AN EPIZOOTIC IN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) CAUSED BY A SORBITOL-POSITIVE SEROVAR 2 STRAIN OF YERSINIA RUCKERI

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AN EPIZOOTIC IN CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) CAUSED BY A SORBITOL-POSITIVE SEROVAR 2 STRAIN OF *YERSINIA RUCKERI*

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ABSTRACT: Enteric redmouth disease is described in chinook salmon (*Oncorhynchus tshawytscha*) at a state hatchery in Sand Ridge, Illinois. Biochemical, isoenzyme, and serological data indicated that the epizootic was caused by a sorbitol-fermenting Serovar 2 strain of *Yersinia ruckeri*. In laboratory experiments the isolate was pathogenic for both brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*).

INTRODUCTION

Enteric redmouth disease, caused by *Yersinia ruckeri* (Ewing et al., 1978), was reported initially among hatchery-reared rainbow trout (*Salmo gairdneri*) from the Hagerman Valley in Idaho (Rucker, 1966); however, other data indicated that the disease was a problem in the United States and Australia before this report was published (Bullock et al., 1977). The subsequent dissemination of this disease throughout the United States was attributed in part to the transport of infected fish (Bullock et al., 1978), which may have become asymptomatic carriers, shed bacteria from their intestines, and established reservoirs for recurrent infections (Busch and Lingg, 1975; Busch, 1978). Enteric redmouth disease has been reported since from fish disease outbreaks in Australia (Llewellynn, 1980), West Germany (Fuhrmann et al., 1983), France (Lesel et al., 1983), and England (Roberts, 1983). *Yersinia ruckeri* has been isolated also from carrier fish in Canada (Wobeser, 1973).

Although *Y. ruckeri* exhibits some pleomorphism (Austin et al., 1982), the bacterium is homogeneous and highly clonal as judged by biochemical (Bullock et al., 1978; O'Leary et al., 1979) and genetic (Ewing et al., 1978; Schill et al., 1984) analyses. The bacterium was divided initially into two serotypes that correlated biochemically with the ability of some strains to ferment sorbitol (O'Leary et al., 1979). Strains of *Y. ruckeri* that did not ferment sorbitol formed a distinct serological group and were considered generally to be virulent for fish (Bullock et al., 1978; McCarthy and Johnson, 1982). Although strains of *Y. ruckeri* that fermented sorbitol were first considered to form a second serotype (O'Leary et al., 1982), later studies indicated that these strains are diverse antigenically (Stevenson and Daly, 1982; Stevenson and Airdrie, 1984).

Most epizootics of enteric redmouth disease are caused by the distinct Serovar 1, which does not ferment sorbitol (Bullock et al., 1978). Therefore, it is believed commonly that sorbitol-fermenting strains of *Y. ruckeri* are of no significant consequence to fish culture. Here, however, we report on an epizootic of enteric redmouth disease in chinook salmon (*Oncorhynchus tshawytscha*) caused by a sorbitol-fermenting strain of *Y. ruckeri*.

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MATERIALS AND METHODS

Case history

In October 1984 eggs of fall chinook salmon were transported from Lake Michigan, Manistee, Michigan, USA, to the state fish hatchery at Sand Ridge, Illinois, USA. Mortalities began on 8 February 1984 and affected fish showed hemorrhages around the eyes, and in the liver and spleen. Bacteria were isolated from the kidneys of dead fish on brain heart infusion agar incubated at 20 C for 24 hr. The bacteria were gram negative, motile rods that were oxidase negative and produced an alkaline over acid reaction in triple sugar iron agar (TSI; Difco). Some gas was evident in the TSI butt in early isolations. The bacteria did not react by fluorescent antibody microscopy with antiserum prepared against a sorbitol-nonfermenting strain of Y. ruckeri. Bacteria were sensitive to oxytetracyline fed at 4 g per 100 lb of fish per day for 10 days. Prophylactic treatments decreased mortality within 6 days after application.

A second outbreak of the disease began on 18 March 1985. The bacteria isolated from affected fish were similar to that already described. The isolate was sent to the National Fish Health Research Laboratory, Kearneysville, West Virginia, USA, for further confirmation.

Biochemical analysis

Bacteria were streaked onto TSA and Shotts-Waltman agar (Waltman and Shotts, 1984), incubated at 25 C for 24 hr, and checked for purity. Individual colonies were cloned by three passages on TSA at 25 C. Cloned bacteria were used for additional biochemical tests. Sorbitol utilization was determined as described by Cipriano and Pyle (1985). All other biochemical tests were performed as described by O'Leary et al. (1979). The following enzymes were analyzed further for isoenzyme variations as described by Schill et al. (1984): aspartate aminotransferase (EC 2.6.1.1), creatine kinase (EC 2.7.3.2), glucosephosphate isomerase (EC 5.3.1.9), isocitrate dehydrogenase (EC 1.1.1.42), malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40), peptidase-glycylleucine (EC 3.4.*.*), phosphoglucomutase (EC 2.7.5.1), and superoxide dismutase (EC 1.15.1.1).

Serological analysis

Formalin-killed whole cell antigens used for microagglutination tests were prepared for a typical sorbitol nonfermenting isolate (11.40) of Y. ruckeri, a sorbitol-fermenting isolate (11.29), and the isolate obtained from chinook salmon (11.86). Antigens were prepared by washing cells from TSA with sterile 0.85% saline (pH 7.2). Cells were sedimented at 10,000 g for 5 min in a Beckman Microfuge 11, and washed three times in saline; after the final centrifugation, the cell concentration was adjusted in 0.5% formalin-saline to a 30% transmittance in a Spectronic-20 colorimeter (Bausch and Lomb) at 525 nm. Each antigen was reacted with antisera prepared in New Zealand white rabbits against Y. ruckeri isolates 11.29 and 11.40. Normal rabbit serum was used as a control. All antisera were obtained from the Biologics Section at the National Fish Health Research Laboratory. Microagglutination assays were performed as described by Maisse and Dorson (1976). Antibody titers were expressed as the last dilution of antiserum that agglutinated the antigen.

Similar whole cell antigens from isolates 11.29, 11.40, and 11.86 were sonicated and reacted with antisera in immunodiffusion assay (Ouchterlony, 1958).

Virulence studies

The uniformity of the sorbitol reaction within isolates 11.40 and 11.86 was determined before the fish were challenged. Initially, 40 colonies per isolate were picked from TSA plates and used to inoculate the sorbitol utilization medium described by Cipriano and Pyle (1985).

Specific-pathogen-free brook trout (Salvelinus fontinalis) were obtained from the White Sulphur Springs (West Virginia) National Fish Hatchery, and specific-pathogen-free Atlantic salmon (Salmo salar) from the Cortland (New York) National Fish Hatchery. Duplicate groups of 10 brook trout (52.0 g) and Atlantic salmon (36.0 g) were challenged by a 60-sec dip exposure to 10^9 cells/ml of isolates 11.40 and 11.86. Challenges were conducted as described by Cipriano (1982). Mortality was recorded for 14 days and the presence of Y. ruckeri in dead fish was confirmed by bacteriological isolation on Shotts-Waltman agar at 25 C.

RESULTS

Results from our biochemical studies were consistent with those of other workers (Ross et al., 1966; Bullock et al., 1978; O'Leary et al., 1979) and the 11.86 isolate was identified as Y. ruckeri. Growth on Shotts-Waltman agar was typical for Y. ruckeri—white colonies (negative fermentation of sucrose) surrounded by a zone of lipolysis (hydrolysis of Tween-80).

TABLE 1.   Number of dead fish in groups of 10 fish each challenged by a 60-sec exposure to two isolates of *Yersinia ruckeri*.

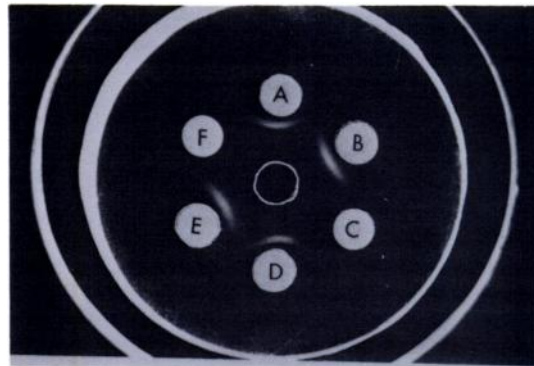|		Species and group			
		Brook trout		Atlantic salmon	
Challenge isolate	Dose (bacteria/ml)	I	II	I	II
Sorbitol +, 11.86	1.4×10^9	7	8	10	10
Sorbitol −, 11.40	3.5×10^9	9	9	7	7

FIGURE 1.   Immunodiffusion reactions between rabbit antiserum to a Serotype II sorbitol-fermenting isolate (11.29) of *Yersinia ruckeri* (center well) and sonicated antigens from *Y. ruckeri* isolates 11.29 (wells A and D), 11.86 (B and E), and 11.40 (C and F).

Individual bacteria were approximately 1.5–2.0 µm long by 0.5–1.0 µm wide. The bacteria produced an alkaline over acid reaction in TSI agar but no gas was produced at 25 C. Results of other biochemical tests were consistent with those reported by other researchers for *Y. ruckeri* (Bullock et al., 1978; O'Leary et al., 1979). No electrophoretic variation was observed at 14 enzyme loci in isolates 11.29, 11.40, and 11.86.

Both microagglutination and immunodiffusion studies indicated that isolate 11.86 was related serologically to the sorbitol-positive isolate 11.29. Antiserum to the 11.29 isolate agglutinated both the homologous antigen (11.29) and 11.86 to a 1:1,024 dilution of serum. However, 11.29 antiserum did not agglutinate the sorbitol-negative isolate 11.40. Conversely, 11.40 antiserum had a 1:4,096 agglutinin titer against homologous antigen (11.40) but did not agglutinate either the 11.29 or 11.86 antigen.

Immunodiffusion tests indicated that the 11.29 antiserum formed two precipitin bands with homologous cell sonicates and that one of these bands was present in the 11.86 antigen. No reaction was observed between 11.29 antiserum and 11.40 antigen (Fig. 1). Although 11.40 antiserum formed two precipitin bands with its homologous antigen (11.40), this antiserum did not react with either the 11.29 or 11.86 antigens.

Before we conducted virulence studies,

we determined the stability of the sorbitol characteristic in isolates 11.40 and 11.86. None of the 40 individual colonies from isolate 11.40, but all of the colonies from isolate 11.86, fermented sorbitol. Experimental challenge studies also indicated that the sorbitol-positive isolate was virulent for salmonid fishes (Table 1). The mean mortality was 90% in brook trout and 70% in Atlantic salmon that were challenged with isolate 11.40—as compared with 75% in brook trout and 100% in Atlantic salmon challenged with isolate 11.86. Bacteria that produced reactions typical of *Y. ruckeri* on Shotts-Waltman agar were isolated from the kidney of each dead fish.

DISCUSSION

Biochemical, isozyme, serological, and virulence tests indicated that the mortality in chinook salmon at the Sand Ridge hatchery was caused by a strain of *Y. ruckeri* that was biochemically and serologically similar to the Serotype II, BC-74 strain described by O'Leary et al. (1982). These findings are particularly important because, until now, field observations have suggested that Serotype II, sorbitol-posi-

tive isolates of *Y. ruckeri*, are not pathogenic for fish (Bullock et al., 1977).

In our study, all biochemical tests were consistent with those for *Y. ruckeri* (Ewing et al., 1978; O'Leary et al., 1979). However, we did find one discrepancy with published reports in the ability of isolate 11.86 to produce gas in TSI agar. Although field biologists had indicated that isolate 11.86 produced gas in TSI, initially we could not confirm this observation. We then observed that isolate 11.86 produced gas in TSI agar at 30 C but showed no evidence of gas production at 15, 20, or 25 C. Further tests with additional Serotype II isolates of *Y. ruckeri* obtained from the reference culture collection at the National Fish Health Research Laboratory also showed differential temperature dependent patterns for the production of gas from glucose (data not shown).

Since the initial epizootic in chinook salmon at the Sand Ridge hatchery, we have confirmed three additional outbreaks of enteric redmouth disease caused by sorbitol-positive strains of *Y. ruckeri*. Collectively, these data indicated that sorbitol-positive Serotype II strains of *Y. ruckeri* can be pathogenic to fish. In addition, a sorbitol-positive strain of *Y. ruckeri* was isolated recently from a single case of clinical disease in humans (Farmer et al., 1985). Our data therefore support the policies of current fish health programs (Amos, 1985) that do not discriminate between serotypic differences of *Y. ruckeri*.

LITERATURE CITED

AMOS, K. H., ed. 1985. Procedures for the Detection and Identification of Certain Fish Pathogens, 3rd Ed. American Fisheries Society Fish Health Section, Corvallis, Oregon, 114 pp.

AUSTIN, B., M. GREEN, AND C. J. RODGERS. 1982. Morphological diversity among strains of *Yersinia ruckeri*. Aquaculture 27: 73–78.

BULLOCK, G. L., H. M. STUCKEY, AND E. B. SHOTTS, JR. 1977. Early records of North American and Australian outbreaks of enteric redmouth disease. Fish Health News 6: 96–97.

————, ————, AND ————. 1978. Enteric red-mouth bacterium: Comparison of isolates from different geographic areas. J. Fish Dis. 1: 351–356.

BUSCH, R. A. 1978. Enteric red mouth disease (Hagerman strain). Mar. Fish. Rev. 40: 42–51.

————, AND A. J. LINGG. 1975. Establishment of an asymptomatic carrier state infection of enteric redmouth disease in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 2429–2432.

CIPRIANO, R. C. 1982. Furunculosis in brook trout: Infection by contact exposure. Prog. Fish-Cult. 44: 12–14.

————, AND J. B. PYLE. 1985. Development of a culture medium for determination of sorbitol utilization among strains of *Yersinia ruckeri*. Microbios Lett. 28: 79–82.

EWING, W. H., A. J. ROSS, D. J. BRENNER, AND G. R. FANNING. 1978. *Yersinia ruckeri* sp. nov., the redmouth (RM) bacterium. Int. J. Syst. Bacteriol. 28: 37–44.

FARMER, J. J., III, B. R. DAVIS, F. W. HICKMAN-BRENNER, A. MCWHORTER, G. P. HUNTLEY-CARTER, M. A. ASBURY, C. RIDDLE, H. G. WATHEN-GRADY, C. ELIAS, G. R. FANNING, A. G. STEIGERWALT, C. M. O'HARA, G. K. MORRIS, P. B. SMITH, AND D. J. BRENNER. 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. 21: 46–76.

FUHRMANN, H., K. H. BOHM, AND H.-J. SCHLOTFELDT. 1983. An outbreak of enteric redmouth disease in West Germany. J. Fish Dis. 6: 309–311.

LESEL, R., M. LESEL, F. GAVINI, AND A. VUILLAUME. 1983. Outbreak of enteric redmouth disease in rainbow trout, *Salmo gairdneri* Richardson, in France. J. Fish Dis. 6: 385–387.

LLEWELLYN, L. C. 1980. A bacterium with similarities to the redmouth bacterium and *Serratia liquefaciens* (Grimes and Hennerty) causing mortalities in hatchery reared salmonids in Australia. J. Fish Dis. 3: 29–39.

MAISSE, G., AND M. DORSON. 1976. Production d'agglutinnines anti-*Aeromonas salmonicida* par la truite arc-en-ciel. Ann. Rech. Vet. 10: 33–40.

MCCARTHY, D. H., AND K. A. JOHNSON. 1982. A serotypic survey and cross-protection test of North American field isolates of *Yersinia ruckeri*. J. Fish Dis. 5: 323–328.

O'LEARY, P. J., J. S. ROHOVEC, AND J. L. FRYER. 1979. A further characterization of *Yersinia ruckeri* (enteric redmouth bacterium). Fish Pathol. 14: 71–78.

————, ————, J. E. SANDERS, AND J. L. FRYER. 1982. Serotypes of *Yersinia ruckeri* and their immunogenic properties. Ore. State Univ. Sea

Grant Coll. Program Publ. No. ORESU-T-82-001, 15 pp.

OUCHTERLONY, O. 1958. Diffusion-in-gel methods for immunological analysis. Prog. Allergy 5: 1.

ROBERTS, M. S. 1983. A report of an epizootic in hatchery reared rainbow trout, *Salmo gairdneri* Richardson, at an English trout farm, caused by *Yersinia ruckeri*. J. Fish Dis. 6: 551–552.

ROSS, A. J., R. R. RUCKER, AND W. H. EWING. 1966. Description of a bacterium associated with red-mouth disease of rainbow trout (*Salmo gairdneri*). Can. J. Microbiol. 12: 763–770.

RUCKER, R. R. 1966. Redmouth disease of rainbow trout (*Salmo gairdneri*). Bull. Off. Int. Epizoot. 65: 825–830.

SCHILL, W. B., S. R. PHELPS, AND S. W. PYLE. 1984. Multilocus electrophoretic assessment of the ge-

netic structure and diversity of *Yersinia ruckeri*. Appl. Environ. Microbiol. 48: 975–979.

STEVENSON, R. M. W., AND D. W. AIRDRIE. 1984. Serological variation among *Yersinia ruckeri* strains. J. Fish Dis. 7: 247–254.

————, AND J. G. DALY. 1982. Biochemical and serological characteristics of Ontario isolates of *Yersinia ruckeri*. Can. J. Fish. Aquat. Sci. 39: 870–876.

WALTMAN, W. D., AND E. B. SHOTTS, JR. 1984. A medium for the isolation and differentiation of *Yersinia ruckeri*. Can. J. Fish. Aquat. Sci. 41: 804–806.

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

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