

CASE STUDY

***Pasteurella skyensis* in Atlantic salmon (*Salmo salar* L.) in Western Norway**

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Pasteurellosis has been an emerging disease in Atlantic salmon (*Salmo salar* L.) in Western Norway in recent years. This paper presents the first case study from Atlantic salmon in Western Norway where the cause of mortality and morbidity was associated with *Pasteurella skyensis*.

Introduction

Pasteurella skyensis has until now, only been reported as a cause of disease in Scotland, where it was first discovered at three separate sites over the course of a four-year period (Birkbeck et al. 2002). Two serotypes, O1 and O2, have so far been described (Reid and Birkbeck 2015). In recent times, *P. skyensis* outbreaks in Scotland have been associated with serotype O2 (Soares et al. 2019).

Pasteurellosis in Atlantic salmon was first diagnosed in Norway in 1989 (Valheim et al. 2000) and has been a rapidly increasing problem in Atlantic salmon in Western Norway over the last three years (Legård and Strøm 2020). All outbreaks have been associated with a particular *Pasteurella* variant for which the name "*Pasteurella atlantica* genomovar *salmonicida*" has been proposed as a preliminary working nomenclature (Gulla et al. 2020). Until the present study, *P. skyensis* had not been identified in Norway.

During a follow up at a farm site in Western Norway by FoMAS, prompted by increased mortality, it was noted that the clinical signs and autopsy findings did not match those typical of *Pasteurella atlantica* genomovar *salmonicida* outbreaks, nor other common disease findings from that particular geographic area.

Case

Anamnesis

FoMAS – Fiskehelse og Miljø AS was initially contacted in August of 2020, because of an increase in mortality at an aquaculture site. Increase in daily mortality was only observed in one cage which had initially started low but increased from 0.04% to 1% within a month. Personnel at the farm had not observed any changes in fish behaviour or appetite and no obvious external changes in dead fish were noted. Fish in the affected cage had been delivered to the site 3-4 months earlier, and fish in other cages at the site were delivered 4-5 months earlier. Approximately three weeks after the initial outbreak, following delousing using thermal treatment of several cages, not including the primary

Table 1. Autopsy findings

Affected organ/tissue/area	Observations
Skin	Dark coloring
Gills	Petechial bleeding
Thymus	Petechial bleeding/erythema
Abdominal wall	Petechial bleeding
Abdominal fat tissue	Petechial bleeding
Abdominal cavity	Clear fluid
Cardiac cavity	Clear fluid
Swim bladder	Petechial bleeding
Fatty tissue behind eyes	Petechial bleeding
Cranium	Petechial bleeding
Liver	Petechial bleeding

affected cage, other cages became affected and displayed increased mortality, but not as severely as the initial cage. Total mortality in other cages within a month post treatment ranged from approximately 0.5% to 2%. There was no cleaning of delousing equipment between cages treated the same day.

Clinical findings

On the day of inspection, several moribund and lethargic fish were seen close to the net pen wall of the affected cage. Affected fish were observed to swim sluggishly, in random patterns, and away from the rest of the group. They also had a very dark skin color and did not respond at all to any attempt at startling them.

Autopsy findings were similar in all examined fish but varied in severity. Affected fish seemed anaemic and the blood appeared “thin”, but this was only by direct visual observation, measurement of hematocrit was not performed. [Table 1](#) and [Figure 1](#) present these findings. Autopsy findings can be summarised as consistent with septicaemia and circulatory failure.

Laboratories contributing to case diagnostics

The following laboratories were used for bacteriological, histological and PCR analysis: the Norwegian Veterinary Institute (NVI), Patogen A/S, and Fish Vet Group A/S (now Pharmaq Analytiq A/S).

Histology

Histological samples were collected according to standard procedure. All samples were fixed using 10% phosphate – buffered formalin (pH 7.2 – 7.4) on site prior to shipment to laboratories. Organs collected were gills, heart, liver, kidney, spleen, pyloric caeca, muscle, skin, thymus, eyes, brain, swim bladder, and pseudobranch. At the laboratory the organs were separately embedded in paraffin, and sections (3 µm) stained with hematoxylin and eosin (H&E) were subsequently examined by light microscopy.

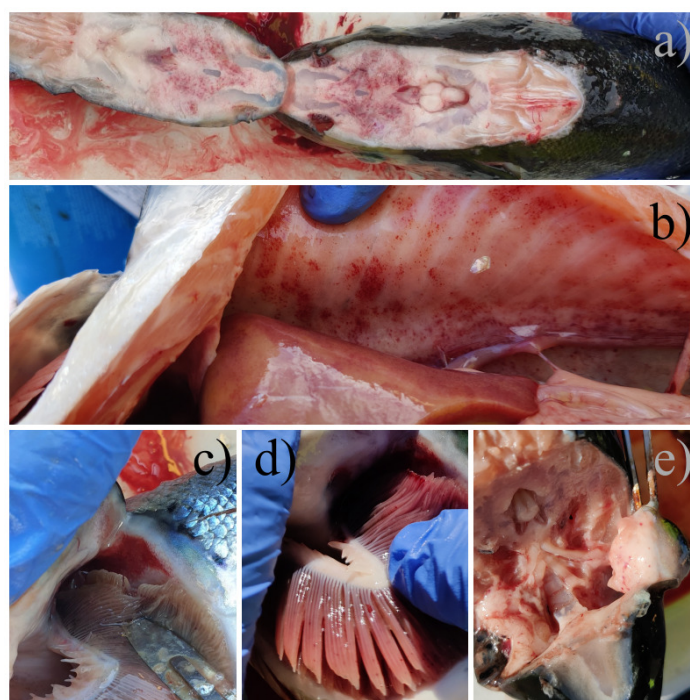


Figure 1. Clinical signs of *Pasteurella skyensis* in Atlantic salmon.

(a) Petechial bleeding in the cranium. (b) Petechial bleeding on the peritoneum and swim bladder. (c) Petechia/erythema on the thymus. (d) Petechial bleeding on the gill filaments. (e) Petechial bleeding in adipose tissue behind the eyes.

PCR

Tissue samples from gills, kidney and heart were collected according to standard procedure and fixed in RNA-later® on site prior to shipment to laboratories for analysis.

Bacteriology

Bacterial samples from anterior kidney were taken by inoculating loop and isolated onto blood agar supplemented with 1.5% NaCl (final concentration 2% NaCl). Bacterial cultures were initially incubated for 24-48 h at approximately 18°C before shipment to the Norwegian Veterinary Institute (NVI).

Matrix Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

Main Spectra Profiles (MSPs) for 4 strains of *P. skyensis* from Scotland kindly donated by Professor H. Birkbeck, University of Glasgow (VIO 9388 and VIO 9390 serotype O2, VIO 9389 and VIO 9392, serotype O1), and 5 strains of *Pasteurella* sp. from Norway (four lump sucker strains [2016: 50-1365 and 50-1366, 2019: 50-3468 and 50-3490], one Atlantic salmon [2019: 50-3309]) were made “in house” according to instructions from Bruker A/S. For identification of suspected isolates, single colonies were transferred with a toothpick as a thin film onto two successive spots on a MALDI target plate. After drying at room temperature, the spots were overlaid with HCCA matrix

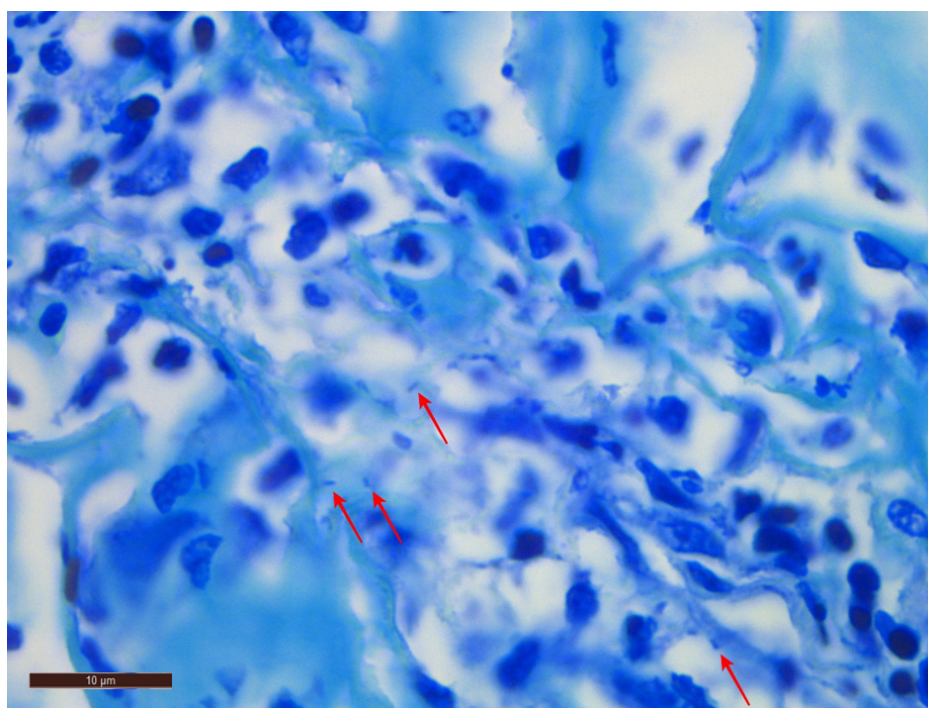


Figure 2. Histopathological sample from connective tissue surrounding the swimbladder stained with May-Grünwald-/Giemsa (MGG 1000x).

Suspected *Pasteurella skyensis* (arrows), inflammatory cells and erythrocytes.

and air-dried. Identification of representative isolates from the case in question was then performed using the standard Biotyper Database supplemented with the in house generated MSPs.

Results

Some preliminary histopathological samples indicated that a systemic bacterial infection could be the cause of mortality. Changes included bleeding, necrosis and/or inflammation of the heart (endocarditis), but findings were otherwise mostly unspecific and inconsistent. Later histopathological findings in three fish sampled in September, from a different cage than the primary outbreak, revealed granulomatous tissue in connective tissue surrounding the swim bladder. There were also some inflammatory cells in different tissues, but this finding was not consistent. Perivascular cuffing with neutrophils were seen, and selected samples from swim bladder tissue stained with May-Grünwald-/Giemsa (MGG) stain showed suspected short bacterial rods consistent with *Pasteurella* spp. (Figure 2).

PCR analysis identified *Paranucleospora theridion*, *Candidatus Branchiomonas cysticola* and Salmon gill poxvirus in 100% of the initially sampled fish and IPN virus in 70% of initially sampled fish, but histological findings did not support these as the causal agents of the observed mortality.

Bacterial cultures were dominated by slow growing pin-point colonies isolated from all initially sampled fish. Later cultures from fish suspected of infection with *P. skyensis* showed identical colonies. MALDI-TOF MS identified these

Table 2. Subsequent autopsy findings

Affected organ/tissue/area	Observations
Skin	Dark coloring and ventral oedema
Gills	Some petechial bleeding
Abdominal cavity	Clear fluid
Cardiac cavity	Opalescent fluid
Heart	Fibrinous epicarditis
Swim bladder	Oedema
Eyes	Exophthalmos
Liver	Fibrinous surface

as *P. skyensis* with scores of between 2.5 and 2.7 against the reference isolates. Whole genome sequencing of two isolates confirmed the identification of *P. skyensis* and clustered the investigated isolates closely with serotype O1 (Personal communication Snorre Gulla, NVI). Thus, infection with *P. skyensis* was confirmed by MALDI-TOF and whole genome sequencing of bacterial cultures from infected fish with macroscopic signs of sepsis. Histological visualisation of bacterial rods required special staining.

Outcome

Pasteurella skyensis was subsequently isolated from fish in most of the remaining cages at the site by bacteriology and confirmed with MALDI-TOF MS. Mortality rates decreased after a few weeks and especially after sea temperatures dropped below 10°C. *P. skyensis* was also isolated from a neighboring aquaculture site, but only in fish delivered from the same hatchery, with the same wellboat one day prior to delivery to the original site. It should be mentioned that there had been movement of boats and personnel between the two sites prior to detection of the disease. After the first isolation of *P. skyensis*, several laboratories developed PCR screening analyses for *P. skyensis* serotype O1 to be used subsequently for screening of the bacterium on nearby aquaculture sites.

No other sites in the area displayed signs of *P. skyensis* infection over the following months, even though PCR screening of kidney tissue was utilised. Later on, infection with *P. skyensis* in neighboring cages at the original site seemed to resolve, and the bacterium could not be cultured from kidney again, only in the original cage. Disease symptoms also changed over the course of the outbreak and are presented in [Table 2](#) and [Figures 3](#) and [4](#). Autopsy findings could be summarised as being consistent with chronic circulatory failure. Septicaemia seemed to have resolved, but darkened skin, anaemia and “thin” blood remained the same.

Discussion

Antibiotic treatment was discussed but was never truly considered an option. The reason being that antibiotic treatment would have to be orally administered and based on experience from examination of dead fish suffering



Figure 3. Subsequent and chronic external signs of *Pasteurella skyensis* infection in Atlantic salmon. (a) & (b) Bilateral exophthalmos and dark coloring of the skin

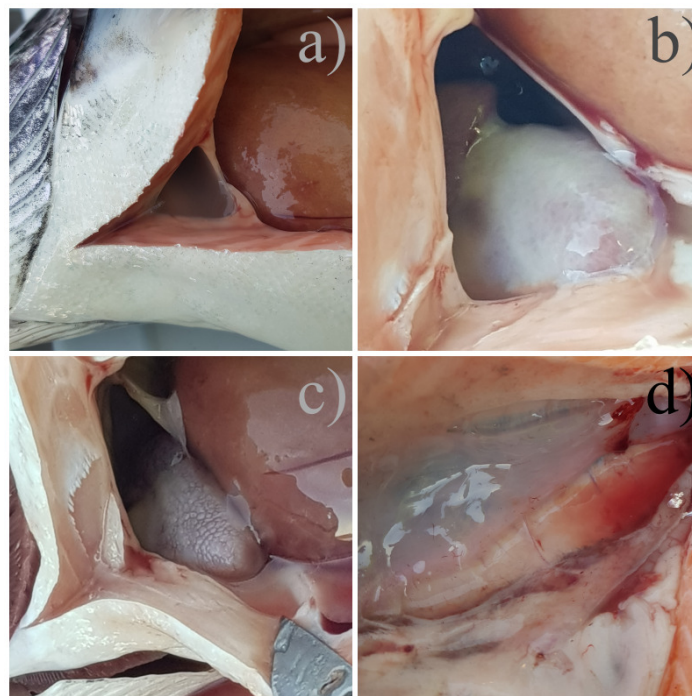


Figure 4. Subsequent and chronic signs of *Pasteurella skyensis* infection in Atlantic salmon. (a) Opalescent fluid in the cardiac cavity. (b) & (c) purulent material covering the surface of the heart. (d) Oedema of the swim bladder, giving it a gelatinous appearance.

from infection with *Pasteurella* sp., moribund fish would likely not ingest any feed at all. Also considering experience from oral administration of antibiotics against *Pasteurella* sp. and *Yersinia ruckeri*, the treatment would likely not cure the fish of *P. skyensis*, only alleviate symptoms for a short period. Fish free from

symptoms could also potentially continue shedding bacteria afterwards. From a One-health perspective, this outcome would not be acceptable, and treatment was therefore not initiated. Instead, focus was put on strengthening biosecurity measures to control the spread of infection between cages and also from the affected site to other nearby sites.

It is unknown if the disease was introduced to the fish prior to delivery, during delivery or just after delivery to sites since the original group was affected at both sites.

Attempts at tracking the source of *P. skyensis* infection were unsuccessful. The bacterium was not suspected of originating at the hatchery as no other groups at the hatchery showed signs or symptoms related to *P. skyensis* and the affected group was not reported to have any clinical findings prior to sea transfer. Prior to the delivery of the smolts to the affected site the wellboat had only been used for transporting smolts along the Norwegian coast, and aquaculture sites that received fish earlier have not reported findings of *P. skyensis*. The wellboat continued smolt delivery afterwards to a nearby aquaculture site which had not reported any suspected infection with *P. skyensis*. Typical biosecurity measures for wellboats delivering smolts is cleaning and disinfection of wells and equipment between aquaculture sites.

Proper biosecurity measures are an important tool to reduce the spread of pathogens. Many vessels travel between many different aquaculture sites within a short time span and over large distances. Many of these are used in handling and treatment of salmon or come into close contact with the salmon which can potentially introduce pathogens to sites. Proper cleaning and disinfection of all vessels and equipment arriving at and leaving aquaculture sites should therefore be highly prioritised. Personnel should limit travel between sites and at the very least, change clothing, disinfect footwear and wash their hands before arriving at another site.

Pasteurella skyensis is suspected of having an affinity for warm temperatures, 14-32 °C, based on growth rates at different incubation temperatures (Birkbeck et al. 2002) in laboratory settings. Infection rate, morbidity and mortality were therefore expected to decrease at lower sea temperatures. Considering the time of year and water temperature expectancy at the aquaculture site, it was decided to take a “wait and see” approach in combination with aforementioned biosecurity measures.

In the period (seven days prior and seven days after) during which mortality spiked at the primary site, the warmest sea water temperatures were recorded that year, approximately 19-14 °C at a water depth of 3-15 meters, respectively. During warm periods, dissolved oxygen in water is usually lower, which might aggravate any condition that causes circulatory failure. Oxygen consumption in salmon increases with rising water temperatures (Lee et al. 2003; Thorarensen and Farrell 2011)

A decrease in water temperature the in following fall and winter period was followed by a rapid decrease in mortality. Mortality was markedly reduced after sea temperature decreased below 12 °C and especially after temperatures at 3-15 meters depth were 12-8 °C, respectively, the mortality returned to expected levels, consistent with clinical course as earlier described by Birkbeck et al. (2002). This might be because the fish are more resilient against the disease at lower temperatures.

Bacteria of the *Pasteurellaceae* family have been isolated from different sea mammals (Reid and Birkbeck 2015) and *P. skyensis* has previously been isolated from the skin of a Humpback whale (Apprill et al. 2011). Different sea mammals such as porpoise (*Phocoena phocoena*) and killer whale (*Orcinus orca*) are commonly seen in the area where the *P. skyensis* infected sites are located. Wild fish were suggested by Birkbeck et al. (2002) as a possible source of infection. Mackerel is a common visitor in western fjords late in summer and thus, one might speculate that marine mammals or wild fish may act as a vector or reservoir for the infection in this case.

The chain of events in the affected site indicates that gross symptoms associated with *P. skyensis* infection may rely on the presence of stressful events as increased mortality in secondary pens occurred following delousing procedures. *P. skyensis*, seems to have a low capability of horizontal spread as obvious signs of disease as well as PCR results and culture attempts were negative (with one exception) in aquaculture sites nearby. Also, within in the affected site the disease seemed to resolve in fish in secondary pens, although fish in the original cage were still infected. We cannot conclude that those same pens are completely free of *P. skyensis*, and symptoms may reemerge once water temperatures increase, but they did not reemerge following delousing treatments during the course of the winter. Part of the lowered mortality might also be explained by lower oxygen requirements of the salmon at these temperatures as they showed signs of circulatory failure which increases sensitivity to low oxygen concentration.

In future cases of *P. skyensis*, one might attempt to alleviate increased mortality during warm periods with suspected suboptimal water quality by one or several of the following suggested measures, such as: increasing oxygen content of water in the pens; reducing oxygen requirements of the affected fish groups, reducing water temperature in the pens.

During the summer months, marine-fouling or net fouling caused mostly by algae, hydroids and mussels, occurs at increased rates. Net fouling reduces water flow through pens which results in reduced oxygen levels. Ensuring adequate cleaning of nets would therefore increase oxygen levels.

To increase the oxygen content of the water in the pens, an aeration system to add pure oxygen to the pen might be used.

To reduce oxygen requirement of the affected fish groups, reduced feeding during the warm periods might have a positive impact as feed intake and increased oxygen requirements of fish are correlated (Eliason, Higgs, and Farrell 2008; Thorarensen and Farrell 2011). Another suggested measure could be to avoid stressful events like handling/delousing or grading of fish.

Reducing water temperature in the pens might be feasible by an aeration system or pump system to bring in water from lower depths where it is usually cooler during the summer in Western Norway (Aure and Østensen 1993). This temperature variation is also supported by environmental measurements performed at the affected site.

Treatment of Atlantic salmon with antibiotics on commercial aquaculture sites has traditionally been performed by oral administration with medicated feed. As mentioned earlier, moribund fish tend to not ingest any feed, and based on personal experience, this is what is usually observed with *Pasteurella* sp. infected fish. The result is therefore that moribund fish will suffer from treatment failure and will continue shedding bacteria. An alternative treatment method would be injection of antibiotics, but this is expected to be very time consuming and logistically challenging on aquaculture sites harboring between 500,000 to 1,500,000 salmon. This strategy is deemed unfeasible at a site of this size but could be considered an option for sites with few but very valuable fish, for example broodfish.

The most important driver of antibiotic resistance (AR) is use of antimicrobials, thus from a One-health perspective, treatment with antibiotics should be used restrictively. Unabsorbed antibiotic in undigested feed and fish feces might represent a threat to aquatic microbiological communities and as much as 70-80% of antibiotics given to fish are excreted into water and spread rapidly (FAO). Thus routine use of antibiotics in salmon farming not only results in the selection of resistant target microorganisms but also non-target microorganisms and AR genes can be dispersed among a wide variety of different bacteria at the aquaculture site and the environment (Higuera-Llantén et al. 2018; van Hoek et al. 2011).

We hope that others find the information conveyed in this paper of use for recognition of symptoms and rapid diagnosis of the disease in the event that it might appear at other aquaculture sites.

The case description shows that routine bacteriology with culturing of bacteria should be included as a standard procedure from fish with increased mortality, especially where autopsy findings are consistent with septicemia. MALDI-TOF MS and whole genome sequencing was proven to be a useful diagnostic tool for identification and discrimination of slow growing halophile *Pasteurella* species.

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