A New Myxozoa *Thelohanellus kitauei*, the Cause of Intestinal Giant Cystic Disease of Carp

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A description is given of a new myxozoan parasite located in the intestinal wall of the cultured carp *Cyprinus carpio* from Japan. The name *Thelohanellus kitauei* n. sp. is suggested for this parasite whose spores are characterized by an egg-shaped balloon-like sack, 33.4 μm by 15.0 μm in size in average. Spores measure 26.3 μm in length and 9.2 μm in breadth and thickness in average in water. The polysporous vegetative form which forms a giant polyp-like swelling in the intestine is inferred to be a single winding tube based on histological observations of the serial paraffin sections of the swellings. A mass of minute spherical disporous form is exceptionally observed in a small visible lesion lying under the mucosa, too.

A new harmful thelohanellosis, the intestinal giant cystic disease of the carp, was first found out in carp farms in Kagoshima Prefecture in 1978 (KITAUE, 1980). Subsequently the same disease was noted in net pens in Lake Ikeda (Kagoshima Pref.), Lake Kasumigaura (Ibaragi Pref.) and Lake Suwa (Nagano Pref.) and also in carp ponds in the Keta (Mie Pref.), Fukuoka (Toyama Pref.) and Shioda (Nagano Pref.) areas. It attracted our attention because it occurred at relatively high incidences and caused much damage to the carp in some irrigation-ponds at Shioda in 1979. In one of the ponds about 4,000 of 56,000 one-year-old carp died from the disease through early August into mid-October. The remained fish were harvested in November and about 45,000 one-year-old carp were newly stocked in the pond in May, 1980, without taking any disinfectant measures against the pond. The just death due to the disease was noticed on July 22, and the death continued till the end of October, 1980 and about 4,000 fish in total died. From a field examination the infection rate was estimated to be about 22.6%, that is, the mortality of the infected fish was about 40% and the mortality of the whole fish was about 9%. The present study was made to identify the myxozoa and to clarify the development and the pathological effect of the parasite.

Materials and Methods

The materials for the study were obtained three times from the aforementioned heavily infected pond at Shioda from September, 1979 through October, 1980.

Five dying fish, 28.0-39.5 cm in body length, were collected on September 18, 1979. All fish had many giant swellings in their intestines. Numerous fresh spores were obtained from some of the swellings and preserved in a physiological salt solution for a cold-blooded animal use. Preserved living spores were placed in water to observe the shape and to measure the size. They were treated with 5% KOH solution to release the polar filament and stained with the Lugol's solution to examine for the iodophilous vacuole.

Before mortality started in late July, 1980, 31 healthy fish which were 14-month-old and 22.4-26.2 cm in body length were caught at random on the feeding-platform on July 11. Seven of the 31 fish examined had visible lesions in the intestines. The number of the visible lesions changed individually from 1 to 16 and amounted to 36 in all. Four of the 36 lesions found in three fish were small in size and laid under the mucosa of the intestine, not forming prominent swellings. The other 32 lesions were all small swellings. The small, not swelled lesions and the small swellings together with the surrounding intestinal tissues

were fixed in 10% formalin and the HE-stained serial paraffin sections were prepared for histological observation.

Seven dying fish, 26.5–38.0 cm in body length, were collected on August 19, 1980. All fish had 3–18 giant swellings in their intestines. Some of the swellings were fixed to prepare the paraffin sections.

The materials are deposited in the National Science Museum, Tokyo. Cat. No. NSMT-Pr 158.

Results and Discussion

Spores

Spores were characterized by having an egg-shaped balloon-like sack with a pointed end which attached to the anterior end of the spore (Fig. 1). The sack consisted of a thin, colorless membrane and contained a colorless, transparent fluid. The sack was distinctly observed with living spores placed in water but was not always clearly observed with HE-stained spores in paraffin sections. Some spores immediately taking out from swellings and put in the physiological salt solution had no sack (Fig. 2-A). Some deformed spores were observed and interestingly the shape of their sacks was the same as that of the spores themselves (Fig. 2-B). The sack often opened at the anterior end in water (Fig. 2-C). These facts suggested that the sack may be formed by separation of an outer membranous layer of the spore.

The spore proper within the sack was pyriform in front and side view (Fig. 1). It had two, very thin shells equal in size and shape (Fig. 2-D). The sutural line was indistinct. The polar capsule was single and pyriform. A coiled polar filament was distinct in the polar capsule and very long when released. A large iodophilous vacuole was con-

spicuous in the sporoplasm (Fig. 2-E). Dimensions of living spores in water on 20 measurements were as follows: length 26.3 (23–29) μm , breadth and thickness 9.2(8–11) μm , sack 33.4(31–35) μm

Fig. 1. Spores of *Thelohanellus kitauei*.

Fig. 2. Spores of *Thelohanellus kitauei*. A: Some spores without any sack placed in the physiological salt solution. $\times 550$. B: Deformed spore. Notice the shape of the sack. $\times 800$. C: Opened spore sack placed in water. $\times 1,300$. D: Divided shells within the sack. $\times 1,700$. E: Iodophilous vacuole in the sporoplasm. $\times 1,300$.

by 15.0(12–17) μm , polar capsule 16.8(14–18) μm by 7.4(6–9) μm , length of polar filament 166,7 (120–197) μm .

The present parasite is correctly identified with the genus *Thelohanellus* by having pyriform spores with one polar capsule and also having a conspicuous iodophilous vacuole in the sporoplasm. Any *Thelohanellus* species which has a balloon-like spore sack has not been reported. In addition any *Thelohanellus* species which is parasitic to the intestines of the carp and forms giant cystic swellings projected into the intestinal lumen has not been known. Moreover the length of the polar filament of this species is longer than those of already known members of the genus *Thelohanellus* and the size of the spore is smaller than *T. rohita* (SOUTHWELL et PRASHAD, 1918) but distinctly larger than *T. andhrae* QUARDI, 1962, *T. catlae* CHAKRAVARTY et BASU, 1948, *T. dogieli* ACHMEROV, 1955, *T. fuhrmanni* (AUERBACH, 1909), *T. misgurni* (KUDO, 1920), *T. notatus* (MAVOR, 1916), *T. oculi-leucisci* (TROJAN, 1909), *T. pyriformis* (THÉLOHAN, 1892), *T. seni* (SOUTHWELL et PRASHAD, 1918), *T. shortti* QUARDI, 1967 and *T. toyamai* (KUDO, 1915), except for the following two species whose original descriptions could not be referred, *T. swellengrebeli* SCHUURMANS et STEKHOVEN from the musculature of *Rhodeus amarus* and *T. unicapsulatus* (GURLEY, 1893) from the skin of *Lebeo niloticus* in Nile. From the above, the present species is recognized as a distinct new species for which the specific name *Thelehanellus kitauei* is proposed.

Vegetative forms and Swellings

Two types of the vegetative form, a minute spherical disporous form and a winding tubular polysporous form, were observed.

The four small, not swelled visible lesions observed in 14-month-old carp on July 11, 1980 were similar in appearance about 0.8–1.2 mm in diameter and observed as a poppy seed under the mucosa of the intestine (Pl. I-A). One of the 4 lesions was of the minute spherical disporous form. Accordingly this form may be an exceptional formation. In this lesion numerous disporous vegetative forms and free spores were found in the tunica propria mucosa and the submucosa (Pl. I-B). The disporous vegetative forms were spherical and measured 20–26 μm in diameter in the paraffin section (Pl. I-C).

In the other three small, not swelled lesions 4–15 cross sections of the immature vegetative form were observed in the sections near the central part of the lesion (Pl. I-D). The vegetative form was 0.04–0.2 mm in diameter and lived in the tunica propria mucosa and the submucosa. Observations of the serial paraffin sections from each lesion clearly demonstrate that this type of the vegetative form have a winding tubular body like a nematode. The both ends of the vegetative form were usually observed at a distance (Pl. I-D).

Small swellings obtained from 14-month-old carp in July, 1980 were variable in shape and size, from a pimple-like body to a soybean-like body (Pl. I-E). Their sizes were measured about 2 mm by 1.5 mm to 6 mm by 5 mm in fresh condition. In paraffin sections of the smallest one, 1.8 mm in width and 1.2 mm in height in the paraffin section, about 60 cross sections of the tubular vegetative form, 0.04–0.24 mm in diameter, were observed (Pl. I-F). In paraffin sections of the largest one, 4.6 mm in width and 3.0 mm in height in the paraffin section, about 300 cross sections of the immature tubular vegetative form, 0.07–0.35 mm in diameter, were observed.

Whereas the giant polyp-like swellings obtained from 15-month-old carp in August, 1980 were as large as a sparrow egg or a pigeon egg (Pl. II-B). The maximum diameter measured was 4.2 cm. In a relatively smaller swelling, 11 mm by 7.5 mm in size in the paraffin section, about 300 cross sections up to 1.05 mm in diameter of the well matured tubular vegetative form were observed. In the sections of giant swellings the both ends of the tubular vegetative form were usually observed to remain near the center and the border of the swelling (Pl. II-D). Numberless spores filled all parts of the matured tubular vegetative form. In addition many spores freed from the vegetative form were found in the section of the giant swelling, however free spores were not observed in the sections of small swellings with the immature tubular vegetative form.

Any connective tissues of the host covering the winding tubular polysporous vegetative form were not observed. The swelling was surrounded by an abnormally developed mucosal tissues of the intestine. From this observation the fate of the giant swelling is considered as follows: The matured tubular vegetative form may break for

itself, then the swelling opens at the top (Pl. II-C) and the spores are shed into the intestinal lumen. The spores released may be discharged through the anus into the water.

Pathology

Giant swellings were formed in various parts of the intestines and numbered up to 22 in dying fish. In all carp in advanced stages of the disease the affected parts of the intestine were expanded markedly with the development of the swellings (Pl. II-A). Atrophie of hepatic cells of the neighbouring hepatopancreas and stagnation or hyperemia of the neighbouring blood vessels are caused by compression of the expanded intestine. Moreover giant swellings blockade completely the intestinal lumen, leading to loss of appetite and starvation of the host. Dying carp show emaciation and anemia more or less.

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Explanation of Plate I.

Small visible lesions and small swellings formed by the vegetative form of *Thelohanellus kitauei*. **A:** Small visible lesion lying under the mucosa of the intestine of a healthy 14-month-old carp in July, 1980. $\times 1.5$. **B:** Section of a small visible lesion. The center of the lesion is a mass of numerous minute disporous vegetative forms. $\times 24$. **C:** Magnified section of the above lesion. Arrows show many spherical disporous vegetative forms. $\times 400$. **D:** Section of an initial lesion caused by a winding tubular vegetative form. Arrows show growing ends of the tubular vegetative form. $\times 50$. **E:** Many small swellings developed in the intestine of a healthy 14-month-old carp in July, 1980. $\times 1$. **F:** Section of the smallest swelling. About 60 cross sections of the immature tubular vegetative form are seen. $\times 2.5$.

Explanation of Plate II.

Affected intestine of the carp and giant swellings formed by *Thelohanallus kitauei*. **A:** Expanded intestine of dying 15-month-old carp in August, 1980. $\times 0.6$. **B:** Giant swellings formed in the above intestine. $\times 0.8$. **C:** Broken swelling found in a dying 15-month-old carp in August. $\times 0.5$. **D:** Part of the section of a giant swelling filled up with a matured single tubular vegetative form. $\times 28$.