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Efficacy of injectable doxycycline in natural cases of canine monocytic ehrlichiosis

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Abstract
Background: Canine monocytotropic ehrlichiosis (CME), caused by *Ehrlichia canis*, is a significant tick-borne disease among canines, particularly in the tropical regions and urban areas of India. The standard treatment for CME is a 28-day course of oral doxycycline. However, some dogs may struggle with oral doxycycline due to its associated side effects. This study aimed to assess the effectiveness of parenteral doxycycline in natural cases of non-myelosuppressive CME within a shorter treatment duration of 15 days.
Methods: To confirm the presence of parasitic DNA, we utilized blood and splenic aspirates and performed 16S rRNA nested PCR. We established non-myelosuppression through bone marrow aspiration cytology, focusing on myeloid and erythroid cells. Among the cases confirmed by PCR, we randomly assigned 22 non-myelosuppressive cases into two groups. Group I received injectable doxycycline at a dosage of 10 mg/kg once daily for 15 days, while Group II received oral doxycycline at the same dosage for 28 days, also administered once daily. Our study highlights the utility of bone marrow aspiration for clinicians in identifying CME patients based on the presence or absence of myelosuppression.
Results: Injectable doxycycline demonstrated minimal side effects and proved to be convenient for dogs. After 15 days of therapy, group I showed negative results for 16S rRNA on nested PCR in both blood and splenic aspirates. Furthermore, erythrocyte and thrombocyte levels significantly ($p \leq 0.05$) improved in the group treated with injectable doxycycline.
Conclusion: Hence, injectable doxycycline therapy effectively eliminated parasitic DNA within a 15-day treatment period and demonstrated its efficacy.

Keywords: Ehrlichiosis, doxycycline, bone marrow, splenic aspiration

1. Introduction
Ticks are the primary source of pathogen transmission to animals and the second most significant source for humans. *Rhipicephalus sanguineus*, also known as the brown dog tick, serves as an excellent example of 'parasite globalization' due to its widespread distribution [1]. Rhipicephalus is the most common tick species found in urban India [2]. Canine monocytotropic ehrlichiosis (CME), caused by *Ehrlichia canis*, is a significant tick-borne disease in dogs [3] with a global presence [4]. CME presents as a multisystemic disease with acute, subclinical, or chronic forms. These phases respond differently to therapy and thus have varying prognoses [5]. However, in some chronically infected dogs, there is a risk of severe bone marrow aplasia, profound peripheral blood pancytopenia, and a high mortality rate, often resulting from septicemia and/or severe bleeding [5, 6, 7]. Therefore, the terms "non-myelosuppressive" and "myelosuppressive" CME may better describe disease severity in a clinical context, irrespective of the disease onset or presumed phase [8]. The recommended treatment protocol for CME, is a 28-day course of oral doxycycline [9]. Some dogs may not tolerate oral doxycycline due to side effects such as anorexia, vomiting, diarrhea, or rapid post-treatment increases in alanine aminotransferase and alkaline phosphatase activities [10, 11]. In such cases, the use and efficacy of oral doxycycline become questionable, and alternative administration routes should be considered [12]. This study aims to assess the efficacy of parenteral doxycycline in natural cases of non-myelosuppressive CME over a short duration of therapy, specifically for 15 days.

The use of injectable doxycycline in this study is intended to mitigate side effects and reduce the duration of treatment.

2. Materials and Methods

2.1 Selection of Case

The study was conducted as a randomized, double-blinded trial involving naturally infected and ailing dogs that were attending the outpatient unit of the Teaching Hospital at Madras Veterinary College in Chennai, Southern India. Dogs with severe tick infestations or a history of tick infestation were screened and isolated separately. Among these dogs, those exhibiting symptoms such as bleeding tendencies, lymphadenopathy, splenomegaly, and clinical signs indicative of CME were identified through routine clinical examinations. All of these animals underwent ultrasonographic examinations to confirm the presence of splenomegaly. Additionally, a total of nine healthy animals from the Chennai city police were included as a control group.

2.2 Sample Collection

Peripheral smears were prepared to detect the presence of parasites in peripheral blood. Two milliliters of blood were collected, and serum separation was carried out with the utmost care to prevent hemolysis. The suspected cases were initially screened using commercial lateral flow assays with spot diagnostic kits. These kits, which detect *E. canis* and *Anaplasma platys* antibodies, were purchased from Bionote in South Korea through Artec Diagnostic Systems in Mumbai. A total of 120 rapid diagnostic kits were acquired for routine screening, and their usage followed the manufacturer's guidelines. A positive result was indicated by the presence of double lines, while a negative sample displayed only one line. Animals that tested positive in the sero-diagnostic tests underwent PCR confirmation using 2 mL of EDTA blood. The collected serum samples were stored at -40°C in aliquots for further analysis.

2.3 Splenic Aspiration

Fine-needle aspirates of the spleen were obtained with the animal in a right lateral recumbent position, employing manual restraint. A 5-ml disposable plastic syringe, handheld and attached to a 23-gauge, 1½ inch needle, was utilized for obtaining aspiration biopsy specimens. The overlying abdominal wall was clipped and surgically prepared. Subsequently, the spleen was located through ultrasonography and the needle was promptly inserted into the organ. After applying suction to the syringe two to three times without relocating the needle, both the needle and syringe were swiftly withdrawn. The minute amount of specimen obtained was placed on glass slides, from which pull smears were created. These were left to air-dry, followed by staining with Wright's-Giemsa stain, and then mounted on slides. The remaining aspirated samples were preserved in EDTA tubes and dispatched for PCR analysis, mirroring the procedure for blood samples.

2.4 Polymerase Chain Reaction

Genomic DNA was extracted using the QIAMP DNA mini kit (Qiagen, Germany). The isolated DNA underwent quantification and purity assessment using the nanodrop technique. PCR amplification was carried out utilizing *E. canis* specific primers targeting the repetitive nucleotide sequences within the 16S rRNA gene fragment such as ECB: 5'-CGTATTACCGCGGCTGCTGGCA-3', ECC: 5'-AGAACGAACGCTGGCGGCAAGCC-3' and ECAN5: 5'-CAATAATTTATAGCCTCTGGCTATAGG A- 3', HE3: 5'-TATAGGTACCGTCATTATCTTCCCTAT-3' as described by reference (13). PCR amplicons, along with known positive and negative controls, as well as a molecular weight marker (a 450 bp ladder), were subjected to electrophoresis in a 2% agarose gel. The gel was then stained with ethidium bromide and electrophoresed using submarine gel electrophoresis equipment. The results were visualized using a UV transilluminator from Fotodyne in the USA. A similar analysis method was applied to splenic and bone marrow aspirates. Post-therapy blood and splenic aspirates for PCR were collected on the 15th day in Group I and on the 28th day in both Group I and Group II.

2.5 Bone marrow Aspiration

Animals that tested positive in both tests were selected for bone marrow aspiration and, if necessary, a biopsy as well. The procedure was conducted using a Jamshidi needle, with a gauge size of either 13G or 11G, depending on the size of the animal. In cases involving weak, anemic, and hypoproteinemic small-sized animals, a 20G hypodermic needle was used to collect the aspiration. Prior to the procedure, the owner's consent was obtained. To ensure the animal's comfort, standard sedation protocols were administered. This involved the use of ketamine at a dosage of 10 mg/kg, along with diazepam at 0.5 mg/kg, or propofol at 4 mg/kg, in combination with isoflurane gaseous anesthesia. Following sedation, the animal was placed in lateral recumbency, and the site was meticulously prepared in an aseptic manner. The proximal femur (Fig 1) was chosen as the point of penetration and aspiration, following the technique described by reference (14). Aspiration was performed using a 20 ml EDTA-coated syringe, involving several brisk withdrawals of the plunger until reaching the 10 ml mark on the syringe. Smears were promptly prepared using the squash preparation technique and left to air dry. The myeloid-to-erythroid ratio in these cases was examined using a modified Leishman stain.

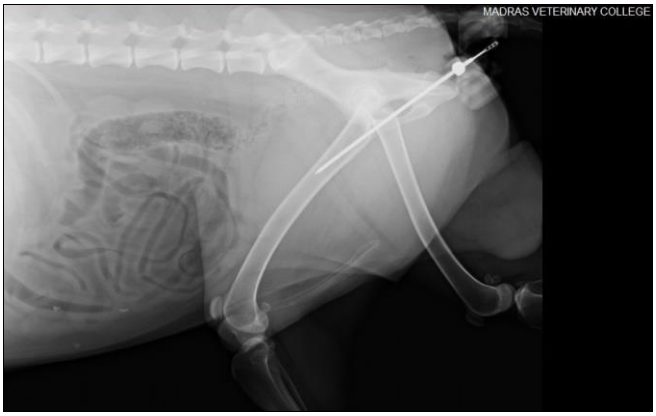

Fig 1: Radiographic view of bone marrow aspiration from femur

2.6 Therapeutic approach

The confirmed non-myelosuppressive cases of CME were randomly divided into two groups. Group one received injectable Doxycycline (Docmycin[1]) treatment at a dose of 10 mg/kg administered daily via intravenous (IV) route for a duration of 15 days. Group II, serving as the control, received standard therapy with oral doxycycline at a dosage of 10 mg/kg daily for a full 28-day course. Clinical specimens were collected both before and after the therapy in both the groups. A total of 300 vials of injectable doxycycline (Docmycin[1])

injection were provided by Alembic Pharmaceuticals Pvt Ltd, as referenced under USO No. 50836/G2/2019 and Proc. No. 2158/G2/2020, dated 05.03.2020. Due to challenges associated with poor owner compliance, post-therapy data was only available for 11 cases in both treatment groups after 28 days of therapy. Throughout the therapy period, parameters such as clinical improvements, hemato-biochemical changes, and the presence of antigen after therapy were meticulously studied. Owners were actively encouraged to report any untoward side effects associated with the doxycycline injection.

For data analysis, serum and whole blood parameters were processed using SPSS software, specifically version 20. One-way ANOVA followed by post hoc multiple comparisons (Tukey test) were employed for comparing parameters. Additionally, post-therapy assessments were conducted using the Paired T-test.

3. Results

During the study period, a total of 120 cases presented with various signs suggestive of hemoprotozoan diseases and were screened for CME using lateral flow assay kits. Among these 120 suspected cases, 62 were provisionally identified as having CME based on either blood smear examination or lateral flow assay results. These cases underwent further confirmation through PCR analysis, which included both blood and splenic aspirate samples. Ultimately, 48 cases were confirmed to be positive for CME based on the results of the 16S rRNA nested PCR (Fig 1). Immediately after confirmation, the owners were advised and followed up regarding tick control measures, which included periodic administration of fluralaner (Bravecto[2]) or sarolaner (Simparica[3]). This was done to prevent reinfection from ticks.

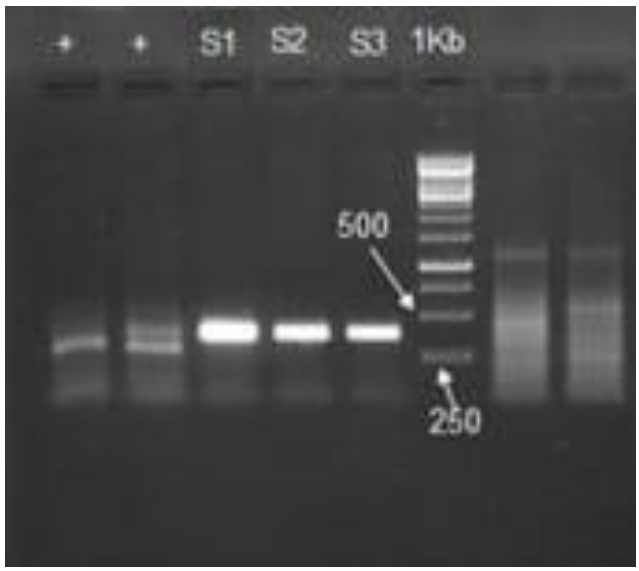

Fig 2: Positive control, blood and bone marrow samples (S1, S2 & S3) tested positive for 16S rRNA nested PCR at 450 bp level

Clinical signs observed in these cases included fever, anorexia, depression, epistaxis, petechial and ecchymotic hemorrhages in the abdomen and inguinal regions (Fig 2 & 3), episcleral hemorrhage (Fig 4), oral and scrotal hemorrhage (Fig 5), prolonged bleeding from vein puncture sites, melena, anemia, lymph node enlargements, and splenomegaly.

Fig 3: Severe petechial and Ecchymotic hemorrhage in abdomen of a dog

Fig 4: Severe Ecchymotic haemorrhage with icterus in inguinal region of a dog

Fig 5: Episcleral haemorrhage in a dog

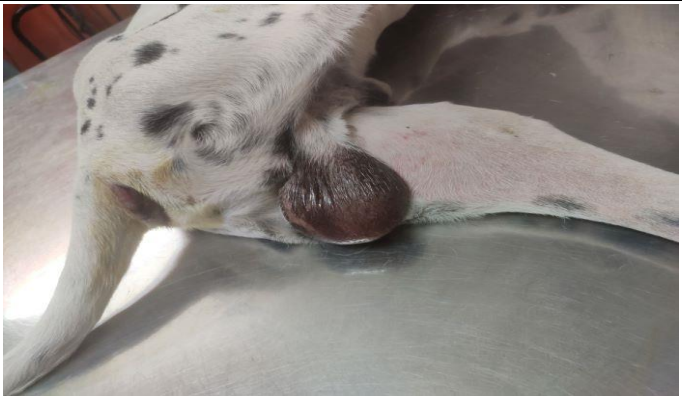

Fig 6: Scrotal haemorrhage in a dog

Many of the smears obtained from splenic aspirations showed the presence of band and mature neutrophils (Fig 6), along with eosinophils and lymphoplasmacytic infiltration, which are indicative of infection.

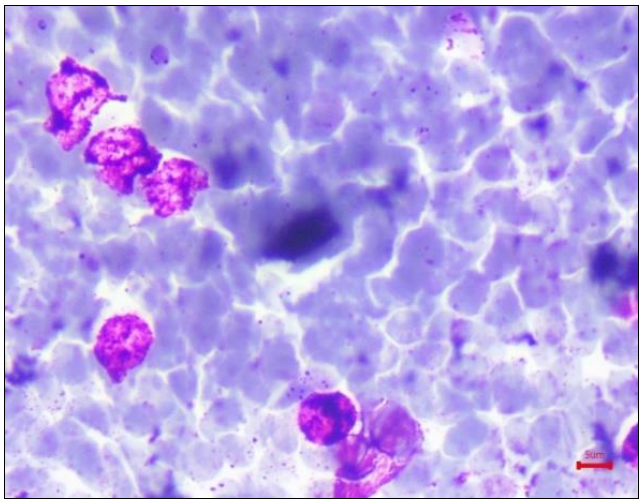

Fig 7: Band neutrophils and Eosinophils in splenic aspirates

For the clinical cases, random division was carried out to assess their hematological parameters with those of healthy animals (Table 1). Significant reductions were observed in erythrocyte count, hemoglobin levels, hematocrit values, and platelet counts in animals affected by CME. Additionally, there was a notable increase in blood urea nitrogen (BUN) and ALT (Alanine Transaminase) values. Bone marrow aspirates and/or biopsy were primarily obtained from the femur, and in some cases, the humerus was used. Samples with a low number of myeloid and/or erythroid cells, with or without the presence of fibrous tissue, were classified as myelosuppressive.

Table 1: Presents the mean ± SE values of hemato biochemical parameters affected by CME and control group

S. No.	Parametrs	Control (n=9)	CME with Docmycin[1] (n=24)	CME with oral doxy (n=24)
1.	Hemoglobin (g/dl)	13.98±0.64[a]	9.07±1.01[b]	10.67±0.40[b]
2.	Hematocrit %	41.80±2.63 [a]	25..58±2.74 [b]	31.96±2.15 [b]
3.	Total erythrocyte count (/µl)	6.48±0.39 [a]	4.16±0.42 [b]	5.04±0.23 [b]
4.	Luekocyte count (/µl)	11522±895	7466±1326	9758±1321
5.	Platelet count (/µl)	301777±29130[a]	51800±8860 [b]	53664±13456 [b]
6.	Neutrophil %	72±0.86	74±1.98	68±2.41
7.	Lymphocyte %	20±0.92	18±1.80	21±2.58
8.	Monocyte %	4.78±0.54 [a]	6.87±0.71 [ab]	7.82±0.84 [b]
9.	Glucose (g/dl)	92.7±4.84	96.3±4.29	97.6±4.88
10.	Total Protein (g/dl)	6.98±0.17	7.10±0.22	6.81±0.31
11.	Albumin (g/dl)	3.17±0.24 [a]	2.71±0.15 [ab]	2.71±0.15 [ab]
12.	BUN (mg/dl)	12.05±1.57 [a]	31.52±7.08 [b]	20.73±3.52 [ab]
13.	Creatinine (mg/dl)	1.29±0.15	1.66±0.32	1.11±0.12
14.	ALT (U/L)	44.77±5.69 [a]	111.9±27.66 [b]	80.76±14.03 [ab]
15.	ALP (U/L)	113.22±28.31	242.2±71.09	206.53±44
16.	Total Bilirubin (mg/dl)	0.41±0.05	1.32±0.41	0.92±0.19
17.	Direct Bilirubin (mg/dl)	0.22±0.03	0.89±0.27	0.66±0.16

Means bearing different superscript differ significantly each other (p≤0.05)

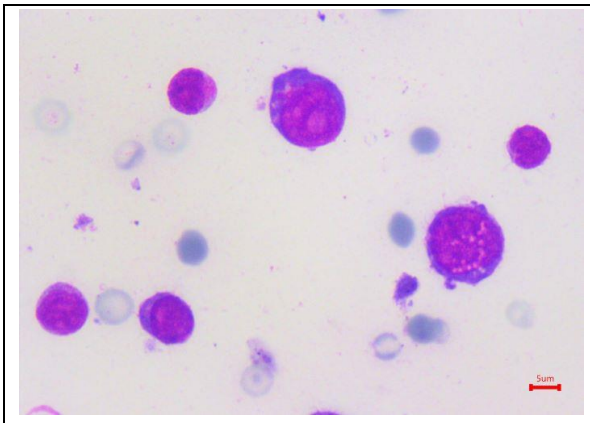

Fig 8: Myeloblast clls in the bone marrow

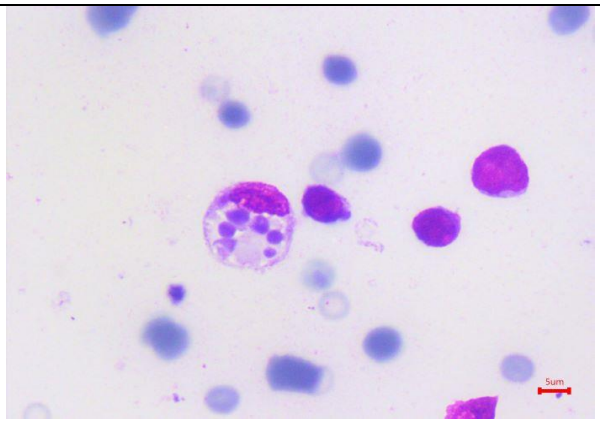

Fig 9: Segmented neutrophilic myeloid cells

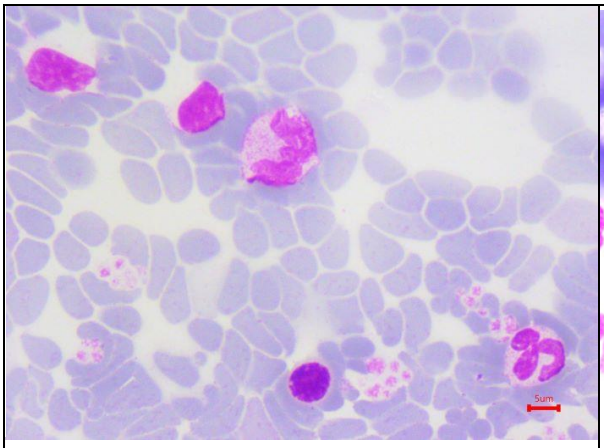

Fig 10: Eosinophilic metamyeloid cells

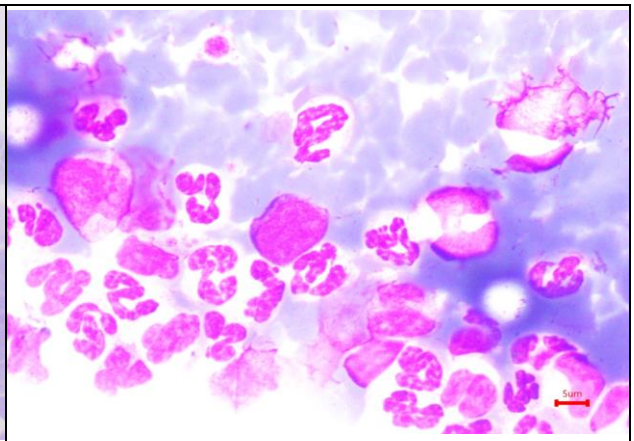

Fig 11: Segmented rubriblast and myeloblast

Therefore, among the 48 cases, 22 exhibited non-myelosuppressive bone marrow. Other cases were excluded either due to poor owner compliance or because of myelosuppression. In non-myelosuppressive samples, the myeloid-to-erythroid ratio was calculated after a careful examination of 200 cells per slide. The aspirate cells were analyzed, and various cell types were identified, as depicted in figures 7-10. However, it's important to note that in none of the cases were parasites identified, despite their positive PCR results.

The myeloid-to-erythroid ratio exhibited a normal distribution with a mean value of 1.33 among sixteen animals, falling within the normal range of 0.75 to 2.53. Notably, four animals displayed a high myeloid ratio with an average value of 3.44. This observation underscores effective granulopoiesis, particularly attributable to neutrophilia. The presence of a substantial number of mature and band neutrophils in cytology further supports this finding. Moreover, it confirms thrombocytosis, which is secondary to chronic inflammation, particularly linked to Canine Monocytotropic Ehrlichiosis (CME).

Conversely, two animals displayed a higher erythroid ratio than myeloid, indicating regenerative anemia. During therapy it's worth noting that despite encouragement for owners to report side effects, no observable side effects were reported with injectable doxycycline. However, vomiting was reported in many animals within the oral therapy group.

In animals treated with injectable doxycycline, a substantial increase was observed in hemoglobin levels, hematocrit values, and platelet counts when compared to their pre-therapy levels (Table 2). Notably, there was a highly significant increase in the total erythrocyte count in animals treated with injectable doxycycline. Additionally, a significant reduction in monocyte levels was noted in the injectable doxycycline-treated group compared to their pre-therapy values. In contrast, among the oral doxycycline group, a significant increase was observed only in platelet numbers. All other parameters displayed differences but without statistical significance.

Table 2: Displays the mean ± SE values of hemato-biochemical changes affected by CME before and after therapy

S. No.	Parametrs	CME with Docmycin[1] (n=11)		CME with oral doxy (n=11)	
		Pretherapy	Post therapy	Pretherapy	Post therapy
1.	Hemoglobin (g/dl)	9.00±0.5	11.23±0.33*	10.97±0.92	11.46±0.85
2.	Hematocrit %	25.8±1.6	32.07±0.93*	29.57±2.41	32.61±1.93
3.	TEC (/µl)	4.13±0.25	5.36±0.18**	4.62±0.38	4.99±0.4
4.	Luekocyte count (/µl)	8093±1405	12025±1658	11788.89±3317.0	11677.78±1184.36
5.	Plateletcount(/µl)	20875±2263	139375±5013*	41911.11±7823.1	96555.55±15949.6*
6.	Neutrophil %	71±0.55	75±1.64	75.44±1.89	73.89±1.02
7.	Lymphocyt%	19±0.53	18.5±1.4	17.56±1.99	20.33±1.52
8.	Monocyte %	9.87±0.83	5.5±0.18*	7.11±0.72	5.56±0.37
9.	Glucose(g/dl	94.37±6.8	90.13±5.58	86.33±4.6	92.22±4.8
10.	Total Protein (g/dl)	6.84±0.23	7.44±.17	7.66±0.32	7.02±0.3
11.	Albumin(g/dl)	2.45±.11	2.63±.12	2.83±0.17	2.16±0.04
12.	BUN (mg/dl)	34.72±10.39	18.80±4.47	20.59±3.46	11.05±0.77
13.	Creatinine(mg/dl)	1.96±.42	1.22±.08	1.27±0.09	0.88±0.12
14.	ALT (U/L)	87.61±21.93	45.75±5.13	83.08±17.22	51.33±8.14
15.	ALP (U/L)	304.0±93.70	91.63±27.42	226.44±44.94	156±24.74
16.	Total Bilirubin (mg/dl)	0.91±.23	0.89±0.134	0.93±0.15	0.87±0.13
17.	Direct Bilirubin (mg/dl)	0.59±0.16	0.52±0.08	0.85±0.15	0.54±0.09

* Significant ** Highly significant (p≤0.05)

4. Discussion

Many owners in our study reported that oral doxycycline caused vomiting and anorexia. This observation is consistent with field observations from several practicing veterinarians (Authors' personal communication). Vomiting, anorexia, and diarrhea are common side effects associated with oral doxycycline therapy, as noted by reference [10]. These side effects often lead to either discontinuation of treatment or alterations in dosing schedules. Furthermore, there is a risk of esophageal strictures, particularly in small dogs and cats, as reported by references [15, 16]. In contrast, injectable doxycycline was well tolerated, with none of the animals

showing any of these side effects. Additionally, the cost of the injections remained within an affordable range. Therefore, the parenteral route was selected, with a reduced duration of treatment. It's important to note that there is no single method for diagnosing Ehrlichiosis. Diagnosis relies on a combination of clinical and hematological indicators, serologic evidence, and molecular confirmation [17]. In our study, diagnosis was established through a comprehensive approach, incorporating clinical signs, hematological changes, smear examination, spot diagnostic lateral flow assays, and PCR analysis conducted on both blood and splenic aspiration samples. Positive serologic test results can be considered as circumstantial evidence of infection. Moreover, a positive result on a snap test in an endemic area is more likely to be a true positive [17]. To optimize the diagnosis of ehrlichiosis, it is recommended to use both serology-based and molecular-based assays [16]. As per the reference [18] the 16S rRNA nested PCR is a highly specific and sensitive method for detecting E. canis DNA in blood samples. It can even detect the presence of Ehrlichia after specific antibiotic therapy [13]. In infected dogs, E. canis DNA can be detected earlier than serum antibodies [19]. During the subclinical phase or carrier state, dogs continue to harbour the rickettsia, presumably in splenic macrophages [20]. PCR performed on splenic samples is considered more sensitive for evaluating ehrlichial elimination compared to blood and bone marrow samples because the spleen retains ehrlichial DNA for a longer duration than blood [21-23]. In our study, splenic aspirates were used to confirm the presence of antigen and the response to therapy through nested PCR. Distinguishing between the acute and chronic phases of ehrlichiosis is not always straightforward in clinical practice because many clinical signs overlap. A complete blood count and bone marrow aspiration can assist in these cases [24]. Dogs with myelosuppression eventually succumb to secondary infections and/or bleeding and do not respond to treatment with the antibiotic doxycycline [20]. The exclusion of myelosuppressive cases from our study was therefore justified. Furthermore, dogs artificially infected with E. canis have shown an initial increase in the myeloid-to-erythroid ratio [25]. Transient pancytopenia can accompany acute E. canis infection, but it is usually associated with bone marrow hypercellularity [26]. Myeloid hyperplasia observed in four animals in our study might be attributed to an initial increase in marrow cells. However, 16 animals had normal marrow cellularity, indicating that these animals were non-myelosuppressive. Significant increase in ALT and BUN values and significant reduction in platelets and albumin values in CME affected cases is seen in our study. These hemato-biochemical alterations were already reported by many authors earlier [16, 27, 24, 4, 11]. In animals treated with injectable doxycycline, there was a highly significant increase in erythrocyte count compared to pre-therapy levels. Additionally, there was a significant increase in platelet numbers and significant reduction in monocyte levels in the injectable doxycycline-treated group when compared to their pre-therapy values. A significant increase in platelet numbers was observed in dogs treated with oral doxycycline. The significant increase in hematocrit and platelet numbers, the absence of parasite DNA in peripheral blood and splenic aspirates, and clinical recovery provide evidence that injectable doxycycline was effective in clearing parasitic DNA. Dogs with pancytopenia and normocellular bone marrow typically respond well to medical treatment [7]. In a study by reference [28], dogs treated with generic doxycycline tablets recovered from CME after 20 days of therapy and tested negative in PCR at 28 days of therapy. Acute experimental CME has been successfully treated with doxycycline at 10 mg/kg, orally, once daily for 16 days (20), and 5 mg/kg, orally, twice daily for 14 days (29). Our study also demonstrates that a 15-day course of injectable doxycycline was effective in eliminating bacteria in natural cases.

Based on our study, we can conclude that bone marrow aspiration aids clinicians in identifying CME patients based on the presence of myelosuppression. Injectable doxycycline demonstrated a lack of notable side effects and proved to be convenient for dogs. After 15 days of therapy, the treated animals tested negative for 16S rRNA in both blood and splenic aspirates. Additionally, there was a significant increase in erythrocyte and platelet levels 28 days post-therapy in this group. Hence, injectable doxycycline therapy effectively eliminated parasitic DNA within a 15-day treatment period and demonstrated its efficacy.

Docmycin[1] Injectable Doxycycline USP, Alembic Pharmaceuticals Ltd, India.

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Conflict of Interest Statement

There is no conflict of interest between and within the authors.

Author's contribution

1. Project Design, Fundraising, Experimental Conduct, with a focus on Bone Marrow and Therapeutic Trials, Data Collection and Analysis, Manuscript Submission
2. Project Design, PCR Analysis of Blood and Spleen Samples, Data Collection and Analysis, Manuscript Submission
3. Fundraising, Experimental Conduct with Emphasis on Therapeutic Trials, Data Collection and Analysis
4. Bone Marrow Analysis, and Data Segmentation
5. Drawing data & analyse it

5. References

1. Berrada ZL, Telford SR. Burden of tick-borne infections on American companion animals. Topics in Companion Animal Medicine. 2009;24:175–181.
2. Rani PAA, Irwin PJ, Coleman GT, Gatne M, Traub RJ. A survey of canine tick-borne diseases in India. Parasites & Vectors. 2011;4:141-149.
3. Chomel B. Tick-borne infections in dogs—An emerging infectious threat. Veterinary Parasitology. 2011;179:294-301.
4. Harrus S, Waner T. Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): An overview. The Veterinary Journal. 2011;187:292–296.
5. Mylonakis ME, Koutinas AF, Breitscwerdt EB, Hegarty BC, Billinis CD, Leontides LS, Kontos VS. Chronic canine ehrlichiosis (*Ehrlichia canis*): A retrospective study of 19 natural cases. Journal of American Animal Hospital Association. 2004;40:174-184.
6. Shipov A, Klement E, Reuveni-Tager L, Waner T, Harrus S. Prognostic indicators for canine monocytic ehrlichiosis. Veterinary Parasitology, 2008;153:131–138.

7. Mylonakis ME, Ceron JJ, Leontides L, Siarkou VI, Martinez S, Tvarijonaviciute A. Serum acute phase proteins as clinical phase indicators and outcome predictors in naturally occurring canine monocytic ehrlichiosis. Journal of Veterinary Internal Medicine. 2011;25:811-817.
8. Mylonakis ME, Harrusb S, Breitschwerdt EB. An update on the treatment of canine monocytic ehrlichiosis (*Ehrlichia canis*). The Veterinary Journal. 2019;246:45–53
9. Neer TM, Breitschwerdt EB, Greene RT, Lappin MR. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. American College of Veterinary Internal Medicine. JVIM. 2002;16:309-315.
10. Schulz BS, Hupfauer S, Ammerm H, Sauter-Louism C, Hartmannm K. Suspected side effects of doxycycline use in dogs-a retrospective study of 386 cases. Veterinary Record. 2011;169:229-232.
11. Villaescusa A, García-Sancho M, Rodríguez-Franco F, Tesouro MÁ, Sainz Á. Effects of doxycycline on haematology, blood chemistry and peripheral blood lymphocyte subsets of healthy dogs and dogs naturally infected with *Ehrlichia canis*. The Veterinary Journal. 2015;204:263–268.
12. Sapadin AN, Fleischmajer R. Tetracyclines: Nonantibiotic properties and their clinical implications. Journal of The American Academy of Dermatology. 2006;54(2):258-265.
13. Lakshmanan B, John J, Gomathinayagam S, Dhinakarraj G. Molecular detection of *Ehrlichia canis* from blood of naturally infected dogs in India. Veterinarski arhiv. 2007;77(4):307-312.
14. Raskin RE, Messick JB. Bone Marrow Cytologic and Histologic Biopsies: Indications, Technique, and Evaluation. Veterinary Clinics North America. 2012;42: 23–42.
15. German AJ, Cannon MJ, Dye C, Booth MJ, Pearson GR, Reay CA, Gruffydd-Jones TJ, Oesophageal strictures in cats associated with doxycycline therapy. Feline Medicine & Surgery. 2005;7(1):33–41.
16. Diniz PPVP, Aguiar DMD, Ehrlichiosis and Anaplasmosis: An Update. Veterinary Clinics North America. 2022;52:1225–1266.
17. Cohn LA, Ehrlichiosis and related infections. Veterinary Clinics North America. 2003;33(4):863-84
18. Wen B, Rikihisa Y, Mott JM, Greene R, Kim HY, Zhi N, *et al*., Comparison of nested PCR with immunofluorescent-antibody assay for detection of *Ehrlichia canis* infection in dogs treated with doxycycline. Journal of Clinical Microbiology. 1997;35:1852–1855.
19. Moroff S, Sokolchik I, Woodring T, Woodruff C, Atkinson B, Lappin MR, *et al*., Use of an automated system for detection of canine serum antibodies against *Ehrlichia canis* glycoprotein 36. Journal of Veterinary Diagnostic Investigation. 2014;26:558–562.
20. Waner T, Hematopathological changes in dogs infected with *Ehrlichia canis*. Israel Journal of Veterinary Medicine. 2008;63:1–8.
21. Harrus S, Kenny M, Miara L, Aizenberg I, Waner T, Shaw S. Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental *Ehrlichia canis* infection. Antimicrobial Agents and Chemotherapy. 2004;48(11):4488-4490.
22. Faria JL, Dagnone AS, Munhoz TD, Joao CF, Pereira WA, Machado RZ. *Ehrlichia canis* morulae and DNA detection in whole blood and spleen aspiration samples. Revista Brasileira de Parasitologia Veterinária, 2010;19:98-102.
23. Rodríguez-Alarcón CA, Beristain-Ruiz DM, Olivares-Muñoz A, Quezada-Casasola A, Pérez-Casio F, Álvarez-Martínez JA, *et al*., Demonstrating the presence of *Ehrlichia canis* DNA from diferent tissues of dogs with suspected subclinical ehrlichiosis. Parasites & Vectors. 2020;13:518-525.
24. Sainz A, Roura X, Miró G, Estrada-Peña A, Kohn B, Harrus S, *et al*. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasites & Vectors. 2015;8:75-95.
25. Reardon MJ, Pierce KR, Acute experimental canine ehrlichiosis. II. Sequential reaction of the hemic and lymphoreticular system of selectively immunosuppressed dogs. Veterinary Pathology. 1981;18:384-395.
26. Mylonakis ME, Koutinas AF, Billinis C, Leontides LS, Kontos V, Papadopoulos O, *et al*., Evaluation of the cytological diagnosis in the acute monocytic ehrlichiosis (*Ehrlichia canis*) of the dog: A comparative study between five cytological methods. Veterinary Microbiology. 2003;91:197-204.
27. Angkanaporn K, Sanguanwai J, Baiyokvichit TO, Vorrachotvarittorn P, Wongsompong M, Sukhumavasi W, *et al*. Retrospective analysis of canine monocytic ehrlichiosis in Thailand with emphasis on hematological and ultrasonographic changes, Veterinary World. 2022;15(1):1-9.
28. Fourie JJ, Horak I, Crafford D, Erasmus HL, Botha OJ. The efficacy of a generic doxycycline tablet in the treatment of canine monocytic ehrlichiosis. Journal of the South African Veterinary Association. 2015;86(1):1193 - 1202.
29. Breitschwerdt EB, Hegarty BC, Hancock SI. Doxycycline hyclate treatment of experimental canine ehrlichiosis followed by challenge inoculation with two *Ehrlichia canis* strains. Antimicrobial Agents & Chemotherapy. 1998;42:362–368.