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Analysis of Bacterial Kidney Disease (BKD) and BKD Control Measures with Erythromycin Phosphate Among Cutthroat Trout (*clarki bouvieri*)

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Abstract

Bacterial kidney disease (BKD) has been isolated from cutthroat trout (Salmo clarki bouvieri) mortalities at the Yellowstone River Trout Hatchery since 1976. Daily mortalities were monitored for presence and extent of BKD. The testes of maturing males was often the best place to isolate the BKD organism. Erythromycin injections worked well to control the BKD pathogen in adult brood fish. Injections were required at 30-35 day intervals. Erythromycin was administered on a practical rather than experimental basis; uninjected controls were not maintained because of limited facilities and chance of reinfection. All eggs collected during spawning were water hardened in Erythromycin. Fish from eggs water hardened in Erythromycin in 1978 have developed for over three years without showing any sign of BKD, and not one BKD organism was found in any fish examined from this lot.

Bacterial kidney disease (BKD) was identified in cutthroat trout mortalities at the Yellowstone River Trout Hatchery from 1976-1980 and is believed to have been the cause of chronically high numbers of mortalities. BKD was first positively identified during a routine fish health inspection in 1976, although accounts of signs and mortalities prior to 1976 hint that the pathogen was present several years before it was positively identified (Dotson, 1977).

BKD is caused by a small gram-positive bacterium, *Renibacterium salmoninarum* (Sanders, Fryer, 1980). The disease is described as a chronic to acute inflamatory reaction usually systemic and historically related to the kidney. However, other organs of the body such as the liver and intestines have been found to contain concentrations of the bacteria which may serve as good locations from which to identify pathogen (Lobb, 1976).

At the Yellowstone River Hatchery predominant signs of the disease were hemorrhagic areas in the musculature, liver, lower gut, and testes of maturing males. Other signs included gray swollen kidneys, external fluid-filled blebs which later resulted in open lesions, small "measle-like" red speckles on body sides, and often popeye. Histological examination revealed that kidneys of infected fish showed areas of massive necrosis of hematopoietic tissue and adjacent renal tubules. Tissue of infected testes were degenerate and devoid of sperm cells with granulomas diffusely scattered throughout the tissue. (Charlie Smith, Fish Cultural Development Center, Bozeman, Mt., personal communication).

The most likely transmission of BKD at the Yellowstone Rivr Hatchery was with the egg. Egg transmission of BKD has been demonstrated by Klontz (1978) and is the basis of the BKD control program at the hatchery.

The Yellowstone River Trout Hatchery is operated by the Montana Department of Fish, Wildlife and Parks. It is a small hatchery but very valuable as it maintains the state's only source of Yellowstone cutthroat trout (*Salmo clarki bouvieri*). It was important to control BKD and eventually eliminate it without destroying the broodstock. The Erythromycin BKD control program was conducted while the hatchery was in full production. The hatchery is not an

experimental facility and because of limited space and chance of reinfection no fish could be held as experimental controls. Earlier work by Klontz (1976) with similar Erythromycin programs using uninjected controls has documented the effectiveness of Erythromycin against BKD. The BKD control program at the Yellowstone River Hatchery is a practical application of that earlier experimental work.

Materials and Methods

A two-part program to control BKD mortalities and eventually eliminate the pathogen was initiated in 1977. The program is described in depth by Hodges (1978). Briefly, the program consisted of two phases: 1) Injections of Erythromycin phosphate in the form of Erythro 200 (a commerical product available from Abbott Laboratories) at a dose of 5 mg active Erythromycin per pound of fish were given to adult fish. Injections were given subcutaneous anterior to the dorsal fin. Needles ranging from 20-gauge, 7/8-inch long to 25-gauge, 1½ inches long were selected depending on the size of the fish. Injections were given at timed intervals in order to best control BKD mortalities. 2) Water hardening eggs in a 3 mg/1 solution of Erythromycin. This part of the program was intended to eliminate egg transmission of the BKD pathogen. Erythromycin for this solution was obtained from a commerical product, Gallimycin, Poultry Formula Improved (PFI). Abbott Laboratories.

The initial identification of BKD was made at the Fish Disease Control Center, Fort Morgan, Colorado by use of the fluorescent antibody technique (FAT). Since that time a few tissue samples have been examined for BKD using the FAT. However, most of the routine checks for BKD among daily morts have been done with Gram staining.

The first BKD control injections were given to a test lot of 117 two-year-old cutthroat (lot 1-75) on March 2, 1977. Results of these injections were most encouraging (Dotson, 1977) and the remainder of lot 1-75 (1,307 fish) were injected in July 1977. Erythromycin injections were then given to the next two lots (1-76, 1-77) to control BKD mortalities. All eggs collected at the hatchery beginning in 1977 with lot 1-77 were water hardened in Erythromycin.

Results and Discussion

Brood lot 1-76 experienced a mortality peak in August of 1978 (Table 1). BKD was diagnosed in 68 percent of the August mortalities (Table 2). This dropped to 50 percent in September and mortalities were only 28. Reduced mortalities followed an Erythromycin injection which these fish received on August 4. Prior to injection the fish had lost color, were listless, and most were off feed.

The mortality peak lot 1-76 experienced in August 1978 consisted of 76 percent males. The appearance of high numbers of males in the mortality count may indicate a greater susceptibility of the male or a greater tolerance of the female to the BKD organism. However, many of the male mortalities showed signs of beginning sexual maturity in the testes. Increased metabolic activity in that organ may have created a desirable environment for the BKD pathogen. Microscopic examination of mortalities revealed far heavier concentrations of the BKD organism in the testes than in any other organ. Gram staining of various organs and tissues revealed

TABLE 1.

TOTAL MORTALITY IN BROOD LOTS 1-76 AND 1-77 AND DATES OF ERYTHROMYCIN INJECTIONS FROM MAY 1978 THROUGH FEBRUARY 1980

	LOT 1-76		LOT 1-77	
Month	Total Mortality	Injection Date	Total Mortality	Injection Date
1978				
May	9			
June	26			
July	70		1	
August	102	8-4	38	8-2
Sept.	28	9-7	72	9-6
Oct.	12		47	10-12
Nov.	9		34	11-15
Dec.	5	12-7	11	12-6
1979				
Jan.	5		5	1-10
Feb.	5	2-7	4	2-8
March	1		4	3-7
April	2		3	4-5
May	1*		8	5-8
June	3		13	6-6
July	3		17	7-3
August	3		15	8-2,31
Sept.	0		7	9-27
Oct.	1		5	10-30
Nov.	3		3	
Dec.	0		10	12-21
1980				
Jan.	3		4	1-29
Feb.	0		0	2-20

*Spawn taking was completed for lot 1-76 by May 25, 1979, and most of the fish in this lot were removed from the station. Only 100 were retained as brood stock.

that the testis was the organ of choice for the pathogen, the kidney was next, and the liver and lower gut of very highly infected fish often had high concentrations of the bacteria. No bacteria was observed in the ovaries of females. BKD was often observed in testes of male mortalities even when no BKD was isolated from the kidney of the same fish.

Brood lot 1-77 had a mortality peak in 1978 very similar to lot 1-76. The first BKD pathogens observed in this lot appeared in July 1978 (Table 1). Losses rose in August and these fish were injected on August 2. Mortality continued to increase in August and September. On September 6 the fish were refusing food, rode high in the water and many crowded the tail screen. Another injection was given on September 9. Mortality began to taper off, but continued monthly injections were necessary to control losses.

Mortalities in lot 1-77 consisted mostly of males as did mortalities in lot 1-76 in the 1978 peak season. Sixty-one percent of the mortalities were males, and in this lot also the testes were observed to contain high numbers of BKD bacteria. The bacteria was more abundant in this lot than any lot previously observed; 81 percent of the August mortalities and 71 percent of September mortalities were confirmed BKD positive.

Incubation period of the BKD organism in the hatchery's 11 degrees C (52 degrees F) water was determined to be 30-35 days. Using this time frame it was decided to inject these fish at intervals of no more than 30 days in order to prevent the anticipated increase in mortality. Monthly injections were initiated and continued through October of 1979.

Mortalities dropped to as few as three-to-four per month after injections with Erythromycin. But in May of 1979 they started to increase. The 1979 mortality peak was in July. Again sharp mortality increases were observed and just as sharply they declined from July to October. However, the most mortalities observed in any one month in 1979 was 20 in July. This is a significantly lower number than was involved during the peak summer BKD season in 1978. Though the mortality increased noticeably, the incidence of BKD did not increase. In May a total of eight mortalities were collected with a 62 percent incidence of BKD, and in July 20 mortalities were found but only 20 percent of those were found to contain BKD. From May to September mortalities were higher than normal but incidence of BKD was never higher than 27 percent. This is believed to be a direct result of Erythromycin injections limiting the numbers and spread of BKD bacteria. In November when total mortality again dropped, the incidence of BKD increased among the mortalities to 33 percent and it was up to 57 percent in December even though there were only seven mortalities.

The increase in mortality between May and September may have been related to BKD directly or indirectly. BKD bacteria are often difficult to locate and we have found that they can accumulate in various organs and go unobserved throughout the rest of the fish. Therefore, mortalities containing BKD may have been overlooked. In many of the slides examined from fish collected from June through September another pathogen was isolated which may have contributed more towards the death of these fish than BKD. Several slide identifications were made of this Gram negative bacterial rod. It was cultured and identified as a motile *Aeromonas sp.* bacteria. This bacteria was not seen after September, and as incidence of *Aeromonas* declined, percentage incidence of BKD increased among mortalities. The *Aeromonas* infection is felt to have been a secondary infection brought on by the injections and handling stress.

During the 1979 mortality peak 67 percent of the mortalities were female. This was inconsistent with past trends when the majority were males. Total mortalities were down from the 1978 season and this may reflect the male mortality which didn't show up in 1979. It appears that the injections had limited the numbers of bacteria and reduced its virulence, especially among males.

Incidence of BKD was so low in October 1979 (12 percent of mortalities) that after injecting the fish on October 30 it was decided to discontinue injections for at least 60 days. This was to allow the fish to recover from monthly handling while they were maturing for the upcoming spawning season. But

TABLE 2.

PERCENT OF MORTALITIES FOUND TO HAVE BEEN INFECTED WITH BKD AT THE YELLOWSTONE RIVER TROUT HATCHERY

Month/Year	Brood Lot 1-76	Brood Lot 1-77	Brood Lot 1-78
June 1978			No. BKD
July	44.44%		
August	68.42%	81.48%	
September	50.0%	70.97%	
October			
November			
December		50.0%	
May 1979		62.50%	
June		26.66%	
July		20.0%	
August		7.14%	
September		23.07%	
October		12.50%	
November		33.33%	
December		57.14%	

All mortalities examined were negative for BKD from 1978-1981

(% values indicate percent of total mortalities positively identified with BKD.)

Not all mortalities were examined for BKD. However, a significant cross section of mortalities were examined for BKD during the peak (high mortality) seasons. These results reflect only months for which enough fish were sampled to be of significant value.

after 50 days mortalities began to appear and microscopic examination revealed heavy concentrations of BKD bacteria among mortalities. No *Aeromonas sp.* bacteria was found. As no other pathogen or cause of death could be ascertained, this increase in mortality is felt to have been directly caused by BKD which was allowed to flourish in the absence of November Erythromycin injections. Injections to lot 1-77 continued on December 24 and BKD mortalities were again brought under control.

Brood lot 1-77 was the first lot to be water hardened on Erythromycin. However, when fish in this lot were 15 months old mortality increased and a high incidence of BKD was observed among mortalities. This first attempt to water harden eggs in Erythromycin most likely failed because the drug and dosage were not correct. On lot 1-77 a pure form of Erythromycin was used, the effectiveness of which s uncertain. The Poultry Formula Improved (PFI) used on all following lots has proven much more effective and accurate to use.

Lot 1-78 was successfully water hardened in PFI. Fish in this lot were water hardened when taken as eggs in 1978 and have developed for nearly four years without showing a single sympton of BKD. All mortalities in this lot have been examined and not one BKD pathogen has been identified. Previous lots exhibited BKD symptoms by the time they were 15 months, but this lot has remained free of the pathogen even after maturing and producing spawn in 1981. During the 1981 spawning season a random sample of 60 fish from this lot was collected and sampled at the Fish Disease Control Center, Fort Morgan. Fecal samples from these fish were examined using high speed differential centrifugation and direct fluorescent antibody procedures. This is one of the most sensitive methods of identifying the BKD pathogen. Results of these tests were negative for BKD also.

Results of the tests conducted on lot 1-78 are most encouraging. This is the first lot reared at the hatchery since the discovery of BKD in 1976 that has developed and spawned successfully without having come down with BKD. Lots succeeding lot 1-78 (lots 1-79 and 1-80) have also developed BKD-free to date. Erythromycin at the Yellowstone River Trout Hatchery has provided a practical means of controlling BKD mortalities through injections of brood fish while disease-free eggs, water hardened in Erythromycin, could be produced. Fish from those eggs have now replaced the original diseased fish and all indications to date are that BKD has been eliminated from the hatchery.

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