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NOTE

First detection of piscine reovirus (PRV) in marine fish species

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ABSTRACT: Heart and skeletal muscle inflammation (HSMI) is a disease that affects farmed Atlantic salmon *Salmo salar* L. several months after the fish have been transferred to seawater. Recently, a new virus called piscine reovirus (PRV) was identified in Atlantic salmon from an outbreak of HSMI and in experimentally challenged fish. PRV is associated with the development of HSMI, and has until now only been detected in Atlantic salmon. This study investigates whether the virus is also present in wild fish populations that may serve as vectors for the virus. The virus was found in few of the analyzed samples so there is probably a more complex relationship that involves several carriers and virus reservoirs.

KEY WORDS: Heart and skeletal muscle inflammation · HSMI · Transmission · Reovirus · Wild marine fish species · Farmed fish · PCR

INTRODUCTION

A severe disease affecting farmed Atlantic salmon *Salmo salar* L. in Norway is heart and skeletal muscle inflammation (HSMI). The etiology of the disease is unknown. However, in 2010, piscine reovirus (PRV) was identified in fish from an outbreak of HSMI as well as in experimentally challenged fish (Palacios et al. 2010). The disease generally affects Atlantic salmon several months after transfer to seawater, when the fish weigh approximately 1 kg. Morbidity is high, as most fish in a sea cage are affected with histopathological lesions in the heart and skeletal muscle. However, the mortality varies from almost insignificant to 20% (Kongtorp et al. 2004). HSMI was first described in 1999 and the number of outbreaks has increased, with 131 registered outbreaks in 2010 (Bornøeet al. 2011). The causative agent of the disease is assumed to be a naked, double-stranded RNA virus related to the *Reoviridae* group, named PRV (Palacios et al. 2010). PRV is detected in both farmed and wild Atlantic salmon. In wild Atlantic salmon PRV has only been detected in low quantities (Palacios et al. 2010). For PRV and other viral pathogens that cause economic losses in aquaculture there is a potential for the transfer of virus from wild to farmed fish and vice versa. Other viral reservoirs and vectors in the marine environment are also possible.

MATERIALS AND METHODS

Here we study the presence of PRV in different species of marine fish sampled at many locations from the west coast to the north of Norway. The set of samples was a representative subset of the catch from 2 trawling expeditions by the Institute of Marine

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Research, Bergen. Sampling was financed and performed by the project 'Viral haemorrhagic septicaemia virus (VHSV) in wild and farmed fish in Norway' (NFR-190245). Briefly, the fish were captured by trawling and 1 g samples of spleen, kidney and brain were pooled and directly frozen in 9 ml Leibovitz (L-15) culture medium supplemented with 4 mM L-glutamine and 100 ng ml^{-1} gentamicin (all from Sigma-Aldrich). Each pool consisted of material from 2 to 5 individuals. Samples were immediately transferred to −80°C for storage. RNA was extracted from 100 µl of mixed tissue homogenate with the NucliSENS easyMAG nucleic acid extraction system (bioMérieux) according to the manufacturer's recommendations. A total of 1627 fish (379 pools) from 37 different species were sampled and screened by means of a PRV-specific RT-qPCR assay (Palacios et al. 2010).

RESULTS

The majority of the 379 pools of samples tested negative for the PRV-specific real-time PCR, but pools from 4 different fish species yielded positive results (Table 1) that had relatively high threshold cycle number (C_t)-values (great silver smelt *Argentina silus*, C_t = 37; capelin *Mallotus villosus*, C_t = 38; Atlantic horse mackerel *Trachurus trachurus*, C_t = 37; and Atlantic herring *Clupea harengus*, C_t = 31).

DISCUSSION

The fish species that tested positive with the PRV-specific real-time PCR are common species found along the Norwegian coast at different periods of the year. *Argentina silus* is a smelt of the Argentinidae family. It is a pelagic species that is common along the Norwegian coastline. The positive pool originated from *A. silus* that were caught by trawl in northern Trøndelag. *Trachurus trachurus* is a pelagic species that is common in west and northern parts of Norway during late summer and autumn. Only one *T. trachurus* was caught and it was in the Andøyfjord. *Mallotus villosus* is a small fish of the smelt family Osmeridae. This is an arctic species, but local stocks are also found along the coast of the northern part of Norway. The positive pool of *M. villosus* was from the Altafjord.

Table 1. Marine fish species, by family, tested for piscine reovirus (PRV). The 'PCR' column indicates number of positive pooled samples (**bold**) out of total pools tested. Number in brackets indicates the total number of fish in the pooled samples

Fish species	PCR
Ammodytidae	
Lesser sand eel *Ammodytes tobianus* (L.)	0/2 (10)
Raitt's sand eel *Ammodytes marinus* (Raitt)	0/2 (6)
Anarhichadidae	
Atlantic wolffish *Anarhichas lupus* (L.)	0/5 (22)
Argentinidae	
Great silver smelt *Argentina silus* (Ascanius)	**1**/38 (176)
Carangidae	
Atlantic horse mackerel *Trachurus trachurus* (L.)	**1**/1 (1)
Clupeidae	
Atlantic herring *Clupea harengus* (L.)	**1**/37 (170)
Cyclopteridae	
Lumpsucker *Cyclopterus lumpus* (L.)	0/1 (1)
Gadidae	
Atlantic cod *Gadus morhua* (L.)	0/18 (78)
Blue whiting *Micromesistius poutassou* (Risso)	0/23 (104)
Greater forkbeard *Phycis blennoides* (Brünnich)	0/2 (6)
Four-bearded rockling *Rhinonemus cimbrius* (L.)	0/1 (1)
Haddock *Melanogrammus aeglefinus* (L.)	0/26 (121)
Common ling *Molva molva* (L.)	0/4 (6)
Norway pout *Trisopterus esmarkii* (Nilsson)	0/64 (336)
Poor cod *Trisopterus minutus* (L.)	0/7 (27)
Pollock *Pollachius virens* (L.)	0/21 (81)
Silvery cod *Gadiculus argenteus* (Guichenot)	0/12 (60)
Whiting *Merlangius merlangus* (L.)	0/12 (54)
Atlantic pollock *Pollachius pollachius* (L.)	0/1 (2)
Blue ling *Molva dypterygia* (Pennant)	0/2 (3)
Lophiidae	
Anglerfish *Lophius piscatorius* (L.)	0/8 (14)
Lotidae	
Tusk *Brosme brosme* (Ascanius)	0/10 (13)
Merluccidae	
European hake *Merluccius merluccius* (L.)	0/7 (25)
Osmeridae	
Capelin *Mallotus villosus* (Müller)	**1**/16 (79)
Phycidae	
Atlantic halibut *Hippoglossus hippoglossus* (L.)	0/1 (1)
Lemon sole *Microstomus kitt* (Walbaum)	0/3 (11)
American plaice *Hippoglossoides platessoides* (Fabricius)	0/2 (10)
European plaice *Pleuronectes platessa* (L.)	0/6 (24)
Witch flounder *Glyptoceophalus cynoglossus* (L.)	0/5 (15)
Scophthalmidae	
Megrim *Lepidorhombus whiffiagonis* (Walbaum)	0/3 (4)
Sebastinae	
Norway redfish *Sebastes viviparus* (Krøyer)	0/17 (83)
Rose fish *Sebastes marinus* (L.)	0/6 (20)
Sebastes sp. (Cuvier)	0/11 (52)
Squalidae	
Spiny dogfish *Squalus acanthias* (L.)	0/1 (5)
Sternoptychidae	
Pearlsides *Maurolicus muelleri* (Gmelin)	0/1 (2)
Triglidae	
Grey gurnard *Eutrigla gurnardus* (L.)	0/2 (4)
Zoarcidae	
Checker eelpout *Lycodes vahlii gracilis* (Sars)	0/1 (1)

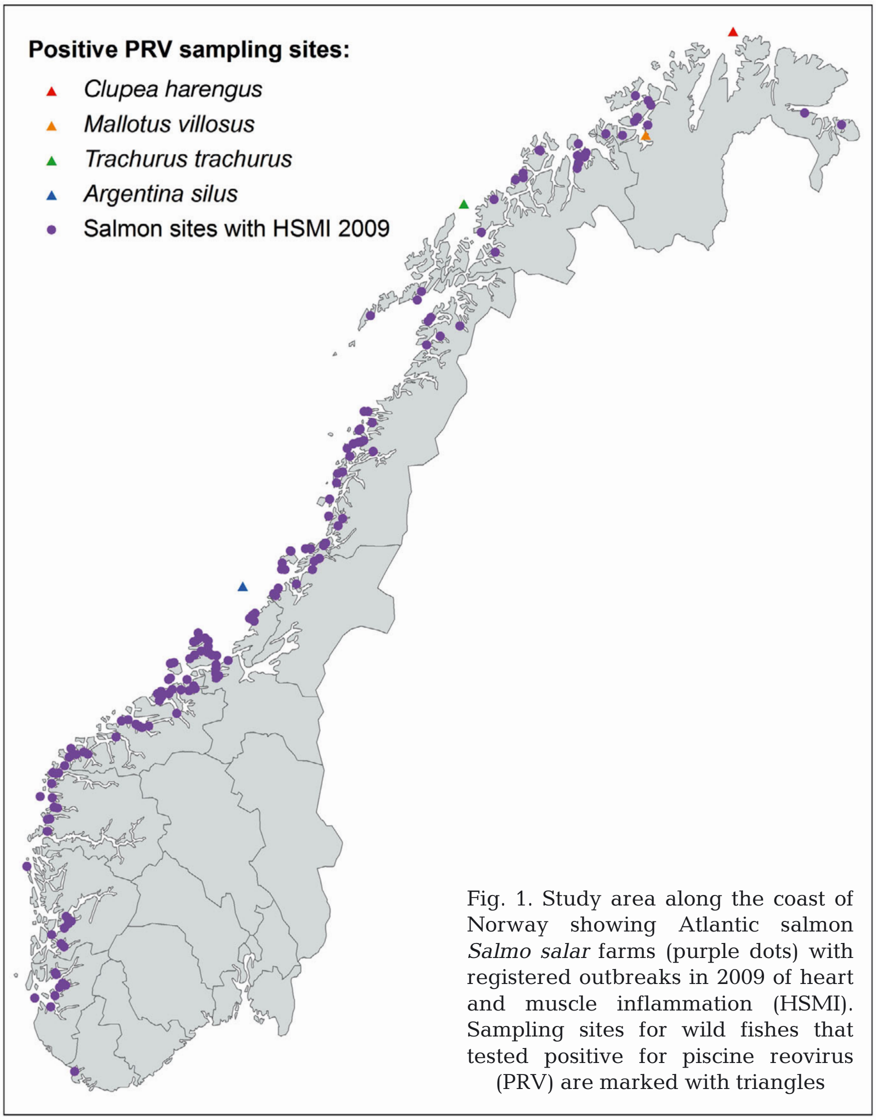

Fig. 1. Study area along the coast of Norway showing Atlantic salmon *Salmo salar* farms (purple dots) with registered outbreaks in 2009 of heart and muscle inflammation (HSMI). Sampling sites for wild fishes that tested positive for piscine reovirus (PRV) are marked with triangles

Clupea harengus, in the family Clupeidae, is one of the most abundant fish species in the world and is common all along the Norwegian coastline. The pool of PRV-positive *C. harengus* was from the Tanafjord. Both *C. harengus* and *M. villosus* are important species in Norwegian fisheries. Interestingly, none of the positive samples were from fish caught close to fish farms that had registered outbreaks of HSMI (Fig. 1).

Few of the tested pools yielded a positive PCR result, which indicated there was a low prevalence of PRV in wild fish. However, underestimation of the real number of carriers owing to the use of fish pools cannot be ruled out. Moreover, to date PRV has not been cultured so it was not possible to decide whether the low levels of PRV RNA detected in the present study represent viral RNA contained in viable viral particles.

It is uncertain whether the species that tested positive for PRV are relevant vectors for the horizontal transfer of PRV to farmed fish. Johansen et al. (2011) have reviewed the existing literature on transmission of viral pathogens from wild fish species to farmed fishes. For several viruses causing losses to the aquaculture industry there was no evidence for transmission of the virus between wild and farmed fish (Johansen et al. 2011). The period of shedding and how long the viral particles can remain infectious in seawater is not known. However, VHSV, another virus affecting fish, has been reported to 'survive' up to 13 d in seawater (Hawley & Garver 2008). It is, therefore, reasonable to assume that there is a complex relationship involving several carriers and virus reservoirs of PRV.

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