

Case 6

MYCOPLASMA HAEMOCANIS INFECTION IN A DOG DETECTED BY BLOOD SMEAR EVALUATION AND REAL-TIME PCR

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Case presentation

An 8-year-old, entire male, Jake Russell terrier dog was presented with one week history of weakness and reluctance to walk.

The dog lived in a rural area with other two dogs and had no travel history outside the UK. Dog's vaccination status was up to date and it had been intermittently treated for ectoparasites and endoparasites. Dog's previous medical history included splenectomy 4 years previously due to a bleeding haematoma after being hit by a car.

Abnormalities on physical examination were pale mucous membranes, a heart rate of 160 BPM and a bounding pulse.

A blood sample was submitted to our laboratory for a complete haematological, biochemical and coagulation profile. Abnormalities on CBC were a marked anaemia (RBC $0.98 \times 10^{12}/L$, reference interval 5 to 8.5; haemoglobin 2.7 g/dl, reference interval 12 to 18; HCT 7.8%, reference interval 37 to 55) with evidence of a moderate regeneration (reticulocyte $176.4 \times 10^9/L$, reference interval < 100) and a very mild monocytosis ($1.33 \times 10^9/L$, reference interval 0 to 1.3). PT and aPTT were within normal limits. No significant abnormalities were detected on biochemistry profile.

A blood smear was evaluated.

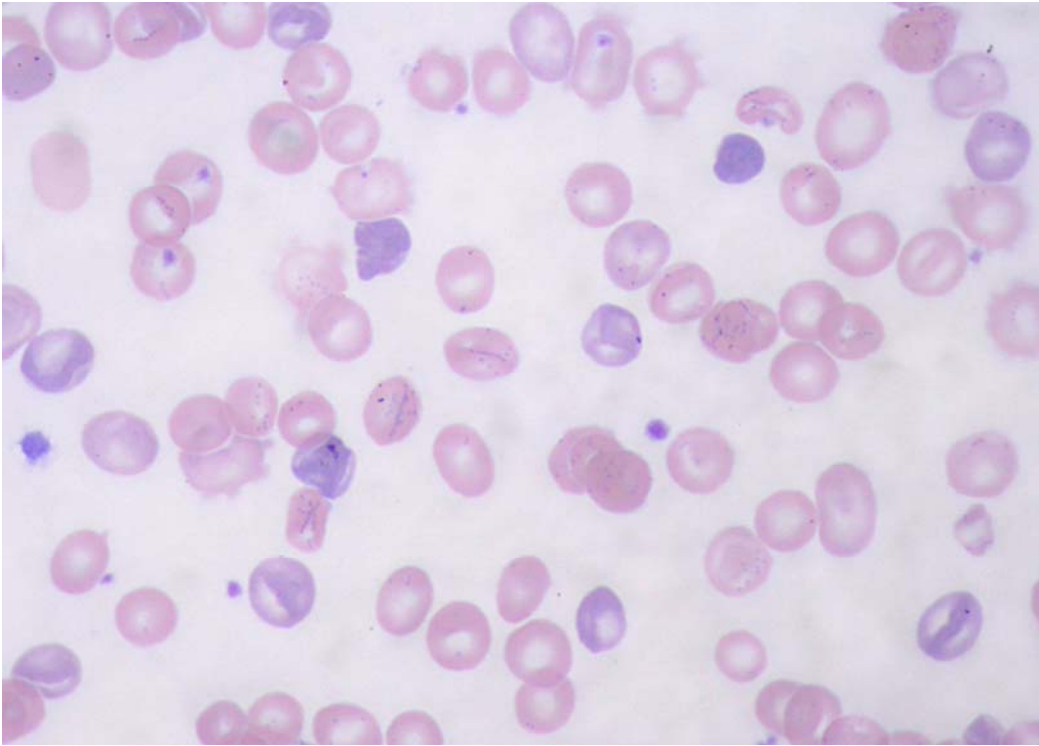


Fig 1 Peripheral blood smear from a dog with marked anaemia.

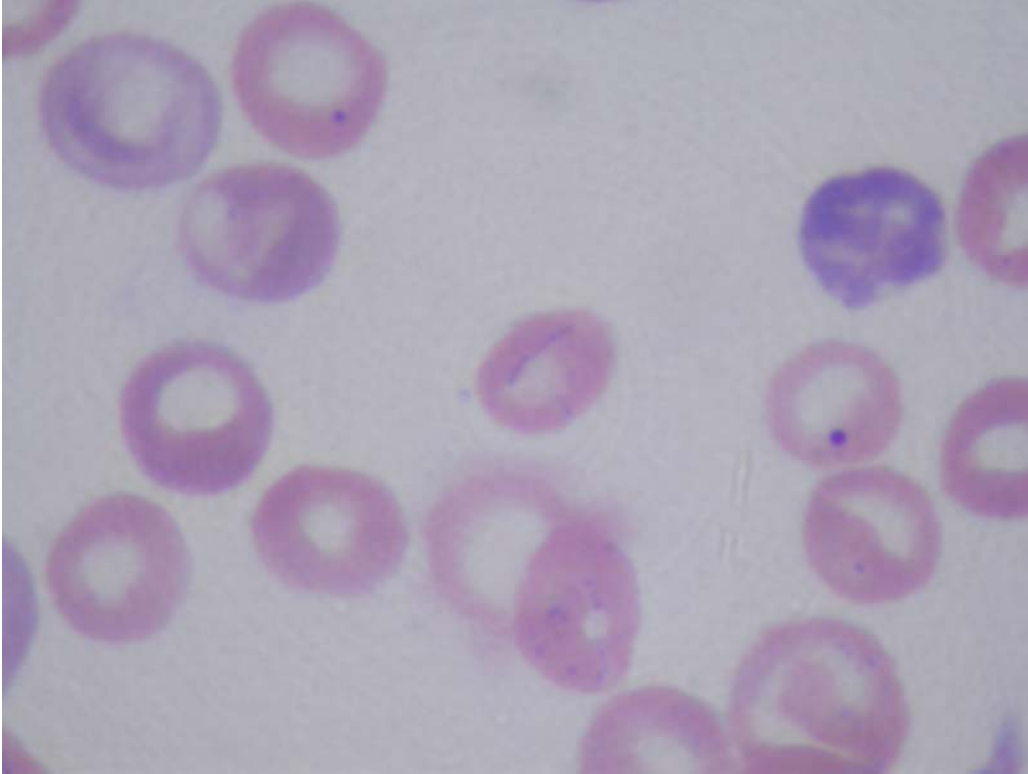


Fig 2 Peripheral blood smear from a dog with marked anaemia.

Blood smear evaluation

On blood smear evaluation red cells showed a moderate increase in polychromasia with polychromatophils and polychromatophilic leptocytes and a mild increase in anisocytosis with a few late normoblasts seen. Occasional Howell Jolly bodies were also noted. Frequent very small rod shaped basophilic structures evident singly and in filamentous chains across the surface of red cells were identified. There was no evidence of spherocytes or autoagglutinates on smear evaluation. A diagnosis of haemolytic anemia due to haemotropic *Mycoplasma* species infection was made.

Additional test results and follow up

An EDTA sample was submitted to Bristol University for real-time PCR identification of the haemotropic *Mycoplasma*. The dog was tested for both *Mycoplasma haemocanis* and *Candidatus Mycoplasma haemotoparvum* and was positive for *Mycoplasma haemocanis*. Treatment with doxycycline and prednisolone was started.

A repeat FBC after 3 weeks of treatment revealed a marked improvement in the extent of anaemia (RBC $2.66 \times 10^{12}/L$, haemoglobin 6.9 g/dl, HCT 22.2%) with signs of marked regeneration (reticulocyte count $457.52 \times 10^9/L$). *Mycoplasma haemocanis* was not identified on peripheral blood smear examination. Real-time PCR remained positive but revealed a reduction in the blood load compared with the previous result.

After 2 months of treatment the anaemia had further improved (RBC $4.77 \times 10^{12}/L$, haemoglobin 9.9 g/dl, HCT 31.5%) and real-time PCR showed a further decrease in the blood load detected. On this occasion the two in-contact dogs that lived with the infected animal were tested by PCR for haemotropic *Mycoplasma* in an attempt to identify the source of infection. These samples however gave negative results.

After 3 months of treatment a further sample was negative for *Mycoplasma haemocanis* by PCR and hematological parameters were nearly within normal limits.

Discussion

In this work we describe the first case of *Mycoplasma haemocanis* infection in a dog in the UK.

Two types of haemotropic mycoplasmas are described in dogs: *Mycoplasma haemocanis* (previously *Haemobartonella canis*) and the more recent isolated *Candidatus Mycoplasma haemotoparvum*^{1,2,3,4}. Haemoplasmas are considered opportunistic bacteria, which parasitize red cells adhering to their surface and depending on the host cell for provision of amino acids, fatty acids, cholesterol and vitamins^{1,2}.

The natural methods of transmission of canine haemotropic mycoplasmas have not been definitively established. *Rhipicephalus sanguineus* is thought to be a vector for this type of infection in naturally infected dogs, although other methods of infection (e.g. transplacental infection, direct blood inoculation and oral ingestion) have been also considered^{1,5,6}.

The interest in haemotropic *Mycoplasmas* has been increased in the veterinary literature in the last few years following the introduction of PCR and real-time quantitative PCR as very sensitive tools in the diagnosis and therapeutic monitoring of this type of infection^{7,8}. A few studies have been recently reported regarding the prevalence of these parasites in different countries^{8,9,10,11}. Haemotropic *Mycoplasmas* have been described worldwide, but higher prevalence may occur in geographical areas where the proposed vector *Rhipicephalus sanguineus* has a widespread distribution^{9,11}.

Although no studies regarding the prevalence of haemotropic *Mycoplasmas* in the UK have been published as yet in the veterinary literature, Warman et al reported that 227 dogs gave negative PCR results for both *Mycoplasma haemocanis* and *Candidatus Mycoplasma haemotoparvum* in a study regarding the possible correlation between haemolytic anemia and haemotropic *Mycoplasma* infections in dogs in the UK¹². This suggests that the prevalence of these types of infection may be low in this country, as reported for other countries where the climate is incompatible with the frequent occurrence of *Rhipicephalus sanguineus*¹¹.

Living in a rural area, our dog had a history of frequent flea and tick infestations, but no travel history to countries where *Rhipicephalus sanguineus* is more prevalent. This may support an association between the exposure of the dog to ticks and haemoplasma infections and the hypothesis of an autochthonous infection.

The dog lives in the south of the UK where *Rhipicephalus sanguineus* has been occasionally identified, however, since the transmission of the infection by *Rhipicephalus sanguineus* has not been completely demonstrated, it is possible to speculate that other species of ticks could be involved.

Two clinical forms of *Mycoplasma haemocanis* infection have been recognized; a chronic latent form in which apparently healthy dogs are asymptomatic carriers of the

bacteria with low or undetectable numbers of organisms observed in peripheral blood and an acute form always reported in immunocompromised or splenectomized dogs and characterized by a rapid development of severe haemolytic anemia and a marked peripheral blood parasitemia^{1,2,13,14,15,16}. The onset of the acute form in splenectomized dogs is usually reported to occur rapidly after surgery^{1,14}. In this case, the acute form occurred 4 years after splenectomy, suggesting likely subsequent infection rather than the development of infection in a previously asymptomatic carrier.

Interestingly the other two dogs living with the infected dog tested PCR negative for both *Mycoplasma haemocanis* and *Candidatus Mycoplasma haemotoparvum*, supporting the fact that specific host immune reaction and an impaired immune system, as was present in the infected dog, may be necessary also for the acquisition of the infection.

Reported clinical signs in the acute form include weakness, anorexia and anemia^{1,2}.

The mechanisms responsible for the pathogenicity of haemotropic mycoplasmas have not yet been completely defined^{1,2,14}. It is evident that haemotropic Mycoplasmas severely alter the shape and the deformability of affected red cells leading to an increased sequestration and phagocytosis of the parasitized erythrocytes but antibody mediated haemolysis has also been reported and may be contributory^{1,2,14}.

In our case there was no evidence of autoagglutination, hyperbilirubinaemia or spherocytosis at the initial presentation and, although a Coombs' test was not performed, the hypothesis of an immune-mediated haemolytic anemia was considered unlikely.

Treatment with doxycycline is recommended for the acute form of *Mycoplasma haemocanis* infection^{1,14}. Concomitant corticosteroid administration is considered useful in limiting haemolysis and erythrocytes phagocytosis¹⁴. It has been reported however that the course of the disease is largely unpredictable and that systemic antimicrobial therapy may not fully eliminate the parasite from the blood leading to the possibility of relapses of the infection later after recovering^{1,11,14}. In our case the dog responded well to therapy, became negative after 3 months and no relapses has occurred 4 months after becoming negative by PCR. The recent introduction of PCR has led to an increase in the diagnostic sensitivity for this type of disease compared to microscopic identification of the bacteria¹¹. This was found also in our case where no *Mycoplasma haemocanis* organisms were microscopically detected in two samples that gave positive results on PCR analysis. Moreover with real-time quantitative PCR more sensitive monitoring of a dog's individual response to treatment is possible¹¹. All these should lead to an improved knowledge of canine haemotropic mycoplasmas in particular assist in determining the route of transmission, the pathogenesis and the outcome after therapy.

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