Vol. 1: 151–153, 1986 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published July 15

NOTE

Serological heterogeneity of recent isolates of *Yersinia ruckeri* from Ontario and British Columbia

J. G. Daly, B. Lindvik* & R. M. W. Stevenson**

Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT: *Yersinia ruckeri*, the enteric redmouth (ERM) bacterium, has been isolated from salmonid fish in Ontario and British Columbia on 16 occasions between 1982 and 1985. The 2 most common serovars, I and II, were found in both provinces. In 2 cases in British Columbia and 1 in Ontario, the isolates belonged to serovar V. This variety of *Y. ruckeri* has been found only once before, in Colorado fish. A serovar III isolate from British Columbia and a new serological variety (serovar VI) from Ontario have also been confirmed as *Y. ruckeri* isolates. The relative importance of these various isolates as disease agents is not clear, and, because of this, they present a problem when fish health management decisions must be made.

The enteric redmouth (ERM) bacterium *Yersinia ruckeri* is designated as a certifiable disease agent by Canadian Fish Health Protection Regulations (Department of Fisheries and Oceans 1984). This designation was given because the pathogen was considered to be limited in geographical distribution. Outbreaks of ERM disease in Canada most often have been linked to imports of fish from areas where the organism is prevalent (Wobeser 1973). However, the organism has been shown to have a wider distribution than can be explained solely on the basis of fish transfers (Bullock et al. 1978).

Previously, we reported a number of *Yersinia ruckeri* isolations from sources in Ontario (Stevenson & Daly 1982), an area in which the associated disease has not been observed. Only one of those isolates belonged to serovar I, the Hagerman type. Busch (1982) suggested that isolates made during routine fish health inspections, from asymptomatically and subclinically infected fish, are often of the second, sorbitol-fermenting, serological variety described by O'Leary et al. (1982). Since 1982, we have isolated and authenticated a number of *Y. ruckeri* cultures from Canadian sources. This report describes the characteristics of these new strains, the significance of which to fish health must still be ascertained. Of particular interest are the *Y. ruckeri* isolates that belong to the new serovar V group (Stevenson & Airdrie 1984), and 2 other unusual serological types.

Methods. Except as outlined below, isolations from fish were made according to procedures specified by the manual of compliance (Department of Fisheries and Oceans 1984). In the case of isolations made at the Fish Health Laboratory, Department of Microbiology, Guelph, cultures were made from the spleen, heart and liver as well as from the kidney, and all organs were enriched in tryptic soy broth at 20°C for 2 d before streaking onto tryptic soy agar plates. Colonies from plates were selected on the basis of morphology and slide agglutination reactions with anti-*Yersinia ruckeri* serum. Identifications were confirmed by biochemical test reactions, using standard tube tests, and API 20E^R test strips (Stevenson & Daly 1982). Serological characterization with cross-absorbed rabbit antiserum raised against formalin-killed cells was carried out as previously described (Stevenson & Daly 1982, Stevenson & Airdrie 1984).

Results and Discussion. The 16 isolations of *Yersinia ruckeri* made from fish in Ontario and British Columbia in 1982 to 1985 are summarized in Table 1. The number of isolations does not readily give values for prevalence of the organism in these provinces because only selected fish populations were examined. For example, only 2 of the Ontario isolates came from inspections of private hatcheries. These were received for confirmation of identity. The other 5 Ontario isolates occurred among 278 fish populations examined during the same period.

* Formerly Fisheries Branch, British Columbia Ministry of Environment, Nanaimo, British Columbia, Canada. Present address: 3406 Hendon St., Abbotsford, British Columbia V2S 4X8, Canada

** Addressee for correspondence

0177-5103/86/0001/0151/$ 05.00

Table 1. Isolations of *Yersinia ruckeri*, 1982 to 1985

Strain code	Serovar[a]	Location of isolation	Year	Fish source[b]	Isolated by[c]
Ontario					
ONT 487	I	Bothwell Creek	1982	Rainbow trout	JD
ONT 518	II	Chatsworth	1983	Lake trout	JD
ONT 534	I	Lake Nipigon	1983	Lake trout	JD
# 30	II	Private hatchery	1983	Rainbow trout	SD
16A	UT	Private hatchery	1984	Rainbow trout	SD
ONT 764	I	Ganaraska River	1985	Rainbow trout	JD
ONT 765	V	Ganaraska River	1985	Rainbow trout	JD
British Columbia					
82-091	II	Trout farm	1982	Rainbow trout	TS
82-077	I	Vedder River	1982	Steelhead trout	TS
YR 40	III	Revelstoke Dam	1982	Dolly Varden trout	BL
83-206	V	Okanagan Lake	1983	Whitefish	BL
83-106	I	Gold River	1983	Steelhead	BL
84-024	V	Nathan Creek	1984	Cutthroat trout	BL
84-081	II	Big Qualicum Hatchery	1984	Chinook salmon	GH
84-025	I	Cowichan River	1984	Steelhead trout	BL
84-247	I	Nicola River	1984	Steelhead trout	BL

[a] Serovar designations are as previously reported (Stevenson & Airdrie 1984). UT = untyped. Serovar III (Bullock et al. 1978) and the previously designated Serovar I′ are identical

[b] Rainbow trout *Salmo gairdneri*; lake trout *Salvelinus namaycush*; steelhead trout *Salmo gairdneri*; Dolly Varden trout *Salvelinus malma*; whitefish *Coregonus clupeaformis*; cutthroat trout *Salmo clarki*; chinook salmon *Oncorhynchus tshawytscha*

[c] JD = J. G. Daly, Fish Health Laboratory, University of Guelph; SD = Samir S. Durzi, Fisheries and Oceans Canada, Ontario Region; GH = G. Hoskins, Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, B. C.; TS = T. A. Shortt and BL = B. Lindvik, both formerly of the British Columbia Fisheries Branch, Nanaimo

The majority of isolates belonged to either serovar I or II. The Ontario strains ONT 487, ONT 534, and ONT 764 were of interest because they were serovar I isolates from fish. The only previous isolation of this serological variety in Ontario was from a muskrat *Ondatra zibethica* by K. Lautenschlager in 1978 (Stevenson & Daly 1982). Serovar I strains do occur in fish in the Great Lakes region, and all 9 isolates of *Yersinia ruckeri* sent by J. W. Warren and D. D. Desens of the Fish Disease Control Center, LaCrosse, Wisconsin, belonged to serovar I (data not shown). The 4 isolations of serovar I strains in British Columbia are not unexpected, as ERM disease was first generally prevalent in the western states of North America.

Serovar V strains were isolated from 3 geographically distinct sources: 2 in British Columbia and 1 in Ontario. Serovar V strains ferment sorbitol, but react only weakly with antisera specific for serovars I, II, or III strains. The only previously known serovar V strain was isolated in 1978, from rainbow trout *Salmo gairdneri* in Leadville, Colorado (Stevenson & Airdrie 1984). We lack any further information about the health of those fish. Both British Columbia isolations (83-206 and 84-024) were from moribund fish. The isolate of serovar V *Yersinia ruckeri* from whitefish *Coregonus clupeaformis* was a pure culture from the kidney of a moribund fish taken from the south end of Okanagan Lake. At the same time, 2 other dead whitefish had been collected from Skaha lake, which is immediately south of Okanagan Lake. In March 1983 there had been a fish kill in Skaha Lake, which apparently only affected whitefish. The extent to which serovar V *Y. ruckeri* may have been the cause of the mortalities is not known. The Ontario isolate, ONT 765, was found in the spleen of a spawning rainbow trout in May 1985. Serovar I *Y. ruckeri* was also found in apparently healthy fish of this same population (ON 764). The significance of serovar V *Y. ruckeri* strains in fish health management is unknown.

The British Columbia strain YR 40 was isolated from the kidney of a mature, unspawned Dolly Varden trout *Salvelinus malma* during routine egg collection. Serologically, it resembles the serovar III strains described by Bullock et al. (1978), but, unlike them, it ferments sorbitol (Stevenson & Airdrie 1984). This serological group has not previously been reported as occurring in fish outside Australia.

Another unusual isolate, 16A, from the kidney of a rainbow trout, was found during a routine inspection of a private hatchery. Previously a serovar II *Yersinia ruckeri* culture (#30) had been isolated from a tongue lesion in a fish from this hatchery. The biochemical

characteristics of 16A were consistent with identification as *Y. ruckeri,* but the organism did not cross-react serologically with any of the known *Y. ruckeri* types (data not shown). This isolate should be considered a new serological variety of *Y. ruckeri* serovar VI, because its identity has been confirmed by genetic analysis (S. A. De Grandis, P. Krell & R. M. W. Stevenson, unpubl.).

Increased surveillance of the health status of fish populations, and increased awareness and ability to recognize and identify *Yersinia ruckeri* likely account for the greater number of isolations in recent years. What is not known is the extent to which *Y. ruckeri,* particularly the recently recognized serological varieties, causes active disease in fish populations. Serovar I strains are most commonly the isolate found in disease outbreaks, and experiments with rainbow trout suggest that serovar II and III strains are less virulent (Bullock & Anderson 1984). However, ERM disease in chinook salmon is often associated with serovar II isolates (O'Leary et al. 1982). Until there is clear information about the relative ability of the different *Y. ruckeri* varieties to cause disease in different fish species, the isolation of the ERM organism from fish will continue to present difficulties in making fish health management decisions.

Acknowledgements. This work was supported by a Science Subvention Program Grant from Fisheries and Oceans Canada. We thank Samir Durzi, Ontario Region, Fisheries and Oceans for his liaison assistance. The Fish Culture Sections of the Ontario Ministry of Natural Resources and the British Columbia Ministry of the Environment provided support for J.G.D. and B.L., respectively.

LITERATURE CITED

Bullock, G. L., Anderson, D. P. (1984). Immunization against *Yersinia ruckeri,* cause of enteric redmouth disease. In: de Klinkelin, P. (ed.) Symposium on Fish Vaccination, Office International des Epizooties, Paris. p. 151–166

Bullock, G. L., Stuckey, H. M., Shotts, Jr., E. B. (1978). Enteric redmouth bacterium: comparison of isolates from different geographic areas. J. Fish Dis. 1: 351–356

Busch, R. A. (1982). Enteric redmouth disease *(Yersinia ruckeri).* In: Anderson, D. P., Dorson, M., . Dubourget, Ph. (ed.) Antigens of fish pathogens. Coll. Fond. Marcel Merieux, Lyon, p. 201–223

Department of Fisheries and Oceans (1984). Fish health protection regulations: manual of compliance. Fish. Mar. Serv. Misc. Spec. Publ. 31 (Revised): 1–43

O'Leary, P. J., Rohovec, J. S., Sanders, J. E., Fryer, J. L. (1982). Serotypes of *Yersinia ruckeri* and their immunogenic properties. Sea Grant College Program Publication No. ORESU-T-82-001, Oregon State Univ., Corvallis, Oregon, p. 1–15

Stevenson, R. M. W., Airdrie, D. W. (1984). Serological variation among *Yersinia ruckeri* strains. J. Fish Dis. 7: 247–254

Stevenson, R. M. W., Daly, J. G. (1982). Biochemical and serological characteristics of Ontario isolates of *Yersinia ruckeri.* Can. J. Fish. Aquat. Sci. 39: 870–876

Wobeser, G. (1973). An outbreak of redmouth disease in rainbow trout *(Salmo gairdneri)* in Saskatchewan. J. Fish. Res. Bd. Can. 30: 571–575

Responsible Subject Editor: Dr. T Evelyn; accepted for printing on April 30, 1986