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Asmita Shrestha
Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal

Rockson Karmacharya
Veterinary Diagnostic and Research Laboratory, Kathmandu, Nepal

Corresponding Author:
Asmita Shrestha
Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal

Ehrlichiosis in a dog: A case study

Asmita Shrestha and Rockson Karmacharya

Abstract
Spike, age of 1 year, local dog, was found with history of anorexia, weak, and high fever. The blood test determined range of Packed Cell Volume (PCV) and hemoglobin below the normal and White Blood Cell count slightly increased. Ehrlichiosis was diagnosed in dog from blood smear, revealing morula of *E. canis*, and also confirmed by rapid test kit. Oxytetracycline at the dose rate 10 mg/kg body weight was administered intravenously with Normal saline slowly. Continuous slow infusion of lactated ringers was provided for three to four hours. Doxycycline capsule was recommended at the rate 10 mg/ kg body weight orally for 14 days twice a day after food. Dexamethasone and Ranitidine were given at dose 2 mg/kg body weight subcutaneously once. After 14 days the dog was recovered and looked healthier. Hemoglobin came to its normal position and PCV was 30.

Keywords: dog, doxycycline ehrlichiosis, *E. canis*

1. Introduction
Ehrlichia was first associated with veterinary diseases in Africa in 1925 by Cowdry who identified *Ehrlichia ruminantium* in cattle and a decade later by Donatien and Lestoquard who described *E. canis* in Algerian dogs [1]. CME (Canine Monocytic Ehrlichiosis) is tick borne disease caused by *Ehrlichia canis*. It is small, coccus, gram negative bacteria with single circular chromosome. *Ehrlichia spp* manage to survive within the vector ticks by invading and replicating in endothelial cells, white blood cells, midgut cells, and salivary glands of the vector ticks [2]. *Ehrlichia* spp has developed strategies to subvert host cell processes ranging from host signaling, modulation of vesicular traffic, protection from oxidative burst, acquisition of nutrients, and control of innate immune activation [2]. The main vector for *E. canis* in Europe is the tick *Rhipicephalus* known as the brown dog-tick [3]. Following an incubation period of one to three weeks, three typical phases of the disease may develop sequentially: acute, subclinical, and chronic [4]. Common clinical signs of Ehrlichiosis include anemia, epistaxis, petechiae, ecchymoses, prolonged bleeding during estrus, hematuria or melena associated with thrombocytopenia, thrombocytopathy, or vasculitis. Ocular signs are also common in CME. The most common are anterior uveitis, corneal opacity, hyphema, retinal vessel tortuosity, chorioretinal lesions, subretinal hemorrhage, retinal detachment, or blindness [5]. The study conducted, determined the prevalence of ehrlichiosis in dogs as 1.8%and 8% , in Kathmandu [6,7]. A dog with *E. canis* can recover after proper treatment during acute phase but the prognosis is grave in chronic phase. The treatment of dog takes longer time period and regular blood test is required to determine the recurrent infection [5]. As, *E. canis* is common in dogs of Kathmandu, the purpose of this case study was to provide general outline of the condition of dog from initial stage of infection to recovery phase and treatment response in dog.

2. Materials and Methodology
2.1 History
Spike, age of 1 year, local dog, was brought with history of anorexia since many days, weak, and high fever. No any vomiting and diarrhea was reported. Finding the pale mucus membrane and pale conjunctiva, owner was suggested to have complete blood profiling test of dog. Blood was collected from jugular vein through 5 ml syringe.

2.2 Complete Blood Test
Approximately, one ml blood was collected in EDTA tube and four ml was collected in serum tube. Automatic analyzer was used to know different biochemical parameters. After complete blood test, following results was obtained.

2.3 Blood smear preparation and leishmann staining
Blood smear was prepared from EDTA blood. A clean slide measuring 75X25 mm and approximately 1 mm thick was taken. Tongue shaped smear of uniform thickness was prepared with the help of spreader on glass slide. After air dry the smear was fixed with ethanol.

Leishmann staining was done according to the steps as follows;

- leishman's stain was poured on slide.
- After two min double the volume of water was added
- After five to seven minutes of pouring water it was washed in a stream of buffered water until pinkish tinge was there (usually washing for two minutes)
- The back of slide was wiped and cleaned
- It was kept upright to dry.

2.4 The Antigen Rapid *E. canis* Ab Test Kit is a chromatographic immunoassay for the qualitative detection of *Ehrlichia canis* antibodies in canine whole blood, serum, or plasma. The Antigen Rapid *E. canis* Ab Test Kit has a letter of "T" and "C" as test line and control line on the surface of the device. A purple test line will be visible in the result window if *E. canis* antibodies are present in the specimen. The specially selected *E. canis* antigens are used in the test band as both capture and detector materials. These enable the Antigen Rapid *E. canis* Ab Test Kit to identify *E. canis* antibodies in canine whole blood, serum, or plasma with a very high degree of accuracy.

2.4.1 Procedure
- Three drops of whole blood diluents was placed in a test tube
- One drop of whole blood of suspected patient was added and mixed in the test tube
- The mixture was placed into the well of rapid test kit.

2.4.2 Interpretation
- Positive result: The presence of only one band ("C") within the result window indicates a negative result.
- Negative result: The presence of two color bands ("T" and "C") within the result window, no matter which band appears first indicates a positive result.
- Invalid test: If the purple color band ("C") is not visible within the result window after performing the test, the result is considered invalid.

3. Result
3.1 Diagnosis
The complete blood profiling gave the values as given in table 1. On the basis of blood test, it was found that the dog was anemic as PCV and Hemoglobin were below the normal, and suffering from thrombocytopenia.

Table 1: Complete blood profiling to determine, blood values, liver function, and kidney function.

Blood parameter	Result	Normal Range
PCV (%)	20	45-55
HGB(gm/dl)	7	12-18
WBC(cells/cumm)	32000	5000-11000
Differential count		
Neutrophil(%)	77	60-77
Monocyte(%)	1	3-10
Lymphocyte(%)	25	12-30
Eosinophil(%)	2	2-10
Basophil(%)	0	00-01
Platelet count(cells/cumm)	115000	150,000-350,000
Biochemistry profile		
Tests	**Result**	**Normal Range**
Alt(U/L)	20	15-110
Alp(U/L)	60	20-200
Total billirubin(mg/dl)	0.4	0.3-0.9
Total protein(gm/dl)	5.6	5-8
Albumin(gm/dl)	1.3	2.5-3.7
BUN(mg/dl)	21	5-30
Creatinine(mg/dl)	1.3	0.7-1.8
Glucose(mg/dl)	81	70-125

Blood smear was prepared and leishmann staining was done where morula of *E. canis* was found.

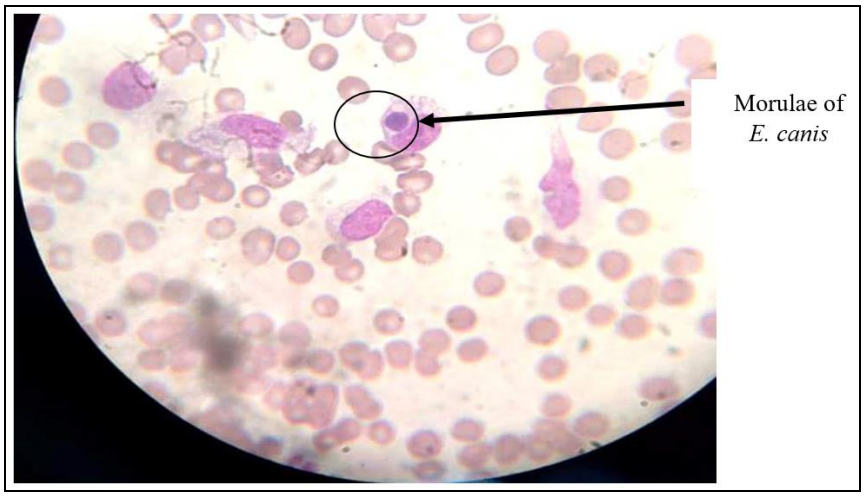

Fig 1: Showing morulae of *E. canis* in blood smear stained with leishmann staining

Fig 2: Rapid test kit For detection of *E. canis* specific antibody

A single red line on C indicates negative result while double line both on C and T indicates positive result. Here only slight red line was seen under T. This indicates having less antibody IgG against pathogen, thus E. canis was also confirmed by rapid test kit.

3.2 Management of the patient

The patient was anorectic so kept on IV ringer lactate fluid immediately. The patient was found to have blood rickettsiae and was suffered with fever so decision was to first control the fever. Oxytetracycline was administered intravenously with Normal saline. Oxytetracycline is bacteriostatic antibiotic and suppresses the protein synthesis and growth of both gram positive and gram negative bacteria. The dose used in dog was 10 mg/ kg body weight. This antibiotic is the preferred drugs for the treatment of protozoa, rickettsiae. Fever was down within few hours and continuous slow infusion of lactated ringers was provided for three to four hours. The deficit losses should be replaced over a long time but ongoing losses should be replaced within three to four hours. Dehydration percent was assessed as five % and deficit volume was calculated as 500 ml. (Davis. *et al*, 2010).

Since it was not possible to bring dog daily to hospital so, doxycycline capsule was recommended. This drug was also provided at the rate 10 mg/ kg body weight orally. This drug was given for 14 days twice a day after food. Only for 14 days was recommended because the decision whether to continue the medicine will be based on repeated blood test and blood smear examination. This drug is quite efficient for penetrating the cells and suppressing the growth of intracellular parasites.

Dexamethosone as a steroidal drug used to suppress inflammatory action. Since in Ehrliciosis the RBC is attacked by the protozoa and is identified as foreign body in reticulo-endothelial system, thus, destroyed by macrophage. So to halt this process Dexamethosone was given intramuscularly to dog. Dexamethasone dose varies depending upon the reason since its use here is for immune suppression it was used at a dose 2 mg/kg body weight subcutaneously once. Ranitidine was then used since it was anorexic for several days. It was given subcutaneously at the dose rate 2mg/kg body weight. After 14 days the dog was recovered and looked healthier. Hemoglobin came to its normal position and PCV was 30. Improvement was observed.

4. Discussion

The most important clinical sign, persistent fever of 103.4˚F, was observed which was similar to the findings of Phuyal [7]. Appetite remains well in early stage and later in chronic stage becomes anorectic. In this case dog was anorectic even in early stage. Profound changes in hematological parameters were observed as reported by Nair [9]. Haemoglobin, packed cell volume, and thrombocytopenia were down and the value was similar to the findings of study done by Phuyal [7]. Thrombocytopenia is observed due to combined direct and indirect effect of Pathogen. Pathogen has direct effect on life span of platelets and indirect effect on bone marrow that suppress erythropoiesis activities. Thus results thrombocytopenia in infected dog. Decrease in level of platelets in severe cases results bleeding due to lack of clotting factor. This types of bleeding was not observed in patient may be due to early stage. In chronic stage this disease relates to various pathological findings like arthritis [9].

Splenomegaly is common findings on radiograph but X- ray was not taken in this case because disease was diagnosed based on the rapid test kit. Usually antibody pathogen specific IgG is detected [9]. The band seen in result well was not so dark. The cause of this light band is due to fewer antibodies. The antibody response may be delayed for several weeks; thus, serologic testing may not be a reliable diagnostic tool early in the course of the disease. Furthermore, antibodies can persist for months or years after infection, making in-house tests for the organisms problematic for confirmation of acute infection, particularly in highly enzootic areas where many dogs may have antibodies to these agents because of previous infections. Therapeutic management included doxycycline @ 10 mg/kg orally for a period of 14 days until blood smear becomes negative and till complete clinical recovery along with supportive therapy but in a study of Shrestha [10], doxycycline @ 5 mg/kg was used. But unfortunately dog died after three days of treatment in case of Shrestha [10]. The reason might be the lower value of Haemoglobin, PCV and glucose level than the level of our dog. According to report of Kottadamane [11], Boxer Dog recovered after 21 days of post treatment which suggests that continuous monitoring, specific, and appropriate supportive therapy as well as owner's compliance is key factors in elimination of the infection from blood in CME affected dog.

5. Conclusion

Presence of morulae in monocyte of the infected dog and Antigen Rapid *E. canis* Ab Test Kit confirms the Ehrliciosis in dog. Doxycycline at the dose rate 10 mg/ kg body weight orally and supportive therapy for 14 days completely removes *Ehrlichia canis* from the blood of infected dog.

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