EFFECTS ON *Anguillicola crassus* AND *Trypanosoma granulosum* INFECTIONS ON PERIPHERAL BLOOD CELLS OF EUROPEAN EEL (*Anguilla anguilla* L)

BY J.H. BOON, YAO ZUXU AND G.H.R. BOOMS

Recent reports have shown that infestation of the European eel, *Anguilla anguilla*, with the swim bladder nematode, *Anguillicola crassus*, is widely spread in Europe, both in natural basins and in intensive eel culture (Peters and Hartmann, 1986; Tarschewski *et al.*, 1987). Some reports indicate that in intensive eel culture, this infection can induce economic losses (Egusa, 1979).

*Trypanosoma granulosum* has also been found in the circulating blood of eels in the Netherlands (Boon, unpublished result, 1988) and Portugal (Eiras, 1989). Mammalian trypanosomiasis is characterized by a haemolytic anaemia resulting in a decreased haematocrit (Hct) (Dwinger, 1985). Granulocytes play an important role in the inflammatory response to parasitic infestations. In eels two types of granulocytes can be distinguished by specific staining methods: granulocytes which have polysaccharides on the cell surface and granulocytes which have peroxidases in cytoplasmic granules. It is possible that simultaneous infections with *A. crassus* and *T. granulosum* will change the Hct values and percentages of (specific) granulocytes in the peripheral blood of the host due to their larval migration and blood sucking (*A. crassus*) and haemolytic (*T. granulosum*) properties. Thus wild-caught eels were investigated to see if infestation by these parasites could be correlated with changes in Hct and granulocytes in the peripheral blood.

Out of 100 wild-caught eels originating from Lake IJssel, the Netherlands, 30 eels were randomly chosen. The mean weight (+/- s.d.) was 23.7g (7.0). The eels were anaesthetized with Hypnodyl® (10 mg/l) and blood was sampled from the *Vena caudalis* (Boon *et al.*, 1989). From each eel, 2 blood smears were made and the Hct was determined. Blood smears were stained either by the May-Grunwald-Giemsa method to determine the relative abundance of lymphocytes, granulocytes and monocytes (Boon *et al.*, 1990) and to estimate the parasitemia of the trypanosome (magnification 1000×); or with 3'3-diamino benzidine (DAB) reagents to identify granulocytes with peroxidases (DAB+) together with the periodic acid schiff (PAS) method to identify granulocytes with polysaccharides (PAS+).

Thereafter these smears were counterstained in Mayer's haemalum. The granulocytes were judged as (1) PAS+DAB+, cells with red cytoplasm and brown granules in the cytoplasm; (2) PAS+DAB-, cells with red cytoplasm without brown granules; (3) PAS-DAB+, cells with lilac-pink cytoplasm and brown granules; and (4) PAS-DAB-, cells with lilac-pink cytoplasm without brown granules. The relative abundance of each group was determined by counting a total of 100 granulocytes at a magnification of 1000× (Boon *et al.*, 1990). After blood sampling the eels were sacrificed, the swim bladder dissected and the number of *A. crassus* was counted macroscopically.

The effects, including quadratic and interaction terms, of the two parasites on the Hct and white blood cells were examined by analysis of variance. Thereafter, the regressions of the number of parasites on the values of the parameters, which were significantly influenced by the parasites, were analysed (Snedecor and Cochran, 1967).

In 70% of the eels one or more specimens of *A. crassus* were observed, with a mean number of 3.1 per eel (range: 0–5). In 89.3% of the eels one or more specimen of *T. granulosum* were found, with a mean number of 33.1 trypanosomes per blood smear (range:0–200).
In 62.5% of the eels both parasites were found. The analysis of variance revealed a significant effect of \textit{A. crassus} on the DAB+PAS+ granulocytes ($p<0.05$) and also the trypanosomes significantly affected the hematocrit ($p<0.01$). No interaction effect nor quadratic effects of these two parasites on the blood parameters measured were found. The analysis of the regressions showed that the Hct was negatively influenced by the \textit{T. granulosum} infection ($y = -0.0002 \times \text{number of trypanosome} + 29.8, p<0.01$), while the \textit{A. crassus} infection positively influenced the number of PAS+DAB+ granulocytes ($y = 1.49 \times \text{number of nematodes} + 27.6, p<0.05$).

From the results of the analysis of variance it can be concluded that the effects of \textit{A. crassus} and \textit{T. granulosum} infections on the investigated blood parameters are independent of each other.

In the present study the results concerning the Hct are comparable with those from previous studies (Boon \textit{et al.}, 1989, 1990). \textit{A. crassus} infection did not induce significant changes of the Hct of wild-caught eels, though even the gut of the (pre)adults of \textit{A. crassus} was found engorged with host erythrocytes (Boon \textit{et al.}, 1990). An explanation for the independence of the Hct from the \textit{A. crassus} infection is probably the time span between infection and sampling of the eel. If the time span is long enough the eel may re-establish the previous Hct level and therefore mask the influence of the infection with \textit{A. crassus} on the Hct. When migrating from the intestine to the swim bladder lumen, \textit{A. crassus} larvae partly damage some internal tissues of the eel and induce an inflammatory response (Haenen \textit{et al.}, 1989). In the present study there was a positive influence on the PAS+/DAP+ granulocytes which may represent a defensive response to the helminths.

In the present study, a high % of eels with a high \textit{T. granulosum} burden was found. This confirmed results of earlier reports in which the eels in Lake IJssel had been found widely and heavily infected by this parasite (Boon, unpublished results, 1967). A concurrent decrease of the hematocrit is thought to be due to the hemolytic characteristics of the parasite. This hypothesis is corroborated by results of Eiras (1983) who described a degeneration of eel erythrocytes as a consequence of \textit{T. granulosum} infection. One might expect that parasitic infection causes an increase in the level of granulocytes in the peripheral blood with a shift in the DAP+/PAS+/DAP-PAS- ratio. However, the percentage of DAP+/PAS+ granulocytes was not influenced by \textit{T. granulosum} in this study, which might be due to a possible immunosuppression induced by the parasites as shown in mammals (Dwinger, 1985).

Effects of \textit{T. granulosum} infections on eel have only been incidentally studied before. The high numbers of trypanosomes found in the present study in relatively small eels, indicates that the importance of \textit{T. granulosum} infection in eel might be underestimated.

The results of the present study support the idea that more attention should be paid to the relevancy of \textit{T. granulosum} infection for eel production.

\textbf{Summary}

Wild-caught European eel (\textit{Anguilla anguilla}) were investigated for the presence of \textit{Anguillicola crassus} in the swim bladder and \textit{Trypanosoma granulosum} in the peripheral blood and the influence of these parasites on the peripheral blood cells was studied. It appeared that \textit{A. crassus} increased the percentage of periodic acid schiff and peroxidase positive granulocytes while \textit{T. granulosum} decreased the hematocrit.

\textbf{References}


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Authors’ address
Department of Fish Culture and Fisheries, Wageningen Agricultural University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.