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SHORT COMMUNICATION

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Infectious salmon anaemia virus detected by RT-qPCR in Norwegian farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792)

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Infectious salmon anaemia (ISA), caused by infectious salmon anaemia virus (ISAV), is a notifiable infection of farmed Atlantic salmon, *Salmo salar* L. The disease is characterized by severe anaemia, infection of the circulatory system, bleeding and variable cumulative mortality.

Natural outbreaks of ISA have only been observed in farmed Atlantic salmon, but ISAV has been detected from wild, apparently healthy sea trout, *Salmo trutta* L. (Raynard et al., 2001). In addition, replication of ISAV, with or without disease development, has been demonstrated in experimentally infected rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Biacchesi et al., 2007; Olsen et al., 2012). It has been suggested that this species could act as healthy carriers and reservoirs of ISAV (Nylund et al., 1997; Snow et al., 2001).

In early February 2015, ISA was diagnosed in Atlantic salmon on a farm site in the Lofoten area in Northern Norway (Farm A). This farm stocked both Atlantic salmon and rainbow trout. The site was in an endemic area where several ISA outbreaks had occurred during the past two years; however, these sites had been fallowed at the time of the current ISAV detections. While the Atlantic salmon showed acute mortality, no morbidity or mortality was observed in the rainbow trout. The Atlantic salmon at farm A had been slaughtered by the first week of March. Sampling was performed from the rainbow trout, as described in Table 1. A second farm (Farm B) was located two kilometres from Farm A and was also stocked with both Atlantic salmon and rainbow trout. The Atlantic salmon in this farm was harvested by the end of October 2014, following a major mortality incident in mid-October with a total mortality of 56% in four netpens. The mortality incident was reported to be caused by sea lice treatment. No suspicion of ISA was reported; however, ISAV was detected in exported salmon fillets, originating from this farm, at entry inspection to China (Xiao et al., 2018). This finding was later confirmed by RT-qPCR testing of stored frozen filets by the Norwegian Veterinary Institute. Except for some moribund fish at the surface during the first sampling point four months after the Atlantic salmon were slaughtered (Table 1), no morbidity or mortality was observed in the rainbow trout.

All tissue samples and swabs were screened by RT-qPCR targeting ISAV segment 8 (Snow et al., 2006) using Ct 40 as the cut-off value. Positive samples were detected at all sampling points in both farms; however, the sample type testing positive and the proportion of positive samples differed (Table 1). This discrepancy of results

TABLE 1 Overview of rainbow trout sampling schemes and laboratory results

Farm	Average weight	Sampling date	ISAV RT-qPCR[a] Positive/analysed (Ct value[b])						Sequencing
			Kidney (RNA*later*)	Heart (RNA*later*)	Pooled heart & kidney (RNA*later*)	Plasma (RNA*later*)	Gill tissue (RNA*later*)	Gill swab (RLT-buffer (QIAGEN))	Skin swabs (RLT-buffer (QIAGEN))	
A	0.8 kg	16/3-2015	1/20 (29)	1/20 (30)		1/20 (38)	6/20 (27–37, median 30)		8/20 (32–38, median 36)	ISAV-HPR-deleted
		18/5–2015			7/10 (15–38, median 35)					ISAV-HPR-deleted
B	4.2 kg	13/2-2015			10/29 (12–36, median 33)					ISAV-HPR-deleted
		16/3-2015	0/10	0/10		0/10	0/10		7/10 (32–38, median 35)	Not performed
		18/5-2015			9/20 (29–38, median 32)					ISAV-HPR-deleted
		4/6-2015 (at slaughter)	0/25	0/25			0/25	0/25	7/25 (33–36, median 35)	ISAV-HPR-deleted

[a]RT-qPCR targeting the matrix gene (segment 8) of ISAV.

[b]The range of positive Ct values where more than one positive sample were recorded are included in brackets.

between sampling points was most likely attributed to different sampling procedures. The three samplings pooling heart and kidney tissues for RT-qPCR testing were performed by the Norwegian Food Safety Authority or a local health service. The three other samplings of separate organs, including mucosal swabs, were done by personnel from the NVI. In the latter samplings, special care was taken not to cross-contaminate samples neither within- or between-single fish.

Assuming that the rainbow trout had been infected by ISAV-infected Atlantic salmon at the respective sites and that the salmon had been infected at least three to four weeks before detection of ISAV, it seems reasonable to conclude that the ISAV infection of rainbow trout was at least initiated in early January 2015 (Farm A) and early October 2014 (Farm B). Thus, it is likely that the ISAV infection of rainbow trout in farm A and B persisted for at least 5 and 8 months, respectively (i.e., until slaughter).

Sequencing of the HE gene and F gene was performed on all sampling points. All ISAV-positive rainbow trout samples were found to be HPR-deleted and identical to the virus detected in the Atlantic salmon (GenBank accession numbers MG976849–MG976854). Also, all virus sequences were closely related to the ISA outbreaks diagnosed in this area in 2013 and 2014.

A total of 32 rainbow trout tissue samples (20 from Farm A and 12 from Farm B) were examined by histology and immunohistochemistry (IHC) (Aamelfot et al., 2012), with only mild non-specific lesions and nephrocalcinosis observed at histopathological examination. No lesions indicating ISA were observed, that is bleedings, necrosis, signs suggesting circulatory disturbances and infected endothelial cells. All samples were IHC negative, that is no indication of an active ongoing infection. Haematocrit levels were all within the normal range (data not shown).

The higher prevalence of ISAV detection by RT-qPCR in skin mucus swabs (22/55) than in gill swabs (0/25) at three samplings may point to an ISAV cell tropism in rainbow trout resembling that previously found in Atlantic salmon infected with ISAV HPR0 (Aamelfot et al., 2016), and during early infection stages with HPR-deleted ISAV in Atlantic salmon (Aamelfot et al., 2015). While samples from mucous surfaces could have been contaminated from the environment, the ISAV relatively short survival time in sea water (Tapia et al., 2013), the lack of other virus hosts than rainbow trout and the epidemiology, all suggests possible virus replication in mucosal epithelium.

In summary, the present results show that rainbow trout are susceptible to ISAV infection during seawater aquaculture conditions. However, no clinical disease was observed in the rainbow trout. Our observations and data did not allow any firm conclusions regarding the role of the rainbow trout for ISAV transmission and persistence during this local ISA epidemic in Atlantic salmon. Because both rainbow trout sites also reared Atlantic salmon and that the first infected farm in the control zone was diagnosed with ISA nearly two years prior to the detections in the rainbow trout, the authors assume that the rainbow trout acquired the ISAV from infected Atlantic salmon. Based on this assumption, our data indicate that the ISAV infection persisted in the rainbow trout populations for

several months. Thus, from a regulatory point of view, sub-clinically infected rainbow trout may contribute to sustaining ISAV infection on a site or in an area. Added to this, the results also support previous experimental findings suggesting rainbow trout as a potential reservoir of ISAV (Nylund et al., 1997; Snow et al., 2001).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Results and data related to this publication will be deposited on the Norwegian Veterinary Institute's Research Data Repository—BIRD and the publication made available upon acceptance via BRAGE. These are open access repository systems for data and publications that fulfil the legal requirements from Norwegian authorities in what concerns free access to science.

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