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Case Report

First Detection of Diffuse and Cerebral *Theileria equi* Infection in Neonatal Filly

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ABSTRACT

Theileria equi is a tick-borne hemoparasite that may cause severe illness in equids. Intrauterine transmission of *T. equi* can occur and may result in abortion, stillbirth, or neonatal piroplasmosis of foals. *Theileria equi* and *Babesia caballi* infection are present in Israel, and subclinical infection with *T. equi* is highly prevalent. Here, we describe a case of a neonatal piroplasmosis that manifested with diffuse and cerebral *T. equi* infection. A Quarter Horse filly was born to a mare chronically infected with *T. equi*. The filly was born weak, could not stand and suckle, and had jaundice, pigmenturia, and died within hours from parturition. High *T. equi* parasitemia was detected in stained blood smears and imprint smears of different organs, further confirmed by polymerase chain reaction and sequence analysis. Intraerythrocytic parasites were also found in brain capillaries. *Theileria equi* infection in brain, to our knowledge, has never been reported previously. In vitro culture from the mare's blood enabled isolation of *T. equi* parasites. Phylogenetic analysis of the *T. equi* 18S rRNA gene from the dam's isolate and from the filly was identical and showed high similarity to previously reported *T. equi* sequences from Israel. Further studies should be done to determine whether the cerebral infection observed here was caused by the phenotypic particularities of the *T. equi* strain involved or resulted from intrinsic/unique characteristics of the immune responses elicited by the parasite in the infected foal.

1. Introduction

Equine piroplasmosis (EP) caused by the apicomplexan hemoprotozoans *Theileria equi* or by *Babesia caballi* is a tick-borne disease of equids affecting horses, mules, donkeys, and zebras. *Theileria equi* and *B. caballi* are transmitted by several species of Ixodid ticks including *Dermacentor, Hyalomma*, and *Rhipicephalus*; coinfection is often observed as the parasites are transmitted by the same ticks [1–3]. Intrauterine transmission of *T. equi* has been reported and can result in abortions, stillbirths, or neonatal piroplasmosis of foals [4]. Equine piroplasmosis is reported in tropical, subtropical, and some temperate regions. In Israel, the reported prevalence of *T. equi* was more than 20% in horses [5,6], the north of Israel was found to be hyper-endemic with prevalence higher than 75%, whereas prevalence in the central lowlands and the northern valleys was lower than 20% [6].

Animal welfare/ethical statement: The work described in this manuscript had been carried out in accordance with the code of ethics for animal experiments.

Conflict of interest statement: The authors declare no conflicts of interest.

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In endemic areas, *T. equi* infection might be subclinical and infected animals become long-term carriers serving as reservoirs, while *B. caballi* infections might spontaneously clear within months [7]. The clinical signs of EP are generally broad and nonspecific. The disease can be subclinical and asymptomatic to severe and even fatal. The clinical signs in the acute form include fever, anemia, jaundice, lethargy, depression, and weight loss. In severe cases, hemoglobinuria and bilirubinemia are present and can result in acute kidney injury [2,8]. Equine piroplasmosis can be diagnosed by microscopy examination of stained blood smears or organ touch (imprint) smears. This method is typically used in the acute form, when the parasitemia is higher and can be microscopically observed. Serological tests can be used to distinguish *T. equi* from *B. caballi* infections and are the preferred method to identify exposure to EP. Molecular approaches such as polymerase chain reaction (PCR) are highly sensitive for detection of parasite DNA, but these methods are mostly reserved for research purposes or for identification of carriers subclinically infected animals.

Hereby we describe a clinical case of transplacental transmission of *T. equi,* inducing a fatal, diffuse, and cerebral *T. equi* infection in a neonatal filly.

2. Materials and Methods

2.1. Case Description

A 12-hour-old Quarter Horse filly was presented in March 2016 at the Koret School of Veterinary Medicine–Veterinary Teaching Hospital with lethargy, severe jaundice, pigmenturia, and refusal to nurse. The mare was a 14-year-old multiparous Quarter Horse with a history of several normal pregnancies and healthy foals. The current pregnancy was diagnosed at day 20 as a twin pregnancy, and one of the embryos was reduced. The rest of the pregnancy and parturition were normal. Physical examination of the filly upon arrival to the hospital revealed hypothermia (35.6°C), and normal heart and respiratory rate. Blood tests results showed severe leucopenia (560/μL) with neutropenia (290/μL), lymphopenia (240/μL), and anemia (5.36×10^6/μL RBC, 45.7 fL MCV, 34.8% MCHC, and 22 PCV). Serum biochemistry results showed significant hypoglycemia (14.49 mg/dL), and elevated liver and muscle enzymes (80 U/L GGT, 1400 U/L AST; 1767 U/L CKL). Microscopic examination of stained blood smears of the filly revealed high parasitemia (<30%). Complete blood count and blood smear of the mare were unremarkable.

The filly was initially treated with intravenous fluids (lactated ringer's) with dextrose (2.5%–5%), flunixin meglumine (0.5 mg/kg, IV), ampicillin (25 mg/kg IV), and amikacin (25 mg/kg IV) as broad spectrum antibiotics. After identification of the severe parasitemia, the filly was treated with 1M imidocarb dipropionate (1 mg/kg IM), planned to be administrated every 6 hours. The filly was unresponsive to treatment and died 11 hours after admission. Postmortem examination was performed at the Kimron Veterinary Institute.

2.2. Sample Collection

As part of a year-long sentinel surveillance of clinically healthy horses, whole blood from the mare had been sampled on three previous occasions (November 2014, February 2015, and May 2015) before the present foaling. Whole blood and serum samples were collected during hospitalization of the present case (March 2016).

Whole blood was also collected from the filly during hospitalization as well as spleen and brain samples during postmortal examination.

Blood was collected from the jugular vein into sterile vacuum tubes containing EDTA and serum tubes. Sera were obtained from clotted blood samples by centrifugation (3,000*g* for 8 minutes) and stored at −80°C until usage. Tissue samples were stored at −80°C until processing.

2.3. Indirect Fluorescent Antibody Test

Serological examination for the detection of *T. equi*– and *B. caballi*–specific antibodies was performed by indirect fluorescent antibody test (IFAT) as previously described on serum extracted from the mare during hospitalization [5].

2.4. Parasite Culture

Isolation of the parasite in culture was attempted from blood taken from the mare during hospitalization. The culture was initiated and maintained as previously reported [9]. The complete medium used contained HL-1 medium with a supplement of 20% horse serum, 2 mM L-glutamine, 0.2 mM hypoxanthine, 10 mM TAPSO, and 1:500 100IU penicillin/mL and 100 μg streptomycin/mL. Passages were performed every 72 hours or early according to the observed parasitemia.

2.5. DNA Extraction and Piroplasm Identification by PCR

DNA was extracted from blood and tissue samples from the mare and filly using a commercial DNA extraction kit (DNeasy Tissue Kit Qiagen, Hildenberg, Germany), according to the manufacturer's instructions.

The identification and genetic characterization of the parasites were performed using primers described in Table 1, targeting the 18S rRNA [10,11], the equine merozoite antigen-1 genes of *T. equi* [10], and the rhoptry-associated protein-1 gene of *B. caballi* [10,12].

All PCRs included a nontemplate control, consisting of the reaction mix and 1 μL of DNase/RNase-free water (Sigma, St. Louis, MO), and positive controls from horses previously identified with *T. equi* or *B. caballi*, and later confirmed by nucleotide sequencing. Reactions were performed in 20 μL of a mixture consisting of 10 μL GoTaq green master mix (Promega, Madison, WI), 7 μL DNase/RNase-free water (Sigma, St. Louis, MO), 1 μM of each primer and 1 μL DNA template, or in 25 μL of a mixture consisting of 1.0 mM of each primer, 200 mM dNTP (Invitrogen, Life Technologies, 5791 Van Allen Way, CA), 2.5 μL buffer, 13.25 μL DNase/RNase-free water, 0.25 μL Taq DNA polymerase (Invitrogen, Life Technologies), and 1 μL DNA template. Polymerase chain reaction products were