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Comparative susceptibility of Atlantic salmon and rainbow trout to *Yersinia ruckeri*: relationship to O antigen serotype and resistance to serum killing.

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Abstract

A study was undertaken to compare the virulence and serum killing resistance properties of Atlantic salmon and rainbow trout *Yersinia ruckeri* isolates. Five isolates, covering heat-stable O-antigen O1, O2 and O5 serotypes, were tested for virulence towards fry and juveniles of both species by experimental bath challenge. The sensitivity of 15 diverse isolates to non-immune salmon and rainbow trout serum was also examined. All five isolates caused significant mortality in salmon fry. Serotype O1 isolate 06059 caused the highest mortality in salmon (74% and 70% in fry and juveniles respectively). Isolate 06041, a typical ERM-causing serotype O1 UK rainbow trout strain, caused mortalities in both rainbow trout and salmon. None of the salmon isolates caused any mortalities in 150-250g rainbow trout, and only serotype O2 isolate 06060 caused any significant mortality (10%) in rainbow trout fry. Disease progression and severity was affected by water temperature. Mortality in salmon caused by the isolates 06059 and 05094 was much higher at 16 ^{o}C (74 and 33%, respectively) than at 12ºC (30 and 4% respectively). Virulent rainbow trout isolates were generally resistant to sera from both species, whereas salmon isolates varied in their serum sensitivity. Convalescent serum from salmon and rainbow trout that had been infected by serotype O1 isolates mediated effective classical pathway complement killing of serotype O1 and O5 isolates that were resistant to normal sera. Overall, strains recovered from infected salmon possess a wider range of phenotypic properties (relative virulence, O serotype and possession of serum-resistance factors), compared to ERM-causing rainbow trout isolates.

Introduction

The Gram negative enterobacterium *Yersinia ruckeri* is the aetiological agent of enteric redmouth (ERM) disease, a chronic to acute haemorrhagic septicaemia of salmonid fish species, in particular rainbow trout (*Oncorhynchus mykiss* Walbaum) and Atlantic salmon (*Salmo salar* L) (Horne and Barnes 1999). A single serogroup (serovar 1 or serotype O1) is responsible for most outbreaks in (rainbow) trout farmed worldwide (Stevenson and Airdrie 1984, Davies 1990; 1991a, Wheeler et al. 2009).

Wheeler et al. (2009) revealed that isolates recovered from UK salmon were genetically and serologically distinct from trout isolates, suggesting that they may have emerged or been introduced separately. Interestingly, the emergence of these novel Atlantic salmon isolates has necessitated the modification of the vaccines used to immunise salmon fry by the inclusion of bacterins prepared from these novel isolates (L. A. Laidler, Marine Harvest (Scotland) Ltd, personal communication). This further suggests that these isolates are divergent to typical rainbow trout isolates. While these isolates were reportedly recovered from fish displaying clinical signs of ERM, their virulence to Atlantic salmon has not been verified.

The complement system plays an important role in the killing and neutralisation of micro-organisms in teleost fish, as in other vertebrate phyla, with effects in teleosts mediated by activation of both classical and alternative pathways (Claire et al. 2002). Davies (1991b) demonstrated that virulent trout isolates possessed serum-resistance factors, which likely allowed them to evade complement-mediated killing in the absence of antibody. To date, no comparable studies have been performed on salmon isolates with salmon sera.

The current study aimed to compare the virulence of five isolates from salmon and trout against salmon and rainbow trout under experimental conditions. We also investigated whether salmon isolates possess serum resistance factors that might explain differences in the virulence of these organisms towards both species.

2.0Materials and methods

2.1 Bacterial strains

The *Yersinia ruckeri* isolates used in this study are described in Table 1.These included nine salmon isolates that had been recovered from Scotland, Norway and Tasmania over a 30 year period and four isolates from rainbow trout. Two further isolates were recovered from eel and whitefish. The isolates represented a wide range of biotypes, OMP types, O serotypes and pulsotypes recovered from other salmonids (Wheeler et al. 2009).

2.2 Fish stocks

Four stocks of disease-free trout and salmon were used for the challenge studies. These included larger trout (150-250g), rainbow trout fry (0.35g), salmon parr (5-10g) and salmon fry (0.4g). Larger trout were challenged in 300L tanks, whereas the rainbow trout fry, salmon fry and salmon parr were challenged in half full 30L tanks. The tanks were supplied with dechlorinated fresh water (0.2-0.4 L min^{-1} for the 30L tanks and 3-4L min^{-1} for the300L tanks), from the Weymouth potable drinking water supply (sourced from local chalk and limestone boreholes). A 12h-day length, 30min dusk/dawn was provided with day light illumination set to provide approximate 200 Lux light at the water surface. Tank water temperatures were maintained to within

0.1 ºC test temperature(16ºC and 12ºC, see below) by central computer control throughout the studies. All fish were reared from surface-disinfected eggs in the experimental facility on separate flow-through systems, under conditions of strict biosecurity. All fish were acclimated in their test tanks to test temperature for a minimum of seven days prior to treatment.

2.3 Preparation of challenge doses

For the first intraperitoneal infection experiments, isolates were recovered from cryopreserved stocks by inoculation onto Tryptone Soya Agar (TSA, Oxoid) and incubated at 22°C for 48h.

Isolated pure colonies were inoculated into Tryptone Soya Broth (TSB; Oxoid, Basingstoke, UK) and incubated for 18-24 h at 22ºC with shaking. The cultures were harvested and washed three times by centrifuging (300g for 15 min) and resuspension in phosphate-buffered saline (PBS). The final suspension was adjusted with PBS to an optical density to give the required challenge dose, based on an A_{550} of 1.0 being equivalent to approximately 1.0 x 10^9 c.f.u. ml^{-1}. Serial ten-fold dilutions (0.1 ml) of each suspension were inoculated onto TSA plates, incubated at 22^{o}C for 48h, and resultant colony forming units (c.f.u.) counted to confirm the challenge doses.

For the bath challenge experiments (see below), similar procedures were followed to produce challenge doses, except that fresh subcultures of isolates recovered from the head kidneys of i.p.-challenged fish in the first experiments were used. These *in vivo*-passaged isolates were maintained on solid media (TSA) at 4ºC and used as challenge inocula source for all four experimental bath challenges.

2.4 Challenge by intra-peritoneal (i.p.) injection

Nine groups of ten salmon parr (5-10g) were each challenged by intraperitoneal injection with each of nine different salmon isolates, (Table 1). Ten salmon were also challenged with the rainbow trout isolate 06041 (Table 1). This virulent ERM-causing UK trout isolate was used as a positive-control strain in a previous study (Davies 1991b). All fish were fasted for 24h prior to treatment, then taken from their tanks and placed in a bucket containing tricaine methane sulphonate (MS222) at strength sufficient to induce light anaesthesia within 2-3 minutes of introduction (approximately 90 mg L^{-1}). Fish were injected into the peritoneal cavity with a 0.1ml dose containing $2.3x10^7$ $\pm 1.4x10^7$ c.f.u., then returned to their original tank and observed to confirm that they had safely recovered from anaesthesia. Supply of feed was resumed 24 h post challenge. Test temperature was 16 ºC for the i.p. challenge experiments.

2.5 Challenge by bath exposure

For salmon and rainbow trout fry and salmon parr in 30 L tanks, fish were challenged *in situ*, flows were stopped and the tank volume was adjusted to 5L. One hundred ml of bacterial suspensions were then added to the tank water to give final concentrations between 1.19×10^7 – 1.3×10^8 c.f.u. ml^{-1} for the salmon parr challenge, and $1.2 – 4.8 \times 10^7$ c.f.u. ml^{-1} for both fry challenges. For challenge of 250-300g rainbow trout (bath challenge experiment 4; Table 2), rainbow trout were transferred into an aerated bucket containing 40L clean tank water. Bacterial suspensions were then added to the bucket to give final concentrations between $8.7x 10^6 – 2.3 \times 10^8$ c.f.u. ml^{-1}.

In all cases, fish were kept in the bacterial suspension for 4h with constant aeration. After 4h, flow was resumed to the tanks where fish had been challenged in the holding tanks and the larger trout were returned to their respective challenge tanks. All fish were challenged at 16 ºC, except for two groups of salmon fry that were challenged at 12ºC in parallel with two of the isolates (06059 and 05094). Experiment duration for the salmon parr and larger rainbow trout challenges (experiment 2 and 4; Table 2) was 33-35 days. The fry challenges were continued for 17 days (this was more than three days after the last mortality in any challenge tank, and approximately ten days after the main peaks of mortalities had subsided). In experiments 1 & 3, two tanks of twenty-five fish were exposed to each isolate. In experiments 2 & 4, two tanks of twelve fish were exposed to each isolate. For experiments 1, 3 and 4, the two tanks of fish challenged with each isolate were each exposed to separately prepared bacterial suspensions on different days.

All the challenge experiments were conducted in compliance with the requirements of the UK's Animals (Scientific Procedures) Act 1989, under a UK Home Office Project Licence. This included prior approval by the Cefas Weymouth Local Review of Ethical Procedures Committee.

2.6Confirmation that challenge mortalities were the result of Y. ruckeri *infection*

Kidney swabs from challenge mortalities and survivors were made onto TSA to detect *Y. ruckeri*. Isolates recovered in pure culture from a minimum of two fish per challenge tank were confirmed as *Y. ruckeri,* based on colony appearance, primary test results and Mono-Yr latex agglutination testing (Bionor Laboratories, Norway)**.**

2.7 Serum killing assays

2.7.1 Non-immune serum killing assay

Non-immune sera were obtained by caudal venipuncture from 350g trout and 400g salmon from disease-free stock. These fish had been reared from eggs in the Cefas Weymouth Laboratory experimental facility on separate flow-through systems under conditions of strict biosecurity. Sera were separated within 4h of collection, pooled by tank and species origin, filter sterilised, divided into 0.4ml samples, and stored at

-80^{o}C.

Isolates were tested for sensitivity to both pooled non-immune (naive) Rainbow Trout serum (PNRTS) and pooled non-immune (naive) Atlantic Salmon serum (PNASS), as described by Davies (1991b) with minor modifications. The test organisms were inoculated onto TSA and grown overnight at 22 ^{o}C. Resultant fresh colonies were then suspended in 10ml PBSa to an optical density of 0.4 ± 0.01 at 610nm (equivalent to approximately $5x10^{8}$ c.f.u ml^{-1}). A total of 0.1ml of this suspension was then added to 0.4ml of test serum and incubated at 22ºC. Duplicate 0.1 ml samples were serially tenfold diluted and dilutions inoculated onto TSA at 0, 1.5 and 3h. Plates were incubated for 48h at 22 ºC and resultant colonies counted. All isolates were also tested with PNRTS and PNASS that had been heated at 46ºC for 20 min to abolish complement activity (Sakai 1981). All isolates were tested in triplicate with both PNRTS and PNASS. Serum-resistant isolates were defined as those that increased in number by 3h. Serum-sensitive isolates were defined as those that decreased in number by 3h.

2.7.2 Plate assay for antibody-mediated (classical complement) killing

Three *Y. ruckeri* isolates (Table 3) were tested for resistance to antibody-mediated (classical complement) killing. The method used was that of Boesen et al. (1999) with minor modifications. Antisera used for the assays were samples of convalescent serum obtained from 5-10g salmon in experiment 2 that survived exposure to serotype O1 isolates 06041, 06059 and 07039, serotype O2 isolate 06060 and serotype O5 isolate 05094. Also included was serum collected from 250g trout that survived exposure to isolate 06041 in experiment 3. All sera were collected 5-6 weeks after exposure to the test organisms. For testing, an approximately 1 x 10^8 cfu ml^{-1} bacterial suspension of each isolate were first prepared, as described for the non-immune serum killing assay. A sterile swab was then introduced into a 1:10 dilution of the suspension in PBS. Surplus moisture was removed from the swab by rotating several times while pressing firmly against the inside wall of the tube. The swab containing the inoculum was then streaked over the surface of a TSA plate. After streaking over the entire surface the plate was rotated 60º and the plate streaked again. Following a second rotation of 60º, the agar surface was streaked for a third time. This was then left to dry for 1h, producing a semi-confluent growth of the test organism. Samples of 3µl heat-decomplemented antisera were placed as drops on the agar and allowed to bind with the bacteria at 10ºC as a source of specific antibody. After 1h, 3 µl of the species-specific pooled non-immune serum (as a source of complement) was added to the previously applied drops of antiserum. The plates were incubated for 48h and examined for zones of clearing. Samples to which 3µl of heat inactivated and normal PNRTS and PNASS were initially added, as opposed to heat-decomplemented antisera, were also included as controls.

2.8 Statistical Analysis

Data for serum-susceptibility were logarithmically transformed and the log10-change of each isolate between 0h and 3h calculated. Two-way ANOVAs were carried out to confirm that the average log-change did not differ between species but did significantly differ between the naïve and heated (negative control) serum, significant for P-values <0.05. All statistical analyses was performed using Stata 10 StataCorp 2007).

3.0 **Results**

3.1 intraperitoneal (i.p.) injection

All ten isolates tested, including the ERM-causing trout isolate 06041, caused high levels of mortality in juvenile salmon by i.p. injection, with 60% or more mortality by the time the experiment was terminated after 5 days (data not shown).

3.2 Bath challenge of salmon

Bath exposure of salmon fry and juveniles to the ERM-causing trout isolate 06041 at 16ºC caused high levels of mortality in both age groups tested (60% and 59% and respectively; Table 2). When tested at 16ºC, the salmon O1 serotype isolate 06059 was also highly virulent to salmon by this challenge route, causing 74% in fry and 63% mortality in parr (Table 2). The other salmon isolates also caused mortality in both size classes at this challenge temperature (16^{o}C). Although there was evidence of both tank-to-tank variation in mortality and apparent differences in susceptibility between fry and older fish. For instance, the serotype O2 isolate 06060 caused 42% mortality in fry, but only 4% older salmon parr that were challenged died. Similarly, 33% fry challenged with the serotype O5 isolate tested 05094 died, while only 13% the 5-10g juveniles exposed to this organism died, and these were all in only one of the two test tanks. Typical clinical signs included inappetance, darkening, exophthalmia, and haemorrhaging around the vent and fin bases. Internally, fish showed severe haemorrhaging of the internal

220 organs and ascites. Mortalities by bath exposure to salmon fry typically showed the pattern seen
221 in Figure 1 for isolates 06059 and 05094. The response for the larger fish challenged was
222 delayed, with mortalities peaking at d 9-10 post-challenge.

223 *3.3 Bath challenge of rainbow trout*

224 Both trout fry and larger 150-250g fish were confirmed as highly susceptible to the serotype O1
225 ERM-causing isolate 06041 by immersion exposure, with an average of 78% of larger fish and
226 34% fry killed (Table 2). The salmon isolates were avirulent or of low virulence towards either
227 size class of trout challenged), with fry seemingly moderately more susceptible than 150-250g
228 fish (Table 2). Although the larger fish were not killed by the other test isolates, a number of
229 individuals, particularly those exposed to isolate 06059, exhibited signs of disease two to three
230 weeks post-exposure. Signs included bilateral exophthalmia, ascites and darkening. When the
231 experiment was terminated five weeks post exposure, all fish appeared healthy.

232 *3.4 Effect of temperature*

233 Both serotype O1 isolate 06059 and serotype O5 isolate 05094 caused much greater mortality
234 in salmon when tested at 16^{o}C (other factors kept constant) compared to 12^{o}C (Figure 1). Isolate
235 06059 caused the highest mortality at both temperatures.

236

237 *3.6 Carriage of Y. ruckeri by survivors*

238 There was some evidence of persistent infection in fish of both species challenged with the
239 different isolates (Table 2). In experiment 4, *Y. ruckeri* was detected five weeks post-challenge in
240 the head kidneys of a higher proportion of larger trout bath-challenged with serum-resistant
241 serotype O1 isolates, compared to those challenged with non-serotype O1 isolates.

242 *3.5 Serum Susceptibility*

243 Five out of the 15 tested isolates grew, or at least did not decrease in viable cell concentration,
244 after 3h incubation in PNRTS (isolates 06018, 06041, 06076,06059, 06051; Table 1). The other

ten isolates were all killed by PNRTS, showing average $\log_{10}$ cell concentration reductions of between -0.12 and –3.4 after 3 h (Table 1). Biotype 1 and biotype 2 examples of rainbow trout serotype O1 isolates tested (isolates 06018, 06041 and 06076) were all resistant to PNRTS and PNASS serum killing. The Norwegian serotype O5 salmon isolate 06051, was resistant to PNRTS killing, but killed by PNASS (Table 1). The other organisms tested were all sensitive to both sera (Table 1). In heated (decomplemented) control serum from both species, all isolates showed a +0.1 to +0.82 $\log_{10}$ c.f.u ml^{-1} increase in cell number after 3h, suggesting that killing was likely complement-mediated (data not shown). Preliminary experiments were also undertaken with salmon serum at 15 ºC. These confirmed there was no difference in PNASS killing effectiveness at this lower temperature (data not shown).

3.5.2 Antibody-mediated serum killing assay

Convalescent serum recovered from trout that had survived exposure to a virulent biotype 2 serotype O1 isolate (06041) mediated effective antibody (likely classical complement) serum killing of 06041 and other PNRTS serotype O1 isolates tested (06018 and the virulent salmon isolate 06059) (Table 3).

For salmon serum, in all cases the homologous antiserum mediated effective killing of isolates that were resistant to PNASS (table 3). Four of the other five convalescent antisera used mediated killing of all the PNASS-resistant isolates (Table 3). The exception being the serotype O2 isolate 06060 antiserum, which failed to kill two typical O1 ERM-causing trout strains (06018 and 06041) (Table 3). Isolates that were killed when incubated in PNASS and PNRTS were also shown to be normal serum sensitive in this assay (data not shown). Although not directly quantified, it was observed that the zones of clearing in the lawns of normal serum sensitive isolates were larger and more intense when both normal serum and antiserum were added together then when only normal serum was added.

Discussion

Major differences between the virulence of different *Y. ruckeri* isolates to salmon and trout were shown, particularly when administered by a more natural exposure route (bath challenge), than by injection. Davies (1991b) also demonstrated host tropism in *Y. ruckeri*, with isolates reportedly virulent to chinook salmon (*Onchorhynchus tshawytscha L.*) and brook trout *Salvelinus fontinalis* (Mitchill, 1814) (Cipriano et al. 1986; Cipriano and Ruppenthal 1987), avirulent to (rainbow) trout, when tested in that species by experimental bath challenge.

The organisms that typically cause ERM in rainbow trout represent a closely-related subgroup of O1 serotype *Y. ruckeri* , the Hagerman Type strain ATCC 29473^{T} -like clonal complex (Davies 1991b; Wheeler et al. 2009). This work confirms that an example of this group (isolate 06041) also caused severe disease in salmon by experimental bath challenge.

Other isolates that affect salmon do not necessarily also cause disease in trout. Although the most virulent isolates were O1 serotype, significant mortalities were also seen in fry challenged with O2 and O5 serotype isolates, though these isolates were not highly virulent to older salmon. There is evidence that serotype O2 and serotype O5 isolates genetically very similar to those tested have been circulating in UK salmon hatcheries for more than 15 years (Wheeler et al. 2009). This is consistent with reports from hatcheries that *Y. ruckeri* outbreaks, particularly those associated with serotype O2 and O5 isolates, normally affect fry rather than older fish. The finding that two isolates were both more virulent when tested at 16ºC than 12ºC is also consistent with reports that farmers typically see *Y. ruckeri* problems in salmon hatcheries when temperatures are greater than 15ºC.

Although serotype O1 isolates are typically noted as more virulent, serotype O2 isolates have been reported as causing disease in chinook salmon (*Onchorhynchus tshawytscha* L) and brook trout (Cipriano et al. 1986; Cipriano and Ruppenthal 1987).

The experiments were not explicitly designed to study carriage of the different isolates by both species (*carriage was only determined in one organ (the head kidney) for 17-35 days post-infection (Table ;)).However it was interesting to note that, even for groups of fish*

Differences of O antigen serotype in *Y. ruckeri* relate to variations in LPS structure (Davies 1989) as is typical for other *Enterobacteriacae.* Variations in O2 and O5 serotype isolate resistance to PNASS did not correlate with virulence. In particular, salmon serotype O5 isolate

05094 was sensitive to PNASS, while a Norwegian serotype O5 isolate tested, 06051, was very resistant. Salmon serotype O2 isolate 06060, that was moderately pathogenic to both trout and salmon fry, was sensitive to PNRTS and did not grow in PNASS. These isolates may possess other factors that allow them to survive, replicate and cause disease in salmon fry. Serotype O1 isolate 06059 was highly virulent to salmon but not to trout, even though the isolate was resistant to killing by naive serum collected from both species.

Davies (1991b) studied the effect of repeated in vivo passage of eight diverse *Y. ruckeri* isolates through rainbow trout and observed no differences in their virulence before the first and after the fourth passage. Attempts to serial passage isolate 06059 through rainbow trout also did not increase the virulence towards that species (data not shown). Trout ERM-causing strains may well possess trout colonisation factor(s) that are absent in isolates such as 06059. Further work is required to determine the causes of observed variations in *Y. ruckeri* PNRTS and PNASS isolate sensitivity.

This is the first demonstration that prior exposure to the pathogen likely mediates effective classical complement killing of *Y. ruckeri* in either species. There was evidence of *in vitro* cross-protectiveness, with both salmon and trout sera prepared from fish that were exposed to isolate 06041 acting as a source of antibody that mediated effective killing of PNRTS and PNASS-resistant *Y . ruckeri* isolates. This is similar to results obtained by Boesen et al. (1999) for PNRTS resistant *V. anguillarum* O1 isolates, though that study also showed that homologous antibody did not mediate killing of PNRTS resistant *V. anguillarum* O2 isolates.

In conclusion, this study confirms hatchery reports that *Y. ruckeri* poses a significant risk to salmon, as well as to rainbow trout. Strains that affect salmon show diversity in their phenotypic properties (relative virulence, serotype and possession of serum-resistance factors), compared to ERM-causing serotype O1 rainbow trout isolates

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Tables

Table 1 Properties of *Y. ruckeri* isolates

Table 2. Summary of experiments 1-4 bath challenge results. For each experiment, groups[a] of different size rainbow trout and Atlantic salmon were exposed to ≥1.0 x 10^7 c.f.u. ml^{-1} of the different *Y. ruckeri* isolates indicated for 4 h at 16ºC.

Table 3 Results of testing two rainbow trout (isolates 06018 and 06041) and one Atlantic salmon (isolate 06059) serotype O1 normal serum resistant isolates for sensitivity to antibody-mediated (classical complement) salmon and rainbow trout serum killing. Isolates were incubated with different heat inactivated convalescent sera (antisera) as a source of antibody, followed by exposure to normal serum from the same species. S = normal serum resistant and antiserum sensitive, R = normal and antiserum resistant .

Figure captions

Figure 1. Cumulative mortality of 5-10g Atlantic salmon exposed to two *Y. ruckeri* isolates at 16 (upper yellow broken lines) and 12 ºC (lower solid blue lines). The isolates tested were serotype O1 isolate 06059 and serotype O5 isolate 05094, both originally recovered from Scottish Atlantic salmon.

Table 1 Properties of *Y. ruckeri* isolates

Isolate	Previous designation	Origin	Year Isolated	Host Species	Pulsotype[a]	O- serotype[a]	Biotype[a]	Normal serum resistance[b]	
								PNRTS	PNASS
06018	ATCC 29473^T	USA	1976	Rainbow trout	39	O1	1	+0.27(R)	+0.56(R)
06041	RD6	UK	nk[c]	Rainbow trout	31	O1	2	+0.27(R)	+0.64(R)
06076		Spain	2001	Rainbow trout	36	O1	2	+0.55(R)	+0.52 (R)
06054	RD194	Canada	nk[c]	Rainbow trout	23	O6	1	-3.4(S)	-0.55(S)
86020		UK	1986	Atlantic Salmon	28	O1	2	-0.39(S)	-1.0(S)
07030	107/02	UK	2002	Atlantic Salmon	17	O1	1	-0.68(S)	-0.68(S)
07039	FVG 269/06	UK	2006	Atlantic Salmon	19	O1	1	-0.86 (S)	-0.08 (S)
06059	RB TW60/05 XLIN (SB2)	UK	2006	Atlantic Salmon	40	O1	1	+0.1(R)	+0.15(R)
07156		Tasmania	2007	Atlantic Salmon	NT	O1	1	-0.19(S)	-2.3(S)
06060	RB TW130/05 XLIN (SB3)	UK	2006	Atlantic Salmon	2	O2	1	-0.12(S)	-1.0(S)
07029	TW92/05	UK	2005	Atlantic Salmon	8	O2	1	-0.86(S)	-0.08(S)
05094	ERM50	UK	1991	Atlantic Salmon	13	O5	1	-1.1(S)	-3.0(S)
06051	RD154	Norway	1985	Atlantic Salmon	16	O5	2	+0.23(R)	-3.7(S)
06050	RD150	Denmark	1985	European eel	12	O7	1	-0.44(S)	-0.06(S)
06043	RD20	Finland	nk[c]	Whitefish	43	O1	1	-0.67(S)	-0.43(S)

[a]Previously characterised by biotype, O antigen serotype and , except 07156 (NT= not typed), pulsed field gel electrophoresis, according to Wheeler et al. (2009).

[b]All isolates were tested, in triplicate, for resistance to PNRTS and PNASS. Values reported are the average $\log_{10}$ change in bacterial concentration (c.f.u. ml^{-1}) after 3h incubation in the respective sera. R = resistant to normal serum killing, S = sensitive to normal serum killing.

[c]nk = not known; all isolated prior to 1986.

Table 2. Summary of experiments 1-4 bath challenge results. For each experiment, groups[a] of different size rainbow trout and Atlantic salmon were exposed to ≥1.0 x 10^7 c.f.u. ml^{-1} of the different *Y. ruckeri* isolates indicated for 4 h at 16ºC. For experiments 1 & 3, two tanks of twenty five fish (n=50) were exposed to each isolate, for experiments 2 & 4, two tanks of twelve fish (n=24) were exposed to each isolate. Experiments 2 and 4 were terminated at 33-35 days post-challenge and experiments 1 & 3 seventeen days post-challenge.

Isolate[a]	O serotype	Origin[b]	Atlantic salmon				Rainbow trout			
			Experiment 1 Fry (0.4g)		Experiment 2 Parr (5-10g)		Experiment 3 (Fry 0.4g)		Experiment 4 (Adult 150-250g)	
			Mortality[c]	Infected Survivors (%)	Mortality[c]	Infected Survivors	Mortality[c]	Infected Survivors	Mortality[c]	Infected Survivors
06041	O1	RBT	60 (16;18)	100	59 (6;8)	20	34 (9;8)	12	78 (7;11)	60
06059	O1	AS	74 (16;21)	10	63 (7;8)	67	0	26	0	24
07039	O1	AS	40 (4;16)	90	17 (1;3)	60	2 (1;0)	40	0	9.1
06060	O2	AS	42 (3;18)	100	4 (1;0)	70	10 (2;3)	36	0	4.2
05094	O5	AS	33 (11; 5)	70	13 (3;0)	33	2 (1;0)	45	0	0
-ve Control group			0	0	0	0	0	0	0	0

[a]Cefas culture collection identification number; other information on the isolates (pulsotype, previous designation, biotype, year of isolation) is as indicated in Table 1.
[b]AS = Atlantic salmon; RBT = rainbow trout.
[c] Mortality expressed as total percentage killed for each isolate, with individual tank totals shown in brackets.

Table 3 Results of testing two rainbow trout (isolates 06018 and 06041) and one Atlantic salmon (isolate 06059) serotype O1 normal serum resistant isolates for sensitivity to antibody-mediated (classical complement) salmon and rainbow trout serum killing. Isolates were incubated with different heat inactivated convalescent sera (antisera) as a source of antibody, followed by exposure to normal serum from the same species. S = normal serum resistant and antiserum sensitive, R = normal and antiserum resistant .

[a]Isolate	[b]Antiserum					
	α-06041 (O1)	α-06059 (O1)	α-07039 (O1)	α-05094 (O5)	α-06060 (O2)	RTα-06041 (O1)
06018	S	S	S	S	R	S
06041	S	S	S	S	R	S
06059	S	S	S	S	S	S

[a]Cefas culture collection identification number; information on the isolates (pulsotype, previous designation, biotype, year of isolation) is as indicated in Table 1.

[b] Antisera recovered from Atlantic salmon and rainbow trout previously bath challenged with different *Y. ruckeri* isolates as indicated (the three serotype O1 isolates tested and a serotype O2 isolate (06060) and a serotype O5 isolate (05094). With the exception of RTα-06041, recovered from surviving rainbow trout, all antisera were from surviving Atlantic salmon.

Figure 1