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Parasitology

**OBSERVATIONS ON *TRYPANOSOMA GRANULOSUM* LAVERAN
ET MESNIL, 1902 (PROTOZOA, KINETOPLASTIDA), A BLOOD
PARASITE OF EEL, *ANGUILLA ANGUILLA* (L.)**

**NIEKTÓRE OBSERWACJE *TRYPANOSOMA GRANULOSUM* LAVERAN
ET MESNIL, 1902 (PROTOZOA, KINETOPLASTIDA) PASOŻYTA KRWI
WĘGORZA, *ANGUILLA ANGUILLA* (L.)**

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The results of a comparative study on *Trypanosoma granulorum* Laveran et Mesnil, 1902 in the peripheral blood of eel, *Anguilla anguilla* from two lakes in north-western Poland are presented. The following parameters were analyzed: dependence of the infection parameters on the host's age and the number of the protozoans in one drop of blood. Hitherto existing data on the morphology of the parasite have been presently supplemented. In addition, the division stages were observed, which indicates that the species can multiply within the fish. Such phenomenon has not been observed in *T. granulorum* in natural conditions.

INTRODUCTION

Protozoans *Trypanosoma granulorum* Laveran et Mesnil, 1902 have been recorded in the European eel in England (Kennedy 1974; Davies et al. 1992), Denmark (Køie 1988 a, b), the Netherlands (Boon et al. 1990), Portugal (França 1907; Eiras 1988; Saraiva and Chubb 1989; Saraiva 1994), former Czechoslovakia (Lom et al. 1989), former Soviet Union (Šul'man 1962, 1984), and Poland (Orecka-Grabda 1986; Własow et al. 1991; Wierzbicka and Orecka-Grabda 1994). The majority of the works represented parasite-fauna surveys, and only some of them provided morphological descriptions of those para-

sites. Detailed analysis of morphometric characters, based on 47 specimens was conducted by Eiras (1988). He also compared his own data with the data of earlier authors. On the other hand, Saraiva (1994), in her study, took into account the seasonality of infection. Data on the impact of *T. granulorum* infection on the blood parameters of the European eel were presented by Boon et al. (1990), while in vitro culture of this species was described by França (1907) and Davies et al. (1992).

The presently conducted study was aimed at: monitoring *T. granulorum* infections in the peripheral blood of the eels from the two lakes of north-western Poland, proving the existence of possible relationship between the infection parameters and the age, and determination of the protozoans' quantities in one drop of blood. In addition, the observations of the collected individuals yielded data supplementing hitherto existing information on the morphology and biology of this species parasitic in the fish.

MATERIAL AND METHODS

The studied fish originated from Lake Dąbie, situated by the Odra River estuary, and from Lake Siecino in the province of Koszalin. The sampling in both lakes took place within spring and fall, i.e. in May, September, and October 1970–1973. From each lake a total of 6 samples was taken. The number of the fish in these samples ranged from 30 to 41. A total of 185 eels *Anguilla anguilla* (L.) from Lake Dąbie and a total of 242 specimens from Lake Siecino have been studied. The length (longitudo totalis) of the fish from Lake Dąbie was 48–92 cm, while that from Lake Siecino it was 38–83 cm. The masses were 190–1190 g and 80–840 g respectively. The eel's age ranged from 2+ to 9+ (without considering the larval period). In the samples from Lake Dąbie, predominated specimens aged 5+ and 6+, while in those from Lake Siecino—age groups 3+ and 5+ were most numerous.

Captured eels were kept alive after being brought to the laboratory for up to 3 days. The blood was sampled from the caudal vein. The blood smears were made using one drop of blood in all cases. These smears, after drying, were stained using the Pappenheim method (May-Grünwald and Giemsa liquids).

In order to verify the exactness of the smear method, live flagellates were counted in single drops of blood from 33 randomly selected eels, using the Bürker chamber. The results were compared with the data from the same fish individuals studied, using stained smear (taking into account the whole surface area). The results obtained, showed higher similarity under low intensity of the infection (Fig. 1). On the other hand the differences under low and maximal infection were sometimes substantially extensive and proved rather the advantage of counting live protozoans. In the further determinations of the infection intensity in all fish, the specimens were counted on the stained slides, with the smear area limited to the area of 20 × 25 mm.

RESULTS

The infection by *Trypanosoma granulosum* Laveran et Mesnil, 1902 in the peripheral blood of the eel, from the studied lakes was variable. The prevalence of the analyzed fish population from Lake Dąbie was substantially higher and it equaled 68%, whereas that from Lake Siecino reached only 24%. The intensity of infection defined as the number of protozoans found on a smear area of 20×25 mm, ranged in the eels of both water bodies from single to mass occurring (above 30 individuals). Generally it was higher in Lake Dąbie, where it averaged in three samples 2.2–6.4 parasites, while in three other—9.5–16.2 specimens. In the latter water body, as many as eight fish exhibited mass infection. On the other hand, the mean intensity of infection of the eels from Lake Siecino in four samples ranged from 0.2 to 2.1 protozoans and only in two samples it reached 8.2 and 16.0 parasites. In the latter sample only, a mass infection was revealed in two cases (one eel contained as many as 117 specimens within the smear area of 20×25 mm).

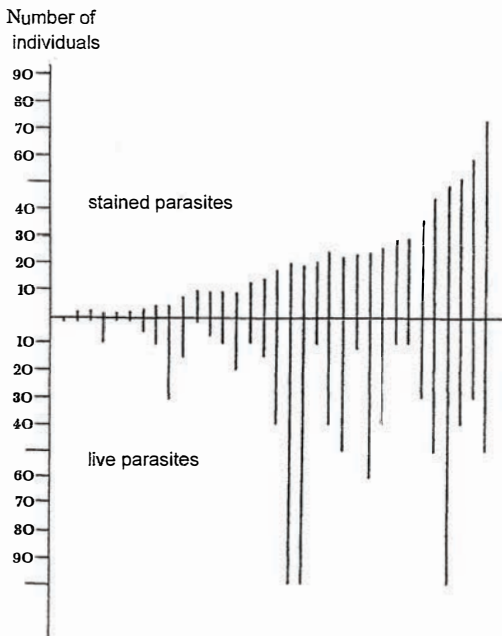


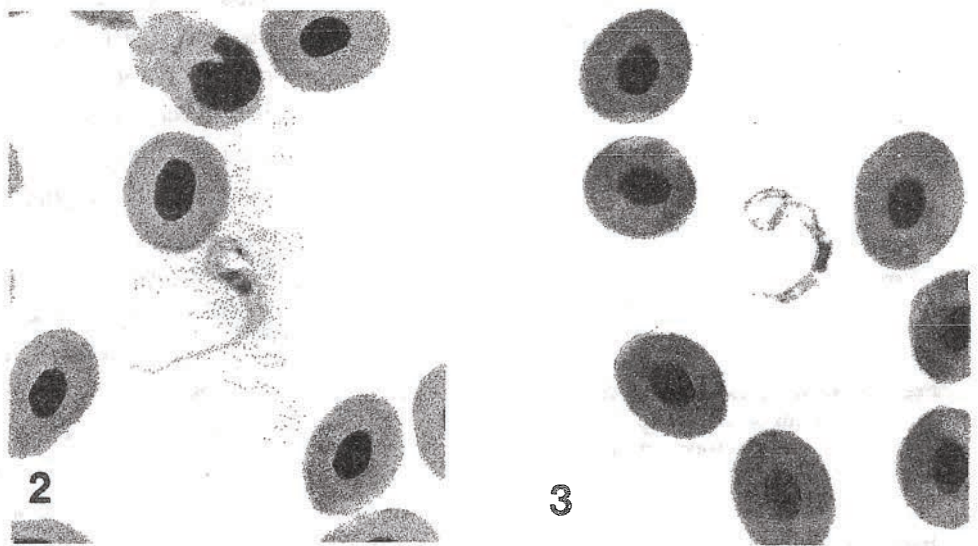
Fig. 1. Graphic representation of the numbers of *T. granulosum* specimens in one drop of the peripheral blood of the eels, counted in two ways: on the stained preparations and *in vivo*

In one drop of blood of the randomly selected eels a maximum of 70 protozoans was found on a stained smear, while as many as 100 parasites was observed in three fish in the blood *in vivo* (Fig. 1). There was even higher intensity—in the blood of an eel from the Szczecin Lagoon—up to 190 trypanosomes in a single drop of blood (Orecka-Grabda 1986).

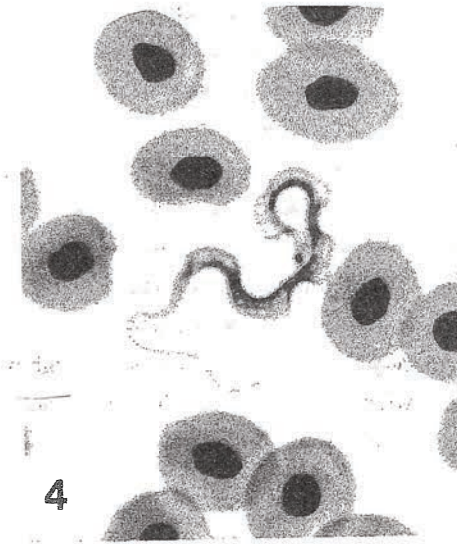
Examination of the peripheral blood smears of the eels from Lake Dąbie and Lake Siecino revealed that all fish in all eight studied age groups i.e. 2+ to 9+ were infected with *T. granulosum*. In addition, in the course of the survey of the eels from the Szczecin Lagoon, the infection by this species was revealed also from the individuals in the age of 1+ and 10+

(data from Orecka-Grabda 1986). The results acquired from both lakes did not show clear relationship between the intensity of infection by *T. granulorum* and the host age. The prevalence in Lake Dąbie ranged in the respective age groups from 58 to 79% (assuming the number of studied fish in a group as 100%). In Lake Siecino it equaled 33–43% and only in the sixth year of life it reached 70%.

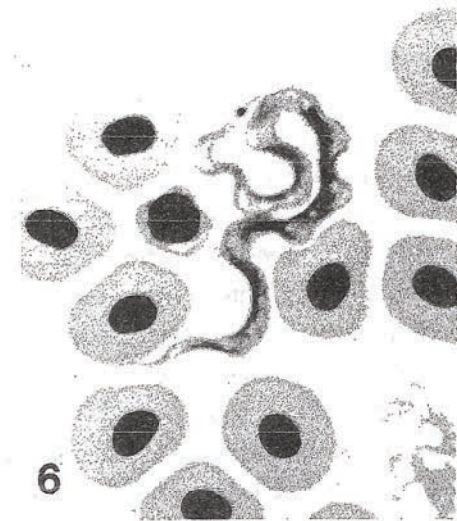
In the peripheral blood of the studied eels, variable in size and shape forms of the flagellates were being encountered. They all represented, however, the trypomastigote type. In the observed protozoans the kinetoplast was always situated close to the posterior end of the cell. On the individual smears, small as well as very large specimens were being encountered. The smallest forms (Figs. 2, 3) had their body lengths within 13.9–20 μm , body widths within 1.8–1.9 μm , and the length of the free flagellum within 10–15 μm . The largest specimens (Figs. 6, 7) reached maximum body lengths of 75–78.4 μm , width 2.7–2.9 μm , and the flagellum length about 30 μm . Besides the described above ultimate forms, there were also relatively numerous intermediate stages (Figs. 4, 5). The smallest protozoans, except for the morphometric differences, were characterized by weakly and uniformly stained cytoplasm, in which sometimes singular stain-resistant vacuoles were observed. The large, as well as intermediate forms were more intensely stained and contained, in their cytoplasm, numerous granules and clearly delimited unstained vacuoles distributed on the whole length (Figs. 4–7). The number of the trypanosomes (on the studied slides) was the highest for the medium-size forms, while very small forms were the most rare.



Figs. 2, 3. The smallest forms of *T. granulorum* in the peripheral blood of eel



Figs. 4, 5. Intermediate forms of *T. granulorum* in the peripheral blood of eel



Figs. 6, 7. The largest forms of *T. granulorum* in the peripheral blood of eel

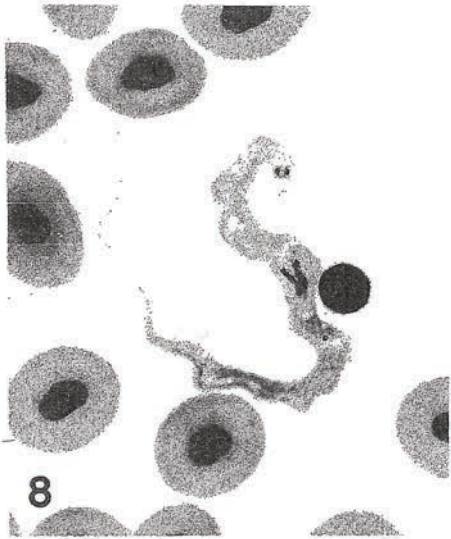


Fig. 8. *T. granulorum* in the peripheral blood of eel; specimen in the division stage

In the studied material, in at least three cases, large trypanosomes were found being at the stage of division, with dual kinetoplast, clearly visible and regrouoped chromosomes in the zonally stained nucleus (Fig. 8). The body length of these specimens equaled 46–49.6 μm , width—3.3–3.5 μm , and the length of free flagellum—17–20 μm . The protozoans at the stage of cell division were slightly wider compared to the other large forms

DISCUSSION

The presently conducted study, on the two lakes, showed clear variability in the infection by the protozoan *Trypanosoma granulorum*. It is undoubtedly connected with various environmental conditions in these water bodies. Environmental impact on the parasite fauna of the eel has been discussed also in the works of Wierzbicka and Orecka-Grabda (1994), Orecka-Grabda and Wierzbicka (1994), and K oie (1988b). Very high intensity of infection of eel by this species was reported by K oie (1988a), Eiras (1988), Saraiva and Chubb (1989), Boon et al. (1990), and Saraiva (1994). The present studies revealed, that in some cases the intensity of infection was very high. It reached maximal number of 190 specimens in one drop of blood and 117 trypanosomes in the smear, in the area of 20 \times 25 mm. Similarly high infection (up to 200 specimens on a smear) were recorded by Boon et al. (1990). Their data concerned small ells (average mass 23.7 g), while the present results were based on big fish. Two different methods for counting the protozoans (in vivo and on stained smears) from the same fish exhibited certain differences (Fig. 1).

It has been revealed, while analyzing relatively abundant, age-variable material from Lake Dabie and from Lake Siecino, that infection by the protozoans *T. granulorum* was not dependent on the eel's age. These parasites were recorded in all studied groups i.e. from 1+ to 10+. Eiras (1988) studied only 23 ells measuring 17.3–52.4 cm and also found no relationship with the host's length. Similarly, Saraiva (1994) did not found significant differences in the infection seasonality of the eel by this species.

The trypanosomes recorded in the peripheral blood of the presently studied eels from both lakes exhibited very wide range of sizes. The maximum values of the body length of the large forms were very much alike those published by Eiras (1988) and Lom and Dyková (1992). On the other hand, the smallest forms according to the above-mentioned authors measured 32 μm (Eiras 1988), 25 μm (Šul'man 1962, 1984, Lom et al. 1989) and 20 μm (Lom and Dyková 1992). In the presently studied material the smallest specimens—of the body length within the range of 13.9–20 μm . The above forms differed from the large and intermediate forms by the ability to stain and by the structure of the cytoplasm, what has been noticed as early as by França (1907) describing *T. granulosum* as var. *magna* Lebailly and var. *parva* Lebailly.

The forms at the stage of division have been found also in the smears of the peripheral blood. These forms are the first records of a *T. granulosum* individual dividing in the blood of the European eel under natural conditions. Multiplication of the flagellates *T. carassi* (Mitrophanov, 1883) in the blood of *Carassius auratus auratus* under experimental condition was described by Lom and Dyková (1992). According to the above-mentioned authors the ultimate forms of the trypanosomes may undergo divisions in the blood of fishes. Multiplication of *T. granulosum* cultured in vitro was achieved by Davies et al. (1992). These were the epimastigote and trypomastigote forms.

There is a likelihood that the observed differences in the size and structure of the cytoplasm of the specimens of *T. granulosum* found, may be linked, among others, with possibility of divisions of the large forms in the blood of eel, what has been recorded in the present material. Unequal longitudinal division of *T. carassi* in the blood of fish under experimental conditions is suggested by the data (Fig. 2.19) published by Lom and Dyková (1992). According to the above-mentioned authors the presence of different forms of *T. granulosum* in the blood of the fish may be attributed to the phenomenon of pleomorphism occurring in this parasite species.

RECAPITULATION

1. The study on the peripheral blood of the eels from two lakes of north-western Poland exhibited distinct variability in the infection of the fish populations from these lakes by the protozoan *T. granulosum*. In some instances, very high infection intensity, reaching 190 flagellates in a drop of blood was recorded.
2. There was no relationship between the infection parameters and the host's age. All the age groups were infected (from 1+ to 10+).
3. Presently recorded, smaller than hitherto described forms and forms at the stage of division evidenced multiplication of *T. granulosum* in the eel's blood.

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STRESZCZENIE

Ocena zarażenia węgorza, *Anguilla anguilla* (L.) pierwotniakami *Trypanosoma granulosum* Laveran et Mesnil, 1902 wykazała znacznie wyższy poziom zarażenia w jeziorze Dąbie (68%) w porównaniu do jeziora Siecino (24%). Wiąże się to niewątpliwie z różnymi warunkami ekologicznymi w tych zbiornikach. W badanym materiale zanotowano w niektórych przypadkach bardzo wysoką intensywność zarażenia, maksymalnie osiągała ona do 190 pierwotniaków w jednej kropli krwi. Nie stwierdzono w nasileniu zarażenia zależności od wieku żywiciela, zarażeniem były objęte wszystkie badane grupy od 1+ do 10+.

Obserwowane trypanosomy we krwi obwodowej węgorzy posiadały bardzo duży zasięg wielkości. Znalezione mniejsze formy od dotychczas opisywanych (w zakresie 13,9–20 µm długości ciała) oraz pojedyncze postaci w stadium podziału, świadczące o pomnażaniu się *T. granulosum* we krwi węgorza; zjawisko nie notowane u tego gatunku w warunkach naturalnych.

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