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ORIGINAL ARTICLE

Classico-molecular targeting of oligopeptidase B, cysteine protease and variable surface glycoprotein (VSG) genes of *Trypanosoma evansi*

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Abstract Trypanosomosis or Surra can rightly be attributed as the most economically important vector-borne haemoprotozoan disease encountering India. Surra infected chronic cases show almost similar types of signs and symptoms often confusing it with other haemoprotozoan infections, thereby, making it prerequisite for the development of aspecific and sensitive technique for its detection in susceptible animals. Blood microscopy and serology suffers from the hands of lack of sensitivity and specificity thereby leaving molecular detection techniques as one of the promising alternative. Alongside, there is utmost need for exploring of new molecular gene targets for the development of a putative alternative for diagnosis and immunoprophylaxsis. The present communication describes the identification and amplification of oligopeptidase B, cysteine protease and variable surface glycoprotein genes of *T. evansi* so as to exploit them in future as potential candidates for immune protection and/or molecular detection.

Keywords Cysteine protease · Oligopeptidase B · *Trypanosoma evansi* · Variable surface glycoprotein

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Introduction

Trypanosomosis or Surra is an important vector-borne protozoan disease that imposes serious constraints on the health and productivity of domestic animals in tropical and sub-tropical regions throughout the world. This causative agent for surra, a haemoflagellate *Trypanosoma evansi*, has established itself in Southeast Asia for close to a century (Luckins 1988). Surra is widely prevalent in different parts of the country and is of significant economic importance in livestock production (Singh and Tiwari 2012) alongside causing deleterious effects on health status of animals (Pandey et al. 2015). The total net benefit from effective surra control for a typical village in a moderate/high risk area was estimated to be US $158,000 per annum (Dobson et al. 2009). Following recovery from primary infection, animals become persistent carriers acting as reservoirs of infection thereby playing a critical role in disease epidemiology. The situation becomes even worse in chronic cases of recurrence under stress conditions or due to overuse of corticosteroids (Kumar et al. 2012). Serological tests like indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) are capable of detecting antibodies in carrier animals and hence are routinely used for monitoring surveillance (Singh et al. 2015; Sudan et al. 2015a, b) and export certification, but they too suffer on the grounds that antibodies can be detected even years after recovery of infection, though no active infection is prevalent, thereby obscuring the exact picture of prevalence of infection at that particular point. PCR is the most accurate tool for the diagnosis of subclinical and latent infections (Sudan et al. 2014; Parashar et al. 2015). This stresses the relevance of molecular PCR-based techniques. Moreover, there is utmost need of exploring newer molecular targets for assessing their role

as a putative candidate for both prophylaxis and diagnosis. The present study was designed to amplify the oligopeptidase B, cysteine protease and variable surface glycoprotein (VSG) genes of *T. evansi* so as to exploit them in future as potential candidates for immune protection and/or molecular detection.

Materials and methods

Collection of blood samples

Blood [1 ml aliquot in clean sterile vacutainers, containing ethylene diamine tetra acetic acid (EDTA)] and stored plasma samples were taken from earlier *T. evansi* confirmed animals (microscopic observation of blood smears).

DNA isolation from whole blood

DNA was isolated using commercial blood genomic DNA purification kit (Banglore GeNei^{TM}) following manufacturers protocol. The concentration of the purified DNA was measured by Nanodrop (Eppendroff).

Primers selection and Polymerase chain reaction (PCR)

Primers corresponding to oligopeptidase B (opd b F and R), cysteine protease (CP F and R) and variable surface glycoprotein (VSG F and R) genes of *T. evansi* were custom synthesized. The PCR reaction was set up into 25 µl volume containing 12.5 µl of 2× DreamTaq Green PCR Master Mix (Thermo scientific) containing DreamTaq DNA polymerase with blue and yellow dyes in reaction mixture buffer, 4 mM $MgCl_2$, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP, and 0.4 mM dTTP, 1.5 µl of each primer (15 p moles), 2 µl of the DNA template and total volume was made up to 25 µl using Nuclease free water. The primer sequence, thermocyclic conditions along with the expected size of amplicons are described in Table 1. The PCR amplicons were later analyzed by agarose gel electrophoresis on 1.5 % agarose gel containing ethidium bromide.

Results and discussion

The kit was able to isolate DNA from both blood and plasma samples as was verified by Nanodrop values. The PCR was laboratory standardized and was able to amplify all the three genes. PCR amplification of opd b, CP, VSG genes of *T. evansi* revealed DNA fragment of 2092, 432, 681 bp lengths, respectively (Fig. 1).

Blood smear examination is known to be of limited value in diagnosis of subacute or chronic cases of trypanosomosis (Sudan et al. 2015a, c). No doubt, the conventional parasitological techniques will always remain important for understanding the biology, ecology and molecular epidemiology of different strains of the parasites yet there is need of a highly sensitive test that can detect the lowest levels of parasitemia (Singh and Tiwari 2012). Herbert and Lumsden (1976) reported non feasibility of microscopic detection in trypanosomiosis when the parasite number is <2,500,000 parasites/ml of blood. In serological tests, antibodies to *T. evansi* infection persist after drug treatment, complicating the differentiation of patent

Table 1 Primer sequence, PCR conditions as well as size of amplified amplicon

Primer	Primer sequence	Amplicon size (bp)	References
Opd b F	5′GGACACATATGATGCAAACTGAACGTGGTCC3′	2092	Morty et al. (2005)
Opd b R	5′TACGCTCATATGCTACTTCCGCAGCAGCGGCC3′		
CP F	5′CTGGGCCTTTTCAACTATC3′	432	Self synthesized
CP R	5′TAACCAACGAGGAGCACAC3′		
VSG F	5′GGGAATTCATGCAAACCAAGGCGCTCGTTGGCGT3′	681	Sengupta et al. (2012)
VSG R	5′CGGGAATTCCTTGATGTTGCTGGTCGCGATTTTGATC3′		

	Initial denaturation	Denaturation	Annealing	Extension	Termination
Thermal cycling profile					
PCR with opd b gene	94 °C, 240 s	94 °C, 45 s ×35 cycles	57 °C, 60 s	72 °C, 60 s	72 °C, 600 s
PCR with CP gene	95 °C, 120 s	94 °C, 45 s ×40 cycles	48 °C, 60 s	72 °C, 300 s	72 °C, 600 s
PCR with VSG gene	95 °C, 120 s	94 °C, 45 s ×35 cycles	60 °C, 60 s	72 °C, 120 s	72 °C, 600 s

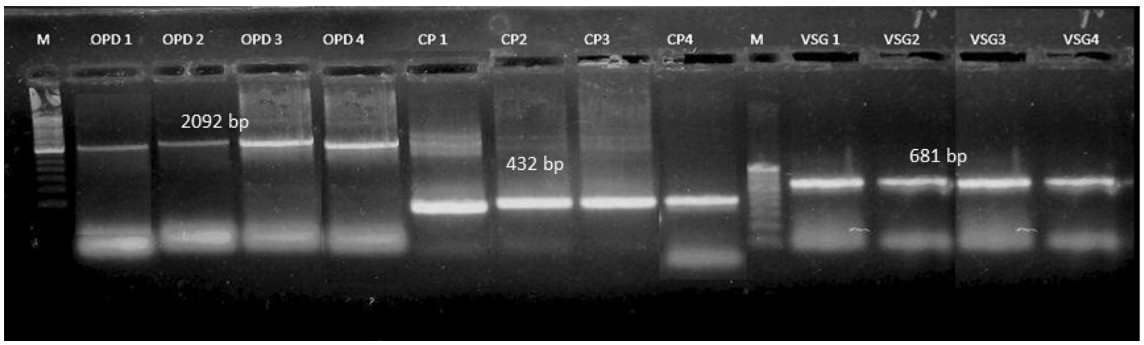

Fig. 1 PCR amplification of ologopeptidase B, cysteine protease and variable glycoprotein genes of *Trypanosoma evansi*

infection from non-patent infections using serological methods. Polymerase chain reaction is free of these hurdles and specifically amplifies genetically defined regions of the genome of the infectious agent. Although the detection of a single DNA molecule is possible, detection levels to a minimum of five trypanosomes by PCR assay are well documented (Artama et al. 1992). Amplification of repetitive *T. evansi* specific DNA sequence was possible even with DNA of a single trypanosome (Viseshakul and Panyim 1990).

There is urgent need to identify several promising candidates or targets for sensitive detection of surra. Proteases, a ubiquitous group of enzymes, are known to play key roles in the life cycle of parasites (McKerrow et al. 2006). Oligopeptidase B, a protease, has been implicated as an important virulence factor in trypanosomosis (Burleigh and Woolsey 2002) and it could become a vital therapeutic target. Oligopeptidase B is essential for proteolyti cleavage of many of the host derived peptides and proteins like kinogen and atrial natriuretic factor in the bloodstream of infected host (Morty et al. 2005). Cysteine proteases are important for parasite survival and are involved in the digestion of exogenous proteins for nutritive purposes (Rosenthal 1999), invasion of host cells and tissues, and modification of host proteins (Caler et al. 1998). VSG undergoes switching in its expression which facilitates the evasion from the host immune response and leads to chronic persistence of the parasite in the host (Barry and McCullogh 2001). It is also a well known fact that the whole VSG molecule is antigenic and that the VSG specific epitopes are usually localized in the N-terminal protein part. Over all, the VSG gene is expressed during early, middle and later stages of the infection of susceptible animals with all *T. evansi* strains (Verloo et al. 2000; Ngaira et al. 2004). Earlier studies suggest that VSG can be used as an antigen for the diagnosis of *T. evansi* infection (Songa and Hamers 1988). Further, it is also known that the immunoreactivity of the glycosylated and deglycosylated VSG are same against the anti-VSG antibody (Reinwald 1985). Hence the protein portion of the VSG is significantly antigenic.

It would be interesting to further investigate the role of all these molecular targets in the specific diagnosis and immune protection of the disease in different mammalian hosts. Further research is thereby warranted on all these three molecules about their roles on diagnosis and/or immunoprotection.

References

Artama WT, Agey MW, Danelson JE (1992) DNA comparison of *Trypanosoma evansi* (Indonesia) and *Trypanosoma brucei* spp. Parasitol 104:67–74

Barry JD, McCullogh R (2001) Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. Adv Parasitol 49:1–70

Burleigh BA, Woolsey AM (2002) Cell signalling and *Trypanosoma cruzi* invasion. Cell Microbiol 4:701–711

Caler EV, de Avalos SV, Haynes PA, Andrews NW, Burleigh BA (1998) Oligopeptidase B-dependent signaling mediates host cell by *Trypanosoma cruzi*. EMBO 17:4975–4986

Dobson RJ, Dargantes AP, Mercado RT, Reid SA (2009) Models for *Trypanosoma evansi* (surra), its control and economic impact on small-hold livestock owners in the Philippines. Int J Parasitol 39:1115–1123

Herbert WJ, Lumsden WH (1976) *Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitemia. Exp Parasitol 40(3):427–431

Kumar H, Gupta MP, Sidhu PK, Mahajan V, Bal MS, Kaur K, Ashuma Verma S, Singla LD (2012) An outbreak of acute *Trypanosoma evansi* infection in crossbred cattle in Punjab, India. J App Anim Res 40(3):256–259

Luckins AG (1988) *Trypanosoma evansi* in Asia. Parasitol Today 4:137–142

McKerrow JH, Caffrey C, Kelly B, Loke P, Sajid M (2006) Proteases in parasitic diseases. Ann Rev Pathol 1:497–536

Morty RE, Pelle R, Vadasz I, Uzcanga GL, Seeger W, Bubis J (2005) Oligopeptidase B from *Trypanosoma evansi*: a parasite peptidase that inactivates atrial natriuretic factor in the bloodstream in infected hosts. J Biol Chem 280:10925–10937

Ngaira JM, Njagi ENM, Ngeranwa JJN, Olembo NK (2004) PCR amplification of RoTat 1.2 VSG gene in *Trypanosoma evansi* isolates in Kenya. Vet Parasitol 120:23–33

Pandey V, Nigam R, Jiaswal AK, Sudan V, Singh RK, Yadav PK (2015) Haemato biochemical and oxidative status of buffaloes naturally infected with *Trypanosoma evansi*. Vet Parasitol. doi: 10.1016/j.vetpar.2015.07.025

Parashar R, Shanker D, Sudan V, Jaiswal AK (2015) PCR-based diagnosis of surra-targeting mini-chromosomal satellite DNA for unraveling the cryptic epizootiology of bubaline trypanosomosis. Indian J Anim Sci 85(4):370–372

Reinwald E (1985) Role of carbohydrates within variant surface glycoprotein of *Trypanosoma congolense:* protection against proteolytic attack. Eur J Biochem 151:385–391

Rosenthal PJ (1999) Proteases of protozoan parasites. Adv Parasitol 43:105–159

Sengupta PP, Balumahendiran M, Balamurugan V, Rudramurthy GR, Prabhudas K (2012) Expressed truncated N-terminal variable surface glycoprotein (VSG) of *Trypanosoma evansi* in *E. coli* exhibits immuno-reactivity. Vet Parasitol 187:1–8

Singh V, Tiwari AK (2012) Bovine surra in India: an update. Ruminant Sci 1:1–7

Singh H, Tewari AK, Mishra AK, Maharana B, Sudan V, Raina OK, Rao JR (2015) Detection of antibodies to *Toxoplasma gondii* in domesticated ruminants by recombinant truncated SAG2 enzyme-linked immunosorbent assay. Trop Anim Health Prod. doi:10.1007/s11250-014-0703-5

Songa EB, Hamers R (1988) A card agglutination test (CATT) for veterinary use based on an early VAT Ro Tat ½ of *Trypanosoma evansi*. Ann Soc Belg Med Trop 68:233–240

Sudan V, Jaiswal AK, Parashar R, Shanker D (2014) A nested polymerase chain reaction based assay for sensitive diagnosis of *Trypanosoma evansi* infection in water buffaloes. Indian J Anim Sci 84(12):26–30

Sudan V, Tewari AK, Singh H (2015a) A native whole cell lysate antigen (WCLA) based ELISA for the sero-surveillance of surra in cattle. Indian J Anim Sci 85(6):16

Sudan V, Tewari AK, Singh H (2015b) i Serodiagnosis of *Toxoplasma gondii* infection in bovines from Kerala, India using a recombinant surface antigen 1 ELISA. Biologicals 43:250–255

Sudan V, Jaiswal AK, Parashar R, Shanker D (2015c) A duplex PCR-based assay for simultaneous detection of *Trypanosoma evansi* and *Theileria annulata* infections in water buffaloes. Trop Anim Health Prod 47:915–919

Verloo D, Holland W, My LN, Thanh NG, Tam PT, Goddeeris B, Vercruysse J, Buscher P (2000) Comparison of serological tests for *Trypanosoma evansi* natural infections in water buffaloes from North Vietnam. Vet Parasitol 29:87–96

Viseshakul N, Panyim S (1990) Specific DNA probe for the sensitivity detection of *Trypanosoma evansi*. Southeast Asian J Trop Med Public Health 21:21–27