Acta vet. scand. 1972, *13*, 591—593.

Brief Communication

MIGRATION INHIBITION TESTING ON PERIPHERAL LEUKOCYTES FROM LIVER FLUKE INFECTED RABBITS

The development of delayed type hypersensitivity in animals infected with Fasciola hepatica was observed by migration inhibition studies on peripheral leukocytes according to the technique of *Aalund et al.* (1970).

Eight adult conventional, female albino rabbits were randomly distributed into 2 groups of 4 rabbits. One of the groups served as a control group. In the second group each rabbit received 50 metacercariae administered perorally in gelatine capsules. Migration studies were initiated 5 months after inoculation when the infection was patent and clinically manifest with anemia, hypoalbuminemia and hyperimmunoglobulinemia. Blood was drawn from an ear vein of each rabbit every second week, and after 2 months the infected rabbits were sacrificed. Twelve, 14, 15 and 31 adult parasites were recovered, respectively.

One volume of heparin stabilized blood was mixed with 0.5 volume of plasmagel* and the leukocytes were harvested by aspirating the supernatant plasma after sedimentation of the erythrocytes at 37°C and 1 × g for 1 hr. The leukocytes were subjected to the migration inhibition test using as antigens extracts with phosphate buffered saline (PBS), 0.015 M-PO_4, 0.15 M-NaCl, pH 8.0, of sonically disintegrated adult Fasciola hepatica and liver fluke eggs, both obtained from infected cattle. The dry matter content of the antigen solutions was 2 mg/ml for Fasciola hepatica antigen and 1 mg/ml for the egg antigen.

Two dose levels, 3 and 5 drops per 1 ml migration chamber, were employed with both antigens in each test. The distribution of the migration indices (I) for the experimental period of leukocytes from the infected and the control group respectively was subjected to chi-square analysis (Table 1). It may be seen that only in the case of egg antigen, 5 drops, was a significant

* A plasma substitute based on modified fluid gelatine. Laboratoire Roger Bellon, Neuilli-Paris, France.

Table 1. Chi-square analysis of the distribution of migration indices.

Antigen	Dose of antigen	Migration index (I)*	Probability**
egg	3 drops	$I < \bar{x} - 1s$	$P = 1.00$
egg	5 -	$I < \bar{x} - 1s$	$0.02 < P < 0.05$
liver fluke	3 -	$I < \bar{x} - 1s$	$0.80 < P < 0.90$
liver fluke	5 -	$I < \bar{x} - 1s$	$0.30 < P < 0.50$
egg	3 -	$\bar{x} - 1s \geq I > \bar{x} - 2s$	$P = 1.00$
egg	5 -	$\bar{x} - 1s \geq I > \bar{x} - 2s$	$0.05 < P < 0.10$
liver fluke	3 -	$\bar{x} - 1s \geq I > \bar{x} - 2s$	$0.80 < P < 0.90$
liver fluke	5 -	$\bar{x} - 1s \geq I > \bar{x} - 2s$	$0.30 < P < 0.50$

* $\bar{x}$ and s are the mean and the standard deviation of the control group of rabbits for the type and dose of antigen indicated in the table.

** Probability of the percentage of indices complying with the specification indicated in the index column being identical for the control group and the infected group of rabbits.

difference ($P < 0.05$) encountered for the criterion $I < \bar{x}$ minus 1 standard deviation. This observation is consonant with a significant difference ($P < 0.05$) between egg antigen (dose 3 drops) and fluke antigen (dose 3 drops) when compared on the basis of observations in the infected group of animals.

During this late phase of the infection there was no significant difference between the control group and the infected group of rabbits in regard to the relative leukocyte yield ($P > 0.40$) and in regard to the planimetric figures of the control migration chambers ($P > 0.98$).

The rabbits of the control group in the first experiment were subsequently infected with 50 metacercariae and studied according to the protocol of the first experiment. Blood was drawn every second week throughout the course of the infection. The rabbits were sacrificed 4 months after the inoculation, and 8, 15, 27 and 37 adult flukes respectively were recovered. Parasite eggs were observed in the feces 8 weeks after inoculation thus indicating that the infection had become patent within the usual period of time. The rabbits subsequently became anemic and dysproteinemic.

All rabbits of the second experiment developed reactivity in the migration inhibition system against the 2 dose levels of egg and liver fluke antigen respectively. A migration index $\geq \bar{x} +$

1 s was recorded as stimulation of migration, and a migration index $\leq \bar{x} - 1$ s was recorded as inhibition of migration. The parameters $\bar{x}$ and s are the mean and the standard deviation of the preinoculation period for the respective type and dose of antigen. A period of approx. 4 weeks with stimulation preceeded the phase with migration inhibition. Three of the rabbits did not exhibit migration inhibition until 105 days after inoculation, while the fourth rabbit showed inhibition from day 42 of the experiment and onwards. The egg and fluke antigens appeared to possess comparable potency in this experiment.

ACKNOWLEDGEMENT

Dr. H. J. Bendixen and Dr. P. Gørtz, The State Veterinary Serum Laboratory, kindly serviced the leukocyte counts. The authors are grateful to Mrs. Kirsten Poulsen for very skilled technical assistance.

O. Aalund
The Department of Forensic and State Veterinary Medicine,

P. Nansen
The Department of Special Pathology and Therapeutics,
The Parasitological Research Group of the Danish Agricultural and Veterinary Research Council,

Royal Veterinary and Agricultural University,
Copenhagen, Denmark.

REFERENCES

Aalund, O., A. B. Hoerlein & H. C. Adler: The migration test on circulating bovine leukocytes and its possible application in the diagnosis of Johne's disease. Acta vet. scand. 1970, *11*, 331—334.

(Received September 18, 1972).

Reprints may be requested from: O. Aalund, Department of Forensic and State Veterinary Medicine, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.