



Fish vet. Ola Nordmann
Valdres

EXERCISE, EXERCISE, EXERCISE

Your reference: Valdres1

Our reference: 2013-04-123456

Date: 20.11.2013

Sampled: 17th of Nov.

Received: 18th of Nov.

Site: 12345 Valdres

Owner: Valdres Fish Farming AS

Samples: Organs from 5 fish on formalin, marked 1-5

Species: Rainbow trout

Anamnesis: Increased mortality in one tank, 1% per day. Rainbow trout ≈250gr. Fresh water ≈10°.

Samples from diseased fish; showing darkening of skin and slow swimming.

Fish 1 & 2 were swimming in circles. Pale gills on fish 3.

Autopsy shows some swollen spleen in all fish and bleedings in the muscle of fish 3 & 5.

Bacteriology testing on blood agar: results will be forwarded

Method (ME01_002): Histopatologi

Examined: gills, heart, kidney, spleen, liver and muscle from 5 fish, marked 1-5

Results: Moderate to severe haemorrhages in the interstitie of kidney in fish 2 and 3.

Some sparse focal haemorrhages in kidney also in the rest of the fish.

Focal haemorrhage also in the muscle of fish 3 and brain of fish 1.

Congestion of blood in the spleen in all fish.

Method (ME01_038): Viral haemorrhagic septikemi (VHS), immunohistochemistry

Examined: kidney from fish 2 and 3

Comment: ongoing test, results in two days

Diagnose: Haemorrhages and circulatory disturbances in all fish
Suspicion of Viral haemorrhagic septicemia (VHS)

Comments: New sampling including samples on transport medium and/or RNAlater is necessary to possibly confirm the diagnosis and further characterize any virus detection.

The circle swimming can indicate a CNS-form of VHS and brains should therefore also be sampled both on formalin and RNAlater.

Renate Johansen
Veterinary

Copy to the Norwegian Food Safety Authority



Fish vet. Ola Nordmann
Valdres

EXERCISE, EXERCISE, EXERCISE

Your reference: Valdres1

Our reference: 2013-04-123456

Date: 22.11.2013

Additional letter with immunohistochemistry results. First letter dated 20.11.2013

Sampled: 17th of Oct.

Received: 18th of Oct.

Site: 12345 Valdres

Owner: Valdres Fish Farming AS

Samples: Organs from 5 fish on formalin, marked 1-5

Species: Rainbow trout

Anamnesis: Increased mortality in one tank, 1% per day. Rainbow trout ≈250gr. Fresh water ≈10°.

Samples from diseased fish; showing darkening of skin and slow swimming.

Fish 1 & 2 were swimming in circles. Pale gills on fish 3.

Autopsy shows some swollen spleen in all fish and bleedings in the muscle of fish 3 & 5.

Bacteriology testing on blood agar: results will be forwarded

Method (ME01_002): Histopatologi

Examined: gills, heart, kidney, spleen, liver and muscle from 5 fish, marked 1-5

Results: Moderate to severe haemorrhages in the interstitie of kidney in fish 2 and 3.

Some sparse focal haemorrhages in kidney also in the rest of the fish.

Focal haemorrhage also in the muscle of fish 3 and brain of fish 1.

Congestion of blood in the spleen in all fish.

Method (ME01_038): Viral haemorrhagic septikemi (VHS), immunohistochemistry

Examined: kidney from fish 2 and 3

Comment: positive marking in the affected areas of kidney in both fish

Diagnose: Haemorrhages and circulatory disturbances in all fish
Suspicion of Viral haemorrhagic septicemia (VHS)

Comments: The positive immunohistochemistry strengthens the suspicion of VHS. We got new samples from the farm sent by the Food Safety Authority today. Results will be finished early next week.

Renate Johansen
Veterinary

Copy to the Norwegian Food Safety Authority



National Food Safety Authority

EXERCISE, EXERCISE, EXERCISE

Your reference: Valdres2

Our reference: 2013-04-123467

Date: 25.11.2013

Sampled: 21th of Nov.

Received: 22th of Nov.

Site: Farm A

Owner: Valdres Fish Farming AS

Samples: Organs from 30 fish on formalin, marked 1-30. Kidney on RNA-later from the same 30 fish. Fish 1-10 from tank A, 11-20 tank B, 21-30 tank C.

Samples from brain were included both on RNAlater and formalin from fish showing symptoms of abnormal swimming (Fish 1-4 in all tanks).

3 pooled kidneys homogenates (10 fish per pool) marked tank A, B, C

Anamnesis: Rainbow trout ≈250gr. Fresh water ≈10°.

Increased mortality in tank A, 2-3% per day. Low mortality in the rest of the 10 tanks of the farm, but some abnormal swimming and darkening of fish in tank B and tank C.

In tank A all sampled fish showed slow swimming, low appetite and exophthalmus. Autopsy revealed enlarged spleen in fish 1,2,4,6,8. Bleeding in muscle in fish 4 and 6. Moderate ascites in all 10 fish.

Autopsy also revealed moderate ascites and enlargement of spleen in fish 1 and 4 in tank B and fish 1 and 2 in tank C.

Method (ME01_002): Histopatologi

Examined: gills, heart, kidney, spleen, liver, muscle and brain from 5 fish per tank (1-5, 11-15, 21-25)

Results:

Tank A: Moderate to severe haemorrhages in the interstitie of kidney in fish 1 and 3.

Some sparse focal haemorrhages in kidney also in the rest of the fish.

Focal haemorrhages also in the muscle of fish 3.

Congestion of blood in the spleen in all fish. Focal haemorrhages in brain of fish 2 and 3.

Tank B: Fish 1 and 3 show some congestion of blood and possible inflammation in the brain.

Fish 1&4 show moderate haemorrhages in kidney and spleen.

Tank C: Fish 1-3 show some focal haemorrhages in the brain.

Fish 1&2 show moderate haemorrhages in kidney along with congestion of blood in the spleen

Method (ME01_038): Viral haemorrhagic septikemi (VHS), immunohistochemistry

Examine: kidney and brain from fish 2&3 tank A, 1&3 tank B, 1&2 tank C

Comment: on-going test

Method (ME03_006): Cell culture isolation for VHSV and IHNV

Examine: 3 homogenates of pooled kidneys from 10 fish marked tank A, B, C

Results: Inoculated on BF-2 and EPC cells 22.Nov. CPE detected in BF-2 cells inoculated with material from tank A after 3 day incubation, and immunofluorescence testing is positive for VHSV.

Comment: No CPE from tank B and C so far but the testing will continue

Method (ME07_188): Infectious hematopoietic necrosis virus, real time RT PCR

Examine: 3 homogenates of pooled kidneys from 10 fish marked tank A, B, C

Results: Negative for IHNV

Method (ME07_204): Viral haemorrhagic septikemi virus, real time RT PCR

Examined: 3 pooled kidneys homogenates (10 fish per pool) marked tank A, B, C.

All 30 kidneys (marked 1-30) and 12 brains (marked 1-4, 11-14, 21-24) on RNAlater.

Results:

Tank A: VHSV detected in all samples.

Tank B: VHSV detected in the homogenate, kidneys from fish 1-6 and all brains 1-4.

Tank C: VHSV detected in kidney and brain from fish 1 and 2. Inconclusive results from the homogenate.

Comments: CT values indicate moderate to high amounts of virus in tank A, low to moderate in tank B and low in tank C. The low amount of virus and the pooling of 10 fish can explain the difficulties of detection of VHSV in the homogenate from tank C.

Diagnose: Viral haemorrhagic septicemia (VHS)
 Viral haemorrhagic septicemia virus (VHSV)

Comments: The samples arrived too late on Friday to get an answer for you before the weekend. The results today are clearly positive for VHSV and sequencing of the virus will be performed tomorrow.



Renate Johansen
Veterinary

Copy to owner and Fish vet. Ola Nordmann



National Food Safety Authority

EXERCISE, EXERCISE, EXERCISE

Your reference: Valdres2

Our reference: 2013-04-123467

Date: 26.11.2013

New letter with additional results. First letter dated 25.11.2013

Sampled: 21th of Nov.

Received: 22th of Nov.

Site: Farm A

Owner: Valdres Fish Farming AS

Samples: Organs from 30 fish on formalin, marked 1-30. Kidney on RNA-later from the same 30 fish. Fish 1-10 from tank A, 11-20 tank B, 21-30 tank C.

Samples from brain were included both on RNAlater and formalin from fish showing symptoms of abnormal swimming (Fish 1-4 in all tanks).

3 pooled kidneys homogenates (10 fish per pool) marked tank A, B, C

Anamnesis: Rainbow trout ≈250gr. Fresh water ≈10°.

Increased mortality in tank A, 2-3% per day. Low mortality in the rest of the 10 tanks of the farm, but some abnormal swimming and darkening of fish in tank B and tank C.

In tank A all sampled fish showed slow swimming, low appetite and exophthalmus. Autopsy revealed enlarged spleen in fish 1,2,4,6,8. Bleeding in muscle in fish 4 and 6. Moderate ascites in all 10 fish.

Autopsy also revealed moderate ascites and enlargement of spleen in fish 1&4 in tank B and fish 1 and 2 in tank C.

Method (ME01_002): Histopatologi

Examined: gills, heart, kidney, spleen, liver, muscle and brain from 5 fish per tank (1-5, 11-15, 21-25)

Results:

Tank A: Moderate to severe haemorrhages in the interstitie of kidney in fish 1 and 3.

Some sparse focal haemorrhages in kidney also in the rest of the fish.

Focal haemorrhages also in the muscle of fish 3.

Congestion of blood in the spleen in all fish. Focal haemorrhages in brain of fish 2 and 3.

Tank B: Fish 1 and 3 show some congestion of blood and possible inflammation in the brain.

Fish 1&4 show moderate haemorrhages in kidney and spleen.

Tank C: Fish 1-3 show some focal haemorrhages in the brain.

Fish 1&2 show moderate haemorrhages in kidney along with congestion of blood in the spleen

Method (ME01_038): Viral haemorrhagic septikemi (VHS), immunohistochemistry

Examine: kidney and brain from fish 2&3 tank A, 1&3 tank B, 1&2 tank C

Results: clearly marking of VHSV in both kidney and brain of all fish

Method (ME03_006): Cell culture isolation for VHSV and IHNV

Examine: 3 homogenates of pooled kidneys from 10 fish marked tank A, B, C

Results: Inoculated on BF-2 and EPC cells 22.Nov. CPE detected in BF-2 cells inoculated with material from tank A after 3 day incubation, and immunofluorescence testing is positive for VHSV.

Comment: Still no CPE from tank B and C so far but the testing will continue

Method (ME07_188): Infectious hematopoietic necrosis virus, real time RT PCR

Examine: 3 homogenates of pooled kidneys from 10 fish marked tank A, B, C

Results: Negative for IHNV

Method (ME07_204): Viral haemorrhagic septikemi virus, real time RT PCR

Examined: 3 pooled kidneys homogenates (10 fish per pool) marked tank A, B, C.

All 30 kidneys (marked 1-30) and 12 brains (marked 1-4, 11-14, 21-24) on RNAlater.

Results:

Tank A: VHSV detected in all samples.

Tank B: VHSV detected in the homogenate, kidneys from fish 1-6 and all brains 1-4.

Tank C: VHSV detected in kidney and brain from fish 1 and 2. Inconclusive results from the homogenate.

Comments: CT values indicate moderate to high amounts of virus in tank A, low to moderate in tank B and low in tank C. The low amount of virus and the pooling of 10 fish can explain the difficulties of detection of VHSV in the homogenate from tank C.

Method (ME07_093): Viral haemorrhagic septikemi virus RT-PCR

Examined: positive kidney samples from all 3 tanks

Results: PCR products from all 3 tanks were sequenced and compared to available sequences in the databank. All sequences (625bp) were 100% identical to VHSV of genotype Id (AY546615).

Diagnose: Viral haemorrhagic septicemia (VHS)
 Viral haemorrhagic septicemia virus (VHSV) genotype Id

Comments: VHSV of genotype Id has not been reported in Norway since 1968 (Vestrefjorden AY546621) and imported eggs from Denmark was then believed to be the source of infection.

The positive detection of VHSV in brain both with rRT-PCR and immunohistochemistry indicates a nervous form of VHS. Prevalence of VHSV in fish showing abnormal swimming is much higher than in fish with no clinical signs. Further sampling should therefore be focused on sampling of fish with clinical signs and brains need to be included in the sampling.

The sequence analyzed from the present VHSV in Valdres are identical to VHSV isolated from rainbow trout from brackish water in Finland 2000 (FI-ka-422, AY546615). Full genome sequencing is needed to further characterize the virus.



Renate Johansen
Veterinary

Copy to owner and Fish vet. Ola Nordmann



National Food Safety Authority

EXERCISE, EXERCISE, EXERCISE

Your reference: Valdres 3

Our reference: 2013-04-123478

Date: 20.12.2013

Sampled: 16th of Des.

Received: 17th of Des.

Site: Lake close to Farm A

Samples: samples from 10 Arctic char (1-10) and 10 Brown trout (11-20).

Gill, heart, kidney, spleen, liver, muscle and brain on formalin.

Kidney and heart on RNAlater.

Pooled samples of kidney and heart on transport medium (5 fish per glass), marked A1, A2, B3, B4.

Anamnesis: The lake is totally covered by ice. The fish were caught by ice-fishing. No signs of disease either externally or by autopsy.

Method (ME01_002): Histopatologi

Examined: gills, heart, kidney, spleen, liver, muscle and brain from all 20 fish

Results: No signs of VHS. A few large parasites were seen both inside the intestines and in the abdominal cavity.

Method (ME03_006): Cell culture isolation for VHSV and IHNV

Examine: 4 homogenates of pooled organs from the 20 fish, marked A1, A2, B3, B4.

Inoculated on BF-2 and EPC cells 17th of Des.

Results: No CPE detected so far but the testing will continue for 2-3 weeks

Method (ME07_188): Infectious hematopoietic necrosis virus, real time RT PCR

Examine: 4 homogenates of pooled organs from the 20 fish, marked A1, A2, B3, B4.

Results: Negative for IHNV

Method (ME07_204): Viral haemorrhagic septikemia virus, real time RT PCR

Examined: Kidney & hearts from 20 fish, marked 1-20

Results: Negative for VHSV

Diagnose: None

Comments: No detection of VHS in the 20 sampled fish.

Renate Johansen
Veterinary



National Food Safety Authority

EXERCISE, EXERCISE, EXERCISE

Your reference: Valdres 4

Our reference: 2014-04-1234

Date: 29.01.2014

Sampled: 20th of Jan.

Received: 23th of Jan.

Site: Lake close to Farm A

Samples: 10 frozen dead Arctic char detected by fishermen. 100-300gr.

Anamnesis: Dead fish mainly of Arctic char and brown trout has been observed in the lake. Earlier samples provided by the Food safety authority from healthy Arctic char and brown trout caught by ice-fishing in the lake has not revealed any deadly diseases (2013-04-123478)

The sampled fish were found dead along the shore of the lake in a ice-free area close an outgoing river. The fish where frozen whole at -20°C for 2 days before shipped to the laboratory on ice.

Method (ME01_001): Autopsy

Examined: 10 frozen Arctic char

Results: The fish was still frozen at arrival with -4°C in the package. Autopsy was performed on the day of arrival and revealed that 5 fish were too decayed for further examination. The 5 remaining fish showed little decay and no external damages. Autopsy revealed enlarged spleen in all fish. Samples were taken from brain, heart, spleen and kidney individually on RNA later and pooled on transport medium (1 fish per glass).

Method (ME03_006): Cell culture isolation for VHSV and IHNV

Examine: 5 homogenates of pooled organs from 5 fish marked 1-5

Inoculated on BF-2 and EPC cells 24.Jan.

Results: CPE detected in BF-2 cells inoculated with material from fish 1 and 3 after 3 day incubation. Immunofluorescence testing shows positive marking for VHSV.

Comment: No CPE from the rest of the fish so far but the testing will continue

Method (ME07_188): Infectious hematopoietic necrosis virus, real time RT PCR

Examine: 5 homogenates of pooled organs from 5 fish marked 1-5

Results: Negative for IHNV

Method (ME07_204): Viral haemorrhagic septikemi virus, real time RT PCR

Examined: Individual organs (brain, heart, spleen and kidney) from fish 1-5 on RNA later

Results: VHSV detected in all organs from fish 1 and 3.

Comments: CT values indicate moderate to high amounts of virus in brain and heart, low in kidney and spleen.

Method (ME07_093): Viral haemorrhagic septikemi virus RT-PCR

Examined: positive heart from fish 1 and 3

Results: PCR products from both fish were sequenced showing 100% identity with VHSV earlier detected in Farm A (genotype Id).

Diagnose: Viral haemorrhagic septicemia virus (VHSV) genotype Id

Comments: Frozen fish are not suited for histopathology. New samples from diseased fish in the lake are needed to reveal if the fish are dying with or from the VHS-virus.



Renate Johansen
Veterinary

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