Veterinary Parasitology 190 (2012) 595–598

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Development of a rapid immunochromatographic test using a recombinant thrombospondin-related adhesive protein of *Babesia gibsoni*

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ARTICLE INFO

Article history:
Received 8 March 2012
Received in revised form 17 June 2012
Accepted 19 June 2012

Keywords:
Babesia gibsoni
Immunochromatographic test
Thrombospondin-related adhesive protein

ABSTRACT

We developed an immunochromatographic test (ICT) with the full-length of thrombospondin-related adhesive protein of *Babesia gibsoni* expressed by the modified expression method. The developed ICT showed high sensitivity, specificity, and kappa value with a reference test (100%, 93.78%, and 0.8976, respectively), indicating that the ICT could be a new practical diagnostic test for *B. gibsoni* infection.

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Babesia gibsoni is an intraerythrocytic parasite that causes hemolytic anemia, fever, splenomegaly, and sometimes death in domestic dogs and wild Canidae (Boozer and Macintire, 2003). Since this parasite has been found in India in 1910, the *B. gibsoni* infection has been emerging in many regions of Asia, Africa, Europe, America, and even Australia (Birkenheuer et al., 2005; Gotsch et al., 2009; Miyama et al., 2005; Muhlnickel et al., 2002). Although Ixodid tick has been known as a transmission route of this disease in general, blood–blood transmission during fighting or blood transfusion from dogs carrying *B. gibsoni* without any symptoms was raised as another route spreading this disease (Brandao et al., 2003; Chauvin et al., 2009; Jefferies et al., 2007; Stegeman et al., 2003). Therefore, screening dogs in not only acute stage with typical symptoms of *B. gibsoni* infection but also chronic stage without clinical illness would be needed to prevent this disease from spreading.

A number of trials have been done to identify a promising antigen for a development of serodiagnostic methods, such as enzyme-linked immunosorbent assay (ELISA) and immunochromatographic test (ICT) (Goo et al., 2009; Jia et al., 2009; Miyama et al., 2006; Verdida et al., 2004, 2005; Zhou et al., 2007). Among the identified antigens, thrombospondin-related adhesive protein of *B. gibsoni* (BgTRAP) appeared to be the best antigen for the serodiagnosis of *B. gibsoni* infection according to the previous study comparing ELISA results of recombinant BgTRAP (rBgTRAP) and other recombinant antigens using sera obtained from animal clinics (Goo et al., 2008; Konishi et al., 2008). ICT is a rapid, simple, and easy method in which the diagnosis is completed within 15 min after dropping a serum sample to the test strip (Luo et al., 2012). Therefore, ICT with rBgTRAP has been expected to be the best combination; however, low expression level of full length of rBgTRAP (rBgTRAPf) in *Escherichia coli* precluded it from development of ICT

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0304-4017/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2012.06.024

with the rBgTRAPf. Although the expression yield of rBgTRAP was recently improved by truncating BgTRAPf, ELISAs with the truncated BgTRAPs, C and N-terminal of BgTRAP (BgTRAPc and BgTRAPn, respectively), were not superior to that with the BgTRAPf in terms of sensitivity and concordance with IFAT results (Narantsatsral et al., 2011). Therefore, we herein introduced stress response inducers such as incubation temperature downshift and an addition of ethanol (Kusano et al., 1999; Thomas and Baneyx, 1996), in order to increase the expression yield of the rBgTRAPf.

Three plasmids, containing a respective open reading frame of BgTRAPf, BgTRAPn, and BgTRAPc in pGEX-4T-3 vector (GE healthcare, UK) were obtained from the previous study (Narantsatsral et al., 2011). The three recombinant proteins, rBgTRAPf, rBgTRAPn, and rBgTRAPc, were expressed as a glutathione *S*-transferase (GST)-fusion protein by a method previously described (Narantsatsral et al., 2011), and simultaneously rBgTRAPf was expressed as a GST-fusion protein by a modified method. Briefly, medium with *E. coli* expressing rBgTRAPf was incubated with 2% ethanol at 37 °C, induced by 0.1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG), and then incubated overnight at 25 °C. After purification, the yield of the resulted protein was compared with that of the truncated rBgTRAPs (rBgTRAPn and rBgTRAPc) and rBgTRAPf expressed by non-modified method. The result appeared that the modified expression method increased the yield of rBgTRAPf as equal as that of the truncated rBgTRAPc (Fig. 1). It is thought that the incubation temperature downshift and the ethanol activated respectively cold shock proteins involved in translational regulation (Etchegaray and Inouye, 1999; Jones et al., 1996) and heat shock proteins functioning as molecular chaperones (Chaudhuri et al., 2006). Subsequently, the rBgTRAPf expressed by the modified method and the rBgTRAPc were used for ICT strip, and then the ICT results were compared with results of ELISAs as well as the reference IFAT.

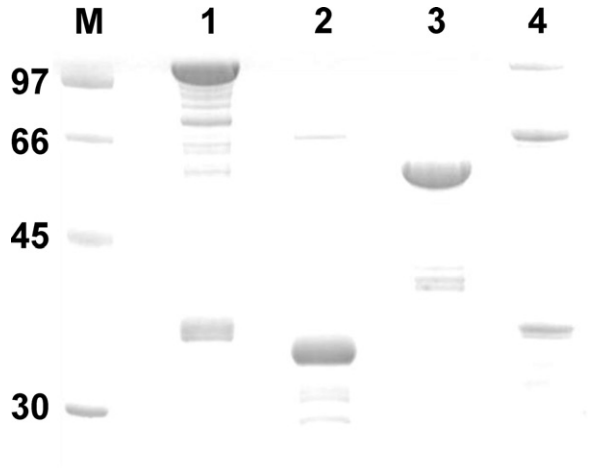

Fig. 1. Effect of incubation temperature downshift and ethanol addition on expression of rBgTRAPf. The expression yield of rBgTRAPf was increased by modified expression method, incubation temperature downshift and ethanol addition (line 1), compared to that of rBgTRAPf expressed by non-modified method (line 4). Concentration of the resulted rBgTRAPf expressed by the modified method was as equal as that of truncated rBgTRAPs (lines 2 and 3). M, low molecular weight marker; line 1, rBgTRAPf expressed by the modified method; line 2, rBgTRAPn expressed by non-modified method; line 3, rBgTRAPc expressed by non-modified method; line 4, rBgTRAPf expressed by non-modified method.

The chromatography strip was prepared as follows. The purified GST-rBgTRAPf and GST-rBgTRAPc (200 μg/ml) in 5 mM phosphate buffer were conjugated with a gold colloid (British BioCell International, UK) (1:10, vol/vol) at pH 7.4 by incubation at room temperature for 10 min. After stabilizing and blocking conjugate particles by 0.05% polyethylene glycol 20000 (PEG) and 1% bovine serum albumin (BSA), the conjugate was diluted in 10 mM Tris–HCl (pH 8.2) with 5% sucrose, sprayed on the glass fiber (Schleicher & Schell, USA), and dried overnight. Mouse

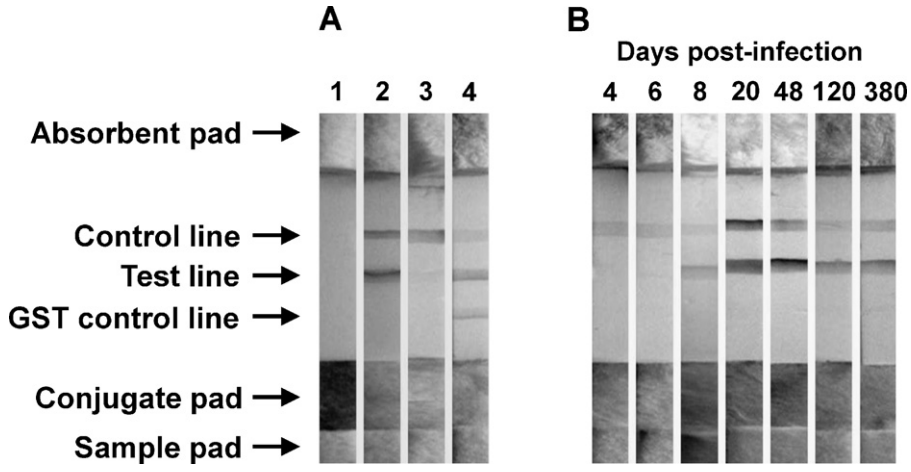

Fig. 2. Rapid immunochromatographic test strips for detection of specific antibodies to *Babesia gibsoni*. (A) Example of pretest (lane 1) and posttests (lines 2–4). Lane 1, strip before application of serum; line 2, strip after application of serum collected from a dog experimentally infected with *B. gibsoni*; line 3, strip after application of serum collected from a healthy dog; line 4, strip after application of anti-GST mouse serum. (B) Specific antibody responses in sequential dog sera obtained from a dog experimentally infected with *B. gibsoni*. The developed ICT detected specific antibodies to *B. gibsoni* in sera collected from 8 to 380 days post-infection.

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