ADAPTATION OF *EIMERIA TENELLA* (LOCAL ISOLATE) SPOROZOITES IN CHICKEN EMBRYOS

Masood Akhtar, M.M. Ayaz, C.S. Hayat, M. Ashfaq[1] and I. Hussain[1]
Department of Veterinary Parasitology and [1]Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Eimeria (E.) tenella (local isolate) oocysts were recovered from the cases of chicken coccidiosis. Oocysts were sporulated in 2.5 per cent potassium dichromate solution at 37°C with 60 per cent humidity. Excystation of the sporulated oocysts was done with sodium hypochlorite. Sporozoites were released by treating with 1 per cent Trypsin (1:250) followed by sodium taurocholate at 41°C. The final concentrations of the sporozoites were maintained at 1.8×10^3–2.0×10^3 per 0.1 ml. Ten chicken embryos (12 days old) were inoculated each with 0.1 ml suspension of sporozoites into the chorio-allantoic membrane (CAM) along with penicillin and streptomycin. The embryos were maintained at 41°C with daily candling. Death of the embryo occurred on 5th day post inoculation in six out of ten embryos. Severe haemorrahages were seen on all dead embryos. Total number of oocysts harvested from the CAM were 6.1×10^4-7.2×10^4 per ml. The remaining four embryos died on the 7th day post-inoculation and had mild haemorrhages. Total number of oocysts harvested were 3.0×10^4-3.5×10^4.

Key words: *Eimeria tenella*, adaptation, chicken embryos

INTRODUCTION

Eimeria (E.) tenella is the most prevalent and pathogenic species causing coccidiosis in poultry. To control the coccidiosis several attempts have been made with variable results. The sonicated vaccine prepared using local isolates gave promising results as evaluated on the basis of cellular, humoral and challenge responses (Akhtar *et al.*, 1998, 2000, 2001; Ayaz, 1999).

It has been reported that sporopzoites of the *E. tenella* inoculated into the chorio-allantoic membrane (CAM) of chicken embryos completed its life cycle (Long, 1966; Shirley *et al.*, 1981). The present paper reports the adaptation of *E. tenella* (local isolate) on chicken embryos to obtain large scale antigenic material.

MATERIALS AND METHODS

Oocysts were recovered from the naturally infected chickens with coccidia and were subjected to sporulation in 2.5 per cent potassium dichromate solution at 37 °C and 60 per cent humidity (Akhtar *et al.*, 1998) and species were identified (Soulsby, 1982). Excystation of *E. tenella* sporulated oocysts was done with sodium hypochlorate at 41°C. The final concentrations of the sporozoites were maintained at 1.8×10^3 - 2×10^3 per 0.1 mL. Ten chicken embryos (12 days old) were inoculated with 0.1 mL suspension of sporozoites into the CAM along with penicillin (2000 IU) and streptomycin (0.05 mg). The embryos were incubated at 41°C for 5-7 days. Candling was performed daily to record the observations.

RESULTS AND DISCUSSION

A complete life cycle of *E. tenella* (local isolate) was established following inoculation of sporozoites via chorioallantoic membrane in chicken embryos (12 days old). The embryos died at different times post inoculation. The first type of deaths occurred two days after infection, there were no haemorrhages and was found to be not associated with parasitemia. This early mortality may be caused by toxic substances and is consistent with the findings of Burns (1959); Rikimaru *et al.* (1961) and Strout and Holman (1965), who have reported that *E. tenella* possesses substances toxic to rabbits. The second type of deaths occurred 5-7 days after inoculation in which considerable haemorrhages were observed on the CAM.

Six out of ten embryos showed death on the 5th day post-inoculation. Severe haemorrahages were seen in all the dead embryos. Total number of oocysts harvested from the CAM were 6.1×10^4-7.2×10^4 per ml. Embryos in remaining four eggs died on the 7th day post-inoculation and had mild haemorrhages. Total number of oocysts harvested were 3.0×10^4-3.5×10^4 per

ml. Total amount of fluid harvested from CAM was 1.5-2.0 mL per embryo.

Endogenous stages (macro- and microgametes) were numerous in epithelial cells of the CAM of embryonated eggs as compared to the number of oocysts recovered. Reasons for low rate of oocysts from macro-and microgametes are not known. It is possible that proportionally fewer microgametocytes develop in the CAM and consequently too few microgametes were available to fertilize the macrogametes (Shirley *et al.*, 1981) but it is very much clear that the merozoites differentiated into at least macrogametocytes which are the direct precursors of oocysts (Naciri-Bontemps. 1976). After 72 hours of incubation of recovered oocyst, 20-30 per cent of them sporulated.

It is concluded that sporozoites of *E. tenella* (local isolate) can be adapted on CAM of chicken embryos to complete its life cycle and to obtain antigenic material on large scale.

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