Detection of Antibodies to *Babesia equi* in Horses by a Latex Agglutination Test Using Recombinant EMA-1

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A latex agglutination test (LAT) using recombinant equi merozoite antigen 1 (EMA-1) for the detection of antibodies to *Babesia equi* was developed. The LAT was able to differentiate very clearly between sera from *B. equi*-infected horses and sera from *Babesia caballi*-infected horses or from normal horses. The LAT results were identical to those of a previously developed enzyme-linked immunosorbent assay. These results indicate that LAT using recombinant EMA-1 might be very useful as a routine screening method for the diagnosis of *B. equi* infection.

* Babesia equi* is a tick-borne hemoprotezoan parasite that causes equine piroplasmosis. The disease is characterized clinically by fever, anemia, and icterus. The disease is endemic in most tropical and subtropical areas of the world (8). Due to the almost worldwide distribution of the various tick vectors, the introduction of carriers into areas or countries where the disease is nonendemic must be prevented (8). Prior to importation to such areas or countries, horses must be shown to be negative for piroplasmosis through serological testing (3, 4). The complement fixation test and the indirect fluorescent antibody test (IFAT) are commonly used for detecting *B. equi* infection. However, these serological tests are generally restricted by antibody detection limits and cross-reactivity (3, 4, 9). Therefore, there is a great need for the development of a rapid, specific, sensitive, and inexpensive serological test.

An immunodominant surface protein, equi merozoite antigen 1 (EMA-1) of *B. equi*, is considered an important candidate for the development of an effective diagnostic reagent (5, 6). Previously, researchers expressed EMA-1 in insect cells by recombinant baculovirus and demonstrated that the enzyme-linked immunosorbent assay (ELISA) using highly purified recombinant EMA-1 as an antigen is useful for detecting *B. equi* infection (10). IFAT and ELISA were performed as described elsewhere (1, 2). IFAT and ELISA were performed as described elsewhere.

To evaluate whether LAT with recombinant EMA-1 can be used for the detection of antibodies to *B. equi* in horses, serum samples from horses experimentally infected with either *B. equi* or *B. caballi* and from normal horses were tested by LAT. Table 1 shows that all serum samples from 10 horses experimentally infected with *B. equi* were positive, whereas serum samples from five normal horses or from five horses experimentally infected with *B. caballi* were negative. In addition, the LAT results were compared with those of the previously developed IFAT (1) and ELISA (10). The LAT results were similar to those of ELISA and IFAT, except that two samples (sera 11 and 14) showed a false positive in IFAT.

Blood samples collected from 40 field horses in central Mongolia (the Ulan Bator region) were investigated by LAT and in vitro culture. As shown in Table 2, 36 (90%) and 12 (30%) samples were identified positively by LAT and in vitro culture, respectively. All 12 (30%) in vitro culture-positive samples were LAT positive. This result indicates that all carrier horses had detectable LAT antibodies. On the other hand, 24 (60%)
TABLE 1. Comparison of LAT with IFAT and ELISA for detection of antibodies to B. equi in horses experimentally infected with either B. equi or B. caballi and in normal horses

| Serum<sup>a</sup> | Specificity | Reciprocal of dilution ratio for: | \(\text{LAT}^b\) | IFAT<sup>c</sup> | ELISA<sup>d</sup> |
|------------------|-------------|----------------------------------|----------------|---------------|---------------|
| 1                | B. equi     | 64                               | 2,560          | 10,240        |               |
| 2                | B. equi     | 64                               | 1,280          | 10,240        |               |
| 3                | B. equi     | 64                               | 1,280          | 5,120         |               |
| 4                | B. equi     | 32                               | 320            | 640           |               |
| 5                | B. equi     | 16                               | 640            |               |               |
| 6                | B. equi     | 16                               | 640            |               |               |
| 7                | B. equi     | 16                               | 160            | 320           |               |
| 8                | B. equi     | 8                                | 320            | 160           |               |
| 9                | B. equi     | 4                                | 160            | 80            |               |
| 10               | B. equi     | 4                                | 80             |               |               |
| 11               | B. caballi  | <4                               | ≤80            | <80           | <80           |
| 12               | B. caballi  | <4                               | <80            | <80           | <80           |
| 13               | B. caballi  | <4                               | <80            | <80           | <80           |
| 14               | B. caballi  | <4                               | ≤80            | <80           | <80           |
| 15               | B. caballi  | <4                               | <80            | <80           | <80           |
| 16               | Normal      | <4                               | <80            | <80           | <80           |
| 17               | Normal      | <4                               | <80            | <80           | <80           |
| 18               | Normal      | <4                               | <80            | <80           | <80           |
| 19               | Normal      | <4                               | <80            | <80           | <80           |
| 20               | Normal      | <4                               | <80            | <80           | <80           |

<sup>a</sup> Serum samples were collected from horses experimentally infected with B. equi or B. caballi (1 to 24 months postinfection) and from normal horses.

<sup>b</sup> LAT was considered positive when agglutination was observed at dilutions of 1:4 and above.

<sup>c</sup> IFAT was considered positive when fluorescence was observed at dilutions of 1:80 and above.

<sup>d</sup> ELISA was considered positive when an optical density at 415 nm equal to or greater than 0.1 was observed at dilutions of 1:80 and above.

in vitro culture-negative samples were identified positively by LAT, indicating that some of the LAT-positive horses might have recovered from a previous B. equi infection.

The results with five independently produced lots of LAT antigen exhibited nearly perfect reproducibility and agreement in lot-to-lot testing (Table 3). In addition, the LAT antigen kept at 4°C was stable for at least 1 year (data not shown).

The results of the present study indicate that highly purified recombinant EMA-1 could be used as an antigen for LAT to provide a simple, rapid, sensitive, specific, and inexpensive alternative to IFAT or ELISA for the detection of antibodies to B. equi in horses. LAT might be very useful in situations in which the time, equipment, and technology required for IFAT or ELISA are not available or are inappropriate.

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TABLE 2. Comparison of LAT with in vitro culture method for detection of B. equi infection in field horses

| Result given by LAT | No. (%) of results given by culture | Total no. (%) of horses |
|---------------------|-------------------------------------|-------------------------|
| Positive            | 12 (30) 24 (60) 36 (90)             | Total no. of horses    |
| Negative            | 0 (0) 4 (10) 4 (10)                 | Total no. of horses    |

TABLE 3. Reproducibility of LAT antigens among different lots

| Serum | Specificity | Reciprocal of dilution ratio given by LAT for: |
|-------|-------------|---------------------------------------------|
| Lot 1 | Lot 2       | Lot 3 | Lot 4 | Lot 5 |
| 1     | B. equi     | 64    | 64    | 64    | 64    | 64    |
| 5     | B. equi     | 16    | 16    | 32    | 32    | 16    |
| 8     | B. equi     | 8     | 8     | 16    | 8     | 8     |
| 11    | B. caballi  | <4    | <4    | <4    | <4    | <4    |
| 16    | Normal      | <4    | <4    | <4    | <4    | <4    |

REFERENCES

1. Avarazed, A., D. T. de Waal, I. Igarashi, A. Saito, T. Oyamada, Y. Toyoda, and N. Suzuki. 1997. Prevalence of equine piroplasmosis in central Mongolia. Onderstepoort J. Vet. Res. 64:141–145.

2. Avarazed, A., I. Igarashi, D. T. de Waal, S. Kawai, Y. Oomori, N. Inoue, Y. Maki, Y. Omata, A. Saito, H. Nagasawa, Y. Toyoda, and N. Suzuki. 1998. Monoclonal antibody against Babesia equi: characterization and potential application of antigen for serodiagnosis. J. Clin. Microbiol. 36:1835–1839.

3. Bose, R., W. K. Jorgensen, R. J. Dalgliesh, K. T. Friedhoff, and A. J. de Vos. 1995. Current state and future trends in the diagnosis of babesiosis. Vet. Parasitol. 57:61–74.

4. Bruning, A. 1996. Equine piroplasmosis: an update on diagnosis, treatment and prevention. Br. Vet. J. 152:139–151.

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

6. Knowles, D. P., L. S. Kappmeyer, D. Stiller, S. G. Hennager, and L. E. Perryman. 1992. Antibody to a recombinant merozoite protein epitope identified horses infected with Babesia equi. J. Clin. Microbiol. 30:122–3126.

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

8. Schein, E. 1988. Equine babesiosis, p. 197–208. In M. Ristic (ed.), Babesiosis of domestic animals and man. CRC Press, Inc., Boca Raton, Fla.

9. Tenter, A. M., and K. T. Friedhoff. 1986. Serodiagnosis of experimental and natural Babesia equi and B. caballi infections. Vet. Parasitol. 28:49–61.

10. Xuan, X., A. Larsen, H. Badai, T. Tanaka, I. Igarashi, H. Nagasawa, K. Fujisaki, Y. Toyoda, N. Suzuki, and T. Mikami. 2001. Expression of Babesia equi merozoite antigen 1 in insect cells by recombinant baculovirus and evaluation of its diagnostic potential in an enzyme-linked immunosorbent assay. J. Clin. Microbiol. 39:705–709.