Evaluation of Blood Glucose As A Biomarker For On Spot Detection of Bovine Trypanosomosis In Emergent Nations

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Abstract

Trypanosomosis or surra has been declared notifiable multispecies animal disease by World Organization for Animal Health. Diagnosis of trypanosomosis is a challenging task in field condition because none of reported signs is pathognomonic resulting in failure of treatment either inappropriate or delay in treatment. In spite of this, low hemoglobin level and hypoglycaemia are consistent hemato-biochemical changes in trypanosomosis. Therefore taking into consideration of both these two changes, tentative diagnosis of trypanosomosis could be done in low resource laboratory situated in rural area. With the development of eco and user friendly pen-side diagnostic, effective management of animal trypanosomosis could only be possible. Out of 123 buffaloes, 21 buffaloes found positive by PCR were showing average temperature 105.13^0F±0.983, blood glucose 32.319±9.760 and haemoglobin 8.60±1.485. Likewise Value of blood glucose (glucometer- 95% CI 68.488 to 77.141 and semiautomatic blood analyzer- CI 64.637 to 74.087) and haemoglobin (95% CI 95.743 to 97.314) were found statistically highly significant (P<0.001) with body temperature. Lower the blood glucose level in fevered animals may suspect the trypanosomosis. In these cases portable blood glucose meter may help the field veterinarian for on spot estimation of blood glucose level of fevered animals. An on spot estimation of lowered glucose level, anaemia and high temperature increases the probability of trypanosomosis. So that timely treatment of infected animal could be possible in rural area without wasting of time to get the report from full equipped laboratory. This might be the first time reported method for early diagnosis of trypanosomosis.

Introduction

Trypanosomosis or surra caused by *Trypanosoma evansi* is a disease of major livestock species and widely distributed in different agro climatic regions of various developing countries including India (Truc et al. 2007). Trypanosomosis is mainly confined to Africa and Asia along with seven countries in South America and four countries in Europe. Pooled prevalence of *T. evansi* at global level in buffaloes and cattle is 28% and 16%, respectively by molecular methods (Aregawi et al. 2019). Overall prevalence of trypanosomosis in Indian buffalo is 4.15% (Kumar et al. 2017). Surra is one of the factors for failure of vaccination against foot and mouth disease, hemorrhagic septicemia and classical swine fever (Payne et al. 1993). Surra has been declared notifiable multispecies animal disease by World Organization for Animal Health (OIE) (Van Vinh Chau et al. 2016).

Diagnosis of trypanosomosis is a challenging task in field condition because none of reported signs is pathognomonic (Stephen 1986) resulting in failure of treatment either inappropriate or delay in treatment. In spite of this, anemia is most reliable sign and haemoglobin level is a good indicator for anemia (Uilenberg 1998) as hemodilution is not a feature in trypanosomosis (Dargie et al. 1979). Likewise hypoglycaemia is consistent biochemical change in trypanosomosis. Therefore taking into consideration of both these two changes, tentative diagnosis of trypanosomosis could be done in low resource laboratory situated in rural area. Total economic loss due to animal trypanosomosis in India and Africa is USD 671.1 million (Kumar et al. 2017) and USD 1-1.2 billion (Swallow 2000) per annum, respectively.

Outbreak of Trypanosomosis in ruminants have been reported from various parts of India (Kumar et al 2012; Shyma et al 2012) and mortality rate in cattle and buffaloes ranging from 20 to 90% in Indian subcontinent (Gill 1991; Kumar et al 2012). Much of the economic losses could be minimized by timely and appropriate treatment which would be only possible with accurate and prompt diagnosis in field condition. With the development of eco and user friendly pen-side diagnostic, effective management of animal trypanosomosis could only be possible. Keeping these in mind blood glucose and haemoglobin level of fevered buffaloes were estimated and correlated with trypanosomosis. This might be the first time reported method for early diagnosis of trypanosomosis.

Materials And Method

A total of 123 buffaloes (*Bubalus bubalis*) having history of high body temperature were selected for the study between period of January 2018 to December 2018. Aseptically 5 ml blood from each buffalo was collected through jugular vein using sterile syringe. Approximately 3 ml blood was transferred in tube containing anticoagulant (10%EDTA) while rest blood (approx. 2 ml) was transferred in tube without anticoagulant (for estimation of serum glucose). Side by side blood glucose levels of studied buffaloes were estimated by Portable glucose meter (The Accu-Chek Instant S System Roche Diabetes Care South Africa (Pty) Ltd). The selected portable glucose meter meant for blood glucose estimation in human as there is no portable glucose meter available for use in farm animals till date. Simultaneously thin blood smears were also prepared from each buffalo by ear vein method at the site of collection and immediately fixed with methanol.

The blood sample were brought to Department of Veterinary Parasitology, College of Veterinary Science and A.H, Mhow, India and blood smears were stained by standard protocol for Giemsa staining and examined under microscope. Screening of blood smears was done under oil immersion lens (1000x) of a compound research microscope and examined minimum 100 microscopic fields to detect *T. evansi*. Molecular diagnosis of *T. evansi* infection in buffaloes were done by Polymerase chain reaction (PCR) as per the procedure of Wuyts et al. (1995) by using following primers TR3 5´ GCG CGG ATT CTT TGC AGA CGA 3´ and TR4 5´TGC AGA CAC TGG AAT GTT ACT 3´, specifically targeting repetitive nucleotide sequence of variable surface glycoproteins of *T. evansi*. The amplified product was run on 1.5% agarose gel stain with ethidium bromide samples for obtaining the 257bp band. Serum blood glucose was estimated using semiautomatic blood analyzer, as per the manufacturer's instructions and using standard kits to counter the reading of portable blood glucometer. Hemoglobin (Hb) of suspected blood was also estimated (Schalm et al. 1975) to correlate the physiological parameters with trypanosomosis. The statistical analysis was done to prove the relation between fever, trypanosomosis, blood glucose level and anaemia (Snedecor and Cochran 1994)

Results

Out of 123 buffaloes, total seven buffaloes were found positive with trypanosomosis by stained blood smear examination while 21 buffaloes were found positive by PCR (Fig. 1).

The buffaloes found positive in blood microscopy were showing average temperature 105.48^{0}F±0.488, blood glucose 20.614 ± 4.761 and haemoglobin 7.20 ± 1.399 (Table 1). Value of blood glucose (glucometer- 95% CI 80.930 to 88.813 and semiautomatic blood analyzer- CI 78.162 to 86.981) and haemoglobin (95% CI 97.066 to 99.506) were found statistically highly significant (P < 0.001) with body temperature (Table 2).

Table 1
Different body parameters in *Trypanosoma evansi* positive (by blood microscopy) buffaloes

	Body Temperature (^{0}F)	**Blood glucose (mg/dl) Semi automatic blood analyzer**	**Blood glucose (mg/dl) Portable glucometer**	**Haemoglobin (g/dl)**
N	7	7	7	7
Mean	105.486	22.914	20.614	7.200
SD	0.488	5.332	4.761	1.399
SEM	0.184	2.015	1.800	0.529

Table 2
Calculated “t” value in between physiological parameters of *Trypanosoma evansi* positive (by blood microscopy) buffaloes

	Body Temperature (^{0}F)	**Blood glucose (mg/dl) Semi automatic blood analyzer**	**Blood glucose (mg/dl) Portable glucometer**	**Haemoglobin (g/dl)**
N	21	21	21	21
Mean	105.133	35.771	32.319	8.605
SD	0.983	10.669	9.760	1.485
SEM	0.214	2.328	2.130	0.324

Similarly 21 buffaloes found positive by PCR were showing average temperature 105.13^{0}F±0.983, blood glucose 32.319 ± 9.760 and haemoglobin 8.60 ± 1.485 (Table 3). Likewise Value of blood glucose (glucometer- 95% CI 68.488 to 77.141 and semiautomatic blood analyzer- CI 64.637 to 74.087) and haemoglobin (95% CI 95.743 to 97.314) were found statistically highly significant (P < 0.001) with body temperature (Table 4).

Table 3
Different body parameters in *Trypanosoma evansi* (by PCR reaction) buffaloes

Parameter 1	Parameter 2	t value	95% CI
Body Temperature (^{0}F)	Blood glucose (mg/dl) using semi automatic blood analyzer	40.803*	78.162 to 86.981
	Blood glucose (mg/dl) Portable glucometer	46.917*	80.930 to 88.813
	Haemoglobin (g/dl)	175.528*	97.066 to 99.506
* P < 0.0001			

Table 4
Calculated "t" value in between physiological parameters of *Trypanosoma evansi* positive (by PCR reaction) buffaloes

Parameter 1	Parameter 2	t value	95% CI
Body Temperature (^{0}F)	Blood glucose (mg/dl) using semi automatic blood analyzer	29.668*	64.637 to 74.087
	Blood glucose (mg/dl) Portable glucometer	34.017*	68.488 to 77.141
	Haemoglobin (g/dl)	248.417*	95.743 to 97.314
* P < 0.0001			

Discussion

Blood smear examination is considered as gold standard for diagnosis of trypanosomosis even after its limited sensitivity. Although this method is easy to conduct without using expensive equipments. This method gives positive results if numbers of trypanosomes are 10^5 or more in one ml of blood (Reid et al. 2001). The another method having highest sensitivity to detect trypanosomes is amplification of parasite DNA using PCR (Agrawal et al. 2018). The study revealed that lower the level of glucose either by portable blood glucose meter or semi auto analyzer increase the chance of trypanosomosis.

Among 123 buffaloes having history of fever, 21 buffaloes found positive for trypanosomosis in PCR based diagnosis. The blood glucose level of all the infected animals found subnormal with average value of blood glucose by portable glucometer and colorimetric methods was 32.32 ± 9.76mg/dl and 35.77 ± 10.67mg/dl. Precision and accuracy of portable glucometer for cattle was documented 95% and 92%, respectively and value for portable glucometer was significantly lower by 8.3% in cattle (Katsoulos et al. 2011). Hypoglycaemia was reported in infected buffalo which occurs due to increase demand of glucose for metabolism of Trypanosoma (Takeet and Fagbemi 2009). Simultaneously motility of *T. evansi*

depends upon the availability of glucose (Marshall 1948; Newton 1978) besides this high fever and hepatocellular damage in host is also responsible for enhanced metabolic rate. This is in corroborated with finding of Sazmand et al. (2011). There is difference in glycolysis for glucose metabolism between *T. evansi* and eukaryotes because end product is pyruvate rather than lactate in glucose metabolism of *T. evansi* (Opperdoes 1987). Glycolysis is efficient energy pathway as conversion of D-glyceraldehyde-3-phosphate (DGAP) to pyruvate yields two ATP molecules and Triosephosphate isomerase (TIM) (Hunt, 2010). End product of glucose by *T .evansi* is pyruvate (Marshall 1948), although it is utilized by host tissue but accumulation in blood is directly proportional to number of *T. evansi*. Hence, if high number of *T. evansi* is there then resulting in to high concentration of pyruvate leading to depletion of alkalosis resulting in acidosis. Resultant of acidosis is the less affinity of hemoglobin with oxygen (Newton 1978) reflected clinically in form of respiratory distress as reported open mouth breathing in infected buffalo. The average value of glucose, haemoglobin was lower in trypanosome positive animals detected by PCR than microscopy detected trypanosomsa positive buffaloes. The normal value of glucose under field condition in buffaloes was reported 57.66 ± 0.949 and no significant difference ($P < 0.05$) in blood glucose levels among buffaloes of different categories of farmers viz Landless, Marginal, Small and Large categories of farmers (Maurya and Singh 2015).

All the PCR positive cases of present study had significantly lower value of haemoglobin (8.605 ± 0.34). In trypanosomosis, decrease in haemoglobin and PCV are hallmark pathological changes in various species of animals (Tabel et al. 1978). In bovine trypanosomosis anaemia is occurred mainly due to haemolytic factors like hemolysis and free fatty acids, immunologic mechanisms, hemodilution, coagulation disorders, depression of erythrogenesis and release of trypanosomal sialidase (Omer et al. 2007; Adamu et al. 2008). Due to activated and elevated mononuclear phagocytic system, erthrophagocytosis occur along with significant reduced half- life of erythrocytes (Adamu et al. 2008). Besides, sialic acid on surface of erythrocytes are cleaved by sialidase and exposed galactosyl residue recognized by D-galactose specific lectins on macrophages resulting in to erthrophagocytosis (Sallau et al. 2008) otherwise removal of sialic acid from the erythrocyte surface is normally an age-dependent process leads to phagocytosis of aged cell (Sallau et al. 2008). Murthy, 1980 reported normal range of Hb in Indian buffaloes was 10.3 ± 0.2g/dl. Agrawal et al. 2020 reported 90.48% of *Trypanosoma* infected buffalo was showing anaemic condition. Hypoglycemic condition in buffaloes need to be differentiated from ketosis. Buffaloes in type I ketosis(occurs at 4–6 weeks after parturition) and type II ketosis (Very early lactation) showed hypoglycemia without hyperthermia and anaemia (Herdt 2019). Babesiosis and theileriosis are other haemoprotozoan diseases in which if hypoglycaemia is occurred but the degree of hypoglycemia; (Theilerioisis; 51.9 ± 3.82mg/dl), (Babesiosis; 56.67 ± 1.87 mg/dl) is less than in trypanosomosis (35.77 ± 10.67mg/dl) (Ganguly et al. 2019).

Needless to say that timely and accurate diagnosis of trypanosomosis would be helpful to minimize the menace of drug resistance (Chitanga et al 2011) and problems is exaggerated by the facts that research for development of new trypanocide drugs has not been occurred from decades. Resistance against trypanocidal drugs have been reported from 21 African countries along with multiple drug resistance from 10 African countries leading to lesser therapeutic option and major impact on economy. Due to

slowdown in development of new antimicrobial (Grace, 2015), it is need of hour that limited chemoprophylactic and chemotherapeutic trypanocidal compounds should be used with caution (Barret 2004). Due to less cost benefit ratio, pharmaceutical companies are not interested to invest in development of new trypanocidal drug (Geerts et al 2010). Therefore timely and accurate diagnosis of trypanosomosis would be helpful for longevity of available trypanocidal drugs. Plethora of literature is available which clearly indicate that hypoglycemia and anemia is valuable feature of trypanosomosis. But till date no literature traced which indicate the use of theses parameter to diagnose the trypanosomosis in field conditions. The inference can be drawn from present study that if temperature is high and blood glucose level is low then such buffaloes should be suspected for trypanosomosis. Additionally further confirmation of trypanosomosis suspected buffaloes could be done in laboratory by estimating haemoglobin and blood smear examination in low resource laboratory in developing nations. Lower the blood glucose level in fevered animals may suspect the trypanosomosis. In these cases portable blood glucose meter may help the field veterinarian for on spot estimation of blood glucose level of fevered animals. An on spot estimation of lowered glucose level, anaemia and high temperature increases the probability of trypanosomosis. So that timely treatment of infected animal could be possible in rural area without wasting of time to get the report from full equipped laboratory.

Declarations

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Competing interests The authors do not have any financial or personal relationship with other people or organizations that could in appropriately influence or bias the content of the paper.

Availability of data and material Data can be shared through the corresponding author.

Code Availability Not applicable

Authors' contributions Each of the authors contributed in different aspects of the study design, data collection, data analyses and manuscript preparation. All of the authors checked and reviewed the final manuscript.

Ethical approval The study was conducted with the prior consent of the owner and sample collection was carried out in humanely manners considering animal welfare as per the standard protocol of the ethical guidelines

Consent to participate Dairy owner provided informed verbal consent and was always present during the sampling of the calf.

Consent for publication Permission has been granted by the university authorities for publication and all the authors have given their consent for publication.

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Figures

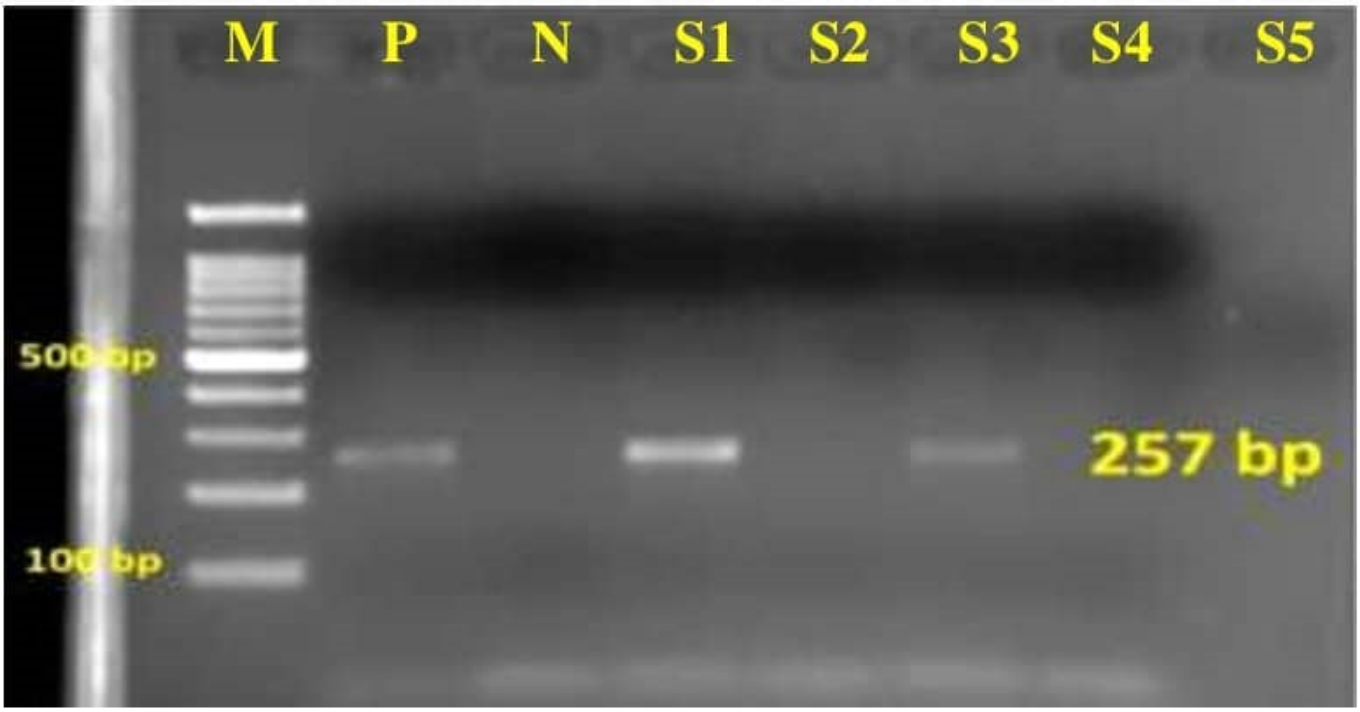

Figure 1

Agarose gel electrophoresis (1.5%) showing the intact band of 257 bp fragment from genomic DNA of Trypanosoma evansi Lane M: 100bp DNA ladder, Lane P: Amplification of T. evansi genomic DNA from the blood of animal positive for infection (Positive control), Lane N: Negative control (No template), Lane S1 & S3: Positive processed field samples, Lane S2, S4 & S5: Negative processed field samples