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

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

3
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23 Key words: tropism;

24

25 **Abstract**

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

45

46 Introduction

47 The Gram negative enterobacterium *Yersinia ruckeri* is the aetiological agent of enteric
 48 redmouth (ERM) disease, a chronic to acute haemorrhagic septicaemia of salmonid fish
 49 species, in particular rainbow trout (*Oncorhynchus mykiss* Walbaum) and Atlantic salmon
 50 (*Salmo salar* L) (Horne and Barnes 1999). A single serogroup (serovar 1 or serotype O1) is
 51 responsible for most outbreaks in (rainbow) trout farmed worldwide (Stevenson and Airdrie
 52 1984, Davies 1990; 1991a, Wheeler et al. 2009).

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

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

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

71

72 **2.0 Materials and methods**

73 *2.1 Bacterial strains*

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

79 *2.2 Fish stocks*

80 Four stocks of disease-free trout and salmon were used for the challenge studies. These included
81 larger trout (150-250g), rainbow trout fry (0.35g), salmon parr (5-10g) and salmon fry (0.4g).
82 Larger trout were challenged in 300L tanks, whereas the rainbow trout fry, salmon fry and
83 salmon parr were challenged in half full 30L tanks. The tanks were supplied with dechlorinated
84 fresh water (0.2-0.4 L min⁻¹ for the 30L tanks and 3-4L min⁻¹ for the 300L tanks), from the
85 Weymouth potable drinking water supply (sourced from local chalk and limestone boreholes). A
86 12h-day length, 30min dusk/dawn was provided with day light illumination set to provide
87 approximate 200 Lux light at the water surface. Tank water temperatures were maintained to
88 within
89 0.1 °C test temperature (16°C and 12°C, see below) by central computer control throughout the
90 studies. All fish were reared from surface-disinfected eggs in the experimental facility on
91 separate flow-through systems, under conditions of strict biosecurity. All fish were acclimated in
92 their test tanks to test temperature for a minimum of seven days prior to treatment.

93 *2.3 Preparation of challenge doses*

94 For the first intraperitoneal infection experiments, isolates were recovered from cryopreserved
95 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 22°C 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 1.0×10^9 c.f.u. ml⁻¹. Serial ten-fold dilutions (0.1 ml) of each suspension were inoculated onto TSA plates, incubated at 22°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 4°C 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 $2.3 \times 10^7 \pm 1.4 \times 10^7$ 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 °C 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 8.7×10^6 – 2.3×10^8 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 °C, except for two groups of salmon fry that were challenged at 12°C 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.

146 2.6 Confirmation that challenge mortalities were the result of *Y. ruckeri* infection

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

151 2.7 Serum killing assays

152 2.7.1 Non-immune serum killing assay

153 Non-immune sera were obtained by caudal venipuncture from 350g trout and 400g salmon from
 154 disease-free stock. These fish had been reared from eggs in the Cefas Weymouth Laboratory
 155 experimental facility on separate flow-through systems under conditions of strict biosecurity.
 156 Sera were separated within 4h of collection, pooled by tank and species origin, filter sterilised,
 157 divided into 0.4ml samples, and stored at
 158 -80°C.

159 Isolates were tested for sensitivity to both pooled non-immune (naive) Rainbow Trout serum
 160 (PNRTS) and pooled non-immune (naive) Atlantic Salmon serum (PNASS), as described by
 161 Davies (1991b) with minor modifications. The test organisms were inoculated onto TSA and
 162 grown overnight at 22 °C. Resultant fresh colonies were then suspended in 10ml PBSa to an
 163 optical density of 0.4 ± 0.01 at 610nm (equivalent to approximately 5×10^8 c.f.u ml⁻¹). A total of
 164 0.1ml of this suspension was then added to 0.4ml of test serum and incubated at 22°C. Duplicate
 165 0.1 ml samples were serially tenfold diluted and dilutions inoculated onto TSA at 0, 1.5 and 3h.
 166 Plates were incubated for 48h at 22 °C and resultant colonies counted. All isolates were also
 167 tested with PNRTS and PNASS that had been heated at 46°C for 20 min to abolish complement
 168 activity (Sakai 1981). All isolates were tested in triplicate with both PNRTS and PNASS.
 169 Serum-resistant isolates were defined as those that increased in number by 3h. Serum-sensitive
 170 isolates were defined as those that decreased in number by 3h.

171 2.7.2 Plate assay for antibody-mediated (classical complement) killing

172 Three *Y. ruckeri* isolates (Table 3) were tested for resistance to antibody-mediated (classical
 173 complement) killing. The method used was that of Boesen et al. (1999) with minor
 174 modifications. Antisera used for the assays were samples of convalescent serum obtained from 5-
 175 10g salmon in experiment 2 that survived exposure to serotype O1 isolates 06041, 06059 and
 176 07039, serotype O2 isolate 06060 and serotype O5 isolate 05094. Also included was serum
 177 collected from 250g trout that survived exposure to isolate 06041 in experiment 3. All sera were
 178 collected 5-6 weeks after exposure to the test organisms. For testing, an approximately 1×10^8
 179 cfu ml⁻¹ bacterial suspension of each isolate were first prepared, as described for the non-immune
 180 serum killing assay. A sterile swab was then introduced into a 1:10 dilution of the suspension in
 181 PBS. Surplus moisture was removed from the swab by rotating several times while pressing
 182 firmly against the inside wall of the tube. The swab containing the inoculum was then streaked
 183 over the surface of a TSA plate. After streaking over the entire surface the plate was rotated 60°
 184 and the plate streaked again. Following a second rotation of 60°, the agar surface was streaked
 185 for a third time. This was then left to dry for 1h, producing a semi-confluent growth of the test
 186 organism. Samples of 3µl heat-decomplemented antisera were placed as drops on the agar and
 187 allowed to bind with the bacteria at 10°C as a source of specific antibody. After 1h, 3 µl of the
 188 species-specific pooled non-immune serum (as a source of complement) was added to the
 189 previously applied drops of antiserum. The plates were incubated for 48h and examined for
 190 zones of clearing. Samples to which 3µl of heat inactivated and normal PNRTS and PNASS
 191 were initially added, as opposed to heat-decomplemented antisera, were also included as
 192 controls.

193

194

195

196 2.8 Statistical Analysis

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

202 3.0 Results

203 3.1 intraperitoneal (i.p.) injection

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

207 3.2 Bath challenge of salmon

208 Bath exposure of salmon fry and juveniles to the ERM-causing trout isolate 06041 at 16°C
 209 caused high levels of mortality in both age groups tested (60% and 59% and respectively; Table
 210 2). When tested at 16°C, the salmon O1 serotype isolate 06059 was also highly virulent to
 211 salmon by this challenge route, causing 74% in fry and 63% mortality in parr (Table 2). The
 212 other salmon isolates also caused mortality in both size classes at this challenge temperature
 213 (16°C). Although there was evidence of both tank-to-tank variation in mortality and apparent
 214 differences in susceptibility between fry and older fish. For instance, the serotype O2 isolate
 215 06060 caused 42% mortality in fry, but only 4% older salmon parr that were challenged died.
 216 Similarly, 33% fry challenged with the serotype O5 isolate tested 05094 died, while only 13%
 217 the 5-10g juveniles exposed to this organism died, and these were all in only one of the two test
 218 tanks. Typical clinical signs included inappetance, darkening, exophthalmia, and haemorrhaging
 219 around the vent and fin bases. Internally, fish showed severe haemorrhaging of the internal

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

3.3 Bath challenge of rainbow trout

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

3.4 Effect of temperature

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

3.6 Carriage of *Y. ruckeri* by survivors

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

3.5 Serum Susceptibility

Five out of the 15 tested isolates grew, or at least did not decrease in viable cell concentration, 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 °C. These confirmed there was no difference in PNASS killing effectiveness at this lower temperature (data not shown).

255 3.5.2 Antibody-mediated serum killing assay

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

260 For salmon serum, in all cases the homologous antiserum mediated effective killing of isolates
 261 that were resistant to PNASS (table 3). Four of the other five convalescent antisera used
 262 mediated killing of all the PNASS-resistant isolates (Table 3). The exception being the serotype
 263 O2 isolate 06060 antiserum, which failed to kill two typical O1 ERM-causing trout strains
 264 (06018 and 06041) (Table 3). Isolates that were killed when incubated in PNASS and PNRTS
 265 were also shown to be normal serum sensitive in this assay (data not shown). Although not
 266 directly quantified, it was observed that the zones of clearing in the lawns of normal serum
 267 sensitive isolates were larger and more intense when both normal serum and antiserum were
 268 added together then when only normal serum was added.

269

270 Discussion

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

277 The organisms that typically cause ERM in rainbow trout represent a closely-related subgroup of
 278 O1 serotype *Y. ruckeri*, the Hagerman Type strain ATCC 29473^T-like clonal complex (Davies
 279 1991b; Wheeler et al. 2009). This work confirms that an example of this group (isolate 06041)
 280 also caused severe disease in salmon by experimental bath challenge.

281 Other isolates that affect salmon do not necessarily also cause disease in trout. Although the
 282 most virulent isolates were O1 serotype, significant mortalities were also seen in fry challenged
 283 with O2 and O5 serotype isolates, though these isolates were not highly virulent to older salmon.
 284 There is evidence that serotype O2 and serotype O5 isolates genetically very similar to those
 285 tested have been circulating in UK salmon hatcheries for more than 15 years (Wheeler et al.
 286 2009). This is consistent with reports from hatcheries that *Y. ruckeri* outbreaks, particularly those
 287 associated with serotype O2 and O5 isolates, normally affect fry rather than older fish. The
 288 finding that two isolates were both more virulent when tested at 16°C than 12°C is also consistent
 289 with reports that farmers typically see *Y. ruckeri* problems in salmon hatcheries when
 290 temperatures are greater than 15°C.

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

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

297 Differences of O antigen serotype in *Y. ruckeri* relate to variations in LPS structure (Davies
 298 1989) as is typical for other *Enterobacteriaceae*. Variations in O2 and O5 serotype isolate
 299 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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372
373

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 $\geq 1.0 \times 10^7$ c.f.u. ml⁻¹ of the different *Y. ruckeri* isolates indicated for 4 h at 16°C.

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 °C (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)

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

^bAll isolates were tested, in triplicate, for resistance to PNRTS and PNASS. Values reported are the average log₁₀ 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.

^cnk = 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 $\geq 1.0 \times 10^7$ c.f.u. ml⁻¹ of the different *Y. ruckeri* isolates indicated for 4 h at 16°C. 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

^aCefas culture collection identification number; other information on the isolates (pulsotype, previous designation, biotype, year of isolation) is as indicated in Table 1.

^bAS = Atlantic salmon; RBT = rainbow trout.

^cMortality 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)	RT α -06041 (O1)
06018	S	S	S	S	R	S
06041	S	S	S	S	R	S
06059	S	S	S	S	S	S

^aCefas culture

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 RT α -06041, recovered from surviving rainbow trout, all antisera were from surviving Atlantic salmon.

collection

Figure 1