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Detection of Antibodies to *Babesia equi* by The Latex Agglutination Test with Recombinant Merozoite Antigen-2

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ABSTRACT

Babesia equi merozoite antigen-2 (EMA-2) expressed by recombinant baculovirus in insect cells was coupled latex beads, and these complexes were used to develop the latex agglutination test for detection of antibodies to *B. equi*. Serum samples from horses experimentally infected with either *B. equi* or *Babesia caballi* and from normal horses were assayed by the test. When the latex agglutination test compared with the enzyme-linked immunosorbent assay, the results of both tests agreed completely, suggesting that the latex agglutination test is suitable for a routine test.

INTRODUCTION

Babesia equi, a member of the phylum Apicomplexa, is a tick-transmitted protozoan parasite of horses (Schein 1988). This parasite causes destruction of red blood cells following invasion, and then induces fever, anaemia and icterus in horses (Knowles 1988). Complete prevention of *B. equi* infection by drug therapy or vaccination is not currently possible (Brüning 1996). The parasite is usually observed during the acute phase of infection in blood smears stained by Giemsa. However, horses that survive the primary infection become lifelong carriers of *B. equi* (Holbrook 1969), and it is much more difficult to demonstrate the presence of parasites in these carrier animals. To date the complement fixation test and indirect fluorescent antibody test (IFAT) are commonly used for detection of *B. equi* infection under considerable cross-reaction and sensitivity. Therefore, there

is a need to develop a more sensitive diagnostic method for detection of carrier animals.

Kappmeyer et al. (1993) characterized a *B. equi* gene encoding a merozoite surface 34 kDa protein termed merozoite antigen-1 (EMA-1). Recently, Knowles et al. (1997) reported that a EMA-2 gene encodes a surface 30 kDa protein. Previously, we expressed recombinant EMA-2 in insect cells by recombinant baculovirus, and developed an enzyme-linked immunosorbent assay (ELISA) for diagnosis of equine piroplasmosis (Tanaka et al. 1999). ELISA offers advantage of high sensitivity but the disadvantage of requiring several hours to complete the test and instruments which are not available in some laboratories. A latex agglutination test could offer the advantages of relatively high sensitivity, specificity, speed and simplicity in situations in which the time and technology required for ELISA were not available or appropriate. The test, unlike ELISA, is direct assay for detection of antibodies, and is based upon cross-linking antigen, which is attached to latex beads with antibodies to form visible aggregates. In this study, we developed the latex agglutination test with recombinant EMA-2 for detection of antibodies to *B. equi* in horses.

MATERIALS AND METHODS

Production and purification of the recombinant EMA-2 have been described previously (Tanaka et al. 1999). Briefly, the EMA-2 gene was cloned and expressed recombinant baculovirus in insect cells. *Spodopera frugiperda* (Sf9) cells infected with AcEMA-2 at a multiplicity of 10 plaque-forming unit/cell in the protein-free Sf-900 medium (GIBCO BRL, USA) for 4 days were harvested and washed twice with phosphated-buffer saline (PBS). The supernatants were filtered through 0.22 μm filter (Millipore Corp., Japan), and then centrifuged at 100,000 g for 2 hrs to remove baculoviruses. The supernatants were dialysed against PBS.

Purified EMA-2 was then covalently coupled to 0.9 mm carboxylated latex beads (Sigma, USA) according to the methods of Mazumder et al. (1988). Carboxylated latex beads were brought to 1 % (vol/vol) and activated overnight at 4°C in PBS containing 2 mg of 1-ehyl-3-(3dimethylamino-propyl) carbodiimide hydrochloride per ml. The activated latex beads were centrifuged at 12,500 g for 5 min and washed once with PBS. The activated latex beads were suspended to 1% (vol/vol) in PBS containing 1 μg EMA-2 per ml. The mixture was allowed to react for 2 hrs at room temperature on a shaker (NR 30 TAITEC, Japan). After centrifugation at 12,500 g for 5 min, the EMA-2 coupled latex beads were suspended to 1% in PBS containing 1% bovine serum albumin (BSA) to block unreacted binding sites on the latex. The EMA-2 latex beads were stored at 4°C

until use.

Serum samples from horses experimentally infected with either *B. equi* or *B. caballi* and negative serum samples were obtained from the Equine Research Institute, The Japan Racing Association, Tochigi, Japan and Onderstepoort Veterinary Institute, Onderstepoort, South Africa. Nine positive sera to *B. equi*, five positive sera to *B. caballi* and eight negative sera were used as serum samples in the latex agglutination test.

For latex agglutination procedure, serum or plasma samples were diluted 1:4 with PBS containing 1% BSA. The diluted serum sample (10 μl) was mixed on a glass slide with an equal volume of the EMA-2 coupled latex beads. The slide was rotated by hand for 5 min, and the agglutination was determined visually on the white paper.

RESULTS AND DISCUSSION

To test specificity and sensitivity of the latex agglutination test, we assayed positive sera to *B. equi* or *B. caballi* and negative sera by the test and compared the results with those determined by ELISA (Tanaka et al. 1999). As a result, the latex agglutination test exhibits 100% specificity (Table 1). All nine positive samples showed agglutination. While all eight negative control samples and all five positive serum samples to *B. caballi* showed no agglutination. These data agreed well with the results of ELISA.

Although several serological tests have been developed for detection of antibodies against *Babesia* infections (Tenter and Friedhoff 1986; Weiland and Reiter 1988; El-Ghaysh et al. 1996), the contamination by red blood cell components in antigen preparations has prevented the development of a specific and sensitive test. Knowles et al. (1992) developed a competitive inhibition ELISA for horse babesiosis with the recombinant EMA-1 expressed by *E. coli*. In the present study, the latex agglutination test with EMA-2 of *B. equi* expressed by baculovirus showed a clear difference between negative and positive serum samples from experimentally infected horses. These latex beads did not show any cross reaction with *B. caballi*-infected horse sera. These results suggest its potential usefulness for a more sensitive and specific identification of *B. equi* infection Thus, the latex agglutination test provides an excellent format for routine serological screening because of its high specificity and low cost and because it is not subject to false-positive results with samples exhibiting nonspecific polar staining often seen by IFAT. On the other hand, the test is not panacea for *B. equi* antibody testing, because it cannot distinguish immunoglobulin classes like IgG or IgM response. Nevertheless, the latex agglutination test is suitable for a routine test.

Table 1 The results of the latex agglutination test compared with ELISA using serum samples from horses experimentally infected with *B. equi*, *B. caballi* and negative horse sera.

Sample No.	Specificity	Latex agglutination[a] (1:4)[b]	ELISA (OD415 nm) (1:100)[b]
1	*B. equi*	+	0.367
2	*B. equi*	+	0.542
3	*B. equi*	+	0.674
4	*B. equi*	+	0.398
5	*B. equi*	+	0.360
6	*B. equi*	+	0.316
7	*B. equi*	+	0.841
8	*B. equi*	+	0.313
9	*B. equi*	+	0.316
10	Negative	-	0.015
11	Negative	-	0.005
12	Negative	-	0.001
13	Negative	-	0.015
14	Negative	-	0.012
15	Negative	-	0.037
16	Negative	-	0.095
17	Negative	-	0.026
18	*B. caballi*	-	0.017
19	*B. caballi*	-	0.017
20	*B. caballi*	-	0.006
21	*B. caballi*	-	0.002
22	*B. caballi*	-	0.014

[a] No latex agglutination: -, Latex agglutination: +

[b] The ratio of serum dilution.

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REFERENCES

Brüning, A. 1996. Equine piroplasmosis: an update on diagnosis, treatment and prevention. *Br. Vet. J. 152:* 139-151.

El-Ghaysh, A., Sundquist, B., Christensson, D. A., Hilali, M. & Nassar, A. M. 1996. Observations on the use of ELISA for detection of *Babesia bigemina* specific antibodies. *Vet. Parasitol. 62:* 51-61.

Holbrook, A. A. 1969. Biology of equine piroplasmosis. *Am. J. Vet. Med. Assoc. 155:* 453-454.

Kappmeyer, L. S., Perryman, L. E. & Knowles, D. P. Jr. 1993. A *Babesia equi* gene encodes a surface protein with homology to *Theileria* species. *Mol. Biochem. Parasitol. 62:* 121-124.

Knowles, R. C. 1988. Equine babesiosis: epidemiology, control and chemotherapy. *Equine Vet. Sci. 8:* 61-64.

Knowles, D. P. Jr., Kappmeyer, L. S., Stiller, D., Hannager, S. G. & Perryman, L. E. 1992. Antibody to a recombinant merozoite protein epitope identifies horses infected with *Babesia equi. J. Clin. Microbiol. 30:* 3122-3126.

Knowles, D. P. Jr., Kappmeyer, L. S. & Perryman, L. E. 1997. Genetic and biochemical analysis of erythrocyte-stage surface antigens belonging to a family of highly conserved proteins of *Babesia equi* and *Theileria* species. *Mol. Biochem. Parasitol. 90:* 69-79.

Mazumder, P., Chuang, H. Y., Wentz, M. W. & Wiedbrauk, D. L. 1988. Latex agglutination test for detection of antibodies to *Toxoplasma gondii. J. Clin. Microbiol. 26:* 2444-2446.

Schein, E. 1988. Equine babesiosis. *In:* Babesiosis of domestic animals and man. Ristic, M. ed., Boca Raton, CRC Press, pp. 197-208.

Tanaka, T., Xuan, X., Ikadai, I., Igarashi, I., Nagasawa, H., Fujisaki, K., Mikami, T. & Suzuki, N. 1999. Expression of *Babesia equi* merozoite antigen-2 by recombinant baculovirus and its use in the EMA-2 for enzyme-linked immunosorbent assay. *Int. J. Parasitol. 29:* 1803-1808.

Tenter, A. M. & Friedhoff, K. T. 1986. Serodiagnosis of experimental and natural *Babesia equi* and *B. caballi* infections. *Vet. Parasitol. 20:* 49-61.

Weiland, G. & Reiter, I. 1988. Methods for the measurement of the serological response to *Babesia. In:* Babesiosis of domestic animals and man. Ristic, M, ed., Boca Raton: CRC Press, pp. 143-162.