Reproductive Characteristic of a Precocious Line of E. Tenella Sporozoite as Material Bioactive in Embyionating Eggs and the Implications of the Findings Appraised

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Original Article

Reproductive Characteristic of a Precocious Line of *E.Tenella* Sporozoite as Material Bioactive in Embyionating Eggs and the Implications of the Findings Appraised

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Abstract

The reproductive characteristics of a precocious line of *E. tenella* sporozoite were investigated in chicken embryos and the implications of the findings appraised. While it was possible to reproduce *E. tenella* in chicken embryos, other species could not be propagated efficiently. The results of this experiments showed that the reproductive index of this line of *E. tenella* in chickens, the propagation in eggs is presently more efficient and yields are variable. There was considerable variation in the oocyst yields among experiments, although the yields within an experiment among individual embryos did not vary greatly. It should be possible to achieve higher oocyst yields and enhanced embryo-adaptation (leading to decreased embryonic mortality) through increased serial passage in eggs, thereby maintaining immunogenicity in the vaccinated host.

Keywords: *E. tenella* sporozoite, chicken embryo, reproductive character.

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Introduction

Eimeria tenella is the most pathogenic species, causing haemorrhagic typhlitis in chickens (McDougald and Reid, 1997). *Eimeria* species reproduce asexually (schizogony or merogony) and sexually (gametogony) in the intestinal cells to produce large numbers of progeny (oocysts), which are excreted in the faeces and subsequently undergo sporulation (sporogony) in the environment to become infective to susceptible chickens (McDougald and Reid, 1997). The ability to complete the life cycle of various species of *Eimeria* in embryonated chicken eggs has significant implications for investigating fundamental aspects of their biology and development, for establishing methods for the testing of anti-coccidial drugs and for the production of live vaccines against avian coccidiosis. Long (1965, 1966) first demonstrated that some species of *Eimeria* could complete their endogenous development in the chorio-allantoic membrane of the chicken embryo. Subsequently, Long (1972, 1973) also showed that the continuous passage of *E. tenella* in embryos resulted in an adaptation, associated with a reduction of pathogenicity, size of the schizonts (asexual stages) stage and a change in the site of development, compared with the original, parent line of the parasite. Serial passage of *E. tenella* in embryonating chicken eggs usually results in the development of a line of parasite which is significantly less pathogenic to chickens compared with wild-type strains while remaining immunogenic (Long *et al.*, 1982). For these reasons, an egg-adapted line of *E. tenella* (derived after some passages) has been included in a commercial vaccine (Livacox®) (Shirley and Bedrnik, 1997; Williams, 2002a,b). In the present study, the reproductive characteristics of this precocious line of *E. tenella* sporozoite were investigated in chicken embryos and the implications of the findings discussed.

Materials and Methods

A precocious line of E. tenella isolated from naturally infected chickens in chicken farm coccidiostat free was maintained in specific pathogen free (SPF) chickens. The parent line of E. tenella was originally identified to species based on the morphometry of sporulated oocysts, prepatent period and location of gross lesions in the caeca (McDougald and Reid, 1997). The biological parameters of the precocious line conformed to those (i.e. shortened prepatent period, smaller and fewer generations in schizogony, lower virulence and reproductive potentials) Oocysts produced in SPF chickens were allowed to sporulate in the caecal content under constant aeration at 30°C for a minimum of 48 h, isolated by flotation using saturated NaCl, washed extensively in water, pelleted by centrifugation (1000 rpm for 10 min) and resuspended and stored in phosphate-buffered saline (PBS) supplemented with benzyl-penicillin (200 IU) and dihydro-streptomycin sulphate (250 mg/ml) at a concentration of 104 oocysts per ml (estimated using a Neubauer haemocytometer; Gasser *et al.*, 1987). Approximately 105 oocysts were washed three times in PBS (by centrifugation) in a tube and then resuspended in 3.3 ml of PBS; a sub-aliquot (300 μl) of oocyst suspension was plated onto horse blood agar (Oxoid®) and incubated aerobically at 37°C for 24 h to examine whether any bacteria were capable of growing (after exposure to antibiotics). The same volume (3 ml) of glass beads (2 mm in diameter) was added to the tube which was vortexed for 2 minutes until >90% of the oocysts had ruptured to release sporocysts (verified by light microscopic examination at 100-times magnification). To release sporozoites from the sporocysts, the suspension (without glass beads) was transferred to a fresh conical 50 ml tube and incubated (under gentle inversion every 20 minutes) at 41°C for 60-90 minutes in 25 ml of PBS containing 1% w/v porcine trypsin and 2% w/v sodium taurocholate (Sigma). The tube was centrifuged, the supernatant aspirated and 50 ml of foetal calf serum (FCS) containing antibiotics (same concentration as used previously) added. The sporozoites were sedimented by centrifugation and re-suspended in 50% v/v FCS in PBS to achieve a final concentration of 5x104 per ml. This

suspension, pre-incubated at 41°C, was used for the inoculation of embryonating chicken eggs.

Fertile eggs from SPF chickens incubated to produce 10 days old embryos were used for the inoculation with different doses of sporozoites (Table 1). The eggs were candled, the air sac marked, and a site free from blood vessels selected for the inoculation. The injection site on the shell was disinfected with iodine, allowed to dry, and a small hole (2 mm; above the air sac) drilled into which a circular dentist drill prick-punch was inserted. The sporozoite suspension was then injected into the allantoic cavity using a sterile 25-guage needle. The hole was sealed with a non-toxic glue, and the eggs incubated at 37.8°C for 4 hours (rotating them every 30 minutes) and subsequently at 41°C for 7 days (rotating them two times daily). Within 24 hours of inoculation, the eggs were „candled" to verify the viability of the embryos, and daily thereafter.

After 168 hours, eggs were cooled to 4°C and the shell over the air sac was fractured and removed to the level of the air sac/allantoic cavity region using sterile forceps. The membrane separating the air sac from the embryo was peeled off. The embryo was removed with the yolk sac and albumen bolus, and the allantoic fluid discarded. The remaining chorio-allantoic membranes were excised and placed into a sterile container. The membranes were pooled, diced finely using scissors, placed into a 50 ml tube and centrifuged at 2000 rpm for 15 min. The membranes were washed in PBS, followed by two washes in PBS containing 2% Tergitol® (BDH Chemicals). They were then transferred to a fresh tube, digested for 60 minutes in 1% trypsin (in PBS) and centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded and the sediment resuspended in 1% v/v potassium dichromate (in 30-40 ml). The remaining tissue residues were removed by sieving (1 mm mesh size). Oocysts were allowed to sporulate (at 30°C under constant aeration) for ≥ 48 hours, after which sporulation was verified microscopically. The sporulated oocysts were sedimented, washed two times in 40 ml of water, incubated for 20 minutes with 3% v/v sodium hypochlorite, washed extensively in PBS containing antibiotics (at the same concentration as used previously) and stored at 4°C. The oocysts in the suspension were enumerated, and the sporulation established (see Table 1).

Results and Discussion

After preliminary experiments, in which it was shown that the precocious line of E. tenella could be propagated in chicken embryos, two independent experiments were conducted employing different inoculation doses. The results of these experiments are shown in Table 1. Using inoculation group of doses of 2.5x 103 and 5 x 103 sporozoites per egg, average of 14680 oocysts and 75650 oocysts were produced per egg, respectively. The oocyst yields equated to reproductive indices varying 46.976 and 121.04, respectively (Figure 1). The mortality of infected chicken embryos was 10% and 20%, respectively. There was no evidènce of bacterial growth before inoculation of eggs with sporozoites or during experimentation. Between 0-168 after inoculation, the mean chicken mortality was 10% and 20% in each doses of sporozoites, post mortem examination of dead embryos indicated there was trauma due to needle damage during inoculation and chorio-allantoic haemorrhage (the allantoic fluid being dark red), which was interpreted to represent damage due to the asexual replication (shizogony) of the parasite (results not shown). Eggs were rotated after inoculation, with the rationale of achieving an even distribution of the sporozoite inoculum and minimizing the high levels of embryo mortality recorded in preliminary studies when eggs were not rotated. Mortality in embryos in eggs not rotated showed extensive haemorrhage in the chorio-allantoic membranes. Hence, current evidence suggests that egg rotation achieves improved reproduction in the chorio-allantoic membranes. Further experiments should compare the effects of different types/speeds of egg rotation on embryo mortality and on the reproductive index for E. tenella

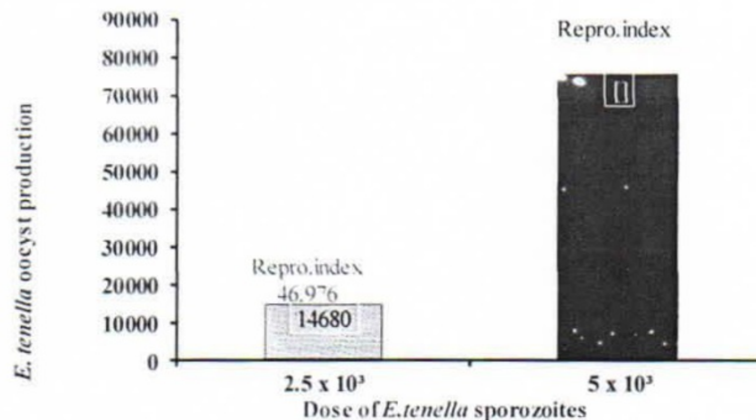

Fig. 1: Inoculation group of doses of 2.5 x 10^3 and 5 x 10^3 sporozoites per egg, average of 14680 oocysts and 75650 oocysts were produced per egg, respectively. The oocyst yields equated to reproductive indices varying 46.976 and 121.04, respectively.

Eimeria tenella is presently being used as an effective vaccine at a dose rate of 150 oocysts per chicken (Gasser, 2007). With the mean inoculation dose of 2.5 x 103 sporozoites, one chicken egg can produced about 98 doses of vaccine. This equates to 10 eggs per 980 vaccine doses. Moreover, with the mean inoculation dose of 5 x 103 sporozoites, one chicken egg can produced about 504 doses of vaccine.

However, compared with the reproductive index of this line of E. tenella in chickens, the propagation in eggs is presently more efficient and yields are variable. In the present study, there was considerable variation in the oocyst yields among experiments, although the yields within an experiment among individual embryos did not vary greatly. Nonetheless, it should be possible to achieve higher oocyst yields and enhanced embryo-adaptation (leading to decreased embryonic mortality) through increased serial passage in eggs, thereby maintaining immunogenicity in the vaccinated host. For instance (using an inoculation dose of 1 x 104 sporozoites per egg), Gasser (2007) reported embryonic mortalities of 3.7% and 6.5% during passages one, 2600 and 5100 sporozoites, respectively. The corresponding reproductive indices were 60 and 141 from respective passages. It is likely that the lower reproductive indices achieved in the present study compared with that of Gasser (2007) relate to an insufficient adaptation to the growth in chicken embryo and precocious nature of E. tenella line. Further experimentation is required to verify whether the pathogenicity or/and immunogenicity of *E.tenella* in chickens change as it adapts to chicken embryos over a large number of passages and to establish its reproductive characteristics in embryos and, subsequently, in chickens. Since the serial passage of E. tenella in embryonating chicken eggs commonly results in the emergence of a line significantly less pathogenic in chickens compared with „field strains" but immunogenic (Long *et al.*, 1982) an embryo-adapted line (after more than 100 passages) has been incorporated into the Livacox® vaccine. Eimeria tenella has the potential to cause moderate caecal lesions in young chickens. Thus, a reduction in its pathogenicity and increase in its reproductive index during chicken embryo-adaptation are likely to enhance its suitability as a vaccine constituent, but these aspects require testing.

The propagation of E. tenella in embryonated chicken eggs may also have implications for fundamental investigations of the developmental biology of this parasite, the parasite-host

relationship and, importantly, for investigating the molecular biology of reproduction and development. This "closed" experimental system (i.e. the chicken embryo) could provide opportunities for functional genomic studies of *E.tenella*, having advantages over the use of chickens. This is particularly pertinent given the Eimeria genome projects presently underway (Shirley *et al.*, 2004). Some improvements need to be made to the propagation approach for the production of E. tenella in chicken embryo because it has the potential to lead to the development of a functional genomic tool. Also, future work should to focus on the in ovo propagation of other species of Eimeria.

Table 1: Propagation of oocysts in embryonating chicken eggs inoculated with different doses of sporozoites of *Eimeria tenella*. Chicken embryo mortalities, oocyst yields and reproductive indices are tabled.

Sporozoites inoculated per egg	Number egg used	Embryo mortality (0-168 hours)	Means of number oocyst per egg	Reproductive Index[a]
$2,5 \times 10^3$	10	1 (10%)	14.680	46.976
5×10^3	10	2 (20%)	75.650	121.04

[a] Reproductive index = (number of oocysts produced multiplied by eight sporozoites each) divided by the total number of sporozoites inoculated.

References

Gasser RB, Eckert J and Rohrer L (1987). Infectivity of Swiss *Giardia* isolates to jirds and mice, and in vitro cultivation of trophozoites originating from sheep. Parasitol. Res., 74: 103-111.

Gasser RB (2007). *Eimeria* vaccines and diagnostics. the Australian Poultry CRC.

McDougald LR and Reid WM (1997). In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM (Eds.), Diseases of Poultry, 10th Edition, Iowa State University Press, Ames, Iowa, pp. 865-883.

Long PL (1965). Development of *Eimeria tenella* in avian embryos. Nat., 208: 509-510.

Long PL (1966). Growth of some species of *Eimeria* in avian embryos. Parasitol., 56: 575-581.

Long PL (1972). *Eimeria tenella*: reproduction, pathogenicity and immunogenicity of a strain maintained in chick embryos by serial passage. J. Comp. Pathol., 82: 429-437.

Long, PL (1973). The growth of avian coccidia in the developing chick embryo and in cultured cells. Parasitol., 66: 55-62.

Long PL, Millard BJ, Batty AF and Davison C (1982). Immunisation against coccidiosis in chickens: tests under simulated field conditions. Avian Pathol., 11(1): 131-144.

Shirley MW and Bedrnik P (1997). Live attenuated vaccines against avian coccidiosis: Success with precocious and egg-adapted lines of Eimeria. Parasitol. Today 13: 481-484.

Shirley MW, Ivens A, Grube RA, Madeira AM, Wan KL, Dear PH and Tomley FM (2004). The Eimeria genome projects: a sequence of events. Trends Parasitol., 20: 199-201.

Williams RB (2002a). Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol., 31: 317-353.

Williams RB (2002b). Fifty years of anticoccidial vaccines for poultry (1952-2002). Avian Dis., 46(4): 775-802.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5