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A COMPARATIVE HAEMATOLOGICAL STUDY IN DOGS INFECTED WITH TWO STRAINS OF *EHRLICHIA CANIS*

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An experiment was conducted to compare haematological changes in dogs associated with two isolates of *Ehrlichia canis*, i.e. one which occurs predominantly in lymphocytes and the other found in neutrophils and eosinophils. The study included complete hemograms (hematocrit, haemoglobin, erthocytes, reticulocytes and total and differential leukocytes). From the haematological data, it was concluded that the disease produced by these two isolates of *E. canis* differed from each other largely in degree of severity. The neutrophilic isolate produced a very mild disorder as compared to the severe illness characterized by anaemia and leukopenia produced by the lymphocytic isolate. Anaemia and leukopenia were not seen in pups infected with neutrophilic isolate. There is a strong evidence that the anaemia in the former case is due to the severe bone marrow depression. In the latter case hematopoietic capabilities were not effected.

INTRODUCTION

Canine ehrlichiosis is a febrile debilitating disease of dogs characterised by anaemia and other signs. Two strains of *Ehrlichia c anis* i.e. lymphocytic isolate in which the organism as a morula is found predominantly in the cytoplasm of lymphocytes and monocytes and in contrast morulae of neutorphilic isolate have been found exclusively in neutrophils, occasionally in eosinophils and never in other leukocytes (Ewing *et al.*, 1963; Ewing *et al.*,1971 and Hayat *et al.*, 1972). The neutrophilic strain was isolated from Arkansas and Okiahoma and produced a milder form of canine ehrlichiosis than the Oklahoma isolate found in 1962 (Ewing *et al.*, 1971; Hayat *et al.*, 1972; Hayat, 1973 and Hayat and Ewing 1973).

The clinical and haematologic changes associated with canine ehrlichiosis

(lymphocytic isolate) were described by Ewing and Buckner (1965). They concluded that severe anaemia produced by *E. canis* was of the normocytic-normochromic type. Dogs with this condition developed severe anaemia and apparently were unable to replace functional erythrocytes rapidly enough to keep pace with the need. The disease produced by the lymphocytic isolate was similar to that described by Bool (1959) who worked with a strain isolated in Netherland Antiles.

Huxsoll *et al.* (1970) reported a highly fatal syndrome termed tropical canine pancytopenia, a newly recognised disease of dogs in the diverse tropical and subtropical areas. Epistaxis was the most dramatic sign of this syndrome and *E. canis* was found consistently in affected dogs. Clinical and haematologic findings concerning this syndrome were reported by Walker *et al.* (1970) and included severe anaemia, leukopenia and thrombocytopenia. The purpose of this investigation was to re-examine the haematological changes in canine Ehrlichiosis caused by lymphocytic isolate and to compare it with neutrophilic isolate.

MATERIALS AND METHODS

A litter of seven pups of mixed breed was used in this experiment. Three of the pups (No. 21,22 and 29) were infected by intravenous inoculation with lymphocytic isolate of *E.canis* obtained from an infected dog. Another batch of three pups from the same lot (No. 27, 28 and 39) was infected with intravenous inoculation of similar quantity of blood containing neutrophilic isolates of *E. canis* obtained from a reservoir dog experimentally infected with this strain. Seventh dog (No. 30) was kept as uninoculated control.

Three millilitres of blood were drawn aseptically from jugular vein of all principals and the unexposed control pre-and post-exposure. The blood was placed in tubes containing 0.04 ml of 16 percent solution of disodium salt of ethylene diaminetetracetic acid (EDTA) as the anticoagulant. All these pups were bled twice a week. The thrice weekly schedule was followed until the recovery of some of these pups.

Haematological methods described by Schalm (1965) were employed to ascertain the packed cell volume (PCV), haemoglobin, total erythrocytes, reticulocytes and total and differential leukocytes. Total red blood cells and total white blood cell determinations were made by using certified pipettes for dilution and the improved "Bright Line" Neubauer haemocytometer for the counting. For differential leukocytic counts films were prepared on cover slips by the method described by Coles, 1967. Smears stained by new methylene blue and counter stained by Wright's stain (buffer 6.8) were used to demonstrate the reticulocytes. The absolute number of reticulocytes among 1,000 erthrocytes was determined and recorded as percentages. Plain capillary tubes were used to determine PCV. Cyanmethaemoglobin method was employed for haemoglobin determination.

RESULTS AND DISCUSSION

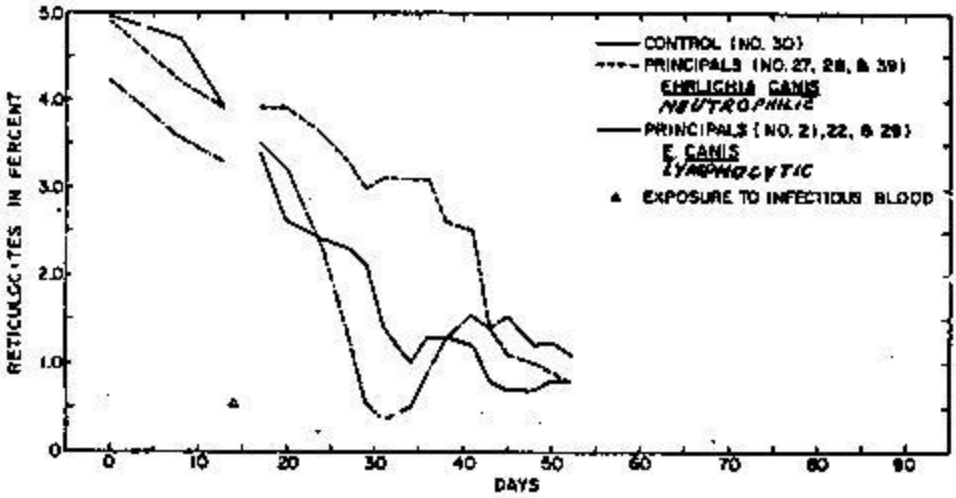
The neutrophilic isolate of *E. canis* produces a very mild disease as compared to that caused by the lymphocytic isolate, which is, in turn milder than the disease produced by the strain or strains isolated in Asia (Ewing *et al* , 1963; Ewing *et al.*, 1971; Hayat *et al.*, 1972; Hayat, 1973; Hayat & Ewing, 1973 and Hayat & Hayat, 1984). Such a finding is also supported by haematological data of this experiment. Figures 1,2,3,4 and 5 depict the haematological changes which developed in six principals (No. 21,22,27,28,29 and 39) suffering from experimentally induced ehrlichiosis and their unexposed littermate control (No. 30).

The PCV, haemoglobin values, erythrocyte and reticulocyte counts can be interpreted to mean that pups infected with the lymphocytic isolate apparently were unable to replace erythrocytes rapidly enough to keep pace with the need and anaemia developed. The development of anaemia corresponds well with the persistence of morulae in the peripheral blood lymphocytes. The PCV, haemoglobin values and total erythrocyte counts decreased soon after exposure and declined to the lowest level 17-22 days after exposure. All values then increased concurrently with the disappearance of fever and parasitemia, only to

Figure 1. Mean packed cell volume of six principals and a control, three principals exposed to *Ehrlichia canis*, neutrophilic isolate and three to *E. canis*, lymphocytic isolate.

Figure 2. Mean haemoglobin values of six principals and a control, three principals exposed to *E. canis*, neutrophilic isolate and three to *E. canis*, lymphocytic isolate.

Figure 3. Mean total erythrocyte counts of six principals and a control, three principals exposed to *E. canis*, neutrophilic isolate and three to *E. canis*, lymphocytic isolate.



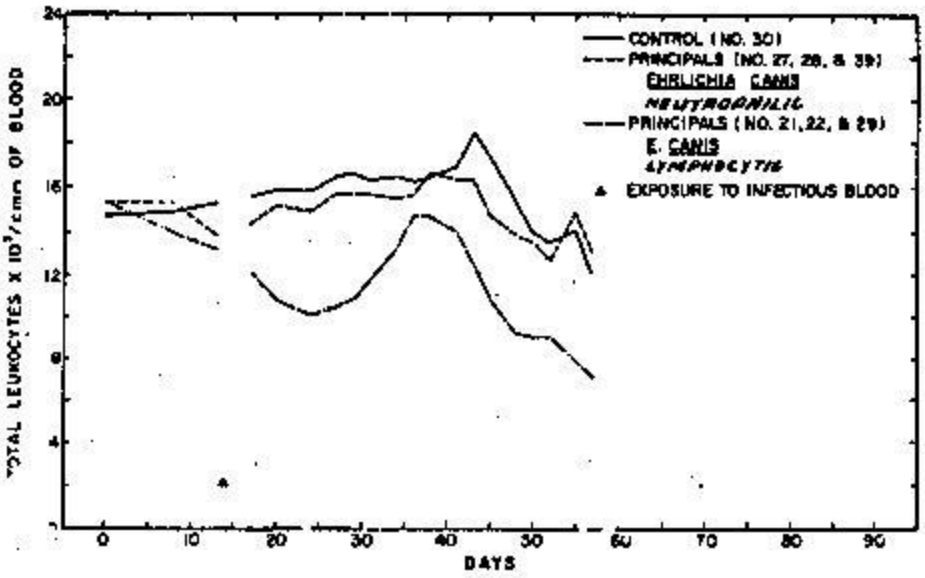


Figure 4. Mean Reticulocyte counts of six principals and a control; three principals exposed to *E. canis*, neutrophilic isolate and three to *E. canis*, lymphocytic isolate.

Figure 5. Mean total leukocyte counts of six principals and a control; three principals exposed to *E. canis*, neutrophilic isolate and three to *E. canis* lymphocytic isolate.

decrease again after a period roughly corresponding to the recurrence of fever and of morulae in the peripheral blood. These findings agree in principle to those of Ewing and Buckner (1965) and Walker *et al.* (1979).

In littermate pups exposed to the neutrophilic isolate, PCV, haemoglobin values and total erythrocytes decreased slightly when compared with control, but the decrease was much less marked than that observed in pups infected with the lymphocytic isolate. Severe anaemia did not develop in any of the pups infected with the neutrophilic isolate. PCV, haemoglobin values and erythrocyte counts reached their lowest level 17 to 31 days post-exposure and then increased quickly reaching the level of the littermate control 24 to 34 days post-exposure.

Reticulocyte counts (Figure 4) in pups infected with the lymphocytic isolate decreased quickly. Anaemia accompanied by a reticulocyte count which was within the normal range indicated that the hematopoietic capabilities of the animals were depressed. This finding was quite different from the results obtained in pups infected with neutrophilic isolate. In this case PCV, haemoglobin values and erythrocyte counts dropped only slightly when compared with the control. The reticulocyte counts remained at a slightly higher level than the control throughout the experiment. This clearly indicates that the hematopoietic capabilities of the host are not depressed by the neutrophilic isolate at any period of the infection. It can be concluded from the present findings, i.e., (a) severe anaemia is accompanied by normal rather than elevated reticulocyte counts in pups exposed to *E. canis*, lymphocytic isolate and (b) absence of anaemia but high reticulocyte counts in those exposed to the neutrophilic isolate and that the anaemia results from the bone marrow depression in one instance and fails to develop in the other. The difference in the response of bone marrow to the organism would appear to be a function of variation in the strain of *E. canis* studied.

The total leukocyte counts (Figure 5) of pups exposed to the lymphocytic isolate decreased slightly for a short period of 3 to 7 days beginning from

10-20 days after exposure and then increased. This increase corresponds to the disappearance of morulae from the peripheral blood. A second decline occurrd 34-38 days post-exposure and persisted till the end of the experiment. The second occurrence of leukopenia was accompanied by recurrence of parasitemia. In contrast, pups exposed to the neutrophilic isolate did not suffer from marked leukopenia, and the leukocyte counts were almost identical to those of the control. Lymphopenia was not marked in any of the pups infected with the lymphocytic isolate at the onset of illness but was observed during the relapse which occurred 31-38 days post-exposure. A slight neutropenia was observed in one pup (No. 39) infected with the neutrophilic isolate in this experiment.

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