Immune-mediated haemolytic anaemia: possible association with *Ancylostoma caninum* infection in three dogs

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ABSTRACT

Immune-mediated haemolytic anaemia (IMHA) may be primary or secondary. In primary IMHA, no underlying cause can be found, whereas secondary IMHA is triggered by an underlying cause, such as neoplasia, infectious diseases, or drugs. This paper describes 3 dogs with typical signs of IMHA that was possibly associated with the intestinal parasite *Ancylostoma caninum*. As intestinal helminths can be difficult to diagnose on faecal examination, it would be pertinent to perform multiple faecal examinations on any animal that has IMHA with no apparent underlying cause, as part of the therapy.

Key words: immune response, intestinal parasites, secondary haemolytic anaemia.

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INTRODUCTION

Immune-mediated haemolytic anaemia (IMHA) may be primary (idiopathic) or secondary[9], and can be classified into 5 classes[11]. IMHA arises when an immune response targets erythrocytes either directly or indirectly. The immune-mediated destruction of erythrocytes is initiated by binding of immunoglobulin G or M and/or complement to the erythrocyte surface, which results in their recognition by specific receptors on the reticulo-endothelial cells. The subsequent destruction is mainly extravascular[5], although intravascular destruction can also occur[13]. In primary IMHA no underlying inciting cause can be found, whereas secondary IMHA is triggered by an underlying cause[5], such as neoplasia, infectious diseases (parasitic, viral, bacterial, rickettsial or fungal), or drugs[8]. This paper describes 3 dogs with IMHA, which was possibly associated with the intestinal parasite *Ancylostoma caninum.*

CASE HISTORIES

Case 1

A 16-month-old female Labrador retriever was presented to the Onderstepoort Veterinary Academic Hospital (OVAH) with a history of anorexia of 2 days' duration. On clinical examination, tachycardia, polypnoea and pale mucous membranes were evident. The dog had a haematocrit of 15 % and the in-saline agglutination (ISA) test was positive. No parasites could be identified on repeated thin capillary blood smear evaluation. On faecal examination, *A. caninum* infestation was present. Regenerative anaemia, left shift leukocytosis, monocytosis, thrombocytopenia, and spherocytosis were evident on full haematological examination (Table 1). Urine analysis and survey thoracic radiographs were both within normal limits. On abdominal ultrasonography the only abnormality was an enlarged normoechoic spleen. Treatment was initiated with 2 mg/kg of prednisolone (Lenisolone, Pharmador) twice a day and 50 mg/kg fenbendazole (Panacur, Hoechst) once a day for 5 days. Over 4 days the haematocrit progressively increased, although the ISA test remained positive. From the 5th day the prednisolone dose was tapered down every 4 days as follows: 2 mg/kg once a day, then 1 mg/kg once day, then ½ mg/kg once a day, and finally ½ mg/kg every second day for 2 weeks. At the end of the treatment period the owner reported that the dog was doing very well.

Case 2

A 2-year-old male German shepherd dog was presented to the OVAH after anorexia, vomition and yellow mucous membranes had been present for 4 days. Abnormalities on clinical examination were splenomegaly and pale and icteric mucous membranes. The dog had a haematocrit of 17 % and the ISA test was positive. On repeated thin capillary blood smear evaluation, no parasites could be identified. Faecal analysis revealed *Ancylostoma caninum* infestation. Urine analysis revealed bilirubinuria as the only abnormality. Haematological examination demonstrated regenerative anaemia, mature neutrophilia with leukocytosis, monocytosis, eosinopenia, thrombocytosis, reticulocytosis and spherocytosis (Table 1). Survey thoracic radiographs were within normal limits, whereas survey abdominal radiographs and ultrasonography showed splenomegaly. The dog was treated with prednisolone at 2 mg/kg twice a day and fenbendazole at 50 mg/kg once a day for 5 days. Over the next 3 days the haematocrit progressively increased and the ISA test became negative. The prednisolone dosage was tapered as for case 1.

Case 3

A 2-year-old male cross-breed dog was presented to the OVAH after anorexia and weakness of 2 days' duration. On clinical examination the dog was febrile and had pale mucous membranes. The dog had a haematocrit of 14 % and the ISA test negative. On repeated thin capillary blood smear evaluation, no parasites were evident but severe spherocytosis was present. *Ancylostoma caninum* infestation was present on faecal analysis. The only abnormality revealed by urine analysis was bilirubinuria. Haematological examination demonstrated regenerative anaemia, left shift leukocytosis, monocytosis, reticulocytosis and spherocytosis (Table 1). Further investigations were not performed owing to financial constraints. The dog was given a whole blood transfusion and treated with prednisolone at 2 mg/kg twice a day and fenbendazole at 50 mg/kg once a day for 5 days. Over the next 4 days the haematocrit increased progressively and the ISA test remained negative. The prednisolone dosage was tapered as for case 1.

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Table 1: **Haematological parameters for 3 dogs with immune-mediated haemolytic anaemia secondary to *Ancylostoma caninum* infestation.**

Parameter	Reference range	Case 1	Case 2	Case 3
Haemoglobin	120–180 g/ℓ	43.6	91	68
Red cell count	5.5–8.5 × 10^{12}/ℓ	0.61	3.29	2.99
Haematocrit	37–55 %	15	17	14
Mean cell volume	60–70 fℓ	77.2	74.4	77.9
Mean cell haemoglobin concentration	32–36 g/dℓ	29.2	37.2	29.1
Red cell distribution width	15–24 %	25.6	20.8	19.1
In-saline agglutination	Negative	Positive	Positive	Negative
White cell count	6–15 × 10^{9}/ℓ	21.2	27.2	49.6
Neutrophils	3–11.5 × 10^{9}/ℓ	11.45	22.03	32.24
Band cells	0–0.05 × 10^{9}/ℓ	2.33	0.27	12.9
Monocytes	0.1–1.35	2.76	3.56	2.48
Lymphocytes	1–4.8 × 10^{9}/ℓ	3.82	1.36	1.98
Eosinophils	0.1–1.25 × 10^{9}/ℓ	0.85	0	0
Thrombocytes	200–500 × 10^{9}/ℓ	12	597	134
Reticulocytes	<1 %	15.83	6.95	17.8
Spherocytes	0 %	30	50	70

DISCUSSION

Immune-mediated haemolytic anaemia is associated with the excessive destruction of erythrocytes and is characterised by moderate to severe anaemia, reticulocytosis, spherocytosis and autoagglutination[5,10,13]. The 3 dogs described in this report had characteristic signs of IMHA (spherocytosis, positive ISA test and regenerative anaemia).

The standard diagnostic method for IMHA is the antiglobulin (Coombs') test, which demonstrates the presence of anti-erythrocyte antibodies or complement[9]. This was not performed in these 3 dogs, as all showed enough evidence of IMHA. The ISA test is considered diagnostic for IMHA[1,11], and a positive result is considered synonymous with a positive Coombs' test[13,15]. The presence of both macroscopic as well as microscopic agglutination in the ISA test strengthens the diagnosis, as does the presence of agglutination on repeated examinations. Two of the dogs in this report had, on a number of occasions, a positive ISA test. Spherocytosis is usually present in IMHA[9], and is considered a reliable diagnostic indicator[5]. A negative spherocyte count does not, however, rule out IMHA, as spherocytes may be absent in complement-mediated destruction[13], and also because spherocytes may be overlooked or miscounted on blood smear evaluation. In this report, 2 dogs had marked (>50 %) and 1 moderate (30 %) spherocytosis. One dog that had marked spherocytosis had a negative ISA test.

In early studies most cases of IMHA in dogs were considered to be primary, whereas later studies have identified underlying causes, such as neoplasia, other immune disorders and chronic infections, such as abscesses, discospondylitis, pyometra, and pyelonephritis[5,8]. Currently, the frequency of primary and secondary IMHA is 60 and 40 % respectively[1,3,12]. This change can be ascribed to improved diagnostic methods. In all the dogs in this report *A. caninum* was considered to be the trigger because no other underlying cause could be identified and complete response occurred with deworming and only a short course of corticosteroids (approximately 4 weeks). Dogs with primary IMHA require protracted immune-suppressive therapy[5,13]. Recent vaccinations and drug therapy have also been implicated in secondary IMHA[4,5]. None of the dogs in this report had been vaccinated recently nor were they on any drug therapy.

IMHA (both primary and secondary) results from a breakdown in immune self-tolerance, with B-cells responding to erythrocyte antigens. As erythrocyte antigens are hidden or cryptic, the appropriate B-cell may encounter the antigen only after membrane damage exposes the antigen, or an inflammatory or infectious process releases new antigens into circulation that cross-react with erythrocyte antigens[5]. *Ancylostoma* can induce both specific and non-specific immune responses[14], as well as result in erythrocyte membrane damage, as they are blood-sucking helminths. The gastrointestinal (GIT) immune response is important in the elimination of many GIT parasites in the dog, with non-specific immunity, self-cure, and humoral and cell-mediated responses being possible mechanisms[7]. Intestinal parasites induce a T-cell response, plasma cell stimulation with subsequent production of immunoglobulins (E, A, G and M), an eosinophilic response, and a non-specific local inflammatory response[7,14,16]. All of these mechanisms can potentially result in an immune response directed at the erythrocyte membrane and consequent secondary IMHA.

It can be suggested that the presence of IMHA and *A. caninum* in the dogs in this report was coincidental. However, *A. caninum* is a common problem at the OVAH, and a review of 203 cases of *A. caninum* failed to show the concurrent presence of IMHA. We therefore believe that the association between IMHA and *A. caninum* in the dogs in this report was not coincidental.

One of the dogs in this report showed severe thrombocytopenia without any obvious clinical signs of haemorrhage such as petechiation, ecchymosis and/or purpura[2]. The most likely cause for the thrombocytopenia in this dog was platelet sequestration, which is frequently associated with splenic- and/or hepatomegaly[2]. The possibility also exists that this dog had concurrent IMHA and immune-mediated thrombocytopenia (IMT), which has been described as Evans' syndrome[8]. In a recent study[12], thrombocytopenia was present in 41 % of dogs with IMHA with none of the dogs showing a haemorrhagic tendency. Thrombocytosis was present in 1 dog in this report, which can be associated with chronic verminosis[2]

The lungworm *Angiostrongylus vasorum* has recently been shown to induce IMT[6]. However, severe poorly-regenerative anaemia was also present. As IMHA was not ruled out by means of the Coombs' and/or ISA tests, it is possible that IMHA was also present in this case.

As intestinal helminths can be difficult to diagnose on faecal examination owing to irregular egg shedding, it would be pertinent to subject any animal that has IMHA with no apparent underlying cause to multiple faecal examinations and to deworm it as part of the therapy.

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