



Electrocardiographic Changes Associated with Trypanosomiasis in Horses

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THE objective of this study is to evaluate the electrocardiographic findings in naturally infected horses by trypanosomiasis as well as to investigate the presence of *Trypanosoma sp.* in northern Iraq. Twenty-two male horses aged 3-7 years and their weights of 340 to 390 kg were examined. All infected horses showed anaemia, also two horses died, with clinical signs suggestive of heart failure. Results showed trypanosomes in blood smears of infected horses, which was confirmed using the conventional polymerase chain reaction (c-PCR) technique. 12 horses (54.54%) are infected of them 7 (31.82%) were infected with *T. evansi* and 5 (22.72%) were infected with *T. vivax*. Clinical, haematological, and electrocardiographic evaluations (ECG) were performed. Serum cardiac troponin (cTnI) biomarker was measured to support ECG findings and a deeper understanding of myocardial tissue damage, and the results were compared with normal values in healthy horses and were found negative for blood smear and PCR. The electrocardiograms showed significant changes which characterized by the length of the P and T waves and the QRS complex in addition to the deviation of the electrical heart axis to the left and tachycardia. The mean values of serum cTnI were significantly higher in *T. evansi* and *T. vivax* of infected horses (0.78 and 17.5 ng/m respectively) compared to healthy horses (0.01 range 0.01-0.03 ng/mL). *T. evansi* and *T. vivax* are responsible for the incidence of heart lesions, which are associated with heart failure, changes in the electrocardiogram, and death of the affected horses.

Keywords: *Trypanosoma evansi*, *T. vivax*, Electrocardiography, Horses, Troponin.

Introduction

Trypanosomiasis is a serious disease in horses characterized by fever, pale mucosa, edema of the limbs, lethargy, loss of appetite, emaciation, dehydration, abortion, and incoordination, followed by paralysis of the hindquarters and lips [1]. *T. evansi* is the causative agent of equine trypanosomiasis (Surra), an unapparent spreading disease in Iraqi horses related to the loss of extensive cattle breeding in the Iraqi pastures. In Iraq, the existence of *T. evansi* and *T. vivax* in cattle was first reported in Mosul city in 2012 [2,3] and later *T. vivax* was found in sheep and goats [4]. To the best of the authors' knowledge, *T. vivax* infection had not been reported in horses in Iraq until this study. However, in Africa, it often causes disease in horses and donkeys in

single or mixed infections with *T. congolense*, *T. evansi*, and *T. brucei* [5].

Although the macroscopic lesions that were described in experimental infections in mice and camels with *T. evansi* [6,7] were noted with generalized edema, anemia, lymphadenopathy, hepatomegaly, splenomegaly, renal enlargement, and striated muscle atrophy, as well as epicardial and endocardial hemorrhage. Any report of electrocardiographic changes in natural or experimental-induced infection in horses by *T. evansi* or *T. vivax* is not available. There are previous studies related to electrocardiography to assess the extent of cardiac changes in Chagas disease in humans and dogs [8,9] and dogs infected with *Trypanosoma brucei* [10] as well as cardiac infection in sheep when

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experimentally infected with *Trypanosoma vivax* [11].

The first to indicate the effect of trypanosomiasis on the heart was the scientist Kimeto *et al.* in 1990 [12] by using electron microscopy to examine tissues prepared from experimentally infected cattle hearts with *T. vivax* to detect the parasite in the heart muscle as well as to relate the severity of inflammatory lesions caused by the parasite outside blood vessels. Other researchers [11] observed the presence of interstitial edema, mononuclear multifocal myocarditis, myocardial necrosis, and degeneration of conduction tissues in the heart of experimentally infected cattle and goat tissues. Although cardiac lesions are diagnosed frequently on histological examination of animals infected with *T. evansi* and *T. vivax*, little is recognized about the functional importance of the lesions or their clinical effects with time.

However, these studies did not indicate the relationship between such lesions and histopathological signs with the development of clinical signs of cardiac dysfunction and death in animals. Therefore, this study aims to evaluate the clinical signs, hematological, cardiac biomarker, and ECG changes in the hearts of horses normally infected with *T. evansi* and *T. vivax* as well as to confirm the presence of trypanosome DNA in the horse blood using the c-PCR technique

Material and Methods

Animal description

This study included twenty-two male horses, 3-7 years old, and their weight ranged from 340 to 390 kg, housed in single stalls at the Equestrian Club (Nadi Alfurusia) in the Shallalat area, Mosul, Iraq. Some of them had been suffering from anorexia and gradual weight loss for two months. The veterinary teaching hospital at the University of Mosul was summoned to examine and treat horses and conduct further diagnosis to find out the causes of their weakness and the death of two of them. Information obtained from the case histories revealed that the horses were indeed in poor physical condition although they were in good health when they were brought to the club. The horses were given supportive treatment for clinical signs during the first visit, and samples were collected for diagnostic tests. But on the morning of the second visit scheduled two days later, two animals were found dead and had previously shown clinical signs of heart failure. At this time, the differential diagnosis for these cases was largely one of hematoparasitism. All the animals underwent clinical, hematological, and molecular evaluations an electrocardiogram (ECG) was recorded, and serum cardiac troponin I (cTnI) was also measured to evaluate cardiac involvement.

Clinical examination

After taking the medical history of the cases and collecting information about them, basic clinical examinations such as body temperature, heart rate, and respiratory rate were performed on all horses, and the clinical signs that appeared on some horses were recorded.

Blood sampling and hematological examination

Six milliliters of jugular vein blood samples were collected from all horses (n=22). Four milliliters of blood were placed into a special tube containing Ethylenediaminetetraacetic acid (EDTA). Two milliliters of the EDTA blood were stored in a cool box for DNA extraction and sent to the Laboratory of Clinical Pathology at the Department of internal and Preventive Medicine, Veterinary Teaching Hospital, University of Mosul, Iraq. These samples were then placed in microtubes and kept at -20 °C until PCR testing. Also, 2 mL of EDTA blood was taken for blood tests including hematocrit (HCT) (%), hemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and erythrocyte indices. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were analyzed using the Abaxis Vet scan HM5CVS2 Hematology Analyzer, eBay.com. Morphological criteria of *Trypanosoma* parasites upon microscopic examination of blood smears stained with Giemsa and acridine orange (GAO) were used to confirm the diagnosis of trypanosomiasis in horses. Using the same sample of venous blood, a thin blood smear (5 µl) was prepared and stained with GAO in a ratio of 2:3 in which 40 ml of Acridine Orange (0.1 mg/ml) (Sigma-Aldrich: No. 318337) was mixed with 60 ml of Giemsa 10% (Bio lab Diagnostics Ltd, India). The mixture of both stains was flooded on each slide and left for 10 minutes on the bench at room temperature before rinsing with buffered distilled water with PH 6.8 for examination by bright field microscopy; then examination by fluorescence microscopy (BX51 Olympus U-RFL-T-Japan) under oil immersion [13].

Cardiac troponin I (cTnI) Assays

Two milliliters of blood were placed in a plain test tube and left to clot for 10-20 minutes at room temperature to obtain serum, then centrifuged at 2,000-3,000 rpm for 20 minutes.

The horse cardiac troponin I (cTnI) Sandwich-ELISA Kit (Sun Long Biotech Co., LTD) was used for the measurement of serum I (cTnI). Absorbance optical density (OD) was read at 450nm using a microtiter

plate reader. The serum I (cTnI) values were compared with the values of ten healthy horses.

DNA extraction and PCR

In the current study, infection of horses with *Trypanosoma sp.* was confirmed by testing whole blood samples of 22 horses using ITS1CF/BR PCR.

From each sample, 250 µl was used to extract genomic DNA using a commercially available kit (Qiagen D Neasy Blood and Tissue kits, Qiagen, Germany). The manufacturer's instructions were followed, and the DNA samples were stored at -80 °C until use. PCR tests were performed using two pairs of primers for analysis

Details on the primers used in the ITS1CF/BR PCR Trypanozoon are F:5'-CCGGAAGTTCACCGATATTG-3' R:5'-TGCTGCGTTCCTTCAACGAA-3' 480 bp [14]. RoTat 1.2 PCR *T. evansi* F:5'-GCGGGGTGTTTAAAGCAATA-3' R:5'-ATTAGTGCTGCGTGTGTTTCG-3' 205 bp according to [15] and TVW primers *T. vivax*-specific, TVW 1: TGAGTGCTCCATGTGCCAC and TVW2: CCACCAGAACACCAACCTGA 150 bp [16]. According to the method of amplification of PCR described by Suganuma et al. [16]. The products were analyzed by electrophoresis in a 1.5% agarose gel and UV illumination after staining the DNA with ethidium bromide.

Electrocardiographic recordings

The cardiac function of horses naturally infected with *T. evansi* and *T. vivax* infections was studied electrocardiographically. Einthoven's triangle-leads electrocardiogram was recorded using six limb leads (I, II, III, AVR, AVL, and AVF). Electrocardiogram was recorded from a standing position for each horse using a portable electrocardiograph (EDAN VET ECG, Model VE-100, 220V-240 V-/ 100V-115 V, 50Hz/60Hz. 0.3 max, Edan Instrment Inc, Shenzhen, P.R. China). The paper speed during ECG recording was 25mm/sec, and the pen sensitivity was 1mm=0.1 mV. The electrodes were placed on the appropriate limbs after applying electrode gel to get good contact. The electrodes were placed as follows: the red electrode was placed behind the elbow on the right forelimb, the yellow electrode was placed behind the elbow on the left forelimb, the green electrode was placed on the left hind limb in front of the stifle, and the black lead was placed on the right hind limb, positioned in front of the stifle [Fig. 1]. The ECGs of twelve horses were analyzed and compared with the ECGs of ten healthy horses.

Electrocardiographic measurements

Heart rate was calculated automatically by the device and checked manually by counting the number of QRS complexes over a period of six seconds and

multiplying them by ten [17]. Using a ruler and calipers, QT and QRS durations, and R and T wave amplitudes were recorded manually. The QT interval was determined from the beginning of Q to the end of T, which can be defined as the return of T to the electrical baseline [18]. The length of the longest and shortest RR intervals with the corresponding QT interval was measured on each ECG. The T amplitude was calculated as the T/R ratio [8]. The corrected QT interval (QTc) was calculated using the Friedrichia formula; $QTc = QT/RR^{(1/3)}$ [10].

Statistical analysis

The data were expressed as mean ± standard deviation. Differences within parameters were evaluated by ANOVA for repeated measures. The statistical significance between the healthy and values of the infected horses was determined using a paired t-test. When $p < 0.05$, and $p < 0.001$, the infection is adjudged to have affected the parameter. All statistical tests were done using SPSS version 23.

Ethical Considerations

The approval of the ethics committee of the college of veterinary medicine at the University of Mosul, Iraq, was obtained to conduct this study. Written informed consent was also obtained from each participant prior to enrollment in the study.

Results

The presence of trypanosomes in the blood was observed in twelve horses (54.54%) out of 22 horses, 7 of them were infected with *T. evansi* (31.82%), and 5 horses (22.72%) were infected with *T. vivax*. The remaining 10 healthy horses were used for comparison of parameters.

Clinical findings

During the clinical examination, the horses infected with *T. evansi* were alert, revealed emaciation, pale pink, oral mucous membrane, hyperthermia 103.64F (39.8°C±1.5) tachycardia (64.0±2.4) beats/minute and significantly increase in respiratory rates (54±2 breaths/minute). Whereas, horses infected with *T. vivax* had a rectal temperature of 101.28F (38.48°C). The heart rate was 98 ± 5.2 beats/min. The mucous membranes were icteric. The cardiac rhythm was irregular, and the heart sounds were of varying intensity on auscultation. Heart murmurs were not detected. A staggering gait, hindquarter weakness, incoordination, ataxia, and paralysis of the hindquarter and lips before death were observed in two horses.

Microscopically examining Giemsa acridine orange (GAO) stained blood smears revealed Trypanosome infection in 12/22 (54.54%) horses. A thin blood smear showed high parasitemia by *T. evansi* which is characterized by the centrally located nucleus,

kinetoplast, undulating membrane, and flagellum [Fig.2 a, b]. A rare and different form of *Trypanosoma species* with a few parasitemia was observed in the other five horses. The size and shape of the trypomastigotes were different from *T. evansi*, and the position of the kinetoplast in the rounded posterior extremity allowed us to classify it as *T. vivax*. [Fig. 2, c, d].

The initial diagnosis of *T. evansi* and/or *T. vivax* in the blood smears was confirmed by PCR testing. No blood parasite was detected in the remaining horses. PCR revealed that 7/22 (31.22%) horses were positive for *T. evansi* [Fig.3]. However, results were positive for 5/22 (22.72%) in other samples using the *T. vivax*-specific primers [Fig.4]. No mixed infection was recorded in this study.

In *T. evansi* infected horses the hematological results revealed a significant decrease ($p < 0.05$) in erythrocytes count, mean corpuscular hemoglobin concentration, hemoglobin concentration levels, hematocrit, the mean corpuscular volume (MCV) were normal with decreased MCHC, indicative of normocytic hypochromic anemia. Hemograms of *T. vivax* horses showed a significant decrease in values of total red blood cell count, hematocrit, and hemoglobin concentration. Erythrocytes indices were normal which means normocytic normochromic anemia. Leukocytosis and monocytosis were recorded in all affected horses when compared to normal values [Table 1]. Mean hematocrit values and standard deviations SD, were assigned according to the status (positive or negative) in the blood smears, and PCR assay. Horses found positive in blood smears, and PCR tests had significantly lower average HCT values than animals that were found negative in both tests. HCT mean values in positive *T. evansi* horses ($30.0 \pm 0.34\%$) and *T. vivax* positive horses ($24.6 \pm 0.21\%$) were significantly lower from negatives ($41.0 \pm 0.35\%$) ($p < 0.05$) [Table 1].

The mean values of serum cTnI were significantly ($P < 0.001$) higher in *T. evansi* and *T. vivax* infected horses (0.78 and 17.5 ng/m respectively) than levels in healthy horses (0.01-0.03 ng/mL). Furthermore, it was found that *T. vivax* infected horses had higher serum concentrations of cTnI than both *T. evansi* horses and healthy horses. High cTnI concentration (>0.03 ng/mL) was detected in a high percentage of horses with *Trypanosoma evansi* (5/7, 71.42%), and horses with a *T. vivax* (3/5; 60%) in comparison with healthy horses (0/10). The mean of cTnI concentrations for trypanosomes-infected animals with cardiac abnormality was 25.32 ng/mL and ranged from 0.28 to 49.80 ng/mL, which was higher than in the healthy horses (0.03 ng/mL) and ranged from 0.01 to 0.03 ng/mL. Out of the 12 infected horses, two with heart failure have died which were

infected with *T. vivax*. The mean cTnI concentration of the dead horses was (24.94ng/mL, ranging from 0.01 to 49.80 ng/mL) was higher than that of the survivors (15.16 ng/mL, ranging from 0.01 to 30.31 ng/mL).

Electrocardiographic Findings

The normal ECG of six leads of the horse was illustrated in [Fig5a,b]. Infected horses had increased heart rates in all six leads. Infected horses showed clinical signs indicating heart failure and electrocardiogram alterations which were characterized by prolonged P, T, and QRS complex durations. Furthermore, a cardiac electrical axis shift to the left was detected with a significant increase in heart rate ($p < 0.05$) in a comparison with normal adult horses have a heart rate of 35 ± 5.2 bpm [Fig.6]. At many infected horses, the QRS complex was significantly (<0.05) wider than those of the healthy horses. *T. evansi* infected horses have a lower variability of their RR index compared to healthy horses. The R wave voltage was reduced and the T wave amplitude (tall T) was increased due to the infection in lead I and lead aVL respectively [Fig.7]. Prolongation QT and QTc of the infected horses were significantly higher ($p < 0.05$ and $p < 0.001$ respectively) than the uninfected ones in leads II and AVF [Table 2]. Ventricular premature depolarization (VPD) [Fig.8]. Notched R wave, ST wave abnormalities, and sinoventricular rhythm were detected in the infected horses [Fig.9,10, 11].

The ECG baseline intervals and characteristics are listed in Table 2. At baseline, QTc prolongation, which was 0.47 ± 0.03 sec. observed in 13% of all horses affected with *T. evansi* and 27% of horses affected with *T. vivax*. These QTc values are considered to indicate an increased risk for arrhythmia. The proportion of major ECG findings indicating heart involvement was significantly ($p < 0.001$) lower in *T. evansi* (53.5%) than in *T. vivax* (69.5%). The QTc interval was prolonged, and the early repolarization changes were of the type ST wave elevation (0.1 mV) concave without notch, and partial right bundle branch block (RBBB), a notch at point J, positive and tall T waves. The PR depression was >0.8 mV and low voltage R was <1.6 mV in leads I, II, and III. All these changes were significantly more frequent than in healthy horses.

Discussion

A natural infection with *T. evansi* and *T. vivax* was reported in horses in Al Shallalat district, Mosul, northern Iraq, resulting in severe disease and the death of two out of twelve animals diagnosed with the infection. The diagnosis of trypanosomiasis caused by *T. vivax* was not expected in Iraqi horses. At first, horses were suspected of being infected with *T. evansi*, which commonly happens in dogs, cattle,

horses, camels, and buffaloes in Iraq [2, 19, 20, 21, 22]. *T. vivax* has been considered emergent in Iraq [3]. Although the results of the blood smear and PCR test were negative for *T. evansi* in five of the 22 horses examined, both methods confirmed their infection with *T. vivax*. The clinical findings observed in this study were consistent with those reported in previous studies in horses infected with *T. evansi*, such as fever, anemia and hindquarter weakness, lethargy, staggering gait, incoordination, and ataxia [23, 24]. Hematological examinations of the *T. evansi* infected horses showed that the horses had normocytic and hypochromic anemia, whereas horses infected with *T. vivax* suffered from normocytic normochromic anemia. These results agree well with previous studies [23,24]. In fact, anemia can be considered the most important and main sign of trypanosomiasis, which is closely related to the degree of parasitism. Anemia is attributed to the engulfing of the erythrocytes and their removal from the blood by the action of mononuclear phagocytosis which explains the decrease in total red blood cells [25]. Another cause of anemia may be due to the toxic substances produced by trypanosomes, in addition to the action of host-trypanosome cellular interference. Due to the adhesion of *T. evansi* and *T. vivax* on the surface of red blood cells, the shape of the erythrocytes changes, as well as the formation of holes or vesicles in the surface of the red blood cells [26,27]. Altered RBCs are susceptible to removal by mononuclear phagocytic activity, which causes a decrease in hematocrit during infection. Also, affected horses were shown to have leukocytosis and mononucleosis as a result of increased activities of the mononuclear phagocytic system to engulf aged and dead erythrocytes due to extensive extravascular hemolysis.

In equines, *T. vivax* frequently causes chronic disease, weight loss, and severe anemia, differing from *T. evansi* which usually causes severe infection and sudden death of the animal [5]. However, in the current study, according to the case history, clinical signs, and ECG changes, it was concluded that the horses had trypanosomiasis and were in a chronic stage before death rather than in an acute form. Moreover, infected horses were kept with other horses, and no high mortality rate was reported for any of the infections in the club. Overall, all infected cases were classified as chronic *T. evansi* with signs of chronic weight loss, impaired performance, fever, and signs of neurological abnormalities including ataxia, incoordination, and hind limb weakness. There is good agreement between these observations with what was recorded by others [20,23,24]. It is rare for chronic trypanosomiasis to be detected in

Iraq because animals do not show clear signs as in acute trypanosomiasis. While blood smear testing may be easy and inexpensive, it lacks a high sensitivity rate because parasitemia is rather low in chronic cases [28]. Definitive diagnosis of infection was possible by PCR because the tests were performed for veterinary training purposes at the teaching hospital. In usual cases, these diagnostic techniques may not be performed because the cost of performing these tests is high and may not be accepted by the owner without a strong suspicion of the etiology. Thus, this may have donated to the absence of information on the species of trypanosomes infected horses in Iraq. Therefore, due to the lack of recent information on trypanosomiasis in Iraqi horses, local horses may be at risk of severe equine disease.

Since the results of ECG can be used as an additional tool to assess the progression of the disease [29,30], it was relied upon in the evaluation of the disease state in this study. Degenerative and inflammatory processes of cells may occur in different organs such as skeletal muscles and central nervous system during trypanosomiasis [31,32]. *T. evansi* and *T. vivax* can be considered responsible for the incidence of heart lesions that manifested as signs of heart failure, changes in ECG, and mortality of affected horses in the current study. The most prominent pathological changes such as degeneration of cardiac myofibers during experimental infection of mice with *T. evansi* isolates derived from naturally infected horses were observed in the myocardium previously [33,34]. In addition, *T. vivax* was diagnosed by PCR in the cardiac tissue of experimentally infected sheep [11]. Since cardiac biomarkers are an easy way to track cardiac injury from progressive myocarditis associated with increased cTnI in serum [35,36], serum cardiac cTnI troponin was measured to support ECG findings and provide a better understanding of cardiac injury. The increase in serum cTnI values in horses infected with *T. evansi* and *T. vivax* compared to levels in healthy horses could be explained by the occurrence of oxidative stress in the heart tissues. This has been proven by other researchers in previous studies [33, 37]. The significance of the ECG findings was interpreted in light of the clinical signs shown by affected horses. An ECG can make a definitive diagnosis if an arrhythmia is suspected during cardiac auscultation [38,39]. The arrhythmia reported in this study may be due to myocardial infarction, a condition reported in experimental *Trypanosoma brucei* infection in dogs [10]. The tachycardia, variability of RR, and prolongation of the QT interval and QTc observed in horses infected with *T. evansi* or *T. vivax* may be attributed to the increased sympathetic activity in infected horses, as

indicated by other researchers [10]. An increase in the QT interval depends on changes in heart rate and the risk of cardiac death is associated with increased heart rate variability [9]. The electrocardiogram of infected horses showed an increase in the length of the T waves, and this increase can be attributed to acute myocardial ischemia, which is a feature of hyperkalemia. Trypanosomes use glucose and oxygen present in the host for their growth and reproduction, which leads to the depletion of these agents and consequently degenerative changes in the host [40]. In the absence of successful treatment, trypanosome infection persists for a lifetime, causing serious cardiac disease and the death of the infected horses.

Conclusion

This is the first report of *T. evansi* and *T. vivax* in Iraqi horses in northern Iraq. It was concluded that infection with *T. evansi* and *T. vivax* causes changes in the electrocardiogram (ECG) and is a cause of

poor performance in horses. Cardiac involvement in horses infected with *T. evansi*, and *T. vivax* as demonstrated by ECG alterations, and an elevation in cTnI. QTc prolongation indicates a risk of fatal arrhythmias.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding statement

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Fig. 1. The positioning of electrodes for ECG recording, using a recording device (EDAN VET ECG) with 4 electrode cables (green, yellow, red and black) and a self-adhesive electrode.

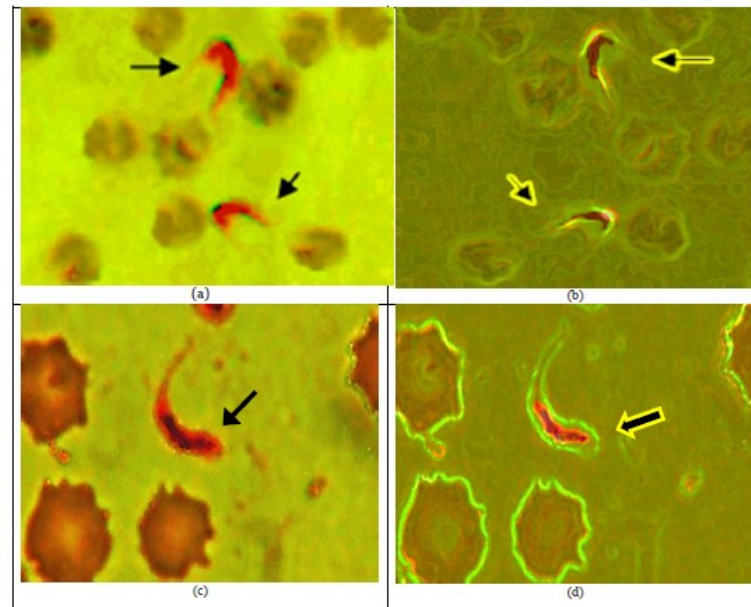


Fig. 2. Bright field microscope images of horse blood smear stained GAO. Predicted *T. evansi* parasites observed in (Size 360x469 pixels). Scale bar—10 μ m (a). Fluorescent image of blood smear from a horse *Trypanosoma evansi* infection. Note the elongated trypanosomes. (Size 360x483 pixels). Scale bar—10 μ m (b). blood smear from a horse *Trypanosoma vivax* in horse blood smear (Size 360x464 pixels). Scale bar—10 μ m (c). Fluorescent image *Trypanosoma vivax* in horse blood smear (Size 360x464 pixels). Scale bar—10 μ m (d).

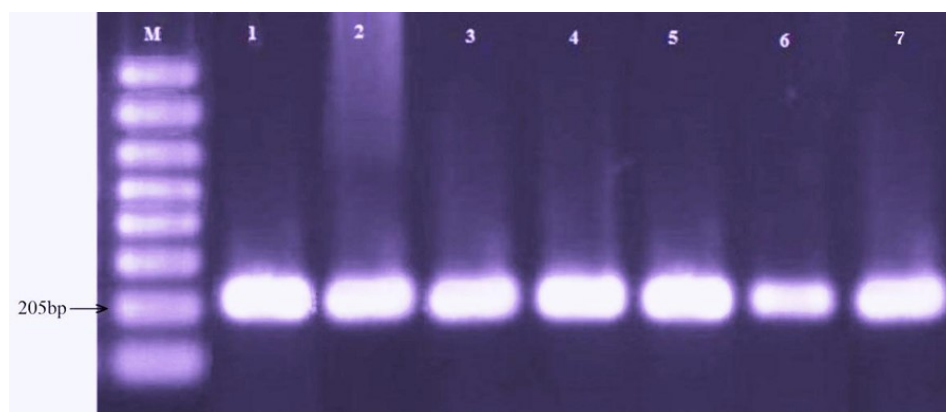


Fig. 3. RoTat 1.2 PCR products. Lane M: molecular weight marker; (lanes 1–7) *T. evansi* DNA on 1.5% agarose gel stained with ethidium bromide.

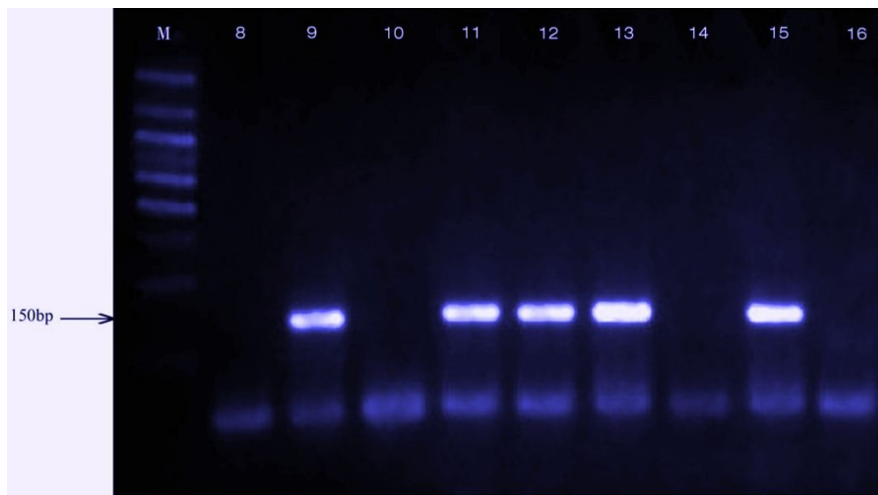


Fig. 4. TVW 1.2 PCR products. Lane M: molecular weight marker; (lanes 9,11, 12,13,15) *T. vivax* DNA on 1.5% agarose gel stained with ethidium bromide.

TABLE 1. Hematologic results of the horses infected with *T. evansi* and *T. vivax*

Hematology parameters	<i>T. evansi</i> infected horses (n=7)	<i>T. vivax</i> infected horses (n=5)	Healthy horses (n=10)	Reference range
RBC ($10^6/\mu\text{l}$)	5.1 \pm 0.20*	4.9 \pm 0.10*	7.3 \pm 0.18	6.0–12.0
HGB (g/dl)	7.7 \pm 0.10*	8.5 \pm 0.12*	13.8 \pm 0.53	11.0–17.0
HCT (%)	30.0 \pm 0.34*	24.6 \pm 0.21*	41.0 \pm 0.35	35.0–55.0
MCV (μm^3)	58.88 \pm 1.34	50.20 \pm 2.60	56.16 \pm 1.21	34.0–58.0
MCHC (g/dl)	25.66 \pm 0.67*	34.55 \pm 0.63	33.65 \pm 0.34	31.0–35.5
WBC ($10^3/\mu\text{l}$)	14.3 \pm 0.93*	11.8 \pm 0.28*	8.7 \pm 0.54	5.4–12.7
Monocyte ($10^3/\mu\text{l}$)	2.0 \pm 0.06*	3.4 \pm 0.01	0.2 \pm 0.02	0.1–0.8
Lymphocyte ($10^3/\mu\text{l}$)	5.0 \pm 0.02	2.3 \pm 0.03	3.6 \pm 0.01	1.5–5.5
Granulocyte ($10^3/\mu\text{l}$)	7.3 \pm 0.01	6.1 \pm 0.01	4.9 \pm 0.01	2.0–8.0

* Mean significant at $P < 0.05$. Values are mean \pm SD for horses. SD: Standard Deviation.

TABLE 2. ECG findings at baseline, by diseased horses with *T. evansi* compared to healthy horses

ECG values	Healthy horses (n= 10) (mean \pm SD)	<i>T. evansi</i> infected horses (n= 7) (mean \pm SD)	<i>T. vivax</i> infected horses (n= 5) (mean \pm SD)	p-value
Heart rate (bpm)	35.0 \pm 5.2	64.0 \pm 2.4	98.0 \pm 5.2	< 0.05
P wave (mV)	0.11 \pm 0.05	0.18 \pm 0.01	0.20 \pm 0.01	<0.001
T wave (mV)	0.16 \pm 0.06	10.0 \pm 0.20	0.14 \pm 0.07	<0.001
ST segment mV	0.31 \pm 0.06	0.35 \pm 0.11	1.19 \pm 0.08	< 0.05
PR interval sec.	0.28 \pm 0.06	0.50 \pm 0.01	0.80 \pm 0.04	< 0.05
R-R sec.	0.32 \pm 0.06	0.80 \pm 0.06	1.00 \pm 0.05	<0.001
QRS sec.*	0.11 \pm 0.01	0.14 \pm 0.01	0.19 \pm 0.01	< 0.05
QT sec.	0.51 \pm 0.04	0.59 \pm 0.06	0.58 \pm 0.08	<0.05
QTc sec.	0.37 \pm 0.025	0.47 \pm 0.03	0.52 \pm 0.01	0.001
ECG findings %		53.5%	69.5%	<0.001

Values of the ECG parameters are expressed as mean \pm SD., n = number of animals., when $p < 0.001$ (highly significant), and $p < 0.05$ (moderately significant) compared to the healthy horses.

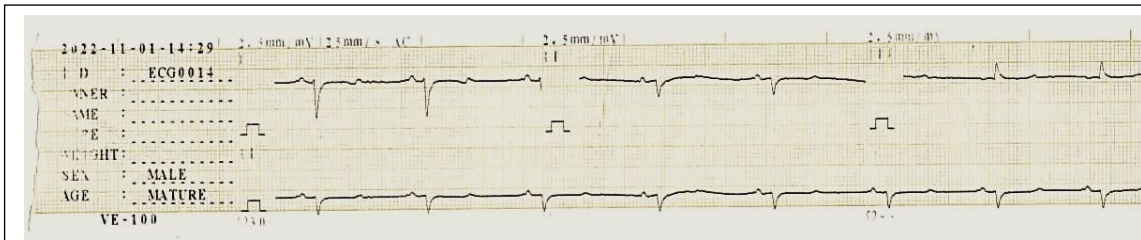


Fig. 5a. ECG from a normal horse illustrating P wave, QRS complex, and T wave morphology in Lead I, II, and III, the line bar indicates 1 second

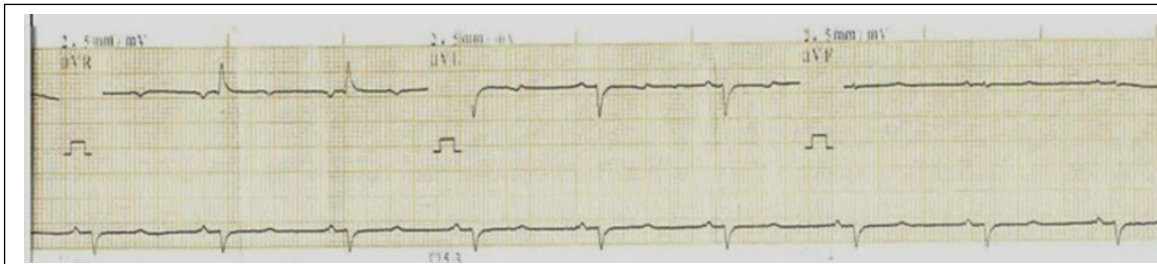


Fig. 5b. ECG from a normal horse illustrating P wave, QRS complex and T wave morphology in Lead aVR, aVL, and aVF, the line bar indicates 1 second

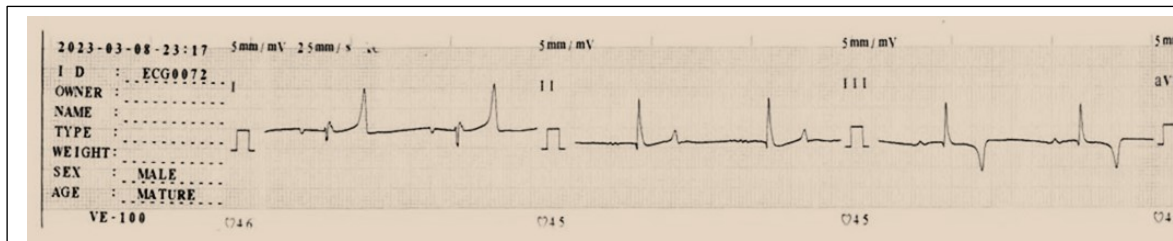


Fig. 6. ECG of *T. evansi* infected horse showed Prolonged P, T, and QRS complex durations as well as a cardiac electrical axis shift to the left (Lead II) and increased heart rate.

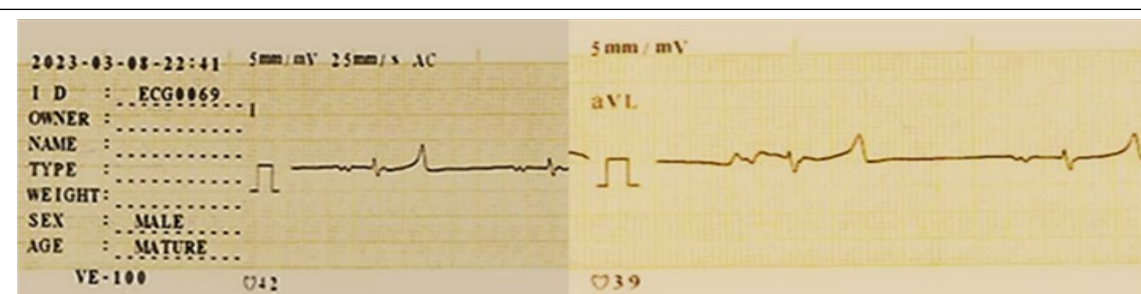


Fig. 7. The R wave voltage was significantly reduced and T wave amplitude was significantly increased (black arrows) by *T. vivax* infection in lead I and lead aVL respectively. The line bar indicates 1 second.

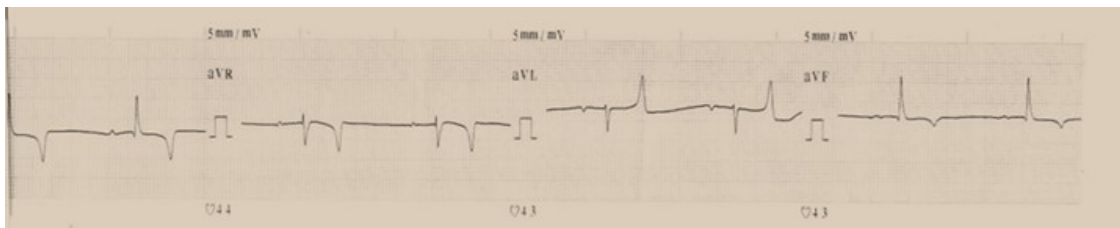


Fig. 8. ECG of a horse infected with *T. evansi* showed ventricular premature depolarization (VPD) (black arrows)

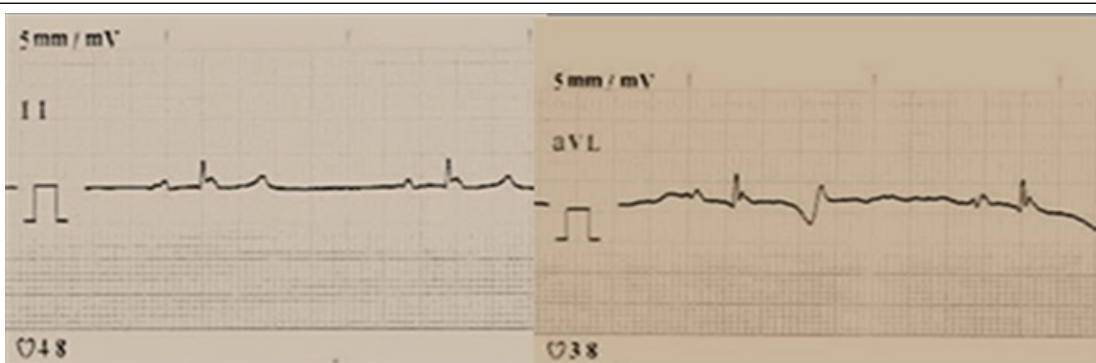


Fig. 9. ECG of a horse infected with *T. evansi* showed early repolarization: J-wave (black arrow) elevation (lead II and aVL).

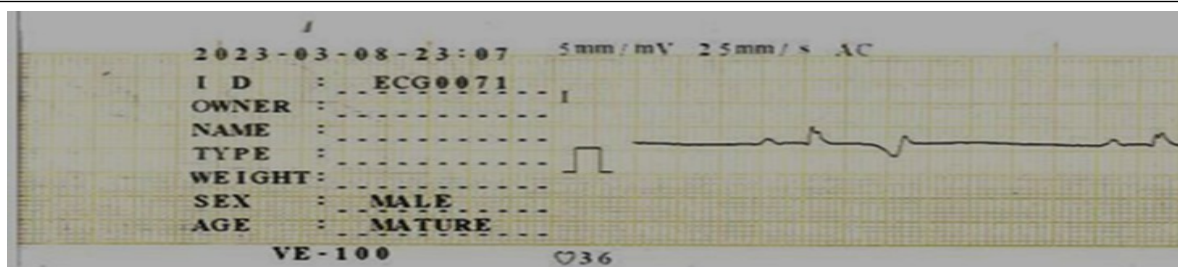


Fig. 10. Right bundle branch block (RBBB), ECG showed notched R wave (black arrows) QRS width of more than 3 small squares and a duration of 0.12 sec. in the horse infected with *T. vivax*.

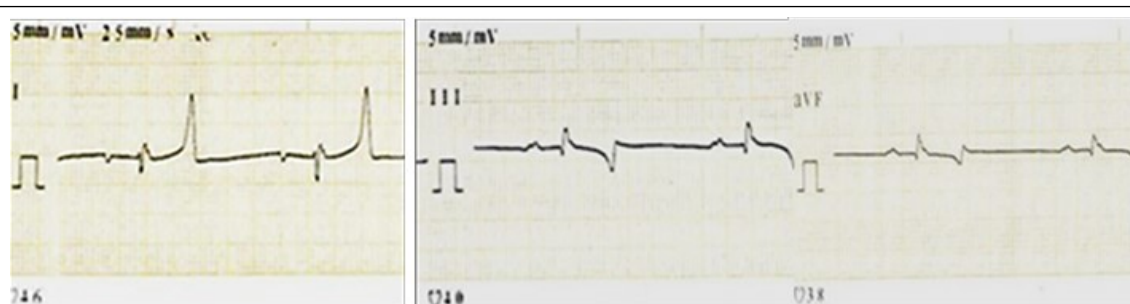


Fig. 11. ECG of a horse infected with *T. vivax* showed inferior Deep Q wave (black arrow) in leads (II, III, aVF) with ST elevation due to Inferior Myocardial infarction (MI).

References

1. Büscher, P., Gonzatti, M.I., Hébert, L., Inoue, N., Pascucci, I., Schnauffer, A., Suganuma, K., Touratier, L. and Reet, N. Equine trypanosomiasis: enigmas and diagnostic challenges. *Parasit. Vec.*, **12** (2), 234-245 (2019).
2. Al-Abadi, B.H. and Al-Badrani, A.B. Cattle blood analyses for parasitic infestation in Mosul, Iraq. *Res. Opin. Anim. Vet. Sci.*, **2**(11), 535-542 (2012).
3. Rhaymah, M.S.H. and AL-Badrani, B.A. Clinical and diagnostic study of trypanosomiasis of cattle in Mosul, Iraq. *Res. Opin. Anim. Vet. Sci.*, **2**(1), 23-26 (2012).
4. Mahmood, M.S. and Alobaidii, W.A. Molecular detection of *Trypanosoma species* in sheep and goats in Mosul city. *Iraq. J. Vet. Sci.*, **36**(2), 445-449 (2022).
5. Da Silva, A. S., Herakles, A. Perez, G., Costa, M.M., França, R.T., De Gasperi, D., Zanette, R.A, Amado, J.A, Lopes, S.A, Teixeira, M.G and Monteiro, S.G. Horses are naturally infected by *Trypanosoma vivax* in southern Brazil. *Parasitol. Res.*, **108**(4), 23–30 (2011).
6. Tejero, F., Arias-Mota, L. L., Roschman-González, A., Aso, P.M. and Finol. H.J. *Trypanosoma evansi*. Ultrastructural Cardiac Microvasculature Changes in Experimental Murine Infections. *Acta Sci. Vet.*, **38**(3), 275-281 (2010).
7. Ghaffar, M., A., El-Melegy, M., Afifi, M. F., El-Aswad, B.E., El-Kady, N. and Atia, A.F. The histopathological effects of *Trypanosoma evansi* on experimentally infected mice *Menouf. Med. J.*, **29** (4), 868–873 (2017).
8. Dvir, E., Lobetti, R.G., Jacobson, L.S., Pearson, J. and Becker, P.J. Electrocardiographic changes and cardiac pathology in canine babesiosis. *J. Vet. Cardiol.*, **6**(1), 15–23 (2004).
9. Blum, J.A, Schmid, C., Burri, C., Hatz, C. and Olson, C. Cardiac Alterations in Human African Trypanosomiasis (*T.b. gambiense*) with Respect to the Disease Stage and Antiparasitic Treatment. *PLoS. Negl. Trop. Dis.*, **3**(2), 383-306 (2009).
10. Ajibola, E. S. and Oyewale, J. O. A. Six Lead Electrocardiogram of *Trypanosoma Brucei* Infected Dogs. *Alexand. J. Vet. Sci.*, **48** (2), 23-32 (2016).
11. Batista, J.S, de Araújo Júnior, H.N, Moura, G.H, de Souza Góis, R.C, de Paiva, K.A, da Silva, J.B, Costa, W.B, de Menezes, M.C, Nunes, F.V, Costa, K.M. and de Medeiros, G.D. Cardiac involvement in trypanosomiasis in sheep experimentally infected by *Trypanosoma vivax* (Ziemman, 1905). *Experim. Parasitol.*, **6**(2), 205-208 (2019).
12. Kimeto, B.A., Mugeru, G.M. and Nyaga, P.N. Hemorrhagic pericarditis in cattle infected with *Trypanosoma vivax*. *Vet. Parasitol.*, **34**(4), 295-301 (1990).
13. Hammed, S.A and Albadrani, B.A. New and Modified Staining Techniques for Rapid Diagnosis of Hemoparasites in Blood Smears of Cows. *IOSR-JAVS.*, **14**(9), 11-20 (2021).
14. Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C. and Dávila, A.M. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. *Parasitol. Res.*, **95**(3), 186-192 (2005).
15. Claes, F., Radwanska, M., Urakawa, T., Majiwa, P.A., Goddeeris, B. and Büscher, P. Variable Surface Glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. *Kinetoplastids Biol Dis.*, **3**(1), 3- 6 (2004).
16. Suganuma, K., Acosta, T.J., Valinotti, M.F.R., Sanchez, A.R., Mossaad, E., Elata, A. and Inoue, N. First molecular survey of animal trypanosomes in Paraguayan horses. *Vet Parasitol. Reg. Stud. Reports.*, **22**(1), 234-245(2022).
17. Verheyen, T., Decloedt, A., De Clercq, D., Deprez, P., Sys, S.U. and van Loon, G. Electrocardiography in horses – Part 2: How to read the equine ECG. *Vlaams. Dierg. Tijdschrift.* **79** (2), 337-344 (2010).
18. Pedersen, P.J., Karlsson, M., Flethøj, M., Trachsel, D.S., Kanters, J.K., Klaerke, D.A. and Buhl, R. Differences in the electrocardiographic QT interval of various breeds of athletic horses during rest and exercise. *J. Vet. Cardiol.*, **18** (3), 255-264 (2016).
19. Faraj, A.A., Hadi, A.M and Al-Amery, A.M. Prevalence trypanosomiasis of stray dogs in Baghdad city, Iraq. *Inter. J. Rec. Sci. Res.*, **6**(11), 7206-7208 (2015).

20. Al-Rubaie, L.S., Al-Qayim, M.A, Al-badrawi, A.A and Al-Rubaie, H.M. Study Iron Homeostasis of Infected and Non-Infected Iraqi Camels with Trypanosomiasis. *Iraq. J. Vet. Med.*, **44** (2), 35–44 (2020).
21. Asal, S.H. and Al-Samarai, F.R. The effect of infection with *Trypanosoma evansi* on some hematological parameters of Iraqi local camels in Wasit province. *Biochem. Cell. Arch.*, **20**(2), 5033-5039 (2020).
22. Alsaad, K.M. Jarad, A. and Lafta, M.H. *Trypanosomiasis evansi* of buffalo at Basrah, Iraq: Clinico-hematobiochemical and diagnostic studies. *Biochem. Cell. Arch.*, **21**(2), 2035-2042 (2021).
23. Mohd Rajdi, N.Z., Mohamad, M.A, Tan, L.P., Choong, S.S., Reduan, M.F., Hamdan, R.H. and Salma, C.W. First case report on the occurrence of *Trypanosoma evansi* in a Siam B Mare in Kelantan, *Malaysia Vet Med Sci.*, **7**(2)303–309 (2021).
24. Elshafie, E.I, Sani, R.A. Sharma, R. and Bubakar, A. Clinical and Hematological Profiles of Malaysian Ponies Experimentally Infected with a Field Strain of *Trypanosoma evansi*. *Open Parasitol. J.*, **6**(1), 8-16 (2018),
25. Mbaya, A., Kumshe, H. and Nwosu, C. O. The mechanisms of anemia in trypanosomosis: A review. In Silverberg D. (Ed.), *Anemia*, **38**(3), 978-953 (2012).
26. Boada-Sucre, A., Spadafora, M.S.R., Tavares-Marques, L.M, Finol, H.J. and Reyna-Bello, A. *Trypanosoma vivax* Adhesion to Red Blood Cells in Experimentally Infected Sheep. *Pathology. Res. Int.*, **2** (1), 3-9 (2016).
27. Rossi, M.S., Boada-Sucre, A. Simoes, M.T. and Spadafora, R. Adhesion of *Trypanosoma evansi* to Red Blood Cells (RBCs): Implications in the Pathogenesis of Anaemia and Evasion of Immune System. *Diagn. Pathol. Open*, **2**(1), 1-10 (2017).
28. Desquesnes, M., Sazmand, A., Gonzatti, M., Boulangé, A., Bossard, G., Thévenon, S., Gimonneau, G., Truc, P., Herder, S., Rave, S., Sereno, D., Waleckx, E, Jamonneau, V., Jacquiet, P., SJittapalapong, S., Berthier, D., Solano, P. and Hébert, L. A Review: Diagnosis of animal trypanosomosis: proper use of current tools and future prospects *Parasit. and Vect.*, **15** (2), 235-238 (2022).
29. Kann, S., Mendoza, G.A.C., Hartmann, M., Frickmann, H. and Kreienbrock, L. Chagas Disease: Medical and ECG Related Findings in an Indigenous Population in Colombia. *Trop. Med. Infect. Dis.*, **8** (3), 297(2023).
30. Aquino, L.P. Machado, R.Z., Alessi, A.C., Santana, A.E. Castro, M.B., Marques, L.C. and Malheiros, E.B. Hematological, biochemical and anatomopathological aspects of the experimental infection with *Trypanosoma evansi* in dogs. *Arq. Bras. Med. Vet. Zootec.*, **54** (1), 12-13 (2002).
31. Galiza, G.J, Garcia, H.A, Assis, A.C, Oliveira, D.M, Pimentel, L.A, Dantas, A.F, Simões, S.V, Teixeira, M.M, and Riet-Correa, F. High mortality and lesions of the central nervous system in trypanosomosis by *Trypanosoma vivax* in Brazilian hair sheep. *Vet Parasitol.*, **182**(2-4),359-363 (2011).
32. Crilly, N.P. and Mugnier, M.R. Thinking outside the blood: Perspectives on tissue-resident *Trypanosoma brucei*. *PLoS Pathog.*, **17**(9), 15-20 (2021).
33. EL-Deeb, W. and ELmoselmany, A. Cardiac and oxidative stress biomarkers in *Trypanosoma evansi* infected camels: Diagnostic and prognostic prominence. *Parasitol.*, **142**(6), 767-772 (2015).
34. Nath, L.C, Anderson, G.A, Hinchcliff, K.W. and Savage CJ. Serum cardiac troponin I concentrations in horses with cardiac disease. *Aust. Vet. J.*, **90**(9), 351-357 (2012).
35. Begg, L.M., Hoffman, K.L. and Begg, A.P. Serum and plasma cardiac troponin I concentrations in clinically normal Thoroughbreds in training in Australia. *Aust. Vet. J.*, **84**(9),336-370 (2006).
36. Baldissera, M.D, Souza, C. F, Bertoncheli, C.M, Silveira, K.L, Grando, T.H, Porto, B.C, Leal, D.B., Silva, A.S, Mendes, R.E, Stefani, L.M. and Monteiro, S.G. Oxidative Stress in the Heart of Rats Infected with *Trypanosoma evansi*. *Korean J Parasitol.*, **54**(3), 247-52 (2016).
37. Gow, N.M. ECG interpretation in the horse. *In Pract.*, **43** (2), 234-237 (2001).
38. Verheyen, T. Equine electrocardiography: exploration of new diagnostic strategies. *PhD thesis*, Faculty of Veterinary Medicine, Ghent University, Belgium. (2012).
39. Tejero, F., Arias-Mota, L. L., Roschman-González, A., Aso, P. M. and Finol, H. J. *Trypanosoma evansi*: ultrastructural cardiac muscle and cardiac microvasculature changes in experimental murine infections. *Acta Scientiae Veterinariae*, **38**(3), 279–285 (2018).
40. Bal, M.S, Singla, L.D, Kumar, H., Vasudev, A., Gupta, K. and Juyal, P.D. Pathological studies on experimental *Trypanosoma evansi* infection in Swiss albino mice. *J. Parasit. Dis.*, **6**(2),260-264 (2012).

التغيرات في تخطيط كهربية القلب المرتبطة بداء المثقبيات في الخيول

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كان الهدف من هذه الدراسة هو تقييم نتائج تخطيط القلب الكهربائي في الخيول المصابة طبيعياً بداء المثقبيات وكذلك التحقق من وجود طفيلي المثقبي في شمال العراق. تم فحص اثنين وعشرون حصاناً من الذكور تتراوح أعمارهم بين 3-7 سنوات وتراوحت أوزانهم من 340 إلى 390 كجم. أظهرت جميع الخيول المصابة فقر الدم. هلك حصانان، وكانت هناك علامات سريرية تشير إلى إصابتهما بقصور في القلب. تم تأكيد تشخيص داء المثقبيات في مسحة الدم وتفاعل البلمرة المتسلسل الخاص بالأنواع في اثني عشر حصاناً بنسبة (54.54%)، منها 31.82% مصابة بالمثقبية إيفانسي و22.72% مصابة بالمثقبية النشيطة. تم إجراء التقييمات السريرية وأمراض الدم وتخطيط القلب الكهربائي (ECG). تم قياس العلامة الحيوية للترابونين القلبي (cTnI) في مصل الدم من أجل دعم نتائج تخطيط القلب وفهم أعماق لأضرار أنسجة عضلة القلب، وتمت مقارنة النتائج مع القيم الطبيعية لعشرة خيول سليمة ووجد أنها سلبية لمسحة الدم وتفاعل البلمرة المتسلسل. أظهرت مخططات القلب الكهربائية تغيرات كبيرة وتميزت بطول موجتي P و T ومركب QRS بالإضافة إلى انحراف محور القلب الكهربائي إلى اليسار وتسارع القلب. كانت القيم المتوسطة لـ cTnI في المصل أعلى بشكل ملحوظ ($P < 0.001$) في الخيول المصابة بالمثقبية إيفانسي و بالمثقبية النشيطة (0.78 و 17.5 نانوغرام/م على التوالي) والتي كانت أعلى منها في الخيول السليمة (0.01)، المدى 0.01-0.03 نانوغرام/م و بمستوى معنوية (> 0.001). مثقبية إيفانسي والمثقبية النشيطة مسؤولان عن حدوث آفات القلب، والتي ترتبط بقصور القلب، والتغيرات في مخطط كهربية القلب، وموت الخيول المصابة.