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Clinical and hematological study of *Trypanosoma brucei* and *Trypanosoma congolense* in cattle in Mosul City, Iraq

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Abstract

An out-break of trypanosomosis was reported and subsequently investigated in Mosul city, Iraq. Twenty seven blood samples were received at the Clinical pathology Laboratory of Veterinary Teaching Hospital, University of Mosul, from fifteen cows (2-5 years old) and twelve calves (10-12 months old) of different farms located around Mosul city. Clinical signs of the infected animals revealed fever, progressive weight loss, anemia, and frequent recumbent position. Identification of trypanosome species were based on their motility using morphological differentiation on Giemsa or Leishman stained that showed trypomastigot which can be classified according to the biometrical data into *T. brucei* and *T. congolense.* A decreased was also observed in some blood parameters. To our knowledge, this is the first record of *T. brucei* and *T. congolense* infection in cattle. This study also suggested that trypanosomosis is associated with introduction of exotic breeds of cattle into Mosul, Iraq.

Key words: Cattle, *Trypanosoma brucei*, *Trypanosoma congolense*, Cattle, Iraq

Introduction

Bovine trypanosomosis is caused by 3 main pathogenic trypanosome, *Trypanosoma brucei*, Trypanosoma *congolense* and *Trypanosoma vivax*, transmitted in nature by tsetse-flies (*Glossina spp.*). The disease occurs wherever tsetse-flies are prevalent, but may also be transmitted mechanically by other hematophagous flies (Maudlin et al., 2004). The disease has a quite wide host range. It can affect cattle, sheep, goats, pigs, dogs and cats. All of these species are susceptible to the trypanosomosis and may suffer syndromes ranging from subclinical mild or chronic infections to acute fatal disease characterized by intermittent fever, anemia, emaciation, drop in milk production, occasionally diarrhea, rapid loss of body condition, reproductive disorders such as irregular oestrus, abortion, retained placenta, neonatal death, infertility and often terminates in death (Seifert, 1996; Mare, 1998). Besides, there is more than 30 species of the wild animals that can be infected with the pathogenic trypanosomes but many of these remain carriers of the organism. Ruminants are widely known as active reservoirs of the trypanosomes. The disease represents a major obstacle not only for increased food production, but also to the agricultural and socioeconomic development endeavors of the communities in tsetse infested areas (Stephen, 1986).

T. brucei causing wasting in cattle (Nagana) and sleeping sickness in humans, has been long linked to the bite of the tsetse-fly. Early papers described trypanosomes as polymorphic (later pleomorphic) and characterized by morphologies present in the mammalian bloodstream and in different compartments of the tsetse-fly. These early studies provided insight into the way in which trypanosomes replicate and the way in which they adapt to new environments by differentiation. Since the turn of the century the species complex of trypanosomes that causes Nagana and can cause sleeping sickness has been named *Trypanosoma brucei* (Plimmer and Bradford, 1899). For reviews of many of these early studies see Duggan (1970), Cox (1996) and Vickerman (1997).

Trypanosomes are flagellated protozoons, the bloodstream forms are elongate and taper towards the anterior end. They are highly motile and are longer and narrower than the (approximately) 10µm diameter erythrocytes which surround them. Trypanosomes

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possess two masses of DNA, a nucleus and a kinetoplast. The kinetoplast, the genome of the trypanosome's single mitochondrion, is visible at the level of the light microscope and is situated at the posterior end of the cell close to the region which subtends the flagellum.

T. brucei causes a widespread zoonosis and includes the only African trypanosomes pathogenic to humans. *T. brucei* is lysed by human serum but not *T. gambiense* and *T. rhodesiense* (Tyler, 1998). In addition there have been various reports of human infections by stocks of *T. brucei* and resistance to human serum is clearly variable (Hajduk et al., 1994). It remains likely that *T. rhodesiense* is a convenient way of referring to some strains of *T. brucei* which have acquired infectivity to humans, but are easily accommodated inside the genetic heterogeneity of *T. brucei.*

Nagana is a disease of high economic importance which has rendered the tsetse belt of sub-Saharan Africa, a region of some 10 million square kilometers, unsuitable for rearing domestic cattle(Danbirni etal., 2010). Two other tsetse transmitted trypanosomes, *T.congolense* and *T.vivax*, are responsible for most of the cases of Nagana in African cattle (Ameen et al., 2008). *T. congolense* is a haematic trypanosome found only in the blood vessels of the infected animal (Alnasri, 2005). It does not localize or multiply outside the blood vessels. Infection with *T. congolense* may result in peracute, acute or chronic disease in cattle, sheep, goats, horses and camels. Pigs often develop a milder disease; chronic disease is common in dogs. The incubation period is followed by intermittent febrile episodes, depression, lethargy, weakness, loss of condition, anaemia, salivation, lacrimation and nasal discharges. As the disease progresses, loss of condition and hair colour changes from black to metallic brown. Anaemia is a prominent sign. Early in the infection, the organisms are readily demonstrated in lymph node smears (Maré, 1998).

The present study reported for the first time the presence of *T. brucei* and *T. congolense* in blood smears of cattle in Mosul city, Iraq.

Materials and Methods

Twelve calves (10-12 years) and fifteen cows (2-5 years) were brought from different farms around Mosul city to the Veterinary Teaching Hospital at Veterinary Medicine College of Mosul University, having signs of fever, pale mucous membranes, anorexia, dullness, reduce weight gain, weight lose, emaciation, chest edema, short and moist cough, moist rales, un-coordinated movements, mucopurulant ocular discharges recumbency and death of two cows. The clinical picture observed in the animals could be compatible with Trypanosomes infection. Blood samples were taken and send to the Clinical pathology Laboratory. Multiple smears were prepared by placing a small drop of blood on a clean glass slide and covered with cover–slip to spread the blood as a monolayer of cells. This was examined by light microscope (200x). Thin blood smears were prepared and stained with Giemsa and Leishman, then examined under a microscope (Coles, 1986).

For the biometrics characterization, 50 trypanosomes were measured using a light microscope connected to a specific software (Image-prp Plus). The biometric data was compiled as described by Hoare (1972). Blood samples were collected from the jugular vein with ethylene diamine tetra acetate (EDTA) as anticoagulant and examined for estimation of total erythrocyte counts (TEC), total leukocyte counts (TLC), packed cell volume (PCV) and hemoglobin concentration (Hb) according to the method of Weiss and Jane (2010). Morphological changes of blood cells, differential leukocyte counts (DLC), platelets counts (PLT) were estimated from blood smears by the method of Coles (1986).

Results

An outbreak of bovine trypanosomosis in Mosul, Iraq caused by *T. brucei* and *T. congolense* was recorded. The results of the study showed that all calves (12) were infected with *T. brucei* (Fig. 1). *T. brucei* is polymorphic, rapidly moving in wet films, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane. Long slender forms (23–30 µm) with a free flagellum, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central (Fig. 2). The kinetoplast is present in front of the posterior extremity. Short stumpy forms (17–22 µm in length) normally without a free flagellum (Table 1). The kinetoplast usually is subterminal. The position of the nucleus varies greatly and it is in some cases in the posterior part of the cell, sometimes so far posterior that the kinetoplast is anterior to it (so-called postero-nuclear forms). There is considerable variation in appearance between short stumpy forms, from broad, squat types (which include the postero-nuclear forms) to a form similar to *T. congolense*, although longer. In stained specimens blue volutin granules are often present in the cytoplasm, often arranged in a line along the margin of the cell. Intermediate forms, varying in length between the two previously mentioned types. A free flagellum, of varying length, is always present. The nucleus is centrally placed. The posterior end is somewhat variable in shape, but usually bluntly pointed. The kinetoplast is close to the posterior extremity. Volute granules are occasionally present but neither as common nor as plentiful as in the short, stumpy forms.

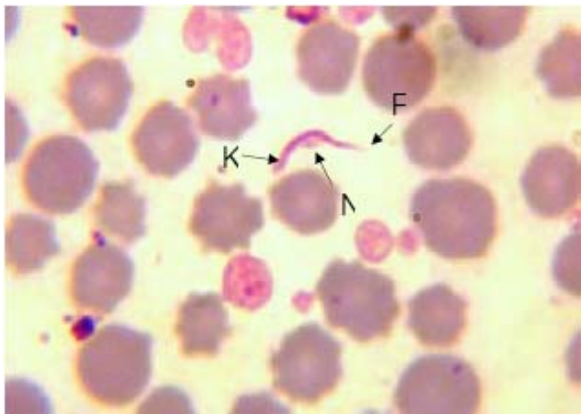

Fig. 1: Blood film with long slender form of Trypanosoma *brucei.* Note the dark sub-terminal Kinetoplas (K), large purple nucleus (N), conspicuous undulating membrane with long flagella (F) .Giemsa stain Oil immersion(1000x).

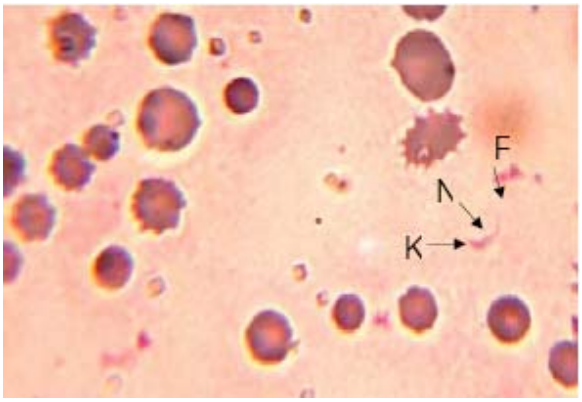

Fig. 2: Blood film with short form of *Trypanosoma brucei* with free flagellum (F), conspicuous undulating membrane, the posterior end is pointed and the nucleus (N) is central with subterminal kinetoplast (K). Leishman stain Oil immersion (1000x)

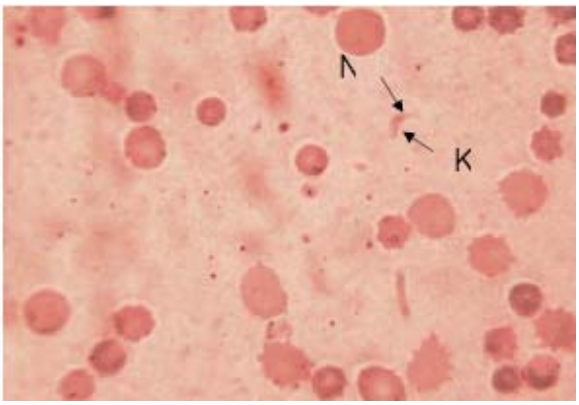

Fig. 3: *T. congolense* (short form) in blood films centrally placed nucleus (N) with kinetoplast (K) situated at the margin of the body, no flagellum and inconspicuous undulating membrane, Leishman stain Oil immersion(1000x).

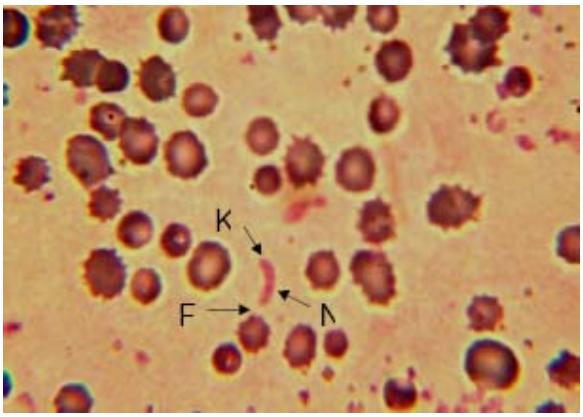

Fig. 4: T. congolense (long form) in blood films centrally placed nucleus (N) with kinetoplast (K) situated at the margin of the body, with very short flagellum (F) and inconspicuous undulating membrane ,Giemsa stain Oil immersion(1000x).

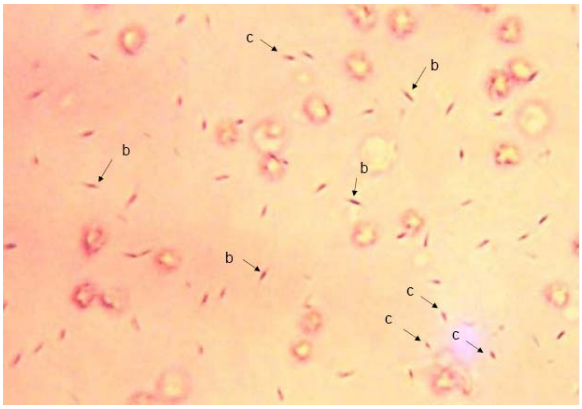

Fig. 5: *T. brucei (b) and T. congolense* (c)

Microscopic examination of blood smears of cows' revealed *T. congolense* in 15 cows, nine of them had a single infection of *T. congolense* while the other six were positive for the two types of trypanosomes (*T. congolense* and *T. brucei*). *Trypanosoma congolense* (Fig. 3) is the smallest of the pathogenic trypanosomes, sluggish in wet films, with a length of 9–22 µm. The blood forms are monomorphic, in that they lack a free flagellum (in the longer forms the shape of the anterior extremity may suggest the presence of a very short free flagellum). The use of the term monomorphic is somewhat misleading in this species due to variation in size and shape between strains. Generally, two variants were seen in blood smears stained by Giemsa, a shorter form (9–18µm), the typical *T. congolense* type and a longer form (up to 25µm), with individuals intermediate in length between the two (Table 2) and (Figure 3). The proportions of long and short forms vary in different cases. In Leishman stained blood smear of *T. congolense*, the cytoplasm stains a diffuse, even, pinkish color and is seldom granular. The nucleus is

Table 1: Measurements (µm±SE) of *Trypanosoma brucei* of cattle

Biometrical data	PK	KN	PN	NA	F	L	PN/KN	PN/NA
Minimum	1.8	2.0	3.2	4.0	7.0	17.0	1.6	0.8
Maximum	6.2	2.6	5.8	6.4	9.0	30.0	2.2	0.9
Mean	4.0	2.3	4.5	5.2	8.0	23.9	1.9	0.85
SE	0.15	0.30	0.16	0.13	0.20	2.1		

PK= Distance from the posterior end to kinetoplast. KN= Distance from Kinetoplast to middle of nucleus; PN= Distance from the posterior end to middle of nucleus. NA= Distance from nucleus to anterior extremity. F= Free flagellum length. L= Total length, including free flagellum.

Table 2: Measurements (µm±SE) of *Trypanosoma congolense* of cattle

Biometrical data	PK	KN	PN	NA	F	L	PN/KN	PN/NA
Minimum	0.7	2.3	2.0	3.6	0.4	9	0.55	0.86
Maximum	2.1	4.7	7.6	8.4	2.2	25.0	1.61	0.90
Mean	1.9	4.2	5.8	3.4	1.7	17.0	1.8	0.75
SE	0.4	1.8	1.5	0.9	0.6	6.0		

PK= Distance from the posterior end to kinetoplast. KN= Distance from Kinetoplast to middle of nucleus.
PN= Distance from the posterior end to middle of nucleus. NA= Distance from nucleus to anterior extremity. F= Free flagellum length. L= Total length, including free flagellum.

Table 3: Initial hemograms from cattle infected with Trypanosomosis (Mean ±SE)

Parameters	Infected calves (*T. brucei*)	Infected cows (T. *congolen)*)	Infected cows (*T. congolense* and *T. brucei*)	Reference range (Mean±SE)
Hb (gm/dl)	9.3±0.3	5.7 ±0.30	7.4±0.01	8-15 (13.5±0.15)
PCV (%)	20.2±2.0	19.5±0.50	15.8±2.3	24-46 (40. 5±0.20)
TEC (cellsx10^6 /µL)	6.3±0.4	5.8±0.25	4.2±0.12	7-14 (12.9±0.25)
TLC(cells x 10^3/ µL)	3.5±0.6	13.2±2.50	3.2±0.15	4-12 (7.8±0.25)
PLT(cells x10^3/ µL)	2.6±0.02	0.04±0.00	1.8±0.05	0.1-0.8 (0.55±0.03)
Neutrophils(x10^3/ µL)	1.35±0.20	5.48±0.80	0.3±0.02	0.6-4.0 (2.3±0.06)
Lymphocytes (x10^3/µL)	4.10± 0.60	2.0 ±0.02	1.6±0.02	2.5-2.7 (2.3±0.03)
Monocytes (x10^3/µL)	0.3± 0.05	0.2±0.02	0.5±0.03	0.25-0.84 (0.52±0.07)
Eosinophiles (x10^3/ µL)	0.00	4.0±0.10	0.00	0-2.4 (1.6±0.03)
Basophiles (x10^3/ µL)	0.00	0.00	0.00	0.00

Reference range source: Meyer and Harvey (2004)

centrally placed. The kinetoplast is of medium size and is usually situated at the margin of the body, just in front of the posterior extremity (marginal and subterminal). The undulating membrane is poorly developed and inconspicuous (Fig. 4).

Microhematocrit Buffy coat technique was used for the detection of infections with both types of trypanosomes (*T.brucei and T.congolense*). The differences between them were obvious (Figure 5). Anemia manifested by the decreased in Hb, TEC, the mean PCV values of the infected animals were significantly lower than those of the reference range, it was 20.2±0.2%. A drop in the mean total leucocyte count. Neutropenia, eosinopenia with lymphocytosis. Thrombocytosis manifested by aa significant increased of platelet count (Table 3). The observed clinical signs in cows with *T. congolense* included the rise in body temperature (41.0-42 Cº), loss of appetite, depression, emaciation, paleness of the mucous membranes, conjunctivitis, corneal opacity, decrease in milk yield, and usually diarrhea. The significant decreased Hb, PCV (19.5±0.5%) and TEC were noticed in affected cows. The total leucocytic count (TLC) revealed leucocytosis associated with lymphopenia, neutrophilia, and eosinophilia with thrombocytopenia (Table 3). Clinical exploration of cows infected with both trypanosomes (*T. cogolense* and *T. brucei*) revealed fever (40.5 – 41.0 ºC), emaciation, dry leather skin, tail alopecia, reduced milk yield (as indicated by the visibly underfed suckling calves), anemia. Mean PCV was 15.8±2.3 %. With significant decreased of Hb and TEC, there was leucopenia due to lymphopenia and neutropenia in comparison with normal range for cattle, with left shift neutropenia and eosinopenia. Thrombocytosis was also recorded (Table 3). The result of the present study was the first reported the presence of *T.brucei T. congolense* in blood smears from naturally infected cattle in the Mosul city Iraq.

Discussion

Trypanosomosis is one of the major constraints for livestock productivity in sub-Saharan Africa (Murray et al., 1982). The animals under investigation were sedentary and were brought from a supposedly tsetse–free area; however, the cattle were trypanosoma infected due to *T. brucei* and *T. congolense*. Only tsetse fly can transmit the infection. Unconfirmed report by

the owners indicated the presence of tsetse flies in the vicinity in recent times. The disease might have occurred due to introduction of a susceptible herd which was imported from Brazil, India, Turkey, Iran and other regions associated with trypanosomiasis. The difference observed in biometrical data could be related to the phase of the disease (acute or chronic). Fairburn (1949) reported that short form of trypanosome was characteristic of the strains causing acute disease in cattle in west Africa, while long form were associated chiefly with strains causing chronic infection in East Africa. When animals become infected with trypanosomosis, their physiology alters (Biryomumaisho et al., 2003). This is due to the wide range of blood biochemical changes and hematological aberrations that occur (Anosa and Isoun, 1980).

The evaluation of blood parameters helps to determine the health status of animal (Coles, 1986), also to establish the degree of damage to hosts tissues as well as the severity of the infection (Allam et al., 2011). Anemia indicated by a drop in PCV values was observed during the course of this infection. It is the most consistent feature of trypanosomosis caused by *T. vivax*, *T. congolense* and *T. brucei* (Anosa, 1983 a&b). The etiology of this anemia is complex, but the most important factor is said to be hemolysis based on a reduction in red cell mass and life span also on the occurrence of erythrophagocytosis, hemosiderosis and sometimes hyperbilirubinemia (Anosa a&b). The depression in the mean leukocyte count and the eventual elevation observed during this infection agrees with findings by Allam et al. (2011). The depression of leukocyte levels could have been the result of immunosuppression, which usually co-exists with trypanosomosis. The decrease in the values of neutrophils seen in this study agrees with findings by Anosa and Isoun (1980), but disagrees with Chaudhary and Iqbal (2000), who observed an increase in the levels of neutrophils in camels infected with *T. evansi*. The decrease could have been a result of overwhelming secondary bacterial infection that comes about as a consequence of immuno-depression in animals infected with trypanosomosis. Also massive amounts of circulating neutrophils are mobilized into the organs that have ongoing severe inflammatory reactions as noticed in this study (Lording and Friend, 1991). The persistent increase in the mean lymphocyte levels contradicts the observations of Chaudhary and Iqbal (2000), who reported a decrease in lymphocytes in camels infected with *Trypanosoma evansi*. The eosinopenia noticed in this study was also observed in goats and sheep infected with *T. vivax* by Anosa and Isoun (1980) and in mice infected with *T. brucei* (Anosa, 1975). The eosinopenia could have resulted from the chronic nature of trypanosomosis (Lording and Friend, 1991). The abnormalities of blood parameters are clear and in a good agreement with the results of experimentally infected gilts with *Trypanosoma brucei* as reported by Allam et al. (2011).

All observed trypanosomes in thin blood smears were identified as *T. brucei* and *T. congolense* which were reported for the first time in Mosul city in Iraq. This study is indicative of changing ecology with regards to the geographical localization of tsetse flies. Therefore, a possibly new risk map of the location and spread of tsetse flies is required.

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