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Challenge model for effluent mediated transmission of diseases between fish species

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ABSTRACT

Waterborne transmission of fish pathogens between different fish species in aquaculture is an important issue. Particularly because there is increased interest in expanding the number of fish species in fish farming. In this study, challenge models for effluent transmission of classical vibriosis and atypical furunculosis between Atlantic salmon, cod and halibut have been evaluated by the use of a tank system designed for transmission via effluent. The system was tested with effluent transmission of classical vibriosis caused by *Vibrio anguillarum* serotype O2a from infected salmon to cod. Subsequently infected cod developed chronic infections with clinical signs such as petechial haemorrhages and ulcerations on the ventral abdominal wall, in addition to fin erosion. However, transmission of *V. anguillarum* serotype O2b could not be demonstrated from infected cod to salmon, as *V. anguillarum* could not be reisolated from moribund or dead salmon. Transmission via effluent of atypical furunculosis from infected halibut to healthy cod and halibut was confirmed. The mortality of cod and halibut in the receiving tanks was low, but physical stress seemed to provoke an outbreak in cod, but not in halibut.

1. Introduction

Farming of Atlantic cod, *Gadus morhua* L. and Atlantic halibut, *Hippoglossus hippoglossus*, are growing industries in Norway. However, today the costal area of Norway is occupied with large facilities for Atlantic salmon, *Salmo salar* L, and new farms will most likely be situated close to them.

Vibriosis is a fatal haemorrhagic septicaemia that affects marine fish species world wide (Toranzo et al., 2005; Austin and Austin, 1987). At present, classical vibriosis is one of the most serious bacterial diseases for farmed cod in Norway (Samuelsen et al., 2006). The disease is caused by *Vibrio anguillarum*, that has a broad host range and several serotypes have been described (Larsen et al., 1994). *V. anguillarum* serotype O1 mainly affects salmonids, turbot (*Scophthalmus maximus*) and halibut while serotype O2 affects both salmonids and marine fish species (Larsen et al., 1994; Knappskog et al., 1993; Myhr et al., 1991; Egidius and Andersen, 1978). *V. anguillarum* serotype O2 is a heterogeneous serotype and can be divided into the sero-subtypes O2a and O2b (Knappskog et al., 1993; Rasmussen, 1987a,b). In addition, there are *V. anguillarum* O2 isolates from cod that differ from serotype O2a and O2b with respect to genetical, biochemical and serological characteristics (Mikkelsen et al., 2007).

Atypical furunculosis is caused by atypical *Aeromonas salmonicida*, a heterogeneous group of bacteria with respect to biochemical and molecular characteristics (Wiklund and Dalsgaard, 1998). Atypical strains of *A. salmonicida* cause haemorrhagic septicaemia and ulcerative disease. The disease have been reported in both farmed and wild fish species e.g. cod, halibut, turbot, spotted wolffish (*Anarhichas minor*) and common wolffish (*Anarhichas lupus*) in addition to salmonids and ornamental fish species (Toranzo et al., 2005; Magnadottir et al., 2002; Hiney and Oliver, 1999; Wiklund and Dalsgaard, 1998; Wiklund et al., 1994). Atypical furunculosis is the major bacterial disease problem for farmed cod in Iceland (Magnadottir et al., 2002) and in halibut farming in Norway (Skjelstad et al., 2008). In addition, the disease has been registered in an increasing number of cod farms in Norway (Skjelstad et al., 2008).

Today, all farmed salmon in Norway are vaccinated with oil based vaccines and are highly protected against at least *V. anguillarum* serotype O1 and O2a, *V. salmonicida* and *A. salmonicida* subsp. *salmonicida*. Farmed cod are vaccinated against classical vibriosis (*V. anguillarum* serotype O1, O2a and O2b) while farmed halibut are not vaccinated.

Horizontal transmission of pathogens is possible through faecal materials, feed particles or the waterborne route. Natural waterborne transmission has been demonstrated for infectious haematopoietic necrosis virus among spawning sockeye salmon, *Oncorhynchus nerka*, in river systems (Mulcahy et al., 1983). Furthermore, infectious diseases may be transmitted between fish species reared in the same geographical area, resulting in outbreaks of diseases or establishment of asymptomatic carriers. For example, the disease

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cold water vibriosis was transmitted from farmed salmon to wild caught cod in neighbouring net pens resulting in outbreaks of disease among the cod after transportation to new locations (Sørum et al., 1990; Jørgensen et al., 1989). Also, nodavirus has been shown to transmit from infected net pen-reared sea bass (*Dicentrarchus labrax*) to sea bream (*Sparus aurata*) (Castric et al., 2001). In addition, waterborne transmissions of pathogens from farmed to wild fish stocks are likely and a recent report presents a summary of possible transmissions of important fish diseases (Raynard et al., 2007). To our knowledge there are only two reports based on using effluent to study horizontal transmission of pathogens between fish species. Effluent transmission of the parasite *Enteromyxum leei* has been shown from gilthead sea bream to red drum (*Sciaenops ocellatus*) (Diamant, 1998) and to sea bass (Sitja-Bobadilla et al., 2007).

The aim of the current study was to establish challenge models for effluent mediated transmission of diseases in order to assess the risk of spreading bacterial diseases between fish species. Transmission of classical vibriosis and atypical furunculosis were performed in a tank system where fish of one species were infected in one tank and the effluent was transmitted to receiving tanks with naïve fish. This system may be used to predict spreading of diseases between new species in fish farming or between wild and farmed fish.

2. Materials and methods

2.1. Experimental facilities for effluent mediated transmission of pathogens

The tank system to study effluent mediated transmission of pathogens is composed of a main tank of 500 L connected to three receiving tanks of 300 L (Fig. 1). The tanks were centrally drained and effluent from the main tank was collected in a separate waste-tank. Here, particles such as faecal materials and feed were removed by sedimentation and clear effluent was pumped through pipelines to the three receiving tanks (Fig. 1). The particles were removed both to make certain that only bacteria suspended in water were transferred and to maintain as good as possible water quality in the receiving tanks. The effluent was passed through an aerator before entering the tanks. The aerator was 3/4 filled with small (9.1 × 7.2 mm) plastic beads (Krüger Kaldnes AS, Sandefjord, Norway) to ensure optimal gas exchange. By the use of a flow meter it was possible to vary the inter-mixing of effluent and clean water. Seawater with salinity of 3.4%, oxygen saturation >80% and fish density of 30 kg m^{-3} were used in all trials. In experiment 3b (Table 2), the aerator was removed and the infective effluent was scattered on the entire water surface to ensure optimal transmission of bacteria. To ensure that the fish in the receiving tanks were infected from bacteria shed from diseased fish in the main tank and not from bacteria leaking from the site of infection, effluent was not transferred from the main tank to the receiving tanks the first day post infection.

2.2. Fish

Different batches of Atlantic cod, *G. morhua* L., produced either at Troms Marine Yngel, (Tromsø, Norway), or at Sagafjord seafarm (Norway) were used in the experiments. Atlantic halibut, *H. hippoglossus* L., were produced at Risør Fisk A/S (Norway). The fish were transported to the Aquaculture Research Station (Tromsø, Norway) for grow-out in tanks at 10 °C with seawater, 24 h light and fed continuously (Dana Feed A/S, Horsens, Denmark). Atlantic salmon, *S. salar* L, postsmolts of the Norwegian salmon breeding strain (Sunndalsøra, Norway) were reared at the Aquaculture Research Station and fed standard feed (Skretting, Norway). All the fish used were healthy and unvaccinated.

The fish were anaesthetized prior to intra peritoneal injections (ip), containing either Metacainum (Norsk Medisinaldepot, Norway) 50 mg L^{-1} water (cod), Benzocain (A.C.D. SA, Belgium) 90 mg L^{-1} (salmon) or 60 mg L^{-1} (halibut). All the fish experiments were approved by the National Animal Research Authority in Norway.

2.3. Bacteria

The bacteria used were all originally isolated from cod in Norway. *V. anguillarum* strain HI 11349 serotype O2a (*Va*-O2a) was provided by the Institute of Marine Research, Norway. *V. anguillarum* strain 2001/09/700 serotype O2b (*Va*-O2b) and the atypical *A. salmonicida* strain 93/09/914 (*aAs*) were received from the Norwegian Veterinary Institute. The various bacterial strains will, from here on, be referred to as *Va*-O2a, *Va*-O2b and *aAs*.

V. anguillarum was grown in Marine Broth (MB-2216, Difco) and atypical *A. salmonicida* in Brain Heart Infusion broth (BHI, Difco) supplemented with 2% NaCl at 12 °C for 24 h. Blood agar (BA, Oxoid

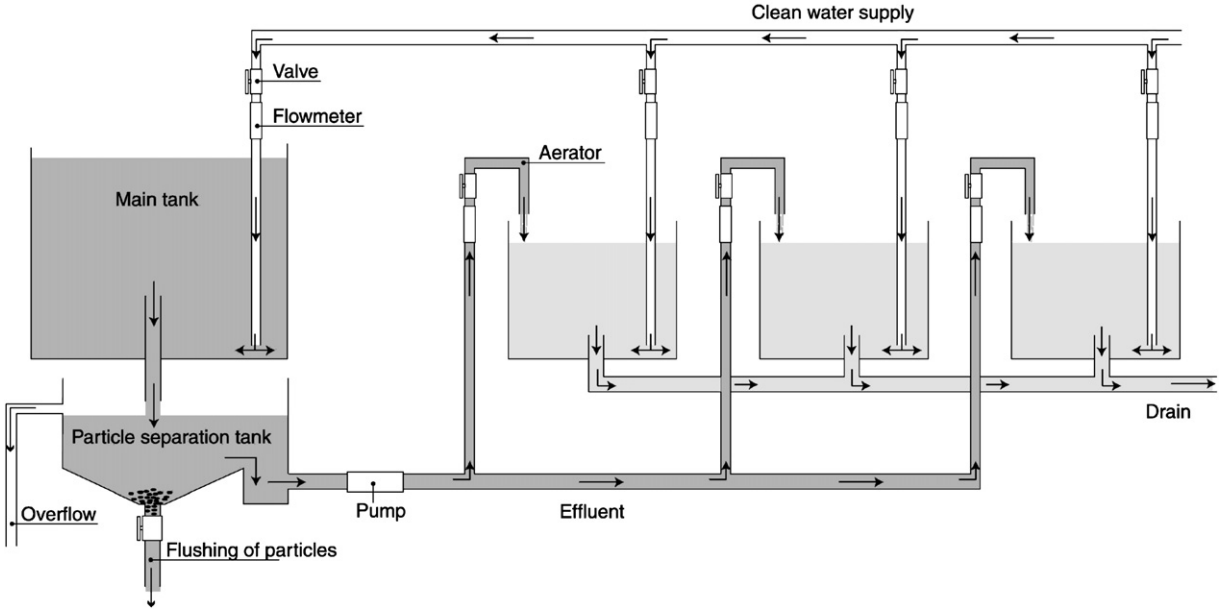

Fig. 1. Tank system for waterborne transmission via effluent of pathogens from a main tank (500 L) with infected fish to receiving tanks (300 L) with naïve fish.