EVALUATION OF A SIMPLIFIED MEMBRANE FILTRATION TECHNIQUE FOR THE DIAGNOSIS OF CANINE FILARIASIS

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In his recent presidential address to the American Society of Tropical Medicine and Hygiene, Dr. Paul Beaver stressed the probability that many filarial infections remain undiagnosed by conventional parasitologic techniques. Whether this is due to a complete absence of a microfilaremia or of a concentration too low to be detected by the 20 cmm.-40 cmm. thick blood film is not known. Identification of these cases is of obvious importance not only for the individual patient's welfare but also to gain comprehensive epidemiologic information of an endemic area. Nor can serologic diagnosis be applied with confidence until parasitologic corroboration is forthcoming. For example, in a skin test survey conducted in New Guinea, Desowitz *et al*, (1966) noted an approximately three-fold greater difference between positive skin-test and microfilaremia rates. This discrepancy can variously be interpreted to mean that in the parasitologically negative-immunologically positive individuals (a) the microfilaremia is too low to be detected by the single 20 cmm. film, (b) the adult worms are either dead or not yet sexually mature, or (c) the skin test reaction reflects an antigenic experience with a non-human filaria such as *Dirofilaria.*

There are a number of methods to increase the sensitivity of parasitologic diagnosis. Edeson (1959) demonstrated that the microfilaremia rate may be increased by 25% or more simply by making a 60 cmm. thick blood film rather than one of 20 cmm. Two techniques have been used to concentrate the microfilaria in relatively large blood samples. The Knotts technique is the older of these procedures. Recently Bell (1967) has introduced the promising method of filtering hemolyzed blood through a Millipore filter. The filter which retains the microfilaria is stained, cleared and examined microsopically. However, both the Knott and Bell procedures are limited for field use by the necessity of electrically operated apparatus. In order to overcome this limitation, we (Chularerk and Desowitz, 1970) have devised a simplified membrane filtration concentration technique which requires only a syringe and Swinney membrane-filter holder. As a preliminary investigation prior to trial in an endemic area of human filariasis, we have evaluated the efficiency of the method for the diagnosis of canine filariasis. The results of this study are presented in this paper.

MATERIALS AND METHODS

Heparinized venous blood was obtained from dogs impounded in the Honolulu Humane Society's kennels. One ml. of each blood sample was concentrated by the membrane filtration technique of Chularerk and Desowitz (1970). Two linear 20 cmm. thick film smears were also made of each blood sample. The smears were dehemoglobinized and stained in Giemsa.

RESULTS AND DISCUSSION

Of the 92 blood samples examined, twenty-nine (31.5%) were found to have microfilariae in the 40 cmm. blood film. In all but one positive blood film, microfilaria were

found in both 20 cmm. smears. Concentration of 1 ml. of blood by membrane filtration revealed an additional 16 positive samples to give a total microfilaremia rate of 47.82%.

In the samples in which the thick films were negative but were found to be positive on filtration, the number of microfilariae counted on the membranes ranged from 1 to 33. Since the concentration technique employs a 50 times greater volume of blood than the 20 cmm. smear it would appear that even one microfilaria in 1 ml. of blood can probably be detected by this method. Larger amounts of blood can be concentrated and it would be of interest to determine the actual volume of blood required before a negative diagnosis can be confidently made.

It is estimated that a trained technician can process 35 to 40 blood samples a day using the technique that we have described. It is felt that this number can be increased appreciably by the use of simple mechanical aids such as a press to facilitate passing the hemolyzed blood through the filter. These accessories are now being designed.

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