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Dr. Anish Kumar
M.V.Sc. Scholar, Department of Veterinary Medicine, College of Veterinary &Animal Sciences Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. TK Sarkar
Professor & Head, Department of Veterinary Medicine, College of Veterinary &Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. PS Maurya
Assistant Professor, Department of Veterinary Parasitology, College of Veterinary &Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Vipul Thakur
Assistant Professor, Department of Veterinary Medicine, College of Veterinary & Animal Sciences Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India)

Dr. MV Jithin
Assistant Professor, Veterinary Clinical Complex, College of Veterinary & Animal Sciences Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Jeny K John
Assistant Professor, Veterinary Clinical Complex, College of Veterinary & Animal Sciences Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Ramakant
Ph.D. Scholar, Department of Veterinary Medicine, College of Veterinary & Animal Sciences Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Corresponding Author:
Dr. TK Sarkar
Professor & Head, Department of Veterinary Medicine, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Therapeutic effects of doxycycline and minocycline in monocytic ehrlichiosis in canine

Dr. Anish Kumar, Dr. TK Sarkar, Dr. PS Maurya, Dr. Vipul Thakur, Dr. MV Jithin, Dr. Jeny K John and Dr. Ramakant

Abstract
A study was carried out to compare the therapeutic efficacy between doxycycline and minocycline on clinical cases of canine monocytic ehrlichiosis (CME). Six CME infected dogs were treated with Doxycycline and six with CME infected dogs were treated with minocycline for 21 days. On the basis of the clinical examinations and laboratory findings (positive for Ehrlichiosis), 12 animals were selected and randomly divided into Groups I (N=6) and II (N=6). To compare the hemato-biochemical status of CME cases, 6 apparently healthy dogs were selected. Group I was administered doxycycline at the dose rate of 10 mg/kg b.wt. P/O OD for 21 days and Group II were administered minocycline at the dose rate of 20 mg/kg b.wt P/O BID for 21 days. Both group I &II supportive treatment were administered with standard dose of pantoprazole and domperidone combination syrup, silymarin syrup and haematenic syrup orally. After treatment with doxycycline and minocycline the haemato-biochemical parameters come back towards normal level on day 21 post therapy. A group of dogs treated with minocycline showed better results on clinical and haemato-biochemical parameters compared to dogs treated with doxycycline. Minocycline could be used for the treatment of *E. canis* infected dogs instead doxycycline.

Keywords: Doxycycline, minocycline, canine, ehrlichiosis

Introduction
Canine ehrlichiosis a tick-borne disease (*Rhipicephalus* sanguineus, the brown dog tick) is caused by *Ehrlichia canis* Gram-negative pleiomorphic obligatory intracellular cocci that infect blood cells of canine (Shukla *et al.*, 2011) [1]. Clinical findings vary depending on acute, subclinical and chronic phase. It is mostly characterized by high fever (104 to 105^{0}F), epistaxis, anorexia, weakness, edema of dependent parts and lymphadenopathy (Singla *et al.*, 2011) [2]. The incubation period of canine ehrlichiosis varies from 8 to 20 days, after which the clinical findings reveal in three phase including acute phase, subclinical phase and chronic phase (Harrus *et al.*, 1997) [3]. Acute canine monocytic ehrlichiosis (CME) is characterized by high fever, depression, anorexia and lethargy. Physical examination reveals lymphadenomegaly, spleenomegaly and hemorrhages exhibited as dermal petechiae, ecchymosis, epistaxis and thrombocytopenia being the prevailing signs (Waner and Harrus, 2013) [4]. The disease is diagnosed on the basis of clinical sign, hematologic abnormalities, demonstration of morulae in peripheral monocyte by blood smears, molecular method of polymerase chain reaction (PCR) and serological method including enzyme-linked immunosorbent assay (ELISA), indirect flurorescent antibody test (IFAT) and rapid immunochromatographic test (RIT).The recommended therapy for canine monocytic ehrlichiosis is doxycycline (10 mg/kg body weight (BW), PO once daily for 28 days), but other drugs such as amicarbalide, chloramphenicol, imidocarb dipropionate and tetracycline have been studied (Neer *et al.*, 2002) [5]. While considered safe and efficient, treatment failure with at different doses of doxycycline has been reported (Harrus *et al.*, 2004) [6]. A few studies recommended that efficacy is related to the stage of infection, chronic infections potentially more difficult to treat than acute or subacute (Harrus *et al.*, 1998) [7]. Minocycline one more drug in the tetracycline family can be used as an alternative to doxycycline in veterinary medical practices. Little information is accessible regarding efficacy of minocycline for treatment of rickettsial diseases (Carris *et al.*, 2015) [8]. The objective of this study was to evaluate the comparative therapeutic efficacy of doxycycline and minocycline in canine monocytic ehrlichiosis.

Materials and Methods

Three to four milliliters of blood was collected aseptically from the cephalic/saphenous vein of dogs in a sterile EDTA coated vial. A thin blood smear was prepared for Giemsa staining and observed under the light microscope at 100X using oil immerse on lens to detect parasitized and abnormal red blood cells (RBCs). The Hb, PCV, TEC, TLC, total platelets count was detected by using fully Automatic haematology analyzers (Mindray BC-30 Vet) using commercial kits. Blood samples were analyzed for various biochemical parameters like total protein, total bilirubin, alkaline phosphatase, AST, and ALT. These analyses were performed using fully automatic haematology analyzers (Mindray BS-240) according to manufacture protocol using commercial kits. Whole blood samples were used for isolation of DNA using commercially available kits. The genomic DNA from the blood samples were extracted by QIAampMini Kit (Murphy *et al.*, 1998) [9]. The Sequence of Oligonucleotide primers used for Genus specific and nested PCR (Species specific) areas mentioned below:

The Sequence of Oligonucleotide primers used for Genus specific and nested PCR (Species specific) areas mentioned below

Ehrlichia canis	**Primer Sequence Primary PCR –Nested PCR**	**Target Gene**	**Product Size**	**References**
Genus specific primers	EC9:5'-AAGGATCCTACCTTGTTACGACTT-3 (FORWARD) EC12:-AATCTAGAGTTTGATCMTGG-3 (REVERSE)	16SrRNA	15000 bp	(Kawahara *et al.*, 1999) [10]
Species specific primers	HE3:5'TATAGGTACCGTCATTATCTTCCCTAT-3 (FORWARD) ECA:5'-CAATTATTTATAGCCTCTGGCTATAGGAA-3 (REVERSE)	16SrRNA	389 bp	(Wen *et al.*, 1997) [11]

Clinical trial

On the basis of the clinical examinations and laboratory results (positive for Ehrlichiosis), 12 animals were selected and randomly divided into Groups I (N=6) and II (N=6).To compare the hemato-biochemical status of CME cases, 6 apparently healthy dogs were selected on the basis of clinical and hematological examinations. Group I was given doxycycline at the dose rate of 10 mg/kg b.wt. P/O OD for 21 days and Group II were given minocycline at the dose rate of 20 mg/kg b.wt P/O BID for 21 days. In both groups supportive treatment were provided with standard dose of pantoprazole and domperidone combination syrup, silymarin syrup and haematenic syrup orally.

Statistically analysis

The IBM- SPSS 20th version software was used to analyze numerical data gathered in the present study. Data were subjective to analysis of variance (ANOVA) using general linear model procedure (SPSS, 2008). Differences among means were tested using Duncan's test according to Snedecor and Cochran (1994) [12].

Ethics approval

The permission for research work was dully approved by the Institutional Animal Ethics Committee (IAEC) vide no. IAEC/SVPUAT/2022/105 dated 24/05/2022.

Result and discussions

The dogs affected with canine monocytic ehrlichiosis (CME) observed characteristic clinical features of anorexia (92.3%), tick infestation (76.9%) pyrexia (61.55%), melena (53.8%), peripheral lymhnodes enlargement (23.07%), epistaxis (15.38%) and corneal opacity (7.69%).

Giemsa stained blood smear examination detected intracytoplasmic inclusion bodies in monocytes. The PCR examinations considered very specific and sensitive tool for detecting *E. canis* infection in dogs during the chronic phase of ehrlichiosis, where as blood smear testing frequently fails to identify the illness. These dogs were found positive for genes specific PCR generating 1500 bp amplicons of *E. canis* and the same samples were tested with the species specific Nested PCR targeting 16S rRNA gene detected positive for *E. canis* producing amplicons of 389 bp on electrophoresis.

In *E. canis* positive cases there was significant (p<0.01) decrease of Hb (gm/dl), PCV (%), TEC ($X10^6$/µl) and total platelet count (10^3/mm^3) as compared to healthy dogs. There was significant (p<0.01) improvement of haemoglobin concentration in doxycycline (12.95±0.22 gm/dl) and minocycline (13.88±0.19 gm/dl) treated dogs on day 21 post treatment as compared with *E. canis* infected dogs on day zero. The PCV value was improved significantly (p<0.01) in dogs treated with doxycycline (39.61±0.59%)) and minocycline (41±0.51%) on day 21 post treatment and returned to normal level. In *E. canis* positive cases there was significant (p<0.01) increase of TLC in groups I (23.65 ± 2.13 10^3/mm^3) & groups II (18.20 ± 1.16 10^3/mm^3) on day zero as compared with healthy control (11.25±0.98 10^3/mm^3). The TLC value in dogs treated with doxycycline (Group-I) and minocycline (Group-II) on day 21 post treatment returned to normal level.

Total protein (gm/dl) level was significantly decreased (p<0.01) in group I (3.59±0.30 mg/dL) and group II (3.95±0.25 mg/dL) as compared with healthy control (6.58±026 mg/dL). It indicates that there was hypo-proteinemia in CME. After day 21 post treatment with doxycycline and minocycline the total prtein level returned towards normal. There was significant increased (p<0.01) of total bilirubin (mg/dl), Alkaline phosphatase (U/L), Alanine Transaminase (U/L) and Aspartate Transaminase (U/L) in CME as compared with healthy dogs. In doxycycline and minocycline treated dogs, there was improvement of total bilirubin on day 21 post treatment and returned to normal level. There was significant improvement of ALP level in minocycline and doxycycline treated dogs as compared with healthy control dogs on day 21 post treatment. The ALT values come to normal level on day 21 of post treatment in doxycycline and minocycline groups. There was improvement of AST level in doxycycline and minocycline treated dogs on day 21. After day 21 post treatment with doxycycline in group- I and minocycline in group -II, all blood smear examination and PCR test were recorded negative.

Discussion

The dogs affected with CME showed the characteristic clinical features of anorexia (92.3%), tick infestation (76.9%), pyrexia (61.55%), melena (53.8%), peripheral lymph node enlargement (23.07%), epistaxis (15.38%) and corneal

opacity (7.69%). Parashar *et al.* (2016) [13] recorded the main clinical symptoms were pyrexia (95.65%), followed by weakness (65.21%), anorexia (56.52%), anaemia (47.82%), epistaxis (52.17%), melena (21.73%), ocular signs (43.47%), jaundice (21.73%) and yellow coloured urine (39.13%). Singh *et al.* (2021) [14] recorded a wide variety of clinical manifestations and the most frequently reported observations were tick infestation (90.2%), melena (75.6%), anorexia (65.9%), pale mucosa (61%), lethargy (53.7%) and lymphadenopathy (48.8%). The minocycline treated dogs recovered clinically within 14 days of post therapy but doxycycline treated dogs become normal after day 21 post treatment. Minocycline can clear or suppress circulating *E. canis* and might be an alternative treatment to the doxycycline (Jenkins *et al.*, 2018) [15]. The Giemsa blood smear examination detected morulae of *E. canis* as intracytoplasmic inclusion bodies of varying sizes and shapes in monocytes. The observations of microscopic study corroborated with the finding of earlier worker (Dhankar *et al.*, 2011; Shukla *et al.*, 2011) [16, 1]. These 12 dogs found genes specific PCR generating 1500 bp amplicons of *E. canis* and tested with the species specific Nested PCR targeting 16S rRNA gene detected positive for *E. canis* producing amplicons of 389 bp on electrophoresis. PCR is more effective in detecting canine ehrlichiosis in early and subclinical stages (Rajagopal *et al.*, 2009) [17]. Kalaivanan *et al.* (2020) [18] reported that PCR technique was more sensitive than traditional microscopic inspection for the diagnosis of CME in dogs.

Dogs infected with CME showed significant (p<0.01) decrease of Hb (gm/dl), PCV (%), TEC (X10^6/µl) and total platelet count (10^3/mm^3) as compared with healthy dogs. In doxycycline and minocycline treated dogs, there was improvement of haemoglobin concentration on day 21 of post treatment and came to normal. Castro *et al.* (2004) [19] and Bhardwaj *et al.* (2013) [20] recorded that there was significant (p<0.01) decrease of Hb concentration. The PCV value in dogs treated with minocycline (Group-II) improved better than doxycycline (Group-I) on day 21 post treatment. There were lower PCV levels in *E. canis* positive cases Oliveira (2000) [21], Castro *et al.* (2004) [19] and Sharma *et al.* (2015) [22]. After 21 days of post treatment with doxycycline (Group-I) and minocycline (Group-II) the TEC returned to normal level. Bai *et al.* (2017) [23], Kottadamane *et al.* (2017) [24], Parashar *et al.* (2016) [13] and Mondal *et al.* (2019) [25] also reported that there was decrease of TEC in canine ehrlichiosis. Harrus and Waner (2011) [26] suggested immunological mechanism may be involved in destruction of erythrocytes causing anemia during acute stage of infection. Bhardwaj *et al.* (2013) [20] reported that due to lower Hb, platelets and TEC levels there could be the epistaxis, petechial hemorrhages and myelosuppression. After 14 days of post treatment the platelets count was enhanced better in minocycline (Group-II) treated animals than doxycycline (Group-I) treated dogs. Oliveira *et al.* (2009) [27], Salem *et al.* (2014) [28] and Singh *et al.* (2014) [29] also reported significant decrease in the level of total platelet count (TPC) in canine ehrlichiosis. Thrombocytopenia in canine ehrlichiosis may be attributed to decreased circulating half life of platelets during acute phase of infection, reduced adhesiveness of platelets due to antiplatelet antibody, plasma inhibiting factor or direct effect of *E. canis* on circulating platelets or endothelial damage and platelet aggregation as suggested by Kuehn and Gaunt (1985) [30]. In *E. canis* positive cases there was significant (p<0.01) increase of TLC in groups I and groups II on day zero as compared with healthy control. The TLC value in dogs treated with minocycline (Group -II) improved better than doxycycline (Group-I) and on day 14 post treatment. This finding also support Waner *et al.* (1997) [31], Das and Konar (2013) [32] and Parmar *et al.* (2013) [33], who claimed that there was leukocytosis in ehrlichiosis positive dogs. Mondal *et al.* (2019) [25] and Kottadamane *et al.* (2017) [24] also claimed that there was leukocytosis in ehrlichiosis infected dogs.

Total protein (gm/dl) level was significantly decreased (p<0.01) in group I (3.59±0.30 mg/dL) and group II (3.95±0.25 mg/dL) as compared with healthy control (6.58±026 mg/dL). It indicates that there was hypo-proteinemia in ehrlichiosis cases. This value returns to normal level on day 21 post treatment with doxycycline and minocycline. Rao *et al.* (2020) [34] and Bhadesiya and Raval (2015) [35] revealed that there was significant decrease of total serum protein and serum albumin in canine ehrlichiosis. There was significant increased (p<0.01) of total bilirubin (mg/dl), Alkaline phosphatase (U/L), Alanine Transaminase (U/L) and Aspartate Transaminase (U/L) CME dogs as compared with healthy dogs. In doxycycline and minocycline treated dogs, there was improvement of total bilirubin on day 21 post treatment and this value comes to normal level. There was significant increase in the levels of serum total bilirubin and ALP in ehrlichiosis infected dogs (Singh *et al.*, 2021) [14]. These finding are attributed to *E. canis* induced inflammation of the sinusoidal endothelium of liver parenchyma (Mylonakis *et al.*, 2010) [36]. There was better improvement of ALP level in minocycline treated dogs than doxycycline treated dogs on day 14 post treatment. The greater lipophilic properties and high tissue concentrations of minocycline could be beneficial in *E. canis* treatment as compared to doxycycline (Chopra and Roberts, 2001) [37]. Mondal *et al.* (2019) [25], Sasanelli *et al.*, 2009) [38] reported that there were elevated activities ALP, ALT and AST in canine ehrlichiosis. Akhtardanesh *et al.* (2010) [39] and Kottadamane *et al.* (2017) [24] also reported high level of ALP in canine ehrlichiosis. Better improvement of ALT values was observed in minocycline treated dogs as compared to doxycycline treated dogs on day 21 of post treatment. Hypoalbuminemia and elevated level of ALT activity were recorded in canine ehrlichiosis (Kottadamane *et al.*, 2017) [24], Bhadesiya and Raval (2015) [35] also recorded that the levels of ALT increased significantly (p<0.01) in dogs with ehrlichiosis than healthy dogs. There was improvement of AST level in Doxycycline and Minocycline treated dogs on day 21. Bai *et al.* (2016) [23] reported that there was significant (p<0.05) rise in mean values of ALT, AST, GGT, total bilirubin, indirect bilirubin, ALP and A/G ratio in affected dogs those of healthy control. After 21 days of post treatment in doxycycline and minocycline groups of dog all blood smear examination and PCR test were recorded negative.

Conclusion

In conclusion there was significant (p<0.01) decrease in Hb (gm/dl), PCV (%), TEC (10^6/ µl), total platelets count (10^3/mm^3) and increased in TLC (10^3/ µl) in CME dogs. There was significant increased total bilirubin (mg/dL), ALP (U/L), ALT (U/L), AST (U/L) and decreased total protein in CME dogs. After treatment with doxycycline and minocycline the haemato-biochemical parameters returned towards normal

level on day 21 post therapy. Minocycline treated group of dogs showed better result in clinical and haemato-biochemical parameters as compared with doxycycline treated dogs. Minocycline can be used for the treatment of *E. canis* infected dogs instead doxycycline.

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