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Short communication

Molecular and serological prevalence of *Babesia bigemina* and *Babesia bovis* in cattle and water buffalos under small-scale dairy farming in Beheira and Faiyum Provinces, Egypt

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ABSTRACT

In order to determine the molecular and serological prevalence of *Babesia bigemina* and *Babesia bovis*, a total of 247 blood samples were collected from cattle and water buffalos in Beheira and Faiyum Provinces in Egypt and examined by nested polymerase chain reaction (nPCR) and enzyme-linked immunosorbent assay (ELISA). In cattle, the prevalence of *B. bigemina* and *B. bovis* was 5.30% and 3.97% by nPCR and 10.60% and 9.27% by ELISA, respectively, whereas those of water buffalos were 10.42% and 4.17% by nPCR and 15.63% and 11.46% by ELISA, respectively. Statistically significant differences in the prevalence of the two infections were observed on the basis of age and health status. Sequencing analysis revealed two genotypes for *B. bovis* spherical body protein-4. In conclusion, the current data provide valuable information regarding the epidemiology of *B. bigemina* and *B. bovis* infections in cattle and water buffalos from Egypt, which can be employed in developing future strategies for disease management and control.

1. Introduction

Bovine babesiosis is a tick borne disease caused by protozoan parasites of genus *Babesia*, order Piroplasmida, phylum Apicomplexa (Bock et al., 2004). Ticks of the Ixodidae family are the main vectors and their geographical distribution influences the epidemiology of the disease (OIE, 2008). *Babesia bigemina* and *Babesia bovis* are particularly economically important in Asia, Africa, Central and South America, Southern Europe and Australia (Spickler and Roth, 2008). Despite advances in understanding *Babesia* spp. biology, genetics and life cycle (Suarez and Noh, 2011; Gohil et al., 2013), there is no safe and efficient vaccine available, and chemotherapeutic options are limited (Mosqueda et al., 2012). Currently, assessment of enzootic stability (Bock et al., 2004) and risk factors such as animal species, breed, age, and ticks distribution are the key elements for effective control of babesiosis. Thus, epidemiological surveys using sensitive and specific diagnostic tools are needed for reliable data on herd situation.

In Egypt, *B. bovis* and *B. bigemina* have been identified in ticks, cattle and water buffalos (*Bubalis bubalis*) using microscopy, indirect immunofluorescent antibody test

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(IFAT) and polymerase chain reaction (PCR) (El Kammah et al., 2001, 2007; Adham et al., 2009; Mazyad et al., 2010; Mahmmod, 2012; Nayel et al., 2012). In spite of their advantages over other diagnostic methods (Goo et al., 2008; Terkawi et al., 2011a; Mosqueda et al., 2012), the enzyme-linked immunosorbent assay (ELISA) and the combination of serological and molecular methods such as PCR/ELISA have not yet been used in babesiosis epidemiological study in Egypt. An epidemiological survey using PCR/ELISA approach and including the main hosts of bovine babesiosis will therefore provide highly desirable information for adequate control and prevention of the disease in Egypt. The present study aimed to investigate the prevalence of *B. bigemina* and *B. bovis* infections in cattle and water buffalos in Beheira and Faiyum Provinces of Egypt through a cross sectional survey based on specific nested PCR and ELISA.

2. Materials and methods

2.1. Blood samples

A total of 247 blood samples from randomly selected Friesian Holstein cross-bred cattle and water buffalos were collected from open public markets in Beheira and Faiyum Provinces (Supplementary data, Fig. S1), respectively. The sampling was carried out during January–July, 2011. Beheira Province lies north to Cairo between 31° North latitude and 30° East longitude whereas Faiyum Province lies south to Cairo between 29° North latitude and 30° East longitude. The climate in Beheira is humid, warm and rainy while in Faiyum it is dry, hot with less rain. The animals are usually moved to fields in daytime and returned back to farmer's stables in the evening. Cattle from 4 to 8 years old were divided into two groups based on their age; young (less than 5 years) and aged (5–8 years). Water buffalos from 5 to 10 years old were divided into two groups based on their age; young (5–7 years) and aged (8–10 years). Based on their body temperature at the sampling time cattle were divided into two groups: fevered and normal. Blood sampling, sera collection and genomic DNA extraction were carried out as described in Terkawi et al. (2012).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2013.08.028.

2.2. Parasites

Argentina strain of *B. bigemina* and Texas strain of *B. bovis* were continuously cultured in bovine red blood cells (RBCs) using a microaerophilous stationary-phase culturing system (Levy and Rustic, 1980). Cultured parasites were harvested when the parasitemia reached 5–10% and used for DNA or RNA extraction.

2.3. Preparation of recombinant proteins

The recombinant proteins including *B. bovis* spherical body protein-4 (BbSBP-4, GenBank accession number AB594813) and *B. bigemina* C-terminal region of rhoptry-associated protein 1 (BbigRAP-1a/CT: 390–480 aa, GenBank accession number M60878) were expressed in *Escherichia coli* BL21 strain (Amersham Pharmacia Biotech, USA), purified from the soluble fractions of *E. coli* lysates using Glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech, USA) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Terkawi et al., 2011a).

2.4. Enzyme-linked immunosorbent assay (ELISA)

ELISAs were performed according to modified procedure described previously (Ibrahim et al., 2009; Terkawi et al., 2011a,b,c). The absorbance was measured at 405 nm using microplate reader (Seac, Radim Company, Italy). ELISAs data were determined on the base of mean optical densities at a value of 405 nm (OD_{405}) for the recombinant antigens (BbigRAP-1a/CT or BbSBP-4) subtracted from those of GST protein. The cutoff values were determined as the OD_{405} value for *B. bigemina* or *B. bovis* negative sera plus three standard deviations; BbigRAP-1a/CT: 0.02 and BbSBP-4: 0.019 in cattle ($n = 22$), and BbigRAP-1a/CT: 0.016 and BbSBP-4: 0.013 in buffalo ($n = 22$). The negative sera were tested and confirmed negative by PCR and nested PCR of its corresponding whole blood samples.

2.5. Nested PCR

Cattle genomic DNA samples were analyzed by nested PCR (nPCR) on the bases of primer sets targeting *B. bigemina* RAP-1a and *B. bovis* SBP-4 genes as previously described (Terkawi et al., 2011a; Cao et al., 2012; Terkawi et al., 2012). Genomic DNA purified from RBCs infected with *Babesia* parasites were used as positive control and DNA from non-infected RBC was used as negative control.

2.6. Cloning and sequencing

Three nPCR positive samples for *B. bigemina* or *B. bovis* obtained from each of the study areas were randomly selected and sequenced for genetic characterization of BbigRAP-1 and BbSBP-4. PCR products (690 bp and 521 bp for *BbigRAP-1* and *BbSBP-4* genes, respectively) were extracted from the agarose gel using QIAquick Gel Extraction Kit (QIAGEN GmbH, Germany), ligated into a pGEM-T Easy Vector (Promega, USA) and transformed into the *E. coli* DH5α-competent cells. A positive inserted plasmid was purified from a single clone using Plasmid Quick Pure (NucleoSpin, MACHEREY-NAGEL GmbH & Co. KG, Germany) and subsequently sequenced using a Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) with pGEM-T Easy Vector primers (pUC/M13). DNA sequences were determined using ABI PRISM 3100 genetic analyzer (Applied Biosystems, USA). Obtained sequences were analyzed using GenBank BLASTn analysis and Clustal X program (Version 2.0.11).

2.7. Statistical analysis

The proportions of agreement were calculated to evaluate the concordance between the nPCR assay and ELISA