Detection of Serum Antibody against Bovine Coccidiosis by Using *Eimeria tenella* Antigen

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ABSTRACT. Antigens of *Eimeria tenella* sporulated oocysts were applied to detect serum antibody of calf infected with *Eimeria*. Polypeptides of *E. tenella* oocyst were strongly proved with the infected bovine serum in immunoblotting. The infected serum also showed higher absorbances than that of normal serum in enzyme linked immunosorbent assay (ELISA) using *E. tenella* oocyst antigen. Suitable conditions for the ELISA were obtained by using soluble antigen of *E. tenella* oocysts at 2.5 µg/ml and bovine serum at 200 fold dilution.—**KEY WORDS:** bovine coccidiosis, *Eimeria tenella* antigen, ELISA.

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Bovine coccidiosis is an important parasitic disease throughout the world, which results in considerable economic loss in beef and dairy industries. To know prevalence of coccidiosis in the field is important for disease control and economic management.

Bovine coccidiosis has been mainly diagnosed by the fecal examination. However, this method has disadvantage in the sensitivity and handling of large number of samples. For understanding of the state of prevalence of bovine coccidia, it is necessary to develop easy and sensitive diagnostic method. ELISA is one of the most sensitive and promising method for processing large number of samples. Although this method has been successfully and widely used for detecting serum antibody in the chicken coccidiosis [1, 8–10], only a few works were performed on bovine coccidiosis since the difficulty to obtain large amount of bovine coccidial antigen. Saatara Oz *et al.* [11] attempted to detect bovine serum antibody by ELISA using *Eimeria tenella* antigen. They successfully showed shift of the titers as the course of infection; however, details of ELISA condition were not shown.

In the present study, we attempted to demonstrate cross reactivity of *E. tenella* antigen to bovine eimerian species and determine the condition of ELISA with *E. tenella* antigen as the serodiagnostic method for bovine coccidiosis. To allow for the practical use, we used the calf serum infected with *E. bovis* and *E. zuernii*. These two were the most important species among bovine *Eimeria* and the mixed infection of these species has often seen in the field.

Oocysts of *E. bovis* were freshly isolated from a calf in Kawatabi farm of Tohoku University and *E. zuernii* oocysts were obtained from Daiichi pharmaceutical & Co., Ltd. A female Holstein calf with no experience of coccidial infection was reared in the clean boxstall. The calf was orally inoculated with sporulated oocysts of 2×10^2 of *E. bovis* at 47 days of age and 3×10^5 of *E. zuernii* at 75 days of age. Immune serum was collected from the calf at 26 days after the last inoculation. Normal serum was obtained from 9-month-old Holstein calf kept under coccidia free condition at National Institute of Animal Health (NIAH), Japan. Immune and normal sera were stored at -20°C after inactivation. Soluble antigen was prepared from sporulated oocysts of *E. tenella* NIAH strain. Oocysts purified by the methods of Wagenbach [14] were sonicated in the sonication buffer (0.1 M Tris-HCl,

pH 6.8 containing 1 mM phenyl methane sulfonyl fluoride and 200 KU/ml aprotinin). The homogenate was mixed with the same volume of extraction buffer (sonication buffer added with 3% nonidet P-40 and 10 mM ethylene diamine tetraacetic acid) and extracted by standing at 4°C for overnight. The supernatant was collected as the soluble antigen and stored at -80°C until use.

Soluble antigen was electrophoretically separated by the method of Laemmli [4] and transferred to nitrocellulose membrane by the method of Towbin *et al.* [12]. Transferred polypeptides were reacted with immune or normal bovine sera followed by horseradish peroxidase conjugated rabbit anti-bovine IgG (Organon Teknika N. V. Cappel Products). The reaction was detected by development of 3,3'-diaminobenzidine tetrahydrochloride. ELISA was performed according to the methods of Voller *et al.* [13] with slight modifications. Wells of 96-well microtiter plate were coated with soluble antigen and reacted with immune or normal bovine sera followed by horseradish peroxidase conjugated rabbit anti-bovine IgG (Organon Teknika N. V. Cappel Products). The reaction was developed with 2,2'-azino-di[3-ethyl-benzthiazoline sulfate(6)] and the absorbances at 405 nm were measured.

Immunoblotting showed the reactivity of *E. tenella* oocyst antigen with *Eimeria* infected bovine serum (Fig. 1). Numerous numbers of polypeptide bands were indicated. Strong reaction was observed in 31, 33, 34–35, 39, 41–43, 59, 74 kDa bands. In contrast, normal calf serum did not indicate any polypeptide bands. From this result, it is clear that there are some *E. tenella* antigens possessing the epitopes similar to those of bovine *Eimeria* antigen. We consider that bovine serum antibody to bovine coccidia can be detected using *E. tenella* antigen.

In ELISA, immune serum showed higher absorbances than that of normal serum. Absorbances of calf serum to serial diluted *E. tenella* antigen were shown in Fig. 2. The values of immune serum rose as increase of antigen concentration. Those were on the plateau over 2.5 µg/ml of antigen. In the case of normal serum, the values were low and constant at every antigen concentration. Absorbances at serial serum dilution were also investigated (Fig. 3). The values of immune serum markedly decreased in higher serum dilution, especially over 200 fold. Normal serum showed lower level of absorbances at every dilution. From these results, suitable condition of the ELISA for detection of bovine serum antibody as follow-

Fig. 1. Immunoblot profiles of *E. tenella* sporulated oocyst antigen proved with calf serum. Lane 1: Molecular weight standard stained with amido black 10B. Lane 2: Normal calf serum. Lane 3: Immune calf serum with *E. bovis* and *E. zuernii*.

Fig. 2. Reactivity of calf serum to serial concentration of *E. tenella* sporulated oocyst antigen. ○: 200-fold diluted immune serum. □: 400-fold diluted immune serum. ●: 200-fold diluted normal serum. ■: 400-fold diluted normal serum.

Fig. 3. Reactivity of serial diluted calf serum to *E. tenella* sporulated oocyst antigen. ○: Immune serum to antigen concentration at 2.5 $\mu\text{g/ml}$. □: Immune serum to antigen concentration at 1.25 $\mu\text{g/ml}$. ●: Normal serum to antigen concentration at 2.5 $\mu\text{g/ml}$. ■: Normal serum to antigen concentration at 1.25 $\mu\text{g/ml}$.

ing; 2.5 $\mu\text{g/ml}$ of soluble antigen from *E. tenella* sporulated oocyst and 200-fold dilution of the serum.

As mentioned above, field screening of bovine coccidiosis has been performed only by the fecal examination [2, 3, 5, 6]. However, this method requires much of time, labor and experience to process large number of samples. In this study, ELISA using *E. tenella* antigen could detect the bovine serum antibody against coccidia. Onaga *et al.* [7] showed that ELISA using *E. tenella* antigen can be applied for field survey of chicken coccidial infection. Although there is disadvantage in identification of eimerian species and it is necessary to investigate much more samples, the present ELISA also has the possibility to detect bovine coccidial infection in the field at large scale. Furthermore, combination with the fecal examination may provide more detailed information of the prevalence. In addition, we are also considering that ELISA in this study may be useful for monitoring of the immune state in bovine immune experiment.

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