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Research Note

Histopathological Studies on Adaptation of *Eimeria tenalla* (Local isolates) on Hen's Embryos through Chorioallantoic Membrane

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Abstract

In the histological sections of the chorioallantoic membrane (CAM), there were abundant small dark colored rounded bodies of gametes and in some cases cluster of small mature and immature shizoints in increasing numbers on day 4-6 post inoculation. Numerous merozoites were also observed in the allontic fluid on day 6-7 post inoculation. In the present studies, gametes like bodies numerous in number and distributed extensively in tissues of CAM were observed 120-134 hours of post-inoculation of sporozoites into chicken embryos. Shizonts and gametes recovered 5-6 days post inoculation of sporozoites of *E. tenalla* (local isolate) into the CAM.

Keywords: Histopathology, *Eimeria tenalla*, Chorioallantoic membrane

Introduction

Members of genus Eimeria are considered species and site specific (Long, 1965). Limited studies have been conducted on the growth of Eimeria species on other than normal hosts (Long, 1965, 1966, 1972; Shirley *et al.*, 1981; Nakai *et al.*, 1992). Sporozoites of Eimeria(E.) tenella (local isolate) have been adapted successfully on chorioallantoic membrane (Akhtar *et al.*, 2002). Present paper reports the histopathological studies on the adaptation of *E. tenella* (local isolate) on chicken embryos through chorioallantoic membrane.

Materials and Methods

Sporulated oocysts of *E. tenalla* (local isolate) maintained at Immunoparasitology Laboratory, Department of Veterinary Parasitology, University of Agriculture, Faisalabad, Pakistan were used in the present studies. Sporulated oocysts were subjected to ex-sporocystation to release sporozoites (Speer *et al.*, 1973). Their concentration was maintained at $1.8x10^3$ to $2x10^3$ per 0.1 mL in normal saline and were inoculated into 10-12 days chicken embryo (0.1mL each) through chorioallantoic membrane (CAM) along with penicillin (2000 IU) and streptomycin (0.05mg).

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Embryos were maintained at 37°C and 70% humidity for 5-6 days. Chorioallantoic fluid was harvested for further studies and chorioallantoic membrane having hemorrhages was collected and preserved in 70 % alcohol for histopathological studies (Bankraft and Steven, 1990). Permanent slides made were observed to record the observations under the low and high power magnifications of inverted microscope and photographs were taken.

Results and Discussion

In the present studies, *E. tenalla* (local isolate) adopted on the 10-12 days hen's embryos through CAM successfully and completed its life cycle following inoculation of sporozoites.

Sporozoites injected into chorioallantoic route, the parasites seen only in the CAM and not in the embryo itself. In the histological sections of the CAM, there were abundant small dark colored rounded bodies of gametes.In some cases, cluster of small mature and immature shizoints were seen in increasing numbers on day 4-6 post inoculation. Numerous merozoites were also observed in the allontoic fluid on day 6-7 post inoculation.

The results of gametes forming bodies of this study are in corrugated with the observations recorded by Long (1965, 1972). Adaptation of *E. tenalla* on CAM of Japanese quail's embryos have also been conducted successfully (Nakai *et al.*, 1992) but its development through histopathological technique have not been studied. In the present study, small dark colored rounded bodies might be the gametes penetrated deep into the tissues of CAM for development and later they may form into zygote. Similar observations have also been recorded previously (Long, 1965). A few cluster of relatively larger bodies observed in the present findings might be the shizonts (Long, 1965 and Vetterling and Doran, 1966).

In the present studies, gametes like bodies numerous in number and distributed extensively in tissues of CAM were observed 120-134 hours of post-inoculation of sporozoites into chicken embryos while in the natural host (chicken) the gametes were observed 84-89 hours post infection (Vetterling and Doran, 1966).

Shizonts and gametes recovered 5-6 days post inoculation of sporozoites of *E. tenalla* (local isolate)

into the CAM in contrary to observations recorded in previous studies where shizonts and gametes recovered 7-9 days and 7-11 day post inoculation of sporozoites into CAM (Long, 1965), respectively. This variation may be due to strain difference as local isolate of *E. tenalla* was used.

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