ELSEVIER

Aquaculture 186 (2000) 193–203

Aquaculture

www.elsevier.nl/locate/aqua-online

Measures applied to control *Renibacterium salmoninarum* infection in Atlantic salmon: a retrospective study of two sea ranches in Iceland

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Accepted 6 December 1999

Abstract

This study describes the success of broodstock culling in controlling *Renibacterium salmoninarum* infection on two sea ranches in Iceland. On both ranches, the overall percentage of positive broodfish was around 35% when the program was initiated. After a few years of broodstock culling, the prevalence figures for broodfish declined and remained below 2.0%. The progeny of the fish subjected to culling, sampled during smoltification, always tested negative for *R. salmoninarum*. As infected broodfish were detected in most years, there was a continuous risk of bacterial kidney disease (BKD) epidemics on both ranches.

Keywords: *Renibacterium salmoninarum*; Bacterial kidney disease; Atlantic salmon; Sea ranching; Broodstock culling; Erythromycin

1. Introduction

Since its first description in Scotland in 1933 (Smith, 1964) bacterial kidney disease (BKD) has been widely reported in wild and farmed salmonids (Fryer and Sanders, 1981; Evelyn, 1993). The prevalence and mortality figures vary and the disease can be

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0044-8486/00/$ - see front matter
PII: S0044-8486(99)00375-0

acute or chronic (Austin and Austin, 1993; Evelyn, 1993). *Renibacterium salmoninarum*, the cause of BKD, is a Gram-positive, fastidious, slow growing diplo-bacillus, which has no known habitat outside the host (Sanders and Fryer, 1980). The bacterium is able to survive and maintain minimal replication within phagocytic cells (Young and Chapman, 1978; Bruno, 1986; Brown et al., 1996; Gutenberger et al., 1997).

R. salmoninarum is transmitted horizontally in fresh (Mitchum and Shermann, 1981) and seawater (Murray et al., 1992). The bacterium is shed in faeces and can survive for a limited time outside the host (Balfry et al., 1996). *R. salmoninarum* can also be transmitted vertically and is the only bacterial pathogen of fish known to utilize that route of infection (Bullock et al., 1978; Evelyn et al., 1986a). The eggs get infected in the coelomic fluid (Evelyn et al., 1986b) or prior to ovulation (Bruno and Munro, 1986). The sperm appears to be of little or no importance in the egg infection process (Evelyn et al., 1986b; Brown et al., 1996).

Control measures against *R. salmoninarum* include routine sanitary precautions, good husbandry, keeping year classes apart and avoiding contact with other farms and salmonid rivers. Rarely, stamping out is employed. Chemotherapy (oral or immersion) is of little use and no vaccine is available (Elliott et al., 1989). Vertical transmission can be avoided by using broodstocks free of *R. salmoninarum*, but for infected stocks, segregation or culling can be considered (Pascho et al., 1991; Elliott et al., 1995; Maule et al., 1996). Injection of female salmon with erythromycin before spawning may interrupt or diminish vertical transmission (Bullock and Leek, 1986; Lee and Evelyn, 1989). The drug accumulates in the yolk where it can persist in therapeutical concentrations for 30–60 days post injection (Bullock and Leek, 1986; Brown et al., 1990; Lee and Evelyn, 1994). Immersion of newly fertilized eggs in iodophor or erythromycin is not effective (Groman and Klonz, 1983; Bullock and Leek, 1986).

In Iceland, BKD was diagnosed for the first time in farmed Atlantic salmon in 1968 (Helgason, 1985) and *R. salmoninarum* has since been detected sporadically in farmed, ranched, and wild populations of salmonid fish (Sigurjónsdóttir et al., 1995). Annual screening of returning wild Atlantic salmon broodfish shows low prevalence figures, fluctuating between 0.0% and 2.5% (Sigurjónsdóttir et al., 1995). A recent 3-year survey of wild Arctic charr and brown trout in Icelandic lakes shows a high prevalence of *R. salmoninarum* antigens in many populations (Jónsdóttir et al., 1998).

Early in 1985, outbreaks of BKD occurred at four different sites in SW Iceland. The disease had spread with infected Atlantic salmon parr from one sea ranch to another and to two land-based farms (Helgason, 1985, Helgason et al., 1992). This led to systematic control of BKD. A regulation was issued in the autumn of 1986, where screening for *R. salmoninarum* in wild, and ranched broodfish and a minimum of 60 fish from each farm throughout the country was required, followed by culling if infected parents were detected. Until 1993, both sexes were screened but thereafter, females only. Other prophylactic measures practiced on the Icelandic fish farms against BKD as well as other fish diseases include the use of ground water for hatching and rearing, and surface disinfection of eggs in active iodidine (100 ppm mg^{-1} l^{-1} for 10 min).

In the present paper, we report the effects of broodstock culling and erythromycin injections (on broodfish) at two sea ranches, K and V. An account of the ranches' history is given below to aid in the assessment of the results presented.

2. History of sea ranches K and V

Sea ranching started on ranch K in 1963. In January 1985, the first cases of BKD were diagnosed and inspection revealed that 15% of the broodfish had macroscopic lesions in internal organs. These were fish of year classes 1980 and 1981, used for spawning the previous autumn. They were destroyed by incineration, along with their progeny. In contrast, smolts of spawning season 1983 were released to the sea in the spring of 1985 although the examination of kidney smears with fluorescent antibody test revealed a 1% prevalence (Helgason et al., 1992). Subsequently, all rearing facilities were thoroughly disinfected. Returning fish selected for brooding in the summer of 1985 were injected intra-muscularly (i.m.) with erythromycin (Gallimycin-200, 11 mg kg^{-1}) 1 month prior to spawning. In the autumn, the prevalence of infection was 15% (isolation on SKDM and FA test) and gametes from all positive fish were destroyed. As of 1986, screening and culling were practiced according to the new regulations. Ranch K was run until 1994.

Ranch V operated between 1983 and 1992. In the spring of 1984, Atlantic salmon parr were transferred from ranch K to ranch V and released after smoltification. Later, it became clear that these fish had harbored *R. salmoninarum.* The ranched fish selected for brooding in 1985 was given two injections i.m. with Gallimycin-200 (11 mg kg^{-1} shortly after capture and 30 mg kg^{-1} 1 month before stripping) but neither screening nor culling was carried out. Screening and broodstock culling were practiced according to regulations as of the year 1986.

The procedures for selecting and keeping the broodfish were the same throughout the study period on ranch K. When the fish started to return from sea in mid-June, females returning after 2 years at sea were selected and kept in an earth canal until late September. Males returning after 1 year were selected throughout the summer and kept there as well. The water in the canal was fresh but occasionally, during high tide, some seawater entered. A mortality of about 10% was observed, mainly due to *Saprolegnia* spp. infections. During the summer, temperature fluctuated between 9°C and 12°C and was generally down to 7–8°C when the fish was moved to concrete raceways in late September. Maturing fish was kept in pathogen-free fresh water at 4–6°C, until stripped in mid-October through November. On ranch V, conditions and husbandry practices were being improved throughout the study period. In 1986 and 1987, returning fish was seined and brought to shore, but from 1988 and onwards, returning fish assembled in a trap of concrete close to the shore. Usually, females returning after 2 years and males returning after 1 year at sea were selected for brooding. Mortalities, caused by *Saprolegnia* spp. infections were highest in 1989. In 1988, atypical furunculosis caused some deaths. During 1986–1988, maturing fish was kept in fresh water at 8–10°C during the summer months, with a weekly bath treatment in seawater to control *Saprolegnia* spp. skin infections. A sea water borhole was taken into use in 1989 and the maturing fish were held at salinity of 3–5 ppm, 20 ppm in 1990 and 12 ppm in 1991. Towards autumn, the water temperature was lowered to 4°C.

On ranch K, some of the fish received erythromycin 2–3 times in 1987 and on ranch V, some of the fish got two injections 1987–1989. These fish were subjected to culling as well. Erythromycin treatment was abandoned on both farms when the percentage of

positives was as high for treated fish as untreated and the effectiveness of culling alone became evident.

3. Materials and methods

3.1. Fish

The sea ranching stocks were originally developed from native Atlantic salmon that returns from the sea during summer and spawns in early winter. The parr undergo smoltification about 18 months later, move on to sea and return 1 or 2 years later. During the study period, female broodfish were selected from the fish returning after 2 years when possible and males returning after 1 year at sea. Hence, broodfish used each year descended from fish used for spawning 3 or 4 years earlier.

On sea ranch K, a total of 4288 broodfish were examined for the presence of *R. salmoninarum* during the years 1986–1994, including 136 erythromycin-treated fish in 1987. The fish received three injections of erythromycin (20–30 mg kg^{-1} with 1-month intervals, the last one about a month before spawning. On ranch V, 6158 broodfish were screened 1986–1992. Of those, 1568 were erythromycin-treated during the years 1987–1989. In 1987, 132 fish (20%) were injected twice with erythromycin, soon after capture (11 mg kg^{-1}) and 1 month before stripping (30 mg kg^{-1}). In 1988 and 1989, 821 and 615 (72% and 35% of the selected broodfish) were injected once with erythromycin (30 mg kg^{-1}) 1 month prior to stripping.

Screening for the presence of *R. salmoninarum* was performed on 2220 smolts spawned 1985–1990 on sea ranch K and 1548 smolts on sea ranch V, spawned 1986–1989.

On sea ranch K, 197, 213, and 92 fish were tested immediately when returning from the sea during the summers of 1986, 1987, and 1988.

Effects of husbandry were studied on ranch K in 1987 by comparing screening results for a total of 659 randomly selected broodfish, kept for several weeks under different conditions. One hundred and fifty-two fish were kept in a brackish lagoon (biomass 0.05 kg m^{3-1}), 213 in an earth canal (biomass 1.0 kg m^{3-1}), 151 in concrete raceway (biomass 20.8 kg m^{3-1}) and 143 received three injections of erythromycin i.m. (20–30 mg kg^{-1}) with 1 month intervals and were kept in an identical concrete raceway (biomass 20.3 kg m^{3-1}). Fisher's Exact test was used to evaluate the results obtained.

3.2. Sampling

Kidney tissue was taken aseptically from the broodfish at the farm site, placed in sterile plastic bag, chilled and sent to the laboratory. Samples for isolation on agar were processed within 24 h while samples for ELISA were either processed upon arrival or frozen and processed later.

Smolts were sent alive to the laboratory where kidneys were sampled and prepared for inoculation on agar.

3.3. Detection methods

Incubation of kidney samples on selective medium (SKDM) (Austin et al., 1983) for 6 weeks and a direct FA test, as described in Guðmundsdóttir et al. (1991), were used for screening in 1985. The incubation time for bacterial isolation on SKDM was lengthened to 12 weeks in 1986 (Benediktsdóttir et al., 1991). Representative strains for bacterial identification were tested on blood agar for the absence of growth. The colonies were confirmed as *R. salmoninarum* by the Gram strain reaction, cell morphology, and direct fluorescent antibody test. Samples from erythromycin-treated fish were screened with the direct FA test. In 1991, a double-sandwich ELISA (Guðmundsdóttir et al., 1993) replaced isolation on SKDM.

4. Results

Broodstock culling and to a limited extent, erythromycin injections, were applied on two sea ranches as preventive measures against BKD. The effects of the strategy as it was actualized each year, can be followed by observing the results for smolts screened 2 years later (Table 1) and for the fish returning after 1 or 2 years at sea, tested as mature broodfish each autumn (Fig. 1).

4.1. Smolts

Altogether, 3768 smolts, of 6-year classes, the progeny of broodfish stripped on both ranches in 1985–1990, were screened for *R. salmoninarum* in early spring, just before release to the sea. This effort peaked in 1989 (smolts from spawning season 1987). A positive sample was never detected.

4.2. Freshly returned fish

For 3 consecutive years (1986–1988), samples from freshly returned fish were tested on ranch K in order to compare the prevalence of *R. salmoninarum* to the prevalence observed in mature broodfish in the autumn. The samples tested in 1986, from freshly returned fish of spawning season 1982 were all negative (Table 1). Fish spawned in

Table 1
Prevalence of *R. salmoninarum* in fish freshly returned from sea. Isolation on SKDM was used for screening

Spawning year	Returning year	Years at sea	No. tested	% Positive
1982	1986	2	42	0.0
1983	1986	1	155	5.2
1984	1987	2	213	2.7
1985	1988	1	92	0.0

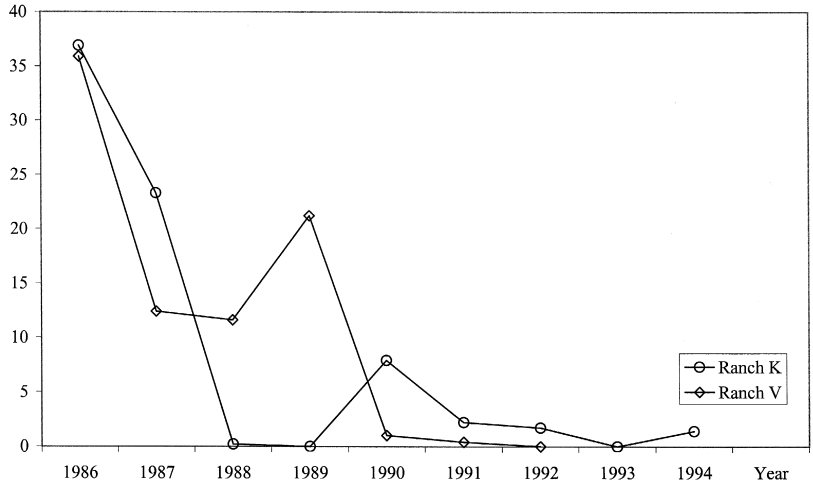

Fig. 1. Prevalence of *R. salmoninarum* in all returning broodfish 1986–1994. On ranch K and ranch V, 1988 and 1990, respectively, were the first years when all returning fish descended from culled parents.

1983 showed 5.2% prevalence in 1986 upon returning after 1 year at sea, and 2.7% were positive when returning in 1987 after 2 years at sea (Table 1). No infection was detected in freshly returned fish in 1988 (spawning season 1985).

4.3. Broodfish

The prevalence of *R. salmoninarum* in all broodfish used for stripping 1986–1994, is shown in Fig. 1.

On ranch K, the prevalence was 36.9% and 23.3% in 1986 and 1987, respectively, and 0.2% in 1988 and 0.0% in 1989. In 1990, percentage of positives rose to 7.9% but between 1991 and 1994, the prevalence was between 0.0% and 2.2%.

On ranch V, the decline of the prevalence percentage came 2 years after ranch K and was down to 1.0% in 1990 (Fig. 1). In the remaining 2 years, prevalence was 0.4% and 0.0%.

Table 2
Prevalence of *R. salmoninarum* in erythromycin treated fish, selected for brooding. An FA test was used for screening. Untreated fish is shown for comparison

Year	Ranch K				Ranch V			
	Untreated		Treated		Untreated		Treated	
	No. tested	% Positive	No. tested	% Positive	No. tested	% Positive	No. tested	% Positive
1987	517	24.9	136	14.7	529	13.0	132	9.8
1988					337	13.1	821	11.0
1989					1161	15.0	615	33.0

Table 3
Prevalence of *R. salmoninarum* in broodfish subjected to different conditions for 12 weeks. Isolation on SKDM was used for screening except for samples from erythromycin treated fish, where an FA test was employed

Habitat	Biomass kg m^{3-1}	No. tested	% Positive
Concrete raceway	20.8	151	41.7
Earth canal	1.0	213	23.0
Brackish lagoon	0.05	152	15.1
Concrete raceway[a]	20.3	143	15.1

[a]Received erythromycin i.m., 20–30 mg kg^{-1}, three times, with 1 month interval.

4.4. Erythromycin treatment

The prevalence of *R. salmoninarum* in broodfish being treated with erythromycin 1987–1989 is shown in Table 2. The prevalence observed ranged from 9.8% to 33.0%. The screening test used to monitor these fish was an FA test and in most samples, bacteria detected in the microscope were few, typically below 10 per smear. The prevalence of untreated fish is shown for comparison.

4.5. Effects of holding

The effects of different holding conditions during the maturing period, on the prevalence of infection in broodfish ready for spawning, were studied on ranch K in 1987. Fish selected for brooding in early summer were kept for a minimum of 12 weeks until sampled (Table 3). The prevalence for untreated fish in the concrete raceway (41.7%) differed significantly ($p < 0.0002$ and $p < 0.0001$) from the prevalence observed in the earth canal (23.0%) or the lagoon (15.1%). The difference between untreated (41.7%) and erythromycin-treated (15.1%) fish in the concrete raceways was similarly highly significant ($p < 0.0001$). The difference observed between fish kept in the earth canal or the lagoon was not significant ($p = 0.0823$) and neither was the difference between erythromycin-treated fish in the raceway and untreated fish kept in the earth canal ($p = 0.0806$) or the lagoon ($p = 1.000$).

5. Discussion

The present paper reports the successful management of *R. salmoninarum* infection, applying broodstock culling. Some of the broodfish were treated with erythromycin.

No positive samples were detected from thousands of smolts tested after the program was launched, demonstrating its effectiveness. The success of the program was also manifested by a rapid decline of infection prevalence in broodfish. Reports of similar programs were not found in the literature but Maule et al. (1996) demonstrated, that by

destroying eggs from heavily infected broodfish, the prevalence of *R. salmoninarum* infection could be reduced in the next generation.

On ranch K, all broodfish were treated with erythromycin in 1985, tested and culled. Before release to sea in 1987, descending smolts tested negative. All fish returning in 1988, when prevalence was 0.2%, was of that origin, as fry spawned in 1984 were destroyed. On ranch V, all broodfish selected in 1985 was likewise treated with erythromycin but neither screening nor culling was carried out. Descending smolts released in 1987 were not tested either, but returning broodfish in 1988 and 1989 showed a prevalence of 11.6% and 21.2%. Here, the picture was complicated by the fact that in 1988, progeny from infected fish spawned in 1984 were also returning. The prevalence declined to 1.0% in 1990, the first year on ranch V, when all returning fish descended from culled fish. The prevalence stayed low on both ranches with one exception (see below). These results show that culling was the key to the success of the program. However, possible effects of erythromycin treatment cannot be deduced accurately since treated fish were either culled as well, or their progeny were returning with descendants of infected fish.

Judging from our experience, sensitivity of the cultivation method is sufficient for a successful broodstock culling program. We infer that lengthening the incubation time from 6 to 12 weeks in 1986 was an important step as it enhances the detection of covert infections (Benediktsdóttir et al., 1991). The switch to a more sensitive screening method ELISA in 1991 might have altered the picture (Guðmundsdóttir et al., 1993) but that did not happen, probably because the prevalence was already down to very low figures. Changing to a faster method, however, simplified the procedure by shortening the time needed to keep gametes of different origins apart.

On ranch K, a prevalence of 7.9% was observed in 1990. There is no obvious explanation for this incident, but it is possible that sea cages with infected salmon, stationed in a bay near ranch K, provided a source of infection. It is also thinkable that infected wild fish, on its way to nearby rivers, brought the bacterium. Such an event is also a possible explanation for the initial infection on ranch K, which had operated for 22 years when BKD was first detected. Annual screening of Atlantic salmon returning to Icelandic rivers shows that they bring moderate risk. The prevalence of infection lay between 0.0% and 2.5% in 1986–1993 (Sigurjónsdóttir et al., 1995). Such information on Atlantic salmon is scarce in published literature but in a survey of the Margaree River in Canada, 37.5% of returning fish tested positive (Paterson et al., 1979).

In fish farming, many factors can induce stress and bring about overt disease in covertly infected fish, making good husbandry important. Two parameters, i.e., crowding and administration of erythromycin, were studied in a small-scale experiment on ranch K in 1987. In June–July, freshly returned fish showed 2.7% prevalence but in the autumn, a 5–15-fold increase was observed. Erythromycin treatment under crowded conditions resulted in a prevalence of 15.1%, the same figure as observed for untreated fish in the lagoon, while the prevalence was 41.7% for untreated fish under crowded conditions. Rearing density has previously been shown to affect the prevalence of *R. salmoninarum* infection (Mazur et al., 1993). The results suggest that the rise in prevalence during the summer is partly due to the activation of an undetectable infection during sexual maturation in the progeny of infected gametes and partly to horizontal

transmission under crowded conditions. Since gametes from erythromycin treated as well as untreated fish that tested positive were destroyed, this experiment did not resolve whether both groups were equally liable to transmit the bacterium to their offspring.

In conclusion, a successive broodstock culling program can be executed on a sea ranch and smolts free of *R. salmoninarum* produced from infected stocks. The practice requires extensive screening and facilities where gametes of different parentage are kept apart while broodfish samples are being tested. A continuous surveillance of the broodstock is required, since *R. salmoninarum* can be introduced into a sea ranch at any given time.

Acknowledgements

Thanks are due to the staff at both sea ranches, especially Ólafur Ásmundsson and Sesselja Guðmundsdóttir. We would also like to acknowledge the cooperation of the Directorate of Freshwater Fisheries and the Fish Disease Committee. The personnel of the Fish Disease Department at Keldur and local veterinarians are also acknowledged for their interest and cooperation.

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