Infectious salmon anaemia

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Summary of general activities related to the disease

1a) Types of test(s) in use/or available, purpose of testing (diagnosis*, surveillance, etc.) and approximate number performed for each purpose

Test	For	Specificity	Research/Surveillance	Total
Conventional RT-PCR on tissues	Virus RNA	ISAV	633	633
Real time RT-PCR on tissues	Quantitation of virus RNA	ISAV	34	34
Virus isolation in cell culture	Infective virus	Group	161	161
Electron microscopy	Virion morphology	Group	0	0
In-situ hybridization on tissues	Virus RNA	ISAV	129	129
ELISA	Antibody	ISAV	0	0
Serum neutralization	Antibody	ISAV	16	16
Virus neutralization	Antigenicity	Serotype	2003 isolates: 74 2004 isolates: 19	93
Passage in CHSE-214 cell line	Cytopathic effects	CHSE phenotype	2003 isolates: 74 2004 isolates: 19	93
DNA Sequencing	Nucleotide sequence	Genotype	2003 isolates: 74 2004 isolates: 19	93

***In accordance with Resolution No. XXVIII, adopted by the OIE International Committee in May 2004, OIE Reference Laboratories are now required by mandate to inform the OIE Central Bureau of confirmed positive diagnostic results for diseases that are reportable to the OIE. Such information will be sent by the OIE Central Bureau to the OIE Delegate of the country concerned before any publication. How many confirmed positive results have you reported to the OIE Central Bureau?**

None.

1b) Agent identification

Agent identification is carried out using:	TO cell culture SHK-1 cell culture ASK-2 cell culture CHSE-214 cell culture RT-PCR
Total number of samples tested:	93

2. Production, testing and distribution of diagnostic reagents

Several hybridoma cell lines that produce monoclonal antibodies (mAbs) specific to ISAV have been established following immunization of mice with purified whole virus preparations. Monoclonal antibody 4A11 which is of IgG_1 isotype and is directed against an epitope on ISAV putative NP protein has recently been shown to be highly sensitive for detection of ISAV in samples. When this mAb was prepared at a concentration of 1-5 mg/ml, and used in ELISA, at a dilution of 1:200 it could detect ISAV in a sample with a total protein concentration of 0.47 µg/well on an ELISA plate (OD of sample was 2.488, background level was 0.300). Monoclonal antibody 4A11 is now available.

3. Research especially related to development of diagnostic methods and vaccines

The long-term goal is to develop novel strategies that will allow effective control of infectious salmon anaemia.

In collaboration with the New Brunswick Department of Agriculture, Fisheries and Aquaculture, all ISAV isolates from disease outbreaks in the Bay of Fundy, New Brunswick, from 1997 onwards, are forwarded to our laboratory for phenotypic, antigenic and phylogenetic characterization (refer to Table in section 1a above for Year 2003 & Year 2004 ISAV isolates). This database is part of a continuous active surveillance for the region in order to gain detailed knowledge about the epidemic strains of ISAV. The phenotypic characterization includes culturing the virus isolates in the CHSE-214 cell line, and any isolates identified with unique properties in virus replication (such as rapidity with which CPE is induced, virus titre, etc.) are flagged for further analysis. Virus neutralization with rabbit antisera raised against selected ISAV isolates shows that there are at least three haemagglutinin-esterase (HE) subtypes of ISAV: (1) North American, (2) European, and (3) isolate U5575-1 (from Nova Scotia). Alignment of deduced amino acid sequences in the highly polymorphic region (HPR) of the HE protein of selected ISAV isolates identified the putative antigenic motif around the common potential N-glycosylation site at amino acid positions 333NIT335. The HE protein in ISAV isolate U5575-1 has an additional unique potential N-glycosylation site at amino acid positions 349NQT351, just next to the predicted transmembrane region (amino acid position 373-394). We hypothesize that the presence of the additional carbohydrate chain in this region results in a new epitope accounting for isolate U5575-1 (and possibly other isolates with this second glycosylation site, for example Norwegian isolate 485/9/97) being antigenically distinct from the European HE subtype.

Work towards developing a vaccine for broad and sterile immunity to ISAV is ongoing. Different formulations are being investigated for reduction in Atlantic salmon mortality and virus persistence, and type of immune response.

An indirect ELISA for detection of antibodies against ISAV in fish serum, developed by Kibenge *et al*. (*Dis Aquat Org* 51:1-11 (2002), demonstrated diagnostic value for ISAV infection when it was used in conjunction with RT-PCR to test a random sample of farmed Atlantic salmon from the Bay of Fundy, New Brunswick. Field trials to validate this assay are planned for June-July 2005. An *in situ* hybridization (ISH) diagnostic procedure for detection of ISAV nucleic acid sequences in formalin-fixed fish tissues (including gill tissue), developed by Moneke *et al*. (*J Vet Diagn Invest* 15:407-417 (2003), has been reported. This assay appears to be more sensitive than immunohistochemical staining of tissues with antibodies against ISAV, and might prove extremely useful in trace-back investigations on tissue samples preserved in formalin or wax blocks, and in situations where either the virus is non-cultivable or in which more than one disease complicates that diagnosis of ISA. Moreover, ISH allows earlier detection of virus replication in cell culture than IFAT or CPE. Most recently, a one-tube real-time RT-PCR using LightCycler technology (Roche Applied Science) and SYBR Green chemistry that quantitatively detects ISAV in biological samples was developed (Munir and Kibenge, 2004, *J Virol Meth* 117:37-47). The result of this real-time RT-PCR assay can be obtained on-line in only 80 minutes, and is 100 times more sensitive than the conventional one-tube RT-PCR. With this test and the passaging of virus in the CHSE-214 cell line, ISAV isolates

can be grouped into three "CHSE phenotypes": (1) CHSE-CPE positive phenotype, (2) CHSE-CPE negative, replicating phenotype, and (3) CHSE-CPE negative, non-replicating phenotype.

Activities specifically related to the mandate of OIE Reference Laboratories

4. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

No activity

5. Preparation and supply of international reference standards for diagnostic tests or vaccines

No activity

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

No activity

7. Provision of consultant expertise to OIE or to OIE Member Countries

A proposal for the establishment of a twinning arrangement with the Aquatic Health Chile Laboratory in Puerto Varas, Chile, has been prepared and discussed with the OIE Delegates of Canada and Chile.

8. Provision of scientific and technical training to personnel from other OIE Member Countries

See section 7 above.

9. Organisation of international scientific meetings on behalf of OIE or other international bodies

No activity

10. Participation in international scientific collaborative studies

See section 7 above.

11. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

■ *Presentations at international conferences and meetings*

Joseph, T., Cepica, A., Brown, L., Ikede, B.O., and Kibenge, F.S.B. 2004. Mechanism of cell death during infectious salmon anaemia virus infection of fish cell lines. 23rd *Annual Meeting of the American Society for Virology, Montreal, Quebec, July 10-14, 2004.*

Kibenge, F.S.B., Joseph, T., Beecroft, R., Qian, B., and Otto, T. 2003. Generation of monoclonal antibodies to infectious salmon anaemia virus antigens. *20th Annual Meeting of the Aquaculture Association of Canada, Victoria, B.C., October 29-November 1, 2003.*

Munir, K., and Kibenge, F.S.B. 2003. Analysis of infectious salmon anaemia virus by real-time RT-PCR. *20th Annual Meeting of the Aquaculture Association of Canada, Victoria, B.C., October 29-November 1, 2003.*

Kibenge, F.S.B., Munir, K., Kibenge, M.J.T., Joseph, T., and Moneke, E. 2003. Biochemistry, aetiopathogenesis and immunology of infectious salmon anaemia virus. 3rd *International Veterinary Vaccines and Diagnostics Conference, University of Guelph, Guelph, Canada, July 13-18, 2003.*

Moneke, E., Kibenge, M.J.T., Groman, D., Johnson, G., Wright, G., Ikede, B.O. and Kibenge, F.S.B. 2003. Infectious salmon anemia: A correlation between histopathological lesions and virus RNA in Atlantic salmon *Salmo salar L.* experimentally infected with Infectious salmon anemia virus. *Annual Fish Health Section and Western Fish Disease Conference, Seattle, Washington, USA, July 15 – 17, 2003.*

MacWilliams, C, Kibenge, F.S.B. and Johnson G. Fatal infectious haemorrhagic disease in rainbow trout experimentally infected with infectious salmon anaemia virus (ISAV). *Annual Fish Health Section and Western Fish Disease Conference, Seattle, Washington, USA, July 15 – 17, 2003.*

Joseph, T. Kibenge, M.J.T., Kibenge, F.S.B. 2003. Apparent Fc receptor-mediated infection of macrophage-like fish cell lines by infectious salmon anaemia virus. *3rd International Symposium on Fish Vaccinology, Bergen, Norway, 9-11 April, 2003.*

■ *Scientific publications*

Moneke, E., Groman, D.B., Wright G.M., Stryhn, H., Johnson, G.R., Ikede, B.O., and Kibenge, F.S.B. 2005. Correlation of virus replication in tissues with histologic lesions in Atlantic salmon experimentally infected with infectious salmon anaemia virus (ISAV). *Veterinary Pathology,*42: (in press May 23, 2004).

Kibenge, F.S.B., Munir, K., Kibenge, M.J.T., Joseph, T., and Moneke, E. 2004. Infectious salmon anaemia virus: the causative agent, pathogenesis and immunity. *Animal Health Research Reviews* 5:65-78.

Joseph, T., Cepica, A., Brown, L., Ikede, B.O., and Kibenge, F.S.B. Mechanism of cell death during infectious salmon anaemia virus (ISAV) infection is cell-type specific. *Journal of General Virology* 85:3027-3036.

Munir, K., and Kibenge, F.S.B. 2004. Detection of infectious salmon anaemia virus by real-time RT-PCR. *Journal of Virological Methods* 117:37-47.

Moneke, E., Kibenge, M.J.T., Groman, D., Johnson, G.R., Ikede, B.O., and Kibenge, F.S.B. 2003. Infectious salmon anaemia virus (ISAV) RNA in fish cell cultures and in tissue sections of Atlantic salmon experimentally infected with ISAV. *Journal of Veterinary Diagnostic Investigation 15: 407-417.*

Joseph, T., Kibenge, M.T., and Kibenge, F.S.B. 2003. Antibody-mediated growth of infectious salmon anaemia virus in macrophage-like fish cell lines. *Journal of General Virology 84: 1701-1710.*

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

Test	*For*	*Specificity*	*Total*
Conventional RT-PCR	Virus RNA	Group	361
Real time RT-PCR	Virus RNA	Group	108
ASK-2/TO/CHSE-214 cell cultures	Virus isolation		89
Electron microscopy	Virion morphology	Group	0
In-situ hybridization	Virus mRNA	Group	0
ELISA	Antibody	Group	130
ELISA	Antigen	Group	0
Serum neutralization	Antibody	Serotype	0
Virus neutralization	Antigenicity	Serotype	69
DNA Sequencing	Nucleotide sequence	Genotype	97

2. Production and distribution of diagnostic reagents

Several hybridoma cell lines that produce monoclonal antibodies (mAbs) specific to ISAV have been established following immunization of mice with purified whole virus preparations. Monoclonal antibody 4A11 which is of IgG_1 isotype and is directed against an epitope on ISAV putative NP protein has been shown to be highly sensitive for detection of ISAV in samples. When this mAb was prepared at a concentration of 1-5 mg/ml, and used in ELISA, at a dilution of 1:200 it could detect ISAV in a sample with a total protein concentration of 0.47 µg/well on an ELISA plate (OD of sample was 2.488, background level was 0.300). Monoclonal antibody 4A11 is now available.

In collaboration with the New Brunswick Department of Agriculture, Fisheries and Aquaculture, all ISAV isolates from disease outbreaks in the Bay of Fundy, New Brunswick, from 1997 onwards, are forwarded to our laboratory for phenotypic, antigenic and phylogenetic characterization (51 Year-2004 ISAV isolates and 18 Year-2005 ISAV isolates). This database is part of a continuous active surveillance for the region in order to gain detailed knowledge about the epidemic strains of ISAV. The phenotypic characterization includes culturing the virus

isolates in the CHSE-214 cell line, and any isolates identified with unique properties in virus replication (such as rapidity with which CPE is induced, virus titre, etc.) are flagged for nucleotide sequence analysis. These isolates are made available to other laboratories nationally and internationally as reference isolates.

Four (4) ISAV reference strains were supplied to National Veterinary Institute, Oslo, Norway.

Three (3) ISAV reference strains were supplied to National Veterinary Services Laboratories, Ames, Iowa, USA.

Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

We participated in an inter-laboratory proficiency test (Ring Test) involving 5 Aquatic Disease Diagnostic laboratories in eastern Canada and Maine, USA, to detect ISAV by RT-PCR on 40 blind tissue samples, and by virus isolation on selected 20 blind tissue samples using ASK-2, TO, SHK-1, and CHSE-214 cell lines. The purpose of the Ring Test was to gain information on how the different labs compare with the different techniques, and to determine the significance of the RT-PCR results. The data gained will be used to discuss the next steps to be taken in order to harmonize the testing protocols and strategies.

Validation of antibody ELISA for the detection of antibodies against ISAV in fish has been initiated. Fish serum samples of “known positive” fish and “known uninfected” fish were collected in 2005. Formal validation of this test in accordance with the OIE Validation Template is anticipated in 2006.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

One vial each of four (4) ISAV reference strains: U5575-1 (Canada), RPC/NB-980-280-2 (Canada), NBISA01 (Canada), and 7833-1 (Chile), were supplied to National Veterinary Institute, Oslo, Norway.

Two vials each of three (3) ISAV reference strains: RPC/NB-980-280-2 (Canada), 810/9/99 (Norway), 301/98 (Scotland) were supplied to National Veterinary Services Laboratories, Ames, Iowa, USA.

RT-PCR primers for Aquareovirus were supplied to Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

5. Research and development of new procedures for diagnosis and control

The long-term goal is to develop novel strategies that will allow effective control of infectious salmon anaemia.

1) Development of a novel oral vaccine against ISAV that can be administered to fish *en masse*. This is part of an on-going strategic research program at the Atlantic Veterinary College, in partnership with several vaccine companies, to develop oral vaccines for aquaculture. There is now technology available that permits oral delivery of vaccines to fish. We will further develop this technology in order to deliver a series of novel ISA vaccines orally. When administered to fish, an orally-administered ISAV vaccine will provide a practical method to combat this devastating disease, permitting multiple vaccinations and therefore inducing intensive and long-term immune protection, without the side effects associated with injectable vaccines.

2) An indirect ELISA for detection of antibodies against ISAV in fish serum, developed by Kibenge *et al*. (*Dis Aquat Org* 51:1-11 (2002), demonstrated diagnostic value for ISAV infection when it was used in conjunction with RT-PCR to test a random sample of farmed Atlantic salmon from the Bay of Fundy, New Brunswick. Formal validation of this antibody ELISA in accordance with the OIE Validation Template is anticipated in 2006.

3) Rapid typing of ISAV isolates using LightCycler technology (Roche Applied Science) and dual labelled (TaqMan) probes. This is an extension of the Real-Time RT-PCR assay developed previously (Munir and Kibenge, 2004, *J Virol Meth* 117:37-47). Interest is now focussed on understanding the molecular basis for the ISAV virulence which requires the identification of the functions of the different viral proteins. We intend to use

this information to identify genetic markers to distinguish between different ISAV phenotypes by virus typing with real-time RT-PCR.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

1) Extrapolation of relative levels of virulence among different ISAV strains is difficult because most experimental studies of ISAV infection in Atlantic salmon have used single ISAV isolates. Recently, we reported on a comparison of the infectivity of different ISAV isolates in three farmed fishes (Atlantic salmon, coho salmon and rainbow trout) using an infectious dose of 10^6 $TCID_{50}$/0.2ml per fish (Kibenge *et al*. 2004, *Ani Hlth Res Rev* 5:65-78). It was found that the most virulent strains caused the highest mortalities in Atlantic salmon (>95% mortality), with the shortest duration (9-12 days). These highly pathogenic ISAV strains also caused mortality in rainbow trout, and were also more aggressive in cell culture, inducing CPE sooner and more completely in most permissive cell lines than the less pathogenic ISAV isolates. The rainbow trout infection phenotype of ISAV is presented as a correlate of ISAV pathogenicity.

2) We reported on the identification of a new HPR group of ISAV in New Brunswick (RPC/NB-04-085-1) at the 30th Annual Eastern Fish Health Workshop, Shepherdstown, West Virginia, USA. June 13-17, 2005.

7. Provision of consultant expertise to OIE or to OIE Member Countries

1) Reviewed and commented on chapter on ISA in the OIE Manual of Diagnostic Tests for Aquatic Animals, Fifth Edition (2006).

2) Reviewed one (1) grant proposal for US Dept of Agriculture.

3) Served as Opponent for Vidar Teis Aspehaug: Characterization of Major Structural Proteins of the Infectious Salmon Anaemia Virus (ISAV). Doctoral Thesis, Department of Biology, University of Bergen, Norway, June 10, 2005.

8. Provision of scientific and technical training to personnel from other OIE Member Countries

No activity

9. Provision of diagnostic testing facilities to other OIE Member Countries

No activity

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

No activity

11. Participation in international scientific collaborative studies

We are participating in Canada's Inter-agency Wild Bird Influenza Survey (2005-2010) in which the laboratory screens wild birds in Atlantic Canada for Avian Influenza virus by real-time RT-PCR and by virus isolation in SPF embryonated chicken eggs. This project is coordinated by the Canadian Cooperative Wildlife Health Centre (CCWHC) and is primarily funded by the Public Health Agency of Canada (PHAC), the Canadian Food Inspection Agency (CFIA), Environment Canada – Canadian Wildlife Service (CWS), and the respective provincial departments. In 2005, we tested 786 cloacal swabs from wild birds in Atlantic Canada and found 333 positive for avian influenza virus. Of the positive samples, 38 were shown to be of the low pathogenic North American H5N2 subtype.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

Kibenge, M.T., Qian, B., Hariharan S., and Kibenge, F.S.B. 2005. Identification of a new HPR group of infectious salmon anaemia virus in New Brunswick: What does it mean? *30th Annual Eastern Fish Health Workshop, Shepherdstown, West Virginia, June 13-17, 2005.*

Kibenge, M.T., Qian, B., Hariharan S., and Kibenge, F.S.B. 2005. Identification of a new HPR group of infectious salmon anaemia virus in New Brunswick. *Annual Meeting of the Canadian Society of Microbiologists, Halifax, Nova Scotia, June 12-15, 2005.*

- ***Scientific publications in peer-reviewed journals***

Kibenge, M.J.T., Munir, K., and Kibenge, F.S.B. 2005. Constitutive expression of Atlantic salmon Mx1 protein in CHSE-214 cells confers resistance to infectious salmon anaemia virus. *Virology Journal, 2:75-80.*

Moneke, E., Ikede, B.O., and Kibenge, F.S.B. 2005. Viraemia during infectious salmon anaemia virus infection of Atlantic salmon is associated with replicating virus in leucocytes. *Diseases of Aquatic Organisms 66:153-157.*

Moneke, E., Groman, D., Johnson, G.R., Wright, G.M., Stryhn, H., Ikede, B.O., and Kibenge, F.S.B. 2005. Correlation of virus distribution in tissues with histologic lesions in Atlantic salmon experimentally infected with infectious salmon anaemia virus. *Veterinary Pathology, 42:338-349*.

- ***Other communications***

Presented an invited seminar titled “Correlates of virulence of infectious salmon anaemia virus (ISAV)” at the National Veterinary Institute, Oslo, Norway, June 8, 2005.

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

Test	*For*	*Specificity*	*Total*
Conventional RT-PCR	Virus RNA	Group	107
Real-time RT-PCR	Virus RNA	Group & viral load	105
ASK-2/TO/CHSE-214 cell cultures at 16°C	Virus isolation		24
Electron microscopy	Virion morphology	Group	20
In-situ hybridization	Virus mRNA	Group	0
ELISA	Antibody detection	Group	0
ELISA	Antigen detection	Group	0
Virus neutralization	Antibody titre	Serotype	0
DNA sequencing	Nucleotide sequence	Genotype	68

2. Production and distribution of diagnostic reagents

Rabbit polyclonal antisera for serotyping of ISAV.
Monoclonal antibodies (mAbs) specific to ISAV.
ISAV isolates.
ISAV RNA and cDNA clones of ISAV gene segments for PCR.

This year no requests were received from other OIE Member Countries for supply of diagnostic reagents.

Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

Participated in the *ad hoc* group for the Norwegian Scientific Committee for Food Safety concerning risk assessment – infectious salmon anaemia. This committee was set up to ensure that the Norwegian Food Safety Authority bases its management of infectious salmon anaemia on internationally accepted knowledge. The

international experts represented Scotland, Canada and the USA (http://www.vkm.no/eway/default.aspx?pid=0&oid=-2&trg=__new&__new=-2:17005).

Participated in the First International Conference of OIE Reference Laboratories and Collaborating Centres that was held at Florianopolis (Brazil) from 3-5 December 2006. This conference, among other things, promoted the updating and setting of standards for methodologies in the fields of diagnostics, vaccine quality and biosecurity.

Participated in a Special Workshop on viral strain differentiation and listing and notification of diseases by strain/genotype, which was held during the First International Conference of OIE Reference Laboratories and Collaborating Centres, held at Florianopolis (Brazil) from 3-5 December 2006.

Validation of antibody ELISA for the detection of antibodies against ISAV in fish has been initiated. Fish serum samples of "known positive" fish and "known uninfected" fish were collected in 2005. Formal validation of this test in accordance with the OIE Validation Template is anticipated in 2007.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

Provided approximately 30 mls of a proprietary ISA virus vaccine to an international fish vaccine manufacturer for a head-to-head evaluation with a commercially available ISA virus vaccine.

Provided one vial of infectious bursal disease virus (IBDV) strain QC2 to Canadian Food Ispection Agency (CFIA) laboratory in Nepean, Ontario, Canada.

5. Research and development of new procedures for diagnosis and control

The long-term goal is to develop novel strategies that will allow effective control of infectious salmon anaemia.

1) Development of a novel oral vaccine against ISAV that can be administered to fish *en masse*. This is part of an on-going strategic research program at the Atlantic Veterinary College, in partnership with several vaccine companies, to develop oral vaccines for aquaculture.

2) Evaluated 6 different ISAV vaccine preparations using 600 Atlantic salmon which were then experimentally challenged with two different virus doses ($10^{3.5}$ and $10^{6.1}$ $TCID_{50}$/0.2 ml/fish). From this fish vaccination/challenge study, one of these preparations is now being tested by an international fish vaccine manufacturer.

3) Collaborated with an international pharmaceutical company on a preliminary study on administration of vaccines through fish feed to Atlantic salmon.

4) Interest in genotyping of ISAV isolates is focused on understanding the molecular basis for the ISAV virulence, which requires the identification of the functions of the different viral proteins. We intend to use this information to identify genetic markers to distinguish between different ISAV phenotypes. Thus we have carried out pathogenicity experiments on selected ISAV isolates (Kibenge *et al.,* 2006, *J Gen Virol* 87:2645-2652), which has enabled us to identify a putative virulence motif ^{352}FNT^{354} adjacent to the second potential *N*-glycosylation site in isolates such as RPC/NB 04-085-1, which are of low pathogenicity in Atlantic salmon (Kibenge *et al.,* manuscript in preparation).

5) Have initiated a research project to determine the interferon gene expression profiles of fish cells when infected with ISAV strains differing in pathogenicity.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

Have studied the phenotypic correlates of pathogenicity for ISAV in salmonid fishes (Kibenge *et al.,* 2006, *J Gen Virol* 87:2645-2652). In this study, we compared 13 different strains of ISAV isolated from different geographical regions between 1997 and 2004, for their infectivity in 3 fish species (Atlantic salmon, *Salmo salar*, coho salmon, *Oncorhynchus kisutch*, and rainbow trout, *O. mykiss*). When the different virus isolates were used at an approximate inoculum dose of 10^6 $TCID_{50}$/0.2 ml/fish, it was found that the most virulent strains had an acute mortality phase in Atlantic salmon that started at 10-13 days post-inoculation and lasted for 9-15 days with a cumulative per cent mortality of $\geq$ 90%. These highly pathogenic strains also caused low mortality in rainbow trout, albeit later in the infection. Viruses with a more delayed or protracted mortality phase, resulting in

cumulative mortalities of 50-89% in Atlantic salmon were considered to be of intermediate pathogenicity, and isolates with ≤ 49% were considered of low pathogenicity. On this basis, 3 of the ISAV isolates showed a high-, 8 an intermediate-, and 2 a low-pathogenicity phenotype in Atlantic salmon. Coho salmon were resistant to all ISAV isolates. These results confirm that there is variation in pathogenicity among ISAV strains for Atlantic salmon and rainbow trout, and that other salmonid species such as coho salmon can carry highly pathogenic strains of ISAV without showing signs of disease. The identified pathogenicity phenotypes may aid in the identification of molecular markers of ISAV virulence.

Used the 13 ISAV strains characterized for ability to kill fish (Kibenge *et al.,* 2006, *J Gen Virol* 87:2645-2652), to search for markers of virulence. Genetic analyses revealed two genotypes of ISAV, North American and European. The European genotype was further separated into two genogroups, real-European and European-in-North America. Deletion/insertion of ≤ 13 amino acids and the presence of two specific motifs in the HE gene (^{349}NQT^{351} motif and/or mutation of the ^{352}FNT^{354} motif) were correlated with reduced cytopathogenicity and reduced virulence for Atlantic salmon. A novel phylogenetic software program, BACKTRACK, we wrote estimated that the North American and European genotypes diverged between 1921 and 1929, whereas the European-in-North America genogroup diverged from the real-European genogroup between 1980 and 1988 (Kibenge et al., 2006, 1st OIE Global Conference on Aquatic Animal Health, Bergen, Norway, October 9-12, 2006).

At an ISA Research Strategy Workshop (in Saint John, New Brunswick, December 6-7, 2006) organized by the salmon industry and government departments, the Canadian Food Inspection Agency (CFIA) agreed to fund a study to determine whether ISA virus in New Brunswick can be eradicated or managed. CFIA is considering making ISA a reportable disease, which would mean that veterinarians, fish farmers and laboratories must inform the CFIA of any suspected outbreak, and if destruction of stock is ordered, then the fish farmers would receive compensation (http://thechronicleherald.ca/NovaScotia/550550.html).

7. Provision of consultant expertise to OIE or to OIE Member Countries

1) Provided good gross photos of ISA for teaching to laboratory at University of California, USA.

2) Participated in the *ad hoc* group for the Norwegian Scientific Committee for Food Safety concerning risk assessment – infectious salmon anaemia. This committee was set up to ensure that the Norwegian Food Safety Authority bases its management of infectious salmon anaemia on internationally accepted knowledge. The international experts represented Scotland, Canada and the USA.

3) Provided advice to a 5th year Veterinary Student at University of the Americas, Chile, about existing vaccines for ISAV.

4) Was a Speaker at the Special Workshop on viral strain differentiation and listing and notification of diseases by strain/genotype, which was held during the First International Conference of OIE Reference Laboratories and Collaborating Centres, held at Florianopolis (Brazil) from 3-5 December 2006. Title of presentation: Distinguishing between different virus species and different strains of a viral species.

8. Provision of scientific and technical training to personnel from other OIE Member Countries

No activity

9. Provision of diagnostic testing facilities to other OIE Member Countries

Number of times	Tentative testing	Confirmatory testing	Member country	Informed OIE
1		Yes	Chile	No*

*Sample was negative for ISAV

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

No activity

11. Participation in international scientific collaborative studies

No activity

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

Kibenge, F.S.B. 2006. Distinguishing between different virus species and different strains of a viral species. Special Workshop on viral strain differentiation and listing and notification of diseases by strain/genotype. *1st International Conference of OIE Reference Laboratories and Collaborating Centres, Florianopolis, Brazil, December 3-5, 2006.*

Kibenge, F.S.B., Kibenge, M.J.T., Wang, Y., Qian, B., Hariharan, S., and McGeachy, S. 2006. Correlates of virulence of infectious salmon anaemia virus. *1st OIE Global Conference on Aquatic Animal Health, Bergen, Norway, October 9-12, 2006.*

Kibenge, M.J.T., Wang, Y., Qian, B., McGeachy, S., and Kibenge, F.S.B. 2006. Virulence phenotypes of infectious salmon anaemia virus (ISAV). *5th International Symposium on Aquatic Animal Health, San Francisco, California, September 2-6, 2006.*

- ***Scientific publications in peer-reviewed journals***

Workenhe, S.T., Wadowska, D.W., Wright, G.M., Kibenge, M.J.T., and Kibenge, F.S.B. 2007. Demonstration of infectious salmon anaemia virus (ISAV) endocytosis in erythrocytes of Atlantic salmon. *Virology Journal, 4:13-21.*

Kibenge, F.S.B., Kibenge M.J.T, Groman, D. and McGeachy S. 2006. *In-vivo* correlates of infectious salmon anaemia virus pathogenesis in fish. *Journal of General Virology, 87 (9):2645-2652.*

- ***Other communications***

Presented an invited seminar titled "Virus-host interactions of infectious salmon anaemia virus, an Orthomyxovirus of fish" at University of Missouri, October 2006.

Infectious salmon anaemia

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

Test	*For*	*Specificity*	*Total**
Conventional RT-PCR	Virus RNA	Group	385
Real-time RT-PCR	Virus RNA	Group & viral load	1,479
ASK-2/TO/CHSE-214 cell cultures at 16°C	Virus isolation	Phenotype	168
Electron microscopy	Virion morphology	Group	0
In-situ hybridization	Virus mRNA	Group	0
ELISA	Antibody detection	Group	5
ELISA	Antigen detection	Group	0
Virus neutralization	Antibody titre	Serotype	0
DNA sequencing	Nucleotide sequence	Genotype	211

*Total includes tests on surveillance, diagnostic, research, and QA samples

2. Production and distribution of diagnostic reagents

Reagents produced:

a. ISAV reference strains for research and diagnostic purposes.

b. ISAV antigens for antibody ELISA.

c. Rabbit polyclonal antisera for serotyping of ISAV.

d. ISAV RNA (whole genome and gene-specific transcripts) preserved in 100% ethanol for use in RT-PCR.

e. ISAV cDNA clones of gene segments preserved in 100% ethanol for use in for RT-PCR.

Reagents supplied nationally:

One (1) vial of ISAV RNA for positive control in RT-PCR was supplied to a laboratory in British Columbia.

Reagents supplied to other OIE Member Countries:

Two (2) vials of ISAV RNA for positive control in 1-step RT-PCR and two (2) vials of ISAV cDNA for positive control in 2-step RT-PCR were supplied to Argentina.

Two (2) vials of ISAV cDNA for positive control in 2-step RT-PCR were supplied to Chile.

Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

Validation of antibody ELISA for the detection of antibodies against ISAV in fish has been initiated. Fish serum samples of "known positive" fish and "known uninfected" fish were collected in 2005-2007. Formal validation of this test in accordance with the OIE Validation Template is anticipated in 2008.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

One (1) vial of ISAV RNA (10 µg in ethanol) for positive control in RT-PCR was supplied to a laboratory in British Columbia.

Two (2) vials of ISAV RNA for positive control in 1-step RT-PCR and two (2) vials of ISAV cDNA for positive control in 2-step RT-PCR were supplied to Argentina.

Two (2) vials of ISAV cDNA for positive control in 2-step RT-PCR were supplied to Chile.

5. Research and development of new procedures for diagnosis and control

The long-term goal is to develop novel strategies that will allow effective control of infectious salmon anaemia.

1) Development of a novel oral vaccine against ISAV that can be administered to fish *en masse*. This is part of an on-going strategic research program at the Atlantic Veterinary College, in partnership with several vaccine companies, to develop oral vaccines for aquaculture.

2) Evaluated 6 different ISAV vaccine preparations using 600 Atlantic salmon which were then experimentally challenged with two different virus doses ($10^{3.5}$ and $10^{6.1}$ $TCID_{50}$/0.2 ml/fish). From this fish vaccination/challenge study, one of these preparations is now being evaluated by an international fish vaccine manufacturer.

3) Interest in genotyping of ISAV isolates is focused on understanding the molecular basis for the ISAV virulence, which requires the identification of the functions of the different viral proteins. We intend to use this information to identify genetic markers to distinguish between different ISAV phenotypes. Such research will help us to establish the significance, if any, of different highly polymorphic region (HPR) groups in clinical disease and virus isolation.

4) Have initiated a research project to determine the interferon gene expression profiles of fish cells (including erythrocytes) when infected with ISAV strains differing in pathogenicity.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

All ISAV isolates made during diagnostic investigations of marine-farmed Atlantic salmon in the Bay of Fundy by the New Brunswick Department of Agriculture and Aquaculture are forwarded to the OIE Reference Laboratory for further phenotypic and genotypic characterizations. 136 isolates were received during this reporting period.

Dr. Fred Kibenge participated in two international workshops in Chile to discuss the 2007 ISA outbreaks in Chile and relate the Canadian experience of ISA: (1) ISAV International Symposium in Chile, Puerto Varas, August 3, 2007, organized by Aquagestión-Fundación Chile, Puerto Montt, Chile; and (2) ISA workshop in Chile, Puerto Varas, November 19-20, 2007, organized by The Chilean Salmon Industry Association A.G. – Intesal-SalmonChile- and the National Service of Fishing – Sernapesca.

7. Provision of consultant expertise to OIE or to OIE Member Countries

1) Confirmed the 2007 ISA outbreaks in Chile. Determined the nucleotide sequence of the Chilean ISAV genome for RNA segments 5, 6 and 7, and showed that the virus was of European genotype and had a unique 11-amino acid insert in the Fusion protein (segment 5), and that the most prevalent HPR group in the outbreaks was HPR7b.

2) Arranged with Sernapesca for January 2008 to organize a ring test of laboratories in Chile to evaluate ISAV RT-PCR.

3) Helped with design of primers and RT-PCR to detect presence of unique Chilean ISAV in farmed fish in Chile following the ISA outbreak of June 2007.

8. Provision of scientific and technical training to personnel from other OIE Member Countries

Dr. Fred Kibenge visited fish diseases diagnostic laboratories in Chile in August and November 2007, and advised on protocols for ISAV isolation and/or detection by cell culture, ISAV detection by RT-PCR, and genotyping of ISAV.

9. Provision of diagnostic testing facilities to other OIE Member Countries

Number of times	Tentative testing	Confirmatory testing	Member country	Informed OIE
7		Yes	Chile	Yes

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

No activity this reporting period

11. Participation in international scientific collaborative studies

Collaborated with the salmon industry and diagnostic laboratories in Chile to characterise the ISAV strains associated with the 2007 Chilean ISA outbreaks. The most prevalent HPR group in these outbreaks is HPR7b. The RNA segment 5 for some Chile ISAV strains has a small insert of 33 nucleotides (or 11 amino acids) relative to ISAV strains from Europe and North America. ISAV RNA segment 5 encodes the Fusion glycoprotein found on the virus envelope. The small insert has 100% sequence identity with RNA segment 2 of European genotype. ISAV RNA segment 2 encodes the ISAV polymerase PB1. The small insert probably occurred through non-homologous recombination between RNA segments 2 and 5 of the same virus.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

Kibenge, F.S.B., Kibenge, M.J.T., Wang, Y., Qian, B., Hariharan, S., and McGeachy, S. 2007. Molecular correlates of ISAV virulence. *32nd Eastern Fish Health Workshop, Gettysburg, Pennsylvania, June 18-22, 2007.*

Kibenge, F.S.B. 2007. Presentation of infectious salmon anaemia virus in Canada. *ISAV International Symposium in Chile, Puerto Varas, August 3, 2007*, organized by Aquagestión-Fundación Chile, Puerto Montt, CHILE.

Kibenge, F.S.B. 2007. Methods of diagnosis of ISAV. *ISAV International Symposium in Chile, Puerto Varas, August 3, 2007*, organized by Aquagestión-Fundación Chile, Puerto Montt, Chile.

Godoy, M., and Kibenge, F.S.B. 2007. ISA-v presence in Chile: Comparative analysis of strains and pathogenicity. *ISA workshop in Chile, Puerto Varas, November 19-20, 2007*, organized by The Chilean Salmon Industry Association A.G. – Intesal-SalmonChile- and the National Service of Fishing – Sernapesca.

Kibenge, F.S.B. 2007. ISA-v research and impacts on salmon farming in Canada. *ISA workshop in Chile, Puerto Varas, November 19-20, 2007*, organized by The Chilean Salmon Industry Association A.G. – Intesal-SalmonChile- and the National Service of Fishing – Sernapesca.

Kibenge, F.S.B. 2007. Canadian regulation to control ISA-v. *ISA workshop in Chile, Puerto Varas, November 19-20, 2007*, organized by The Chilean Salmon Industry Association A.G. – Intesal-SalmonChile- and the National Service of Fishing – Sernapesca.

- ***Scientific publications in peer-reviewed journals***

Workenhe, S.T., Wadowska, D.W., Wright, G.M., Kibenge, M.J.T., and Kibenge, F.S.B. 2007. Demonstration of infectious salmon anaemia virus (ISAV) endocytosis in erythrocytes of Atlantic salmon. *Virology Journal, 4:13.*

Kibenge, F.S.B., Kibenge, M.J.T., Wang, Y., Qian, B., Hariharan, S., and McGeachy, S. 2007. Mapping of putative virulence motifs on infectious salmon anaemia virus surface glycoprotein genes. *Journal of General Virology, 88:3100-3111.*

Kibenge, F.S.B., Xu, H., Kibenge, M.J.T., Qian, B., and Joseph, T. 2007. Characterization of gene expression on genomic segment 7 of infectious salmon anaemia virus. *Virology Journal, 4:34.*

MacWilliams, C., Johnson, G.R., Groman, D., and Kibenge, F.S.B. 2007. Morphologic description of infectious salmon anaemia virus (ISAV)-induced lesions in rainbow trout, Oncorhynchus mykiss (Walbaum). *Diseases of Aquatic Organisms, 78:1-12.*

- ***Other communications***

Activities in 2008

Infectious salmon anaemia

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

Test	*For*	*Specificity*	*Total**
Conventional RT-PCR	Virus RNA	Group	2,162
Real-time RT-PCR	Virus RNA	Group & viral load	5,694
SHK-1/ASK-2/TO/CHSE-214 cell cultures at 16°C	Virus isolation	Phenotype	140
Electron microscopy	Virion morphology	Group	2
In-situ hybridization	Virus mRNA	Group	0
ELISA	Antibody detection	Group	16
ELISA	Antigen detection	Group	0
Virus neutralization	Antibody titre	Serotype	0
DNA sequencing	Nucleotide sequence	Genotype	503

***** Total includes tests on surveillance, diagnostic, research, and QA samples

2. Production and distribution of diagnostic reagents

Reagents produced for ISA:
a. ISAV antigens for antibody ELISA.
b. Rabbit polyclonal antisera for serotyping of ISAV.
c. ISAV RNA (whole genome and gene-specific transcripts) for RT-PCR.
d. ISAV cDNA clones of gene segments for RT-PCR.
e. Histology slides of ISA in Atlantic salmon.

Reagents supplied nationally (including use by my laboratory):
a. ISAV antigens for antibody ELISA: 3 vials
b. Rabbit polyclonal antisera for serotyping of ISAV: none
c. ISAV RNA (whole genome and gene-specific transcripts) for RT-PCR: 10 vials (one per gene)
d. ISAV cDNA clones of gene segments for RT-PCR: 10 vials (one per gene)
e. Histology slides of ISA in Atlantic salmon: none.

Reagents supplied to other OIE Member Countries:
Eleven (11) vials of ISAV RNA for positive control in RT-PCR were supplied to Chile.
Four (3) vials of ISAV cDNA clones were supplied to Republic of Korea.
Eight (8) histology slides of ISA in Atlantic salmon were supplied to Republic of Korea.

Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

Validation of antibody ELISA for the detection of antibodies against ISAV in fish has been initiated. Fish serum samples of "known positive" fish and "known uninfected" fish were collected in 2005-2007. Formal validation of this test in accordance with the OIE Validation Template is anticipated in 2009.

Have organized round robin QA testing (ring test) to evaluate ISAV RT-PCR involving laboratories in Chile.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

Eleven (11) vials of ISAV RNA for positive control in RT-PCR were supplied to Chile.
Four (3) vials of ISAV cDNA clones were supplied to Republic of Korea.
Eight (8) histology slides of ISA in Atlantic salmon were supplied to Republic of Korea.

5. Research and development of new procedures for diagnosis and control

Routine laboratory diagnosis of ISAV infection is primarily by RT-PCR because of the high sensitivity and rapid turnaround time of the test. The OIE Reference Laboratory for ISA at AVC has described methods for highly reproducible absolute viral load measurements using external standard curves generated and quantitative (Q)RT-PCR with SYBR® Green I chemistry or with TaqMan® probe chemistry (Workenhe *et al*., 2008, *J Virol Meth* 154:128-134).

In 2007, the laboratory helped to confirm the ISA outbreaks in Chile (Godoy *et al*., 2008, *BMC Vet Res* 4:28). In order to describe the molecular characteristics of the virus so as to understand its origins, how ISAV isolates are maintained, spread, and virulence characteristics, we determined the nucleotide sequences of the ISAV RNA segments 5 and 6 of the new Chilean ISAV isolates and their phylogenetic relationships with selected European and North American isolates that are representative of the genetic diversity of ISAV (Kibenge *et al.,* manuscript in preparation). Because there is presently no universally accepted nomenclature system for designation of genetic relatedness between ISAV isolates, this study utilized a novel sequence analysis method to generate a phylogenetic tree that is proposed as the basis for the uniform nomenclature and genotyping for the ISAV species.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

Dr. Fred Kibenge participated in two international workshops in Chile to discuss the 2007-2008 ISA outbreaks in Chile: (1) International ISA-PD workshop in Chile, Puerto Montt, June 06, 2008, sponsored by Biovac, Puerto Montt, Chile; and (2) ISA workshop in Chile, Puerto Montt, November 06, 2008, organized by Marine Harvest S.A. Chile.

Since the first ISA outbreak in Chile in July 2007, the OIE Reference Laboratory for ISA at AVC has sequenced 51 new Chile ISAV isolates on RNA segment 5 and 79 new isolates on RNA segment 6, and has performed an extensive comparative analysis of ISAV F and HE sequence data, including isolates sampled from Norway, Faroe Islands, Scotland, USA, and Canada. Details of this study are in a report (Kibenge *et al.,* manuscript in preparation). Our findings suggest that the 2007-2008 ISA outbreaks in Chile were caused by virus that was already present in Chile that mutated to new strains.

7. Provision of consultant expertise to OIE or to OIE Member Countries and Territories

Have worked very closely with government officials and the Atlantic salmon industry in Chile on detection and control of ISA, and to characterize the virus responsible for the 2007-2008 ISA epizootic in Chile. In January 2008, the laboratory implemented round robin QA testing of fish diagnostic laboratories in Chile.

8. Provision of scientific and technical training to personnel from other OIE Member Countries and Territories

Two personnel from Chile took a 2-week training course offered by the OIE Reference Laboratory for ISA at AVC on diagnostics of ISAV infections.

9. Provision of diagnostic testing facilities to other OIE Member Countries and Territories

Number of times	Tentative testing	Confirmatory testing	Member country	Informed OIE
Numerous		Yes	Chile	No

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

In July 2008, the OIE Reference Laboratory for ISA at AVC hosted an ISAV Special Session at the Annual Meeting of the Fish Health Section of the American Fisheries Society that was held at UPEI. The session focused on "*ISAV phenomics*" in order to provide a better understanding of ISAV strain identification, and the biological correlates (if any) of the different genotyping schemes available and/or proposed. In addition, the lab offered a post-conference workshop to 11 participants on the "*Theory and Practice of Real-time RT-PCR for Virus Detection and Quantitation*".

11. Participation in international scientific collaborative studies

Mitigation of infectious salmon anaemia (ISA) by vaccination and genetic selection is a four-year project (2008-2012) to be undertaken by Novartis Animal Health Canada Inc. Aqua Health Business, which is located in Victoria, Prince Edward Island, Canada. This project represents a partnership between Novartis, AVC/UPEI (OIE Reference Laboratory), Cooke Aquaculture Inc., New Brunswick, New Brunswick Research and Productivity Council (RPC), and the Norwegian Veterinary Institute (NVI), Norway.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

Godoy, M and Kibenge, F.S.B. 2008. Status of ISA in the world, impact in fresh water production. Second Freshwater conference, Chile2008, Organized by Skretting, November 14, 2008.

Kibenge, F.S.B. 2008. The Phylogeography of infectious salmon anaemia virus. International ISA workshop in Chile, Puerto Montt, June 06, 2008, sponsored by Marine Harvest, Puerto Montt, Chile.

Kibenge, F.S.B. 2008. Infectious salmon anaemia virus (ISAV) phenomics. 2008 Annual Meeting of the Fish Health Section of the American Fisheries Society, July 9-12, 2008, University of Prince Edward Island, Charlottetown, PEI, Canada.

Kibenge, F.S.B. 2008. Molecular epidemiology of infectious salmon anaemia virus in Chile. International ISA-PD workshop in Chile, Puerto Montt, June 06, 2008, sponsored by Biovac, Puerto Montt, Chile.

Kibenge, F.S.B. 2008. Diagnostic considerations of infectious salmon anaemia virus using molecular biology techniques. International ISA-PD workshop in Chile, Puerto Montt, June 06, 2008, sponsored by Biovac, Puerto Montt, Chile.

- ***Scientific publications in peer-reviewed journals***

Workenhe, S.T., Kibenge, M.J.T., Iwamoto, T., and Kibenge, F.S.B. 2008. Absolute Quantitation of Infectious Salmon Anaemia Virus Using Different Real-time Reverse Transcription PCR Chemistries. *Journal of Virological Methods, 154:128-134.*

Godoy, M.G., Aedo, A. Kibenge, M.J.T., Groman, D.B., Yason, C.V., Grothusen, H., Lisperguer, A., Calbucura, M., Avendaño, F., Imilán, M., Jarpa, M., and Kibenge, F.S.B. 2008. First detection, isolation and molecular characterization of infectious Salmon anaemia virus associated with clinical disease in farmed Atlantic salmon (*Salmo salar*) in Chile. *BMC Veterinary Research 4:28.* [Highly accessed article].

Workenhe, S.T., Kibenge, M.J.T., Wright, G.M., Wadowska, D.W., Groman, D., and Kibenge, F.S.B. 2008. Infectious salmon anaemia virus replication and induction of alpha interferon system genes in Atlantic salmon erythrocytes. *Virology Journal 5:36*.

Godoy, M., Kibenge, F., Aedo, A., Kibenge, M., Groman, D., Grothusen, H., Lisperguer, A., Calbucula, M., Aveldaño, F., Imillan, M., and Jarpa M. 2007. Primera detección, aislamiento y caracterización Molecular de ISAv en Salmon del atlántico Salmo salar de cultivo en Chile. *Salmo Ciencia, 2(2): 47-55*.

- ***Other communications***

None.

13. Inscription of diagnostic kits on the OIE Register

i) Did you participate in expert panels for the validation of candidate kits for inscription on the OIE Register? If yes, for which kits?

No.

ii) Did you submit to the OIE candidate kits for inscription on the OIE Register? If yes, for which kits?

No.

Activities in 2009

Infectious salmon anaemia

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

Test	For	Specificity	Total*
Conventional RT-PCR	Virus RNA	Group	2,228
Real-time RT-PCR	Virus RNA	Group & viral load	1,375
SHK-1/ASK-2/TO/CHSE-214 cell cultures at 16°C	Virus isolation	Phenotype	12
Electron microscopy	Virion morphology	Group	0
In-situ hybridization	Virus mRNA	Group	0
ELISA	Antibody detection	Group	147
ELISA	Antigen detection	Group	0
Virus neutralization	Antibody titre	Serotype	5
DNA sequencing	Nucleotide sequence	Genotype	615

***** Total includes tests on surveillance, diagnostic, research, and QA samples

2. Production and distribution of diagnostic reagents

Reagents produced for ISA:
a. ISAV antigens for antibody ELISA.
b. Atlantic salmon serum (positive control) for ISAV antibody ELISA.
c. Rabbit polyclonal antisera for serotyping of ISAV.
d. ISAV RNA (whole genome and gene-specific transcripts) for RT-PCR.
e. ISAV laboratory proficiency panels for real-time RT-PCR.
f. ISAV cDNA clones of gene segments.
g. Histology slides of ISA in Atlantic salmon.

Reagents supplied nationally (including use by my own laboratory):
a. ISAV antigens for antibody ELISA: 10 vials.
b. Atlantic salmon serum (positive control) for ISAV antibody ELISA: 3 vials.
c. Rabbit polyclonal antisera for serotyping of ISAV: none.
d. ISAV RNA (whole genome and gene-specific transcripts) for RT-PCR: 10 vials (one per gene)

e. ISAV laboratory proficiency panels for real-time RT-PCR: 3 panels (48 vials per panel).
f. ISAV cDNA clones of gene segments: 10 vials (one per gene).
g. Histology slides of ISA in Atlantic salmon: none.

Reagents supplied to other OIE Members:
a. ISAV antigens for antibody ELISA: 20 vials to Chile.
b. Atlantic salmon serum (positive control) for ISAV antibody ELISA: 1 vial.
c. Rabbit polyclonal antisera for serotyping of ISAV: 2 vials to Chile.
d. ISAV RNA (whole genome and gene-specific transcripts) for RT-PCR: 4 vials of segment 8 cRNA to Chile.
e. ISAV laboratory proficiency panels for real-time RT-PCR: 27 panels (48 vials per panel) to Chile; 1 panel to Denmark, and 1 panel to Singapore.
f. ISAV cDNA clones of gene segments: 4 vials (one per gene for 4 different genes) to Chile; 1 vial to Taiwan (ROC).

Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

Validation of antibody ELISA for the detection of antibodies against ISAV in fish has been initiated. Formal validation of this test in accordance with the OIE Validation Template is anticipated in 2010.

In collaboration with Sernapesca (National Fisheries Service, Chile), the lab implemented round robin QA testing (ring test) to evaluate ISAV RT-PCR of fish diagnostic laboratories in Chile in 2008. This year the lab set up Phase II of this Ring Test of fish diagnostic laboratories in Chile, a lab in Denmark, and a lab in Singapore.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

Twenty (20) vials of ISAV antigens for antibody ELISA were supplied to Chile.
One (1) vial of Atlantic salmon serum (positive control) for ISAV antibody ELISA was supplied to Chile.
Two (2) vials of rabbit polyclonal antisera for serotyping of ISAV were supplied to Chile.
Four (4) vials of ISAV RNA for positive control in RT-PCR were supplied to Chile.
Four (4) vials of ISAV cDNA clones were supplied to Chile; one (1) vial was supplied to Taiwan (ROC).

5. Research and development of new procedures for diagnosis and control

Routine laboratory diagnosis of ISAV infection is primarily by RT-PCR because of the high sensitivity and rapid turnaround time of the test. The OIE Reference Laboratory for ISA at AVC has described methods for highly reproducible absolute viral load measurements using external standard curves generated and quantitative (Q)RT-PCR with SYBR® Green I chemistry or with TaqMan® probe chemistry (Workenhe *et al*., 2008, *J Virol Meth* 154:128-134).

In 2009, the laboratory confirmed an ISA outbreak in PEI, Canada. The virus isolated from this outbreak was sequenced on all the 8 ISAV RNA genomic segments; it was identified as ISAV of North American genotype. The RNA segment 6 sequences of amplified directly from clinical samples and from the virus isolates had nucleotide sequence identity of 94-95% to other isolates of North American genotype, in contrast to all previous isolates of North American genotype in the OIE Reference Lab database which are more similar, having a nucleotide sequence identity of >98% (Kibenge *et al*., 2001, *Journal of General Virology 82:2869-2879*). The viruses responsible for this outbreak have a 9-amino acid deletion in the highly polymorphic region (HPR) of the haemagglutinin-esterase (HE) protein, and are thus unique ISAV isolates not previously reported. A phylogenetic tree using segment 6 sequences of reference isolates from Norway, Faroe Islands, Scotland, USA, and Canada showed that the isolates cluster separately from all previous isolates of the North American genotype (Kibenge *et al.,* manuscript in preparation). These new ISAV isolates may not be detected by some of the RT-PCR procedures

currently used, and may also be antigenically distinct. Research is on-going to obtain complete phenotypic characterization of these new ISAV isolates.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

Since the first ISA outbreak in Chile in July 2007, the OIE Reference Laboratory for ISA at AVC has sequenced 51 new Chile ISAV isolates on RNA segment 5 and 79 new isolates on RNA segment 6, and has performed an extensive comparative analysis of ISAV F and HE sequence data, including isolates sampled from Norway, Faroe Islands, Scotland, USA, and Canada. Details of this study are in a report (Kibenge *et al.,* 2009, *Virology Journal, 6:88*). Our findings suggest that the ISA outbreaks in Chile, which started in 2007, were caused by virus that was already present in Chile that mutated to new strains.

One report of positive results for ISA on samples received in the OIE Reference Laboratory for ISA at AVC from the outbreak in PEI, Canada, was sent to the OIE.

7. Provision of consultant expertise to OIE or to OIE Members

Helped Dr. Barrie D. Carnat to write ISA information summary published on line at the OIE web site (http://www.oie.int/eng/ressources/en_diseasecards.htm).

Have worked very closely with government officials and the Atlantic salmon industry in Chile on detection and control of ISA, and to characterize the virus responsible for the 2007-2010 ISA epizootic in Chile. In July 2009, the laboratory implemented Phase II round robin QA testing of fish diagnostic laboratories in Chile; Labs in Denmark and Singapore also participated.

8. Provision of scientific and technical training to personnel from other OIE Members

Submitted an OIE Laboratory Twinning proposal with Lab in Valparaiso, Chile.

9. Provision of diagnostic testing facilities to other OIE Members

Number of times	Tentative testing	Confirmatory testing	Member country	Informed OIE
Numerous		Yes	Chile	No

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

Member of the scientific committee for the International Symposium on Infectious Salmon Anaemia September 13-15, 2010, in Oslo, Norway (Scientific Committee Chair is Dr. Knut Falk). The meeting is supported by OIE. The programme will include scientific sessions and a one day workshop focusing on risk management. Keynotes will be given by international recognized speakers. The goal of this conference is to provide updates on ISA research, biosecurity and disease control strategies. The symposium is organized by a steering committee, an executive committee and a scientific committee. The mandate of the scientific committee is to: (1) Suggest a scientific programme for the symposium, and (2) Suggest keynote speakers to be approved by the executive committee.

11. Participation in international scientific collaborative studies

Mitigation of infectious salmon anaemia (ISA) by vaccination and genetic selection is a four-year project (2008-2012) being undertaken by Novartis Animal Health Canada Inc. Aqua Health Business, which is located in Victoria, Prince Edward Island, Canada. This project represents a partnership between Novartis, AVC/UPEI (OIE Reference Laboratory), Cooke Aquaculture Inc., New Brunswick, New Brunswick Research and Productivity Council (RPC), and the Norwegian Veterinary Institute (NVI), Norway.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

Kibenge, F., Kibenge, M., Simard, S., Riveroll, A., Pallapothu, M., and Salonius, K. Development of a DIVA system for an infectious salmon anaemia (ISA) virus vaccine using a qRT-PCR test based on segment 6 of the ISA virus. 14th *European Association of Fish Pathologists International Conference on Diseases of Fish and Shellfish, Prague, Czech Republic, September 14-19, 2009.*

Kibenge, F.S.B., Godoy, M.G., Wang, Y., Kibenge, M.J.T., and Gherardelli, V. 2009. Phlogeography of infectious salmon anaemia virus (ISAV). *World Association of Veterinary Laboratory Diagnosticians-14th International Symposium, Madrid, Spain, 17-20 June 2009.*

Scientific publications in peer-reviewed journals

Workenhe, S.T., Hori, T.S., Rise, M.L., Kibenge, M.J.T., and Kibenge, F.S.B. 2009. Infectious salmon anaemia virus (ISAV) isolates induce distinct gene expression responses in the Atlantic salmon (Salmo salar) macrophage/dendritic-like cell line TO, assessed using genomic techniques. *Molecular Immunology, 46:2955-2974.*

Kibenge, F.S.B., Godoy, M.G., Wang, Y., Kibenge, M.J.T., Gherardelli, V., Mansilla, S., Lisperger, A., Jarpa, M., Larroquete, G., Avendaño, F., Lara, M. and Gallardo, A. 2009. Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences. *Virology Journal, 6:88.* [**Highly accessed article**].

Other communications

None.

13. Inscription of diagnostic kits on the OIE Register

i) Did you participate in expert panels for the validation of candidate kits for inscription on the OIE Register? If yes, for which kits?

No.

ii) Did you submit to the OIE candidate kits for inscription on the OIE Register? If yes, for which kits?

No.

OIE Reference Laboratory Reports

Activities in 2010

Name of disease (or topic) for which you are a designated OIE Reference Laboratory:	**Infectious Salmon Anaemia (ISA)**
Address of laboratory	**Department of Pathology an Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, CANADA, C1A 4P3**
Tel.: Fax:	**(902) 566-0967** **(902) 566-0851**
e-mail address: website:	**kibenge@upei.ca** **http://www.upei.ca/~avc/html/oie.html**
Name of Head of Laboratory (Responsible Official):	**Dr. Frederick S.B. Kibenge**
Name of OIE Reference Expert:	**Dr. Frederick S.B. Kibenge**
Name of writer of this report (if different from above):	

Please note the text in red font is given for guidance and should be deleted from your report.

Part I: Summary of general activities related to the disease

The Atlantic Veterinary College (AVC) is one of six faculties of the University of Prince Edward Island (UPEI), in Charlottetown, Canada. The university has approximately 4,000 full-time students. AVC offers a 4-year Doctor of Veterinary Medicine degree to Atlantic Canadian and international students, which is accredited by the Canadian Veterinary Medical Association, the American Veterinary Medical Association, and recognized by the Royal College of Veterinary Surgeons in the United Kingdom. AVC is recognized by other veterinary colleges around the world for its expertise and extensive research and service in aquaculture and fish health. The OIE Reference Laboratory for ISA at AVC is located within the UPEI Aquatic Virology Collaborating Centre, one of UPEI's six Centres of Expertise.

1. Test(s) in use/or available for the specified disease/topic at your laboratory

Test	For	Specificity	Total*
Conventional RT-PCR	Virus RNA	Group	408
Real-time RT-PCR	Virus RNA	Group & viral load	0
SHK-1/ASK-2/TO/CHSE-214 cell cultures at 16°C	Virus isolation	Phenotype	0
Electron microscopy	Virion morphology	Group	0
In-situ hybridization	Virus mRNA	Group	0
ELISA	Antibody detection	Group	1705
ELISA	Antigen detection	Group	0
Virus neutralization	Antibody titre	Serotype	0
DNA sequencing	Nucleotide sequence	Genotype	253

* Total includes all tests on surveillance, diagnostic, and research samples

2. Production and distribution of diagnostic reagents

Type of reagent	***Amount supplied nationally*** (*including for own use*)	***Amount supplied to other countries***
ISAV antigens for antibody ELISA	100 ml	20 ml to USA
Atlantic salmon serum (positive control) for ISAV antibody ELISA	50 ml	10 ml to USA
ISAV Rabbit polyclonal antisera	30 ml	
ISAV RNA for RT-PCR	10 vials (one per gene)	
ISAV laboratory proficiency panels for real-time RT-PCR	2 panels (48 vials per panel)	
ISAV cDNA clones of gene segments	10 vials (one per gene)	
Histology slides of ISA		

Part II: Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

Validation of antibody ELISA for the detection of antibodies against ISAV in fish has been initiated. Formal validation of this test in accordance with the OIE Validation Template is anticipated in 2011.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

20 mls of ISAV antigens for antibody ELISA and 10 mls of Atlantic salmon serum (positive control) for ISAV antibody ELISA were supplied to USA.

5. Research and development of new procedures for diagnosis and control

Routine laboratory diagnosis of ISAV infection is primarily by RT-PCR because of the high sensitivity and rapid turnaround time of the test. The OIE Reference Laboratory for ISA at AVC has optimized the TaqMan® real-time RT-PCR detection of ISAV (Godoy *et al*., 2010, *Dis Aquatic Organ* 90:25-30), and recently co-authored co-authored guidelines for scientific rigor in fluorescence-based quantitative real-time PCR experiments (Bustin *et al*., 2010, *BMC Mol Bio* 11:74).

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

Since the first ISA outbreak in Chile in July 2007, the OIE Reference Laboratory for ISA at AVC has continued to characterize new Chile ISAV isolates. Our recent findings suggest that ISAV HPR7b has been largely replaced by HPR0 in the Chilean salmon industry.

7. Provision of consultant expertise to OIE or to OIE Members

Have worked very closely with government officials and the Atlantic salmon industry in Chile on detection and control of ISA, and to characterize the virus responsible for the 2007-2010 ISA epizootic in Chile. In July 2009, the laboratory implemented Phase II round robin QA testing of fish diagnostic laboratories in Chile; A final report of this ringtest was submitted to Sernapesca in October 2010.

Attended the meeting for Launching the Network of the National Laboratories of the Veterinary Services of the Americas which was held in Panama, Republic of Panama, 3 - 5 November 2010. The meeting was organized by the OIE Sub Regional Representation for Central America based in Panama. Full members of the network are the National Laboratories of Veterinary Services in the OIE Member Countries of the Americas and Caribbean regions. The Reference Laboratories and Collaborating Centres of the OIE are Observer members.

8. Provision of scientific and technical training to personnel from other OIE Members

OIE Laboratory Twinning Project between AVC and Pontificia Universidad Catolica de Valparaiso, Chile, was launched on October 15, 2010 in Valparaiso, Chile. The first training workshop was held from October 12-15, 2010 in Valparaiso, and included training in Taqman RT-PCR assay for ISAV.

9. Provision of diagnostic testing facilities to other OIE Members

Number of times	Primary diagnostic testing	Confirmatory testing	Member country	Informed OIE
68		Yes	Chile	No

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

Member of the scientific committee for the International Symposium on Infectious Salmon Anaemia September 13-15, 2010, in Oslo, Norway (Scientific Committee Chair was Dr. Knut Falk). The meeting was supported by OIE. The programme included scientific sessions and a one day workshop focusing on risk management. Keynotes were given by international recognized speakers. The goal of this conference was to provide updates on ISA research, biosecurity and disease control strategies. The symposium was organized by a steering committee, an executive committee and a scientific committee. The mandate of the scientific committee was to: (1) Suggest a scientific programme for the symposium, and (2) Suggest keynote speakers to be approved by the executive committee.

11. Participation in international scientific collaborative studies

Mitigation of infectious salmon anaemia (ISA) by vaccination and genetic selection is a four-year project (2008-2012) being undertaken by Novartis Animal Health Canada Inc. Aqua Health Business, which is located in Victoria, Prince Edward Island, Canada. This project represents a partnership between Novartis, AVC/UPEI (OIE Reference Laboratory), Cooke Aquaculture Inc., New Brunswick, New Brunswick Research and Productivity Council (RPC), and the Norwegian Veterinary Institute (NVI), Norway.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- ***Presentations at international conferences and meetings***

Kibenge, M.J.T., Godoy, M.G., Wang, Y., Gherardelli, V., Olmos, P., and Kibenge, F.S.B. 2010. Tracing the spread of ISAV by use of genotypically assigned types. *First Chilean Biosecurity Conference in Salmon Farming, Puerto Montt, Chile, October 18-19, 2010.*

Rimstad, E., Falk, K., Mjaaland, S., and Kibenge, F.S.B. 2010. Characterization of infectious salmon anaemia virus (Plenary). *OIE International Symposium on Infectious Salmon Anaemia, Oslo, Norway, September 13-15, 2010.*

Godoy, M., Kibenge, F.S.B., Gherardelli, V., Olmos, P., Ovalle, L., Munoz, G., Kibenge, M., Wang, Y., Gallardo, A., Lara, M., and Avendano-Herrera, R. 2010. Virulence markers in haemagglutinin-esterase (HE) protein (Highly Polymorphic Region) and fusion protein (Q266→L266) in infectious salmon anaemia virus (ISAV) in Chile. (Oral). *OIE International Symposium on Infectious Salmon Anaemia,Oslo, Norway, September 13-15, 2010.*

Kibenge, F.S.B., Olmos, P., Almonacid, J., Gherardelli, V., Gaggero, A., Kibenge, M., Avendano-Herrera, R., and Godoy, M. 2010. Observations on the behaviour of Chilean strains of infectious salmon anaemia virus in cell culture (Poster). *OIE International Symposium on Infectious Salmon Anaemia, Oslo, Norway, September 13-15, 2010.*

Olmos, P., Kibenge, F.S.B., Gaggero, A., Kibenge, M., and Godoy, M. 2010. Detection of infectious salmon anaemia virus strains by shell vial assay. (Poster). *OIE International Symposium on Infectious Salmon Anaemia, Oslo, Norway, September 13-15, 2010.*

Kibenge, F.S.B. 2010. Virus-host interactions of infectious salmon anaemia virus, an orthomyxovirus of fish. *PROYECTO FIP 2008-66 Workshop, Bivalve molluscs and copepods as possible vectors of high risk diseases in salmonids, Valparaiso, Chile, August 18, 2010,* sponsored by Pontificia Universidad Catolica de Valparaiso, Chile.

Kibenge, F.S.B. 2010. Salmon farming in the marine virus environment. *PROYECTO FIP 2008-66 Workshop, Bivalve molluscs and copepods as possible vectors of high risk diseases in salmonids, Valparaiso, Chile, August 18, 2010*, sponsored by Pontificia Universidad Catolica de Valparaiso, Chile.

Kibenge, F.S.B. 2010. Infectious salmon anaemia virus in Rainbow trout under controlled conditions. *Puerto Varas, Chile, July 9, 2010,* sponsored by Aquagestion S.A., Puerto Montt, Chile.

Kibenge, F.S.B. 2010. Solving a fish mystery : The origin of ISA in Chile. *Puerto Varas, Chile, July 9, 2010.* sponsored by Aquagestion S.A., Puerto Montt, Chile.

Kibenge, F.S.B. 2010. Meaning of presence of ISAV HPR0 group, world situation. *Background to manage Chile ISAV-HPR0, Puerto Montt, Chile, July 9, 2010.* sponsored by Aquagestion S.A., Puerto Montt, Chile.

- ***Scientific publications in peer-reviewed journals***

Kulshreshtha, V., Kibenge, M., Salonius, K., Simard, N., Riveroll, A., and Kibenge, F. 2010. Identification of the 3' and 5' Terminal Sequences of the 8 RNA Genome Segments of European and North American Genotypes of Infectious Salmon Anaemia Virus (an Orthomyxovirus) and Evidence for Quasispecies Based on the Non-Coding Sequences of Transcripts. *Virology Journal 7:338.*

Workenhe, S.T., Rise, M.L., Kibenge, M.J.T., and Kibenge, F.S.B. 2010. The fight between the teleost fish immune response and aquatic viruses. *Molecular Immunology 47:2525-2536*.

Cornejo, I., Sepúlveda F.V., Kibenge, F.S.B., and Young J.I. 2010. Isolation of the Atlantic salmon ß-actin promoter and its use to drive expression in salmon cells in culture and in transgenic zebrafish. *Aquaculture 309:75-81*.

Bustin, S.A., Beaulieu, J-F., Huggett, J., Jaggi, R., Kibenge, F.S.B., Olsvik, P.A., Penning, L.C., Toegel, S. 2010. MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Molecular Biology 11:74*. [**Highly accessed article**].

Godoy, M.G., Kibenge, F.S., Kibenge, M.J., Olmos, P., Ovalle, A., Yañez, R., and Avendaño-Herrera. 2010. TaqMan® real-time RT-PCR detection of infectious salmon anaemia virus (ISAV) from formalin-fixed paraffin-embedded Atlantic salmon *Salmo salar* tissues. *Diseases of Aquatic Organisms 90:25-30*.

- ***Other communications***

None

13. Inscription of diagnostic kits on the OIE Register

i) Did you participate in expert panels for the validation of candidate kits for inscription on the OIE Register? If yes, for which kits?

No.

ii) Did you submit to the OIE candidate kits for inscription on the OIE Register? If yes, for which kits?

No.