Francisellosis in fish: an emerging challenge

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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[1], being only second to carp in global aquaculture production[2]. Atlantic cod (*Gadus morhua*) production peaked at 22.7 tons in 2009 and dropped to 4.3 tons in 2013[3]. Francisellosis has been reported in farmed tilapia from Taiwan (1992), United States (2003), Costa Rica (2009), Indonesia (2009), United Kingdom (2010), and Brazil (2012)[4] with mortalities of 30–75%[5,6] and up to 95%[7]. 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[7]. Initially thought to be a *Rickettsia*-like organism[5,6,8] or *Piscirickettsia*-like organism[8,9], the pathogen was later confirmed as a γ-Proteobacteria in the family Francisellaceae, order Thiotrichales[7]. *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[7,8] (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*[7,10]. 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[7]. *Fno* infected hybrid striped bass (*Morone chrysops* × *M. saxatilis*)[8] and *Francisella halioticida*[11] infected the giant abalone (*Haliotis gigantea*)[7]. Recently, disease in marine ornamental fish species (wrasses and damselfish) was associated with *Fno*[12].

The gross pathology is typified by visceral granulomatosis causing splenomegaly and renomegaly 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 exopthalmia and skin haemorrhages and scale loss[7,8]. Histopathology in affected organs feature granulomas consisting of vacuolated macrophages with the *Francisella* organisms, associated central necrosis and fibrous encapsulation[5,7–9,12,13], in the sub-acute (7 days post challenge[5]) to chronic disease. Acute disease has been experimentally replicated causing 100% mortality by 72 h post intraperitoneal inoculation of approximately 10^7 colony-forming unit (cfu) per fish where bloody ascites, increased melanomacrophage centres but no granulomas were observed[6]. For tilapia, epizootics typically occur in cooler, winter water temperatures with higher mortalities at 15°C than 30°C[7] or no mortalities at 26.5–29.2°C[8]. Francisellosis causes more mortalities as water temperatures increase towards 20°C in summer for Atlantic cod[7]. Epidemiologically, piscine Francisellae cause disease in both fresh and marine waters and morbidity can be extremely elevated for Atlantic cod and tilapia[8]. Fish pathogenic *Francisella* can enter a viable but non-culturable state in cold water after 30 days at 8°C and 16 days at 12°C[8], meaning that they are non-virulent. A reservoir of the *F. philomiragia* in the aquatic protozoan *Acanthamoeba castellanii* and the aquatic biofilm has been reported[14] with implications of transmission to fish.

Francisella are 0.1–1.5 µm, strictly aerobic, facultatively intracellular, non-motile, Gram-negative coccobacilli[7] to pleomorphic

spherical[6,9,15,16], 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 (CHAH) or cysteine heart agar with 5% sheep blood (CHAB) or Thayer-Martin media[6–9]. 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]. Polymixin B (100 U/mL) and/or ampicillin (50 µg/mL) maybe 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[7,17]. 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-acetyl-β-D-glucosamide (ONAG), P-nitrophenyl-β-D-galactopyranoside (PNPG), leucine arylamidase, and N-acetyl-β-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* (Altlantic cod and Atlantic salmon)[7,18]. 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-214[5]) and tilapia ovary cells (TO)[9]. Similarly *Fnn* can be grown using salmon head kidney (SKK-1) and Atlantic salmon kidney (ASK)[8]. Serological testing using antiserum raised against *Fnn* detects *Fno* as well, with *F. philomiragia* agglutinating slightly to the *Fnn* antiserum[7] 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[6,19], 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[13,15,17]. *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[8]. 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 *iglC*[*] 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* with these being isolated also from aquatic environments[15,19], bacteriological culture conditions to exclude these zoonotic Francisellae from fish samples is an important exercise. This will avoid inadvertent human infection from aquatic or aquaculture environments.

References

1. Roldan, M.A.M. (2014) Development of a vaccine against *Francisella noatunensis* subsp. *orientalis* in red Nile tilapia (*Oreochromis niloticus*). Thesis, Institute of Aquaculture, University of Stirling.
2. Soto, E. *et al*. (2016) Dynamics of piscine francisellosis differs amongst tilapia species (*Oreochromis* spp.) in a controlled challenge with *Francisella noatunensis* subsp. *orientalis*. *J. Fish Dis.* doi:10.1111/jfd.12461

3. FAO Fisheries and Aquaculture Department (2004) Cultured Aquatic Species Information Programme: *Gadus morhua*. Text by Håkon Otterå. Rome. Updated 1 January 2004. http://www.fao.org/fishery/culturedspecies/Gadus_morhua/en (accessed 11 May 2016).
4. Leal, C.A.G. *et al*. (2014) Outbreaks and genetic diversity of *Francisella noatunensis* subsp *orientalis* isolated from farm-raised Nile tilapia (*Oreochromis niloticus*) in Brazil. *Genet. Mol. Res.* **13**, 5704–5712. doi:10.4238/2014.July.25.26
5. Chen, S.C. *et al*. (1994) Systemic granulomas caused by a rickettsia-like organism in Nile tilapia, *Oreochronuis niloticus* (L.), from southern Taiwan. *J. Fish Dis.* **36**, 681–684.
6. Soto, E. *et al*. (2009) *Francisella* sp., an emerging pathogen of tilapia, *Oreochromis niloticus* (L), in Costa Rica. *J. Fish Dis.* **32**, 713–722. doi:10.1111/j.1365-2761.2009.01070.x
7. Birkbeck, T.H. *et al*. (2011) Review article: *Francisella* infections in fish and shellfish. *J. Fish Dis.* **34**, 173–187. doi:10.1111/j.1365-2761.2010.01226.x
8. Colquhoun, D.J. *et al*. (2011) *Francisella* infections in farmed and wild aquatic organisms. *Vet. Res.* **42**, 47. doi:10.1186/1297-9716-42-47
9. Mauel, M. (2010) Francisellosis. *In* AFS-FHS (American Fisheries Society-Fish Health Section). FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens. 2014 edn. http://afs-fhs.org/bluebook/bluebook-index.php
10. Mikalsen, J. *et al*. (2007) *Francisella philomiragia* subsp. *noatunensis* subsp. nov., isolated from farmed Atlantic cod (*Gadus morhua* L.). *Int. J. Syst. Evol. Microbiol.* **57**, 1960–1965. doi:10.1099/ijs.0.64765-0
11. Brevik, O.J. *et al*. (2011) *Francisella halioticida* sp. nov., a pathogen of farmed giant abalone (*Haliotis gigantea*) in Japan. *J. Appl. Microbiol.* **111**, 1044–1056. doi:10.1111/j.1365-2672.2011.05133.x
12. Camus, A.C. *et al*. (2013) *Francisella noatunensis* subsp. *orientalis* infection in Indo-Pacific reef fish entering the United States through the ornamental fish trade. *J. Fish Dis.* **36**, 681–684. doi:10.1111/jfd.12071
13. Wenger, J.D. *et al*. (1989) Infection caused by *Francisella philomiragia* (formerly *Yersinia philomiragia*). A newly recognized human pathogen. *Ann. Intern. Med.* **110**, 888–892. doi:10.7326/0003-4819-110-11-888
14. Verhoeven, A.B. *et al*. (2010) *Francisella philomiragia* biofilm formation and interaction with the aquatic protist *Acanthamoeba castellanii*. *Biol. Bull.* **219**, 178–188.
15. Friis-Møller, A. *et al*. (2004) Problems in identification of *Francisella philomiragia* associated with fatal bacteremia in a patient with chronic granulomatous disease. *J. Clin. Microbiol.* **42**, 1840–1842. doi:10.1128/JCM.42.4.1840-1842.2004
16. Gonçalves, L.A. *et al*. (2016) Complete genome sequences of *Francisella noatunensis* subsp. *orientalis* strains FNO12,FNO24 and FNO190: a fish pathogen with genomic clonal behavior. *Stand. Genomic Sci.* **11**, 30. doi:10.1186/s40793-016-0151-0
17. Mailman, T.L. *et al*. (2005) *Francisella philomiragia* adenitis and pulmonary nodules in a child with chronic granulomatous disease. *Can. J. Infect. Dis. Med. Microbiol.* **16**, 245–248. doi:10.1155/2005/486417
18. Bordevik, M. *et al*. (2008) *Francisella* sp., an emerging disease problem for the global fish farming industry? Pharmaq poster presentation at Seventh symposium on Disease in Asian Aquaculture (DAA VII), 22–26 June, Taipei, Taiwan.
19. Berrada, Z.L. *et al*. (2010) Diversity of *Francisella* species from Martha's Vineyard, Massachusetts. *Microb. Ecol.* **59**, 277–283. doi:10.1007/s00248-009-9568-y
20. Gürcan, Ş. (2014) Epidemiology of tularemia. *Balkan Med. J.* **31**, 3–10. doi:10.5152/balkanmedj.2014.13117
21. Leblebicioglu, H. *et al*. (2008) Outbreak of tularemia: a case-control study and environmental investigation in Turkey. *Int. J. Infect. Dis.* **12**, 265–269. doi:10.1016/j.ijid.2007.06.013

Biography

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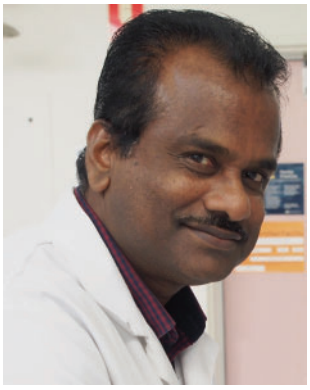
Providencia rettgeri septicaemia in farmed crocodiles



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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