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NEUTROPHILIC ISOLATE OF EHRLICHIA CANIS AS A CAUSE OF THROMBOCYTOPENIA IN DOGS

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A study was undertaken to observe the effects of 1970 Oklahoma isolate of *Ehrlichia canis* on the thrombocytes of dogs. Four pups of mixed breeding at approximately six weeks of age were exposed to 1970 Oklahoma isolate of *Ehrlichia canis* by injecting 5 ml. of infective blood intravenously from a reservoir dog. Two pups from the same litter served as uninoculated controls. The thrombocyte counts decreased in the 4 principals and the lowest level was reached 29-31 days after the exposure. The values then returned to the pre-exposure level. No increase in the size of thrombocytes occurred in the principals.

INTRODUCTION

Canine ehrlichiosis caused by *Ehrlichia canis* (Donatien and lestoquard 1935), was first recognized in the United States Oklahoma in 1962 (Ewing 1964 a and 1964 b) more than twenty five years after its discovery in the old world (Donatien and lestoquard 1935). The 1962 Oklahoma isolate of *E. canis* was found to be quite pathogenic for young pups and often produced a fatal disease under experimental conditions. *Ehrlichia canis* occurs in the cytoplasm of leukocytes as a characteristic morula which is actually an aggregate of organisms. A relatively non-pathogenic strain of *E. canis* was reported from Arkansas by Ewing, et al. (1971). The organisim in this case was found primarily in neutrophils rather than in lymphocytes and monocytes and produced a milder form of canine ehrlichiosis than the Oklahoma isolate found in 1962. A similarly less pathogenic neutrophilic strain of *E. canis* was isolated by the author in Oklahoma in 1970 (Hayat, et al. 1972).

There are some reports in the literature stating the role of *E. canis* in the causation of thrombocytopenia. A severe thrombocytopenia was seen in a dog suffering from a syndrome in which the main sign was epistaxis (Bobin, et al. 1962). Depression of all cellular components of the blood including

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thrombocytes in ehrlichiosis was observed in Singapore (Wilkins, et al. 1967 and Huxsoll, et al. 1970). A decrease in thrombocytes was observed in canine ehrlichiosis by workers in California (Gribble 1969). A severe thrombocytopenia was observed, 9-14 days postexposure in dogs infected with 1962 Oklahoma isolate of *Ehrlichia canis*. The thrombocyte count decreased to 21,000/cmm in one pup. Thrombocytopenia persisted throughout the study, a period of four months in 2 pups. (Hayat, 1973).

Inspite of the above mentioned studieds there is a complete lack of knowledge, on the effects of the recently discovered less pathogenic neutrophilic strain of *E. canis* on the thrombocytes of dogs. It is therefore the purpose of this investigation to study the effects of this 1970 Oklahoma isolate of *E. canis* on thrombocytes of dogs. Throughout this paper the terms "1962 Oklahoma isolate" and "lymphocytic isolats" will be used interchangeably as will the terms "1970 Oklahoma isolate" and "neutrophlic isolate." In neither case are the white blood cells named the only ones parasitized by that strain of *E. canis*. They are, however, by far the predominant cell type found to harbour morulae in the respective strains.

MATERIALS AND METHODS

Six pups used for this experiment were a mixture of Chow and Collie and were approximately 6 weeks of age when pre-exposure observations were begun. The dogs were vaccinated to protect against canine distemper and infectious hepatitis. The pups were found infected with *Ancylostoma caninum* and *Toxocara canis* and were treated for ancylostomiasis by subcutaneous injection of dinitrophenol[1] and for *Toxocara canis* infection by oral administration of piperazene citrate.[2]

After pre-exposure studies, four pups were exposed to 1970 Oklahoma isolate of *E. canis* (Figure 1) by injecting 5 ml. of infective blood from a reservoir dog. All the pups were housed in clean quarters which excluded all other animals except arthropods. Ticks were never found on any of the pup or in the room.

The pups were bled from the juggular vein for thrombocyte count. One ml. of blood for thrombocyte count was placed in sterile silicone tubes immersed in an ice water bath maintained at approximately 0°C. Dilutions were made directly from the tube by using either red blood cells (RBC) or

1. DNP; American Cyanamid Company; Princeton, New Jersey
2. Parlomate; Ormont Drug and Chemical Co; Inc; Englewood, New Jersey.

white blood cells (WBC) diluting pipettes. WBC pipettes were used to make 1 : 20 dilution during period of severe thrombocytopenia and RBC pipettes were employed for 1 : 100 dilution when thrombocyte values were not so depressed. Platelets were enumerated by using flat bottomed counting chamber and phase contrast microscopy.

RESULTS AND DISCUSSION

Data on the thrombocyte count is presented in Figure 2. The lines on the graph represent three point averages of the mean values of the principals and of control animals. The thrombocyte count was higher in both controls (# 7 and 10) than in any principal during the pre-exposure period, a bias which could not be avoided because principals were randomly selected from the same litter. During the postexposure period, the thrombocyte counts increased in both controls but stayed within the normal ranges for dogs stated by schalm (1965). At the same time, thrombocyte counts decreased in the 4 principals and the lowest level was reached 29-31 days after expoture. The values then gradually returned to the pre-exposure level. In no case did the thrombocyte counts of either principals or controls approach the subnormal range stated by schalm (1965). Gribble (1969) in his studies of canine ehrlichiosis, reported that thrombocytopenia occurred on days 4 through 12 postexposure and that less than 50,000 thrombocytes/cmm were a frequent observation.

In the present study the thrombocyte numbers were not as drastically decreased as were those of horses studied by gribble (1969). It is difficult to evaluate the differences in effect of these two strains on thrombocytes because two different species of hosts are involved, and the toxonomic relationship between the two isolates of *Ehrlichia canis* is not known. Comparing these results with the earlier data on 1962 Oklahoma isolate (Hayat, 1973), it seems evident that the reduction in the number of thrombocytes as a result of 1970 Oklahoma isolate is not as great as that produced by 1962 Oklahoma isolate. This may be due to the fact that 1970 neutrophilic isolate is mildly pathogenic and it depresses the bone marrow by an unknown mechanism but not to a point to produce recognizable thrombocytopenia as does that 1962 Oklahoma isolate.

No increase in the size of thrombocytes occurred in the principals in the present study. This is in sharp contrast to earlier study in which there was a definite increase in the size of thrombocytes in pups infected with the 1962 Oklahoma isolate (Hayat 1973).

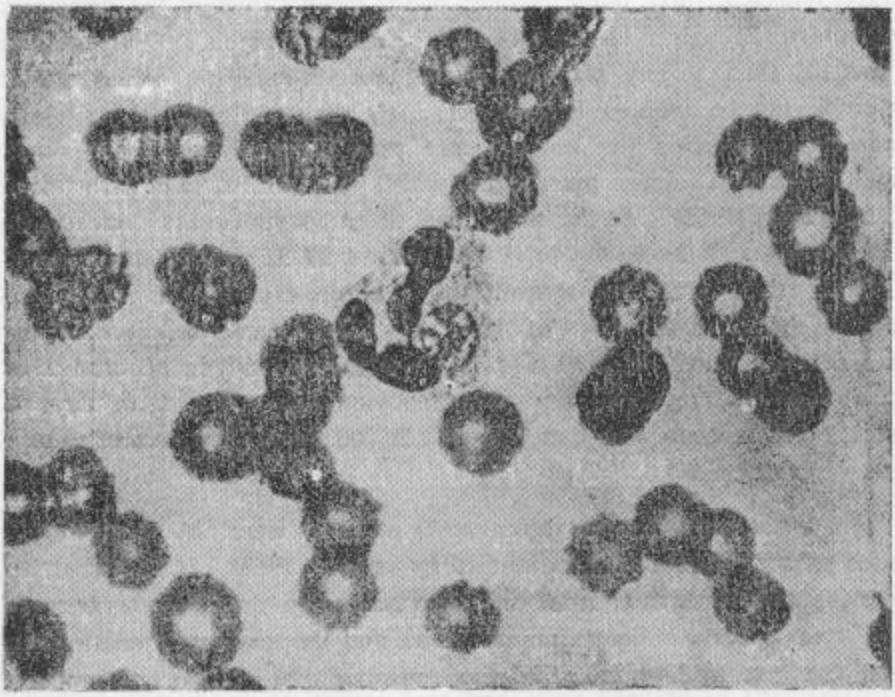

Figure 1. Neutrophil containing morula of *Ehrlichia canis*, 1970. Oklahoma isolate.

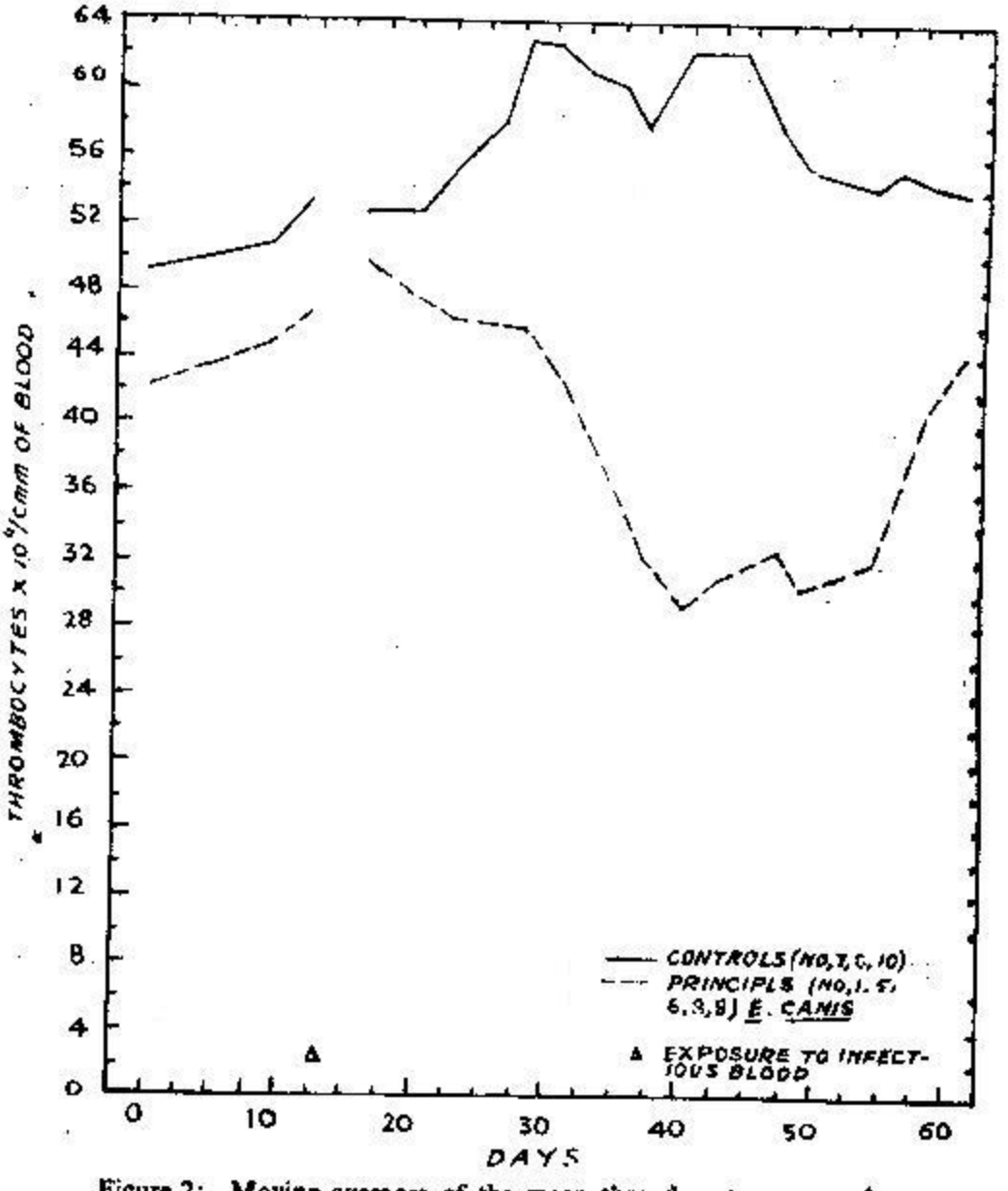

Figure 2: Moving averages of the mean thrombocyte counts of four principals and two controls; principals exposed to *E. canis*, 1970. Oklahoma isolate.

LITERATURE CITED

1. Bobin, A., C. Chabassol, J. de Brux, A. Fiehrer, G. Guillot, C. Michel, and L. Pigoury. 1952. Syndrome Hemorragique Thrombotique Thrombocytopenique du Chien on Tunisie. Rev. pathol. Gen. 62 : 317-334.

2. Donatien, A. and F. Lestoquard. 1935. Existence en Algerie d'une Rickettsia du Chien. Bull. Soc. Pathol. Exot. 28 : 418-419.

3. Ewing, S. A. 1964a Correspondence. J. Amer. Vet. Med. Assoc. 144 : 4.

4. Ewing, S. A. 1964b. Recognition of a canine Rickettsiosis in Oklahoma and its Differentiation from Babesiosis. Ph.D. Tnesis. Oklahoma State University, Stillwater, Oklahoma.

5. Ewing, S. A., W. R. Roberson, R. G. Buckner, and C. S. Hayat. 1971 A New Strain of *Ehrlichia canis*. J. Amer. Vet. Med. Assoc. 159 : 1771-1774.

6. Gribble, D. H. 1969. Equine Ehrlichiosis. J. Amer. Vet. Med. Assoc. 155 : 462-469.

7. Hayat, C. S. S. A. Ewing and R. G. Buckner. 1972. A new strain of *Ehrlichia canis* in Oklahoma Okl. Vet. 24 : 19-21.

8. Hayat, C. S. 1973. *Ehrlichia canis* as a cause of thrombocytopenia in dogs. Pak. J. Sci. Res. 25: 193—196.

9. Huxsoll. D. L., P. K. Hildebrandt, R. M. Nims, and J. S. Walker. 1970. Tropical Canine Pancytopenia. J. Amer. Vet. Med. Assoc. 157 : 1627-1632.

10. Wilkins, J. H., R. S. Bowden and G.T. Wilkinson. 1967. A New Canine Disease Syndrome, Vet. Rec. 81 : 57-58.