# EVALUATION OF PERIPHERAL BLOOD LYMPHOCYTE SUBSETS IN FAMILY-OWNED DOGS NATURALLY INFECTED BY *Ehrlichia canis*

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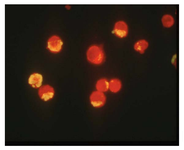
## OBJECTIVES

It has been suggested that clinical manifestations, histopathological lesions and even infection maintenance in the course of canine monocytic ehrlichiosis (CME) are directly related with the immune response developed by the host[1-3]. However, the immunopathogenesis of the disease remains poorly understood[4].

The main **goal** of the present study was to evaluate, therefore, the effect of *E. canis* infection on blood lymphocyte populations distribution in dogs with naturally occurring CME.

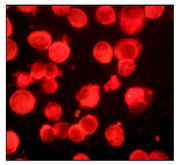
## METHODS

*a.* ***Animals.*** A total of 84 dogs were included in this study:



- Thirty-seven dogs with naturally occurring CME were included in the first group after the exclusion of other vector-borne diseases (anaplasmosis, neorickettsiosis, leishmaniosis). None of the animals had previously received treatment against *E. canis*.

Fig 1. Positive inmunofluorescent antobody test (IFA) to *E. canis*



- Forty-seven healthy dogs were used as controls. They were clinically healthy, did not present any abnormality in physical exam nor alterations in haemataology and blood chemistry and were also negative against *Ehrlichia/Anaplasma/Neorickettsia* spp. and *Leishmania* spp. by serology and PCR.

Fig 2. Negative IFA result to *E. canis*

Blood was obtained from each dog by cephalic or jugular venipuncture and collected in EDTA and heparin anticoagulant tubes for haematology, biochemical analysis, serology and PCR against *Ehrlichia/Anaplasma/ Neorickettsia* spp. and *Leishmania* spp. and flow cytometry.

*b.* ***Flow cytometry.*** Blood lymphocyte subsets were analyzed by multiparametric flow cytometry using a FACSCalibur flow cytometer.

Monoclonal antibodies to canine lymphocyte cell surface antigens were obtained from AbD Serotec and include: anti-canine CD3 (clone CA17.2A12) conjugated with FITC, anti-canine CD4 (clone YKIX302.9) conjugated with PE, anti-canine CD8 (clone YCATE55.9) conjugated with Alexa Fluor 647, anti-canine CD21 (clone CA2.1D6) conjugated with PE and anti-canine MHC class II (clone YKIX334.2) conjugated with FITC.

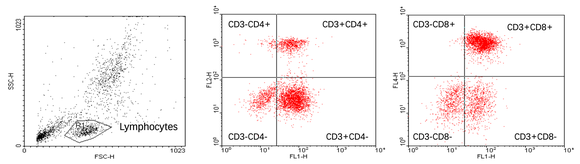



Fig 3. Flow cytometry analysis of canine peripheral blood

The use of this panel of antibodies, exposing each blood sample to three combinations of monoclonal antibodies, allowed the characterization of different lymphocyte subpopulations: T, Th, Tc, B and those that express MHC class II.

*c.* ***Statistical analysis*** Analysis of data was performed using the t-student test or Wilcoxon test, considering a level of significance of $p<0.05$. Statistical analysis was performed using the Statgraphics (Centurion XVI version) software.

## RESULTS

When comparing with healthy dogs, animals with CME showed higher relative values of T cells (72.21% in dogs with ehrlichiosis and 65.69% in healthy dogs, p=0.000) and Tc cells (27.98% in CME and 19.75% in healthy dogs, p=0.042), and higher absolute number of Tc cells in peripheral blood (1117/µl in CME and 506/µl in healthy animals, p=0.007).

On the other hand, the percentage of Th cells (32.91% in CME and 37.91% in healthy dogs, p=0.017) and the relative and absolute values of B cells (14.05% and 355/µl in CME and 18.41% and 527/µl in healthy dogs, p=0.010 and p=0.009, respectively) were higher in healthy animals than in CME affected dogs.

There were not statistically significant differences between *E. canis*-infected dogs and healthy animals in relative and absolute values of MHCII+ and CD3-CD21- lymphocytes, in absolute values of T and Th cells and in the ratio CD4/CD8.

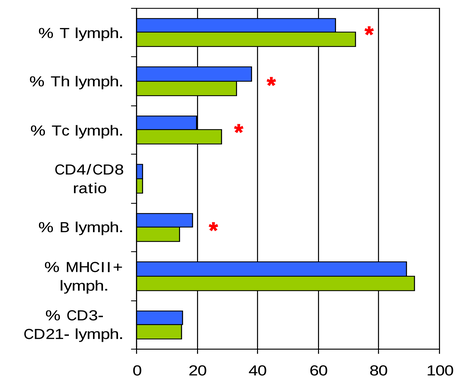



Fig. 4. Mean relative counts of peripheral blood lymphocytes subsets and CD4/CD8 mean ratio in healthy dogs (blue) and in dogs with CME (green).

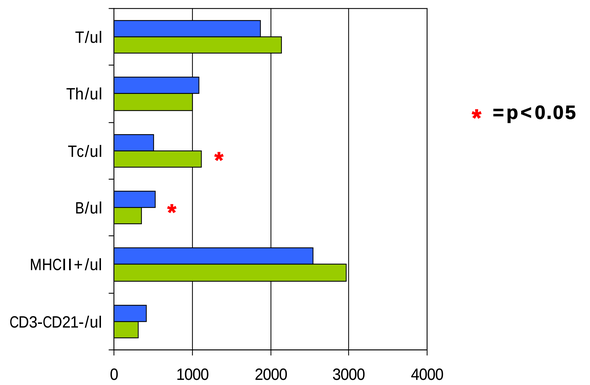



Fig. 4. Mean absolute counts of peripheral blood lymphocytes subsets in healthy dogs (blue) and in dogs with CME (green).

## CONCLUSIONS

Naturally occurring infection by *E. canis* in the dog appears to affect the lymphocyte subpopulations distribution in peripheral blood. The significance of these changes on the pathogenesis of natural infection by *E. canis* needs further evaluation.

## REFERENCES

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