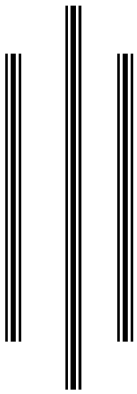
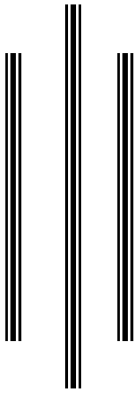
STUDY ON PREVALENCE OF *EIMERIA* SPECIES IN LAYER CHICKEN OF KATHMANDU AND LALITPUR DISTRICTS



A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER IN SCIENCE

BY

RAKESH PRASAD JAYSWAL



TO

THE CENTRAL DEPARTMENT OF ZOOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

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RECOMMENDATION

It is our pleasure to mention here that Mr. **Rakesh Jayswal** carried out the thesis work entitled "**STUDY ON PREVALENCE OF *EIMERIA* SPECIES IN LAYER CHICKEN OF KATHMANDU AND LALITPUR DISTRICTS**" under our supervision and guidance. It is his original work and brings out useful results and findings in the concerned field.

We strongly recommend this thesis for the partial fulfillment of the requirements for the Master's Degree of Science in Zoology with special paper Parasitology.

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LETTER OF APPROVAL

On the recommendation of supervisor **Dr. Ranjana Gupta** and Co-supervisor **Dr. Swoyam Prakash Shrestha**, this thesis of **Mr. Rakesh Jayswal** is approved for examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for M.Sc. degree in Zoology with special paper Parasitology.

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ACCEPTANCE CERTIFICATE

This thesis presented by **Mr. Rakesh Jayswal** entitled "STUDY ON PREVALENCE OF *EIMERIA* SPECIES IN LAYER CHICKEN OF KATHMANDU AND LALITPUR DISTRICTS" has been approved for partial fulfillment of the requirements for the degree of Master of Science in Zoology with Parasitology as a special paper.

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ABSTRACT

The present study was conducted in Machhegaun, Futung and Gothatar of Kathmandu district and Khumaltar of Lalitpur district from June 2002 to May 2003 with an aim to determine age- wise, site-wise and month- wise prevalence of coccidiosis as well as to identify the disease causing *Eimeria* species in Layer chicken. Five different species viz. *E. tenella, E. acervulina, E. necatrix, E. maxima* and *E. brunetti* responsible for coccidiosis were reported and the general prevalence was 24 percent. The highest number of positive cases (40%) was found with mixed infection. Species-wise infection with *E. tenella* was recorded to be the highest (25%), followed by *E. acervulina* (15%), *E. necatrix* (10%). *E. maxima* (7%) and *E. brunetti (3%)*. Clinical symptoms of coccidiosis were diarrhoea, sometimes with blood tinged chocolate colour or bloody droppings. Post mortem lesion were intestine balloning, pin point haemorrhage, haemorrhagic enteritis, caecal distention and bloody ingesta.

There were altogether 96 cases of coccidiosis of which 25%were caecal, 35% intestinal and 40% mixed. Prevalence of coccidiosis was recorded in all the 12 months and four seasons, of the study period.The highest (38%) prevalence rate was found in the month of July and the lowest (5.71%) in the month of October. The difference in monthly prevalence was insignificant ($\chi 2=19.675$, $p<0.05$). Similarly season wise prevalence showed the highest (35%) prevalence rate in the spring season followed by autumn (12%), winter (23%) and summer (25%), The difference in season-wise prevalence was insignificant ($\chi 2= 7.815$, $p> 0.05$). The age- wise prevalence was the highest (34.66%) in 61 weeks above chicken, followed by 30% in the 46-60 weeks age group, 22.5% in the 31-45 weeks age group, 17.14% in the 0-15 weeks age group and 15.78% in the 16-30 weeks age group. The difference in age- wise prevalence was found to be insignificant ($\chi 2= 9.488$,$P> 0.05$). Site- wise prevalence of coccidiosis was the highest 29.14% in Khumaltar, followed by 22.5% in Gothatar, 18.66% in Futung and 18.57% in Machhegaun. The difference in site- wise prevalence was found to be insignificant ($\chi 2= 7.15$, $P >0.05$).

Sporulation of oocyts of *Eimeria* species was completed after 72 hours period at 24^0 c to 26^0c, 48 hours period at 30°c and one week or more period below 20°c in an aerobic condition.

From histopathological sections, various degree of destruction of villi, loss of inflammatory cells and lymphocytes in the intestinal lumen and hemorrhages the intestinal epithelial tissue were found. Intestinal wall was also found to be thickened due to infection of above mentioned *Emeria* species. Caecal coccidiosis was found to be more pathogenic in comparison to intestinal coccidiosis.

Hence in poultry coccidia are ubiquitous but disease is most likely when young stocks are concentrated under condition which permit the accumulation and sporulation of large number of oocyst. The five species of *Eimeria* identified in this study are all pathogenic.

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LIST OF ABBREVIATIONS

ASI	Anterior Small Intestine
AHRD	Animal Health Research Division
C-SDR	Cause Specific Death Rate
CVL	Central Veterinary Laboratory
FCR	Food Conversion Ratio
GDP	Gross domestic product
GI tract	Gastro-intestinal tract
GLS	Gross Lesion Score
IAAS	Institute of Agriculture and Animal Science
L.S.	Longitudinal section
M	Mucosa
MSI	Middle Small Intestine
MLS	Microscopic Lesion Score
MU	Muscularis
OCS	Oocysts Count Score
PSI	Posterior Small Intestine
SM	Submucosa
T.S.	Transverse section
V.D.C.	Village Development Committee

I

INTRODUCTION

In Nepal, poultry farming is one of the commercial branch of agriculture practice. It is the main source of livelihood both in the rural and urban farming communities. It has gained tremendous momentum day by day for the fulfillment of chicken and egg demand of the country. Chicken is also used extensively as a delicious food. The egg is also rich in vitamins like A, D, B_1, B_2 and pantothenic acid. The chicken meat contains a high quality of thiamine, riboflavin and pantothenic acid (Naidu, 1967).

The other advantage of poultry farming is poultry manure which contains high proportion of nitrogen, phosphorous and potassium. The feather has decorative value. The down feathers and filoplumes are best suitable for sleeping bags and high altitude trekking jackets. The poultry meat is quite popular in Nepal and poultry farming may be a full or part time occupation requiring minimum labor and money.

The Layer is commercially developed for egg production; about 300 to 330 eggs are produced by Layer per annum up to 72 weeks of age. The common breeds of Layer chicken in Nepal are *Keystone, Hyaline, Astrolop, New Ham share* and *Babcock.*

Poultry farming commenced in Nepal from early sixties only after, when some improved breeds like Astrolop, Wewhamshire pullets were imported from abroad(Canada, North America) With these improved Layer chicken breeds, government as well as private hatcheries came in existence to fulfill the demand of Layer chickens. In the last decade the poultry industry has experienced massive growth and expansion of over 10% and 18% per annum for eggs and meat respectively, Now, the industry shares 3% of the total GDP and 8 percent of the agriculture GDP of the country. It indicates that the industry had been transformed into one of the main sustainable agro-livestock enterprises. In Nepal, total number of fowl is 23023979and meat production and egg production of chicken is 15881 M.T. and 560033000 in number respectively (Central Bureau of Statistics, 2004).

There are two major genera of coccidia belonging to phylum protozoa. *Isospora,* which infects to the dogs, rabbits, cats and humans and may cause mortality if not treated

in time. The other genera *Eimeria* is responsible for the coccidiosis in poultry, rabbits, goats and other herbivorous animals. *Eimeria* oocysts have four sporocysts containing two sporzoites each whereas *Isospora* oocysts have two sporocysts containing four sporozoites each. In both the cases the mature oocysts contain eight sporozoites

Infection with a single species of coccidia are rare in natural conditions, mixed infection being the rule; never the less, in many outbreaks the clinical entity can be ascribed principally to one spices , or occasionally a combination of two or three. *Eimeria tenella* is the most pathogenic and important species, followed by *Eimeria necatrix*. Many coccidiostatic drugs have been directed against *E.tenella*, with the result that other species are increasingly incriminated as a cause of poultry coccidiosis. *Eimeria brunetti* may be markedly pathogenic but is uncommon. *E. maxima* and *E. acervulina* are increasingly common but are of moderate pathogenicity , and *E. mitix* and *E. praecox* are considered to be less pathogenic than the others.

Eimeria species infections are ubiquitous and the limit to their distribution is the host (Jordan and Pattion, 1996). On the basis of affected organ, the disease is classified as intestinal coccidiosis affecting the intestine and caecal coccidiosis affecting the large intestine (caeca) At least nine species of *Eimeria* are to occur in poultry viz. *E. acervulina, E. mitis, E. maxima, E. necatrix, E. brunetti, E. temella, E. hagani E. praecox*, and *E. mivati*. Each species has its own specific site of invasion in the intestine of chicken. *E. tenella* attack the double caeca, while *E. acervulina* affect the double portion of intestine. Similarly *E. necatrix* and *E. maxima* affect the major portion of the small intestine. The species specific site of predilection in the intestine provides great value for their identification.

The species of *Eimeria* are identified of the morphological basis taking in view of their shape, size and internal structure. Based on the organ affected, the disease is classified as intestinal coccidiosis, affecting the small intestine and caecal coccidiosis, affecting the caeca of large intestine.

The intestinal coccidiosis occurs in chicken aged between 5 to 20 weeks. It occurs in both acute and chronic forms. In the acute form the chicken dies without showing any clinical signs while in the chronic forms the chicken shows anorexia, becomes inactive for some days and ultimately dies (bloody droppings).

In the host, the parasite grows and multiplies intracellular in epithelial and sub epithelial cell of the gut wall (Gordan and Jordan, 1982) . The *Eimeria* are highly host specific, coccidiosis in Turkey is a less serious problem than in chicken. Similarly, they have species specific predilection site on the gut of chicken, which provide a basis for their identification.

Ingestion of viable sporulated oocysts through contaminated feed and water is only natural method of transmission. Human are second in transport and dissemination of oocysts to chicken. Mechanical transmission is commonly affected by manure clinging to shoes or by fomites from one person to another.

Coccidia are cosmopolitan in distribution (Hufshed, 1992) and only the range of appropriate hosts evidently limits the geographical range of individual species. In the external environment, the limit of factors for survival of oocysts include the presence of oxygen, water and suitable temperature. The viability of oocysts and their survival on the ground is one of the most important factors affecting the cause of the disease. Infected chicken discharge large number of oocysts in their dropping in the fresh state. The occysts are not infective until further development take place. Sporulation takes one or two days under optimum temperature of 25^0c to 32^0c with high humidity but fail to sporulate under adverse environmental condition.

Exposure to temperature of 45 - 50^0c for about one day or short- term exposure when oocysts are sporulated they are resistant to low temperature, but not freezing, relatively resistant to dry condition and resistant most of the bacterial disinfectants. They are susceptible to temperature above 56^0 c and are readily killed by ammonia gas and methyl bromide gas.

Since the use of anticoccidial drugs stanted, the loss encured by the coccidial infection in chiken has been controlled to certain extent. Still the loss due to this disease in the developed country like U.S.A. is about 650 million dollors per year (www.anticoccidiosisdrugs.com2005).

Coccidiosis can occur at any stage of life and any season when condition are favorable. But chicken reared outdoors under ranged conditions, outbreaks of coccidiosis are more common in spring and summer than under cool fall and winter condition. Out breaks may occur at any season of the year, if confinement rearing is practiced. Day old

chicken are susceptible to coccidiosis but may develop minimal infections because occysts are not excysted in very young chicken (Rose, 1967).

Various species of *Eimeria* are known to cause coccidiosis in chicken but pathogenecity varies with the species (Urguhart, 1987). Severity of the disease also depends upon the number of oocyst ingested and also on the frequency of ingestion of occysts. The disease is likely to occur only under condition of high stocking density and unhygienic environment. A single viable sporulated oocysts swallowed by a bird is capable of massive and very rapid multiplication so that within 5 days it can cause complete destruction of more than a million of the cells lining the digestive tract (Sekar and Eajendran, 1997).

In conclusion, coccidiosis is one of the important diseases in intensive farming system. In free range system coccidiosis outbreaks are common in spring and summer than in winter. If confinement rearing is practiced out break may occur at any season of the year. It is considered to be a disease of poor management. Due to indiscriminate use of anticoccidial drugs in feeds and water, drugs resistance against this disease is a major problem. Hence, it is strongly felt that, a study is essential for proper identification of different species of *Eimeria* and the knowledge, regarding the prevalence of disease in different age groups. The assessment of the damage caused by the parasites in the host and their identification will greatly help in formulating the treatments and control strategy in the future to enhance the poultry industry (Layer&Broiler family) in the country.

II

AIMS AND OBJECTIVES

The aim of study is to identify the various species of *Eimeria* and to know the epidemiological condition of the disease.

General Objective

- To determine the epidemiological study on the prevalence of coccidiosis in Layer chicken of Kathmandu and Lalitpur districts.

Specific Objectives

- To identify the *Eimeria* species in Layer chicken of Kathmandu and Lalitpur district.
- To determine the month- wise, season- wise, age- wise and site- wise prevalence of coccidiosis in Layer chicken
- To assess the damage caused by the parasite in the Layer chicken.

III

LITERATURE REVIEW

History of Coccidiosis

Coccidiosis caused by *Eimeria* species has been recognized since (1891), different scientists recorded nine main economically important *Eimeria* species of chickens.

Raillet and Lucet (1891) and Fontham (1909) recorded *Eimeria tnella* which invaded epithelium and submucosa of the caecal pouches causing 'bloody' caecal coccidiosis. This species was highly pathogenic and caused 100 percent mortality in artificially infected chicken.

E. acervulina was examined by Tyzzer (1929) and found that this species invades the epithelial cell of the anterior small intestine especially the duodenal loop. He reported *E. acervuina* is non pathogenic. He also examined *E. mitis* infected predominately the anterior half of small intestine without producing any gross lesions.

Tyzzer (1929) further examined *Eimeria maxima* which parasitized the middle portion of intestine. He reported that infection of this parasite caused a hemorrhagic enteritis, weight loss, poor conversion ratio, reduced egg production and decreased yellow pigment in the skin.

The most important pathogenic species of chicken coccidiosis was *Eimeria necatrix* reported by Johnson (1930). He found that this species invaded the jejunum producing lesions and balloning of the middle intestine. This species caused higher mortality rather than any other species except *E. tenella*. He also recorded *Eimeria praecox,* winch Infected the upper intestine. This species was mild pathogenic causing depression and loss in weight gain.

Levine (1938) reported *Eimeria hagani* as a moderately pathogenic species of chicken from New York State. He also reported *Eimeria brunetti* in the lower portion of small and large intestine in (1942). In severe infection, he

found this species localized in the anterior portion of the small intestine and neck of the caeca.

Coccidiosis Research in Global Perspectives

Clarke (1979) investigated on periodicity of caecal defecation in chicks inoculated with *E. tenella*. The chicks were illuminated for 12 hr./day (7.00 hr.-19.00 hr.) and faeces were collected at 3 hr. intervals for up to 27 days during the periods 7.00 - 10.00 hr., 10.00 -13.00 hr., 13.00 -16.00 hr. and 16.00 -19.00 hr. Caecal defecation were occured in appropriately 44%, 19%, 55% and 91% of the total number of days respectively.

Gordon and Jordan (1982) studied the specific characters for seven species of chicken coccidia *(Eimeria* species) from UK and presented in tabular form. The figures and description made by them still serve as a main basis for species identification all over the world.

Long and Reid (1982) summarized the specific characters for nine different species of chicken *Eimeria* from USA and presented in a tabular form. The finding was based on the research work by themselves and other workers in this field.

Umeche and Eno (1987) surveyed on parasites of chickens from poultry farms in Calabar, Nigeria. A total of 150 chickens were examined and observed ten species of parasites. Among them *E. tenella* (64%) was found most prevalent and coccidiosis was reported to be a serious problem.

Pavlovic and Dragica (1989') studied parasitic fauna of poultry industry in the Republic of Serbia. *E. tenella. E. necatrix, E. maxima, E. brunetti* and *E. acervulina* of coccidial oocysts were diagnosed in 75 flocks.

Isolation of five species of *Eimeria* from chicken in Bangladesh was earned out by Karim and Trees (1990). Five species of *Eimeria* namely *E. acervulina, E. tenella, E. maxima. E. brunetti* and E. *necatrix* were identified in chickens on the basis of lesion seen at post mortem examination of naturally

infected birds and on dimension of oocysts and the lesions seen in chicks experimentally infected with single oocyst derived strains.

Kim *et al.* (1990) reported a rapid method for the purification of chicken coccidian oocysts in Korea. They obtained oocysts of chicken coccidia by using a gravity floatation and sodium hypochlorite Cleaning techniques. Out of 5 species of coccidia tested, the recovery rates of pure cocysts was the highest as 90.5% in *E. acervulina* and followed by 74.1% in *E. mitis,* 67.5% in *E. brunetti,* 66.3% in *E.tenella* and 64.1% in *E.maxima.*

Shiotani *et al.* (1992) studied on distribution of oocysts, sporocysts and sporozoites of *E. tenella* and *E. maxima* in the digestive tract of chicken in Osaka. At one hour after the oral inoculation of *E. tenella* oocysts, the number of sporocysts in the caecum was $3.4x10^6$ and decrease gradually there after and the number of sporozoites in the caecum increased and remained at a high level unit 12 hours after the inoculation was found. Small number of sporocysts and sporozoites of *E.tenella* were found in other intestinal sites. A great number of *E. maxima* sporozoites especially in the jejunum were found 2 hr. after inoculation

Fitz-coy *et al.* (1992) studied on the effect of *E. mitis* on egg production of single comb white leghorn hen in the Department of Agriculture University Md. Eastern Shore. They reported egg production was significantly reduced temporarily but most birds return to production within 14 days.

Majara (1993) studied on coccidian parasites of indigenous domestic fowls *(Gallus gallus domesticus)* from rural areas of Oyo State, Nigeria. He reported 66% positive oocysts samples in 400 fecal samples collected. He identified 6 *Eimeria* species viz. *E. tenella, E. necatrix, E. acevulina, E. brunetti, E. maxima* and *E. mitis* by employing the morphological and shape index criteria. *E. acervullna* and *E. tenella* were the most prevalent species with percentage occurrence of 41 and 28 respectively.

Franco (1993) surveyed on avian coccidiosis from two layer farms in Campinas, Brazil. Five *Eimeria* species were detected viz. *E. acervulia, E. mitis, E. tenella, E. maxima and E. necatrix* on the basis of site of the parasite in the

intestinal tract, type of the lesions, size of the oocysts, prapatent period of the oocysts and development stages of the parasites. The numbers of oocyst present in fecal samples of 8-16 weeks age group were found higher than 24 - 42 weeks age groups.

Karim and Begun (1994) studied the usefulness of oocyst morphology and biological characteristics in the identification of *Eimeria* species of chicken at Bangladesh Agriculture University and reported that oocyst dimension when used by analyzing regression coefficients of width of length, combined with prepatent period and site of the infection can be I used for identification of species. They identified seven species of *Eimeria* viz. *E. tenella, E necatrix, E.maxima, E. brunetti, E. acervulina, E praecox* and *E. mitts* in artificially infected chicken.

Grault *el al.* (1994) studied on sporulation of oocysts in *E. acervulina* under different environmental conditions. They recorded maximum sporulation percentage in both dry (22.6%) and clammy litter (19.5%) was higher ($P<0.05$) than in pure faces (11.6%). They also recorded 68% sporulation of oocysts under optimum condition (29^0c aeration in 2% potassium dichromate solution). Sporulation at 33°c proceeded at a faster pace than at 22°C ($P<0.05$) with respect to relative humidity arid substrate. They concluded that temperature is the important factor in sporulation of *E, acervulina* during a flock cycle.

Williams (1995 a) studied the fate of ingested oocysts of *E. tenella* during the prepatent period in susceptible chicks in UK. He found that the recovery rates of sporulated oocysts were less (2-9%) than unsporulated oocysts (15-21%) from chicks inoculated at all ages tested from 2 to 28 days. The higher recovery rates of unsporulated oocysts indicated that they were more resistant to disruption in the gizzard than sporulated oocysts. He also found that the length of oocysts recovery period in the oldest birds were 10 -15% of those in the youngest.

Williams (1995 b) also studied physical condition and survival of *Eimeria acervulina* oocysts in poultry house litter in UK. He reported physical condition of oocysts is not reliable indication of their viability. He found that the

surface temperature (25-28°c) of litter and moisture content (31.0 to 62.1 %) were not limiting factors for oocyst sporulation.

Tsuji *et al.* (1997) studied on discrimination of eight species of *Eimeria* species using the two steps polymerase chain reaction (PCR). A method was developed for the discrimination of 8 species of chicken using two steps PCR. They reported that this method should be useful for discrimination of the parasite species for diagnosis or epidemiological study of chicken coccidiosis.

Thebo *et al.* (1998) studied on identification of E*imeria* species in domesticated fowl in Sweden. They reported seven *Eimeria* species from dead birds on the basis of oocyst morphology, location, characteristics of intestinal lesions, morphology of parasite endogenous stages, prepatent time and isoenzyme electrophoresis.

Lunden *et al.* (2000) studied on *Eimeria* infections in litter based, high stocking density system for loose housed laying hens in Sweden. They reported outbreaks occurred when the birds were 19 to 32 weeks old. The maximum oocysts counted were found 4 to 8 weeks after introduction to laying house. They further reported the raising pullets without any coccidiosis, to increase their chance to develop immunity against coccidiosis were not found to decrease the risk of coccidiosis during the production period.

Mattiello *el al.* (2000) studied on confirmation of 7 species of *Eimeria* in ten poultry farms (broiler breeder pullets, layer pullets and broiler) in Argentina. Species were identified by prepatent period, oocyst size, location and appearance of lesions in the intestine, microscopic examination of mucosal smears and histology (to confirm *E. brunetti).* On this basis *E. praecox* was found in two samples, *E. mitis* in two, *E. acervulina* in nine, *E. maxima* in seven, *E. necatrix in* three. *E. tenella* in seven and *E. bruetti* in four.

Basith and Rajavelu (2000) studied seasonal prevalence of coccidiosis in chicken at Tamil Nadu, India. They reported high occurrance of disease of 64.80% in 9 to 20 weeks age groups followed by 21.79% in 21 to 72 weeks age groups and 13.4% in 0 to 8 weeks age groups. They recorded 9.5% cases of coccidiosis during

summer season at 29°C - 37°C and, rainfall (0-99 mm.) and 11.7% cases of coccidiosis during cold season at 24°C-32^{0}C and nil rainfall. They also reported prevalence rate 26.26% during southwest monsoon period of June to September at 29.7°C-34°C and rainfall (97-187.5mm) and 53.07% during northeast monsoon .period at 21^{0}C-33°C and rainfall (57-157.5 mm).

Diaz *et al.* (2001) investigated on frequency of *Eimeria* species in some parts of poultry area of Tahuacan in the state of Puebla in Maxico. They found the mean age of maximum oocysts elimination in the chickens was of 40 days. Average frequency of *Eimeria* species in the area was ' *tenella* 40%, *E. brunetti* 25.6% *E. maxima* 20.6% and *E. acervulina* 13.8%.

Safari (2003) studied the prevalence and economic impact of poultry coccidiosis in small scale and large scale poultry farms in urban and Pen Urban areas of Ethiopia. They detected coccidiosis oocysts in 220 (80.3%), 8 (6.8%), 11 (14.8%), 231 (72.9%) and 73 (81.1%) of fecal samples which were collected from large scale broiler farms and large scale layer farms. They recorded *E. acervulina* (34.4%), *E. necatrix* (4%), *E. maxima* 8.6%), *E. tenella* (32.8%) and 20% mixed species. They reported lesion due to *E. tenella* and *E. necatrix* were characterized by haemorrhagic thyphlitis and enteritis, respectively and due to *E. acervulina* and *E. maxima* were characterized by mucoid enteritis.

Coccidiosis Research in National Perspectives

Thakuri and Rai (1996) carried out a study at Pakhribas Agriculture Central. Dhankuta to identify the species of *Eimeria* infecting village chicken in the eastern hills of Nepal. They identified four species of *Eimeria* namely *E. acervulina, E, brunetti, E. tenella* and *E. maxima* on the basis of oocyst morphology (dimension and shape) and postmortem lesions seen in the experimentally infected chickens.

Singh *et. al.* (2001) carried out a study on incidence of coccidiosis chicken in three commercial poultry farms in Lalitpur district for one year and found 41.5% were caecel coccidiosis, 36.6% were intestinal coccidiosis and 21.9% were mixed coccidiosis among total 123 cases of coccidiosis. They also

analyzed cases of coccidiosis recorded at Central Veterinary Laboratory. Total cases of coccidiosis were 133 of winch 71 (53.4%) were intestinal and 62 (46.6%) were caecal coccidiosis. The morbidity and mortality percentage were 5.53°c and 6.29% respective. The highest incidence of coccidiosis was 45.8% recorded in 4-8 week age group followed by 39.1% in 0-4 week age group.

Aryal (2001) conducted an epidemiological studies on *Eimena* species in natural outbreak of coccidiosis at institute of Agricultural and Animal Science, Rampur and its vicinity. He found 64% mixed infection, 12% *E. tenella*, 8% *E. necairix* and 4% *E. brunetti*. Highest incidence rate (41.7%) of coccidiosis was recorded in broiler of 2-4 weeks and 34% was recorded in 4-6 weeks age group of layer chicken. He reported the highest incidence (44%) during summer followed by during winter (24%) and the lowest incidence was in autumn (13.33%) season.

Pant (2003) carried out a study on identification of coccidian parasite in chicken from 45 farms of 7 districts. He identified two *Eimena* species viz. *E. tenella* and *E. maxima*. He reported the prevalence of *E. tenella* and E. *maxima* were 97.77% and 2.22% respectively.

REGION

IV

MATERIALS AND METHODS

Glass wares:- (i) Conical flask (ii) Petridishes

(iii) Measuring cylinders (iv) Glass rods

(v) Slides (vi) Cover slips

Chemicals:-

i) Potassium dichromate 2% v) Acid alcohol 1%

ii) Methylene blue vi) Methanol

iii) Formalin vii) Hydrochloric acid

iv) Glycerol viii) Carbol fuchsion

Equipment

i) Electric balance iv) Refrigerator

ii) Incubator v) Electric microscope

iii)Calibrated eyepice micrometer

Study Duration

The duration of the present study was one year from June 2002 to May 2003.

Study Area

Kathmandu valley lies in Central Development Region which covers an area of 395sq km and has 3 small administrative districts viz Kathmandu, Lalitpur and Bhaktapur. The altitude of these districts is in average of 1350m. The climate is temperate type with an average annual temperature of 19^0 c and rainfall of 152mm.

The current study was conducted in 3VDCs, of Kathmandu district namely Machhegaun, Futung and Gothatar and in Khumaltar of Lalitpur.

Place	Flock size	Samples size
Machhegaun	200	70
Futung	650	75
Gothatar	200	80
Khumaltar	3026	175

The present work was divided into following parts:

1. Stool sample collection, preservation and examination
2. Stool culture and examination
3. Postmortem examination
4. Histopathological examination

Stool sample collection, preservation and examination

From above selected sites at least 4 poultry farms were randomly selected. From these poultry farms 400 stool samples (dropping) were collected by random sampling method. These samples were preserved in preservative solution (2% potassium dichromate solution). Stools were examined by smear methods.

Staining for Oocysts of Coccidia (*Eimeria* species)

Oocysts of *Eimeria* species passed in droppings are oval, spherical or subspherical, measuring (18.3-24.6⎪14.6-18.8) µm in diameter. They can be concentrated by either direct concentration method with the help of centrifuging machine by 2000-rpm speed till 5 minutes or using modified formaline ether technique. These oocysts of *Eimeria* species were stained by modified Ziehl-Neelsen technique.

Process of Modified Ziehl-Neelsen technique

A thin faecal smear was made and left it to air dry. Then it was fixed in methanol for 2 to 3 minutes. The fixation of formalin vapor was performed to reduce infectivity. The smear was stained with cold carbol-fuchsin for 5 to 10 minutes. It was then differentiated in 1% hydrochloric acid-ethanol until colour ceases to flood out. It was rinsed with tap water. It was further counterstained with 0.25% malachite green

(methylene blue) for 30 seconds. Then it was rinsed with tap water and was left for air dry. For making permanent slides, it was mounted with D.P.X.

Stool Culture and Examination

For detailed morphological study of oocysts, they were cultured (sporulated) in the laboratory. For this, these droppings were kept in 2% potassium dichromate solution in the ratio of 1:5 (1 part dropping and 5 parts of potassium dichromate solution) under aerobic condition at room temperature. These sporulated oocysts were examined under microscope and their shape and size were observed. The size of the sporulated oocysts was measured using a calibrated eyepiece micrometer under microscope at 100x magnification. The size (length and width) and shape of about 10 sporulated oocysts from each positive sample were recorded for identification of *Eimeria* species.

Characteristics of *Eimeria* species.

Character	*E.acervulina*	*E.necatrix*	*E.maxima*	*E.tenella*	*E.bruntti*
Zone Intestine parasitized	Anterior small intestine (ASI)	Middle small intestine (MSI)	Middle small intestine (MSI)	Caeca	Posterior small intertine (PSI)
Post mortem lesion	Transverse whitish band on duodenal loop.	Petechial haemorrhage in MSI	Thickened intestine with pink	Distended caeca by blood	Mucold bloody enteritis
	Market mucold enteritis	Ballooning of intestine.	Mucoid exudates	Erosion of caecal mucosa	Coagulation necrosis
	Intestinal wall.	Mucoid blood filled exudates		Clotted blood in caecal pouch	Yellowish white caseous material
Oocysts shape	Ovoid	Oblong Ovoid	Ovoid	Ovoid	Ovoid
Length | width	Average 18.3 |14.6 (17.7-20.2) (13.7-16.3	Average 20.4 |17.2 (13.2-22.7) (11.3-18.3)	Average 30 |20.7 (21.5-42.5) (16.5-29.8)	Average 20.2 |19.0 (19.5-26.0) 16.5-22.8)	Average 24 |18.8 (20.7-30.3) (18.1-24.2)

Long and Reid, (1982)

Plate No. 1 Stool Sample Collection from Layer Farm Machhegaun.

Plate No.2 layer Farm in Futung.

Plate No. 3 Stool Sample Collection from Layer Farm Gothatar.

Plate No. 4 Stool Sample Collection from Layer Farm Khumaltar.

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Plate No. 5 Stool Samples from the Filed Under Process of Sporulation.

Plate No.6 Samples from Post Mortem Layer for histopathological study.

Plate No. 7 Microscopic examination of AHRD, Tripureshwor

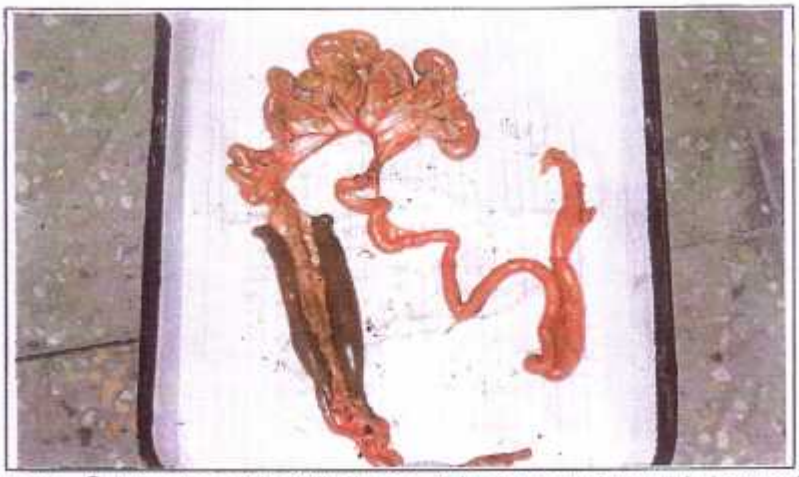

Plate No. 8. Mixed coccidiosis (ballooning of duodenum and caeca) in Layer chicken

Plate No. 9. Caecal coccidoisis in 3 weeks Layer chicken.

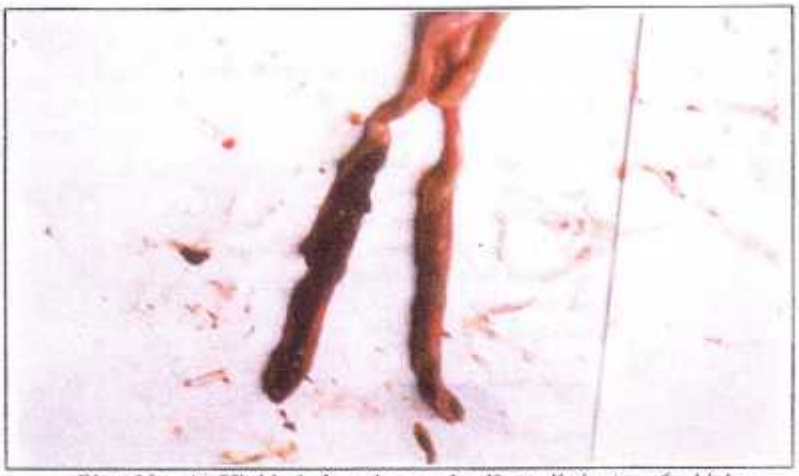

Plate No. 10 Highly infected caeca by *E.tenella* in Layer chicken.

Post Mortem Examination

Post mortem examination was carried out on the chickens that were brought to the Animal Health Research Division, Veterinary Complex, Tripureshwar, for the disease diagnosis. Post mortem examination was carried out

Necropsy examination:- On the basis of gross lesion and hemorrhages in the gastrointestinal tract. The history of the case, clinical symptoms, nature of macro lesion on the gastrointestinal (GI) tract were the main criteria for diagnosis of the disease. Samples were collected from intestinal contents, mucosal scrapings of the intestinal lesion and also from four different sites of the intestinal mucosa i.e. anterior small intestinel (ASI), mid small intestine (MSI), posterior small intestine (PSI) and caeca.

Microscopic examination of Mucosal scrapping

Superficial scrapping of intestinal mucosa were made using a scalpel blood during necropsy. The scrapping were then mounted on a glass slide and diluted with equal amount of normal saline and covered with a coverslip. The slides were examined under low (10x) and higher (40x) objectives for observing the oocysts. Finding the oocysts, in sufficiently large number on the slides of intestinal lesions confirms coccidiosis. The oocysts of different shape and size and lesions on more than one site of intestine i.e. ASI, MSI, PSI and caeca confirmed mixed infections.

Histopathology

The degree of damage of the intestinal wall due to coccidiosis was performed by histopathological method. For detail anatomical study, infected and normal part of the intestinal tract were collected and preserved in 10% buffer formaline for histological examination. The samples were washed, sectioned and stained with Haematoxylene and Eosin.

Process of Tissue preparation of Histopathology

Collection

About one inch long infected gastro-intestinal tracts were collected in normal saline. In case of mixed infections samples from all parts of GI tracts were collected.

Fixing and Hardening

The samples collected were fixed and hardened in 10% buffer formalin so that it could be cut into thin sections. After fixation, the excess of fixative was washed by tap water from the tissues to prevent the interference with subsequent process.

Dehydration: Ascending series of Alcohol were used for dehydration.

Impregnation: For this purpose, paraffin wax was used to impregnate the tissues.

Cuttings: Microtome was used for cutting the impregnated tissues in the block.

Mountings: The paraffin ribbons containing the sectioned tissues were mounted on a grease free side.

Removal of paraffin wax

The wax of melting point 58^0c was taken. The slide were kept in an oven for removing the wax. It was also removed by immersing the slides in a jar containing xylene, a wax solvent.

Staining, Dehydration, clearing and mountings

For staining the material, double staining method (Haematoxylene and Eosin) and for dehydration alcohol series were used. Then the material was mounted in D.P.X.

Data Tabulation, analysis & interpretation. The collected data was tabulated, analysed & Interpretation was drawn in text and statistically by $\Re^2$ and 't' - test.

V

RESULTS

The present study ' Study on prevalence of *Eimera* species in Layer chicken of Kathmandu and Lalitpur districts' was conducted from June, 2002 to May, 2003. The results were analysed and discussed according to the objectives of the study.

1. General prevalence of coccidiosis

The total number of dropping examined were 400 of which coccidiosis positive samples were 96. The percentage of positive droppings for coccidiosis was 24.

2. Month-wise prevalence

The month- wise prevalence of coccidiosis with respect to temperature, relative humidity and rainfall in one year period has been presented in table no. 1 and illustrated in fig 1.

Table no.1 shows that the prevalence of coccidiosis was high (38%) in rainfall 544.80 mm, relative humidity 85.45 precent and temperature 24.50^0c in the month of July. Then there was a gradually decrease in the prevalence rate of coccidiosis from August to October. The lowest prevalence rate was in the month of October (5.71%) in rainfall, 15.00mm, relative humidity 82.70 and temperature 20.10^0c. In the month of November the prevalence rate increased slightly.

The Chi-square test indicated that the difference in monthly prevalence of coccidiosis was insignificat

($\Re^2$ = 19.675, P <0.05).

The student's 't' test indicated that the difference in monthly prevalence of coccidiosis was insignificant (t = .197, P>0.05).

Table No. 1

Monthly prevalence of coccidiosis from June 2002 to May 2003, in Layer chicken.

S.N.	Months	Average temperature (^{0}c)	Average RH %	Average rain faill (mm)	No. of droppings examined	No. of droppings positive for caccidiosis	Positive %
1	June	24.45	79.50	227.40	40	14	35
2	July	24.50	85.45	544.80	50	19	38
3	August	24.40	85.70	499.90	30	9	30
4	September	23.15	83.40	148.00	40	7	17.5
5	October	20.10	82.70	15.00	35	2	5.71
6	November	16.10	85.95	26.50	35	4	11.42
7	December	13.10	82.20	68.40	30	6	20
8	January	10.55	85.70	19.5	25	5	20
9	February	11.90	87.50	0.00	35	10	28.57
10	March	16.75	74.75	86.00	30	7	23
11	April	20.95	69.35	33.00	25	6	24
12	May	22.00	76.75	158.80	25	7	28

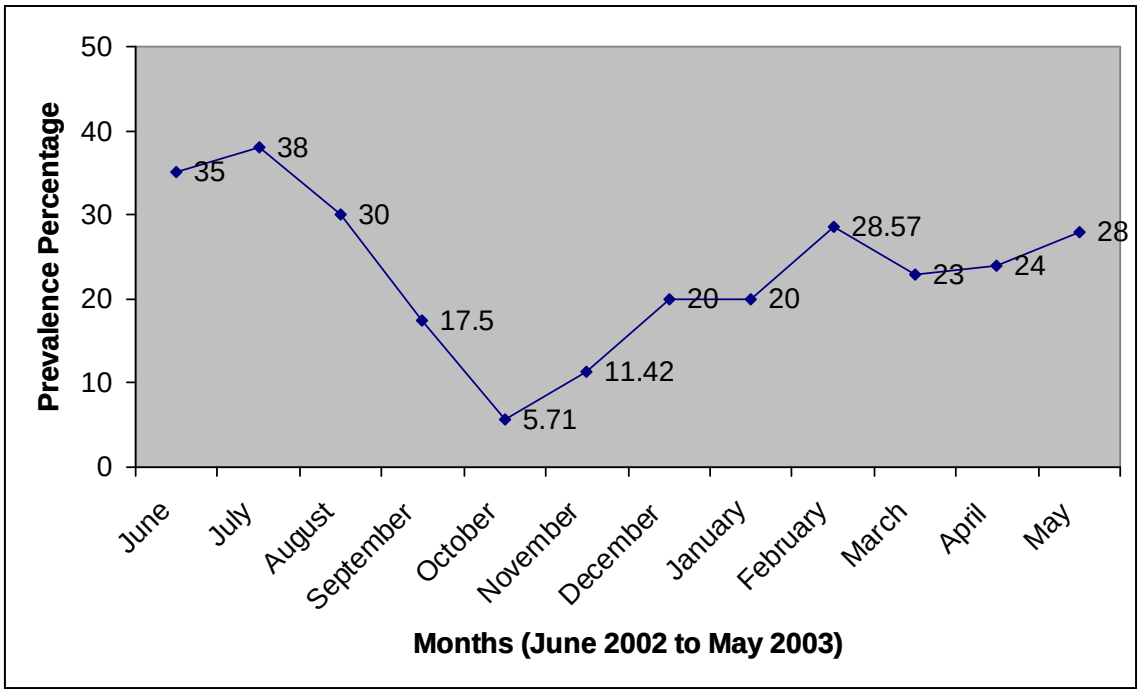

Fig. 1: Month-wise prevalence of coccidiosis in Kathmandu and Lalitpur districts.

3. Season-wise prevalence of coccidiosis in layer chicken.

The seasonal variation in prevalence rate of coccidiosis has been in the table no. 2 and illustrated in Fig. 2. The prevalence of coccidiosis was the highest during spring (Rainy) season (35%) and the lowest during Autumn season (12%). The prevalence of coccidiosis in the winter and summer gradually increased were (23%) and (25%) respectively. The chi-squire test indicated that the difference in season-wise prevalence of coccidiosid was insignificant (χ^2 = 7.815, P >0.05). The student's 't' test indicated that the difference in season-wise prevalence of coccidiosis was insignificant (t = 1.15, P>0.05). However, the prevalence of the disease was found to be higher when the climate is hot and humid during rainy season.

Table No. 2

Seasonal prevalence of coccidiosis from June 2002 to May 2003 in Layer chicken.

S.N.	Season	Total no. of droppings examined	No. of droppings positive for coccidiosis	Positive %
1	Spring (rainy) (June-Aug.)	120	42	35
2	Autumn (Sep. Nov.)	110	13	12
3.	Winter (Dec- Feb.)	90	21	23
4.	Summer (March- May)	80	20	25

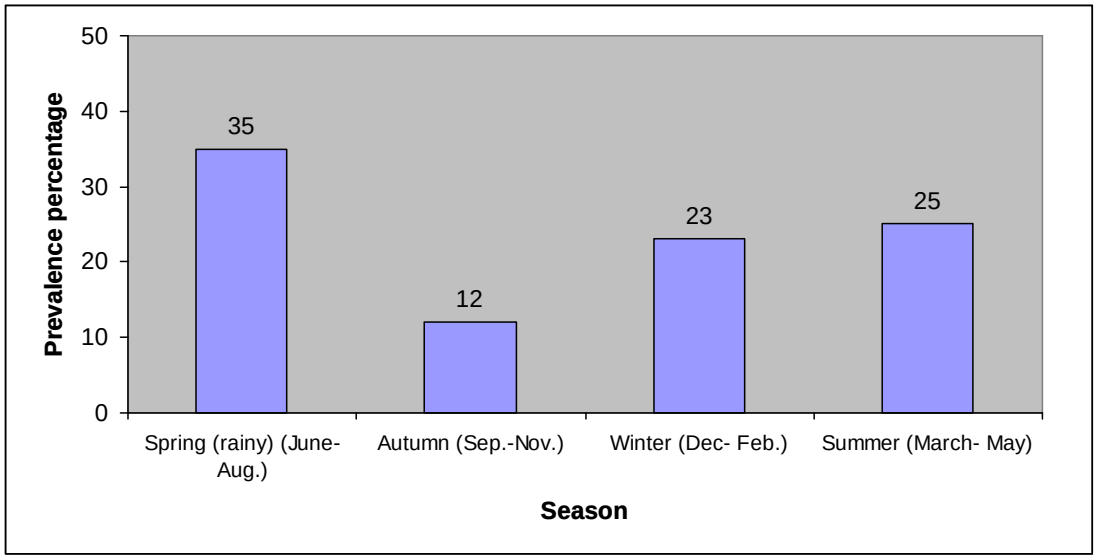

Fig. 2: Season-wise prevalence of coccidiosis of Layer chicken.

4. Age-wise prevalence of coccidiosis in Layer chicken.

The age wise prevalence of coccidiosis in Layer is presented in Table No. 3 and Fig. no. 3. The age group of studied Layer chicken were categorized into difference of 15 weeks, up to 61 weeks and above. The table 3 shows that the prevalence of coccidiosis was the highest in 61 to above week age group of Layer (34.66%) chronically affected with high morbidity and less mortality. The lowest prevalence of coccidiosis was in 16 to 30 weeks age group of Layer (15.78%). The mortality rate was found to be high between(0-15) weeks age group. The prevalence rate of coccidiosis in Layer gradually increased from 22.5% in 31 to 45 weeks age group up to 34.66% in 61- above age group. The chi-squire test indicated that the difference in age-wise prevalence of coccidiosis was insignificant (χ^2 = 9.488, P > 0.05). The student's 't' test indicated that the difference in age-wise prevalence of coccidiosis was insignificant (t = .17, P>0.05).

Table No. 3

Age-wise prevalence of coccidiosis in Layer chicken

S.N.	Age of Layer chicken (in weeks)	Total no. of droppings examined	No. of droppings positive for coccidiosis	Positive %
1	0-15	70	12	17.14
2	16-30	95	15	15.78
3	31-45	80	18	22.5
4	46-60	80	14	30.00
5	61-Above	75	26	34.66

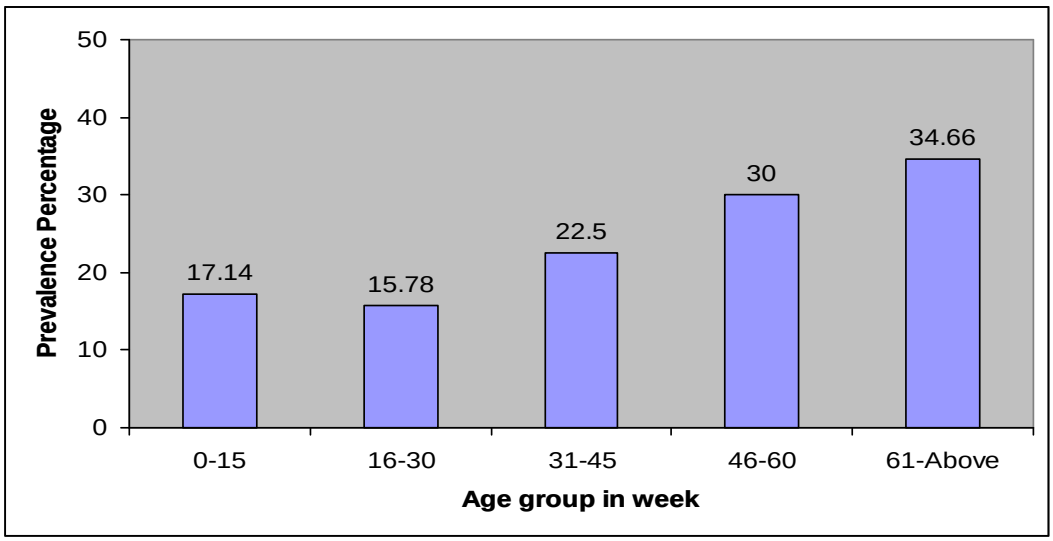

Fig. 3: Prevalence of coccidiosis in different age of Layer chicken.

5. Site- wise prevalence of coccidiosis in Layer chicken.

The site wise prevalence of coccidiosis in Layer chicken from June 2002 to May 2003, is presented in Table no. 4 and Fig. 4 For this study four sites were choosen Machhegaun (Champadevi), Futung (Balaju), Gothatar in Kathmandu and Khumaltar in Lalitpur districts. It revealed that the highest positive case was recorded from Khumaltar (29.14%) and the lowest positive case was recorded from Machhegaun (18.57%). From Futung and Gothatar the positive cases recorded were 18.66% and 22.5% respectively. The chi- squire test indicated that the difference in site-wise prevalence of coccidiosis was insignificant (χ^2 = 7.15, P>0.05). The student's 't' test indicated that the difference in site-wise prevalence of coccidiosis was insignificant (t = .23, P>0.05).

Table No. 4

Site-wise prevalence of coccidiosis in Layer chicken.

S.N.	Study area	Total no. of droppings examined	No. of droppings positive for coccidiosis	Positive %
1	Machhegaun (Champadevi)	70	13	18.57
2	Futuing (Balaju)	75	14	18.66
3	Gothatar	80	18	22.5
4	Khumaltar	175	51	29.14

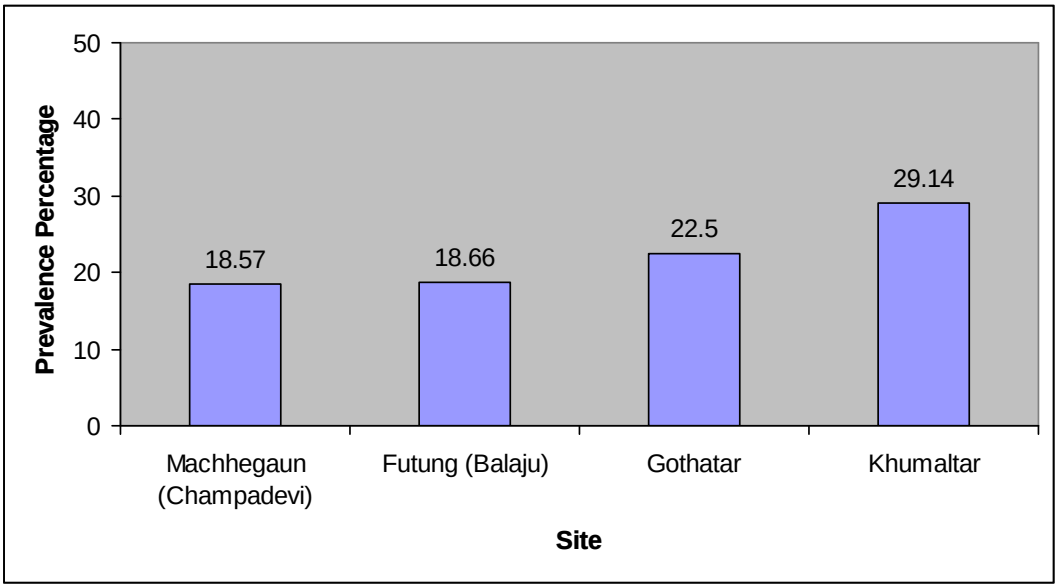

Fig. 4 : Site-wise prevalence of coccidiosis in Kathmandu and Lalitpur districts.

6. Post mortem examination result for the identification of *Eimeria* species.

Post mortem examination was carried out in 30 dead Layer chicken. Their types of infections were observed in the gastro intestinal tract.

a) Intestinal infection:- There was a greyish white pin head sized foci type lesions in small and large numbers. Small intestine was marked ballon-like and filled with clotted or unclotted blood.

b) Caecal infection:- There was marked congestion and thickening of the caecal wall. Caeca was found to be enlarged containing clotted or unclotted blood.

c) Mixed (intestinal and caecal) infection:- In this type of infection both intestine and caeca were found infected.

The pie chart shows that among 30 Layer chicken examined 25% were caecal, 35% were intestinal and 40% were mixed coccidiosis. The post mortem lesion observed in the different locations of intestine and caeca on layer chicken Fig. no.5

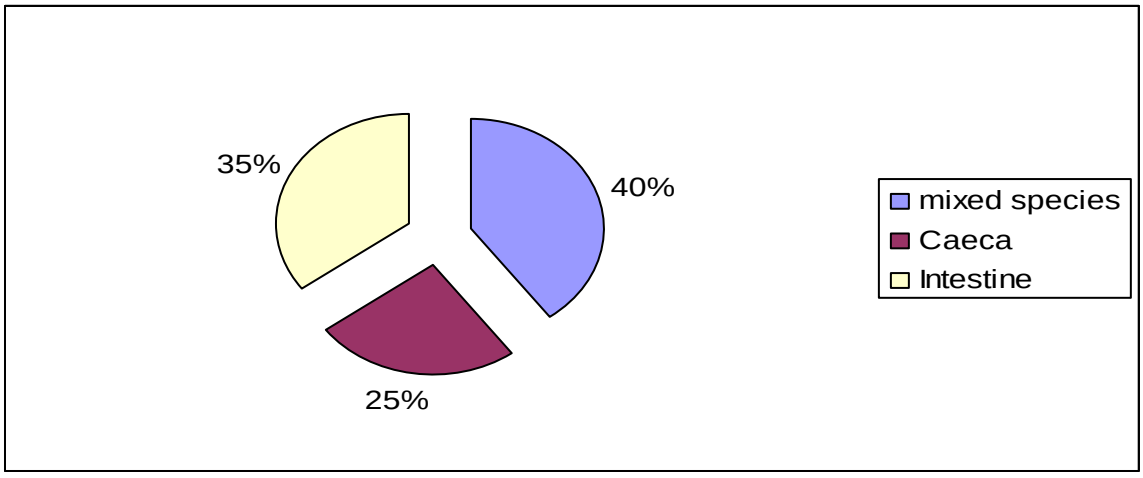

Fig. 5: Prevalence of different types of coccidiosis in Layer chicken.

Microphotographs showing different Species of *Eimeria* at 100Xmagnification

Plate No.11 Oocyst of *E. tenella*

Plate No.12 Oocyst of *E.acervulina*

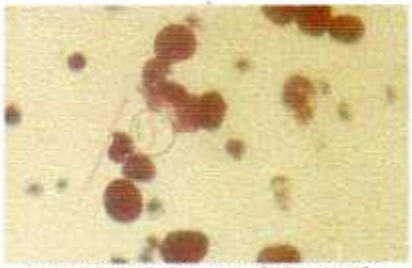

Plate No.13 Oocyst of *E.necatrix*

Plate No.14 Oocyst of E.maxima

Plate No. 15 Oocyst of *E.burnetti*

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7. Identification of different species of *Eimeria* in Layer chicken.

Eimeria species were identified by the examination of gross lesions, caecal and intestinal scrapings and oocysts morphology (shape and size).

Altogether 5 different *Eimeria* species were observed. They were *E. tenella, E.acervulina, E. necatrix, E. maxima* and *E.brunetti.*

Post mortem examination was carried out in 30 dead Layer chicken. The highest prevalence of coccidiosis percentage (40%) was found in mixed species. The prevalence rate of mono species infection due to *E. tenella, E. acervulina, E. necatrix, E. maxima and E. brunetti* were 25%, 15%, 10%, 7% and 3% respectively.

Table No. 5

Prevalence of different *Eimeria* speices in Layer chicken.

S.N.	Species of *Eimeria*	Frequency	Positive%
1	*E.tenella*	7	25
2	*E. acervulina*	5	15
3	*E.necatrix*	3	10
4	*E.maxima*	2	7
5	*E. brunetti*	1	3
6	Mixed species	12	40

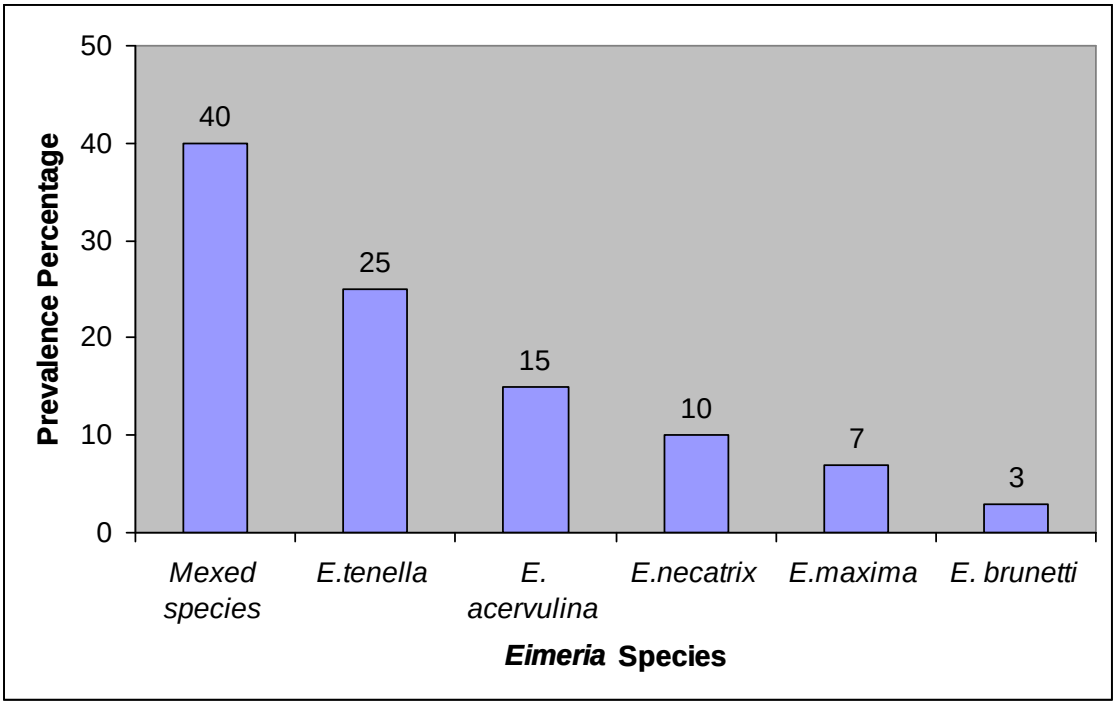

Fig. 6 : Prevalence of different *Eimeri*a species in Layer chicken.

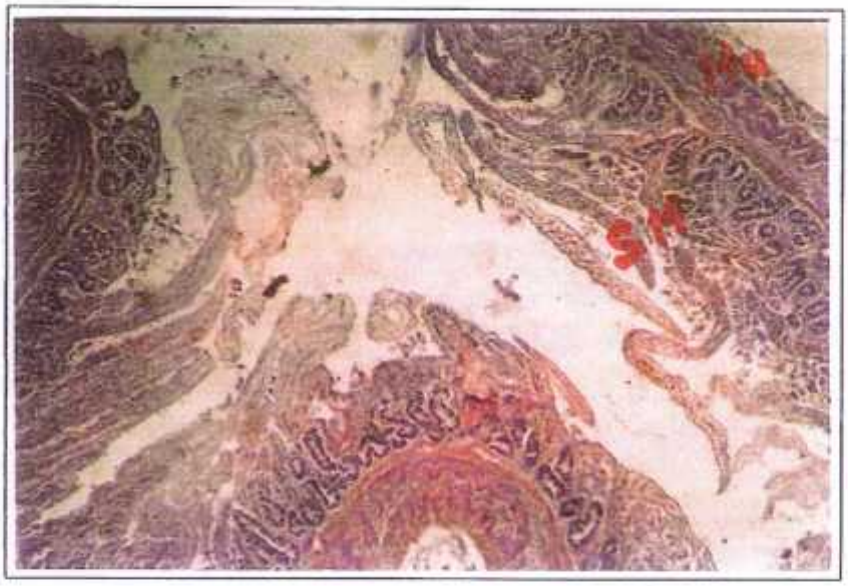

Plate No. 16 Histopathological section of intestine of Layer chicken infected with *Eimeria* species showing destruction of sub-mucosal layer.

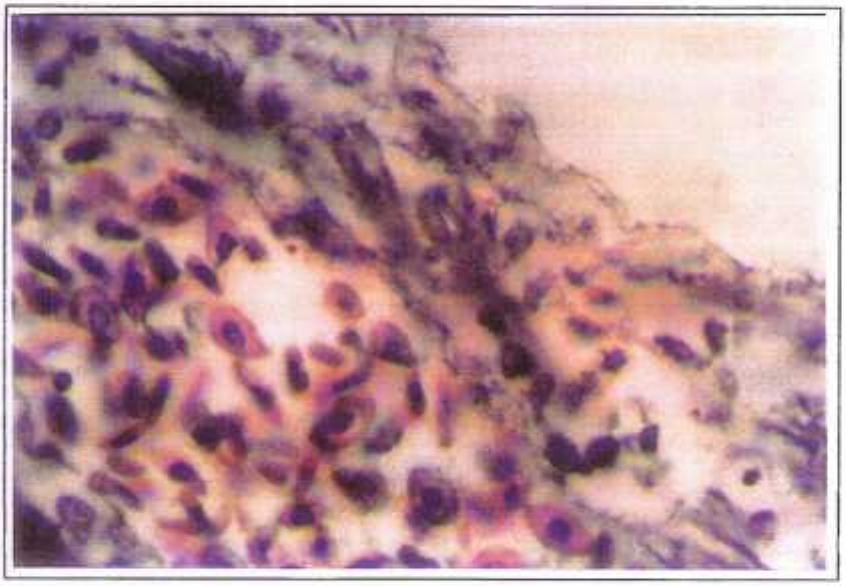

Plate No. 17 Histopathological section of intestine of Layer chicken infected with *Eimeria* species showing acute haemorrhage (clumping of R.B.C.) in mucosal layer at 100x magnification.

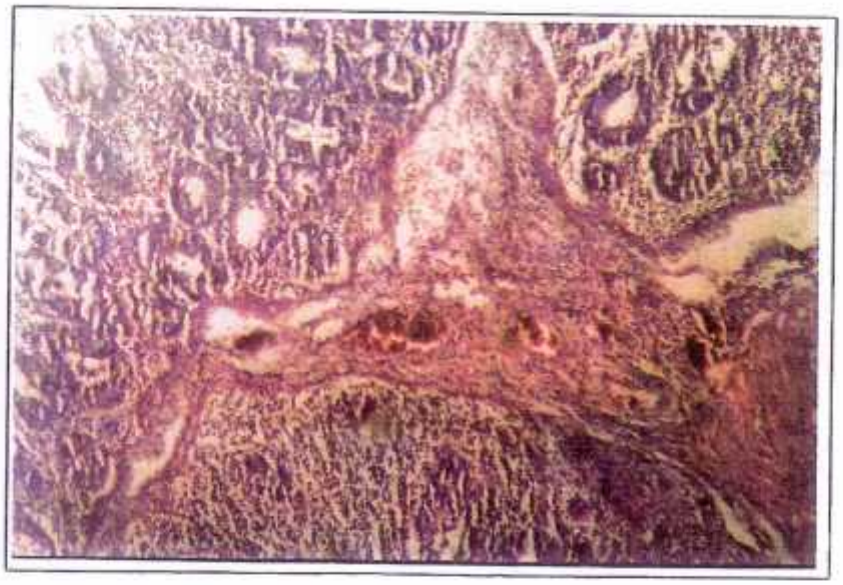

Plate No. 18 L.S. of caeca infected with *E.tenella* at 100x magnification.

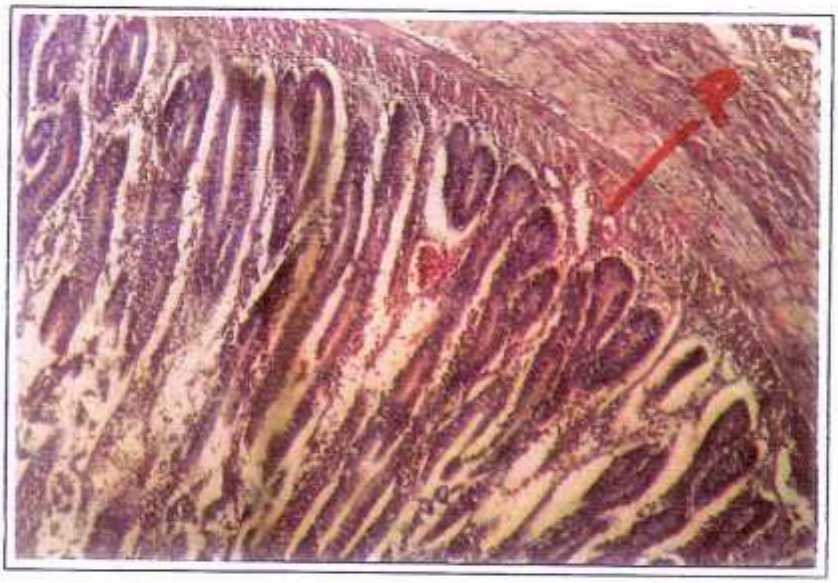

Plate No. 19 T.S. of caeca infected with *E.tenella* in sub-mucosa layer at 100x magnification.

8. Histopathological Report

- Histopathogical lesion showed the various degrees of destruction of the villi and the loss of inflammatory cells and lymphocytes in the intestinal lumen.
- Progressive increase in the granulocyte leucocytes in the submucosa and lamina propia was observed.
- In one case the massive number of heterophil leucocytes, lymphocytes and pyroninophilic cells infiltration in tunica propria, lamina propria and muscularis mucosa was seen.
- Intestinal wall was also to thickened.
- Infection of *Eimeria acervulina* showed the morphological changes in the infected epithelial cells. These changes revealed the enlargement and abnormal appearance of the principle cell mitochondrion in epithelial cells of chicken.
- Haemorrhages of the intestinal epithelial tissues also occured due to these parasites as clumping of erythrocytes were seen in the histopathological sections of the caeca.

VI

DISCUSSION

Coccidiosis is one of the main problem in poultry farming in Nepal. *Eimeria* species is the main causative agent of coccidiosis, which widely occurs through out Nepal. Heavy morbidity and mortality due to coccidiosis have significantly affected the poultry industry.

In the present study, which was carried out in Futung, Gathatar, Machhegaun of Kathmandu district and Khumaltar of Lalitpur, the prevalence rate of coccidiosis was found to be 24% The disease was due to 5 *Eimeria* species namely *E. tenella, E. necatrix, E. acervulina, E. brunetti and E. maxima*. This finding is some- what similar to the study conducted by (Adhikari 2004).

These identified *Eimeria* species have been reported earlier by Thakuri and Rai (1996), in the local chickens of eastern hills of Nepal except *E. necatrix,*. It is correlated to the report mentioned in Bangladesh by Karim and Begum (1994), in Argentina by Dougald *et al.* (1997), in Sweden by Thebo *et al*. (1998), in Rampur and its vicinity by Aryal (2001), and in 7 districts of Nepal by Pant (2003). The findings agree with the statement made by Macpherson (1978), that the same species of *Eimeria* all over the world will infect domestic poultry. The morphological (shape and size) characteristics, location and nature of intestinal lesions correspond to the description made by Gordon and Jordon (1982), and earlier works.

The monthly prevalence found in the present study was highest (38%) in the month of July, with the average temperature 24.50^0c and rainfall 544.80mm and humidity 85.45. The lowest prevalence was found in the month of October (5.71%) with the average temperature 20.10^0c and rainfall 15.00mm, This might be due to effect of favorable environment for the sporulation of oocysts. Hence the present study showed that the favorable environment for the multiplication of *Eimeria* species is when the temperature ranges between 25 to 30^0c with enough rainfall of 544.80mm and relative humidity of 85.45.

The month-wise study carried out by Chio *et. al* (1986) showed that the highest prevalence rate of coccidiosis was 50% in the months of June and March, respectively. This high prevalence rate of coccidiosis might be due to effect of favorable environment for the sporulation of oocysts.

The seasonal prevalence found in this study was the highest (35%) during spring (rainy) season with temperature 25 to 30^0c, rainfall 544.80 to 499.90mm and humidity 85.70 to 79.50, which helped to increase the sporulation rate of the oocysts. The lowest prevalence was recorded during autumn (12%).Hence the factors effecting coccidiosis during autumn was found to be unfavorable.

The finding of Basisth and Rajavelu (2000) in Tamil Nadu, India. showed that the season-wise prevalence rate was the highesst (33%) during summer season and lowest (14%) during autumn season in Layer flock.

The age-wise highest prevalence of coccidiosis was found in the 61-above week age group (34.66%) chronically affected with high morbadity and less mortality. This might be associated with crowding factor and loss of immunity power. The mortality rate was found to be high between (0-15) weeks age group. Lower prevalence rate (15.78%) in (16-30) weeks age group might be associated with time, immune status and requirement for completion of *Eimerial* life cycle.

The study conducted by Panda *et al.* (1997) in India revealed that the prevalence rate of coccidiosis was highest (48%) in Layer of 31-45 days age group than that of 0-15 days age group (6%). The high prevalence of coccidiosis might be associated with crowding factor. The increase in the age of the birds and more developed mechanical grinding action of the gizzard ruptures the oocysts to liberate the sporocysts.

The site-wise prevalence rate also depends upon the management condition of the farm. From the study, the management condition was better in Machhegaun in comparison to Futung . So the prevalence rate was lowest in Machhegaun (18.57%) and highest in Khumaltar (29.14%). The highest prevalence rate in Khumaltar was due to poor management condition. The government farm was lacking in proper hygiene and sanitation. The disease is dependent on management problem.

The prevalence of coccidiosis was found highest (24%) in Layer chicken (Farooq 1999)in mud/ mud + brick type floor than that of concrete type floor.

The prevalence of coccidiosis in cage and deep litter system of layer chicken were 3 % and 8 % respectively. This finding suggests that there is comparatively more chances of coccidiosis in deep litter system. The result might be associated with every time contact with litter of Layer, reared in deep litter system. No literature regarding the comparative prevalence of coccidiosis in cage and deep litter system was found from Chitwan district (Adhikari 2004).

In the study,carried out by Franco (1993) in Brazil five species of *Eimeria viz E. acervulina, E. maxima, E. brunetti, E. necatrix and E. tenella* were identified on the basis of lesion seen, affected site and morphological characteristics from Layer.

The mixed coccidial infection (50%) and only with *E. tenella* infection (25%) were found most prevalent in Layer in this study. The highest prevalence of *E. tenella* might be due to its highly pathogenic and predominate nature.

The frequency of five *Eimeria* species were mixed (50%), *E. tenella* (25%), *E. necatrix* (10%), *E. acervulina* (5%), *E. maxima* (5%), *E. brunetti* (5%) respectively in Layer. This finding was reported by Diaz *et al.* (2001).

The post mortem report in this study revealed was very much similar to the report given by Long and Reid (1982) which is as follos:

- Transverse whitish band on duodenum by the infection of *E. acervulina,*
- Thickened MSI with mucoid exudates during *E. maxima,*
- Distended caeca by blood and erosion of caecal mucosa in *E.tenella* infection and
- Mucoid bloody enteritis and coagulation necrosis in PSI by the infection of *E. brunetti*.

In the present study histopathological section revealed that the submucosa was found to be infiltrated with lymphoid cell and there was an increase in number of granulocytic leucocytes in the submucosa and lamina propria. Similar type of result was also given by Ruff and Reid (1977). They reported that with *E. tenella*

and *E*. *necatrix*, the granulocyte- cell response becomes most obvious when the first generation merozoites leave the glandular tissues and migrate into the tunica propria and begin to develop into the large second generation schizonts which is the characteristic of this species.

In the present report, in one case hemorrhage in the muscalaris mucosa was also found accompanied by a thickening of the intestinal wall. This is in support of the result given by Long *et al*. (1968). From the study it was found that caecal coccidiosis infection was more pathogenic when compared to intestinal coccidiosis. Natt and Herrick (1955), Joyner and Davis, (1960), reported that with *E. tenella* infection, erythrocyte numbers are frequently depressed by more than 50% but with *E. necatrix* infections, the packed erythrocyte volume is not depressed to such an extent.

VII

CONCLUSION

- The mean length and width of oocysts, nature of intestinal lesions and histopathological changes can identify *Eimeria* species.
- Poultry coccidian are ubiquitous but disease is most likely when young stocks are concentrated under conditions, which permit the accumulation and sporulation of large number of oocysts.
- Coccidiosis may occur at any age, season, month and site.
- In this study the highest prevalence rate of coccidiosis was found (38%) in the month of July and lowest (5.17%) in the month of October.
- Regarding season- wise infection of coccidoisis the highest (35%) prevalence was recorded in spring season and the lowest (12%) in autumn season.
- Age - wise prevalence was found to be the highest (34.66%) in 61 and above weeks age group of chicken and lowest (15.78%) in 16 to 30 weeks age group of chicken.
- Five different species were observed viz *E. tenella, E. acervulina, E. necatrix, E. maxima* and *E. brunetti*.
- The five species of *Eimeria* identified in this study were all pathogenic.
- *Eimeria tenella* was found to be more pathogenic showing symptoms like weight loss, soft and watery faeces with blood, severe dehydration before death. So it is the predominant and highly pathogenic species causing heavy economic loss among chicken by increasing mortality rate.
- Post mortem lesions were intestine balloning, pin point hemorrhage, hemorrhagic enteritis, caecal distention and bloody ingesta.
- Sporulation of oocysts of *Eimeria* species completed after 72 hours period at 24^0c to 26^0c, 48 hours period at 30^0c and one week or more period below 20^0c in aerobic condition.
- Histopathological lesion revealed that caecal coccidiosis is more pathogenic in comparison to intestinal coccidiosis.

VIII

RECOMMENDATIONS

The result of this study suggest that outbreak of coccidiosis may occur at any season and age of the chicken. Coccidiosis is the management based problem. The outbreak of coccidiosis depends upon the presence of favorable environment for oocysts sporulation. Therefore, the commercial poultry raisers in this area need to be aware of this situation. For this following points should be noted to make poultry house free from coccidiosis infection.

1. Dust, flies, pests and children may cause contamination, so their entry into the poultry house must be avoided.
2. Before stocking, scrubbing with hot detergent must be used by following chemicals.
    a. 10% Ammonia gas which kills coccidiosis in 45 min .
    b. Methyl bromide 5 mg/liter for 20 hours has lethal effect on occysts of *Eimeria* species.
3. For improving hygienic measure, general cleanliness to be observed and young and adult stock must be kept separately.
4. The litter should be kept dry since wet litter farms are ideal for sporulation of coccidial oocysts. This can be done by removing wet spots or forking fresh litter.
5. Overcrowding must be avoided.
6. High stocking density should be avoided.
7. Cage system should be used for better prevention of coccidiosis.

IX

REFERENCES

Anderson, W.I., Reid, W.M. and Jnce, K.J. (1976). Effect of high environmental temperatures on caecal coccidiosis in Athens. ,*Dep. Pout. Sci, Univ. GA. Athens, Ga 30602, U.S.A., Poult. Sci., 55* (4): 1429-1435.

Aryal, M.P. (2001). Epidemiological study on *Eimeria* species in natural outbreak of chicken coccidiosis at IAAS Ram pur and its vicinity. *Advances in Agricultural Research in Nepal at Society of Agricultural Scientists (SAS), Nepal,* 168-175.

Basist, S. A. and Rajavelu, G. (2000). Seasonal Prevalence of coccidiosis in chicken at Namakkal (Tamil Nadu). *Indian Veterinary Journal,* 77: 163-164.

Bohara, K . Dhakal, I.P., Bhattarai, T.C. and Adhikari, D.R (1996). Outbreak of newcastle disease in commercial chicken in Nepal. *Bulletin of Veterinary Science and Animal Husbandary Nepal,* 24: 100-104.

Central Bureau of Statistics (2003). Statistical Year Book of Nepal. HMG *National Planning Commission, Kathmandu, Nepal*.

Central Epidemioiogical Unit (2002). *Annual Epidemioiogical Bulletin.* Central Veterinary Laboratory and Animal Disease Control Section. Tripureshwor, Kathmandu, Nepal.

Chio, S.H. , Kim, K. S. and Kim, Y.H. (1986). Epizootiology study on the Coccidiosis of broiler chicken in Korea. *Res Rep off Rural Dev*. (Livest vet) Suwen, 26(2): 44-52.

Clarke, P.L. (1979). Coccidial infection with *Eimeria tenella* and caecal defecation in chicks. *Br. Poult. Science,* 20(3): 317-322.

Dhakal, I.P. (2001). Common poultry diseases and. their management in Nepal. *A Report of National Symposium on Poultry Production and Health,* 1-11,

Dhakal, I.P., Poudei, R.P. and Parajuli, N. (2000). Occurrence of poultry diseases in Chitwan valley of Nepal. *Nepalese Vet. J.,* 26: 27-35.

Diaz, M.R., Velarde, F.I. and Gal van, P.O. (2001). Frequency of *Eimeria* species in some farms of the poultry areas or Tehuacan in the state of Puebla in Mexico. *Veterinaria Mexico, 32(2):* 103-108.

District Livestock Services Office (2001). *Annual Progressive Report.* District Livestock Services Office, Bharatpur, Chitwan, Nepal, 58.

Farooq,M., Durranil, F.R. Waheedullah, W., Sajjad,A. and Asghar, A. (1999). Prevalence of Coccidiosis in broilers in the subtropical environment. *http:/www.com/vet/broilers.htm.*

Fitz-coy, S.H. and Edgar, S.A. (1992). Effect of *E. mitis* on egg production of single-comb white leghorn hens. *Avian Dis.* 36(3): 718-721.

Franco, R.M.B. (1993). Survey of avian coccidiosis from two layer's poultry farms in Campinas. Brazil. *Arguivo- Brasileiro dc Medicina Veiennana c Zoolechla,* 45(6): 557-571.

Gordon, R.F. and Jordan, F.T.W. (1982). Poultry Diseases. Second edition. *Bailliere Tindall.* 69-177.

Grault, E.A.M., Henken, A.M.. Ploeger, H.W., Noordnuizen, J.P\T.M. and Vertommen, M.H. (1994). Course of sporulation of oocysts in *Eimeria acervulina* under different environmental conditions (Dep. Animal Husbandary, Ague, University, PO box 338, 6700 AH wageim-gen Net). *Parasitology.,* 108(5): 497-502.

Hufshed, M.S. (1992). Diseases of Poultry, *8th Edition Parima Educational Book Agency*, New Delhi, 691-708.

Jha, V.C., Singh, U.M. and Hokonohara, S. (1999). An outbreak of avian encephalomyelitis in chicks at Kavreplanchowk district in Nepal. *Annual report (1998/99), Animal Health Research Division*, Tripureshwor, Kathmandu, 36-39.

Johnson, D. (1930). Director's Biennial Rpt. for 1928-1930. *Oreg. Agr. Exp. Sta.*

Johnson, M. and Rajeswari,k. (1990). Coccidiosis epidemiology and economic loss. *Poultry Guide,* 27 (l): 77-80.

Jordan, F.T.W. and Pattison, M. (1996). Poultry Diseases, 4th Edition. W.B. *Saunders Company LTD*. London NW 17DX.

Joyner, L.P. and Davies, S.F.M. (1960). Detection and Assessment of sublethl infection of *Eimeria tenella and Eimeria necatrix Exp. Parasitol.*9:243-249.

Karim, M.J. and Trees, A.J. (1990). Isolation of five species of *Eimeria* from chickens in Bangladesh, *Trop. Anim. Health Prod,* 22(3): 153-159.

Karim, Md. J. and Begun, N. (1994). Morphological and biological characterization of chicken *Eimeria* with special reference to species identification, *vet. Review* Pakhribas Agriculture Centre (PAC). 9(1) and 10(1): 7-9.

Kim, K.E., Lee, S.H., Chung, G.S.. Kwon, J.H., Choi, S.H., Youn, H.J., Kim, S.H. and Namgoong. S. (1990). A rapid method tor the purification of chicken coccidian oocysts. *Res Rep Rular Dev And (Suweon).* 32(2 vet.): 33-36.

Levine, N.D. (1970). Taxonomy of the Sporozoa. .*J. Parasitol* 56 (11): 208-209.

Lohani, M. N. and Amatya, J. L. (2000). Demand and supply of raw materials for livestock and poultry feed. *Nepal Veterinary Journal,* 26: 41-45.

Long, P.L. and Harton, S.C. (1968). Coccidia and Coccidiosis in the domestic fowl. *Parasitol.* 6:313-325.

Long, P.L. and Reid, W.M. (1982). *Research report 404*, College of Agricultural Experiment Station; University of Georgia, USA.

Lunden, A., Thebo, P., Gunnarsson, S., Hooshmand-Rad, P., Tausan, R. and Uggla, A. (2000). *Eimeria* infections in litter based, high stocking density system for loose housed laying hens in Sweden. *British Poultry Science,* 41(4): 440-147.

Macpherson, I. (1978). Avian Coccidiosis, British Poultry Science Ltd.Edinburgh, Scotland, 465-494.

Mahato, S.N. and Thapa, P.B. (1992). Seroprevalence of some viral infections in chicken in Koshi Zone. *Veterinary Review.* I: 26-28.

Majara, O.M. (1993). Coccidian parasites of indigenous domestic fowls *(Gallus gallus domesticus)* from rural areas of Oyo state, Nigeria. (Dep. Vet. Microbial.Parasitol. Univ. Ibadon, Ibadon NIG). *Tropical Veterinarian,* 11(1-2): 9-13.

Mattiello, R., Boviez, D. and Me Dougald, L.R. (2000). *Eimeria bruntti* and *Eimeria necatrix.* in chickens of Argentina and confirmation of seven species of *Eimeria. Avian Diseases,* 44:711-714.

Mc Dougald, L.R., Fuller, L. and Mattiello, R. (1997). A survey of coccidian on 43 poultry farms in Argentina. *'AvianDiseases,* 41: 923-929.

Naidu, P.M.N. (1967). Poultry Keeping in India. *Indian council of Agriculture Research,* New Delhi.4(7)36-37.

Natt,M.P, and Herrick, C.A. (1955). The effect of caecal Coccidiosis on the blood cell of the domestic fowl . *Poult. Sci.* 34:1100-1106.

Panda,D.N., Mishra, A., Mishra, S.C. and Panda, B.K. (1997). Incidence of Coccidiosis in broiler bird in and around Bhubaneswar, Orrisa (Department of Parasitology,Orrisa Veterinary College, Bhubaneswar,751003). *Indian Veterinary Journal,* 74:430-431.

Pant, G.R. (2003). Investigation of coccidiosis in poultry (Under publication).

Pant, G.R. and Mailla, S.M. (2001). Poultry disease situation in Nepal Proceeding of workshop on livestock disease investigation. 28th June 2001. Central Veterinary Laboratory, Tripureshwor. Nepal, 16-18.

Pavlovic, I. and Dragica, N. (1989). Parasitic fauna of poultry industry in the Republic of Serbia. *Vet G/as,* 45(3/4): 245-247.

Pradhan, A., Jha, V.C. and Hokonohara, S. (1998). Study on chronic respiratory disease. Animal Health Research Division. Tripureshwor, Kalhmandu. 11-13.

Rajeshwari, and Kumar, K. (1994). Coccidiosis: epidemiology and economic loss. *Poultry Guide,* 26(10): 11-16.

Reddy, A.S. and Kumar, K. (1994). Clinical aspects of coccidiosis. *Poulty Guide,* 31(8): 11-12.

Rose, M.E. (1967). The influence of age of host on infection with *Eimeria tenella.J. Parasilol,* 53: 924-929.

Ruff, M.D. and Reid, W.M. (1977). Coccidiosis and Intestinal pH in chicken. *Avian Dis*. 19:52-58.

Safari, M.K. (2003). Studies on prevalence and economy impact on poultry coccidiosis in different production systems in Debre Zeit and Addis Abana Ethiopia. *http://www.vetmed. fu-berlin. del p-*5 *safari.htm.*

Sekar, R.and Eajendran, K. (1977). Intestinal diseases of rabbit and its control. *Pashudhan,* 12(4):3.

Shiotani, N., Baba, E., Fukata, E., Arakawa, A. and Nakanishi, T. (1992) Distribution of oocysts, sporocysts and sporozoites of *Eimeria tenella* and *E. maxima* in the digestive tract of chicken, *vet. Parasitol,* 42(1/2): 17-22.

Singh, R.A. (1985). Poultry Production. Kalyani Publishers, New Delhi.

Singh, U.M., Shrcstha, S.P. and Sharma, K. (2001). Incidence of coccidiosis in chicken. *Advances in Agricultural Research in Nepal at Society of Agricultural Scientists* (SAS), Nepal, 276-280.

Thakuri, K.C. and Rai, K. (1996) Identification of *Eimeria* species found in local chicken of eastern hills of Nepal. *Veterinary Review,* 9(1): 5-6, Pakhribas Agriculture Centre, Dhankutta, Nepal.

Thebo, P.. Lunden, A., Uggla, A. and Hooshmand, R.P. (1998). Identification of seven *Eimeria* species in Swedish domestic fowl. *Avian Pathology,* 27(6): 613-61 7.

Tsuji, N., Kawazu, S.I., Ohta, M., Kamio, T., Isobe, T., Shimura, K. and Fujisaki, K. (1997). Discrimination of eight chicken *Einieria* species using the two polymerase chain reaction, (Natl. lust. Anmi. Health, Tsukuba, Ibaraki, Japan). *Journal of Parasitology,* 83(5): 966-970.

Tyzzer, E.E. (1929). Parasitic Protozoa. Gregarines Haemogregarines. Coccidian. Plasmodia and Hemoproteids 3: 42(Ed.) *Kreir, J.P. Academic Press, New York.*

Tyzzer, E.E., Theiler, H. and Jones, E. (1929). Coccidiosis in gallinaceous birds. 11 A comparative study of species of *Eimeria* of the chicken. *Am. J. Hyg.,* i 5:3 19-332.

Umeche, A. and Eno, R.O.A. (1987). A survey of parasites of chickens from poultry farms in Calabar, Nigeria. *Rev Latmoan Microbial.* 29(2): 133-136.

Urguhart, G.M. (1987). *Veterinary Parasitology,* Longman Scientific and Technical,. Longman Group, UK. Limited, Essex CM 202 JE, England, 221-223.

Williams, R.B. (1995 a) The fete of ingested oocysts of *Eimeria tenella* during the prepatent period in susceptible chicks. *Applied Parasitoogy,* 36(2): 83-89.

Williams, R.B. (1995 b). Physical condition and survival *of Eimeria acervulina* oocysts in poultry house jitter. *Applied Parasitology,* 36(2): 90-96.

Williams, R.B. (1999). A compartmentalized model for the estimation of the cost of coccidiosis to the world's poultry production industry. *International Journal for parasitology\4d: * 209-1229.

Annex 1

Post mortem report of coccidiosis in Layer chicken

S.N.	*Eimeria* species	Site of predilection	Main symptoms of the disease.
1	*Eimeria tenella*	Caeca	⁾ Caeca was found to be filled with fluid, blood or coagulate blood or necrotic material. ⁾ Thickening of caecal wall was found
2	*Eimeria acervulina*	Duodenum	⁾ Haemorrhagic appearance in mucosa was found. ⁾ Small round or elongated whitish lesion was found.
3	*Eimeria maxima*	Terminal part of small intestine	⁾ Middle portion of the intestine was distended and a mucoid intestinal content tinged with blood was found.
4	*Eimeria brunetti*	Tereminal part of intestine as well as rectum and caeca	⁾ The affected intestinal wall was found with a few haemorrhagic lesion.
5	*Eimeria necatrix*	middle part of intestine	⁾ Haemorrhage and massive swelling of the intestine was found.

Annex 2

Different species of *Eimeria* identified in Layer chicken

Intestinal Part/location	Post mortem lesions	Characteristics of oocysts				Species of *Eimeria* identified
		Size (μ m)		Shape index	Shape	
		Mean length ± SD (Range	Mean width ± SD (Range		Ovoid	
Anterior small intestine	Treansverse whitish band on doudenal loop	17.93±2.61 (13.76-20.64)	14.12±2.59 (10.32-17.20)	1.27	Ovoid	*E.acervulina*
Middle small intestine	Thickened intestine wall. patechiae	29.41±3.25 (24.08-37.84)	22.28±3.25 (17.20-27.52)	1.32	Ovoid	*E. maxima*
Middle small intestine		20.10±2.6 (17.20-24.08)	16.32±2.43 (13.76-20.64)	1.23	Oblong Ovoid	*E.necatrix*
Caeca		20.81±2.34 (17.20-24.08)	17.12±2.27 (13.76-20.64)	1.21	Ovoid	*E.tenella*
Posterior small intestine		23.75±2.85 (20.64-27.52)	19.52±1.98 (17.52-24.08)	1.22		*E. brunetti*

Annex 3

Coccidia: The Parasite Causing Coccidiosis

1 .Taxonomic position

Phylum: Protozoa

Sub phylum: Apicomplexa. Levine. 1970

Class: Sporozoasida. Leuckart, 1879

Sub class: Coccidiasina. Leukcart, 1979

Order: Eucoccidiosida, Leger and Debasa, 1910

Suborder: Eimeriorina. Leger. 1911.

Family: Eimeriidae. Minchin, 1975.

Genus: *Eimeria.* Schneider, 1975.

The major characteristics of members of the *Eimeria* are:

a) The structure of the sporulated oocysts which always contain four sporocysts each containing two sporozoites.

b) Marked host specificity; (here being very few exceptions to the general rule that species from one animal do not develop in closely related hosts).

c) Marked species specificity in which host resistance acquired to one species does not protect against infection with another.

d) Marked predilection for development within specific sites in the host.

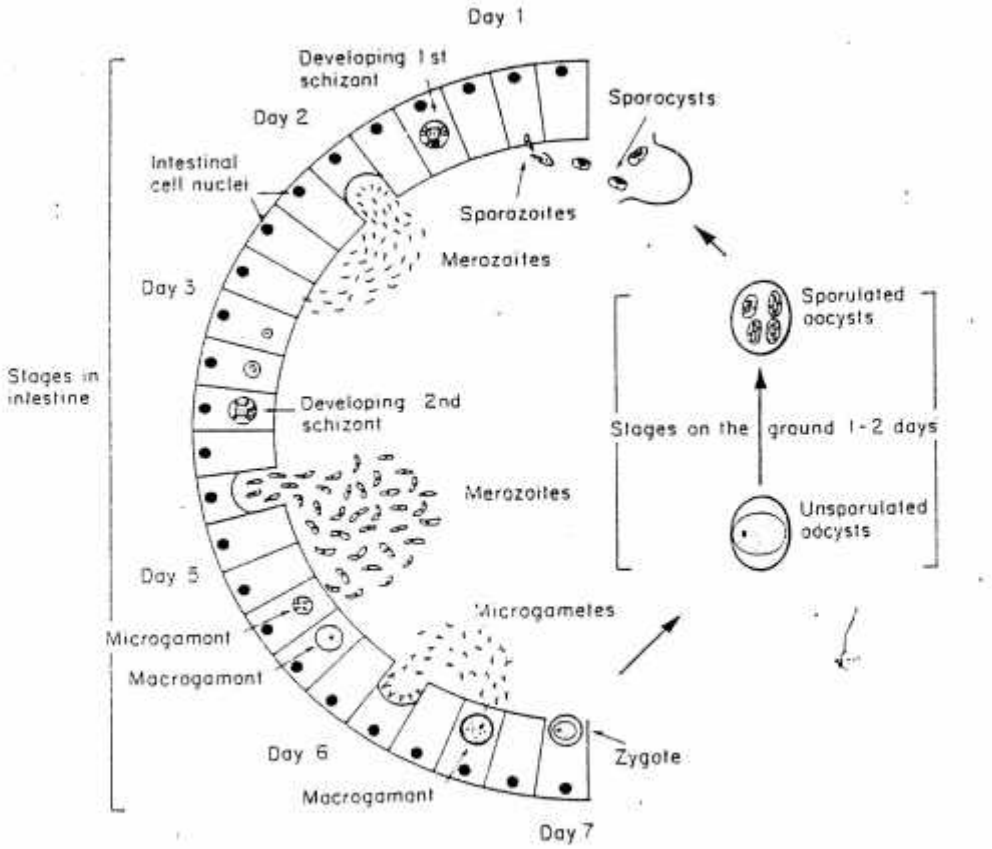

Fig. . Life cycle of *E. tenella*, typical of the genus *Eimeria*.

Life Cycle

Life cycle of *Eimeria* species does not require any intermediate host. Oocysts with sporozoites within are ingested by the chicken through feed, water or litter. The digestive enzymes (bile salts and trypsin) dissolve the wall of the oocysts to literate sporozoites. These released sporozoites involve the epithelial cells of the mucosa of the intestine and develop into round bodies having a nucleus, which are known as trophozoites. The trophozoites grow in size and their nucleus divides to form first generation schizonts. The nucleus of the first-generation schizont develop into sickle shaped bodies called merozoites which are placed closely packed and parallel to each other, looking somewhat like the segments of an orange. The merozoites break the schizont and the epithelial cells and hence invades other epithelial cells The merozoites grow as trophozoites and develop again into schizonts: known as second generation schizonts which literate merozoites. The merozoites develop in new epithelial cells into sexual phase of the parasite. Some of the merozoites develop into male cells, microgametocytes and others into female cells macrogamatocytes. Small male microgamates are expelled to fertilize the larger intracellular female macrogametes to become the zygotes (oocysts) that are discharged in the host's droppings. A single ingested oocyst can form about one hundred fifty thousands new oocysts in a chicken. These oocysts may survive for about 200 days to 2 year in the suitable environment. Invasion of host cells is most important step, which has to be repeated sevral times through rounds of asexual and sexual reproduction in case of *Eimeria* species of chicken.