STUDIES ON AN ICHTHYOSPORIDIUM INFECTION IN FISH: TRANSMISSION AND HOST SPECIFICITY

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STUDIES ON AN ICHTHYOSPORIDIUM INFECTION IN FISH: TRANSMISSION AND HOST SPECIFICITY

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STUDIES ON AN ICHTHYOSPORIDIUM INFECTION IN FISH: TRANSMISSION AND HOST SPECIFICITY

The causative agent of an epizootic among rainbow trout (Salmo gairdneri) was described in an earlier paper by Rucker and Gustafson (1953). This protist of uncertain classification was assigned to the genus Ichthyosporidium, Caullery and Mesnil, (1905). The specific identity is confused by the mulip-licity of species assigned to the genus and to the synonymous genus, Ichthyophonus. The organism we have here appears to be most generally like Ichthyosporidium hoferi (Plehn and Mulsow 1911), as described by Ellis (1930), and the similar form described by Robertson (1909). It differs from the organism studied by Fish (1934), and Sindermann and Scattergood (1954), in the sites of the lesions, the host range, and the characteristic "hyphal" forms. The serious nature of this disease, as previously demonstrated, directed our observations and experiments towards information relating to transmission, host specificity, and prognosis. Some information on the transmission and host range of other closely related organisms has been established. An infection was produced in flounders by Fish (1934) through feeding diseased herring; in Salmonidae by Neresheimer and Clodi (1914) by feeding parasitized tissue; and in tench, carp, and perch by Pettit (postscript, Neresheimer and Clodi 1914) through feeding and also by letting the fish live together with infected salmonoids.

MATERIALS AND METHODS

The infective agent was from the same stock of fish as that recorded in the previous paper by Rucker and Gustafson (1953) and was maintained in the laboratory by holding naturally infected fish in troughs. Infections in both stock fish and experimentally infected fish were determined most easily by examination of liver and kidney smears. In most cases, these organs showed overwhelming infections with large numbers of spheres (fig. 1) and "hyphal" stages (fig. 2) which were most common in chronic, well-established infections. The organism could also be found in the spleen, heart, and peritoneal surface, and occasionally in adjacent body musculature, wall of the stomach, and other portions of the gut. These sites were less favorable than the kidney and liver in determining whether or not a fish was infected. Skin lesions commonly produced by the organism described by Sindermann and Scattergood (1954) were not seen. No brain infections were observed, which is unlike the findings of Plehn and Mulsow (1911).

Material for parenteral inoculation was prepared by blending fresh, infected viscera with an equal amount of saline in a blender for a few seconds. Material for feeding was prepared by mincing fresh infected viscera composed mostly of kidney and liver, with knives. Fish were maintained in individual troughs in running fresh water, where the temperatures ranged from 50° to 60° F.

TRANSMISSION EXPERIMENTS

The information on the transmission experiments is summarized in Table 1.

Parenteral -- The first attempts to transmit the disease in our laboratory were made by intraperitoneal inoculation into young fish which came from stocks in which the disease had never been known. The fish used were rainbow trout (Salmo gairdneri), silver salmon (Oncorhynchus kisutch), sockeye salmon (O. nerka), and squawfish (Ptychocheilus oregonensis).

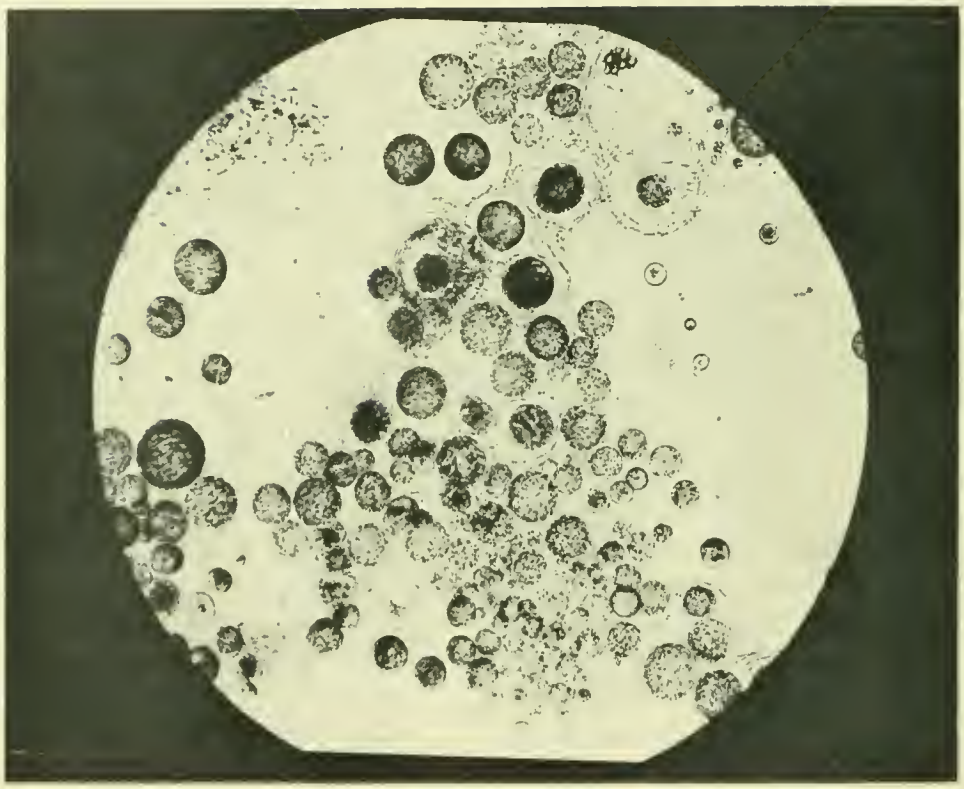

Fig. 1. Photomicrograph of a water mount of fresh liver material from a rainbow trout infected with Ichthyosporidium sp. Mag. X 100.

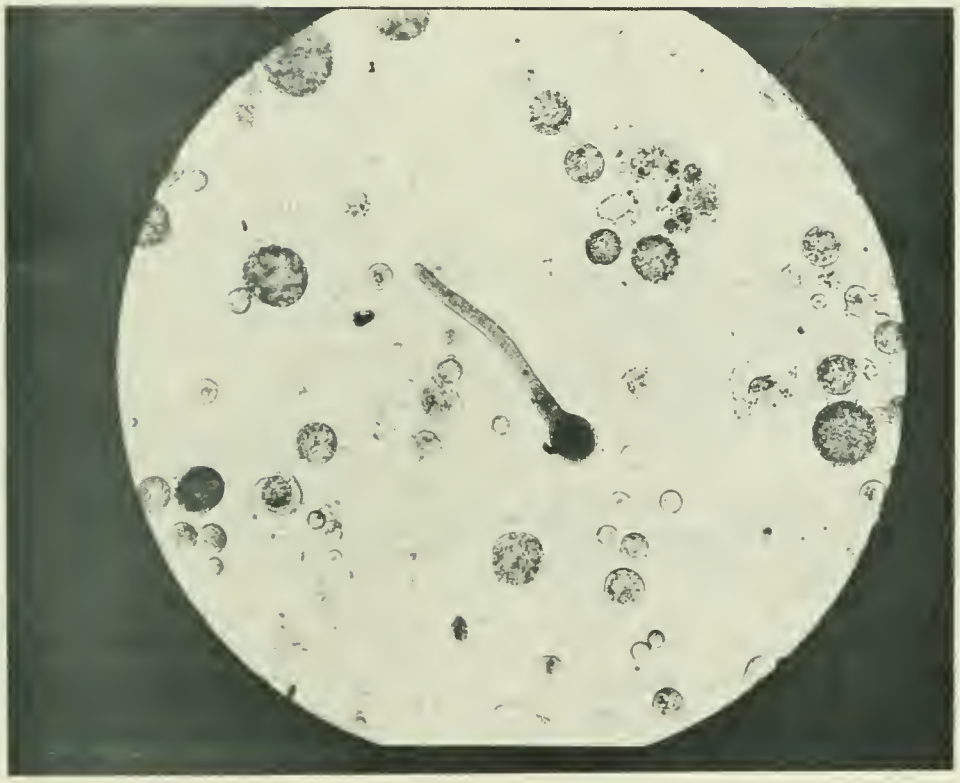

Fig. 2. Photomicrograph of a water mount of fresh liver material from a rainbow trout infected with Ichthyosporidium sp. showing the "hyphal" stage. Mag. X 100.

Rainbow trout -- Of twenty 5-inch rainbow trout inoculated, six died shortly after inoculation; of the remaining 14, all proved to be positive for Ichthyosporidium between the 18th and 40th day.

Silver salmon -- Of eight 3-inch silver salmon which were inoculated by this process, one died early in the experiment and was negative; the other seven, which died of the disease between the 19th and 25th day, were all positive for the infection.

Sockeye salmon -- Of twenty 3-inch sockeyes inoculated, 14 (all negative) died in the first 2 weeks; four of the remaining six were positive for the infection and died of the disease between the 19th and 25th day.

Squawfish -- Six inoculated squawfish, about 3 inches in length, proved to be negative for the infection after 4 weeks of incubation.

Feeding -- After we had established the fact that the disease could be maintained in the laboratory by inoculation, we sought mechanisms more nearly similar to transmissal in nature. The first of these was by feeding. The fish used were rainbow trout, silver salmon, sockeye salmon, chinook salmon (O. tshawytscha), cottoids (Cottus asper), goldfish (Carassius auratus), guppies (Lebistes reticulatus), squawfish, and catfish (Ameiurus nebulosus). Fresh viscera from diseased fish were used as the infectious material.

Rainbow trout -- The infected material fed to rainbow trout usually resulted in a high percentage of positive cases. However, in two well-documented cases, none of the trout were infected. The trout used in all experiments were from three different stocks of fish, but there was no correlation with infectiousness of the organism and source of the experimental fish. In one series, 1-day-old viscera were fed twice at an interval of 3 days to twenty 5-inch rainbow trout: six fish, all negative, died before the 34th day after the first feeding; 12 fish were found to be positive, the first dying on the 30th day after feeding; the two remaining fish were sacrificed on the 62d day and found to be negative. In another series, which was fed only once with viscera from the fish killed the previous day, all 17 fish died of the infection, the first on the 22d day, and the last on the 98th day. This series is illustrated in figure 3.

Chinook salmon -- Of a group of 30 chinook-salmon fingerlings, 12 were found to be positive; the first died on the 35th day and the last on the 62d day after ingesting the infectious material. During this time, 18 died and were negative as regards to this infection; 16 of these presumably died of an intercurrent infection.

Sockeye salmon -- Of a group of fifteen 2-inch sockeye salmon, five died from the Ichthyosporidium infection between the 15th and 25th day after ingestion; 10 died before the 12th day. The latter showed the organism as spheres in the stomach and intestine, but these were not considered to be significant in view of the long incubation period required by this organism. In one experimental attempt to infect sockeye salmon fingerlings with viscera which had been frozen at -40°F. for 9 days, all 15 of these fish were found to be negative when examined between the 45th and 50th day

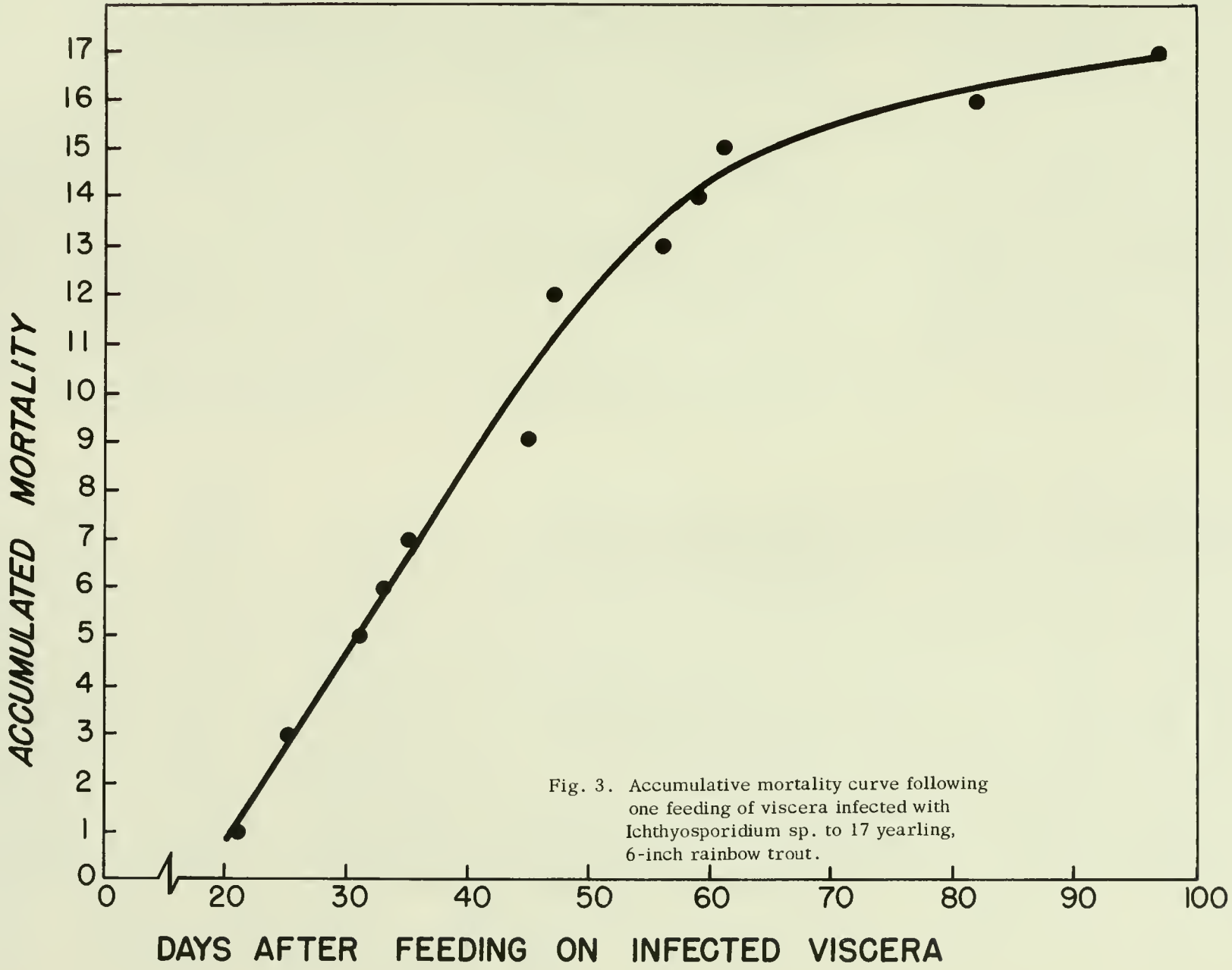

Fig. 3. Accumulative mortality curve following one feeding of viscera infected with Ichthyosporidium sp. to 17 yearling, 6-inch rainbow trout.

Silver salmon -- Of ten 3-inch silver salmon, six, all negative, died within 17 days after ingesting the infectious material. The remaining four, which were all positive, died between the 26th and 55th day. These experiments indicate that salmon as well as trout are subject to Ichthyosporidium infections.

Cottoid -- The possibility of fish other than the salmonids carrying this infection was investigated because of the experience with the previous epizootic in which trout ponds were infected. The water source in this case was a large spring which flowed through a wooded area and provided adequate cover for escaped and native fish. Seven 3-inch cottoids (Cottus asper) were fed fresh diseased viscera six times over a period of 2 weeks. The first of these cottoids died after 8 days and showed an early infection in the kidney. The others, which were all infected, died or were sacrificed between the 19th and 158th day.

Other fish -- Similar attempts to infect groups of 2 goldfish, 10 guppies, 13 squawfish, and 3 catfish, by administering multiple feedings of infected viscera, were all negative, whereas control rainbow trout produced positive infections.

Contact -- In a third experimental approach, information was sought on the spreading of the infection to healthy fish from infected fish in the same water

Rainbow trout -- Ten stock rainbow trout with a natural infection of Ichthyosporidium were put into the upper compartment of a trough, separated by a screen from ten Ichthyosporidium-free rainbow trout in the lower half of the trough. The dead fish were removed from the upper compartment as soon as they were discovered. Three of the trout in the lower half were sacrificed between the 47th and the 66th day, and found to be negative. On the 168th day, two were dead; one of these was positive for Ichthyosporidium. On the 191st day the last five were sacrificed; of these, four were positive. Thus, it is demonstrated that indirect contact between fish can transmit the infection

DISCUSSION

It is apparent that several routes of infection were achieved with the Ichthyosporidium species with which we have worked. The intraperitoneal inoculation with homogenated infected tissue is primarily an implantation of a pathogen from one tissue to another. This would be brought about by the spherical forms normally found in the tissues. The feeding experiments and those allowing contact between infected and uninfected fish are close to situations most likely to occur in nature. Feeding infected viscera would be the equivalent of cannibalism or feeding on dead fish which had died of the disease.

Irregular results derived from feeding experiments can be partly accounted for by several variables. First, specificity would be suggested by the variation between response of salmonids and various fish which were not found to be susceptible. Relative specificity might be suggested by the data presented for the different species of salmon. But the variability in response of different groups of rainbow trout would throw some doubt on the homogeneity of the inoculum. This would bring up the question of the life cycle of this organism and the stages which would be ineffective by mouth.

Variability of the morphology of the organism has been recognized by all workers reporting on this group. The spheres which

were found in the tissues vary tenfold in their diameters, and the internal organization varies from completely syncytial protoplasm to quite discrete bodies within the parent sphere. Besides this, there are the forms which protrude in a germ tube or hypha from the spheres which are stimulated by means not well known. In the condition under study here, hyphal forms are found in the tissues of fish very recently killed and fixed shortly after death, as well as in tissues which have been allowed to age, either within the fish, or in vitro, under refrigeration. It is impossible to determine which of these many forms are the infective stages, or whether they all are. We have observed that in the viscera of trout which had been held for several days or weeks, or even months, there is a definite progression in the more complete subdivision of protoplasm of the spheres and within the hyphal stages. There seems to be a high percentage of hyphal stages within the aged tissues. The spheres, which were the prominant stage in the inoculum being fed, could be found unchanged in the intestinal contents of the experimental fish. These experimental fish, which had died of other causes, were examined shortly after feeding.

Commonly occurring lesions in the wall of the stomach, usually in the distal end, would tend to support the theory that a direct invasion of the host tissue at this site is the normal circumstance when the inoculum is eaten. All parasites seen within the host tissue at this site were spherical in shape, and were arranged with several in a cluster as though derived from a common parent organism. This would allow for progressive infection through the wall of the stomach and a transperitoneal spread through adjacent viscera, rather than through being blood-borne. In the intraperitoneally inoculated fish, found to be positive, the contents of the rectum included spheres typical for this disease indicating that the reverse migration is possible. Transmission of the disease, through water, from chronically chronically infected fish to uninfected fish would suggest that stages are able to leave a fish, whether these are spheres or other stages.

The one experiment on freezing viscera would suggest that this might be a reasonable mechanism for control of Ichthyosporidium infections in marine fish and other fish viscera which are being fed to hatchery stock.

SUMMARY

Transmission experiments demonstrated that Ichthyosporidium sp., occurring in rainbow trout, can be transmitted by three different methods: through intraperitoneal inoculation of fresh viscera homogenate into susceptible fish; through the feeding of fresh infected viscera; and through indirect contact between living carriers and susceptible fish. The host range has been demonstrated to include rainbow trout, chinook salmon, sockeye salmon, silver salmon, and a cottoid. Fish which were refractory were the goldfish, guppy, squawfish, and catfish.

LITERATURE CITED

Ellis, Marjory F.
1930. Ichthyophonus hoferi, a flounder parasite new to North American waters. Proc. and Trans. of the Nova Scotian Institute of Science, 17 (3): 185-192.

Fish, F. F.
1934. A fungus disease in fishes of the Gulf of Maine. Parasitology, 26 (1): 1-16.

Neresheimer, E., and C. Clodi.
1914. Ichthyophonus hoferi, der Erreger der Taummelkrankheit der Salmoiden. Arch. für Protist, 34 (3): 217-248.

Plehn, M., and K. Mulsow.
1911. Der Erreger der Taummelkrankheit der Salmoiden. Centr. für Bakt. u. Parasitenk. I Abt. Bd. 59:63-68.

Robertson, M.
1909. Notes upon a Haplosporidian belonging to the genus Ichthyosporidium. Proceed. Royal Phys. Soc. of Edinburgh, 17(5):175-187.

Rucker, R. R., and Paul V. Gustafson.
1953. An epizootic among rainbow trout. Prog. Fish-Cult., 15 (4): 179-181.

Sindermann, Carl J., and Leslie W. Scattergood.
1954. Diseases of Fishes of the Western North Atlantic. II. Ichthyosporidium disease of the sea herring (Clupea harengus). Maine Dept. Sea and Shore Fisheries, Res. Bull. No. 19:1-40.

TABLE 1.

Inoculation Route		Number of fish at start	Number of deaths before first positive	Days to first positive	Number of positives
PARENTERAL					
	Rainbow Trout	20	6	18	14
	Silver Salmon	8	1	19	7
	Sockeye Salmon	20	14	19	4
	Squawfish	6	0	0	0
FEEDING					
	Rainbow Trout	20	6	30	12
	Chinook Salmon	30	1	35	12
	Sockeye Salmon	15	10	15	5
	Silver Salmon	10	6	26	4
	Cottoid (Bullhead)	7	0	8	7
	Goldfish	2	---	---	0
	Guppy	10	---	---	0
	Squawfish	13	---	---	0
	Catfish	3	---	---	0
CONTACT					
	Rainbow Trout	10	4	168	5