

TRYPANOSOMIASIS EVANSI OF BUFFALO AT BASRAH, IRAQ - CLINICO-HEMATOBIOCHEMICAL AND DIAGNOSTIC STUDIES

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ABSTRACT : *Trypanosoma evansi* infection in buffaloes at Basrah, Iraq was diagnosed. The study was conducted on (86) animals, 3-5 years old, of both sexes, show signs of anemia and lassitude. Ten clinically healthy buffaloes were considered as controls. The infection was diagnosed based on Giemsa stained blood smears and confirmed by Sandwich Elisa with an infection rate of (87.2%) and the parasite has appeared in classical and truncated forms with a thin posterior extremity. Diseased buffaloes show different clinical signs and a significant increase was a notice in the body temperature, respiratory, and heart rate of diseased buffaloes compared with the control group, On the other hand, a statistically significant decrease has been indicated in ruminal contractions. The results of the hematological analysis revealed a significant decrease in the values of Hemoglobin concentration, Red blood cells and Packed cell volume in diseased buffaloes which reflect Normocytic Normochromic anemia. Moreover, a significant increase in total leukocyte count was also indicated due to a significant increase in lymphocytes. The examination of clotting factor parameters show a significant decrease in total Thrombocytes count in diseased animals than in controls, In contrast, a significantly high value was encountered in Thrombocytes volume, Thrombocytes distribution width, Clotting time, Prothrombin time and activated partial thromboplastin time in *Trypanosoma evansi* infected buffaloes compared with healthy controls. Results of acute-phase response indicated a significant increase of Haptoglobin in diseased buffaloes. However, a significant decrease was found in Fibrinogen time of infected animals with *T. evansi* compared with a control group.

Key words : *Trypanosoma evansi*, ELISA, hematobiochemical changes, Iraq.

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INTRODUCTION

Trypanosomiasis is one of the most important infectious diseases infected animals and human being caused by a protozoan parasite belonging to the genus, *Trypanosoma* and transmitted periodically by the tsetse fly, Where it can be said that it is one of the most important global diseases that causes a lack of production especially in African animals, Therefore, it could prevent the use of the land to feed the sudden increase in population (Jaiswa *et al*, 2015; Giordani *et al*, 2016). Surra is the name of the disease caused by *Trypanosoma evansi*, affects a large number of domesticated and even wild type of animals, however is very common in Africa, Asia, as well as the Central and South part of America. The main host could different geographically. However, cows, buffaloes, horses and camels could be infected (Aregawi *et al*, 2018).

The disease is arthropod-borne. Since, different species of blood-sucking flies, including Tabanids and Stomoxes are acting in carrying and transferring the infection from animals to other animals mechanically (Desquesnes *et al*, 2013).

It was documented that considerable variations in the pathogenesis of the causative agent were mentioned (Thompson *et al*, 2014). As, the disease could present in different forms mainly the acute and the chronic form, Whereas, the chronic type might persist for a longer time for up to several months, or possibly years. The disease is predominantly fatal in some animals like horses and camels, but it has also caused mortalities in buffalo, cattle, llamas and dogs. However, these hosts might develop mild or even a subclinical infection (Silva *et al*, 1995). Moreover, some wild animals such as capybara, coati and wild deer's could also be infected and show signs, but

they may also become a carrier. Further, stress and stressors such as pregnancy and/or abortion, malnutrition, and heavy work could increase the susceptibility of the infection (Santos *et al*, 2018).

It was documented that *Trypanosoma evansi* is a form of *Trypanosoma brucei* which lost its kinetoplasts (part of the mitochondrial). Therefore, its capability to promote and develop cyclically in *Glossina* spp could be altered. Therefore, *Trypanosoma evansi* the causative parasite takes another curve, which is the mechanical transmission for movement and the transmission from one host to another. On the other hand, because of this type of transmission (the mechanical type), The parasite does not just stick to transfer via tsetse fly but it will alter to another vector such as *Stomoxys*, *Chrysops*, *Tabanus* and *Lyperosia* (Lai *et al*, 1999; Nakayima *et al*, 2012).

It has been shown that infection with *Trypanosoma evansi* characterized by increase body temperature which directly associated with the average of the parasitemia together with progressive anemia, fatigue, tiredness, and loss of condition, which mostly not sufficiently pathognomonic for the clinical diagnosis of the disease. Moreover, episodes of pyrexia and parasitemia could also be indicated. Furthermore, Edema, especially of the lower parts of the body has been also mentioned in diseased animals (Rodríguez *et al*, 2012; Constable *et al*, 2017). On the other hand, Abortion has been reported in buffalos and camels (Jittapalapong *et al*, 2009; Nguyen *et al*, 2013). Moreover, urticarial like plaques, petechial hemorrhages of the serous membranes, and nervous manifestations are sometimes observed in horses (Laha and Sasmal, 2009).

The clinical diagnosis of the disease may not be sufficient to confirm the infection therefore, Laboratory methods for identifying the causative agent are needed. In the early infection, as the parasitemia is too many, the examination of stained blood smears, and also lymph smears as well as wet blood films, might detect the parasite. Furthermore, the use of a thick type of blood smears, and other procedures like a parasite concentration and inoculation of laboratory animals are also required when the parasitemia is too low in more chronic cases (Constable *et al*, 2017). Ngaira *et al* (2003) and Desquesnes *et al* (2009) added that sequences of DNA are available for diagnosis by PCR, which could be very sensitive and more required nowadays than the microscopic detection of the parasite. However, it might give false-negative results when the parasitemia is too low, in these cases, suspicion of potential carriers can only be confirmed by serological examination, as, the infection gives rise to specific antibody responses.

However, the most relevant are enzyme-linked immunosorbent assays (ELISA), immunofluorescence test, and card agglutination test.

Infection of buffaloes with *Trypanosoma evansi* at Basrah, Iraq are infrequent and little information has been provided. Therefore, the current study was aimed to investigate local buffalo breeds with Clinico-hematobiochemical and diagnostic studies.

MATERIALS AND METHODS

Study design and examined animals

The study was conducted on 86 local buffalo breeds, 3-5 years old, of both sexes, show signs of anemia, lassitude, edema of lower parts of the body. Moreover, Ticks were found infested in different parts of the animal body. Ten clinically healthy local buffalo breeds were considered as the control group. The infection with *Trypanosoma evansi* was firstly detected and diagnosed based on Giemsa stained blood smears (thin and thick blood smears) and was confirmed by ELISA test (Sandwich enzyme-linked immune-sorbent assay) according to the manufacture instructions from (SunLong Biotech Co. Ltd). Routine and complete clinical examinations have been applied to all animals, Besides, carrying out some laboratory tests, to exclude them from the infection with gastrointestinal and blood parasites.

Hematological examinations and samples

In the current study, ten milliliters (10ml) of blood were withdrawn from each buffalo via jugular vein puncture. (2.5 mL) of blood which mixed with Ethylenediaminetetraacetic acid to evaluate the concentration of Hemoglobin (HB), Total erythrocytes count (RBC), the packed cell volume (PCV), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), the total leukocyte counts (TLC), total thrombocytes count, using the full digital cell counter (USA). Moreover, differential leukocyte counts were calculated according to Weiss and Wardrop (2010), using, Giemsa-stained blood smears. Furthermore, The clotting factor parameters were calculated according to manufacture instructions of (Biolabo / France) using the plasma and including a calculation of the thrombocytes distribution width, the mean thrombocytes volume, the prothrombin time, the activated partial prothrombin time, Moreover, The clotting time was also calculated and according to Dayyal (2016).

Biochemical analysis and Evaluation of acute phase response

It includes

- Evaluation of, AST, CK and Total protein (Serum

samples extracted from the blood were tested spectrophotometrically according to the manufactures instructions of Roche Diagnostics, Indianapolis, GMBH, Germany.

- Evaluation of serum Haptoglobin (Sandwich Elisa Method) according to the manufacture instructions of Biotechnology co-China.
- Evaluation of Fibrinogen time (according to the manufacture instructions of (Biolabo / France) (using the plasma).

Statistical analysis

Data were analyzed and the significance of variations has been statistically analyzed using (SPSS) student t-test (Leech *et al*, 2013).

RESULTS

Diseases buffaloes exhibited clinical signs of loss of appetite (94.18%), weakness, lethargy and unable to rise (Fig. 1) (76.74%), pale mucus membranes (70.93%), ticks were found infested different parts of the animal body (69.76%), decrease milk production of lactating animals (52.53%), edema of legs (38.37%) (Fig. 2, Table 1).

Statistical significant high values ($p < 0.05$) are noticed in the body temperature, respiratory and heart rate of infected buffaloes compared with a control group, On the other hand, a statistically significant decrease ($p < 0.05$) has been indicated in ruminal contractions of diseased buffaloes than of controls (Table 2).

Trypanosome evansi was firstly detected and diagnosed based on Giemsa stained blood smears (thin and thick blood smears) and the parasite has appeared in a form of classical and truncated forms with thin posterior extremity together with truncated forms whose posterior extremities are truncated just below the kinetoplast location (Fig. 3 and 4).

Moreover, confirmative diagnosis has been done using ELISA test (Sandwich enzyme-linked immunosorbent assay) with an infection rate of 87.2% as 75 animals were given positive results out of 86.

In the current study, the results of the hematological analysis revealed an obvious reduction ($P < 0.05$) in the concentration of hemoglobin (HB), the erythrocytes cell count (RBC) and the packed cell volume (PCV) in diseased buffaloes compared with a control group, which reflects Normocytic Normochromic type of anemia. Moreover, it has been shown that leucocytosis was also indicated as a result of high values which indicated ($P < 0.05$) in lymphocyte cells (Table 3).

Furthermore, the examination of clotting factor parameters shows a significant decrease ($P < 0.05$) in total



Fig. 1 : Weakness, lethargy and unable to rise.



Fig. 2 : Edema of legs.

Table 1 : Clinical signs of diseased buffaloes with *T. evansi* infection.

Clinical signs	n = 86 (%)
Loss of appetite	94.18
Weakness, lethargy and unable to rise	76.74
Pale mucus membranes	70.93
Ticks were found infested different parts of the animal body	69.76
Decrease milk production of lactating animals	52.32
Edema of legs	38.37

Thrombocytes count in diseased buffaloes than in controls In contrast significant high values ($P < 0.05$) was also encountered in thrombocyte cells volume, the values of the distribution width of thrombocytes, the clotting time, the prothrombin time values and the activated partial thromboplastin time in *Trypanosoma evansi* infected buffaloes compared with healthy control animals (Table 4).

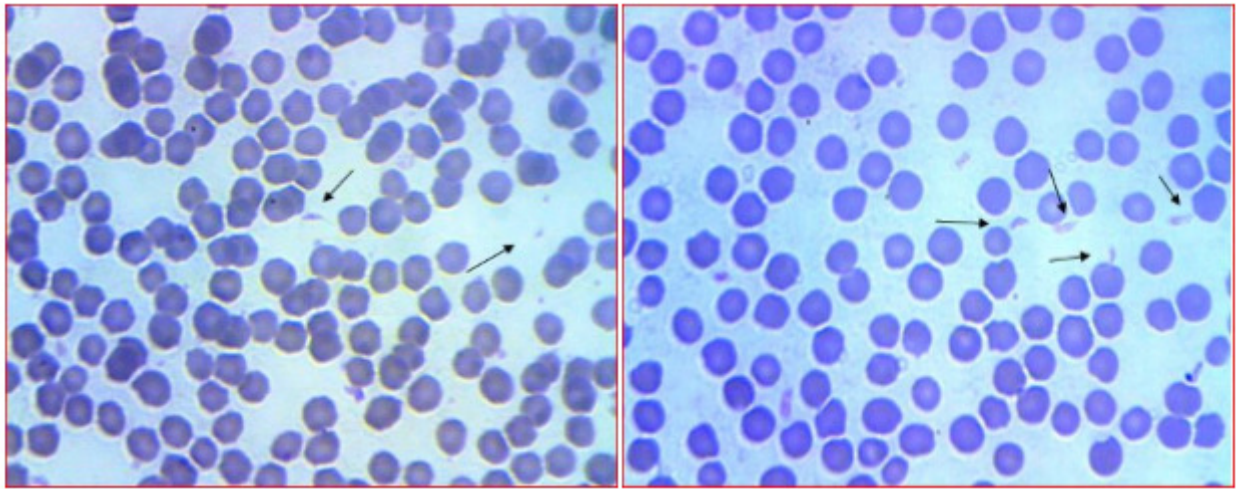


Figure 3 and 4 : *Trypanosoma evansi* in Giemsa stained blood smears 100x.

Table 2 : Body temperature, respiratory rate, heart rate, and ruminal contractions of diseased buffaloes and controls. *Mean ± standard error. * (P<0.05).*

Clinical parameters	Control group n= 10	Diseased n= 86
Body temperature °C	38.33 ± 0.65	40.5 ± 1.31*
Respiratory rate/min	22.45 ± 4.34	72.3 ± 9.2*
Heart rate / min	57.4 ± 3.4	119.3 ± 11.4*
Ruminal contractions/5 mints	3.52 ± 0.72	1.65 ± 1.42*

Table 3 : Changes in blood indices of diseased buffaloes and control group.

Blood indices	Control group n=10	Diseased n=86
Hb g/dl	13.3 ± 1.72	9.52 ± 1.22*
RBC × 10 ⁶	7.43 ± 1.56	5.32 ± 1.23*
PCV %	33.4 ± 4.32	24.71 ± 6.52 *
MCV fL	44.95 ± 4.53	46.44 ± 7.67
MCHC %	39.8 ± 3.78	38.69 ± 2.32
TLC × 10 ³	10.83 ± 3.83	14.21 ± 4.56 *
Lymphocytes/Absolute	4787 ± 334.23	8938 ± 432.23 *
Nutrophils/Absolute	4261 ± 422.12	4224.264 ± 535.13
Monocytes/Absolute	570 ± 362	572 ± 314
Eosinophiles/Absolute	388 ± 12	390 ± 13
Basophiles/Absolute	82 ± 61	81 ± 64

Mean ± standard error * (P<0.05)

As for the biochemical analysis, the results of the present study show that a significant rise (P<0.05) was indicated in values of AST and CK, However, values of total protein were drooped in infected animals compared with the control group, Besides, results of the acute phase response of the current study show a clear increase of Haptoglobin values (P<0.05) in infected buffaloes compared with the control group, However, low significant values (P<0.05) was found in Fibrinogen time of infected animals with *T. evansi* compared with a control group (Table 5).

Table 4 : Clotting factors parameters of diseased buffalo and control group.

Clotting factor parameters	Control group n= 10	Diseased n=86
Total Thrombocytes count×10 ³	577.332±46.331	313.411±52.23*
Thrombocytes volume/fl	12.521±4.247	15.189±3.726*
Thrombocytes distribution width %	12.551±1.661	21.192±6.583*
Clotting time/min	3.422±1.531	4.811±2.871*
Prothrombin time/Sec	12.172±2.351	22.531±4.122*
Activated partial thrombo-plastin time/sec	52.433±5.344	61.352± 12.765*

Mean±standard error. * (P<0.05).

Table 5 : Biochemical changes and acute phase response of diseased buffaloes and control group.

Parameters	Control group n= 10	Diseased n=86
AST U/L	56.43 ± 9.87	176.87 ± 18.11*
CK U/L	254 ± 12.54	634 ± 22.71*
Total protein g/dl	6.71 ± 0.53	5.22 ± 1.02*
Haptoglobin g/dl	0.024 ± 0.005	0.077 ± 0.013*
Fibrinogen time/Sec	12.83 ± 6.43	36.42 ± 8.77*

Mean ± standard error. * (P<0.05)

DISCUSSION

Generally, it can be said that the more severity and the more incidental occurrence of *Trypanosoma evansi* infection in different areas could depend on various events. However, in some regions, practically there was no economic livestock development can be achieved because of the infection. Therefore, the effect of the disease on the agricultural and veterinary levels noticed at the flock level causing decrease milk production of lactating animals, reduced live animal production as well

as reduce the efficiency of animals used for cultivation (Desquesnes *et al*, 2013; Jaiswa *et al*, 2015).

It has been shown that the causative parasite is known to infect a different variety of mammals, including some type of wild of animals. As the main difference from other infectious trypanosome spp. is the loss of its maxicircle kinetoplast DNA. *T. evansi* does not develop in its vector (Aregawi, 2019). It will be transferred mechanically via special vectors from the genera *Tabanus* and *Stomoxys*. The mechanical type of transmission of this parasite could depend on the existence of the protozoan in the transmitter mouth cavity. Nonetheless, the less period of blood-sucking fly between a diseased and a non-diseased animal, the major the transmission success of the causative protozoa (Aregawi *et al*, 2015).

It has been shown that *T. evansi* infection was investigated during many past years. However, the epidemiological criteria of this trypanosomiasis still difficult to understand in different areas and financial financing is ignored on the effects of this infectious disease in people who depend on their domestic animals. In last year's, a lot of investigations was applied on the distribution rate of the disease among the both domestic and non-domestic wild type of animals (Giordani *et al*, 2016).

Diseased buffaloes show several clinical signs which are also mentioned by Thompson *et al* (2014), Constable *et al* (2017) and Aregawi *et al* (2019). *T. evansi* infection was reported in cattle, buffaloes, horses, sheep, camels, and dogs. Clinically manifested by acute, progressive, and severe anemia in most infected animals. However, diseased animals show signs of high body temperature, edema of subcutaneous tissues, obvious anemia reflected by the pale of mucus membranes, blindness some times, weakness and lethargy, with clear changes of hemogram (Desquesnes *et al*, 2013; Thompson *et al*, 2014). Further, Jaiswal *et al* (2015) added that, in buffaloes and cattle, trypanosomiasis could be asymptomatic. However, a peracute or acute infection could also be seen with more mortalities or with obvious clinical signs. The visible manifestations in buffaloes and cattle are mild to severe sometimes especially in Asian and African cases, which are characterized by acute form with intermittent high body temperature, anemia, decrease animal production and loss of body weight.

It has been shown that one of the most important pathological characteristics of *T. evansi* infection which is summarized by Desquesnes *et al* (2013), Constable *et al* (2017) and Aregawi *et al* (2019) is that, the parasites

are inoculated by biting flies into the host while feeding. These organisms might enter the blood through direct way or via the lymph stream and multiplication occur in the bloodstream and all body fluids and initiate the characteristic intermittent parasitemia. The main characteristic of the disease is anemia. However, tissue damage and immunosuppression will also be indicated. Anemia is due to increased erythrophagocytosis, hemolysis and dysheamopoiesis. In cases of severe infections the increasing parasitemia and disseminated coagulation of blood vessels with hemorrhage resemble septicemia. A chronically infected animal may be got concurrent secondary and fatal microbial infections as a result of immunosuppression.

It was explained that the main cause of wasting in trypanosome infecting animals is that, over the time of infection, the appetite becomes changeable, which decreases due to the intensity of body temperature. And could become pronounced, almost until death and thus because of severe weakness the animal cannot be rise. The declared wasting is therefore not caused by starvation. There is the consumption of the body fat reserves during the fever attacks, but there are also severe degenerative changes of the muscle fibers and other tissue cells and there is an increase of protein breakdown in muscles causing atrophic degeneration. Moreover, the decreasing supply of oxygen also has a good role, Furthermore, the significant high of AST in the present study may enhance these results as well (Madhanmohan *et al*, 2003; Arunachalam *et al*, 2008). Furthermore, weakness and lethargy which are represented by infected animals might occur because of decreased muscle masses indicated by decrease values of serum creatinine kinase, probably related to the poor body condition, since, the enzyme creatine kinase is a diagnostic marker for myopathies. Creatinine Kinase is a type of protein that is especially active in skeletal and heart tissue muscles, and also the brain tissue (Smith, 2004).

Anemia is the main and important sign of this disease, in fact, its closely related to the degree of parasitemia. It is of hemolytic type. Therefore, the red blood cells will be engulfed and removed from the bloodstream by the action of phagocytosis. The degenerative and inflammatory processes of cells might occur in different organs such as skeletal muscles and the central nervous system. However, the more prominent pathological effects could be seen in the myocardial muscles where there are separation and degeneration of the muscle fibers (Urquhart *et al*, 1996).

Moreover, Madhanmohan *et al* (2003) confirm that, typically, trypanosomiasis is a wasting type of diseases.

As, there is a gradual loss of animal condition related to increasing anemia and weakness to the point of severe emaciation, followed by collapse and death is the final result in most cases. On the other hand, Nakajima *et al* (2012) was also added that healthy animals whom not been subjected to any physiological harms (such as long transport or walking for long distance between farms) usually suffer less than those animals whom suffering from nutritional deficiencies and having to spend more of their energy.

In the current study, *Trypanosome evansi* has been diagnosed based on Giemsa stained blood smears and the parasite has appeared in a form of classical and truncated forms with thin posterior extremity together with truncated forms whose posterior extremities are truncated just below the kinetoplast location same results was also confirmed by Jaiswa *et al* (2015), Aregawi *et al* (2019). Moreover, the final diagnosis of the disease has been confirmed by the use of Sandwich enzyme-linked immune-sorbent assay. As it was documented that, The sandwich ELISA will measure the antigen between two layers or strata of the antibodies. The intended or targeted antigen must have at least two antigenic sites for binding the specific antibodies. Monoclonal and or polyclonal antibodies can be applied for capturing and detecting the specific antibodies in this sandwich ELISA mechanism. Monoclonal antibodies will be granted a single epitope that allows quantification of small differences in antigen, On the other hand, A polyclonal is applied as the capture antibody to pull down the antigen as much as possible. This test can remove the sample purification step before analysis and it considered as more sensitive than the direct or the indirect Elisa (Elgert, 2009; Rayulu *et al*, 2010).

A significant decrease in hemogram was indicated in the current study which reflected a Normocytic normochromic type of anemia represented by the pale of mucus membranes of diseased buffaloes, same results were also indicated by Madhanmohan *et al* (2003), Arunachalam *et al* (2008), Constable *et al* (2017).

In the present study, Leucocytosis has been detected in diseased buffaloes compared with the control group, same results were also mentioned by Linhares (2006). As high leukocytes number can be indicated as a reflex difference of inflammation or infection, nevertheless, in these circumstances. This reflection could be arranged by several molecules, which are liberated as a response to stimulation that comprises growth or survival agents like the granulocyte-macrophage colony-stimulating factor, the colony-stimulating factor, adhesion molecules and various cytokines. On the other hand, due to the

request on the white blood cells producing layers in the bone marrow have increased to the degree at which there is an inadequate number of mature cells to be sent to the bloodstream, as the infection subsides, the number of younger forms and the total leukocytes count depress and finally could return to normal, during the stage of restoration following an inflammatory process (Debacq *et al*, 2002; Salgado *et al*, 2011).

A significant difference of the clotting factor parameters has been also indicated in diseased buffaloes of the present study compared with the control healthy buffaloes. As, decrease platelet count might occur in a regular pattern when trypanosome infection was in acute form, although, the low values of platelets will not always indicate a severe hemorrhage (Davison *et al*, 2000). It was also indicated by the results of the present study. The exact reason for thrombocytopenia might unclear completely. However, the damage of megakaryocytes and the reduction of thrombocytes production by megakaryocytes as well as the increased consumption of the platelet cells in the periphery and functional defects of the thrombocytes have all been indicated as such a contributing factor (Pantanowitz, 2003).

In the present study, the results were also indicated hypoproteinemia in *Trypanosoma evansi* infected buffaloes which could be the reason for the development of subcutaneous edema of the lower parts of the body indicated in diseased animals, same results were also indicated by Kaneko *et al* (2008) and Migri *et al* (2016), who concluded that the reduction in total protein count which could result from starvation, malabsorption, destruction of proteins due to fever. However, it could be attributed to stress and dehydration which might affect the hepatic parenchyma causing hepatic depletion resulting in less protein synthesis.

Animals with *T. evansi* infection reflect a significant increase in haptoglobin which was also found by El-Bahr *et al* (2016). Increase haptoglobin values could indicate the unspecific and complex innate reaction that occurs very fast after tissue harm, As, the pro-inflammatory cytokines could be released initially at the site of an insult and will be responsible for the starting of local and systemic defenses. It was mentioned that the major mediators of acute-phase protein synthesis in the liver, are Tumor necrosis factor-alpha (TNF- α), inflammatory cytokines, the interleukin-6 and interleukin-1-beta. On the other hand, The main and important functions of acute-phase protein and response are an enhancement of phagocytosis, activation of the immune system, clearance of the product of inflammations. Nevertheless, it has been thought that the acute phase protein response is more active and

sensitive than leukocyte count as a marker of inflammations and are more stable than cellular components. Moreover, they also confirmed that the acute phase response has a faster response rather than alterations in leukocyte count in situations where new leukocytes must be generated by the bone marrow (Jain *et al*, 2011; Tothova *et al*, 2014). On the other hand, the hypo-fibrinogenemia and the prolonged clotting time indicated in the infected buffaloes might suggest the prevalence of petechial hemorrhages, which could sometimes detect on the ocular mucous membranes. However, the thrombocytopenia might also play a present role due to bone marrow depressed activities and over a long way, sequestration of the thrombocytes, which indicated due to disturbance of homeostatic system and could be terminated with infarction due to micro-thrombosis of an important tissue such as the lung, the brain as well as the intestine (Rebar *et al*, 2005).

CONCLUSION

Limited information on the impact of *Trypanosoma evansi* infection among livestock in endemic countries, has been provided, which made the disease of great importance at the medical, veterinary, and economic level because it has great effects on diseased animals, which often end with the death of the infected animal. Therefore, the course of the disease must be monitored continuously and all preventive and curative measures must be taken to reduce it and to reduce the economic loss that may result.

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