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Veterinary Parasitology 137 (2006) 28–35
veterinary parasitology
www.elsevier.com/locate/vetpar

Expression of C-terminal truncated and full-length *Babesia bigemina* rhoptry-associated protein 1 and their potential use in enzyme-linked immunosorbent assay

Suthisak Boonchit [a], Andy Alhassan [a], Bun Chan [a], Xuenan Xuan [a], Naoaki Yokoyama [a], Mamoru Ooshiro [b], Will L. Goff [c], Suryakant D. Waghela [d], Gale Wagner [d], Ikuo Igarashi [a,*]

[a] *National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan*
[b] *Okinawa Prefectural Institute of Animal Health, Naha, Okinawa 900-0024, Japan*
[c] *Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA 99164, USA*
[d] *Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843, USA*

Received 15 January 2005; received in revised form 7 December 2005; accepted 7 December 2005

Abstract

Recombinant antigen-based enzyme-linked immunosorbent assay (ELISA) was developed for the serological diagnosis of *Babesia bigemina* infection by using a full-length *B. bigemina* rhoptry-associated protein 1 (rRAP-1) and the truncated C-terminal RAP-1 (rRAP-1/CT). While the rRAP-1 showed cross reactivity between *B. bigemina*- and *Babesia bovis*-infected bovine sera, the rRAP-1/CT was highly specific to *B. bigemina*-infected bovine sera and proved useful in the detection of sequential sera collected from an experimentally infected cow during the acute and latent infection. The high yield of soluble rRAP-1/CT and its diagnostic specificity demonstrate its potential in the diagnosis of *B. bigemina* infection. Its usefulness for epidemiological investigation is currently being evaluated.

Keywords: *Babesia bigemina*; Rhoptry-associated protein 1; Recombinant antigen; Enzyme-linked immunosorbent assay

1. Introduction

Babesia bigemina is a tick-borne intraerythrocytic protozoan parasite that causes bovine babesiosis in tropical and sub-tropical areas and constitutes one of the most important diseases affecting cattle industry worldwide (McCosker, 1981; Kuttler, 1988). While the acute infection can readily be diagnosed by direct microscopic examination of Giemsa-stained blood smears, in subclinical cases, this may be impractical due to low levels of parasitemia (Bose et al., 1995). Serological tests such as the immunofluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA) have

* Corresponding author. Tel.: +81 155 49 5641; fax: +81 155 49 5643.
E-mail address: igarcpmi@obihiro.ac.jp (I. Igarashi).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2005.12.025

been used in the detection of sub-clinical cases and epidemiological surveys (Weiland and Reiter, 1988). Unlike ELISA, the interpretation of IFAT has disadvantages of low sample throughput and subjectivity, and is affected by cross reactivity with *Babesia bovis* (Fuginaga et al., 1980; Wright, 1990; Bose et al., 1995).

The detection of specific antibodies (Abs) by ELISA based on the native crude *Babesia* antigens requires mass production of the parasite either from experimentally infected animals or cultures, and is therefore laborious, expensive, and limited in terms of both specificity and assay-reproducibility (Bose et al., 1995). Thus, emphasis has shifted to the characterization of *Babesia* antigenic components and their use in diagnostics. Several *Babesia* recombinant antigens have been expressed in *Escherichia coli* and evaluated purportedly to replace native parasite antigens to improve the sensitivity and specificity of serological tests (Ikadai et al., 1999; Tebele et al., 2000; Hirata et al., 2002; Huang et al., 2003; Goff et al., 2003). The utilization of recombinant antigen(s) creates improved standardization of tests and reduces the production cost.

Among several proteins of *B. bigemina* merozoites, rhoptry-associated protein 1 (RAP-1) (McElwain et al., 1987; Machado et al., 1993) has been well characterized for its immunogenicity and conservation among different geographic isolates (Figueroa et al., 1990; McElwain et al., 1987, 1991; Suarez et al., 1994; Vidotto et al., 1995). Suarez et al. (1991) earlier described a relatively conserved N-terminal region of RAP-1 in *B. bigemina* and *B. bovis*. In a related study, using the full-length RAP-1 antigen, Boonchit et al. (2002) have noted cross reactivity between *B. bigemina* and *B. bovis* in ELISA, and associated such cross reaction to the high degree of sequence identity in the first 300 amino acids of RAP-1 as earlier documented by Suarez et al. (1991). In view of these earlier findings, we evaluated and compared the diagnostic potential of the recombinant full-length and the truncated C-terminal of *B. bigemina* RAP-1 protein in ELISA.

2. Materials and methods

2.1. Parasites

B. bigemina, Argentina strain that has been continuously cultured in vitro with bovine erythrocytes employing the microaerophilous stationary-phase culture system (Vega et al., 1985) in our laboratory was used. When the level of parasitemia reached 5–10%, the infected erythrocytes were washed three times with phosphate-buffered saline (PBS), and the pellets were stored at −80 °C until use.

2.2. Cloning of the full-length and the C-terminal truncated genes of RAP-1 (p58)

B. bigemina genomic DNA was extracted from *B. bigemina*-infected erythrocyte pellets with phenol–chloroform as previously described (Boonchit et al., 2002) and used as a template DNA in PCR. Oligonucleotide primers were designed based on the DNA sequence of *B. bigemina* RAP-1 (p58) gene (Gene Bank accession no. M60878) with restriction enzyme-compatible ends for the subsequent DNA cloning. The nucleotide sequences (nt 186–1625 and 1352–1625) coding the entire RAP-1 (p58) and the Carboxy Terminal Variant Type 1 (CT1) which is highly conserved among strains were obtained by PCR using a pair of primers, RAP-1-1 (5′-ACGCGGCCGCAAATGTACAGCTAAATTGCTGT-TA-3′; the underlined sequence contains an *Not*I restriction site) and RAP-1-3 (5′-ACGTCGACAA-CAATGAGGAGGAGCTTCTTGGGTGTGT-3′; the underlined sequence contains a *Sal*I restriction site), and another pair of primers, RAP-1-2 (5′-ACGCGG-CCGCCCGTTGTGCCGATAAAG-3′; the underlined sequence contains an *Not*I restriction site) and RAP-1-3. The PCR conditions were 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and then extension at 73 °C for 2 min. Each of the PCR-amplified DNA was digested with restriction enzymes, *Sal*I and *Not*I, and then ligated to a similarly digested pGEX-4T expression plasmid (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England), resulting in the generation of pGEX-RAP-1 and pGEX-RAP-1/CT, which contain the full-length RAP-1 and the C-terminal RAP-1 fragments, respectively.

2.3. Expression and purification of the recombinant proteins

The plasmids, pGEX-RAP-1 and pGEX-RAP-1/CT were transformed in *E. coli* (strain DH5α). Each