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SOME STUDIES ON *EIMERIA TENELLA* INFECTING CHICKEN

AThesis

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For

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2018

Cairo University
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Abstract

Our study was directed to collect caecal samples from 600 broiler chicken during the period from January to December 2015 from Cairo and Giza chicken farms. The caeca were scraped for *Eimeria tenella* oocysts collection which were propagated in 49 one day old chicks. Weeklv antibodv titre was measured in the sera of infected chicks using Enzyme-linked immunosorbent assay and Agar Gel Precipitation Test and these serological tests revealed the presence of antibody against *Eimeria tenella* which was found in 68% of the examined samples. Maternal immunity was notice in the 1st week of age and decreased till reaching the 4th week in our study. The propagated oocysts were identified morphlologically using micrometer and molecularly using Polymerase Chain Reaction. The molecular identification of *Eimeria tenella* was targeting the Internal Transcribed Spacer 1(ITS-1) region and the amplified fragment consists of 278 bp. the phylogenetic analysis was also done and revealed 99.6% identity with other global isolates on the Genbank. Transmission electron microscopy used to detect the ultrastructural components of *Eimeria tenella* endogenous stages in caecal tissue. Immunohistochemical study was conducted on the infected caecal tissue and revealed a positive reaction Histopathological examination was conducted on the infected caecal tissue and revealed that *Eimeria tenella* infection. On conclusion , results of the present study denotes that identification and confirmation of infection with *Eimeria tenella* could be performed using traditional and molecular methods as well as antibodies against *Eimeria tenella* could be detected serological tests (ELISA and AGPT). Commercial ELISA must be applied in further study as a reference method to measure sensitivity and specificity of our ELISA results and also maternal immunity must be subjected to further studies.

Summary

Coccidiosis is the most important parasitic infection in poultry farms worldwide. *Eimeria tenella* is considered the most pathogenic species that affect caecal tissue of chicks causing severe economic losses in poultry farms. In this study, samples were collected from poultry farms during the period from January to December 2015. It has been revealed that *Eimeria tenella* was found in 68% of the examined samples. The highest prevalence of infection was reported during the summer (88%) compared to 44% in winter. Monthly survey revealed that the highest prevalence (100%) was found during May and August, while the lower prevalence was seen in December (24%). The collected oocysts were propagated in 49 one day old chicks. Weekly antibody titre was measured in the sera of infected chicks using ELISA and the serological test revealed the presence of antibody against *Eimeria tenella* in the sera of experimentally infected chicks appeared from the first week post infection, reaching plateau at the 3rd week and decreasing till the 7th week. The propagated oocysts were identified morphlologically using micrometer and molecularly using PCR. The morphological identification revealed that the collected oocysts have 1.2- 1.4 *µm* shape index, the average length was 20.7 *µm* and the average width was 15-25 *µm*.The molecular identification of *Eimeria tenella* was targeting the Internal Transcribed Spacer 1(ITS-1) region. Agar gel electrophoresis revealed that the amplified fragment consists of 278 bp which sequenced in Germany and the phylogenetic analysis was also done and proved (99.6%) identity between the isolate of the present work which has accession number (MF034720) and other isolates on the Genbank. Transmission electron microscopy used to detect the ultrastructural components of *Eimeria tenella* endogenous stages in caecal tissue of the experimentally infected chicken. In the present study, 49 day- old broiler chicks were experimentally infected with a dose of 7000 sporulated oocysts of *Eimeria tenella*. Thus, infected caecal tissue were removed from the chicks and the examination revealed the presence of immature and mature macrogametocytes, microgametocytes, mature merozoites, immature oocysts, schizonts. Immunohistochemical study was conducted on the infected caecal tissue and revealed a positive reaction in direct immuno peroxidase and immunofluoresent tests. Histopathological examination was conducted on the infected caecal tissue of the experimentally infected chicks with *Eimeria tenella* and it revealed that *Eimeria tenella* infection were characterized by aggregation of desqumated epithelial cells, RBC.S with some developmental stages of *Eimeria* in ceacal lumen marked proliferaton of epithelial

cells, dilation and necrosis of mucosal gland, infiltration and focal aggregation of inflammatory cells mainly lymphocytes ,eosinophil's ,and heterophiles. Haemorrhage was also noticed. Necrosis being more sever where massive aggregations of schizonts are localized .Multifocal and interstatial edema at the submucosa associated with various intra lesional forms of the parasite.

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I-Introduction

Poultry plays a very important role for man kind through food supply, income and employment generation. Huge number of man power work in poultry industry so it is considered an important part of our income. Poultry meat and eggs are considered as a good source of vitamins, minerals and protein. Poultry meat is considered lower in cost than other types of meats. Also, it has great acceptability to all religion.

Poultry industry is adversely affected by the protozoan parasite of genus *Eimeria* , phylum Apicomplexa has an economic importance stimulated great deal of research.

Coccidiosis is one of the most important protozoan disease caused by *Eimeria* species including *Eimeria tenella* that adversly affect poultry industry **(Williams, 2002)**. Recently coccidiosis causes heavy economic losses estimated to be two billion US dollars a year world wide (**Zang and Zeng, 2005).** *Eimeria tenella* is the most pathogenic species affecting chicken charachterized by bloody diahrrea and lower weight gain (**An *et al*, 2001).**

The short and direct life cycle as well as the high reproductive potential of *Eimeria* in poultry often leads to severe outbreaks of disease in small backyard flocks or in the modern poultry house, **(McDougald and Fitz-Coy, 2008**). *Eimeria tenella is* a protozoan that multiplies in the intestinal tract and causes tissue damage, resulting in the interruption of feeding, digestive processes, nutrient absorption, dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease pathogens like *clostridium, salmonella* and *E.coli* (**Takimoto *et al*, 1984).** The parasite is host specific, found in the caecum of the birds, where it develops and multiplies intracellularly. Transmission of parasites among broilers is by fecal - oral route through the ingestion of sporulated oocysts, causing severe lesion of caeca, body weight loss and hemorrhagic diarrhea **(Dalloul and Lillehoj, 2005).**

Subclinically, it is manifested by poor performance, impared feed conversion, poor flock uniformity and poor growth **(Fetterer and Allen,**

2000).

The problem of this disease is very difficult to combat because different species of coccidian exist in field and poultry may infected with different species because the immunity that developed after infection is specific only to one species and *Eimeria* life cycle is very complex as it involves several developmental stages within host cell.

Identification of *Eimeria* is very important for controlling and management of the parasite. The traditional methods for identification needs experience and have serious limitations due to this subjective nature and overlapping characters among different species. Mixed infection is also a problem for the discrimination of *Eimeria* and it is based on morphological features of the sporulated oocyst, sporulation time and location/ scoring of the pathological lesions in the intestine (**McDougald *et al*, 1997).** Immunohistochemistry is considered a method of diagnosis that refers to detect *Eimeria tenella* antigen (protein) in the cells of a tissue section by exploiting the principle of Ab binding specially to *Eimeria tenella* in biological tissue. This test is widely used in basic research to understand the existence and distribution of *Eimeria* in different part of caecal tissue depending on Ag-Ab interaction **(Bayaz *et al*, 2009).**

Molecular identification using Polymerase Chain Reaction and sequencing have some advantages over traditional methods, that they rely on targeting different regions of *Eimeria* species genome **(Stucki *et al*, 1993)**. So PCR and sequencing are useful for identification, detection or charachterization of *Eimeria tenella*.

Enzyme-linked immunosorbent assay is widely used to measure antibody titre against *Eimeria tenella*. Although ELISA test has not yet become a routine method in most parasitological labs, it requires only a small amount of Ag and has short reaction time, it is likely that ELISA is useful for large scale Ab screening for parasites. For these reasons the present study was conducted to develop a sensitive ELISA kit for detecting Ab against *Eimeria* and surveing the seroprevalence for infection due to this pathogen **(Kiani and**

Farhang, 2008).

The aims of the present study are :-

1- Studing the prevalence of *Eimeria tenella* infection in chicks.

2- Molecular identification of the isolated *Eimeria tenella* using PCR and DNA sequencing.

3- Serological tests were used to detect the antibodies against *Eimeria tenella* in the sera of the infected chicks.

4- Identification of *Eimeria tenella* inside the infected caecal tissue using immunohistochemistry and transmission electron microscope.

5- Detection of tissue damage in the caecum of the infected chicks caused by *Eimeria tenella* stages through histopathological examination.

II- Review of literature

II .1- Prevalence of *Eimeria tenella* infection in local native chicks:-

Abdel-Malek (1997) reported that in Port Said out of 350 examined chicken (85.34%) were found infected with *E. tenella*.

Lunden *et al.* (2000) mentioned that coccidiosis was diagnosed in 11 flocks (19.3%) from 9(31%) of the farms. The outbreaks occurred when the birds were 19 to 32 weeks old. *E. tenella* was found in 3 of the out breaks and reported high incidence of infection in Winter in Swedan.

Razmi and Kalideri (2000) survyed the prevalence of sub clinical coccidiosis in broiler chickens in Khorasan, Iran. The farm level prevalence of subclinical coccidiosis was 38%. An increased risk of infection in the broiler was associated with the larger farm, with older chickens, and if the chicken farm were sampled in winter or spring. The peak oocyst score in the litter occurred at>6^{th} week of age. Most farms had 12% of *E. tenella.*

Al- Natour *et al.* (2002) mentioned that after post mortum and parasitological examination of 6 chicks out of 200 broiler farms *E.tenella* was the most prevalent species 39% in Northen Jordan.

Ayaz *et al.* (2003) conducted a study on broiler chicken in Faisalabad Pakistan. They found that prevalence of infection with *Eimeria tenella* was 50%.

Abdel-Satar (2004) mentioned that caecal coccidiosis was the most common type in Upper Egypt 59.3%.

Lobago *et al.* (2005) recorded that at Kombolcha poultry multiplication and research center, Ethiopia was conducted from November 2002 to April 2003. The incidence of coccidiosis and the distribution of *Eimeria* spp. In dead chickens 1- 60 days. Out of 965 dead birds, 370 (38.34) were found to have clinical coccidiosis and *Eimeria tenella* prevalence was 40.8%.