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Insight into trypanosomosis (Surra) of Indian livestock: Recent updates

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ABSTRACT

Surra, caused by *Trypanosoma evansi*, is an economically important disease of a wide range of domestic and wild animals, and is most widely distributed. It is a potentially fatal disease causing huge economic losses to the livestock owners in terms of morbidity, mortality, abortion, infertility, reduced milk yield and also by interfering with vaccination programme in India. Due to sub clinical nature of the disease, it has been underestimated in cattle and buffaloes. Emergence of atypical cases of human trypanosomiasis has created an alarming situation and indicates a possible zoonotic threat in future. Accurate diagnosis of surra is extremely essential to identify animals for treatment, to assess the prevalence of the disease and to avoid indiscriminate usage of trypanocidal drugs. Diagnosis of surra still suffers from low sensitivity and specificity. There is an urgent need for sensitive cost effective pen-side diagnostic that can be applicable and affordable to smallholder farmers in endemic regions. The present review addresses various aspects of surra with special emphasis on disease epidemiology, emerging issues, current diagnostic trends, chemotherapeutics and preventive measures to limits its prevalence in livestock.

Key words: Chemotherapy, Diagnosis, Epidemiology, Livestock, Surra, Trypanosomosis

Trypanosoma evansi is a salivarian haemo-flagellate of both intra and extra vascular fluids of mammals causing a devastating disease called trypanosomosis or Surra throughout the tropical and subtropical regions of the world (Desquesnes *et al*. 2013). It has a vast range of hosts receptive and susceptible to the infection. The hemoparasite exhibits highly variable clinical effects, depending on the host and the geographical area. It is a kinetoplastic maxicircle deleted descendent of *T. b. brucei*. It is the most prevalent trypanosome of livestock in India, although isolated cases of *T. equiperdum* and *T. theileri* were also encountered (Ruprah 1985). *T. evansi* has the ability to periodically switch its major surface glycoprotein (VSG) producing relapses of parasitaemia. It receives several common name in different areas such as *surra, purana*, *dubla, tebarsa, makhi ki bimari, dance makhi no rog*, *chakri* and g*altia* (Gill 1991, Singh *et al*. 2018). Surra is a potentially fatal disease causing significant economic losses to livestock owners in terms of morbidity, mortality, abortion, infertility, reduced milk yield, interference with vaccination programme etc. (Singh and Raisinghani 1990, Kurup and Tewari 2012). Emergence of human cases of trypanosomosis has opened new vistas in the field of emerging zoonotic diseases. Absence of pathognomonic signs of the disease necessitates (Singh and Chhabra 2008) several laboratory (from conventional to molecular) techniques to be carried out for diagnosis of Surra but each technique has its limitations and advantages for some species of animals and needs elaborate standardization. Additionally, there is an urgent need for understanding the disease and its complex epidemiology in order to develop diagnostics, drugs and vaccines for its effective control. The present review addresses overall aspects of trypanosomosis (surra) with special emphasis on disease epidemiology, emerging zoonotic issues, trends in its diagnosis, treatment, prevention and control measures which would help in limiting its prevalence in livestock.

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Disease epidemiology

History and geographical distribution: Trypanosoma (*Trypanozoon*) *evansi* (Steel 1885) Balbiani, 1888, is the first pathogenic mammalian trypanosome isolated from infected camels and equids in Dera Ismail Khan district of Punjab, in 1880, by a British veterinarian Griffith Evans (Evans 1880). *T. evansi* is considered to be derived from *T. brucei brucei* (cyclically transmitted by *tse-tse* flies) but has lost the ability to undergo cyclical development in *tse-tse fly* due to the loss of the maxicircles of kinetoplastic mitochondrial DNA. The disease caused by this haemoparasite has spread from Africa through the Arabian peninsula to a large geographical area spanning from Iran to Indonesia (Hoare 1972, Luckins 1988, Lai *et al*. 2008, Field and Carrington 2009). Now-a-days, its geographical

distribution is continuous from the northern part of Africa through the Middle East to South-East Asia. Surra in India is very old with records dating back from VIII centuries BC (Hoare 1972) with prevalence in almost all over the country, where environment for the breeding of the fly vectors is most suitable (Bhatia *et al*. 2006). It was believed to be brought to the Latin America by the Spanish conquerors where vampire bats (*Desmodus rotundus*) were involved with the spread of the infection (Hoare 1972). Occurrence of *T. evansi* infection had also been reported from Spain and France (Gutierrez *et al*. 2006, Desquesnes *et al*. 2008, Desquesnes *et al*. 2009). It is so far absent from Australia (Reid 2002). *T. evansi* could only be eradicated from areas if detected very early and controlled. Once *T. evansi* reached to an enzootic level, it is not possible to eradicate, most likely due to the existence of a wide domestic and wild reservoir, the ability to be transmitted by nonspecific mechanical vectors present all over the world and its ability to disseminate silently through healthy carriers.

Prevalence and host range in domesticated animals: India is considered to be the major source from where the disease has disseminated throughout the continent of Asia and Islands of Indian Ocean (Singh *et al*. 2018). In the Indian sub-continent, the disease is mainly endemic and most of the epizootics have occurred particularly in bovines with a high mortality rate ranging from 20 to 90% (Gill 1991). Though surra in cattle is thought to be widely prevalent in the entire south-east Asia, the prevalence data for surra in cattle has been inadequate from the Indian subcontinent. Considerable variation in degree of endemicity is correlated to prevalence of the fly vector, size of susceptible host population, prevailing agro-climatic conditions as well as the sensitivity of the diagnostic test applied (Singh and Tewari 2012, Singh and Chhabra 2008). Semi-intensive nature of animal husbandry practices in India with scattered animal population in the form of unorganized herds of bovine pose a threat to other susceptible species, viz. camels and horses reared in the vicinity. Surra has been detected in animals of arid and semi-arid regions of countries with warm and temperate climate (Singh 1989). The incidence of surra is higher in north and northwestern parts as compared to eastern and southern parts of India. Significant prevalence had been reported in animals in Haryana, Punjab, Rajasthan, Madhya Pradesh, Uttar Pradesh, Gujarat, Maharashtra, Tamil Nadu, Kerala, Asom, Andhra Pradesh and Karnataka etc. Outbreaks of acute disease were recorded in these animals especially in the high endemicity states like Haryana and Punjab (Batra *et al*. 1994, Gupta *et al*. 2003, Jindal *et al*. 2005). In Bihar, the incidence of bovine trypanosomosis was fairly high in cattle, viz. 58.86% and 41.14% in buffaloes (Sinha *et al*. 2006). Lower prevalence rate was reported from Andhra Pradesh, viz. 1.42% in cattle and 2.71% in buffaloes by blood smears in Guntur district (Das *et al*. 1998) and 7.28% in East Godavari district (Bhaskar Rao and Hafeez 2005). In Karnakata, prevalence rate was 42.12% in cattle, 39.78% in buffaloes and 2.15% in goats (Krishnappa *et al.* 2002). Using a monoclonal antibody based latex agglutination test (LAT), Shyma *et al*. (2012) reported a very high seroprevalence of *T. evansi* in 60.23% bovines at Karnal. From Mathura, among equines, 100% morbidity and 66.6% mortality was recorded (Kumar *et al*. 1994). Laha and Samal (2008) recorded higher positivity (12.74%) in a horse stable in eastern India. An outbreak in ponies was documented from Jammu (Raina *et al*. 2000). There is an increase in incidences of trypanosomiasis outbreaks in camels after the advent of Indira Gandhi Canal and irrigation of vast tracts of arid land in Western Rajasthan (Pathak and Khanna 1995). Earlier studies reported the endemicity of cameline surra in 18 districts of Rajasthan (Raisinghani and Lodha 1989) with prevalence of 7.5% by the wet-blood/Giemsa stain smears and 76 (31.66%) positive for antigen using double antibody sandwich ELISA (Pathak *et al*. 1993) from Western Rajasthan. On the basis of clinical observations in camels alone, a prevalence rate of 20.37% had been reported from Bikaner district of Rajasthan (Singh *et al*. 1997). Reports of natural trypanosomiasis in goats are scarce (Jana and Jana 2005) and are rare in sheep (Rao *et al*. 1987). Apparently, *T. evansi* infections are uncommon in goats and sheep (Gill 1991). Amongst dogs, 4.68% prevalence was recorded in Ludhiana (Singh *et al*. 1993). Incidence among dogs in and around Kolkata city was found rather less (Chowdhury *et al*. 2005). Cases of trypanosomiasis had also been documented from native dog breeds (Krishnamoorthy and Manohar 2005). Exotic breeds are found to be more prone and usually experience acute fatal disease (Dakshinkar and Bhojne 2001).

Trypanosomosis in wild animals: Trypanososmosis affects wild animals throughout the globe. The innate ability of the wild animals to co-exist with trypanosomes without showing clinical signs contributes appreciably to their reservoir status (Mbaya *et al*. 2008). Wild animals to some extent exhibit moderate level of trypanotolerance by controlling excessive proliferation of parasite alongside limiting its pathogenic effect (d'Iteren *et al*. 1998). Early on records of natural infections of trypanosomes in wild animals all over the globe included the finding of *T. evansi* in Indian elephants (*Elaphus maximus*) (Evans 1910). Among wild animals, incidence in hyena in Delhi zoo and Chitals in Bhilai zoo were cited (Arora 1994). There are a lot of reports regarding trypanosomosis in tigers (Sinha *et al*. 1971, Upadhye and Dhoot 2000, Gupta *et al*. 2009), from India. An outbreak of 'surra' in tigers at Ranthambore National Park with a fatal case report in a male tiger (Ramachandraiah *et al*. 1995) and an outbreak in circus tigers in Andhra Pradesh involving 5 adults and 4 cubs (Bhaskar Rao *et al*. 1995) were described. As many as 12 tigers died at Nandankanan zoo in Odisha due to trypanosomosis (Parija and Bhattacharya 2001). Trypanosomosis in a circus tigress was reported from Chittoor, Andhra Pradesh (Devasena and Shobhamani 2006). Besides tigers, jaguars, leopards, wolves, fox and jungle cat are the other carnivores reported to be suffered from trypanosomosis (Sudan *et al.* 2017). Herbivores like

sambar (*Cervus unicolor*), spotted deer (*C. axis*) and wild feral cattle (Pathak *et al*. 1988, Singh 1998) are the other notifiable reservoirs of trypanosomiasis. The high incidence in wild carnivores apparently supports the hypothesis that feeding of infected tissues can also be a possible mode of transmission (Bhatia *et al*. 2006). A case was also documented on trypanosomosis from mithun (*Bos frontalis*) in Asom (Rajkhowa *et al*. 2003).

Transmission: The non-cyclical transmission of *T. evansi* is aided by haematophagus biting flies like *Tabanus*, *Stomoxys*, *Haematopota*, *Chrysops*, *Lyperosia*, *Hippobusca* flies. Efficiency of transmission is reliant on degree of parasitaemia, intensity of fly challenge and the intermission between 2 successive feedings. In Indian subcontinent, the outbreaks of surra occur during the rainy season and post monsoon season reaching climax in October and November months correlating the high density of the insect vector (Singh and Singla 2012). Transmission can be vertical, horizontal, iatrogenic, and per-oral. Carnivores can also become infected after feeding on infected tissues when the oral mucosae are damaged. There is also likelihood of sexual transmission of *T. evansi* (Singla *et al*. 2003). Potential of leeches for transmission of *T. evansi* especially buffalo leech in Asia should be explored (Desquesnes *et al.* 2013). Vertical or transplacental transmissions of trypanosomiasis are also reported in several instances (Rao *et al*. 2001, Pathak and Kapoor 1999). The vampire bat (*Desmodus rotundus*) in latin America acts as a host, reservoir, and biological vector of the parasite in which the trypanosome may be transmitted from biter to bitten or vice versa. They can also contaminate livestock, acting as permanent vectors, capable of infecting their host for a pretty long period (Desquesnes *et al*. 2013).

Antigenic variation a means for immune evasion

Persistence and lethality in trypanosomes infection is attributed to antigenic variation which involves changes in the identity of the variant surface glycoprotein (VSG) that forms a dense cell surface coat to shield invariant surface antigens from immune recognition (Singh *et al*. 1995, 1997). In fact, during infection, most of the circulating trypanosomes are successfully destroyed by VSG-specific antibodies generated by the host. But the problem is that there are always survivors because a minority of parasites evade clearance by switching expression to antigenically distinct VSGs and not recognized by the current wave of antibodies. It is a highly complicated survival strategy. It involves stochastic switches between the transcriptions of one of an expected thousand variant surface glycoprotein (VSG) genes. Switching involves either transcriptional control, resulting in switching between different VSG expression sites or DNA rearrangement events slotting previously inactive VSG genes into an active VSG expression site (Morrison *et al*. 2009).

Pathogenesis

The degree of pathogenicity depends host species, the virulence of the *T. evansi* strain and the dose received by the host. Anaemia is a major component of pathology of *Surra*. The mechanism or pathophysiology of anaemia is complex and multifactorial in origin which primarily compromised the cellular integrity of erythrocytes leading to either haemolytic anaemia or enhanced erythrophagocytosis. Loss of sialic acid from erythrocytic membranes may predispose the erythrocytes for phagocytosis and development of anaemia. Tizard (1985) attributed anaemia to phospholipases in excretory/ secretory (E/S) products of *T. brucei*. It is probable that erythrocytes may acquire trypanosomal antigen, which may result in the immunological reaction and complement mediated destruction of erythrocytes. Other factors that promoted haemolytic anaemia in trypanosomosis were trypanosome autolysates, platelet aggregation, undulating pyrexia, oxidative stress, lipid peroxidation, nutritional and hormonal imbalances, disseminated intravascular coagulation, idiopathic and tumor necrosis factors (TNF) and bone marrow nitric oxide (NO) activity (Mbaya *et al*. 2012). The lysosomal secretory proteinases, phospholipases and other hydrolytic enzymes of trypanosomes are considered potentially important factors in the development of the diseases. The E/S proteases released into the blood stream may degrade the host tissue proteins and contribute to the pathogenesis.

Atypical human trypanosomoses due to T. evansi in India and abroad: The classical human trypanosomoses are human African trypanosomosis (HAT) or sleeping sickness (caused by *Trypanosoma brucei gambiense* or *T. b. rhodesiense*) and Chagas disease, the Latin American human trypanosomosis (*T. cruzi*). Atypical human infections caused by *Trypanosoma* species (*T. b. brucei, T. vivax, T. congolense, T. evansi, T. lewisi*) that normally are restricted to animals had been reported.

The host range of *T. evansi* is restricted to non-human animals because of susceptibility to cytolysis by the trypanolytic factor in normal human serum (NHS).The key players involved in NHS-mediated trypanolysis are the primate-specific apolipoprotein L-I (apoL1) and haptoglobin-related protein (Hpr) which are associated with a minor subfraction of HDLs (high density lipoproteins) and an IgM/ apolipoprotein A-I (apoA1) complex, respectively, termed trypanosome lytic factor (TLF) 1 and TLF2. The TLF1-Hpr-haemoglobin (Hb) complex binds to the trypanosome haptoglobin (Hp)-Hb receptor, which triggers efficient uptake of TLF1 and subsequent trypanosome lysis. Here Hpr acts as TLF ligand while the lytic activity is mainly due to apoL1, a Bcl-2-like pore-forming protein (Vanhollebeke and Pays 2010).

Three cases of human *T. evansi* had been reported from the India during the last decade. In 1977, the first case reported an accidental infection by a syringe containing *T. evansi* infected blood. It was confirmed morphologically by microscopy. The patient was treated with Atoxyl (an arsenic derivative used in HAT treatment before the currently used melarsoprol drug and got cured (Truc *et al.* 2013). In India, the first parasitologically and

immunomolecularly confirmed atypical case of human trypanosomosis caused by *T. evansi*, was reported in September 2004 from a 45 years old male herdsman staying in village Shivani block Sindewahi in Chandrapur district of Maharashtra (Joshi *et al*. 2005, Vanhollebeke *et al*. 2006, True *et al*. 2007). The infection was due to a frameshift mutation in both Apo-L1 alleles in the patient (Vanhollebeke *et al*. 2006). In West Bengal (2005), the 1st atypical human trypanosomosis was reported from a 40 year old female suffering from headache, fever and died within two days. The blood examination revealed *Trypanosoma* spp. and the area was prevalent only for the *T. evansi* among livestock. In absence of proper diagnostic aids and based on aforesaid facts, it was presumed that the *Trypanosoma* spp. observed in the patient could be *T. evansi* (Parashar *et al.* 2016).

In addition to these cases, there are other reports of human infected with *T. evansi* from Sri Lanka (1999) and Egypt (2010) confirmed morphologically by microscopy and the patients got cured without any treatment (Truc *et al.* 2013). So a new network has been created to coordinate information and research on atypical human infections caused by animal trypanosomes (NAHIAT). The NAHIAT (Network on Atypical Human Infection by Animal Trypanosomes) was created in May 2011. It is coordinated by the Institute of Research for Development (IRD) and the Center for International Collaboration on Agricultural Research for Development (CIRAD) with the support of FAO, OIE, WHO, and a number of international research institutes and universities. Although *T. evansi* is still not considered to be a zoonotic disease, it is wise to be cautious.

Diagnosis

It is difficult to diagnose surra since clinical signs are not specific and varied. In absence of pathognomonic clinical signs, the definitive diagnosis of surra involves several laboratory analyses by using parasitological, serological and molecular tools (Singh *et al*. 1994, 2014).

Parasitological techniques: Trypanosomes can be detected microscopically in fresh or fixed and stained smears prepared from blood or lymph nodes of infected animals. However, it is of limited sensitivity (approximately 10^5 parasites/ml for wet blood film). Therefore, various concentration techniques have been developed to increase the sensitivity of microscopic examination, viz. microhematocrit centrifugation, quantitative buffy coat technique and mini-anion-exchange centrifugation technique that can detect parasitemia as low as 100–200 trypanosomes/ml (Desquesnes *et al*. 2013). Inoculating laboratory rodents for cryptic infection can reveal infection with very high sensitivity. It lowers the minimum level of parasitaemia detected to 20–50 parasites/ml (Desquesnes *et al*. 2013). But a high lag time in diagnosis and the cost and ethical considerations precludes this technique for the routine diagnosis.

Molecular techniques: A range of nucleic acid amplification-based molecular techniques has been developed to improve the detection of pathogenic trypanosomes. A PCR-based *T. evansi* detection technique was developed with a sensitivity of 0.5 pg of parasite DNA or one single parasite in 10 µl of blood sample (Wuyts *et al*. 1994). In India, PCR was first employed (Basagoundar *et al*. 1998) for detection of *T. evansi* in infected camel blood samples. Omanwar *et al*. (1999) determined the sensitivity and specificity of PCR using oligonucleotide primers constructed from *T. evansi* repetitive DNA sequences and revealed that it could amplify template DNA of *T. evansi* derived from buffaloes, camels and horses to a threshold sensitivity level of 0.5 pg and can detect DNA from as few as five organisms in 10 µl crude blood samples. PCR technique reported to be more sensitive after an experiment conducted on 217 camels in Rajasthan (Singh *et al*. 2004) and also in captive and wild animals (Shailaja *et al*. 2005). Later, various workers have used PCR for sensitive and specific detection of *T. evansi* in camel, donkey and dogs (Ravindran *et al*. 2008). In another study, Shahardar *et al*. (2009) targeted ribosomal DNA for detection of *T. evansi* in Indian dromedary. Bal *et al*. (2014) conducted an investigation to evaluate the sensitivity of PCR based method for detection of *T. evansi* and to know its efficacy of corresponding trypanocidal treatment. Shyma *et al*. (2013) carried out a survey with the aim of detecting *T. evansi* in cattle, buffaloes and equines in Haryana by PCR technique and revealed this technique to be highly sensitive to the conventional parasitological method. PCR based on invariant surface glycoprotein 75 gene can be useful in the detection of carrier status of surra in animals (Rudramurthy *et al*. 2013). Sudan *et al*. (2015) developed a PCR-based diagnostic tool of surra-targeting mini-chromosomal satellite DNA for unraveling the cryptic epizootiology of bubaline trypanosomosis.

Several variants of PCR-based diagnostic assays have been developed which include the use of species-specific primers, single and nested PCRs, and real-time PCR. Sudan *et al*. (2014) described nested polymerase chain reaction (nPCR) for detection of *T. evansi* in water buffaloes (*Bubalus bubalis*). Isothermal reactions, such as LAMP (Loop mediated isothermal amplification) were developed for detection of *T. evansi* strain B in Kenya. The assay analytical sensitivity is approximately 0.1 tryps/ml while that of classical PCR test targeting the same gene is approximately 10 tryps/ml (Njiru *et al*. 2010). Konnai *et al.* (2009) developed a real-time PCR assay for the detection and quantification of parasites in water buffaloes using specific primers for the *T. evansi* Rode Trypanozoon antigen type (RoTat) 1.2 Variable Surface Glycoprotein (VSG) gene. Sharma *et al*. (2012) standardised and validated a real time PCR assay using TaqMan primer and probe targeting the internal transcribed spacer 1 (ITS-1) region of rRNA for *T. evansi*. The minimum detection limit of the assay for purified trypanosomal DNA was 0.01 ng (≈ 0.33 genomic DNA of *T. evansi*) whereas for whole blood the minimum detection limit was 0.1 ng (≈ 6.12 genomic DNA).

Serological techniques: Several serological tests have been developed to detect circulating antigen/antibodies in

the serum samples of *T. evansi* infected/ suspected animals.

Card agglutination test for Trypanosoma evansi (*CATT*): Infection with *T. evansi* results in production of circulating antibodies against several surface antigens of the parasite. Such antibodies can be demonstrated in the serum or plasma of the infected animal by direct agglutination. The CATT-antigen is a freeze dried suspension of purified, fixed and stained bloodstream form trypanosomes expressing a predominant variable antigen type of *T. evansi* (RoTat 1.2). The test was developed, standardised and validated at the Laboratory of Serology, Institute of Tropical Medicine, Antwerp, Belgium. It is an OIE recommended test for serodiagnosis of surra (Bajyana and Hamers 1988). Chaudhri *et al*. (1995) validated CATT in *T. evansi* infected crossbred cattle and buffaloes of India, pre- and post-treatment with quinapyramine prosalt. The test was found to be highly sensitive and did not reveal cross reactivity with other haemoparasites. It could detect agglutination titres from day 14 post infection (PI) till death in experimentally infected calves (Chaudhri *et al*. 1996). Hilali *et al*. (2004) assessed the CATT/*T. evansi* for detection of antibodies against *T. evansi* in experimentally and naturally infected buffaloes. Anti-*T. evansi* antibodies were detected in buffalo samples by CATT/*T. evansi* which were declared negative by parasitological examination. Singla *et al*. (2013) in their serodiagnostic studies on surra in cattle and buffaloes revealed CATT/ *T. evansi* test as a pen-side test with higher field applicability.

Surra Sero K-SeT: It is a new immunochromatographic test for serodiagnosis of *T. evansi* infection in domestic animals. The *Surra Sero K-SeT* makes use of recombinant variant surface glycoprotein rVSG RoTat 1.2, expressed in the yeast *Pichia pastoris*. The Surra Sero K-SeT displayed somewhat lower specificity but overall higher sensitivity when compared with CATT/ *T. evansi*. Hence, this can be viable alternative for the CATT/ *T. evansi* for sensitive detection of antibodies against *T. evansi* in domestic animals (Birhanu *et al*. 2015).

Slide enzyme linked immunosorbent assay (*sELISA*): sELISA, a modified ELISA technique was used for specific detection of antibodies in *T. evansi* infected animals. This test replaces the micro ELISA plates with glass slide fixed whole *T. evansi* organisms. Direct immunostaining of the fixed whole *T. evansi* organisms (antigen) on microscopic glass slide with sera followed by incubation with anti-species IgG-HRPO conjugate and substrate diaminobenzidine tetrahydrochloride resulted in brown coloured surface reactivity in positive cases whereas the organisms remain colourless in negative cases. This can be seen under simple microscopy and nullifies the need of an expensive ELISA reader (Sivajothi *et al*. 2012). This could emerge as a test of preference for the detection of antibodies against *T. evansi* infection in cattle and buffaloes in developing countries.

Colloidal dye immunobinding assay: Recently, colloidal dye immunobinding technique was developed for diagnosis of *T. evansi* in dogs. Briefly, the whole cell lysate antigen was coated on the nitrocellulose membrane of the flow-through device. Protein A colloidal gold was used as detector. The bound antibodies were visualised by the addition of detector which imparts pink colour to the membrane. The assay has been validated with wet blood film examination. The test is a satisfactory alternative for use in clinical laboratories which lacks the advanced equipment for screening of *T. evansi* infection in dogs (Sivajothi *et al*. 2015).

Disease eradication requires sensitive diagnostic tools and efficient treatment strategies. Since antibody based detection cannot differentiate between active infection and cure, immunodiagnostics based on antigen detection are preferable (Singh *et al*. 1995).

Latex agglutination test: Nantulya (1994) developed and validated a monoclonal antibody based latex agglutination test (Suratex®) for the detection of circulating invariant trypanosomal antigens in surra. This test requires a little training of the field workers, a cavity slide, the latex reagent and a few drops of blood from suspected animals to infer the result. Rayulu *et al*. (2007) developed a monoclonal antibody based latex agglutination test (MAb-LAT) to detect the circulating antigens of *T. evansi* in the sera of domestic animals. MAb was produced for the first time against a surface antigen of an Indian isolate of *T. evansi* and used in development of MAb-LAT for screening of buffalo sera samples collected from different regions of Haryana. The diagnostic sensitivity and diagnostic specificity were recorded as 95.38 and 59.74% for LAT using microhaematocrit technique (MHCT) as reference test and 90.33 and 88.30% using Ag-ELISA as reference test. Later, monoclonal antibody based latex agglutination test was used for detection of *T. evansi* antigen in sera samples of cattle (Shyma *et al.* 2012a), buffaloes (Shyma *et al*. 2012b), and equines (Shyma *et al*. 2011) and the results were compared with PCR of their corresponding blood. The results revealed a good correlation between LAT and PCR and found both the tests more sensitive than parasitological methods. Singh *et al*. (2017) investigated the seroprevalence of *T. evansi* in buffaloes using monoclonal antibody based-latex agglutination test (TE-LAT) in south western semi arid plane zone of Uttar Pradesh. Overall seroprevalence of 18.56% and suspected seroprevalence of 52.02% were obtained indicating endemicity of surra in buffaloes of the region.

Antigen capture ELISA: Detection of circulating trypanosomal antigens in the blood of infected animals ELISA (Ag-ELISA) was a major improvement in immunodiagnosis and also a useful tool to provide a direct indication of active infection (Singh and Chhabra 1993). In India, Swarnkar *et al*. (1993) developed double antibody sandwich ELISA for the detection of *T. evansi* antigens in surra suspected cattle and buffaloes of Rajasthan and found it to be highly sensitive. Rayulu *et al*. (2009) developed an Ag-ELISA using monoclonal antibodies (mAbs) as capture antibodies for the detection of specific circulating trypanosomal antigens in the sera of domestic animals.

Nanotechnology based approach: Recombinant nanobodies are heat-stable, small-sized (15 kDa), antigen-specific, single-domain, variable fragments derived from heavy chain-only antibodies. Using phage display and bio-panning techniques, recent research had developed a cross-reactive nanobody (Nb392) targeting all trypanosome species and isolates that targets a conserved kinetoplastid Paraflagellar Rod protein (PFR). Nb392 is an excellent marker for the PFR and can be useful in the diagnosis of trypanosomiasis (Obishakin *et al*. 2014).

Chemotherapy

Control of surra mainly depends on chemotherapy and chemoprophylaxis. Curative drugs are meant to cure individual infected animals, not to protect the whole herd or group for a longer period. Prophylactic drugs are used where the risk is so high that the health of the herds cannot be maintained by individual application of curative compounds and when the infected animals cannot be reached e.g. inaccessibility during the rainy season, trade cattle moving to distant markets etc. Diminazene aceturate is the most extensively used curative trypanocide@ 7 mg/kg body weight against surra in ruminants followed by isometamidium chloride (both curative and preventive), cymelarsan (for curative treatment of camels), suramin, and quinapyramine (curative and/or preventive) (Desquesnes *et al.* 2013). Its use in horses and dogs is limited due to poor efficacy and tolerance in these species. Isometamidium chloride (IMC), a member of phenanthridine family, can be used for curative (0.5 mg/kg bw) and preventive (1 mg/kg bw) treatment of surra in ruminants and horses, *via* intramuscular or subcutaneous injection. Further, DA and IMC constitutes a "sanative pair," which means that once resistance develops to one of the drugs, the other drug can be used to control the infection. Melarsomine dihydrochloride (Cymelarsan) is the latest trypanocide to be discovered. It is used to control Surra at a dose rate of 0.25 mg/kg bw, 0.25–0.5 mg/kg bw, 0.5 mg/kg bw, and 0.75 mg/kg bw in camels, horses, cattle and buffaloes respectively. Quinapyramine methyl-sulphate, a member of aminoquinaldine derivatives can be used to treat the infection by subcutaneous injection at a dose of 5 mg/kg bw. A more effective mixture of quinapyramine chloride and quinapyramine sulphate (quinpyramine prosalt) can be used as a curative/ preventive drug against *T. evansi* in buffalo, horse and camel (Singh *et al.* 1987, 1989, 1990). The drug has chemoprophylactic effect which can last up to 4 months. Its use should be restricted to horses and camels only. Quinapyramine is not recommended in cattle, because it may induce cross-resistance to both IMC and DA. Diminazene aceturate (Berenil), quinapyramine sulphate and chloride (Antrycide prosalt, Triquin), quinapyramine sulphate (Triquin S, Antrycide) are the mostly used drugs for trypanosomiasis in India and most trial reports are based on these drugs while, suramin and cymelarsan are not commercialized. Currently, the use of isometamidium hydrochloride for treatment of *T. evansi* is on the rise (Kumar *et al.* 2009).

Recent research findings have shown new hopes for successful treatment of trypanosomosis with nanotechnology based approach. Nerolidol nanospheres have shown promising trypanocidal efficacy against diminazene aceturate resistance *T. evansi* infection (Baldissera *et al*. 2016a). The association of Diminazene aceturate with α-Bisabolol and solid lipid nanoparticles containing α-Bisabolol (SLN-B) can be used as an alternative to improve the therapeutic effectiveness of D.A. and for treatment of infected animals with *T. evansi* and can be used as an alternative to traditional chemotherapeutics (Baldissera *et al.* 2016b).

Prevention and control

The vaccination approaches by using dominant surface proteins have not been successful, mainly due to antigenic variation of the parasite surface coat. Since VSG no longer remains an impressive choice as a vaccine candidate, emphasis is laid on testing of various other invariant molecules, viz. paraflagellar rod proteins, beta tubulins, flagellar pocket antigens etc. for their immunoprotective potential (Singh *et al*. 1995, Maharana *et al*. 2011a, b, 2013, 2014, 2015, Kurup and Tewari 2012, Tewari *et al*. 2015). However the protection was not absolute. Currently, the control measures are centered on adopting following preventive measures.

Treatment of infected animal: Treatment of affected/sick animals with effective drug after early diagnosis. This reduces the risk of infection and development of carrier status and helps in the complete cure of disease. As trypanosomes cause immunosupression resulting in vaccination failure, the animals must be treated for surra, before adopting any vaccination programme.

Chemoprophylaxix of animals at disease risk: Trypanocides must be selected keeping in mind the problem of drug resistance in the area concerned. Use of chemoprophylactic (suramin, quinapyramine methyl sulphate and chloride) drugs must be practised before the onset of rainy season in the endemic areas, where fly population reached the peak due to heavy rainfall, etc. This will protect the susceptible animals during the high-risk period. Quinapyramine salts are the drug of choice which gives protection from existing infection as well as further infection for 3 months. Morever, regular disease monitoring and surveillance in apparently healthy animals are also required.

Fly control: Control of flies by various physical methods, viz. regular removal of dung and moist beddings, stacking of manure in compact heaps (helps in killing larvae of *Stomoxys, Liperosia*), avoiding animal grazing during bright sunshine, bush clearing over ditches and water bodies, dung management etc.

Control of flies by chemical methods: Spraying/dipping of insecticide over animals during fly season, spraying kerosene over water bodies (prevents tabanus flies from skimming over water bodies). Mass campaign for

eradication of *Tabanus* and other biting flies by spraying insecticides. It has limited value under Indian conditions.

International trading of animals: The following guidelines could be helpful to avoid the introduction of infected animals into non-infected areas (Desquesnes *et al.* 2013).

- Two quarantines should be applied for the international trade from an infected country to a non-infected country. This includes four weeks quarantine each at the exporting and importing farm.
- To qualify for trading, an animal should originate from a non-infected farm in a non-suspected area and be negative to surra tests twice at a 3–4 week interval during each of the quarantines.
- A farm is considered to be non-suspected areas if there have been no reports of surra in the previous three years within a 30 km radius of the farm.
- A non-infected farm is a farm located in a non-suspect area, which only permits the introduction of animals that are negative to the surra tests and that originate from non-infected farms located in a non-suspect area. A farm can be said to as non-infected farm if all animal species on the farm are negative to surra tests twice at a three months interval. Additionally to maintain the status of non-infected farm, all the animal species on the farm must be negative to surra tests when tested every 10–12 months.

Alternative control strategies: Herbal drugs as alternative control strategies are gaining popularity because of several advantages such as limited side effect, better patient tolerance, relatively less expensive and wider acceptance. Previous research revealed that aqueous extract of *Acacia albida* stem bark have antitrypanosomal activity (Ndidi *et al*. 2015). Previous research reported that the aqueous extract of *G. hombroniana* (seaushore mangosteen) has a potential antitrypanosomal activity through the inhibition of kinetoplast division, as one of the possible mechanisms of its antitrypanosomal effect. This plant could serve as a possible source of new antitrypanosomal compounds (Dyary *et al*. 2015). Trypanocidal activity of free and nanoencapsulated curcumin against *T. evansi* was tested *in vitro*. Results revealed better parasitaemia control, good antioxidant activity and a protective effect on liver and kidney functions of *T. evansi*-infected adult male Wistar rats (Gressler *et al*. 2015). Essential oil of achyrocline satureioides was effective in reducing the number of trypanosomes. Association of this natural product with a trypanocidal drug may augment its curative effect (Baldissera *et al*. 2014a). *In vitro* trypanocidal activity of macela (*Achyrocline satureioides*) extracts against *T. evansi* was also evaluated (Baldissera *et al*. 2014b). Alchornedine a new guanidine alkaloid from the leaves of *Alchornea glandulosa* was found effective against trypanosomiasis (Barrosa *et al*. 2014). As alternative vaccination approaches, different parasitic molecules have been attempted. Heat shock protein 90 (Hsp90) from protozoan parasites can be a potential drug target of trypanosomiasis (Rochani *et al*. 2014). The GPI-anchor of the VSG as an alternative strategy with antidisease potential has been identified. Using liposomes as slow delivery system, the GPI administered prior to the infection had been shown to result in a better control of the parasitemia and a longer lifespan of the infected mice which could be associated with a reduced TNF production and an increased level of IL-10, along with the expression of alternatively activated macrophage (Naessens 2006). An alternative approach for a vaccine is to direct the immune response to parasite antigens such as cysteine proteinases (C.P) that plays a crucial role in the pathology of the disease. This target been identified in the infectious cycle of trypanosomes such as cruzain from *T. cruzi*, rhodesain or brucipain from *T. brucei rhodesiense* and congopain from *T. congelense*. Immunoprophylactics based on C.P. may not affect the survival of the parasite, but would neutralise pathogenic factors, thereby lessening the pathological effects and may contribute to mechanisms of trypanotolerance (Auty *et al*. 2015).

Conclusion

Information is scanty on the impact surra among livestock in India. Coordinated efforts should be initiated for systematic documentation of real prevalence of Surra throughout the country and to assess its impact on host population dynamics and demographics, the economic losses due to infection and social impact on livestock owners. Despite biotechnological advancement, there is still lack of a highly sensitive, cheap, simple diagnostic tool to distinguish infected and non-infected animals. There is an urgent need for pen-side test that can be applicable and affordable to smallholder farmers in endemic regions. Government should take initiatives for setting up of a centralized referral facility to liaise, coordinate and standardize the findings emanating from state laboratories and efforts should be made for early diagnosis and treatment in order to reduce additional economic loss to the animal owners. With limited option for chemotherapeutics, research is required for new formulations of effective therapy to combat drug resistance. Though a plethora of publication has enriched the knowledge on different facets of biology as well as molecular basis of antigenic variation in *T. evansi*, devising a foolproof strategy for blocking the sequential expression of variable antigenic surface epitopes *in vivo* has not yet been successful. Therefore research should be focused for identification and application of invariant antigens as futuristic immunoprophylactic targets. Further the genetic diversity of trypanosomes and its relationship with variations in virulence and pathogenicity of parasite strains also demand investigation. Vector management and change in husbandry practices to reduce exposure to biting flies merits attention.

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