Cryptobia iubilans infection in juvenile discus

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In spring 1998, a Florida tropical fish producer (farm A) reported that approximately 20% to 40% of the 2- to 5-week-old discus (Symphysodon aequifasciatus) at the farm had become sick and died, along with approximately 5% of the 2- to 3-month-old juveniles. The facility had recently undergone restructuring, which had included adding a separate grow-out recirculating system and modifying the existing breeder system. The recirculating system tanks used for the grow-out phase each contained established sponge filters for biological filtration that were derived from the breeder system tanks. Water quality parameters had been unstable for several weeks with high total ammonia nitrogen (TAN) and nitrite concentrations. The target pH for raising the juvenile discus was 5.8 to 6.2, but the measured pH was 5.2 to 5.7. Because acidic pH and resulting low alkalinity greatly reduce the growth and metabolic efficiency of nitrifying bacteria, it was thought that the acidic pH was having a negative effect on the biofilters. Therefore, the water was changed weekly in an attempt to increase the pH and decrease the TAN and nitrite concentrations. The producer had also added some other South American cichlids to 1 of the breeder systems in the facility during the winter and spring of 1998.

Three moribund discus (23 to 26 mm total length [TL]; the length of a fish measured from the front of the jaw that is most anterior to the end of the longest caudal ray]) were submitted to the University of Florida's Tropical Aquaculture Laboratory in Ruskin for necropsy and microbiologic evaluation. Major findings were intestinal parasites (hexamitid flagellates, presumably Spironucleus spp) and a moderate number of granulomas in stomach tissue from the fish. Microbiologic culture of brain and kidney specimens on tryptic soy agar (TSA) with 5% sheep's blood at 30°C did not yield any bacterial growth after 48 hours. The problems at the farm were attributed to poor water quality and Spironucleus infection.

Two months later, discus were still becoming sick and dying even though water quality had stabilized, and 3 to 4 weeks after that, the producer began to notice even higher morbidity and mortality rates. At this time, most life stages were affected, including fry (2 to 4 weeks old), juveniles (1 to 6 months old), and breeders (1 to 3 years old). The morbidity rate was highest among the 2- to 4-week-old fish, with clinical signs including darkening, lateral recumbency, lethargy, and moving slowly in the water column; mortality rate was 70% to 90%. Fish that were > 2 months old, including breeders, had high morbidity rates but low mortality rates (2% to 5%). These fish became dark, had increased mucus production and respiratory rates, and became laterally recumbent for 1 or more weeks before recovering.

At this time, an additional 6 moribund 2- to 4-week-old discus (12 to 17 mm TL) from the same affected systems at farm A were submitted to the Tropical Aquaculture Laboratory for evaluation. Gross abnormalities included a larger-than-normal amount of mucus on the gills, skin, and fins. Fin erosions were also evident, and low numbers of moneganeas (family Dactylogyridae) were seen in gill samples. Hexamitid flagellates, presumably Spironucleus spp on the basis of morphology, movement, and location, and low numbers of nematodes (Camallanus spp) were seen in the lumen of the intestine. In the lumen and within the mucosa and submucosa of the stomach and intestines from most of the fish were...
numerous other flagellates tentatively identified as *Cryptobia iubilans* (order Kinetoplastida, family Bodonidae). These flagellates were ovoid and had 2 flagella of unequal length that emerged from the anterior end. The flagellates’ movements were slow and undulant. Other important findings included a few to many granulomas in stomach tissue and a few granulomas in the intestinal tract. The gastric granulomas were considered consistent with *C iubilans* infection but not with *Spironucleus* infection. Stomach samples were submitted for histologic examination, scanning electron microscopy, and transmission electron microscopy. Bacterial culture of kidney and brain specimens again did not yield any bacterial growth after 48 hours.

Histologic evaluation of multiple affected fish revealed various degrees of granulomatous gastritis, with some fish having only a few, individual granulomas and others having multiple, coalescing granulomas (Fig 1). Large foci consisting of macrophages surrounded by fibroblasts and having cores frequently containing degenerate cells and nuclear debris were found in the submucosal layers. Lymphocytic cells and occasional heterophils were seen in the tissues surrounding the granulomas, and in severely affected fish, this infiltrate resulted in marked thickening of the submucosal layer. The mucosal layer in the most severely affected fish was eroded or ulcerated. Large, plump macrophages filled with a fine, eosinophilic granular material and often containing several vacuoles were seen. Some of these vacuoles were seen to contain slender, elongate, ovoid flagellated structures, but detailed morphology was difficult to discern.

Scanning electron microscopy of specimens from affected fish from farm A revealed numerous elongate *C iubilans* trophozoites lying on and penetrating into the gastric mucosa (Fig 2). The posterior flagellum appeared to be free, contrasting with the recurrent position previously reported for *Cryptobia* spp.1,2 Parasites measured 10 to 13 µm long and 2 µm wide, excluding the flagella.

Transmission electron microscopy confirmed that flagellates were present in vacuoles in host cells (Fig 3). The flagellates were confirmed to be members of the order Kinetoplastida on the basis of detection of a kinetoplast (ie, a single mitochondrion in which DNA is amplified into a large mass), paraxial rod (ie, a lattice-like structure along the axoneme in the flagellum), and a cytoskeleton composed of microtubules lying beneath the body surface. Particular taxonomic features consistent with *C iubilans* included an anterior beak-like rostrum, triangular kinetoplast, and an incomplete cell corset dominated by a large dorsal sheet of microtubules, along with the fact that the cytostome and cytopharynx were accompanied by microtubules and the posterior part of the cytopharynx was accompanied by only 4 microtubules.

In addition to the *C iubilans* that were seen, numerous meronts and gamonts identified as *Cryptosporidium*...
spp were seen. These were spherical structures up to 5 μm in diameter that were covered with microvilli and situated at the tip of mucosal epithelial cells in the stomach. Identity of these structures was confirmed by detection of a unique attachment-feeding organelle at the interface between the parasite and host cell (Fig 4). Sporulated oocysts in parasiathorophorous vacuoles were found deep in the epithelial cells and contained at least 3 sporozoites. Findings were consistent with descriptions of Cryptosporidium spp identified in another species of cichlid, the tilapia (Oreochromis aureus X O. niloticus), in Israel.1

The histologic and electron microscopic findings suggested that C iubilans infection was likely the most important health problem at farm A, although the other parasites that were seen were believed to have contributed to the high morbidity and mortality rates. Therefore, treatment with metronidazole (10 mg/g of food or 6.6 mg/L of water) for 5 days was recommended to eliminate the Spironucleus infection, treatment with formalin (12 to 25 mg/L of water) once every 7 to 10 days for a total of 3 treatments was recommended to eliminate the monogeneans, and treatment with levamisole (2 mg/L of water) once a week for 3 treatments was recommended to eliminate the Camallanus infection.

Unfortunately, there are no treatments clinically proven to be effective against C iubilans infection. Therefore, several chemicals were recommended for experimental use, including nitrofurazone (a nitrofurazone effective against trichomonads, amoebas, and some coccidia) and metronidazole, dimetridazole, and fenbendazole (effective against other flagellates). The producer treated various groups of fish for approximately 5 days each, testing 2 different bath treatments and 2 different gel diets. However, affected fish were not aggressive feeders, and many were anorectic. Bath treatments were administered once a day with a 70% to 100% water change before reaplication of the medication. Treatments that were evaluated included nitrofurazone at a concentration of 11 mg/L and metronidazole at a concentration of 13 mg/L. Dietary treatments that were evaluated included dimetridazole (10 mg/g of food) and fenbendazole (0.5 to 1.0 mg/g of food). Fish in each of the 4 groups were euthanatized at the end of the treatment period. At necropsy, most fish from all 4 treatment groups still had moderate to heavy C iubilans infestations.

Although morbidity and mortality rates in fish > 2 months old decreased, sporadic cryptobiosis in younger fish (2 to 4 weeks old) continued. Because this breeder was producing other species of fish more lucratively, he elected to expand production of these other species and reduce production of discus.

In summer and fall 1998, a New York discus producer (farm B) experienced problems with stunted juvenile discus housed in indoor, static aquaria with airstones. Fish were thin and lethargic and had high respiratory rates, but the mortality rate was low.

Five fish (21 to 110 mm TL) were submitted to the University of Florida’s Fish Disease Diagnostic Laboratory in Gainesville, Fla, for evaluation. Fish were thin and dark and had swollen gills. Histologic examination of gill biopsy specimens revealed moderate to heavy infestations of monogeneans that had contributed to severe hyperplasia of the secondary lamellae. Examination of the stomach and intestinal tract revealed moderate to heavy infestations of C iubilans and the nematode Camallanus spp. In the smaller fish (21 to 25 mm TL), numerous multilocular granulomas were seen in the stomach, intestinal tract, liver, spleen, anterior kidney, and posterior kidney. In the larger fish (97 to 110 mm TL), granulomas were found only in the stomach. However, few fish of either size were examined, making it impossible to draw any conclusions about these apparent size differences. Bacterial culture of specimens from the posterior kidney, liver, and spleen did not yield any growth. Acid-fast bacteria were not identified in any histologic sections stained with special stains. Cryptobia iubilans infestation was considered to be the most important disease problem in these fish, but no specific treatment was provided because effective treatments for C iubilans infestation have not been described. Bath treatments with formalin (12 to 25 mg/L of water for 24 hours) and levamisole (1 to 2 mg/L of water for 24 hours) were recommended to eliminate the monogeneans and Camallanus spp.

In spring 2000, an ornamental fish producer in Florida (farm C) reported a high number of deaths among 5- to 7-week-old discus housed in a 908-L, flow-through system. In 1 week, 280 of a group of 300 (93%) fish died. Although the system had only been in place for about 5 weeks, the producer insisted that water quality was fine and refused to submit water
specific treatments were recommended for
not seen in histologic sections stained with special
rescens
infestation.

intestines. Granulomas were observed in the stomach.

10o to 16oC water to be dripped into a 473-L tank for
temperature shock when a heater malfunction caused
aquaria were thin and lethargic and had high respira-
tory rates. Water-quality data were unremarkable,
farm D were submitted to the Fish Disease Diagnostic
laboratory. Results did not yield any growth, and acid-fast bacteria
were not seen in histologic sections stained with special
cysts. Treatment with formalin (12 to 25 mg/L of
water) was recommended to eliminate the
sporocysts are passed in the feces.
merogony, gametogony, and sporogony subsequently occur. Fully sporu-
lated sporocysts are passed in the feaces.

Although 52 species of the genus Cryptobia have been identified in
fish, the actual number may be substantially less, owing to subleties in the
taxonomy of this genus. Most (n = 40) species that have been identified are hemoflagellates, but 5 are con-
sidered ectoparasitic, and 7 are found in the gastroin-
testinal tract.19 Of the 7 species that reportedly affect
the gastrointestinal tract, only C. iubilans is considered
truly parasitic and pathogenic.11 Cryptobia iubilans is believed to have a direct life cycle.11

In fish from farm A in the present report, organ-
isms identified as Cryptosporidium spp were found in
addition to C. iubilans but were considered to be less
important. Cryptosporidium spp also have a direct life
cycle, and after oocysts are ingested, liberated sporo-
zeites enter epithelial cells where merogony, gameto-
gony, and sporogony subsequently occur. Fully sporu-
lated sporocysts are passed in the feaces.

Research with a population of wild-caught cichlids held in isolation demonstrated that C. iubilans may be a
part of the normal fauna of these fish, becoming more
pathogenic only under certain conditions.6 Wet-mount
preparations of stomach tissues from recently import-
ed wild-caught P. zebra contained fewer, smaller granu-
lomas, and these were found in only 2 of 9 fish necrop-
sied. However, after the fish were held in isolation for
several months, those that remained (5/5) had gastric
granulomas that were much larger and in greater over-
all number in each fish, and examination of gastric tis-
sue by means of transmission electron microscopy
revealed C. iubilans in these granulomas.5 Some
researchers consider C. iubilans to be a facultative para-
site,12 and the 4 production facilities discussed in the
present report all had additional factors (eg, water-
quality problems and external or other internal para-
sites) that likely contributed to the illness and death of
the fish.

Granulomatous gastritis has been associated with
gastrointestinal cryptobiosis in a number of cichlid
species, including Astronotus ocellatus, Aulonocara
jacobi, Aulonocara stuartgranti, Cichlasoma
(Herichthys) meeki, Cichlasoma nigrofasciatum,
Cichlasoma octofasciatum, Cyphotilapia frontosa,
Dimidiochromis compressiceps, Haplochromis milomo
(VC-10), Cichlasoma (Herichthys) cyanoguttatum, Cichlasoma
(Herichthys) nicaraguense, Cichlasoma (Herichthys)
syn-
pilum, Oreochromis esculentus, Pseudotropheus zebra, and
Symphysodon spp. Interestingly, we have not seen similar disease signs in angelfish (Pterophyllum scalare), another blackwater cichlid of South American origin, even though angelfish develop many other diseases commonly seen in discus. In fact, we have only rarely seen any flagellates in the stomach of this species.

It is likely that many cases of granulomatous gastritis and gastroenteritis in cichlids caused by Cryptobia iubilans have been misdiagnosed because live parasites were not seen. Although the epidemiology of the disease has not been determined, our field observations of active cases of the disease suggest that the rapid time course of acute infection in susceptible populations or the slow spread with chronic infections may account for the low prevalence of active infections. Differences in morbidity and mortality rates have been seen among different species, as well as among different age classes of the same species.

It is also possible that the clinical importance of C. iubilans infestations in cichlids has been overlooked because of infestations with other distantly related, enteric protozoal parasites. Flagellates commonly found in the intestinal tract of many cichlid species, including discus, that may be confused with C. iubilans are members of the order Diplomonadida, family Hexamitidae. These flagellates are known as hexamitids and include Spirorhabdos spp and Hexamita spp. Spironucleus vortens has been isolated from the intestinal tract of angelfish, and S. vortens or a closely related species is commonly found in numerous wild-caught and captive-bred cichlids.

The hexamitids can easily be differentiated from C. iubilans without the use of electron microscopy on the basis of their typical location, lesions that they induce, movement, and appearance. The appearance of the body of both types of small flagellates can easily be observed in fresh preparations to which a clear viscous medium has been added. The hexamitids are typically found in the lumen of the intestinal tract; do not incite a granulomatous response; have a more linear, directed movement than C. iubilans; have a pear-shaped body (broader anteriorly) that is approximately 12.5 to 20 µm long and 5.0 to 11.2 µm wide when the organism is alive; bear 6 anterior locomotory flagella; and have 2 trailing posterior flagella.

In fish with active infestations, C. iubilans is most commonly found in the stomach but may also be found in the intestinal tract and other organs. Cryptobia iubilans typically incites a substantial granulomatous response, especially in the stomach, with large numbers of organisms in parasitophorous vacuoles in macrophages. Cryptobia iubilans movements are slow and undulant, and in our experience, they have more of a wiggle during movement than do the hexamitids. The organism has only 2 flagella and tends to be more elongate in early infection and more oval, tear-drop shaped, or round in chronic infection.

Another important clinical difference between hexamitid and C. iubilans infection is treatment efficacy. The hexamitids can easily be controlled with metronidazole administered in a bath or the feed and recent producer reports of resistance of hexamitids to metronidazole may be attributable to misdiagnosis of C. iubilans infection. On the other hand, successful treatment for gastrointestinal cryptobiosis in cichlids has not been reported in the literature. Steinighagen et al. reported success in the field with 2-amino-5-nitrothiazole but did not perform any controlled studies. In our experience here in the United States, a major contributing factor to the lack of field trials has been the difficulty in finding active cases of the disease or populations with a high enough prevalence of the disease for clinical trials. Other problems, as exemplified by findings in the present report, are the high mortality rate and severe anorexia, which limit the potential for field trials of chemotherapeutic agents.

Because of the morbidity and mortality rates associated with C. iubilans infestation among the 4 producers described in the present report, we performed clinical trials on the efficacy of 6 chemotherapeutic agents, including 2-amino-5-nitrothiazole; primaquine and chloroquine (2 antiprotozoal drugs used in human medicine to treat Plasmodium falciparum infestation and amoebiasis), nitrofurazone, metronidazole, and dimetridazole.

Fish populations with active C. iubilans infestations were used for these clinical trials. Stomachs from an average of 6 fish from suspect populations were examined to determine the prevalence of C. iubilans infestation in the population. Fish were classified as positive or negative for active C. iubilans infestation on the basis of results of examination of the entire stomach. If 1 or more motile C. iubilans were observed on wet mounts from stomach tissues, the fish was considered positive for active infestation; otherwise, fish were considered negative for active infestation.

In each of the clinical trials, the number of fish used and prevalence of active infestations varied. Because large numbers of fish from populations with a high (>30%) prevalence of active infestations were difficult to acquire, the number of replicates in certain trials was limited. All trials were carried out in 38-L tanks with the following water-quality variables: temperature, 25° to 27°C; dissolved oxygen concentration, 7.2 to 7.6 mg/L; salinity, 0.2 g/L; conductivity, 416 µS; TAN concentration, 0.1 to 0.2 mg/L; nitrite concentration, 0.01 to 0.03 mg/L; pH, 8.0 to 8.2; alkalinity, 120 mg/L; and hardness, 239.4 to 256 mg/L. Trials involving bath treatment were 3 days long; trials involving oral treatments were 4 days long. The number of fish that died during each trial was recorded. Remaining fish at the end of each trial were examined and classified as positive or negative for active C. iubilans infestation on the basis of results of microscopic examination of wet mounts of the entire stomach.

The first clinical trial used 30 Aulonocara jacobfreibergi. Bath treatments were used because the fish were anorexic. Fish were randomly assigned to 1 of 5 treatment groups with 6 fish/group. Fish in each group were treated with primaquine (16 mg/L for 24 hours, repeated daily for 3 days), chloroquine (21 mg/L for 24 hours, repeated daily for 3 days), dimetridazole (80 mg/L for 24 hours, repeated daily for 3 days), or nitrofurazone (11 mg/L for 24 hours, repeated daily for 3 days) or were not treated (control). For each group, a 100% water change was performed every 24 hours.
All primaquine- and chloroquine-treated fish were dead after 3 days. All diminazazole-treated fish were alive after 3 days, and on day 4, no _C. iubilans_ organisms were seen in any of the 6 fish. All nitrofurazone-treated fish were alive after 3 days, but on day 4, _C. iubilans_ organisms were seen in 5 of the 6 fish. All control fish were alive, and all 6 fish were positive for _C. iubilans_ infestation. Prevalence of _C. iubilans_ infestation at the end of the treatment period was significantly (χ², 6.167; P < 0.05) different among the diminazazole, nitrofurazone, and control groups; by inspection, this difference was most likely attributable to the diminazazole group. Primaquine and chloroquine appeared to be toxic, but death of fish treated with these drugs might have been related to severity of the disease rather than to the drug dosage used. Because only 1 replicate of 6 fish/treatment was used, additional trials of longer duration and involving more replicates are required.

The second clinical trial used 55 _Haplochromis_ (Pimelodochromis) mimo. Bath treatments were used because these fish were also anorectic. One tank of 10 fish and 2 tanks of 9 fish were randomly assigned to be treated with 2-amino-5-nitrothiazol (10 mg/L for 24 hours, repeated daily for 3 days), and 3 tanks of 9 fish were maintained as untreated controls. For each group, a 100% water change was performed every 24 hours. Six treated (21%) and 10 control (37%) fish died; these percentages were not significantly different. At the end of the treatment period, 10 of the 22 (45%) remaining fish that had been treated with 2-amino-5-nitrothiazol and 13 of the remaining 17 (76%) control fish were positive for _C. iubilans_ infestation; these percentages were not significantly different.

The third clinical trial used 375 oscars (_Astronotus ocellatus_). Fish were acclimated to a commercial diet for approximately 3 days prior to the start of the trial and then randomly assigned to 1 of 5 groups, with each group consisting of 3 tanks with 25 fish/tank. Fish in each group were treated with metronidazole (20 mg/g of food), diminazazole (4.4 mg/g of food), or 2-amino-5-nitrothiazol (4.4 mg/g of food) or were maintained as untreated controls. During the trials, fish were fed approximately 3% of their body weight daily (divided into 2 feedings/d) for 4 days. Fish were observed to consume all food fed. A representative sample of 16 fish from each tank (48 fish/treatment group) was examined for the presence or absence of _C. iubilans_ in stomach tissue. Data were analyzed by use of a generalized logistic model that revealed a significant (Wald χ², 9.64; P = 0.047) difference in prevalence of _C. iubilans_ infestation among treatment groups at the end of the treatment period. This difference was primarily attributable to a difference in prevalence between the control and 2-amino-5-nitrothiazol groups. The 95% confidence interval for prevalence of infestation in control fish (25% to 52%) overlapped only slightly with the 95% confidence interval for prevalence of infestation in fish treated with 2-amino-5-nitrothiazol (6% to 23%), but overlapped extensively with confidence intervals for the other treatment groups. There was no significant difference in mortality rates among treatment groups (Wald χ², 2.77; P = 0.597). Future studies using higher oral dosages and longer treatment periods for 2-amino-5-nitrothiazol are warranted.

In summary, findings for producers described in the present report together with findings of previous reports suggest that the flagellate _C. iubilans_ is an important parasite of discus and many other cichlids that typically induces granulomatous gastritis, as well as granulomatous disease in other organs, including the kidney, spleen, liver, mesenteric fat, swim bladder, eye, heart, and gonads. Morbidity and mortality rates in a population appear to be linked to a number of variables, including water quality, the presence of other parasites and bacteria, diet, species, size, and age of the fish, but epidemiologic studies are required to determine the relative importance of these variables. Survivors may suffer from chronic wasting, probably as a result of damage to gastric tissue and other organs. Optimization of husbandry appears to be an important tool in preventing and alleviating the severity of the disease. Equally important is the use of appropriate diagnostic testing, including transmission electron microscopy to detect and identify the parasite when live organisms are no longer present. The more common, related flagellates _Spiroplana_ spp induce different lesions and, unlike _C. iubilans_, readily respond to treatment with metronidazole. Thus, the 2 flagellates must be differentiated, although this can often be done at the light microscopic level with live specimens. Results of our clinical trials suggest that treatment with diminazazole or 2-amino-5-nitrothiazol may help reduce the prevalence of infestation with _C. iubilans_, but additional studies are needed to determine the optimal dose and duration of treatment. Other potential treatments should also be examined; however, anorexia will limit the effectiveness of any oral treatments.

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**References**

