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ISOLATION OF *AEROMONAS SALMONICIDA* FROM SEA LAMPREY (*PETROMYZON MARINUS*) WITH FURUNCLE-LIKE LESIONS IN LAKE ONTARIO

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ABSTRACT: For the past six decades, parasitic sea lampreys (*Petromyzon marinus*) have caused devastating losses to salmonid fisheries in the Great Lakes. To reduce the number of sea lampreys, the Great Lakes Fishery Commission began a large-scale program based on trapping male sea lampreys, sterilizing them, and releasing sterile males back into streams to compete with fertile males for spawning females. The transfer of lampreys among lakes can potentially lead to the transfer of various pathogens, and this has raised major concerns regarding the possibility of resident fish populations becoming infected by introduced pathogens. During a health inspection of sea lampreys collected from Lake Ontario, lampreys with obvious furuncle-like lesions (1–2 cm in diameter) were noticed. Most of the furuncles occupied the dorso-lateral musculature, and *Aeromonas salmonicida* subsp. *salmonicida* was isolated from the kidneys. This bacterium was cultured from kidneys of 2.5% of the sea lampreys collected from two locations within the Lake Ontario watershed in 2004. The identity of bacterial colonies was presumptively verified with biochemical reactions and confirmed with polymerase chain reaction. This is the first report of *A. salmonicida* infection in sea lamprey in the Great Lakes basin associated with furunculosis.

Key words: *Aeromonas salmonicida*, furunculosis, Great Lakes, *Petromyzon marinus*, sea lamprey.

INTRODUCTION

Furunculosis, caused by *Aeromonas salmonicida*, is a serious bacterial disease of salmonids worldwide (Austin and Austin, 1999). The disease signs vary according to the stage of infection. In peracute infections, the disease is characterized by sudden death without premonitory clinical signs. In its acute form, the disease is characterized by septicemia with external and internal hemorrhagic lesions; the chronic form of the disease is characterized by formation of external furuncle-like lesions and congestion of visceral organs (Cipriano and Bullock, 2001). Although the pathogen has been primarily known as a salmonid pathogen, there are increasing reports of outbreaks in nonsalmonids such as goldfish (*Carrasius auratus*) (Humphrey and Ashburner, 1993), turbot (*Scophthalmus maximus* L.) (Toranzo and Barja, 1992), halibut, (*Hippoglossus hippoglossus* L.) (Gudmundsdottir et al., 2003), cod (*Gadus morhua* L.) (Cornick et al., 1984), sand-eels (*Ammodytes lances* [Cuvier]) and *Hyperoplus lanceolatus* (Lesauvege) (Dalsgaard and Paulsen, 1986), and wrasse (Labridae) (Frerichs et al., 1992; Laidler et al., 1999). Recently, El Morabit et al. (2004) isolated the organism from the sea lamprey (*Petromyzon marinus*) in Europe, and they reported that it was pathogenic to cultured rainbow trout when experimentally challenged.

In the Great Lakes basin, a number of nonindigenous species have invaded the system and caused serious ecologic and economic losses (Lupi and Hoehn, 1998). The sea lamprey, one of these invasive species, has been incriminated as an important factor contributing to the collapse of the lake trout (*Salvelinus namaycush*) and the lake whitefish (*Coregonus clupeaformis*) fisheries in the Great Lakes during the 1940s and 1950s; these fisheries are still depressed, despite ongoing sea lamprey chemical control since 1958 (Smith and Tibbles, 1980).

To further reduce the number of sea lamprey and limit their spread, the Great Lakes Fishery Commission (GLFC) began a large-scale experimental program based on trapping male sea lampreys, sterilizing them, and subsequently releasing the sterile males back into streams where they compete with fertile males for spawning females. Currently, sea lamprey males are collected from different areas in the Great Lakes basin, transported to a sterilizing facility in Hammond Bay, Michigan, and then released into selected river systems basin-wide. The relocation of lampreys to different sites has raised concerns regarding the translocation of pathogens and the possibility of resident fish populations becoming infected. To prevent this, the GLFC initiated a disease screening program to determine whether the sea lamprey might be contributing to the within-basin spread of fish diseases.

In the current study, we report the isolation of *A. salmonicida* infection in sea lampreys showing skin lesions consistent with furunculosis in the Great Lakes. To our knowledge, this is the first report of a natural infection of *A. salmonicida* in sea lampreys in the Great lakes basin.

MATERIALS AND METHODS

Fish and sample collection

In midsummer of 2004, 118 adult sea lampreys were caught as two individual lots from Duffins Creek (58 fish) and Humber River (60 fish) in eastern Ontario, Canada. They were held separately and brought alive to the Aquatic Animal Health Laboratory (AAHL) at Michigan State University, East Lansing, Michigan, USA. Lampreys were kept in well-aerated, chilled (10 C) water tanks until examined. At the AAHL, the sea lampreys were inspected for the suitability of larger numbers to be subsequently transferred from the two streams to a nonisolated lamprey-sterilizing facility at Hammond Bay. From there, the sterilized males would be transferred to a target stream.

Lampreys were euthanized with an overdose of MS 222 (tricaine methane sulfonate, Finquel-Argent Chemical Laboratories, Redmond, Washington, USA), and then they were dissected under aseptic conditions. Kidneys were removed aseptically and placed in sterile 7.5 cm × 18.5 cm Whirl-Pak® bags (Nasco, Fort Atkinson, Wisconsin, USA) to which Hank's balanced salt solution (Sigma-Aldrich, St. Louis, Missouri, USA) was added at a ratio of 1:4 (weight/volume). The tissues were homogenized for 120 sec using a high-speed Biomaster Stomacher-80 (Wolf Laboratories Limited, Pocklington, York, UK).

Isolation and identification

Aliquots (100 µl) of stomached kidney tissue were spread onto tryptic soy agar (TSA, Remel, Lenexa, Kansas, USA) and Coomassie brilliant blue agar (CBBA) (Cipriano and Bertolini, 1988). Coomassie brilliant blue agar was prepared by adding 0.1 g of Coomassie brilliant blue (Sigma-Aldrich) powder to 1 l of TSA agar. Inoculated TSA and CBBA plates were incubated at 22 C for 24–48 hr. Representative colonies were selected and restreaked for isolation on TSA and again incubated at 22 C for 24–48 hr. Identification tests were subsequently performed on these pure isolates.

Bacterial isolates were presumptively identified using conventional biochemical tests, including catalase with 3% hydrogen peroxide solution; cytochrome oxidase with Pathotec oxidase strips (Remel); motility in motility test medium (BD Biosciences, Sparks, Maryland, USA); citrate utilization using Simmons citrate (Remel), sugar utilization using triple sugar iron (TSI, Remel), oxidation; and fermentation of glucose using OF Basal media with glucose as the sole carbohydrate source (BD Biosciences), esculin hydrolysis using bile esculin agar (Remel), presence of poly A layer using uptake of Coomassie blue on Coomassie Brilliant Blue Agar, and presence of brown diffusible pigment after 48 hr of growth on TSA. Further biochemical testing was performed using API20E and 20NE tests (BioMérieux Inc., Durham, North Carolina, USA), with isolates incubated at 15 C and results interpreted at 48–72 hr according to manufacturer's instructions.

Antibiotic sensitivity testing was done using the Kirby-Bauer antibiotic disc diffusion method (Bauer et al., 1966). The following antibiotic discs were all purchased from Remel: tetracycline, sulfamethaxozole-trimethoprim (Romet 30), novobiocin, erythromycin, and azithromycin. Florfenicol was purchased from BD Biosciences.

Chromosomal DNA was extracted from 100 µl of bacterial suspension (single colony of each of the isolated bacteria suspended in

100 μl of sterile saline) using DNeasy tissue extraction kit (QIAGEN., Valencia, California, USA) according to manufacturer's instructions. The extracted DNA was amplified using oligonucleotide primer set specific for *A. salmonicida* subsp. *salmonicida* (Miyata et al., 1996). The sequences of the two primers were primer-1 (5′-AGC CTC CAC GCG CTC ACA GC-3′) and primer-2 (5′-AAG AGG CCC CAT AGT GTG GG-3′).

The controls consisted of a polymerase chain reaction (PCR) mixture without DNA template (negative control) and with DNA extracted from known *A. salmonicida* subsp. *salmonicida* (positive control). Thermal cycling was done in a Robocycler Gradient 96 (Stratagene, La Jolla, California, USA) using 10 ng of template DNA, 0.2 mM each of dNTP, 16 pmol each of primer, 5 μl of 10× reaction buffer, and 1.25 units of *Taq* DNA polymerase (Invitrogen, Carlsbad, California, USA) in a volume of 50 μl of reaction mix. Thirty thermal cycles were done at 94 C for 30 sec for denaturation, 60 C for 30 sec for annealing, and 72 C for 60 sec for extension. The samples were allowed one extension cycle at 72 C for 5 min after thermal cycling (Miyata et al., 1996). The PCR products were electrophoresed in 2% agarose gel (Invitrogen), stained with ethidium bromide, visualized with ultraviolet light, and photographed using Kodak EDAS System (Eastman Kodak, Rochester, New York, USA). Samples were considered positive when a 512-bp band was detected.

Treatment trial

Guided by the antibiogram study, tetracycline (OTC) was chosen to be used in a water bath for treatment of Duffins Creek lamprey at a dose of 100 mg per gallon for 10 days. Treated lamprey then retested for the presence of lesions or bacteria in the internal organs or blood.

RESULTS

Clinical examination revealed the presence of skin lesion in sea lamprey from Duffins Creek but not from Humber River. Clinical signs occurred in the form of localized swellings on the dorsal fin and dorsal muscle. Some lampreys showed shallow ulcerations. During a routine health inspection before relocation during midsummer 2004, 118 sea lampreys were tested for bacterial pathogens. Of these, *A. salmonicida* was identified in only three (2.5%) of the samples. The three isolates were presumptively identified as *A. salmonicida* using the following biochemical profile: Gram-negative short bacilli, nonmotile, catalase positive, cytochrome oxidase positive, citrate utilization negative, TSI K/A, poly A layer positive (blue colonies on CBBA), glucose fermentative, esculin hydrolysis positive, and presence of brown diffusible pigment on TSA (Table 1). Further identifications using BioMérieux API20E and 20NE rapid test strips were used (Table 2). Consistent with *A. salmonicida* subsp. *salmonicida*, all isolates produced a 512-bp band when tested by PCR.

The antibiogram, which was based on the diameter of inhibition zone per each antibiotic as per manufacturer's recommendation, revealed high sensitivity of the three *A. salmonicida* isolates to OTC, Florfenicol, Romet 30, and Novobiocin. Intermediate sensitivity to erythromycin and azithromycin also was observed. Lampreys from Duffins Creek were sub-

TABLE 1. Conventional biochemical tests[a] used to identify the isolated *A. salmonicida* species.

Isolate no.	M	Cat	CO	Cit	TSI	CBBA	O/F	E	Brown diffusible pigment on TSA
4	−	−	+	−		Blue	+/+	+	+
24	−	−	+	−		Blue	+/+	+	+
25	−	+	+	−	K/A	Blue	+/+	+	+
A.s.s.s.[b]	−	−	+	−		Blue	+/+	+	+

[a] M = motility; Cat = catalase; CO = cytochrome oxidase; Cit = citrate (Simmons); O/F = glucose, oxidase/fermentation; E = esculin.

[b] A.s.s.s. = *Aeromonas salmonicida* subsp. *salmonicida* positive control.

TABLE 2. API 20E and API 20NE results for the *A. salmonicida* isolates from the sea lamprey.

Test	Isolate 4	Isolate 24	Isolate 25
Nitrates to nitrites	+	+	+
Indole production	−	−	−
Glucose fermentation	+	+	+
Arginine dihydrolase	−	−	−
Urease	−	−	−
Esculin hydrolysis	+	+	+
Gelatin hydrolysis	+	+	+
Paranitrophenyl B-D-galactopyranoside	−	−	−
Glucose assimilation	−	−	−
Arabnose assimilation	−	−	−
Mannose assimilation	−	−	−
Manitol assimilation	−	−	−
N-Acetyl glucosamine assimilation	−	−	−
Maltose assimilation	−	−	−
Gluconate assimilation	−	−	−
Capric acid assimilation	−	−	−
Adipic acid assimilation	−	−	−
Malate assimilation	−	−	−
Citrate assimilation	−	−	−
Phenylacetic acid assimilation	−	−	−

jected to treatment with a full course of OTC, and then they were retested for the presence of lesions or bacteria in the internal organs or blood. No bacteria were isolated from the treated lampreys.

DISCUSSION

Despite the fact that *A. salmonicida* has been described primarily as a salmonid pathogen, there are increasing reports of isolation of the bacteria from diseased nonsalmonid species (Cornick et al., 1984; Toranzo and Barja, 1992; Humphrey and Ashburner, 1993; Gudmundsdottir et al., 2003). Furthermore, the bacteria were isolated for the first time from sea lampreys in Europe in 2004, but in this case there was no associated disease (El Morabit et al., 2004). To our knowledge, this is the first report of *A. salmonicida* infection in sea lamprey in the Great Lakes basin, and the first report of *A. salmonicida*-associated lesions in this species.

Affected lampreys showed furuncle-like lesions on the dorsal musculature and fins similar to what is observed in salmonids. Some lampreys developed erosions to shallow ulcerations. Infected lampreys were most probably in the chronic stage of infection, because the bacteria were isolated only from kidney and not from blood. The acute form of the disease is characterized by septicemia, and the organism can be isolated from multiple organs. In addition, sea lampreys developed furuncle-like lesions on skin, a result corroborated by Bernoth et al. (1997) and Austin and Austin (1999).

The morphologic criteria and biochemical profile of the *A. salmonicida* isolates from sea lamprey coincided with those described for *A. salmonicida*. (Table 2). Additional testing using BioMérieux API20E and 20NE rapid biochemical tests supported the results and tentatively identified the three isolates as typical *A. salmonicida* subsp. *salmonicida*; this was confirmed by PCR (Miyata et al., 1996; Byers et al., 2002). The application of OTC to treat the infected Duffins Creek sea lampreys suggests that OTC could be effectively used for treatment of *A. salmonicida* infection in lampreys before relocation procedures.

This study reports the first natural infection of *A. salmonicida* in sea lamprey with lesions similar to what reported in salmonids during furunculosis. Sea lampreys may act as a source of infection to cohabitating salmonids and contribute in the spread of the disease between lakes; however, it is difficult to assess the potential impact with regard to the occurrence of furunculosis in salmonid populations in the Great Lakes basin. Therefore, further studies are necessary to categorize *A. salmonicida* subsp. *salmonicida* isolated from sea lampreys and to assess their pathogenicity to salmonids and nonsalmonids. Such information is needed to fully understand the risks and benefits of the relocation program.

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LITERATURE CITED

AUSTIN, B., AND D. A. AUSTIN. 1999. Bacterial fish pathogens: Diseases of farmed and wild fish. 3rd Edition. Springer-Parxis, London, UK, 457 pp.

BAUER, A. W., W. M. M. KIRBY, J. C. SHERRIS, AND M. TURCK. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45: 493–496.

BERNOTH, E. M., A. E. ELLIS, P. J. MIDTLYNG, G. OLIVIER, AND S. PETER. 1997. Furunculosis Multidisciplinary fish disease research. Academic Press, New York, New York, 529 pp.

BYERS, K. H., N. GUDKOVS, AND M. J. CRANE. 2002. PCR-based assays for the fish pathogen *Aeromonas salmonicida*. I. Evaluation of three PCR primer sets for detection and identification. Diseases of Aquatic Organisms 49: 129–138.

CIPRIANO, R., AND J. BERTOLINI. 1988. Selection for virulence in the fish pathogen *Aeromonas salmonicida* using Coomassie brilliant blue agar. Journal of Wildlife Diseases 24: 672–678.

———, AND G. L. BULLOCK. 2001. Furunculosis and other diseases caused by *Aeromonas salmonicida*. Fish Disease Leaflet 66. National Fish Health Laboratory, Kernsville, West Virginia, USA.

CORNICK, J. W., C. M. MORRISON, B. ZWICKER, AND G. SHUM. 1984. Atypical *Aeromonas salmonicida* infection in Atlantic cod, *Gadus morhua* L. Journal of Fish Diseases 7: 495–499.

DALSGAARD, I., AND H. PAULSEN. 1986. Atypical *Aeromonas salmonicida* isolated from diseased sand-eels, *Ammodytes lancea* (Cuvier) and *Hyperoplus lanceolatis* (Lesauvege). Journal of Fish Diseases 9: 361–364.

EL MORABIT, A., S. GARCÍA-MÁRQUEZ, AND Y. SANTOS. 2004. Is sea lamprey a potential source of infection with *Aeromonas salmonicida* for wild and farmed fish? Bulletin of the European Association of Fish Pathologists 24: 100–104.

FRERICHS, G. N., S. D. MILLER, AND C. MCMANUS. 1992. Atypical *Aeromonas salmonicida* isolated from healthy wrasse (*Ctenolabrus rupestris*). Bulletin of the European Association of Fish Pathologists 12: 48–49.

GUDMUNDSDOTTIR, S., S. LANGE, B. MANGNADOTTIR, AND B. K. GUDMUNDSDOTTIR. 2003. Protection against atypical furunculosis in Atlantic halibut, *Hippoglossus hippoglossus* (L.): Comparison of a commercial furunculosis vaccine and an autogenous vaccine. Journal of Fish Diseases 26: 331–338.

HUMPHREY, J. D., AND L. D. ASHBURNER. 1993. Spread of the bacterial fish pathogen *Aeroomonas salmonicida* after importation of infected goldfish, *Carassius auratus*, into Australia. Australian Veterinary Journal 70: 453–454.

LAIDLER, L. A., J. W. TREASURER, A. N. GRANT, AND D. I. COX. 1999. Atypical *Aeromonas salmonicida* infection in wrasse (Labridae) used as cleaner fish of farmed atlantic salmon, *Salmo salar* L., in Scotland. Journal of Fish Diseases 22: 209–213.

LUPI, F., AND J. P. HOEHN. 1998. A partial benefit-cost analysis of sea lamprey treatment options on the St. Marys River. A report submitted to the Great Lakes Fishery Commission. Department of Agricultural Economics, Michigan Sate University, East Lansing, Michigan.

MIYATA, M., V. INGLIS, AND T. AOKI. 1996. Rapid identification of *Aeromonas salmonicida* subspecies *salmonicida* by the polymerase chain reaction. Aquaculture 141: 13–24.

SMITH, B. R., AND J. J. TIBBLES. 1980. Sea lamprey (*Petromyzon marinus*) in lakes Huron, Michigan and Superior: History of invasion and control, 1936–1978. Canadian Journal of Fisheries and Aquatic Sciences 37: 1780–1801.

TORANZO, A. E., AND J. L. BARJA. 1992. First report of furunculosis in turbot (*Scophthalmus maximus*) rearing in floating cage in north-west of Spain. Bulletin of the European Association of Fish Pathologists 12: 147–149.

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