DOI: https://doi.org/10.54393/mjz.v5i03.151

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Original Article

OPEN ACCESS

Prevalence and Chemotherapy of Canine Monocytic Ehrlichiosis in Lahore, Pakistan

Moez Iftikhar[1], Syed Saleem Ahmed[1], Muhammad Ijaz[1], Asfand Yar Khan[2*], Muhammad Muneeb[2], Mehlib Hussain[2], Danial Ahmad[2], Sajid Zaman[2] and Hizbullah[2]

[1]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan

ARTICLE INFO

Keywords:
Ehrlichia canis, Microscopy, Immunochromatography, Ticks, Rifampicin

How to cite:
Iftikhar, M., Ahmed, S. S., Ijaz, M., Khan, A. Y., Muneeb, M., Hussain, M., Ahmad, D., Zaman, S., & Hizbullah, . (2024). Prevalence and Chemotherapy of Canine Monocytic Ehrlichiosis in Lahore, Pakistan: Prevalence and Chemotherapy of Canine Monocytic Ehrlichiosis . MARKHOR (The Journal of Zoology), 5(03), 33-39. https://doi.org/10.54393/mjz.v5i03.151

***Corresponding Author:**
Asfand Yar Khan
Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan
dr.asfandkhan888@gmail.com

Received Date: 27th July, 2024
Acceptance Date: 21st September, 2024
Published Date: 30th September, 2024

ABSTRACT

Preventing animals from vector-borne diseases is of prodigious concern in the current era. **Objective:** To estimate the prevalence of Canine Monocytic Ehrlichiosis (CME) (*Ehrlichia canis*) in Lahore, Pakistan. **Methods:** For initial diagnosis, microscopy was performed. Later on, anti-*E. canis* antibodies were detected through immunochromatography (ICA) based *Ehrlichia canis* Ab tests (Quicking biotech ®). A total of 100 dogs showing clinical signs of CME were tested, and an overall seroprevalence rate of 29% was noticed. The success rate of microscopy was observed to be 3% in the present study. **Results:** Occurrence of CME was not statistically associated with age, sex, and breed ($p>0.05$), while body condition ($p<0.001$) and tick infestation ($p=0.004$) were significantly associated with CME. Moreover, blood parameters, including Hb, RBC count, WBC count, and Total platelet count, were decreased ($p<0.001$) in the infected animals. Rifampicin was found to be more effective in diminishing clinical signs and normalization of blood parameters as compared to Doxycycline. **Conclusions:** Dogs in Lahore are at great risk of acquiring CME due to increased tick population and lack of awareness among owners. For rapid diagnosis and timely treatment against the infection, veterinarians can use ICA-based rapid test kits as an effective tool. In addition, Rifampicin is found to be more effective than Doxycycline, and may be preferred for the treatment of Canine Monocytic Ehrlichiosis.

INTRODUCTION

Canine Monocytic Ehrlichiosis (CME) poses a significant threat to the dog population of the world. It is caused by *Ehrlichia canis*, an obligate, intracellular, and pleomorphic gram-negative proteobacterium of the genus Anaplasma and family *Anapalasmataceae* [1]. *E. canis* is transmitted through a Brown tick named *Rhipicephalus sanguineus* of the Ixodidae family prevalent worldwide [2]. *E. canis* multiplies in the salivary glands, hemocytes, and midgut of the tick after feeding on the blood of an infected animal [3]. *E. canis* was first identified in Algeria in 1935 by Donatien and Lestoquard. Later, the disease was reported in Tunisia [4]. Infections due to *E. canis* have been commonly reported from tropical and subtropical areas of the world [5]. In Pakistan, *Ehrlichia* infections are commonly reported in dogs. Literature revealed that the prevalence of *E. canis* in Lahore was 48%, with an overall prevalence of 28% in Punjab, Pakistan [6]. Canine Monocytic Ehrlichiosis obtained global attention in 1970 due to the high mortality rate of German Shepherd dogs owned by the army during the Vietnam War [7]. *E. canis* primarily attacks immune cells, in particular monocytes, resulting in a cytoplasmic membrane-bound cluster of bacteria known as morulae [8]. This disease is multi-systemic and affects multiple organs and has three forms, including acute, subclinical, and chronic [9]. During the acute form, major signs in dogs are pyrexia, anorexia, mild anemia, lymphadenopathy, epistaxis, splenomegaly, and ophthalmic lesions. Moreover, the complete blood count of an infected dog

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shows drastic changes, including severe thrombocytopenia and mild leukopenia during the acute phase [10]. In subclinical CME, thrombocytopenia of the mild category, along with a notable decline in leucocytic count, is observed [11]. The chronic form of disease is more lethal and involves severe pancytopenia, anemia, weakness, paralysis, and death [12]. Microscopic examination of stained thin blood smear, PCR, and ELISA are the technical aids for confirmatory diagnosis [13]. Inclusions of this rickettsial organism are present in the cytoplasm of monocytes, detected when blood is stained with Romanovsky stains. Inclusions are present as dense granules that are identified after staining. Multiplication of organisms observed in macrophages and endothelial cells can be identified by microscopy of stained blood smears [14]. The inclusion bodies vary in shape and size. They can be round or oval, and the size of the smaller spherical structure can be 0.2µm -0.4µm, and of the larger ovoid structures can range between 2.5µm -3µm in diameter. The success rate of morulae identification through blood smear is very low, and it is a difficult and time-consuming route [15]. Microscopy of blood smears stained with Giemsa stain appeared to be an insensitive technique in the subclinical form of CME. Although PCR, IFA, and ELISA are more specific and sensitive but they could be expensive as well as time-consuming, moreover require specialized personnel to process samples. Immunochromatography is a rapid and comparatively cheaper diagnostic technique that could prove convenient in field practices such as veterinary clinics. Sensitivity and specificity of immunochromatography-based tests are reported to be 79.2% and 95.3% respectively [16]. Multiple clinical and experimental trials have shown that dogs with acute infection recovered subsequently to specific treatment with effective antibiotics administered for the recommended period. Even with the recommended dose of antibiotics, some dogs are found to be subclinical carriers if treated for a shorter duration. The drug of choice for treatment of Ehrlichiosis is Doxycycline at the dose rate of 5mg/kg twice daily (BID) for 4 weeks [17]. Rifampicin, known to be an inhibitor of DNA-dependent RNA polymerase, specifically its B subunit, has been found effective in the clearance of subclinical, clinical, as well as chronic CME. Rifampicin 15mg/kg twice daily (BID) for 21 days was found effective in dogs that did not respond after Doxycycline therapy [8]. Evaluation of therapeutic efficacy is also a significant challenge. Usually diminishing of clinical signs and normalization of hematological parameters are considered as indicators of the therapeutic efficacy [17].

This study aimed to assess the current status of CME in Lahore, Pakistan, to develop a better and alternative route for rapid diagnosis, along with a more effective line of treatment.

METHODS

A cross-sectional observational study was conducted from April 2020- September 2020. Blood samples were collected as per recommendations and permission from the Ethical Review Committee of the University of Veterinary and Animal Sciences (UVAS), Lahore. The current study was carried out at the Pet center (UVAS), government hospitals, and private veterinary clinics in Lahore. A total of 100 pet dogs' samples were collected. Sample size was calculated using an expected prevalence of 33%, a 95% confidence level, and a 10% margin of error. Dogs showing any of the following signs, i.e., fever, lethargy, hematuria, anemia, vomiting, and epistaxis, were included in this study. Inspected dogs were classified into 3 groups concerning age: a)<1 year, b)1-3 years, and c)>3 years. Data regarding owner, animal, management, environment, etc., were recorded on a predesigned questionnaire at the time of sample collection. Sample collection was done in two ways. Convenience sampling was used. Initially, thin blood smears (triplet) were made by using ear tip venipuncture and were air-dried on the spot. Then, blood samples were drawn from the cephalic vein into 3 ml gel-clot activator and EDTA-coated vacuum vials separately. The samples were transported to the Medicine laboratory, UVAS, in an ice box. The sera were centrifuged and preserved (-40°C) for further serological assay. The blood smears were fixed in a 10% ethanol solution, and Giemsa stain was used for staining of the blood smears. After 15 min, the stain was wiped out with water, and the smears were air-dried and observed at 100X oil immersion lens. Then smears were examined for the presence of intracytoplasmic inclusion bodies. For serological diagnosis of CME, a commercially available Quicking *Ehrlichia canis* Ab test kit (Quicking biotech. Cat No. W81132) was used according to the manufacturer's instructions. The result was interpreted as follows: Positive: Appearance of both C band and T band, no matter T band was clear or faint. Negative: If only a clear C band appeared. Invalid: When no color band appeared in the C zone, no matter whether the T band appeared. For hematology, 10 dogs were equally divided into two groups based on serological diagnosis, i.e., group A: CME-infected dogs, group B: healthy dogs. Blood samples were analyzed (total erythrocyte count; TEC, total leukocyte count; TLC, hemoglobin; Hb, platelet count; PC, and packed cell volume; PCV) using a hematological analyzer (Model no. DW-3680/DW-36). Hematology was performed at day 0, 7, 14, and after completion of the treatment protocol at day 21. Chemotherapy was conducted on 10 dogs positive for Ehrlichiosis based on microscopy and serological testing, and were divided into 2 equal groups, A and B. Doxycycline

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@ 5 mg/kg body weight twice daily (BID) and Rifampicin @ 15 mg/kg body weight twice daily (BID) PO were used to treat dogs of group A and B, respectively, for 21 days. The efficacy of the drug used was measured on the basis of the disappearance of clinical signs and hematological parameters noticed at day 0, 7, 14, and 21, after initiation of treatment. Data analysis was conducted using appropriate statistical methods. Serological prevalence was calculated using the Thrusfield formula. Therapeutic trials against ehrlichiosis were evaluated using the chi-square test. Hematological data were analyzed using the Student's t-test, while repeated-measures ANOVA was employed to assess hematological parameters in therapeutic trials. All statistical analyses were performed using SPSS version 20.0, with a p-value of <0.05 considered statistically significant.

RESULTS

Out of 100, dog samples were found positive (29%) for *Ehrlichia canis*. Prevalence was marginally found higher (p=0.848) in dogs of age 1-3 years, followed by the group of age >3 years and <1 year, respectively. *E.canis* infection was noted to be numerically more prevalent (p=0.739) in male dogs as compared to the female (Table 1).

Table 1: Prevalence of CME According to Age and Sex of Animal

Variables	No. of samples	Positive	Negative	p-Value
Age				
<1 Year	17	4 (23.53%)	13 (76.47%)	0.848
1-3 Years	62	19 (30.64)	43 (69.36%)	
>3 Years	21	6 (28.57)	15 (71.43%)	
Sex				
Male	63	19 (30.16%)	44 (69.48%)	0.739
Female	37	10 (27.02%)	27 (72.8%)	

Presence of ticks and emaciated body condition were noted as significant determinants for CME (p=0.004; p<0.001, respectively)(Table2).

Table 2: Association of Tick Infestation and Body Condition with Prevalence of CME

Variables	Category	No. of samples	Positive	Negative	p-Value
Tick Infestation					
Ticks infestation	Yes	57	23 (40.35%)	34 (59.65%)	0.004
	No	43	6 (13.95%)	37 (86.05%)	
Ticks history	Yes	72	27 (37.50%)	45 (62.50%)	0.003
	No	28	2 (7.14%)	26 (92.86%)	
Body Condition					
Body Condition	Healthy	52	4 (7.69%)	48 (92.31%)	<0.001
	Emaciated	48	25 (52.08%)	23 (47.92%)	

Clinical signs such as fever (p=0.642), vomiting (p=0.222), splenomegaly (p=0.786), and lethargy (p=0.462) were noted as non-significantly associated with CME, whereas epistaxis (p=0.001) and anemia (p=0.025) were noted to be significantly associated. Thrombocytopenia was shown by 23 (p=0.025) dogs positive for ehrlichiosis and was noted to be statistically significant (Table 3).

Table 3: Association of Clinical Manifestations and CME

Variables	Category	Positive (%)	Negative (%)	p-Value
Fever	Present	17 (30.90)	38 (69.10)	0.642
	Absent	12 (26.67)	33 (73.33)	
Vomiting	Present	09 (39.13)	14 (60.87)	0.222
	Absent	20 (25.97)	57 (74.03)	
Mucous Membranes	Pink	08 (17.78)	37 (82.22)	0.025
	Pale	21 (38.18)	34 (61.82)	
Epistaxis	Present	11 (61.11)	07 (38.89)	0.001
	Absent	18 (21.95)	64 (79.05)	
Splenomegaly	Present	07 (25.00)	19 (75.00)	0.786
	Absent	22 (29.73)	52 (70.27)	
Lethargy	Lethargic	08 (24.24)	25 (75.76)	0.462
	Active	21 (31.34)	46 (68.66)	

p-value<0.05 indicates significance.

Hematological parameters are severely affected by *E.canis*. There is a significant decrease in HB, PCV, RBCs, and Thrombocytes (Table 4).

Table 4: Blood Parameters of Diseased and Healthy Dogs

Parameters	Diseased dogs	Healthy dogs	p-Value
WBCs	8.48 ± 1.67	10.76 ± 0.46	<0.001
RBCs	2.94 ± 0.46	6.48 ± 0.61	<0.001
Hb	7.40 ± 1.34	15.80 ± 1.92	<0.001
PCV	28.00 ± 1.58	41.80 ± 2.38	<0.001
Thrombocytes	44 ± 7.86	313 ± 23.55	<0.001
Monocytes	5.74 ± 0.86	6.44 ± 0.96	0.261
Lymphocytes	20.08 ± 0.84	23.76 ± 3.48	0.051
Granulocytes	66.40 ± 3.13	68.80 ± 3.49	0.286

p-value<0.05 indicates significance

Characteristic *E.canis* morulae were found in the stained microscopic blood slides of only 3 positive dogs. While 29 dogs tested positive serologically using Quicking *Ehrlichia canis* Ab tests (Quicking biotech, Cat No. W81132). Results of Immunochromatographic Assay (ICA) for detection of *Ehrlichia canis* antibodies in canine serum samples using the Quicking *Ehrlichia canis* Ab test kit were shown (Figure 1).

DOI: https://doi.org/10.54393/mjz.v5i03.151

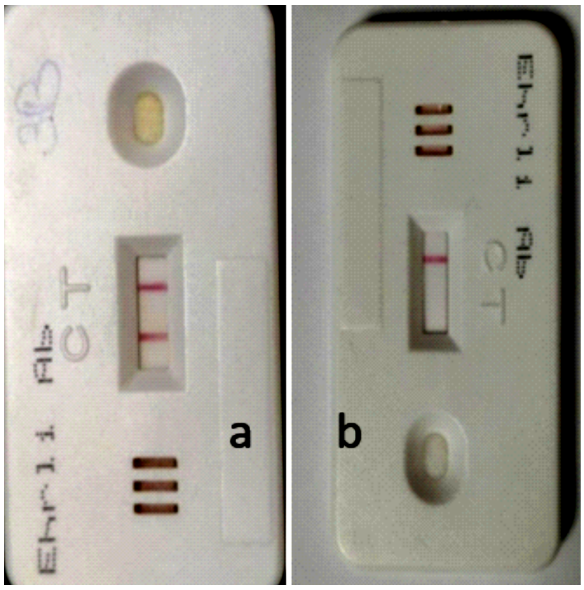

Figure 1: a) Rapid Test Kit Showing Positive Test Result and (b) Rapid Test Kit Showing Negative Test Result

Results of the Rifampicin-treated group showed that all dogs in the group had normalized temperature, controlled vomiting, and epistaxis by day 14. Anorexia and blood parameters were normalized by day 21. Blood parameters, including Hb, RBCs, platelets, and PCV, were noted to be in normal range by day 21, showing 100% efficacy in the elimination of infection. Results of the Doxycycline-treated group showed that 4 dogs out of 5 in the group had normalized temperature, controlled vomiting, epistaxis, and anorexia by day 21. Blood parameters of 4 dogs got normalized, falling in the lower limits of the normal ranges, while 1 dog remained with abnormal hematology after the completion of the protocol. Blood parameters, including Hb, RBCs, platelets, and PCV, were noted to be in normal range by day 21, depicting efficacy of 80% (Table 5).

Table 5: Comparison of Blood Parameters of Dogs Treated with Doxycycline and Rifampicin

Parameter	Groups	Day 0	Day 7	Day 14	Day 21	CI 95 %	p-Value
Hb	Group A	7.56 ± 0.89	7.920 ± 0.83	9.180 ± 1.11	10.78 ± 1.65	10.94-12.33	<0.001
	Group B	7.60 ± 1.517	9.960 ± 1.11	11.740 ± 0.56	13.06 ± 0.39		
	Healthy	15.22 ± 1.92	15.440 ± 1.51	15.620 ± 1.57	15.56 ± 1.53		
RBCs	Group A	3.10 ± 0.69	3.860 ± 0.68	4.680 ± 1.02	4.98 ± 1.11	4.75-5.38	<0.001
	Group B	3.04 ± 0.18	4.080 ± 0.22	4.920 ± 0.26	5.88 ± 0.24		
	Healthy	6.48 ± 0.61	6.640 ± 0.57	6.680 ± 0.52	6.44 ± 0.38		
Platelets Count	Group A	44.20 ± 5.89	151.40 ± 36.07	279.40 ± 97.11	279.80 ± 59.88	258.19-297.43	<0.001
	Group B	44.60 ± 7.86	166.00 ± 6.96	362.00 ± 20.29	323.00 ± 33.04		
	Healthy	410.00 ± 46.16	420.40 ± 39.93	431.80 ± 34.29	421.20 ± 29.97		
PCV	Group A	29.60 ± 2.07	33.00 ± 3.39	35.20 ± 4.09	36.80 ± 4.38	37.22-40.58	<0.001
	Group B	30.00 ± 2.91	36.60 ± 1.67	41.60 ± 1.67	43.00 ± 0.71		
	Healthy	45.60 ± 4.56	44.40 ± 3.51	45.20 ± 3.96	45.80 ± 3.35		

Abbreviations: Hb; Hemoglobin, RBCs; Red blood cells, PCV; Packed cell volume. Values differ significantly where p-value<0.05

DISCUSSION

Canine Monocytic Ehrlichiosis is reported throughout the globe [1] in tropical as well as subtropical areas [5]. Increased occurrence of CME is reported because its vector population density is high throughout the world [18]. In Pakistan, prevalence and molecular characterization of E.canis have been reported [6], but to date, there is no data available regarding the efficacy of the treatment protocol. Therefore, this is a novel study regarding serological prevalence and association of risk factors with dynamics of the disease in dogs of District Lahore, Pakistan. Overall prevalence of CME in this study, on the basis of immunochromatography-based rapid test kits, was 29%. Current findings are supported by the previous studies, which reported PCR-based prevalence to be 30% in India [19] and ELISA-based prevalence to be 30.98% in Iran [20]. In contrast, a higher prevalence of 48% in Lahore [6] and 57.7% in India [21] has been reported. On the other hand, a 9.6% prevalence has been documented on the basis of Immunochromatography in Iran [16]. The plausible reason for variation in the prevalence of CME in different regions of the world may be associated with geographical climate, socioeconomic status of the population, vector load, and infection status. Analysis of risk factors showed that it is socioeconomic and climatic factor that plays a vital role rather than the immunological status of the animal [22]. A higher number of male dogs tested positive for the disease as compared to female dogs in the current study, but this association was noted to be non-significant, indicating no relationship between prevalence of disease and sex of animal, as supported by similar studies in which more male

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dogs were reported positive for the disease than female dogs [6]. On the other hand, Selim *et al.* reported a higher prevalence of disease in female dogs as compared to male dogs [23]. Adult animals were infected at a higher rate as compared to young animals in the current study, which is also supported by [24]. This increased infection rate in adult animals could be explained by increased exposure to the vector over time. Contrary to this, Botros *et al.* reported a higher incidence of CME in young animals than in adults [25]. Canine Monocytic Ehrlichiosis is a tick-borne ailment, and that is why the rate of infection in dogs with tick infestation and a history of previous tick infestation is higher. In the current study, animals with tick infestation tested positive at a higher rate as compared to those without ticks. This finding is also supported by [10]. In this study, animals with poor body condition had a higher infection rate as compared to animals with good body condition. This finding is also supported by [24]. Microscopic identification of *E. canis* is comparatively problematic, as the success rate of this technique is very low. In the current study, only 3 samples out of 29 positive samples and overall, 100 samples tested were found positive on the basis of microscopy, with a success rate of only 3% which is supported by [26] who reported a success rate of microscopic examination to be 2.33%. Serological diagnosis through immunochromatography assay (ICA) based rapid test kits provided better results and are a better and quicker tool to diagnose the disease at early stages, and these results are supported by [16]. The values of RBCs, WBCs, PCV, Hb, and Thrombocytes were significantly decreased in Ehrlichia-infected dogs in the current study. Anemia is characterized by decreased RBCs, HB, and PCV. The findings of the current study were supported by Parashar *et al.* who screened 46 infected dogs and found similar results as in the current study [27]. Thrombocytopenia, the characteristic feature of CME, can be explained by low production of thrombocytes due to aplastic anemia, annihilation of thrombocytes as a result of an immune response, sequestration, and depletion of the cells as a result of an inflammatory response. Severe thrombocytopenia can be a predictor of clinical as well as subclinical ehrlichiosis. This statement is also supported by Moonarmart *et al.* who reported that every 10000 decrease in thrombocytes increases the 15% probability of an animal being positive for ehrlichiosis [10]. Rifampicin, a DNA-dependent RNA polymerase inhibitor, is considered an alternative to Doxycycline in the treatment of ehrlichiosis in dogs. In the current study, Rifampicin was found to be more effective in diminishing clinical manifestations along with normalization of hematological parameters as compared to Doxycycline against canine ehrlichiosis. All 5 animals treated with Rifampicin showed complete recovery after completion of the treatment protocol. These rectifications of clinical signs in response to Rifampicin were supported by Schaefer *et al.* who reported complete recovery of 2 Ehrlichia-infected dogs after completion of the Rifampicin regimen [28]. Akhtardanesh *et al.* also reported similar results of all dogs treated with Rifampicin tested negative and clinical signs diminished after a week [29]. However, Doxycycline is considered as drug of choice against ehrlichiosis in dogs, but in the current study, 4 dogs out of 5 recovered when treated with Doxycycline. These findings are well supported by Mylonakis *et al.* who reported failure in clearance of infection when treated with Doxycycline against CME [8].

CONCLUSIONS

The prevalence of Canine Monocytic Ehrlichiosis in Lahore, Pakistan, is found to be 29%. Rifampicin is found to be more effective than Doxycycline (drug of choice for Ehrlichiosis), and may be preferred for treatment of Canine Monocytic Ehrlichiosis.

Authors Contribution

Conceptualization: MI[1]
Methodology: MI[1], SSA, AYK
Formal analysis: MI[1], MI[2], AYK, MM
Writing review and editing: SSA, AYK, MM, MH, DA, SZ, H

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Ferrolho J, Antunes S, Vilhena H, Anastácio S, Ramalho de Sousa S, Frouco G et al. The Complexities of Canine Monocytic Ehrlichiosis: Insights into Ehrlichia canis and Its Vector Rhipicephalus sanguineus. Microbiology Research. 2025 Apr; 16(4): 85. doi: 10.3390/microbiolres16040085.

[2] Zhang G, Naeem MI, Akhtar T, Younus M, Nisa QU, Ameer T *et al.* Ehrlichiosis: Tick-Borne Malady. One Health Triad, Unique Scientific Publishers, Faisalabad, Pakistan. 2023; 3: 69-77. doi: 10.47278/book.oht/2023.79.

[3] Groth M, Skrzydlewska E, Dobrzyńska M, Pancewicz S, Moniuszko-Malinowska A. Redox Imbalance and Its Metabolic Consequences In Tick-Borne Diseases. Frontiers in Cellular and Infection Microbiology. 2022

DOI: https://doi.org/10.54393/mjz.v5i03.151

Jul; 12: 870398. doi: 10.3389/fcimb.2022.870398.

[4] Masika SJ. Prevalence and Genetic Characterization of Anaplasma Phagocytophilum in Olive Baboons and Vervet Monkeys in Laikipia County, Kenya (Doctoral dissertation, Uon). 2021. doi: 10.21203/rs.3.rs-355087/v1.

[5] Marshet B and Dessie D. A Review on Canine Ehrlichiosis and Its Zoonotic Implications. International Journal of Veterinary Sciences and Animal Husbandry. 2020; 5(3): 36-42.

[6] Malik MI, Qamar M, Ain Q, Hussain MF, Dahmani M, Ayaz M *et al.* Molecular Detection of Ehrlichia Canis in Dogs from Three Districts in Punjab (Pakistan). Veterinary Medicine and Science. 2018 May; 4(2): 126-32. doi: 10.1002/vms3.94.

[7] Aziz MU, Hussain S, Song B, Ghauri HN, Zeb J, Sparagano OA. Ehrlichiosis in Dogs: A Comprehensive Review About the Pathogen and Its Vectors with Emphasis on South and East Asian Countries. Veterinary Sciences. 2022 Dec; 10(1): 21. doi: 10.3390/vetsci10010021.

[8] Mylonakis ME and Theodorou KN. Canine Monocytic Ehrlichiosis: An Update on Diagnosis and Treatment. Acta Veterinaria (Beograd). 2017; 67(3): 299-317. doi: 10.1515/acve-2017-0025.

[9] Checa R, Peteiro L, Pérez-Hernando B, de la Morena M, Cano L, López-Suárez P *et al.* High Serological and Molecular Prevalence of Ehrlichia Canis and Other Vector-Borne Pathogens in Dogs from Boa Vista Island, Cape Verde. Parasites and Vectors. 2024 Sep; 17(1): 374. doi: 10.1186/s13071-024-06437-9.

[10] Moonarmart W, Sungpradit S, Rawangchue T, Suphaphiphat K, Suksusieng S, Jirapattharasate C. Clinical History and Hematological Findings among Canines with Monocytic Ehrlichiosis. Southeast Asian Journal of Tropical Medicine and Public Health. 2014; 45(1): 157.

[11] Jaheen AH, Kubesy AA, Rakha GM, Salem SI, El-Sherif MA. Diagnostic Value of Procalcitonin, C-Reactive Protein, and Leukocyte Count in Canine Ehrlichiosis and Canine Demodicosis. Comparative Clinical Pathology. 2022 Jun; 31(3): 529-36. doi: 10.1007/s00580-022-03350-4.

[12] Ramakant RK, Verma HC, Diwakar RP. Canine Ehrlichiosis: A Review. Journal of Entomology and Zoology Studies. 2020; 8(2): 1849-52.

[13] De Villiers L, Schoeman JP, Penzhorn BL, Molini U, de Villiers M, Makgabo SM *et al.* Prevalence, Diagnostic Evaluation, and Disease Associations of Vector-Borne Pathogens in Domestic Dogs Across Namibia: A Multi-Modal Approach. Parasites and Vectors. 2025 Jul; 18(1): 275. doi: 10.1186/s13071-025-06906-9.

[14] Rikihisa Y. The tribe Ehrlichieae and Ehrlichial Diseases. Clinical Microbiology Reviews. 1991 Jul; 4(3): 286-308. doi: 10.1128/CMR.4.3.286.

[15] Woody BJ and Hoskins JD. Ehrlichial Diseases of Dogs. Veterinary Clinics of North America: Small Animal Practice. 1991 Jan; 21(1): 75-98. doi: 10.1016/S0195-5616(91)50009-7.

[16] Razi Jalali MH, Mosallanejad B, Avizeh R, Alborzi AR. Seroprevalence of Ehrlichia canis in dogs referred to Veterinary Hospital of Shahid Chamran University of Ahvaz, Iran. Archives of Razi Institute. 2010 Sep; 65(1): 21-6.

[17] Sainz Á, 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 and Vectors. 2015 Feb; 8(1): 75. doi: 10.1186/s13071-015-0649-0.

[18] Iatta R, Sazmand A, Nguyen VL, Nemati F, Ayaz MM, Bahiraei Z *et al.* Vector-Borne Pathogens in Dogs of Different Regions of Iran and Pakistan. Parasitology Research. 2021 Dec; 120(12): 4219-28. doi: 10.1007/s00436-020-06992-x.

[19] Bai L, Goel P, Jhambh R, Kumar P, Joshi VG. Molecular Prevalence and Haemato-Biochemical Profile of Canine Monocytic Ehrlichiosis in Dogs in and Around Hisar, Haryana, India. Journal of Parasitic Diseases. 2017 Sep; 41(3): 647-54. doi: 10.1007/s12639-016-0860-8.

[20] Baharie Yazdi M, Pourmahdi Borujeni M, Mosallanejad B, Gharibi D. Seropervalence and Risk Factors of Canine Ehrlichiosis in Urban and Rural Dogs in Ahvaz. Iranian Veterinary Journal. 2018 Sep; 14(3): 5-13.

[21] Kukreti K, Pandey L, Das M, Rastogi A, Dubey R, Sharma P. Prevalence of Canine Monocytic Ehrlichiosis in Canine Population Across India. Archives of Razi Institute. 2018 Jun; 73(2): 87-93.

[22] Stich RW, Blagburn BL, Bowman DD, Carpenter C, Cortinas MR, Ewing SA et al. Quantitative Factors Proposed to Influence the Prevalence of Canine Tick-Borne Disease Agents in the United States. Parasites and Vectors. 2014 Sep; 7(1): 417. doi: 10.1186/1756-3305-7-417.

[23] Selim A, Abdelhady A, Alahadeb J. Prevalence and First Molecular Characterization of Ehrlichia Canis in Egyptian Dogs. Pakistan Veterinary Journal. 2020 Jul; 41(1): 117-21. doi: 10.29261/pakvetj/2020.061.

[24] Dhliwayo S, Chihambakwe B, Taonezvi K, Chikerema SM, Tivapasi MT, Pfukenyi DM. Seroprevalence of Canine Ehrlichiosis and Microscopic Screening for Canine Babesiosis in Dogs in Harare, Zimbabwe, 2016-2017. Veterinary Medicine International. 2019; 2019(1): 4130210. doi: 10.1155/2019/4130210.

DOI: https://doi.org/10.54393/mjz.v5i03.151

[25] Botros BA, Elmolla MS, Salib AW, Calamaio CA, Dasch GA, Arthur RR. Canine Ehrlichiosis in Egypt: Sero-Epidemiological Survey. 1995.

[26] Milanjeet M, Harkirat Singh HS, Singh NK, Singh ND, Chanchal Singh CS, Rath SS. Molecular Prevalence and Risk Factors for the Occurrence of Canine Monocytic Ehrlichiosis. Veterinarni Medicina. 2014; 59(3): 129–136. doi: 10.17221/7380-VETMED.

[27] Parashar R, Sudan V, Jaiswal AK, Srivastava A, Shanker D. Evaluation of Clinical, Biochemical and Haematological Markers in Natural Infection of Canine Monocytic Ehrlichiosis. Journal of Parasitic Diseases. 2016 Dec; 40(4): 1351-4. doi: 10.1007/s 12639-015-0688-7.

[28] Schaefer JJ, Kahn J, Needham GR, Rikihisa Y, Ewing SA, Stich RW. Antibiotic Clearance of Ehrlichia Canis from Dogs Infected by Intravenous Inoculation of Carrier Blood. Annals of the New York Academy of Sciences. 2008 Dec; 1149(1): 263-9. doi: 10.1196/ annals.1428.087.

[29] Akhtardanesh B, Ghanbarpour R, Sharifi H. Comparative Study of Doxycycline and Rifampin Therapeutic Effects in Subclinical Phase of Canine Monocytic Ehrlichiosis. Comparative Clinical Pathology. 2011 Oct; 20(5): 461-5. doi: 10.1007/s0058 0-010-1019-0.