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IN VIVO AND *IN VITRO* STUDIES ON THE HOST SPECIFICITY OF *Trypanoplasma salmositica*

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Abstract: The host specificity of *Trypanoplasma salmositica* was studied by experimental inoculation into 13 species of common teleosts *(Notropis cornutus, Notropis heterolepis, Notropis spilopterus, Nocomis biguttatus, Rhinichthys atratulus, Semotilus atromaculatus, Carassius auratus, Ambloplites rupestris, Lepomis gibbosus, Etheostoma nigrum, Hypentelium nigricans, Ictalurus melas,* and *Eucalia inconstans). T. salmositica* was not recovered at 14 and 40 days post inoculation. However, large numbers of the parasite were recovered from *Salmo gairdneri* and *Cottus cognatus,* thus indicating that sculpins might be reservoir hosts in certain areas where salmonids and sculpins occur in close proximity.

Using a modified *In Vitro* Plasma Incubation Technique, it was shown that the plasma of refractory fishes had lytic ability and the titer ranged from 1:4 to 1:8. Undiluted fresh plasma of goldfish and of the northern hog sucker lysed about 500 parasites within 30 to 60 mins at 4 C. This lytic ability of fresh plasma was heat labile and partial coagulation of the blood also reduced the lytic titer.

This study showed that *Trypanoplasma salmositica* was more host specific than originally reported. It was suggested that the mechanism responsible for providing the innate immunity to this parasite in some fishes was the Alternate Pathway of Complement Activation.

INTRODUCTION

Trypanoplasma salmositica (Katz) has been recorded from all species of Pacific salmon, *Oncorhynchus* spp.[4] It was considered to be non-host specific and has been reported from many teleosts including *Rhinichthys cataractae* and *Gasterosteus aculeatus,*[1] however, this was based on examination of blood smears. Because of the pleomorphic nature of these haemoflagellates[1,2] experimental inoculations would be necessary to determine more precisely the host range of these organisms.

Since *T. salmositica* has been shown to produce a disease (anemia, exophthalmia, abdominal distension with ascites, and splenomegaly) in trout[5] the purposes of the present study were (i) to test the host specificity (i.e. potential reservoir host) of *T. salmositica* by blood inoculations, and (ii) to ascertain the mechanism of resistance in some refractory fishes using an *in vitro* technique.

MATERIALS AND METHODS

Numerous species of fresh water teleosts were seined from streams in the Guelph area. Previous studies (Woo, unpubl.) have shown that no haemoflagellate was found in fishes from these streams. Goldfish *(Carassius auratus)* were obtained from a local pet store and rainbow trout *Salmo gairdneri)* fingerlings from a hatchery. The fishes were held in 120 l circular plastic tanks supplied with air and a continuous flow (260 ml/min) of well water (about 12 C) and fed commercially prepared fish food.

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The history and origin of the strain (T4 C60 Cl 1) of *T. salmositica* used has been adequately documented in an earlier study.[3] Approximately 5×10^3 organisms were injected into the peritoneal cavity of the experimental fish. Rainbow trout were injected as positive controls since these salmonids produce specific clinical signs following long-term infections. Trout always were inoculated last to ensure the infectivity of the inoculum.

The fishes were examined 14 to 40 days post-injection. The caudal peduncle was severed, blood collected into heparinized capillary tubes and a wet mount preparation made. The examination of wet mounts consisted of scanning 20 low power (eye piece 10×, and objective 10×) fields. The presence or absence of haemoflagellates was recorded. The capillary tubes were kept at 4 C to be used as inoculum for trout to ensure that any organism detected was indeed *T. salmositica* and not a natural infection since these fishes were collected from the field.

The *in vitro* plasma incubation technique[4] was used to test the mechanism of natural immunity in refractory fishes. A micropipette was used to dispense 0.025 ml of phosphate buffer (pH 7.2) into the wells of a microtiter plate. Twofold serial dilution of the plasma was made using a microdiluter.

The technique was modified as follows: *Preparation of organisms:* 0.5 cc heparinized blood from a heavily infected rainbow trout was diluted with an equal amount of phosphate buffer (pH 7.2) and 0.1 cc heat inactivated horse serum added. This mixture was cold centrifuged (1000 g) for 10 min. The supernatant containing large numbers of organisms was diluted with phosphate buffer such that there were approximately 500 parasites per 0.025 ml.

Plasma preparation: Freshly collected plasma stored at 4 C and heat inactivated plasma were used. The heat inactivation was at 37 C for 30 min. This inactivates the complement.

Examination of parasite: Following the 3 h incubation period at 4 C the total contents of each well was examined under a microscope by wet mount preparations.

The time required for activating the lytic factors in goldfish and northern hog sucker plasma was determined by further modification of the plasma incubation technique. A drop of plasma was placed with a drop of the organism suspension on a standard microscope slide. The coverslip was ringed with vaseline. These preparations were kept at 4 C and examined every 15 min.

Rainbow trout plasma served as positive controls in all the *in vitro* procedures.

RESULTS

In vivo Inoculations

None of these experimentally inoculated fishes[2] (*Notropis cornutus* 8; *Notropis heterolepis* 3; *Notropis spilopterus* 4; *Nocomis biguttatus* 14; *Rhinichthys atratulus* 6; *Semotilus atromaculatus* 4; *Carassius auratus* 5; *Ambloplites ruperstris* 4; *Lepomis gibbosus* 5; *Etheostoma nigrum* 5; *Hypentelium nigricans* 9; *Ictalurus melas* 4; *Eucalia inconstans* 11) were infected with *T. salmositica* when their blood was examined at 14 and 40 days post inoculation. Large numbers of the parasite were found in the blood of 5 *Salmo gairdneri* and 4 *Cottus cognatus*. None of the control fishes (not inoculated) were infected. The haemoflagellates recovered from the sculpins were infective to trout.

In vitro Studies

Live *T. salmositica* were recovered from all wells of serially diluted rainbow

[2] Fish species followed by number inoculated.

trout plasma (controls) after the 3 h incubation at 4 C. However, the fresh untreated plasma of the refractory fishes (Table 1) lysed all the organisms with end points ranging from 1:4 to 1:8 depending on fish species. At the dilution next to the end point, the parasite was present in large numbers. Heat treatment of the plasma inactivates the lytic activity of the plasma and organisms were seen in all the wells as in the case of the controls. Partial coagulation of the plasma reduced the end point from 1:8 to 1:4 in untreated goldfish plasma.

The time required to lyse *T. salmositica* during the plasma incubation in undiluted plasma varied slightly among individuals of the refractive species (Table 2). In rainbow trout plasma (control) and in heat inactivated northern hog sucker plasma no lysis of the organism occurred. Partial coagulation of goldfish blood resulted in an increase in the time required for lysis. In general, approximately 30 to 60 mins was required for the lytic activity to destroy about 500 *T. salmositica* in 0.025 ml of goldfish and northern hog sucker plasma.

DISCUSSION

The *in vivo* study shows that *T. salmositica* may be more host specific than originally reported[1] since most of

TABLE 1. *In vitro* incubation of *Trypanoplasma salmositica* in serially diluted plasma of fishes.

Fish species	No. of fish	Treatment**	Plasma dilutions* 1:2	1:4	1:8	1:16
Rhinichthys atratulus	5	a	-	-	+	+
	2	b	+	+	+	+
Notropis cornutus	12	a	-	-	+	+
	12	b	+	+	+	+
Ambloplites rupestris	8	a	-	-	+	+
	10	b	+	+	+	+
Carassius auratus	10	a	-	-	-	+
	10	b	+	+	+	+
Hypentelium nigricans	6	a	-	-	+	+
	6	b	+	+	+	+
Ictalurus melas	7	a	-	-	+	+
	8	b	+	+	+	+
Semotilus atromaculatus	3	a	-	-	+	+
	3	b	+	+	+	+
Lepomis gibbosus	10	a	-	-	+	+
	10	b	+	+	+	+
Cyprinus carpio	4	a	-	-	+	+
	4	b	+	+	+	+
Moxostoma carinatum	6	a	-	-	-	+
	4	b	+	+	+	+
Salmo gairdneri	12	a	+	+	+	+
	12	b	+	+	+	+

*+ living *T. salmositica* in preparations
- no living *T. salmositica* detected
**a freshly collected plasma at 4 C
b heat inactivated plasma

TABLE 2. Time requirement for *in vitro* lysis of *T. salmositica* in plasma of some fishes.

Species tested	Plasma treatment	Time (min)* 15	30	45	60	75	90
1. *Carassius auratus*	none	+	+	-	-	-	
	none**	+	+	+	+	-	
	none	+	+	+	-	-	
2. *Hypentelium nigricans*	none	+	+	+	-	-	
	none	+	+	-	-	-	
	none	+	+	-	-	-	
	heat inactivated	+	+	+	+	+	+
	heat inactivated	+	+	+	+	+	+
	heat inactivated	+	+	+	+	+	+
3. *Salmo gairdneri*	none	+	+	+	+	+	+
	none	+	+	+	+	+	+
	none	+	+	+	+	+	+

*+ living *T. salmositica*
- no living *T. salmositica* detected
**Partially coagulated

the inoculated fishes were not infected. It appears then that reliance on data from blood smear examinations is insufficient in determining the host range of *Trypanoplasma* spp. since species are difficult to distinguish morphologically. Consequently it is likely that the haemoflagellates recorded by Becker and Katz in other teleosts were of different species.

The *in vitro* results support those obtained by the *in vivo* study. These results further suggest that complement is involved in the lysis of *T. salmositica* as was found earlier by Bower and Woo[3] for *Trypanoplasma catostomi.*

Complement can be activated in one of two ways. The classical pathway of complement activation is by the combination of antigen with specific antibody. This requires previous exposure of the fish to the antigen. However, it is unlikely that the goldfish obtained from a pet store had prior exposure to *T. salmositica.* Lysis must then be due to activation of the alternate pathway as was suggested earlier.[4] This pathway can be blocked by heat inactivation.

This reaction is also dependent on ions (Mg^{++} and Ca^{++}). Coagulation of blood also requires these ions. This explains the lower lytic titer in the partially coagulated goldfish plasma.

The modified *in vitro* plasma incubation test used to ascertain the time required for complement activation showed a degree of individual variation. In general, this process appears rapid, requiring only 30 to 60 min for complete lysis of approximately 500 organisms in 0.025 ml plasma.

A high degree of host specificity of *T. catostomi* for white sucker was demonstrated by Bower and Woo.[3] It is possible that other species of *Trypanoplasma* spp. are also highly specific. The alternate pathway of complement activation appears to be the mechanism providing natural immunity in the refractive fish. This may be the basis of the high specificity displayed by some species of *Trypanoplasma.* Study of host specificity may help to distinguish species since it is difficult to distinguish them morphologically.

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LITERATURE CITED

1. BECKER, C.D. and M. KATZ. 1965. Infections of the haemoflagellate, *Cryptobia salmositica* Katz, 1951, in freshwater teleosts of the Pacific coast. Trans. Am. Fish. Soc. 94: 327-333.
2. BOWER, S.M. and P.T.K. WOO. 1977a. Morphology and host specificity of *Cryptobia catostomi* n.sp. (Protozoa: Kinetoplastida) from white sucker *(catostomus commersoni)* in southern Ontario. Can. J. Zool. 55: 1082-1092.
3. ——— and ———. 1977b. *Cryptobia catostomi:* Incubation in plasma of susceptible and refractory fishes. Exp. Parasit. 43: 63-68.
4. KATZ, M., J.C. WOODEY, C.D. BECKER, P.T.K. WOO and J.R. ADAMS. 1966. Records of *Cryptobia salmositica* from sockeye salmon from the Fraser river drainage and from the state of Washington. J. Fish. Res. Board. Can. 23: 1965-1966.
5. WOO, P.T.K. 1979. *Trypanoplasma salmositica:* Experimental infections in rainbow trout, *Salmo gairdneri*. Exp. Parasit. 47: 36-48.

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