B-5277
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CASE REPORT Indian Journal of Animal Research

Idiopathic Immune Mediated Haemolytic Anaemia (IMHA) in a 2.5-Month-old Puppy: A Case Report

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10.18805/IJAR.B-5277

ABSTRACT

Background: A 2.5-month-old puppy was presented to the Veterinary Clinical Complex of the College of Veterinary Sciences and AH, Jalukie, Nagaland with a complaint of emesis for one week, inappetence, icterus of the lower abdominal area and other visible mucous membrane. Detailed clinical history was collected and clinical examination was conducted.

Methods: 4 ml blood peripheral blood was collected from the cephalic vein in the Lev-Lock blood clot activator vacutainer to harvest serum for biochemical analysis. Another 2 ml of whole blood was also collected in Lev-Lock K3 EDTA vacutainer for complete blood count, blood smear examination for blood parasites, morphological changes in the RBC and IDEXX SNAP 4Dx Plus test kit was also used for diagnosis of the blood parasites test.

Result: Peripheral blood smear examination and IDEXX SNAP 4Dx Plus test were found to be negative for blood parasites, but spherocytosis could be observed in the blood smears. A complete blood count (CBC) examination revealed severe anaemia of macrocytic normochromic type, neutrophilic leucocytosis and thrombocytopenia. Serum biochemistry revealed a decrease in blood urea nitrogen (BUN) and creatinine with normal alanine transaminase (ALT). Treatment with whole blood transfusion, corticosteroid and other supportive therapy was successful in this case. Based on the clinical presentation, laboratory findings and its response to the treatment with prednisolone, it was diagnosed as an idiopathic immune-mediated haemolytic anaemia (IMHA) with an extravascular mechanism of haemolysis. A follow-up review after 1 year and 4 months post-treatment reported that the dog was still alive and healthy.

Key words: Blood transfusion, Idiopathic Immune Mediated Haemolytic Anaemia (IMHA), Spherocytosis, Steroid, Puppy.

INTRODUCTION

In canine, one of the most common manifestations of the immune-mediated disease is the Immune mediated haemolytic anaemia. IMHA may either occur as an idiopathic (primary) form or a secondary form. Idiopathic IMHA is immune mediated destruction of RBCs resulting in loss of self-tolerance against endogenous (self) antigens and not associated with any cause, whereas the immune reaction that is associated with an exciting cause *viz*. foreign (non-self) antigen such as blood parasites infection, infectious disease, neoplastic condition, chronic inflammation, toxins, drugs, vaccines is termed as secondary IMHA (Jutkowitz *et al*., 2013). In men and cats, two-third or more of the cases of IMHA are secondary. However, most cases of IMHA in dogs are idiopathic (Day, 1999). Jutkowitz *et al*. (2013) stated that the signs presented by IMHA dogs were either acute or chronic and the most common signs in chronic form were lethargy, weakness, inappetence, vomiting, diarrhoea and pigmenturia. The acute form is characterized by sudden collapse. Most of the dogs have a relatively slow onset of anaemia which may take days to weeks, with clinical signs attributed to loss of RBC mass following extravascular haemolysis. A proportion of dogs have an acute onset (1-2 days) of severe haemolytic anaemia with intravascular haemolysis, erythrocyte autoagglutination, collapse, icterus, hemoglobinemia and haemoglobinuria (Pedersen, 1999). The first line of the treatment regimen for IMHA was reported to be prednisolone at 2 mg/kg/day (Jutkowitz *et al*., 2013). Blood transfusions are beneficial and can be very helpful in improving the clinical condition as well as the Hb and PCV of dogs suffering from IMHA (Khan *et al*., 2021).

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How to cite this article: Ozukum, S., Kuotsu, N., Kuotsu, K., Laltlankimi, Devi, N.B., Chutia, T., Ndang, H. and Das, G. (2024). Idiopathic Immune Mediated Haemolytic Anaemia (IMHA) in a 2.5-Month-old Puppy: A Case Report. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5277.

Submitted: 02-12-2023 **Accepted:** 19-03-2024 **Online:** 11-05-2024

MATERIALS AND METHODS

A 2.5-month-old male puppy, weighing 2.8 kg of the non-define local breed was the patient for this study. The puppy was brought to the Veterinary Clinic, College of Veterinary Sciences, CAU, Jalukie, Nagaland, with a complaint of emesis for the past one week, anorexia and lethargic

(Fig 1-a). The puppy was neither vaccinated nor dewormed and there was no medical history of the previous usage of any other drugs. Physical examination revealed severe dehydration with icteric mucous membrane (Fig 1-b,c), subnormal body temperature of 94°F, swollen popliteal and mandibular lymph nodes with the presence of ticks on the body surface.

Blood collection

4 ml of blood was collected from the cephalic vein in the Lev-Lock blood clot activator vacutainer to harvest serum for biochemical analysis. Another 2 ml of whole blood was also collected in Lev-Lock K3 EDTA vacutainer for complete blood count, blood smear examination for blood parasites, morphological changes in the RBC and for IDEXX SNAP 4Dx Plus test.

Blood parasite examination

A peripheral blood smear was prepared by collecting one drop of blood from the ear vein directly into a glass slide and preparing the smear immediately, followed by air drying and staining with Leishman stain for examination of blood parasites under microscope.

IDEXX SNAP 4Dx Plus test kit was also used for diagnosis of the blood parasites. The IDEXX SNAP 4Dx Plus test detects highly specific antibodies for *Dirofilaria immitis, Anaplasma* spp*, Ehrlichia* spp and *Borrelia burgdorferi*.

Blood smears for RBC structure

Blood smear was prepared and stained by 66 Iliyan Manev, Victoria Marincheva, Petar Stamberov Romanowsky-Giemsa technique. The microscopic examination was performed under oil emersion with Olympus CX-31 for detection of any abnormal structures of the RBC.

Complete blood count

For haematological analysis, Hb, PCV, RBC, WBC, Platelets, Neutrophils, Lymphocytes, MCV, MCHC were analysed by using Melet Schloesing 4S haematological analyser, France.

Blood biochemistry

Biochemical parameters like BUN, Creatinine, Total bilirubin, SGPT/ALT were analysed using Transasia's Erba EM 200 automated biochemistry analyzer, India.

RESULTS AND DISCUSSION

Both peripheral blood smear and IDEXX 4DX Plus snap test were negative for haemo-protozoal and rickettsial infection. Complete blood count examination of the blood sample that was collected on day one, revealed severe anaemia (Table 1) of macrocytic normochromic type (RBC= 1.04 $\times$ 10^6/µl; Hb=3.2 g/dl; PCV=8.3%; MCV= 80.7fl: MCHC=38.5 g/dl), neutrophilic leucocytosis (Neutrophils=15.81 $\times$ 10^3/µl; WBC= 27.01 $\times$ 10^3/µl) and thrombocytopenia (Platelets = 80 $\times$ 10^3/µl). Serum biochemistry revealed (Table 1) a decrease in BUN (6.5 mg/dl) and creatinine (0.46 mg/dl) with normal ALT (1.77 u/L).

The treatment protocol was as follows:

Fig 1(a): Lethargic puppy.

Fig 1(b): Icteric gums.

Fig 1(c): Icteric conjunctiva.

- ✓ Day 1 and Day 2 (while awaiting for the blood reports): Infusion Forlyte- P fluid @100ml IV ; Inj. dexamethasone @ 0.5 ml IV at stat ; Inj. tribivet (Vitamin B1, B6, B12) @ 0.8 ml IV o.d ; Inj. Pantoprazole @ 1 mg/kg IV b.i.d ; Inj. ondansetron @ 0.5 mg/kg IV b.i.d ; Inj. oxytetracycline @10 mg/kg IV o.d ; Inj. metronidazole @ 15 mg/kg IV o.d.
- ✓ Day 3 (on getting the haematological report for CBC, IDEXX 4DX Plus snap test, blood smear and biochemical analysis): Inj. pheniramine maleate @ 0.5 ml IM half hour before the transfusion; Inj. dexamethasone @ 0.5 ml IV half hour before the transfusion; Whole blood @ 100 ml IV at stat; Inj. Pantoprazole @ 1 mg/kg IV b.i.d; Inj. oxyte tracycline @ 10 mg/kg IV o.d; Inj. metronidazole @ 15 mg/kg IV o.d. Immediate whole blood transfusion on the puppy was done along with the other follow-up treatment on the 3rd day of the treatment on getting the blood reports.
- ✓ Day 4 to Day 9 : Infusion Forlyte- P fluid @100 ml IV once; Inj. pantoprazole@ 1 mg/kg IV b.i.d; Inj. oxytetracycline @10 mg/kg IV o.d; Tablet Doxycycline @ 15 mg/kg orally od for 5 days from day 5 to day 9; Syrup Multivit (Multivitamin) @ 2 ml bid for 5 days; Tablet Limcee (Vitamin C) @ 1/2 tab orally b.i.d for 5days; Tablet Pantoprazole 10 mg @ ½ tab orally po bid half hour before food for 5 days.
- ✓ Day 10- Day 24 : Tablet Limcee (Vitamin C) @ 1/2 tab orally b.i.d for 14 days; Syrup Multivit (Multivitamin) @ 2 ml bid for 14 days; Suspension Albomar (Albendazole) @ 25 mg/kg orally o.d po for 3 days.
- ✓ Day 25- Day 38: Syrup Multivit (Multivitamin) @ 2 ml bid for 14 days; Tablet Prednisolone @ 4 mg/kg orally bid for 7 days, followed by od for another 7 days.
- ✓ Day 39- Day 63: Tablet Prednisolone (at tapering dose) @ 4 mg/kg orally o. d for 7 days; @ 4 mg/kg orally o. d alternate days for 7 days; @ 4 mg/kg orally o. d orally every 2 days for 7 days; @ 4 mg/kg orally o. d orally after 3 days.
- ✓ Following the blood transfusion, on the 4th day it was noted that the puppy was more active, there was no sign of vomition, started taking little amount of food and water, rectal temperature also increased from 94°F (on the day 1) to 100.5°F (on day 4), but ulcers in the gums were noticed and was still icteric (Fig 2 a, b). Hence, a course of doxycycline tablet (from day 5th to day 9th of the treatment) was prescribed along with Limcee tablet, multivit syrup and Pantop tablet for 5 days and was advised for a review after 5 days.

The puppy was brought to the clinic again for the review after 5 days (the 10th day of the treatment) wherein it was observed that the puppy had regained his appetite but the ulcers in the gums still persisted and it was slightly icteric. So, the puppy was made to continue with the Limcee tablet, multivit syrup and was dewormed with Albomar suspension. A second review was advised after 14 days.

On the 2nd review (25th day of treatment), it was observed that the ulcers in the mouth have completely healed (Fig 3) but the visible mucous membrane was still pale and slightly icteric. Hence, the blood sample was collected again for CBC in which it was revealed that although there was an increase in RBC, PCV, Hb and platelets, the puppy was still anaemic of normocytic normochromic type (Table 2) and a blood smear examination was done in which

Table 1: Haematological and biochemistry result on the first day.

Haemogram and biochemistry	Result	Normal range
Hb (g/dL)	3.2	12-18
PCV (%)	8.3	37-55
RBC (10^6/µl)	1.04	5.5-8.5
WBC (10^3/µl)	27.01	6-17
Platelets (10^3/µl)	80	120-600
Neutrophils (10^3/µl)	15.81	3-13.6
Lymphocytes (10^3/µl)	10.9	0.4-6.4
MCV (fl)	80.7	58-73
MCHC (g/dl)	38.5	28-40
BUN (mg/dl)	6.5	8-28
Creatinine (mg/dl)	0.46	0.5-1.7
Total bilirubin (mg/dl)	0.26	0-0.3
SGPT/ALT (u/L)	17.7	10-114

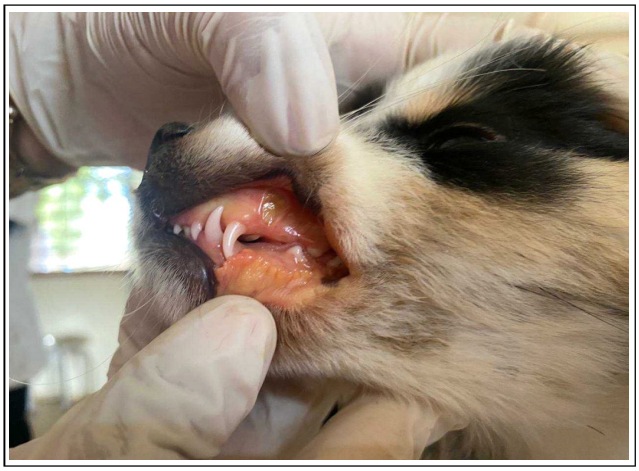

Fig 2(a): Icteric gum with ulcer.

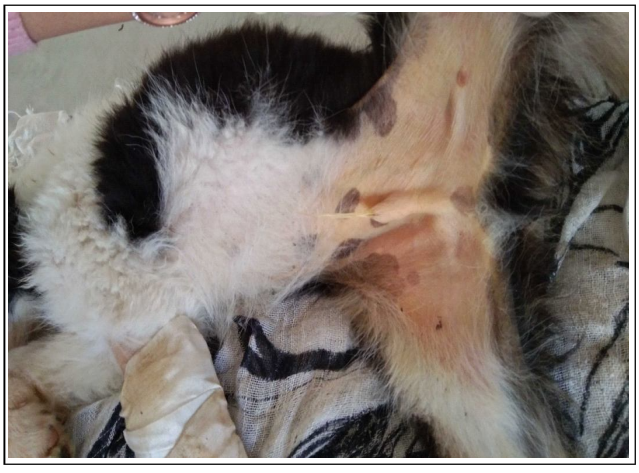

Fig 2(b): Icteric abdominal region.

spherocytosis along with codocytes and echinocytes could be observed in the blood (Fig 4). Based on the blood smear findings and CBC result, prednisolone treatment was started on the 25th day of the treatment which was continued for 42 days, with tapering doses. On day 43 of the treatment (18th day of prednisolone treatment) the CBC result showed a significant increase in the Hb, RBC, PCV, platelets and decrease in WBC (Table 2), with no visible icterus (Fig 5 a, b) and marked improvement of the puppy. A review of the puppy over the call was done, after the completion of the prednisolone treatment and again after 1 year and 4 months post treatment. The dog was found to be still alive and healthy (Fig 6).

Based on the clinical presentation, laboratory findings and its response to the treatment with prednisolone, it was diagnosed as an idiopathic immune-mediated haemolytic anaemia with the extravascular mechanism of haemolysis. Lack of haematuria indicates a possible extravascular mechanism of haemolysis which might have been mediated by opsonization with immunoglobulin molecules on the RBC surface which induces the phagocytosis of the target cells through Fc receptors of the macrophages (Barcellini, 2015). Initially, the line of treatment was directed towards blood parasites induced IMHA due to the evidence of ticks infestation during the clinical examination. However, repeated negative result in both peripheral blood smear and IDEXX 4DX Plus snap test for haemoprotozao and rickettsia, absence of any previous medication with other drugs, but the presence of spherocytosis, macrocytic normochromic anaemia, leucocytosis and thrombocytopenia were highly suggestive of idiopathic immune mediated haemolytic anaemia (Christine, 2012). Hence, the treatment was diverted to canine idiopathic IMHA by using immunosuppressive doses of prednisolone as prednisolone was found to be effective in the management of canine idiopathic IMHA (Lachungpa *et al.*, 2020).

Severe anaemia was seen in the present case, which was consistent with previous studies (Burgess *et al.*, 2000) in which severe anaemia has been reported in 81 to 98% of cases. Thrombocytopenia (Platelets= 80 $\times$ 10^3/µl) as seen on day one, could be related to concurrent immune-mediated destruction of platelets. It has been previously reported as a risk factor for increased mortality in dogs with IMHA (Weinkle *et al.*, 2005). The leukocyte profile on the day of presentation indicated a value above the normal range. A similar increase in leukocytes was also reported by Manev

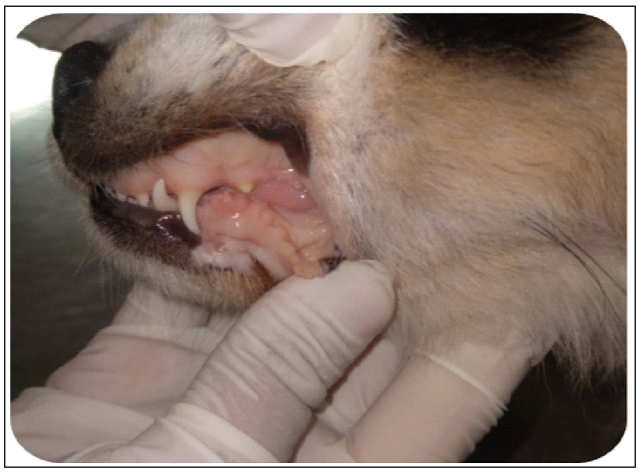

Fig 3: Healed gum which is still pale and slightly icteric.

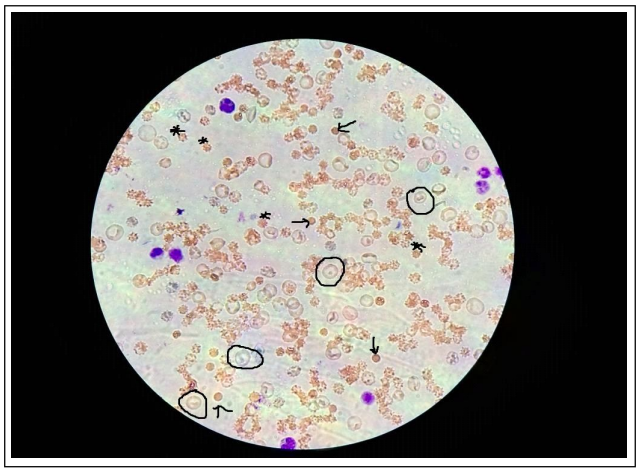

Fig 4: Spherocytosis (arrow), Codocytes (circle), Echinocytes (asterisk).

Table 2: Comparison of Haematological results before blood transfusion, after transfusion and after prednisolone treatment.

Haemogram	Before blood transfusion (on day 1)	After 22 days of blood transfusion (Day 25th of the treatment)	On the 18th day of Prednisolone treatment (Day 43rd of the treatment)	Normal range
Hb (g/dL)	3.2	4.4	7.2	12-18
PCV (%)	8.3	11.9	20.7	37-55
RBC (10^6/µl)	1.04	1.78	2.97	5.5-8.5
WBC (10^3/µl)	27.01	20.66	26.9	6-17
Platelets (10^3/µl)	80	120	350	120-600
Neutrophils (10^3/µl)	15.81	8.36	6.82	3-13.6
Lymphocytes (10^3/µl)	10.96	12.04	19.63	0.4-6.4
MCV (fl)	80.7	66.9	70	58-73
MCHC (g/dL)	38.5	36.9	34.7	28-40

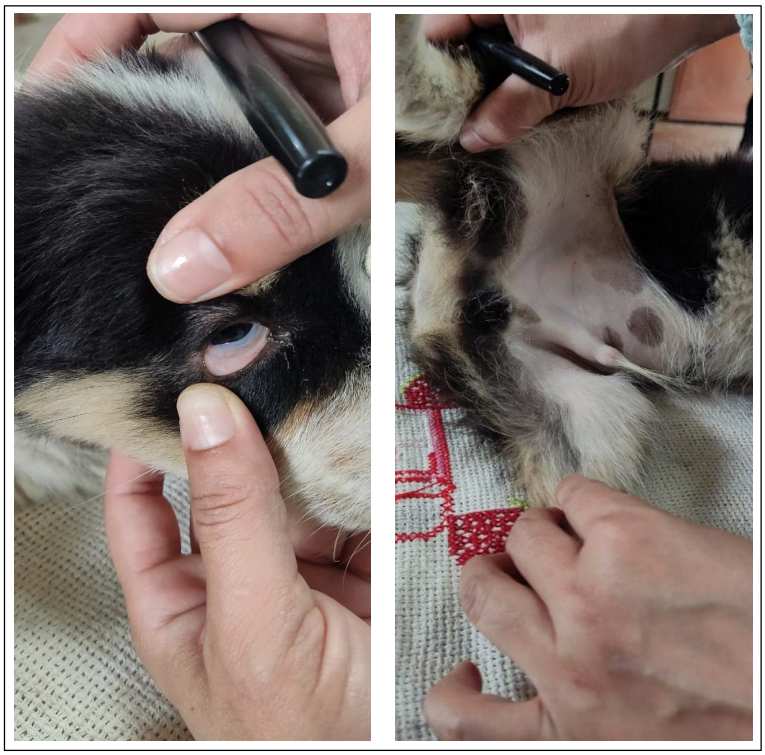

Fig 5: (a) Healthy conjunctiva with no signs of Icterus (b) No signs of icterus in the abdominal regional.

Fig 6: The latest picture of the dog that was taken after 1 year and 4 months post treatment.

et al. (2018). Extreme leukocytosis in IMHA is a common but transient accompanying finding and is based on the functional reactivity of the bone marrow (Cohn, 1991).

During the 18th day of treatment with prednisolone, the blood picture showed an increase in the Hb, RBC, PCV, platelets and a decrease in WBC (Table 2). The improvement might be due to decreased production of antibodies thereby reducing lymphocytes in the circulation pool (Dowling, 1995) or by reduced erythrophagocytosis of opsonized RBC (Piek, 2011). A similar result was also recorded by Lachungpa *et al*. (2020) after using prednisolone. Lachungpa *et al*. (2020) found that in prednisolone treatment group there was a significant increase in the means of Hb, RBC, PCV, platelets and a significant decrease in means of WBC, PT, APTT, BUN and Total Bilirubin levels as compared to the group that was treated with a combination of prednisolone and azthioprine. In all the dogs which survived, there was a significant increase in the means of Hb, RBC, PCV and platelets and a decrease in mean WBC, PT and APTT. Prednisolone reduce the phagocytic action of macrophages by reducing the expression of surface receptors which detect immunoglobulin on RBC and they also stabilize the lysosomal membrane of macrophages, decrease complement activation, block chemotaxis and prevent the synthesis of prostaglandin and leukotriene (Dowling, 1995). It was also observed that the increase in the Hb, RBC, PCV and platelets after the prednisolone treatment was quite significant in comparison to the increase of the same mentioned blood parameters after the whole blood transfusion (Table 2). The first indication of response to prednisolone therapy is stabilization of the hematocrit, followed by a slow increase to normal values over several weeks (Day, 1996).

Many reports show that Idiopathic IMHA is primarily considered a disease of middle-aged to older dogs and rare in dogs below 1 year of age (Burgess *et al*, 2000). Hence, Idiopathic IMHA was initially overlooked in our case, as the patient was just a 2.5 months old puppy.

CONCLUSION

The aim of the treatment regime was to achieve a long-term remission so that the immunosuppressive drug doses could be gradually withdrawn and discontinued eventually without the condition relapsing. In this case, the immunosuppressive drug treatment was done for 42 days which started initially with prednisolone tablet @ 4 mg/kg, twice a day for 7 days followed by weekly tapering of the drugs till the 42nd day, after which it was stopped completely. A final review of the case done over the call after 1 year and 4 months post treatment found that the dog was still alive, healthy and there was no sign of relapsing.

ACKNOWLEDGEMENT

The authors are thankful to the College of Veterinary Sciences and Animal Husbandry, CAU, Jalukie, Nagaland for providing all the necessary facilities to carry out this case study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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