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Immune Mediated Haemolytic Anaemia Associated with Haemoparasitic Infections in Dogs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

A haematological condition known as Immune-Mediated Haemolytic Anaemia (IMHA) affects dogs and is caused by antibodies directly destroying red blood cells in the bloodstream. It can be primary (idiopathic) or secondary (caused by neoplastic or infectious illnesses). An attack on circulating red blood cells by the immune system is the hallmark of IMHA, a potentially fatal autoimmune disease. Though the disease occurs naturally in both humans and dogs, it is significantly more prevalent in dogs. The aim of this review was to methodically assess the available data on the disease's clinico-pathology, its therapy and utilize the results to derive recommendations that may be implemented in broader veterinary medicine.

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1. INTRODUCTION

Primary (idiopathic or autoimmune) or secondary immune mediated haemolytic anaemia (IMHA) are the two possible types. Primary IMHA, the most prevalent form of IMHA in dogs, is a traditional autoimmune disease for which there is no known underlying cause. Animals in their young adult and middle age frequently suffer from this illness. Additionally, IMHA may develop as a side effect of numerous inflammatory, malignant, or infectious conditions. IMHA has also been observed to be triggered by a number of drugs. Animals of any age or breed can be affected by secondary IMHA, and individuals exhibiting symptoms unusual for initial IMHA, like elderly animals, should be highly suspected. In contrast to dogs, cats typically have secondary IMHA. Therapeutic differentiation between primary and secondary IMHA is crucial because secondary IMHA frequently reacts poorly to treatment or recurs unless the underlying cause is recognized and eliminated [1]. As a result, the basic causes, symptoms and clinico-pathological findings, diagnosis and treatment of IMHA associated with haemoparasitic infections in dogs is the focus of this review.

2. ETIO-PATHOLOGY OF IMMUNE MEDIATED HAEMOLYTIC ANAEMIA ASSOCIATED WITH HAEMOPARASITIC INFECTIONS IN DOGS

In the 1960s, a case series consisting of 19 dogs with IMHA was reported. However, the ailment was first documented in Veterinary Medicine in the 1970s. [2]. IMHA, a Type II hypersensitivity immune reaction where the body's own immune system recognises its RBCs as antigen/ foreign objects and acts against it leading to destruction of cells and consequently results in anaemia. The anti-RBC antibodies can be either immunoglobulin IgG or IgM. In case of high levels of antibodies, the complement system is also activated and leads to formation of membrane attack complexes. This refers to extravascular haemolysis, which occurs when red blood cells (RBC) are destroyed in the spleen, liver, and other immune system organs, and intravascular haemolysis, which occurs when RBCs are destroyed within the vasculature. Less commonly, antibodies are also directed against marrow RBC precursors, resulting in non-regenerative anaemia [3]. Antibodies that target normal components on the surface of erythrocytes are generated in conjunction with the development of non-associative IMHA. Autoantibodies have been found to target glycophorin, a glycoprotein that spans the plasma membrane, as one of the most prevalent membrane antigens on RBCs. The etiology of associative IMHA is multifactorial. T and B cells are stimulated to make antibodies by the alteration of antigens and molecular mimicry of the RBC membrane or linked with normal RBC membranes. Associative IMHA has been linked to protozoan, viral, bacterial, rickettsial, parasitic, and neoplastic diseases, among other known and hypothesized causes [4]. Similarly, Whitley and Day [5] clarified that the antibody on the surface of erythrocytes may perhaps not bind to own self-antigen but rather to self-antigen that has been altered or exposed by hapten (drug or disease) or to non-specifically attached foreign antigens; this is known as secondary IMHA. The intricate pathophysiology of IMHA is caused by both a systemic inflammatory response, which is marked by an increase in acute phase proteins, and high white blood cell count, the degree of which is correlated with the severity of PM examination disease in several organs. Lobetti and Schoeman [6] also stated that IMHA (both primary and secondary) is an outcome of interruption in immune self-tolerance where B cells respond to RBC antigens. As erythrocyte antigens are hidden or cryptic, the proper B cell may not come into contact with them until membrane damage has revealed them, or until an infectious or inflammatory activity has released fresh antigens into the bloodstream that interact with erythrocyte antigens. Ong et al. [7] as well described that in secondary IMHA, antibodies are specific to a neo-antigen or a foreign antigen (drug or infectious agent) linked to the surface of erythrocytes. Bystander haemolysis is the cause of RBC destruction because the causative antibody does not recognize normal RBCs. A multitude of illnesses, such as systemic lupus erythematosus (SLE), neoplasia, blood parasites, and the use of specific medications, can result in IMHA; the majority of individuals are thought to be idiopathic [8].

Zandvliet et al. [9] described those immune mediated diseases like IMHA, thrombocytopenia, glomerulonephritis, and polyarthritis are induced due to antigenic stimulation by parasites and

hypergammaglobulinemia because of ehrlichiosis. Among immune mediated diseases of dogs, IMHA is the most popular one with findings of anaemia, packed cell volume of 12-14%, reticulocytosis, and a left shift leukocytosis. Mortality may reach upto 50% in 2 weeks' time and is due to failure of kidney and liver, coagulation disorders and inflammatory response [2]. Bovens [10] found that the dogs with IMHA had a marked inflammatory reaction that included leukocytosis with left shift and monocytosis when compared to the other unhealthy dogs. There was decrease in coagulation factor activities but increased in the acute phase proteins VIII and fibrinogen. According to Nassiri et al. [11], the hematologic and biochemical test results for IMHA in dogs included mean haematocrit (21.4±1.4%), spherocytosis in seven dogs (54%), polychromasia in five dogs (38.5%), thrombocytopenia in seven dogs (54%), and hyperbilirubinemia in eight dogs (80%) out of the ten dogs that were assessed. Thirteen canines were examined for Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), with results showing increases in six (46.2%) and eight (61.5%), respectively.

Kim et al. [12] noted that as a result of increased platelet loss from immune-mediated destruction, haemorrhages, or disseminated intravascular coagulation, 50–70% of dogs with IMHA also have concomitant thrombocytopenia. Development of thromboembolism is an important complication in dogs with IMHA. Factors which seem to contribute are changes in coagulability, endothelial integrity, and blood flow [13]. McManus and Craig [14] noted moderate to high WBC count, neutrophilic with left shift, and toxic alteration in neutrophils in IMHA dogs have the possibility for moderate to alarming tissue damage, that might complicate treatment and degrade prognosis. Thongsahuan et al. [15] conveyed that *E. canis* infection may result to anaemia due to antibody production against RBCs, in combination with IMHA. Oriá et al. [16] discussed that ehrlichiosis is said to be as one of the most potentially serious disease in domestic and wild dogs, due to its severity. The pathogenesis of ehrlichiosis commences with 8 to 20 days of incubation period and is followed by acute, subclinical and chronic phase of the disease. After entering the bloodstream and lymphatic system during the acute phase, the parasite replicates in the mononuclear phagocyte cells of the liver, spleen, and lymph nodes. The rickettsias are spread by the cells to other organs that interact with endothelial cells to cause a vasculitis. The pathophysiology of ehrlichiosis is supported by histological observations of perivascular cuffing of vessels in the lungs, kidneys, spleen, meninges, and ocular tissues, as well as significant plasma cell infiltration of multiple parenchymal organs. Persistent hypergammaglobulinemia during the whole course of the illness, as well as positive autoagglutination and direct antiglobulin tests in infected animals, further point to an immunogenic mechanism.

Dubie et al. [17] likewise discussed that the tick acts as vector of *E. canis* and gets transferred between hosts at the time of blood meals. Once it is transmitted, mononuclear phagocytic cells are targeted and infected by *E. canis*. Most infected are the monocytes, within the human or canine. Dogs infected with *E. canis* typically exhibit host hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia as the main biochemical abnormalities. Marshet and Dessie [18] noted that ehrlichial organisms enter the canine host by the tick vector's bite, travel through the bloodstream, enter cells, and spread to other organs. Once inside tissues, they proceed to infiltrate, survive, and multiply in cells. Intravascular coagulation and vasculitis can result from circulating infected cells. Platelets are destroyed as a result of this and a change in cell-mediated immunity. Similarly, clinical leukopenia and anaemia may result from the destruction of leukocytes and erythrocytes along with a decrease in RBC synthesis.

Waner and Harrus [19] deliberated that *E. canis* are extensively scattered throughout the body organs of infected dogs as indicated by pathological and molecular studies. The ubiquity of the organism may result to its association in the pathology in a variety of organs. Pathology of several organs and related clinical signs has in fact been encountered in many natural and experimental cases. All body systems are affected by the severe lymphoplasmacytosis that is impacting the parenchymal organs and the widespread surface bleeding that is indicative of CME, which results in a wide range of clinical symptoms. According to the finding's, Neelawala et al. [20] urge veterinarians to pay close observation to dogs with secondary IMHA, concurrent haemoparasitism, reduced RBC counts on diagnosis and those with persistent anaemia to reduce the risk of relapse as 10% of the patients in canine babesiosis after

antibabesial treatment is observable for recurrence of babesiosis. Ayoob et al. [21] discussed that Babesiosis is caused by haemoprotozoa of the genus *Babesia.* Babesial organisms mostly spend their life cycle inside the definitive host's RBCs, which can lead to haemolysis along with systemic problems. Direct observation of the organism on blood smear gives a definitive diagnosis. Antibody test which gives a positive result on serologic exposure may indicate that the animal is with or without active infection.

Shah et al. [22] described that haemolytic anaemia is the main symptom of babesiosis, and thrombocytopenia is frequently seen in affected dogs. Both intravascular and extravascular haemolysis are blamed for anaemia. RBC breakdown mechanisms include erythrophagocytosis, reduced RBC life span, and enhanced osmotic fragility. Due to parasite antigens on the RBC surface secondary immune mediated destruction occurs, parasite-induced membrane impairment, and possibly other membrane-associated antigens. Oxidative impairment, sludging, damaged haemoglobin function, and sequestration of RBC also likely occur. In canine babesiosis, Solano-Gallego et al. [23] reported weight loss, glomerulonephritis, acute or chronic nephropathy, coagulation disorders (disseminated intravascular coagulation), icterus from liver disease, immune-mediated haemolysis or thrombocytopenia, haemoconcentration, shock, metabolic and/or respiratory alkalosis, and/or acidosis, gastrointestinal disorders (vomiting or diarrhoea), ocular lesions (uveitis or blindness), pancreatitis, myalgia, ascites, rhabdomyolysis, and respiratory issues (oedema or acute respiratory distress).

Tóthová et al. [24] also reported that in some cases of babesiosis, after initial parasitaemia, the immune system may not eliminate the infection, and chronic carrier state remains without clinical signs of the illness. Months to years later relapses may occur and many complications like glomerulonephritis and polyarthritis may develop. Nalubamba et al. [25] moreover observed that thrombocytopenia, regenerative anaemia, and leukopenia (neutropenia and lymphopenia) initially after infection followed by increase WBC count and neutrophilia with a left shift a few days after infection are the clinicopathological pictures in Babesia cases. Dhliwayo et al. [26] likewise reported dogs with Babesiosis showed more pronounced in thrombocytopenia and hypoalbuminemia only while anaemia was more marked in dogs with babesiosis along with positive to *Ehrlichia* spp. antibodies.

3. CLINICAL SYMPTOMS OF IMMUNE MEDIATED HAEMOLYTIC ANAEMIA ASSOCIATED WITH HAEMOPARASITIC INFECTIONS IN DOGS

Manev et al. [27] reported that a dog which showed weakness and anorexia for few days with tachycardia, pale mucous membranes, and a moderate hepatomegaly on palpation was diagnosed for primary IMHA. Similarly, Schoeman [28] noted that physical examination of dogs affected with IMHA shows tachypnoea, splenomegaly, hepatomegaly, pale mucous membranes, jaundice, pigmenturia (haemoglobinuria or bilirubinuria), fever, and lymphadenopathy. During physical examination, Jaundice is commonly and easily observed abnormality. When the serum bilirubin level surpasses 2 to 3 mg/dl, jaundice is usually first noted on the mucous membranes and when bilirubin concentrations are higher it later affects the skin. Paes et al. [29] noted that clinical symptoms of IMHA in dogs are equivalent to those observed in cats' vague signs which are primarily informed by the owner are weakness, anorexia, pigmenturia, vomiting and diarrhoea. Clinical signs like icterus, systolic heart murmur and cranial abdominal organomegaly are also frequently observed, each exist in 50% of the patients with IMHA. Increased heart rate and rapid breathing were present in roughly 30% of the dogs with IMHA.

Burgess et al. [30] also reported that haematocrit <25% was present in 59 (98%) dogs. 25 dogs (42%) had a regenerative response whereas 35 dogs (58%) had a non-regenerative anaemia respectively, at the time of presentation. Thrombocytopenia was seen in 41 (68%) dogs. 9 of 34 dogs (26%) had an extended Prothrombin Time (PT), 19 of 34 (56%) had an extended PT clotting time, and 12 out of 34 (35%) had abnormal fibrinogen concentrations. Likewise, Dantas-Torres [31] stated that dogs infested by different tick-borne pathogens typically shows clinical signs such as high fever, loss of appetite, vomiting, drowsiness, pale mucous membranes, and weight loss. Das and Konar [32] also reported that anorexia and weight loss occurred due to parasitic infestation. Epistaxis and increased corneal opacity were frequently observed in ehrlichiosis. Bleeding occurred continuously from nasal cavity because of

thrombocytopenia. In Ehrlichiosis, Babesiosis, and Hepatozoonosis, anaemia and neutrophilic leukocytosis were noted; these conditions may be caused by acute forms of the parasite diseases or by a combination of parasitic infestations. Haemoglobinuria or coffee colour urine is a characteristic clinical symptom of babesiosis when RBC loss occurs.

Sarma et al. [33] also reported that concomitant infection with *Ehrlichia canis* and *Babesia gibsoni* in a dog revealed clinical observation which was dull and depressed, pale mucous membrane, dehydration and staggering gait with hind limb weakness and presence of ticks. Popliteal lymph node and liver was found to be enlarged on palpation. Nakaghi et al. [34] reported that the clinical signs most frequently observed in *E. canis* were fever, apathy, anorexia, pale mucous membrane, haemorrhages, lymphadenopathy, splenomegaly, and uveitis. Gonde et al. [35] reported among the affected animals, an ample range of clinical signs were seen, including uncommon observations of paraplegia, blindness, ocular haemorrhage, IMHA, ascites, and skin lesions. Anisocytosis and nucleated erythrocytes were seen in blood films signifying regenerative anaemia. There was marked decrease in Hb, TEC, PCV and thrombocytes in blood parameters of the affected dogs. *B. gibsoni* affected animals showed significant decrease in lymphocytes. Marked increase in serum bilirubin, ALT, ALP, BUN and creatinine were seen in the affected dogs.

Köster et al. [36] also observed lethargy, fever, and variable degrees of haemolytic anaemia with associated signs in most babesia infections and were mainly reported in summer and/or spring. Most dogs become chronically infected in acute phase with no or only poorly characterized signs. Depending on the *Babesia* spp. involved it gives different outcome of infections. Solano-Gallego et al. [23] conveyed that the clinical manifestations of canine babesiosis have been reported to range widely, from subclinical disease to serious ailment. These presentations include fever, pallor, jaundice, splenomegaly, weakness, and collapse associated with intravascular and extravascular haemolysis, systemic inflammation, thrombocytopenia hypoxic injury, and pigmenturia. Furthermore Sainz et al. [37] described diarrhoea as atypical sign in babesiosis and thrombocytopenia as a constant finding in both ehrlichiosis and babesiosis. The clinical indications of an Ehrlichiosis infection might vary depending on the strain, the dog's immune system, and the existence of concurrent infections with other tick- or flea-borne illnesses. Nonspecific symptoms may include fever, weakness, anorexia, splenomegaly, hepatomegaly, lymphadenomegaly, or weight loss. Additional symptoms that have been reported include vomiting, diarrhoea, intolerance to exercise, pain, oedema (in the tail, scrotum or hind legs), dyspnoea and/or cough (related to pneumonia), skin ulcers, pale mucous membranes, anaemia, nose bleed, ecchymoses, petechiae, prolonged bleeding during oestrus, and haemorrhage or blood in stool linked to thrombocytopenia, vasculitis, or thrombocytopathy. Derakhshandeh et al. [38] also reported clinical cases of ehrlichiosis in dogs display symptoms such as fever, diarrhoea, anaemia, staggering gait, debilitated condition, and presence of ticks were recorded during the study period. Procajlo et al. [39] described that in the chronic form of ehrlichiosis, extreme malnourishment, hepatic and splenic tumors, IMHA, a bleeding disorder characterized by nosebleeds, blood in the stool, skin bruises, polyarthritis, and endocarditis. Freire et al. [40] also noted that during subclinical phase dogs infected with ehrlichiosis remains asymptomatic, although the microorganism persists intracellularly, progressing to chronic phase, featuring among the chief signs of mucosal pallor, spontaneous bleeding, petechiae, splenic and hepatomegaly. Additionally, Stephanie et al. [41] described a variety of clinical and hematologic abnormalities which include Coomb's positive anaemia, hypergammaglobulinemia, pancytopenia, polyarthritis because of immune complex deposition in joints, lymphadenopathy and plasma cell infiltration into tissues have been observed in canine ehrlichiosis.

Gahalot et al. [42] reported that dogs with pale or whitish mucous membrane, pancytopenia, thrombocytopenia, epistaxis, ecchymotic haemorrhages, neurological signs and dogs previously expose to ticks, are to be suspected for Ehrlichiosis. Barman et al. [43] observed that inappetence, fever, weakness, anaemia, scanty faeces, haemoglobinuria, shrunken eyeball with mild corneal opacity and reluctant to walk, increased capillary refill time to three seconds, pale mucous membrane of penile, second degree of dehydration, splenomegaly and partial hepatomegaly on palpation in an *E. canis* affected dog. Saravanan et al. [44] reported that autoimmune disorder like immune mediated thrombocytopenia (ITP) and IMHA in dogs are

cause by babesia infection and it may occur concurrently or individually. Distended abdomen, hepatosplenomegaly, petechial haemorrhage on ventral abdomen revealed on physical examination and ticks were noticed on ear and inter-digital space.

4. DIAGNOSIS OF IMMUNE MEDIATED HAEMOLYTIC ANAEMIA ASSOCIATED WITH HAEMOPARASITIC INFECTIONS IN DOGS

Furlanello and Reale [45] noted that within all immune disorders, differentiating between primary (autoimmune) and secondary (neoplasia-related effects, infections) reasons is crucial for clinicians as they might result in intravascular or extravascular erythrocyte destruction through different mechanisms. An accelerated immune destruction of RBCs must be demonstrated for IMHA diagnosis. Icterus is evidence that clinically suggest haemolytic anaemia and hyperbilirubinuria suggest regenerative anaemia. And to support a diagnosis of immune mediated haemolysis, one or more of the following three hallmarks must be present *i.e*., marked spherocytosis, persistent agglutination and a direct Coomb's test result [1].

In contrast Warman et al*.* [46] stated that dogs with concurrent or underlying diseases and those with primary IMHA displayed notably distinct patterns for Coomb's test reactivity. However, according to Woodward and White [47] IMHA diagnosis in dogs does not have a gold standard; instead, it entails determining if the anaemia is severe regenerative or pre-regenerative, showing indications of immune-mediated destruction, and ruling out other possible causes. Arthropod-transmitted pathogens, such as species of *Anaplasma, Babesia, Bartonella*, *Mycoplasma*, and *Ehrlichia* spp., are linked to IMHA in specific geographic areas. It is stated that screening tests identify no substantial abnormalities in 70–75 percent of instances, and the case is labelled as idiopathic. The prognosis is considered fair to guarded for both associative and non-associative IMHA, with death rates reaching as high as 51%.

Fleischman [48] described that in dogs, autoagglutination and the presence of spherocytes point to IMHA. A low haematocrit combined with one or more of the following conditions—autoagglutination, spherocytosis, osmotic fragility, and a positive Coomb's test result—supports the diagnosis of IMHA. Persistent agglutination helps diagnose IMHA by demonstrating the occurrence of anti-RBC immunoglobulin on the surface of RBCs, but it does not distinguish between primary and secondary IMHA. Scott-Moncrieff et al. [49] reported primary IMHA with presence of either spherocytosis or spontaneous persistent agglutination, and haematocrit of 25% or less (reference range, 37% to 55%). Out of 20 dogs with primary IMHA two dogs had increased prothrombin time. A complete blood count (CBC) with evaluation of RBC and white blood cell (WBC) morphology, platelet count, reticulocyte count, direct Coomb's test, serum biochemical profile, urinalysis, urine culture, heartworm antigen test, antibody titres for *E. canis* and *Rickettsia rickettsii*, radiographs of the thorax and abdomen, and abdominal ultrasonography were also performed so as to confirm the diagnosis of IMHA and rule out secondary causes. Additionally, measurements of unconjugated and total conjugated bilirubin were made.

Sumathi et al. [50] reported prothrombin time was estimated to detect coagulation disorders with human commercial kits. Human PT kits were used as there were no canine specific kits available and because of their high sensitivity for canine plasma. The range of PT value was 12.12±0.57 second. Manev et al. [27] noted that the identification of self-agglutination in vitro, laboratory results, and some common RBC abnormalities found on a blood smear—such as spherocytosis, polychromasia, and anisocytosis—are the basis for the diagnosis of IMHA. The Coomb's test, according to Wardrop [51], can identify complement and immunoglobulin on the surface of erythrocytes, which makes it useful for assisting with the diagnosis of immunization-mediated haemolysis.

In veterinary medicine, with species-specific reagents, the Direct Antiglobulin Test (DAT) has also been employed, primarily for the diagnosis of IMHA in dogs. Similar to human medicine, the DAT is still the most sensitive and specific method for diagnosing canine IMHA, and it appears to be resistant to artifacts from transfusions, immunosuppression, and storage [52].

Moraes et al. [53] reported that IMHA dogs had macro-autoagglutination, hyperglobulinemia and bilirubinuria in haematological and clinical observations. Dogs with IMHA had 9-10% of spherocytosis, 29-30% of dogs had leukocytosis, 39-40% neutrophilia, and 72%

thrombocytopenia. Most cases of IHMA (74-75%) were attributed to infectious diseases and associated with *Ehrlichia* sp. (secondary IMHA), 20-22% of dogs with IMHA had azotaemia, and 50-52% had raised urine protein creatinine ratio. Fernandez et al. [54] also reported pregnancy associated IMHA, where the dog was presented with inappetence, lethargy, and progressive regenerative anaemia with spherocytosis. An in-house saline agglutination test revealed considerable agglutination, and a urinalysis revealed haemoglobinuria.

For haemoparasites detection Laha et al. [55] stated that *B. canis* and *B. gibsoni* are the two species of *Babesia* that cause canine babesiosis. Based on their sizes, they can be distinguished morphologically. The large type of *Babesia*, *B. canis*, is 4-5 µm long. They have a pyriform form with rounded ends and points at one end. Up to 16 different organisms, or several infections, can be discovered in a single RBC. The tiny (1.5–2.5 µm) type of *Babesia* is called *B. gibsoni*. They absence of usual pyriform shapes but their trophozoites are annular or oval and signet ring forms may also occur. Salem and Farag [56] reported that fever and anaemia should be the presumptive diagnosis for canine babesiosis, while thrombocytopenia is considered the hallmark of the disease and microscopic examination remains the most rapid confirmatory method. Bhat et al. [57] reported a case in a dog with a history of lethargy, fever, tachycardia, tachypnoea, and haematuria. Blood smear examination was done, and it was found to be positive for *B. gibsoni*. The majority of veterinarians in India appear to find blood smears to be the most convenient and straightforward diagnostic test, despite being a valuable diagnostic tool. Lee et al. [58] reported outcomes of haematological analysis revealed severe haemolytic anaemia and thrombocytopenia in the *B. gibsoni* infected dogs. Nevertheless, the blood smears showed very low levels of parasitaemia in 29 infected dogs.

Sunitha et al. [59] also reported a case in German shepherd dog with a past characterized by overall malaise, anorexia, and weakness. Upon clinical examination, the animal's temperature was recorded at 104°F, and its oral and conjunctival mucous membranes were pale. Hb value of 3%, haematocrit of 20%, and total RBC count of 1.1 million/cubic millimetre were found by haematological analysis. The case was identified as having *Babesiosis* as a result of *B. gibsoni* infection based on clinic-haematological results and a blood smear examination. According to Bhadesiya and Raval [60], the Immunocomb® quick diagnostic kit was used to analyze blood samples from a few canines in the Gujarat's Anand region is known for having anti-*E. canis* antibodies. According to haematology, dogs with ehrlichiosis had far lower mean values for haemoglobin, total RBC counts, platelet counts, and PCV when compared to healthy dogs. When comparing dogs with ehrlichiosis to healthy dogs, the mean levels of neutrophils increased, eosinophils reduced, basophils declined, and lymphocytes decreased among other differential leucocyte counts. Comparing dogs with ehrlichiosis to healthy dogs, serum biochemistry also showed a significant increase in SGPT, SGOT, and creatinine levels, but a decrease in total protein levels.

Zoia et al. [61] reported in dogs with IMHA, the plasma mean platelet component (MPC) concentration at first examination may be helpful in predicting prognosis. Kaewmongkol et al. [62] reported that parameters of blood, blood smear analyses, particular PCR from blood samples, and commercial test kits (Snap 4Dx) were also used to define *E. canis* infections. Dhliwayo et al. [26] suggested that the most practical diagnostic method for clinical babesiosis in dogs is blood smear examinations and microscopy evaluation, which remain the simplest and most accessible diagnostic tests for the majority of laboratories.

Kaewmongkol et al. [63] detected *E. canis* in considerably greater number of severe anaemia cases (PCV<15%) than moderate or mild anaemia cases (PCV 16-29%) ($P<0.05$) and these severe anaemia cases were 7-fold more at risk of having *E. canis* infections. Gospodinova et al. [64] found that rapid antibody detection test for *E. canis* gave 64.9% positivity out of 48 samples and by immunofluorescence assay (IFA) only 59.5% samples gave positive result. Chandrashekar et al. [65] observed that the quick detection test's sensitivity and specificity for finding antibodies against *E. canis* were 96.2% and 100%, respectively, which were comparable to the findings of a comparable commercial ELISA. A crossbreed dog, age ten, exhibiting positive in-saline agglutination and antiplatelet antibodies. It was found to be *Anaplasma phagocytophilum* DNA using PCR. *A. phagocytophilum*-associated IMHA was the diagnosis made [66].

Based on seasonality, Park et al. [67] reported that tick-borne illnesses like ehrlichiosis or babesiosis were proposed as potential causes of

Coomb's positive IMHA. Engelbrecht et al. [68] reported seven anaemic dogs with macroscopic slide agglutination that broke up with saline washing, a positive Coomb's test result, and spherocytosis had a secondary IMHA due to leishmaniasis, ehrlichiosis, babesiosis, hepatic carcinoma, liver necrosis or phenobarbital treatment. The results of this case series document the common occurrence of IMHA and concur with other published studies.

5. MANAGEMENT OF IMMUNE MEDIATED HAEMOLYTIC ANAEMIA ASSOCIATED WITH HAEMOPARASITIC INFECTIONS IN DOGS

Humans and dogs currently depend on use of broad-spectrum immunosuppressive drugs therapy for autoimmune diseases. Currently, immunotherapies of particular interest include adoptive transfer of regulatory T cells (Tregs), low-dose recombinant interleukin 2 to promote Treg proliferation and activation, peptide antigen administration by subcutaneous or sublingual routes to establish tolerance, and monoclonal antibodies that produce selective depletion of the B cell compartment to lower autoantibody production [69]. McCullough [70] noted that treatment of IMHA may be satisfying but many patients do not show satisfactorily to glucocorticoids alone and need additional immunosuppressive therapy. Some patients may even result to acute severe anaemia and die within the initial few weeks of treatment. Relapses may occur even if they survive.

Wang et al. [71] found that when glucocorticoids and mycophenolate mofetil (MMF) are combined, the short-term results are comparable and there may be less negative side effects than with other immunosuppressive regimens used to treat this illness.

The majority, if not all, of the body's cells are impacted by glucocorticoids when they attach to the intracellular cytoplasmic GC receptor. The GC-receptor complex binds to DNA GC response regions that affect gene transcription when it is translocated to the nucleus. It is assumed that the cellular actions of GCs are dose dependant. To target macrophage function, GCs limit the expression of the Fc receptor, decrease sensitivity to antibody-sensitized cells, and decrease antigen processing at immunosuppressive dosages. With repeated use, GCs caused T cell death and decreased T cell activity. In certain people, the generation of B-cell antibodies may be suppressed [72].

MMF, an immunosuppressive medication, is a prodrug of mycophenolic acid (MPA). More than 20 years ago, the first MPA-based medication to be approved for sale was initially intended to prevent organ rejection in recipients of human transplants. It is hydrolyzed in the intestines to the parent chemical, mycophenolic acid, and then inhibits T and B lymphocytes' generation of inosine monophosphate dehydrogenase (IMPDH), which is how it achieves its immunosuppressive effects. By inhibiting IMPDH, less guanosine triphosphate is produced, which eventually results in less DNA being produced. Additional ways that MMF can treat inflammatory and immune-mediated disorders include inhibiting the development of dendritic cells and reducing the recruitment of monocytes to the site of inflammation [72,73].

Swann et al. [74] reported about a comparison of protocol for treating IMHA in dogs where they used prednisolone alone and a combination of prednisolone (1.4mg/kg/d) and MMF (10mg/kg/d) and found effective. Si et al. [75] also noted improvement in the haematological parameters following the addition of MMF to the treatment regimen. For the initial three days of treatment, just prednisolone at an immunosuppressive dose of 3.6 mg/kg has been used; for the next five days, cyclosporine is added. This has been associated with severe haemolysis. Park et al. [76] reported that dogs treated with MMF, and prednisolone were recovered and displayed good prognosis. For initial treatment, MMF is recommended in canine IMHA. Oggier et al. [77] reported that the addition of MMF to prednisolone for the treatment of dogs with acute IMHA was well tolerated and seemed to positively affect the course of the disease. West and Hart [78] reported that while only one dog was put to sleep due to progressive IMHA, five dogs with idiopathic IMHA survived for two weeks after diagnosis, and the authors came to the conclusion that MMF may be useful in treating dogs with IMHA, the GI toxicity linked to the prescribed dosage was found to be clinically limiting. While Strzok et al. [79] noted that in dogs with suspected primary immune-mediated thrombocytopenia, MMF with corticosteroids appeared to be just as effective as cyclosporine plus corticosteroids; additionally, side effects were less frequent and therapy costs were lower in the MMF group.

For haemoparasitic management, Mittal et al. [80] observed clinical relapses were frequently seen, making chemo-sterilization with regularly prescribed anti-protozoal medications unsuccessful in treating the infection caused by the tiny type of Babesia (*B. gibsoni*). Eddlestone et al*.* [81] testified that the treatment done with doxycycline in dogs for *E. canis* was found to be effective as *E. canis* DNA could not be spotted in the tissues and blood after treatment. Platelet counts were seen within reference intervals, and *E. canis* antibodies got reduced.

The long-acting tetracycline doxycycline, which is generated from oxytetracycline, is used to stop the creation of proteins in bacteria. It is lipophilic and able to penetrate the lipid bilayer of bacteria, just like minocycline. It prevents bacterial protein synthesis by reversibly binding to the 30-S and perhaps the 50-S ribosomal subunits. This prevents aminoacyl transfer ribonucleic acid (tRNA) from binding to messenger ribonucleic acid (mRNA). The liver concentrates it in bile, and the faeces and urine contain large amounts of it in a physiologically active form. The stated biological half-life ranges from 12 to 24 hours to 18 to 22 hours. Doxycycline, when used with other supportive drugs, can help dogs recover from CME successfully and quickly. Doxycycline has a bacteriostatic effect by preventing aminoacyl tRNA from attaching to bacterial ribosomes during protein synthesis. Moreover, it has a favourable impact on the concentration of erythroid cell corpuscular haemoglobin (MCHC) and platelet proliferation [82].

Wulansari et al*.* [83] noted clindamycin's effectiveness for the treatment of experimentally infected dogs with *B. gibsoni*. Clindamycin treatment gradually decreased parasitaemia levels and brought about morphological changes that indicated degeneration of parasites. Additionally, it lessened the listlessness, anorexia, and anaemia that are clinical signs of a Babesia infection. In another case, Almendros et al*.* [84] found that the combination of enrofloxacin, metronidazole, and doxycycline was generally successful in 85-86% of cases when diminazene diaceturate was administered, and in 83-84% of cases when it was not administered. For canine babesiosis, the mean recovery period was 24.2 and 23.5 days, respectively.

The use of clindamycin-metronidazole-doxycycline with appropriate dose rate for 10 days has some benefits. The innate immunity is boosted by the combination, and it is known as the Marshall Protocol. Clindamycin has been proposed to enhance clinical state by stimulating cellular and humoral immunity against Babesia infection. Antibiotics containing tetracyclines, such doxycycline and minocycline hydrochloride, have been found to be effective against Babesia parasites *(B. canis and B. divergens).* One such nitroimidazole molecule is metronidazole, which is used often. The combination therapy of clindamycin, metronidazole, and doxycycline is an effective alternative treatment strategy for chronic clinical babesiosis with fewer side effects, despite the fact that metronidazole was reportedly used as part of the combination therapy and no activity was seen in in-vitro studies of *B. gibsoni* [85]. During a 6-week course of prednisolone and cyclosporine administration, the experimentally infected dogs that had been treated with doxycycline with or without imidocarb for *E. canis* infections during the acute or subclinical phases failed to develop clinical or clinicopathological evidence for reactivated infection [86].

The drugs indicated for secondary IMHA (*B. gibsoni*) were given treatment of underlying causes with prednisolone. Combine therapy with doxycycline @ 5 mg/kg bodyweight PO BID for 28 days, clindamycin @ 25mg/kg bodyweight. PO BID, and metronidazole @ 15mg/kg bodyweight. PO BID. Prednisolone @ 2mg/kg bodyweight I/M or PO BID for 5 days followed by 1mg/kg bodyweight PO BID for next 5 days PO. The drug was tapered to 0.5mg/kg bodyweight PO SID for next 5 days [87]. Maheshwarappa et al*.* [88] also reported treatment of ehrlichiosis with doxycycline along with prednisolone and other supportive therapy. The dog exhibited obvious improvement in condition, after 4 weeks of therapy.

It is difficult to predict the course of IMHA in dogs, and few reliable prognostic markers have been found [89]. Manev and Marincheva [90] discussed that expected complications in IMHA are disseminated intravascular coagulation (DIC) and thromboembolism which are more often manifested as pulmonary embolism. Prognosis is guarded even with intensive therapy. The primary indicators of death in dogs with idiopathic IMHA at the time of diagnosis are elevated levels of plasma urea and bilirubin, thrombocytopenia, and petechiae. When treated with prednisolone and azathioprine, the assessed half-year survival for dogs that survived the first two weeks was

92.5%, and the predicted one-year survival was 69%. Mellett et al. [91] also described that in dogs, thromboembolic illness is a common side effect of primary IMHA. Furthermore, it is believed that pulmonary thromboembolism (PTE) and venous thrombosis account for 80% of the mortality in dogs with primary IMHA.

6. CONCLUSION

More high-quality research will be needed to examine both established and cutting-edge treatment regimens for IMHA associated with haemoparasitic infections in dogs as the quality of the evidence currently available to guide clinical decisions in this regard is typically quite low.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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