SERODIAGNOSIS OF EQUINE PIROPLASMOSIS IN EGYPT USING CELISA

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Abstract

This study was done to obtain a descriptive insight about the prevalence of piroplasmosis in Egypt. One hundred and seventeen blood films of horses and donkeys were examined for evidence of piroplasm stages in stained blood smears while sera of the same animals were processed using the competitive inhibition ELISA (cELISA) to detect antibodies for T. equi and B. caballi. On clinical examination, all animals were apparently health except four cases of horses showed fever (40 C°-41 C°), loss of appetite, increased respiratory rate, conjunctivitis, lacrimation and enlarged lymph nodes. T. equi were detected in nine horses (11.2%) and two donkeys (5.4%) using Giemsa-stained blood smears. Serological examination revealed that eleven horses (13.75%) and two donkeys (5.4%) had antibodies against T. equi using cELISA. B. caballi were not detected by examination of Giemsa-stained blood smears and cELISA.

INTRODUCTION

Equine piroplasmosis (EP) ranges among the most important tick-borne protozoan infection in equine (horse, donkey, mule and Zebra) caused by Theileria equi (T. equi) and Babesia caballi (B. caballi) of the Order Piroplasmida. Theileria equi was previously designated as Babesia equi but compelling evolutionary, morphological, biochemical, and genetic evidence supports its reclassification as a Theileria (OIE 2009). The disease is found in most tropical and subtropical areas of the world as well as in temperate zones (Schein 1988 and Bruning, 1996). Equine piroplasmosis has caused important economic losses in the horse industry, being a serious threat to the horse raising industry and international movement of horses (Friedhoff et al., 1990). The genera of ticks that transmit piroplasm in equine include, Hyalomma, Rhipicephalus, Dermacentor and Boophilus (Battseseg et al.,2002). Several factors may play an important role in the spread of the disease: the relocation of infected horses and ticks through national and international movement together with the geographic expansion of the tick-vectors due to climate warming and respective creation of new areas eco logically permissive for these vectors.

Clinical signs of the disease in equine are fever, anemia, icterus, hemoglobinuria, odema, increased respiratory and heart rates, colic, constipation followed by diarrhea,

depression and even death (de Waal, 1992). B. caballi causes a less severe disease, as only about 1% of the red blood cells are infected. In contrast, T. equi infects up to 20% of red blood cells, leading to more severe clinical signs. Piroplasmosis can occur in peracute, acute, subacute and chronic forms. Documented case fatality rates vary from 10–50%. Foals can be infected in utero, and can be aborted or born anemic and weak (de Waal and Heerden, 2004). Sub-clinical infected animals are of major concern, as they can be carriers of the organism. (Hailat et al., 1997). Thus there is a real need for the diagnosis of both clinical and sub-clinical infections.

The diagnosis of piroplasmosis is based on clinical manifestations, the presence of tick vectors, history of the area and the demonstration of either the parasites or antibodies to the parasites in the host. However, for purpose of confirmation or specific diagnosis, the demonstration of the parasites or antibodies to the parasites is more often achieved, using several techniques including Giemsa-stained blood smear, CFT, IFAT, ELISA and PCR. Examination of Giemsa-stained blood smear is useful in diagnosing acute infection with both T. equi and B. caballi and cannot identify chronic carriers. The serological diagnosis based on the detection of circulating antibodies of current and previous exposure to infection agents. The competitive inhibition ELISA (cELISA) was demonstrated to be of great value, validated and recommended by OIE and approved by the USDA as the official diagnosis assay (OIE,2008).

The objective of this study is to compare Giemsa stain blood smears and cELISA for diagnosing piroplasmosis in order to confirm seronegativity and to identify seropositive animals whose require a negative piroplasmosis certificate to move and participate in racing. Determining the prevalence and distribution of equine piroplasmosis in Egypt.

MATERIALS AND METHODS

Animals and collection of samples:

Eighty horses and thirty-seven donkeys were sampled. All the animals were clinically healthy at the moment of sample collection except four cases of horses. Whole Blood samples were collected from jugular venipuncture and placed in sterile tubes. One tube containing ethylenediamine tetraacetic acid (EDTA) at a concentration of 1 mg/ml-1) were used immediately for blood smears. Thin blood smears were fixed with absolute methanol, stained with Giemsa and then examined under light microscopy (magnification 1000×; 100 fields) for evidence of the T. equi and B. caballi (Shute, 1966). The second tube without anticoagulant, the blood was left at room temperature to coagulate. Then serum samples were obtained by

centrifugation at 3000 rpm for 10 minutes and were stored at –20 °C until serological analysis.

Serological examination (cELISA)

Collected serum samples were tested to detect antibodies for T. equi and B. caballi by using a commercially available competitive enzyme linked immunosorbant assay (c-ELISA) kit (VMRD, USA). The test was performed following the instructions of manufacturer (USDA, 2005). The mean optical density (OD) at 620 nm was determined for all wells using a microplate reader (Expert plus UV-G020 151- Austria). The percent inhibition for each test sample was determined using the mentioned formula in the instructions. A sample was considered positive when the percent inhibition was ≥40%.

RESULTS

Clinical findings:

All animals were apparently health except four cases of horses showed fever (40 C°-41 C°), loss of appetite, increased respiratory rate, conjunctivitis, lacrimation and enlarged lymph nodes.

Microscopical findings:

Of 117 blood samples examined by microscopic observation of Giemsa-stained blood smears, T. equi were detected in nine horses (11.2%) and two donkeys (5.4%). Intra-erythrocytic stages of Theileria were detected as spherical, oval, ring form and pear shaped organisms measuring 2.5 - 3 µm long (Fig. 1A). Four pear-shaped stages form a tetrad, the characteristic arrangement called Maltese cross measuring 1.5 - 2 µm long (Fig.1B). The schizonts were detected in the lymphocytes with two forms macroschizonts and microschizonts. Macroschizont measured 3.5 x 2.3 um (Fig.2A). Microschizont measured 5.75x3.5 um (Fig.2B). B.caballi were not detected by examination of Giemsa-stained blood smears.

CELISA results:

Among the sera collected from 80 horses and 37 donkeys 13 (11.1%) were found positive for T. equi by the cELISA . The average prevalence of the infection for T. equi by cELISA was showed in Table (2). All samples proved to be positive by thin blood smears examination were also positive by

cELISA except one case in horses was negative by cELISA. Whereas, no erythrocytic stages were seen in 3 horse samples by microscopic examinations were proved to be

positive by cELISA. None horses and donkeys were found to be positive for B. caballi antibodies.

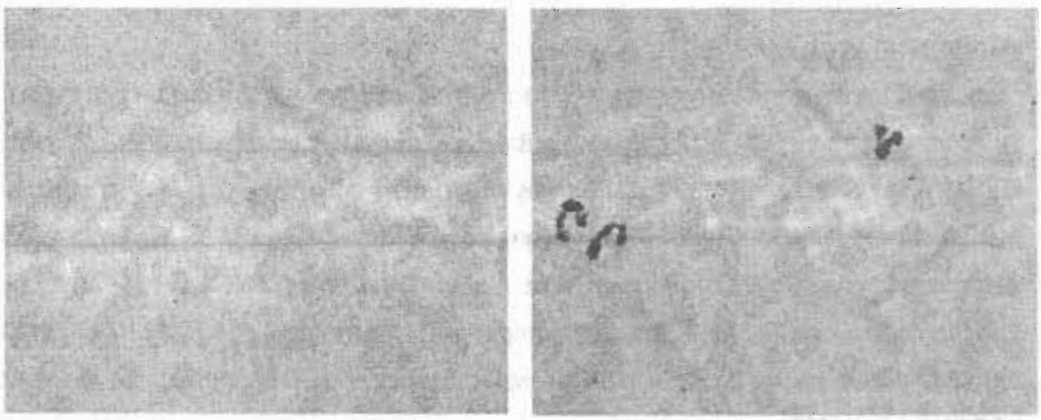

Fig.(1): Intra-erythrocytic stages of T. equi in Blood smears.

A: ring form.

B: Maltese cross

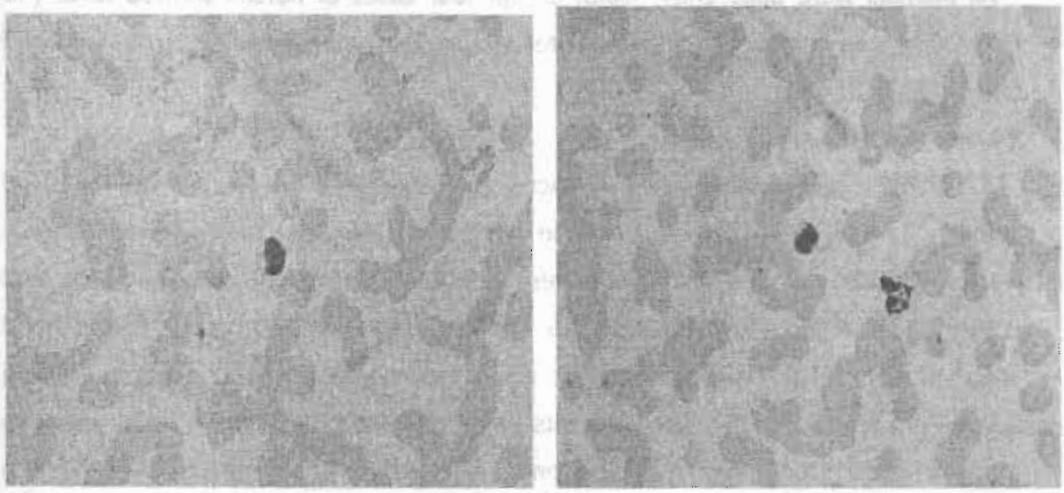

Fig.(2): Intralymphocytic stages of T.equi in Blood smears.

A: Macroschizonts

B: Microschizonts

Table 1. Prevalence of Theileria equi and Babesia caballi infections in horses and donkeys by microscopical examination.

Animal species	No. of examined animals		T. equi		B. caballi	
	Symptomatic	Asymptomatic	Positive with ME	%	Positive with ME	%
Horse	4	76	9	11.2	-	-
Donkeys	-	37	2	5.4	-	-

Table 2. Prevalence of antibodies against Theileria equi and Babesia caballi infections in horses and donkeys by cELISA.

Animal species	No. of examined animals	T.equi		B.caballi	
		Positive with cELISA	Seropositive rate%	Positive with cELISA	Seropositive rate %
Horse	80	11	13.75%	-	-
Donkeys	37	2	5.4	-	-

Table 3. Comparison between the results of blood smears examination and cELISA to determine the prevalence of T. equi in horses and donkeys.

Test	Positive		Negative	
	Number of animals	%	Number of animals	%
Blood smears	11	9.4	106	90.6
cELISA	13	11.1	104	88.9

DISCUSSION

Equine piroplasmosis is of great importance due to the international movement of horses, especially for horses that travel to equestrian sport events Moreover; some countries maintain stringent restrictions that prevent import of horses serologically positive for piroplasma species (BRÜNING, 1996).

Reported clinical sings in four horses were fever (40 C°-41 C°), loss of appetite, increased respiratory rate, conjunctivitis, lacrimation and enlarged lymph nodes. These observations concurred with reports by Ibrahim et al. (2011), Rashid et al. (2009), Hailat et al.(1997) and Freidhoff(1982), who described the Theileria infection in equines in relation to the history of fever, anaemia, anorexia and weakness.

No clinical sings were observed on examined donkeys in this study. These results agreement with that recorded by Kumar et al 2009 Donkeys usually remain asymptomatic carriers with positive antibody titers throughout life. Further studies are required to determine the epidemiologic situation of equine piroplasmosis in donkeys.

Microscopic examination of the Gemsia stained blood smears with oil immersion lens revealed the presence of small intra-erythrocytic pear-shaped merozoites, spherical and/or ovoid stages of T. equi in 9 blood samples of examined horses (4 showing symptoms and five healthy) with a prevalence rate of 11.2 % while blood smear of the other 108 (88.8%) of examined horses appeared free from developmental stages of T. equi. The present results of the microscopic examination were in agreement with those was corroborated by of Ibrahim et al (2011), KATIE 2010 and Rashid et al. (2009) who recorded the prevalence of T. equi was 18%, 14% in Egypt and 30% in Pakistan respectively. On the other hand, Farah et al. (2003) and Salim et al.(2008) who reported the prevalence rate of T.equi was 38.8%in Egypt and 63.5% in Sudan. These rates were higher than that mentioned in this study. These differences may be related to different factors such as parasitaemia levels, the age of infection, different localities that carried out by these studies and prevalence of tick vector between these regions, where climatic factors as temperature and humidity influence the habitat for ticks.

In the present study, Theileria was observed as oval, spherical and pear-shaped within red blood cells in blood smears. This finding was in agreement with those of Rashid et al. (2009) and Guimaraes et al. (1997). Intralymphocytic forms (macroschizonts and microschizonts) were observed in this study as mentioned by Schein (1988) Brüning and (1996).

Despite microscopic examination is highly specific, it lacks sensitivity, especially in the diagnosis of subclinical infections when parasitemia becomes too low to detect positive cases (BÖSE et al., 1995). So, serological methods are commonly used for detecting T. equi and B. caballi infections. More recently, the cELISA using a recombinant protein and a specific monoclonal antibody (MAb) has been described and might be a very useful test for the mass screening of serum samples for the diagnosis of T. equi and B. caballi infections (HIRATA et al., 2003 and XUAN et al., 2001). The cELISA used in the present study has been shown to be a suitable serological assay for detection of antibodies to T. equi and B. caballi in equine, and it is internationally recommended for certification purposes (OIE, 2008).

In the present study, of eighty horses and thirty-seven donkeys, eleven horses and two donkeys were seropositive for T. equi with a seropositivity rate 13.74% and 5.4% in horses and donkeys respectively.

Serologic data about equine piroplasmosis is very limited In Egypt Although, there are 5,700 purebred Arabian horses registered in Egypt (EAO, 2011) and the movement of horses between countries require a certificate proving they are free of piroplasmosis antibodies.

Several studies carried out in other countries indicated a wide range of seroprevalence for equine piroplasmosis. Prevalences reported include 40% for T. equi and 28.3% for B. caballi by IFAT in spain (Camacho et al., 2005). In Italy, 12.4% for T. equi and 17.9% for B.caballi by IFAT (Moretti et al., 2009). Alsaad et al. (2010) detected antibodies for T. equi in 86.58% of horses and B. caballi detected in 54.39% in Basrah. The difference in the prevalence of equine piroplasmosis among countries may due to differences in sensitivity of diagnostic tests used, number of equines examined and the occurrence of vectors. Other studies reported a prevalence of T. equi infection were similar to that found in the present study Kouam et al.(2010) recorded, 11% for T. equi in Greece.

In our study, none of the horse samples were found positive for B. caballi antibodies. This suggests that horses and donkeys in Egypt limited by the T. equi infection. This result is in agreement with sigg et al.(2010) who reported that horses from Germany, Italy, Poland, Hungary, Czech Republic and Austria were found to have antibodies against T .equi only

In one sample the result of the microscopic examination were positive and the results of cELISA negative. This suggests that being a recent infection might not have the time necessary for an antibody response to the infection. Weiland (1986) observed that horses artificially infected with T. equi and B. caballi delayed of 3 to 20 days to produce antibodies, which might corroborate with our observation. In other hand, 3 animals were observed positive results for cELISA and negative for microscopic examination, probably these horse are not anymore in the acute phase of the infection or the sample are not well examined.

Four cases that appeared the symptoms of the disease were positive for both microscopic examination and cELISA antibodies. This result suggests that the symptoms observed on the animals are specific symptoms for T. equi.

The results obtained from this study indicated that the rate of T. equi infection in horses is relatively higher than in donkeys, which can be explained due to the small number of examined donkeys compared to horses.

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التشخيص السيرولولجي لمرض البيروبلازموزيس في الخيول بمصر باستخدام الإليزا التنافسية

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أجريت هذه الدراسة للحصول على رؤية وصفية حول انتشار داء البيروبلازموزيس في الخيول بمصر. تم فحص مسحات دم من مائة وسبعة عشر من الخيول والحمير للحصول على اطوار الطفيل في مسحات الدم المصبوغ في حين تمت فحص سيرم من نفس الحيوانات باستخدام الإليزا التنافسية (cELISA) للكشف عن الأجسام المضادة للثيلريا اكواي والبابيزيا كابلي. وأظهرت النتائج أولا بالفحص السريري، ان جميع الحيوانات التي تم فحصها ظاهريا سليمة ما عدا أربع حالات من الخيول فقد ظهر عليهم الاعراض الاتية الحمى (٤٠ درجة مئوية -٤١ درجة مئوية) وفقدان الشهية وزيادة معدل التنفس والتهاب الملتحمة ودموع، وتضخم الغدد الليمفاوية. وبفحص مسحات الدم المصبوغة بالجيمسا فقد تم الكشف عن الثيلريا اكواي في تسعة خيول (١١.٢٪) واثنين من الحمير (٥.٤٪) . كما كشف الفحص السيرولوجي عن وجود أجسام مضادة للثيلريا اكواي في ١٣.٧٥٪ من الخيول و٥.٤٪ من الحمير باستخدام الإليزا التنافسية. ولم يتم الكشف عن البابيزيا كابلي سواء بفحص مسحات الدم المصبوغة بالجيمسا أو باستخدام الإليزا التنافسية.