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Short communication

Gene expression associated with immune response in Atlantic salmon head-kidney vaccinated with inactivated whole-cell bacterin of *Piscirickettsia salmonis* and pathogenic isolates

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ABSTRACT

Piscirickettsiosis is the most challenging disease present in the Chilean salmon industry. The aim of this study was to describe the expression of genes associated with immune response of Atlantic salmon intraperitoneally infected with LF-89 and EM-90 *Piscirickettsia salmonis* and vaccinated with inactivated whole-cell bacterin of *P. salmonis*. The fish infected with PS-LF-89 showed an anti-inflammatory response, whereas this finding was not observed in the PS-EM-90-infected fish and vaccinated fish. Fish infected with both *P. salmonis* isolates showed *mhc1-mhc2*, *cd4-cd8b* and *igm* overexpression, suggesting that *P. salmonis* promotes a T $CD4^+$ and T $CD8^+$ cell response and a humoral immune response. The vaccinated-fish exhibited *mhc1*, *mhc2* and *cd4* overexpression but a significant downregulation of *cd8b* and *igm*, suggesting that the vaccine supported the $CD4^+$ T-cell response but did not induce an immune response mediated by $CD8^+$ T cells or a humoral response. In conclusion, the expression pattern of genes related to the humoral and cell-mediated adaptive immune response showed up-regulation in fish infected with *P. salmonis* and down-regulation in vaccinated fish. The results of this study contribute to our understanding of the immune response against *P. salmonis* and can be used in the optimization of SRS prevention and control measures.

1. Introduction

Piscirickettsiosis (also known as Salmonid Rickettsial Septicaemia, SRS) is the most challenging disease present in the Chilean salmon industry [1]. The isolates LF-89 and EM-90 show genomic differences that would determine different degrees of virulence, pathogenesis and immune response [2–5]. The control of SRS has focused mainly on chemotherapy (florfenicol and oxytetracycline) and vaccination. Although the available vaccines based on bacterins, recombinant subunits and/or live-attenuated have not prevented SRS in Chile, they have delayed onset of the first outbreak [1,6,7]. The control of *P. salmonis* may require the stimulation of adaptive cellular immunity, although the mechanisms by which this process is directed are still poorly understood [1].

Fish have a specific cell-mediated immunity that is characterized by antigen presentation and the participation of T $CD4^+$ and $CD8^+$ lymphocytes [8–10]. *Piscirickettsia salmonis* induces IL-10 overexpression and reduces IL-12 expression *in vitro* and *in vivo* [4,11], which could be a strategy to promote intracellular survival and replication. Different expression patterns of immune relates genes in Atlantic salmon infected with *P. salmonis* suggests strong innate response stimulation but an inhibition of the adaptive response [4,5,12,13]. However, comparative *in vivo* studies of the immune response of Atlantic salmon intraperitoneally (i.p.) infected with both most representative isolates of *P. salmonis* and vaccinated fish have not been described. Consequently, the aim of this study was to describe the expression of genes associated with innate and adaptive immune responses of Atlantic salmon infected with LF-89 and EM-90 isolates and vaccinated with the inactivated whole-cell bacterin of *P. salmonis*.

2. Material and methods

To evaluate the expression of immune response genes, we used head-kidney samples obtained from Atlantic salmon i.p. infected with LF-89 and EM-90 *P. salmonis* (PS-LF-89 and PS-EM-90, respectively), as described by Rozas-Serri et al. [4]. Briefly, the challenge was performed using an infectious dose of $10^{5.6}$ u.f.c./0.1 ml of the LF-89 and EM-90 isolates. Two parallel tanks supplied with 1,000 L of 12 °C water with

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Table 1
Genes, primers, efficiency, correlation coefficients, primer concentrations and optimal annealing temperatures for reference and target genes.

Gene name	Primers sequence (5'→3')	Accesion number	Tm (°C)	Primers concentration (nM)	Efficiency	R^2
ifng	CTAAAGAAGGACAACCGCAG CACCGTTAGAGGGAGAAATG	AY795563	56	500	1,95	0,9948
tnfa	AGGTTGGCTATGGAGGCTGT TCTGCTTCAATGTATGGTGGG	NM_001123589 NM_001123590	56	250	2,13	0,9925
il1b	ATCACCATGCGTCACATTGC GTCCTTGAACTCGGTTCCCA	NM_001123582	58	500	2,05	0,9968
il2	CATGTCCAGATTCAGTCTTCTATACACC GAAGTGTCCGTTGTGCTGTTCTC	AM422779	58	750	2.01	0.9948
il6	CCTTGCGGAACCAACAGTTTG CCTCAGCAACCTTCATCTGGTC	DQ866150	58	750	2.10	0.9964
il8	GGCCCTCCTGACCATTACT ATGAGTCTACCAATTCGTCTGC	NM_001140710	56	500	2,01	0,9974
il10	CGCTATGGACAGCATCCT AAGTGGTTGTTCTGCGTT	EF165029	55	250	2,00	0,9982
il12b	CTGAATGAGGTGGACTGGTATG ATCGTCCTGTTCCTCCG	BT049114	55	250	2,10	0,9990
il15	TTGGTTTTTGCCCTAACTGC CAGGTCCATCGCACTCTTTT	EG792923	56	250	1,99	0,9937
il18	ATGACATTGACAGGCCCAGAGGAA GTTGCTCCAGTGGTTTGGCAGAAA	NM_001141408	60	750	1,97	0,9941
mhc1	CTGCATTGAGTGGCTGAAGA GGTGATCTTGTCCGTCTTTC	AF508864	60	250	1,99	0,9976
mhc2	TCTCCAGTCTGCCCTTCACC GAACACAGCAGGACCCACAC	BT049430	60	250	2,03	0,9964
cd4	GAGTACACCTGCGCTGTGGAAT GGTTGACCTCCTGACCTACAAAGG	NM_001124539	60	500	2,01	0,9728
cd8b	CGCACACACCTCAACAACTC ATTGATGCGCAGTGTGAAAG	AY693394	56	500	1,94	0,9453
igm	TCTGGGTTGCATTGCCACTG GTAGCTTCCACTGGTTTGGAC	CA039888	60	250	2,09	0,9980
β-actina	ACGAGAGGTTCCGTTGTCC GCAAGACTCCATACCGAGGA	BG933897	60	250	2,10	0,9989
ELF-1α	CCCCTCCAGGACGTTTACAAA CACACGGCCCACAGGTACA	NM_001123629	60	750	2,00	0,9969

15 ppt salinity were used for each isolate. Each tank contained 55 fish that received i.p. injections of 0.1 ml of each inoculum. A separate tank housed 150 uninfected control fish that received i.p. injections of 0.1 ml of sterile saline solution (0.9%). Other tank was conformed with 150 fish that were i.p. injected with 0.1 ml of an inactivated whole-cell bacterin of *P. salmonis* (each 0.1 ml contained 5.7×10^5–2.5×10^6 *P. salmonis*, oil adjuvant and formaldehyde as an inactivating agent). These fish were not challenged with *P. salmonis* later. Five fish were sampled from each tank at 1 (12 Degree-Days), 3 (36 DD), 5 (60 DD), 7 (84 DD) and 14 days post-inoculation (DPI) (168 DD). Head-kidney samples of 0.5 cm^3 were obtained from each fish and fixed in RNA Later™ (Ambion, Austin, TX, USA).

The RNA extraction and relative quantification of the immunes relates genes was evaluated in head-kidney of fish of each experimental group by normalized RT-qPCR as described by Rozas-Serri et al. [4] (Table 1). Briefly, differential expression of selected genes was determined with the Real-Time PCR StepOnePlus™ system (Applied Biosystems, Life Technologies, Waltham, MA, USA) using the Brilliant II SYBR Green qPCR Master Mix kit (Agilent Technologies, Santa Clara, CA, USA). Each amplification reaction was performed in a final volume of 15 µl, consisting of 7.5 µl of buffer, 250 nM to 750 nM primers depending on the gene (Table 1), 300 nM ROX (50 nM) and 2 µl of cDNA diluted 1:10. The PCR program consisted of a 10-min activation and denaturation step at 95°C, followed by 45 cycles of 15 s at 95°C, 30 s at the annealing temperature of the corresponding primers and an additional 15 s extension at 72°C. Five biological replicates were used, and each qPCR reaction was run in duplicate, including a negative control without reverse transcriptase to check for genomic DNA contamination and a negative control without template to check for primer dimers. Relative expression results were analysed using amplification efficiencies as described by Pfaffl et al. [14]. ELF1A and ß-actin were selected as housekeeping for gene normalization as described by Rozas-Serri et al. [4]. Linear regression models were used to evaluate the interaction of time and gene expression using Stata statistical software, version 13 (StataCorp LP, College Station, TX, USA).

To understand the progression of SRS pathogenesis, the histoscore SRS (hsSRS) described by Rozas-Serri et al. [3] was used. The hsSRS of each fish at each sampling time (n = 5) was used to calculate the mean hsSRS ± standard deviation (SD) of each group at each sampled time point. In addition, the relative quantification of mRNA transcribed from the 16S rRNA gene of PS-LF-89 and PS-EM-90 was performed using RT-qPCR as described by Rozas-Serri et al. [3,4]. The relative abundance values of the 16S rRNA transcript of *P. salmonis* are expressed as log10-fold (log-fold) values. The effect of time and of *P. salmonis* isolates and their interaction on the gene expression was evaluate by descriptive analyses and linear regression models using Stata statistical software, version 13 (StataCorp LP, College Station, TX, USA).

2.1. Key resource table

Resource	Source	Identifier
Chemical formaldehyde		

3. Results

Fish infected with PS-LF-89 or PS-EM-90 isolates showed no significant differences in cumulative mortality or in time to mortality between replicates (Fig. 1a). PS-LF-89 fish showed the presence of 16S rRNA transcripts in kidney at 5 dpi and this value exponentially increased at 7 and 14 dpi (Fig. 1b), whereas the same transcripts were detected in PS-EM-90-infected fish at 3 dpi, and significantly increasing

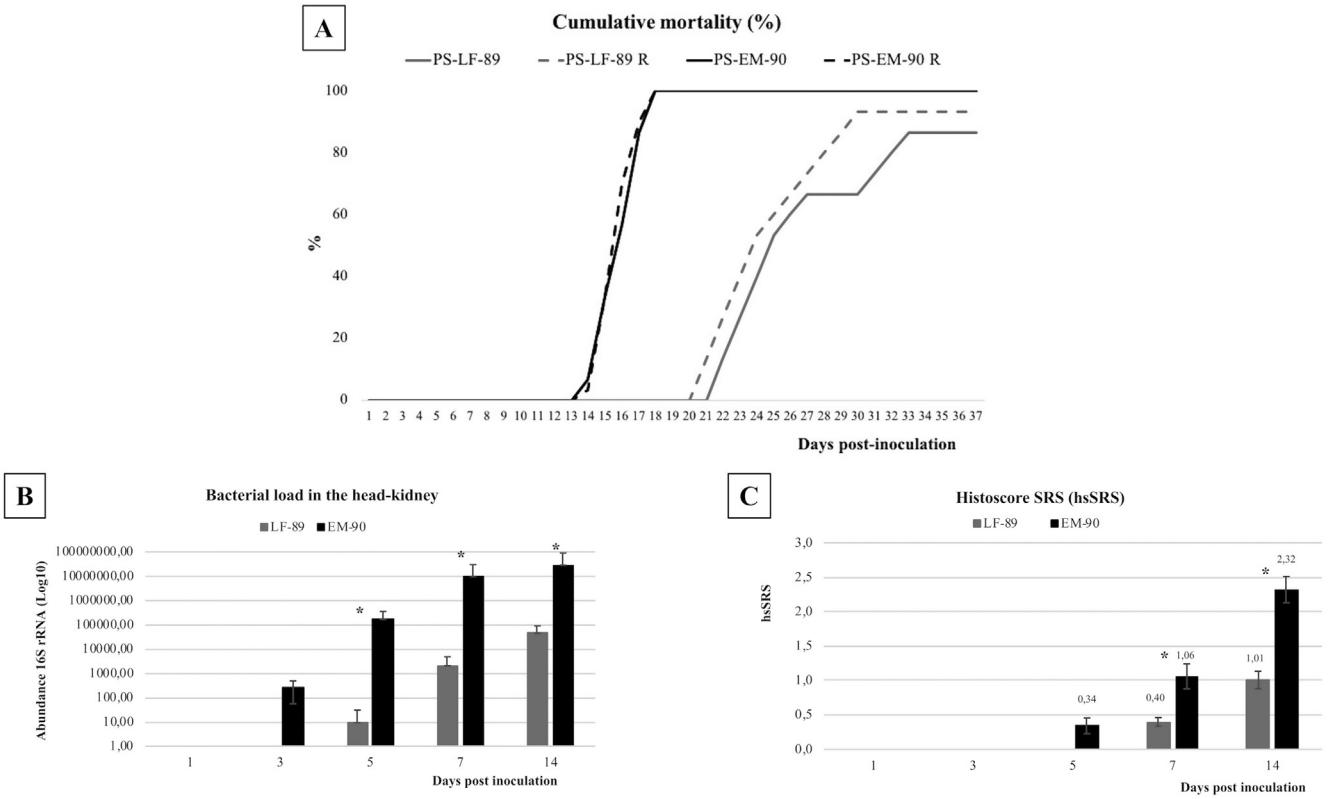

Fig. 1. Mortality rates, bacterial load and tissue damage in fish infected with PS-LF-89 and PS-EM-90 isolates. (A) Cumulative mortality percentage in i.p post-smolt Atlantic salmon infected with PS-LF-89 and PS-EM-90 isolates. (B) RNA abundance (log_{10}) of the 16S rRNA transcripts of PS-LF-89 and PS-EM-90 isolates in head kidney. (C) Histoscore SRS evolution considering histopathological lesions at the early stages of SRS infection (0–14 dpi). Each time point represents the relative amount of the bacterial reference gene in five fish reported as mean log_{10} fold ± standard error of the mean (SEM). *p < 0.05.

in comparison with PS-LF-89 fish to at 5 and 7 dpi (p = 0.0092) (Fig. 1b). *Piscirickettsia salmonis* transcripts were not detectable in the head-kidney of either vaccinated and PBS injected fish. The hsSRS of PS-EM-90 fish was significantly higher and increased more rapidly than PS-LF-89 fish, increasing from 0.34 ± 0.11 at 5 dpi to 2.32 ± 0.19 at 14 dpi (p = 0.0081) (Fig. 1c). The hsSRS showed a significant positive correlation with bacterial growth expressed as the abundance of 16S rRNA gene transcripts of *P. salmonis*, but PS-EM-90 fish showed a higher degree of association between hsSRS and bacterial load (P = 0.9476, p = 0.0000).

A positive correlation of the overexpression of IFNγ, IL-2, IL-10, IL-12ß, MHC-II and CD4 was seen in the PS-LF-89 (Table 2)- and PS-EM-90-infected fish (Table 3) at 14 dpi, but the proinflammatory response in the PS-EM-90-infected fish was more exacerbated (Fig. 2). In addition, the fish infected with PS-LF-89 showed an anti-inflammatory response, whereas this finding was not observed in the PS-EM-90-infected fish. Conversely, a positive correlation of the expressions of downregulation of IFNγ, IL-2, IL-12ß, MHC-I and CD8 was seen in the vaccinated fish at 14 dpi (Table 4, Fig. 2). The proinflammatory response in these fish was more exacerbated than in the fish infected with PS-LF-89 but was slighter than in the PS-EM-90-infected fish. The PS-EM-90 isolate promoted a more significant imbalance between IL-10 overexpression and IL-12 under expression than the PS-LF-89 isolate, and both groups of infected fish showed a greater imbalance in IL-10 and IL-12 than the vaccinated fish. An anti-inflammatory response in the vaccinated fish was not observed.

At the same time, vaccinated-fish exhibited *mhc1*, *mhc2* and *cd4* overexpression but a significant downregulation of *cd8b* and *igm*, suggesting that the vaccine supported the T $CD4^+$ cell response, but did not induce an immune response mediated by T $CD8^+$ cells and a humoral response. PS-LF-89 and PS-EM-90-infected fish showed a significantly higher expression of *mhc1* and *mhc2* at 14 dpi than the vaccinated fish (Fig. 2). The upregulation of *cd4* remained in all of the groups until 14 dpi (Fig. 2), but the upregulation of *cd8b* was conserved in both infected fish until 14 dpi (Fig. 2), whereas the vaccinated-fish exhibited a significant downregulation of *cd8b* between 7 and 14 dpi (Fig. 2). However, the fish infected with PS-EM-90 showed a significantly higher expression of *cd8b* than the PS-LF-89-infected fish at the 14 dpi (Fig. 2). The upregulation of *igm* was conserved until 14 dpi in both of the infected-fish groups (Fig. 2), whereas the vaccinated-fish exhibited a downregulation of the transcript between 7 and 14 dpi (Fig. 2). Taking together, these results are not as expected from a vaccination strategy and could partly explain the relative field efficacy of vaccines, although this requires further investigation.

4. Discussion

The primary immune tissue in fish is the head kidney, which has haematopoietic, phagocytic, antigen processing and IgM production capabilities [4,5]; for this reason, the expression of genes associated with the immune response was analysed in head kidney in this study. The cumulative mortality was significantly higher in fish i.p infected with PS-EM-90 than in those i.p infected with PS-LF-89, whereas the mean time to mortality was significantly lower in PS-EM-90 fish. At the same time, fish infected with PS-EM-90 showed higher bacterial load and more severe histological lesions than PS-LF-89 infected fish. Therefore, the host response would depend on the degree of virulence of the bacteria, consistently with findings observed in *P. salmonis* [3–5] and *Francisella* infections [15,16].

Fish infected with either of the two *P. salmonis* isolates showed *mhc1-mhc2* and *cd4-cd8b* overexpression, suggesting that *P. salmonis* would promotes a T $CD4^+$ and T $CD8^+$ cells response and a humoral

Table 2
Correlations in the expression of various genes in the head kidney of fish infected with PS-LF-89. *p < 0.05; **p < 0.005; ***p < 0.0005.

Gene	cd4	cd8	il10	il12b	il15	il18	il1b	il2	il6	il8	ifng	igm	mhci	mhcii	tnfa
cd4	1														
cd8	0,0379	1													
il10	0,7971***	0,1849	1												
il12b	0,8378***	0,1594	0,7506***	1											
il15	0,5653**	0,5529**	0,3967*	0,6066***	1										
il18	0,7698***	0,3261*	0,7191***	0,8155***	0,7661***	1									
il1b	0,7697***	0,1855	0,6541***	0,6295***	0,7923***	0,702***	1								
il2	0,8832***	0,1178	0,735***	0,8423***	0,5721**	0,867***	0,6574***	1							
il6	0,6605***	−0,028	0,4192*	0,7196***	0,2052	0,5091**	0,2093	0,684***	1						
il8	0,7286***	0,1504	0,7238***	0,8808***	0,6075***	0,7461***	0,6903***	0,6575***	0,5358**	1					
ifng	0,7844***	0,1322	0,5734**	0,7862***	0,4162*	0,5968**	0,3862*	0,7182***	0,7067***	0,4866**	1				
igm	0,4019*	0,5482**	0,6057***	0,6622***	0,4796**	0,6537***	0,2786	0,4745*	0,3506*	0,6891***	0,495**	1			
mhci	0,5169**	0,3217*	0,6203***	0,7972***	0,5706**	0,8388***	0,4288*	0,6554***	0,4528**	0,8024***	0,4815**	0,8668***	1		
mhcii	0,6905***	0,109	0,6148***	0,8407***	0,4356*	0,68***	0,4253*	0,7512***	0,7617***	0,782***	0,5611**	0,5177**	0,6296***	1	
tnfa	0,6995***	−0,106	0,6444***	0,7743***	0,324*	0,5721**	0,5596**	0,6391***	0,5565**	0,8881***	0,4357**	0,515**	0,6273***	0,763***	1

Table 3
Correlations in the expression of various genes in the head kidney of fish infected with PS-EM-90. *p < 0.05; **p < 0.005; ***p < 0.0005.

Gene	cd4	cd8	il10	il12b	il15	il18	il1b	il2	il6	il8	ifng	igm	mhci	mhcii	tnfa
cd4	1														
cd8	0,3736*	1													
il10	0,3479*	0,0896	1												
il12b	0,8758***	0,13	0,3531*	1											
il15	0,7072***	0,5291**	0,4463*	0,5456**	1										
il18	0,8482***	0,154	0,5825**	0,8843***	0,6542***	1									
il1b	−0,185	−0,206	−0,048	−0,174	−0,201	−0,181	1								
il2	0,7223***	0,5225**	0,4612*	0,6502***	0,8111***	0,6682***	0,12	1							
il6	0,4084*	−0,08	0,6373***	0,4225*	0,3519*	0,5135**	0,6272***	0,5839**	1						
il8	0,758***	0,3081*	0,4612*	0,7016***	0,7647***	0,7883***	0,2996	0,8409***	0,7552***	1					
ifng	0,8527***	0,3317*	0,247	0,8172***	0,6733***	0,7555***	0,0275	0,7286***	0,5393**	0,8365***	1				
igm	0,8752***	0,327*	0,5458**	0,8358***	0,7983***	0,8874***	−0,19	0,7904***	0,4796**	0,82***	0,7681***	1			
mhci	0,7782***	0,1835	0,1457	0,7394***	0,7789***	0,749***	−0,137	0,7033***	0,299	0,7566***	0,7567***	0,835***	1		
mhcii	0,8973***	0,371*	0,5121**	0,7115***	0,7788***	0,8197***	−0,184	0,732***	0,43*	0,7568***	0,6927***	0,9227***	0,7627***	1	
tnfa	−0,033	−0,212	0,0891	−0,055	−0,027	0,0111	0,951***	0,2095	0,729***	0,4419*	0,139	−0,048	0,0239	−0,031	1

Gene	P. salmonis isolate	Relative expression, head-kidney (days post-inoculation, dpi)				
		1	3	5	7	14
Mediators of innate immunity and proinflammatory citokines						
IFNγ	LF-89	7,44	2,70	2,49	3,05	2,47
	EM-90	7,69	1,60	4,90	1,30	2,82
	Vaccinated	8,38	2,59	3,23	1,69	1,68
TNFα	LF-89	5,08	1,00	-1,58	-5,64	2,43
	EM-90	3,21	-2,11	14,25	-1,54	1,46
	Vaccinated	2,58	0,96	1,15	1,14	1,44
IL-1β	LF-89	7,83	1,72	1,65	2,57	6,13
	EM-90	1,72	1,59	25,20	2,10	3,33
	Vaccinated	4,38	1,42	2,00	-1,13	2,68
IL-2	LF-89	37,80	7,93	1,90	2,19	4,40
	EM-90	2,95	-2,07	1,73	-1,20	2,08
	Vaccinated	31,75	8,32	9,50	2,31	-5,74
IL-8	LF-89	3,17	-2,62	-1,90	-2,37	2,07
	EM-90	3,72	-3,77	2,52	-1,80	1,76
	Vaccinated	2,18	-1,85	-1,54	-2,99	1,53
IL-10	LF-89	28,32	2,57	1,79	2,57	2,73
	EM-90	45,18	4,78	6,43	1,47	4,10
	Vaccinated	3,78	1,84	3,44	8,84	1,91
IL-12β	LF-89	5,22	-1,04	-1,86	-1,16	2,11
	EM-90	8,02	2,12	1,84	1,70	-1,45
	Vaccinated	5,70	-1,18	1,18	-1,56	-1,57
IL-15	LF-89	104,48	26,95	33,46	17,95	113,79
	EM-90	146,56	15,57	47,44	12,61	126,55
	Vaccinated	183,78	48,73	62,83	5,79	1,17
IL-18	LF-89	230,88	70,92	48,14	48,83	109,11
	EM-90	498,11	105,12	100,95	154,70	83,69
	Vaccinated	462,86	88,41	105,18	28,98	25,65
Cell-mediated immunity & Humoral immunity						
MHC-I	LF-89	18,30	7,70	3,08	3,20	10,83
	EM-90	18,12	6,60	9,84	9,75	11,35
	Vaccinated	18,28	7,07	7,52	2,49	2,94
MHC-II	LF-89	4,52	1,90	2,17	1,08	2,23
	EM-90	3,65	2,11	1,59	1,24	2,31
	Vaccinated	2,16	1,81	2,85	1,49	1,96
CD4	LF-89	11,04	3,44	4,09	4,72	5,33
	EM-90	9,94	4,74	3,72	2,30	4,57
	Vaccinated	6,22	3,44	5,98	2,23	6,31
CD8β	LF-89	1,98	1,36	2,81	-2,17	2,23
	EM-90	2,98	1,23	1,14	1,06	5,54
	Vaccinated	2,76	2,65	2,27	-3,98	-1,70
IgM	LF-89	7,35	3,38	3,22	1,16	4,41
	EM-90	15,84	4,14	3,87	3,31	6,22
	Vaccinated	2,99	3,85	5,78	-1,11	-1,36

Fig. 2. Relative expression of genes related to the innate immune response and to the adaptive humoral and cell-mediated immune responses determined by RT-qPCR. Each box represents the average expression level in five fish, which is presented as the fold change ± SEM compared with the average expression level in five control fish. Statistically significant differences between fish infected by PS-LF-89 and PS- EM-90, and vaccinated fish are indicated by *$p < 0.05$, **$p < 0.005$ and ***$p < 0.0005$.

immune response, but these results were not consistent with findings described in fish infected by cohabitation with the same *P. salmonis*, which showed a significant downregulation of *il12b*, *il15*, *il18*, *igm* and *cd8* [4]. In addition, the expression of *cd8b* showed a significant positive correlation with the PS-LF-89 bacterial load in the head kidney expressed as abundance of the 16S rRNA ($p = 0.009$).

Interestingly, these differences could indicate that in the challenge model (i.p vs cohabitation), it is very important how the fish are infected with *P. salmonis* in terms of implementing the immune response and combating the infection, which should be considered in vaccine efficacy and/or genetic resistance trials. Challenge models using baths and cohabitation more faithfully represent the conditions of natural exposure and provide predictable results for mortality; however, these models do not allow precise control of the dose or infection time [3]. Infection with *P. salmonis* mainly occurs through horizontal transmission [3,17,18]. *Piscirickettsia salmonis* was detected in the gills of cohabitant fish at 21 days post-inoculation (dpi), confirming that the gills are the main entry point of the bacterium into the host [3].

The proinflammatory response was significantly exacerbated in fish infected by PS-EM-90 compared with fish infected by PS-LF-89, a finding that is probably associated with the higher pathogenicity of PS-EM-90 [3,4]. The increase in proinflammatory gene expression was seen in the early stage similar to the findings described in Atlantic salmon infected with *P. salmonis* via i.p [13]. and in rainbow trout

Table 4
Correlations in the expression of various genes in the head kidney of vaccinated fish. *p < 0.05; **p < 0.005; ***p < 0.0005.

Gene	cd4	cd8	il10	il12b	il15	il18	il1b	il2	il6	il8	ifng	igm	mhci	mhcii	tnfa
cd4	1														
cd8	0,4019*	1													
il10	−0,042	−0,162	1												
il12b	0,58**	0,4587**	0,1268	1											
il15	0,575**	0,6401***	0,1043	0,834***	1										
il18	0,5374**	0,5547**	0,0936	0,8103***	0,9802***	1									
il1b	0,6275***	0,2947	−0,019	0,6453***	0,7372***	0,8078***	1								
il2	0,4859*	0,6122***	0,0809	0,7355***	0,9398***	0,9592***	0,7576***	1							
il6	0,1904	0,2747	0,082	0,4606*	0,6054***	0,6007***	0,4252*	0,7029***	1						
il8	0,7625***	0,2824	−0,128	0,7657***	0,7042***	0,738***	0,7349***	0,6719***	0,3255	1					
ifng	0,5913**	0,6026***	0,1833	0,7476***	0,9608***	0,9472***	0,7451***	0,8972***	0,6149***	0,6555***	1				
igm	0,2903	0,7117***	−0,178	0,143	0,4134*	0,2866	0,1131	0,3432*	0,2598	−0,054	0,3751*	1			
mhci	0,6215***	0,6336***	0,0713	0,8099***	0,9669***	0,9654***	0,7993***	0,9648***	0,6253***	0,7199***	0,9266***	0,3813*	1		
mhcii	0,5093**	0,4308*	−0,131	0,1859	0,2893	0,2037	0,2701	0,1231	0,1826	0,1126	0,4156*	0,5871**	0,2551	1	
tnfa	0,5673**	0,3141*	−0,023	0,5987***	0,3601*	0,3341*	0,4104*	0,2999	0,2733	0,4492*	0,4222*	0,1034	0,4317*	0,5349*	1

infected with *Yersinia ruckeri* [19]. One of the other noticeable differences was the significant decrease in IL-15, IL-18 and IL-10 expression in the vaccinated fish at 14 dpi vs. the PS-LF-89-infected fish, which was similar to the findings described in Atlantic salmon infected with *P. salmonis* to selectively inhibit IL-12p40 production [4,12,13].

Vaccinated-fish exhibited a *cd4* overexpression but a down-regulation of *cd8b* and *igm*, suggesting that the vaccine supported the T $CD4^+$ cell response. These findings are consistent with the decreased expression of *cd8b* reported in Atlantic salmon i.p. infected with *P. salmonis* LF-89 [13] and the downregulation of *cd8b* and *igm* in fish infected with *P. salmonis* by cohabitation [4].

5. Conclusions

The expression pattern of the genes related to the humoral and cell-mediated adaptive immune response in fish infected with *P salmonis*, regardless of pathogenicity, and in vaccinated fish was different. While the fish infected showed upregulation of the immune related genes, the vaccinated fish showed a down-regulation of the same genes. The findings from these experiments provide valuable insights into the inflammatory and adaptive immune responses in fish following vaccination and will hopefully allow for the rational design of new vaccines to provide long-term protection in fish.

Ethics approval and consent to participate

All animal procedures were conducted in strict accordance with the recommendations in the Guide of Use of Animals for Research of Universidad Austral de Chile and were approved by the Committee on the Ethics of Animals for Research. The animals were anaesthetized with benzocaine prior to handling and marking. Euthanasia was performed using an overdose of anaesthesia. All efforts were made to provide the best growing conditions and to minimize suffering.

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Authors' contributions

MRS designed the study, performed the challenge and collected data; MRS and AP analysed and interpreted data; AP and LM performed the quantitative real-time PCR validation; MRS. wrote the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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List of abbreviations

CD	cluster of differentiation
DD	degree days
DPI	days post-inoculation
IFN	interferon

Ig	immunoglobulin
IL	interleukin
IP	intraperitoneally
MHC	major histocompatibility complex
PS-LF-89	*Piscirickettsia salmonis* LF-89 strain
PS-EM-90	*Piscirickettsia salmonis* EM-90 strain
RT-PCR	reverse transcription polymerase chain reaction
SRS	Piscirickettsiosis or Salmonid Rickettsial Septicaemia
TNF	tumour necrosis factor

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.08.031.

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