Transfer of serum and cells from *Yersinia ruckeri* vaccinated doubled-haploid hot creek rainbow trout into outcross F1 progeny elucidates mechanisms of vaccine-induced protection

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**Abstract**

*Yersinia ruckeri* is a well-established bacterial pathogen for many salmonid species, against which a formalin-killed bacterin vaccine has been effective in reducing disease outbreaks. Previous studies have reported conflicting results about the protective value of the systemic humoral response to *Y. ruckeri* vaccination. Here we directly demonstrate that plasma contains the long-term protective component elicited by both immersion and intraperitoneal injection vaccination of rainbow trout. A total of 0.5 μL of plasma from vaccinated fish provided almost complete protection against experimental challenge. Conversely, the cells obtained from peripheral blood conferred little or no protection in naïve recipients. The protective component of immune sera was IgM based on size exclusion chromatography and recognition by monoclonal antibody Warr 1–14. Immune plasma generated against a *Y. ruckeri* biotype 1 strain protected equally against challenges with *Y. ruckeri* biotype 1 and 2 strains. These results illustrate the importance of the humoral IgM response against *Y. ruckeri* and the use of doubled haploid rainbow trout (*Oncorhynchus mykiss*) and transfer of plasma/serum and cells into F1 outcross progeny as a model system for dissection of the mechanism(s) of vaccine-induced protection.

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1. Introduction

*Yersinia ruckeri* was first described and isolated from rainbow trout (*Oncorhynchus mykiss*) (Rucker, 1966; Ross et al., 1966), and is the etiological agent of enteric red mouth disease (ERM). Prior to extensive vaccination and antibiotic use, which have been very effective at controlling ERM outbreaks, losses ranged from 30 to 70% (Busch, 1978; Horne and Barnes, 1999). The most virulent forms of *Y. ruckeri* have been ascribed to serovar O1 (Gravningen et al., 1998), for which a formalin-killed bacterin vaccine was developed. More recently, several outbreaks have occurred in trout that have been immunization vaccinated (Austin et al., 2003; Fouz and Amaro, 2006; Arias et al., 2007). Outbreaks in immunization vaccinated fish have been associated with alternative serovar O1 strains. These strains have been classified as biotype 2 (BT2) and are lacking flagella motility and secreted lipase activity (Evenhuis et al., 2009; Welch et al., 2011). It has been postulated that these strains have reduced sensitivity to the immersion vaccine (Austin et al., 2003). Thus, in order to better understand potential vaccine failures, it is first necessary to characterize the mechanisms of protective immunity elicited by *Y. ruckeri* BT1 vaccination.

Several studies using rainbow trout have attempted, using passive immunization, to determine whether protective serum factors are elicited by *Y. ruckeri* vaccination, but have reported conflicting results. Olesen (1991) demonstrated that humoral factors may play a significant role in protective immunity in rainbow trout by passively immunizing naïve fish with serum from immersion-vaccinated rainbow trout. Raida et al. (2011) suggested that circulating IgM may be the protective component against *Y. ruckeri* and demonstrated increases in circulating IgM titers, reduced bacteremia and a significantly increased bactericidal effect of plasma from vaccinated rainbow trout. In contrast, Raida and Buchmann (2008a,b) earlier reported that they were unable to confer protection by passive immunization of naïve recipients, after either injection or immersion vaccination. In addition, Cipriano and Ruppenthal (1987) demonstrated a strong antibody response to *Y. ruckeri* vaccination but was unable to confer passive protection.
to naïve brook trout or demonstrate cross-serovar protection. Additionally, passively transferred maternal immunity against yer- siniosis did not occur in Atlantic salmon fry (Lillehaug et al., 1996). Thus, mechanism(s) of protective immunity against *Y. ruckeri* induced by vaccination are still poorly understood.

Previous research has shown that genes associated with both the humoral and cellular components of the adaptive immune sys-

2. Materials and methods

2.1. Fish and fish culture

A doubled haploid line of rainbow trout derived from a single fish obtained from a domesticated strain in Hot Creek, California (HC) (Young et al., 1996) provided donors and recipients for the initial adoptive transfer and passive immunization study. These fish are considered analogous to inbred lines of mice and would reduce complica-

2.2. Bacteria, vaccination and challenge

*Y. ruckeri* strains CSF007-82, a serovar 1 BT 1 strain, and YRNC10, a serovar 1 BT2 strain (Welch and Wiens, 2005; Evenhuis et al., 2009), were used as challenge strains. Bacteria were grown for 72 h at 28 °C in tryptic soy broth, and viable cell numbers were quantified using direct plate counts.

Intraperitoneal (IP) injection and immersion vaccinations were performed using a formalin-killed bacterin derived from the CSF007-82 strain. Intraperitoneal injections used 100 μL of undiluted bacterin and immersion vaccinations were done with a 1:10 dilution of the bacterin in spring water for ~2 min with vig-orous aeration. The first study immunized fish with a 2 min immersion vaccination followed by 100 μL IP injection 2 weeks later. Blood and plasma were collected 30 days after the primary immersion vaccination. Subsequent vaccinations consisted of a single immersion or IP injection with no boost to determine varia-

2.3. Peripheral blood leukocyte and sera collection

Peripheral blood and plasma/sera were collected by caudal vein puncture using 20 Gx1″ vacuette (Greiner Bio-One) and heparin-

ized vacutainers (Becton Dickinson). Peripheral blood leukocytes (PBLs) were isolated by centrifugation (300 × G for 20 min) on 1.077 Histopaque (Sigma–Aldrich). Plasma was taken from unvac-

inated and vaccinated fish by centrifugation of peripheral blood at 300 × G for 10 min and pooling. Serum was generated by collecting blood every 2 months after the initial vaccination protocol; the col-

lected blood was allowed to coagulate overnight at 4 °C and stored at −20 °C until needed.

In some cases the IgM surface-positive cells were separated from the PBLs by magnetic bead separation (Quadro MACS, Miltenyi Biotec) using the Warr 1.14 antibody (U.S. Veterinary Immune Re-
agent Network), generated to the heavy chain of the rainbow trout IgM protein, and the manufacturer’s protocol. Cell numbers were determined by using a hemocytometer and adjusted using 1 × phosphate buffered saline (PBS) pH 7.2. IgM negative and IgM positive populations were confirmed by PCR using primers (IgM F 5′-caacggctggaagctacat-3′ and IgM R 5′-agacggtgtcgcagat att-3′) directed to the IgM heavy chain. This primer set does not distinguish between the secreted and membrane-bound IgM
forms. The PCR was performed using an initial 94 °C denaturation step lasting 10 min followed by 30 cycles at 94 °C for 1 min, annealing at 55 °C for 1 min and elongation at 72 °C for 1 min. Products were analyzed by tris–acetate-EDTA (TAE) + 1% (w/v) agarose gel electrophoresis. Gels were precast with 1 x SYBR Safe DNA gel stain (Invitrogen) and visualization of PCR products was accomplished by ultraviolet transillumination.

2.4. Adoptive transfer and passive immunization

Between 1 x 10^6 and 1 x 10^7 cells per recipient fish were transferred by IP injection. Passive immunization recipients were given up to 200 μL of plasma/serum by IP injection. Transfers of immune cells or sera were performed 2 days prior to Y. ruckeri challenges.

2.5. Agglutination assay and ELISA

Approximately 1 x 10^8 CFU/mL of formalin-killed CSF007-82 Y. ruckeri were mixed with 1:2 serial diluted plasma from vaccinated fish in 96-well round bottom plates, for a total volume of 200 μL. Formalin was removed from the killed bacteria prior to addition to the plates by washing 3 x with 1 x PBS at 300 x g at 4 °C. Plates were parafilm sealed and mixtures were incubated overnight at 15 °C.

2.6. CellTrace staining

CellTrace™ (Invitrogen) CFSE cell staining was performed using manufacturer’s protocols. Vaccinated PBLs were taken from HC or outbred donors and IP injected into F1 (NCCCW A X HC) recipients. Two days later, 100 μL of the CSF007-82 bacteria or PBS was IP injected into the recipients. PBLs from 3 recipients were isolated on days 1, 5, and 8 post-bacterium injection, pooled and ~1 million cells were analyzed by fluorescence activated cell scanning (FACS) for changes in CFSE positive cell numbers on a FACs Caliber (Becton Dickinson) machine.

2.7. Size exclusion gel filtration and ELISA of vaccinated serum

Approximately 400 μL of hyper-immune sera was loaded into a 50 cm x 1 cm column filled with pre-swollen CL-6B Sepharose (Sigma–Aldrich). A Biologic LP system was set to flow 0.5 mL/min and readings were taken on an ELISA plate reader at a wavelength of 450 nm. Y. ruckeri specific antibody ELISAs were performed in a similar manner, with the exception being the 96-well microtiter plates were initially coated with 100 μg of total CSF007–82 or YRNC10 lysates (overnight cultures were freeze-thawed ~10 times) and the primary antibody was the sera in question, followed by Warr’s 1.14 secondary and the goat anti-mouse HRP conjugated antibody.

Passive immunization was performed by pooling 10 consecutive fractions. For example, fractions 1–10 were pooled and used for passively immunizing one group of recipients. Thirteen different pooled samples were used in this manner. Recipients were challenged 2 days later as previously described.

2.8. Statistical analysis

GraphPad version 5 software was used to analyze survival by Kaplan–Meier analysis, and statistical differences were determined using both the log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests. A P-value of <0.05 was deemed significant.

3. Results

3.1. Adoptive transfer of IgM negative PBLs and passive immunization of boosted serum

To compare the protective abilities of the IgM surface-negative (IgM−) PBL populations of vaccinated and unvaccinated donors to their corresponding plasma fractions, we MACS-separated surface IgM−PBL away from the total PBL population to eliminate the production of new Y. ruckeri-specific antibodies from the donor population. Confirmation of the IgM negative population was performed by PCR, and no IgM mRNA transcript was present (Supplementary Data, Fig. 1). Recipients (HC) (n = 8), weighing approximately 150 g by PCR, and no IgM mRNA transcript was present (Supplementary Data, Fig. 1). All fish given the plasma from unvaccinated fish were dead by day 7 post challenge but significant protection was conferred in the passively immunized recipients (P value < 0.001). There was no statistical difference in survival (P value = 0.14) between the vaccinated fish (100%) and the fish passively immunized with vaccinated plasma (75%).

A 1:2 serial dilution of the plasma from vaccinated fish was used to determine the agglutination titer. The plasma showed agglutination out to ~1:64 dilution but lost observable agglutinating ability by 1:128 dilution (Supplementary Data, Fig. 2). This is in contrast to no agglutinating seen with the plasma obtained from unvaccinated fish.

3.2. Dilution of plasma and protection against Y. ruckeri biotype 1 & 2

When the serial-diluted plasma was passively transferred to F1 (NCCCW A X HC) naïve recipients (n = 30), approximately 2 g in size, nearly complete protection was seen with 0.5 μL of plasma obtained from vaccinated fish, regardless of the Y. ruckeri biotype used for the challenge (Fig. 2). Protection decreased when only 0.25 μL of plasma were passively transferred, with 66% survival against BT1 and 80% survival against BT2. The protective ability of the plasma was further decreased when the volume transferred was reduced to 0.125 μL, with 48% survival against BT1 and 57% against BT2. At the lowest plasma dilution, 0.125 μL of plasma, 48% survival was still achieved against a BT1 challenge, as compared to 29% survival in recipients that received PBS (P value = 0.03). No difference in survivability was observed in recipients transferred the lowest dilution of plasma, 0.125 μL, or...
PBS and challenged with the BT2 Y. ruckeri, 57% versus 54% respectively (P value = 0.79) (Fig. 2). An ELISA assay demonstrated equivalent recognition of both BT1 and BT2 whole cell lysates. (Supplementary Data, Fig. 3) suggesting specific IgM binding is equivalent to both biotypes in vitro but in vivo the plasma appeared to provide different levels of protection depending on the biotype the fish were exposed to.

3.3. Protection of serum from vaccinated fish over time

We next addressed whether long term protection is elicited following vaccination. Approximately every 2 months post vaccination, serum was collected from vaccinated fish and stored at −20°C. After the 8 month collection, 0.5 µL serum from each collection was used to passively immunize naïve recipients (NCCCWA X HC approximately 4 gr in size) (n = 45). The two challenged control groups had no survivors by 21 d post challenge (Fig. 3), but fish that received serum from 4, 6, and 8 months post vaccination exhibited survival rates of 75%, 56%, and 30%, respectively. A hyper-immune serum positive control group had 88% survival. These data demonstrate that antibodies are induced and maintained for a long duration but that the magnitude of the protective response diminishes over time.

3.4. Immersion vaccination versus injection vaccination

To determine if there is a variation in protection due to the route of vaccination, two single step vaccinations protocols were compared: immersion (Fig 4A) and IP injection (Fig 4B). Naïve F1 recipients (8 g in size) were passively immunized with plasma or adoptively transferred cells from donor HC fish vaccinated by either immersion or injection Y.ruckeri bacterin, to evaluate the differences that may occur due to route of vaccination. Total PBL populations from donor fish were MACS sorted, based on the presence of surface IgM, into IgM+ or IgM− cells and approximately 1 × 10^7 cells were transferred to each recipient fish. A group of recipients received unsorted PBLs from either the immersion or injected vaccinated donors. The number of recipients was variable depending...
on the number of cells recovered for each group. Separation of IgM− cells was confirmed by PCR and the lack of IgM mRNA message (Supplementary Data, Fig. 1).

The recipients that received the plasma had the highest survival rate regardless of the vaccination protocol with 93% for the immersion-plasma recipients and 98% for the injection-plasma recipients surviving relative to the controls (Fig. 4). The immersion-plasma and injection-plasma recipients groups both had significantly higher survival (P value of < 0.0001) than fish that received the total PBL population for the immersion-vaccinated fish and the surface IgM+ leukocytes for the injection-vaccinated fish. Recipients transferred total PBL, from immersion-vaccinated donors, were significantly better survivors than the control unvaccinated challenged fish (P value of 0.019), with 42% survival compared to 23% survival respectively. The surface IgM+ cell recipients from the injection-vaccinated donors did not reach significance compared to the unvaccinated challenged group (P value = 0.07). These data suggest that the major protective component resides in the plasma and not the cells regardless of vaccination protocol used. To determine if the donor cells were being eliminated in the recipient fish, total PBLs from outbred or immersion-vaccinated HC donors were CSFE stained prior to adoptive transfer to recipient F1 (HC X NCCWA) fish. Two days after the adoptive transfer of CSFE-stained PBLs, the recipients were boosted with either 100 μL of PBS or Y. ruckeri vaccine. Less than 2.5% of the of the total PBL population from recipients given outbred donor cells were present in all the groups tested (Supplementary Data, Fig. 4). The HC donor cells were present in the greatest numbers 5 d post boost with 8% present in the PBS group and 20% present in the Y. ruckeri vaccine-challenged group. By 8 d post boost the HC donor cells were almost completely gone from the circulating PBLs in recipients boosted with PBS, but in the vaccine-challenged recipients, 12% of the circulating PBLs were CSFE positive. In the scatter plots for all the groups tested, step-wise reduction of CSFE stain intensity was observed indicating cell division was not occurring and that the cells were not dividing in response to the post transfer vaccine injection.

3.5. Size exclusion separation of Hyper-immune sera

Separation of hyper-immune sera by a size-exclusion chromatography was used to identify the protective fraction. Total IgM separated into two distinct peaks with the first peak between fractions 15 and 40 and the second between fractions 60 and 90 (Fig. 5). The dip in IgM binding between fractions 30 and 60 may be due to competition for surface binding sites within the microtiter plate with serum albumin because these fractions contained the greatest amount of total protein based on BCA analysis (data not shown). Yersinia ruckeri-specific IgM binding occurred between fractions 15 and 80 with the peak binding occurred between fractions 20–30 (Fig. 5). After fraction 30, the Y. ruckeri-specific binding slowly dropped off to negligible amounts after fraction 80. Pools of 10 consecutive fractions were used to test for the retention or loss of protective activity after passive immunization to naive recipients (n = 20) against a subsequent Y. ruckeri challenge. Pooled fractions 21–30 retained the most protection closely followed by pooled fractions 11–20 and 31–40 with 5%, 10%, and 15% mortality, respectively (Fig. 5). Protection bottomed out with the pooled fractions 71–80, which exhibited 94% mortality. The protective component of the hyper-immune sera coincided with the 1st total IgM peak and the Y. ruckeri IgM-specific peak.

4. Discussion

Herein we show that the circulating protection induced by Y. ruckeri vaccination resides within the plasma/sera and little to no protection can be conferred from circulating PBLs by using a dou-

![Fig. 4](image-url) Protection of recipient/host fish after adoptive transfer or passive immunization from unvaccinated (Unvacc.) immersion vaccinated (Imm.) or injection vaccinated (Inj.) and challenged with Y. ruckeri (serovar 1 biotype 1) (Yr) 2 days after plasma or cell transfer. Recipient/host groups received PBS ( ), unvaccinated plasma ( ), vaccinated plasma ( ), total vaccinated PBL ( ), vaccinated IgM surface + cells ( ) or vaccinated IgM surface – cells ( ). Significant differences are indicated with a bracket and symbol.

![Fig. 5](image-url) Size exclusion of hyper-immune sera to Y. ruckeri. The X axis represents individual 0.5 mL fractions. The left Y axis, O.D.450, corresponds to the ELISA results for amount of total IgM (dotted line ) and the amount of Y. ruckeri specific IgM (solid line with closed circles ). The right Y axis corresponds to the% mortality indicated by the solid line with open triangles . The alternating white and shaded areas indicate the pooled fractions used for passive immunization.
bled haploid line of rainbow trout and their outbred F1 progeny as donor and recipients, respectively. Also, the protection correlates to the IgM from fractions 15–40, based on size exclusion separations, ELISA and challenge assays. IgM that corresponded to fractions 60–90 conferred little to no protection (Fig. 5). This suggests that the larger form of IgM that separates in earlier fractions is the protective form of IgM.

Adoptive transfer and passive immunization is a well-established in vivo assay for mammalian immunological research (Jeneway et al., 2005) providing the ability to compare and contrast the cellular and humoral components of the adaptive immune response and to determine which confers protection. The adoptive transfer part of this assay requires the use of a genetically related, inbred line of donors and highly related recipients, to prevent graft rejection. Studying the cellular component of the adaptive immune system in vivo is limiting in fish due to the low number of inbred lines and their lower fecundity (Parsons and Thorgaard, 1985; Quillet, 1994). Several androgenetically derived lines of rainbow trout (Parsons and Thorgaard, 1985; Scheerer et al., 1991) are known to accept skin grafts (Ristow et al., 1996). These cloned lines have been used in prior studies dealing with immune function (Ristow et al., 1995; Zimmerman et al., 2004) and host response to disease (Ristow et al., 2000a,b). These fish lines are considered analogous to inbred lines of mice and a useful tool for fish disease studies. Herein we make use of a cloned, androgenetically derived rainbow trout line (HC) (Young et al., 1996) and compare the two arms of the adaptive immune response that occur after vaccination to the bacterial pathogen Y. ruckeri. This rainbow trout line was proven to be isogenically identical by DNA fingerprinting (Young et al., 1996) and able to accept skin grafts (Ristow et al., 1996).

The HC line has been used previously for in vitro immunological studies to look at innate cellular activity and shown to have high cytotoxic activity to xenogenic targets (Zimmerman et al., 2004). To increase the number of individual recipients, we used F1 progeny, using Outbred oocytes (NCCCW A) Crossed to HC spermatozoa. To our knowledge this is the first report of the use of an androgenetically derived cloned line of rainbow trout use as donors and F1 progeny as recipients.

The circulating leukocytes, regardless of the presence of surface IgM, were unable to confer total protection to naïve recipients at any cell concentration tested here (Fig. 1 and Fig. 4). Though the survival of the total-PBL transferred group, 42%, from the immunization vaccinated protocol was significantly better than the unvaccinated control fish 23% (P value 0.03), this improvement was minimal when compared to the survival of the plasma transferred from immunization-vaccinated group which exhibited 93% (Fig. 4). This was the only instance where the cellular transferred component was better than the unvaccinated controls again suggesting that the major component of protection resides in the plasma. Hyperacute rejection can occur when allogenic tissues or cells are transferred to an unrelated recipient, which could affect the ability of immune donor leukocytes to protect naïve recipients. The potential loss of a protective cell-mediated immune response is not due to the elimination of transferred cells in the recipients as the CSFE stained donor cells were still present in recipients eight days after they were boosted with the Y. ruckeri vaccine (Supplementary Data, Fig. 4). This study specifically investigated the circulating protection induced by multiple vaccination protocols but does not eliminate the possibility that cellular protection is harbored in other lymphoid tissues. The adoptive transfer of immune leukocytes has been used to look at the protective capabilities against Vibrio anguillarum in rainbow trout (Viele et al., 1980) with varying success. Viele et al. (1980) showed that immune leukocytes from the pronephros conferred some protection, but little protection was seen from leukocytes transferred from the spleen or thymus tissues. Still the passive immunity displayed in Viele’s study would suggest that the humoral response was the major component of protection against Vibrio anguillarum. Also, a two day interval between adoptive cell transfer and Y. ruckeri challenge may not have allowed for production of enough specific protective antibodies. The type and specificity of an adaptive immune response may also be dependent on the pathogen being investigated: An adoptive transfer study performed in crucian carp against the crucian carp hematopoietic necrosis virus (CHNV) showed that there was an inverse relationship between cytotoxic activity and viral loads and that the adoptive transfer of immune leukocytes prevented a CHNV infection (Somamoto et al., 2002). Also, an oral immunization of crucian carp to CHNV produced a protective cellular response (Somamoto et al., 2013) and produce cellular cytotoxic activity to CHNV-infected syngeneic cells but not to syngeneic cells infected with eel virus from America (Sato and Okamoto, 2010). As Y. ruckeri survives in large numbers extracellularly with a small percentage of viable bacteria found intracellularly (Welch and Wiens, 2005), this pathogenesis strategy may lead to a robust humoral response versus a strong cell-mediated response.

This study shows that the most significant protective component of the adaptive immune system after Y. ruckeri vaccination, was in the plasma/serum indicating the importance of the humoral response to a Y. ruckeri challenge (Fig. 1 and Fig. 4). This protection could be maintained after diluting the plasma to 0.5 µL/ ~2 g fish (Fig. 2), a volume of transferred serum much less than that used in other studies which have transferred as much as 100SatoµL of immune sera, in fish ranging from 0.2 g to 25 g in size (Cipriano and Ruppenthal, 1987; Olesen, 1991; Raida and Buchmann, 2008a,b), suggesting that either the concentration of the circulating factor conferring protection is high or very potent. The serum-derived protection in this study was retained over several months (Fig. 3). Long-term, up to 18 months post injection, circulating humoral response to a pathogen in HC rainbow trout was also shown to an attenuated strain of infectious hematopoietic necrosis virus by western blot but no characterization of protection was conducted (Ristow et al., 2000a,b). This study shows that protection to Y. ruckeri after an immersion vaccination and IP injection boost is measurable, but is reduced, over an 8 month period.

The commercially available ERM vaccine has been effective by immersion vaccination since its introduction nearly 3 decades ago (Stevenson, 1997), but recent ERM outbreaks, due to virulent BT2 strains, have occurred in immersion-vaccinated fish (Austin et al., 2003; Fouz and Amaro, 2006; Arias et al., 2007). The emerging BT 2 Y. ruckeri may be using the loss of flagella as a mechanism for subverting the immersion vaccination protection induced by the BT1 strain (Evenhuis et al., 2009; Welch et al., 2011). This study shows that a 2 min immersion vaccination followed by IP injection boost of the serovar O1 BT1 ERM vaccine will elicit a protective immune response to both the BT1 and BT2 challenges (Fig. 2). This protection to both biotypes can be passively conferred to naïve recipients at a small volume and there is no difference in protection when a single vaccination delivered by immersion or IP injection is used against BT1 strain (Fig. 4).

We isolated the protective component from sera by size exclusion chromatography and were able to show that the most protective fractions, which exhibited up to 84% survival, corresponded to the fractions that also contained the highest amount of Y. ruckeri-specific antibodies. These results support the previous work by Kai da et al. (2011), which showed bactericidal effect of plasma from vaccinated fish and that IgM is responsible for protection against Y. ruckeri. IgM may play an important role in protective circulating immunity but is thought to be more relevant in mucosal immunity (Zhang et al., 2010). Future studies directed at the mucosal immune response and comparing the circulating to the mucosal humoral response to Y. ruckeri would be interesting.
5. Conclusion

Little or no protection was observed from the circulating cellular components of vaccinated fish when adoptively transferred to naïve recipients, but robust protection was passively transferred after immersion vaccination when combined with an IP injection boost. Protection was also exhibited against the BT2 Y. ruckeri strain. A single immersion or injection vaccination protocol conferred almost complete passive protection to naïve recipients to the serovar 1 BT1 Y. ruckeri. The protective component of the sera corresponds to IgM when serum is separated by size exclusion. This study also supports the use of androgenetically derived clonal lines of rainbow trout as models for immune and host versus pathogen research.

Acknowledgments

The authors thank Ryan Lipscomb, Jen Harper and Travis Moreland for expert technical assistance and Mark L. Richardson for critical comments on the manuscript. This work was supported by Agricultural Research Service CRIS Project 1930-32000-005 “Host-Pathogen and Environmental Interactions in Cool and Cold Water Aquaculture”. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture. USDA is an equal opportunity provider and employer.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jci.2013.12.004.

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