LIMB OEDEMA ASSOCIATED WITH CANINE EHRLICHIOSIS AND ITS THERAPEUTIC MANAGEMENT*

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Abstract

Six Ehrlichia canis positive dogs with limb oedema were selected and routine haematobiochemical observations were made. All other possible causes of limb oedema were ruled out by orthopaedic examination of the limbs, thoracic radiography, ECG and abdominal ultrasonography. Histopathological results of skin biopsy from oedematous limbs were suggestive of cutaneous vasculitis. All the dogs were treated with doxycycline per os and in addition, prednisolone was administered to the vasculitic dogs. All the dogs responded to therapy and oedema subsided within a week.

Keywords: *E. canis, cutaneous vasculitis, limb oedema*

Canine monocytic ehrlichiosis (CME) is caused by obligate intracellular parasites of the genera Ehrlichia and transmitted by the brown dog-tick, *Rhipicephalus sanguineus*. The disease is manifested by a wide variety of clinical signs and three phases have been recognized: acute, subclinical and chronic (Waner and Harrus, 2013). The most commonly observed haematological abnormalities are thrombocytopaenia and anamia (Sainz *et al*., 2015). Uncommon signs like cutaneous vasculitis due to immune reactions was also reported in association with canine ehrlichiosis (Innera, 2013). This paper describes six cases of ehrlichiosis with limb oedema which was associated with cutaneous vasculitis.

Materials and Methods

Six dogs with limb oedema without any orthopaedic abnormalities and which were diagnosed positive for ehrlichiosis by buffy coat smear examination were selected for the study (Fig. 1). To rule out cardiac abnormalities, electrocardiography and thoracic radiography were performed. Abdominal ultrasonography was performed to evaluate any organ abnormality. Haematological and biochemical evaluations were conducted in all the cases on both 0^{th} and 30^{th} day of observation. For further diagnosis, a skin biopsy was taken from the odematous area using 6 mm punch biopsy needle as per the method of Miller *et al*. (2013). Treatment was given based on the histopathology result and the response was studied.

Statistical analysis of the haematological and biochemical data before

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and after therapy were carried out using paired t test described by Kaps and Lamberson (2009) with computer software, SPSS version 21.0.

Results and Discussion

All the six cases of ehrlichiosis were diagnosed by buffy coat smear examination. According to Woddy and Hoskins (1991), buffy coat smears were preferred for detection of intracellular *E. canis* morulae (Fig. 2). Electrocardiography and thoracic radiography did not reveal any cardiac abnormalities. According to Waner and Harrus (2013), splenomegaly was a prominent finding in acute and chronic stages of ehrlichiosis. In the present study only one dog revealed splenomegaly and this might be due to diffuse proliferation of lymphocytes and plasma cells in spleen during the acute stage of infection. Splenomegaly was not prominent in other dogs.

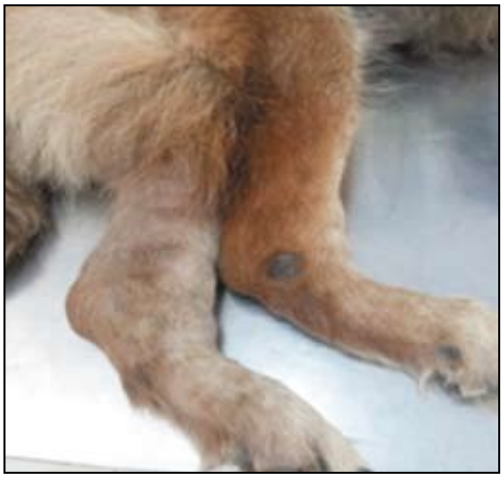

Fig. 1. Limb edema

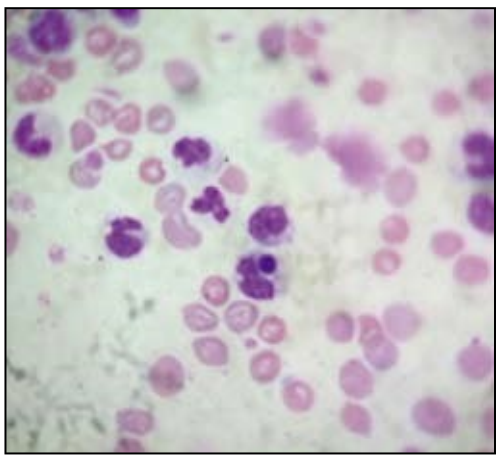

Fig. 2. Buffy coat smear (1000x)-morulae within monocyte

Histopathology of skin biopsy from two cases were suggestive of cutaneous vasculitis which corroborates with the findings of Innera (2013). According to the author, cutaneous vasculitis was an immune mediated dermatosis characterized by an aberrant immune response directed towards the blood vessels and pitting oedema might be the only finding in the early stages of the disease. Infection with ehrlichia might cause direct injury to vessel wall or there might be formation of antigen-antibody complexes on the vascular endothelium or activation of B or T cells,which subsequently resulted in vasculitis. According to Shumaker (2015) histologic features of a deep skin biopsy sample only can confirm the diagnosis. The histopathological changes associated with vasculitis included variable degrees of neutrophilic, eosinophilic and mononuclear cell invasion of vessel walls, endothelial cell swelling, fibrinoid degeneration, red blood cell extravasation and occasional leukocytoclasia within or near the vessel walls. In the present study the infiltration of inflammatory cells especially mononuclear cells were seen around small blood vessels in the subepithelial and subcutaneous area which were suggestive of small vessel vasculitis (Fig. 3).

All the cases were treated with doxycycline @ 5 mg/kg bodyweight twice daily orally for 21 days according to the treatment protocol recommended by Harrus *et al*. (2012). Tetracyclines exert a variety of

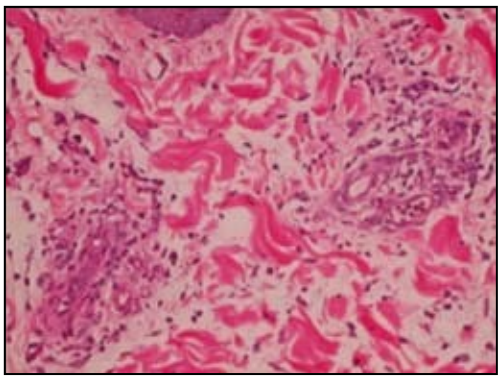

Fig. 3. Skin-H&E (400 x)-Mononuclear infiltration around small blood vessels

anti-inflammatory and immunomodulating properties by themselves or in combination with niacinamide (Innerå, 2013). In addition, prednisolone was administered in two cases with histopathological lesions suggestive of vasculitis @ 1 mg/kg body weight twice daily orally for the first week followed by tapering to 25 per cent every week. This was in accordance with Sainz *et al*. (2015) who suggested the administration of glucocorticoids, if immune mediated complications occur with ehrlichiosis.

Haemato-biochemical parameters of diseased dogs before and after therapy are as in table 1. The mean TEC of animals were 5.53 ± 0.28 and 5.98 ± 0.22 million/cmm on 0^{th} and 30^{th} day respectively. The mild anaemia present during the 0^{th} day might be due to subclinical infection and TEC after therapy showed significant increase ($p<0.05$) indicating response to therapy. Similarly, increased total protein and globulin that were recorded before therapy were significantly decreased ($p<0.05$) after therapy. This corroborates with the reports of Harrus *et al*. (2012) who stated that most of the dogs with ehrlichiosis developed hyperproteinemia due to hypergammaglobulinemia, and hyperimmune mechanisms which play an important role in the pathogenesis of ehrlichiosis. The decreased globulin levels after therapy indicated the clearance of ehrlichial antigen which proved the efficacy of doxycycline and prednisolone in reducing the limb oedema associated with ehrlichiosis. All the cases showed a positive response to therapy. Haemato-biochemical parameters improved after 30 days of observation and blood and buffycoat smear examination were negative for ehrlichia. Oedema subsided during the first week of therapy in all cases, but in one case recurrence was reported one-month post therapy.

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Table 1. Haemato-biochemical parameters of diseased dogs before and after therapy

Parameters	Before therapy	After therapy	t value
TEC (million/cmm)	5.53 ± 0.28	5.98± 0.22	0.030*
Hb (g/dl)	12.77 ± 0.87	13.55± 0.51	0.123
VPRC (%)	37.47 ± 2.00	39.15± 1.54	0.067
TLC (10^3/cmm)	17.42 ± 2.08	14.68 ± 0.90	0.174
Platelet (10^5/cmm)	2.96 ± 0.40	3.26 ± 0.24	0.162
Total protein (g/dl)	8.42 ± 0.43	7.86± 0.30	0.026*
Albumin (g/dl)	3.22 ± 0.08	3.25± 0.11	0.691
Globulin (g/dl)	5.19 ± 0.38	4.62± 0.22	0.028*
AG ratio	0.60 ± 0.06	0.71 ±0.02	0.072
ALT (IU/l)	31.01 ± 6.76	32.6 ± 4.97	0.591
ALP (IU/dl)	113.86 ± 21.58	160.03 ± 48.15	0.379
Total bilirubin (mg/dl)	0.53 ± 0.06	0.34 ± 5.93	0.374
Direct bilirubin (mg/dl)	0.25 ± 0.02	0.25 ± 0.02	0.891
BUN (mg/dl)	15.13 ± 1.37	17.83 ±1.83	0.270
Creatinine (mg/dl)	1.25 ± 0.10	1.30 ±0.37	0.873

* Represents significance at 5 % level

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