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Short Communication

Prevalence of piscine myocarditis virus (PMCV) in marine fish species

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Cardiomyopathy syndrome (CMS) is an inflammatory disease of the heart primarily affecting farmed Atlantic salmon, *Salmo salar* L. (Ferguson, Poppe & Speare 1990). The disease mainly appears in fish 12–15 months after transfer to sea water, and pathological signs include inflammation of the endocardium and spongiosum of the atrium and ventricle. Highest mortality rates are seen in fish weighing 2–5 kg, and the cause of death is generally rupturing of the atrium or sinus venosus. The disease has been reported from locations all along the Norwegian coastline since the 1980s (Amin & Trasti 1988) and has also been observed in Scotland (Rodger & Turnbull 2000), the Faroe Islands (Sande & Poppe 1995) and Canada (Brocklebank & Raverty 2002). CMS causes substantial financial losses for the fish farming industry (Brun, Poppe, Skrudland & Jarp 2003), and CMS-like lesions have also been observed in wild Atlantic salmon (Poppe & Seierstad 2003).

The causative agent is most likely a naked, double-stranded RNA virus related to the *Totiviridae* group, provisionally named piscine myocarditis virus (PMCV) (Lovoll, Wiik-Nielsen, Grove, Wiik-Nielsen, Kristoffersen, Faller *et al.* 2010; Haugland, Mikalsen, Nilsen, Lindmo, Thu, Eliassen *et al.* 2011), and thus far, the virus has been found exclusively in farmed Atlantic salmon sampled from pens with CMS. Using a PMCV-specific real-time PCR assay (Lovoll *et al.* 2010), we have screened for the presence of PMCV in more than 30 species of marine fish (Table 1) sampled off the central, western coastline of Norway from Trondheim to the Varanger fjord. The set of samples was a representative subset of the catch from a single trawling expedition. Sampling was performed and financed by the project 'Viral haemorrhagic septicaemia virus (VHSV) in wild and farmed fish in Norway' (NFR-190245), and more details on the sampling will be published later along with other results from this project. Organ samples of spleen, kidney and brain were pooled and directly frozen in transport medium (Leibovitz culture medium supplemented with 4 mM L-glutamine and 100 ng mL^{-1} gentamicin; all from Sigma-Aldrich). Each pool consisted of material from 1 to 5 individuals of the same fish species sampled at the same time and place. For some of the samples included in the pools, kidney tissue from individual fish was also stored on RNAlater (Qiagen AB). Nucleic acids from the organ pools were extracted from 100 uL of tissue homogenate using the NucliSENS easyMAG nucleic acid extraction system (bioMérieux, Inc.) according to the manufacturer's recommendations. RNA from individual fish kidneys was extracted using an RNeasy Mini kit (Qiagen AB).

A total of 342 pools (1501 individuals) representing 32 species were screened. The majority of the samples were negative, but 11 of 38 pools from *Argentina silus* gave a positive PCR result (Table 1).

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Table 1 PCR results for piscine myocarditis virus for different species [number of positive pools/number of pools tested (number of individual fish represented by the pools)]

Family/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	
Atlantic argentine, *Argentina silus* Ascanius	11/38 (176)
Carangidae	
Atlantic horse mackerel, *Trachurus trachurus* (L.)	0/1 (1)
Clupeidae	
Atlantic herring, *Clupea harengus* L.	0/37 (170)
Gadidae	
Atlantic cod, *Gadus morhua* L.	0/14 (64)
Blue whiting, *Micromesistius poutassou* (Risso)	0/23 (104)
Greater forkbeard, *Phycis blennoides* (Brünnich)	0/1 (3)
Four-bearded rockling, *Rhinonemus cimbrius* (L.)	0/1 (1)
Haddock, *Melanogrammus aeglefinus* (L.)	0/26 (121)
Common ling, *Molva molva* (L.)	0/3 (5)
Norway pout, *Trisopterus esmarkii* (Nilsson)	0/64 (336)
Poor cod, *Trisopterus minutus* (L.)	0/4 (12)
Pollock, *Pollachius virens* (L.)	0/14 (52)
Silvery pout, *Gadiculus argenteus* (Guichenot)	0/12 (60)
Whiting, *Merlangius merlangus* (L.)	0/12 (54)
Lophiidae	
Anglerfish, *Lophius piscatorius* L.	0/6 (10)
Lotidae	
Tusk, *Brosme brosme* (L.)	0/9 (11)
Merluccidae	
European hake, *Merluccius merluccius* (L.)	0/1 (2)
Osmeridae	
Capelin, *Mallotus villosus* (Müller)	0/16 (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.	0/4 (17)
Witch, *Glyptoceophalus cynoglossus* (L.)	0/4 (10)
Scophthalmidae	
Megrim, *Lepidorhombus whiffiagonis* (Walbaum)	0/2 (3)
Triglidae	
Grey gurnard, *Eutrigla gurnardus* (L.)	0/2 (4)
Zoarcidae	
Checker eelpout, *Lycodes vahlii gracilis* (Sars)	0/1 (1)
Sebastinae	
Norway redfish, *Sebastes viviparus* Krøyer	0/15 (73)
Rod fish, *Sebastes marinus* (L.)	0/6 (20)
Sebastes sp.	0/11 (52)

This would indicate an approximate prevalence of PMCV in *A. silus* of 6.6% (95% confidence interval: 3.3–11.6%). To confirm the presence of PMCV in *A. silus*, a total of 30 kidney samples that were found from the positive RNA pools were subsequently screened and nine of these samples were positive.

Owing to the inherent bias introduced when choosing a particular sampling location and method, it is worth noting that in this case a relatively large number of *A. silus* were caught and subsequently screened. It is therefore possible that PMCV is present in some of the other species tested, albeit with a lower prevalence. The pooling of RNA might also have diluted some of the samples below level of detection.

Argentina silus is a smelt in the family Argentinidae. It is a pelagic species that generally forms schools close to the sea bottom, at depths down to 600 m. It is common all along the Norwegian coastline, and during autumn and winter, it migrates closer to the shore and more shallow

water. If this species is a relevant vector for PMCV and thus involved in the epidemiology of CMS, one can imagine horizontal transmission of the virus in fish pens. It is unknown how much virus is shed by infected fish or how long viral particles can remain infectious in sea water, so the possibility of a more complex relationship involving more carriers or virus reservoirs can, of course, not be excluded.

Argentina silus is also used as an ingredient in fish feed (see statistics for 'vassild' at the Statistics Norway webpage; http://www.ssb.no/). Investigations on possible survival of PMCV in fish feed production are therefore needed to exclude a possible oral transmission of the virus.

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