

# The Morphological Endogenous Development of EimeriatenellaWild Strain in Primary and Secondary Infection in Chickens

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## The Morphological Endogenous Development of *Eimeriatenella* Wild Strain in Primary and Secondary Infection in Chickens

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### Abstract

The endogenous development of *E. Tenella* wild strain was observed in primary and secondary infected chicken to know protective immunity development on host. Twenty broiler chicks at three weeks old were infected with  $5 \times 10^3$  oocyst of *E. tenella*. Morphological parasitic endogenous development was observed at 5 days pi, oocyst production calculated at 6 to 11 days pi for primary infection. Two weeks pi, the same procedure was performed for secondary infection. Total oocyst production at primary infection was higher than at secondary infection. Endogenous development disabilities of parasites occur as a result of protective immunity generated at the first antigen exposure.

**Key words:** endogenous development, *E. tenella*, protective immunity

One of pathogenic *Eimeria* species in chicken is *E. tenella* that causes cecal coccidiosis. The assessment of protective immunity to cecal coccidiosis particularly due to *E. tenella* wild strain can be assessed by knowing host response to antigen exposure. One of the evaluation of antigen exposure of cecal coccidiosis can be done by observation of histomorphology of cecum as site of endogenous development of parasite and the damage caused in initial as well as repeated infection.

### Materials and Methods

Oocysts of *E. tenella* wild strain were obtained from the non commercial chicken farm around Surabaya city. This strain was propagated in chicks; oocysts were preserved in 2.5%  $K_2Cr_2O_7$  solution to induce sporulation at 28°C in

incubator for 4-7 days.

A total of twenty pathogen-free male broiler chicks (CP 707) aged 3 weeks were infected orally with a suspension of  $5 \times 10^3$  oocysts *E. tenella* per chick. All chickens housed in clean individual cages and fed with a standard diet without coccidiostat and tap water ad libitum. At 5 days post infection (pi), five chickens were sacrificed to observe morphological endogenous development of parasites by histopathological changes occurred due to primary infection, while oocyst production calculation was done at 6 to 11 days post infection on other infected chickens. Two weeks pi, the same procedure was performed for secondary infection.

### Results and Discussion

The temporal pattern of oocyst output per day was initiated at day 6 to day 11 post infection. Oocyst first appeared on the six days pi, then reached peak on the nine days pi before numbers declined rapidly and the fewest oocysts were detected on 11 days pi. Basically, the same pattern of daily oocyst output was seen in both *E. tenella* primary and secondary infected chicken, but the *E. tenella* secondary infected chicken, oocyst output per day and/or as well as totally were significantly lower than *E. tenella* primary infection.

The total numbers of oocysts produced of *E. tenella* primary infected chicken in this study was  $[4.7 \pm 0.17] \times 10^6$  per chick and *E. tenella* secondary infection was  $[1.3 \pm 0.025] \times 10^6$  per chick. Total of oocyst production in *E. tenella* secondary infected chicken were significantly decreased ( $p < 0.01$ ) about 72 % compared with primary infected chicken and/or initial infected chicken.

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Endogenous development of *E. tenella* (schizogony and gametogony) in *E. tenella* secondary infected chicken was suppressed and/or incomplet. Several generations of schizont appeared degenerated consequently un-break schizont, damaged cecal mucosal epithelial cell has not occurred and automatically there were no bleeding in cecum (Fig. 1). Many abnormal endogenous developments of parasites such as gametogony results in disturbance of syngamy of microgamete and macrogamete. Thus, oocyst formation was not perfectly continued. In contrast, endogenous development of parasites in *E. tenella* primary infected chicken occurred well and no inhibition (Fig. 1). Well development of numerous in acellular schizonts containing merozoites and immature macrogametocytes in the border epithelial cells of cecum at 120 hours (Fig. 1). So, after 72 and 120 hours of *E. tenella* infectio in chicken, severe inflammatory process was observed in the lamina propria. The period of five days pi is the time when the second generation schizogony is in progress and most of the third trophozoites and immature schizonts were morphologically degenerated in the *E. tenella* secondary infected chicken, whereas in the primary infected chicken, the parasites were histologically normal as well as the multinucleated immature schizonts (Fig. 1). The term of immunity will be used to refer to host that have been previously infected with the coccidia and have subsequently recovered from the disease.

Infection with one species of *Eimeria* induces protective immunity in the host that is long lasting and exquisitely specific to that particular species (Tang *et al.*, 2018). While a large number of inoculating oocysts is generally required to generate an immune response against *Eimeria*, some exceptions have been noted, e.g. *E. maxima* is highly immunogenic and requires only a small number of oocysts to induce almost complete immunity. The early endogenous stages of the parasite life cycle are considered to be more immunogenic than the later sexual stages (Tang *et al.*, *loc. cit*) although Song *et al.*, (2015) and Ahmad *et al.*, (2016) showed that immunization with recombinant gamete associated antigen, induced partial protection against challenge infection. Immunity to *Eimeria* is stimulated by the initial developing stages of parasite, particularly the schizonts, and subsequently boosted and maintained by multiple

reexposure to oocysts in the litter. Thus, the recycling of infection following administration of live oocysts is critical for the development of protective immunity (Chapman *et al.*, 2005).

The inherent difference in reproductive potential is high for *E. tenella* and *E. acervulina*, and low for *E. maxima*. Immunity, which is specific to each coccidian species, results in decreased production of oocysts after ingestion of infective oocysts (Arabkhazaeli *et al.*, 2011). The histopathological analyses confirmed more extensive presence of lesions, observed with the light microscope, where more inflammatory cells occur in chickens infected with *E. tenella* than in other *Eimeria* species. This criterion was used previously by Debou-loukane *et al.*, (2018) who identified 5 *Eimeria* species: *E. acervulina*, *E. tenella*, *E. maxima*, *E. brunette* and *E. necatrix*; based on a lesion seen at

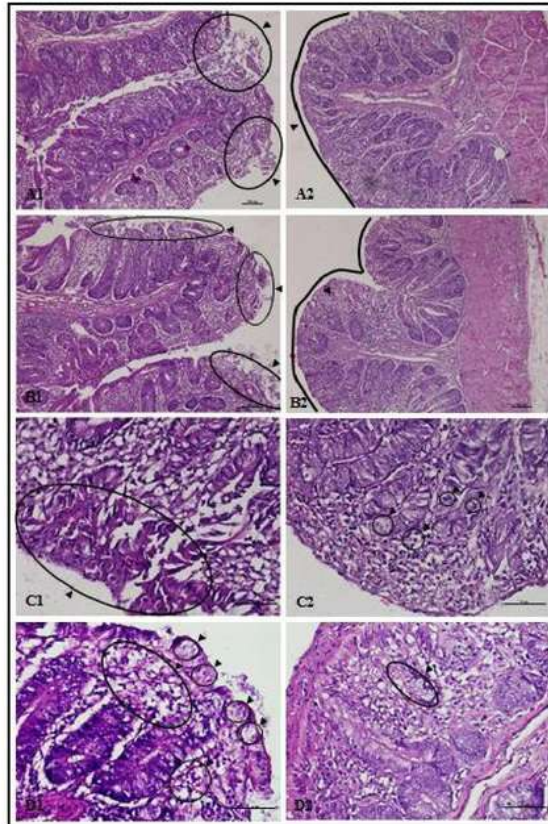


Fig 1. Morphological comparison of endogenous development stages of *E. tenella* between primary infection [A1, B1(x100); C1, D1(x400), H&E] and secondary infection [A2, B2(x100); C2, D2(x400), H&E].

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post mortem examinations of naturally infected birds, dimensions of oocyst and lesion seen in experimentally infected chicks with single oocyst. *E. tenella* showed considerable numbers of oocyst in lamina propria of caecum beside severe hemorrhage and complete desquamation of epithelium and edema of muscular tissue which agreed with the finding of You (2014).

It was discovered that daily inoculations of small numbers of oocysts over twenty days produced a stronger immunity than when a large number of oocysts was given in a single dose (Marugan-Hernandez *et al.*, 2016). This discovery was labeled as a trickle infection. The trickle infection suggests that for chickens *Eimeria* species protective immunity is developed only after the bird has been infected repeatedly by the parasite through cycling. It was generally found that the motile sporozoites played an important role in conferring an immune response while the gametogonic stages elicited little protective immunity (Blake and Tomley, 2014). Protective immunity is generally regarded as the prevention of oocyst production and absence of clinical signs in birds challenged by the parasites (Blake and Tomley, *loc. cit.*). Once protective immunity is developed for a certain *Eimeria* species (and sometimes strain) the bird is immune against further infection with this parasite (Shivaramaiah *et al.*, 2014). *Eimeria maxima* best demonstrates acquired immunity; a single infection initiated with only a few oocysts results in the development of almost complete (>99.99%) immunity (Blake *et al.*, 2017).

### Summary

The endogenous development of *E. Tenella* wild strain was observed at primary and secondary infected chicken to know protective immunity development on host. Total oocyst production at primary infection was higher than at secondary infection. Endogenous development disabilities of parasites in secondary infection occur as a result of protective immunity generated the first antigen exposure in primary infection.

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