460 D

A preliminary study on Ehrlichia sp., Moshkovski 1945, (Rickettsiales: Rickettsiaceae) of dogs: Light microscopic studies of the parasite

Parasites of the genus *Ehrlichia*, generally, infect leukocytes of dogs. Diagnosis of ehrlichiosis in dogs is based on microscopy and clinical features. Reports from Sri Lanka document infections in monocytes and more recently, from lymphocytes. Microscopic observations however, are often confounded by the appearance of many types of morphologically distinct inclusions within leukocytes. Therefore, this study attempted to investigate the light microscopic structure of the inclusions found in leukocytes of dogs, using different staining methods. Blood and buffy coat smears of dogs, clinically diagnosed with ehrlichiosis (Group A, n = 10) and clinically healthy (Group B, n = 10), were studied (April – November 2001, Kandy District)

using different stains and modifications of staining procedures. Stains used were Leishman 1901, Harris' Heamatoxylin 1900, Ziehl-Neelsen 1883, Freifeld, modified Ziehl-Neelsen and modified Ziehl-Neelsen with methylene blue as counterstain (new preparation).

Three types (type I, II and III) of morphologically distinct inclusions were observed in the lymphocytes of Group A, and all were not of parasite origin. Type I was large, oval or round in shape (0.6 – 4.2 μm), consisting of a cluster of bodies (morulae). Only 1 – 3 such inclusions per cell were found within 2 – 10% of lymphocytes of Group A (8/10). Type II (< 0.6 μm) appeared as either large (n < 10 per cell) or small sized granules (n > 10 per cell), present in 5.4 – 29 % of lymphocytes of Group A (10/10) and 7.6 – 18.5% of Group B (4/10). Type III (< 0.6 μm) was like type II, but was found within a vacuole like structure, which stained lighter than the lymphocyte cytoplasm of Group A (3/10). Modified Ziehl-Neelsen (with methylene blue counterstain) differentiated morulae from granules. With this stain the organisms stain pink against a blue background, where as the granular structures stain blue. In general, Group A lymphocytes were larger, with displaced nuclei and more cytoplasm. Modified Ziehl-Neelsen method (methylene blue) together with Leishman stain could be used to differentiate the parasites from granules (which are found in the lymphocytes of the healthy animals as well). Size of the lymphocytes may also prove useful for the identification of parasites. Further studies are underway to study the biology of the parasite.