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A study on diagnosis of immune mediated hemolytic anaemia in dogs

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Abstract

The present communication describes Immune-mediated hemolytic anemia (IMHA) in five dogs. IMHA is predominantly a type II hypersensitivity reaction. Clinical signs and diagnosis of IMHA was based on the presence of spherocytosis on blood smear examination, saline agglutination, and confirmed by canine-specific coomb's reagent. The age of dogs suffering from immune-mediated hemolytic anemia ranged from 1-2 years with a mean age of 1.8 years and one female and 4 males were presented. Hemato-biochemical studies revealed anaemia, thrombocytopenia, hyperbilirubinemia, and elevation of alanine aminotransferase and aspartate aminotransferase enzymes along with severe anemia. Successful whole blood transfusion was done in four patients one dog collapsed before transfusion.

Keywords: IMHA, coombs test, spherocytes, prednisolone

Introduction

Immune-mediated hemolytic anemia is one of the most common immune-mediated diseases in dogs. IMHA is considered a predominantly type II hypersensitivity reaction in which anti-RBC antibodies, including IgG, IgM, and IgA, attach directly or indirectly to various components of the RBC membrane. The disease's clinical manifestations are the result of a spontaneous autoimmune reaction directed toward normal glycoprotein molecules on the erythrocyte's surface (Balch and Mackin 2007) [2]. Antibodies and complement molecules in the immune system directly or indirectly target RBCs of all ages for destruction via macrophage phagocytosis in the spleen or liver (extravascular hemolysis) or complement-mediated cytolysis within the circulation, resulting in IMHA (intravascular hemolysis). IMHA is divided into two main types, primary and secondary. IMHA is a classic example of an autoimmune disorder with no identifiable underlying cause and is the predominant form of IMHA but it may also be secondary to causes including infectious, inflammatory, or neoplastic diseases, drugs, and vaccines. IMHA is primarily a disease of middle-aged to older dogs. A confirmatory diagnosis of IMHA is difficult to make, however, the diagnosis (Bennett *et al* 1981 and Burgess *et al* 2000) [4, 5]. It may occur at any age but is rare in dogs younger than 1 year. The predisposed breeds include the cocker spaniel, springer spaniel, and Old English sheepdog (Burgess *et al* 2000; Klag *et al* 1993; Mason *et al* 2003) [5, 7, 8]. The first line of treatment regimen includes immunosuppressive doses of corticosteroids is supported by the presence of spherocytes, RBC agglutination, positive results from a Coombs' test, and the absence of a detectable underlying cause of hemolytic anemia. The overall mortality rate associated with IMHA is high, despite awareness of the disease and new drug treatment approaches and afterward combination with azathioprine or cyclosporine.

Materials and Methods

Five severely anemic dogs were included in the study. Physical examination was performed and a blood sample was collected from cephalic or saphenous using a 22G needle. The dogs were vaccinated and regularly dewormed. There was no medical history of the previous usage of other drugs. Anamnesis included severe weakness and anorexia from the past few days. The CBC was done on an automatic blood counting analyzer ADVIVA 2120 Hematology System, Siemens). The biochemical profile included total protein, albumin, alanine transaminase (ALT), total bilirubin (TB), and creatinine was done on VITROS DT60 II chemistry system (Ortho-Clinical Diagnostics, Johnson and Johnson Company, New Brunswick, NJ, USA). Blood smears were prepared from an EDTA sample and stained by Leishman stain and

subjected to microscopic examination to detect the different hemoprotozoan and rickettsial infections viz., *Babesia gibsoni*, *Babesia canis vogeli*, *Hepatozoon canis* and *Ehrlichia canis*, etc. Agglutination assay was performed via the standard technique with washed RBCs from the patient which are mixed with saline on a slide and read macro- and microscopically for agglutination. Canine coombs test was performed with canine-specific coomb's test (consisting of anti-IgG, IgM, and C3) in all canine cases Follow up of cases was taken at 3rd and 7th-day interval (Table 1).

Statistics Descriptive statistics were performed, calculating the mean, the range, the maximum and minimum of age, duration of clinical signs, PCV, duration of treatment (Microsoft® Excel, Munich, Germany).

Results and Discussion

The clinical examination showed icteric mucous membranes, tachycardia, and tachypnea in all the dogs. Body temperature was within the normal reference range for all the five dogs (38.6 °C). The hematological and biochemical profile at Day 1 (D1) is showed in Table 1. The laboratory data showed severe regenerative anemia (Fig1) and moderate thrombocytopenia. The leukocyte profile on the day of presentation indicated values at the slight upper reference range. Biochemical parameters determined hyperbilirubinemia with high ALT, normal BUN, and creatinine levels. The PCR was used for the diagnosis of hemoprotozoan diseases (Ehrlichiosis, Anaplasmosis, Babesiosis,) which were all negative. Blood smears showed marked anisocytosis, polychromasia, and spherocytosis (Fig 2). The self-agglutination assay revealed macro and microscopically RBCs agglutination (Fig 3 and 4). Coombs test was performed for the confirmatory diagnosis of IMHA in dogs which showed positive results in all the patients (Fig 5). The urine analysis established no hematuria, but bilirubinuria was recorded in three dogs. Based on the clinical presentation and laboratory findings primary IMHA was diagnosed. Immediate whole blood transfusion was performed in four out of five dogs immediately upon the diagnosis of IMHA. One dog collapsed within one hour of the presentation. On the 14th day, 3 out of 5 dogs (60.00%) survived up to 1 year of presentation. The dogs were treated as per the following protocol

- Day 1: Whole blood transfusion once Dexamethasone – 0.5 mg/kg i.v. Pantoprazole– 1 mg/kg i.v. q24 h, Enrofloxacin – 5 mg/kg i.v. q24 h
- Day 2 to Day 5: Prednisolone – 2 mg/kg p.o. q12 h, Enrofloxacin – 5 mg/kg s.c. q24 h. Pantoprazole– 1 mg/kg i.v. q24 h
- Day 6 to Day 15: Prednisolone – 1.5 mg/kg p.o. q12 h, Ranitidine – 0.5 mg/kg p.o. q12 h
- Day 15 to Day 30: Prednisolone – 1 mg/kg p.o. q12 h, Ranitidine – 0.5 mg/kg p.o. q12 h

Primary IMHA is a common disease in dogs. pIMHA is diagnosed in an average of 15 dogs per year (Weingart *et al* 2019) [13]. IMHA is a common cause of anemia in dogs causing phagocytosis of erythrocytes opsonized by IgG, IgM, and/or complement (Moraes *et al* 2017) [9]. The diagnosis of IMHA is based on the clinical presentation, laboratory findings (severe anemia), strong self-agglutination reaction, and presence of spherocytes on the blood smear. The type of anemia is usually macrocytic, normo to polychromic with an appropriate regenerative response. The absence of hematuria

indicates extravascular RBC destruction, which is mediated by the opsonization of immunoglobulin molecules on the RBC surface, which causes phagocytosis of the target cells via macrophage Fc receptors (Barcellini, 2015) [3]. Extreme leukocytosis in IMHA is a common but transient accompanying sign and is based on the functional reactivity of the bone marrow (Cohn 1991) [6]. After the diagnosis of IMHA is made many dogs with IMHA receive blood products that aim to improve the delivery of oxygen to peripheral tissues to achieve cardiovascular stability (Swann and Skelly 2016). Many dogs respond well to prednisolone alone for immunosuppression, and no evidence exists adding a second immunosuppressant is beneficial (Piek 2017) [10]. The therapeutic properties with immunosuppressive glucocorticoid doses aim at halting erythrocyte lysis rapidly (Al-Ghazlat 2009) [1]. The mortality rate of IMHA after 90 days of treatment, the mortality was 8.2% (5/61) (Weingart *et al* 2019) [13]. The goal of treatment was to achieve long-term remission so that immunosuppressive drug doses could be decreased and ultimately stopped.

Table 1: Hemato-biochemical profile of dogs confirmed with IMHA

Variable	Day 0	Day 3	Day 7
Hemoglobin (g/dL)	2.66 ±0.54	4.22 ±0.92	6.66± 1.63
PCV (%)	8.80 ±1.66	13.75 ±2.46	20.75 ±5.62
TEC (×10 ⁶ /μl)	1.41±0.32	1.61±0.42	3.29±0.91
TLC (×10 ³ /μl)	24.06±7.39	22.32±7.82	15.30±53.54
Platelet (x10 ³ /μl)	84.80±4.49	102.75±45.67	-
Total bilirubin (mg/dL)	9.50±5.47	7.25±1.43	-
ALT (IU/L)	321.00±23.25	-	-

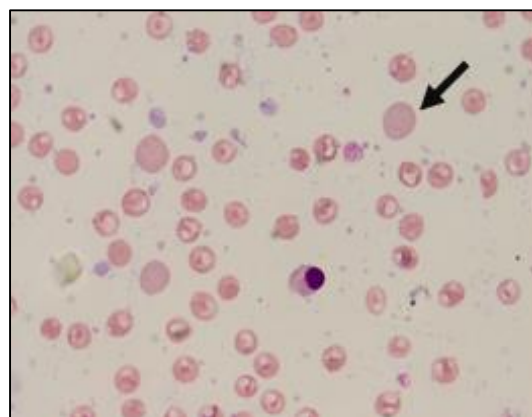


Fig 1: Blood smear (arrow) showing polychromatophilic RBCs 100 X

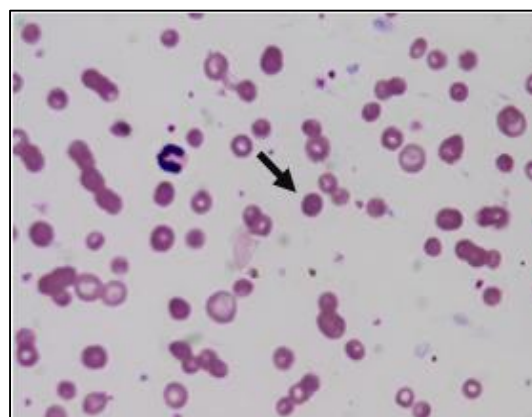


Fig 2: Spherocytosis (arrow) along with agglutination (line) 100X

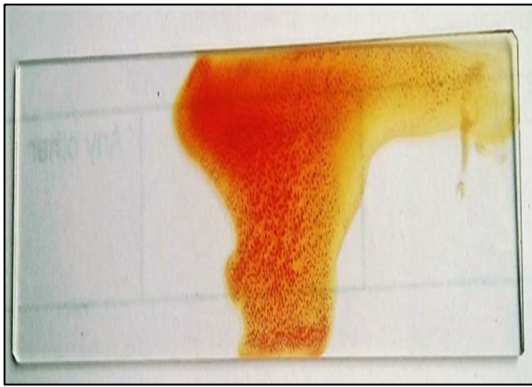


Fig 3: Positive slide agglutination test indicating IMHA

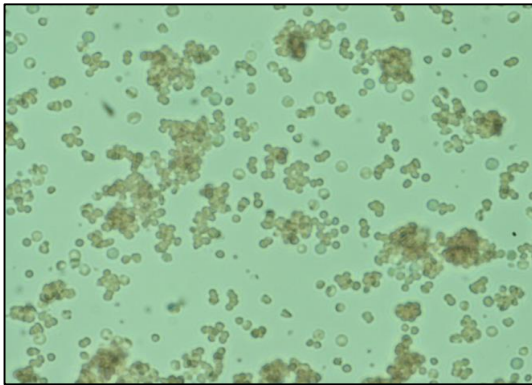


Fig 4: Positive saline agglutination test indicating IMHA 40 X

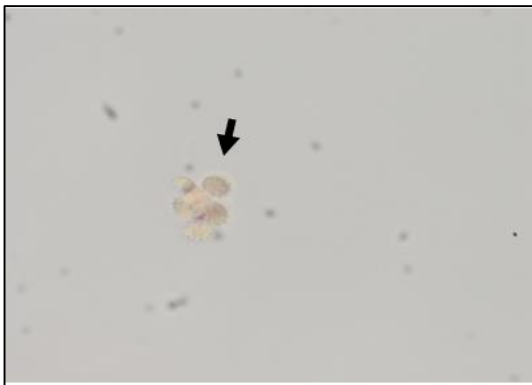


Fig 5: Arrow indicating positive Coombs test showing a clump of RBCs confirming IMHA 100X

autoimmune haemolytic anaemia in the dog. *The Veterinary Record* 1981;109(8):150-153.

5. Burgess K, Moore A, Rand W, Cotter SM. Treatment of immune-mediated hemolytic anemia in dogs with cyclophosphamide. *Journal of Veterinary Internal Medicine* 2000;14(4):456-462.
6. Cohn L. *Journal of Veterinary Internal Medicine*, 1991;5(2):95-104.
7. Klag AR, Giger U, Shofer FS. Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986-1990). *Journal-American Veterinary Medical Association* 1993;202:783-783.
8. Mason N, Duval D, Shofer FS, Giger U. Cyclophosphamide exerts no beneficial effect over prednisone alone in the initial treatment of acute immune-mediated hemolytic anemia in dogs: a randomized controlled clinical trial. *Journal of veterinary internal medicine* 2003;17(2):206-212.
9. Moraes LF, Takahira RK, de Assis Golim M. Avaliação hematológica e da função renal em cães com AHIM. *Acta Scientiae Veterinariae* 2017;45:1-12.
10. Piek CJ. Immune-mediated hemolytic anemias and other regenerative anemias. In: Ettinger SJ, Feldman EC, Cote E, editors. *Textbook of Veterinary Internal Medicine*. 8th edn. Elsevier Saunders, St. Louis 2017, 829-837.
11. Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988-1996). *Journal of the American Animal Hospital Association* 1999;35(5):384-391.
12. Swann JW, Skelly BJ. Evaluation of immunosuppressive regimens for immune-mediated haemolytic anaemia: a retrospective study of 42 dogs. *Journal of Small Animal Practice* 2011;52(7):353-358.
13. Weingart C, Thielemann D, Kohn B. Primary immune-mediated haemolytic anaemia: a retrospective long-term study in 61 dogs. *Australian Veterinary Journal* 2019;97(12):483-489.
14. Weinkle TK, Center SA, Randolph JF, Warner KL, Barr SC, Erb HN. Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 cases (1993–2002). *Journal of the American Veterinary Medical Association* 2005;226(11):1869-1880.

Conclusions

IMHA is a very serious and fatal disease the diagnosis of IMHA is done with blood smear examination and coombs tests. Blood transfusions are beneficial and can be very helpful to improve the clinical condition as well as the Hb and PCV of dogs suffering from IMHA.

References

1. Al-Ghazlat S. Immunosuppressive Therapy for Canine Immune-Mediated Hemolytic Anemia. *Compend Contin Educ Vet* 2009;31(1):33-41, 44; quiz 44.
2. Balch A, Mackin A. Canine immune-mediated hemolytic anemia: pathophysiology, clinical signs, and diagnosis. *Compend* 2007;29(4):217-225.
3. Barcellini W. New Insights in the Pathogenesis of Autoimmune Hemolytic Anemia. *Transfus Med Hemother*. Sep 2015;42(5):287-93. doi: 10.1159/000439002. Epub 2015 Sep 7.
4. Bennett D, Finnett SL, Nash AS, Kirkham D. Primary