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Diagnostic value of the recombinant tandem repeat antigen TeGM6-4r for surra in water buffaloes

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ABSTRACT

Trypanosoma evansi infection, or surra, is currently affecting various species of animals, especially water buffaloes. Since diagnosis is an important aspect of surra control, development of novel diagnostic antigens is of interest to implement and improve the currently utilized methods. Our study evaluated the tandem repeat antigen TeGM6-4r in *T. evansi* antibody detection in water buffaloes. TeGM6-4r-based ELISA was performed with 20 positive and 8 negative controls and 484 field samples from water buffaloes in Northern Vietnam. To examine cross-reactivity, sera from Japanese cattle that had been experimentally infected with *Theileria orientalis* ($n = 10$), *Babesia bovis* ($n = 3$), *Babesia bigemina* ($n = 7$) and *Trypanosoma theileri* ($n = 59$) were included in the study. The sensitivity of the test was 80%. TeGM6-4r did not react with *Theileria* or *Babesia* infected sera, however it showed cross reactivity with 11/59 *T. theileri* infected samples. The reference test, CATT/*T. evansi* also reacted with 3/59 *T. theileri* infected sera. The lysate antigen-based ELISA reacted with 4/59 *T. theileri*, 9/10 *Theileria* and 3/10 *Babesia* infected sera. In contrast, TeGM6-4r-based ELISA was 86.3% sensitive and 58.3% specific in the screening of field samples. The average seroprevalence of *T. evansi* infection among water buffaloes in Northern Vietnam was 27.1% by CATT/*T. evansi* and 53.7% by TeGM6-4r. Seroprevalence in the five surveyed provinces ranged from 17.4% to 39.8% in the reference test, and 47.3% to 67.3% in the recombinant antigen based test. The finding indicated that the disease is still widely endemic in the area and that surveillance programs need to be carried out regularly to better control surra. We proposed TeGM6-4r as a useful serodiagnostic antigen for the detection and epidemiological surveillance of *T. evansi* infection among water buffaloes.

1. Introduction

Serodiagnosis of *Trypanosoma evansi* infection, or surra, mostly relies on the lysate antigen of the parasite. Although lysate antigen production has been standardized, it is usually difficult for the standard protocol to be accepted and strictly adhered to by laboratory workers (Reid and

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Copeman, 2002; OIE, 2012). Furthermore, the lysate antigen shows cross-reactivity in diagnostic tests, leading to false-positive results. However, recombinant antigens can be developed to be highly specific and they have the additional advantage that laboratory animal usage is reduced. Several recombinant antigens of *T. evansi* were introduced, including the variable surface glycoprotein, RoTat 1.2 (Lejon et al., 2005), the invariant surface glycoprotein, ISG 75 (Tran et al., 2009) and tandem repeat (TR) protein GM6 (Thuy et al., 2012). TR protein GM6 is a cytoskeletal protein, located at the connection site between the microtubules of the membrane skeleton and the flagellum of the parasite. The protein is recognized in the early phase of infection when many parasites are destroyed by the host immune response (Müller et al., 1992; Imboden et al., 1995). We reported that recombinant TbbGM6 from *Trypanosoma brucei* was highly antigenic to water buffalo sera that had been experimentally infected with *T. evansi* and that it held qualities that would make it useful as a diagnostic antigen (Thuy et al., 2012). To continue from our findings, it was necessary to further evaluate and validate GM6 for its future application to the serodiagnosis of *T. evansi* infection in animals, especially water buffaloes. The world population of water buffaloes is estimated at 168 million, 161 million of which are in Asia (Michelizzi et al., 2010), where surra is endemic and remains a major constraint for water buffalo productivity (Luckins, 1988; Davison et al., 2000; Villareal et al., 2013). The present study, which involved a water buffalo surra survey that was conducted in five provinces in Northern Vietnam utilizing TeGM6-4r-based ELISA, proposed the recombinant TR antigen "TeGM6-4r" as a novel diagnostic antigen candidate for surra in water buffaloes. The results from the survey are useful for gaining a greater understanding of the occurrence of surra and preventing epidemics in the study areas.

2. Materials and methods

2.1. Cloning and sequencing of the TeGM6-4r

The gene encoded TeGM6-4r was amplified by conventional PCR using genomic DNA from *T. evansi* Tansui strain and the primer set 5′-GGA TCC ATG GAG CTT GCT AAA-3′ and 5′-GAA TTC CTA ATG TGA ATG CTC-3′ (underlined nucleotides are restriction sites of *Bam* HI and *Eco* RI). The PCR mixture (50 μl) contained 1.5 mM $MgCl_2$, 2 mM of each dNTP, 5 pmol of each primer and 1 unit of *Taq* DNA polymerase (Invitrogen Japan, Tokyo). Reactions were conducted for 30 cycles, at 94 °C for 30 s (denaturation), 54 °C for 30 s (annealing), and 7 °C for 1.5 min (extension). PCR products containing various numbers of repeat units were separated by agarose gel electrophoresis. DNA fragments consisting of 4-repeat domains were extracted from the gel (Japan-QIAGEN K.K., Tokyo) and ligated into the vector pCR2.1, then transformed into *Escherichia coli* DH5α following the TA cloning procedure (Japan-QIAGEN K.K., Tokyo). After digestion with *Eco* RI restriction enzyme, direct sequencing of the inserts was carried out using the PCR primers, BigDye Terminator Ready Mix (Applied Biosystems, Life Technologies, Carlsbad, CA) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Life Technologies, Carlsbad, CA). Nucleotide and amino acid sequences were identified and analyzed using Genetyx version 8.0 (Genetyx Co., Tokyo) and BLAST (http://blast.ncbi.nlm.nih.gov/).

2.2. Expression of recombinant TeGM6-4r

The expression procedure of the recombinant tandem repeat protein has been previously described (Goto et al., 2006). In brief, the gene fragment encoding TeGM6-4r was inserted into pET-28a vector (EMD Biosciences, San Diego, CA), then transformed into *E. coli* BL 21. The transformed *E. coli* BL 21 was cultured in SOB medium (BD, Sparks, MD) to an OD_{600} of 0.4–0.6. The expression of the recombinant protein was initialized by adding 1 mM isopropyl-thio-β-galactosidase (IPTG) and maintained for 3 h. The recombinant TeGM6-4r was purified in soluble form using Ni-NTA agarose (Japan-QIAGEN K.K., Tokyo) in accordance with the manufacturer's instructions. The integrity and purity of the protein were evaluated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was determined by bicinchoninic acid (BCA) assay (Thermo Fisher Scientific Inc., Waltham, MA). Purified recombinant protein was stored until use at −80 °C.

2.3. Preparation of trypanosome cell lysate antigen

Trypanosome cell lysate antigen was produced from the *T. evansi* Tansui strain, which was propagated using HMI-9 medium as previously described (Hirumi et al., 1997). The preparation procedure was described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2012).

2.4. Positive and negative sera

Twenty positive and 8 negative serum samples were obtained from Vietnamese water buffaloes. Positive serum samples were collected at day 87 or 94 from 12 water buffaloes that had been experimentally infected with *T. evansi* (field isolate from a buffalo in Ha Tay province, Vietnam in 1998). The surra status of each animal was confirmed by microscopic examination of thin blood smear and buffy coat, mouse inoculation test and CATT/*T. evansi* (Institute of Tropical Medicine, Antwerp, Belgium), according to the OIE manual for surra diagnosis (OIE, 2012). In addition to the 8 negative control sera from healthy water buffaloes, the study also used serum samples collected from Japanese cattle that were experimentally infected with *Theileria orientalis* ($n = 10$), *Babesia bovis* ($n = 3$), *Babesia bigemina* ($n = 7$) and *Trypanosoma theileri* ($n = 59$) as negative controls infected with non-related hemoprotozoan parasites. The infections were confirmed through observation of parasitemia and specific-antibody detection in cattle blood and sera. Handling of the experimental animals strictly accorded to the guidelines on Animal Experimentation of Department of Animal Health of Vietnam and the