Aquaculture 473 (2017) 538–544

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Atlantic salmon winter-ulcer disease: Combining mortality and skin ulcer development as clinical efficacy criteria against *Moritella viscosa* infection

Christian Karlsen [a], Ragnar Thorarinsson [b,*], Christian Wallace [c], Kira Salonius [d], Paul J. Midtlyng [e]

[a] Nofima, Ås, Norway
[b] Elanco Animal Health, Norway
[c] VESO Vikan, Namsos, Norway
[d] Previwo AS, Kjeller, Norway
[e] Norwegian University of Life Sciences, School of Veterinary Medicine, Oslo, Norway

ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form 25 January 2017
Accepted 29 January 2017
Available online 10 February 2017

Keywords:
Moritella viscosa
Winter-ulcer
Atlantic salmon
Protective immunity

ABSTRACT

The bacterium *Moritella viscosa* causes classical winter-ulcer disease in seawater-farmed salmonids. Despite widespread vaccination with multi-component vaccines containing the *M. viscosa*-antigen, disease outbreaks continue to be reported in Norway. In trials reported here, commercially available vaccines containing *M. viscosa*-components were used to vaccinate groups of Atlantic salmon (*Salmo salar* L.). In two well-controlled experimental studies, bath challenge with *M. viscosa* was carried out after 600, 1000, or 1500 degree days (dd) post immunization. A field study was also carried out comparing four commercial vaccines to evaluate protection against *M. viscosa* under commercial farming conditions. In order to increase the resolution of the clinical outcome of these *M. viscosa* infections studies, we used mortality and expanded the phenotypic parameters to also include skin ulceration status of survivors. The experimental laboratory challenge studies showed that immunization is effective at 600 dd and significantly protects against the clinical consequences (both mortality and skin ulceration) of *M. viscosa* infection, with relative protection reaching 91% compared to saline controls and 65% compared to a vaccine formulation lacking *M. viscosa* antigen. *M. viscosa* challenge was confirmed but induced only minor clinical consequences to the vaccinated groups in the field trial. The results demonstrate that the experimental bath challenge model discriminates between non-specific and specific vaccine protection against *M. viscosa*. The model was further successful in documenting protection utilizing cutaneous ulcer development, the most prevalent clinical manifestation of *M. viscosa* infection. Although the degree of protection is significantly different between the vaccines on test, immunization constitutes an important management tool providing protection against *M. viscosa* infections in marine farmed Atlantic salmon.

1. Introduction

Outbreaks of ulcerative disease in farmed Atlantic salmon (*Salmo salar* L.) occur across the North Atlantic region when seawater temperature drops below 8–10 °C (Benediktsdottir et al., 1998; Bruno et al., 1998; Lunder, 1992; Whitman et al., 2000). The aetiology of skin disorders or ulceration is complex but ulcerative outbreaks or classical winter-ulcer disease conventionally refers to infection with the bacterium *Moritella viscosa* where superficial skin lesions develop into chronic skin ulcers that may be followed by terminal septicemia (Benediktsdottir et al., 1998; Lunder et al., 1995). However, numerous bacterial species are recurrently reported isolated from ulcers, most commonly *Aliivibrio wodanis* and *Tenacibaculum* spp. (Benediktsdottir et al., 1998; Lunder et al., 2000; Olsen et al., 2011). How other bacterial species may be implicated in ulcer pathology is largely unknown. It is demonstrated that *A. wodanis* secretes toxins cytotoxic to fish cell lines, and is able to co-infect (with *M. viscosa*) Atlantic salmon (Karlsen et al., 2014b). *Tenacibaculum* may invade scarified skin and co-infect ulcers caused by *M. viscosa* (Olsen et al., 2011). Recently *Tenacibaculum finnmarkense* has been suggested as a new species within *Tenacibaculum*, pathogenic to Atlantic salmon (Småge et al., 2015).

Two major phenotypic and genotypic clades ('typical' and 'variant') have been identified in *M. viscosa* (Grove et al., 2010). 'Typical' *M. viscosa* are isolated from Atlantic salmon farmed in Norway, Scotland and the Faroe Islands. 'Variant' *M. viscosa* are isolated from Atlantic salmon farmed in Iceland and Canada and from Norwegian farmed trout. Antigenic heterogeneity indicate further that *M. viscosa* is serologically diverse (Heidarsdottir et al., 2008), which in part is based on the

* Corresponding author at: Elanco Animal Health, c/o Eli Lilly Norge AS, P.O. Box 6090, Etterstad N-0601, Norway.
E-mail address: ragnar.thorarinsson@elanco.com (R. Thorarinsson).

http://dx.doi.org/10.1016/j.aquaculture.2017.01.035
0044-8486/

variation in the major protective antigen MvOmp1 (Bjornsson et al., 2011). It is suggested that lineages within *M. viscosa* have evolved compatibility factors that adapt 'typical' *M. viscosa* to host-specific virulence (Karlsen et al., 2014a). Little is known of *M. viscosa* virulence but extracellular products (ECPs) are both cytotoxic to fish cells and lethal to Atlantic salmon (Bjornsdottir et al., 2011; Tunsjø et al., 2009). It is further suggested that the status of skin health is important for the susceptibility to infections (Karlsen et al., 2012). Current prevention is to avoid management that may result in injuries predisposing to ulcers, vaccination, and removal of infected fish (Bornø and Linaker, 2015; Hjeltnes, 2014).

The bacterial components of the multivalent component vaccines used in the Norwegian aquaculture of Atlantic salmon consists of inactivated bacteria. Following the inactivation by formalin, the bacterins are emulsified in an oil adjuvant for intraperitoneal administration. Adjuvants enhance the immune response but may also induce prolonged inflammation leading to adverse reactions (Midtlyng et al., 1996). Historically, the first monovalent water-based vaccines with *M. viscosa* antigen did not stimulate significant immunity against *M. viscosa* infection (Baalsrud and Lunder, 1993). This changed in the mid 90s with the introduction of polyvalent oil-adjuvanted vaccines containing *M. viscosa* (Greger and Goodrich, 1999). Today almost all Norwegian farmed salmon are vaccinated against *M. viscosa*. However, ulcers still constitute a health problem during seawater rearing causing elevated baseline mortality, where the majority of cases occur as episodic outbreaks (Aunsmo et al., 2008). Clinical disease is of particular significance in the north of Norway (Bornø and Linaker, 2015).

The first challenge study reported in this article was aimed to evaluate vaccine-induced immunity from a new waterborne challenge model (Løvoll et al., 2009; Maira et al., 2006). The second challenge trial aimed to evaluate vaccine-induced protection by the comparison of relative percentage survival (RPS), prevalence of cutaneous ulcers, and antibody responses to *M. viscosa* of all multivalent six component vaccines licensed for sale in Norway. The experimental trial was further designed to investigate extended longevity of protection, how the antibody response developed related to degree days (dd) and to which degree it correlated with protection. Vaccinated groups were compared to fish injected with saline and a *M. viscosa* negative vaccine control, Lipogen Duo (LD). In addition, we here summarize data from a third (field) study where four vaccine formulations were evaluated for protection under clinical (commercial) conditions with respect to weight development and differences in skin ulceration at harvest.

2. Materials and methods

2.1. Fish and vaccination

Challenge studies were performed at the experimental test facility at VESO Vikan (Namsos, Norway) utilizing Atlantic salmon (SalmoBreed strain in study 1 and Aquagen strain in study 2). The experimental fish were confirmed negative for antibodies against *Aeromonas salmonicida*, *Vibrio salmonicida*, *Vibrio anguillarum* serotype O1 and 02, *Vibrio ordalii*, *M. viscosa* and infectious pancreatic necrosis virus (IPNV) prior to enrollment. A total of 438 (~35.6 g) healthy unvaccinated parr in study 1 were first anesthetized with metacain (Finquel vet. ScanVacc) before being marked, group by group, by fin clipping or ink-tattoo and vaccinated intraperitoneally according to the experimental design presented in Table 1. After six weeks in 12 ± 1 °C fresh water, the fish were acclimatized and transferred to seawater with a holding temperature of 9 ± 1 °C, before being subjected to bath challenge twelve days later.

Study 2, utilized 1440 unvaccinated healthy parr, (1200 for experimental challenge and 240 for serology testing). Individual weights of 50 fish were recorded prior to vaccination and the average weight calculated to 42 ± 6.4 g. Weight estimation was further based on bulk weights of 3 × 50 fish with an average weight of 39.5 ± 2.1 g (5.3%), indicating a similar body-size (weight) distribution. Fish were anesthetized with benzocaine and individually passive integrated transponder (PIT) tagged two weeks prior to vaccination. Intraperitoneal injections of vaccines or saline (control) were performed group by group according to Table 1. All six groups were pooled in each of three tanks during immunization. The fish groups in tank 1 were challenged at approx. 1000 dd post vaccination. At approx. the same time, blood plasma samples were taken for antibody analysis from the fish groups residing in tank 2. The fish groups in tank 3 were maintained longer and challenged at approx. 1500 dd post vaccination. In tank 3, (1500 dd groups) blood plasma sampling was carried out using 20 individuals from each group leading to a reduced number of challenged fish. The tanks were supplied with fresh water with a holding temperature of 12 ± 1 °C. After five weeks with a photoperiod regime of 12:12 fish were smoltified using continuous light. The 1000 dd challenge groups were adapted to seawater during the 420–936 dd period post-vaccination. The 1500 dd challenge groups were adapted to seawater during the 936–1439 dd period post-vaccination. The photoperiod regime of constant light was continued after smoltification. The fish were then further acclimatized to 8 °C before experimental bath challenged at 1038 and

Table 1
Experimental vaccinated fish bath challenged to *Moritella viscosa* (adapted from Karlsen et al., 2015).

Immunization period[a]	Injection group[b]		No. of fish	Challenge dose cfu ml^{-1}	Acc. mort.[c]	Survivors with ulcerative category			RPS_{Term} % (*p*-value)[d]	RPP vs. LD (*p*-value)
						Score 0	Score 1	Score 2		
Study 1 600 dd	1:	Pentium Forte Plus (PFP)	110	2.5×10^5	26	n.d.	n.d.	n.d.	75 (<0.0001)	n.d.
	2:	Alpha Ject 6-2 (AJ6-2)	109		47	n.d.	n.d.	n.d.	55 (<0.0001)	n.d.
	3:	Norvax Compact 6 (NC-6)	108		41	n.d.	n.d.	n.d.	60 (<0.0001)	n.d.
	4:	Saline (0.9% NaCl)	111		106	n.d.	n.d.	n.d.	n.a.	n.d.
Study 2 1000 dd	1:	Pentium Forte Plus (PFP)	110	4.2×10^6	41	47	16	6	50 (<0.0001)	37 (0.0001)
	2:	Norvax Minova 6 (NMin-6)	109		65	19	16	9	20 (0.018)	9 (0.08)
	3:	Alpha Ject 6-2 (AJ6-2)	109		54	20	23	12	44 (0.0001)	10 (0.06)
	4:	Alpha Ject Micro 6 (AJM-6)	107		42	41	16	8	48 (<0.0001)	32 (<0.0001)
	5:	Saline (0.9% NaCl)	110		82	14	9	5	n.a.	n.a.
	6:	Lipogen Duo (LD)	107		80	10	4	13	n.a.	n.a.
Study 2 1500 dd	1:	Pentium Forte Plus (PFP)	87	1.1×10^6	4	67	13	3	91 (<0.0001)	65 (<0.0001)
	2:	Norvax Minova 6 (NMin-6)	88		28	41	14	5	36 (0.015)	19 (0.08)
	3:	Alpha Ject 6-2 (AJ6-2)	89		15	52	19	3	66 (<0.0001)	37 (0.001)
	4:	Alpha Ject Micro 6 (AJM-6)	87		9	52	20	6	79 (<0.0001)	39 (0.0006)
	5:	Saline (0.9% NaCl)	89		57	16	13	3	n.a.	n.a.
	6:	Lipogen Duo (LD)	86		43	29	8	6	n.a.	n.a.

[a]Degree days (dd). [b]Intraperitoneal injections of 0.1 ml (AJM-6, 0.05 ml). Commercial vaccine producers: PFP and LD, Novartis/Elanco; AJ6-2 and AJM-6, Pharmaq; NC-6 and NMin-6, MSD Animal Health. All six-component vaccines contain inactivated *Aeromonas salmonicida* subsp. *salmonicida*, *Aliivibrio salmonicida*, *Listonella anguillarum* serotype O1, *L. anguillarum* serotype O2a, *M. viscosa*, and infectious pancreatic necrosis virus. LD contains inactivated *A. salmonicida* subsp. *salmonicida* and L. *anguillarum* serotype O1. [c]Accumulated mortality (acc. mort.) [d]Control injection RPS vs. saline for the 600 dd groups, RPS vs. LD for the 1000 dd and 1500 dd groups. "n.d." = not determined, "n.a." = not applicable.