Equine piroplasmosis in Kelantan, Malaysia: Clinico-hemato-biochemical alterations in subclinically and clinically infected equids

Al-Obaidi, Q.T.[1*], Mohd Mokhtar, A.[1], Al-Sultan, I.I.[1], Azlinda, A.B.[2] and Mohd Azam, K.G.K.[1]
[1]Faculty of Veterinary Medicine, University Malaysia Kelantan, Pengkalan Chepa, 16100, Kota Bharu, Kelantan, Malaysia
[2]School of Medical Science, University Science Malaysia, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia
*Corresponding author e-mail: d12e003f@siswa.umk.edu.my and / or qaes_talb@yahoo.com

Abstract. Equine piroplasmosis (EP) is global disease of equids affect the international movement of horses and their industry. This work was conducted on a random collection of blood samples from a total of 306 equids (horses and ponies) comprising both clinically healthy (n=276) and clinically suspected animals (n=30) for EP from 53 stables in eight districts at Kelantan, Malaysia. Competitive-inhibition enzyme linked immunosorbent test (cELISA) was applied to detect the antibodies for *Theileria equi* and *Babesia caballi* and their titers in the serum. Hemato-biochemical parameters were analyzed from blood and serum samples from clinical and subclinical cases in comparison to healthy animals (n=25) a control group. The overall prevalence of EP infections (*T. equi*, *B. caballi* and *both infections*) in subclinical and clinical infected equids was 70.26 and 9.80 per cent, respectively. The subclinical infection in equids was statistically higher than that of clinical infection (P<0.05). Higher titration of *T. equi* antibodies in clinical infected cases ranged from 1/160 to 1/1280 in (n=27), whereas in *B. caballi* the titres ranged from 1/160 to 1/640 (n=26). Manifestation in clinical cases was the acute onset of the disease, with significant Hemato-biochemical changes. Whereas, equids with subclinical infection appeared healthy with absence of clinical signs and non-significant Hemato-biochemical alterations were seen as compared to clinical cases and healthy control groups.

INTRODUCTION

Equine piroplasmosis is one of the most common and important infectious tick-borne disease in equids including horses, ponies, donkeys, mules and zebras (Laveran, 1901; de Waal & Van Heerden, 2004). A single or concurrent infection of Apicomplexal hemoprotozoan parasites *Babesia caballi* and *Theileria equi* (previously *Babesia equi*) (Mehlhorn & Schein, 1998) results in peracute, acute, subacute, chronic and subclinical disease in equids (Ueti *et al.*, 2008). The infections with *T. equi* are clinically severe and more common than that caused by *B. caballi* (Sigg *et al.*, 2010; Sumbria *et al.*, 2016a). Six genera of ixodid

ticks have been implicated as competent vectors for piroplasms in equids include *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* (Scoles & Ueti, 2015).

In Malaysia EP is a notifiable disease by the department of veterinary services and Malaysian Veterinary Research Institute (MVRI). However, there is no registered report in OIE document (OIE, 2014a). *T. equi* and *B. caballi* are endemic in many tropical and subtropical areas of the world, as well as in temperate climatic zones (Hailat *et al.*, 1997). EP causative agents can be readily transmitted by carrier equids or by infected ticks into originally piroplasmosis-free countries, in this aspect many countries have

decided strict importation regulations restricting the movement of piroplasm seropositive horses cross their border (Friedhoff et al., 1990). The disease varies from subclinical condition to severe and potentially fatal illness depends upon the equids immunity and the virulence of the single or mixed infective pathogens (Brüning, 1996). The incubation period in natural infection with the EP is 12-19 days caused by T. equi and 10-30 days when it is caused by B. caballi (Friedhoff & Soulé, 1996). The clinical signs of piroplasmosis are variable and often nonspecific (Bashiruddin et al., 1999). In a rarely peracute form of the disease is manifested by a sudden onset of signs, which lead to collapse and sudden death within 24-48 hours (Russell et al., 2005; Donnellan & Marais, 2009). The more frequent, acute form of the disease is often characterized by fever, usually greater than 40°C, loss of appetite and weight, severe sweating, congested of mucous membranes with petechial haemorrhage, incoordination, nervous signs, pale and/ or icteric mucous membranes, peripheral edema, panting, depression, colic with signs of diarrhoea and /or constipation, haemoglobin urea, muscle tremor, coughing, dehydration with a rough coat and presence of ticks on different body parts (Ibrahim et al., 2011; Salama, 2016). Moreover, increase in the respiratory rate, heart rate and capillary refilling time in the infected equids (Alsaad et al., 2010). Abortion or neonatal infections can occur in pregnant mares (Lewis, 1999; Chhabra et al., 2012). Furthermore, in the chronic form of disease usually showing nonspecific and variable clinical signs such as mild in appetence, poor performance, loss of body weight and spleen enlarged on rectal examination (Radostitis et al., 2008). In the subclinical infection, an animal looks healthy, unapparent or no clinical signs with low parasitemia. These animals can act as carriers and source of dissemination of the parasites to the ticks and susceptible equine population (Bahrami et al., 2014). Recovered equids from acute infection usually remain carrier of B. caballi for one to four years, whereas in T. equi may remain carriers for many years or for lifelong (Friedhoff et al., 1990). Tick vectors feeding on subclinical infected horses with low T. equi parasitemia (2×10^3 – 10^6 ml^{-1} of blood) can successfully transmit the parasites to the non-infected horses (Ueti et al., 2008).

The clinical diagnosis of EP can be based on observation of clinical signs on infected equids. Although, the clinical manifestation of EP probably nonspecific and confused with other diseases like trypanosomiasis, equine granulocytic anaplasmosis, equine infectious anaemia etc. (de Waal & van Heerden, 2004; Rothschild & Knowles, 2007). Therefore, the diagnosis should be confirmed by direct methods which include the observation of intraerythrocytic forms of the parasite after Geimsa stained blood or organ smears and by polymerase chain reaction (PCR) technique (Qablan et al., 2013).

Several serological methods are used for diagnosis EP such as a complement fixation test CFT, immunofluorescence antibody test (IFAT), indirect enzyme linked immunosorbent assay (iELISA), competitive-inhibition ELISA test (cELISA), latex agglutination test (LAT) and immunechromate- graphic test (ICT) (OIE, 2014b). The cELISA test has been an important, accurate and easily diagnostic method for detection of T. equi and B. caballi antibodies that could be used as a screening test (Alsaad et al., 2010). The test has less operating error and also facilitated the testing of large numbers of animals for epidemiological studies (El-Ghaysh et al., 1996). The OIE recommended the use of cELISA test as a test for international horse sport (OIE, 2014b). cELISA test appears to be reliable and proper in justifying the seroprevalence of EP as well as identifying the carrier cases (Alsaad et al., 2012).

Studies of EP in Kelantan-Malaysia are very scarce and little information has been reported. Accordingly, this study was designed to determine the prevalence of T. equi and B. caballi and concurrent infections by both the parasites in clinically and subclinically infected equids. Moreover, investigate for the clinical-hemato-biochemical parameters alterations of clinical and subclinical infection equids.

MATERIAL AND METHODS

Animals and study areas

In the period between September 2013 till March 2014, the required number of sampling to study the status of the prevalence of the EP, by taking the expected prevalence of 1% with a confidence level (95%) in a population size 658 equids found in Kelantan state (DVSK, 2012). For this we used the formula: $n = [1-(\alpha)^{1/D}]\,[N-(D-1)/2]$.

Where n: required samples, α: 1-confidence level (0.05), D= estimated minimum number of diseased animals in the population (population size x the minimum expected prevalence), N: population size (Stevenson, 2008). It was calculated the minimum number of equids needed to be tested for this study were 258 animals. To set the number of equids 53 stables were visited.

A total of 306 equids randomly selected from stables comprising both clinically healthy animals (n=276) and clinically suspected infected animals (n=30). Twenty five equids from the healthy cases served as a control group. All were randomly selected from stables in eight districts of Kelantan, Malaysia, includes: Kota Bharu, Pasir Mas, Machang, Bachok, Pasir Puteh, Tumpat, Tanah Merah and Gua Musang. The careful clinical examination was carried out for all equids and fecal samples were screened for internal helminths infestation using standard techniques (Zajac & Conboy, 2012).

Blood sample collection and hematological examination

A 10 millilitre (ml) of venous blood was collected from jugular vein using 18-G needle into two vacutainer® tubes (5 ml each), one with anticoagulant (ethylene diamine tetraacetic acid) and another without anticoagulant. The tubes with anticoagulant were used for blood parameters performed i.e. total erythrocyte counts (TECs), haemoglobin concentration (Hb), packed cell volume (PCV), the mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), Thrombocytes count, total leukocytes counts (TLCs) and differential leukocytes counts (DLCs) by using a Haematology analyzer (Mythic 18VET/France). Erythrocyte sedimentation rate (ESR) estimation worked out using Westergren pipette. Reticulocytes count has performed by mixing 2 drops of blood with 2 drops of new methylene blue 0.5%, 20 minutes later a thin blood smear prepared and examined under microscope. The percentage of reticulocytes was calculated following the equation of Jain (2000):

$$\text{Reticulocyte } \% = \frac{\text{number of reticulocyte}}{\text{number of calculating reticulocytes}} \times 100$$

The tubes without anticoagulant were used for serum separation using a centrifuge at 2500 RPM for 15minutes and stored in -20ºC until use (Kouam *et al.*, 2010).

Biochemical analysis

Serums used for biochemical analysis includes: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALKP), blood urea nitrogen (BUN), total bilirubin, total protein, albumins, globulins, calcium, phosphorous, glucose and creatinine using special cassettes for each in a Chemistry analyzer (Vet Test, Arachem/USA).

Competitive-inhibition ELISA test

Serum samples also were analyzed using commercial c-ELISA kits (VMRD, Inc., Pullman, and WA99163 USA) were used for detection of *T. equi* and *B. caballi* antibodies in serum samples according to manufacturer's instruction. In this assay was used recombinant *T. equi* equine merozoites antigen 1 (EMA-1)-coated plate and a specific monoclonal antibody (MAb) that defines this merozoites surface protein epitope. A similar was used recombinant *B. caballi* rhoptry-associated protein 1 (RAP-1)-coated plate and a specific MAb reactive with a peptide epitope of a 60 kDa *B. caballi* antigen. The principle of the assay was detected the antibodies in serum against to the antigen-coated plate by inhibiting of primary monoclonal antibody. The strong colour developments indicate little or no inhibition of primary monoclonal antibody binding therefore absence of *T. equi* or *B. caballi*

antibodies in sample serum. The week colour developments due to inhibition of primary monoclonal antibody binding to antigen on the soled face indicate presence of *T. equi* or *B. caballi* antibodies in sample serum. Three aliquots negative control and two of a positive control were used per plate and optical densities (ODs) measured at a wavelength to 630nm using an ELISA automatic plate reader (BioTek® ELx808, USA). Results were expressed as percentage of inhibition (%I) using the formula: % I = 100 [1-(Sample O.D. ÷ NC O.D.)]. The interpretation of results: If %I $\geq$ 40 the samples were considered positive and if %I < 40 the samples were considered negative.

Determination of antibody titers for *T. equi* and *B. caballi*

A method was developed to standardize the titration of antibodies for *T. equi* and *B. caballi*, using the positive control serum in the cELISA kits. A serial diluent $\log_{10}$ for the positive control serum in the commercial c-ELISA kits competitive-inhibition ELISA for *T. equi* and *B. caballi* provide by VMRD, Inc, Pullman, and WA99163 USA, was applied (as mentioned above) with the serum samples separated from the blood for screened equids to get standardize optical density for each diluent of the positive control serum and optical density reading for each serum sample.

Statistical analysis

Statistical analysis was done by using a two-sided Chi-square to analyzed the sero-prevalence associated with the status of equids, with a 95% confidence interval and using one way analysis of variance (ANOVA) followed by post hoc test (Duncan) to compare the subclinical infected equids with clinically infected and the clinically healthy (control group), in hematological and biochemical parameters, using IBM-SPSS statistics version19 program. All the significant difference was determined at the (P<0.05).

RESULTS

Seroprevalence of EP in the clinical cases was (9.80%); for *T. equi* (8.82%), *B. caballi* (8.49%) and for both infections (7.51%). Whereas, in the subclinical cases was (70.26%); for *T. equi* (42.48%), *B. caballi* (54.57%) and for both infections (26.79%). Subclinical cases were statistically highly significant in prevalence than clinical infected equids in all types of infections (P<0.05) (Table 1). The antibody titres of the clinical infected equids were 1/160 (n=3), 1/320 (n=8), 1/640 (n=10), 1/1280 (n=6) whereas, in subclinical infected equids with *T. equi* were 1/5(n=6), 1/10(n=18), 1/20(n=23), 1/40(n=51), 1/80(n=32). The antibody titres of the clinical cases infected with *B. caballi* were 1/160(n=8), 1/320(n=16), 1/640(n=2), while in subclinical cases were 1/5(n=62), 1/10(n=27), 1/20(n=44), 1/40 (n=22), 1/80(n=12). These were indicated to higher antibody titres of *T. equi* and *B. caballi* in the clinically infected equids (Figure 1 and 2).

Clinical cases were suffering from acute form of the disease and exhibited depression, loss of appetite, emaciation (Figure 3A),

Table 1. Status of equine piroplasmosis in 306 equids using cELISA test

| Equids status | No. of equids | prevalence % | cELISA | | |
			T. equi No. Positive (%)	*B. caballi* No. Positive (%)	Both infections No. Positive (%)
Healthy	61	19.93	0	0	0
Subclinical infection	215	70.26	130 (42.48)[a]	167 (54.57)[a]	82 (26.79)[a]
Clinical infection	30	9.80	27 (8.82)[b]	26 (8.49)[b]	23 (7.51)[b]

Values significantly different (P < 0.05) between equids status are labelled with the vertically different letters ([a, b]).
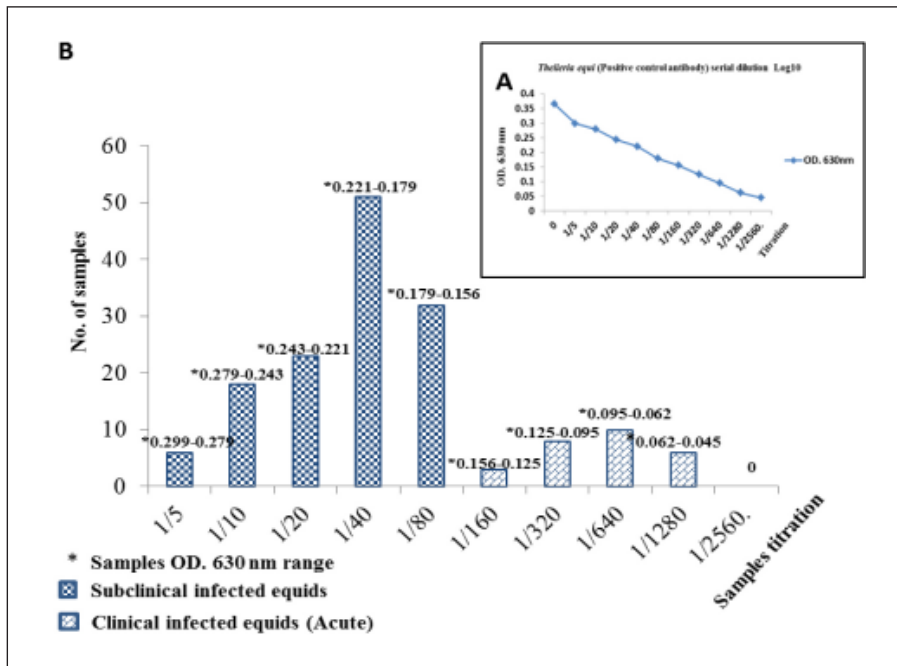
Figure 1. **A)** Determination the optical density of serial dilution $\log_{10}$ positive control serum for *T. equi* in cELISA kit, used as standerd; **B)** The antibody titres in clinical and subclinical infected equids.
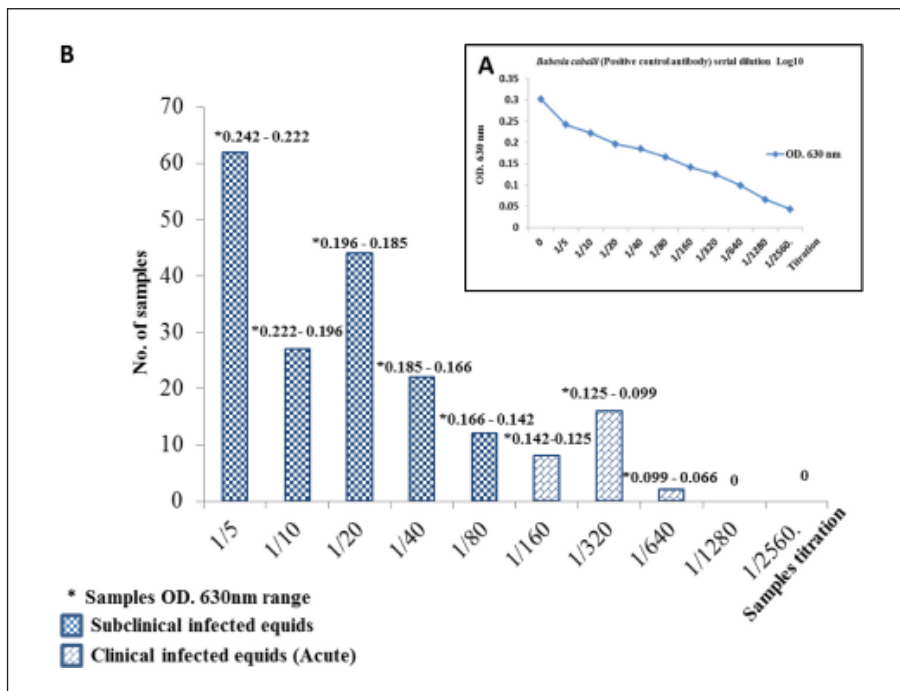

Figure 2. **A)** Determination the optical density of serial dilution $\log_{10}$ positive control serum for *B. caballi* in cELISA kit, used as standerd; **B)** The antibody titres in clinical and subclinical infected equids.

congestion of mucous membranes with petechial haemorrhages on the 3rd eyes lid and conjunctivae (Figure 3B), pale and/icterus mucous membrane (Figure 3C), severe sweating, difficulty in movement and in coordination with edema of fetlock joint, hemoglobin urea, muscles tremor, coughing, colicky signs, diarrhoea and/or constipation, nervous signs, dehydration with rough coat and presence of ticks on different body parts (Figure 3D). The various percentagesof each clinical sign were recorded in the clinical infected equids (Figure 4). While subclinical cases appear healthy with no clinical signs.

Figure 3. **A)** Loss of the body weight (Emaciation) and depression; **B)** Pale of mucous membrane in the 3rd eye lid; **C)** Petechial haemorrhage of mucous membrane in the 3rd eye lid; **D)** Presence of ticks under the ear.
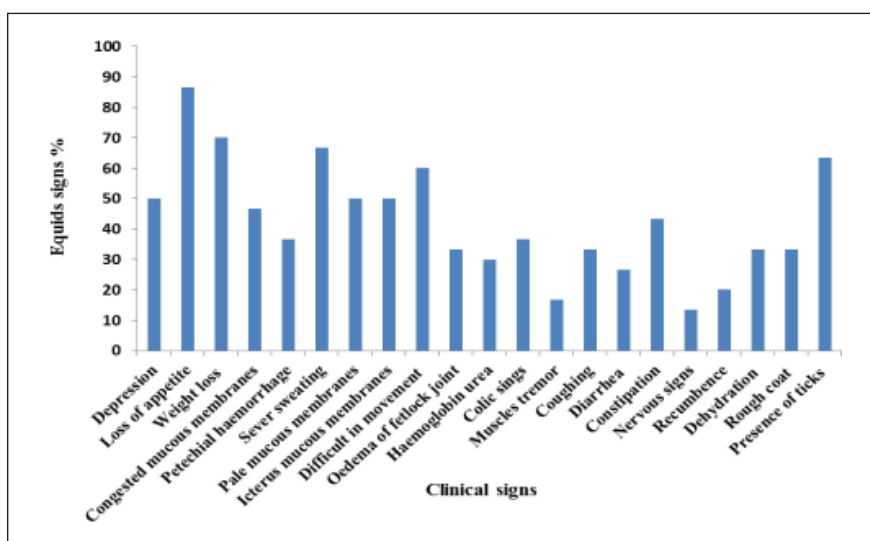

Figure 4. Percentage of clinical signs in the naturally infected equids (30 Cases).

Moreover, statistically significant increase in the body temperature (40.2ºC), respiratory rates (44.33/ min), heart rates (76.25/min) and capillary refilling time (3.9/Sec) in the clinical cases (P<0.05). While, no significant different in these clinical parameters in the subclinical infected equids compared with the control group (Table 2). Examinations of the fecal samples showed that all equids examined are free from internal helminths.

The haemogram of clinical cases showed statistical significant decrease in TEC, Hb, PCV, thrombocytes, MCHC and significant increase in MCV reflecting to macrocytic hypochromic type of anaemia (P<0.05), along with statistical significant increase in the ESR and reticulocytosis (P<0.05). An increase in the TLCs indicated by neutrophilia and lymphocytosis (P<0.05). It has been observed that no statistical significance for these hematological parameters in the subclinical cases compared with the control group (Table 4).

Biochemical analysis of serum from clinical cases observed marked statistical significant increase of AST, ALT, ALKP, BUN and total bilirubin (P<0.05). Whereas, significant decrease in the total protein (albumins and globulins), calcium, phosphorous, glucose and creatinine (P<0.05). No significant biochemical changes seen in subclinical cases in comparison with the clinical cases and control group (Table 5).

DISCUSSION

Equine piroplasmosis is considered as a global problem for the horse industry. The current study demonstrates for the first time cases of acute clinical infection in Kelantan, Malaysia. The latest studies on the prevalence of the disease in Peninsular Malaysia were conducted on clinical healthy equids, claimed that the infection rate of *T. equi* and *B. caballi* was 0% (Nill) in Kelantan. Researchers used a microscopic examination method only (Chandarwathani *et al.*, 1998). Antibodies seropositive of *T. equi* (20%) and *B. caballi* (1%) was detected in 12 states of Malaysia by cELISA test (Zawida *et al.*, 2010). Highly significant prevalence (P<0.05) in subclinical cases (70.29%) compared to clinical ones (9.80%) had been detected and recorded in the current research work. Similar prevalence rate has been registered for both aforementioned parasites by Alsaad *et al.* (2012) in Iraqi equids. The acutely infected horses with the parasites may be finally recovered, but remain subclinically infected with low parasitemia (Kappmeyer *et al.*, 2012). Successfully transmit *T. equi* to healthy horses during ticks feeding on persistent infection cases with low parasitemia (Ueti *et al.*, 2008). Alanazi *et al.* (2014) noted that horses with subclinically infected act as an important reservoir for the parasites. The acute clinical cases had higher antibody titres for *T. equi* and *B. caballi*, whereas subclinical

Table 2. The clinical parameters in naturally infected equids compared with the subclinical infected and control group

Parameters	Healthy animals (Control) Mean ± S.E	Clinical infected Mean ± S.E	Subclinical infected Mean ± S.E
Body temperature / ºC	37.26 ± 0.11[a]	40.2 ± 0.16[b]	37.50 ± 0.18[a]
Respiratory rate / min	17.90 ± 0.40[a]	44.33 ± 0.87[b]	18.03 ± 0.51[a]
Heart rate / min	46.76 ± 1.55[a]	76.25 ± 3.59[b]	46.67 ± 1.03[a]
Capillary refilling time / Sec	1.11 ± 0.12[a]	3.9 ± 0.19[b]	1.22 ± 0.13[a]

Mean values ± Standard error (S.E.) significantly different (P < 0.05) between equids status are labelled with the horizontal different letters ([a, b]).

Table 3. Hematological changes in naturally infected equids compared with the subclinical infected and control group

Parameters	Healthy animals (Control) Mean ± S.E	Clinical infected Mean ± S.E	Subclinical infected Mean ± S.E
RBC x 10^6 µl	8.85 ± 0.40[a]	5.22 ± 0.24[b]	8.98 ± 0.21[a]
HB mg/100 ml	12.10 ± 0.14[a]	6.92 ± 0.22[b]	11.90 ± 0.21[a]
PCV %	36.98 ± 0.85 [a]	24.16 ± 0.51[b]	36.75 ± 0.92[a]
MCV / µm^3	34.49 ± 0.59[a]	38.23 ± 0.94[b]	34.71 ± 0.60[a]
MCHC mg/dl	36.17 ± 0.97[a]	30.82 ± 0.31[b]	35.94 ± 0.94[a]
ESR mm/20 min	24.48 ± 1.69[a]	78.27 ± 2.70[b]	25.82 ± 2.28 [a]
Thrombocytes x 10^3 µl	478.96 ± 18.28[a]	277.40 ± 12.06[b]	475.23 ± 16.19[a]
Reticulocytes %	0.00 ± 0.0[a]	3.10 ± 0.13[b]	0.04 ± 0.01[a]
WBC x 10^3 µl	8.87 ± 0.25[a]	12.45 ± 0.32[b]	9.03 ± 0.24[a]
Lymphocyte x 10^3 µl (%)	4.65 ± 0.25[a] (45.66 ± 1.52)	7.37 ± 0.38[b] (87.94 ± 0.67)	4.99 ± 0.28[a] (44.93 ± 0.72)
Nutrophile 10^3 µl (%)	4.82 ± 0.20[a] (47.62 ± 0.43)	6.29 ± 0.25[b] (64.53 ± 0.72)	4.84 ± 0.20[a] (46.24 ± 0.63)
Monocyte x 10^3 µl (%)	0.66 ± 0.06[a] (5.63 ± 0.1)	0.74 ± 0.07[a] (5.71 ± 0.2)	0.68 ± 0.03[a] (5.20 ± 0.2)
Basophile x 10^3 µl (%)	0.67 ± 0.47[a] (7.84 ± 0.2)	0.72 ± 0.64[a] (7.58 ± 0.22)	0.71 ± 0.05[a] (6.77 ± 0.1)
Eosinophil x 10^3 µl (%)	0.40 ± 0.05[a] (3.27 ± 0.2)	0.43 ± 0.04[a] (3.17 ± 0.1)	0.40 ± 0.24[a] (3.26 ± 0.1)

Mean values ± standard error (S.E.) significantly different (P < 0.05) between equids status are labelled with the horizontal different letters ([a, b]).

Table 4. Biochemical changes in naturally infected equids compared with the subclinical infected and control group

Parameters	Healthy animals (Control) Mean ± S.E	Clinical infected Mean ± S.E	Subclinical infected Mean ± S.E
AST U/L	264.6 ± 6.18[a]	370.43 ± 13.19[b]	264.26 ± 6.38[a]
ALT U/L	20.92 ± 0.59[a]	38.93 ± 0.97[b]	20.70 ± 0.57[a]
ALKP U/L	145.51 ± 5.02[a]	227.83±10.34[b]	146.83 ± 4.53[a]
BUN mg/ dl	21.24 ± 0.54[a]	55.96 ± 1.79[b]	21.53 ± 0.50[a]
Total bilirubin mg / dl	1.70 ± 0.55[a]	2.85 ± 0.16[b]	1.62 ± 0.46[a]
Total protein g / dl	7.58 ± 0.09[a]	3.22 ± 0.11[b]	7.63 ± 0.09[a]
Albumins g/ dl	3.22 ± 0.17[a]	1.57 ± 0.10[b]	3.11 ± 0.15[a]
Globulins g / dl	4.63 ± 0.16[a]	1.85 ± 0.10[b]	4.40 ± 0.16[a]
Calcium mg/ dl	11.24 ± 0.28[a]	6.61 ± 0.39[b]	10.92 ± 0.25 [a]
Glucose mg/ dl	131.88 ± 6.17[a]	68.16 ± 2.87[b]	129.26 ± 5.87[a]
Phosphorous mg/ dl	6.86 ± 0.23[a]	3.52 ± 0.17[b]	6.59 ± 0.28[a]
Creatinine mg/ dl	1.62 ± 0.10[a]	0.63 ± 0.07[b]	1.67 ± 0.08[a]

Mean values ± standard error (S.E.) significantly different at (P < 0.05) between equids status are labelled with the horizontal different letters ([a, b]).

cases have low titres. This observation was in accord with Holbrook *et al.* (1972) and Friedhoff & Soule (1996). They stated that the antibody titre in acute infection is 1/160 or above, while remain at low levels (1/80 or less) during horses in carrier's stage. Careful clinical examination of clinical cases highlight the acute signs of EP, these signs were the same as reported by Alsaad *et al.* (2010); Garba *et al.* (2011); Salib *et al.* (2013) and Alsaad, (2014). Haemorrhages detected on the 3rd eye lid and conjunctivae may be due to thrombocytopenia (Schalm *et al.*, 1975). The excessive destruction and removal of infected RBCs by reticulo-endothelial macrophages change the colour of mucous membranes to become icteric and increase levels of bilirubin in urine of infected equids when enquire a progressive type of anemia (Alsaad, 2009). The difficulty in movement, incoordination and muscle tremors was due to generalized weakness and hypocalcaemia, which is in agreement with Radostitis *et al.* (2008). The fetlock joint edema could be explained due to the differences between arterial hydrostatic pressure and venous osmotic pressure causing fluids escape from vessels which accumulates in the distal parts of the body. Moreover, hypoprotenemia may also play a role in the dialysis and the accumulation of oedematous fluids (Romerrio & Dyson, 1997).

The haemoglobinurea was seen in clinical infected equids, it occurs due to intravascular destruction of RBCs and release of hemoglobin, which in turn passed through the kidney and discolored the urine to brownish or dark coffee like color; this was in agreement with Alsaad *et al.* (2010). Many reasons for destruction of RBCs as a result of infections, including the increase intracellular pressure of infected cells during the multiplication of the protozoa, the toxic mechanism by hemolytic factor produced by parasite and increase erythrocyte fragility due to protozoal consumption of the phosphorus component or disturbance in the accumulation of proteins and fats of cell membrane lead to more distraction (de Gopegui *et al.*, 2007; Zobba *et al.*, 2008). The colic signs in the infected animals may occur due to disturbances of intestinal movements either in the form of diarrhoea or constipation, these signs were also mentioned by Hailat *et al.* (1997) and Alsaad (2009). Hepatic insufficiency lead to lack of bile salt secretion results digestive disturbances, frequent haemoglobin urea might cause glomerulo- nephrosis and renal damage and the microthrombosis in intestinal capillaries all those reflecting colicky signs (Radostitis *et al.*, 2008).

In the current study nervous signs were recorded in EP manifested by walking in a circle, ataxia, mild tonic-clonic spasms and paralysis of hind limbs, which agreement with Soulsby, (1982) and Russell *et al.* (2005). The dehydration was seen in diseased equids, which may be due to the lack of body fluids, resulting in a urea or oligurea, increase thirst and rough hair coat (Lewis, 1999). The detection of ticks on different body parts of infected equids refers to the fact that ticks were important transmitters of piroplasmosis, this agrees with Kouam *et al.* (2010).

The increase in body temperature coincides with the appearance of the parasite in the circulating blood consistent with Soulsby, (1982). The severity of fever depends on the virulence of the parasite, stage of the disease, type of lesions and generalized infection (Krause, 2002). The body temperature raises in the infected equids due to release the endogenous pyrogens after cellular lysis which stimulates the thermoregulatory centers of the hypothalamus (Radostitis *et al.*, 2008).

The increase in heart and respiratory rate, which has been observed in infected equids, was due to hypoxia (hypoxicanemia) which occurs as a compensatory mechanism. Results of blood analysis confirm the anemia that has been reported by other researchers (Zobba *et al.*, 2008; Alsaad *et al.*, 2010). The result of hemogram was showed decrease in RBCs, Hb, PCV, thrombocytes which reflected to anemia. These finding were consistent with Zobba *et al.* (2008); Alsaad *et al.* (2010) and Sumbria *et al.* (2016b). The type of anemia may differ according to the severity and stage of the disease, in this study macrocytic hypochromic observed

may be due to significant increase in the MCV and MCHC values of infected cases (Ibrahim *et al.*, 2011; Sumbria *et al.*, 2016b). Anemia has been reported in naturally infected foals with EP in Mosul (Alsaad, 2009). The advancement of anemia in equids is an ideal clinical signs of *T. equi* infections (de Waal & van Heerden, 2004). There are three mechanisms for hemolytic anemia; mechanical hemolysis by parasites intra erythrocyte binary fission, auto-immunity of the anti-erythrocytic autoantibodies enhancing more erythrophagocytosis (infected and uninfected erythrocytes) and the toxic effect by producing of hemolytic factor from the parasites or may also inhibit the hemopoitic system (de Gopegui *et al.*, 2007; Zygner *et al.*, 2007). In addition, the direct effect of the parasites in the infected erythrocytes causes lipid peroxidase and oxidative stress, may be leading to incriminate the life span of infected RBCs and erythrolysis (Sellon, 1997; Kumar *et al.*, 2009). The increase in the ESR values in the infected equids was agreed with Alsaad *et al.* (2010). There is a correlation between the ESR value and intensity of anemia due to infection, the increase in the sedimentation of RBCs will occur when anemia more intense (Jain, 2000). The appearance of reticulocytes in the blood circulation point out that reticulocytosis synchronize with the presence of regenerative anemia and recorded the highest numerical value (5.6 – 5.7%) on days 23-25 post infection (Maxis, 2011). Significant increase in TLCs due to neutrophilia and lymphocytosis observed in infected equids has been also recorded by Ibrahim *et al.* (2011) and Javed *et al.* (2014).

The increase in AST, ALT, ALKP, BUN, total bilirubin and decrease of total protein level in the clinically infected animals were signaling for the damage of skeletal and heart muscles, hepatocytes, renocytes and erythrocytes. These enzymes are liable to be released and detected during the pathological situation. These findings were in agreement with the literature (Zobba *et al.*, 2008; Alsaad *et al.*, 2010; Sumbria *et al.*, 2016b). Results were demonstrated hypocalcaemia, hypo-phosphatemia and hypoglycemia in the clinically infected equids may be due to starvation, malabsorption and hepatic depletion, which were consistent with Alsaad *et al.* (2010) and Sumbria *et al.* (2016b).

This research proves and records the existence of subclinical infection form of equids that appeared healthy without clinical signs and/or significant hematological and biochemical changes when compared with the clinical infection cases of equids. This result was consistent with Kappmeyer *et al.* (2012); Alanazi *et al.* (2014) and Bahrami *et al.* (2014), while it is inconsistent with Laus *et al.* (2015) who observed higher prevalence of piroplasms associated with the clinical and hematochemical alterations in non-symptomatic donkeys.

CONCLUSIONS

Acute cases of EP in high antibody titres were diagnosed in Kelantan, Malaysia and recorded for the first time. Further, a high prevalence of subclinical cases was recorded with low antibody titres and silent clinical signs with no changes in haematological and biochemical parameters. As these subclinical infected equids can as nidus for spread of infection hence these should be monitored for strategic control of the disease in the country.

REFERENCES

Alanazi, A.D., Said, A.E., Morin-Adeline, V., Alyousif M.S. & Šlapeta, J. (2014). Quantitative PCR detection of *Theileria equi* using laboratory work flows to detect asymptomatic persistently infected horses. *Veterinary Parasitology* **206**: 138-145.

Alssad, K.M. (2009). Acute Babesiosis in foals. *Journal of Animal and Veterinary Advances* **8**: 2585-2589.

Alsaad, K.M. (2014). Evaluation of hemogram, acute phase response, acid base balance and blood gas analysis in new born foals infected with Babesiosis. *Journal of Animal & Plant Sciences* **24**(3): 738-742.

Alsaad, K.M., Alsaad, E.A. & Al-Derawie, H.A. (2010). Clinical and diagnostic study of equine babesiosis in drought horses in some areas of Basrah Province. *Research Journal of Animal Science* **4**: 16-22.

Alsaad, K.M., Hassan, S.D. & Al-Obaidi, Q.T. (2012). Detection of *Babesia equi* and *Babesia caballi* antibodies in horses and donkeys in Mosul, Iraq. *Research Opinions in Animal and Veterinary Sciences* **2**: 291-294.

Bahrami, S., Ghadrdan, A.R., Mirabdollahi, S.M. & Fayed, M.R. (2014). Diagnosis of subclinical equine theileriosis in center of Iran using parasitological and molecular methods. *Tropical Biomedicine* **31**: 110-117.

Bashiruddin, J.B., Camma, 'C. & Rebe^lo, E. (1999). Molecular detection of *Babesia equi* and *Babesia caballi* in horse blood by PCR amplification of part of the 16S rRNA gene. *Veterinary Parasitology* **84**: 75-83.

Brüning, A. (1996). Equine piroplasmosis, an update on diagnosis treatment & prevention. *British Veterinary Journal* **152**: 139-151.

Chandarwathani, P., Yusof, M., Slamah, B. & Rohaya, M.A. (1998). A survey on equine piroplasmosis in selected areas in peninsular Malaysia. *Journal Veterinary Malaysia* **10**: 25-26.

Chhabra, S., Ranjan, R., Uppal, S.K. & Singla, L.D. (2012). Transplacental transmission of *Babesia equi* (*Theileria equi*) from carrier mares to foals. *Journal of Parasitic Diseases* **36**: 31-33.

de Gopegui, R.R., Penalba, B., Goicoa, A., Espada, Y., Fidalgo, L.E. & Espino, L. (2007). Clinico-pathological findings and coagulation disorders in 45 cases of canine babesiosis in Spain. *Veterinary Journal* **174**: 129-132.

Department of Veterinary Services Kelantan (DVSK), 2012. Census for livestock in Kelantan. pp: 53-58.

de Waal, D.T. & van Heerden, J. (2004). Equine piroplasmosis. In: Infectious Diseases of Live Stock, Coetzer, J.A.W. (editor.). Oxford University Press, Cape town, South Africa, pp: 244-245.

Donnellan, C.M. & Marais, H.J. (2009). Equine Piroplasmosis. In: Infectious Disease of Horses. Mair, T.S. & Hutchinson, R.E. (editors.), UK, EVJ Ltd. pp: 333-340.

El-Ghaysh, A., Sundquist, B., Christensson, D.A., Hilali, M. & Nassar, A.M. (1996). Observations on the use of ELISA for detection of *Babesia bigemina* specific antibodies. *Veterinary Parasitology* **62**: 51-61.

Friedhoff, K.T. & Soulé, C. (1996). An account on equine babesiosis. *Revue Scientifique et Technique* (*Office International des Epizooties*) **15**: 1191-1201.

Friedhoff, K.T., Tenter, A.M. & Muller, I. (1990). Haemoparasites of equines: Impact on international trade of horses. *Revue Scientifique et Technique* (*Office International des Epizooties*) **9**: 1187-1194.

Garba, U.M., Sackey, A.K.B., Tekdek, L.B., Agbede, R.I.S. & Bisalla, M. (2011). Clinical manifestations and prevalence of piroplasmosis in Nigerian royal horses. *Journal of Veterinary Advances* **1**(1): 11-15.

Hailat, N.Q., Lafi, S.Q., Al-Darraji, A.M. & Al-Ani, F.K. (1997). Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. *Veterinary Parasitology* **69**(1-2): 1-8.

Holbrook, A.A., Frerich, W.M. & Allen, P.C. (1972). Laboratory diagnosis of equine piroplasmosis. Proceedings of the 3rd International Conference on Equine Infectious Diseases, S. Karger Press, Basel, Switzerland, pp: 467-75.

Ibrahim, A.K., Gamil, I.S., Abd-elbaky, A.A., Hussein, M.M. & Tohamy, A.A. (2011). Comparative molecular and conventional detection methods of *Babesia equi* (*B. equi*) in Egyptian Equine. *Global Veterinaria* **7**: 201-210.

Jain, N.C. (2000). Schalm's veterinary hematology. 5th edition. Philadelphia, Lea & Fibiger, pp: 610-612.

Javed, K., Ijaz, M., Muddassir, M.A., Khan, I., Mehmood, K. & Ali, S. (2014). Prevalence and hematology of tick borne hemoparasitic diseases in equines in and around Lahore. *Pakistan Journal of Zoology* **46**: 401-408.

Kappmeyer, L.S., Thiagarajan, M., Herndon, D.R., Ramsay, J.D., Caler, E., Djikeng, A., Gillespie, J.J., OT Lau, A., Roalson, E.H., Silva, J.C., Silva, M.G., Suarez, C.E., Ueti, M.W., Nene, V.M., Mealey, R.H., Knowles, D.P. & Brayton, K.A. (2012). Comparative genomic analysis and phylogenetic position of *Theileria equi*. *BMC Genomics* **13**: 603.

Kouam, M.K., Kantzoura, V., Gajadhar, A.A., Theis, J.H., Papadopoulos, E. & Theodoropoulos, G. (2010). Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece. *Veterinary Parasitology* **169**: 273-278.

Krause, P.J. (2002). Equine Babesiosis. *Medical Clinics of North America* **86**: 361-373.

Kumar, S., Kumar, R. & Sugimoto, C. (2009). A perspective on *Theileria equi* infections in donkeys. *Japanese Journal of Veterinary Research* **56**: 171-180.

Laus, F., Spaterna, A., Faillace, V., Veronesi, F., Ravagnan, S., Beribé, F., Cerquetella, M., Meligrana, M. & Tesei, B. (2015). Clinical investigation on *Theileria equi* and *Babesia caballi* infections in Italian donkeys. *BMC Veterinary Research* **11**: 100.

Laveran, A. (1901). Contribution a2 l2etude du Piroplasma equi. Comptes rendusdes se´ances de la Societe de Biologie et de ses Filiales 1901. **12**: 385-388.

Lewis, B.D. (1999). Transplacental transmission of *Babesia equi* in horses and chemotherapy of small Babesia species. PhD thesis, University of Pretoria, Pretoria, South Africa.

Maxis, E. (2011). Jubb, Kennedy and Palmer's pathology of domestic animals. 6th edition. New York, Academic press. pp: 117-125.

Mehlhorn, H. & Schein, E. (1998). Re description of Babesia equi Laveran, 1901 as *Theileria equi*. *Parasitology Research* **84**: 467-475.

OIE, (2014a). World Animal Health Information Database (WAHID). www.oie.int/wahis _2/public /wahid. php/ Country information/Animal situation.

OIE, (2014b). Equine piroplasmosis terrestrial manual 2014. Updated:2014. http://www. oie.int/fileadmin/Home/fr/ Health_standards/tahm/2.05.08_ EQUINE_PIROPLASMOSIS.pdf. Accessed in May 2014.

Qablan, M.A., Oborník, M., Petr•elková, K.J., Sloboda, M., Shudiefat, M.F., Hoøín, P. Lukeš, J. & Modrý, D. (2013). Infections by *Babesia caballi* and *Theileria equi* in Jordanian equids: epidemiology and genetic diversity. *Parasitology* **140**: 1096-1003.

Radostitis, O.M., Gay, C.C., Blood, D.C. & Hinchliff, K.W. (2008). Veterinary medicine. A text book of the diseases of cattle, sheep, goats and horses.10th edition. London, Philadelphia, Toronto. Elsevier.St. Louis, MO. pp. 1483-1498.

Romerrio, J.M. & Dyson, S.J. (1997). The diffusely filled limp. In: Current therapy of equine medicine. Robinson, N.E. (editor). W.B. Saunders Company. pp: 23.

Rothschild, C. & Knowles, D. (2007). Equine piroplasmosis. In: Equine infectious diseases. Sellon, D.C. & Long, M.T. (editors). Elsevier, St. Louis, MO. pp: 465-473.

Russell, Z.E., Holly, M., Bruce, E.L. & Kenneth, S.L. (2005). Equine Babesiosis-A review. *Veterinary Clinical Pathology Clerkship Program*. (https://www.google.com/ #q=Equine+ Babesiosis-A+review.+ Veterinary+Clinical+Pathology+Clerkship +Program.).

Salama, A.O. (2016). Clinical, haematological and therapeutic studies on babesiosis in Arabian horses in the Qassim region, central of Saudi Arabia. *Journal of Applied Animal Research*. To link to this article: http://dx.doi.org/10.1080/ 09712119.2015.1124339.

Salib, F.A., Youssef, R.R., Rizk, L.G. & Said, S.F. (2013). Epidemiology, diagnosis and therapy of *Theileria equi* infection in Giza, Egypt. *Veterinary World* **6**: 76-82.

Schalm, O.W., Jain, N.C. & Corral, E.J. (1975). Veterinary hematology. 3rd edition. Philadlphia,Lea & Fibirger. pp. 44, 349. 604-608.

Scoles, G.A. & Ueti, M.W. (2015). Vector ecology of equine piroplasmosis. *Annual Review Entomology* **60**: 561-581.

Sellon, D.C. (1997). Hemolytic animea. In: current therapy of equine medicine Robinoson, N.E. (editor). 4th edition. Philadelphia, Saunders Company. pp. 276-282.

Sigg, L., Gerber, V., Gottstein, B., Doherr, M.G. & Frey, C.F. (2010). Seroprevalence of *Babesia caballi* and *Theileria equi* in the Swiss horse population. *Parasitology International* **59**: 313–317.

Soulsby, E.J.L. (1982). Helminthes, arthropods and protozoa of domesticated animals. 7th edition. London, Baillere Tindal & Cassel, pp: 698-724.

Stevenson, M. (2008). An introduction to veterinary epidemiology. New Zealand, Palmerston North, Massey University, EpiCentre, IVABS, pp: 65-67.

Sumbria, D., Singla, L.D. & Sharma, A. (2016a). *Theileria equi* and *Babesia caballi* infection of equids in Punjab, India: a serological and molecular survey. *Tropical Animal Health and Production* **48**: 45-52.

Sumbria, D., Singla, L.D., Kumar, S., Sharma, A., Dahiya, R.K. & Setia, R. (2016b). Spatial distribution, risk factors and haematobiochemical alterations associated with *Theileria equi* infected equids of Punjab (India) diagnosed by indirect ELISA and nested PCR. *Acta Tropica* **155**: 104-112.

Ueti, M.W., Palmer, G.H., Scoles, G.A., Kappmeyer, L.S. & Knowles, D.P. (2008). Persistently infected horses are reservoirs for intrastadial tick-borne transmission of the apicomplexan parasite Babesia equi. *Infection and Immunity* **76**: 3525–3529.

Zajac, A.M. & Conboy, G.A. (2012). Veterinary clinical parasitology. 8th edition. UK. Wiley-Blackwell. pp. 3-39.

Zawida, Z., Chandarwathani, P., Nurulaini, R., Khor, S.K., Adnan, M., Premaalatha, B., Zaini, C.M., Jammnah, O., Azizah, D., Norlida, A. & Noor Suhaila, S. (2010). Prevalence of protozoan diseases in local horses in peninsular Malaysia. *Journal of Comparative Microbiology, Immunology and Infectious Diseases* **31**: 48-50.

Zobba, R., Ardu, M., Nicollini, S., Chessa, B., Manna, L., Cocco, R. & Parpaglia, M.L.P. (2008). Clinical and laboratory findings in equine piroplasmosis. *Journal of Equine and Veterinary Science* **28**: 301-8.

Zygner, W., Gojska, O., Rapacka, G., Jaros, D. & Wedrychowicz, H. (2007). Hematological changes during the course of canine babesiosis caused by large Babesia in domestic dogs in Warsaw (Poland). *Veterinary Parasitology* **145**: 146-151.