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HISTOPATHOLOGICAL CHANGES OF BROILERS IMMUNIZED WITH SONICATED OOCYSTS AGAINST *EIMERRIA TENELLA*

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ABSTRACT
Eimerria (*E*.) remains one of the major problems for poultry industry throughout the world. The histological changes of chickens immunized against *E. tenella* were observed in broilers. A total of 60 broiler chicks were divided into 3 equal groups. The 1[s] and 2[nd] groups remain as control, while the 3[th] group was inoculated with 0.2 ml of mature sonicated oocyst by I/M injection at day one and repeated by same route at 21 days old. After that, the 2[nd] and 3[th] groups were challenged by 50,000 viable sporulated oocysts of *E. tenella* at day 28. The results revealed that there was a mild lesion graded as 2 lesion score, with inflammatory cells influxes into the sub mucosa, thickened mucosa and sub mucosal layer with slightly congestion of blood vessel caused by *E. tenella* in an immunized group compared with control positive which was safe from severe lesion in the cecum caused by *E. tenella* graded as 4 lesion score with pathological lesions observed in the cecum section showed that necrotized epithelial cells denuded from the mucosal layer and severe hemorrhage in the lamina propria. Also, the crypt cells were highly invaded with the developmental stages of *E. tenella* schizonts and gametocytes that their morphology is almost disappeared. This study indicates I/M immuinzation of sonicated oocyst showed some evidence of reducing negative performance impact associated with coccidiosis.

KEY WORDS: *Eimeria tenella;* histopatology; broilers.

INTRODUCTION

In the modern commercial poultry industry, increases in diseases and infections by enteric pathogens are common due to intense rearing systems. One disease of particular concern is coccidiosis [1]. Coccidiosis remains one of the major problem for poultry industry throughout the world [2, 3]. It caused by *Eimeria* species parasitize epithelial cells of the intestinal lining, causing pathological changes varying from local destruction of the mucosa to systemic effects such as blood loss, shock, and death [4]. Seven species of *Eimeria* are generally accepted to be causative agents of avian coccidiosis, *E. tenella* is found to be the most prevalent and pathogenic species throughout the world [5]. Vaccines have been used in the poultry industry for more than 50 years, primarily in broiler breeder and replacement layer flocks [6]. Vaccination with live or attenuated parasites is a practical alternative to drugs for coccidiosis control. The immune response to inactivated sonicated vaccine was used in chickens. Parental inoculation of dead antigen is although capable of stimulating circulating antibodies against coccidiosis antigen. In spite of these limitations, vaccination remains the most efficient means of preventing disease and reducing economic losses [7]. From a general point of view and the importance of poultry production, the purpose of the present study histopathological observations of *E. tenella* immunized with whole sonicated oocyst in commercial broilers chicks.

MATERIALS AND METHODS

Experimental Birds and Housing

The experiment was done in the poultry house of Pathology and Poultry Diseases Department, College of Veterinary Medicine, Baghdad University, poultry house, after cleaning and disinfecting. One-day-old, broiler chicks were used in this experiment with commercial diet free from anticoccidial.

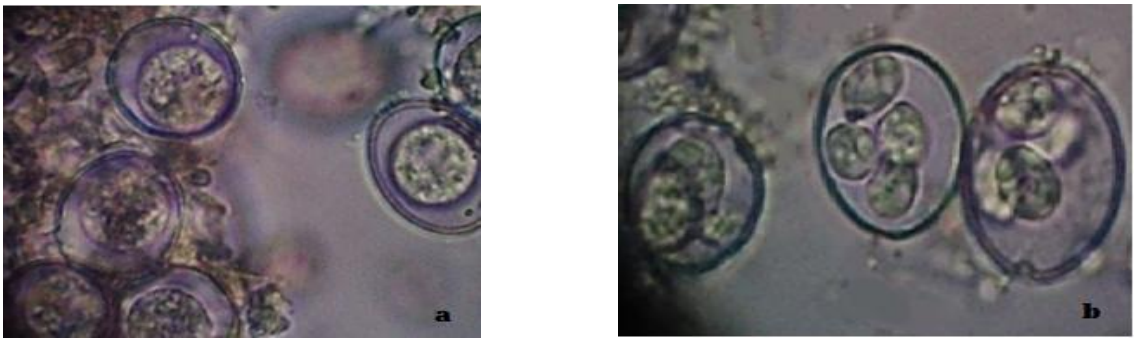


FIGURE 1: shows unsporulated (a) and sporulated (b) Oocysts of *E. tenella* (400X)

Oocysts preparation of *Eimeria tenella*

Oocysts were collected directly from the infected birds, scrapings were made from the lesions and rinsed into a beaker with potassium dichromate solution (% 2.5) to release the unsporulated oocysts, then oocysts were stored at 4°C [8]. Oocysts must undergo sporulation before they are infective as in Figure 1. Sporulation was optimized at 30°C with forced ventilation [9]. The collected oocysts washed by distil water 3-4 times and centrifuged on 3000 rpm for 10 minutes to remove the potassium dichromate. The oocysts were counted using the hemocytometer method [10].

Sonicated antigen and vaccine preparation

The sonication was applied in Biotechnical Center in Baghdad University; the sporulated oocysts collected in potassium dichromate solution were washed 3-4 times by physiological saline solution (pH 7.2) and concentration to 5000-6000 per ml. The washed sporulated oocysts were subjected to ultra sonication by Soniprep150 (SONY Company) for 2 by 30 seconds in jacketed vessel with cool water. The inactivated vaccine was prepared from sonicated suspension through treating with 0.3 percent formalin (33% formaldehyde) for 96 hours at 37°C [11, 7] and stored at 4°C until use.

Experimental design

A total of 60 newly hatched commercial broiler chicks. Upon arrival, the chicks were divided randomly into three equal groups of 10 chicks each. The 1^{s} and 2nd groups remain as control groups, while the 3th group was inoculated with 0.2 ml of sonicated oocyst by I/M injection at day one and repeated by same route at 21 days old. The 2nd and 3th groups were challenged by 50,000 viable sporulated oocysts of *E. tenella* at day 28. The uninfected control group was inoculated with distilled water. All groups feed by basal diet from day one to the end of experiment (35 day). Clinical signs of the test groups were recorded twice in a day until day 7 post infection. On day 8 post infections, all chicks in all experimental groups were scarified for cecal lesion score based on the procedures described by Conway *et al*. [12]. A score of zero is normal and a score of 4 is assigned for the most severe and dead birds due to coccidial infection.

Histopathological examination

Classical lesions were taken for histopathological preparation. Haematoxylin and eosin (H&E) staining was used to demonstrate the developmental stages in the cecum [13]. Tissues sampled was fixed in 10% neutral buffered formalin, sectioned at 5-6µm thicknesses and stained with haematoxylin- eosin stain.

RESULTS & DISCUSSION

The lesions score of broilers during the experimental period (mean±SD) are illustrated in Figures 3, 4. Lesion scores for challenged groups, the non-immunized control group had the highest mean lesion scores with an average of 3.20, while means for I/M group had the lowest mean lesion score of 0.40. No lesion was observed in the control negative group (non- challenged). The procedures for killing birds and techniques for postmortem examination are based on the technique discussed by Zander [14]. The entire length of the external serosal surface of the cecum needs to be examined under light.

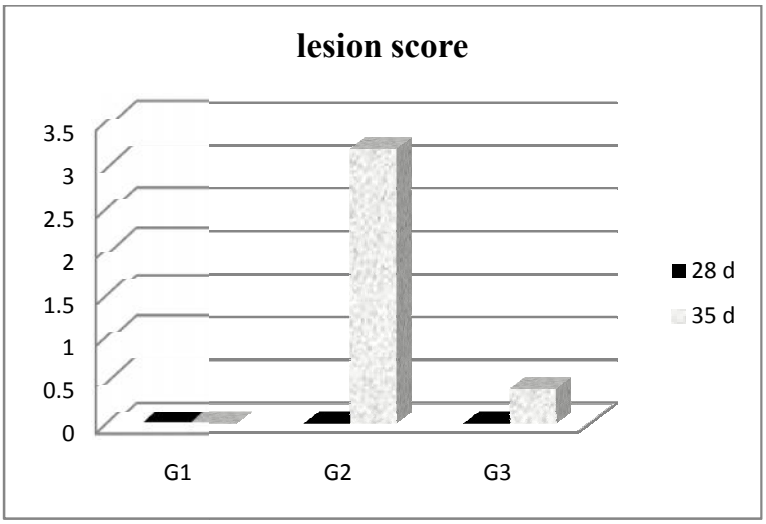


FIGURE 3: lesion scores of broilers during the experimental

In examining the serosal surface a search should be made for whitish plaques or petechiae. Presence of mucus, blood, casts, or cores and presence of cheesy coagulation necrosis should be noted. Presence of blood in the caeca suggests a diagnosis of *E. tenella*. Lesion scoring is a technique developed to provide a numerical ranking of gross lesions caused by coccidian [15] cited by Conway *et al*. [12]. the lesions are scored 1upto 4 based on the key identification characteristics discussed by Conway *et al*. [12], during this study equal challenge of 1 x 10^5 sporulated oocysts was used in all experimental groups. However, this concentration was not sufficient to promote lesions with scores above 2 in vaccinated group. Consequently, vaccines could confer immunity against coccidiosis.

FIGURE 4: Cecum of the chickens of the experimental groups showed; **1** normal appearance (control negative) graded as zero lesion score, **2** mild lesion caused by *E. tenella* challenged (immunized group) graded as 2 lesion score, **3** Lesion in the cecum caused by *E. tenella* with hemorrhage and eroded mucosal layer graded as 4 lesion score.

Histopathological examination

The principal histopathological lesions observed in these birds at 7 day after infection was consistent with those associated with infection induced by *E. tenella* [16]. During this study, *E. tenella* induced-lesions were mild in the immunized group Figure 5 characterized principally by inflammatory cells influxes into the sub mucosa and thickened mucosa and sub mucosal layer with slightly congestion of blood vessel necrotized and denuded epithelial mucosa with slightly congestion of blood vessel. That mean, low to moderate levels of *E. tenella* infestation were apparent in the cecum from vaccinated group compared with non-vaccinated group.

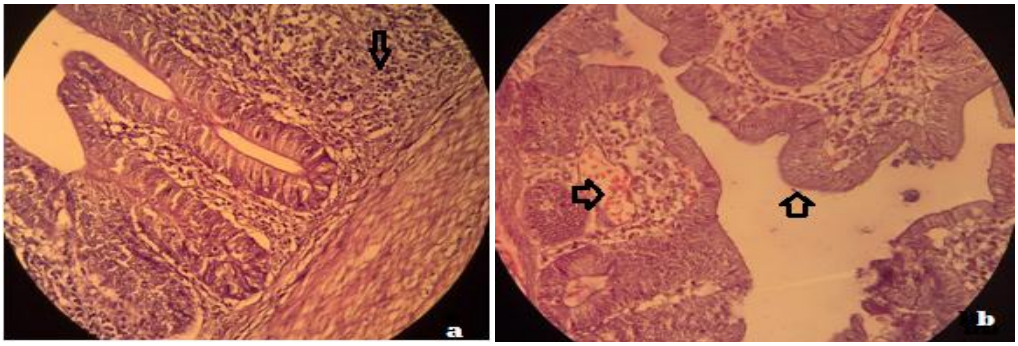


FIGURE 5: Histological sections prepared from the cecum of the immunized group with; (a) inflammatory cells influxes into the sub mucosa, (b) thickened mucosa and sub mucosal layer with slightly congestion of blood vessel (400xH&E).

On other hand, after challenge *E. tenella* induced-lesions were very severe in the non-immunized group Figure 6. The general effects include changes in the cellular kinetics and morphology of the villi. The pathological changes are mainly due to the second generation schizonts [17]. By the fifth or sixth day the caeca are dilated, the contents containing unclotted and partly clotted blood, schizonts. In primary infection, numbers of heterophils and mast cells were increased during the acute inflammation process which indicates mast cells play a role as primary inflammatory cells [18]. Heterophils predominated when necrosis was extensive; otherwise, mononuclear cells were the main inflammatory cells [19, 8].

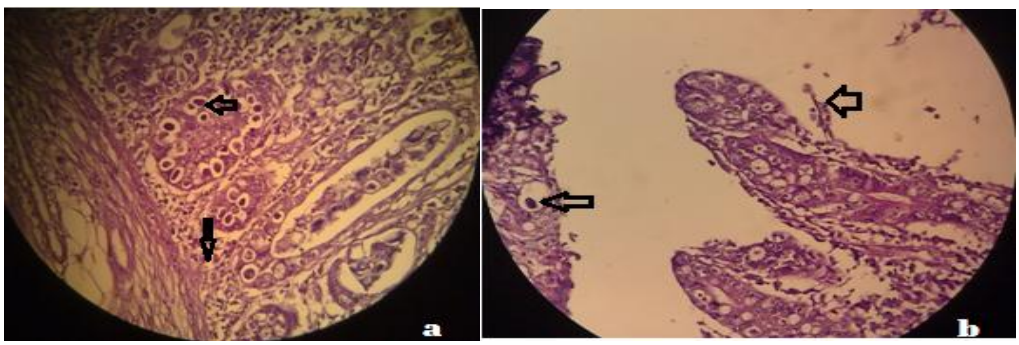
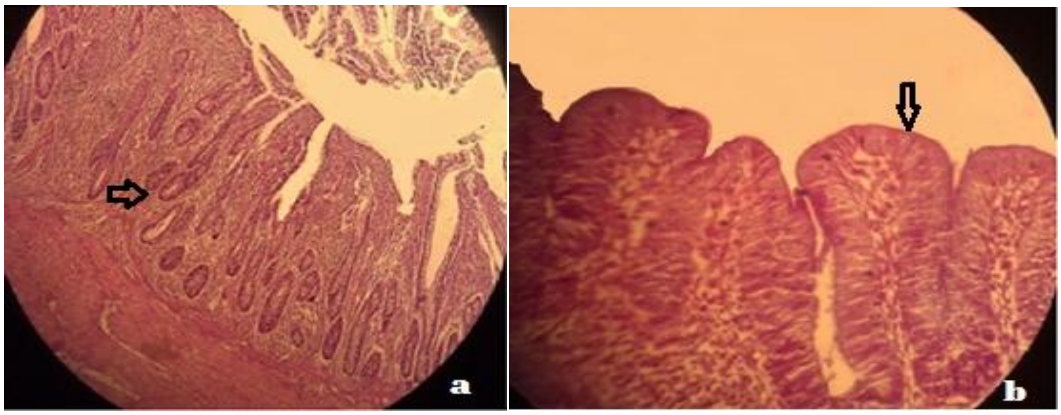


FIGURE 6: Histological sections infected with *E. tenella* prepared from the cecum of the non-vaccinated group with; (a) Pathological lesions observed in the cecum section and the crypt cells which were highly invaded with the developmental stages of *E. tenella* schizonts and gametocytes that their morphology is almost disappeared, high inflammatory cells infiltration into the sub mucosa and thickened sub mucosal layer lamina propria was observed, (b) shows necrotized and denuded epithelial mucosa and the crypt cells are invaded by developmental stage schizonts (400xH&E).

In some cases, parasites in various stages of development were transmurally located throughout the mucosa, with necrosis being more severe where there were massive accumulations of schizonts with merozoites. *E. tenella* induced lesions demonstrated severe villous atrophy and fusion of villi, discrete hemorrhage, marked proliferation of epithelial cells of crypts, foci of intense mononuclear infiltrate at the sub mucosa membrane, multifocal and discrete interstitial edema at the submucosa emuscular membranes associated with various intralesional forms of the parasite within epithelial cells [20]. The control negative group in this study showed normal histology apperance charactarized by normal mucosa, sub mucosal layer and crypt cells Figure 7.



In this study, the sonicated sporulated oocysts were used as an antigen to stimulate the immune response against *E. tenella* in broiler chicks and this gave a successful result to protect the broilers against felid coccidiosis strain due to stimulate superior immune response [21, 7] and reduce the pathological changes. In conclusion, the present study shows that vaccination status, used may exert a significant influence on the host antibody response to Eimeria and protect chickens against severe histopathological changes.

FIGURE 7: Histological sections prepared from the cecum of the control group with normal histology mucosa, sub mucosal layer and crypt cells a (100xH&E), b (400xH&E).

REFERENCES

[1]. De Gussem, M. (2007) Coccidiosis in poultry: Review on diagnosis, control, prevention and interaction with overall gut health. p.p 253–261 in Proc. 16th Eur. Symp. on Poult. Nutr. World's Poultry Science Association, Beekbergen, the Netherlands.

[2]. Dalloul, R.A. and Lillehoj, H.S. (2006) Poultry coccidiosis: Recent advancements in control measures and vaccine development. Expert Review of Vaccines 5, 143-163.

[3]. Hafez, H.M. (2011). Enteric diseases of poultry with special attention to Clostridium perfringens. Pakistan Veterinary Journal 31: 175-184.

[4]. Yun, C. H., Lillehoj, H. S. and Lillehoj, E. P. (2000) Intestinal immune responses to coccidiosis. Developmental & Comparative Immunology 24, 303-324.

[5]. Shirley, M.W. and Bedrinck, P. (1997) Live attenuated vaccine against avian coccidiosis: success with precocious and egg adopted lines of *Eimeria*. Parasitolol. Today 13, 481-84.

[6]. Chapman, H.D., Cherry, T.E. Danforth, H.D. Richards, G. Shirley, M.W. and Williams, R.B. (2002) Sustainable coccidiosis control in poultry production: The role of live vaccines. International Journal Parasitology 32, 617-629.

[7]. Bahram, A.M. and Bahrami, A. (2006) Immune response of chicken to an experimental sonicated coccidian oocyst vaccine. Archives of Razi Institute 61, 43-48.

[8]. Reid, W. M. (1978) Coccidiosis. In Diseases of Poultry, 7th ed., ed. M. S. Hofstad, B.W. Calnek, C. F. Helmboldt,W. M. Reid, and H. W. Yoder, Jr. Ames, IA: Iowa State University Press. pp784-815.

[9]. Davis, L.R. (1973) Techniques. In The Coccidia, ed. D. M. Hammond and P. L. Long, Baltimore, MD: University Park Press. pp 411–58.

[10]. Long, M.A., Handwerger, B. S., Arnos, D. B. and Yunis, E. J. (1976) The genetics of cell mediated lympholysis. Journal of Immunological Methods 117, 2092-2099.

[11]. Fu, H.M. and Lee Y.C. (1976) Immunological studies on chemically attenuated oocysts of chicken caecalcoccidisis. Journal of Chinese Society of Veterinary Science, 2, 51–55.

[12]. Conway, D.P., Sasai, K., Gaafar, S.M. and Smothers, C.D. (1993) Effects of Different Levels of Oocyst Inocula of *E. acervulina*, *E. tenella*, and *E. maxima* on Plasma Constituents, PVC, Lesion Scores and Performance in Chickens. Avian Diseases 37, 118-123.

[13]. MAFF, Ministry of Agriculture, Fisheries, and Food (1979) Manual of veterinary Parasitological

Laboratory Techniques. Technical Bulletin No. 18, 2nd edition. London: Her Majesty's Stationary Office. pp 71-76.

[14]. Zander, D.V. (1978) Principles of Disease Prevention: Diagnosis and Control. In: Hofstad,M.S., Calnek, B. W., Helmboldt, C. F., Reid, W. M. and Yoder, Jr, H. W. (ed.), Diseases of Poultry, 7th Edition. USA, Iowa State University Press/Ames, Iowa, pp.3-48.

[15]. Johnson, J.K. and Reid, W.M. (1970) Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology 28, 30-36.

[16]. McDougald, L. R., Reid, W. M. (1997) Coccidiosis. In:Calnek, B.W. Diseases of poultry. 10th ed. Ames: IowaState University Press, pp.865-882.

[17]. Soulsby, E.J.L. (1982) Helminths, Arthropods and Protozoan's of Domesticated Animals, 7th edition. Bailliere Tindall, London. pp 411–458.

[18]. Petrone, V. M., Constantino, C. F. and Pradal- Roa P. (2002) Identification andQuantification of Granulocytes in Cecal Mucosa and Submucosa of Chickens Experimentally Infected with Eimeria tenellaand Salmonella enteritidis. British Poultry Science 43, 653-661.

[19]. Mesfin, G. M., Bellamy, J. E. C. and Stockdale, P. H. G. (1978) The Pathological Changes Caused by Eimeria falciform is Varpragensis in Mice. Canadian Journal of Comparative Medicine l42, 496-510.

[20]. Shirley, M. W., Smith, A. L. and Tomley F. M. (2005) The biology of avian Eimeria with an emphasis on their control by vaccination. Advances in Parasitology 60, 285-330.

[21]. Hong, Y.H., Lillehoj, H.S., Lillehoj, E.P. and Lee, S.H. (2006) Changes in immune- related gene expression and intestinal lymphocyte subpopulations following *Eimeria maxima* infection of chickens. Veterinary Immunology Immunopathology 114, 259-272.