RENIBACTERIUM SALMONINARUM

AN UNCOMMON "BUG", ALL TOO COMMON IN SALMONIDS

Trevor Evelyn

Emeritus Scientist

Dept. Fisheries & Oceans, Canada

Renibacterium salmoninarm (Rs): history

- Rs first described in wild Atlantic salmon in the 1930s in Scotland (Smith 1964)
- In USA, BKD first described in three species of cultured trout in Massachusetts (Belding & Merrill 1935) and California (work of J.H. Wales cited by Earp et al 1953)
- In Canada, Rs first described in 1937 in cutthroat trout in British Columbia (work of D.C.B. Duff cited by Evelyn 1986)

Renibacterium salmoninarum (RS) : geographic range

- Presently, Rs has been reported from most areas of the world where salmonids occur: the UK, western Europe (Denmark, Finland, France, Germany, Italy, Norway, Sweden, Spain, Yugoslavia, Iceland), Japan, North America, and South America (Chile).
- No reports to date of BKD from Australia, New Zealand, and eastern Europe.

The Disease: external signs

- Sometimes none, or one or more of the following:
- Pale gills (indicative of anemia)
- Exophthalmia
- Abdominal distension (due to the accumulation of ascitic fluid)
- Skin blisters with turbid or clear fluid and/or shallow ulcerations
- Hemorrhages, particularly around the vent

The Disease: internal signs

- Creamy-white granulomatous lesions in the kidney and, less frequently, the spleen and liver
- Turbid fluid in the abdominal and/or the pericardial cavities
- Swollen kidney; enlarged spleen
- Hemorrhages on the abdominal walls and viscera
- Membranous layer on one or more of the visceral organs
- More rarely, cavities in the muscle, filled with blood-tinged caseous or necrotic material

Control of Rs infections via chemotherapy during the growth & maturation of salmonids

- 1950s -- a number of sulphur drugs tested but they provided only temporary BKD control
- 1959 to 2005 -- focus has been on using antibiotics: injected antibiotic mixtures of penicillin, streptomycin & terramycin (DeCew 1972) or of erythromyin (Peterson 1982; Groman & Klontz 1983; Sakai et al 1986); or oral erythromycin (Wolf & Dunar 1959; Austin 1985; Moffit & Shreck 1988), penicillin, clindamycin, spiramycin, rifampicin or kitasamycin (Austin 1985) or azithromycin (Strom et al 2000; Fairgrieve et al 2005),. Once again, treatments provided only temporary BKD control although azithromycin offers promise of improved control.

Vaccination as a means of controlling Rs infections in Pacific salmon

- Evelyn 1971,1984: Sockeye & coho: Antibodies but no protection following vaccination by various routes with killed Rs cells or extracellular products, with and without adjuvant
- Baudin Laurencin et al 1977: Coho: Antibodies but no protection with injected killed Rs cells plus adjuvant
- Kaattari et al (1987, 1988); Yui & Kaattari (1987) : Coho: Feeble and inconsistent protection with various injected renibacterial preparations adjuvanted with highly immunogenic/mitogenic vibrio fractions

Vaccination as a means of controlling Rs infections in Pacific salmon (cont'd)

- Piganelli et al 1999: Coho: Oral vaccination with p57-depleted Rs cells yielded protection (as measured in terms of reduced levels of p57 in tissues following bath challenge)
- Rhodes et al 2005: Chinook: No protection on challenge following vaccination with a live *Arthrobacter* sp. or a killed avirulent Rs strain, with or without genetic adjuvants but a combination of the live and killed vaccines showed therapeutic value versus a pre-existing Rs infection.

Vaccination as a means of controlling Rs in Atlantic salmon (As) & rainbow trout (Rt)

- Paterson et al 1981: As: Some protection (as measured in terms of reduction in lesions) with injected killed Rs cells plus adjuvant
- Bruno & Munro 1984: As: Antibodies but no protection with injected killed Rs cells plus adjuvant
- McCarthy et al 1984: Rt: Some protection with injected pH-lysed Rs cells
- Sakai et al 1989; 1993; 1995: Rt: Some protection with injected variously killed Rs cells and pH-lysed Rs cells with or without immunostimulant

Vaccination as a means of controlling Rs in Atlantic salmon (As) & rainbow trout (Rt) (cont'd)

- Pascho et al 1997: Rt: some protection with injected killed Rs cells but not if adjuvant present
- Griffiths et al 1998: As: Reduction in Rs shedding with injected live cells of *Arthrobacter* sp. or with an attenuated Rs strain (RsTSA1)
- Daly et al 2001: As: Promising protection with injected live attenuated Rs strains, especially strain RsTSA1
- AquaHealth Ltd., Charlottetown, PEI, Canada.: Markets a lyophylized live anti-Rs vaccine (Renogen) intended for use in As

The Bacterium

- A small (0.5 x 1.0 μm), Gram-positive, non-acid-fast, PAS-positive rod. In fish tissues it occurs extra-cellularly & intra-cellularly and it appears to survive and replicate in both locations
- Non-sporing and non-motile
- Grows best but slowly at 15-18 C; not at all at 25 C. In contrast, other Gram-positive fish pathogens grow well at 30 C
- Fastidious in growth requirements (requires cysteine). Unlike other Gram-positve fish pathogens, will not grow on standard laboratory media such as tryptic soy agar or brain heart infusion agar

The Bacterium (cont'd)

- Isolates biochemically uniform in their characteristics (Austin et al 1983; Goodfellow et al 1985; Bruno & Munro 1986). Unlike Rs, other Gram-positive fish pathogens are biochemically active with a large number of substrates, including carbohydrates
- Isolates serologically homogeneous when tested with polyclonal antisera (Bullock et al 1974; Getchell et al 1985). Other Gram-positive fish pathogens do not react with anti-Rs antisera
- Various serological (FAT, ELISA) & nucleic acid-based (including PCR) tests now available for detecting, identifying, and quantifying Rs

Susceptibility of various non-salmonid fishes to experimental challenge with Rs

- <u>Intraperitoneal challenge</u>
- Pacific herring — Yes* — Traxler & Bell (1988)
- Sable fish — Yes* — Bell et al (1990)
- Pacific lamprey — No — Bell & Traxler (1986)
- Shiner perch — Yes* — Evelyn (unpubl. data)
- Carp — No — Sakai et al 1988
- <u>Gastric lavage challenge</u>
- Common shiner — Yes — Hicks et al (1986)
- Flathead minnow — Yes — Hicks et al (1986)

- *These three species appeared refractory to infection by association with Rs-infected salmon

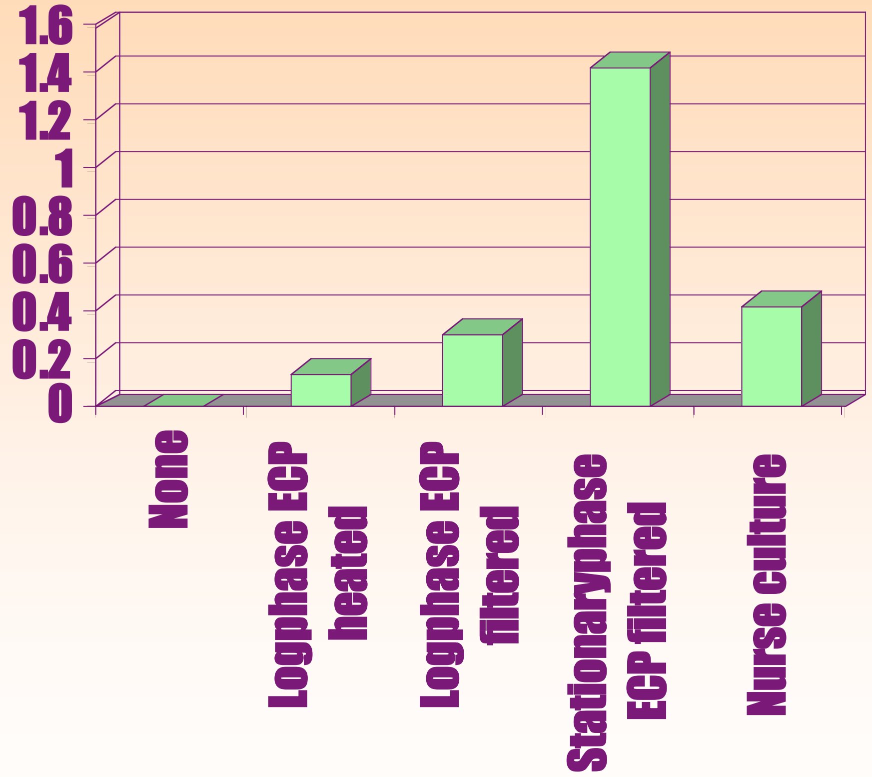
Survival of Rs in sterile & non-sterile systems

- Sterile river water 28 days Austin & Rayment 1985
- Non-sterile river water 4 days Austin & Rayment 1985

- Filter-sterile seawater 7 days Balfry et al 1996
- Non sterile seawater 1 day Balfry et al 1996

- Non sterile freshwater sediments 21 days Austin & Rayment 1985

Short-term growth (OD at 21 days) of a primary Rs isolate on variously supplemented KDM2 medium using a low inoculum (340 Rs cells/spot) Evelyn & Prosperi-Porta (1992)



Source of Rs infections

- Other infected salmonids

Transmission of Rs (other than by injection or skin abrasion)

- Horizontal: via fresh & sea water under natural conditions (Pippy 1969; Mitchum & Sherman 1981; Sakai & Kobayashi 1992) and experimentally by immersion in Rs-containing fresh and sea water, via association with infected fish, and via ingestion of infected material (Wood & Wallace 1955; Murray et al 1992; Balfry et al 1996; Mesa et al 1998; O'Farrell et al 2000)
- Vertical: via internally infected eggs (Bullock et al 1978; Evelyn et al 1984,1986; Bruno & Munro 1986)

Route of Rs entry into host fish

- Has not been established
- Rs has unique ability of inciting its uptake by cells not normally considered to be actively phagocytic (e.g., thrombocytes, oocytes, endothelial cells of the collecting ducts of the kidney)
- It seems probable that uptake occurs mainly via epithelial cells of the gill and gastrointestinal tract.
- This hypothesis is supported by the fact that Rs infections can be established by exposure to infected water and by feeding and because these epithelial cells have been shown to take up a number of other bacterial pathogens (Evelyn 1996)

Survival of Rs in salmonid serum/plasma

- Sakai et al 1989
  Rainbow trout serum:
  --- normal, unheated: 1% (in 3 hrs @ 20 C)
  --- heated 30 min@ 45 C: 25% (in 3 hrs @ 20 C)

- Evelyn, T (unpublished)
  Coho salmon plasma:
  --- normal, unheated 100% (in 5 hrs @ 15 C) ---
  immune, unheated 97% (in 5 hrs @ 15 C)

Benefits to Rs of entry into host cells

- In ova, permits vertical transmission of Rs
- In phagocytes, Rs can, under certain circumstances, survive, replicate, & eventually destroy the phagocytes
- In thrombocytes and endothelial cells, Rs cells may be protected against humoral anti-microbial substances

Renibacterium salmoninarum (Rs): virulence factors

- No acutely lethal factors exhibiting proteolytic, hemolytic, or cytolytic activity detected in Rs ECP (Bandin et al 1991; 1992)

- This may explain why Rs-infected fish can survive to spawn while carrying the large numbers of Rs cells that favor egg infections and vertical transmission

Renibacterium salmoninarum (Rs): Virulence factors (cont'd)

- p57 protein (cell associated and free) (Getchell et al 1985)
- 100-kDa protease (hydrlyzes p57 protein -- & perhaps host proteins?) (Rockey et al 1991)
- a dermatotoxic factor of uncertain function (Bandin et al 1992)
- Two cytolysins (may help account for tissue destruction and reduced hematocrits seen with BKD?) Evenden et al 1990; Grayson et al 1995)
- Iron reductase (enzyme may serve iron acquisition function in Rs) (Grayson et al 1995)
- Lysozyme resistance (permits intra-ovum survival)

Properties of the Rs p57

- Responsible for hydrophobicity, autoagglutination, & hemagglutination (Daly & Stevenson 1987; Bruno 1988)
- Accounts for ready attachment to host cells, e.g., sperm cells, leukocytes, & red blood cells (Daly & Stevenson 1989; Wiens & Kaattari 1991; Gutenberger et al 1997)
- Suppresses antibody production, respiratory burst activity, & macrophage bacteriocidal activity (Turaga et al 1987; Kaattari et al 1988; Siegel & Congleton 1997)
- Induces immunosuppression (immunotolerance?) in young fish (Brown et al 1996)
- Results in p57-antibody complexes that induce a hypersensitivity response resulting in granulomas and glomerulonephritis (Kaattari 1989; Sami et al 1992)

Possible mechanisms of p57-mediated immunosuppression

- p57- mediated agglutination of leukocytes may remove leukocytes involved in antibody formation and macrophage activation
- p57 attachment to leukocytes may facilitate their destruction by attached p57- bearing Rs cells or may render the leukocytes targets for destruction by macrophages
- On the surface of red blood cells and lymphocytes the cells may serve as phagocyte-blockading agents, compromising the anti-microbial efficiency of macrophages

Rs infection: effect on T-cell activity

- Jansen et al 2003: immersion challenge of naïve Rt with live Rs depresses lymphocyte response to a T- cell mitogen Con-A but not to a B-cell mitogen LPS
- Above findings occurred at temperature of 15 C, a temperature permissive for T-cell activity (Hardie et al 1994)

Rs infections: the effect of water temperature

- Rs causes high mortality rates at low water temperatures (given enough time). High water temperatures permitted increased survival (Sanders et al 1978)
- Experience in the Scotland with As & Rt and in the Pacific Northwest with spring Chinook salmon reinforces the above findings and indicates that temperatures of 10 C and above permit recovery from BKD or suppression of BKD epizootics (Bruno 1986, 1988; Hamel 2005)

Possible reason why low water temperatures favor BKD outbreaks

- Effective control of intracellular pathogens is normally effected by cell mediated immunity
- Killing/inhibition of pathogen by macrophages is usually involved
- Effective killing/inhibition by macrophages usually requires macrophage activation by T-cell-produced macrophage activating factors (Hardie et al 1996)
- Responsible T-cells in fish are inhibited by low water temperatures (Bly & Clem 1992; Hardie et al 1994)

Evidence that Rs cells can stimulate disease resistance & activate macrophages

- Amend & Johnson 1984: Coho vaccinated with an Rs/As bacterin had higher resistance to As challenge than those vaccinated with As bacterin alone
- Nikl et al 1991: Same results as above with coho
- Sakai et al 1993: Rt vaccinated with Rs bacterin yielded macrophages with increased phagocytic & chemiluminescence activity
- Siegel & Congleton 1997: Chinook macrophages treated *in vitro* with live or killed Rs cells showed increased killing of As

Future needs

- Need to devise an effective anti-Rs vaccine (will likely have to be one that up-regulates enhanced macrophage activity better than current ones do)

- Need to explain why Rs cells treated with antibody and complement for 16 hrs survive better in naïve Rt macrophages than those treated for only 3 hrs (Bandin et al 1995) (does the 16 hr treatment result in the production of a virulence factor that might be used in vaccines?)

Future needs (cont'd)

- Need to explain why certain attenuated forms of Rs produce protection but still produce the p57 molecule. Does full virulence require yet another molecule that should be incorporated into anti-Rs vaccines (Daly et al 2001) ?

- Unlike T-cells, macrophages are not temperature sensitive within the physiological range of salmonids. Are there new possibilities for activating them directly? (Hardie et al 1994)

Control of Rs by avoidance in seawater netpen farms

- Use seedstock derived from Rs-free parents and reared, if possible in Rs-free water
- Use only one year class per farm; if not feasible, hold different age groups as far apart as possible & athwart dominant line of tidal flow
- Locate farm well away from other farms & major salmon rivers
- Use prophylactic treatments for grow-out fish and brood fish
- Use Rs-resistant stocks as they become available
- Use sanitary precautions at all times

Control of Rs by avoidance in freshwater salmonid hatcheries

- Use eggs derived from Rs-free broodstock; if not feasible, use surface-disinfected eggs derived from broodfish that show only low-level Rs infections and that are then injected, pre-spawning, with a suitable antibiotic
- Rear progeny in Rs-free water and/or use a water supply with a water temperature of 10 C or higher
- Rear only Rs-resistant strains of salmonids unless adaptation to a local drainage is important