NOTE

# Prevalence of salmonid alphavirus in Scottish fish farms from 2006 to 2007

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**Abstract**
During routine inspections of farms in 2006 and 2007 virological and molecular samples were collected from fish to test for SAV. A total of 104 (30 freshwater and 74 marine) sites were tested for SAV. A prevalence of 18% was recorded from 2006 to 2007.

Pancreas disease (PD) in Atlantic salmon, *Salmo salar* L. and sleeping disease (SD) in rainbow trout, *Oncorhynchus mykiss* were first described in Scotland in 1984 (Munro et al., 1984) and in France in 1994 (Boucher and Laurencin, 1994). The PD and SD causative agent, an alphavirus referred to as salmonid alphavirus (SAV), was not identified nor isolated until 1995 for PD (Nelson et al., 1995), and not until 1997 for SD (Castric et al., 1997). Currently PD has been diagnosed in the UK, Ireland and Norway (McLoughlin and Graham, 2007). There have also been reports of PD in Atlantic salmon reared in North America but no agent was identified or characterized (Kibenge et al., 2000; Kent and Elston, 1987). SD virus has also been isolated from rainbow trout in Germany, Italy, Scotland and Spain (McLoughlin and Graham, 2007).

Comparison of genome sequences revealed that the virus that causes PD and SD are closely related subtypes of the same species from the family *Togaviridae* (Weston et al., 2002). Historically, isolates from rainbow trout reared in freshwater are assigned to subtype 2, whilst isolates from Atlantic salmon in Scotland and Ireland belong to subtype 1 (Weston et al., 2005). SAV isolates from Norwegian Atlantic salmon fall into a third subtype (Hodneland et al., 2005; Weston et al., 2005). Fringuelli et al. (2008) identified three further subtypes (4, 5 and 6) which represent PD isolates from Ireland and Scotland and the first occurrence of a subtype 2 SAV isolate originating from Atlantic salmon.

Pancreas disease is responsible for significant losses to the industries of Ireland, Norway and Scotland. In Ireland an estimated €12 million loss was reported in 2003-2004 and the economic impact in Norway is estimated to be €100 million per year (Ruane et al., 2008). In Ireland, 59% of sites in 2002 experienced PD (McLoughlin et al., 2003) increasing to 13 of 21 (62%) sites in 2003 and 12 of 14 (86%) sites in 2004. A reduction in the number of active sites may have contributed to this apparent

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increase (Rodger and Mitchell, 2007). Sleeping disease is endemic in areas of France and reported mortality rates vary (Boucher and Laurencin, 1994; Graham et al., 2003; Graham et al., 2007). In contrast, there has been little focus on PD or SD in Scotland since the early 1990's. McVicar (1987) reported that PD prevalence had increased from the 1980's onwards with 19% of marine sites reporting PD outbreaks in 1985. A serological survey in 1998 confirmed the presence of antibodies to salmon pancreas disease virus in Scotland; however only 8 sites were tested so no comparison on prevalence could be made to previous data (McLoughlin et al., 1998)

Unlike other viral diseases SAV is not screened for as part of an official surveillance scheme in Scotland however it is routinely tested for as a result of diagnostic investigations, meaning that information on the current prevalence of SAV in Scotland is lacking. The aim of this study was to gather data on SAV positive sites in Scotland between 2006 and 2007 to investigate the prevalence and epidemiological significance of alphaviruses on Scottish fish farms.

Tissues were collected from Scottish Atlantic salmon and rainbow trout farms during routine site inspections between 2006 and 2007 and prevalence determined. Kidney, heart and spleen from up to 30 fish were pooled in transport media (Leibovitz-15 medium (Lonza, Basel, Switzerland) supplemented with 10% Newborn calf serum (Invitrogen, Paisley, UK) v/v, 200 U/ml penicillin-streptomycin (Invitrogen, Paisley, UK) 1 mg ml-1, gentamycin (Sigma, Dorset, UK), 200U/ml polymixin B sulphate (Sigma, Dorset, UK ) adjusted to a pH of 7.4 with NaCl for virus isolation. Pools of kidney from up to 30 fish were collected into RNA Later (Ambion, Inc) for molecular analysis.

Virus culture, RNA extraction and real-time RT-PCR were performed as described previously (Snow et al., 2010). Any cultures showing CPE were confirmed by real-time PCR performed on RNA extracted from the cell cultures.

A total of 104 (30 freshwater and 74 marine) Scottish farm sites (Figure 1) were tested for SAV. In total 19 sites tested positive for SAV by molecular and virological methods. Table 1 details the number of sites for each species tested and the number of positive sites detected. From this data a prevalence of 18% ($p>0.10$) can be calculated. There is no evidence of a significant difference

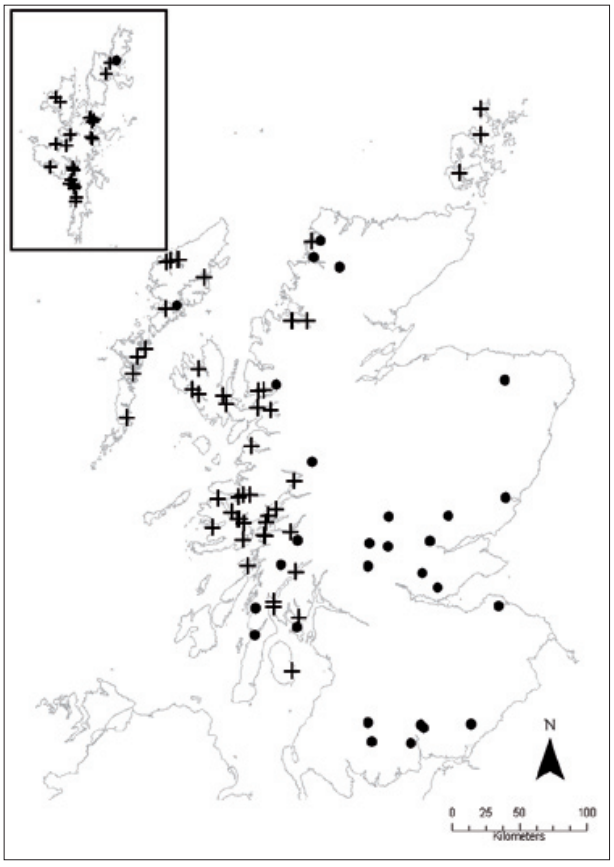


**Figure 1.** Location of sites tested for SAV between 2006-2007. + seawater, ● freshwater.

**Table 1.** Numbers of trout and salmon sites tested for salmonid alphavirus (SAV) and the number of positives detected. FW=Freshwater, SW=Seawater.

| Species | Environment | No. tested | No. positive |
|---|---|---|---|
| Rainbow trout | FW | 23 | 6 |
| | SW | 1 | 0 |
| Atlantic salmon | FW | 7 | 0 |
| | SW | 73 | 13 |
| Total | | 104 | 19 |

**Table 2.** Details of the origins of the 11 isolates sequenced in this study. AS=Atlantic salmon, RT= Rainbow trout. (Extracted from Snow et al., 2010).

| Subtype | Virus | Year | Species | Country | Source |
|---|---|---|---|---|---|
| I | SCO 07-920 (3) | 2007 | AS | Scotland | CHSE-214 |
| | SCO-07 765 (1) | 2007 | RT | Scotland | CHSE-214 |
| | SCO 07-887 (1) | 2007 | AS | Scotland | CHSE-214 |
| II | SCO 07-501 (1) | 2007 | AS | Scotland | Kidney |
| | SCO-07-292 (1) | 2007 | RT | Scotland | Kidney |
| | SCO 06-396 (2) | 2006 | RT | Scotland | CHSE-214 |
| | SCO-07-200 (15) | 2007 | RT | Scotland | CHSE-214 |
| | SCO-07-110 (1) | 2007 | RT | Scotland | Kidney |
| | SCO-07-376 (1) | 2007 | AS | Scotland | Kidney |
| IV | SCO-07-256 (4) | 2007 | AS | Scotland | Kidney |
| V | SCO-07-496 (1) | 2007 | AS | Scotland | CHSE-214 |

in prevalence between Atlantic salmon and rainbow trout species ($p>0.10$) or freshwater and seawater environment ($p>0.10$).

Eleven isolates identified as part of the prevalence survey (Table 2) were analysed based on the partial E2 gene nucleotide sequence (357bp) as previously reported by Snow et al. (2010). All isolates could be classified within the subtypes previously identified by Weston et al. (2005), Hodneland et al. (2005) and Fringuelli et al. (2008). The first isolation of a subtype 1 SAV was sequenced from freshwater rainbow trout in October; the fish were 40-50g.

This survey was undertaken to provide an estimate of SAV prevalence in farmed salmonids in Scotland between 2006 and 2007. There were approximately 400 active sites holding either Atlantic salmon or rainbow trout at this time and SAV was detected, using virological and/or molecular techniques, from 18% of the tested sites over a two year period. From the limited data, there was no evidence of difference in infection between Atlantic salmon and rainbow trout or freshwater and seawater environments. Unfortunately the power of these comparisons is considered to be low due to insufficient data. This result is comparable to a previous study

conducted in Scotland which found 19% of marine salmon farming sites to be affected by PD using histological diagnosis (McVicar, 1987). However, this is lower than results reported in Ireland which found 59% of marine sites to be affected by PD in 2002 increasing to over 90% by 2007 (Ruane et al., 2008). The majority of sites surveyed were only tested once during the 2 year period, hence SAV infections occurring during the production cycle either before or after the site visit would not have been detected. SAV has also been shown to be less persistent in kidney material than other tissues such as heart and gill where the virus was detected by real-time PCR up to 42 weeks after infection (Graham et al., 2010). In addition the detection of antibodies against SAV would have improved our understanding of past infection, in this context Graham et al. (2005) reported that antibodies can be detected up to 36 weeks after the virus can no longer be isolated. However, blood samples are not taken routinely during fish health inspections by this laboratory.

Fish that were confirmed as positive for SAV did not always exhibit signs of pancreas or sleeping disease and in some cases no infection was recorded on site either previously or subsequent to the site inspection. Previous work has demonstrated that sub-clinical infections are possible and that the occurrence of clinical disease appears to be dependent on a number of factors; for example stress, viral load, fish strain and temperature (Graham et al., 2006; McLoughlin and Graham, 2007; McLoughlin et al., 2003; McLoughlin et al., 2006).

Sequencing data, as previously reported by Snow et al. (2010), collected as part of this study contributes to previous findings on subtype and agrees with Fringuelli et al. (2008) and Hodneland et al. (2005). Six isolates of subtype 2 from marine Atlantic salmon were identified as well as the first isolation of subtype 1 in freshwater rainbow trout. Subtype 1 has previously only been isolated from Atlantic salmon. Although the source of this infection is unknown it is evident from this study that transfer of viral subtypes between marine and freshwater and between species may occur.

No clear picture of SAV epidemiology could be deduced from our data. There is no pattern to subtype and no link between similar or identical viral isolates geographically or by company. As SAV is not notifiable in the UK it is not routinely screened for, meaning less data is collected for SAV than other pathogens thus hindering interpretation of its distribution. It is known that the virus can transmit horizontally (McLoughlin and Graham, 2007) implying that the role of industry practices in the transfer of SAV could potentially be important.

In conclusion, this study has provided an estimate of the prevalence of SAV in salmonids throughout fish farms in Scotland. Characterization of an additional 11 isolates contributes to updating the understanding regarding the distribution and variation of SAV in Scotland. It also includes the first isolation of a freshwater SAV subtype 1 from rainbow trout. The unidentified links between sites emphasises that the complicated epidemiology of SAV is further compounded by other factors such as lack of routine identification, control and reporting.

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