NOTE ***Parasitology***

Diagnosis of Equine Piroplasmosis in Brazil by Serodiagnostic Methods with Recombinant Antigens

Xuenan XUAN[1)], Akiko NAGAI[1)], Badgar BATTSETSEG[1)], Shinya FUKUMOTO[1)], Levi Hakwale MAKALA[1)], Noboru INOUE[1)], Ikuo IGARASHI[1)], Takeshi MIKAMI[2)] and Kozo FUJISAKI[1)]*

[1)]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555, and [2)]College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252–8510, Japan

(Received 8 May 2001/Accepted 28 June 2001)

ABSTRACT. Serum samples from horses in the States of Sao Paulo and Mato Grosso do Sul, Brazil were examined for diagnosis of equine piroplasmosis by both the latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) with recombinant antigens. Of the 47 samples analyzed, 38 (81%) and 42 (90%) samples were positive for *B. equi* infection and *B. caballi* infection, respectively. In addition, 35 (75%) samples were positive for both *B. equi* and *B. caballi* infections. These results indicate that equine piroplasmosis is widespread and therefore a cause for serious concern in the States of Sao Paulo and Mato Grosso do Sul, Brazil.
KEY WORDS: ELISA, equine piroplasmosis, LAT.

J. Vet. Med. Sci. 63(10): 1159–1160, 2001

Equine piroplasmosis is caused by two tick-borne haemoprotozoan parasites, *Babesia equi* (*B. equi*) and *Babesia caballi* (*B. caballi*). The diseases are characterized clinically by fever, anemia, and icterus, and are endemic in most tropical and subtropical areas of the world [1, 8]. Due to the almost worldwide distribution of the various tick vectors, the introduction of carriers into non-endemic areas or countries must be prevented [8]. Prior to importation to non-endemic areas or countries, horses must be shown to be negative for piroplasmosis through serological testing [1, 8, 10]. The complement fixation test (CFT) 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 [1, 8]. Recently, we developed a latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) with recombinant antigens to diagnose *B. equi* infection and *B. caballi* infection, respectively, and demonstrated that both methods are highly specific and sensitive [3, 4, 11, 12]. In the present study, we investigated the prevalence of equine piroplasmosis in Brazil by the LAT and ELISA.

LAT with recombinant equi merozoite antigen-1 (EMA-1) expressed in insect cells by baculovirus for diagnosis of *B. equi* infection in horses was performed as described previously [11, 12]. ELISA with recombinant P48 protein expressed in *Escherichia coli* by pGEX vector for diagnosis of *B. caballi* infection in horses was carried out as described elsewhere [3, 4].

A total of 47 blood samples were taken from horses on five farms in the States of Sao Paulo and Mato Grosso do Sul, Brazil in December, 2000. In the both states, the predominant climate is tropical, with temperatures ranging between 17 and 35°C in summer and 10 and 25°C in winter. Sub-zero temperatures are uncommon. As shown in Tables 1 and 2, 38 (81%) and 42 (90%) samples were positive for *B. equi* infection and *B. caballi* infection by LAT and ELISA, respectively. The ages of positive horses ranged from 1 to 10 years. In addition, 35 (75%) samples were positive for both *B. equi* and *B. caballi* infections. On the other hand, 15 (32%) samples were positive for *Babesia* parasites by thin blood smear examination. All the 15 smear-positive samples were positive for *B. caballi* infection by ELISA and 12 of them were positive for *B. equi* infection by LAT.

Previous studies have shown that the prevalence of equine piroplasmosis in Brazil is serious [2, 5–7]. Kerber *et al.* examined serum samples from horses in the States of Sao Paulo and Mato Grosso do Sul, Brazil, and showed that the seropositive rates for *B. equi* and *B. caballi* were 37% and 27%, respectively [5]. The prevalence of equine piroplasmosis recorded here is significantly higher than that of reported by Kerber *et al*. [5]. This may be due to differences in the serological tests used, since previous study used CFT, which is less sensitive than ELISA and LAT, and therefore can give false negative results for equine piroplasmosis.

In the present study, the recombinant antigens, EMA-1 and P48, were expressed by the genes from *B. equi* USDA strain and *B. caballi* USDA strain, both isolated in U.S.A. Previous reports have shown that the natural tick vectors of equine piroplasmosis in the U.S.A. are different from those in Brazil, that is, New World Dermacentor species in the U.S.A. [9], and suspected *Boophilus microplus* species in Brazil [7]. This apparent difference in vector species, may reflect differences in parasite character at the molecular level. Although it has been reported that the differences observed by IFAT using antigens from USDA strains and Brazilian isolates were not significant [2], there is a need to compare the EMA-1 gene and P48 gene of the USDA strains

* CORRESPONDENCE TO: PROF. DR. FUJISAKI, K., National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555, Japan.

Table 1. Detection of antibodies to *B. equi* and *B. caballi* in horses from the States of Sao Paulo and Mato Grosso do Sul, Brazil by serodiagnostic methods with recombinant antigens

Farm[a]	Horse No.	Age[b)]	*B. equi*[c)]	*B. caballi*[d)]	Smear[e)]	Farm	Horse No.	Age	*B. equi*	*B. caballi*	Smear
A	1	U	+	+	+		24	5	+	+	–
	2	U	+	+	–		25	7	+	+	+
	3	U	–	–	–						
	4	U	–	+	–	E	26	4	+	+	–
	5	U	+	–	–		27	5	+	+	+
	6	U	+	–	–		28	6	+	+	–
	7	U	+	+	–		29	2	+	+	–
	8	U	–	+	–		30	6	+	+	–
	9	U	+	+	–		31	2	+	+	+
	10	U	+	+	–		32	3	–	+	+
							33	4	+	+	–
B	11	U	+	+	–		34	1	+	+	+
							35	1	+	+	–
C	12	10	+	+	–		36	2	+	+	–
	13	9	+	+	–		37	3	+	+	–
	14	3	+	+	–		38	6	+	+	+
	15	9	+	+	–		39	1	+	+	+
	16	8	+	–	–		40	1	–	+	+
	17	4	+	+	–		41	1	+	+	+
							42	1	+	+	–
D	18	3	+	+	–		43	7	+	+	+
	19	8	–	+	–		44	5	–	+	+
	20	8	+	+	+		45	2	+	+	+
	21	7	–	–	–		46	3	+	+	+
	22	6	+	+	–		47	3	+	+	–
	23	6	–	+	–						

a) Farm A is located in Sao Paulo State; farms B-E are located in Mato Grosso do Sul State.
b) U: unknown.
c) Antibodies to *B. equi* were detected by LAT with recombinant EMA-1 antigen expressed in insect cells (3, 4). LAT was considered positive when agglutination was observed at dilutions of 1:4 and above.
d) Antibodies to *B. caballi* were detected by ELISA with recombinant P48 antigen expressed in *E. coli* (11, 12). ELISA was considered positive when an optical density at 415 nm equal to or greater than 0.2 was observed at dilutions of 1:80 and above.
e) Smear examination was considered positive when Babesia-like parasites were observed in Giemsa-stained thin blood films.

Table 2. Prevalence of equine piroplasmosis in the States of Sao Paulo and Mato Grosso do Sul, Brazil

	B. equi+	*B. equi*–	Total
B. caballi+	35 (75%)	7 (15%)	42 (90%)
B. caballi–	3 (6%)	2 (4%)	5 (10%)
Total	38 (81%)	9 (19%)	47 (100)

and Brazilian isolates.

ACKNOWLEDGEMENT. This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Bruning, A. 1996. *Br. Vet. J.* **152**: 139–151.
2. Heuchert, C. M., de Giulli, V. Jr., de Athaide, D. F., Rose, R. and Friedhoff, K. T. 1999. *Vet. Parasitol.* **85**: 1–11.
3. Ikadai, H., Osorio, C. R., Xuan, X., Igarashi, I., Kanemaru, T., Nagasawa, H., Fujisaki, K., Suzuki, N. and Mikami, T. 2000. *Int. J. Parasitol.* **30**: 633–635.
4. Ikadai, H., Xuan, X., Igarashi, I., Tanaka, S., Kanemaru, T., Nagasawa, H., Fujisaki, K., Toyoda, Y., Suzuki, N. and Mikami, T. 1999. *J. Clin. Microbiol.* **37**: 3475–3480.
5. Kerber, C. E., Ferreira, F. and Pereira, M. C. 1999. *Onderstepoort J. Vet. Res.* **66**: 123–127.
6. Pfeifer, B. I., Rose, R., Peymann, B. and Friedhoff, K. T. 1995. *Vet. Parasitol.* **58**: 1–8.
7. Ribeiro, M. F., Costa, J. O. and Guimaraes, A. M. 1999. *Vet. Res. Commun.* **23**: 385–390.
8. Schein, E. 1988. Equine babesiosis. p. 197–208. *In*: Babesiosis of Domestic Animals and Man. Ristic, M., ed. CRC Press, Inc., Boca Raton, Fla.
9. Stiller, D. and Coan, M. E. 1995. *Vet. Parasitol.* **57**: 97–108.
10. Tenter, A. M. and Friedhoff, K. T. 1986. *Vet. Parasitol.* **20**: 49–61.
11. Xuan, X., Igarashi, I., Tanaka, T., Fukumoto, S., Nagasawa, H., Fujisaki, K. and Mikami, T. 2001. *Clin. Diagn. Lab. Immunol.* **8**: 645–646.
12. Xuan, X., Larsen, A., Ikadai, H., Tanaka, T., Igarashi, I., Nagasawa, H., Fujisaki, K., Toyoda, Y., Suzuki, N, and Mikami, T. 2001. *J. Clin. Microbiol.* **39**: 705–709.