Case report/Fallbericht

Equine Piroplasmosis – a case of severe Babesia caballi infection associated with acute renal failure

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A 14 year-old gelding was diagnosed with acute equine piroplasmosis caused by Babesia (B) caballi infection using IFAT, microscopy and nested PCR. The horse was presented with a high fever (40.8°C), apathy and inappetence. Clinical examination revealed tachycardia (96 beats/min), tachypnoea (20 breaths/min), icteric mucous membranes, and sternal and preputial oedema. Blood tests showed moderate anaemia and thrombocytopaenia, severe azotaemia and increased plasma free haemoglobin. Urinary tests revealed a highly decreased glomerular filtration rate (GFR) and an increased γ-GT:creatinine ratio indicating acute renal failure. Intraerythrocytic merozoites of B. caballi were detected in the blood smear. The patient was treated with imidocarb dipropionate (Carbesia®) for B. caballi infection and dopamine to correct the GFR. However, the horse continued to deteriorate and the animal’s owner decided to euthanize before the second dose of Carbesia. The necropsy revealed acute renal failure, which was caused mainly by membranoproliferative glomerulonephritis, in addition to moderate inflammatory signs in the brain, liver and myocardium. B. caballi can cause severe multiple organ dysfunction, and parameters such as plasma-free haemoglobin, urinary GFR and γ-GT:creatinine ratio could be helpful indicators of an upcoming renal problem in horses.

Keywords: equine piroplasmosis, Babesia caballi, acute renal failure, nested PCR, cELISA

Zusammenfassung


Schlüsselwörter: Equine Piroplasmose, Babesia caballi, akutes Nierenversagen, PCR, cELISA

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Introduction

Equine piroplasmosis is a tick-borne parasitic disease caused by the protozoans *Babesia* (*B.*) *caballi* and/or *Theileria* (*T.*) *equi* (Waal, 1992; Wise et al., 2013). Southern Europe is considered one of the most endemic areas regarding equine piroplasmosis. In one seroprevalence study, 17.9% of the horses tested positive to *B. caballi* in north and central Italy (Moretti et al., 2010). Infected horses can develop different forms of clinical signs. On rare occasions, the horse might be found dead due to peracute infection. In acute conditions, horses usually present with high fever, pale or icteric mucous membranes, dyspnoea, tachypnoea, tachycardia and anorexia. Additional symptoms, such as colic, haemoglobinuria, head and limbs oedema and sweating, can also be seen. Transient fever, anorexia, weight loss, mild anaemia, splenomegaly and weakness are noticed in chronic piroplasmosis (Zobba et al., 2008; Wise et al., 2013). Although the exact mechanism of haemolysis remains unknown, theories such as mechanical rupture of the erythrocyte’s membrane as a result of the release of merozoites, extravascular haemolysis as the infected and non-infected red blood cells (RBCs) are removed from the circulation by mononuclear phagocytic system, and haemolytic factors released by the causative agent have been put forward (Beard et al., 2013; Wise et al., 2013). Acute renal failure secondary to piroplasmosis has been described in horses as a result of haemolysis and subsequent pigment nephropathy induced by haemoglobin, in addition to renal hypotension secondary to systemic inflammatory reaction to infection (Waal, 1992; Wise et al., 2013). Dopamine has been used in human critical care medicine to correct haemodynamic disorders and renal failure, as it increases the renal blood flow (Singh et al., 2013). However, some other reports have not recommended dopamine usage in such patients because of its side effects, such as extravasation necrosis and gangrene (Kellum et al., 2011). Equine piroplasmosis is a rare disease in Germany, in fact, only one clinical case of *T. equi* has been reported in a mare in the last 15 years (Scheidemann et al., 2003). The seroprevalence in central Germany of *T. equi* was 6.1% measured by an indirect fluorescent antibody test (IFAT) and 3.2% by complement-enzyme linked immune sorbent assay (cELISA). Only one horse out of 314 tested horses which were located in central Germany had antibody titre against *B. caballi* using cELISA, but no horse out of 314 had antibodies using IFAT (Pikalo et al., 2016). Recent studies regarding seroprevalence of *B. caballi* revealed high percentage (10.3%) of seropositive, asymptomatic horses in Central-Southern Italy (del Pino et al., 2016), and about 1.97% of the horses in Turkey are seropositive and symptomatic (Kizilarslan et al., 2015). Horses treated with imidocarb dipropionate became also sero-negative by cELISA within 201 days (Tamzali, 2012). The aim of this report was to describe the clinical signs, diagnostic procedures and treatment of a horse with severe acute renal failure caused by *B. caballi* infection.

Case report

History

A 14 year-old brown draft Breton gelding was referred to the Large Animal Medicine Department, Faculty of Veterinary Medicine, University of Leipzig on 20 October, 2015, with a six-day history of high fever (up to 41.7°C), anorexia and depression. The gelding was treated by the referring veterinarian with Trimethoprim and Sulfadiazine (Equibactin®), metamizole (Metapyrin), flunixin meglumine and intravenous fluid therapy without any improvement. The horse had been vaccinated against tetanus and equine influenza five months previously. The horse was born in southern France and used as a show horse in France, England, Germany and Italy. The gelding was in quarantine for a month (between April and May) in Ohio State, USA, in 2012. After this the horse was living in the region of Berlin until admission. Based on owner’s history no ticks were observed.

Clinical examination

The horse was pyrexic (40.8°C), tachycardic (96 beats/min), slightly tachypnoeic (20 breaths/min) and depressed at presentation. Severe sternal and preputial oedema and enlargement of the right side of the submandibular area were detected (Fig. 1). The conjunctiva and sclera were icteric, and the nasal and oral mucous membranes were pale pink to slightly icteric with normal capillary refill time. Submandibular lymph nodes were lobed, elastic, movable and of a normal size. No ticks were observed on the general clinical examination. Bilateral intestinal borborygmi were moderately reduced. The rectal examination was normal. The body temperature returned to normal range on the third day, and the horse started to eat hay, however, he remained tachycardic. On the fourth day, the patient started to show signs of severe azotaemia, in addition to leukocytic toxic changes. Differential diagnoses included equine piroplasmosis, equine infectious anaemia, equine viral arteritis, equine anaplasmosis, purpura haemorrhagica, immune-mediated haemolytic anaemia and red maple leaf toxicity.

Clinicopathological examination

The clinicopathological data are listed in Table 1 and 2. An erythrocyte osmotic fragility test and serum proteins electrophoresis were performed to rule out RBCs membrane disorders and gammopathies, respectively. The haemato-
logical and serum biochemical parameters are listed in Table 1. The patient’s condition deteriorated on the eighth day as moderate level of merozoites of *B. caballi* (round-shaped intraerythrocytic bodies) appeared in the blood (Fig. 2) and toxic-changed neutrophils increased, which lasted until the horse was euthanized. The diagnosis of *B. caballi* infection was confirmed after the serum antibody titre proved positive (1:160) (Fig. 3) using an immunofluorescent antibody test (IFAT). The IFAT was conducted using the Testkits Mega Screen® Fluobabesia caballi ad us. vet. (anti-Babesia caballi IgG-Antibodies) and Mega Screen® Fluotheliera equi ad us. vet. (anti-Theileria equi IgG-Antibodies) (Diagnostik Megacor, Hörbranz, Austria), according to the manufacturer’s instructions. No antibody titre against *T. equi* was detected using IFAT. The cELISA was conducted using the Babesia caballi antibody test kit cELISA and the Theileria equi antibody test kit; cELISA (Veterinary Medical Research and Development (VMRD), Pullmann, Washington, USA) was used following the manufacturer’s instructions. The cELISA for *B. caballi* also showed a high antibody titre. The cELISA for *T. equi* could not detect any antibody titre. DNA was extracted from EDTA blood using the DNaseasy blood and tissue kit (Qiagen, Hilden, Germany), as described in the manufacturer’s protocol and a nested PCR was performed using primers described by Ano et al. (2001). Aagarose gel electrophoresis of nested PCR products showed a double band (presumably first and second round PCR product) in the range of ≥ 300 bp in the EDTA blood sample collected on day ten, but not in the one collected on day 13 (Fig. 4). Sequencing of both the upper and lower band with the nested PCR primers resulted in a consensus sequence with 100% identity to *B. caballi* 18S rRNA, as determined by sequence comparison using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

### TABLE 1: Haematological and serum biochemical findings of the patient upon admission, day 7, day 10, and day 12 of hospitalisation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Reference range</th>
<th>At admission</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>haematocrit</td>
<td>L/L</td>
<td>0.22–0.35</td>
<td>0.16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>erythrocyte</td>
<td>x 10⁹/L</td>
<td>5.2—13.0</td>
<td>5.2</td>
<td>7.9</td>
<td>6.3</td>
<td>7.0</td>
</tr>
<tr>
<td>neutrophil</td>
<td>x 10⁹/L</td>
<td>0.2—0.5</td>
<td>0.21</td>
<td>0.24</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>lymphocyte</td>
<td>x 10⁹/L</td>
<td>1.0—1.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>eosinophil</td>
<td>x 10⁹/L</td>
<td>0—0.10</td>
<td>0.16</td>
<td>0</td>
<td>0.13</td>
<td>0.21</td>
</tr>
<tr>
<td>total protein</td>
<td>g/L</td>
<td>59.0–81.4</td>
<td>59.4</td>
<td>69.0</td>
<td>66.7</td>
<td>64.7</td>
</tr>
<tr>
<td>albumin</td>
<td>g/L</td>
<td>23.0–35.1</td>
<td>25.6</td>
<td>21.9</td>
<td>-</td>
<td>19.0</td>
</tr>
<tr>
<td>sodium</td>
<td>mmol/L</td>
<td>135—144</td>
<td>129</td>
<td>139</td>
<td>135</td>
<td>138</td>
</tr>
<tr>
<td>chloride</td>
<td>mmol/L</td>
<td>93.6—111.4</td>
<td>91.0</td>
<td>104.0</td>
<td>94.2</td>
<td>92.4</td>
</tr>
<tr>
<td>potassium</td>
<td>mmol/L</td>
<td>2.0—4.70</td>
<td>4.04</td>
<td>2.41</td>
<td>2.24</td>
<td>2.47</td>
</tr>
<tr>
<td>creatine kinase</td>
<td>IU/L</td>
<td>181—652</td>
<td>132</td>
<td>-</td>
<td>1120</td>
<td>-</td>
</tr>
<tr>
<td>lactate dehydrogenase</td>
<td>IU/L</td>
<td>293.8–822.8</td>
<td>4075.0</td>
<td>2812.0</td>
<td>13141.0</td>
<td>14130.0</td>
</tr>
<tr>
<td>cholesterol</td>
<td>mmol/L</td>
<td>1.60—3.03</td>
<td>3.26</td>
<td>3.09</td>
<td>3.12</td>
<td>-</td>
</tr>
<tr>
<td>free haemoglobin</td>
<td>µmol/L</td>
<td>0.09–2.01</td>
<td>59.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>glucose</td>
<td>mmol/L</td>
<td>2.18–6.05</td>
<td>6.36</td>
<td>9.40</td>
<td>4.89</td>
<td>6.86</td>
</tr>
</tbody>
</table>

### TABLE 2: Urinary examination results of the patient on days 6 and 10 of hospitalisation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference range</th>
<th>At admission</th>
<th>Day 6</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>protein</td>
<td>&lt; 0.4</td>
<td>-</td>
<td>0.43</td>
<td>1.11</td>
</tr>
<tr>
<td>specific gravity</td>
<td>1.030—1.050</td>
<td>1.008</td>
<td>1.014</td>
<td></td>
</tr>
<tr>
<td>red blood cells</td>
<td>Per high power field</td>
<td>&lt; 5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>white blood cells</td>
<td>Per high power field</td>
<td>&lt; 5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>glomerular filtration rate (Creatinine clearance)</td>
<td>ml/min/1.73 m²</td>
<td>1.20—2.38</td>
<td>0.19</td>
<td>0.50</td>
</tr>
<tr>
<td>creatinine</td>
<td>mg/dL</td>
<td>&lt; 0.75</td>
<td>36.7</td>
<td>11.9</td>
</tr>
<tr>
<td>electrolytes</td>
<td>µmol/L</td>
<td>44.23–82.73</td>
<td>313.0</td>
<td>85.7</td>
</tr>
<tr>
<td>sodium</td>
<td>mmol/L</td>
<td>140.0–240.0</td>
<td>149.0</td>
<td>146.0</td>
</tr>
<tr>
<td>chloride</td>
<td>mmol/L</td>
<td>90.0–110.0</td>
<td>91.0</td>
<td>104.0</td>
</tr>
<tr>
<td>creatinine</td>
<td>mg/dL</td>
<td>&lt; 0.4</td>
<td>0.43</td>
<td>1.11</td>
</tr>
</tbody>
</table>

Urinary examination

The urinary findings might have been altered because the horse was under intravenous fluid therapy during its entire period of hospitalisation. Urinary examination on the sixth day revealed light yellow, clear, watery urine. On the tenth day, the horse developed red, watery, and slightly turbid urine, and a urine strip test indicated moderate haemoglobinuria (Fig. 5); urine sediment analysis showed low grade erythrocyte and leukocyte counts in addition to renal epithelia. The glomerular filtration rate was estimated by measuring the amount of urine, serum and urine creatinine concentrations within a 24 h
period. This was evaluated on two different days to determine the effect of dopamine. Urinary examination findings are listed in Table 2.

**Treatment and outcome**

The horse was treated initially with antibiotics, amoxicillin (Amoxisel®, Selectavet, Holzolling, Germany; 10 mg/kg, i. v., q12 h for 7 days), gentamicin (Gentamicin 50®, aniMedica GmbH, Senden-Bösensell, Germany; 6.6 mg/kg, i. v., q24 h for five days), in addition to antipyretic, flunixin (Finadyne®, MSD Animal Health Innovation GmbH, Schwabenheim an der Selz, Germany; 1.1 mg/kg, i. v., q12 h for four days) and intravenous 0.9% saline solution therapy which was given continuously in an amount of 50–75 ml/kg b. w./24 h. An amount of 250 mg dopamine was dissolved in saline and administrated as a 500 ml solution to correct the glomerular filtration rate. The horse’s heart rate was closely monitored during the dopamine therapy. The horse was treated once with imidocarb dipropionate (Carbesia®, MSD Animal Health, Beaucouzé, France; 4 mg/kg, i. m.). The decision for euthanasia was made by the owner before administering the second dose of imidocarb dipropionate three days later because of the lateral recumbency and deterioration in the condition of the patient. The horse was euthanized 13 days after admission and two days after the first treatment with imidocarb. Postmortem examination revealed dark red kidneys with multifocal moderate membranoproliferative glomerulonephritis, high-grade multifocal chronic non-purulent interstitial nephritis and moderate diffuse interstitial and perirenal oedema; moderate hepatic portal, sinusoidal and centrilobular fibrosis and hepatic haemosiderosis, moderate cerebral leptomeningeal fibrosis with low grade perivascular mononuclear infiltration; moderate subacute mononuclear myocarditis; right sub-mandibular osteofibroma and active bone marrow (sternum) erythropoiesis.

The final diagnosis of acute *B. caballi* infection and multiple organ dysfunctions was made.

**Discussion**

The current study reports the first fatal acute equine *B. caballi* infection in Germany. The cause of thrombocytopenia in depressed horses with fever had been related to anaplasmosis or equine infectious anemia as an equine European emerging disease in the region of central Germany. But now piroplasmosis should be included in this differential diagnosis (Pikalo et al., 2016). The disease is endemic among equine species in tropical and subtropical areas (Uilenberg, 2006; Abutarbush et al., 2012; Salim et al., 2013; Vieira et al., 2013), and in temperate areas (Moretti et al., 2010; Butler et al., 2012; del Pino et al., 2016). Considering that the patient travelled to some of the endemic areas and the incubation period of *B. caballi* is 10–30 days, it is unlikely that the horse was infected in one of these areas (western France), according to his passport, because the horse spent the last three years only in the region of Berlin. Some horses in central Germany show titers against *B. caballi* without clinical signs (Pikalo et al., 2016). The reason of this infection could be a superinfection and/or an immunosuppression because this horse had a severe lymphocytopenia. The clinical and laboratory findings represent important parts of the diagnostic process. The current patient developed the typical signs of acute equine piroplasmosis in addition to lymphocytopenia and immune complex-mediated glomerulonephritis. Signs of haemolytic anaemia (pale mucous membranes, anaemia, increased lactate dehydrogenase (LDH) level and free haemoglobin, low haemoglobin and haematocrit) were constant, indicating ongoing inflammatory reaction against a considerably large number of infected RBCs, which eventually resulted in the accumulation of immune complexes in the renal glomeruli and development of renal failure. The RBC count, haemoglobin and haematocrit values in an experimental *B. caballi* infection in ponies fluctuated every three to four days associated with recurrent parasitaemia in acutely infected animals (Allen et al., 1975). Uraemia has been reported to be a cause of anaemia, as the nitrogenous waste products interfere with the erythrocytes membrane and ion channels (Finco and Groves, 1985). Although hyperbilirubinaemia is a common sign of intra- and extravascular haemolysis, not all horses infected with *B. caballi* show hyperbilirubinaemia (Zobba...

FIGURE 5: Haemoglobinuria (red urine) observed from the 14 year-old draft Breton gelding on day ten.

et al., 2008). In the current case, normal conjugated bilirubin ruled out bone-marrow and hepatic involvement, and decreased haematocrit, normal total protein and moderate indirect hyperbilirubinaemia indicates haemolysis. Moderate thrombocytopenia is a common finding in B. caballi infection, secondary to increased consumption of the thrombocytes, immune-mediated platelet destruction and/or splenic sequestration (Sellon and Wise, 2009; Wise et al., 2013). Since there was no splenomegaly in the clinical and post-mortem examinations of this horse, the most likely causes of thrombocytopenia were increased use of the thrombocytes and immune complexes, which were formed in the circulation due to the infection, attached to the surface of the platelets, and the mononuclear phagocytic system removed the platelets involved. The patient developed acute renal failure as he showed severe decreased glomerular filtration rate secondary to membranoproliferative glomerulonephritis, and increased \( \gamma \)-GT:creatinine ratio and significant proteinuria indicated proximal tubular epithelia damage due to acute renal failure. The cause of membranoproliferative glomerulonephritis in horses is usually immune-mediated, and \( \gamma \)-GT is normally secreted by the brush border of the proximal convoluted tubules’ epithelia (Walbridge, 2009). Acute glomerulonephritis is an uncommon condition in horses, and most of the cases are caused by deposition of immune complexes in the basement membrane or mesangium, which leads to activation of the complement, the latter causing neutrophilia and platelet aggregation (Klauser et al., 1989). It has been reported in some cases of Streptococcus equi subsp. zooepidemicus and equine infectious anaemia infections (Divers et al., 1992; Waldridge, 2009). Hyponatraemia, hypochloraemia, hypokalaemia, hypocalcaemia and hyponatraemia are all signs of chronic interstitial nephritis, as the renal tubules have become dysfunctional to reabsorbed sodium, chloride, potassium, calcium and magnesium, which was confirmed in these electrolytes’ fractional excretion tests. The fractional excretions of the electrolytes mentioned previously, except calcium, improved on day ten, presumably because of the intensive intravenous fluid therapy that helped to remove the haemoglobin from the nephrons, which discoloured the urine on the same day and let the renal tubules regenerate. As the reabsorption of the calcium is mainly controlled by metabolic factors, such as the parathyroid hormone, the horse’s nutritional status could not help him to reabsorb the filtered calcium. Hepatic and myocardial lesions may have contributed to the increased LDH levels in the last two days before euthanasia. In this patient, hepatic lesions had probably taken place as a result of the systemic inflammatory process, in addition to the imidocarb treatment’s side effect. The latter may also have been attributed to the cardiac and central nervous system lesions. Wise et al. (2013) stated that the most serious complications of the imidocarb therapy are periportal hepatic necrosis and renal tubular necrosis, and the patients treated should be closely monitored. To summarise: B. caballi infection in horses can be fatal in a non-endemic area, as the patient may develop disseminated intravascular coagulation and multiple organ dysfunctions. The treatment protocol using imidocarb in endemic regions for B. caballi and T. equi infections includes 2.2 mg/kg bw i. m. two applications with a one- to two-day interval. In non-endemic regions, using four times 4 mg imidocarb/kg bw i. m. every three days is recommended for clearing (sterilizing) B. caballi and T. equi infections (Tamzali, 2012).

Various diagnostic tests can be used alone or in combination to diagnose equine piroplasmosis (Wise et al., 2013). Detection of parasite DNA by nested PCR is more sensitive than microscopic detection of parasites in blood smears (Fritz, 2010). Thus, PCR is a useful tool for the rapid detection and identification of B. caballi and T. equi infections in blood and as a supplement to microscopy and serology for augmenting diagnostic results (Fritz, 2010; Rothschild, 2013).

The IFAT and the cELISA are serological methods to detect antibodies against B. caballi or T. equi. Positive results with the IFAT will be detected at days 3–20 post infection (Wise et al., 2013). The cELISA is more sensitive and more specific than the IFAT (Mans et al., 2015). With the increasing availability of PCR in laboratory settings, however, one should be cautious to base a diagnosis on a positive cELISA or IFAT result alone, because antibody positive results could indicate past infections of unknown age or after clearance of piroplasms (Wise et al., 2013). A negative cELISA or IFAT result could be a false negative in the early phase of infection. A positive PCR result demonstrates current active parasitaemia (Butler et al., 2012). Furthermore, PCR could be used to check for a therapeutic response and/or as a prognostic marker. Based on
the negative PCR result of the second EDTA plasma sample taken from the patient two days after the first treatment with imidocarb, it could be assumed that the imidocarb treatment had already cleared the B. caballi infection (Tamzali, 2012). In experimental infections with B. caballi, the horses were PCR-negative five days after treatment with the 4 mg imidocarb protocol mentioned above. These experimentally infected and treated horses became seronegative by cELISA within 201 days (Tamzali, 2012). Therefore, it is suggested that both, nested PCR and cELISA, should be performed in cases of clinical suspicion of equine piroplasmosis.

**Conflict of interest**

The authors declare that there is no protected, financial, occupational or other personal interest in a product, service and/or a company which could influence the contents or opinions presented in the manuscript.

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