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Infectious pancreatic necrosis virus isolated from hake, *Merluccius merluccius*, from Scotland

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Wild marine fish caught from a sea loch on the West Coast of Scotland were tested for infectious pancreatic necrosis virus (IPNV). One pool of 10 hake was found to be IPNV positive from 449 fish tested. This is believed to be the first isolation of IPNV made from hake caught in northern European waters. This was the only positive pool obtained from the study, illustrating a low prevalence of IPNV in wild marine fish caught from this location.

The infectious pancreatic necrosis virus (IPNV) has been isolated from a number of fresh water and marine species of fish (Munro et al., 1976; Mortensen et al., 1993; Diamant et al., 1988). IPNV is the causative agent of clinical infectious pancreatic necrosis (IPN). This is currently the most serious viral disease of farmed Atlantic salmon (*Salmo salar*) in the EU (Ariel & Olesen, 2002) and causes varying levels of mortality in the marine and freshwater life stages of this species (Smail et al., 1992). The prevalence of IPNV in Scottish salmon farms has steadily increased each year since 1996 by 10% in marine and 2 – 3% in fresh waters and by 2001 had reached up to 80% in some salmon producing regions of Scotland (Murray et al., 2003). The prevalence of IPNV in Scottish wild freshwater fish was reported to be less than 1% by Munro et al., (1976). There is limited published information on IPNV from wild marine species in Scotland, with one isolation being reported from common dab (*Limanda limanda*), (Diamant et al., 1988).

Due to the diversification of aquaculture in Scotland, the cultivation of marine species such as cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) is increasing. Determining the prevalence of IPNV in wild marine species is therefore important in view of the potential for the transfer of disease agents between wild and farmed fish.

The study was carried out in a sea loch, situated in Argyll on the West Coast of Scotland in December 2002 (Figure 1). This sea loch is approximately 5km long, up to 800m wide with a maximum depth of around 40m.

Demersal trawling was carried out from the fisheries research vessel *Aora* operated by the University of Glasgow to catch wild marine fish. The catch was sorted into individual species, kept cool on ice and taken to a shore-based laboratory for sampling.

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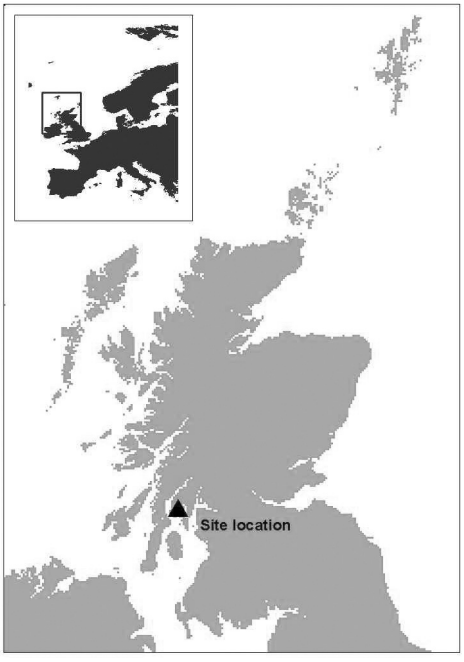

Figure 1. Location of the study site.

Due to the high numbers of fish to be tested, most individuals were grouped in pools of 10, although other pool sizes were taken depending on fish numbers (Table 1). For each pool, 5mm^3 sections of kidney tissue were aseptically removed from each fish. The pooled tissue sections were placed into a sterile 2ml dry tube that was snap frozen and stored at –80^{0}C.

Prior to virological screening, all tissue samples were thawed at 20^{0}C for 2 hours. The tissue culture methodology employed was the standard virological testing method described by Munro et al., (2004). The ELISA used was a double antibody sandwich ELISA using polyclonal antibody to the A2 (Sp) serotype to capture antigen and the monoclonal antibody CE4, which cross reacts to A2, A3 (Ab) and A5 (Te) serotypes as the secondary antibody (Ross et al., 1991).

A total of 449 wild marine fish (48 pools) were caught and sampled (Table 1). An isolation of IPNV was made from one pool containing 10 hake from a total of 6 pools (58 fish). All hake were between 10 and 17 cm in fork length with a mean fork length of 13.6cm. The virus was

Species	Number of fish	Pool size	Number of pools
Cod *(Gadus morhua)*	129	10 (One pool of 9)	13
Grey gurnard *(Eutrigla gurnardus)*	4	4	1
Haddock *(Melanogrammus aeglefinus)*	40	10	4
Hake *(Merluccius merluccius)*	58	10 (One pool of 8)	6
Norway pout *(Trisopterus esmarki)*	1	1	1
Plaice *(Pleuronectes platessa)*	40	10	4
Pollack *(Pollachius pollachius)*	2	2	1
Poor cod *(Trisopterus minutus)*	24	10 & 14	2
Saithe *(Pollachius virens)*	1	1	1
Whiting *(Merlangius merlangus)*	150	10	15
Total	**449**		**48**

Table 1. Species, numbers and pool sizes of fish sampled.

detected on the second passage and ELISA confirmed this isolation as serogroup A IPNV. No clinical signs of disease were observed in any of the fish sampled.

The isolate was grown on CHSE-214 cells, and observed microscopically for the onset of CPE, the infected cell monolayers were harvested and a 400µl aliquot was used for RNA extraction. RNA was extracted using TRIzol reagent (LifeTechnologies) according to manufacturer's recommendations.

A 1180bp portion of the VP2 region of segment A was amplified using primers A1 and A2 (Blake et al., 1995) in reaction mixtures prepared according to the method of Mjaaland et al. (2002). Amplification reactions were subjected to 45 cycles of 94^0C for 1 minute, 54^0C for 1 minute and 72^0C for 1 minute followed by an extension step of 5 minutes at 72^0C.

PCR products were purified using the Wizard PCR prep DNA purification system (Promega, Southampton, UK) according to manufacturer's instructions. These were quantified on a 1.0% (w/v) agarose gel alongside known molecular weight mass markers. The PCR products were sequenced using primers A1/IPN AF (5'-ACGAACCCCCAGGACAAAG-3') and A2/IPN AR (5'-TTGACCCTGGTGATCGGCTT-3') at 3.2pmol. Reactions were carried out using BigDye™ terminator cycle sequencing ready reaction mix followed by electrophoresis on an ABI 377 sequencer (Perkin-Elmer, Warrington, UK). The sequences were analysed using Sequencher™ 3.0 software (Gene codes corporation, Ann Arbor, MI, USA).

A partial sequence of the VP2 gene consisting of 895bp was obtained from the hake isolate (Accession number AJ877117) and compared with a Scottish farmed Atlantic salmon isolate (Accession number AJ877116) obtained from the same geographic area and IPNV Sp (Accession number AF342728). A summary of the nucleotide and deduced amino acid sequence similarity of the three isolates are shown in (Table 2). Within the VP2 region, at the nucleotide level, the hake isolate (AJ877117) exhibits 99.55% similarity to the farmed salmon isolate (AJ877116) and 97.65% similarity to the salmon Sp isolate (AF342728). At the amino acid level, similarity was 99.66% and 97.65% respectively. This difference resulted in one amino acid change from isoleucine in the farmed salmon isolate to valine in the hake.

This study reports the isolation of IPNV from hake. This is the first reported isolation of IPNV from this species and indicates hake as a potential reservoir of IPNV.

The isolate was classed as a serogroup A birnavirus and this group also includes the Scottish salmonid isolations (Hill & Way 1995). Therefore, in this study, the isolation is referred to as IPNV rather than an aquatic birnavirus.

		Nucleotides		
		AJ877117	AJ877116	AF342728
Amino Acids	AJ877117	-	99.55	97.65
	AJ877116	99.66	-	97.43
	AF342728	97.65	97.99	-

Table 2. Sequence similarity of the VP2 gene from genome segment A. AJ877117 (Hake isolate), AJ877116 (Farmed Atlantic salmon isolate) and AF342728 (Sp from GenBank).

Tissue culture was used as the diagnostic method due to the high specificity. The ELISA is shown to only produce positives with IPNV serogroup A isolations. In addition, the use of virological screening confirms that the isolate is infective and enables further studies on genetic characteristion and virulence because an isolate can be re-grown. In this study, fish were pooled for screening as a logistical requirement and it is known that this reduces the likelihood of detecting virus at low prevalence. Screening the samples using RT-PCR would potentially be more sensitive than tissue culture, however, at present a suitable diagnostic PCR capable of distinguishing IPNV from other aquatic birnaviruses has not been validated. Therefore, tissue culture was used for surveillance of wild fish for IPNV in Scotland.

Genetic analysis of the hake isolate supported the serological classification and confirmed this isolate was IPNV. The hake isolate exhibited a high degree of nucleotide similarity (99.55%) to the farmed salmon isolate from the same geographic location. At the amino acid level, there was one amino acid difference between the two isolates, from isoleucine in the farmed salmon isolate to valine in the hake. It is unlikely that there would be any difference in characteristics such as virulence between these isolates because both amino acids are hydrophobic non-polar.

There have been few isolations of IPNV made from wild marine fish. IPNV (serogroup A) has previously been recorded in wild saithe (*Pollachius virens*) by Mortensen et al. (1993). These were caught close to a farm producing turbot, (*Psetta maxima*) during clinical IPN mortalities on site. Diamant et al. (1988), isolated the virus from common dab, caught in an area remote from aquaculture. Hake are a shoaling species being found inshore and offshore and sometimes close to aquaculture facilities. Due to the large numbers of salmon farms and the recent diversification into the culture of marine species in Scotland, this highlights a potential for horizontal transmission between individuals within the shoals and among farmed fish.

The IPNV isolation identifies hake as a potential new reservoir of this virus. Future research could concentrate on identifying other host species of marine fish and virus distribution within these populations. This would facilitate a better understanding of the risks of IPN infection to the marine aquaculture industry.

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