Immunosuppressive Efficacy of Prednisolone in combination with Azathioprine for Immune Mediated Haemolytic Anaemia in Dogs.

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***Abstract*— Immune-mediated haemolytic anaemia (IMHA) is a serious and often life-threatening condition in dogs. The primary focus of treating IMHA lies in immunomodulation, aiming to reduce erythrophagocytosis and inhibit the production of immunoglobulins. The current study aimed to investigate clinical manifestations and haemato-biochemical changes in dogs affected by IMHA as well as assessing response to treatment for this condition using Prednisolone as an immunosuppressive regimen. Six dogs positive for saline agglutination test, marked spherocytosis and Coombs' test were selected for the study.The frequent clinical signs observed in IMHA affected dogs included lethargy, inappetence, anorexia, exercise intolerance, and tachycardia. Haematological investigation revealed anaemia, thrombocytopenia and leucocytosis with shift to left. Serum biochemical findings revealed hyperbilirubinemia, elevated ALT, AST, BUN and ALP levels. Abdominal ultrasonography and radiography revealed hepatomegaly and splenomegaly. Thoracic radiography revealed cardiac enlargement and pulmonary congestion. IMHA affected dogs were treated with Prednisolone in combination with azathioprine along with standard treatment to primary cause and haemato-biochemical changes were recorded. The IMHA affected dogs displayed a mortality rate of 33.33% and recovery rate of 66.67% when treated with prednisolone in combination with Azathioprine as an immunosuppressive regimen.**

***Index Terms*— IMHA, Dogs, Prednisolone, Azathioprine, Immunosuppressive**

I. INTRODUCTION

Immune-Mediated Haemolytic Anaemia (IMHA) is one among the prevalent immune-related disorders observed in dogs (Piek, 2011). This condition arises when the immune system erroneously targets and destroys the dog's own red blood cells which are essential for oxygen transport in the body. IMHA is characterized as a type II hypersensitivity reaction involving the production of autoantibodies that adhere to the surface of red blood cells. The antibodies, primarily of the IgM class, trigger the activation of the complement system through the classical pathway. This activation results in the formation of a membrane-attacking complex, leading to subsequent intravascular haemolysis (Barcellini, 2015). Opsonization of red

blood cells with IgG molecules initiates extravascular haemolysis (Mackin, 2014).

In cases of primary IMHA, the immune response leads to the production of antibodies, predominantly IgG and IgM, directed against the dog's own normal red blood cells (RBCs). Conversely, in secondary IMHA, antibodies are generated against RBCs with altered surface antigens. These alterations occur due to interactions with secondary factors like drugs, infectious diseases, neoplasia and inflammatory processes (McAlees, 2010).

In an endeavour to augment immunosuppression, allow more rapid initial disease control and tapering of the glucocorticoid, a number of second-line drugs have made their way into routine treatment of dogs with IMHA, including azathioprine and mycophenolate mofetil. Despite numerous past efforts, significant uncertainty remains regarding the most effective immunosuppressive regimen to employ when treating an animal diagnosed with IMHA. Considering these facts, the study entitled "Therapeutic efficacy of prednisolone in combination with Azathioprine as an immunosuppressive regimen for immune-mediated haemolytic anaemia in dogs" was undertaken.

II. MATERIALS AND METHODS

The anaemic dogs presented to the Veterinary Clinical Complex, Krantisinh Nana Patil College of Veterinary Science, Shirwal with clinical signs such as pale or icteric mucous membrane, weakness, lethargy, anorexia, exercise intolerance, tachypnoea, tachycardia were screened by saline agglutination, spherocyte count and coombs' test. Six dogs positive for saline agglutination test, marked spherocytosis (+2 or more) and coombs' test were selected for the study. The selected dogs were subjected to detailed clinical examination and haemato-biochemical parameters were recorded. To investigate the primary cause for IMHA in the selected dogs, peripheral blood smear examination for the presence of haem protozoans, fecal examination, thoracic & abdominal radiography for presence of any neoplasm were carried out.

2.1 Haemato-biochemical study:

The blood collection procedure was performed in an aseptic manner. A total of 4 ml of blood was aseptically collected. 2 ml out of the 4 ml of blood was transferred to sterile EDTA vials for haematological examination and 2 ml to sterile vials containing clot activators for serum examination. The haematological parameters (Haemoglobin, Total Erythrocyte Count, Total Leukocyte Count, Differential Leukocyte count, Packed Cell Volume, Platelets Count) were estimated with the help of Auto blood analyzer machine- Abacus ABJ Vet-5 and the serum biochemical parameters (Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, Total bilirubin, Blood Urea Nitrogen, Creatinine, Total Protein, Albumin) were quantified with the help of Alta ADX- CHEM-220 Semi Auto Biochemistry Analyzeron day 0 (pre-treatment) in both IMHA affected and healthy control dogs and on day 14and 28 (post-treatment) in IMHA affected dogs.

2.2 Treatment protocol:

The selected dogs were treated with Injection Prednisolone @ 2 mg/kg B.wt IM BID for 5 days followed by Tab. Prednisolone @ 1 mg/kg B.wt PO BID for next 5 days, again followed by tapering dose @ 0.5 mg/kg B.wt PO once daily for next five days, this was administered in combination with Tab Azathioprine @ 1 mg/kg B.wt PO BID for first 5 days followed by 0.5 mg/kg B.wt PO BID for next five days, again followed by tapering dose @ 0.5 mg/kg B.wt PO once daily for next five days. To assess the response to treatment, post-treatment evaluations were performed on 14^{th} and 28^{th} day using a full clinical examination, complete blood count and haemato-biochemical assessment. The results of the study were analysed statistically by using online statistical software called SPSS (Software Packages for Social Sciences).

III. RESULTS AND DISCUSSION

3.1 Clinical Signs associated with IMHA in dogs:

Among the six IMHA affected dogs, all (100%) dogs exhibited lethargy. Additionally, 66.66% dogs exhibited anorexia, 50.00% dogs exhibited tachycardia, 33.33% each exhibited exercise intolerance, inappetence, tachepnoea, pigmenturia, haematochezia and ascites. Furthermore, 16.67% each exhibited diarrhoea, vomiting, epistaxis, constipation and dyspnoea. The clinical signs reported in IMHA affected dogs are presented in Table 1.

Table 1 Clinical signs associated with IMHA in dogs

Sr. No.	Clinical signs	IMHA affected dogs (n=06)	
		Number	Per cent
1.	Lethargy	6	100
2.	Anorexia	4	66.66
3.	Tachycardia	3	50.00
4.	Exercise intolerance	2	33.33
5.	Inappetance	2	33.33
6.	Tachypnoea	2	33.33
7.	Pigmenturia	2	33.33
8.	Haematochezia	2	33.33
9.	Ascites	2	33.33
10.	Diarrhoea	1	16.67
11.	Vomiting	1	16.67
12.	Epistaxis	1	16.67
13.	Constipation	1	16.67
14.	Dyspnoea	1	16.67

In the present study, more specific signs of haemolysis were documented. Additionally, symptoms such as exercise intolerance, tachypnoea, tachycardia, and dyspnoea were observed due to severe or acute anaemia. The Present findings, along with nonspecific signs, were consistent with those reported by Burgess et al. (2000), Balch and Mackin (2007), Pieket al. (2011) Jutkowitz (2013), Mills (1997) & Jackson and Kruth (1985) who reported similar nonspecific signs in cases of IMHA.

3.2 Physical examination:

Physical examination findings in IMHA affected dogs are presented in Table 2.

Table 2 Physical examination findings associated with IMHA in dogs

Sr. No.	Physical examination findings		IMHA affected dogs (n=06)	
			Number	Per cent
1.	Mucous Membranes	Pale	3	50.00
		Icteric	2	33.33
		Congested	1	16.67
2.	Enlarged lymph nodes		6	100.00
3.	Splenomegaly		5	83.33
4.	Hepatomegaly		3	50.00

On physical examination, all 18 dogs affected by IMHA (100%) displayed enlarged lymph nodes (shown in Plate 4.5) upon presentation. Mucous membranes were observed to be pale/papery white (shown in Plate 4.1) in 10 dogs (55.55%), icteric (shown in Plate 4.2) in 6 dogs (33.33%), and congested in 2 dogs (11.11%). Abdominal palpation revealed splenomegaly in 15 dogs (83.33%) and hepatomegaly in 9 dogs (50%), which were subsequently confirmed by radiography and ultrasonography. Other physical findings included petechial haemorrhages in mucous membranes (Plate 4.3), variations in the quality and strength of pulse, tachycardia, and tachypnoea primarily result from compensatory responses of the cardiovascular and respiratory systems to anaemia, aimed at preventing tissue hypoxia. These findings, documented in our study, are consistent with observations made by Balch and Mackin (2007) and Archer and Mackin (2013). Severe anaemia results in reduced peripheral circulation, manifesting as paleness, while hyperbilirubinemia secondary to intravascular haemolysis leads to jaundice (Day, 1999). Hepatomegaly and splenomegaly, observed in some cases, have also been reported by Klag et al. (1993) and Burgess et al. (2000). The spleen and liver are common sites for extramedullary haematopoiesis, and they play a crucial role in clearing antibody-labelled red blood cells (Jutkowitz, 2013). Enlargement of peripheral lymph nodes may be attributed to the immune response seen in cases of IMHA or infectious diseases such as babesiosis, ehrlichiosis, and mycoplasmosis (Greene, 2012), conditions diagnosed in all the cases in our study.

3.3 Clinical Parameters:

The clinical parameters, including temperature, heart rate, and respiratory rate were recorded in IMHA-affected dogs, alongside six clinically healthy dogs serving as the control group. Standard methods were employed for data collection in both groups and the results are illustrated in Table 3 & figure 14.

Table 3 Clinical parameters in IMHA-positive dogs and healthy control dogs

Parameters	IMHA-positive dogs (n=06)	Healthy Control (n=06)	t-value
Temperature (^{O}F)	104.51 ± 0.13	101.63 ± 0.659	7.817**
Heart Rate (Beats per minute)	122.83 ± 18.34	100.50 ± 11.095	2.552*
Respiration Rate (Breaths per minute)	31.667 ± 6.186	21.67 ± 4.446	3.376**

Significant at 0.05 level; ** Significant at 0.01 level

The mean rectal temperature recorded in the affected dogs (104.35 ± 0.13°F) was found to be significantly higher than that measured in the healthy control group (101.63 ± 0.26°F). Similarly, the heart rate of IMHA-affected dogs was significantly higher (120.83 ± 3.60 beats per minute) as compared to the healthy control group (100.5 ± 4.52 beats per minute). Likewise, the mean respiratory rate of IMHA-affected dogs (29.00 ± 1.60 breaths per minute) was also significantly higher than that of the healthy control group (21.67 ± 1.81 breaths per minute). All of the dogs in present study, involved secondary IMHA, triggered by infectious agents capable of stimulating the production of endogenous pyrogens. This, in turn, leads to the release of prostaglandin E2 (PGE2), which raises the set point in the hypothalamus (Greene, 2012).

3.4 Haematological Parameters:

The IMHA-affected dogs and healthy control dogs were subjected to evaluation for various hematological parameters including Haemoglobin, TEC, PCV, TLC, Neutrophils & Platelets, as per Table 4.

Table 4 Haematological values in IMHA-positive dogs and healthy control dogs

Parameters	IMHA-positive cases (n=6)	Healthy Control (n=06)	t-value
Haemoglobin (g/dl)	6.08 ± 2.17	14.02 ± 1.10	7.976**
TEC (10^6/µl)	2.94 ± 0.93	6.77 ± 0.53	8.737**
PCV (%)	19.77 ± 4.324	38.70 ± 3.47	8.357**
TLC (10^3/µl)	18.25 ± 0.56	11.82 ± 2.24	6.802**
Neutrophils (%)	83.66 ± 5.01	65.67 ± 3.14	7.460**
Platelets (Lacs/µl)	0.99 ± 0.22	3.22 ± 0.64	7.959**

significant at 0.05 level; ** significant at 0.01 level

In the IMHA-affected dogs, the mean haemoglobin values were significantly lower (5.58 ± 0.41 g/dl) as compared to the healthy control group (14.017 ± 0.45 g/dl) with a p-value of <0.01. Similarly, the mean PCV value in the IMHA-affected dogs (17.49 ± 0.98%) was significantly lower (p<0.01) than that of the healthy controls (38.70 ± 1.41%). Additionally, the mean total erythrocyte count was significantly lower (p<0.01) in the IMHA group (2.74 ± 0.18 × 10^6/µl) compared to the healthy control dogs (6.77 ± 0.22 × 10^6/µl). On the other hand, the total leukocyte count in IMHA-affected dogs was significantly higher (p<0.01) at 18.85 ± 0.22 x 10^3/µl compared to healthy controls (11.82 ± 0.91 x 10^3/µl). Neutrophil levels were significantly elevated (p<0.01) in IMHA-affected dogs (81.78 ± 1.29%) compared to healthy controls (65.67 ± 1.28%). Additionally, band neutrophils showed a significant increase (p<0.05) in IMHA-affected dogs (5.72 ± 0.77%) compared to healthy controls (2.00 ± 0.36%). Platelet count was significantly decreased (p<0.01) in IMHA-affected dogs (0.95 ± 0.06 lacs/µl) compared to healthy controls (3.22 ± 0.22 lacs/µl). However, lymphocytes, monocytes, eosinophils, and basophils did not show significant differences compared to the healthy control dogs.

In IMHA, the presence of anti-erythrocyte membrane antibodies leads to complement fixation and the formation of a membrane attack complex. This complex results in intravascular haemolysis through osmosis or detection of these cells by macrophages in the spleen or liver, ultimately causing complete or partial erythrophagocytosis, as proposed by Day (2010). Burgess et al. (2000), McAlees (2010), and Piek (2011) all observed similar changes indicating anaemia in the hemogram of dogs with IMHA. Previous research by Burgess et al. (2000) and Carr et al. (2002) also noted thrombocytopenia with counts below 200,000/µl. In the present study, all cases exhibited secondary IMHA attributed to vector-borne haemoparasites, potentially contributing to thrombocytopenia. This aligns with the suggestion of Jutkowitz (2013) opining tick-borne diseases or consumptive coagulopathy as possible causes of thrombocytopenia. Leucocytosis was noted in studies by Burgess et al. (2000) and McAlees (2010) & McManus and Craig (2001).

3.4 Biochemical Parameters:

The IMHA-affected dogs and healthy control dogs were subjected to evaluation for various biochemical parameters including Total protein, albumin, globulin, Albumin: Globulin ratio, total bilirubin, ALT, AST, ALP, creatinine and BUN using standard kits as per Table 5.

Table 5 Biochemical values in IMHA-positive dogs and healthy control

Parameters	IMHA-positive cases (n=18)	Healthy Control (n=06)	t-value
Total protein (g/dl)	7.26 ± 0.81	6.29 ± 0.49	2.510*
Total bilirubin (mg/dl)	1.69 ± 0.79	0.31 ± 0.08	4.252**
SGPT/ALT (IU/L)	119.00 ± 52.13	61.00 ± 16.35	2.602*
ALP (IU/L)	163.83 ± 72.12	67.17 ± 5.23	3.274**
Creatinine (mg/dl)	1.33 ± 0.57	0.93 ± 0.30	1.528
BUN (mg/dl)	50.46 ± 24.70	19.83 ± 4..708	4.301**

* Significant at 0.05 level; ** significant at 0.01 level

The elevated levels of total protein in some cases may be indicative of a heightened immune response typical in IMHA. Significantly higher mean bilirubin value was seen in the IMHA affected dogs on the day of presentation when compared to healthy control dogs. This condition may be attributed to factors such as acute intravascular haemolysis, severe anaemia leading to hypoxic liver damage, and impaired bilirubin conjugation and excretion, as suggested by Klag et al. (1993). The mean values of ALT, and ALP activities were significantly elevated as compared to those of the healthy control group. This aligns with findings reported by Burgess et al. (2000), suggesting liver dysfunction due to hypoxia induced by severe anaemia. A comparable pattern of elevated BUN levels was observed by Swann and Skelly (2013), which could be attributed to pre-renal, renal, or gastrointestinal factors stemming from hypoxia or thromboembolism.

3.5 Diagnosis:

i. Blood Smear Examination: Amongst 6 dogs affected by IMHA, all were found to be positive for Babesia gibsoni organisms upon blood smear examination conducted on the day of presentation. The higher prevalence of Babesia gibsoni infection in our hospital may be one of the contributing factors to the increased incidence of secondary IMHA observed in our study. Standard treatment protocols were administered, including Clindamycin (25 mg/kg BW, BID) + Doxycycline (10 mg/kg BW, OD) + Metronidazole (15 mg/kg BW, BID) orally for 21 days for babesiosis.

ii. Faecal Examinations: Among the 6 dogs included in the study, faecal examinations were conducted for all. Results showed that one dog tested positive for Ancylostoma caninum and none of the other dogs tested positive for oocysts of any internal parasites or the parasites themselves. Standard treatment protocols including Praziquantel (5 mg/kg BW) + Pyrantel pamoate (14.4 mg/kg BW) + Fenbendazole (50 mg/kg) as a combination drug orally once to treat Ancylostomum caninum infestation, was offered aiming to eliminate the underlying primary cause.

iii. Radiography Examination: Out of 6 IMHA-affected dogs, common thoracic radiographic findings included pulmonary congestion (2 dogs) and cardiac enlargement (2 dogs). Regarding abdominal radiographic findings, few IMHA-affected dogs displayed hepatomegaly (3 dogs), splenomegaly (5 dogs) and gas-filled intestinal loops.

3.6 Treatment response:

Treatment response was assessed based on the remission of clinical signs, haematological & biochemical parameters on day 14th and 28th post-treatment.

Table 6: Effect of prednisolone in combination with azathioprine on haematological values in IMHA-positive dogs

Parameters	Prednisolone + Azathioprine			
	Day 0	Day 14	Day 28	F-Value
Haemoglobin (g/dl)	6.08 ± 0.89	8.19 ± 0.78	9.62 ± 0.53	4.855*
TEC (10^6/µl)	2.94 ± 0.38	4.27 ± 0.45	5.43 ± 0.15	10.635**
PCV (%)	19.77 ± 1.77	23.46 ± 2.25	29.67 ± 1.14	6.707*
TLC (10^3/µl)	18.26 ± 0.23	14.24 ± 1.57	10.34 ± 0.73	15.964**

Neutrophils (%)	83.67 ± 2.04	70.40 ± 2.54	68.75 ± 0.85	16.218**
Platelets (Lacs/µl)	0.99 ± 0.09	1.68 ± 0.24	2.08 ± 0.07	12.821**

NS: non-significant at 0.05 level; * significant at 0.05 level; ** significant at 0.01 level

Among the dogs that survived throughout the research study, noteworthy findings emerged. Specifically, there was a highly significant increase ($p<0.01$) in the mean values of total erythrocyte count (TEC) and platelets, alongside a very significant ($p<0.01$) decrease in the mean values of total leukocyte count (TLC) and neutrophils. Additionally, a significant ($p<0.05$) decrease in the mean value of band neutrophils was observed. Furthermore, there were significant ($p<0.05$) increases in the mean values of haemoglobin (Hb) and packed cell volume (PCV). Notwithstanding, no significant differences were noted in the mean values of monocytes and eosinophils. Remarkably, there was a highly significant ($p<0.01$) increase in the mean level of lymphocytes, while the mean level of band neutrophils showed no significant difference as depicted in table 6 & figure12.

In the present study, a significant increase in mean values of Hb, TEC, PCV and platelets was observed post treatment on day 14th & 28th was in accordance with Piek et al. (2011) and Lachungpa et al. (2020) both documented a comparable significant rise in PCV, Hb, TEC, and platelets after 25 days post-treatment. This enhancement could potentially stem from the selective suppression of T-cell function, involving both cell-mediated immunity and T-lymphocyte-dependent antibody synthesis, as induced by azathioprine similarly recorded by Cajanding, 2018, Balch and Mackin, 2007. A significant reduction in mean values of total leucocyte count and neutrophils was observed. A similar outcome was reported by Lachungpa et al. (2020), potentially stemming from the inhibition of DNA and RNA synthesis, resulting in decreased proliferation of immune cells and manifesting its immunosuppressive effect. Furthermore, recent findings suggested that azathioprine metabolites contribute to the depletion of the T-cell population by inducing apoptosis in activated T cells, thereby leading to a more pronounced immunosuppression.

Table 7 Effect of prednisolone in combination with azathioprine on serum biochemical values in IMHA-positive dogs

Parameters	Prednisolone + Azathioprine			
	Day 0	Day 14	Day 28	F-Value
Total protein (g/dl)	7.27 ± 0.33	7.01 ± 0.22	6.88 ± 0.08	0.562 ^{NS}
Total bilirubin (mg/dl)	1.69 ± 0.32	0.94 ± 0.18	0.68 ± 0.14	4.233*
SGPT/ALT (IU/L)	119.00 ± 21.27	112.60 ± 11.40	111.75 ± 11.74	0.056 ^{NS}
ALP (IU/L)	163.83 ± 29.45	229.00 ± 18.42	323.75 ± 43.65	6.616*
Creatinine (mg/dl)	1.34 ± 0.23	1.23 ± 0.19	1.18 ± 0.18	0.148 ^{NS}
BUN (mg/dl)	50.46 ± 10.09	40.20 ± 4.64	28.75 ± 5.07	1.804 ^{NS}

NS: non-significant at 0.05 level; * significant at 0.05 level; ** significant at 0.01 level

The combination treatment of prednisolone and azathioprine resulted in a significant ($p<0.05$) decrease in the mean activity levels of total bilirubin indicating decreased immune mediated haemolysis. Conversely, there was a notable ($p<0.05$) rise in the mean activity levels of ALP as depicted in table 7 & figure 13. However, there were no statistically significant variances observed in the mean values of total protein, ALT, creatinine, and BUN which was in accordance with Lachungpa et al. (2020) who also reported similar observations, noting comparable trends in their findings.

3.6 Recovery and Mortality Rates:

Among the six dogs treated with prednisolone in combination with azathioprine immunosuppressive regimen, four (66.67%) dogs survived, while one dog died within two weeks after starting the treatment and one dog died after two weeks of treatment, resulting in a recovery rate of 66.67 percent and a mortality rate of 33.33 percent as shown in table 8 & figure 15. It is noteworthy that mortality predominantly occurs within the first two weeks following diagnosis (Piek et al., 2011), a trend mirrored in the present study. Mortality in dogs with IMHA may stem from various

factors, including thromboembolism (Scott-Moncrieff et al., 2001; Carr et al., 2002), tissue hypoxia leading to subsequent necrosis due to severe anaemia, liver and kidney failure, inflammation, and disseminated intravascular coagulation (McManus and Craig, 2001; Piek et al., 2011). As necropsy examinations could not be conducted in the present study, the precise cause of death remained undetermined.

Table 6. Recovery and Mortality Rates

Treatment group	No. of dogs	Recoveries	Deaths	Recovery rate (%)	Mortality rate (%)
Prednisolone + AZA	6	4	2	66.67	33.33

IV. CONCLUSION

Most prevalent clinical manifestations noted in dogs affected with IMHA included lethargy, anorexia, decreased food intake, exercise intolerance, tachycardia, pigmenturia, haematochezia and tachypnoea. Dogs suffering from IMHA exhibited significant haematological alterations like anaemia with significantly low level of haemoglobin, total erythrocyte count, packed cell volume and thrombocytopenia, while characterised by leukocytosis & neutrophilia. Biochemical alterations in IMHA affected dogs revealed bilirubinemia, while high level of ALP. Dogs treated with the combination of prednisolone and azathioprine exhibited a recovery rate of 66.67% & a mortality rate of 33.33%.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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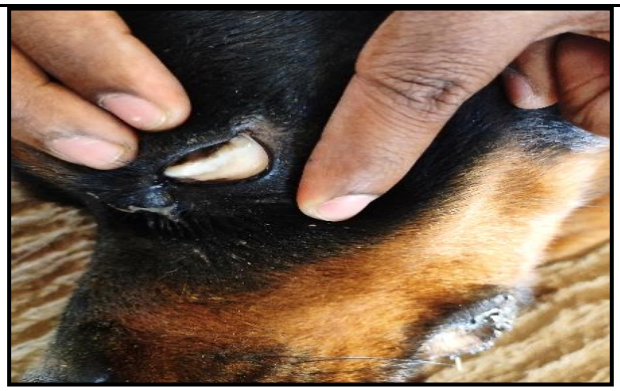


Fig. 1: Pallor conjunctiva

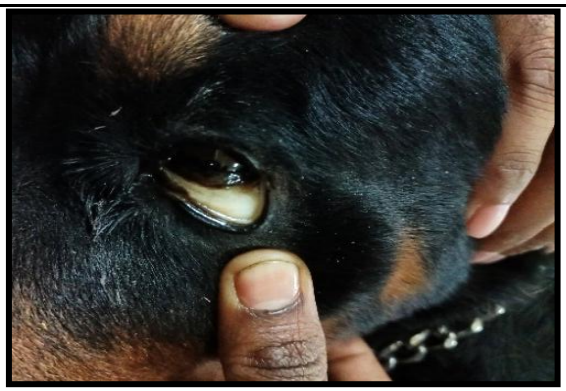


Fig. 2: Icteric conjunctiva

Fig. 3: Petechial haemorrhages in conjunctiva

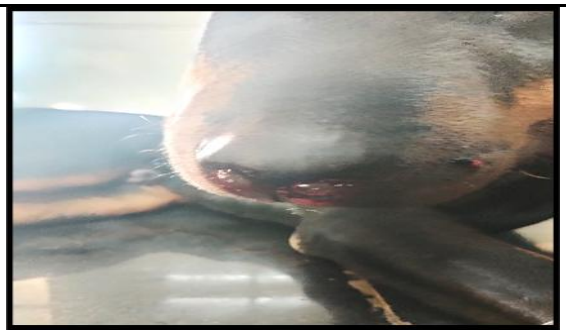


Fig. 4: Epistaxis

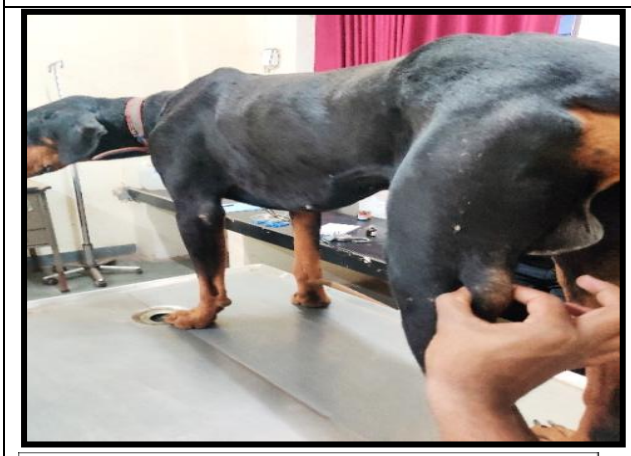


Fig. 5: Enlarged lymph node (popliteal)

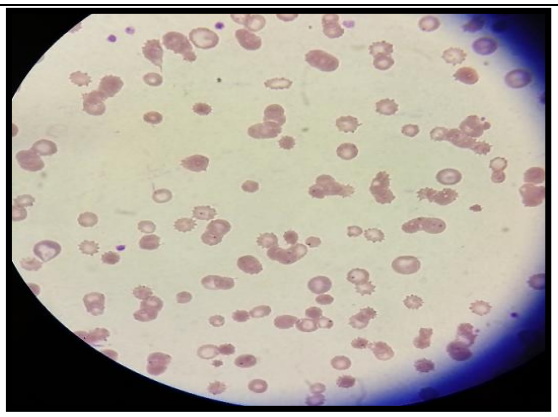
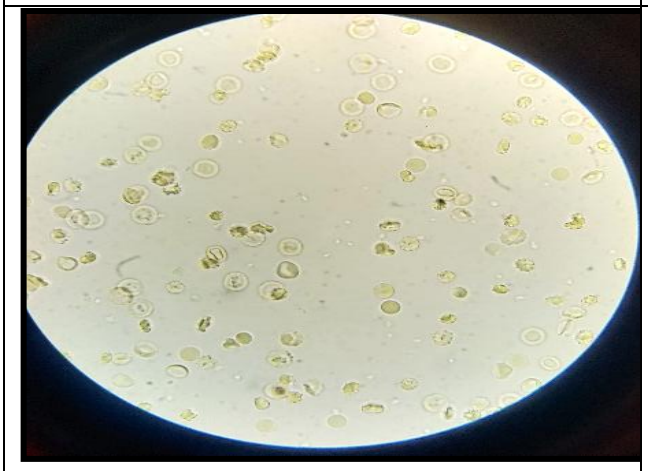


Fig. 6: Intra- erythrocytic piroplasms of *Ba*besia *gibson*i in Blood smear



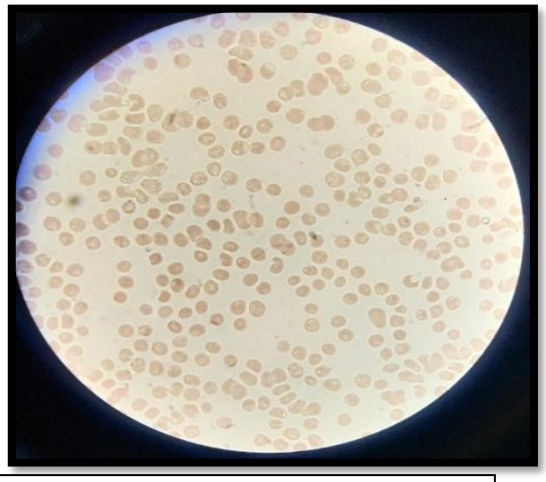


Fig. 7: Marked spherocytosis seen in blood smear of IMHA affected dogs

Fig. 8: Macro agglutination in Saline agglutination test

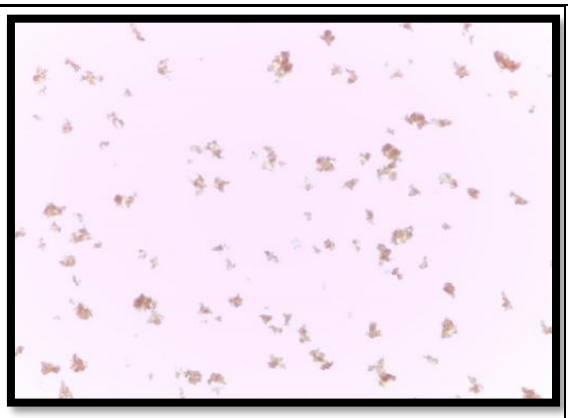


Fig.9: Micro agglutination in Saline agglutination test

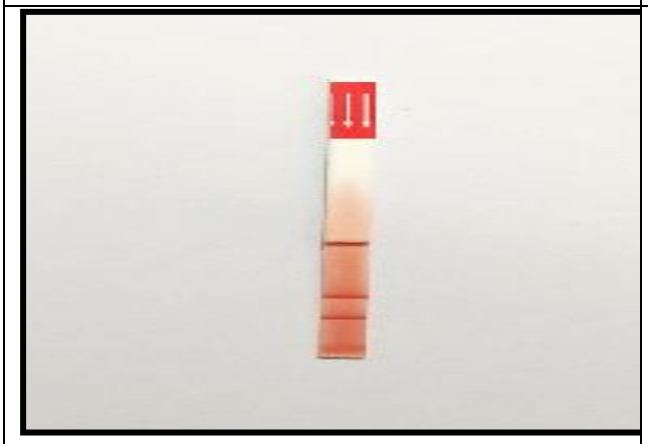


Fig. 10: Positive Coombs' test

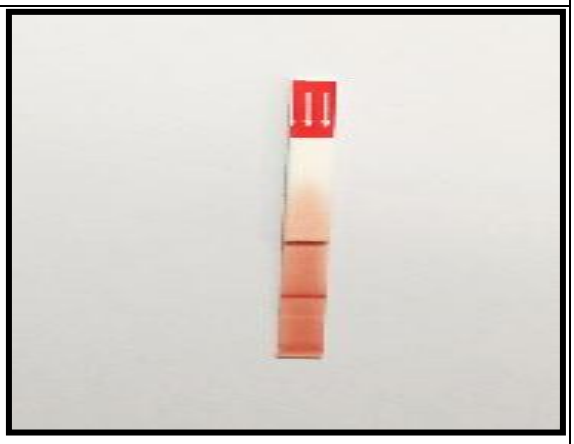


Fig.11: Negative Coombs' test

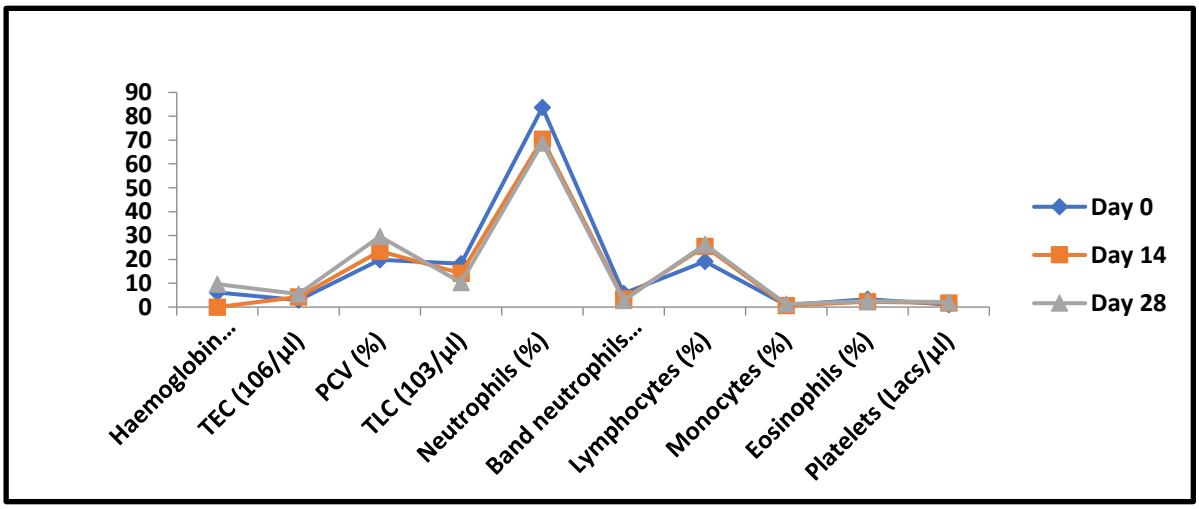


Fig.12: Effect of prednisolone in combination with azathioprine on haematological values in IMHA-affected dogs

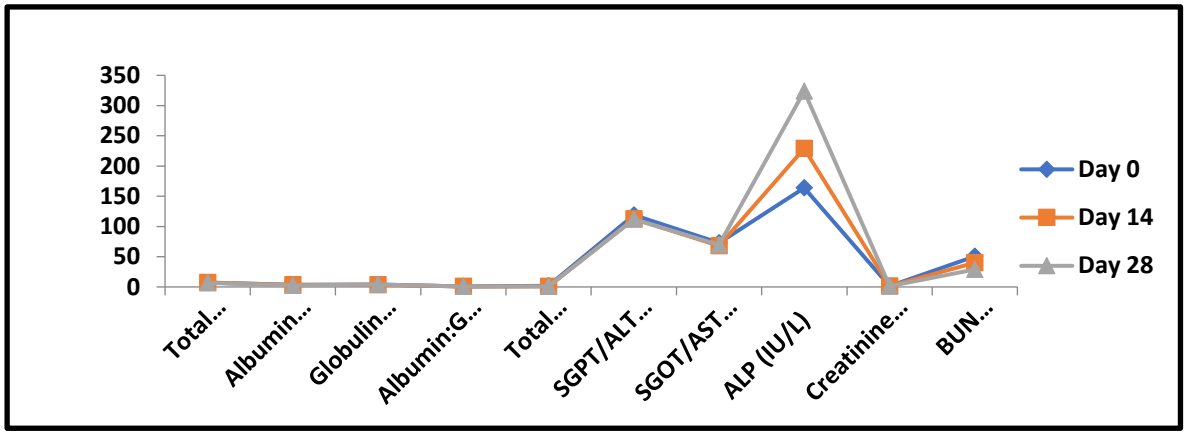


Fig. 13 Effect of prednisolone in combination with azathioprine on serum biochemical values in IMHA-positive dogs

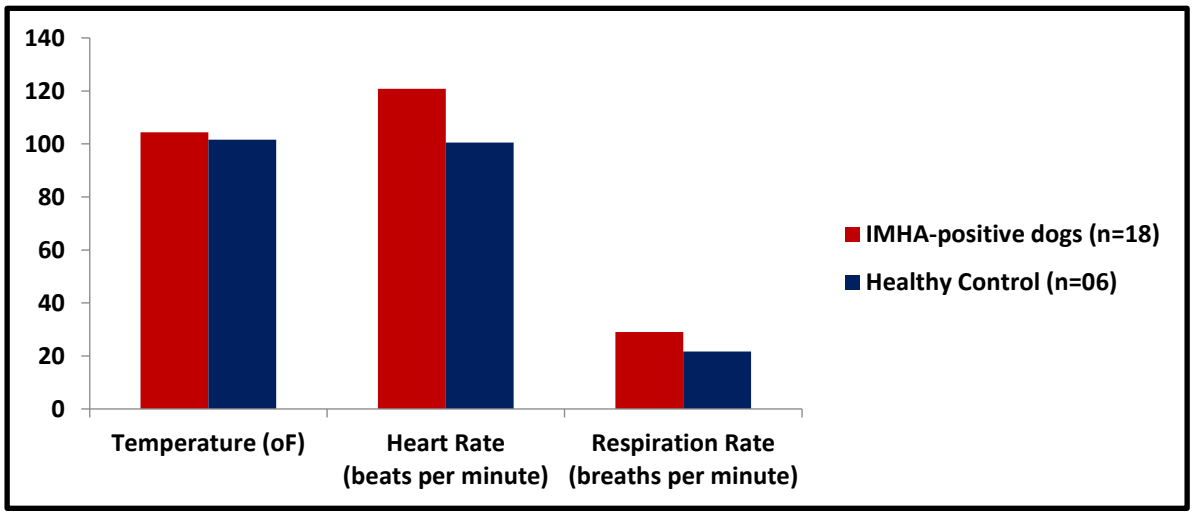


Fig. 14 Clinical parameters in IMHA-positive dogs and healthy control dogs

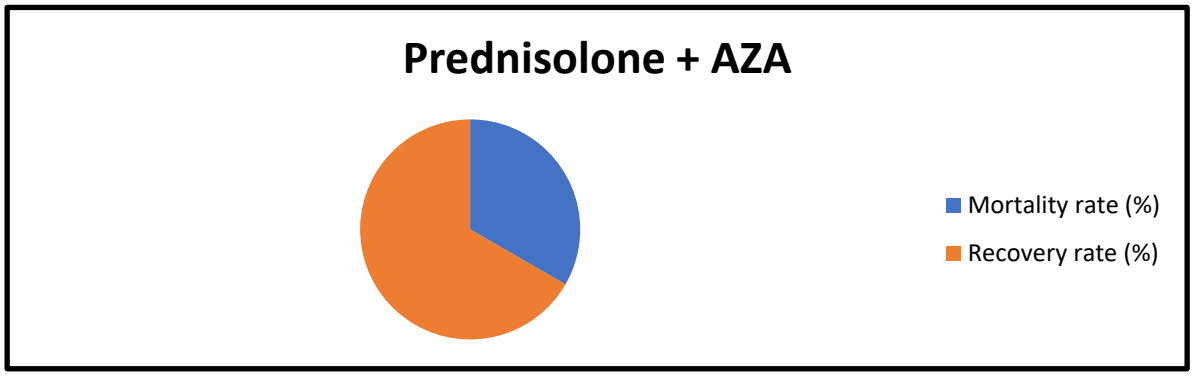


Fig.15: Mortality associated with IMHA in dogs