



ELSEVIER

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Molecular cloning and characterization of *Babesia orientalis* rhoptry-associated protein 1

Qian Yu^{a,b}, Lan He^{a,b,*}, Wen-Jie Zhang^{a,b}, Jian-Xi Cheng^{a,b}, Jin-Fang Hu^{a,b},
Xiao-Yan Miao^{a,b}, Yuan Huang^{a,b}, Li-Zhe Fan^{a,b}, Muhammad Kasib Khan^{a,b},
Yan-Qin Zhou^b, Min Hu^{a,b}, Jun-Long Zhao^{a,b,*}

^a State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China

^b Key Laboratory of Animal Epidemical Diseases and Infectious Zoonoses, Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, Hubei, China

ARTICLE INFO

Article history:

Received 16 June 2014

Received in revised form 13 August 2014

Accepted 14 August 2014

Keywords:

Babesia orientalis

Rhoptry-associated protein

Antigenicity

Diagnosis

ABSTRACT

The rhoptry-associated protein 1 (RAP-1) gene of *Babesia orientalis* was obtained from a cDNA expression library by immunoscreening with *B. orientalis*-infected water buffalo sera. The nucleotide sequence of the cDNA was 1732 bp with an open reading frame (ORF) of 1434 bp, encoding a polypeptide of 478 amino acid residues with a predicted size of 52.5 kDa. The ORF was cloned into a pGEX-KG plasmid and subsequently expressed as a GST-fusion protein. The recombinant RAP-1 of *B. orientalis* (rBoRAP-1) was purified and evaluated as an antigen using Western blotting. The native BoRAP-1 was recognized by the antibodies raised in rabbits against rBoRAP-1. Strong immunofluorescence signals were observed in erythrocytes infected with *B. orientalis*. Phylogenetic analysis revealed that *B. orientalis* fell into a *Babesia* clade and most closely related to *Babesia bovis* and *Babesia ovis*, which was similar to the previous reported trees based on 18S rRNA and HSP70 genes. The present study suggests that the BoRAP-1 might be a potential diagnostic antigen, and the RAP-1 genes can aid in the classification of *Babesia* and *Theileria* species.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Tick-transmitted intraerythrocytic babesial parasites cause significant morbidity in humans and domestic animals, manifested predominantly by anemia (Homer et al., 2000; Kjemtrup and Conrad, 2000). It is considered to be

the second most commonly found parasites in the blood of mammals after trypanosomes (Schnittger et al., 2012).

Babesia orientalis is a protozoan parasite transmitted by *Rhipicephalus haemaphysaloides*, which only infects water buffalo (Liu et al., 1997). Babesiosis, caused by this parasite, is one of the most severe diseases in water buffalo, which results in large economic losses in the central and southern China (Chen, 1984; Liu and Ma, 1987; Zhongling et al., 1986). The clinical symptoms of this disease are fever, anemia, icterus and hemoglobinuria (He et al., 2009; Liu et al., 2005). *B. orientalis* was distinguished from *Babesia bigemina* and *Babesia bovis* in water buffalo and classified as a new species, according to the differences of morphology, transmission and pathogenicity (Liu et al., 1997).

* Corresponding authors at: State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Shizishan Street, Wuhan 430070, Hubei, China. Tel.: +86 27 87281810.

E-mail addresses: helan@mail.hzau.edu.cn (L. He), zhaojunlong@mail.hzau.edu.cn (J.-L. Zhao).

The *Babesia* RAP-1 gene was first described in *B. bovis* (Goff et al., 1988) and sequentially observed in many other *Babesia* species, including *Babesia bigemina*, *Babesia divergens*, *Babesia caballi*, *B. bovis*, and *Babesia canis* (Dalrymple et al., 1993; Ikadai et al., 2000; McElwain et al., 1991; Skuce et al., 1996; Suarez et al., 1991; Zhou et al., 2007). Babesial RAP-1 was tested in several vaccine trials either as an individual candidate or in combined form with other recombinant protein antigens. Experimental evidence suggested that the cattle immunized with RAP-1 elicited strong T-cell and B-cell immune responses (Norimine et al., 2003). As a component of multi-antigen vaccine, three protective antigens, including RAP-1 were identified in *B. bovis* (Wright et al., 1992).

Apicomplexan parasites utilize several rhoptry proteins during invasion into and development within the host cell (Preiser et al., 2000; Reduker et al., 1989). An *in vitro* erythrocyte-binding assay confirmed that *B. bovis* RAP-1 was able to bind with the bovine and equine erythrocytes surface, and the binding affinity was weakened by anti-RAP-1 MAB (Yokoyama et al., 2002). *B. bovis* RAP-1 was expressed in sporozoites, and specific RAP-1 antisera could inhibit the sporozoites invasion into host cells (Mosqueda et al., 2002). These studies strongly support that RAP-1 plays a functional role in the biology and development of *Babesia* parasites.

The genetic organization of RAP-1 family is highly complexed in some *Babesia* species, containing several closely linked genes encoding unique proteins (Dalrymple et al., 1993; Sam-Yellowe, 1996; Skuce et al., 1996; Suarez et al., 1998). Despite of the complexity of the gene loci, all the members of rhoptry-associated protein family have well conserved features, including a N-terminal localized signal peptide, several tandem repeat regions in the C-terminal, four strictly conserved cysteine residues and a 14 amino-acid motif (Dalrymple et al., 1996; Norimine et al., 2003; Sam-Yellowe, 1996; Suarez et al., 1991). The well conserved RAP-1 is also highly immunogenic and has been observed to induce high titer of antibodies in *Babesia*-infected animals. Thus, the RAP-1 has also been widely used to develop serological diagnostic methods for *Babesia* parasites (Boonchit et al., 2002; Ikadai et al., 2000; Zhou et al., 2007).

In the present study, the gene encoding rhoptry-associated protein 1 (RAP-1) was identified from a *B. orientalis* cDNA library, which was further confirmed through phylogenetic analysis of gene sequence. The encoding gene was cloned and expressed in *Escherichia coli* and evaluated its potential use as a diagnostic and vaccine candidate.

2. Materials and methods

2.1. Parasites and experimental animals

B. orientalis strain was previously isolated from water buffalo in Hubei province, China and preserved in liquid nitrogen in State Key laboratory of Agricultural Microbiology, Huazhong Agricultural University, China (Liu et al., 1995).

Two 1-year-old water buffaloes were purchased from *Babesia* free area and confirmed to be *B. orientalis* free by real-time PCR (He et al., 2011). The water buffaloes were splenectomized and subcutaneously injected with 4 ml of *B. orientalis*-infected blood (Wuhan strain, percentage parasitized erythrocytes (PPE) 1%). Blood samples were collected daily to monitor the parasitemia until it reached up to 3%.

All animal experiments described in this article were carried out in compliance with the regulations (no. 5 proclaim of the Standing Committee of Hubei People's Congress) approved by the Standing Committee of Hubei People's Congress, P. R. China. The animal protocols were approved by Laboratory Animals Research Centre of Hubei province and the ethics committee of Huazhong Agricultural University (permit number: 4200696657).

2.2. Preparation of genomic DNA

The blood from experimentally infected water buffalo was collected in EDTA tubes (BD Vacutainer blood collection tubes, USA). The genomic DNA was extracted from 200 μ l of *B. orientalis*-infected blood using a QIAamp DNA mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA samples were stored at -20°C until further use.

2.3. Cloning and sequencing of the BoRAP-1 gene

A cDNA expression library of *B. orientalis* merozoites, constructed previously (Liu et al., 2009), was screened with the serum of *B. orientalis*-infected water buffalo. One of the positive clones expected to be RAP-1 were chosen for further analysis.

To amplify the *B. orientalis* RAP-1 gene from gDNA, a pair of primers was designed based on BLAST analysis of *B. orientalis* genome sequence (unpublished data). The forward primer RAP1-F was 5'-TAC TCA CCT ATA AAA GCC TCT TGC C-3', and the reverse primer RAP1-R was 5'-CTG TAT GTG TCA AAA AGG GA-3'.

The amplified PCR product from both gDNA and cDNA were electrophoresed using 0.8% ethidium bromide-stained agarose gel and purified by TIANGel Midi purification Kit (TIANGEN, China). The purified PCR products were ligated into pMD18-T vector (TaKaRa, Japan), and three positive clones from each sample were sequenced using a Dye Terminator Cycle Sequencing reaction in an ABI PRISM 377 DNA sequencer (Sangon, China). The vector primers M13 (–47) and M13 (–48) were used for sequencing.

2.4. Bioinformatics analysis of BoRAP-1

The isolated gene of *B. orientalis* RAP-1 was given the name "BoRAP-1". The deduced amino acid (aa) sequence of BoRAP-1 was subjected to BLASTp analysis in GenBank (Benson et al., 2012). The homologous sequences of related intra-erythrocytic species (Table 1), including BoRAP-1 sequence were aligned using MAFFT version 7 (Katoh and Frith, 2012; Katoh and Standley, 2013) and manually edited using BioEdit version 7.1.11 (Hall, 1999). A

Download English Version:

<https://daneshyari.com/en/article/5803056>

Download Persian Version:

<https://daneshyari.com/article/5803056>

[Daneshyari.com](https://daneshyari.com)