Parasitol Res (2012) 110:733–739
DOI 10.1007/s00436-011-2632-9

ORIGINAL PAPER

Tandem repeat protein as potential diagnostic antigen for *Trypanosoma evansi* infection

Nguyen Thu Thuy · Yasuyuki Goto · Zhao-Rong Lun · Shin-Ichiro Kawazu · Noboru Inoue

Received: 1 May 2011 / Accepted: 1 July 2011 / Published online: 17 September 2011

Abstract *Trypanosoma evansi* infection (surra) causes significant losses in livestock production in tropical and sub-tropical areas. The current ELISA recommended by OIE for diagnosis of the disease is based on trypanosome lysate antigen. However, antigenic variation and unstable nature of cell lysate antigen make it difficult to standardize the assay. Thus, there are needs to develop recombinant antigen-based ELISA that improve stability, sensitivity, and specificity of the test. Since tandem repeat (TR) proteins of trypanosomatid parasites generally possess high antigenicity, they have been considered to be the promising antigens for trypanosomosis and leishmaniosis. In this study, IgG responses against 14 recombinant TR proteins of trypanosomes were examined by ELISA. Serum samples were obtained from three water buffaloes experimentally infected with *T. evansi*. Since *Trypanosoma congolense* GM6 (TcoGM6) elicited highest IgG responses to all water buffaloes, we further bioinformatically and molecular biologically identified *Trypanosoma brucei brucei* GM6 (TbbGM6) and *T. evansi* GM6 (TeGM6) TR genes, respectively. As expected, predicted amino acid sequences of TbbGM6 and TeGM6 were identical while the nucleic acid sequence homology between TbbGM6 and TcoGM6 was 63.8%. All buffaloes became clearly positive in recombinant TbbGM6 (rTbbGM6)-based ELISA at 48 days post-infection, suggesting that rTbbGM6 is usable as a serodiagnostic antigen for chronic *T. evansi* infection.

N. T. Thuy · S.-I. Kawazu · N. Inoue (✉)
OIE Reference Laboratory for Surra, OIE Collaborating Centre,
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine,
Obihiro, Hokkaido 080-8555, Japan
e-mail: ircpmi@obihiro.ac.jp

Y. Goto
Laboratory of Molecular Immunology, Graduate School of
Agricultural and Life Sciences, The University of Tokyo,
1-1-1 Yayoi,
Bunkyo-ku, Tokyo 113-8657, Japan

Z.-R. Lun
Center for Parasitic Organisms,
State Key Laboratory of Biocontrol, School of Life Sciences,
Sun Yat-Sen (Zhongshan) University,
Guangzhou 510275, People's Republic of China

Introduction

Diagnosis of *Trypanosoma evansi* infection (surra) mainly relies on the identification of the parasites (OIE 2008). However, sensitivity of parasitological diagnosis is limited due to low parasitemia in infected animals. Molecular diagnostic assays have been developed to replace or complement the parasitological diagnosis (Desquesnes and Davila 2002; Thekisoe et al. 2005). Sensitivity of PCR detection methods reach 10 parasites/ml of blood (Desquesnes et al. 2001; Desquesnes and Davila 2002; Claes et al. 2004), and that of loop-mediated isothermal amplification assays is 0.1 parasite/ml blood (Reid and Copeman 2002; Ngaira et al. 2003). Since most of the molecular tests have not been validated yet, serological diagnosis is still retained as a common method.

OIE recommends use of trypanosome lysate antigen in ELISA for serological diagnosis of surra (OIE 2008). However, preparation of the lysate antigen requires in vitro (cell culture) or in vivo (experimental animals) systems to produce sufficient number of the parasite, and procedures for antigen preparation has not been completely standardized (Reid and Copeman 2002; OIE 2008; Tran et al. 2009). Therefore, development of antibody detection ELISA for

surra using defined antigens, such as recombinant proteins, has long been needed.

The recombinant variable surface glycoprotein RoTat1.2 was successfully expressed by insect cells, showing no differences to the native antigen in serological diagnostic tests of *T. evansi* infection in dromedary camels (Lejon et al. 2005). Nevertheless, it failed to detect *T. evansi* type B which lacks or does not express RoTat1.2 (Ngaira et al. 2005; Tran et al. 2009). Another promising recombinant protein is the invariant surface glycoprotein 75 (ISG75) presenting in approximately 5×10^4 molecules on trypanosome cell surface (Tran et al. 2009). However, limited number of available antigen candidates drives an urgent need of identification for novel antigens to improve serological diagnostics of surra.

Tandem repeat (TR) proteins of trypanosomatid parasites are often targets of B cell responses (Kemp et al. 1987; Reeder and Brown 1996; Goto et al. 2007). A number of *Leishmania* species and *Trypanosoma cruzi* TR proteins have been characterized and demonstrated their immunological dominance (Goto et al. 2007, 2008, 2010). Currently, rK39 of *Leishmania* and B13, CRA, TcD, and TcE of *T. cruzi* have been employed effectively in serodiagnosis of the diseases (Sundar et al. 1998; Ozerdem et al. 2009; Hernandez et al. 2010). Likewise, *Trypanosoma brucei* is also rich in proteins with large TR domains, and recently identified TR proteins showed reactivity to sera of trypanosome-infected mice (Goto et al. 2007, 2011). These results are encouraging us to further study on TR proteins of *T. evansi,* evolutionally closely related species to *T. brucei*, as candidate antigens for serological diagnosis of surra. Thus, the objective of this study is to screen bioinformatically identified TR proteins as candidate antigens for a serological diagnostic test of surra using infected sera obtained from water buffalo (*Bubalus bubalis*), a major natural host of *T. evansi*. Since *T. evansi* genome sequence data are not completed yet, we used the whole genome sequence data of *T. brucei* and *Trypanosoma congolense* which belong to the same genus of *T. evansi*. Genes encoding TR proteins were searched using the Tandem Repeats Finder program (Benson 1999). Fourteen TR proteins, nine of them were previously reported (Muller et al. 1992; Imboden et al. 1995; Goto et al. 2011) and five were newly identified in this study, were screened by ELISA for detecting IgG antibodies in sera from the water buffaloes experimentally infected with *T. evansi*.

Materials and methods

Identification and selection of TR genes DNA sequences data from *T. brucei brucei* (TbruceiTreu927Annotated CDS_TriTrypDB-2.2.fasta1), *Trypanosoma brucei gambiense* (TbruceiGambienseAnnotatedCDS_TriTrypDB-2_2) and *T. congolense* (TcongolenseAnnotatedCDS_TriTrypDB-2_2) were obtained from TriTrypDB (http://www.tritrypdb.org/). The Tandem Repeats Finder program was used to find out DNA sequences including TR as described previously (Benson 1999; Goto et al. 2007, 2008). In this study, the genes were regarded as TR if the scores obtained from the Tandem Repeats Finder analysis were 500 or higher. A high score indicates that the gene contains a large TR sequence and the repeat domain is highly conserved among the copies.

Production of recombinant TR proteins Procedure of recombinant protein expression was described previously (Goto et al. 2007, 2011). In brief, seven TR proteins of *T. brucei gambiense* were produced in a previous study (Goto et al. 2011). Nucleotides encoding partial TR domains of seven *T. congolense* proteins and *T. brucei brucei* GM6 were synthesized by GenScript USA. The synthesized genes were inserted into the pET-28a vector (EMD Biosciences) that express a gene-of-interest as 6-histidine-tagged recombinant protein, followed by transforming into *Escherichia coli* Rosetta (EMD Biosciences). Recombinant TR proteins were purified as soluble proteins using Ni-NTA agarose (QIAGEN) as previously described (Goto et al. 2011) or insoluble proteins from inclusion bodies under denaturing condition using Ni-NTA agarose according to the instructions of the manufacturer. Protein concentration was determined by bicinchoninic acid assay (Thermo Fisher Scientific), and purified recombinant TR proteins were stored at −80°C until use.

Trypanosome lysate antigens Bloodstream forms of *T. brucei brucei* (GUTat3.1) and *T. evansi* (Tansui) were propagated by in vitro culture (Hirumi et al. 1980) and utilized as the sources of trypanosome lysate antigens. Preparation of *T. brucei brucei* and *T. evansi* lysate antigens was described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (2008).

ELISA Recombinant TR antigens were screened by ELISA of which procedures were recommended in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (2008). Each well of microplates (Maxisorp, Nalgene-Nunc) was coated with 1 μg of trypanosome lysate antigen or 200 ng of each TR antigen and incubated for 4 h at room temperature. Then, antigen-coated wells were washed five times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T), once with PBS and incubated with blocking solution (PBS-T containing 1% bovine serum albumin (BSA)) for overnight at 4°C. Serum samples diluted at 200 times with PBS-T containing 0.1% BSA were applied (100 μl) into each well and incubated at room temperature for 2 h. According to the manufacturer's

instructions, horseradish peroxidase-conjugated protein G (Invitrogen) and tetramethylbenzidine (Kirkegaard & Perry Laboratories) were utilized for detection of antigen–antibody reaction. Finally, 50 μl of stop solution (1 M phosphoric acid) was added and the absorbance was read at 450 nm.

Water buffaloes and serum samples Three water buffaloes, which were both microhematocrit method and CATT/*T. evansi* (ITM, Belgium) negative, were experimentally infected with *T. evansi* cpogz strain. Each water buffalo was inoculated intravenously with 1×10^4 parasites. Sera were collected from the jugular vein by using heparinized syringe at five time points, namely 22 days before infection (pre), 8, 15, 22, and 48 days post-infection (DPI). Parasitemia was examined by microscopy (wet blood film method) each time after serum collection. All water buffaloes were handled in accordance with Animal Welfare guidelines in Sun Yat-Sen (Zhongshan) University, China.

Cloning and sequencing of T. evansi GM6 Gene encoding *T. evansi* GM6 was amplified using the following PCR primers: 5′-ATG GAG CTT GCT AAA CTG AA-3′ and 5′-CTA ATG TGA ATG CTC GTT CAT-3′. The PCR mixture (50 μl) contained 1.5 mM $MgCl_2$, 2 mM of each dNTP, 1 μM of each primer, and 5 unit of *Taq* DNA polymerase (Invitrogen). Reactions were conducted for 30 cycles, at 94°C for 30 s (denaturation), 54°C for 30 s (annealing), and 72°C for 1.5 min (extension). PCR products containing different TR units of GM6 were separated by gel electrophoresis. DNA fragments consisting of one and two repeat domains were extracted using gel extraction method (QIAGEN) and ligated into vector pCR2.1-TOPO (Invitrogen), then transformed into *E. coli* DH5α. After the digestion with *Eco*RI restriction enzyme, direct sequencing of the inserts was carried out with the abovementioned PCR primers and BigDye Terminator Ready Mix (Applied Biosystems) using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Nucleotide and amino acid sequences were identified and analyzed by using Genetyx version 8.0 (Genetyx) and BLAST (http://blast.ncbi.nlm.nih.gov/).

Results

Experimental infection

In order to produce a series of negative and positive control sera in a natural host for screening of TR antigens, three water buffaloes were experimentally infected with *T. evansi*. Sera were collected from the jugular vein by using heparinized syringe at five time points described in "Materials and methods." Although wet blood films were examined by microscopy each time after serum collection, all animals did not show parasitemia. Nevertheless, increasing antibody responses against trypanosome lysate antigen detected by *T. brucei* and *T. evansi* lysate ELISAs clearly indicated progression of the infection (Fig. 1). Sera collected before the infection was used as negative controls, and the cutoff values (mean + 3SD) of *T. brucei* lysate ELISA and *T. evansi* lysate ELISA were calculated as 0.21 and 0.44, respectively. Based on the cutoff values, serum samples from all water buffaloes were positively detected from 15 DPI onward.

Identification of TR proteins

As listed in Table 1, 14 TR proteins were examined in this study for their reactivity against the infected water buffalo sera. Since the whole genome sequence of *T. evansi* are not completed at present, *T. brucei brucei* and *T. brucei gambiense* were considered as the first candidates to search for the TR antigens. In a previous study, *T. brucei*

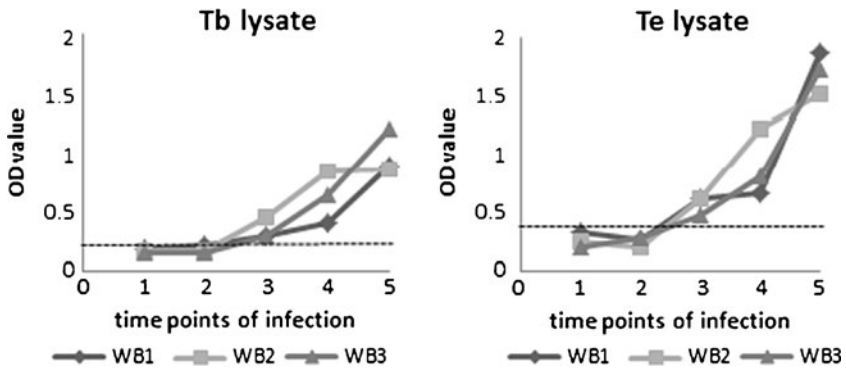

Fig. 1 IgG antibody to *T. evansi* detected in water buffalo sera by ELISA using *T. brucei brucei* (*Tb lysate*) and *T. evansi* (*Te lysate*) lysate antigens. *WB1*, *WB2*, and *WB3* indicate water buffalo no. 1, 2, and 3, respectively. *Numbers shown below the x-axis* indicate 22 days before infection (*1*), 8 (*2*), 5 (*3*), 22 (*4*), and 48 (*5*) DPI. Cutoff values (shown by the *broken lines*) of Tb lysate ELISA and Te lysate ELISA were 0.21 and 0.44, respectively

Table 1 TR proteins screened in this study

	Name[a]	Ref.[b]	Gene ID	PS	CN	Score
1	TbgMARP	a, c	Tbg972.10.12630	114	57.3	12,689
2	TbgI2	a, b	Tbg972.3.6010	405	15.5	9,986
3	TgbI17	a, b	Tbg972.10.13390	57	12.7	1,331
4	Tbg2	a	Tbg972.7.3660	105	73.5	9,671
5	Tbg4	a	Tbg972.7.2590	195	15.5	5,847
6	Tbg5	a	Tbg972.10.1660	237	12	5,623
7	Tgb6	a	Tbg972.7.4640	186	14.3	5,180
8	TcoGM6	c	TcIL3000.11.1030	204	4	1,585
9	TcoMARP	c	TcIL3000.10.8840	114	18.7	4,159
10	Tco2		TcIL3000.0.13300	42	115.5	7,611
11	Tco3		TcIL3000.0.50300	84	12.4	2,032
12	Tco5		TcIL3000.0.57170	162	15.5	4,485
13	Tco9		TcIL3000.0.20560	96	9	1,616
14	Tco11		TcIL3000.0.04370	27	60.1	2,717

PS period size, *CN* copy number

[a]Names of previously characterized proteins or those of recombinant proteins designed for this study

[b]Antigenicity of the proteins were examined/characterized in the indicated references ((a) Goto et al. 2011; (b) Imboden et al. 1995; (c) Muller et al. 1992)

gambiense TR proteins were produced and analyzed (Goto et al. 2011). The *T. brucei gambiense* TR proteins used in the present study are highly conserved (97–100%) in *T. brucei brucei* (Goto et al. 2011). In addition, TR proteins from *T. congolense* were included to this study as belonging to the same genus as *T. evansi*. The genome sequence data of *T. congolense* were surveyed by using computational method. When employing a TR cutoff score of 500, 283 out of 13,459 analyzed genes (2.1%) were identified as genes encoding a TR protein, and 89 out of the 283 genes had the score of 2,000 or higher. The 89 TR genes include the genes encoding GM6 and MARP, which were previously reported antigens of *T. congolense* (Muller et al. 1992). Unlike *T. brucei*, the whole genome sequence of *T. congolense* has not been completed, some annotated genes, such as TcIL3000.0.43110, do not contain either a start codon or a stop codon. Therefore, in order to assure the selection of functional *T. congolense* protein coding genes for serological evaluations, we utilized EMBL EST database for secondary screening to obtain evidence of gene expression. For example, although Tco3 and Tco9 ranked relatively low by TR prediction (scores of 2,032 and 1,616, respectively) (Table 1), they did possess evidences of gene expressions in the bloodstream form that is the developmental stage of African trypanosomes in their mammalian hosts. These genes were therefore selected for serological evaluations.

Screening of TR proteins

For preliminary screening of TR proteins by ELISA, sera collected at 22 days before infection (pre) and 48 DPI from the three water buffaloes experimentally infected with *T. evansi* were used. Figure 2 shows the reactivity of those serum samples against 14 TR proteins. From seven TR proteins of *T. brucei gambiense,* only Tbg5 and TbgMARP demonstrated the OD values greater than 0.5 in serum samples from water buffalo no. 3 and 1, respectively. TbgI2 and TbgI17 were also antigenic, but weak as indicated by low OD values. Among seven TR proteins of *T. congolense,* TcoMARP, Tco11, and TcoGM6 were able to detect antibody to *T. evansi* in the water buffalo sera. Among those, antigenicity of TcoGM6 was the strongest with OD values greater than 0.5 in serum samples from all of the three water buffaloes. Thus, TcoGM6 was further investigated for its potential as a serodiagnostic antigen.

Amino acid sequence analyses of GM6 TR domains

Amino acid sequences of GM6 homologs from different Trypanosomatidae parasites obtained from TriTrypDB were aligned to examine the conservation of GM6 (Fig. 3). TcoGM6 shared only 63.8% identity to TbbGM6, a closer subspecies to *T. evansi*. It suggests that TbbGM6 has stronger serological cross reactivity than that of TcoGM6 against *T. evansi*-infected sera. Hence, partial cloning and sequence analysis of TR unit of TeGM6 was conducted. The result revealed that the cloned TR unit was identical to the same portion of TbbGM6, indicating that TR unit was highly conserved between the *T. brucei brucei* and *T. evansi* (Fig. 3). Although functional significance and antigenicity were unknown, hexapeptide domain consists of PEGVPL was conserved among *Trypanosoma* and *Leishmania*.

Antibody responses against GM6 TR domains

Consequently, two copies of TbbGM6 and TcoGM6 TR units were expressed as recombinant TR proteins and their

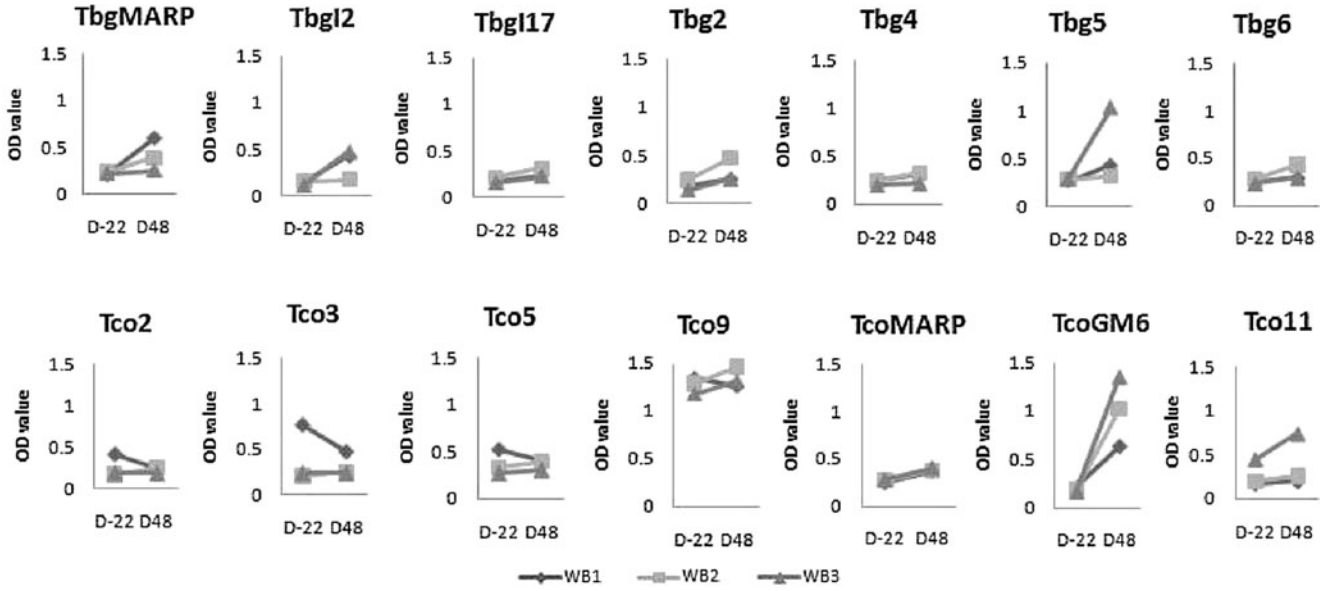

Fig. 2 Screening of 14 TR proteins from *T. brucei gambiense* and *T. congolense*. *WB1*, *WB2*, and *WB3* indicate water buffalo no. 1, 2, and 3, respectively. *D-22* and *D48* indicate water buffalo sera obtained at 22 days before infection and 48 DPI, respectively

antigenicity were evaluated by ELISA using series of *T. evansi*-infected water buffalo sera obtained at pre, 8, 15, 22, and 48 DPI. Serum samples at pre were employed as negative controls to calculate the cutoff values (mean + 3SD) of TbbGM6- and TcoGM6-based ELISAs, giving 0.31 and 0.17, respectively. As compared with the trypanosome lysate antigen-based ELISA (Fig. 1), individual differences in antibody responses were obvious in both TbbGM6- and TcoGM6-based ELISAs (Fig. 4). Water buffaloes no. 2 and 3 became positive to both TbbGM6 and TcoGM6 on 15 DPI, whereas water buffalo no. 1 took longer (48 DPI) to turn positive. TbbGM6 demonstrated higher OD values at 15 DPI than TcoGM6 in water buffalo no. 2 and 3.

Discussion

In this study, seven TR proteins of *T. brucei gambiense* and seven TR proteins of *T. congolense* were screened by ELISA using *T. evansi*-infected water buffalo sera. Trypanosome lysate antigen-based ELISA were utilized as the standard test (OIE 2008). The trypanosome lysate antigen-based ELISA was able to detect the antibody responses in

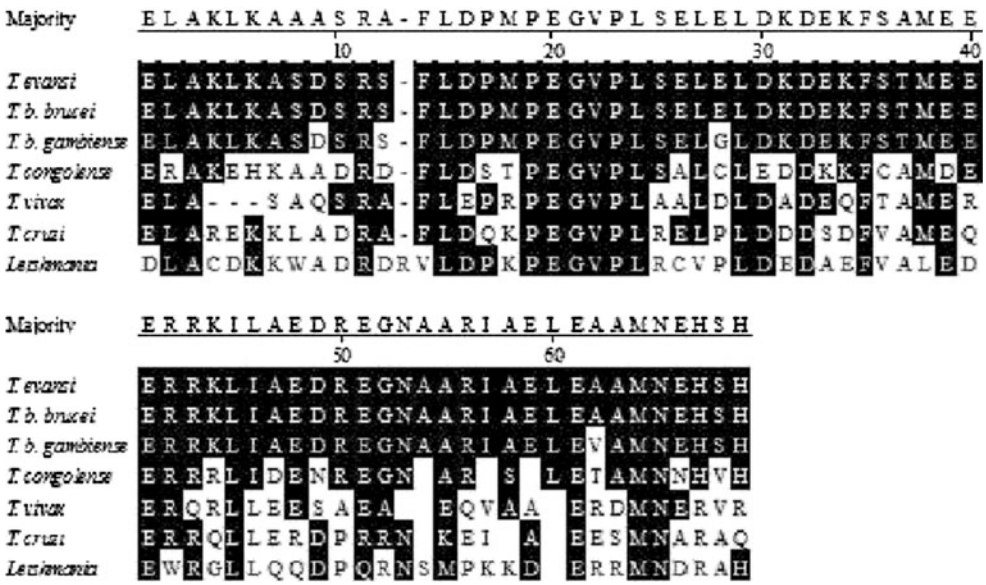

Fig. 3 TR unit of GM6 are highly conserved in *Trypanosoma* and *Leishmania*. Single TR unit of GM6 consists of 68 amino acids. The *dark color* indicates identical amino acids more than two species

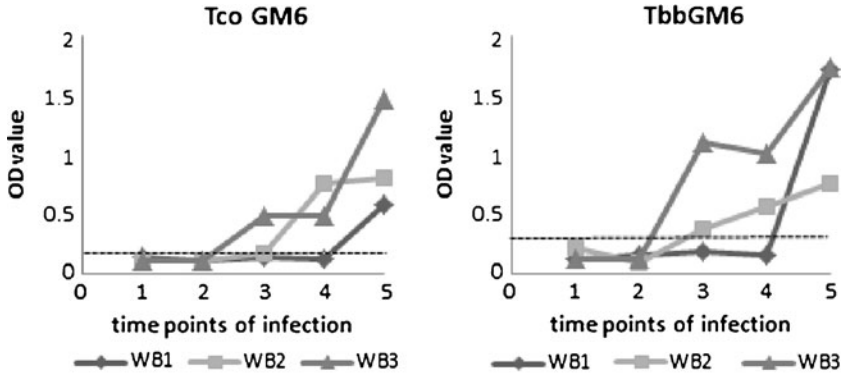

Fig. 4 Immuno-reactivity of recombinant TcoGM6 and TbbGM6 to water buffalo sera collected at five time points of infection. *WB1*, *WB2*, and *WB3* indicate water buffalo no. 1, 2, and 3, respectively. *Numbers shown below the x-axis* indicate 22 days before infection (*1*), 8 (*2*), 5 (*3*), 22 (*4*), and 48 (*5*) DPI. Cutoff values (shown by the *broken lines*) of TcoGM6-ELISA and TbbGM6-ELISA were 0.17 and 0.31, respectively

the three water buffaloes experimentally infected with *T. evansi* from 15 DPI onward while microscopy of wet blood smear was negative throughout the experiment. Additionally, *T. brucei* and *T. evansi* lysate antigens demonstrated similar patterns in ELISA to detect IgG antibody to *T. evansi*; all water buffaloes were positively detected from 15 DPI onward and the OD values reached the highest level at 48 DPI. Although OD values of *T. brucei* lysate was slightly lower, it could be a substitute to *T. evansi* lysate in the case *T. evansi* resource is not available.

The water buffalo sera responded to all seven TR proteins from *T. brucei gambiense*, observed through the increase of OD values. TbgI2, TbgI17, and TbgMARP were previously reported having strong immunological reactivity against sera from cattle infected with *T. brucei brucei, T. congolense* or *Trypanosoma vivax* (Muller et al. 1992; Imboden et al. 1995). However, TR regions of these antigens showed low reactivity to *T. evansi*-infected water buffalo sera (Fig. 2). Similarly, TR regions of Tbg4 and Tbg6 which could detect antibodies in mice infected with *T. brucei gambiense* did not show high reactivity to the water buffalo sera (Goto et al. 2011). Taken together, serological reactivity of TR antigens varies by trypanosome and host species. In addition, except for TbgI17, Tbg4, Tbg6, and TcoMARP, the levels of antibody responses to each TR protein obviously varied among the water buffaloes (Fig. 2). Such variation of antibody responses in trypanosome infection was also observed in *T. evansi* experimentally infected sheep (Onah et al. 1999). This phenomenon could be a considerable obstacle in the screening of a suitable diagnostic TR antigen which is able to elicit strong antibody responses in most of all *T. evansi*-infected individuals.

TcoGM6, a flagellum-associated protein, can be recognized by the immune system when trypanosomes are destroyed in an early phase of the infection (Muller et al. 1992). Through the screening of TR antigens, TR region of TcoGM6 demonstrated highest reactivity to the sera obtained from *T. evansi*-infected water buffaloes (Fig. 2). Like trypanosome lysate antigen-based ELISAs which could detect anti-*T. evansi* IgG responses from 15 DPI (Fig. 1), OD values of TcoGM6- and TbbGM6-based ELISAs also started increasing from 15 DPI onward in serum samples from water buffaloes no. 2 and 3 (Fig. 4). Recombinant GM6-based ELISAs could detect antibody response better than trypanosome lysate antigen-based ELISAs in sera of water buffalo no. 3 on 15 DPI. However, both TcoGM6 and TbbGM6 were not able to detect antibodies in sera of water buffalo no. 1 up to 48 DPI. Although differences of antibody responses in individual water buffaloes in TcoGM6 and TbbGM6 were observed, all water buffaloes eventually became positive on 48 DPI. Since the number of repeat unit affects the binding affinity between TR antigens and antibodies (Goto et al. 2010; Valiente-Gabioud et al. 2011), reactivity of the recombinant GM6 antigens, which consisted of two repeat units, might be improved by increasing number of the repeat. Taking into consideration both advantages and disadvantages of the recombinant GM6 over trypanosome lysate antigens, GM6 could be a good candidate serodiagnostic antigen for chronic *T. evansi* infection. Since GM6 was conserved among trypanosomatid parasites, cross-reaction is possible among trypanosome species (Fig. 3). Both TcoGM6 and TbbGM6 showed serological cross reactivity to water buffalo sera indicated by high OD values in ELISA. Previous study also characterized two antigens from *T. evansi* which were able to detect antibody to *T. vivax* effectively (Camargo et al. 2004). These findings suggest that antigens from one species may be used as the diagnostic antigens and/or vaccine candidates for the infections of other species belonging to the same genus *Trypanosoma* (Li et al. 2009).

In conclusion, recombinant TR protein, GM6, exhibits proficient characteristics of being a candidate diagnostic antigen. Since TbbGM6 and TeGM6 were identical in amino acid sequences, either of these TR proteins are candidate diagnostic antigens in the development of recombinant antigen-based serological diagnostic methods for both surra and nagana. However, further study need to be conducted with a larger number of serum samples to evaluate and optimize the assay condition.

Acknowledgments The authors thank Dusit Laohasinarong, Jose Angeles, and Hassan Hakimi for technical assistances. This study was partly supported by the Ministry of Education, Cultural, Sports, Science and Technology and Japan Society for the Promotion of Science.

References

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Camargo RE, Uzcanga GL, Bubis J (2004) Isolation of two antigens from *Trypanosoma evansi* that are partially responsible for its cross-reactivity with *Trypanosoma vivax*. Vet Parasitol 123:67–81

Claes F, Radwanska M, Urakawa T, Majiwa PA, Goddeeris B, Büscher P (2004) Variable Surface Glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. Kinetoplastid Biol Dis 3:3

Desquesnes M, Davila AM (2002) Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. Vet Parasitol 109:213–231

Desquesnes M, McLaughlin G, Zoungrana A, Davila AM (2001) Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. Int J Parasitol 31:610–614

Goto Y, Coler RN, Reed SG (2007) Bioinformatic identification of tandem repeat antigens of the *Leishmania donovani* complex. Infect Immun 75:846–851

Goto Y, Carter D, Reed SG (2008) Immunological dominance of *Trypanosoma cruzi* tandem repeat proteins. Infect Immun 76:3967–3974

Goto Y, Carter D, Guderian J, Inoue N, Kawazu S, Reed SG (2010) Upregulated expression of B-cell antigen family tandem repeat proteins by *Leishmania* amastigotes. Infect Immun 78:2138–2145

Goto Y, Duthie MS, Kawazu S, Inoue N, Carter D (2011) Biased cellular locations of tandem repeat antigens in African trypanosomes. Biochem Biophys Res Commun 405:434–438

Hernandez P, Heimann M, Riera C, Solano M, Santalla J, Luquetti AO, Beck E (2010) Highly effective serodiagnosis for Chagas' disease. Clin Vaccine Immunol 17:1598–1604

Hirumi H, Hirumi K, Doyle JJ, Cross GA (1980) In vitro cloning of animal-infective bloodstream forms of *Trypanosoma brucei*. Parasitology 80:371–382

Imboden M, Muller N, Hemphill A, Mattioli R, Seebeck T (1995) Repetitive proteins from the flagellar cytoskeleton of African trypanosomes are diagnostically useful antigens. Parasitology 110(Pt 3):249–258

Kemp DJ, Coppel RL, Anders RF (1987) Repetitive proteins and genes of malaria. Annu Rev Microbiol 41:181–208

Lejon V, Claes F, Verloo D, Maina M, Urakawa T, Majiwa PA, Büscher P (2005) Recombinant RoTat 1.2 variable surface glycoprotein as antigen for diagnosis of *Trypanosoma evansi* in dromedary camels. Int J Parasitol 35:455–460

Li SQ, Yang WB, Ma LJ, Xi SM, Chen QL, Song XW, Kang J, Yang LZ (2009) Immunization with recombinant actin from *Trypanosoma evansi* induces protective immunity against *T. evansi*, *T. equiperdum* and *T. b. brucei* infection. Parasitol Res 104:429–435

Muller N, Hemphill A, Imboden M, Duvallet G, Dwinger RH, Seebeck T (1992) Identification and characterization of two repetitive non-variable antigens from African trypanosomes which are recognized early during infection. Parasitology 104 (Pt 1):111–120

Ngaira JM, Bett B, Karanja SM, Njagi EN (2003) Evaluation of antigen and antibody rapid detection tests for *Trypanosoma evansi* infection in camels in Kenya. Vet Parasitol 114:131–141

Ngaira JM, Olembo NK, Njagi EN, Ngeranwa JJ (2005) The detection of non-RoTat 1.2 *Trypanosoma evansi*. Exp Parasitol 110:30–38

OIE (2008) Manual of diagnostic tests and vaccines for terrestrial animals, 6th edn. OIE, Paris

Onah DN, Hopkins J, Luckins AG (1999) Changes in peripheral blood lymphocyte subpopulations and parasite-specific antibody responses in *Trypanosoma evansi* infection of sheep. Parasitol Res 85:263–269

Ozerdem D, Eroglu F, Genc A, Demirkazik M, Koltas IS (2009) Comparison of microscopic examination, rK39, and PCR for visceral leishmaniasis diagnosis in Turkey. Parasitol Res 106:197–200

Reeder JC, Brown GV (1996) Antigenic variation and immune evasion in *Plasmodium falciparum* malaria. Immunol Cell Biol 74:546–554

Reid SA, Copeman DB (2002) Evaluation of an antibody-ELISA using five crude antigen preparations for the diagnosis of *Trypanosoma evansi* infection in cattle. Vet Parasitol 104:79–84

Sundar S, Reed SG, Singh VP, Kumar PC, Murray HW (1998) Rapid accurate field diagnosis of Indian visceral leishmaniasis. Lancet 351:563–565

Thekisoe OM, Inoue N, Kuboki N, Tuntasuvan D, Bunnoy W, Borisutsuwan S, Igarashi I, Sugimoto C (2005) Evaluation of loop-mediated isothermal amplification (LAMP), PCR and parasitological tests for detection of *Trypanosoma evansi* in experimentally infected pigs. Vet Parasitol 130:327–330

Tran T, Claes F, Verloo D, De Greve H, Büscher P (2009) Towards a new reference test for surra in camels. Clin Vaccine Immunol 16:999–1002

Valiente-Gabioud AA, Veaute C, Perrig M, Galan-Romano FS, Sferco SJ, Marcipar IS (2011) Effect of repetitiveness on the immunogenicity and antigenicity of *Trypanosoma cruzi* FRA protein. Exp Parasitol 127:672–679