Veterinary Parasitology 186 (2012) 490–496

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

Short communication

Occurrence of *Theileria* and *Babesia* species in water buffalo (*Bubalus babalis*, Linnaeus, 1758) in the Hubei province, South China

Lan He[a,b], Hui-Hui Feng[a,b], Wen-Jie Zhang[a,b], Qing-Li Zhang[a,b], Rui Fang[a,b], Li-Xia Wang[a,b], Pan Tu[a,b], Yan-Qin Zhou[b,c], Jun-Long Zhao[a,b,**], Marinda C. Oosthuizen[d,*]

[a] *State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei, Wuhan 430070, China*
[b] *College of Veterinary Medicine, Huazhong Agricultural University, Hubei, Wuhan 430070, China*
[c] *Key Laboratory Preventive Veterinary of Hubei Province, Huazhong Agricultural University, Hubei, Wuhan 430070, China*
[d] *Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, South Africa*

ARTICLE INFO

Article history:
Received 3 May 2011
Received in revised form 3 November 2011
Accepted 7 November 2011

Keywords:
Theileria
Babesia
Water buffalo
Reverse line blot hybridization assay
Phylogenetic tree

ABSTRACT

The presence and prevalence of tick-borne haemoparasites in water buffalo from the Hubei province, south China was investigated using the reverse line blot (RLB) hybridization assay and phylogenetic analysis of the parasite 18S rRNA gene. *Theileria buffeli* (19.1%) was the most frequently found species in all of the locations, followed by *Babesia orientalis* (8.9%), *Babesia bovis* (1.0%) and *Babesia bigemina* (0.7%). Only 12 (3.9%) of the samples had mixed infections. Eleven samples with single infections were selected for further characterization using 18S rRNA gene sequence analysis. Phylogenetic analysis showed that the eight *T. buffeli* 18S rRNA gene sequences obtained grouped into four clusters, of which three grouped with the known *T. buffeli* types B and D. The remaining five grouped separately from the previously describe *T. buffeli* types, constituting new *T. buffeli* types. The two *B. bigemina* 18S rRNA gene sequences obtained grouped closely with *B. bigemina* Kunming; this serves as the first report of *B. bigemina* in the Hubei province. The *B. orientalis* Daye 18S rRNA gene sequence obtained grouped closely with the previously reported *B. orientalis* Wuhan strain and with *Babesia* sp. Kashi 1 and Kashi 2.

1. Introduction

Theileria and *Babesia* species, collectively known as piroplasms, are tick-transmitted apicomplexan parasites that infect wild and domestic animals worldwide (Mehlhorn and Schein, 1984). They are an important constraint to livestock production in developing countries, and are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock by-products (Uilenberg, 2001).

In China, *Theileria annulata*, *Theileria mutans* and the *Theileria buffeli*/*Theileria sergenti*/*Theileria orientalis* group have historically have been reported as the three most economically important bovine *Theileria* species (Yang et al., 1964), of which *T. annulata* is considered to be the most pathogenic and *T. sergenti* the most prevalent (Liu et al., 2010). More recently *T. sinensis*, a benign *Theileria* species of cattle and yaks, was discovered in the central part of Gansu Province (Bai et al., 2002). *T. annulata*, transmitted by ticks of the genus *Hyalomma*, causes Mediterranean or tropical theileriosis in cattle and domestic water buffalo with a mortality rate of 10–90% (Levine, 1985). The benign

* Corresponding authors at: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa. Tel.: +27 12 529 8390; fax: +27 12 529 8312.
** Corresponding authors at: State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Shizishan No. 1, Wuhan, Hubei, 430070, China. Tel.: +86 27 87281810; fax: +86 27 87280408.
E-mail addresses: zhaojunlong@mail.hzau.edu.cn (J.-L. Zhao), marinda.oosthuizen@up.ac.za (M.C. Oosthuizen).

0304-4017/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2011.11.021

T. buffeli/T. sergenti/T. orientalis group has a worldwide distribution. The taxonomy and nomenclature of this group is complex and is usually based on the geographic origin of the parasite (Chae et al., 1998). *T. sergenti* causes bovine theileriosis in cattle in Japan and Korea (Hagiwara et al., 2005) and is also known to cause disease, and even death, in water buffalo and cattle in China (Jin et al., 2007). *T. buffeli*, a less pathogenic *Theileria* species infecting cattle and water buffalo, is considered to be a common parasite of cattle in many parts of the world (Neitz, 1957). It causes benign bovine theileriosis with a mortality rate of less than 1% (Levine, 1985). In China, *T. mutans* has only been reported in cattle from the GuiZhou province and XinJiang Uygur Autonomous Region (Lv et al., 1995).

Five *Babesia* species, namely *Babesia bigemina*, *Babesia bovis*, *Babesia major*, *Babesia orientalis* and *Babesia ovata*, have been identified in bovine in China. *B. bovis* and *B. bigemina* are regarded as the major causative agents of bovine babesiosis in China (Yin et al., 1997). They infect both cattle and water buffalo, and are transmitted by the one-host tick *Rhipichephalis micropius*. *B. major* and *B. ovata* are large *Babesia* species infective to cattle, and are transmitted by *Haemaphysalis punctata* and *Haemaphysalis longiconis*, respectively (Liu et al., 2008). It is difficult to discriminate between *B. major* and *B. ovata*; traditionally the discrimination was based on the tick vectors or morphology and pathogenicity (Bai et al., 1990; Higuchi et al., 1991). *B. major* was first reported in 1988 in China in the Henan province and is generally not regarded as such an important pathogen, although it frequently occurs in combination with other tick-borne haemoparasites and exerts a synergistic pathogenicity (Yin et al., 1997). *B. ovata* was not identified in China until 1990 (Bai et al., 1990). It causes anemic diseases among grazing cattle, and is regarded to be of relatively low virulence (Tsuji et al., 1999). *B. orientalis* causes water buffalo babesiosis, one of the most important diseases of buffalo in central and south China (Liu et al., 2005). It has recently been shown to have spread to the north of the Yangtse River, posing a serious threat to the water buffalo industry (He et al., 2009).

In this study, a survey was conducted in the Hubei province, south China to determine the occurrence of *Theileria* and *Babesia* species in water buffalo (*Bubalus babalis*, Linnaeus, 1758) using the reverse line blot (RLB) hybridization assay. The objective was to investigate the presence and prevalence of haemoprotozoan parasites in field water buffalo. The full-length parasite 18S rRNA gene of selected samples were cloned, sequenced and subjected to phylogenetic analysis.

2. Materials and methods

2.1. Samples collection and DNA extraction

A total of 304 EDTA blood samples were collected from water buffalo (*B. babalis*, Linnaeus, 1758) from nine geographic locations in the Hubei province, south China. The genomic DNA was extracted from 200 μl of blood using the QIAamp blood and tissue extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA was eluted in 100 μl elution buffer and stored at −20 °C until further analysis. The standard positive control DNA of *B. orientalis*, *B. bigemina*, *B. bovis* and *T. buffeli* were preserved in our lab.

2.2. PCR amplification and reverse line blot hybridization assay

The V4 hypervariable region of the parasite 18S rRNA gene was amplified using the *Theileria* and *Babesia* genus-specific primers RLB-F2 (5′-GAC ACA GGG AGG TAG TGA CAA G-3′) and RLB-R2 (5′-biotin-CTA AGA ATT TCA CCT CTGACA GT-3′) (Gubbels et al., 1999). A touch-down PCR was followed and the PCR products were analyzed with the RLB hybridization assay as previously described (Gubbels et al., 1999; Nijhof et al., 2005). The genus and species-specific oligonucleotide probes included on the membrane are listed in Table 1. The 18S rRNA gene sequences of *Babesia occultans* (EU376017), *B. orientalis* (AY596279), *Babesia* sp. (Xinjiang) (DQ159073), *Theileria sinensis* (EU277003), *Theileria luwenshuni* (AF081136, AY262119, AY262118, AY262117, and AY262115) and *Theileria uilenbergi* (AY262120, AY262116, and AY262121) were used to design RLB probes for the detection of these species. These were also included on the RLB membrane.

2.3. Cloning and sequencing of 18S rRNA gene

Eight *T. buffeli*/*T. sergenti*/*T. orientalis* positive samples, one *B. orientalis* positive sample and two *B. bigemina* positive samples were chosen for cloning and subsequent sequencing. The complete 18S rRNA gene was amplified using the universal primers forword-P1 (5′-AAC CTG GTT GAT CCT GCC AGT AGT CAT-3′), and reverse-P2 (5′-GAT CCT TCT GCA GGT TCA CCT AC-3′) using the condition as described by Liu et al. (2005). The approximately 1 800 bp amplified products were purified and then ligated into the pMD18-T vector (TaKaRa Biotechnology), and the recombinant clones were sequenced using the ABI PRISM 377 DNA sequencer following the manufacturer's instructions. The primers forward-P1, reverse-P2 (Liu et al., 2005), RLB-F2 and RLB-R2 (Gubbels et al., 1999) were used to obtain the full-length 18S rRNA sequences.

2.4. Phylogenetic analysis

The sequences were assembled and edited using GAP4 of the Staden package (version 1.6.0 for Windows) (Staden et al., 2000). A search for homologous sequences in GenBank was performed using BLASTn (www.ncbi.nlm.nih.gov/BLAST/). The sequences were aligned using MAFFT 6 employing the FFT-NS-i algorithm (Katoh et al., 2002). The alignment was manually edited using BioEdit 7.0.9.0. (Hall, 1999). The best-fit model of nucleotide substitution was determined by JmodelTest 0.1.1 (Posada, 2008) selected by AIC calculations. A general time reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites (TIM1 + I + G) substitution mode was used in PAUP* v4b10 (Swofford, 2002) to explore Neighbor-joining, parsimony and maximum likelihood methods. MrBayes v3.1.2