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DIAGNOSIS AND TREATMENT OF A CAT WITH IMMUNE MEDIATED HEMOLYTIC ANEMIA (IMHA)

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ABSTRACT

To explore the diagnosis and treatment of immune-mediated hemolytic anemia (IMHA) in cats, we diagnosed and treated a cat with IMHA. We made a detailed observation and recorded the treatment process for reference to the treatment of IMHA in cats. Our diagnosis was based on the cat's clinical manifestations, and our diagnosis was based on laboratory tests and ultrasound results. We ruled the possibility of common infectious and parasitic diseases. The cat was diagnosed with hemolytic anemia, fatty liver, hepatocyte injury, and cholestasis. The condition did not improve after symptomatic treatment for five days. But the cat gradually improved after using immunosuppressants and was finally diagnosed to be suffering from IMHA. After 32 days of treatment, the cat was cured and discharged. This paper describes a case study that can serve as the reference for diagnosing and treating feline IMHA.

***Keywords*:** Cat, Immune-mediated hemolytic anemia (IMHA), Diagnosis, Treatment

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1. INTRODUCTION

Immune-mediated hemolytic anemia (IMHA) is one of the most common and serious hemolytic diseases in dogs, whereas it is rare in cats (Kopke et al. 2019). It is a disease in which autoantibodies, complements or both attach to the erythrocyte membrane, resulting in destruction of red blood cell immunity and eventually hemolysis (Borchert et al. 2020). There are two types of pathogenesis of hemolytic anemia. The first type is primary. The second type is secondary, which can be due to tumors (e.g., lymphosarcoma, and lympholeukemia), viruses (e.g., feline leukemia virus and feline immunodeficiency virus), parasitic diseases, use of certain drugs (e.g., β-lactams), vaccination, etc.(Kohn et al. 2006; Paes et al. 2010). Cats with IMHA can show acute or chronic symptoms, including anorexia, drowsiness, depression, mucosal jaundice, dehydration, and hepatosplenomegaly.

Although IMHA is rare in cats, it is a potentially fatal disease in cats. Because it may result in severe disease (Swann et al. 2016).Since many pet owners allow their cats to eat freely, and do not control their cats' indoor and outdoor access, pet owners may not be able to notice the clinical symptoms of cats (drowsiness, weakness, anorexia, etc.) in time (Paes et al. 2010; Rudloff 2017). Therefore, in the early stage of IMHA, pet owners are likely to ignore the symptoms of mild anorexia, drowsiness, etc. If the affected cat is not treated in time, IMHA may lead to vomiting, dehydration, jaundice, and even coma or death. However, because these symptoms are not specific, cats with IMHA are very likely to be misdiagnosed in clinic which can make treatment difficult.

In recent years, with the rapid improvement of people's living standards, the number of pets has increased year by year. Although the domestic pet diagnosis and treatment industry is already well established, the diagnosis and treatment of IMHA in cats is still a difficult immune disease in veterinary medicine. Even with the use of effective preventive measures and the optimal treatment, it is still impossible to avoid deaths in some cases. Through the observation and analysis of the process of treating a cat with IMHA in a pet hospital, we studied the diagnosis and treatment of cats with IMHA, hoping to provide some reference for the diagnosis and treatment of cats with IMHA in the future, so as to improve the clinical cure rate of feline IMHA.

2. MATERIALS AND METHODS

2.1. Clinical information

A ten-year-old, short-hair cat with a body weight of 2.36kg and regularly vaccinated and dewormed which has been emaciated and lost appetite in the past two months, vomiting and retching frequently in the past week. We conducted clinical and laboratory tests on the cat. We observed the cat's mental state, measured the cat's body

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temperature to determine whether it is normal or not, and scored the cat's body condition. At the same time, the cat was examined by whole body palpation and cardiopulmonary auscultation.

2.2. Routine blood test

The dry hematology analyzer from IDEXX was used to examine the cat's routine blood test before and after treatment. The serum biochemical indices of different tissues of the cat before and after treatment were examined by IDEXX automatic biochemical analyzer.

2.3. Imaging examination

The BLS series full digital ultrasound diagnosis system of Xuzhou Bessel Electronic Technology Co., Ltd. (Xuzhou, Jiangsu, China) was used to examine the abdomen of the cat before and after treatment.

2.4. Special inspection

Special inspection was conducted for the detection of *Mycoplasma haemophilus* the California strain, *Mycoplasma haemophilus* the Ohio strain, feline pancreatitis (fpL), feline immunodeficiency disease (FIV) and feline leukemia (FeLV).

2.5. Therapeutic methods

2.5.1. Blood transfusion

The cat was treated with blood transfusion on the first day and the second day.

2.5.2. Symptomatic treatment and supportive therapy

The prescriptions for day 1 and 2 are as follows: compound sodium chloride injection (100ml), 5% glucose (50ml), 10% KCl (3ml), amoxicillin clavulanate potassium (0.05ml/kg) and compound vitamin B (1ml) were injected intravenously. Vetplus Samylin (0.5g/kg), ursodeoxycholic acid (15mg/kg) and diphenhydramine (1mg/kg) were taken orally.

The prescriptions for day 3 to 5 are as follows: compound sodium chloride injection (100ml), 5% glucose (50 ml), 10% KCl (3 ml), amoxicillin clavulanate potassium (0.05ml/kg) and compound vitamin B (1ml) were injected intravenously. Vetplus Samylin (0.5g/kg), ursodeoxycholic acid (15mg/kg), Ganjing Buxuesu Oral Liquid (0.5ml/kg, twice daily), mirtazapine plaster 1/8 tablet (Once every two days) were taken orally.

The prescriptions for day 6 to 9 are as follows: compound sodium chloride injection (100ml), 5% glucose (50ml), 10% KCl (3ml), amoxicillin clavulanate potassium (0.05ml/kg) and compound vitamin B (1ml) were injected intravenously. Vetplus Samylin (0.5g/kg), ursodeoxycholic acid (15mg/kg), Ganjing Buxuesu oral liquid (0.5ml/kg, twice daily), mirtazapine plaster 1/8 tablet (once every 2 days), prednisolone (0.5-2.5mg/kg, twice daily), Synbiotic (2.5mg/kg, twice daily) and lactulose 1 ml (thrice daily) were taken orally.

The prescriptions for day 10 to 13 are as follows: compound sodium chloride (100ml), 5% glucose (50ml) 10% KCl (3ml), amoxicillin clavulanate potassium (0.05ml/kg) were injected intravenously. Vetplus Samylin (0.5g/kg), ursodeoxycholic acid (15mg/kg), Ganjing Buxuesu oral liquid (0.5ml/kg, twice daily), mirtazapine plaster 1/8 tablet (once every 2 days), prednisolone (0.5-2.5mg/kg, twice daily), Synbiotic (2.5mg/kg, twice per day) and lactulose 1ml (three times per day) were taken orally.

The prescriptions for day 14 to 22 are as follows: amoxicillin clavulanate potassium (0.05ml/kg) was injected intravenously. Vetplus Samylin (0.5g/kg), ursodeoxycholic acid (15mg/kg), Ganjing Buxuesu oral solution (0.5ml/kg, twice daily), prednisolone (0.5-2.5mg/kg, twice daily) and lactulose 1ml (3 thrice daily) were taken orally. Stopped using ursodeoxycholic acid on the 23rd day and stop using lactulose on the 24th day (the other medicines in the prescriptions are the same as those for day 14 to 22).

The prescriptions for day 25 to 32 are as follows: amoxicillin clavulanate potassium (0.05 ml/kg) was injected intravenously, Vetplus Samylin (0.5g/kg), Ganjing Buxuesu Oral Liquid (0.5ml/kg, twice daily) and prednisolone (0.5-2.5mg/kg, twice daily) were taken orally. After discharge, continue to use the same drug to prevent disease relapse, and feed a can of Hill's® Prescription Diet® a/d® Canine/Feline (g/jar) according to the vomiting condition of the infected cat every day, and wait until 24 hours after vomiting if vomiting occurs.

3. RESULTS

3.1. Diagnosis results

According to clinical and laboratory examination, it was determined that the cat suffered from hemolytic anemia, accompanied with fatty liver, hepatocyte injury and cholestasis.

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3.2. Clinical ailments

Before treatment, the cat had a body temperature of 37.9°C. Its body condition score was 2/9. The cat showed severe jaundice, poor skin elasticity, shortness of breath, no obvious abnormality in heart and lung auscultation and no obvious abnormality in palpation of abdomen, extremities, and superficial lymph nodes. After treatment, the body temperature of the affected cat returned to normal, the skin elasticity was normal, and the visual mucosa gradually changed from yellow to pale and finally to pink.

3.3. Hematological studies

Before treatment (Table 1), the results showed that the red blood cell, hematocrit, and hemoglobin of the cat decreased significantly, indicating severe anemia, and the increase of reticulocyte suggested that the cat was regenerative anemia. The increase in white blood cells, neutrophils, lymphocytes, and monocytes suggests severe inflammation in cats. After treatment (Table 2), red blood cells, hematocrit and hemoglobin increased day by day, suggesting that the symptoms of anemia in the cat were alleviated. However, leukocytes, neutrophils and monocytes were still at a high level, suggesting that inflammation still existed in cats.

Table 1: Results of the routine blood test before treatment

Indices	Measured Values	Normal Range
Red blood cell count (10^{12}/L)	2.01↓	6.54-12.0
Hematocrit (%)	8.8↓	30.3-52.3
Hemoglobin (g/dL)	2.7↓	9.8-16.2
Red Cell volume Distribution Width (%)	33.2 ↑	15.0-27.0
Reticulocyte (k/μL)	69.7↑	3.0-50.0
Leukocyte (10^9/L)	28.99↑	2.87-17.02
Neutrophil (10^9/L)	20.75↑	2.30-10.29
Lymphocyte (10^9/L)	6.92↑	0.92-6.88
Monocytes (10^9/L)	1.23↑	0.05-0.67

↓: fall; ↑: elevated.

3.4. Serum biochemistry

Before treatment (Table 3), blood glucose, total protein, globulin, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase, glutamate tyrosine transpeptidase, total bilirubin, total cholesterol, amylase, and creatine kinase increased whereas creatinine decreased in cats (Table 3). Results suggested that the cat suffered from hepatocyte injury, hemolytic anemia, and cholestasis. After treatment (Table 4), glutamic pyruvic transaminase, glutamic oxaloacetic transaminase and total bilirubin decreased significantly, suggesting a good prognosis of the cat's liver injury.

Table 2: Results of the routine blood test after treatment

Indices	Blood Collection Days							
	4	6	9	12	18	26	32	37
Red blood cell count (10^{12}/L)	3.9↓	1.9↓	2.1↓	3.1↓	3.8↓	4.4↓	4.0↓	5.2↓
Hematocrit (%)	18↓	10↓	11↓	15↓	15↓	18↓	16↓	23↓
Hemoglobin (g/dL)	6.0↓	2.8↓	3.2↓	4.3↓	4.8↓	5.7↓	5.2↓	7.3↓
Red Cell volume Distribution Width (%)	34↑	40↑	30↑	28↑	29↑	25↑	22↑	22↑
Reticulocyte (k/μL)	52↑	137↑	129↑	256↑	20.0	24.7	42.4	109.4↑
Leukocyte (10^9/L)	11.53	49↑	25↑	33↑	30↑	39↑	32↑	39↑
Neutrophil (10^9/L)	2.1↓	35.0↑	20.↑	6.24	25.7↑	25↑	22↑	34↑
Lymphocyte (10^9/L)	7.2↑	10.6↑	3.53	24↑	3.64	13↑	7.7↑	3.19
Monocytes (10^9/L)	1.9↑	3.02	1.4↑	2.1↑	0.94↑	1.2↑	2.6↑	0.9↑

↓: fall; ↑: elevated.

3.5. Ultrasonic examination

Abdominal ultrasound examination before treatment (Fig. 1A) showed: the outline of the liver of the affected cat was smooth, the liver tip was blunt and round, the echo of the parenchyma was obviously enhanced, no abnormal nodules were found, the gallbladder was filled, the wall was smooth, and no abnormal echo was found in the cavity. The outline of the spleen was smooth, the echo of the parenchyma was uniform, and no abnormal nodules were found. The outline of both kidneys was balanced, and the boundary of the cortex and medulla was clear. The size of the left kidney was about 4.13x2.41 cm, and the size of the right kidney was about 3.66x2.15cm. No obvious structure and abnormal echo were found in bladder, pancreas, and stomach. The results of abdominal ultrasonography after treatment (Fig. 1B): the outline of the liver was smooth, the tip of the liver was sharp, the

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echo of the parenchyma was uniform, the gallbladder was filled, the wall was smooth, and no abnormal echo was found in the cavity. The outline of the spleen was smooth, the echo of the parenchyma was uniform, and no abnormal nodules were found. The outline of both kidneys was balanced, and the boundary of the cortex and medulla was clear. The size of the left kidney was about 3.70x2.31cm, and the size of the right kidney was about 3.55x1.98cm. No obvious structural and echo abnormalities were found in bladder, pancreas, and stomach.

Table 3: Results of biochemical examination before treatment

Indices	Measured value	Normal range
Blood glucose (mmol/L)	11.53 ↑	3.95-8.84
Creatinine (μmmol/L)	50 ↓	71-212
Urea (mmol/L)	9.8	5.7-12.9
P (mmol/L)	1.53	1.00-2.42
Ca (mmol/L)	2.30	1.95-2.83
Total protein (g/L)	96 ↑	57-89
Albumin (g/L)	31	23-39
Globulin (g/L)	65 ↑	28-51
ALB/GLOB	0.5	0.5-1.4
Glutamic-pyruvic transaminase (U/L)	342 ↑	12-130
Glutamic oxaloacetic. Transaminase (U/L)	167 ↑	0-48
Alkaline phosphatase (U/L)	270 ↑	14-111
Transglutaminase (U/L)	5 ↑	0-4
Total bilirubin (μmmol/L)	118 ↑	0-15
Total cholesterol (mmol/L)	6.07 ↑	1.68-5.81
Amylase（U/L）	1856 ↑	500-1500
Pancreatic lipase (U/L)	479	100-1400
Creatine kinase (U/L)	395 ↑	0-134

↓: fall; ↑: elevated.

Table 4: Results of biochemical examination after treatment

Indices	Blood Collection Days							
	4	6	9	12	18	26	32	37
Albumin (g/L)	24	27	25	23	27	30	None	None
Glutamic-pyruvic transaminase (U/L)	181↑	217↑	270↑	134↑	491↑	660↑	140↑	100
Glutamic oxaloacetic transaminase (U/L)	91↑	None	None	None	None	268↑	51↑	62↑
Total bilirubin (μmmol/L)	26↑	180↑	120↑	25↑	13	8	5	None

↓: fall; ↑: elevated; None: lack of data.

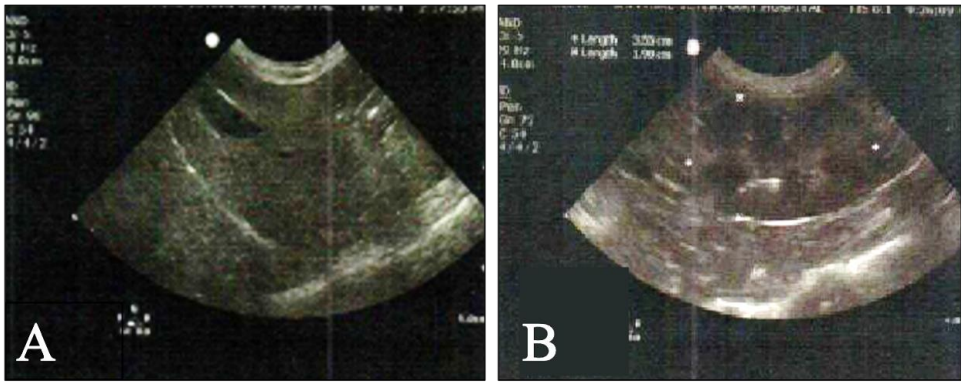

Fig. 1: Results of ultrasound imaging. (A) ultrasound scan of liver before treatment; (B) ultrasound scan of liver after treatment.

3.6. Microbial analysis

The results of special inspection are as follows: Negative for *Mycoplasma haemophilus* the California strain; negative for *Mycoplasma haemophilus* the Ohio strain; negative for fpL; negative for FIV; and negative for FeLV.

4. DISCUSSION

The cat was diagnosed with severe anemia and fatty liver at the initial diagnosis. Our results showed that the RBC count before treatment was 2.01 x 10^{12}, the hematocrit value was 8.8% and the hemoglobin content was

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2.7g/dL. The significant decrease in all three indicators indicated that the cat had severe anemia. Besides, the reticulocytes in the cat's blood were increased, with a value of 69.7k/μL, suggesting that the cat was suffering from regenerative anemia. So, the cat was treated with blood transfusion in the first two days. Blood transfusion therapy can quickly supplement the microcirculatory blood volume and body fluid volume of diseased animals, supplement red blood cells, plasma, coagulation factors and platelets, maintain blood pressure, enhance blood oxygen carrying capacity and blood coagulation, and stimulate hematopoietic function, so that it can improve the tolerance of animals (Terra 2010; Balakrishnan et al. 2016). In addition, diphenhydramine may be used during blood transfusions to prevent allergic reactions in cats.

In this case, the biochemical results showed an increase in total protein and globulin in the cat, with values of 96g/L and 65g/L, respectively, which may be due to a severe inflammatory response in the cat. Because the capillary permeability increases after inflammation with consequent leak of fluid, electrolytes, and proteins (Kumar 2010). That was the reason why we could detect a lot of total protein and globulin in the blood. We also found that the creatinine content significantly decreased, and its value was 50μmol/L. The decrease in creatinine content could be due to the false decrease caused by the increase of bilirubin (118μmmol/L) (Sharkey and Radin 2010). Therefore, combined with the clinical characteristics of the cat and the increased total bilirubin and reticulocyte, the cat was diagnosed with hemolytic anemia disease. In addition, we found the increased of the ALT and AST, with values of 342 U/L and 167 U/L. According to the report, that the increase of ALT and AST content indicated hepatocyte injury (Liu et al. 2000; Sandeep 2006). The clinical sign of increase in alkaline phosphatase is specific to cats. Once it increases, it indicates poor bile discharge (Center et al. 1986). Obviously, according to the results of the examination, we diagnosed that the cat also had liver injury. So in our treatment, we focused on the treatment of anemia, inflammation and liver injury.

Because the cat was only treated for anemia, inflammation and liver injury at first. The treatment included fluid replacement, correcting electrolytes, protecting liver, and retreating jaundice, and improving resistance and blood transfusion. But the condition of the cat did not improve after 5 days of treatment, so after rethinking the diagnosis process and re-examination, it was suspected that the original treatment was incorrect. After treatment with steroids (prednisolone), the cat's symptoms began to improve gradually, and eventually the cat was diagnosed with IMHA. This is because the role of glucocorticoids in the disease is to reduce the production of red blood cell antibodies and prolong the life of red blood cells (Oggier et al. 2018). It has been reported that the autocoagulation reaction of normal saline can be used to make a preliminary diagnosis, and the suspected immune-mediated hemolysis can be further confirmed by direct Coomb's test (MacNeill et al. 2019). Due to our inexperience at the beginning of the treatment, this was not confirmed by the Coombs test.

In addition, ultrasound examination of the visceral tissue revealed that the cat may also have fatty liver. At present, the pathophysiological mechanism of fatty liver is still unclear. It has been reported that primary fatty liver is usually caused by obesity, persistent reduced food intake, environmental stress and prolonged anorexia (Webb 2018). The cat was not obese and there were no other potential factors that could cause fatty liver, so the reason for the cat suffering from fatty liver is that immune-mediated hemolytic anemia caused ischemic lesions of the liver. Secondary, hepatocyte hypoxia leads to the disturbance of fat metabolism and the synthesis and transformation of lipoproteins depend on fat oxidation, so that result in the accumulation of triglycerides in the liver (Isaza et al. 2020). The most important thing in the treatment of fatty liver is nutritional support. When cats have mild appetite, appetite enhancers can be used to promote diet. If cats still cannot take in sufficient nutrition satisfying daily nutritional requirements after using appetite enhancers, nasal feeding intubation is needed. Cats with fatty liver should be fed high-protein, medium-fat and low-carbohydrate foods to meet their resting needs, and too much carbohydrate may lead to high blood sugar and vomiting.

During the treatment of IMHA, the recurrence and aggravation of the disease are related to drug withdrawal or drug reduction. It has been reported that ursodeoxycholic acid can reduce AST and ALT (Pietu et al. 2012; Hu et al. 2019). Therefore, the rebound of AST and ALT on the 26th day of treatment is considered to be the result of discontinuation of ursodeoxycholic acid. Although the cat has a healthy appearance after recovery, stress factors and other diseases can lead to immune dysfunction and make the disease relapse. Therefore, after the sick cat was discharged from the hospital, the pet owner should be told to reduce the stress reaction.

Conflict of Interest Statement: The authors declare that there are no conflicts of interest.

Conclusion: Animal medical personnel are proficient in the professional knowledge of immune diseases, which is very important for the diagnosis and treatment of sick animals. In addition, careful care of sick animals contributes to the prognosis of the disease and prevents the disease from worsening or causing complications.

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Author's Contribution: Ping Liu conceived and designed experiments. Lujia Huang, Xinlei Qian, Cong Wu, Pei Liu, Yulan Zhao, Zheng Xu and Ping Liu were involved in the operation. Lujia Huang prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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