Review Article

IMMUNE MEDIATED HAEMOLYTIC ANAEMIA ASSOCIATED WITH HAEMOPARASITIC INFECTIONS IN DOGS

Abstract:A haematological condition known as immune-mediated haemolyticanaemia (IMHA) affects dogs and is caused by antibodies directly destroying red blood cells in the bloodstream.It can be primary or idiopathic and secondary (which is due to infectious or neoplastic disorders).An attack on circulating red blood cells by the immune system is the hallmark of immune-mediated haemolyticanaemia, 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'sclinico-pathological, its therapy and utilize the results to derive recommendations that may be implemented in broader veterinary medicine.

Keywords: immune mediated haemolytic anaemia, dog, haemoparasites

Introduction

IMHA can be either primary (idiopathic or autoimmune) or secondary. In dogs, primary IMHA is the most common type of IMHA and it is a classic autoimmune illness without an identified underlying cause. The condition typically affects young adult and middle-aged animals. IMHA can also occur secondary to a wide range of infectious, inflammatory or neoplastic processes. Various medications have also been reported to trigger IMHA. Secondary IMHA affects animals of any age or breed, and should be strongly suspected in patients with a signalment atypical for primary IMHA, such as geriatric animals. Unlike the dog, IMHA in the cat is most commonly secondary. Distinction between primary and secondary IMHA is therapeutically important because secondary IMHA will often respond poorly to treatment, or recur, unless the underlying cause is recognized and eliminated [1].As a result, the basic causes, symptoms and clinico-pathological findings, diagnosis and treatment of immune mediated haemolytic anaemia associated with haemoparasitic infections in dogs is the focus of this review.

Etio-pathology of Immune Mediated Haemolytic Anaemia Associated with Haemoparasitic Infections in Dogs

The first case series of 19 dogs with IMHA was described in the 1960s. But the condition was reported in Veterinary Medicine around 1970s [2]. IMHA, a Type II hypersensitivity immune reaction where the body’s own immune system recognises its RBCs as antigen/ foreign objects and act against it leading to destruction of cells and consequently results in anaemia. The anti-RBC antibodies can be

either immunoglobulin IgG or IgM. And in case of high levels of antibodies, the complement system is also activated and lead to formation of membrane attack complex. This marks to intravascular haemolysis when RBCs are destroyed within the vessels and extravascular haemolysis when RBCs are destroyed within the spleen, liver, and other organs of immune system. Less commonly, antibodies are also directed against marrow RBC precursors, resulting in non-regenerative anaemia [3]. Non-associative IMHA development is related with the production of antibodies specific to normal molecules on the surface of RBCs. A glycoprotein called as Glycophorin which extends across the plasma membrane, has been proposed as one of the most common red blood cells (RBC) membrane antigens targeted by autoantibodies. The pathogenesis of associative IMHA is complex. The modification of antigens and molecular mimicry of the RBC membrane or associated with normal RBC membranes activate T and B cells to produce antibodies. Bacterial, viral, rickettsial, parasitic, protozoan, and neoplastic diseases are included in the documented and hypothesized causes of associative IMHA [4]. Whitley and Day [5]likewise explained that the antibody on the erythrocyte surface may not be bound to native self-antigen but to self-antigen modified or exposed by hapten (drug or pathogen) or to non-specifically attached foreign antigens and this is called as secondary IMHA. Involvement of both a systemic inflammatory response characterised by rise in acute phase proteins and a leucocytosis result in complex pathophysiology of IMHA and this magnitude correlates with the severity of PM examination pathology in multiple 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 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.Ong *et al*. [7] as well described that in secondary IMHA, antibodies have specificity for a foreign antigen (an infectious agent or a drug) that is associated with the RBC surface, or for a neo-antigen. RBC destruction is due to bystander haemolysis as the causative antibody is not specific to the normal RBC. IMHA occur secondary to a variety of conditions, including systemic lupus erythematosus (SLE) neoplasia, and blood parasites, as well as administration of certain drugsMost patients are considered idiopathic [8].

Zandvliet*et al*. [9] described those immune mediated diseases like immune mediated haemolytic anaemia, thrombocytopenia, glomerulonephritis, and polyarthritis are induced due to antigenic stimulation by parasites and hypergammaglobulinemia because of ehrlichiosis. Among immune mediated diseases of dogs, Idiopathic immune mediated haemolytic anaemia (IMHA) is the most popular one with findings of anaemia, packed cell volume of 12-14%, reticulocytosis, a left shift

leucocytosis. 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 decreased in Coagulation factor activities but increased in the acute phase proteins FVIII and fibrinogen. Nassiri *et al*. [11] also reported findings of hematologic and biochemical test for IMHA in dogs, which include mean Haematocrit (21.4±1.4%), anisocytosis in 8 dogs (61%), spherocytosis in 7 dogs (54%), polychromasia in 5 dogs (38.5%), thrombocytopenia in 7 dogs (54%), hyperbilirubinemia in 8 dogs (80%) out of the 10 dogs which were evaluated. And 13 dogs that was evaluated for ALT and ALP showed increased in 6 (46.2%) and 8 (61.5%), respectively.

Kim *et al*. [12] noted that concurrent thrombocytopenia occurs in 50-70% of dogs with IMHA, possibly because of increased platelet loss caused by disseminated intravascular coagulation, haemorrhage, or immune mediated destruction.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 marked leukocytosis, neutrophilic Weinkle with left shift, and toxic alteration in neutrophils in IMHA dogs have the possibility for moderate to severe 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 immune mediated haemolytic anaemia (IMHA).Oriá*et al*.[16] discussed that ehrlichiosis is considered as one of the most potentially fatal 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. During the acute phase, the parasite enters the bloodstream and the lymphatic system and then stays in mononuclear phagocyte cells of the spleen, liver and lymph nodes, where it will replicate. The rickettsias are spread by the cells to other organs that interact with endothelial cells to induce a vasculitis. Histological findings of extensive plasma cell infiltration of various parenchymal organs and perivascular cuffing of vessels in the lungs, kidneys, spleen, meninges, and ocular tissues provides evidence that immunogenic mechanisms perform a role in the pathogenesis of ehrlichiosis. Positive autoagglutination and direct antiglobulin tests in infected animals, as well as persistent hypergammaglobulinemia in all stages of the disease, also indicates immunogenic mechanism.

Dubie *et al.*[17]likewise discussed that the tick acts as vector of *E. canis*and gets transferrred 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. Host

hypoalbuminemia, hyperglobulinemia and hypergammaglobulinemia are the predominant biochemical abnormalities found in dogs infected with *E. canis*.Marshet and Dessie [18] also noted that ehrlichial organisms travel through the circulation, invade cells and disseminate to various tissues after entering to the canine host through the bite of the tick vector. They continue to invade, persist, and replicate in cells once they are in tissues. Circulating infected cells can lead to vasculitis and subsequent intravascular coagulation. This in combination with an altered cell-mediated immunity; leads to the destruction of platelets. Likewise, destruction of leukocytes and erythrocytes in combination with decrease of RBC production may cause clinical leukopenia and anaemia respectively. Waner and Harrus[19] deliberated that *E. canis* are widely distributed throughout the body organs of infected dogs as indicated by pathological and molecular studies. The ubiquity of the organism may result to its involvement 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. The intense lymphoplasmacytosis which are affecting parenchymal organs and the general surface bleeding characterizing CME affect all body systems, subsequent in a great variety of clinical signs. According to the finding's, Neelawala*et al.*[20]urge veterinarians to pay attention to dogs with secondary IMHA, concurrent haemoparasitism, low 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 hemoprotozoa of the genus Babesia. The life cycle of the babesial organism is mostly spend within the RBC of the definitive host, resulting in haemolysis, with or without systemic complications. Direct visualization 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 the predominant feature of babesiosis is haemolytic anaemia, and thrombocytopenia is common in infected dogs. Anaemia is attributed to extra- and intravascular haemolysis. Mechanisms of RBC destruction include increased osmotic fragility, decreased RBC life span and erythrophagocytosis. Secondary immune mediated destruction occurs due to parasite antigens on the RBC surface, parasite-induced membrane damage, and possibly other membrane-associated antigens. Oxidative damage, impaired haemoglobin function, sludging, and sequestration of RBC also likely occur.Solano-Gallego *et al.*[23] reported weight loss, acute or chronic nephropathy, glomerulonephritis, coagulation disorders (disseminated intravascular coagulation), jaundice 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 problems (oedema or acute respiratory distress) in canine babesiosis.

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

Clinical symptoms of Immune Mediated Haemolytic AnaemiaAssociated with Haemoparasitic Infections in Dogs

Manev *et al.*[27]reported that a dog which showed weakness and anorexia for few days with pale mucous membranes, tachycardia and a moderate hepatomegaly on palpation was diagnosed for primary IMHA. Similarly Schoeman[28]noted that physical examination of dogs affected with IMHA reveals pale mucous membranes, tachypnoea, splenomegaly, hepatomegaly, icterus, pigmenturia (haemoglobinuria or bilirubinuria), fever, and lymphadenopathy. During physical examination, Jaundice is commonly and easily observed abnormality. When the serum bilirubin level exceeds 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 in dogs, clinical symptoms of IMHA are comparable to those seen in catsVague signs which are mainly reported by the owner are weakness, anorexia, pigmenturia, vomiting and diarrhoea. Clinical signs like icterus, cranial abdominal organomegaly and systolic heart murmur are also frequently observed, each exist in 50% of the patients with IMHA. Increased heart rate and rapid breathing were present in approximately 30% of the dogs with IMHA.

Burgess *et al*. [30]also reported that haematocrit <25% was present in 59 (98%) dogs. 35 dogs (58%) had a non-regenerative anaemia, whereas 25 patients (42%) had a regenerative response respectively, at the time of presentation. Thrombocytopenia was seen in 41 (68%) dogs. Nine of 34 dogs (26%) had an extended PT, 19 of 34 (56%) had an extended a PTT clotting time, and 12 out of 34 (35%) had abnormal fibrinogen concentrations. Likewise,Dantas-Torres [31] stated that dogs infected by different tick-borne pathogens typically shows clinical signs such as high fever, drowsiness, loss of appetite, pale mucous membranes, vomiting, 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. Anaemia and neutrophilic leukocytosis were observed in Ehrlichiosis, babesiosis and hepatozoonosis parasitic diseases which may be due to acute form and mixed parasitic infestation. Haemoglobinuria or coffee colour urine is a characteristic clinical symptom of babesiosis when red blood cell loss occurs.

Sarma *et al*. [33]also reported that Concomitant infection with *Babesia gibsoni and Ehrlichiacanis* 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 *Ehrlichiacanis*were apathy, anorexia, pale mucous membrane, fever, lymphadenopathy, splenomegaly, haemorrhages, and uveitis.Gonde *et al*. [35] reported alarge variation of clinical manifestations including rare findings of paraplegia, blindness, ocular bleeding, immune mediated haemolytic anaemia (IMHA), ascites and skin lesions were observed among the affected animals. Anisocytosis and nucleated erythrocytes were seen in blood films signifying regenerative anaemia. There was significant 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, AKP, BUN and creatinine were seen in the affected dogs.

Köster *et al*.[36]also observed fever, lethargy, and varying degrees of haemolytic anaemia with associated signs in most babesia infections and were mainly reported in spring and/or summer. During acute phase most dogs become chronically infected 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 clinically canine babesiosis has been found to result in a wide range of presentations from subclinical disease to serious illness characterised by fever, pallor, jaundice, splenomegaly, weakness, and collapse related with intravascular and extravascular haemolysis, hypoxic injury, systemic inflammation, thrombocytopenia and pigmenturia. FurthermoreSainz *et al*. [37] described diarrhoea as atypical sign in babesiosis and thrombocytopenia as a constant finding in both ehrlichiosis and babesiosis.Depending on the strain, the immune response of the dog, and the presence of concomitant infections with other tick- or flea-borne pathogens, the clinical signs of an Ehrlichiosis infection can be variable. Nonspecific signs, like fever, weakness, lethargy, anorexia, lymphadenomegaly, splenomegaly, hepatomegaly, or weight loss may be included. Other signs have also been described, including vomiting, diarrhoea, pain, exercise intolerance, oedema (in hind legs,

tail, or scrotum), cough and/or dyspnoea (associated with pneumonia), serous or mucopurulent oculo-nasal discharge, abortion or neonatal death, skin ulcers, pale mucous membranes, due to anaemia, epistaxis, petechiae, ecchymoses, prolonged bleeding during oestrus, haematuria or melena associated with thrombocytopenia, thrombocytopathy, or vasculitis. Derakhshandeh*et al*. [38] also reported clinical cases of ehrlichiosis in dogs showing symptoms such as fever, diarrhoea, staggering gait, anaemia, debilitated condition, and presence of ticks were recorded during the study period. Procajlo*et al*.[39] described that in the chronic type of ehrlichiosis, severe emaciation, tumors of the liver and spleen, autoimmunological disorders such as immune mediated hemolyticanemia (IMHA), a bleeding disorder with symptoms such as nosebleeds, blood in stool, bruising of the skin, polyarthritis and endocarditis are also observed. Freire *et al.*[40]also noted that Dogs infected with ehrlichiosis remain asymptomatic during subclinical phase, although the microorganism persists intracellularly, progressing to chronic phase, featuring among the main signs of mucosal pallor, petechiae, spontaneous bleeding, splenic and hepatomegaly. Additionally,Stephanie *et al*. [41] described a variety of clinical and hematologic abnormalities which include Coomb's positive anaemia, hypergammaglobulinemia, pancytopenia, polyarthritisbecause 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, neurogical 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 haemolytic anaemia (IMHA) and immune mediated thrombocytopenia (ITP) are cause by babesia infection in dogs and it may occur individually or concurrently. Distended abdomen, hepatosplenomegaly, petechial haemorrhage on ventral abdomen revealed on physical examination and ticks were noticed on ear and inter-digital space.

Diagnosis of Immune Mediated Haemolytic AnaemiaAssociated with Haemoparasitic Infections in Dogs

Furlanello and Reale [45]noted that within all immune disorders, it is mandatory for the clinician to discriminate between primary (*i.e.*, autoimmune) and secondary (*i.e.*, consequences of neoplasia, infections) causes that can elicit with various mechanisms, intravascular or extravascular destruction of erythrocytes. 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 primary immune mediated haemolytic anaemia and those with concurrent/underlying disease significantly showed different pattern for Coomb's test reactivity. However,according to Woodward and White[47] there is no gold standard for the diagnosis of IMHA in dogs rather diagnosis involves identifying a severe regenerative or pre-regenerative anaemia with evidence of immune mediated destruction and elimination of other causes of anaemia. In some geographical regions, IMHA is associated with arthropod transmitted pathogens, including species of Anaplasma, Babesia, Bartonella, Mycoplasma and *Ehrlichia* spp. It is reported that in 70%–75% of cases screening tests reveal no significant abnormalities and the case is classified as idiopathic. The prognosis for both associative and non-associative IMHA is considered fair to guarded, with mortality rates of up to 51%.

Fleischman [48]described that in dogs, the presence of spherocytes and autoagglutination suggests immune mediated haemolytic anaemia (IMHA). The diagnosis of IMHA is supported by a low haematocrit with one or more of the following: autoagglutination, positive Coomb's test result, spherocytosis, and osmotic fragility. Persistent agglutination reveals the presence on the surface of RBCs of anti-RBC immunoglobulin and facilitates IMHA diagnosis but it does not indicate whether the IMHA is primary or secondaryScott-Moncrieff *et al*. [49] reported primary IMHA with haematocrit of 25% or less (reference range, 37% to 55%), presence of either spherocytosis or spontaneous persistent agglutination. Out of 20 dogs with primary IMHA two dogs had increased prothrombin time. And to confirm the diagnosis of IMHA and rule out secondary causes, the following tests were performed: 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 *Ehrlichiacanis* and *Rickettsia rickettsii*, radiographs of the thorax and abdomen, and abdominal ultrasonography. Measurement of total conjugated bilirubin, and unconjugated bilirubin were also done.

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. PT range value was 12.12±0.57 second. Manev *et al*. [27]noted that diagnosis of IMHA is based on laboratory findings, detection of self-agglutination in vitro and some typical RBC alterations detected on a blood smear: spherocytosis, polychromasia and

anisocytosis. Wardrop [51] described that the Coomb's test can detect both immunoglobulin and complement on the surface of RBCs, and as such can be of value as an aid in the diagnosis of IMHA.

In veterinary medicine, the Direct antiglobulin test (DAT) has also been used with speciesspecific reagents mainly in the diagnosis of IMHA in dogs. The DAT remains the most sensitive and specific tool to specifically diagnose canine IMHA like human medicine and seems resilient to storage, immunosuppression, and transfusion artefacts [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] alsoreported pregnancyassociated immunemediated haemolytic anaemia, where the dog was presented with lethargy, inappetence, and progressive regenerative anaemia with spherocytosis. Marked agglutination was identified in an in-house saline agglutination test and a urinalysis identified haemoglobinuria.

For haemoparasites detection Laha *et al*. [55]stated that canine babesiosis is caused by two species of Babesia *viz: B. canis* and *B. gibsoni*. They are morphologically differentiated base on their size. *B. canis*is a large form (4–5 μm long) of Babesia. They are pyriform in shape, pointed at one end, and round on the other. In a single RBC, multiple infection *i.e*., more than one organism up to 16 may be found. *B. gibsoni* is small form (1.5–2.5 μm) of Babesia. They lack 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 fever, lethargy, tachycardia, tachypnoea, and haematuria. Blood smear examination was done, and it was found to be positive for *Babesia gibsoni*. Blood smear is a useful diagnostic tool, and it seems to be the easiest and most accessible diagnostic test for most veterinarians in India. Lee *et al*.[58] reported results of haematologic analysis revealed severe haemolytic anaemia and thrombocytopenia in the *Babesia gibsoni* infected dogs. However, the blood smears of 29 infected dogs showed very low levels of parasitaemia.

Sunitha *et al*. [59]also reported a case in German shepherd dog with a history of weakness, anorexia, and general malaise. On clinical examination the animal was found to have temperature of 104°F and pallor of conjunctival and oral mucus membrane. Haematological analysis revealed Hb value of 3 g%, Haematocrit 20% and total RBC count 1.1 million/cubic millimetre. Based

on clinic-haematological findings and blood smear examination, the case was diagnosed as Babesiosis due to *Babesia gibsoni* infection. Bhadesiya and Raval [60] reported that blood samples of some dogs of Anand region of Gujarat state were screened for detection of anti-*Ehrlichiacanis* antibodies using Immunocomb® rapid diagnostic kit. Haematology evinced that the mean values of haemoglobin, total RBC counts, platelet count and PCV in dogs with ehrlichiosis decreased significantly in comparison to healthy dogs. Among differential leucocyte count, mean values of lymphocytes decreased, neutrophils increased, eosinophils decreased, and basophils decreased significantly in dogs with ehrlichiosis in comparison to healthy dogs. Serum biochemistry also revealed significant increase in SGPT, SGOT and creatinine levels but decrease in total protein levels in dogs with ehrlichiosis as compared to healthy dogs.

Zoia *et al*. [61]reported plasma mean platelet component (MPC) concentration at initial examination may be useful for predicting prognosis in dogs with IMHA.Kaewmongkol*et al*.[62] reported that *E. canis* infections were also characterized by blood parameters, blood smear examinations, specific PCR from blood samples, and commercial test kits (Snap 4Dx). Dhliwayo *et al.*[26] reported use of ImmunoComb® Canine *Ehrlichia* Antibody Test Kit (Biogal-Galed Laboratories, Israel) to detect *Ehrlichia* spp. antibodies (IgG) from the collected dog sera and suggested that the useful diagnostic tool for clinical babesiosis in dogs is blood smear examinations and microscopy evaluation continue to be the easiest and most accessible diagnostic test for most laboratories.

Kaewmongkol*et al.*[64]detected *Ehrlichiacanis* in significantly 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 IFA only 59.5% samples gave positive result. Chandrashekar *et al*. [65] observed that sensitivity and specificity of the rapid detection test for detection of antibodies against *E. canis* were 96.2% and 100%, respectively and was like results for a similar commercial ELISA.A 10-year-old crossbreed dog with positive in-saline agglutination and the presence of antiplatelet antibodies. With PCR, it was detected to be DNA of *Anaplasmaphagocytophilum.* It was diagnosed as IMHA associated with *Anaplasmaphagocytophilum*.

Park *et al*. [66] discussed that tick-borne diseases such as ehrlichiosis or babesiosis were suggested to be possible causes of Coomb's positive IMHA based on seasonality. Engelbrecht *et al*.[67] reported seven anaemic dogs with a positive Coomb's test result, macroscopic slide agglutination that broke up with saline washing, and spherocytosis had a secondary IMHA due to

ehrlichiosis, babesiosis, leishmaniasis, 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.

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. Immuno-therapies of particular interest currently include monoclonal antibodies that produce selective depletion of the B cell compartment to lower autoantibody production, administration of peptide antigens by subcutaneous or sublingual routes to establish tolerance, adoptive transfer of regulatory T cells (Tregs), and administration of low dose recombinant interleukin 2 to encourage proliferation and activation of Tregs[68]. McCullough [69] noted that treatment of IMHA may be satisfying but many patients do not respond satisfactorily to glucocorticoids alone and need additional immunosuppressive therapy. Some patients may even result to acute severe anaemia and die within the first few weeks of treatment. Relapses may occur even if they survive.

Wang *et al*. [70] found that the combination of glucocorticoids and mycophenolate mofetil (MMF) provides similar short-term outcomes and potentially fewer adverse side effects compared with other immunosuppressive protocols used to treat this disease.

Glucocorticoids bind to the intracellular cytoplasmic GC receptor and affect most, if not all, cells of the body. When the GC-receptor complex is translocated to the nucleus, it gets bind to DNA GC response elements influencing gene transcription. The cellular effects of GCs are taken to be dose dependent. GCs target macrophage function by down regulating Fc receptor expression, lowering responsiveness to antibody-sensitized cell, and decreasing antigen processing at immunosuppressive doses. T cell function was suppressed and apoptosis of T cells was induced by GCs and with chronic use B-cell antibody production may be inhibited in some patients [71].

Immunosuppressive drug like Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA). The first MPA based product to obtain marketing approval over two decades ago, was originally use for the prophylaxis of organ rejection in human transplant patients. It produces its immunosuppressive effects by inhibiting inosine monophosphate dehydrogenase (IMPDH) production by T and B lymphocytes after being hydrolysed in the intestines to the parent compound, mycophenalic acid. By blocking IMPDH, guanosine triphosphate production is lowered ultimately resulting to reduction in DNA production. Other mechanisms that contribute to MMF's effectiveness in treating inflammatory and immune mediated diseases are through its suppression of dendritic cell maturation and lowering in monocyte recruitment into the site of inflammation [71, 72].

Swann et al. [73]reported about a comparison of protocol for treating IMHA in dogs where they used prednisolone alone and a combination of prednisolone (1.4 mg/kg/d) and mycophenolate mofetil (10 mg/kg/d) and found effective. Si *et al*. [74] also noted haematological improvement after the introduction of mycophenolate mofetil in the therapeutic protocol. Severe haemolysis has been observed with the use for the first three days of treatment with only prednisolone in an immunosuppressive dose of 3.6 mg/kg and along with cyclosporine for the following 5 days. Park *et al*. [75]reported that dogs treated with mycophenolate mofetil, and prednisolone were recovered and showed good prognosis. For initial treatment, mycophenolate mofetil is recommended in canine IMHA. Oggier*et al.*[76] 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[77] reported that five dogs diagnosed with idiopathic IMHA survived two weeks from the time of presentation, and only one dog was euthanized because of progressive IMHA. The authors concluded that MMF may have potential in treating dogs with IMHA. However, the GI toxicity associated with the dosing regimen used was found to be clinically limiting. While Strzok *et al*. [78] noted that combination of mycophenolate mofetil and corticosteroids appears to be as effective as cyclosporine and corticosteroids in the treatment of presumed primary immune mediated thrombocytopenia in dogs and side effects were less common and cost of therapy was lower in the mycophenolate mofetil group.

For haemoparasitic management,Mittal *et al*.[79] observed that the infection by small form of Babesia (*B. gibsoni*) is posing an important therapeutic challenge and chemo-sterilization by commonly prescribed anti-protozoal drugs was not achieved as clinical relapses were often observed. Eddlestone *et al.*[80] testified that the treatment done with doxycycline in dogs for *E. canis* was found to be effective as *E. canis* DNA could not be detected in the blood and tissues after treatment. Platelet counts were seen within reference intervals, and *E. canis* antibodies got reduced.

Doxycycline is a long-acting tetracycline derived from oxytetracycline and it is used to inhibit bacterial protein synthesis. It is lipophilic like minocycline and can pass through the lipid bilayer of bacteria. It reversibly binds to the 30-S ribosomal subunits and possibly the 50-S ribosomal subunits, blocking the binding of aminoacyl transfer ribonucleic acid (tRNA) to the messenger ribonucleic acid (mRNA) and inhibiting bacterial protein synthesis. It is concentrated in the bile by the liver and excreted at high concentrations in a biologically active form in the urine and faeces. The biological half-life reported varies from 12-24 h to 18-22 h. Successful quick recovery of dog from CME can be done with use of Doxycycline along with other supportive medications. Doxycycline inhibits attachment of aminoacyl tRNA to the bacterial ribosomes during protein synthesis, thus

exhibits a bacteriostatic action. Furthermore, it also has a positive effect on the proliferation of platelets and erythroid cell corpuscular haemoglobin concentration *i.e.*,MCH and MCHC [81].

Wulansari *et al.*[82] noted clindamycin's effectiveness for the treatment of experimentally infected dogs with *Babesia gibsoni*. Clindamycin treatment gradually decreased parasitaemia levels and brought about morphological changes that indicated degeneration of parasites. It also reduced the clinical symptoms characteristic of *Babesia* infection, including anaemia, anorexia, and listlessness. In another instance Almendros *et al.*[83] reported the overall effectiveness of enrofloxacin- metronidazole-doxycycline combination in association with administration diminazenediaceturate was 85-86% and without administration of diminazenediaceturate was 83-84%, respectively. And mean recovery time was 24.2 and 23.5 days, respectively for canine babesiosis.

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. It been suggested that clindamycin stimulate humoral and cellular immunity against *Babesia* infection and results in improvement in clinical condition. Tetracycline antibiotics such as doxycycline and minocycline hydrochloride have been known to exhibit activity against Babesia parasites (*B. divergens* and *B. canis*). The commonly used metronidazole is a nitroimidazole compound. Although metronidazole was reported to have been used as part of the combination therapy, no activity was seen in in-vitro studies of *B. gibsoni*, nevertheless, an effective alternative treatment strategy for chronic clinical babesiosis with fewer side effects is the combined therapy of clindamycin, metronidazole, and doxycycline. In their study of 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 during a 6-week course of prednisolone and cyclosporine administration [84].

The drugs indicated for secondary IMHA (*Babesia gibsoni*) were given treatment of underlying causes with prednisolone. Combination therapy with clindamycin @ 25 mg / kg b.wt. PO BID, metronidazole @ 15 mg/kg b.wt. PO BID and doxycycline @ 5 mg/kg b.wt. PO BID for 28 days. Prednisolone @ 2 mg/kg b.wt. I/M or PO BID for 5 days followed by 1 mg/kg b.wt. PO BID for next 5 days PO. The drug was tapered to 0.5 mg/kg b.wt. PO SID for next 5 days [85]. Maheshwarappa*et al.*[86] also reported treatment of ehrlichiosis with doxycycline along with prednisolone and other supportive therapy. After 4 weeks of therapy, the dog showed marked improvement in condition.

The outcome prediction in dogs with immune mediated haemolytic anaemia (IMHA) is challenging and few prognostic indicators have been consistently identified [87]. Manev and Marincheva[88]discussed that expected complications in IMHA are DIC and thromboembolism which

are more often manifested as pulmonary embolism. Prognosis is guarded even with intensive therapy. The presence of increased plasma urea and bilirubin concentration, thrombocytopenia, and petechiae are the main predictors for mortality in dogs with idiopathic IMHA are at the time of diagnosis. The estimated half-year survival for dogs that survived the first 2 weeks was 92.5% and the 1-year survival was 69%, when they were treated with prednisolone and azathioprine. Mellett *et al*. [89] also described that thromboembolic disease is a popular complication of Primary IMHA in dogs. And 80% of the deaths in dogs with primary IMHA are thought to be due to venous thrombosis and pulmonary thromboembolism (PTE).

CONCLUSION

More high-quality research will be needed to examine both established and cutting-edge treatment regimens for immune mediated haemolytic anaemia 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.

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