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1 A case of transplacental transmission of *Theileria equi* in a foal in Trinidad

2

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17 Abstract

18 Equine piroplasmosis due to *T. equi* and *B. caballi* is endemic in Trinidad. A case  
19 of equine piroplasmosis due to *T. equi* was diagnosed in a Thoroughbred foal at 10 hrs  
20 post-partum. A high parasitaemia (63 %) of piroplasms was observed in a Wright-  
21 Giemsa® stained thin blood smear from the foal. In addition, the 18S rRNA gene for  
22 *Babesia/Theileria* was amplified from DNA extracted from the blood of the foal and the  
23 mare. Amplified products were subjected to a reverse line blot hybridization assay  
24 (RLB), which confirmed the presence of *T. equi* DNA in the foal. The mare was negative  
25 by RLB but was positive for *T. equi* using a nested PCR and sequence analysis.

26 In areas where equine piroplasmosis is endemic, severe jaundice in a post-partum  
27 foal may be easily misdiagnosed as neonatal isoerythrolysis. Foals with post-partum  
28 jaundice should be screened for equine piroplasmosis, which may be confirmed using  
29 molecular methods if available.

30 Key words: Neonatal foals, equine piroplasmosis, reverse line blot, Trinidad

31 Introduction

32 Equine piroplasmosis is a tick-transmitted haemoparasitic disease of equids  
33 caused by the protozoa *Theileria equi* or *Babesia caballi*. Ixodid tick vectors of the  
34 genera *Rhipicephalus*, *Hyalomma* and *Dermacentor* are known to transmit the disease (de  
35 Waal, 1992). Both parasites are distributed world wide, however several countries restrict  
36 the importation of horses that are serologically positive for the disease (Magnarelli et al.,  
37 2000) . The clinical signs of equine piroplasmosis are often variable and non-specific.  
38 Acute infection results in fever (40°C), depression, reduced appetite, pallor, icterus,  
39 dyspnoea, petechiation, sweating, colic, eyelid and distal limb oedema and incoordination  
40 (Zobba et al., 2008). Massive destruction of erythrocytes results in haemoglobinuria.  
41 Chronic infection tends to result in variable clinical signs (de Waal, 1992).

42 Equine piroplasmosis can also be transmitted iatrogenically via the common use  
43 of blood contaminated syringes and needles and this route is thought to be responsible for  
44 the 2008 outbreak of the disease in several horses in Florida (Florida Department of  
45 Agriculture and Consumer Services Division of Animal Husbandry, 2009).  
46 Transplacental transmission of *T. equi* has been reported and although suspected,  
47 evidence of *B. caballi* infection via vertical transmission *in utero* is lacking (Allsopp et  
48 al., 2007). Infection of foals by *T. equi in utero* can result in abortions, full term still  
49 births or the birth of live foals with neonatal piroplasmosis (Allsopp et al., 2007; Phipps  
50 and Otter, 2004). Equine piroplasmosis due to *B. caballi* and *T. equi* has been reported in  
51 adult horses in Trinidad using molecular (Rampersad et al., 2003) and serological  
52 (Asgarali et al., 2007) techniques. This is the first report of a case of transplacental  
53 transmission of *T. equi* in Trinidad.

54 Case description:

55           Diplomacy, a 12-year old thoroughbred mare had a normal pregnancy and  
56 parturition. The foal was born weak and severely icteric (Figure 1) with blood tinged  
57 urine. Blood from the foal was collected into an EDTA tube for routine haematological  
58 analysis at 10 hrs postpartum. A thin blood smear was stained with Wright-Giemsa ® and  
59 examined under light microscopy. Piroplasms in red blood cells (RBC) were evident  
60 (Figure 2). A stress leukon of lymphopaenia with thrombocytopaenia was evident (Table  
61 1). The foal died 24 hrs after birth. Microscopic examination of a Wright-Giemsa ®  
62 stained peripheral blood smear from the mare was negative for piroplasms.

63 Materials and methods

64 *Reverse line blot (RLB)*

65           DNA was extracted from 100 µL of EDTA blood from the foal and the mare  
66 using the DNeasy blood and tissue kit (Qiagen Sciences Maryland, USA) according to  
67 the manufactures instructions. The 18S rRNA gene for *Babesia /Theileria* was amplified  
68 using primers F2 and R2 as described previously (Gubbels et al., 1999). DNA of *B. canis*  
69 *rossi* was used as a positive control and PCR grade water (Sigma St. Louis MO, USA)  
70 was used as a negative control. The amplified products were then subjected to the reverse  
71 line blot (RLB) hybridization process (Gubbels et al., 1999).The oligonucleotide probes  
72 that were attached to the membrane for the RLB are listed in Table 2.(Matjila et al., 2005;  
73 Nagore et al., 2004)

74 *Nested PCR*

75           DNA extracted from the blood of the mare was then shipped to the University of  
76 Georgia , USA and subjected to a nested PCR for the *Babesia* or *Theileria* 18S rRNA

77 gene using primary primers 5.1 and B (Medlin et al., 1989; Yabsley et al., 2005) followed  
78 by a secondary reaction using primers F and R as described (Gubbels et al., 1999). To  
79 prevent and detect contamination, DNA extraction, primary and secondary amplification,  
80 and product analysis were done in separate dedicated areas. Two negative water controls  
81 were included in each set of DNA extractions and 1 water control was included in each  
82 set of primary and secondary PCR reactions. A blood sample from a white-tailed deer  
83 (*Odocoileus virginianus*) naturally infected with *Theileria cervi* was used as a PCR-  
84 positive control. To confirm identity, the positive sample was sequenced. The amplicon  
85 was purified with a commercial gel-purification kit (QIAGEN, Valencia, CA) and  
86 submitted to Clemson University Genomics Institute (Clemson, SC) for bi-directional  
87 sequencing using the F and R primers.

#### 88 Results

89 A high parasitaemia (63%) was noted in the thin blood smear from the foal. There  
90 were on average 1-3 organisms per infected RBC. The RBC and haematocrit (HCT)  
91 levels were on the low end of the reference interval(Weiss and Wardrop, 2010). Based on  
92 the results of the RLB, the foal was infected with *T. equi* only (Figure 3). The mare tested  
93 negative by RLB but an amplicon of ~ 550 bp was obtained by nested PCR analysis. The  
94 nucleotide sequence (504 bp) obtained from the mare was 99.8% similar to *T. equi*  
95 sequences from equines from Spain (AY 150062) and South Africa ( EU 642508,  
96 Z15105).

#### 97 Discussion

98 Clinical signs of icterus and haemoglobinuria as well as reductions in the number  
99 of red blood cells and platelets, reduction in haemoglobin concentration and leukopaenia

100 are common in equine babesiosis (de Waal, 1992). Hyperbilirubinaemia is due to  
101 haemolytic anaemia. Haemolysis may be a result of mechanical damage to erythrocytes  
102 by trophozoite intra-erythrocytic binary fission and toxic damage by haemolytic factors  
103 produced by the parasite. The foal was anaemic, however the degree of true anaemia may  
104 not be reflected on the foal's haemogramme because of the sharp increase in RBC  
105 parameters at birth. During the first 12 hrs, transfusion of placental blood to the foal from  
106 the mare increases the foals RBC parameters which then decline sharply over 12-24 hrs  
107 (Weiss and Wardrop, 2010). Mild anisocytosis is also commonly observed.(Weiss and  
108 Wardrop, 2010).

109         Our case has demonstrated that carrier mares can transmit *T. equi* to their foals  
110 and such foals born at term can have an overwhelming parasitaemia. If icterus is not  
111 observed immediately after birth, these cases of neonatal piroplasmosis could be  
112 mistaken for neonatal isoerythrolysis.

113         Allsopp *et al* (2007) suggested that neonatal equine piroplasmosis may be  
114 acquired during days 40 – 150 of gestation which corresponds to the histotrophic when  
115 the embryo is exposed to a mixture of uterine gland secretions, desquamated epithelial  
116 cells and maternal erythrocytes (histotroph). A study which used medically induced  
117 abortions in *T. equi* serologically positive mares, detected *T. equi* DNA in all aborted  
118 foetuses. In that study, the earliest abortion was at day130 of gestation (Allsopp et al.,  
119 2007). However, microscopic examination of peripheral blood smears was not performed  
120 in that study, hence the level of parasitaemia in the aborted foals and mares was  
121 unknown. A report of transplacental transmission of *T. equi* has been described in  
122 asymptomatic horses with no known natural exposure to the tick vector, thus indicating

123 that such carrier animals may act as reservoirs to establish equine piroplasmosis in areas  
124 where competent tick vectors may be present (Phipps and Otter, 2004). The severely  
125 icteric foal in our study had a 63% parasitaemia of *T. equi*, however, no piroplasms were  
126 observed on light microscopy of Wright-Giemsa® stained peripheral blood smears of the  
127 mare. The RLB was also not sensitive enough to detect *T. equi* DNA in the blood of the  
128 mare but *T. equi* was detected using a nested PCR. The negative RLB result in the mare  
129 in our study may be due to a very low level of parasitaemia which was below the limit of  
130 detection of the RLB. The detection limit for the RLB for bovine piroplasms was  
131 estimated by Gubbels *et al* to be  $10^{-6}$  % which corresponded to 3 parasites per  $\mu$ l of blood  
132 (Gubbels et al., 1999).

133         The economic importance of transplacental transmission of equine piroplasmosis  
134 on the thoroughbred industry in Trinidad needs further investigation as many cases may  
135 be misdiagnosed. We therefore recommend that all icteric foals born in endemic areas  
136 should be screened for neonatal piroplasmosis. This is the first reported case of neonatal  
137 piroplasmosis in Trinidad.

138 Acknowledgements:

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140 Figure 1 Icteric sclera and conjunctiva of neonatal foal at 10 h post- partum

141 Figure 2 Red blood cells (RBC) of foal infected with *T. equi* piroplasms. There were 126

142 infected RBCs per high power field (HPF). There are approximately 200 RBC per HPF

143 (100x oil objective).

144 Figure 3 RLB hyperfilm result for foal diplomacy (b) (1, *Babesia/Theileria* genera probe;

145 2, *Babesia canis rossi*; 3, *Babesia caballi*; 4, *Theileria equi*). a, b, c (equine samples), d

146 positive control DNA containing *B. canis rossi*, e (negative control)

147

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190

Table 1 The foal's haematological parameters at 10 hours post- partum  
Variable – Reference interval for  
Thoroughbred foals at 24 hrs<sup>1</sup>

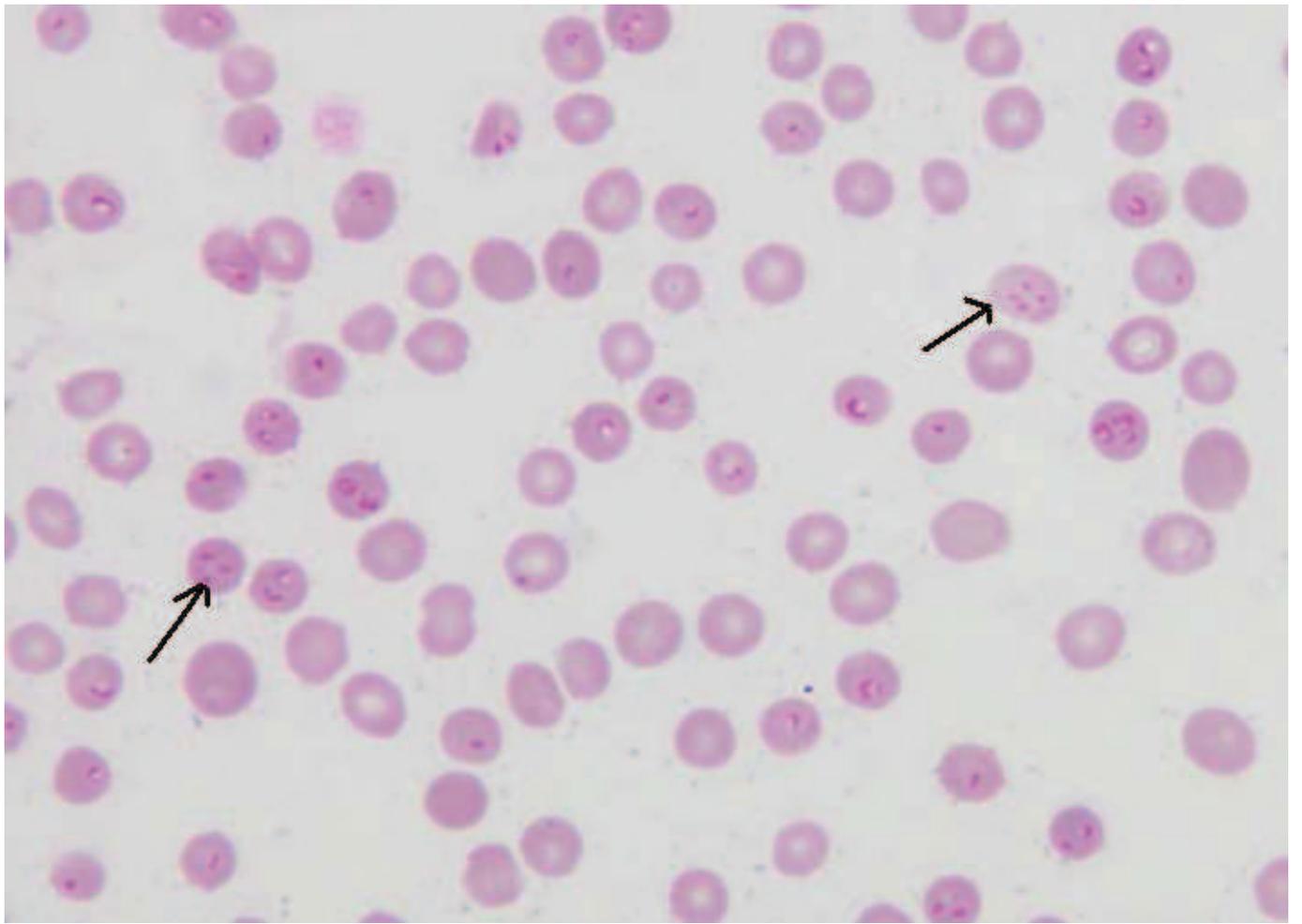
RBC (x 10 <sup>12</sup> / L) – ( 9.1 – 11.9)	7.4
Hgb ( g/L) – (129.0-155.0)	107.0
HCT(L/L) – (0.38 - 0.46)	0.34
MCV (fl) – (36.0 – 44.0)	46.0
MCH(pg) - (13.0 – 15.0)	14.4
Anisocytosis	2+
MCHC (g/L) – (320.0 – 360.0)	312.0
WBC (x 10 <sup>9</sup> / L) – (6.0 – 12.0)	5.0
Neutrophils (%) – (58.0-79.0)	74.0
(x 10 <sup>9</sup> / L) – (4.0-9.5)	3.7
Bands ( %) – (0 – 3.0)	4.0
(x 10 <sup>9</sup> / L) – (0 - 0.1)	0.2
Metamyelocytes (%) – (0)	1.0
(x 10 <sup>9</sup> / L) - (0)	0.05
Lymphocytes (%) – (15.0 – 35.0)	10.0
(x 10 <sup>9</sup> / L) – (1.3-3.1)	0.5
Monocytes (%) – (1.0-7.0)	9.0
(x 10 <sup>9</sup> / L) – (0 -0.6)	0.45
Eosinophils (%) - (1.0-10.0)	0
(x 10 <sup>9</sup> / L)- ( 0 - 0.8)	
Platelets (x 10 <sup>9</sup> / L) – (100.0 – 600.0)	58.0
Protein (g/L) – (53.0 – 71.0)	68.0
Fibrinogen (g/L) – (2.1- 3.3)	5.0

1 RBC = red blood cell, Hgb = haemoglobin, HCT = haematocrit, MCV = mean  
2 corpuscular volume, MCHC = mean corpuscular haemoglobin concentration, WBC =  
3 white blood cell. <sup>1</sup> Adapted from Schalm's Veterinary Hematology, Sixth Edition (ed  
4 Weiss and Wardrop, 2010)





Figure 1 Icteric sclera and conjunctiva of neonatal foal at 10 hrs postpartum



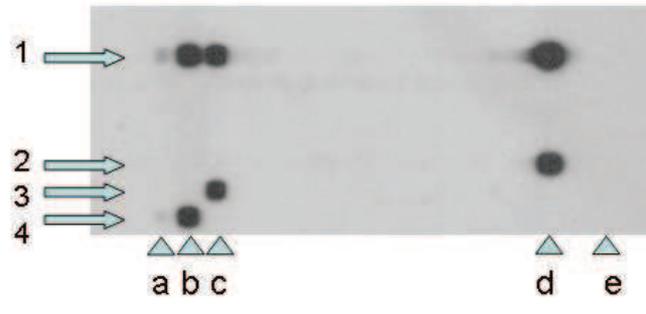


Table 2. 5'-3' sequence of oligonucleotide probes used in this study<sup>2</sup>

Oligonucleotide probe	5'-3' sequence of oligonucleotide probe	Reference
<i>Babesia/Theileria</i> genera	TAATGGTTAATAGGARCRGTTG	(Gubbels et al., 1999)
<i>Babesia caballi</i>	CGGGTTATTGACTTCGCTTTTTCTT	(Nagore et al., 2004)
<i>Babesia canis rossi</i>	CGGTTTGTTGCCTTTGTG	(Matjila et al., 2005)
<i>Theileria equi</i>	TCTGCTGTTTCGTTGACTG	GenBank Accession no. Z15105, AY150062

1

2 <sup>2</sup>R = A or G

3