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MORPHOLOGICAL AND MORPHOMETRICAL STUDIES OF *TRYPANOSOMA DANILEWSKYI* STRAIN FCC-1.

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ABSTRACT

Morphological and morphometrical study was conducted on blood stream forms of *Trypanosoma danilewskyi* strain F Cc-1, isolated from laboratory-infected single breed juvenile common carp. A group of ten fish was intraperitoneally inoculated with 1000 live trypanosomes per fish. At the peak of parasitemia thin blood smears were prepared on precleaned glass slides on days 20, 30, 40 and 50 post infections (p.i). Blood smear was air dried, fixed with methanol and stained with May-Grünwald Giemsa stain. Stained trypanosomes were examined under light microscope at X 1000 magnification in the immersion oil. Morphology of trypanosome was recorded with a camera mounted on the microscope. From well stained slides 200 good specimens of *T. danilewskyi* FCc-1 were selected. For morphometric parameters, linear measurements in microns and nuclear area in square microns were taken by using Videoplan (Image Analysis System Kontron Bildanalyse, Germany). Morphometric measurements of *T. danilewskyi* strain FCc-1 were compared with reference strain of *T. danilewskyi* (Caa-1) already described earlier. The data revealed that *T. danilewskyi* strains FCc-1 and Caa-1 are the same.

Key words: morphometry, *T. danilewskyi* strain FCc-1, common carp, species identification, reference strain Caa-1.

INTRODUCTION

Traditionally fish trypanosome identification is based on the host species where it is isolated from (Mandal, 1977) and its morphological characters when compared with already described species (Smirnova, 1970). Other features like cytoplasmic granules are also considered as an important diagnostic parameter (Woo, 1981; Woo and Black, 1984). As a result of host based species identification, at least 149 species of fish trypanosomes have been documented (Lom, 1979; Zajicek, 1990). Host specificity is another characteristic in some fish trypanosomes, for instance *Trypanosoma platessae* is very much host specific while others (e.g. *T. cobitis*) are not. Host specificity is however, not so strict that it could be applied for species identification (Zajicek, 1990; Zajicek and Peckova, 1990; Overath *et al.* 1999).

The results of carefully executed cross infection experiments indicate some degree of reliability in combination with morphological and morphometrical diagnosis (Lom, 1979; Woo, 1981; Zajicek and Peckova, 1990 and Zajicek, 1990; Ahmed, 1994; Overath *et al.* 1999). It is further required a thorough and careful study of morphological and morphometrical measurements to be sure about the species concerned. The present study was conducted to evaluate the morphological and morphometrical measurements of *Trypanosoma danilewskyi* strain FCc-1(maintained through syringe passages), isolated from experimentally infected juvenile common carp for complete description and identification in comparison with *Trypanosoma danilewskyi* strain Ccc-1 already described by Woo (1981) and re-described by Woo and Black (1984).

MATERIALS AND METHODS

Single breed juvenile common carps (6 months old) were used in the laboratory infection of *Trypanosoma danilewskyi* strain FCc-1. Blood stream forms were used for morphological and morphometrical studies.

Morphological study of *T. danilewskyi* strain FCc-1: Blood stream form (BSF) trypanosomes were separated from the infected blood using a DEAE-cellulose column (Ahmed *et al.* 2001), washed with PSG (phosphate buffered saline with glucose, pH 7, filter sterilized), centrifuged at 5000 rpm for 5 minutes, re-suspended in PSG and their counts/ml were estimated by the Matching Method (Herbert and Lumsden, 1976). A group of ten fish were intraperitoneally inoculated with 1000 live trypanosomes per fish of *T. danilewskyi* strain FCc-1. Parasitemia was monitored at 10 days interval and thin blood smears were prepared on precleaned glass slides on days 20, 30, 40 and 50 post infections (p.i). Slides were air dried, fixed with methanol and stained with May-Grünwald Giemsa stain (Merck, Germany) according to

Möhr (1981). Slides were examined under light microscope at X 1000 magnification in immersion oil. Trypanosome morphology was recorded with a camera mounted on top of a microscope. Emission of light was controlled with a photocontrol unit (Leica Wild MPS48) integrated with microscope.

Morphometrical study of *T. danilewskyi* strain FCc-1: Twenty well stained slides from each sampling time (day 20, 30, 40 and 50 p.i) were selected. Camera lucida drawings of 200 well stained specimens (50 from each sampling time) were made. The measurements of trypanosomes (linear measurements in microns and nuclear area in square microns) were taken by using Videoplan (Image Analysis System Kontron Bildanalyse, Germany). Each morphometrical character was measured three times per cell and their mean was taken. One-way analysis of variance was applied to compare various means of morphometrical measurements followed by Bonferoni (Dunn) t test by using SAS/ETS® Software (SAS Institute Inc. 2001).

RESULTS

Morphology of *T. danilewskyi* FCc-1

Living trypanosomes: Live trypanosome appeared light pale in color under the light microscope in wet blood preparations. They were long, slender and constantly rotating by flickering their anterior flagellum. The body was frequently twisting or folding upon itself.

Fixed and stained trypanosomes: May-Grünwald Giemsa stained specimens appeared elongated and usually coiled or S-shaped (figure 1). The cytoplasm of trypanosome stained in light blue coloration and showed small granules of red-violet color. The undulating membrane stained pale blue. The nucleus appeared oval shaped, stained light red and positioned in the anterior half of the whole cell. The kinetoplast was a small refractile, stained densely blue-violet and situated next to the basal body.

Morphometry of *T. danilewskyi* FCc-1: During the acute trypanosome infections, the cell dimensions showed variability but slender forms with less variability predominate with a body length (PA) of 22.86 ±0.25 µm and width (BW) of 2.4 ±0.15 µm. One free flagellum (AF) was 14.6 ± 0.57 µm long and anteriorly directed. The kinetoplast was at the distance (PK) of 1.36 ±0.12 µm from posterior tip and kinetoplast index (PK/PA) was 0.06 ±0.03 µm. The oval-shaped nucleus measured 2.6 ±0.1 µm (NuL) and 1.7 ±0.09 µm (NuW) and positioned in the anterior half of the body (nuclear index, 0.42 ±0.07) (table 1). The morphometric measurements of *T. danilewskyi* strain Caa-1 originally isolated by Lom (1973) from goldfish, was described by Woo (1981) and re-described by Woo and Black (1984), these data are given in table 2 for comparison. There was no significant difference in all measurements of strain FCc-1 except NA ($P<0.05$) from the measurements given by Woo (1980) and significantly different ($P<0.05$) at 4 measurements (KN, NA, PA and AF) from the measurements documented by Woo and Black (1984).

Table 1. Morphometric measurements of *T. danilewskyi* strain FCc-1 from an experimentally infected juvenile common carp, *Cyprinus carpio* at various interval post infection.

Morphological Characters	Days post-infection				
	20	30	40	50	Mean
PK	1.34±0.05	1.34±0.04	1.36±0.04	**a**1.44±0.05**bx**	1.36±0.12
KN	12.0±0.23	11.84±0.16	11.97±0.25	**a**11.2±0.28**bx**	11.8±0.32
NA	10.4±0.14	**a**9.72±0.2	**a**8.83±0.14**b**	**a**9.17±0.18**b**	**a**9.58±0.21**x**
PN	13.34±0.15	13.18±0.14	13.33±0.21	**a**12.64±0.23**bx**	13.16±0.31
PA	22.87±0.28	22.75±0.28	22.44±0.27	**a**23.62±0.22**bx**	22.86±0.25**y**
AF	14.38±0.28	14.85±0.34	14.05±0.26**b**	**a**15.34±0.50**x**	14.61±0.57
BW	2.39±0.07	2.46±0.07	2.49±0.08	2.42±0.08	2.40±0.15
NuL	2.55±0.05	2.63±0.05	2.52±0.05	**a**2.80±0.05**bx**	2.60±0.10**y**
NuW	1.70±0.04	1.76±0.05	1.63±0.04**b**	1.61±0.06**b**	1.68±0.09
NuArea	3.42±0.12	3.66±0.15	3.22±0.07**b**	3.52±0.16**x**	3.45±0.22
PK/PA (KI)	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.02	0.06±0.03
NA/PA (NI)	0.46±0.01	**a**0.43±0.01	**a**0.41±0.01	**a**0.39±0.01**b**	0.42±0.07
PN/PA	0.58±0.21	0.58±0.21	0.59±0.24	0.54±0.22	0.58±0.28
BW/PA	0.10±0.01	0.11±0.04	0.11±0.01	0.10±0.01	0.11±0.05
AF/PA	0.63±0.01	0.66±0.02	0.63±0.01	0.65±0.02	0.64±0.08
n	50	50	50	50	200

Measurements are in µm or µm². Given values are mean and ± SEM. Abbreviations: PK, distance of kinetoplast to the posterior end; KN, distance of kinetoplast to the middle of the nucleus; NA, distance of anterior end to the middle of the nucleus; PN, distance of posterior end to the middle of the nucleus; PA, body length excluding flagellum; AF, length of free flagellum; BW, body width; NuL, length of the nucleus; NuW, width of the nucleus; NuArea, area of the nucleus; KI (PK/PA), kinetoplast index; NI (NA/PA), nuclear index; BW/PA, ratio of body width and length; AF/PA, ratio of free flagellum and body length. **a** = significant difference with day 20; **b** = significant difference with day 30; **x** = significant difference with day 40; **y** = significant difference with day 50. n is number of reading. P< 0.05

Table 2. Morphometric measurements of *T. danilewskyi* strain FCc-1 described in this study and compared with reference strain of *T. danilewskyi* strain Caa-1 described by Woo (1981) and re-described by Woo and Black (1984).

Characters	*Trypanosoma danilewskyi* strains		
	Caa-1 described by Woo (1981) from *C. auratus*	FCc-1 isolated and described in 1990 from *C. carpio*	Caa-1 re-described by Woo and Black (1984) from *C. auratus*
PK	1.7±0.61	1.36±0.12	1.2±0.06
KN	10.7±2.69	11.8±0.32§	8.0±1.5
NA	8.8±0.34	*9.58±0.21§	6.5±1.44 ¤
PA	21.2±3.64	22.86±0.25§	15.8±2.7
AF	14.3±2.30	14.61±0.57§	11.36±1.58
BW	2.3±0.40	2.40±0.15	2.30±0.40
NuL	2.5	2.60±0.10	2.23±0.34
NuW	1.6	1.68±0.09	1.40±0.32
PK/PA(KI)	0.08	0.06±0.03	0.07±0.01
PN/PA	0.58	0.57±0.33	0.58±0.77
BW/PA	0.11	0.11±0.08	0.15±0.14

The data of *T. danilewskyi* strain FCc-1 is given for comparison. The symbol * indicates significant difference from the data of Woo (1981), § indicates significant difference from the data of Woo and Black (1984) and ¤ indicates significant difference between the data of Woo and Woo and Black. For legend see table 2. P< 0.05.

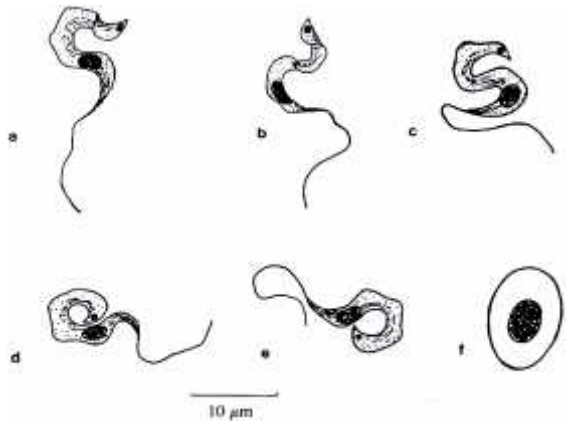

Figure 1: Camera lucida drawings from May-Grünwald Giemsa stained preparation of *T. danilewskyi* strain FCc-1 form the blood of infected common carp. Legend: a-b, elongated; c, S-shaped; d-e, coiled trypanosome and f, red blood cell.

DISCUSSION

It is difficult to differentiate between similar species and subspecies of fish trypanosomes only on the basis of host from where they are isolated. Because the morphological characters, the basic structural parameters of fish trypanosomes are very much similar among freshwater trypanosome species of closely related fish host species (Zajicek, 1990; Ahmed, 1994; Ahmed *et al*., 2001). However, in some cases the morphological and morphometrical studies are very important to differentiate among the species only where the differences are quite distinct, for instance the size of *Trypanosoma granulosum* isolated from freshwater eel is twice bigger than the size of *T. danilewskyi* isolated from goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*).

The isolate, *T. danilewskyi* strain FCc-1 was found morphologically and morphometrically same as *T. danilewskyi* strain Caa-1 described by Woo (1981). This strain, *T. danilewskyi* Caa-1 was originally isolated from goldfish (*C. auratus*) and described during the laboratory infection in common carp by Lom (1979). The same strain was requested by P. T. K. Woo, Laboratory of Parasitology, University of Guelph, Canada. It was used to infect laboratory breed goldfish. It was maintained in goldfish by syringe passages and then described in 1981. After 4 years the same strain was re-described by Woo and Black (1984) from experimentally infected goldfish, the morphometric measurements decreased and a

significant (P<0.05) difference was observed only in NA (distance between nucleus and anterior tip). The differences in few morphometrical parameters, as interpreted by the authors, were because of the influence of fish host on the morphometry of the *T. danilewskyi* strain Caa-1.

T. danilewskyi strain FCc-1 was isolated from a naturally infected common carp, cloned in laboratory bred juvenile common carp and described during the subsequent syringe sub-passage. The strain FCc-1 was morphometrically found similar in size as *T. danilewskyi* Caa-1 except NA that was significantly (P< 0.05) larger. The similar pattern of change in morphometric measurements in fish trypanosomes was noted by Woo and Black (1984) when they inoculated *T. danilewskyi* Caa-1 into goldfish and other fish species like *Barbus conhus*, *Danio malabaricus*, *Catostomus commersoni*, *Etheostoma caeruleum*, *Notropis cormitus* and *Perca flavescens.* The morphometric measurements significantly decreased as compared to the measurements given by Woo (1981).

The present study concluded that *T. danilewskyi* strain FCc-1 is same as other strain of *T. danilewskyi* like Caa-1, Cc-Fr and Cac-BR according to Lom (1979), Woo (1981), Woo and Black (1984), Ahmed (1994) and Ahmed *et al*., 2001. Based on the morphometrical measurements it is very much similar to the natural blood flagellate of common carp, crucian carp and goldfish. These results are in accordance with Zajicek and Peckova (1990) and Zajicek (1991) who put forwarded the idea that the fish trypanosome species are very much similar to each other when only morphological and morphometrical measurements are considered. It is therefore recommended that a through morphological and morphometrical studies alongwith biochemical studies, be conducted to evaluate the minor differences between closely related trypanosomes species.

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