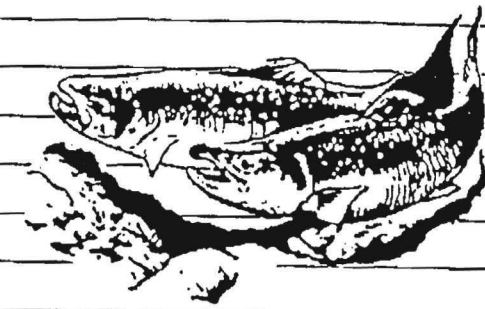
Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY



Number 68
1994

Bacteria Give False-Positive Results Using BKD Diagnostic Tests

The National Broodstock Program (NBP) was established in 1970 to provide disease-free trout eggs to the National Fish Hatchery system of the U.S. Fish and Wildlife Service (USFWS). The Erwin National Fish Hatchery (NFH) plays a key role in the NBP, supplying 15 million rainbow trout eggs from six genetic strains annually. The Erwin NFH (Erwin, Tennessee) operates under the USFWS Fish Disease Control Policy that includes annual inspections by fish health biologists. On 5 November 1993, ovarian fluid samples from adult brood rainbow trout (*Oncorhynchus mykiss*; lot #RBT-ARD-91-ENN) were collected for routine disease monitoring.

Ovarian Fluid Tested for Viruses and Bacteria

Ovarian fluid samples from 150 fish were combined into pools of five (30 total pools). Each pooled sample was evaluated for viruses by cell culture and for the presence of the bacterium *Renibacterium salmoninarum*, the cause of bacterial kidney disease (BKD), by a direct fluorescent antibody technique (DFAT) using polyvalent anti-*R. salmoninarum* sera. Observation of the DFAT slides revealed the presence of fluorescing cells, many not consistent with *R. salmoninarum* morphology. Identical results were obtained using three different sources of polyvalent antisera, each prepared using whole-cell antigens (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland; National Fish Health Research Laboratory, Leetown, West Virginia; and Micrologix International, Victoria, British Columbia).

Bacteria Yield Positive Results in Serologic Tests

Cross-reacting bacteria present in the ovarian fluid were quantitated by a membrane-filtration fluorescent antibody technique (MF-FAT) by using Kirkegaard & Perry antiserum (Table). Each of the four ovarian fluid samples were also evaluated by a second serologically based test—an enzyme-

linked immunosorbent assay (ELISA). The results of this assay, which incorporated reagents from Kirkegaard & Perry Laboratories, indicated that two of four samples (50%) were positive (Table).

To confirm these false-positive results, ovarian fluid was cultured using tryptic soy agar (TSA) incubated at 25°C. Four bacterial isolates were then tested by DFAT for cross-reactivity using all three sources of polyvalent antisera. Two of four exhibited positive fluorescence with both the Kirkegaard & Perry and the National Fish Health Research Laboratory antisera. No reactivity was observed with the Micrologix International antiserum.

Identity of Cross-reacters Confirmed

Cross-reacting bacteria were biochemically identified by using 50 standard substrate utilization and growth tests. Bacteria were identified as *Pseudomonas fluorescens* and *Pasteurella ureae*. We believe this to be the first detection of a mixed co-contamination of fish tissue by a glucose-nonfermenter and glucose-fermenter that cross-react with R. salmoninarum antisera by DFAT and ELISA. These results question the reliability of using commercially available polyvalent antisera for the detection *R. salmoninarum* and the diagnosis of BKD. Polyvalent antisera made against whole-cell preparations of *R. salmoninarum* have been responsible for several cases of false-positive DFAT results. We believe that increasing the availability of monoclonal antisera prepared against defined antigens would decrease occurrences of cross-reactivity with unrelated genera of bacteria.

These findings also illustrate the limitations of ELISA when results are questionable. Because of the lack of morphological data, as can be obtained by DFAT, the ELISA can unknowingly provide false-positive results. The use of monoclonal-based ELISA reagents is not widespread, but these reagents are available and their use should be encouraged. The ELISA, however, is best suited for screening and segregating large samples of fish (i.e., spawning Pacific salmon), but should not be depended on as a confirmatory test. Confirmation of *R. salmoninarum* infections must ultimately involve in vitro culture before management decisions are made. Followup testing at Erwin NFH will assist in determining any long-term presence of these cross-reacting bacteria at the hatchery.

For further information contact

Jeffrey D. Teska
Leetown Science Center
National Fish Health Research Laboratory
1700 Leetown Road
Kearneysville, West Virginia 25430
(304)725-8461

or

Norm P. Heil
U.S. Fish and Wildlife Service
Warm Springs RFC—Fish Health Laboratory
Route 1, Box 515
Warm Springs, Georgia 31830
(706)655-3382

Table. ELISA (threshold value = 0.286) and MF-FAT results of Erwin NFH brood rainbow trout *(Oncorhynchus mykiss)* ovarian fluid (OF) samples using polyvalent anti-*R. salmoninarum* serum.

Sample	Mean OD reading	ELISA result	Mean bacteria/mL
OF-13	0.162	Negative	1.63×10^4
OF-16	0.189	Negative	5.88×10^3
OF-17	0.320	Positive	1.40×10^5
OF-19	0.589	Positive	5.85×10^4