



Prevalence and Pathology Associated with Coccidian Parasite *Eimeria tenella* in Organised and Unorganised Poultry of Kashmir Valley, India

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Abstract

This study was carried out from January 2018 to December 2019 to know the prevalence and the pathology associated with the coccidian parasite *Eimeria tenella* in both organized poultry farms and unorganized backyard chickens of Kashmir valley. For this study a sample size of 400 intestinal caecae were collected from birds belonging to both organized farms and unorganized village backyard poultry of Kashmir valley. The overall prevalence of the parasite during a two year study was found to be 29.75%. Higher prevalence (35.5%) was recorded in backyard poultry as compared to the poultry birds in various organized farms (24%). Mixed infection of *E. tenella* with other Eimerian species was also found to be comparatively higher in unorganized chicken than the organized poultry. The study revealed that the prevalence of Eimerian infection to be highest (76.47%) during September months of both the years of study. Diagnosis of the coccidian species was done based on the identification of oocysts by morphometry. Infected caecae were preserved and subjected to histological studies which revealed disruption of epithelial lining and congestion of blood vessels. Submucosa of caecum reveals severe haemorrhage, muscular oedema and necrosis. Clusters of oocysts and microgametes were found to be lying free in the lamina propria of caecum. Sections revealed many intracellular schizonts containing merozoites with severe submucosal hemorrhage in caecum. Infected sites revealed massive infiltration of various heterophils and mononuclear cells. Overall histological changes cause reductions in the absorptive surfaces, with resultant decreased nutrient absorption, dehydration, blood loss and increased susceptibility to secondary infections.

Keywords: Coccidiosis; *Eimeria tenella*; Pathology

Introduction

Coccidiosis is considered to be one of the major poultry farm diseases throughout the world [1]. Clinical and subclinical forms of the disease are jointly responsible for huge economic losses in the poultry industry [2]. Intracellular protozoan parasites belonging to the genus *Eimeria* are responsible for causing coccidiosis in Chicken [3]. These parasites reside and multiply in the intestinal mucosa. The disease is characterized by enteritis, dysentery, drooping wings, emaciation, poor growth, low production [4] and responsible for high mortality rate and increased morbidity [5].

Mortality rate is found to be highest among young chicks as most of the *Eimeria* species infect chicks between the age of 3 weeks to 18 weeks. Seven species of *Eimeria* have been recognized which vary in their degrees of pathogenicity. These include *E. acervulina*, *E. maxima*, *E. brunetti*, *E. necatrix*, *E. mitis*, *E. praecox* and *E. tenella*.

Eimeria tenella is the most ubiquitous [6] and the most pathogenic coccidian parasite [7-9]. Its infection results in 100 % morbidity and very high mortality due to its severe damage of the digestive tract of the host [10]. *Eimeria tenella* specifically infect epithelial

cells of crypts of Lieberkuhn of caecum, resulting in formation of haemorrhagic lesions of varying severity, influenced by parasite load, size and age of the bird, as well as host genotype and previous infection history. The lesions resulting from the infection of this parasite alter nutrient absorption, triggering several changes in the metabolism of carbohydrates, lipid, protein and macro and trace mineral [11]. In view of lack of specific information available regarding the prevalence and tissue damage caused by coccidian parasite *Eimeria tenella* in poultry sector of Kashmir, the present study was designed to have first hand authentic information about the prevalence of the protozoan in both organized and unorganised types of poultry farms and also report on the extent of tissue damage caused by the parasite at the site of its location posing serious economic threats to the poultry sector of Kashmir valley.

Materials and Methods

Collection and preservation of intestinal caecae

A total of 400 samples of intestinal caecae were collected from both organized farms (200 samples) and backyard poultry (200 samples) belonging to various districts covering almost entire Kashmir valley. Birds for sampling were selected on the basis of physical parameters like dullness, ruffled feathers, less growth and diarrhoea. After sacrificing the birds by slaughtering, viscera was removed and grossly examined for lesions. Caecae having gross lesions were collected and fixed in 10 % Neutral buffer formalin. The particulars like age of birds and the farm management practices were recorded. The age group of studied flocks of birds was between 3-18±1.5 weeks which is the most vulnerable age group for coccidian infection (Shiraz, *et al.* 2011).

Wet smear method

Deeper scrapings were taken from caecum and wet smears were prepared for microscopic examination of coccidian oocyst [12].

Impression smear cytological examination

Impression smears were made from caecum, which were then stained with Giemsa stain [13].

Morphometry of oocyst

Identification of particular Coccidian species was done on the basis of morphological features of Oocyst like shape, size, colour, appearance of the wall, presence or absence of micropyle and micropylar cap and sporulation time [14-17].

Histopathological examination of caecal lesions

For histological examination, representative tissue samples of caecum were collected in 10 % Neutral buffer formalin and then processed for paraffin embedding. The sections were cut at 5 µm thick and were then stained by Harris haematoxylin and eosin method [18].

Data entry and analysis

A computer program (SPSS 10.05 for windows; SPSS Inc., Chicago, Illinois, USA) was used for analysis of data. The chi-square test was used to observe any statistical significance. Differences were considered significant when P values of less than 0.05 were obtained.

Ethical clearance

The study was cleared by the Research ethics committee of Government S P College, Cluster University Srinagar. Unofficial meetings were held with the owners of organized poultry farms and with the household owners rearing backyard poultry in order to explain the protocol of the study and informed consent form was taken from them.

Results

The infection percentage of *Eimeria tenella* in backyard chicken was observed to be higher i.e 35.5% as compared to organized poultry farm where the infection rate was only 24% (Table 1). Among the organized poultry, the prevalence based on the management system was found to be 37.2% and 12.3% in poor and good management system respectively with significant difference between poorly and properly managed chicken ($p < 0.05$). The study also revealed that in unorganized backyard poultry, *E. tenella* showed

mixed infection with *E. maxima* in 15.49% of infected birds and coinfection with *E. acervulina* in 2.81% of infected birds. On the other hand, in organized poultry only *E. tenella* and *E. maxima* co-infection was reported in 6.25% of infected cases. Monthwise prevalence studies reveal highest prevalence rates during September months of both the years of study with 76.47% prevalence in unorganised birds and 64.7% in organized birds and no infection

was reported in January months of both the years of study (Table 2). The infection was observed in all the seasons of year (Table 3) but the prevalence was significantly higher ($p < 0.05$) in the autumn (48% in organized and 62% in unorganized birds) followed by summer (32% in organized and 50% in unorganized birds), spring (12% in organized and 24% in unorganized) and least prevalence was reported in winter (4% in organized and 6% in unorganized).

| Farm Management | Caecal samples collected | Positive samples (%age of infection) | P value | Number of samples with mixed infection of <i>E. tenella</i> and <i>E. maxima</i> (% age of mixed infection) | Number of samples with mixed infection of <i>E. tenella</i> and <i>E. acervulina</i> (% age of mixed infection) |
|-------------------|--------------------------|--------------------------------------|---------|---|---|
| Organized farms | 200 | 48 (24%) | 0.03 | 3 (6.25%) | Nil |
| Unorganized farms | 200 | 71 (35.5%) | | 11 (15.49%) | 2 (2.81%) |
| Total | 400 | 119 (29.75%) | | 14 (11.76%) | 2 (1.68%) |

Table 1: Prevalence of *Eimeria tenella* mono-infection and co-infection in organized and unorganized backyard poultry of Kashmir valley.

| Month and Year of collection | | Total number of samples screened from organized farms | Total number of positive samples (Prevalence %) | Total number of samples screened from unorganized backyard poultry | Total number of positive samples (Prevalence %) |
|------------------------------|-------|---|---|--|---|
| Jan | 2018 | 8 | 0 (0) | 8 | 0 (0) |
| | 2019 | 9 | 0 (0) | 9 | 0 (0) |
| | Total | 17 | 0 (0) | 17 | 0 (0) |
| Feb | 2018 | 9 | 1 (11.11) | 9 | 1 (11.11) |
| | 2019 | 8 | 0 (0) | 8 | 1 (12.5) |
| | Total | 17 | 1 (5.88) | 17 | 2 (11.76) |
| March | 2018 | 8 | 0 (0) | 8 | 1 (12.5) |
| | 2019 | 8 | 1 (12.5) | 8 | 2 (25) |
| | Total | 16 | 1 (6.25) | 16 | 3 (18.75) |
| April | 2018 | 9 | 1 (11.11) | 9 | 2 (22.22) |
| | 2019 | 8 | 1 (12.5) | 8 | 3 (37.5) |
| | Total | 17 | 2 (11.76) | 17 | 5 (29.4) |
| May | 2018 | 8 | 1 (12.5) | 8 | 2 (25) |
| | 2019 | 9 | 2 (22.22) | 9 | 2 (22.22) |
| | Total | 17 | 3 (17.64) | 17 | 4 (23.52) |
| June | 2018 | 8 | 2 (25) | 8 | 3 (37.5) |
| | 2019 | 8 | 1 (12.5) | 8 | 3 (37.5) |
| | Total | 16 | 3 (18.75) | 16 | 6 (37.5) |
| July | 2018 | 9 | 4 (44.44) | 9 | 6 (66.66) |
| | 2019 | 8 | 2 (25) | 8 | 4 (50) |
| | Total | 17 | 6 (35.29) | 17 | 10 (58.82) |

| | | | | | |
|------------|-------------|-----|-----------|-----|------------|
| Aug | 2018 | 8 | 4 (50) | 8 | 5 (62.5) |
| | 2019 | 9 | 3 (33.33) | 9 | 4 (44.44) |
| | Total | 17 | 7 (41.17) | 17 | 9 (52.94) |
| Sep | 2018 | 9 | 6 (66.66) | 9 | 7 (77.77) |
| | 2019 | 8 | 5 (62.5) | 8 | 6 (75) |
| | Total | 17 | 11 (64.7) | 17 | 13 (76.47) |
| Oct | 2018 | 8 | 4 (50) | 8 | 5 (62.5) |
| | 2019 | 8 | 3 (37.5) | 8 | 5 (62.5) |
| | Total | 16 | 7 (43.75) | 16 | 10 (62.5) |
| Nov | 2018 | 8 | 3 (37.5) | 8 | 4 (50) |
| | 2019 | 9 | 3 (33.33) | 9 | 4 (44.44) |
| | Total | 17 | 6 (35.29) | 17 | 8 (47.05) |
| Dec | 2018 | 8 | 0 (0) | 8 | 1 (12.5) |
| | 2019 | 8 | 1 (12.5) | 8 | 0 (0) |
| | Total | 16 | 1 (6.25) | 16 | 1 (6.25) |
| Jan to Dec | 2018 | 100 | 26 (26) | 100 | 37 (37) |
| | 2019 | 100 | 22 (22) | 100 | 34 (34) |
| | Grand Total | 200 | 48 (24) | 200 | 71 (35.5%) |

Table 2: Monthwise prevalence of *Eimeria tenella* in organized and unorganized backyard poultry of Kashmir Valley.

| Season and Year of Collection | | Number of samples examined (Organized/Unorganized) | Number of positive samples (Organized/Unorganized) | Prevalence (%) (Organized/Unorganized) |
|-------------------------------|-------|--|--|--|
| Spring (Mar, Apr, and May) | 2018 | 50 (25/25) | 7 (2/5) | 14 (8/20) |
| | 2019 | 50 (25/25) | 11 (4/7) | 22 (16/28) |
| | Total | 100 (50/50) | 18 (6/12) | 18 (12/24) |
| Summer (June, July, and Aug) | 2018 | 50 (25/25) | 23 (10/14) | 46 (40/56) |
| | 2019 | 50 (25/25) | 17 (6/11) | 34 (24/44) |
| | Total | 100 (50/50) | 40 (16/25) | 40 (32/50) |
| Autumn (Sep, Oct and Nov) | 2018 | 50 (25/25) | 29 (13/16) | 58 (52/64) |
| | 2019 | 50 (25/25) | 26 (11/15) | 52 (44/60) |
| | Total | 100 (50/50) | 55 (24/31) | 55 (48/62) |
| Winter (Dec, Jan and Feb) | 2018 | 50 (25/25) | 3 (1/2) | 6 (4/8) |
| | 2019 | 50 (25/25) | 2 (1/1) | 4 (4/4) |
| | Total | 100 (50/50) | 5 (2/3) | 5 (4/6) |

Table 3: Season wise prevalence of *Eimeria tenella* in organized and unorganized backyard poultry of Kashmir Valley.

Oocysts were seen in wet smear examination of positive faecal samples, where as Eimerian oocysts and schizonts were observed in impression smear examination. The post-mortem examination revealed lesions of caecal coccidiosis. Gross pathology included ballooning of caeca [Figure 1(A)] and severe infection was associated with petechial hemorrhage in caecum [Figure 1(B)]. In severe cases of caecal coccidiosis, characteristic bloody mass was observed on opening of the caeca. Histological studies revealed presence of oocysts at different stages of development in the epithelial cells of submucosal glands of caecum (arrow head) and microgametes (arrow) were found to be free within lamina propria with massive infiltration of heterophils and mononuclear cells [Figure

2(A)] when compared to normal caecum. Presence of numerous intracellular schizonts containing merozoites (arrow) can be seen with severe submucosal hemorrhage [Figure 2(B)]. Intense congestion of blood vessels of sub-mucosa, fibrosis and oedema with degeneration of glandular epithelium harbouring coccidian life-cycle stages and also shedding of oocysts in the glandular lumen was observed. Moreover, blood clots and fibrino-caseous materials were observed in the lumen of caeca. In some cases, different developmental stages of the parasite were transmurally located throughout the mucosa and necrosis was found to be more severe in the regions where there were massive accumulations of schizonts with merozoites. Histological studies of lesions demonstrated fusion of

villi and severe villous atrophy, marked proliferation of epithelial cells of crypts, foci of intense mononuclear infiltrate at the sub mucosa membrane.

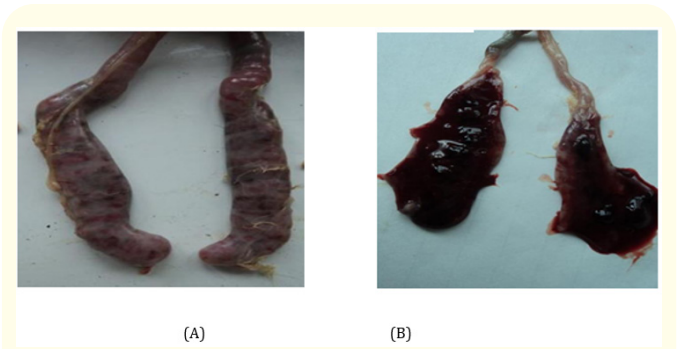


Figure 1

- (A) Photograph showing distension and ballooning of caecum in caecal coccidiosis.
- (B) Photograph of Caecum with petechial hemorrhage during caecal coccidiosis.

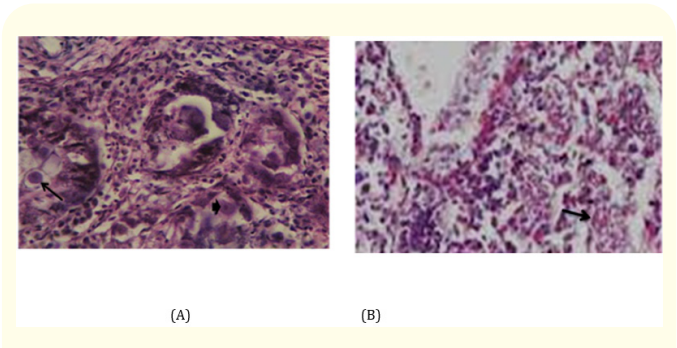


Figure 2

- (A) Photomicrograph of caecum showing presence of oocysts (arrow head) and microgametes (arrow) free within lamina propria with massive infiltration by heterophils and mononuclear cells (H&E x 400).
- (B) Photomicrograph of Caecum showing numerous intracellular schizonts containing merozoites (arrow) can be seen with severe submucosal hemorrhage (H&E x 400).

Discussion and Conclusion

Higher rates of infection in unorganized chicken compared to organized birds observed in the current study is in agreement with the findings of S. Sharma, *et al.* [19] who also reported higher coccidian infection percentage (53.61%) in unorganized farms compared to organized farms (25.55%) in Jammu region of J&K. This difference in infection percentage in two patterns of poultry raising can be attribute to the fact that backyard birds are freely foraging thereby get more exposed to the parasitic infections compared to organized birds. Co-infection of *Eimeria tenella* with other species of *Eimeria* observed in both types of poultry systems may

be attributed to the bad management conditions where birds are exposed to a high-risk infection by different pathogens at the same time. Higher prevalence rates in poorly managed organised farms may be due to poor poultry management practices like overcrowding, leaking water troughs, accumulation of droppings and absence of proper disposal of litter. Highest infection rates observed in the present study during autumn with higher temperature and humidity are in line with the observations of Sharma, *et al.* [19] but unlike their observations lowest prevalence was recorded in winter instead of summer. This high prevalence in the autumn season in Kashmir could be attributed to sudden drop in temperature after hot summer and increase in humidity, which is conducive for sporulation of oocysts for easy dispersion and transmission. Lowering of host resistance during higher temperatures buttresses the parasitic infections in the hosts during warm moist periods. The lowest infection during winter can be attributed to the harsh winter peculiar in Kashmir which suspends the development of parasitic stages.

Gross and histological changes due to *Eimeria tenella* infection observed in this study coincide with those of other workers [20-24]. These authors have also reported enlargement of caecum with clotted blood, haemorrhages throughout the mucosal lining of caeca and change in coloration from reddish to milky white. Infiltration of heterophils and mononuclear cells observed in this study were similar to those of Rasheda and Bano [25]. They have also reported severe oedema and sub-mucosal erosion, glandular tissue cells infiltration and atrophy of villi. Findings of several merozoites, schizonts and microgametes in the epithelial cells were also reported by earlier workers [26,27].

Conflict of Interest

There was no conflict of interest in this study.

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