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INFECTIVITY AND HOST SPECIFICITY OF *T. DANILEWSKYI* STRAIN FCC-1

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ABSTRACT

The parasite *Trypanosoma danilewskyi* strain FCc-1 was isolated from and maintained in juvenile common carps and was also cultivated in various culture media. The present study was conducted to understand its ability of both blood stream forms (BSF) and culture forms (CF) to cause infection in common carp and also other fish species. In the first experiment 3 groups of juvenile carps, each containing 20 fish were inoculated with 1000, 5000, 20,000 live *T. danilewskyi* strain FCc-1 per fish in all the treatment groups (groups A, B and C respectively), while the control group (D) only received 0.2 ml of PSG (phosphate buffered saline with glucose, pH 7, filter sterilized). The development of parasitemia (onset of infection) showed a similar pattern in all inoculated groups, although in group A, the peak of infection only appeared one week later compared with the two other groups (42 days instead of 35 days). In a second experiment 9 groups, each containing 20 juvenile carps, were inoculated with 10,000 live trypanosomes (CF) per fish, but each group receiving a different strain; *T. danilewskyi* strain FCc-1, *T. danilewskyi* (Cac BR), *T. danilewskyi* (Caa 1), *T. danilewskyi* (Tt 1902), *T. scardinii* (Se BL), *T. percae* (Pf FR), *T. boissoni* and *T. triglae* (groups E to L) and ninth group M (control) received only 0.2 ml PSG). In group E the CF infection developed similar as the BSF one in the first experiment, while in groups F and G a significantly lower number of trypanosomes was detected in the fish blood, displaying very low infection peak. In all other groups (H-L) no trypanosomes were detected in the blood until day 98 p.i. In a third cross infection experiment, 8 groups belonging to different freshwater fish species (common carp, crucian carp, rudd, roach, perch, African catfish, brown bullhead and European eel) were inoculated with 10,000 live trypanosomes (BSF) per fish. The *T. danilewskyi* strain FCc-1 involved was able to infect common carp and crucian carp, but not the other fish species. In the fourth experiment trypanosomes were recovered from rudd and re-inoculated in 2 fish species; namely common carp and rudd. The infection developed in both hosts but in rudd the infection peak was very low. From these experiments it appears that *Trypanosoma danilewskyi* strain FCc-1 is not strictly host specific and is able to infect other fish species belonging to the Cyprinidae. Such infection is however not possible with fish hosts belonging to the Percidae, Anguillidae, Ictaluridae and Clariidae.

Key words: Infectivity; Host specificity; Cross infections, Trypanosomes; Common carp

INRODUCTION

Trypanosoma danilewskyi is natural bloods flagellate of common carp in Europe and causes fish mortalities and infections (Lom, 1973, 1979; Woo, 1981 b; Ahmed, 1994). Generally this parasitic species is not host specific (Islam and Woo, 1991 c) as it has been isolated from common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus gibelio*). Some piscine hemoflagellates like *Trypanosoma platessae* are strictly host specific (Cotterll, 1977) while others like *T. cobitis* are not (Letch, 1979; Letch and Ball, 1979).

In laboratory-infection experiments, live trypanosomes are transferred into infection free hosts by either potential natural vectors like *Piscicola geometra* and *Hemiclepsis marginata* or inoculated by a syringe in the peritoneum cavity. After inoculation trypanosomes migrate and are found in the peripheral blood a few days later. Cross infection experiments performed by Lom (1973) revealed that *T. danilewskyi* isolated from common carp caused infection in common carp, crucian carp, goldfish, tench, bream, and many other similar hosts by syringe passage, while *T. acerinae* and *T. remaki* were not able to infect goldfish. *Trypanosoma carassii* isolated from *Carassius carassius* caused severe infections in common carp and a number of other cyprinids and some non-cyprinids (Nazrui Islam and Woo, 1991; Lom and Dykova, 1992; Overath *et al.* 1999). *Trypanosoma salmositica* caused infection in various species of salmonids other than the principal host, *Oncorhynchus mykiss*. Some cryptobia species, e.g. *Cryptobia iubilans,* were not host specific, while *C. dahlii* was limited to its principal host, the lumpfish *Cyclopterus lumpus* (Woo, 1987).

The present study was performed to investigate the infectivity of *Trypanosoma danilewskyi* strain FCc-1, both blood stream forms (BSF) as well as culture forms (CF), in juvenile common carp. Also other strains and *Trypanosoma* species were tested. Cross infection

experiments were also performed to investigate the potential of cross infectivity in different hosts of the same fish family (Cyprinidae) as well as in hosts belonging to other fish families (Percidae, Anguillidae, Ictaluridae and Clariidae).

MATERIALS AND METHODS

Six-month old juvenile common carp (10.5 ±2.7 cm) were purchased from the Experimental Fish Facility, Department of Zodiac, Agricultural University Wageningen, The Netherlands. Since laboratory bred fingerlings of various fish species are difficult to obtain, the fingerlings of crucian carp (*Carassius carassius*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), perch (*Perca fluviatilis*) and brown bullhead (*Ictalurus nebulosus*) were collected from natural fish ponds. Small eel (*Anguilla anguilla*) and laboratory bred African catfish (*Clarias gariepinus*) were obtained from the Fish Culture Facility of the Laboratory of Aquatic Ecology and Evolutionary Biology, KU Leuven, Belgium. Fishes collected from natural ponds were kept in glass aquaria at 20°C, screened 3 times, with monthly intervals upon trypanosome infection, before being used for experimental infection. Blood from artificially infected fishes was examined weekly and parasitemia estimated by the Matching Method (Herbert and Lumsden, 1976) and Hematocrit Centrifuge Technique (HCT) (Woo, 1969) wherever necessary. *Trypanosoma danilewskyi* strain FCc-1 was maintained in juvenile common carp by syringe sub-passages (Ahmed, 1994). Blood stream forms were separated from the infected carp blood by using a DEAE cellulose column (Ahmed *et al.* 2001), washed with PSG (phosphate buffered saline with glucose, pH 7, filter sterilized), centrifuged (5000 rpm for 5 minutes), re-suspended in PSG and their counts/ml was estimated by Matching Method (Herbert and Lumsden, 1976). The reference trypanosome species *T. danilewskyi* (Cc FR), *T. danilewskyi* (Cac BR), *T. danilewskyi* (Caa 1), *T. danilewskyi* (Tt 1902), *T. scardinii* (Se BL) and *T. percae* (Pf FR) were provided with kind courtesy of Dr. J. Lom, Ceské Budejovice, Czech Republic and *T. boissoni* and *T. triglae* (Table 5.1) were a generous gift from Dr. D. Le Ray, Laboratory of Protozoology, Institute of Tropical Medicine and Hygiene, Antwerp, Belgium. The trypanosome species/strains were cultured (Ahmed *et al.* 2001) and used for laboratory infection experiments.

Experimental infection (Exp.1): Infectivity of blood stream form (BSF) of *T. danilewskyi* strain FCc-1 in juvenile common carp: Three groups of trypanosome free juvenile carp, each containing 20 fish (10.5 ±2.7 cm), were maintained in glass aquaria (50 liters water) at 20° C, pH 7.1 - 7.4 and DO 6 - 7.5 mg/l in flow through system at flow rate 4 liter/hour. All fish were injected intraperitoneally with BSF trypanosomes belonging to *T. danilewskyi* strain FCc1@ 1000 trypanosomes/fish in group A, 5,000 trypanosomes/fish in group B and 20,000 trypanosomes/fish in group C. Each fish of the control group D received a similar volume (0.2 ml) of PSG (phosphate buffered saline with glucose, pH 7, filter sterilized) per fish. Parasitemia was estimated by the Matching Method (Herbert and Lumsden, 1976) after weekly examination of peripheral blood from all fish.

Experimental infection (Exp.2): Infectivity of culture forms (CF) of *T. danilewskyi* strain FCc-1 and other reference trypanosome species or strains in juvenile common carp: This experiment was performed to investigate the infectivity of culture forms of several trypanosome species in trypanosome free juvenile common carp. Nine groups of carp (10.6 ±2.65 cm), each containing 20 fish, were maintained at 20°C in glass aquaria (similar conditions as exp. 1). Culture forms of *T. danilewskyi* strain FCc-1 and the eight other reference trypanosome strains or species (Table 1) were washed with PSG, centrifuged, re-suspended in PSG, their numbers estimated, and inoculated as 10,000 trypanosomes/fish in all fish groups except control which received only 0.2 ml of PSG. The development of parasitemia was monitored as in exp. 1.

Cross infection (Exp. 3): The infectivity of blood stream form (BSF) of *T. danilewskyi* strain FCc-1 in various freshwater fish species: Eight groups (10 fish each) of trypanosome free fingerlings belonging to different fish species with length range of 9 – 13.4 cm (Table 2) were maintained in separate glass aquaria at 20°C (similar conditions as exp. 1). All fishes were inoculated with 10,000 trypanosomes/fish of *T. danilewskyi* strain FCc-1 (BSF). The development of parasitemia was weekly monitored as in exp.1.

Cross infection (Exp. 4): Re-infection of *T. danilewskyi* strain FCc-1 in common carp recovered from rudd after cross infection (exp. 3).: Using cross infections this experiment was performed to investigate the infectivity of *T. danilewskyi* strain FCc1 when recovered from inoculated rudd (*S. erythrophthalmus*) being a different host than the principal one. Two groups of trypanosome free fingerlings, one of common carp and a second one of rudd, each containing 5 fish, with an average length of respectively 10.5 cm and 10.1 cm were inoculated with 100 trypanosomes/fish (very small number of trypanosomes was observed in the blood of rudd only at the peak of infection; 21 days p.i in experiment 3) of *T. danilewskyi* strain FCc-1 (BSF). Parasitemia was monitored weekly as in exp. 1.

One way analysis of variance (ANOVA) was applied in order to compare levels of parasitemia among the individuals of juvenile common carp and other fish

species followed by Bonferroni (Dunn) t test using SAS/ETS® Software (SAS Institute Inc. 2001).

RESULTS

Experimental infection (Exp. 1): Infectivity of blood stream form (BSF) of *T. danilewskyi* strain FCc-1 in juvenile common carp: In group A, which received 1000 trypanosomes per fish, the infection developed slowly and the peak (7.52 ± 0.39 log 10 trypanosomes/ml) was obtained on 42 days post infection (p.i). In group B and C, receiving higher inoculum, the infections developed faster and the respective peaks (7.68 ± 0.25 and 7.81 ± 0.13 log10 trypanosomes/ml) were obtained on day 35 p.i (Fig. 1). After the peak, parasitemia decreased in all groups and only a few fish still revealed trypanosomes in their blood 98 days p.i. There was non-significant difference in disappearance of trypanosomes in groups A - C. No trypanosomes were found in group D (control).

Experimental infection (Exp. 2): Infectivity of culture forms (CF) of *T. danilewskyi* strain FCc-1 and other reference trypanosome species or strains in juvenile common carp: All common carps inoculated with the culture form (CF) of *T. danilewskyi* strain FCc-1 developed infection in a similar way as the blood stream forms (BSF, exp. 1). The peak of infection of *T. danilewskyi* FCc1 (7.76 ± 0.12 log10 trypanosomes/ml) was obtained 42 days p.i and trypanosome were still detected in some fishes afterward (see exp.1). Among the reference trypanosome species/strains (CF) only two were found in the blood of common carp; *T. danilewskyi* (Cac BR) presenting an infection peak (31.6 ±2.3 trypanosomes/ml) on 14 days p.i. and *T. triglae* (11.5 ±1.8 trypanosomes/ml) on 21 days p.i. They disappeared from the blood on 42 and 70 days p.i respectively. There was significant difference ($p<0.01$) in the disappearance of *T. danilewskyi* (Cac BR) and *T. triglae* when compared with *T. danilewskyi* FCc-1. The other six reference trypanosomes (*T. danilewskyi* (Cc FR), *T. danilewskyi* (Caa 1), *Trypanosoma* (Tt 1902), *T. scardinii* (Se BL), *T. percae* (Pf FR), *T. boissoni*), which include two *T. danilewskyi* species/strains were not found in the blood of any inoculated common carp (Fig. *2*).

Cross infection (Exp. 3): Infectivity of the blood stream form (BSF) of *T. danilewskyi* strain FCc-1 in various freshwater fish species: In juvenile common carp the inoculation with *T. danilewskyi* strain FCc-1 resulted in an infection, the peak (7.75 ± 0.12 log10 trypanosomes/ml) was obtained on day 35 p.i (Fig. 3). In crucian carp (*C. carassius*) the infection developed slower (statistically insignificant) compared with juvenile common carp and the peak of infection (7.39 ± 0.12 log 10 trypanosomes/ml) was only obtained on day 49 p.i. In rudd (*S. erythrophthalmus*), roach (*R. Rutilus*) and perch (*Perca fluviatilis*) trypanosomes were represented in the blood and maximum number of trypanosomes /ml were 105 ±1.3 79.5 ± 2.4 and 9.5 ± 3.1 respectively (significantly low, $p<0.001$) were found on day 14 p.i. (Fig. 3). In eel 3.3 ± 0.1 trypanosomes/ml, brown bullhead 1.2 ± 1.07 trypanosomes/ml and African catfish 3.2 ± 1.3 trypanosomes/ml were found on day 7 p.i (Figure 3). No trypanosomes were found on and after 42 day p.i. in rudd and roach, 35 days p.i. in perch and 28 days p.i. in eel. In common carp and crucian carp small number of trypanosome (2.53 ± 0.17 and 3.55 ± 0.19 trypanosomes/ml respectively) were detected in their blood on 98 days p.i. as observed in experiment 1.

Cross infection (Exp. 4): Re-infection of common carp with *T. danilewskyi* strain FCc-1 recovered from inoculated rudd of experiment 3: After re-infection of juvenile common carp with *T. danilewskyi* strain FCc1 recovered from inoculated rudd (*S. erythrophthalmus*) of experiment 3, infection developed and the peak (7.05 ±0.33 log 10 trypanosomes/ml) was obtained on day 49 p.i. In injected rudd trypanosomes were recuperated from the blood and the maximum number (135 ±1.4 trypanosomes/ml) was obtained on day 21 p.i (Figure 4).

Table 1. List of reference trypanosome species and strains (culture forms) used to evaluate their infection ability in juvenile common carp.

Trypanosome species/strains	*No. of Subculture*		*Culture media*	*Date of isolation*	*Host species*
	initiated	*Inoculated*			
T. danilewskyi (FCc-1)	isolation	17	L4NHS	19.07.90	*Cyprinus carpio*
T. danilewskyi (Cc FR)	81	101	L4NHS	04.05.87	*Cyprinus carpio*
T. danilewskyi (Cac BR)	2	21	L4NHS	12.10.87	*Carassius carassius*
T. danilewskyi (Caa 1)	20	39	L4NHS	09.06.86	*Carassius auratus*
Trypanosoma (Tt 1902)	138	158	L4NHS	19.10.85	*Tinca tinca*
T. scardinii (SE BL)	26	46	L4NHS	25.04.87	*S. erythrophthalmus*
T. percae (PF FR)	36	56	L4NHS	04.05.87	*Perca Fluviatilis*
T. boissoni	44	67	Tobie	04.02.67	*Zanobatus atlanticus*
T. triglae	43	65	Tobie	21.12.67	*Trigla lineata*

Table 2. List of various freshwater fish species used in cross infection of *T. danilewskyi* strain FCc-1.

Fish families and common names	*Scientific names*	*Size (cm)**
Cyprinidae		
Common carp	*Cyprinus carpio*	10.5 ±2.7
Crucian carp	*Carassius carassius*	12.3 ±3.2
Roach	*Rutilus rutilus*	11.1 ±2.5
Rudd	*Scardinius erythrophthalmus*	10.1 ±1.2
Percidae		
Perch	*Perca fluviatilis*	9.0 ±1.3
Anguillidae		
Eel	*Anguilla anguilla*	15.8 ±4.5
Inctaluridae		
Brown bullhead	*Ictalurus nebulosus*	12.3 ±1.5
Clariidae		
African catfish	*Clarias gariepinus*	13.4 ±1.3

* mean ± SEM

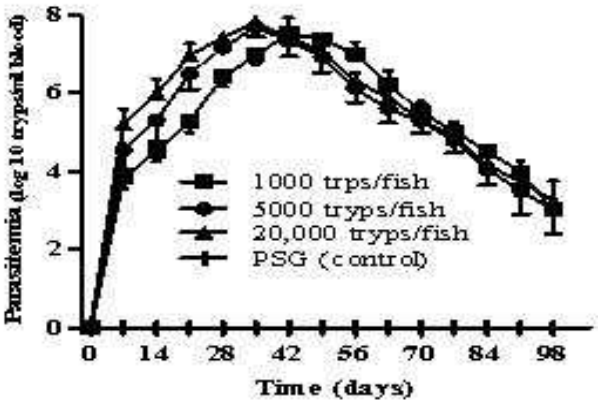

Figure 1. Development of parasitemia in juvenile common carp when inoculated with *Trypanosoma danilewskyi* strain FCc-1 (BSF) at different inoculum size (exp. 1).

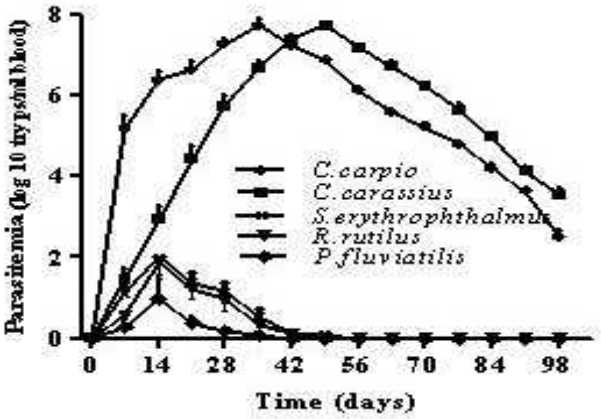

Figure 3. Infectivity of *T. danilewskyi* strain FCc-1 (BSF) in juvenile common carp and other host species belonging to the same Cyprinidae family and other fish families (exp. 3).

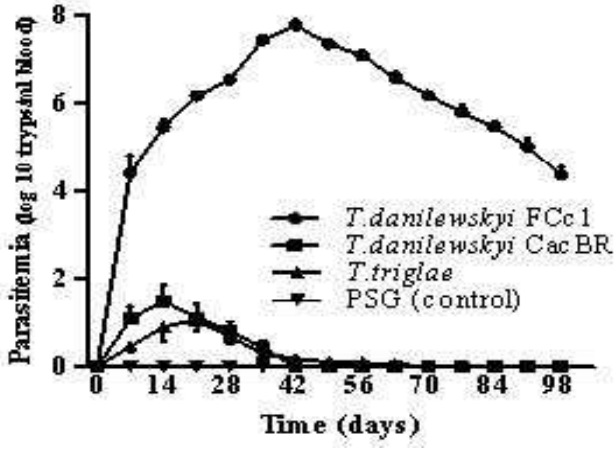

Figure 2. Development of parasitemia in juvenile common carp when inoculated with culture forms (CF) of *T. danilewskyi* strain FCc-1 and some reference trypanosome species/strains (exp. 2).

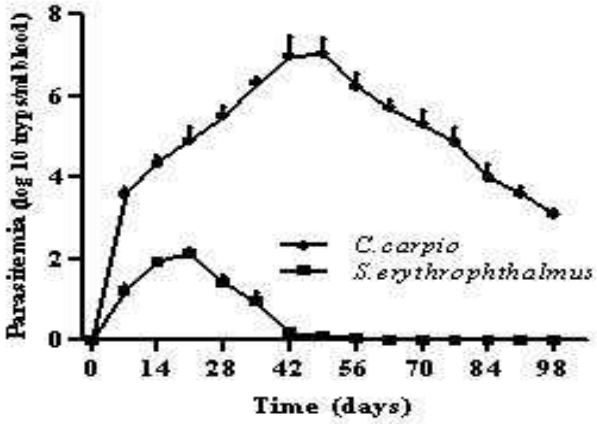

Figure 4. Infectivity of *T. danilewskyi* strain FCc-1 (BSF) in juvenile common carp and rudd after being recovered from rudd, a non principal host (exp. 4).

DISCUSSION

Studies concerning trypanosome (BSF) infection in fishes revealed that the size of inoculum is not correlated with the number of parasites at the peak of infection but has slightly delayed the onset of the peak of infection (Lom, 1973, 1979). While in the similar studies, culture forms (CF) of *Trypanosoma danilewskyi* (various strain) were also found infective to juvenile common carp. And Lom (1979) described that the culture forms (CF) of *T. danilewskyi* (originally isolated from common carp) were infective to goldfish (*Carassius auratus*) until 35th subculture. The latent time for the 35th subculture was however extremely long (6 months) and the peak of infection was lower than observed in normal infections. Among all reference trypanosome species/strains (CF) tested for cross-infection, only *T. danilewskyi* (Cac BR) originally isolated from crucian carp was infective to juvenile common carp (present study) and the highest number (31.6 ±0.23 trypanosomes/ml) was obtained on day 14 p.i. The trypanosomes disappeared however much faster from the blood as compared to *T. danilewskyi* strain FCc-1. On the other hand when *T. triglae* (CF) was inoculated in juvenile common carp (100 trypanosomes/fish) a increased number of trypanosomes were found in the blood, indicating that *Trypanosoma triglae* has retained its infectivity for a very long time (i.e. until subculture 65) in a host other than the principal host because cell division among trypanosomes was observed and the number of trypanosomes found in the blood was more (non significant difference) than the inoculated (Ahmed, 1994). Therefore *T. triglae* is not host specific and not confined to a limited number of hosts species belonging to the particular teleost family of the Triglidae. Originally *T. triglae* was isolated (Ranque, 1967) from a marine fish, *Trigla lineata*. Fish trypanosomes are considered as host specific or at least it is accepted that a marine fish trypanosomes will not cause infection in freshwater fish species. *T. triglae* was cultured in BSL (Brain heart infusion with rabbit blood) and cryopreserved at the 17th subculture. Later its cultivation was done on Tobie's medium and cryopreserved at 43rd subculture. Its culture was re-started from a stabilate of subculture 43rd again on Tobie's medium and continued until the subculture 65th in our laboratory and tested for its ability to cause infection (present study).

T. danilewskyi strain FCc-1 appears not to be strictly host specific because it has infected hosts other than the principal host, although the development of parasitemia as well as the peak of infection was not the same. From the present investigations it is clear that in crucian carp infection developed in a similar way as in juvenile common carp although the peak was reached with a two week delay, while in other hosts like rudd (*S. erythrophthalmus*), roach (*R. rutilus*) and perch (*P. fluviatilis*), the infection developed not only slower but the parasitemia was also significantly ($p < 0.001$) lower than in common carp. These finding are in accordance with Lom (1973, 1979) and Woo and Black (1984) who conducted experiments with *Trypanosoma danilewskyi,* originally isolated from common carp. According to these authors, this parasite has extended its host range and caused similar infection in fishes like crucian carp and goldfish while the infection caused in other similar fish hosts like tench, roach, rudd and bream. The level of parasitemia was very low which means host specificity of freshwater fish trypanosomes is not so strict or absent among hosts similar fish families. It is evident from the studies of Nazrui Islam and Woo (1991), Lom and Dykova (1992) and Overath *et al.* (1999) that *Trypanosoma carassii* (syn. *T. danilewskyi*) isolated from *Carassius carassius* was found to be infective to juvenile common carp and number of other cyprinids as well as some non-cyprinids. In another study (Wita *et al.*, 2001), the differences as well as similarities based on the mode of infections in fishes (vector transmitted laboratory infection) between *Trypanosoma abramis* of bream (*Abramis brama*) and *T. carassii* isolated from different cyprinids found in various lakes of Poland are very much similar morphologically as well as onset of infection. Thus it is evident that freshwater fish trypanosomes are least/not host specific among fishes of same/similar families.

While in the present cross infection study, *T. danilewskyi* strain FCc-1 was unable to produce infection in the fish hosts like brown bullhead (*I. nebulosus*), African catfish (*C. gariepinus*) and eel (*A. anguilla*), only a small number of flagellates was found in their blood which might be due to due to the migration of some inoculated trypanosomes, that reached the bloodstream but not because of multiplication of flagellates (Ahmed, 1994). Those trypanosomes were detected only by Hematocrit Centrifuge Technique (HCT). In other studies Lom (1973, 1979) and Woo and Black (1984), documented that *T. danilewskyi* recovered from a susceptible host (rudd) were infective to goldfish and dividing forms observed, were similar to those present in the natural host (common carp). This study confirmed that *T. danilewskyi* strain FCc-1 was infective to rudd. When trypanosomes from its blood were inoculated in common carp, they were also infective in the same way as those from common carp to common carp. These results are further supported by similar findings from Cross infection experiments of Khan (1977), (Letch, 1979, 1981), Woo and Black (1984) further confirm our findings.

From the present study it is clear that *Trypanosoma danilewskyi* strain FCc-1 (both BSF and CF) were found to be infective to juvenile common carp, crucian carp and to some extent rudd. It is further concluded that *T. danilewskyi* strain FCc-1 is not host

specific within family while fish species from different fish families are found to be resistant to its infection.

Conclusion: The present study has revealed that *T. danilewskyi* strain FCc-1 is not strictly host specific and retains its ability to infect freshwater fish species other than the principal host (*C. carpio*) i.e. members of the fish family Cyprinidae. Such infections are however not possible in fish hosts belonging to fish families like Percidae, Anguillidae, Ictaluridae and Clariidae.

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