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Research Note

Antigenicity of Second Generation Merozoites of *Eimeria tenella*

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Eimeria tenella of *Eimeriidae* is the common and the most pathogenic species of 9 species of chicken coccidia (Fernando, 1982). It infects the ceca and causes hemorrhagic enteritis (Fernando, 1982; Long *et al.*, 1963; McDonald *et al.*, 1988) and even death in young birds (Pellerdy, 1974).

Eimeria tenella has a complex life cycle and each stage of the life cycle induces various extent of the immune response in the chicken (Fernando, 1982; Long *et al.*, 1963; McDonald *et al.*, 1988). The degree of the immunological response depends upon the dose of the primary infection as well as the age of the birds (Fernando, 1982; Long *et al.*, 1963; McDonald *et al.*, 1988; Pellerdy, 1974). Second generation merozoites of *E. tenella,* one of the asexual stage, is considered to be more immunogenic than the other developmental stages such as sporozoites and sexual stages (Long *et al.*, 1963; McDonald *et al.*, 1988; Rose and Hesketh, 1976; Whitmire *et al.*, 1989). In the present study, the antigenicity of second generation merozoites and sporulated oocysts of *E. tenella* was studied by using immunoblotting technique.

Isolation and purification of second generation merozoites was carried out by the methods of Davis (1973); Fernando *et al.* (1984) and McDonald *et al.* (1988) with slight modification. Briefly, each of fifteen commercial broiler chickens (Arbor-Acres) at 14 days of age were inoculated orally with 1×10^5 sporulated oocysts of *E. tenella.* The line of the parasite was originated in National Institute of Animal Health, Japan. Chickens were sacrificed 94–96 hr after infection. Second generation merozoites were harvested from the caecal mucosa and purified by centrifugation in a 70% Percoll solution gradient (Pharmacia). Purified merozoites were sonicated with glass beads and stored at −20°C until use. After sporulation of fresh oocysts by incubation at 29°C for three days, oocyst antigen was prepared as described by Rose (1977) and Reduker and Speer (1986) with slight modification. The soluble merozoite antigen and oocyst antigen were dissolved in sample buffer containing 2% sodium dodecyl sulfate (SDS), 6.25 mM Tris, pH 6.8 and 10% glycerin, with or without 2-mercaptoethanol at ratio 2.4×10^6 merozoites/20 μl and 1.2×10^6 oocysts/20 μl sample buffer. After boiling for 3 min, samples were electrophoresed in 0.1% SDS discontinuous 12.5% polyacrylamide gel as described by Laemmli (1970).

The immunoblotting procedure was performed by the techniques of Towbin *et al.* (1979). After electrophoresis, the gel was applied to a sheet of nitrocellulose membrane (Trans-Blot Transfer medium, Bio-Rad, Richmond, CA) and electrotransferred for 45 min, 20 volt by transfer apparatus (Trans-Blot Cell, Bio-Rad, Richmond, CA) as described by Bjerrum and Heegaard (1988) with slight modification. Following transfer, strips were then exposed with antisera diluted 1:30 in TBS containing 1% gelatin for 2

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hr at room temperature, and then rinsed with distilled water. The nitrocellulose membrane was washed twice with TBS containing 0.05% Tween 20 for 10 min each and binding of serum immunoglobulin was detected by incubating the strips with horseradish peroxidase (HRP)-conjugated rabbit anti-chicken IgG (H + L chains, Cappel) as described previously (Wisher, 1986). After rinsing with distilled water, the strips were rinsed again with TBS containing 0.05% Tween 20 twice for 10 min each. Finally, the strips were developed with TBS containing 0.072% HRP color development reagent (4-chloro-1-naphthol, Bio-Rad, Richmond, CA), 16.6% methanol and 0.015% hydrogen peroxide. The reaction was stopped by soaking with distilled water twice for 10 min each.

For the preparation of hyperimmune sera, groups of 5 single comb white leghorn chickens which were kept in specific-pathogen free (SPF) condition were used. They were orally inoculated with 5×10^4 sporulated oocysts at 14 and 28 days of age. Eleven days after the last inoculation, sera were collected from infected chickens and heated at 56°C for 30 min for inactivation of the complement. The sera were pooled in group (equal volume from each chicken) for immunoblotting and stored at −20°C until use. At the first infection, all chickens exhibited blood in the feces on the fourth day of infection; however, no clinical signs were observed at the second infection.

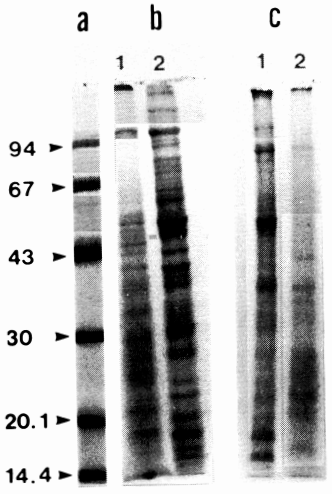

Fig. 1 Polypeptide profile of second generation merozoites and sporulated oocysts of *Eimeria tenella*. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were performed under reducing (b) or non-reducing conditions (c), and gels were stained by Coomassie brilliant blue. Lane a, molecular weight standard; lane b1, sporulated oocysts; lane b2, second generation merozoites; lane c1, second generation merozoites; lane c2, sporulated oocysts.

In the SDS-PAGE analyses under reducing conditions, as shown in Fig. 1b (lanes 1 and 2), merozoites showed larger number of polypeptide bands than oocysts. Of band stained intensely, 8 band (95, 56, 52, 49, 44, 31, 27 and 17 kD) were observed in both merozoites and oocysts. Two band (65 and 40 kD) were detected only from merozoites and three (51, 34 and 19 kD) were only from oocysts.

In the SDS-PAGE under non reducing conditions, as shown in Fig. 1C (lanes 1 and 2), fewer bands were observed than the SDS-PAGE under reducing conditions. Five (88, 61, 41, 20 and 15 kD) of intensely stained bands were common. Two bands (57 and 17 kD) were observed only in merozoites and three (48, 24 and 22 kD) were only in oocysts.

Since non-reducing conditions were considered to be better to preserve antigenicity than reducing conditions in SDS-PAGE as described by Raff *et al.* (1991), antigenicity of the parasite were investigated by western blotting methods using SDS-PAGE under non-reducing conditions. Merozoites showed seven bands of antigens (95, 66, 29, 28, 25, 23 and 14 kD) (Fig. 2, lane B) while oocysts showed five (95, 25, 23, 21 and 14 kD) (Fig. 2, lane C). Of these bands, four bands were common (95, 25, 23 and 14 kD) and three were specific for merozoites (66, 29 and 28 kD) and one was specific for oocysts (21 kD). These results indicate that there are common antigens between merozoites and oocysts or between merozoites and sporozoites; moreover, suggest the existence of specific antigens for merozoites and oocysts, which correspond with McDonald *et al.* (1988), Danforth and McAndrew (1987). The former showed common antigens between second generation merozoites and sporozoites of

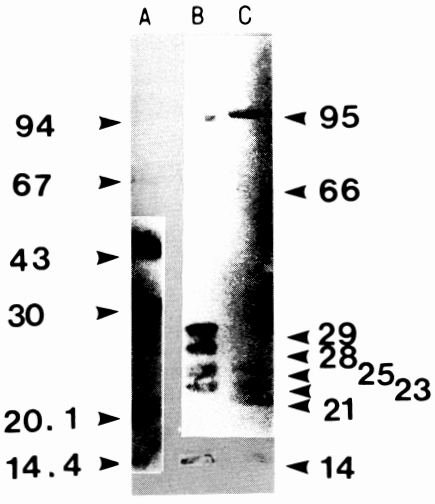

Fig. 2 Immunoblot profile of second generation merozoites (lane B) and sporulated oocysts (lane C) of *Eimeria tenella*. After SDS-PAGE under non-reducing conditions, blotted polypeptides were probed by hyperimmune chicken serum. Molecular weight standards (lane A) were stained with Amido Black 10B.

E. tenella and specific antigens of these stages by the western blotting methods, the latter produced two monoclonal antibodies reactive against both merozoites and sporozoites of *E. tenella* and a monoclonal antibody reactive against not merozoites but sporozoites. Therefore, these data indicate that *E. tenella* has stage specific and stage-common antigens. The difference results obtained between we and McDonald *et al.* (1988) may be caused by different strain of *E. tenella* used.

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