1 ml. of formaldehyde in 9 ml. of saline water in a wider mouth glass bottle).

(ii) The tissues were dehydrated by passing them through the ascending grades of alcohol i.e. 30, 50, 70, and 90 per cent keeping these for half an hour in each.

(iii) Clearing of tissues was done by dipping the tissues in xylene No. I for half an hour and xylene No.II for 6 hours.

(iv) After clearing of the tissues, the paraffin embedding were performed by putting the tissues in the melted paraffin over for about 2 to 3 hours and tissue block was made.

(v) Sections of 5-7/u thickness were cut with the help of a rotatory microtome and stained with standard Haemotoxylene Eoxin (HE) method (Lillie 1965).

(vi) For histological studies, the sections were mounted with cover slip by DPX mountant dried up and were examined under binocular research microscope.

Haematological studies

Following haematological parameters were studied in coccidia infected quail on zero day (prior to medication) to 21st day (after medication).

i) Haemoglobin percentage (Hb%).

ii) Total leucocyte count (TLC).

iii) Differential leucocyte count (DLC).

Blood collection

The anticoagulant mix ure was prepared by mixing the following compounds:-

Potassium Oxalate	-	0.8 gm.
Ammonium Oxalate	-	1.2 gm.
Distilled water	-	100 ml.

The mixture of above two salts is known as "Heller pouls oxalate mixture".

Small sterilised empty neutral glass vials were filled with 0.25 ml. of anticoagulent mixture and were dried in an over below 60°C as described by Sharma (1967).

(ii) Collection of blood: Two ml. blood was collected from each and every bird or haematological studies in group (Amprosol),(Coxiquin) and (infected control) from their wing vein (Archer et al., 1977) with the help of dispos ble syringe and hypodermic needle. The skin was also sterilised with rectified spirit after proper removal of feathers. Then blood was collected through the syringe and placed in a neutral glass vial (Borosil) containing dried anticoagulent as described above. It was shaken thoroughly during the collection to mix properly with anticoagulent (Sahai, 1960). During pricking of needle

adequate prec aution was taken to prevent formation of haematoma.

Haemoglobin Estimation

Estimation of haemoglobin was done through haematin method adopted by using Sahlis haemoglobinometer. In this method, blood was diluted with N/10 HCl and colour was matched with standard colour on haemoglobinometer. This done on the Principle that haemoglobin of red cells gets converted into acidhaematin when it comes in contact with the N/10 HCl. The detailed techniques are given below.

(i) A little quantity of N/10 Hcl was put into a well washed empty graduated tube of the haemoglobinometer.

(ii) With the help of special pipette (Marked for volume of 20 C.mm.), the oxalated blood was taken up to the mark (20 C. mm.) . This can be adjusted by tapping by the finger.

(iii) The blood was drawn from the special pipette into the graduated tube which was containing a little quantity of N/10 Hcl. Pipette was raised slightly and the blood was taken by suck and blow method in the acid without causing any air bubbles so that the whole blood of the pipette is immersed in the acid.

(iv) The tube was shaken gently to mixed the blood and acid thoroughly with glass rod (Stirror).

(v) For matching of the colour, extra N/10 Hcl was added drop by drop till the colour of the graduated tube was matched with the side tubes (Comparator) of the haemoglobinometer.

(vi) Lastly, the value of haemoglobin was taken directly from the scale on the graduated tube which gives the result in gm/100 ml. The reading of upper level of the fluid in the tube was noted for accurate estimation.

ESTIMATION OF BLOOD CELLS COUNT (TOTAL LEUCOCYTE COUNT)

The frequent variations in the characteristics of avian blood create difficulties when performing haematological examination by techniques routinely adopted to study the mammalian Blood. These characteristics include the presence of nucleated erythrocytes and leucocytes (in case of mammals, erythrocyte and platelets are non-nucleated and leucocyte in nucleated) and intensely red heterophils granules colour similar to those in eosinophil. Heterophil is the counterpart of the mammalian neutrophil and thrombocytes appear to function in haemostasis in birds which is analogous to the platelets in mammals. Although the term thrombocytes is synonymous in mammalian haematology the specific term thrombocyte is used in avian species only. Birds and mammals also differ in mechanism of coagulation, particularly in intrinsic system where birds appear to be lacking in coagulation factor XI and XII. Therefore, the avian blood

cells whether they are erythrocytes or leucocytes are nucleated, the method which is used in total leucocyte count in mammalian blood is not used in this case. In mammals two types of diluting fluids (Hayme's and Turck's fluid) are used for erythrocyte and leucocyte cells, respectively in which one type of cell is dissolved and in the avian species only one type of diluting fluid is used. The remaining another cell is counted. But, in the procedure Diluting fluid is made as follows: Natt and Herick 1959):-

Sodium sulphate	-	2.50 gm.
Sodium chlorid	-	3.88 gm.
Potassium hydrogen phosphate	-	0.25 gm.
Disodium hydrogen phosphate	-	2.91 gm.
Formalin (37%)	-	7.50 ml.
Methyl violet 28 or 6 B	-	0.10 gm.

All the above components were dissolved in distilled water in the sequence given above and diluted to total volume of 1000 ml in volumetric flask. After standing it for overnight the solution was filtered through fine filterpaper and the p^H was adjusted to 7.4 This diluting fluid was used for counting leucocytes.

Blood Sample Preparation for cell count

The haemocytometer pipette was filled up to desired mark with the oxalated blood and then, the diluting fluid was drawn up to desired mark given in the pipette. Air bubbles were avoided at the time of drawing, the

diluting fluid in the pipette. The diluted blood was shaken throughly before placing it in the haemocytometer (Sahai 1960).

Total Leucocyte Count (TLC)

Blood was sucked upto 0.5 mark of the WBC pipette and was diluted with the diluting fluid upto 11 mark, (diluted to 20 times). The pipette was shaken and a few drop of diluted blood was discarded and then a drop of diluted blood was allowed to trickle in space between cleaned coverslip and groove of the counting chamber (Neubaurs) of haemocytometer.

The counting of white blood cells were done from four corner squares of the haemocytometer under the microscope. The leucocytes (White blood cells) could be identified by refractile appearance and due to voilet colour taken on account of diluting fluid.

The white blood cells were counted in four cornor squares under high power (40x) objective of the microscope. To obtain the number of leucocytes in one C mm. of blood, the total number of cells counted was multiplied by 50 (fifty). The calculation was done as per Archer's (1965) formula .

Total No. of cells (TLC)

$$\frac{\text{No. of cells counted in 4 chambers x dilution}}{\text{volume assayed}}$$

Area of one large square = 1 sq. mm.

Depth of one large square = 0.1 sq.mm.

Therefore, the volume of fluid in one large square =

$$1 \times 0.1 = 0.1 \text{ C mm.}$$

So the volume in 4 large squares

$$= 4 \times 0.1 \text{ C mm.} = 0.4 \text{ C mm.}$$

Dilution = 1:20

Therefore, T L C (C mm.)

$$= \frac{\text{No. of W.B.C. counted in 4 chambers} \times 20}{4}$$

Or, the T.L.C. per C. mm. of blood

= No. of Leucocytes in 4 large counting chamber x 50.

Differential Leucocyte Count (DLC)

Accurate leucocyte differential counts require that the cells distribution be uniform on the slide to be examined. The method of making a smear probably has the greatest influence on the distribution of the cells. So, keeping this fact in mind the present procedure of counting differential leucocyte has been detailed as follows:

The blood film was prepared on a greese free microscopic slide and stained with Leishman's stain as per the method described by Sahai (1960).

The stained blood film slide was seen under low power objective of microscope to see whether the film was homogeneously stained or not and then examined under

an oil immersion objective with a drop of microscopic cedar wood oil. Cells were counted from each edge and the percentage of different cells were recorded.

Criteria for differentiating the Leucocytes

Leucocytes were differentiated on the basis of the description described by Olson (1937) cited by strukie (1965).

(a) Heterophils

In the blood of quail this type of leucocyte sometimes designated as polymorphonuclear pseudoeosinophilic granulocytes. Such cells are designated as heterophils. For identification of the characteristics features of these cells, were the presence of many rod shaped or spindle shaped acidophilic crystalline bodies in the cytoplasm was observed. The nucleus was polymorphic with varying degree of lubrication.

(b) Eosinophils

These cells are also known as polymorphonuclear eosinophillic granulocyte having the same size as that of the heterophils. But for identification, the characteristics features was that the granules were spherical and relatively large.

(c) Basophils

These cells were known as polymorphonuclear basophillic granulocytes also having about the same size and shape as the heterophils but the nucleus was weakly

of quails were made and the infected cases were marked with numbered tape in their leg. A total of 30 quails, which were clinically suffering from coccidial parasitic infection were selected and divided randomly into 3 groups each constisting of 10 quails. One of these acted as control and the other two as treatment groups. The treatment groups were given Amprolium and Toxiquin. All the haematological test were carried on 0 day and 21 day before and after treatment. Thus, finally six groups were formulated as shown in table I below.

The average egg count per gram (E.P.G.) of faeces were evaluated before treatment and on 7th, 14th, 21st day of post treatment and the percentage efficacy was calculated on the basis of the decline in the rate of E.P.G. during post treatment.

The E.P.G. of the faecal samples were calculated according to the modified 'Stoll's egg counting techniques" by adopting the following steps:-

(a) Approximately 10 or 12 small sized (3-5 mm. diameter) glass beads were put in 120 ml. glass stoppered bottle with 45 ml. of decinormal caustic sode ($NaOH$) solution.

(b) 3 gms. of faeces were well stirred and thoroughly mixed and put in the bottle.

(c) The stopper was fitted to the bottle and shaked throughly until all the faecal matters were broken down.

basophillic in reaction and round or oval in shape. The cytoplasm was abundant and devoid of colour. Deeply basophillic granules around the cytoplasm may be seen.

(d) Monocytes

These cells were very difficult to be distinguished from the larger lymphocytes. But for identification, the characteristics features was that the monocytes were larger cells with relatively more cytoplasm than the large lymphocytes. The cytoplasm of these cells had a blue grey tint. The nucleus was usually irregular in outline.

(e) Lymphocytes

These are most numerous in the quail blood, and hence they constitute the majority of the leucocytes in the blood of the quail. There was a wide variation in the size and shape of these cells. The cytoplasm was usually basophillic . It may consist of a narrow rim bordering one side of the nucleus, as in the small lymphocytes, or it may constitute the major portion of the cells, as in the larger lymphocytes. The nucleus was usually round and may have a small indentation.

EVALUATION OF CHEMOTHERAPEUTIC AGENTS

For the administration of drugs under this experiment, a large scale screening of the faecal samples

of quails were made and the infected cases were marked with numbered tape in their leg. A total of 30 quails, which were clinically suffering from coccidial parasitic infection were selected and divided randomly into 3 groups each constisting of 10 quails. One of these acted as control and the other two as treatment groups. The treatment groups were given Amprolium and Coxiquin. All the haematological test were carried on 0 day and 21 day before and after treatment. Thus, finally six groups were formulated as shown in table I below.

The average egg count per gram (E.P.G.) of faeces were evaluated before treatment and on 7th, 14th, 21st day of post treatment and the percentage efficacy was calculated on the basis of the decline in the rate of E.P.G. during post treatment.

The E.P.G. of the faecal samples were calculated according to the modified 'Stoll's egg counting techniques" by adopting the following steps;-

(a) Approximately 10 or 12 small sized (3-5 mm. diameter) glass beads were put in 120 ml. glass stoppered bottle with 45 ml. of decinormal caustic sode (NaoH) solution.

(b) 3 gms. of faeces were well stirred and thoroughly mixed and put in the bottle.

(c) The stopper was fitted to the bottle and shaked throughly until all the faecal matters were broken down.

(d) The mixture was then poured through a wire mesh screen with an aperture of 0.15 mm. and the strained fluid was kept in a bowl. The debris left on the wire mesh was discarded.

(e) The filtrate of faeces was well stirred and 0.15 ml. of the fluid was taken by means of a graduated pipette.

(f) The measured quantity of fluid was ejected into a McMaster egg counting chamber and covered with a 22 x 22 mm. cover glass. The whole of the 0.15 ml. sample of the faecal suspension was examined under the low power magnification of the microscope and all the eggs seen were counted with a hand tally counter.

(g) The figures obtained from the count (i.e. the total no. of oocysts persent in 0.15 ml. of diluted faeces) were multiplied by 100 to give the number of oocysts (eggs) per gram of the original faecal sample.

(h) After recording their average E.P.G. just prior to treatment, different drugs were used as per the experimental plan. The E.P.G. was measured on 7th, 14th and 21st days post drug treatment for their efficacy.

After generation of data the various standard statistical analysis was done.

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TABLE-1(a): EXPERIMENTAL COCCIDIOSTAT TRIALS IN QUAILS INFECTED WITH COCCIDIA.

Group No.	Control/ treated	No.of quails	Average EPG at the start (0 day) of the treatment 1 x 10	Drugs used for chemotherapy	Dose	Route
1.	2.	3.	4.	5.	6.	7.
I.	0 day infected control	10	1.878	Nil.	Nil.	Nil.
II.	21 day infected control	10	2.02	Nil.	Nil.	Nil.
III.	0 day Amprosol treated	10	1.87	Amprolium	1.2 gm/L of drinking water	Orally
IV.	21 day Amprosol treated	10	0.052	Amprolium	1.2 gm/L of drinking water	Orally
V.	0 day Coxiquin treated	10	1.88	Sulphaquinoxaline + Amprolium (Coxiquin)	0.5 gm/L of drinking water	Orally
VI.	21 day Coxiquin treated	10	0.045	Sulphaquinoxaline + Amprolium (Coxiquin)	0.5 gm/L of drinking water.	Orally

N.B.:- 1. Amprosol (Galaxo)

2. Coxiquine (Vetcare)

RESULTS
AND
OBSERVATIONS

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Incidence of Coccidiosis in Japanese quail

An extensive survey for the prevalence and incidence of coccidia in Japanese quail was conducted in Patna during the period from March 1997 to Feburuary 1998. Altogether 400 faecal samples and 200 intestinal scrapings of the quail chicks obtained mainly from Central Poultry Farm, Patna and also from local poultry dealers were examined. The presence of coccidial species Eimeria uzura and Eimeria bateri were observed in majority of the infected cases.

The prevalence of coccidia in Japanese quail is presented in Table -1 which varies from 80 (20%) out of 400 birds in faecal samples to 42 (21%) out of 200 birds in intestinal scrapings. However, there was no significant variation on the incidence of coccidiosis collected from faecal samples and intestinal scrapings.

The seasonal incidence of coccidia in Japanese quail examined through faecal samples is depicted in Table - 2. The incidence of coccidial infection during spring, summer, rainy and winter was noted to be 18, 16, 21 and 25 per cent, respectively. The value of χ^2_3 df. revealed non-significant difference among various seasons denoting that it did not influence the incidence of coccidia infection in quail.

The seasonal incidence of coccidia in Japanese quail examined through intestinal scrapaings is presented in Table-3. Fifty birds each in all the four seasons were examined, of which 10 (20%), 8 (16%), 11 (22%) and 13 (26%) birds were found infected during spring, summer, rainy and winter seasons. respectively. Coccidial infection in Japanese quails was found during all the four seasons. However, there was also no effect of season on the incidence of coccidiosis in quails examined through intestinal scrapings.

The prevalence of coccidia examined through faecal samples in different age groups of quails has been presented in Table-4. It was observed that 29 (14.5%) birds were found to be infected in age groups upto 4 weeks of age while only 11(5.5%) birds were observed to be infected at the latter ages (above 4 weeks). The Chi-square test of independancy revealed significant difference (P/0.01) among the age groups suggesting that birds at early age (upto 4 weeks) were more susceptible to coccidial infection.

The prevalence of coccidia at different age groups in Japanese quail examined through intestinal scrapings presented in Table -5 showed that 13% of the birds were found infected upto 4 weeks of age and 8% in the age group above 4 weeks. However, the value of X_1^2 df. (1.33) revealed that there was no significant effect of age groups on the incidence of coccidiosis in Japanese quails.

Total Leucocyte Count (TLC)

The mean value along with their standard error and Co-efficient of variation percentage of total leucocyte count in different groups has been shown in Table-8. The analysis of variance showing the effect of different groups on total leucocyte count has been shown in Table-9. The statistical analysis revealed highly significant ($P<0.01$) difference of various groups on total leucocyte count. However, there were no significant differences between the among mean total leucocyte count of groups I, II & V besides these values also did not differ statistically with group II. There was a significant ($P<0.05$) decline of 0.67 (1000/ml.) of TLC on 21 days amprosol treated group from 0 day amprosol treated group. Besides the mean TLC(1000/ml) of 21 days coxiquin treated group also declined significantly ($P<0.05$) from 0 day Coxiquin treated group.

Differential Leucocyte Count (DLC)

Heterophil precentage

The mean and CV % of heterophil in different groups of birds is presented in Table 10 and the analysis of variance for the effect of different groups on heterophil percent is summarised in Table 11. The means of heterophil in groups I, III and V were observed to be 27.13, 27.2 and 27.0 percent on 0 day while the corresponding values of groups II, IV and VI on 21st day were noted as 26.2, 27.8

Haematological studies

A total of thirty birds naturally infected with coccidia were divided into three groups (each group consisting of 10 birds) and were examined for the effect of coccidia on haematological parameters like haemoglobin percentage, total leucocyte count (TLC) and differential leucocyte count (DLC) . The group I worked as the infected control where no drug was given while group II received. Amprosol was given @ 1.2 g/1 of water for 4-5 days oraly. In group III Coxiquin was given @ 0.5 g/1 of water for 5-7 days oraly. All the haematological parameters were studied twice, the day before (0 day) and after twenty first day of the administration of the drugs.

Haemoglobin percentage; (Hb. gm./100 ml)

The mean along with its standard error and Co-effecient of variation percentage in different groups of birds have been shown in Table -6. The haemoglobin percentages in group I, III and V on 0 day were 9.25 $\pm$ 0.12, 9.21 $\pm$ 0.13 and 9.23 $\pm$ 0.14 while the corresponding values of groups II, IV and VI on 21 days were noted as 9.15 $\pm$ 0.11, 9.31 $\pm$ 0.12 and 9.31 $\pm$ 0.14.

The analysis of variance showing the effect of different groups on haemoglobin percentage have been shown in Table - 7. The statistical analysis did not reveal any significant effect of various groups on haemoblobin percentage.

The seasonal incidence of coccidia in Japanese quail examined through intestinal scrapaings is presented in Table-3. Fifty birds each in all the four seasons were examined, of which 10 (20%), 8 (16%), 11 (22%) and 13 (26%) birds were found infected during spring, summer, rainy and winter seasons. respectively. Coccidial infection in Japanese quails was found during all the four seasons. However, there was also no effect of season on the incidence of coccidiosis in quails examined through intestinal scrapings.

The prevalence of coccidia examined through faecal samples in different age groups of quails has been presented in Table-4. It was observed that 29 (14.5%) birds were found to be infected in age groups upto 4 weeks of age while only 11(5.5%) birds were observed to be infected at the latter ages (above 4 weeks). The Chi-square test of independancy revealed significant difference (P/0.01) among the age groups suggesting that birds at early age (upto 4 weeks) were more susceptible to coccidial infection.

The prevalence of coccidia at different age groups in Japanese quail examined through intestinal scrapings presented in Table -5 showed that 13% of the birds were found infected upto 4 weeks of age and 8% in the age group above 4 weeks. However, the value of X_1^2 df. (1.33) revealed that there was no significant effect of age groups on the incidence of coccidiosis in Japanese quails.

Total Leucocyte Count (TLC)

The mean value along with their standard error and Co-efficient of variation percentage of total leucocyte count in different groups has been shown in Table-8. The analysis of variance showing the effect of different groups on total leucocyte count has been shown in Table-9. The statistical analysis revealed highly significant ($P<0.01$) difference of various groups on total leucocyte count. However, there were no significant differences between the among mean total leucocyte count of groups I, II & V besides these values also did not differ statistically with group II. There was a significant ($P<0.05$) decline of 0.67 (1000/ml.) of TLC on 21 days amprosol treated group from 0 day amprosol treated group. Besides the mean TLC(1000/ml) of 21 days coxiquin treated group also declined significantly ($P<0.05$) from 0 day Coxiquin treated group.

Differential Leucocyte Count (DLC)

Heterophil precentage

The mean and CV % of heterophil in different groups of birds is presented in Table 10 and the analysis of variance for the effect of different groups on heterophil percent is summarised in Table 11. The means of heterophil in groups I, III and V were observed to be 27.13, 27.2 and 27.0 percent on 0 day while the corresponding values of groups II, IV and VI on 21st day were noted as 26.2, 27.8

and 27.9. The analysis of variance did not reveal any significant effect of different groups of birds on heterophil percentage.

Eosinophil percentage

The means ± S.E. and C.V. % of eosinophil of different groups of birds is presented in Table 12 and the analysis of variance for the effect of different groups on eosinophil percentage have been shown in Table-13. The statistical analysis as indicated by F values revealed highly significant ($P<0.01$) effect of various groups on eosinophil per cent. The mean eosinophil per cent in different groups ranged from 3.95 to 6.13. The highest and lowest values were observed in 2 21 day infected control and 21 day coxiquin treated group. There was no significant difference between the mean eosinophil % in group I and II. There was decline of 0.85 percent of mean eosinophil in 21 day amprosol treated group from 0 day amprosol treated group. However, the difference was observed to be non-significant. There was also significant ($P<0.05$) decline of 1.15 % of eosinophil in the mean value of 21 day coxiquin treated group from the 0 day coxiquine treated group.

Basophil percentage

Means ± S.E. and C.V.% of basophil in different groups of birds have been presented in Table 14. The average percentage of basophil in groups I, III and V were observed to be 0.09, 0.04 and 0.09 on 0 day while the corresponding values of groups II, IV and VI on 21st day were noted as

0.09, 0.04 and 0.04. The analysis of variance for the effect of different groups on Basophil percent is presented in Table 15. The analysis of variance revealed non-significant effect of different groups of birds on basophil percent.

Lymphocyte Percentage

Means with S.E. and C.V.% in different groups of birds have been presented in Table 16. The analysis of variance showing the effect of different groups on lymphocyte percent has been depicted in Table -17. It revealed highly significant ($P<0.01$) effect of different group on lymphocyte per cent.

The mean lymphocyte per cent among diffrent group ranged from 57.0 to 59.8 in 21 day infected control group. The mean lymphocyte per cent of 0 day infected group and 21 day infected group did not differ significantly. Mean lymphocyte % in 0 day Amprosol treated group differed significantly with mean lymphocyte per cent of 21 day amprosol treated group. However, a significant ($P<0.05$) increase of 1.5 per cent of mean lymphocyte in 21 day coxiquin treated group from 0 day coxiquin treated group was observed.

Monocyte percentage

Mean with S.E. and C.V. per cent of monocytes in different groups of birds have been depicted in Table 18. The average percentage of monocyte in infected control,

Amprosol treated and coxiquin treated groups I, III and V were observed to be 9.04, 8.89 and 9.18 on 0 days while corresponding values of groups II, IV and VI on 21 day were recorded as 10.24 , 7.90 and 8.99 . The analysis of variance for the effect of coccidial infection in different groups of birds on monocyte per cent is presented in Table 19 which did not show any significant effect of coccidial infection in different groups of birds on monocyte per cent.

Histopathological studies

The Japanese quail which were naturally infected with coccidia were kept in three different groups consisting of 10 chicks each. The group I was maintained as infected control whereas chicks of group II and III were treated with Amprosol and coxiquin. The birds from each group were sacrificed after 21 days of experiment. The alimentary canal of non-treated chicks were found to be highly infected with coccidia (Fig. 1).

In heavily infected group i.e. control group the duodenum showed the mucoid degeneration alongwith infiltration of heterophils (Fig. 2) and also showed elongated villi with vacuoles in the lining epithelium, the lamina propria also appeared widened (plate-3) . In the upper part of small infestine degeneration and disquamation of lining epithelium of villi were noticed and lamina propria appeared thin (Plate-4). The mucous gland of small intestine appeared active and the epithilium showed vacuolation (Plate-5).

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Fibroblastic proliteration of the interconnective villus tissues were observed. The villi appeared tall with disquamation of lining epithelium (Plate-6). There were also infiltration of heterophils in the lamina propria and the villi were elongated and hyperplastic. Mild to moderate degree of necrosis and disquamation of su perficial epithelial lining of villi were observed. The lining epithelium of the villi showed proliferative changes with marked hypertrophy. the gland located in the villi showed hypertrophy with occasional disruption. Fibroblastic proliferation of interconnectine villus tissue alongwith monomuclear cell intiltration and mucosal atrophy were seen. H & E section of caeca revealed infilteration of heterophils in the mucosal layer (Plate-7). Superficial necrosis and disquamation of epithelial lining cells of the villi were present. The superficial villus epithelium showed hypertrophic changes. The intervillus tissue revealed tissue monocuclear cell infilteration and mild congestion of the blood vessels.

In lightly infected group (drugs treated) the changes seen in duodenum and upper part of small intestine had less activity of mucus secreting cells though the villi appear elongatod. There is mild infilteration of inflammatory cells in the villi. Lamina propria and submuscularis mucosa did not show any marked changes. In the caeca though the mucosa showing glandular clustures surrounded by slightly leucocytic infilterated area. superficial necrosis and disquamation of epithelial lining

cells of the villi were less noticed and mild in appearance monomuclear cell infiltration in the intervillous tissue were also noticed.

Efficacy of Chemotherapeutic Agents

The efficacy of chemotherapeutic agents like Amprosol, and Coxiquin was tested against coccidial infection in Japanese quail through the estimation of average number of egg per gram (E.P.G.) in faeces. The drugs were applied seperately in two different groups and E.P.G. was estimated at 0 day (before application of the drug), 7th, 14th, and 21st day after applications of the drugs (Table -20). The effic-acy of the drugs was compared against the group-I which was kept as control (naturally infected with coccidia). The average number of E.P.G. on 0 day prior to the application of the drugs in group-II (Amprosol treated) Gr - III (Cosiquin treated) were, found to be 1.878 x 10^6 and 1.870 x 10^6 respectively. After 7th day of the application of the drugs it was observed that number of E.P. G. was decreased heavily in treated groups where as it was found to be increased in control group. The average E.P.G. were estimated to be 0.212 x 10^6 and 0.180 x 10^6 in Amprosol and Coxiquin treated groups, respectively and value was 1.90 x 10^6 in control group.

It was further observed that the average number of EPG decreased in the subsequent days and the value was

0.052×10^6 in Amprosol treated group and 0.045×10^6 in Coxiquin treated group on 21st day. On the other hand, the average number of E.P.G. was further increased in control group and became 2.02×10^6 on 21st day. From the study, it was observed that both the drugs were highly effective against the coccidial infection in Japanese quail but the Coxiquin was more effective than Amprosol . The efficacy of Coxiquin was estimated to be 94.33 per cent and that of Amprosoal was 93.34 per cent.

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TABLE-1 : The prevalence/incidence of coccidia in Japanese quail through examination of faecal samples and intestinal scrapings.

Source	No. of samples examined	No. of birds infected	% of infection	Calculated x_1^2 df.
Faecal samples	400	80	20	0.08^{NS}
Intestinal scrapings	200	42	21	

NS - Non-Significant

FIGURE - 1 : The prevalence/incidence of coccidia in Japanese quail through examination of faecal samples and intestinal scrapings.

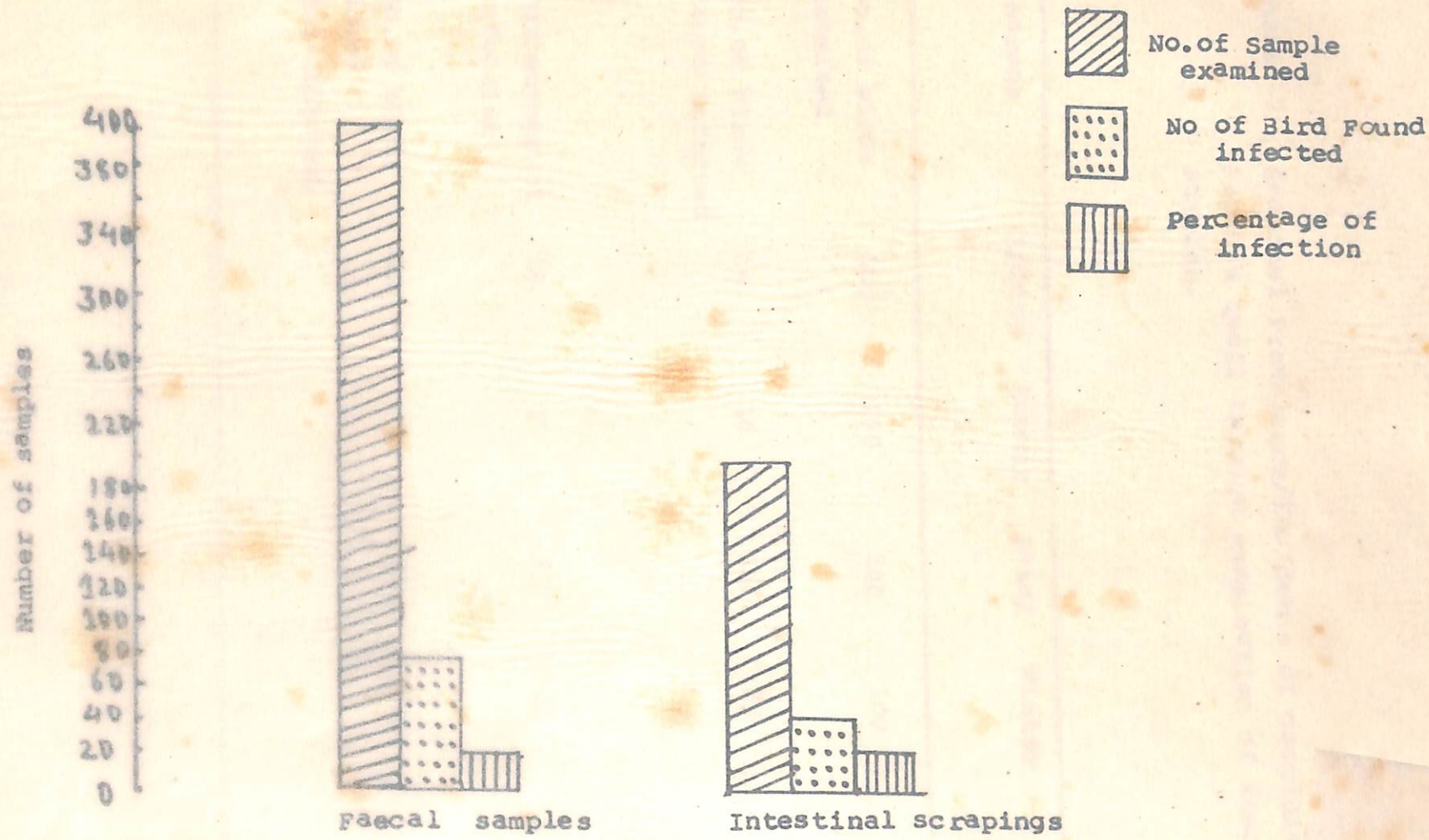


FIGURE - 1 : The prevalence/incidence of coccidia in Japanese quail through examination of faecal samples and intestinal scrapings.

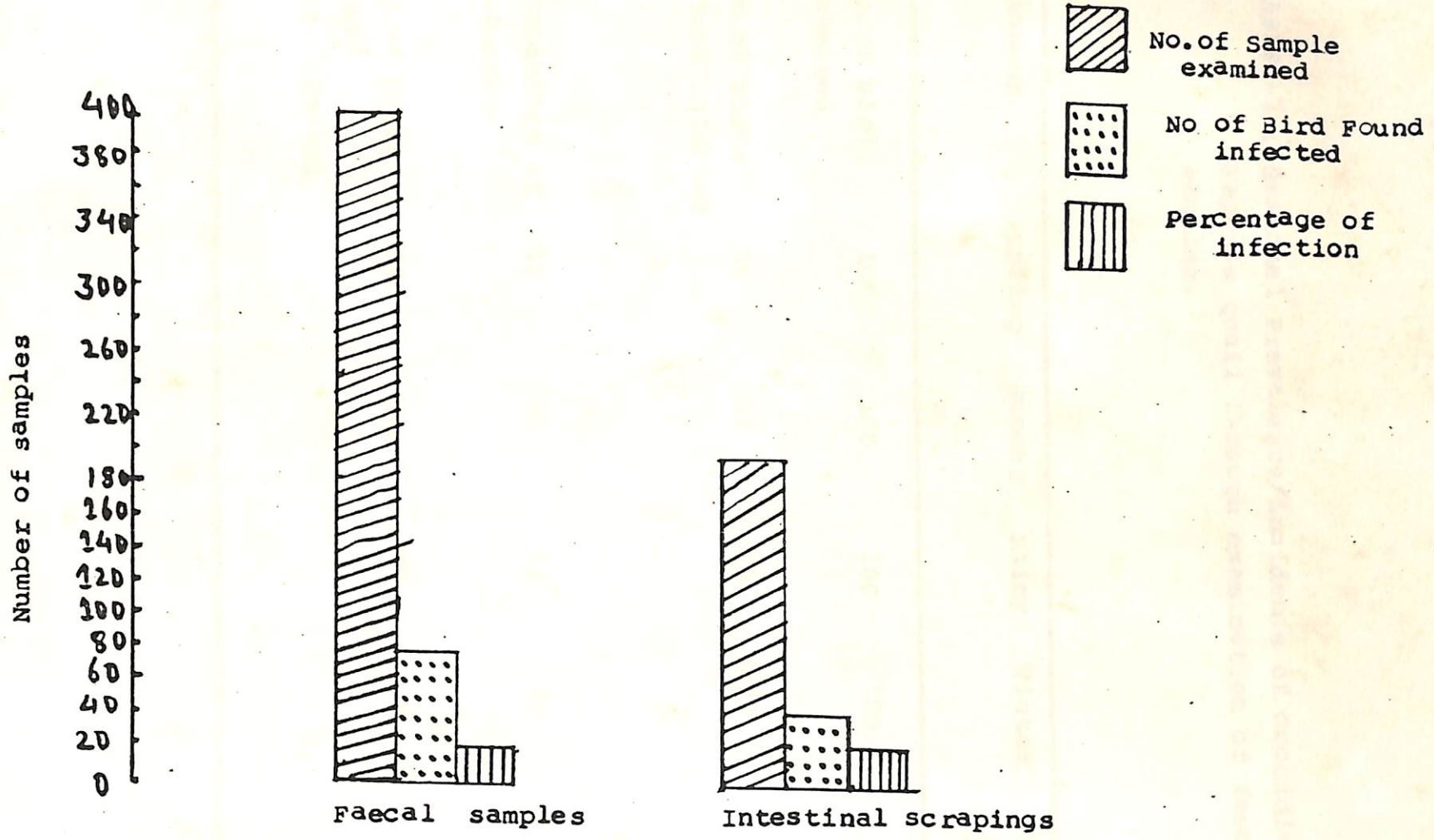
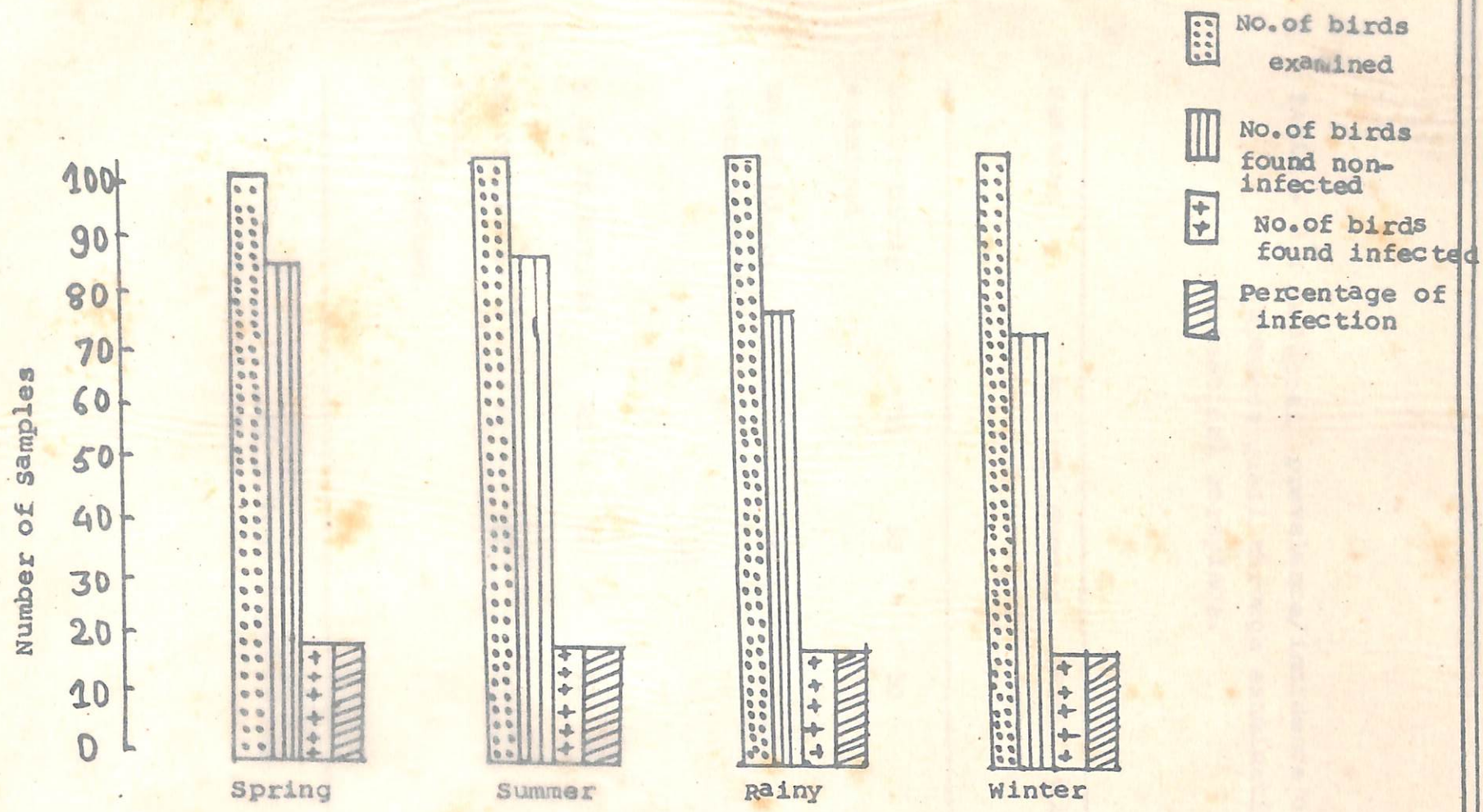


TABLE -2 : Seasonal Prevalence/incidence of coccidia of Japanse quail through examination of faecal samples.

Seasons	Spring	Summer	Rainy	Winter	Calculated x_1^2 df.
No. of birds examined	100	100	100	100	
No. of birds found infected	18	16	21	25	
					2.88 NS
Percentage of infection	18	16	21	25	
No. of birds found Non-infected	82	84	79	75	

FIGURE - 2 : Seasonal Prevalence/incidence of coccidia of Japanese quail through examination of faecal samples.



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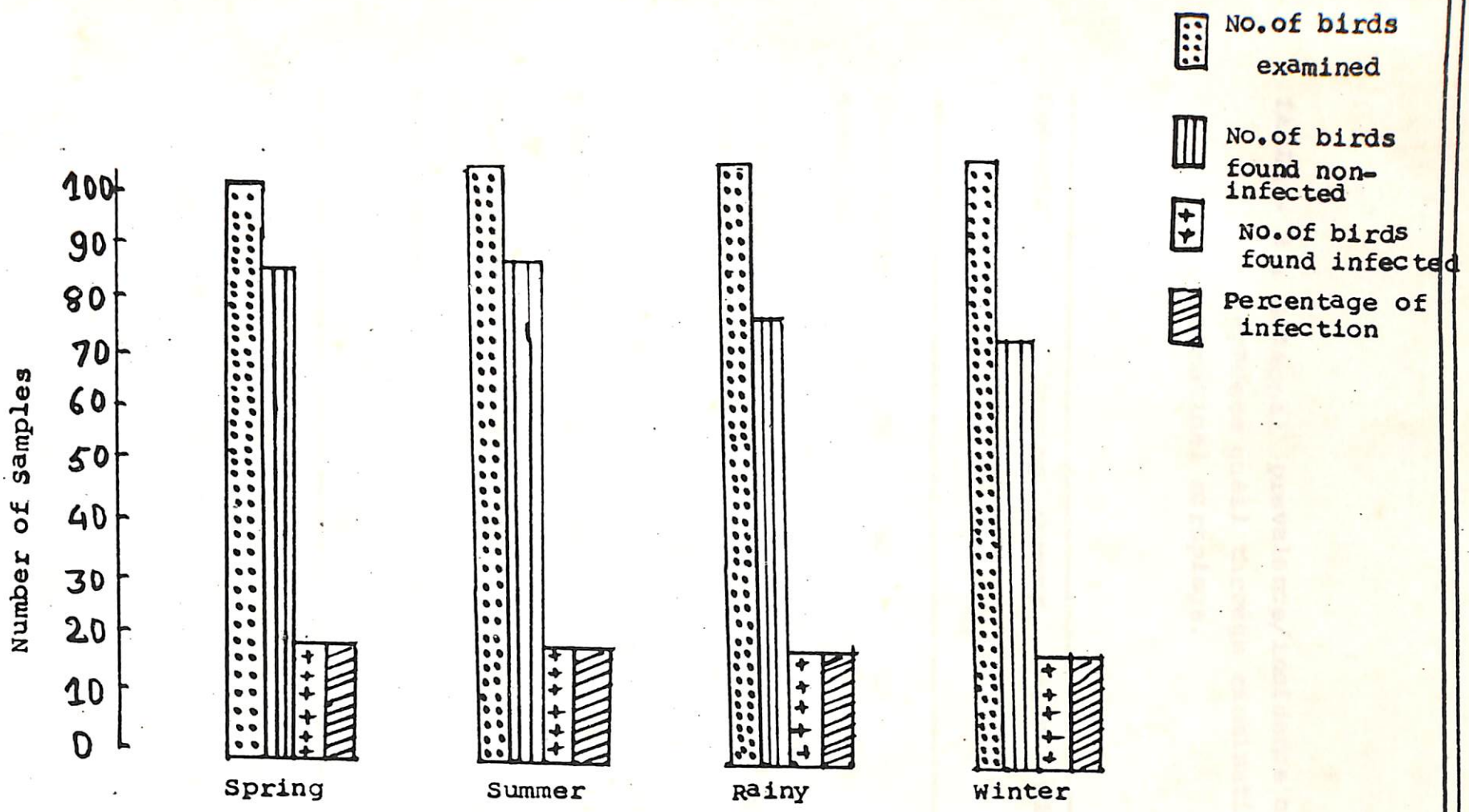


TABLE - 3 : Seasonal prevalence/incidence of coccidia in Japanese quail through examination of intestinal scrapings.

Seasons	Spring	Summer	Rainy	Winter	Calculated x_1^2 df.
No.of birds examined	50	50	50	50	
No.of birds found infected	10	8	11	13	1.57 NS
% of infection	20	16	22	26	
No.of birds found non-infected.	40	42	39	37	

NS - Non- significant.

FIGURE - 3 : Seasonal prevalence/incidence of coccidia in Japanese quail through examination of intestinal scrapings.

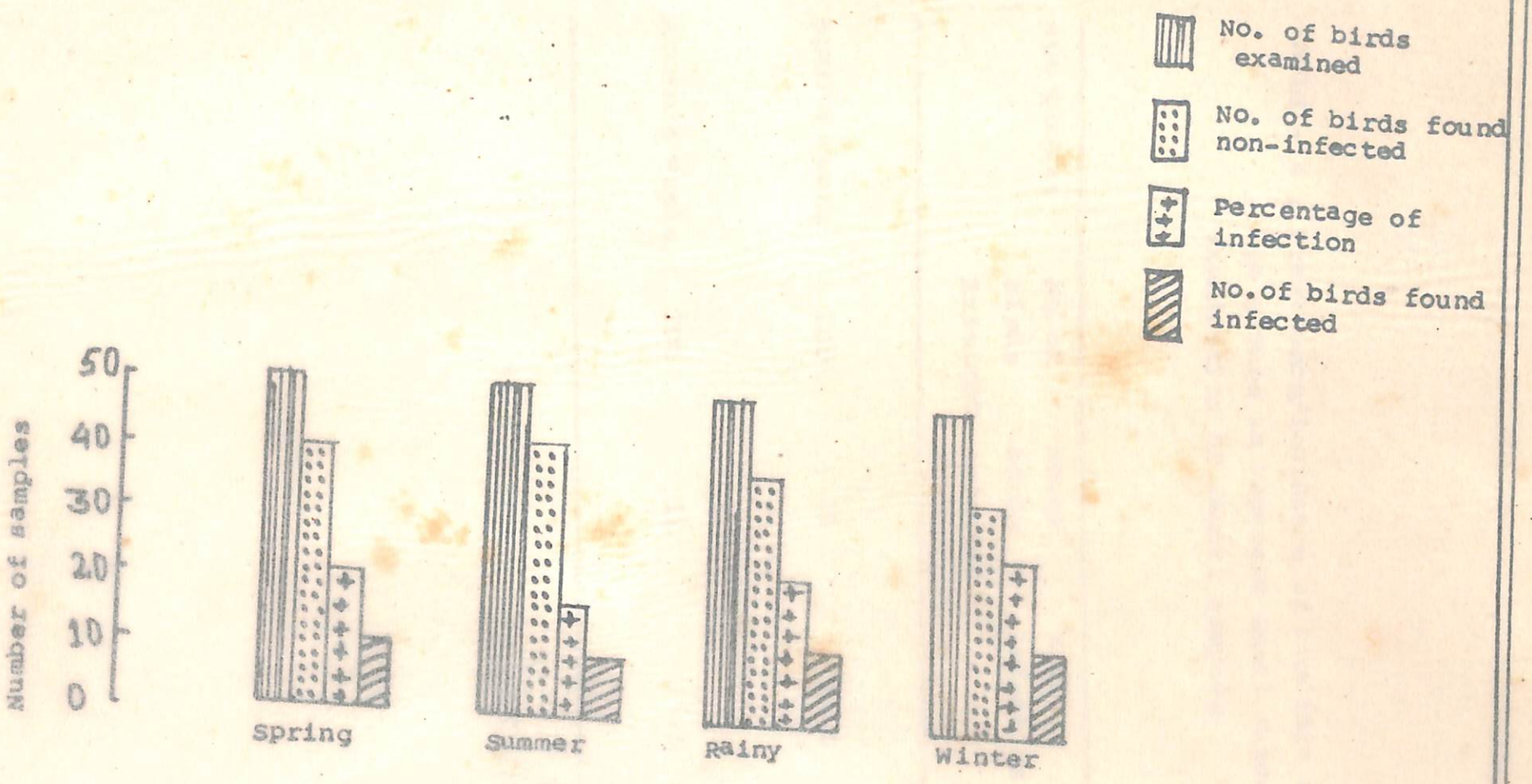


FIGURE - 3 : Seasonal prevalence/incidence of coccidia in Japanese quail through examination of intestinal scrapings.

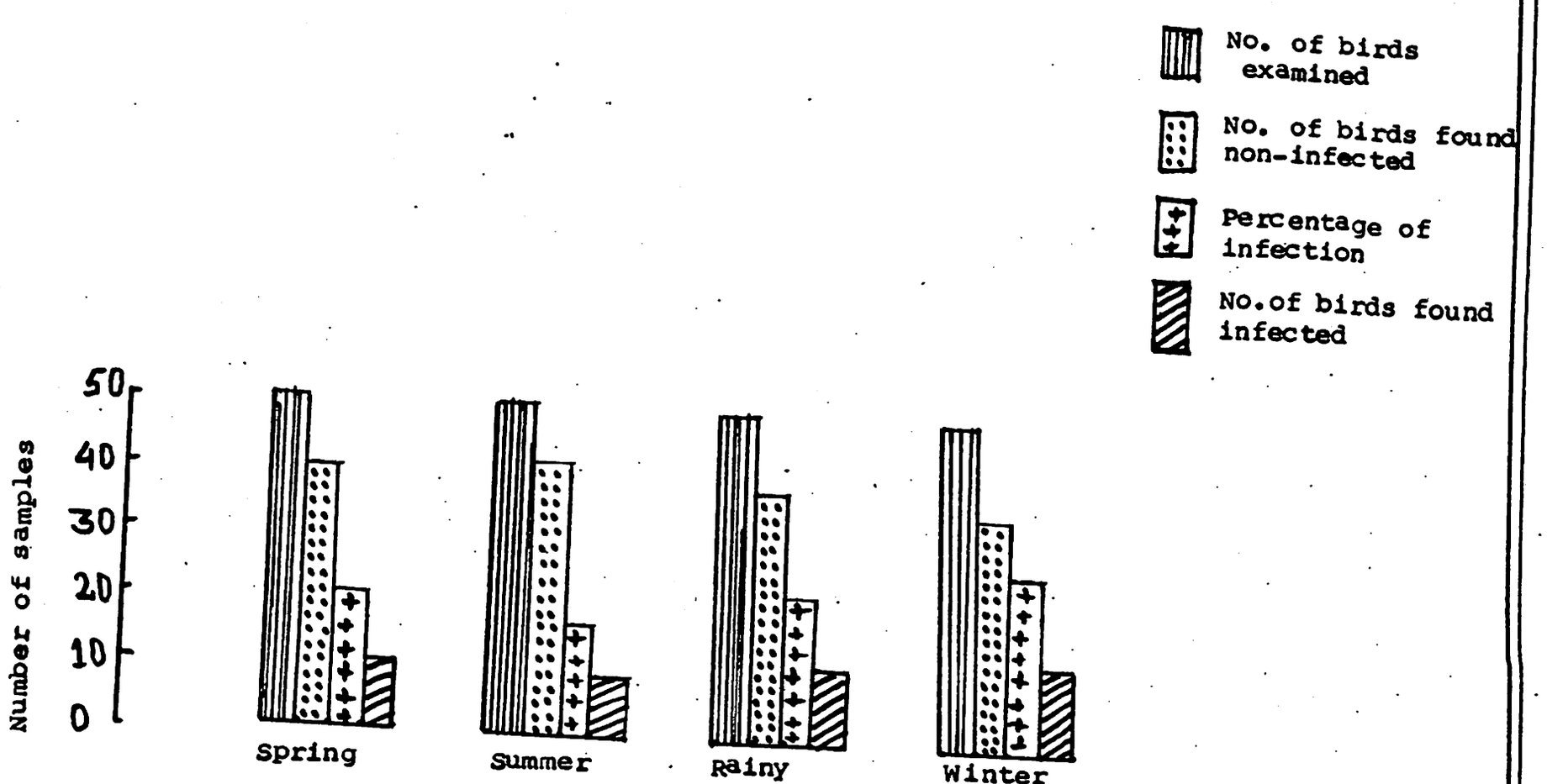
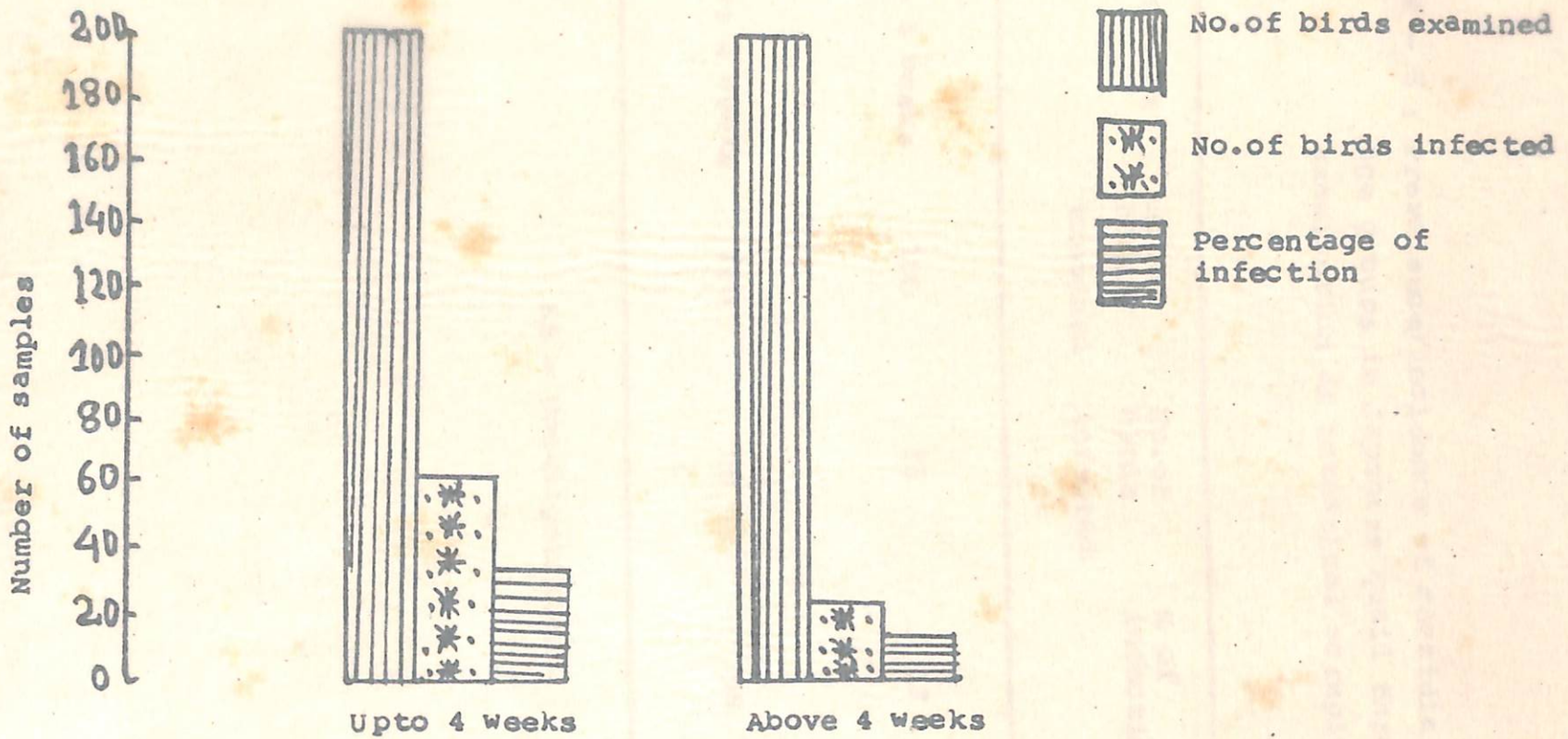


TABLE - 4 : Prevalence/incidence of coccidia in different age groups in Japanese quail through examination of faecal samples.

Age groups	No.of birds Examined	No.of birds infected	% of infection	Calculated x_1^2 df.
Upto 4 weeks	200	29	14.5	
				9.00 **
Above 4 weeks	200	11	5.5	

** significant at (P/0.01)

FIGURE - 4 : Prevalence/incidence of coccidia in different age groups in Japanese quail through examination of faecal samples.



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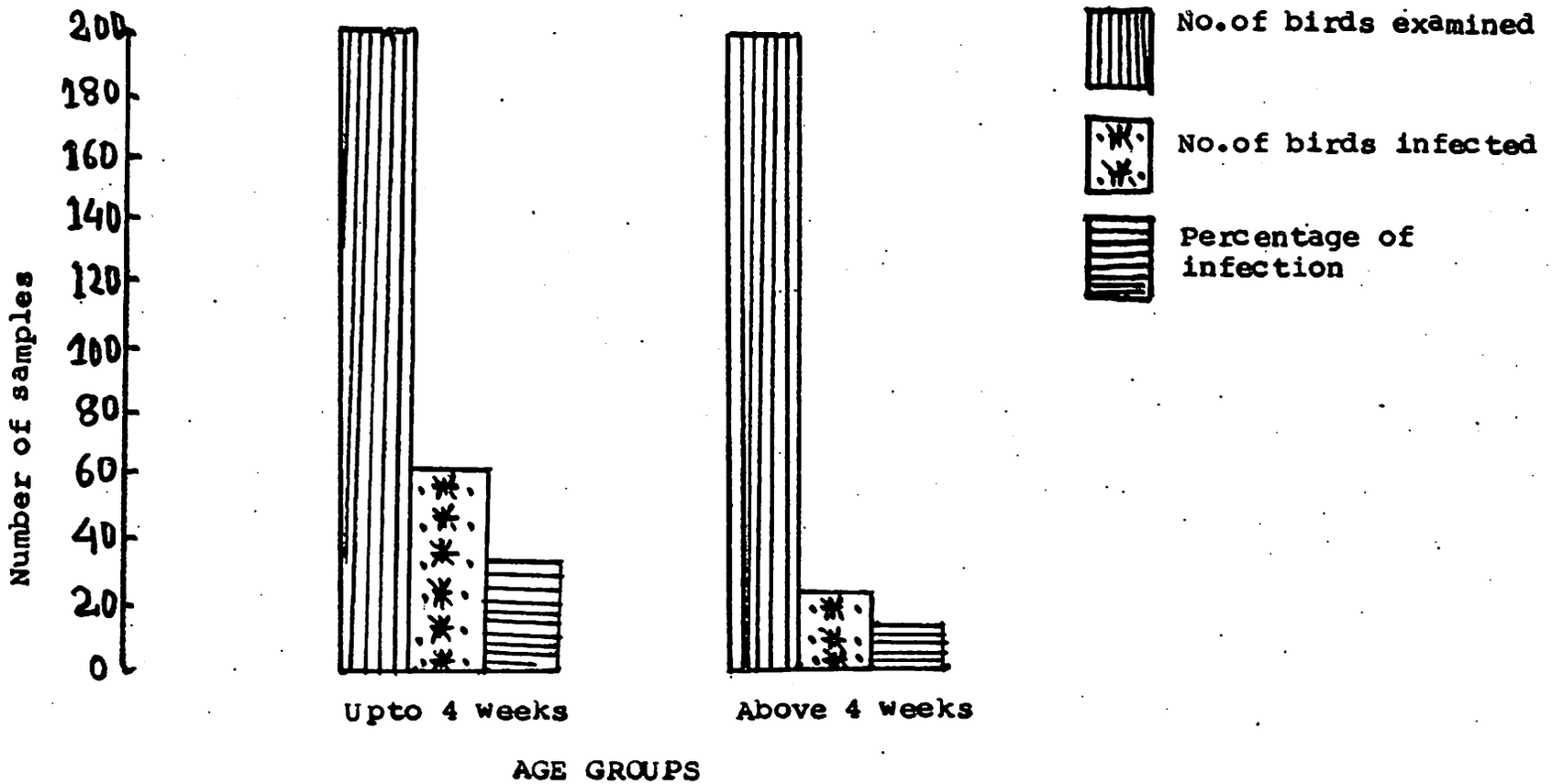

TABLE - 5 : Prevalence/incidence of coccidia in different age groups in Japanese quail through examination of intestinal scrapings.

Age groups	No.of birds examined	No.of birds infected	% of infection	Calculated x^2_1 df.
Upto 4 weeks	100	13	13	1.33 NS
Above 4 weeks	100	08	08	

NS - Non-Significant.

FIGURE - 5 : Prevalence/incidence of coccidia in different age groups in Japanese quail through examination of intestinal scrapings.

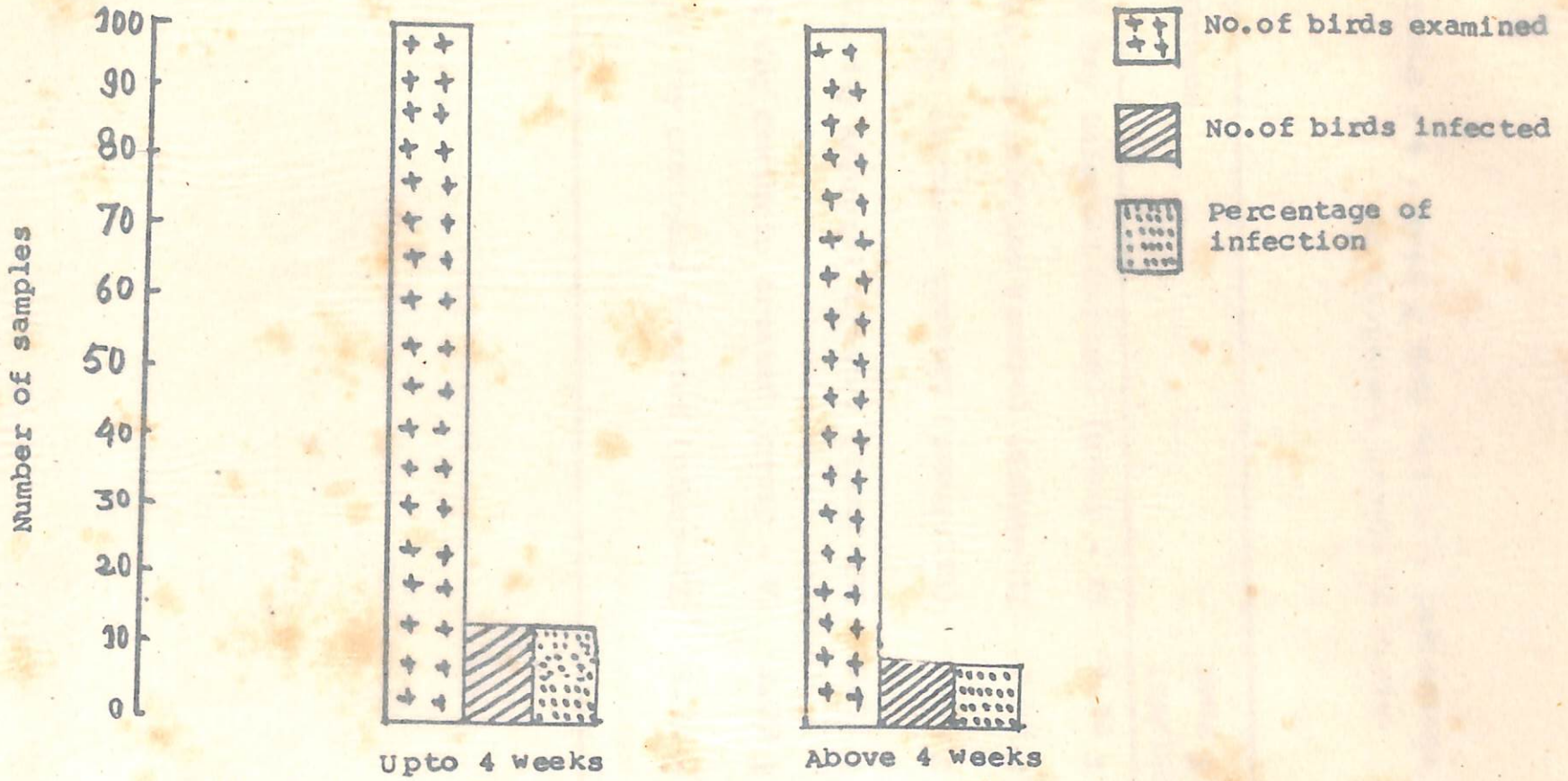

FIGURE - 5 : Prevalence/incidence of coccidia in different age groups in Japanese quail through examination of intestinal scrapings.

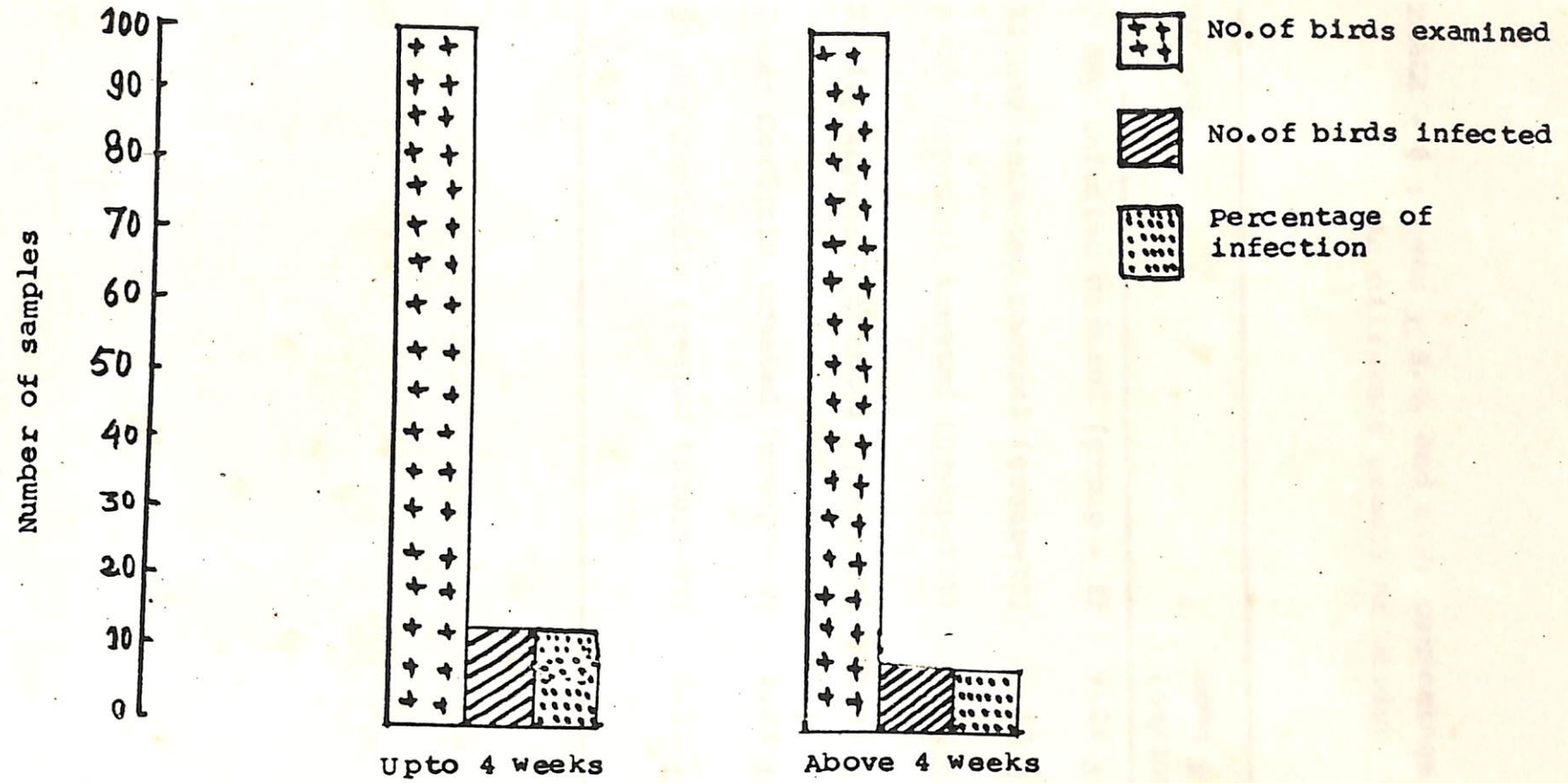

TABLE - 6 : Mean ± S.E. and C.V. percentage of Haemoglobin in different groups of birds.

Groups	Mean ± S.E. (gm/100 ml)	C.V.%
0 day infected control (group - I)	9.25 ± 0.12	4.14
21 day infected control (group-II)	9.15 ± 0.11	3.90
0 day Amprosol treated (group-III)	9.21 ± 0.13	4.59
21 day Amprosol treated (group-IV)	9.31 ± 0.12	4.22
0 day Coxiquin treated (group - V)	9.23 ± 0.14	4.95
21 day Coxiquin treated (gfoup-VI)	9.31 ± 0.14	4.81

TABLE - 7 : Analysis of variance showing the effect of different groups on Haemoglobin percent (Hb%).

Sources of variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	0.18	0.04	0.22 NS
Within groups	54	9.15	0.17	

NS - Non- Significant.

TABLE - 8 : Mean ± S.E. and C.V. percentage of total leucocyte count (1000/ml.) (TLC) in different groups of birds.

Groups	Mean ± S.E. (1000/ml.)	C.V.%
0 day infected control (group-I)	13.60^{a} ± 0.21	4.96
21 day infected control (group-II)	13.90^{a} ± 0.53	3.79
0 day Amprosol treated (group-III)	13.65^{a} ± 0.17	3.93
21 day Amprosol treated (group-IV)	12.08^{b} ± 0.16	3.85
0 day Coxiquin treated (group-V)	13.58^{a} ± 0.16	3.72
21 day Coxiquin treated (group-VI)	12.65^{c} ± 0.13	3.26

Means with different superscripts differ significantly ($P<0.05$).

TABLE - 9 : Analysis of variance showing the effect of different groups on Total leucocyte count (TLC).

Sources of variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	12.994	2.598	9.204**
Within groups	54	6.349	0.282	

** significant at ($P < 0.01$).

TABLE - 9 : Analysis of variance showing the effect of different groups on Total leucocyte count (TLC).

Sources of Variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	12.994	2.598	9.204**
Within groups	54	6.349	0.282	

** significant at (P/0.01).

E - 10 : Mean ± S.E. and C.V. percentage of Heterophil in different groups of birds.

ps	Mean ± S.E.	C.V.%
y infected control (group-I)	31.39 ± 0.41 (27.13)	4.11
ay infected control (group-II)	30.78 ± 0.30 (26.2)	3.13
y Amprosol treated (group-III)	31.46 ± 0.31 (27.20)	3.09
ay Amprosol treated (group-IV)	31.82 ± 0.19 (27.80)	1.89
y Coxiquin treated (group-V)	31.30 ± 0.29 (27.00)	2.89
ay Coxiquin treated (group-VI)	31.88 ± 0.29 (27.90)	2.86

ures in parentheses indicate percentage corresponding Arcsin $\sqrt{\text{percentage}}$.

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ıy Amprosol treated (group-IV)	31.82 ± 0.19 (27.80)	1.89
r Coxiquin treated (group-V)	31.30 ± 0.29 (27.00)	2.89
ıy Coxiquin treated (group-VI)	31.88 ± 0.29 (27.90)	2.86

ıres in parentheses indicate percentage corresponding ırcsin $\sqrt{\text{percentage}}$.

TABLE - 11 : Analysis of variance showing the effect of different groups on Heterophil percent.

Sources of variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	7.96	1.59	0.000017^{NS}
Within groups	54	49.99	0.93	

TABLE - 13 : Analysis of variance showing the effect of different groups on Eosinophil percent .

Sources of variation	D.F.	C.S.S.	M.S.	F.
Between group	5	49.79	9.96	3.99**
Within groups	54	134.74	2.49	

** Significant at ($P < 0.01$)

TABLE - 14 : Mean ± S.E. and C.V. percentage of Basophil in different groups of birds.

Groups	Mean ± S.E.	C.V.%
0 day infected control (group-I)	1.722 ± 0.88 (0.09)	161.02
21 day infected control (group-II)	1.72 ± 0.88 (0.09)	161.02
0 day Amprosol treated (group-III)	1.15 ± 0.77 (0.04)	210.82
21 day Amprosol treated (group-IV)	1.15 ± 0.77 (0.04)	210.82
0 day Coxiquin treated (group-V)	1.72 ± 0.88 (0.09)	161.02
21 day Coxiquin treated (group-VI)	1.15 ± 0.77 (0.04)	210.82

(Figures in parentheses incidate percentage corresponding to Arcsin $\sqrt{\text{percentage}}$).

TABLE - 15 : Analysis of variance showing the effect of different groups on Basophil percent.

Sources of variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	4.94	0.99	0.15^{NS}
Within groups	54	365.72	6.77	

NS :- Non-Signfficant.

TABLE - 16 : Mean ± S.E. and C.V. percentage of lymphocyte in different groups of birds.

Groups	Mean ± S.E.	C.V.%
0 day infected control (group-I)	$49.61^{ab} \pm 0.38$ (58.0)	2.40
21 day infected control (group-II)	$49.03^{ab} \pm 0.33$ (47.0)	2.15
0 day Amprosol treated (group-III)	$49.84^{bc} \pm 0.24$ (58.4)	1.53
21 day Amprosol treated (group-IV)	$50.53^{ce} \pm 0.22$ (59.6)	1.36
0 day Coxiquin treated (group-V)	$49.78^{abc} \pm 0.23$	1.46

TABLE - 17 : Analysis of variance showing the effect of different groups on Lymphocyte percent.

Sources of variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	18.35	3.67	4.53**
Within groups	54	43.75	0.81	

** Significant at ($P < 0.01$).

TABLE - 19 : Analysis of variance showing the effect of different groups on Monocyte percent.

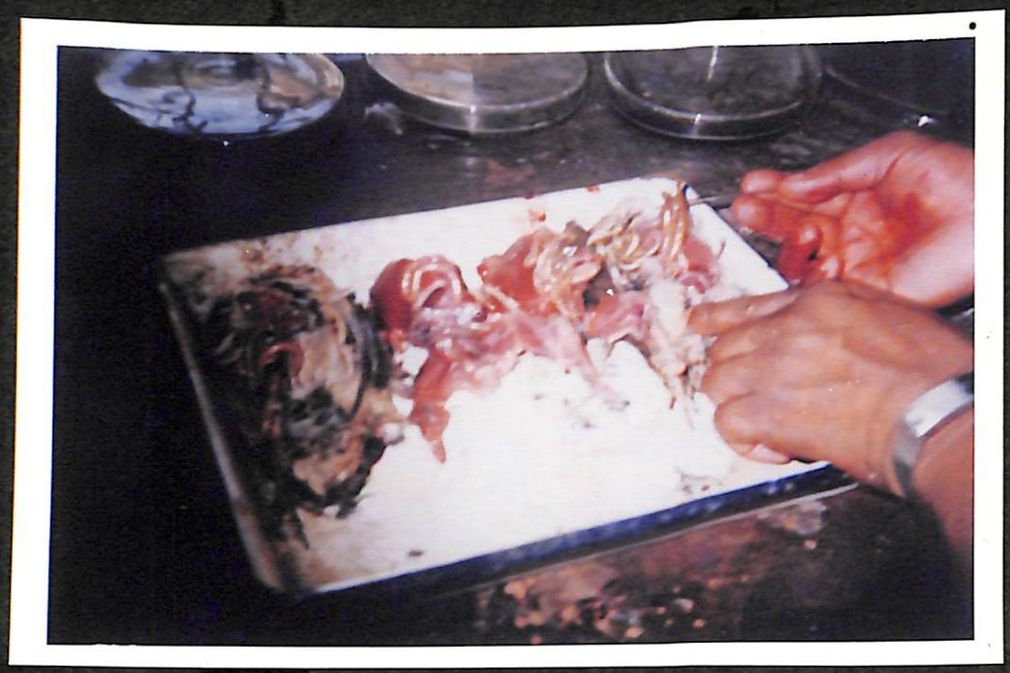
Sources of variation	D.F.	C.S.S.	M.S.	F.
Between groups	5	38.54	7.71	0.98^{NS}
Within groups	54	425.60	7.88	

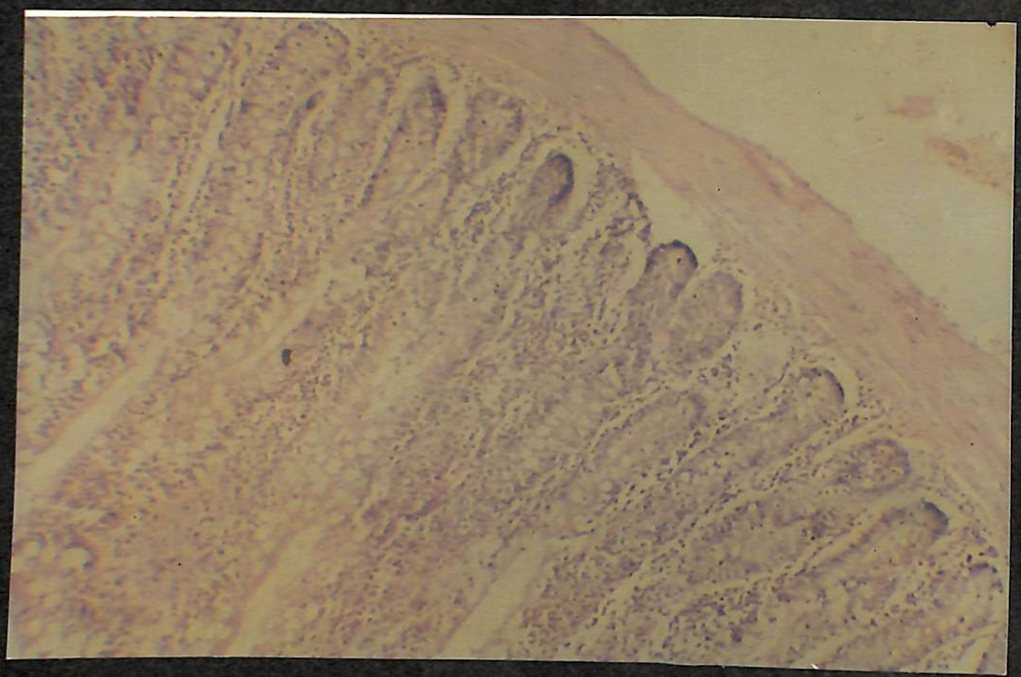
NS :- Non-Significant.

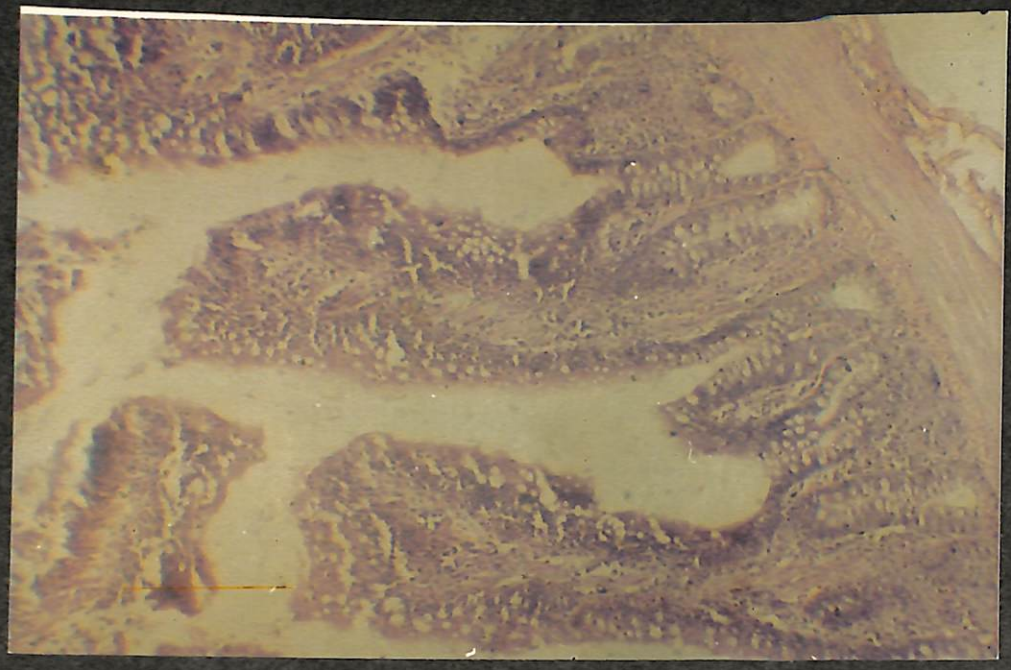
TABLE - 20 : Percentage efficacy of Amprosol and Coxiquin against coccidial infection in Japanese quail.

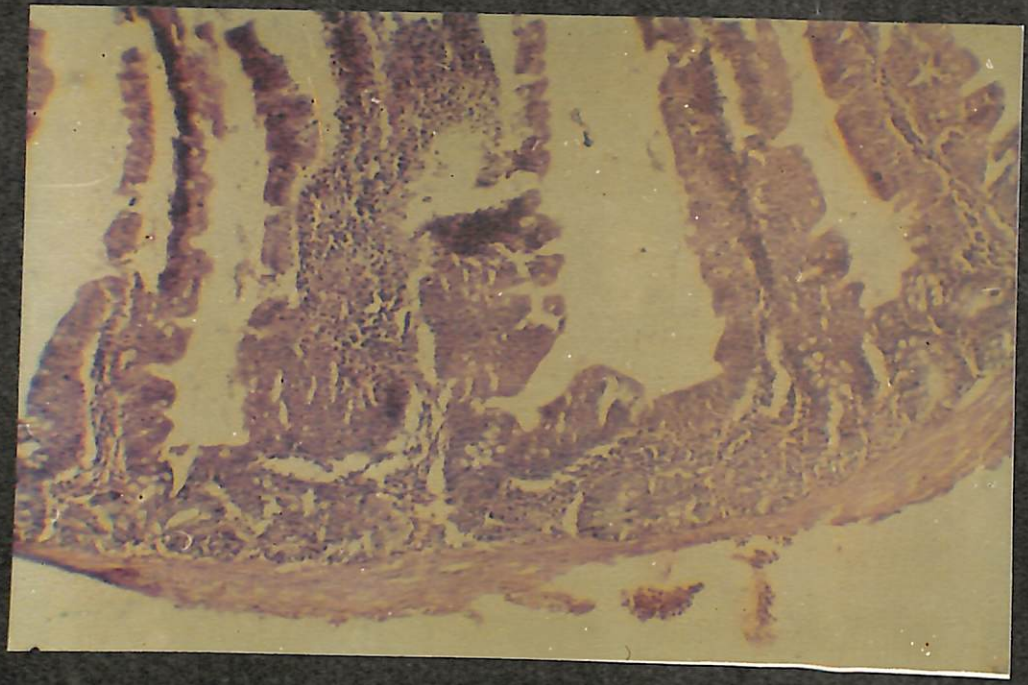
Group	No.of Quails	Drugs used	Dose by oral route.	Average EPG ($1x10^6$) before treatment 0 day	Average E.P.G.($1x10^6$) after treatment			% efficacy
					7th day	14th day	21st day	
Amprosol treated	10	Amprosol	1.2 gm/1 of drinking water for 5 7 days	1.878	0.212	0.110	0.052	93%
Coxiquin treated	10	Coxiquin	0.5 gm/1 of drinking water for 5 - 7 day	1.870	0.180	0.092	0.045	94.34%
Infected control	10	-	-	1.880	1.900	1.960	2.020	-

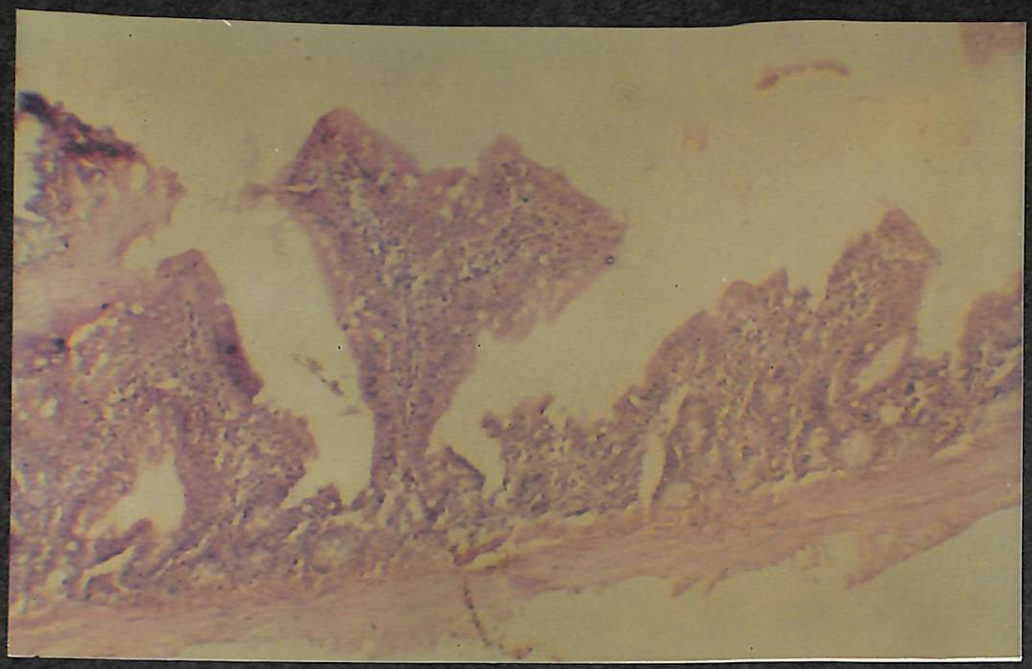
PLATE/PHOTOGRAPH

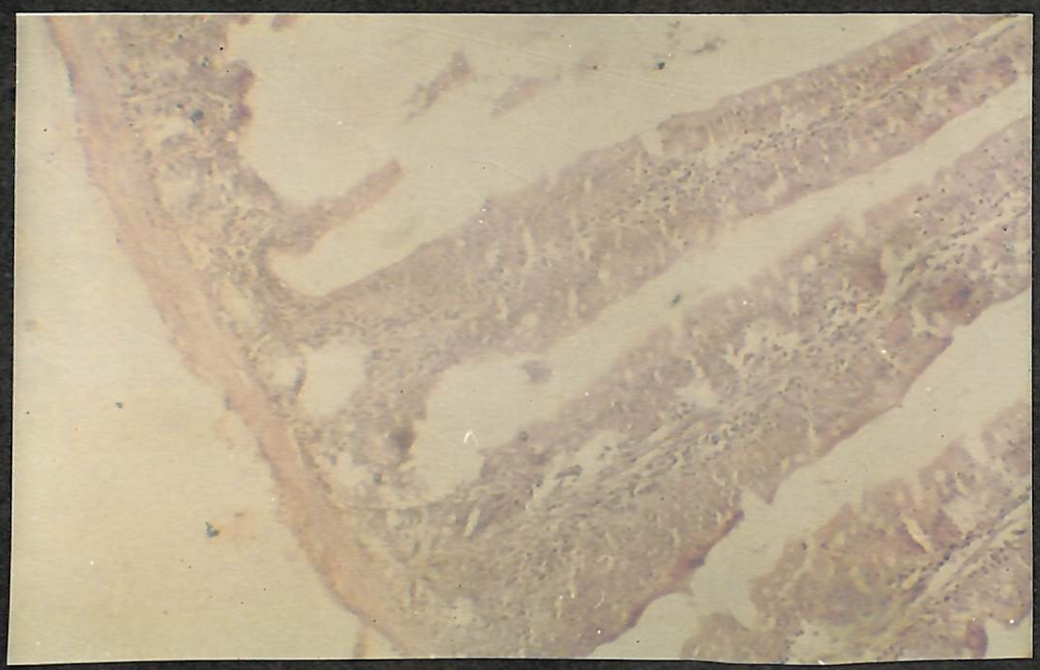


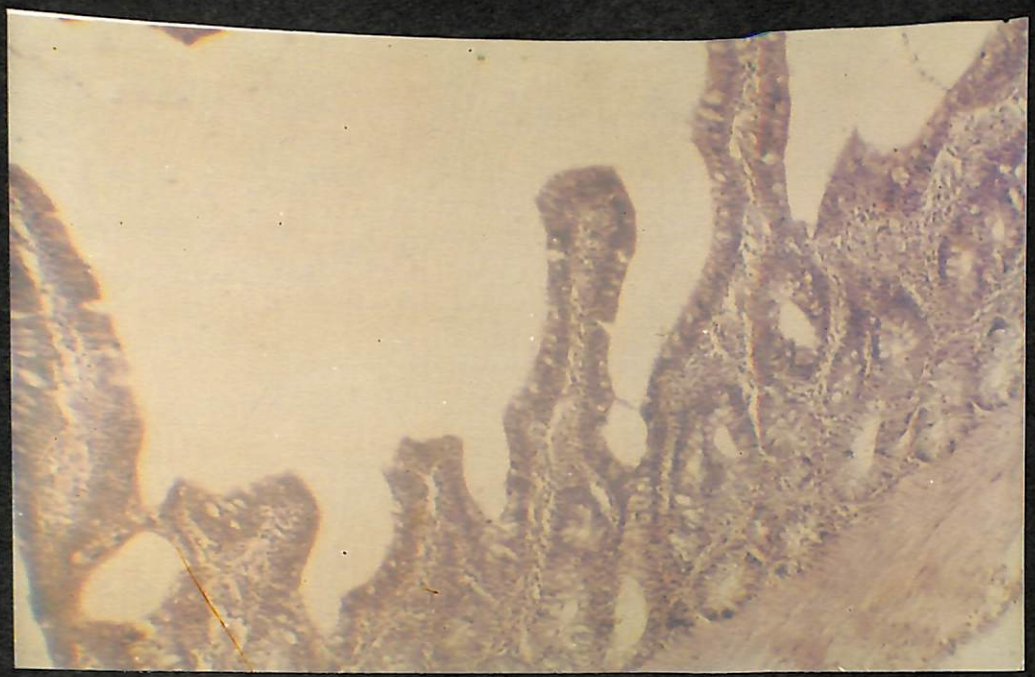


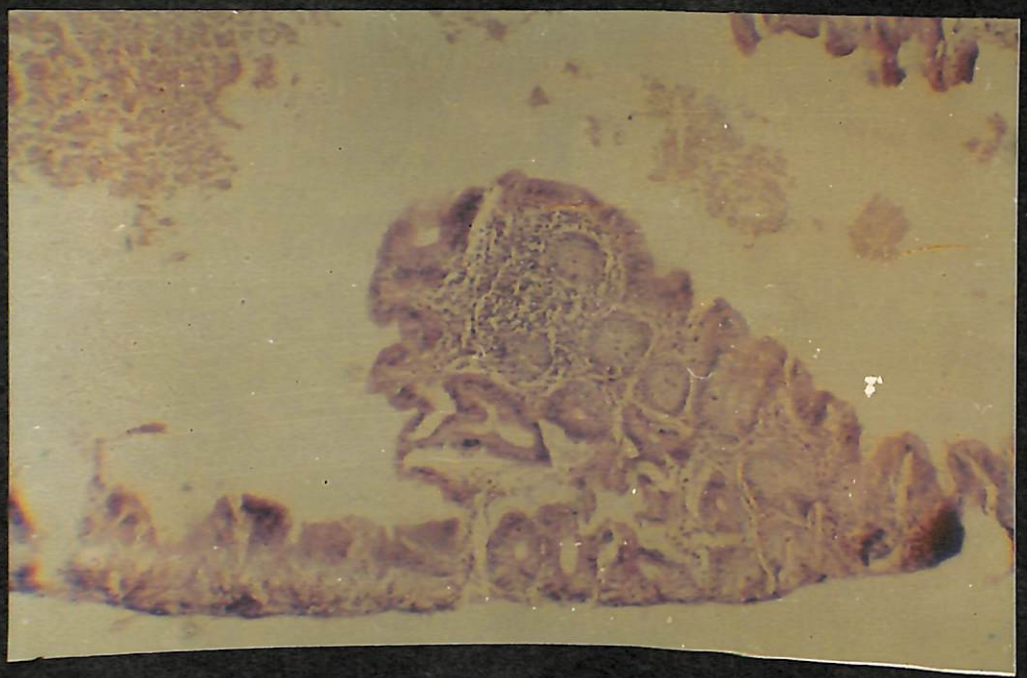


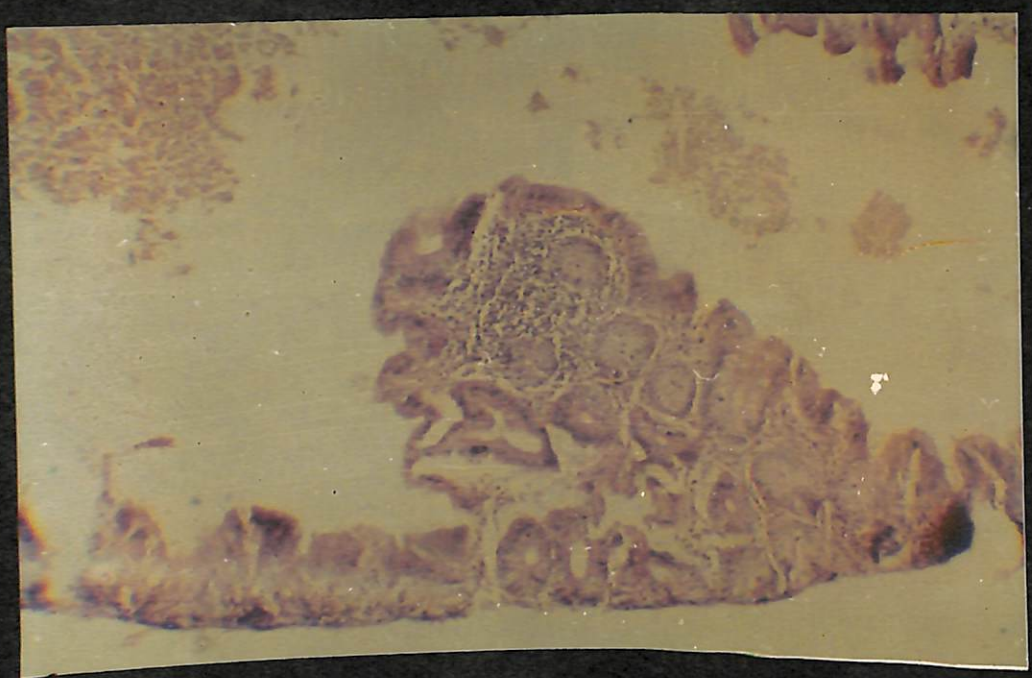


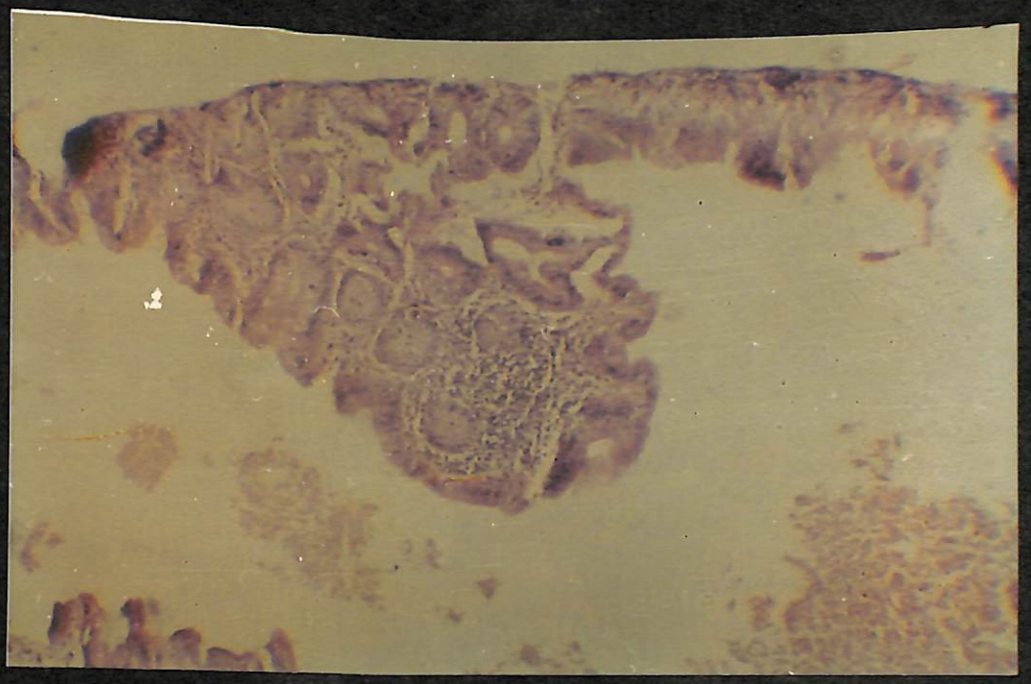


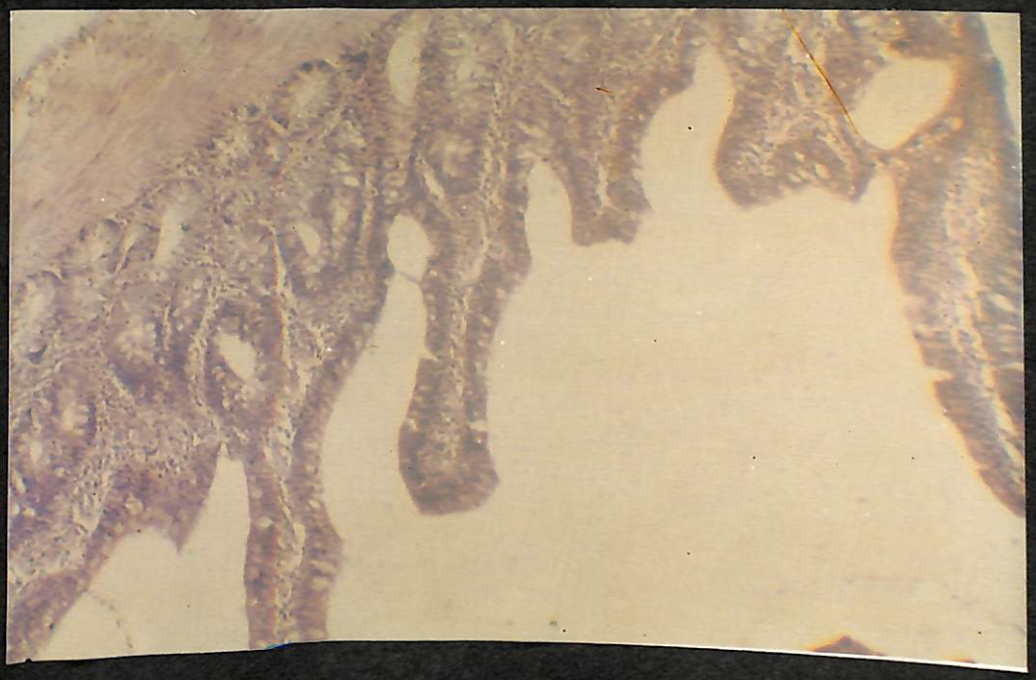


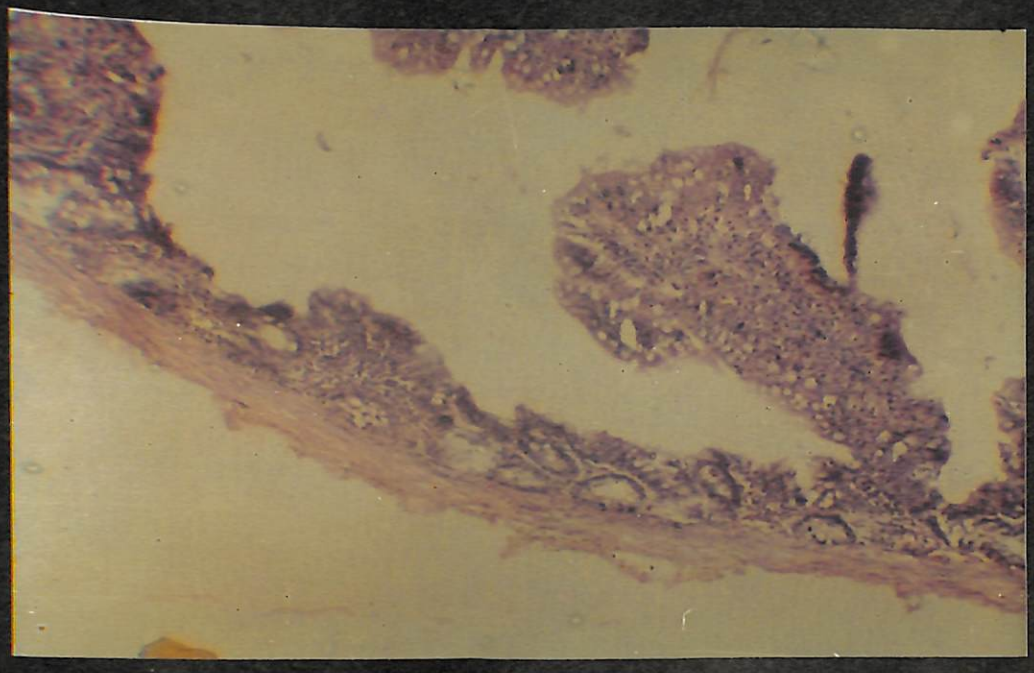


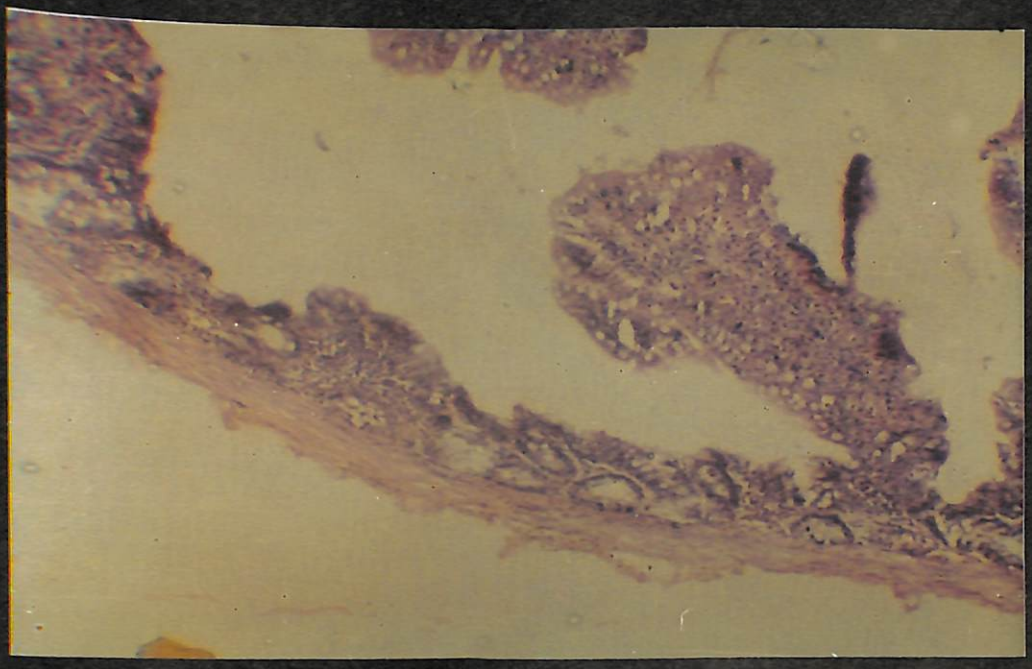


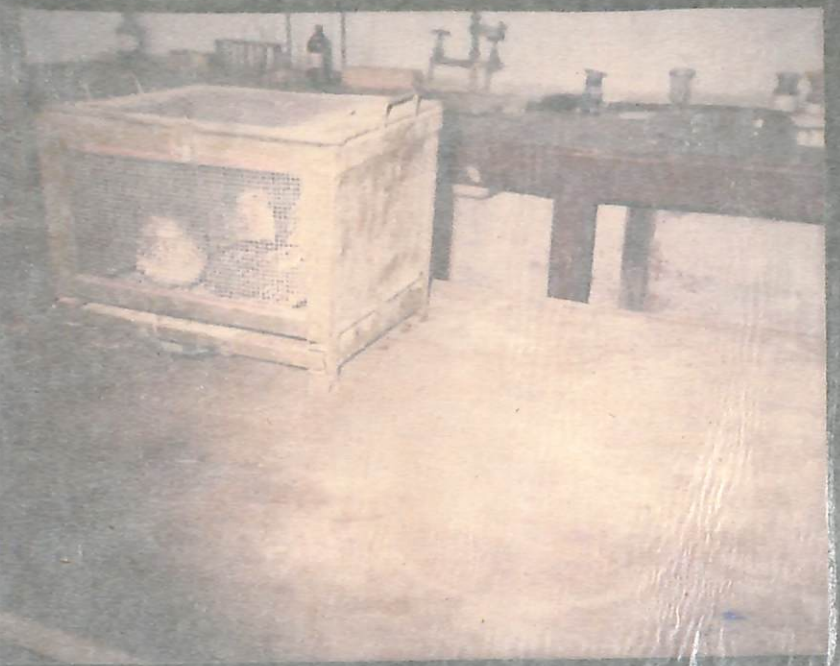


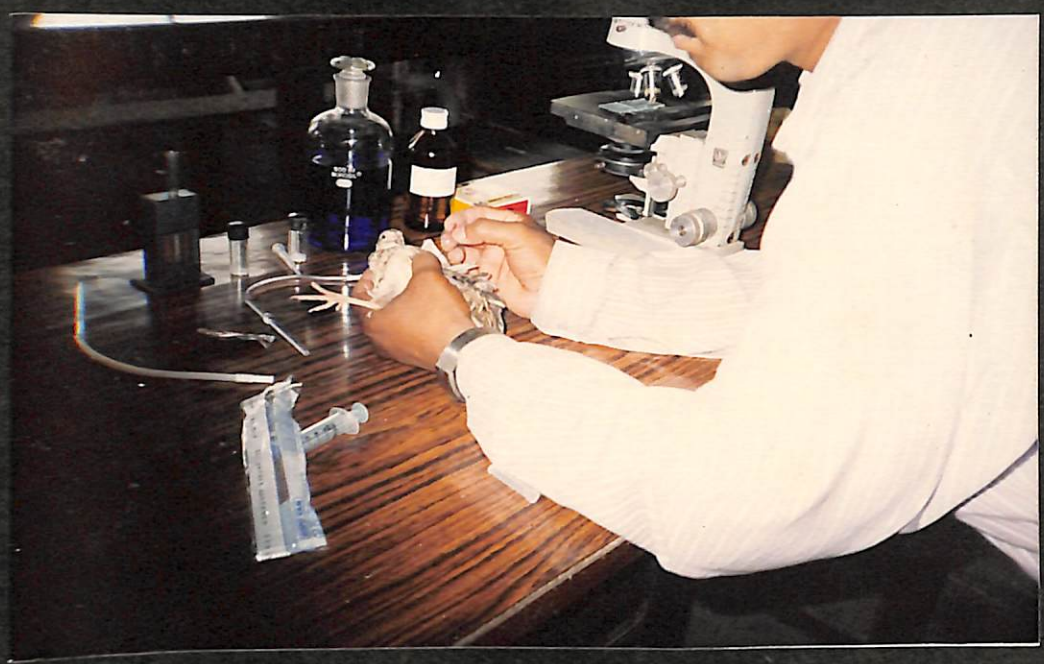












DISCUSSION

D I S C U S S I O N

D I S C U S S I O N

In the present study the prevalence and seasonal incidence of coccidia in Japanese quail was conducted in Patna during the period from March'1997 to Feb. 1998. For which 400 faecal samples and 200 intestinal scrapings were collected mainly from Central Poultry Farm Patna and also from local market.

INCIDENCE/PREVALANCE

The present investigation revealed that two species of coccidia namely Eimeria uzura and E. bateri were commonly isolated and the incidence of coccidial infection was noted to be 20 and 21 per cent from faecal samples and intestinal scrapings respectively . Similar prevalence of coccidiosis

study highest incidence of coccidial infection was observed during winter (25 and 26% from faecal samples and intestinal scrapings) while lowest was noted during summer (16% for both faecal samples and intestinal scrapings). The incidence of coccidia varies in between during rainy and spring seasons (Table 2 and 3). Statistical analysis did not show any significant difference denoting that season may not have much influence on the incidence of coccidial infection in Japanese quail. Panda et al. (1989) also showed highest incidence during winter and lowest during summer in Japanese quail.

In the present study the influence of age in the incidence of coccidial infection was investigaged through examination of faecal samples (Table 4) and intestinal scrapings (Table 5). The study revealed that the percentage of infection was significantly higher ($P<0.01$) in quail upto 4 weeks of age group as compared to quails of above 4 weeks of age group when faecal samples were examined. Though the incidence was higher in age group upto 4 weeks when examination of intestinal scrapings but it was non-significant as compared to quails of above 4 weeks. Panda et al. (1989) also showed higher incidence of death rate in birds of younger age groups compared to adult birds.

Haematological studies and effect of anti-coccidial agents

The available literatures do not show much studies on the effect of coccidial infection in Japanese quail. While

many studies were conducted on poultry. Hence in the present study comparision of the effect of coccidial infection on japanese quail obtained in compared with that of poultry.

In the present study, the infected control showed slight decrease in haemoglobin per cent on 21st day as compared to 0 day, while a minor increase was noted on 21st day in group (IV) (Amprolium treated) and in group (VI) (Coxiquin treated) as compared to 0 day, through the data are not significantly different. This showed that coccidial infection may not have significant effect on haemoglobin. In poultry Arnastauskiene et al. (1977) noted decreased R.B.C. count and haemoglobin values in experimental coccidiosis in chicks in contrast to the present study. Similar decline in haemoglogin was noted by Dakashinkar et al. (1985) and Dlariu et al. (1994) in chicken.

In the present study, the total leucocyte count estimated at 0 day Vs 21st day in infected control did not show significant difference while drugs treated groups showed significant reduction in TLC after 21st day. This denote that due to the effect of anticoccidial agents TLC might have reached to its normal count, while it remained higher in infected control even on 21st. day. This further showed that coccidial infection may possibly increase the T.L.C. count. Available leteratures did not show the effect of chemotherapeutic agents on TLC and DLC

in Japanese quail as well as the effect of coccidia on TLC and DLC.

Studies on differential leucocyte count showed no significant change in heterophil, basophil and monocyte count either due to coccidial infection or due to the effect of anticoccidial agents (Table-10, 14 and 18). No significant change was noted in eosinophil count between 0 day and 21st day in infected control as well as in Amprosol treated group. However, significant ($P \leq 0.05$) reduction in eosinophil was noted after 21 days of coxiquin treated birds as compared to 0 day value (Table 12). Above data showed the possible increase in eosinophil count due to coccidial infection and coxiquin may be effective in bring down the eosinophil count to normal levels. On the other hand, amprosol and ci coxiquin treated group showen significant increse in lymphocyte percent and the increase was more significant in coxiquin treatment group.

Histopathological studies:

In infected control where have infection occured showed higher degree of pathological changes. Mucoid degeneration alongwith infilteration of heterophils with alongated villi were the main feature. The lining epithlium of the villi contained vacuoles. There was widening of the Lamina propria, degeneration and disquamation of epithelial lining cells of villi & moderate degree of necrosis. These changes were also observed by Tsutsumi (1972), Rao (1988) in

Japanese quail infected with coccidia. In chicken also coccidia caused more or less similar histopathological changes in the intestine and caecum (Anwar 1981, Lozanov 1983). After 21 days of treatment with Amprosol and Coxiquin the microscopic lesions were not so prominant suggesting that the drugs were effective against the coccidia. Histopathological examination showed changes in duodenum, upper part of small intestine and caeca which were similar to that of infected control but to a milder degree. This showed that both the drugs are effective in coccidial infection of Japanese quail. The present oversevations is in complete aggrement with the findings of Rao (1988) who also observed mild histopathological changes in infected Japanese quail when they were treated with various anticoccidial agents.

Efficacy of Chemotherapeutic agents against coccidia

In the present study the efficacy of two chemotherapeutic agents namely Amprosol and Coxiquin (Aprosol + Sulphaquinoxaline) were tested through the counting of average number of egg per gram (EPG) in faeces. After 7th day of the application of the drugs it was observed that number of E.P.G. was decreased repadly in drug treated group where as it was found to be slightly increased in control group. The average E.P.G. further declined on 14th and 21st day in drug treated groups while it was slightly increased further in control group. This showed that both Amprosol and coxiquin were effective

in coccidial infection in Japanese quail but coxiquin was more effective than Aprosol. The effecacy of coxiquin was calculated to be 94.34% and that of Amprosol to be 93% . Similarly Tsutsumi and Tsunoda (1972) showed that Amprolium and 0.0125 - 0.12% in feed for 7 consecutive days resulted complete supression of oocyst in bird infected with 10^4 E. tsunodai Panda (1978). Similarly Rao (1988) showed that Amprolium afforded 93.7% protection in Japanese quail infected with E. uzura.

The present study also showed similar rate of protection of Amprosol in Japanese quail against coccidia. Samad et al. (1993) showed that sulphaquinoxaline was more effective than other sulphonamides in the treatment of E. tenella infection in chicken. In the present study also combination of Amprosol with sulphaquinoxaline (Coxiquin) was found to be more effective (94.33%) as compared to Amprosol (93.34%).

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SUMMARY

SUMMARY

S U M M A R Y

During the present investigation the prevalence and seasional incidence of coccidiosis in Japanese quail was conducted in Patna from 400 faecal samples and 200 intestinal scrapings. In addition haematological parameters like haemoglobin percentage (Hb%), Total leucocyte count (T.L. C.) and Differential leucocyte count (D.L.C.) in naturally infected birds control and in drugs treated group i.e. Amprosol and Coxiquin groups were also investigated . Histopathological studies and efficacy of the above noted chemotherapeutic agents (Assessed by average egg per gram (E.P.G.) were also conducted.

The present investigation revealed that two species of coccidia (Eimeria uzura and E. bateri)were present in quails and the incidence of coccidiosis was similar in faecal samples (20%) and intestinal scrapings (21%). The seasonal incidence of coccidiosis was higher during winter while the lowest was noted during summer and fluctuated in between during rainy and spring season. However, statistical analysis did not show any significant difference during various seasons,. However, the influence of age in the incidence of coccidiosis revealed that the percentage of infection was significantly higher ($P < 0.01$) in quail upto 4 weeks of age group as compared to quails of above 4 weeks of age group when faecal samples were examined. The examination of intestinal scrapings although showed higher incidence in age group upto 4 weeks but it was not significant

as compared to quails of above 4 weeks.

Studies on haemoglobin percent (Hb%) showed insignificant decrease in Hb% on 21st day as compared to 0 day in infected control while an insignificant increase in Hb% was noted on 21st day in Amprolium treated and Coxiquin treated birds as compared to 0 day. This showed that coccidial infection may not have much effect on haemoglobin. In the present studies the total leucocyte count estimated at 0 day Vs 21st day did not showed significant difference in infected control while significant reduction in TLC was observed in drugs treated groups on 21st day. This denotes that due to effectiveness of anticoccidial agents TLC might have reached to its normal count while it remained higher in infected control even on 21st day. This further indicates that coccidial infection may possibly restore the TLC count to its normal value. Studies on differential count showed non-significant change in heterophil, basophil and monocyte count either due to Coccidial infection or due to the effect of the anticoccidial agents (Amprolium & Coxiquin). No significant change was noted in Eosinophil count between 0 day and 21st day in infected control while significant decrease in eosinophil count was noted on 21 day, in Amprosol & Coxiquin treated birds as compared to 0 day values. The above data showed the possible increase in eosinophil count due to coccidial infection and the effects of the above drugs in bringing

down the eosinophil count to normal level.

Histopathological investigation showed heavy infection in infected control where higher degree of pathological changes were observed in duodenum sm small intestine (particularly upper part) and caecum . Pathological changes such as mucoid degeneration, infiltration of heterophils, elongated villi containing vacuoles in the lining epithelium modrate, degree of necrosis etc. After 21 day treatment with Amprosol and coxiquin in naturally infected quails mild degree of infection was noticed. Histopathological examination showed changes in duodenum and upper part of small intestine and caccum which was similar to that of infected control but to a mild degree. This showed that both the drugs may be effective in coccidiosis in Japanese quail.

In the present investigation, the efficacy of two chemotherapeutic agents viz. Amprosol and coxiquin (Amprosol + Sulphaquinoxaline) were tested by counting the average number of eggs per gram (EPG) in faeces. After 7th day of treatment with the above drugs that E.P.G. was decreased rapidly in drug treated groups while it was found to be slightly increase in infected control. The average E.P.G. further declined on 14th and 21st day in drug treated groups while it was marginally increased further in infected control. This showed that both Amprosol and

Coxiquin were effective in treating the coccidial infection in Japanese quail but coxiquin was comparatively more effective. The efficacy of coxiquin was calculated to be 94.34% and that of Amprosol to be 93%.

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B I B L I O G R A P H Y

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