Published in final edited form as:

Parasitol Int. 2011 December ; 60(4): 538–540. doi:10.1016/j.parint.2011.09.003.

Serological characterizations of tandem repeat proteins for detection of African trypanosome infection in cattle

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Abstract

Serological diagnosis is a useful method to detect African trypanosome infection in livestock animals. Currently available serological tests utilize whole parasites or crude antigens, and recombinant antigens may improve reproducibility/standardization and reduce production costs. With a goal of identifying such recombinant proteins, we computationally identified proteins with tandem repeat (TR) domain from the parasite proteomes and evaluated their potential for serological diagnosis of African trypanosome infections in cattle. Among those tested, Tbg4 demonstrated the best performance with 92% sensitivity, followed by TbbGM6 (85%), TcoGM6 (85%), Tbg2 (65%) and Tbg5 (65%). Although further evaluations such as investigating cross-reactivity to other infections are needed, our data indicate the potential of these antigens for detection of African trypanosome infection in cattle.

Keywords

African trypanosome; tandem repeat; serological diagnosis

Animal African trypanosomiasis (AAT), also known as Nagana, affects 37 countries, most in Africa, where tsetse infest 10 million square kilometers [1]. Although AAT can affect various mammals, it is particularly important in cattle, representing a major obstacle for the economic development in Africa [2]. Serology has been used for detection of African trypanosome infections in livestock animals [1]. Indirect fluorescent antibody test and enzyme-linked immunosorbent assay (ELISA) are routinely used for the detection of anti-*Trypanosoma* antibodies in cattle [1]. To date, such serological tests use either whole parasites or crude parasite lysate as the antigen, requiring large-scale in vitro or in vivo cultivation of parasites followed by antigen processing that could bring batch-to-batch

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variations. In contrast, recombinant antigens such as ISG75 and HSP70/BiP have shown promise in detection of trypanosome infection in animals [3, 4]. Proteins with tandem repeat (TR) domain have been identified as targets of B-cell response from various protozoan parasites including *Trypanosoma cruzi* and *Leishmania* species [5, 6]. Based on these reports, we have developed a computational method to search genes encoding TR protein as a tool for identification of antigens with serological significance [7–9]. In this presented study, we evaluated African trypanosome TR proteins for their potential to detect infection of the parasites in cattle.

A total of 12 TR proteins were then evaluated by ELISA for ability to antibodies in cattle with African trypanosome infection (see supplementary data for recombinant protein production and ELISA protocols). Cattle serum samples, collected in the Butaleja district, eastern Uganda during the early dry season (July, 2006) [10], were used. Within eastern Uganda, cattle in the Butaleja district and the Tororo district (less than 40 km distant from Butaleja) have been studied for infecting *Trypanosoma* species; in both districts, *T. brucei* subspecies dominated the infecting species over *T. congolense* and *T. vivax*, the species virulent to cattle [10, 11]. Blood samples from these animals had been examined for parasitemia by microhematocrit centrifuge test (MHC). Among them, parasitemia was confirmed in 18 cattle (designated here as Tryp+). PCR was carried out to determine the infecting species; 5 were positive for *T. brucei*, 1 was positive for both *T. brucei* and *T. congolense*, whereas the remaining 12 were negative in the PCR analyses. Eight cattle negative by MHC but positive by PCR analysis were also included into the Tryp+ group; 5 were positive for *T. brucei*, 2 were positive for *T. congolense*, and 1 was positive for both *T. vivax* and *T. congolense*. Sera from 20 cattle in Hokkaido, Japan were kindly provided by Dr. Naoaki Yokoyama (Obihiro University of Agriculture and Veterinary Medicine) and used as controls from a non-endemic area and therefore *bona fide* negative (non-endemic controls: NEC). Fifty cattle negative by both MHC test and PCR from the same endemic area (endemic controls: EC) were also analyzed. For each antigen, the cutoff value was calculated as the mean + 3SD of NEC samples. Both *T. congolense* lysate and *T. b. gambiense* lysate showed 100% sensitivity (Figure 1 and Figure S1). These lysate antigens were produced using the procyclic form (insect stage) where VSGs are not expressed, indicating 100% sensitivity is achievable without using VSGs as antigens. In contrast, any of TR antigens could not achieve 100% sensitivity in detecting trypanosome infection. Among recombinant antigens, Tbg4 showed the best sensitivity (92%) followed by TbbGM6 (85%), TcoGM6 (85%), Tbg2 (65%) and Tbg5 (65%), as shown in Figure 1. Other antigens had their sensitivity 50% or less (Figure S1). These results may be due to heterogeneity in responses to individual antigens. Figure 2 shows antigen recognition patterns by the parasite-positive cattle. A good example is Tbg117; the antigen could not detect antibodies even in the highest responder to lysate antigen (No. 18), whereas the lowest responder (No. 1) showed the strongest response to this antigen. In the case of human visceral leishmaniasis, rK39, the TR protein of the related trypanosomatid parasite *Leishmania infantum*, dominates humoral response during active disease, making its diagnostic potential very high [6]. In contrast, antibody responses during active tuberculosis are heterologous [12]. Therefore, it is anticipated that one component does not always serve as a perfect serodiagnostic antigen. Even in this situation, achievement of higher sensitivity may be possible by using multiple antigens complementing each other, as demonstrated for tuberculosis and Chagas' disease [12, 13]. Of course, careful considerations are need for selection and optimization of antigen mixture as using multiple antigens may compromise specificity of the test.

Fifty EC cattle were analyzed for antibodies to *T. congolense* lysate, Tbg2, Tbg4, Tbg5, Tbg6, TbbGM6 and TcoGM6. By using the cutoff value calculated as the mean + 3SD of NEC samples, 38 (76%) of the EC cattle were positive for *T. congolense* lysate (Figure 1).

To other antigens, the positive rates ranged from 36% to 72%. Ratios of positivity between Tryp+ and EC groups were also diverse, ranging from 1.08 (Tbg5) to 1.92 (Tbg4). The reason why a large portion of EC cattle were positive by ELISA while being negative by MHC and PCR may be explained by low sensitivity of parasitological diagnosis and PCR method, subclinical infections, or false positive reaction in serology. Although PCR generally gives high sensitivity, that of PCR methods used in this study population was only 33% even in parasitologically-confirmed cases, as described above. Therefore, a certain portion of EC cattle in the study area may be infected with African trypanosomes. More sensitive methods like loop-mediated isothermal amplification need to be included in future studies in order to understand the true status of those serologically-positive EC cattle. The lysate antigen detected a higher number of EC cattle as antibody-positive than did Tbg4. Although we do not have direct evidence, Tbg4 is theoretically a more specific diagnostic antigen than lysate antigen because the repeat motif of Tbg4 is trypanosome-specific and because lysate antigens are generally cross-reactive [14]. In addition, defined antigens like HSP/BiP have demonstrated more rapid decline in antibody titers after successful treatment than lysate antigens [15]. Therefore, although further studies are needed, serology using TR antigens may serve as a tool complementing existing methods for more comprehensive epidemiological studies on African trypanosome infections. Because immunochromatographic test would be the most desired format for application in remote areas where AAT occurs, future studies should be directed to prioritization of the TR antigens for optimal ICT development, which may be different from that of ELISA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by KAKENHI (20405041, 21380186 and 21880007), the National Institutes of Health grant AI082800-01, a Cooperative Research Grant (23-joint-10) of National Research Center for Protozoan Diseases, Obihiro University of Agricultural and Veterinary Medicine, and a grant for the Obihiro University of Agriculture & Veterinary Medicine Global COE program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Highlights

- Tandem repeat (TR) proteins are targets of B-cell response in protozoan diseases.
- Computationally identified TR proteins are antigenic during African trypanosomiasis.
- TR antigens of African trypanosomes are applicable for diagnostic tests for cattle.

Figure 1. Detection of antibodies to trypanosomal TR proteins in Ugandan cattle

Selected TR proteins of African trypanosomes as well as *T. congolense* lysate were evaluated for serological reactivity by ELISA using individual sera from Ugandan cattle showing parasitemia or positive by PCR (Tryp+, ●) those from the same endemic area negative by both MHC and PCR analyses (EC, ▲), or Japanese cattle (NEC, ○), diluted at 1:200. Bars represent mean OD values for individual groups. Dashed lines represent cutoff values for individual antigens, calculated as the mean + 3SD of OD values of NEC. Percentages indicate the ratios of positive cattle in individual groups.

Figure 2. Variable recognition of TR antigens by cattle with African trypanosome infection
 ID numbers of MHC-positive cattle (shown on the top) were assigned based on the reactivity to *T. congolense* lysate (from low to high). Reactivity of an animal serum sample to an antigen is expressed with a color indicator (light green for more than mean + 3SD; blue for more than mean + 5SD; black for more than mean + 10SD, as referred to on the bottom).