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Reliability of Some Clinical Parameters for Field Diagnosis of African Animal Trypanosomosis in Cattle

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SUMMARY

Presumptive and inaccurate diagnosis of cattle trypanosomosis among field veterinarians has led to misuse of trypanocides, development of drug resistance, toxicities and huge economic losses. This study assessed the reliability, specificity and sensitivity of some identified trypanosomosis associated signalments (anaemia using FAMACHA® guide, body condition score (BCS), superficial lymph nodes enlargement and jugular pulsation) as field diagnostic parameters. Blood and faecal samples were collected from 273 subjectively selected cattle for the determination of packed cell volume (PCV) and screening for trypanosome, and for helminth egg using McMaster technique, respectively. Data obtained from assessment of packed cell volume based on BCS, FAMANCHA score, superficial lymph nodes enlargement and jugular pulsation were analyzed using descriptive statistics and Chi square, while comparison of data using independent student t-test and one-way ANOVA was similarly conducted. Of the screened cattle, 16.5% (45/273) were positive for trypanosome. Single infection due to *Trypanosoma* spp. was found in 13.5% (37/273) cattle, while 2.9% (8/273) had trypanosome concurrently with *Babesia* spp. or helminth infections. The prevalence of *Trypanosoma* infection was higher in animals with mild or moderately pale mucous membrane, slight emaciation, palpable superficial lymph nodes and pulsating jugular vein. The mean PCV of *Trypanosoma* infected cattle (27.65±0.056) was lower than in non-infected cattle (31.30±0.36) ($p<0.05$). Based on the jugular pulsation status, enlargement of the superficial lymph nodes, trypanosomosis state and Famancha category, there was no significant ($p<0.05$) variation in the PCV of the examined cattle. A negative and low correlation (r = -0.054) existed between BCS and FAMACHA® anaemia score. Each of the clinical diagnostic parameters showed poor sensitivity when employed separately, but the sensitivity improved when applied together and showed 80% specificity to *Trypanosoma* infection. The results of the present study showed that *Trypanosoma* infected cattle were associated with anaemia, emaciation, weight loss, jugular pulsation and lymphadenophathy.

Key Words: Trypanosomosis, Diagnosis, Cattle, Anaemia, Treatment.

INTRODUCTION

African animal trypanosomosis (AAT) is a chronic debilitating disease associated with fever and anaemia resulting in loss of condition and cachexia in animals (Uilenberg, 1998). The disease in cattle is mainly caused by *Trypanosoma congolense* and *T. vivax* and to a lesser extent by *T. brucei* particularly with the sub specie *T. brucei brucei* (Lai *et al*., 2008). Trypanosomosis in livestock is endemic in Nigeria with its abundance being reported in several agro-ecological zones covering about 80% of the total land mass (Fajinmi *et al*., 2011). This may therefore translates into huge economic losses and capable of limiting the growth of livestock industry in the country (Omotainse *et al*., 2004; Fajimi *et al*., 2011).

Transmission of infection can be biological, by *Glossina* species and or mechanical, through biting flies such as *Tabanus* and *Stomoxys* as vectors.

The clinical signs observed in trypanosomosis are the results of the pathogenesis of the infecting *Trpanosoma* species which result in haemolytic anaemia and cardio-vascular collapse (Anosa, 1988). The breakdown of fat reserve and protein in the muscle cells and other tissues results in loss of condition (Urquhart *et al*., 1996; Prowse, 2005). Lymph nodes enlargement in trypanosomosis is associated with plasma cell hyperplasia and hypergammaglobulinaemia primarily due to an increase in immunoglobulin M (IgM) (Singla *et al.*, 1992; Bal *et al.*, 2012). There is also the separation and degeneration of the muscle fibers in the myocardium, resulting in congestive heart failure, passive congestion of liver and spleen, which leads to jugular vein distension and pulsation (Urquhart *et al*., 1996).

The anaemia seen in most infections is the most consistent clinical sign and is often used as a dependable indicator of trypanosomosis (Singla *et al.,* 1997). The methods used for the evaluation of anaemia are also limited, although the highly subjective clinical examination of mucous membrane is the widely used (Grace *et al*., 2007). In South Africa, FAMACHA® anaemia guide was developed to assess anaemia in sheep infected with haemonchosis. The FAMACHA® anaemia guide is made up of high resolution photos of different shades of redness inside the lower eyelid of infected sheep. Each stage of anaemia on the chart is numerically designated from categories 1-5 (Bath *et al*., 2001).

The widely used and the commonly available diagnostic test for trypanosomosis; the light microscopy of different preparations (wet mount, thin and thick blood films and buffy coat technique) is time consuming and unsuitable for use under field condition, in addition to being fraught with low sensitivity and specificity (Eisler *et al.,* 2004; Kumar *et al.,* 2012), while more innovative techniques such as the immunological and molecular methods are expensive and require highly technical facilities and skills (Sharma *et al.,* 2012). Consequently, there is need for the development of diagnostic methods that are simple, affordable and easily applicable by smallholder farmers in tryanosomosis endemic areas. The disease; African animal trypanosomosis has been presumptively diagnosed by pastoralists and field veterinarians based on one or more of clinical parameters consisting of anaemia status, body condition score, superficial lymph nodes status and jugular pulsation. Therefore, this study was designed to determine the reliability of these clinical

parameters of trypanosomosis (Spickler, 2018) for possible diagnosis in the field.

MATERIALS AND METHODS

Study Location

The study was carried out on cattle sampled from Odeda Local Government Area of Ogun State, which lies between 7°13′N3°31′E and 7.217°N coordinates in the transitional zone between the tropical rainforest and the derived Savannah zones of South western Nigeria (Sanusi and Babatunde, 2017). Ogun State has an area of 16,726Km ^{2} expanse of land with an annual temperature range of 21.8^{0}C to 33.2^{0}C and low lying forest area with an average rainfall of 1,445 mm which has a bimodal pattern and peaks in June to September.

Study Animals and Sample Size

A total of 11 White Fulani cattle herds located within three settlements (Odeda, Alabata and Osiele), in Odeda Local Government area of Ogun State were purposely selected for the study after thorough examination. From the 11 selected herds, a total of 273 cattle (25 heads each from 10 herds and 23 animals from one herd), were sampled following initial assessment of the animals' general health condition. The minimum required sample size was calculated using the Epi info software with a 5% degree of precision and a confidence level of 95% and expected prevalence of 15.9% as reported by Dauda *et al*. (2017)

Ethical Approval and Clinical Assessment of Study Animals

This study was approved by the ethics committee of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. Physical examination of the studied animals was done at the farm after restraint according to standard procedure (Hassan and Hassan, 2003). The following parameters were evaluated:

Body Condition Score

This was carried out by the use of specific landmarks (Wildman *et al*., 1982). It consisted of a scoring system ranked 1 through 5 on a scale and measured by appearance, palpation of back and hind quarters. Category 1 animals denote severe cachexic animals, while 5 corresponds to obesity.

Anaemia Assessment Based on FAMACHA© Chart

The colour of the conjunctival mucosa of the lower eyelid was the guide used as previously described (Van Wyk and Bath, 2002).

Jugular Pulsation

The jugular furrow was examined to assess distension of the jugular vein and check for evidence of pulsation. This was reported as present or absent.

Superficial Lymph Nodes Enlargement

The superficial lymph nodes especially the prefemoral and prescapular were examined for enlargement. This was reported as visibly enlarged or not visibly enlarged.

Blood and Faecal Samples Collection

Five (5) ml of venous blood was collected from each animal by jugular venipuncture into an ethylene diamine tetraacetic acid (EDTA) sample bottle. Faecal sample was collected from each study animal directly from the rectum into a plain universal sample bottle. The samples of blood and faeces were transported in ice packed containers to the Veterinary Parasitology Laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta for haematological and parasitological analyses within two hours of collection. All collected faeces were examined for the determination of possible presence of gastrointestinal parasites, while

the presence of haemoparasites was determined by examination of blood. These examinations were done to avoid the effect of concurrent infection.

Haematological Analysis

Packed Cell Volume (PCV)

The PCV of each blood sample was determined using the method described by Dacie and Lewis (1995).

Haemoparasitic Detection

The presence of trypanosomes in the blood sample was determined using buffy coat method as described (Gupta and Singla, 2012; OIE, 2017)

Faecal Sample Analysis

Faecal egg counts were determined using the modified McMaster method (Zajac and Conboy, 2012).

Data Analysis

Descriptive statistics using cross-tab analysis was used to present the data obtained. Comparison of data was done using Chi-square, independent student t-test or one-way ANOVA. All data analysis was done on the statistical package for Social science (SPSS, 2012) version 21 software. A p-value <0.05 was considered significant.

RESULTS

Out of the 273 cattle sampled, 45 (16.5 %) were positive for *Trypanosoma* spp, out of which seven (2.56%) were co-infected with either *Babesia* spp. or strongyle. The prevalence of infection in females (17.5%) was significantly (p>0.05) higher than in the males (7.2%). Mean PCV was found to be lower in trypanosome infected cattle (27.56±0.76) (Table I) than the non-infected (31.36±0.36), with statistically significant (p<0.05) variation.

The body condition scores of trypanosome infected cattle showed that 82.2% had poor body condition (3, 2, and 1). Eighty percent (80%) of infected cattle with poor BCS had corresponding anaemic FAMACHA© chart categories (3, 4, and 5). The only non-anaemic category (2) based on the FAMACHA© chart had only one infected cattle corresponding to BCS 3 (slight emaciation). The mean PCV of infected cattle in FAMACHA© categories 2, 3, 4 and 5 were (31.00±0.00), (27.85±0.98), (26.85±0.98) and (35.33±0.69) respectively (Table II). Overall, there was an inverse relationship between the BCS and FAMACHA© scores. No significant (p>0.05) relationship was observed between the BCS category and their mean PCV. PCV was found to be highest in the BCS category 2 (moderate emaciation) and was lowest in the BCS category 3 (slight emaciation) (Table III).

A total of 73.3% of infected cattle had enlarged pre-femoral lymph nodes with no statistical significance (p>0.05) between trypanosome infection and lymph nodes enlargement (Table IV).

Cattle with jugular pulsation had lower mean PCV than those without. There was however no statistical significant association (p>0.05) between the anaemic state of animals and jugular pulsation. The number of infected cattle (53.3%) with jugular pulsation was not significantly (p>0.05) higher than those without (Table V).

TABLE I: Packed cell volume (PCV) of 273 cattle examined for validation of some parameters for possible field diagnosis of African Trypanosomosis

Trypanosomosis Status	Number of Animals	PCV (%)
Positive Cattle	45	27.60±0.76[a]
Negative Cattle	228	31.40±0.36[b]
Total	273	30.70±0.22

Different superscripts in columns differed significantly ($p<0.05$)

TABLE II: Packed cell volume (PCV) based on Famancha score of 273 cattle examined for validation of some clinical parameters for possible field diagnosis of African Trypanosomosis

Famancha® Score Category	Trypanosomosis Status (n)	PCV (%)	Total PCV (%)
2	Positive (1)	31.00±0.00	30.69±1.23[a]
	Negative (15)	32.06±1.16	
3	Positive (27)	27.85±0.98	30.81±0.46[a]
	Negative (107)	31.39±0.49	
4	Positive (13)	26.85±1.61	31.37±0.56a
	Negative (96)	31.53±0.58	
5	Positive (4)	25.33±0.67	27.46±1.48[a]
	Negative (10)	30.50±2.80	

The same superscripts in columns did not differ significantly ($p<0.05$)

TABLE III: Packed cell volume (PCV) based on body condition score of 273 cattle examined for validation of some clinical parameters for possible field diagnosis of African Trypanosomosis

Body Condition Score	Trypanosomosis Status (n)	PCV (%)	Total PCV (%)
4	Positive (8)	27.63±0.93	31.60±0.66[a]
	Negative (37)	31.69±0.70	
3	Positive (17)	26.47±1.28	31.11±0.48[a]
	Negative (96)	31.43±0.51	
2	Positive (9)	28.89±1.89	31.18±0.64[a]
	Negative (52)	32.21±0.67	
1	Positive (11)	27.70±1.93	27.90±1.34[a]
	Negative (43)	28.25±1.56	

The same superscripts in columns did not differ significantly ($p<0.05$)

TABLE IV: Packed cell volume (PCV) based on lymph node status of 273 cattle examined for validation of some clinical parameters for possible field Diagnosis of African Trypanosomosis

Lymph Node Status	Trypanosomosis Status (n)	PCV (%)	Total PCV (%)
Visibly Enlarged	Positive (33)	26.21±0.90	31.18±0.55[a]
	Negative (139)	31.58±0.45	
Not Enlarged	Positive (12)	30.42±0.96	30.71±0.43[a]
	Negative (89)	31.09±0.58	

The same superscripts in columns did not differ significantly ($p<0.05$)

TABLE V: Packed cell volume (PCV) based on jugular pulsation of 273 cattle examined for validation of some clinical parameters for possible field diagnosis of African Trypanosomosis

Jugular Pulsation	Trypanosomosis Status (n)	PCV (%)	Total PCV (%)
Present	Positive (24)	27.04±0.94	31.01±0.43[a]
	Negative (137)	31.28±0.47	
Absent	Positive (21)	27.90±1.25	30.65±0.54[a]
	Negative (91)	31.54±0.57	

The same superscripts in columns did not differ significantly ($p<0.05$)

DISCUSSION

The overall prevalence of trypanosomosis in cattle in this study is comparable to those reported by Ahmed *et al.* (2007) and Takeet *et al*. (2013) in Nigeria, Mamoudou *et al*. (2006) in Cameroon and Dinede and Aki (2016) in Ethiopia. The PCV of both infected and non-infected cattle in this study is in agreement with the reports of various workers (Enwezor *et al*., 2009; Bitew *et al.*, 2010; Tafese *et al.*, 2012), who observed anaemia as the most consistent clinical finding in AAT. Rowland *et al.* (2001) observed that, improvement in the PCV of trypanosome infected cattle corresponds with a decrease in the rate of detection of typanosomes in the blood. Hence, the mean PCV could be a determinant in the screening and detection of *Trypanasoma* spp. in AAT endemic areas.

The range of anaemic state in trypanosome infected cattle for FAMANCHA categories 3, 4 and 5 was higher compared to anaemia range in haemonchosis, for which the chart was originally designed. This may be due to the fact that anaemia in acute haemonchosis becomes apparent shortly after infection and is characterized by a more progressive and dramatic fall in the PCV than that caused by trypanosomosis (Radostits *et al*., 2006). This development may not be unconnected with the fact that there is loss of whole blood in haemonchosis involving decrease in plasma protein concentration, platelet number and protein–fibrinogen ratio primarily due to haemorrhagic nature of the parasite compared to the anaemia observed in trypanosomosis due to haemolysis.

About 62.2% of infected cattle had ocular mucous membrane colour that corresponded to the borderline category. Thus, at least 60% probability exists that cattle infected with the trypanosome parasite will have a FAMACHA© chart category of 3 (borderline). The FAMACHA© category 3 can therefore be set as the maximum guide point for trypanosome infected cattle. Overall, the FAMACHA test performance was not very good as it proved more specific than sensitive. The poor performance of the FAMACHA in this study, expound that such a test may be most useful for confirming the results of previous tests. A screening test is required to have a high degree of sensitivity to allow for more false positive than false negative cases. High sensitivity of such test is preferable because false negative results are more unacceptable than false positive, given the low cost of treating healthy animals (false positives) and the potential high risk of not treating anaemic animals (false negatives) (Majekodunmi, 2012).

The finding of 82.2% of the infected cattle with varied degrees of emaciation agrees with the earlier finding of Abebe and Wolde (2009), who reported that animals in poor body condition had higher trypanosome prevalence. The poor body condition, probably resulted from the debilitating

nature of the disease as reported by Radostits *et al*. (2006).

The lack of significant relationship between BCS and PCV in this study disagrees with the earlier report of Cabiddu *et al*. (1999) who observed a positive correlation between BCS and PCV. Our findings showed that, as BCS improved, PCV values did not match with the expected corresponding increase, and FAMACHA scores did not decrease accordingly. However, negative correlation between BCS and FAMACHA© score was established during the study, suggesting that the BCS may not be a perfect determinant of PCV in the animals sampled.

Although, there was no significant difference between trypanosome infection and lymph node enlargement, the high proportion of trypanosome infected cattle with lymph node enlargement in this study agreed with the findings of Ikede and Losos (1972), who reported that the lymph nodes of cattle experimentally infected with trypanosome were edematous and grossly enlarged to about three times the normal size from generalized lymphoid hyperplasia. However, a wide range of other disease conditions have been known to cause enlargement of lymph nodes in cattle (McGavin and Zachary, 2006), making it difficult to establish an exclusive nexus between lymph node enlargement and trypanosome infection. Furthermore, the test performance of lymph node enlargement as an index for diagnosis of trypanosomosis in this study was poor, as it had only 19.1% sensitivity. Nevertheless, it implies that up to 70% of cattle with lymph node enlargement in trypanosome endemic regions may be infected with the parasite.

There was low sensitivity of the jugular pulsation in the diagnosis of this disease in all the sampled cattle from this study, as only 14.9% was found. Although, Davis *et al.* (2002), and De Morais and Schwartz (2005), reported the debilitating pathophysiology of trypanosomosis in causing a severe myocardial dysfunction frequently accompanied by decreased filling and dramatic decline in systolic function of the right cardiac output, and manifested by peripheral edema and jugular vein distension, jugular pulsation cannot be entirely designated as a sign of secondary cardiovascular damage due to underlying diseases such as trypanosomosis. This is in view of the low sensitivity which also translates to poor test performance.

Conclusively, this study had shown that the FAMACHA is a reasonable test for anaemia in cattle but could be improved if adapted specifically for use in cattle. The poor performance of the individual indices used for clinical diagnosis of trypanosomosis in this study, have reiterated the fact that there are no pathognomonic clinical signs of the disease. Other potential causes of anaemia, poor body condition, lymph node enlargement and jugular pulsation in trypanosome endemic areas make the clinical diagnosis of trypanosomosis difficult using these indices. However, use of all the four indices together gives very high specificity (at least 80%) in diagnosing trypanosomosis in cattle.

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