Francisellosis is a bacterial disease with increasing economic impacts in the culture of tilapia and Atlantic cod since emerging in 1992. Two main strains – *Francisella noatunensis* subsp. *orientalis* (*Fno*) and *F. noatunensis* subsp. *noatunensis* (*Fnn*), have been identified, causing both acute and chronic granulomatous systemic disease. The piscine host range is increasing and *Francisella* culture should be included in routine diagnosis. Differentiation from the major zoonotic *F. tularensis* and opportunistic zoonotic *F. philomiragia* when dealing with environmental soil and water samples from fish farms is important. Diagnosis can be challenging but presentation of granulomatous pathology in fish should require use of cysteine supplemented selective media, culture at 15–28°C or culture in fish cell lines and specific PCR to exclude piscine *Fno* or *Fnn*. Control of infections in fish rely on appropriate antibiotic selection although in the long term an effective commercial vaccine that includes the pathogenic species of *Francisella* is required.

Tilapia (*Oreochromis niloticus*) production has increased from 2.6 million tons in 2005 to an estimated 4.5 million tons in 2014, being only second to carp in global aquaculture production. Atlantic cod (*Gadus morhua*) production peaked at 22.7 tons in 2009 and dropped to 4.3 tons in 2013. Francisellosis has been reported in farmed tilapia from Taiwan (1992), United States (2003), Costa Rica (2009), Indonesia (2009), United Kingdom (2010), and Brazil (2012) with mortalities of 30–75% and up to 95%. During 2004–2005, outbreaks in farmed Atlantic cod in Norway resulted in approximately 40% losses, presenting a major impediment to the expansion of cod aquaculture. Initially thought to be a *Rickettsia*-like organism or *Piscirickettsia*-like organism, the pathogen was later confirmed as a γ-Proteobacteria in the family *Francissiaceae*, order *Thiotrichales*. *Francisella noatunensis* subsp. *orientalis* (*Fno*) causes francisellosis in tilapia (a fresh and warm water fish species) and *F. noatunensis* subsp. *noatunensis* (*Fnn*) in Atlantic cod (a marine and cold water fish species). *F. philomiragia* subsp. *noatunensis* subsp. nov. and *F. piscicida* were two different names proposed for the organisms isolated from Atlantic cod in Norwegian disease outbreaks, however it has been resolved to be synonymous with *Fnn*. Infections associated with *F. philomiragia/Fnn* in Atlantic salmon (*Salmo salar*), *Francisella* spp. in three-line grunt (*Parapristipoma trilineatum*) and ornamental cichlid species are reported. *Fno* infected hybrid striped bass (*Morone chrysops x M. saxatilis*) and *Francisella baio-ticida* infected the giant ahalone (*Haliotis gigantea*). Recently, disease in marine ornamental fish species (wrasses and damselfish) was associated with *Fno*.

The gross pathology is typified by visceral granulomatosis causing splenomegaly and remenegomy due to multiple whitish-tan nodules with similar lesions in liver, gills or muscle. The degree and range of organ involvement differ between species. In Atlantic cod, emaciation, haemorrhagic skin and heart nodules also occur while in tilapia, gills can have the nodules in addition to exophthalmia and skin haemorrhages and scale loss. Histopathology in affected organs feature granulomas consisting of vacuolated macrophages with the *Francisella* organisms, associated central necrosis and fibrous encapsulation, in the sub-acute (7 days post challenge) to chronic disease. Acute disease has been experimentally replicated causing 100% mortality by 72 h post intraperitoneal inoculation of approximately 107 colony-forming unit (cfu) per fish where bloody ascites, increased melanomacrophage centres but no granulomas were observed. For tilapia, epizootics typically occur in cooler, winter water temperatures with higher mortalities at 15°C than 30°C or no mortalities at 26.5–29.2°C. Francisellosis causes more mortalities as water temperatures increase towards 20°C in summer for Atlantic cod. Epidemiologically, piscine Francisellae cause disease in both fresh and marine waters and morbidity can be extremely elevated for Atlantic cod and tilapia. Fish pathogenic *Francisella* can enter a viable but non-culturable state in cold water and be stored in multiple species, including hybrid striped bass (*Morone chrysops x M. saxatilis*), Atlantic salmon (*Salmo salar*), the aquatic protozoan *Acanthamoeba castellanii* and the aquatic biofilm has been reported with implications of transmission to fish.

*Francisella* are 0.1–1.5 µm, strictly aerobic, facultatively intracellular, non-motile, Gram-negative coccobacilli to pleomorphic.
spherical 5,10,15,16, halophilic 15 or freshwater 6 organisms. Culture of Fno and Fnn from kidney, spleen, blood or granulomatous lesions is made on enriched blood agar plates supplemented with 0.1% cysteine and 1% glucose, cysteine heart agar with 1% haemoglobin (CAHA) or cysteine heart agar with 5% sheep blood (CHAB) or Thayer-Martin media 6-8. The organism fails to grow on trypticase soy agar (TSA) supplemented with 5% sheep blood 6 and can be easily overgrown with or inhibited by contaminant or secondary bacteria 6,8,9. Polymyxin B (100 U/ml) and/or ampicillin (50 μg/mL) may be added to reduce these bacteria 6. Incubation temperature is 15–20°C for Fnn and 25°C for Fno 9 with Fnn growing poorly at 30°C and Fno preferring 28°C 4,8. Colonies develop slowly, taking up to 30 days but may appear as smooth, white to greyish within 3–6 days 9. Differentiation can be made from the zoonotic F. tularensis and F. philomiragia in that these organisms can grow at 35–37°C while Fnn and Fno do not 8. Further, F. philomiragia does not have an essential requirement for cysteine to grow 6,17. Biochemical reactions for Fno and Fnn are the same, with negative reactions for cytochrome oxidase activity, acid production from sucrose, β-galactosidase and no enzymatic activity for O-nitrophenyl-N-D-glucosamide (ONAG), P-nitrophenyl-β-D-galactopyranoside (PNPG), leucine arylamidase, and N-acetyl-β-D-glucosaminidase 7. However, Fnn metabolises D-glucose but does not have indoxyl phosphate (IDP) 7,18 activity, while Fno is the reverse for these tests 7. Molecular testing based on the G1,L1 primers targeting the internal transcribed sequence (ITS) with Eub A and Eub B primers targeting 16S rRNA, followed by sequence homology analysis is able to differentiate Fno (in tilapia and three-line grunt) from Fnn (Atlantic cod and Atlantic salmon) 2,18. Of note, Fnn shows 99.3% and Fno shows 98.6% 16S rRNA similarity to F. philomiragia, but they are more genetically dissimilar to F. tularensis 7,8,16. Cell culture isolation has been demonstrated for Fno using chinook salmon embryo (CHSE-2145) 2 and tilapia ovary cells (TO) 7. Similarly Fnn can be grown using salmon head kidney (SKK-1) and Atlantic salmon kidney (ASK) 8. Serological testing using antisera raised against Fnn detects Fno as well, with F. philomiragia agglutinating slightly to the Fnn antisera 8 but there was no cross reaction with monoclonal antibody against F. tularensis.

In terms of zoonotic risk, F. tularensis is a major environmental and tick or insect vector-borne human pathogen causing pneumonic tularemia 2,19, with F. tularensis subsp. tularensis being the most virulent strain and of biological weapon concern 6,7. Recently, tularemia in Turkey has been associated with beaver, muskrat and voles which infect surface waters suggesting that the aquatic environment is an important risk factor in its epidemiology 20,21. F. philomiragia is a rare disease and is associated with immune-compromised patients as in chronic granulomatous disease (CGD) and in near drowning events causing pneumonia or fever-bacteraemia 15,16,17. F. philomiragia has been isolated from brackish water in an area where repeated tularemia cases occurred 19. Therefore, it may be prudent to consider that zoonotic species of Francisella could be transmitted through the aquatic environment when dealing with aquatic environmental samples, including those from fish farms. To date, Fno and Fnn are considered to have negligible zoonotic risk as they cannot grow at 37°C and for Fno in tilapia, there has been no documented case of human infection despite it being a major aquaculture product processed for human consumption 7,8.

Control of clinical infections of francisellosis in tilapia has been reported with 30–50 mg/kg oxytetracycline over a 10–14 day treatment, but the high infectivity, a low infective concentration, high morbidity and inappetance in severely infected fish may render sustainable management ineffective 5. Isolates may be resistant to trimethoprim-sulfamethoxazole, penicillin, ampicillin, cefuroxime and erythromycin, gentamicin and ciprofloxacin 7. Florfenicol at 15 mg/kg has been demonstrated experimentally to improve survival to challenge with Fno, and it is suggested that this antibiotic could penetrate intracellularly to clear the organism 7. To date there is no commercial vaccine for piscine francisellosis although development work based on attenuation of Fno by mutation of the IgG gene provided effective protection in tilapia 7,8. Formalin-killed Fno bacterin with a mineral oil adjuvant provided a relative percentage survival (RPS) value in tilapia of 100% at day 27 post intraperitoneal challenge, with a specific antibody response at 15, 30 and 45 days post vaccination 1.

There are a number of key issues with piscine francisellosis:

- improving the efficiency of definitive diagnosis to mitigate the inadvertent dissemination of infected carrier fish hosts. This will require veterinary pathologists and microbiologists to be up-to-date regarding the case presentation of the disease. As a standard approach, fish with granulomatous disease should be subject to Francisella sp. exclusion, as part of the differential diagnoses.
- research into the epidemiology (in particular the diversity of reservoir host species) and virulent factors or genes of Fno and Fnn as part of the process for development of commercial vaccine products. This is important as warm water and cold water francisellosis are likely to present different scenarios in terms of disease management.
- finally, regarding the zoonotic risk of F. tularensis and F. philomiragia these being isolated also from aquatic environments 15,16, bacteriological culture conditions to exclude these zoonotic Francisellass from fish samples is an important exercise. This will avoid inadvertent human infection from aquatic or aquaculture environments.

References

**Providencia rettgeri septicaemia in farmed crocodiles**

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Bacterial septicaemia is a major cause of morbidity and mortality in farmed saltwater crocodiles (*Crocodylus porosus*) in the Northern Territory. *Providencia rettgeri* is the most common aetiological agent. Efficacy of antibiotic treatment is dubious and there are high levels of resistance to antibiotics commonly used by farms, underlining the need for exploration of new approaches to managing the disease.

Saltwater crocodile farming is a growing industry in Australia, with an annual gross value of over $50 million, the main product being high quality skins for the luxury leather market. In the Northern Territory, there are several farms, the largest having approximately 40 000 crocodiles. Berrimah Veterinary Laboratories (BVL) is situated within 30 km of the four largest farms, facilitating a close collaborative relationship. Each year, BVL receives from 50–100 farmed crocodile

**Biography**

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