

MOLECULAR CONFIRMATION AND THERAPEUTIC RESPONSE TO MINOCYCLINE IN DOGS WITH CANINE MONOCYTIC EHRLICHIOSIS

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ABSTRACT

Canine monocytic ehrlichiosis (CME), caused by Ehrlichia canis, is a tick-borne disease associated with multisystemic involvement and hematobiochemical alterations. The present study evaluated diagnosis, therapeutic response and clinical outcome in eight infected dogs. Diagnosis was established by peripheral blood smear examination and confirmed by polymerase chain reaction. All dogs received minocycline at 10 mg/kg intravenously every 12 h for 4 days, followed by oral administration at the same dose for 24 days. Supportive therapy included silymarin, platelet-enhancing agents and additional treatment as required. Clinical and hematobiochemical improvements occurred, with complete recovery in all dogs by day 28.

Key words: *Ehrlichia canis*, minocycline, PCR

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INTRODUCTION

Canine monocytic ehrlichiosis (CME) is an important tick-borne disease of dogs caused mainly by the obligate intracellular bacterium *Ehrlichia canis*. It is transmitted primarily by the brown dog

tick, *Rhipicephalus sanguineus* and occurs widely in tropical and subtropical regions (Aziz *et al.*, 2023). The disease may occur in acute, subclinical, or chronic forms, with clinical severity varying among affected animals. Common manifestations include fever, lethargy, anorexia, lymphadenopathy, thrombocytopenia, anemia and bleeding tendencies, while severe or chronic infections may result in pancytopenia, weight loss, ocular abnormalities and neurological signs (Kabir *et al.*, 2024). Tick infestation, outdoor exposure, inadequate ectoparasite control and poor tick-prevention practices are important

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risk factors for infection. Diagnosis is commonly based on clinical findings, blood smear examination, serological tests and molecular techniques. Although morulae may be detected microscopically, their occurrence is often low, particularly in chronic infections. Serological tests may also show cross-reactivity, whereas polymerase chain reaction (PCR) provides more specific evidence of *E. canis* infection (Kukreti *et al.*, 2018). Minocycline, a tetracycline-class antimicrobial, has potential as an alternative therapeutic option for CME. Its greater lipophilicity and higher tissue concentrations may offer therapeutic advantages over doxycycline, particularly in *E. canis* infections involving the central nervous system (Jenkins *et al.*, 2018 and Palerme, 2026). In view of these potential pharmacological advantages, the present case series was undertaken to molecularly confirm *E. canis* infection and evaluate the clinical and hematobiochemical response to minocycline therapy in naturally infected dogs

MATERIAL AND METHOD

A total of eight dogs were presented with various clinical signs. Five dogs had a history of lethargy, inappetence, vomiting, tick infestation and melena, while two dogs exhibited epistaxis along with hematemesis and one dog had a history of coughing accompanied by epistaxis. On physical examination, all eight dogs were found to be febrile, with body temperatures reaching up to 104°F. Pale mucous membranes, conjunctival congestion and lymphadenopathy were

consistently observed in all cases. Approximately 1 ml of blood was collected in EDTA tubes for haematology and 4 ml of blood was collected in serum tubes for rapid immunochromatographic testing and biochemical analysis. Peripheral capillary blood smears were stained with Giemsa stain and examined under oil immersion (100×) for detection of morulae. Molecular detection of *E. canis* was performed as per the standard protocol described by Murphy *et al.* (1998) and Singh *et al.* (2018). To conduct the PCR assays, genomic DNA was isolated from whole blood using a QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany). PCR was carried out using primers ECAN5: 5'-CAATTATTTATAGCC TCTGGCTCTGGCTATAGGA-3' HE3: 5'-TATAGGTACCGTCATTATCTTCCCTAT-3' and 389 base pair (bp) product specific for *E. canis* visualized in 1% agarose gel electrophoresis. PCR reactions followed initial denaturation at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 55°C for 2 min, extension at 72°C for 1.5 min, final extension at 72 °C for 8 min performed in 37 cycles.

Statistical analysis

Changes across different various intervals were analysed using two-way ANOVA and the means were compared using Post Hoc, Duncan's Multiple Range Test (Snedecor and Cochran, 1994). Data were analyzed using IBM SPSS Statistics Version 30.0 (IBM Corp., Armonk, NY, USA). Statistical significance was considered at $p < 0.05$.

RESULT AND DISCUSSION

In all the cases peripheral blood smear examination of Giemsa-stained blood smears revealed the presence of *E. canis* morulae within monocytes (Fig. 01). The haematological and biochemical findings of the eight dogs diagnosed with CME are presented in Table 1. Anaemia and thrombocytopenia were prominent findings at day 0, with mean PCV and haemoglobin values of $25.71 \pm 1.19\%$ and 8.58 ± 0.43 g/dL, respectively. Platelet count was markedly reduced ($111.62 \pm 13.86 \times 10^3/\mu\text{L}$) and showed a significant progressive increase to 195.12 ± 11.40 and $260.62 \pm 10.79 \times 10^3/\mu\text{L}$ on days 14 and 28, respectively. The mean WBC count decreased from 19.34 ± 4.61 to 15.46 ± 0.73 and $12.24 \pm 0.71 \times 10^3/\mu\text{L}$ on days 14 and 28, respectively. Neutrophil, monocyte and lymphocyte percentages showed no significant changes during treatment, whereas eosinophils increased significantly by day 28. Biochemical analysis revealed significant reductions in AST, total bilirubin, globulin, BUN and creatinine following treatment. ALT also decreased from 61.44 ± 26.52 to 47.42 ± 8.01 U/L, although the change was non-significant. Total bilirubin decreased from 0.42 ± 0.10 to 0.11 ± 0.04 mg/dL. Albumin increased significantly from 1.87 ± 0.25 to 2.65 ± 0.19 and 2.76 ± 0.17 g/dL on days 14 and 28, respectively, while total protein remained statistically unchanged.

PCR amplification produced the expected specific 389-bp product on 1% agarose gel electrophoresis, confirming *E. canis* DNA in the tested samples (Fig

02). All eight confirmed dogs were treated with intravenous normal saline according to dehydration status and minocycline (Divaine® Cipla Ltd., Mumbai) @10 mg/kg body weight every 12 h for 4 days, followed by oral tablets minocycline (Minoz®50, Sun Pharmaceutical Industries Ltd., Baddi, Solan, Himachal Pradesh, India) at the same dose for 24 days. Supportive therapy included NSAIDs for pyrexia during the initial treatment period, silymarin (Revell® Sava Healthcare India), platelet-enhancing agents (Imu-late® Neo Kumfurt Solutions Pvt. Ltd. Navi Mumbai) as per standard dose and other symptomatic medications (pantoprazole and domperidone) according to individual requirements.

Persistent fever (up to 104°F), inappetence and anorexia were prominent clinical findings, consistent with observations reported by Espino-Solis *et al.* (2023). The observed anaemia and thrombocytopenia were comparable with previous reports (Jeevitha *et al.*, 2024). Thrombocytopenia in ehrlichiosis may be associated with increased platelet destruction, sequestration, endothelial dysfunction and immune-mediated mechanisms. Elevated ALT and AST suggested hepatocellular involvement, in agreement with reports of hepatic alterations in canine ehrlichiosis (Abhijith *et al.*, 2026). Minocycline therapy was associated with progressive clinical and haematobiochemical improvement, with complete recovery by day 28. Pillco *et al.* (2025) reported comparable therapeutic effects of minocycline and doxycycline, suggesting that minocycline may serve as an alternative when doxycycline is

unavailable. Its greater lipophilicity and tissue penetration may provide additional therapeutic advantages. However, larger controlled studies are required to establish its efficacy conclusively in *E. canis* infection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

Table 1. Haematobiochemical findings in CME-affected dogs

Parameter	0 th Days	14 th Days	28 th Days
PCV (%)	25.71 ± 1.19 ^a	36.94 ± 1.20 ^b	41.10 ± 1.23 ^b
Hemoglobin(g/dl)	8.58 ± 0.43 ^a	12.19 ± 0.51 ^b	13.64 ± 0.70 ^b
WBC (thou/cu.mm)	19.34 ± 4.61 ^{ab}	15.46 ± 0.73 ^a	12.24 ± 0.71 ^b
Neutrophil (%)	64.17 ± 5.10 ^a	60.56 ± 2.67 ^a	62.54 ± 1.01 ^a
Monocyte (%)	4.09 ± 1.04 ^a	4.66 ± 0.58 ^a	4.91 ± 0.50 ^a
Lymphocyte (%)	28.34 ± 4.51 ^a	20.45 ± 1.46 ^a	19.04 ± 1.23 ^a
Eosinophil (%)	2.15 ± 0.77 ^{ab}	2.30 ± 0.43 ^a	3.85 ± 0.41 ^b
Basophil (%)	0.10 ± 0.07 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Platelet count (thou/cu.mm)	111.62 ± 13.86 ^a	195.12 ± 11.40 ^b	260.62 ± 10.79 ^c
ALT (U/L)	61.44 ± 26.52 ^a	60.36 ± 15.54 ^a	47.42 ± 8.01 ^a
AST(U/L)	42.60 ± 12.18 ^a	29.29 ± 8.58 ^b	28.66 ± 5.68 ^{ab}
Total bilirubin (mg/dl)	0.42 ± 0.10 ^a	0.11 ± 0.04 ^b	0.11 ± 0.04 ^b
Total protein (gm/dl)	6.34 ± 0.34 ^a	6.07 ± 0.28 ^a	6.11 ± 0.26 ^a
Albumin (gm/dl)	1.87 ± 0.25 ^a	2.65 ± 0.19 ^b	2.76 ± 0.17 ^b
Globulin (gm/dl)	4.47 ± 0.21 ^a	3.41 ± 0.38 ^b	3.35 ± 0.33 ^b
BUN (mg/dl)	26.99 ± 2.45 ^a	16.25 ± 1.45 ^b	16.02 ± 1.42 ^b
Creatinine (mg/dl)	1.52 ± 0.16 ^a	1.10 ± 0.13 ^b	1.01 ± 0.07 ^c

Means bearing uncommon superscripts within a row (a, b, c) differ significantly at $p < 0.05$.

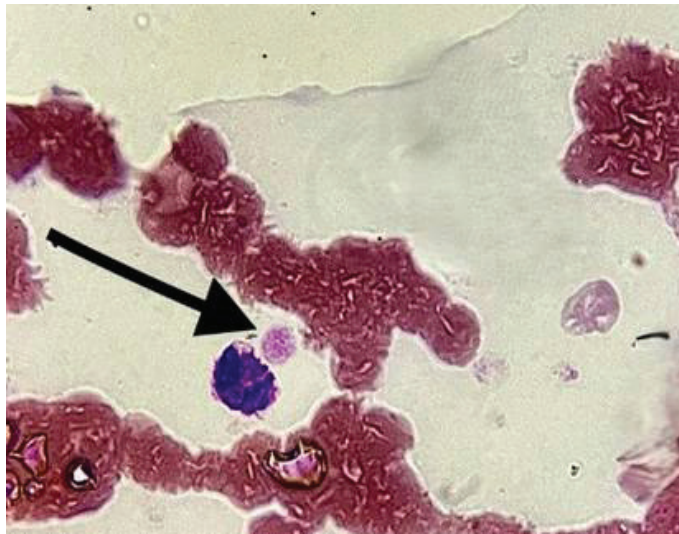


Fig.1. Arrow indicate Morulae stage of *E. canis* in monocyte as morulae from peripheral blood smear of dog



Fig.2. PCR amplification of *E. canis* showing 389-bp of PCR product Lane No - Sample Description

- 1. Ehrlichia canis – negative control
- 2. Ehrlichia canis – positive sample
- 3.. Ehrlichia canis - Positive control
- M. DNA Ladder – 100 bp

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