







## Mixed infection of Bartonella and Eperythrozoon in a dog – case report

[Infecção mista de Bartonella e Eperythrozoon em um cão – relato de caso]

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### ABSTRACT

A March male Golden, weighing 7.6kg, presented with gradual weight loss, high body temperature, depression, poor appetite and thirst, and vomiting before consultation. The results showed that the erythrocytes, hematocrit, hemoglobin, and platelets were lower than the reference values. The diagnosis of mixed infection with haematocrit and eosinophilic bodies was confirmed by real-time fluorescence PCR of whole blood, which was positive for haematocrit and eosinophilic bodies. The dog was treated with doxycycline and ceftriaxone, and the dog fully recovered after 2 weeks with blood transfusion, symptomatic treatment, and supportive therapy. This indicates that the disease can be treated well by a comprehensive treatment approach.

Keywords: dogs, blood baltons, erythrocytes, real-time fluorescence quantitative PCR, diagnosis and treatment

### RESUMO

Um March Golden macho, pesando 7,6kg, apresentou perda de peso gradual, temperatura corporal alta, depressão, falta de apetite e sede, e vômitos antes da consulta. Os resultados mostraram que os eritrócitos, hematócrito, hemoglobina e plaquetas eram menores que os valores de referência. O diagnóstico de infecção mista com hematócrito e corpos eosinófilos foi confirmado por PCR de fluorescência em tempo real de sangue total, o que foi positivo para hematócrito e corpos eosinófilos. O cão foi tratado com doxiciclina e ceftriaxona, e o cão recuperou-se completamente após duas semanas com transfusão de sangue, tratamento sintomático e terapia de suporte. Isto indica que a doença pode ser bem tratada através de uma abordagem de tratamento abrangente.

Palavras-chave: cães, balões de sangue, eritrócitos, PCR quantitativa de fluorescência em tempo real, diagnóstico e tratamento

### INTRODUCTION

Baltons is a short, morphologically diverse, Gram-negative, oxidase-negative, human-animal commensal rickettsia with complex nutritional requirements, that is highly adapted to most mammals, widely parasitized by dogs, cats, rodents etc., and transmitted by blood-sucking arthropods (fleas, flies, lacewings, lice, etc.) (Pitassi *et al.*, 2015; Alvarez-Fernandez *et al.*, 2018; Donovan *et al.*, 2018; Ansil *et al.*, 2021). The bacterium is highly hemophilic and mainly parasitic on the surface of canine red blood cells

and then damages them (Ernst *et al.*, 2020; Ansil *et al.*, 2021). When infected, dogs become depressed, lie down and are reluctant to move around, while anemia, vomiting, diarrhea and enzymatic changes in heart, liver and kidney functions occur (Alvarez-Fernandez *et al.*, 2018). In severe cases, dogs with prolonged anemia and oxygen deprivation cause panting, red tongue and bruised skin, and collapse (Alvarez-Fernandez *et al.*, 2018).

Erythroblastosis is a human-animal syndrome caused by the parasitic growth of erythrocytes on

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the surface of red blood cells, plasma, and bone marrow fluid. Erythroblastosis in dogs is clinically characterized by anemia, jaundice, and fever, with a high rate of infection, but most of them pass insidiously and show acute clinical signs under stressful conditions (Zhang J *et al.*, 2019).

The diagnosis and treatment of a case of canine Baltons disease with ehrlichiosis admitted is described below for clinical reference (Hui J *et al.*, 2022).

### CASUISTRY

A March male Golden, weight 7.6kg, was found to be in a concerning mental condition, poor appetite, vomiting and cough by the owner

before consultation. The preliminary examination revealed that the affected dog had 38.7°C body temperature, emaciated body condition, abnormalities in the hind limbs to be reluctant to walk, rapid breathing, rapid heartbeat, and slightly red tongue and oral cavity, all of which suggested anemia.

### RESULTS

From Table 1, it can be seen that: leukocytosis, erythrocytes, hematocrit, hemoglobin, mean erythrocyte volume, mean erythrocyte hemoglobin volume, platelets, and mean platelet volume were reduced, among which the total number of erythrocytes was much lower than the normal value, suggesting severe anemia (Raimundo *et al.*, 2019).

Table 1 Results of routine blood examination

Item	Examination result	Reference value
WBC (white blood cell)/ ( $\times 10^9 \cdot L^{-1}$ )	54.64↑	5.05-16.76
LYM(lymphocytes)/ ( $\times 10^9 \cdot L^{-1}$ )	8.83↑	1.05-5.10
MONO (monocyte count) / ( $\times 10^9 \cdot L^{-1}$ )	1.67↑	0.16-1.12
NEU (neutrophil) / ( $\times 10^9 \cdot L^{-1}$ )	43.92↑	2.95-11.64
BASO (basophils) / ( $\times 10^9 \cdot L^{-1}$ )	0.14↑	0.00-0.10
HCT (hematocrit) /%	5.1%↓	37.3-61.7
RBC (red blood cells) / ( $\times 10^{12} \cdot L^{-1}$ )	0.98↓	5.65-8.87
HGB (hemoglobin)/ (g/dL)	1.7↓	13.1-20.5
MCV (mean erythrocyte volume) (fL)	52.0↓	61.6-73.5
RDW (coefficient of variation of red blood cell volume distribution width) / (%)	36.3%↑	13.6-21.7
MCH (mean red blood cell hemoglobin volume)/(pg)	17.3↓	21.2-25.9
RLT (platelets) / ( $\times 10^9 \cdot L^{-1}$ )	138↓	148-484
MPV (mean platelet volume) /(fL)	18.2↑	8.7-13.2

Table 2 showed that alanine aminotransferase activity was higher than the reference value and total serum protein was lower than the reference value, suggesting liver damage; albumin content was lower than the reference value, suggesting insufficient albumin synthesis due to liver damage; globulin content was higher than the reference value, suggesting antigenic stimulation; creatinine content was lower than the reference value, suggesting decreased muscle

metabolic rate in the affected dog; lipase was higher than the reference value, suggesting possible pancreatic damage and the need for Further examination was required (Raimundo *et al.*, 2019).

Table 3 showed that total carbon dioxide and partial pressure of carbon dioxide are lower than normal, suggesting that the dog has a fever (Raimundo *et al.*, 2019).

*Mixed infection...*

Table 2 Results of blood biochemical examination

Item	Result	Reference value
ALKP (alkaline phosphatase) /(U/L)	177	23-212
ALT (glutamate transaminase)/(U/L)	221↑	10-125
AMYL (amylase) /(U/L)	655	500-1500
ALB (serum albumin) /(g/dL)	2.2↓	2.3-4.0
BUN (blood urea nitrogen) /(mg/dL)	14	7-27
CA (total serum calcium) /(mg/dL)	9.0	7.9-12.0
CHOL (cholesterol) /(mg/dL)	119	110-320
CREA (blood creatinine) /(mg/dL)	0.1↓	0.5-1.8
GGT (glutamyl transpeptidase) /(U/L)	25↑	0-11
GLOB (immunoglobulin) /(g/dL)	1.9↓	2.5-4.5
GLU (blood glucose) /(mg/dL)	140	74-143
LIPA (lipase) /(U/L)	3561↑	200-1800
PHOS (phosphatase esterase) /(mg/dL)	2.7	2.5-6.8
TBIL (total bilirubin) /(mg/dL)	<0.1	0.0-0.9
TP (total serum protein) /(g/dL)	4.1↓	5.2-8.2

Table 3. Results of blood gas analysis

Item	Examination result	Reference value
BUN (urea nitrogen) /(mg/dL)	16	10-26
Na (sodium) /(mmol/L)	141	139-150
GLU (blood glucose) /(mg/dL)	139↑	60-115
K (potassium) /(mmol/L)	3.1↓	3.4-4.9
Cl (chlorine) /(mmol/L)	109	106-127
TCO <sub>2</sub> (total carbon dioxide) /(mmol/L)	15↓	17-25
AnGap (anion gap) /(mmol/L)	20	8-25
HCT (red blood cell cumulative pressure) %	<15↓	35-50
PH (acidity/alkalinity)	7.515↑	7.35-7.45
PCO <sub>2</sub> (partial pressure of carbon dioxide) /(mmHg)	18.2↓	35-38
HCO <sub>3</sub> (bicarbonate) /(mmol/L)	14.7↓	15-23
BEecf (residual base) /(mmol/L)	-8↓	-5-0

Red blood cell pressure was much lower than normal, indicating severe anemia in the dog.

It also reflects the disturbance of ionic balance and acid-base imbalance of the body.

The blood smear showed that the erythrocytes varied in size and morphology, and there was a large amount of short rod-shaped and oval alkaline material at the edge of the erythrocytes, platelets were obviously reduced, neutrophils were mainly lobulated, and monocytes and lymphocytes were significant increased.

Real-time fluorescence quantitative PCR was used to analyze the affected dogs' whole blood, and the results indicated that Baltons and canine ehrlichiosis were present. However, the results of Babesia and Ehrlichia tests were negative.

The whole blood of the affected dog was taken and tested for C-reactive protein (CRP). As shown in Figure 1 and Table 4, the CRP concentration of the affected dog was normal(Furquin *et al.*, 2021;Cruz *et al.*, 2022).

Table 4. Results of CRP

Item	Examination result	Reference value
Concentration	<5.00mg/L	0-10.00 mg/L

Combined with the clinical symptoms and laboratory diagnosis, the diagnosis of canine Baltons with Erythrocytes mixed infection was confirmed.

The dog was given 200 ml of blood after blood type matching, followed by expansion of blood volume with albumin, 4mL/kg by body weight, diluted with 5% glucose solution intravenously once a day for 5 days. Subcutaneous injection of 2mL of glycohemavir was given for 14 days.

Doxycycline, 10mg/kg per body weight orally daily for 14 days; prednisone (glucocorticoid), 2mg/kg per body weight orally daily for 14 days; ceftriaxone 400mg diluted with 0.9% saline intravenously for 5 days; metronidazole 35ml intravenously for 5 days.

Anti-inflammatory and cough suppression: mix saline 3mL, mucosolvan 1.2mL, dexamethasone 0.5ml and nebulize for 14 days; guggenheim, orally daily, 1ml/kg according to body weight for 14 days. rehydration and enrichment: water soluble vitamin C2ml added to 5% glucose dilution intravenously for 5 days; lactic acid Grenadine 50mL intravenously for 5d. elimination of weakness: quick oral Calcium supplementation daily, 0.3mL/kg according to body weight for 14 days.

Subcutaneous injection of vitamin B12 0.75mg, Kotex (compound Butafos, 100mL/bottle) 1mL for 14days. Ferrous sulfate, 50mg/kg orally according to body weight, twice a day for 14 days.

After 5 days of treatment, the dog's appetite was restored, its mental condition was improved, its tongue color and oral mucosa were still acceptable, and cough subsided. After 14 days of treatment, the dog recovered its spirit and appetite, stopped coughing, and the hind limb deformity was fully covered. 2 months later, the dog was re-examined, no obvious abnormality was seen, and it recovered to health.

## DISCUSSION

Baltonellosis is infected by scratching, biting, or blood-sucking of mites, ticks, and fleas. Baltonellosis enters the body of dog, it mostly parasitizes the canine red blood cells. Baltons are very tiny, and the sinking of the erythrocyte envelope is a relatively common phenomenon after parasitization, and the cell body is affected and ruptured or deformed, which is the main cause of anemia in affected dogs; at the same time, extensive infiltration and aggregation of leukocytes in the endothelium of blood vessels and the surrounding mesenchymal fractional nuclei, and fragmentation-like pathological changes in the epithelium of blood vessels; due to tissue damage and bacterial invasion, the endothelial cells of capillary walls produce bacterial due to tissue damage and bacterial invasion, endothelial cells of capillary wall produced bacterial vesicles and local tissue inflammatory cells proliferated to form granuloma-like pathological changes. Serum biochemical tests revealed the enzymatic changes in heart, kidney and liver functions, and the germs were able to erode the heart and liver of the affected dogs, leading to functional disorders, vomiting and diarrhea, and the best time to treat the affected dogs was missed because the symptoms occurred similar to those of canine microvirus. When a dog suffers from this infectious disease and is disturbed by an external insect vector, a stress immune response is generated, resulting in a decrease in the body's resistance, and usually the cure rate for this disease is relatively low. Prevention is important in the treatment of this disease, and parasitic infestation is a reason for this disease that must be disregarded. In this case, Baltong's infected dog had symptoms such as impaired heart function, fever, cough, and gastrointestinal discomfort. Usually, the affected dog's liver is enlarged by these effects, and analysis of the dog's serum reveals a significant increase in glutamate transferase, which leads to liver failure and eventually death.

### *Mixed infection...*

The filamentous structures of the erythrocyte appendages are embedded in the cell membrane of the erythrocytes causing small, deep depressions and deformations in the erythrocyte membrane, resulting in morphological, structural, and functional changes. These deformed red blood cells are removed as they pass through the lymph nodes and spleen. In the case of latent infection, a dynamic balance is maintained between the number of erythrocytes infected by Erythrocytes and the number of erythrocytes with a functional and normal defense system of the body. Under high intensity stress conditions, the resistance of the body decreases, and the number of deformed erythrocytes increases dramatically due to the strengthening of glucose metabolism at the invasion site. On the one hand, many deformed erythrocytes are removed by the body, leading to anemia; on the other hand, changes in the structure and shape of the erythrocyte membrane can lead to exposure of previously obscured antigens or changes in existing antigens, causing immune hemolysis, and resulting in anemia. In addition, there are changes in blood physiological and biochemical indicators such as decreased blood glucose and significantly elevated glutamate transferase, and extensive tissue damage such as liver function damage and pathological damage to the myocardium of the affected dog, resulting in serious consequences such as metabolic disorders and disruption of acid-base balance, which eventually led to the death of the affected dog.

This case showed signs of severe anemia, and it was necessary to perform not only routine blood tests but also to observe the morphology of blood cells when making the diagnosis to prevent missing the diagnosis of blood disorders. Because certain pathogens appear periodically in the blood, general microscopy is only effective for the acute onset of the disease. Serological methods are also not suitable for confirming the diagnosis of disease due to their own limitations. In contrast, PCR assays have high specificity and sensitivity, so whole blood was drawn from this case for fluorescent quantitative PCR testing. In this case, by investigating her life history, it was suspected that the severe anemia could be caused by *Haematobium bartonii*, *Babesia*, *Ehrlichia*, and canine epizootic infection. Finally, the case was identified as a mixed infection of *Babesia haematobium* and canine ehrlichiosis by

fluorescent quantitative PCR. In the treatment of this case, symptomatic treatment, prevention of infection, and promotion of appetite can improve the cure rate of this disease. The affected dog should always be matched for treatment. For blood sources, the health of the blood source should be ensured to avoid cross-infection.

With the rapid development of scientific research and clinical technology, the understanding of these two pathogens has become more and more extensive, but there are still many unresolved issues, such as the mode of transmission of the pathogens, effective clinical diagnostic methods, the development of efficient therapeutic drugs, and the prospect of genetically engineered vaccines in disease control. Therefore, in-depth studies using new research tools are needed in the future.

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### **REFERENCES**

- ALVAREZ-FERNANDEZ, A.; BREITSCHWERDT, E.B.; SOLANO-GALLEGO, L. Bartonella infections in cats and dogs including zoonotic aspects. *Parasit. Vectors*, v.11, p.624, 2018.
- ANSIL, B.R.; MENDENHALL, I.H.; RAMAKRISHNAN, U. High prevalence and diversity of Bartonella in small mammals from the biodiverse Western Ghats. *PLoS Negl. Trop. Dis.*, v.15, p.e0009178, 2021.
- CRUZ, T.N.A.O.; GONÇALVES, L.R.; FURQUIM, M.E.C. *et al.* Threat under cats' claws: Molecular detection and risk factors for zoonotic Bartonella species in blood and claw samples from cats in Brazil. *Acta Trop.*, v.232, p.106496, 2022.
- DONOVAN, T.A.; BALAKRISHNAN, N.; CARVALHO BARBOSA, I. *et al.* Bartonella spp. as a possible cause or cofactor of feline endomyocarditis-left ventricular endocardial fibrosis complex. *J. Comp. Pathol.*, v.162, p.29-42, 2018.

- ERNST, E.; QUOROLLO, B.; OLECH, C. *et al.* Bartonella rochalimae, a newly recognized pathogen in dogs. *J. Vet. Intern. Med.*, v.34, p.1447-1453, 2020.
- FURQUIM, M.E.C.; AMARAL, R.; DIAS, C.M. *et al.* Genetic diversity and Multilocus Sequence Typing Analysis of Bartonella henselae in domestic cats from Southeastern Brazil. *Acta Trop.*, v.222, p.106037, 2021.
- HUI, J.; RYAN, K.A.; RADEMACHER, N. *et al.* Osteomyelitis associated with Bartonella henselae infection in a young cat. *J. Feline Med. Surg. Open Rep.*, v.8.n.2, 2022.
- MAZUREK, L.; WINIARCZYK, S.; ADASZEK, L. Feline bartonellosis key issues and possible vectors. *Ann. Parasitol.*, v.64, p.309-315, 2018.
- PITASSI, L.H.; PAIVA DINIZ, P.P.; SCORPIO, D.G. *et al.* Bartonella spp. bacteremia in blood donors from Campinas, Brazil. *PLoS Negl. Trop. Dis.*, v.15, p.e0003467, 2015.
- RAIMUNDO, J.M.; GUIMARÃES, A.; AMARO, G.M. *et al.* Molecular survey of bartonella species in shelter cats in Rio de Janeiro: clinical, hematological, and risk factors. *Am. J. Trop. Med. Hyg.*, v.100, p.1321-1327, 2019.
- STEPANIĆ, M.; DUVNJAK, S.; REIL, I. *et al.* First isolation and genotyping of Bartonella henselae from a cat living with a patient with cat scratch disease in Southeast Europe. *BMC Infect. Dis.*, 19, p.299, 2019.
- ZHANG, J.; ZHANG, X.; WANG, H.; ZHOU, Z. First known case of Eperythrozoon endocarditis in China. *J. Int. Med. Res.*, v.48, n.2, 2019.