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THE PATHOGENICITY OF *EIMERIA* SPECIES ISOLATED FROM CHICKEN IN THUA THIEN HUE PROVINCE

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Abtract: The present study was conducted to clarify the effect of local isolated *Eimeria* species on chickens in central Vietnam. Oocysts of *Eimeria* species were isolated from feces suspected to be infected with coccidiosis in 3 farms in Huong Thuy district, Thua Thien Hue province. A total of 54 2-weeks old chickens were randomly allocated to 2 groups: 3 replicates containing 9 chickens in each replicate. The chickens in group 1 were orally inoculated with 2×10^4 isolated oocysts of *Eimeria* species, while the chickens in group 2 were inoculated with PBS as a control. Growth performance, oocyst output, gross lesions and histopathological lesions were measured at 5, 10 and 28 days post infection. The chicks in group 1 showed general signs of ruffled feathers, anorexia, huddling together with diarrhea and/or bloody dropping from 4–7 days post-infection. Feed intake, growth performance and body weight in group 1 of chicks were lower compared with control chicks. Oocyst was detected in the feces of chicks in group 1 from 6–14 days post-infection, among them, the number of oocyst reaching the peak at 8 day post-infection. At day 5 post infection, the most damaged part of intestine is the ceca of chickens with a large amount of blood in the ceca content. The histopathological lesions was detected clearly by HE staining at day 5 and 10 post-infection. In conclusion, the local isolated oocysts of *Eimeria* species have high virulence to the chickens. The present study provide useful information related to the pathogenicity of *Eimeria* species which may contribute for coccidiosis diagnosis and treatment in poultry production.

Keywords: *Eimeria,* coccidiosis, infection, central Vietnam

1 Introduction

Coccidiosis is one of the most important diseases in the poultry industry caused by *Eimeria*, a protozoan parasite genus consisting of 9 species, *Eimeria praecox, E. acervulina, E. mitis, E. mivati, E. hagani, E. maxima, E. brunetti, E. tenella, E. necatrix* (Joyner et al.,1974). Among them, *E. acervulina, E. necatrix, E. tenella, E. maxima* and *E. brunetti* are the major species infecting chickens. The parasites invade and develop inside the intestinal epithelial cells of chickens causing the intestinal barrier destruction which leads to bloody feces excretion, reduction of feed intake, weight losses and death in case of heavy infections, consequently to massive economic losses for the farmers (Zaman *et al*., 2015).

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Thua Thien Hue province is located in central part of Vietnam with tropical monsoon climate, where coccidiosis, as the rule, occurs frequently. According to the observation in the farms around Thua Thien Hue province, there is the high rate of chickens have symptoms of coccidiosis such as weight loss, bloody feces excretion. However, the information of pathogenicity of *Eimeria* species is still limitted in Vietnam, particularly in the central area. The present study is, therefore, to reveal the affects of coccidiosis on chickens, which may be useful for coccidiosis diagnosis and prevention. In addition, this information is considered as references to compare with the data base of pathogenicity of *Eimeria* species in other countries around the world.

2 Materials and Methods

Isolation of *Eimeria* oocysts

Eimeria oocysts were isolated from collected dropping which clinically diagnosed to be infected with coccidiosis from 3 farms in Huong Thuy district, Thua Thien Hue province by flotation method with saturated saccharose. The oocyst were then kept for sporulation in 2.5% potassium dichromate solution at room temperature within 2 days and stored at 4 ^{o}C till use (Rosa et al., 2015).

Eimeria infection

A total of 70 3F-Viet 103 chickens were purchased from the commercial company at 2 days old and raised until 2 weeks old in our facility. 54 chickens were chosen and randomly allocated to 2 treatments: *Eimeria* treated group and control group. The experimental units were repeated 3 times with 9 chicks each. Isolated oocysts which have been mentioned above were used for *Eimeria* infection. For use, the potassium dichromate solution was washed out with distilled water by centrifugation twice (2500 rpm), and the number of oocysts then were counted by a McMaster chamber using light microscopy (Foreyt *et al*., 2001) The chickens in group 1 and 2 were orally inoculated with 1ml of 2×10^4 *Eimeria* sporulated oocysts and PBS, respectively.

Oocyst output

Feces was collected daily from *Eimeria* treated chickens and control chickens from 5-15 days post infection. The feces in each cage was mixed well, then 2gram feces per each cage was collected into 15ml centrifuge tubes and were kept in the refrigerator until counting by McMaster chamber (Long *et al.*, 1967).

Growth performance

Feed intake, body weight, and body weight gain were weekly measured individually by the electronic scale in the morning before feeding.

Gross lesions and Histopathological lesions

Three chicks per treatment were sacrificed at 5, 10 and 28 day post *Eimeria* inoculation by cervical dislocation method. Collected ceca were fixed for 24 hours in 10% formalin. 3–5 mm from the middle part of the cecum was cut, embedded in paraffin, cut as cross-sections at a thickness of 6 μm by a microtome, and stained with Hematoxylin and Eosin (HE). Five cross sections per chick then were examined using light microscopy.

Data analysis

The data of growth performance including feed intake, body weight, and body weight gain were analyzed by Microsoft Excel 2007 and Minitab 16.2.3.0. Paired t-tests were conducted to compare the control and the *Eimeria* treated groups. A probability value of $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 Oocyst output

According to the figure 1, the *Eimeria* treated chickens discharged the oocysts during 9 days (from 6 to 14 days post infection). The number of oocysts were highest at 8 days post-infection (dpi) then gradually reduced until finish at 14 dpi, while no oocyst was detected in control chickens.

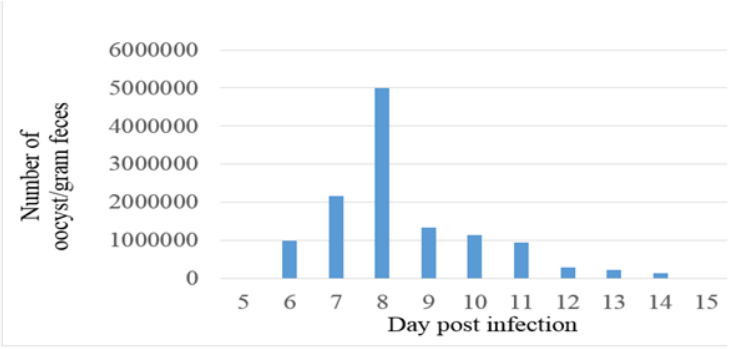


Figure 1. Fecal oocyst output

3.2 Gross lesions

The results showed that ceca were the most damaged part in the gastro-intestinal system of *Eimeria* treated chickens. The ceca of infected chickens were damaged with large amount blood in the ceca content at 5dpi then seem were recovered with stop bleeding but still diarrhea and slightly thickened at 10 dpi and 28 dpi. Meanwhile, the ceca of control chickens were in normal condition.

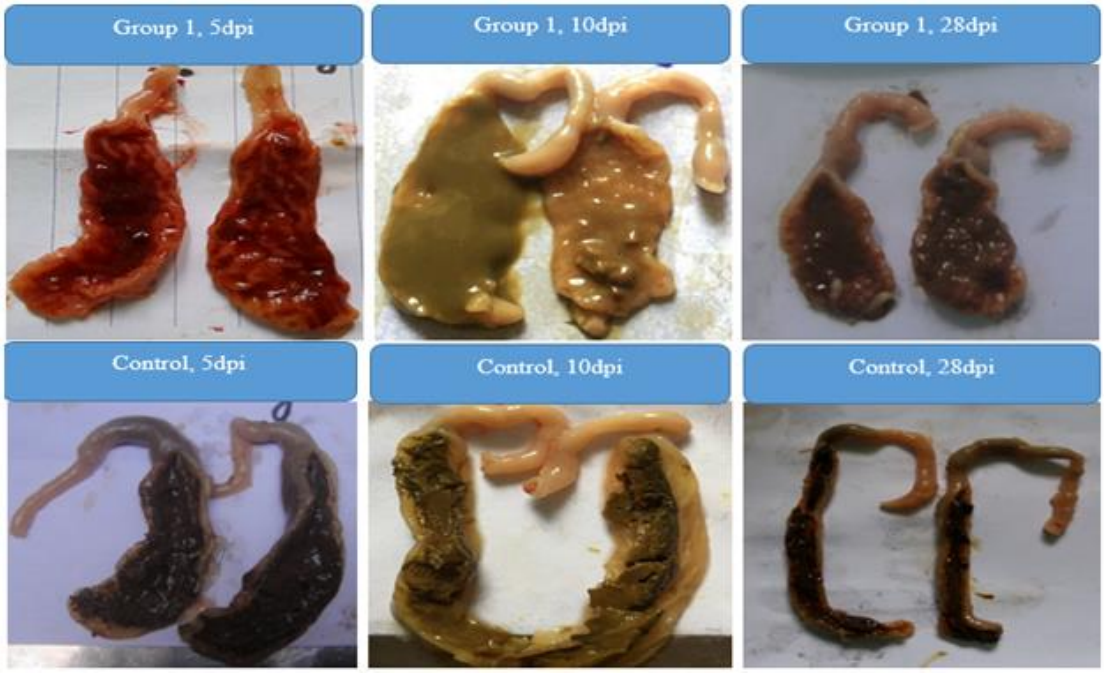


Figure 2. Morphological changes in ceca during *Eimeria* infection

3.3 Growth performance

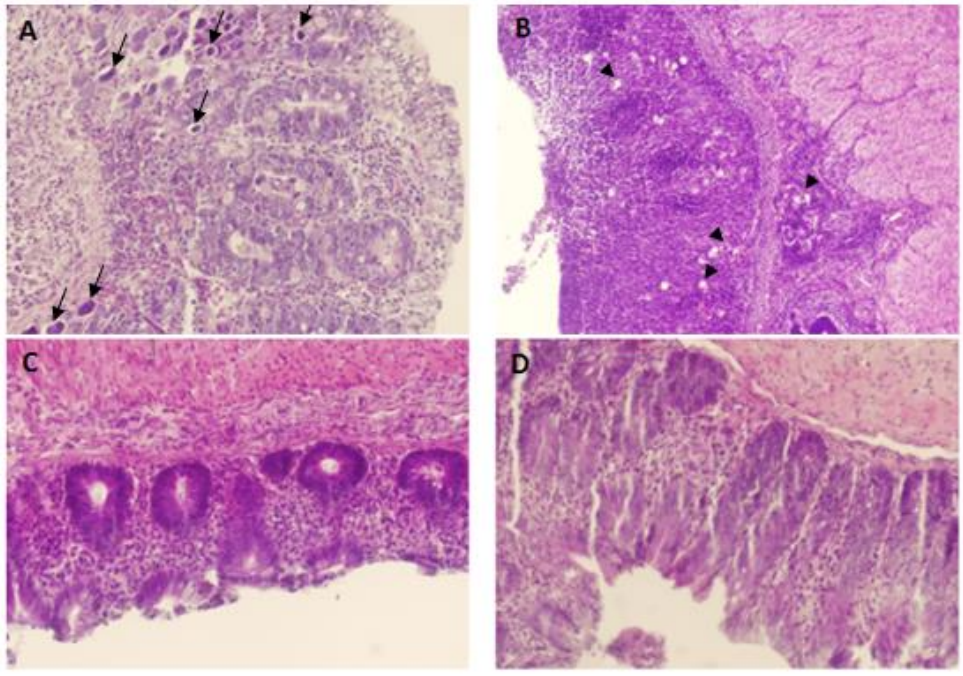
Overall, the tendency of growth performance including feed intake, body weight gain,and body weight was lower in infected chickens than control during this experiment. Among them, Feed intake was statistically significantly higher in control than in treated chickens at 6 weeks old. Body weight of treated chickens was also significantly lower than in control at 5 and 6 weeks old.

Table 1. Growth performance of chickens

Weeks old	Group 1 (Infection)	Group 2 (Control)	P
Feed intake (gram/broiler/week)			
4	241,33 ± 9,01	257,67 ± 5,51	0,056
5	329,67 ± 45,08	374,33 ± 16,07	0,181
6	490,00 ± 20,00	610,00 ± 47,32	0,016
7	728,7 ± 55,30	873,70 ± 194,90	0,283
Body weight gain (gram/broiler/week)			
4	84,76 ± 35,16	116,33 ± 16,65	0,231
5	105,33 ± 38,55	134,00 ± 18,33	0,309
6	142,33 ± 8,74	180,33 ± 29,01	0,096
7	161,00 ± 65,55	172,67 ± 67,52	0,840
Body weight (gram/broiler)			
3	328,67 ± 3,79	325,00 ± 18,03	0,231
4	413,33 ± 31,39	441,33 ± 9,07	0,212
5	518,67 ± 11,15	575,33 ± 14,36	0,006
6	661,00 ± 19,08	755,67 ± 41,19	0,023
7	822,00 ± 84,5	928,00 ± 107,8	0,250

3.4 Histopathological lesions

At 5dpi, there were alarge amount of *Eimeria* in second schizonts (picture A,arrow) were detected from cross sections of the infected chickens. As consequence, the high number of infiltrated inflammatory cells and mucosal destruction were seen in *Eimeria* treated group (Picture A) as well. At 10 dpi, oocyst was observed in the mucosal area of ceca of infected chickens (picture B, arrowhead). Furthermore, normal structure of crypts at 10 dpi also was damaged compare with control sections.



A: Eimeria treated 5dpi; B: Eimeria treated 10 dpi; C: Control 5dpi; D Control 10 dpi

Arrow: Eimeria in second schizont stage; Arrowhead: Oocyst of Eimeria

Figure 3. Histopathological lesions in the ceca of chickens by HE staining

4 Discussion

The chickens infected with mixed *Eimeria* species showed the general symptoms of coccidiosis infection which have been reported such as ruffled feathers, anorexia, huddling together with loose dropping and/or bloody dropping from 4–7days post-infection (data not shown). In the north of Vietnam, there were 6 species of *Eimeria* have been reported including *Eimeria tenella*, *E.acervulina*, *E.brunetti, E. maxima, E. mitis* and *E. necatrix*. Among them, the prevalence of *E. tenella* species was highest, 96,55% (Hoan *et al.*, 2014). In the present experiment, our results also suggest that *E. tenella* is the dominant species in central of Vietnam because ceca were the most damaged part of intestine which was reported as the specific site of *E. tenella* development. The dominant prevalence of *Eimeira tenella* among *Eimeria* species was also reported in China, India, Saudi Arabia, Sudan (Lan *et al.*, 2017, Thenmozhi *et al.*, 2013, Al-Quraishy *et al.*, 2009, Sudan *et al.*, 2017).

The isolated *Eimeria* species from central Vietnam showed high virulence. The evidences consist of reducing feed intake, body weight and body weight gain in *Eimeria* treated chickens compare with those of control. In addition, the ceca of infected chicks were destructed due to

the proliferation and movement of *Eimeria* in endogenous stages (Emilio *et al.*, 2014). Consequence, the ceca contained a large amount of blood in cecal content at 5dpi. The tissue destruction also has been observed clearly in HE sections with massive infiltrated inflammatory cells (figure 3).

The farmers usually diagnose coccidiosis based on blood in the stool because it is difficult to test the presence of oocysts in the fecal samples in their farm facilitis. General, the farmers may consider that chickens were recovered when they stop excrete blood in the stool. However, the results in the present study showed that although there is no blood in the feces (from 8-14 dpi) but still a large amount of oocyst shedding (figure 1). Because, the parasites first invade in to host epithelium cells and undergo 3 times of schizont formation which contains about 900, 350, 16 merozoites respectively. The development and movement of the schizonts lasted for 5-6 days causing tissue destruction and the presence of blood in the feces. After that, the immature oocysts have been formed in sexual stages and be discharged to environment along with feces (Matsubayashi *et al.*, 2012). Therefore, periodic vaccination is neccesary for coccidiosis control in the farms. Besides, collecting and treating the feces of infected chickens in a correct time and manner greatly contributes to limiting the spread of this disease.

1 Conclusion

In this study, the effect of local isolated *Eimeria* species on chickens in Thua Thien Hue province have been identified. The development of *Eimeria* species greatly damage the intestinal system of infected chickens lead to bleeding, particularly at 5 days post infection. Therefore, the infected chickens reduced feed intake, growth performance and body weight chicks. After complete the development in host epithelial cells, the parasite was then discharged to the environment together with feces in oocyst form. Oocyst can be survied in the enviroment for long time and can be spread to healthy chickens through the food and water intake.The present study provide useful information related to the pathogenicity of *Eimeri*a species which may contribute for coccidiosis diagnosis and treatment in poultry production.

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