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Assessment of drug efficacy in the treatment of canine Ehrlichiosis: A comprehensive evaluation and monitoring approach

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Abstract
The present study was conducted to know the therapeutic efficacy of drugs against ehrlichiosis in dog population in and around Jabalpur, (M.P.) A total of 4875 dogs (2925 male and 1950 female) were screened which were presented at Veterinary Clinical Complex (V.C.C.), College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur, Madhya Pradesh, from July 2021 to December 2021. Among dog population, 207 dogs (110 male and 97 female) exhibited clinical signs suspected of ehrlichiosis. The therapeutic trial revealed that the most efficacious regimen was elicited by treatment group G_2 (Doxycycline), followed by G_3 (Rifampicin) and lowest in G_4 (*Crotalus horridus*) due to improvement in the general condition of dogs after 21 days of treatment which was based on restoration of haemato-biochemical values towards normalcy and blood smear examination (Ehrlichia negative).

Keywords: Dog, ehrlichiosis, therapeutic efficacy

Introduction
Ehrlichiosis is a globally distributed rickettsial disease of dogs caused by *Ehrlichia* spp. It is an obligate intracellular, pleomorphic or coccal, gram-negative bacterium that is transmitted by tick vectors. There are two forms of Ehrlichiosis that occur in dogs: Monocytic form and Granulocytic form. The monocytic form is caused by *Ehrlichia canis* and its tick vector is *Rhipicephalus sanguineus* (Fuente *et al*., 2008) [4]. Canine Monocytic Ehrlichiosis (CME) has three clinical forms: acute, sub-clinical and chronic. The acute form is characterized by thrombocytopenia, non-regenerative anaemia and haemorrhagic tendencies. This form is manifested clinically by high fever, depression, lethargy and anorexia. The sub-clinical form is characterized by persistent bacteremia with no overt clinical signs and normal haematological parameters. The chronic form is characterized by pancytopenia due to suppression of bone marrow and lymphadenomegaly. The acute phase of canine monocytic ehrlichiosis is more severe than canine granulocytic ehrlichiosis (Anderson *et al*., 1992) [2]. Clinically the dogs are lethargic, weak and anorexic (Waner and Harrus, 2013) [13]. The granulocytic form (CGE) is caused by *Ehrlichia ewingii* and its tick vector is *Amblyomma americanum* (Fuente *et al*., 2008) [4] and *Dermacentor variabilis*. The tick *Rhipicephalus sanguineus* is often associated with dogs in tropical and sub-tropical urban areas. The greater prevalence of Ehrlichiosis in urban settings such as Mumbai (27.2%) and Delhi (39.5%) have a tropical and sub-tropical climate respectively as compared to the rural Sikkim (0%) and Ladakh (0%) which have a temperate and dry-arid climate respectively (Abd Rani *et al*., 2011) [1].
Diagnosis of Ehrlichiosis can be done on the basis of blood smear examination, cell culture, serology and molecular detection by PCR. In serology, the Indirect Fluorescent Antibody Test (IFAT) is recommended to confirm a diagnosis of ehrlichiosis. Dot-ELISA kits for the detection of *E. canis*-IgG antibodies are commercially available. Western immunoblot is a more specific test, which can distinguish between infections with the different organisms causing ehrlichiosis, anaplasmosis, or neorickettsiosis as well as between *Ehrlichia* spp., for example *E. canis* and *E. ewingii* (Straube, 2010) [10].

Dogs with Ehrlichiosis have been conventionally treated with Tetracycline @ 22mg/kg body weight given every 8 hours or doxycycline @ 5 mg/kg body weight every 12 hours administered daily for 4 weeks. Following therapeutic elimination of *Ehrlichia spp*. dogs do not develop protective immunity and can be reinfected when re-introduced to a vector-competent tick (Breitschwerdt, 1998) [3]. Even after clearance of infection, bone marrow regeneration may require up to 120 days following treatment. In general, the prognosis of acute infection is good but chronic infection is guarded. Keeping the above factors, the present study was designed to evaluate therapeutic efficacy of different drugs against ehrlichiosis in dogs.

Materials and Methods

The proposed work was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh. For this study, a total of 4875 dogs which were brought to Veterinary Clinical Complex (V.C.C.), College of Veterinary Science & A.H., Jabalpur (M.P.) were screened for ehrlichiosis for a period of six months i.e. from July 2021 to December 2021. Complete history of the dogs regarding age, breed, sex, mucous membrane and various symptoms like bleeding tendencies/epistaxis, intermittent fever, arthritis (swelling of legs), labored breathing and lethargy for at least 1-2 weeks or longer.

A total of 207 dogs were suspected for ehrlichiosis on the basis of clinical signs, out of which 27 dogs were diagnosed positive by blood smear examination. Thin blood smear was prepared and fixed with methanol for 30 seconds, allowed to dry and stained in 1 in 20 dilution of Giemsa's stain for 30-40 minutes. Subsequently, the smear was washed with distilled water and allowed to dry and was examined under oil immersion objective (1000X) of compound microscope. Blood collection was done (ehrlichia negative) on day 0 (pre-treatment) and subsequently on day 7th and 21th (post-treatment). A total of 18 positive cases of ehrlichiosis were placed into three groups i.e. G2, G3 and G4 for therapeutic study. Six apparently healthy dogs were kept as healthy control group (G_1). Each treatment group comprised of six dogs. Animals were treated by the following drugs (Table 01).

Table 1: Drugs and dosage in different groups

Groups	Drugs	Dose, route and duration
G1	Apparently healthy control group	-
G2	Doxycycline	10 mg/kg, PO, BID for 21 days
G3	Rifampin	15 mg/kg, PO, BID for 21 days
G4	*Crotalus horridus* (200C)	4 Pills PO, OD for 21 days

Symptomatic and supportive therapy were given according to clinical condition. Therapeutic response evaluation was done on the basis of blood smear examination (ehrlichia negative) on day 0 (pre-treatment) and on day 7 and day 21 (post treatment). Statistical analysis was done by methods as described by Snedecor and Cochran (1994) [9]. Means were compared by using Fisher's pair wise comparison based on the least significant difference at 5% level of significance.

Results

Doxycycline is the most common antibiotic against the bacterial infection transmitted by ticks due to its antimicrobial properties and pleiotropic functions. It has also immunomodulatory and anti-inflammatory properties associated with its effects on blood leukocyte proliferation and functions, synthesis of cytokine and matrix metallo-proteinase activity. The doxycycline is acted by inhibiting the synthesis of protein that prevents phagosomes with lysosomes. It is the most suitable drug than oxytetracycline because it has higher lipid solubility than other tetracycline resulting in better absorption of doxycycline from the gastro-intestinal tract leading to better tissues penetration. Results revealed that Group G_2 (Doxycycline @ 10mg/kg Bodyweight, PO, q12 h for 21 days) showed negative blood smear in 1 dog (16.66%) on 7th day, however, on 21st day, all 6 dogs (100%) were found negative for ehrlichiosis by blood smear examination. Similar findings were documented by Shukla *et al*. (2011) [8], Gel *et al*. (2007) [5] and Petrov *et al*. (2018) [6].

Rifampicin is an inhibitor of the B subunit of DNA-dependent RNA polymerase enzyme and results in drug binding in the polymerase subunit deep within the RNA/DNA channel facilitating direct blocking of the elongating RNA in Ehrlichia organism. Group G_3 (Rifampin @ 15mg/kg Bodyweight, PO, q12 h for 21 days) showed negative blood smear in 1 dog (16.66%) on 7th day, however, on 21st day, 4 dogs (66.66%) were found negative for ehrlichiosis by blood smear examination. Similarly, Schaefer *et al*. (2008) [7] and Theodorou *et al*. (2013) [11] reported the same.

The results revealed that Group G_4 (*Crotalus horridus* 200CH@ 4 pills PO, q 24 h for 21 days) did not revealed negative blood smear in any dogs on 7th day, however, on 21st day 3 dogs (50%) were negative for ehrlichiosis in blood smear examination. Tungnunga *et al*. (2016) [12] also documented the efficacy of *Crotalus horridus* in the treatment of ehrlichiosis.

Table 2: Evaluation of therapeutic response in different treatment groups

Groups	Blood smear examination (absence of Ehrlichia)			
	Day 7	Recovery (%)	Day 21	Recovery (%)
Doxycycline (G2)	1	16.66	6	100.00
Rifampin (G3)	1	16.66	4	66.66
Crotalus horridus (G4)	0	00	3	50.00

Conclusion

On the basis of recovery pattern and absence of Ehrlichia organism in blood smear, in dogs of group G_2 (Doxycycline) was found to be most efficacious followed by group G_3 (Rifampin) and least in group G_4 (*Crotalus horridus*).

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