

Vaccination against streptococcal infections in farmed fish



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Aquaculture produces more than 50% of fish for human consumption and, in spite of major improvements since the adoption of injectable vaccines in the 1990s, bacterial diseases still account for considerable losses, particularly in tropical and warm temperate species. Streptococcosis, caused predominantly by *Streptococcus iniae* and *S. agalactiae*, manifests as a generalised septicaemia and meningitis followed by rapid mortality. Vaccination against streptococcal infections is difficult as a result of multiple, poorly defined serotypes and consequent vaccine escape (reinfection of previously vaccinated animals). However, genomics applied to reverse vaccinology is providing novel insights into diversity among these aquatic pathogens and is identifying cross-serotype targets that may be exploited for new generation streptococcal vaccines for aquaculture.

Aquaculture reached a significant milestone in 2013 as global production for food use overtook beef production for the first time and now accounts for more than 50% of the global seafood supply¹. Aquaculture has wrestled with social license throughout its rapid growth in developed economies, including Australia, through the 1980s and 90s^{2,3}. Objections have been raised around environmental issues such as eutrophication of marine sediments, escape of domesticated fish into wild stocks and pressure on wild fisheries for fishmeal for aquaculture diets. Disease transmission between farmed and wild stock and high antibiotic use for controlling bacterial infections in farmed fish have also attracted attention. Granted, antibiotic use was high in salmonid aquaculture in the 1980s and early 90s, with aquaculture outstripping both human and terrestrial animal use in Norway⁴. However, the widespread adoption of oil-adjuvanted injectable vaccines in salmonid aquaculture

during the mid-1990s all but eliminated antibiotic use from salmonid production^{4,5}. Nevertheless, most current and future expansion of finfish aquaculture is occurring in warm temperate and tropical regions where farmed species and the diseases from which they are at risk are not yet adequately controlled by vaccination.

Streptococcal infections occur in warm-temperate and tropical waters wherever fish are farmed and occasionally cause wild fish kills⁶. While there are a number of streptococcal species that cause disease in fish, the most prevalent and damaging are *S. iniae* and *S. agalactiae*. Infection of fish by either pathogen results in rapid onset of generalised septicaemia, meningitis often associated with bilateral exophthalmia (Figure 1a–d) and death with mortalities often exceeding more than 70% within a few days of infection in experimental models.

Streptococcus iniae was first isolated in 1972 from an abscess on a captive Amazon freshwater dolphin *Inia geoffrensis* from which it derives its name⁷. In aquaculture, the major species affected by *S. iniae* are rainbow trout in Israel⁸, grouper in Taiwan⁹, tilapia, catfish and hybrid bass in the USA¹⁰ and, in Australia, barramundi¹¹. Vaccination against *S. iniae* is accomplished using formalin inactivated bacterins by intraperitoneal injection of fish under general anaesthetic in tilapia¹², trout¹³, grouper⁹ and barramundi (Figure 1e, f)¹⁴. But such vaccines are serotype-specific^{14–16} and in the absence of a robust serotyping scheme or typing antisera, vaccine failures may occur^{13,14} where the prevalent strain does not match the serotype of the vaccine used. Serotype is defined by the polysaccharide capsule in *S. iniae*^{8,14,17,18} and antigenic changes result from non-synonymous mutations in a limited repertoire of the genes in the capsular operon that alter monomer composition,

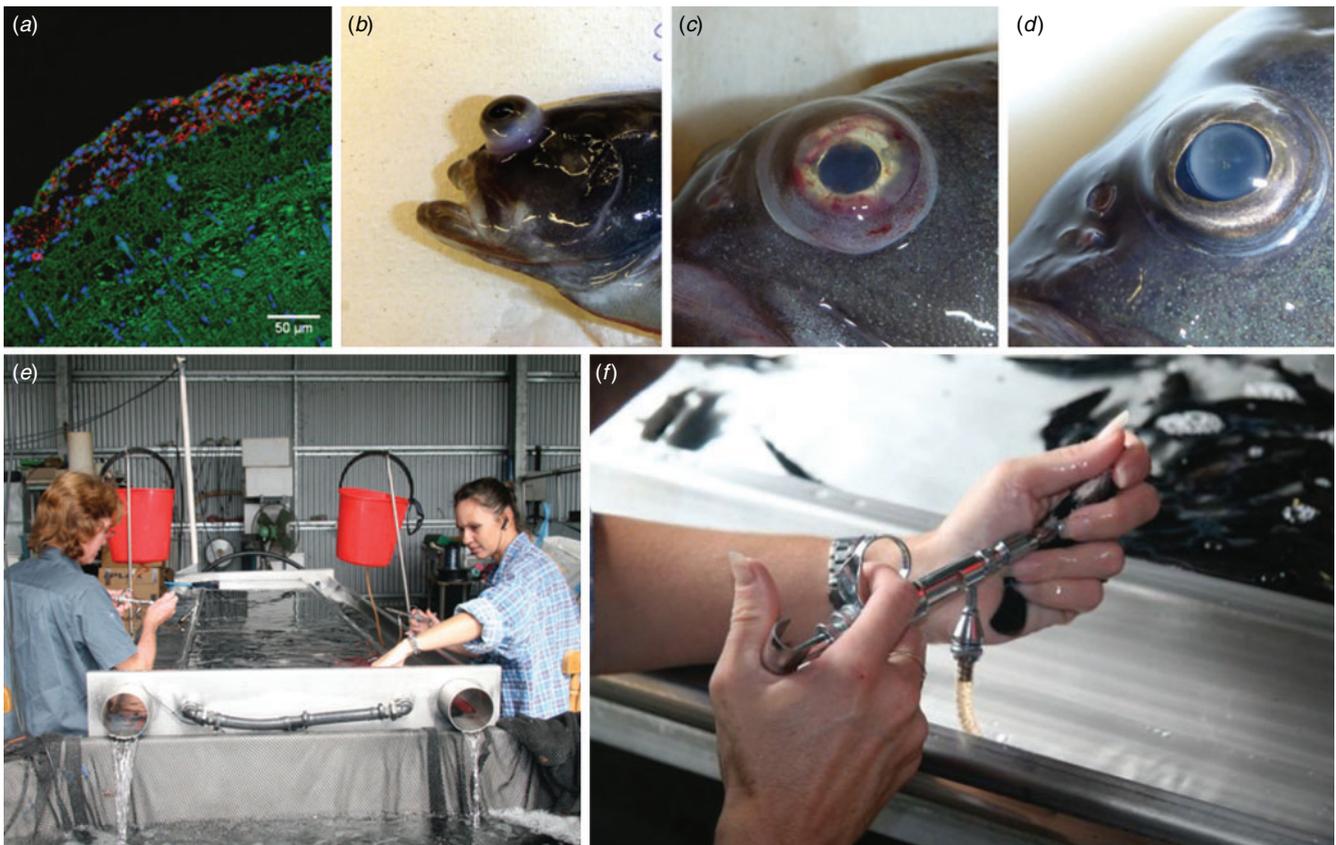


Figure 1. (a) Infection of the meninges shown by immunohistochemistry of infected brain section. Blue: DAPI stained tissue; red: rabbit anti-GBS polysaccharide capsule of *S. agalactiae*. (b) Exophthalmia and associated meningitis. (c) Corneal haemorrhage and (d) corneal opacity. Photographs a–d from acute infection of giant grouper *Epinephelus lanceolatus* with *S. agalactiae* ST261 serotype 1b (Photos: Dr Jerome Delamare-Deboutteville). (e, f) Vaccination of barramundi against *S. iniae* by intraperitoneal injection. (e) Vaccination table with central anaesthetic pool and flowing, oxygenated water along the side channels to a recovery tank. Vaccines are contained in sterile blood-bags on ice in the buckets. (f) Vaccines are delivered directly into the peritoneal cavity of the fish under anaesthesia using a self-refilling syringe. Needles must be changed frequently and regularly de-scaled to prevent injury of the fish, which can lead to infection of the injection pore. (Photos: Andy Barnes).

polymer chain length and quantity of the capsule¹⁴. When a vaccine is deployed, new serotypes periodically arise through mutations in variable capsular genes. These serotypes may be already present in the pool of extant strains co-existing on the farm (serotype replacement, adaptation through standing variation) or originate under immune pressure (adaptation through *de novo* variation). Whole genome sequencing coupled with fluctuation analysis (a statistical method of measuring mutation rate in bacteria based on frequency with which resistance to antibiotic occurs in highly replicated laboratory experiments) suggests both are likely to occur, with a role for hypermutators (variants with greatly elevated mutation rates) facilitating adaptation to the immune host (Figure 2). To overcome the inherent plasticity in the capsular structure, several proteins have been proposed as potential vaccine immunogens based on analysis of the first available genome sequences²¹, but these have not been uniformly effective^{22–24}. With the falling cost of whole genome sequencing, using informatics to identify surface associated and secreted proteins that are conserved across different capsular serotypes has become a fast and cost-effective route to new

experimental vaccines that may be cross-protective across multiple serotypes.

Streptococcus agalactiae is a Lancefield group B *Streptococcus* (GBS). Most fish-pathogenic isolates fall into either (multilocus sequence type) ST260 or ST261 with capsular serotypes Ia and Ib. There have also been reports of fish disease caused by the broad host range ST7 but these have been associated with environmental contamination from terrestrial sources^{25,26}. The ‘true’ fish pathogens are quite distinct from their terrestrial con-specifics, with substantially reduced genomes, depleted virulence factor repertoire and reduction of carbohydrate metabolic pathway genes²⁶. Indeed, fish pathogenic GBS was classified as a separate species, *S. ‘difficilis’*, until these isolates were later assigned to the species *S. agalactiae* based on whole cell protein analysis in the late 1990s²⁷ and confirmed by DNA : DNA hybridisation in 2005²⁸. Infection and mortality caused by GBS is one of the most significant issues facing tilapia culture globally. Injectable vaccines are effective but type-specific²⁹. Once again the potential for exploiting the falling costs of whole genome sequencing for design of cross-serotype protective

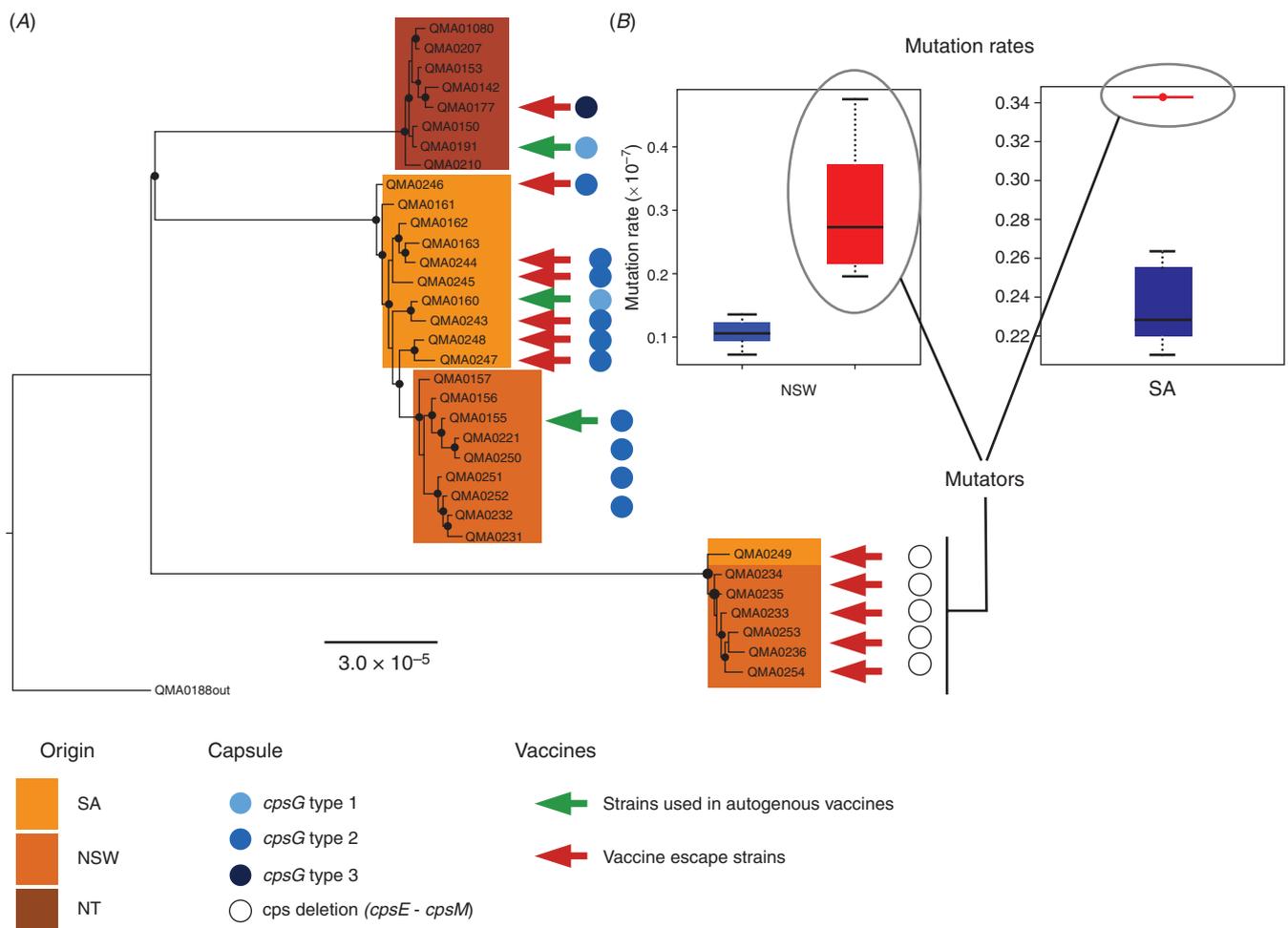


Figure 2. Evidence for role of *S. iniae* mutators in reinfection of vaccinated barramundi in Australia. A) Rooted maximum likelihood tree (RAxML v 8.1.3; GTR+GAMMA model) of *S. iniae* isolates from vaccination cases in Australia based on alignment of core genome single nucleotide polymorphisms (SNPs), filtered for regions of recombination (using Gubbins¹⁹) and corrected for ascertainment bias²⁰. Green arrows indicate strains used to vaccinate the fish on the different farms whilst red arrows indicate strains subsequently isolated from vaccinated fish in which disease had reoccurred. The capsular serotype, defined in most of these cases by mutations in *cpsG*, which controls glucose:galactose ratio in the surface polysaccharide, is indicated by blue circles. The extended branch length supporting the *cps* defective isolates (white circles) from vaccinated fish in NSW and SA is indicative of a much faster nucleotide substitution rate in these strains and evidence that they are likely mutators. This is supported in (B), which shows experimentally determined mutation rates for these isolates (red) and other isolates from the same farm (blue). Taken together this is supportive of adaptation by both standing and de novo variation with a role for mutators in the latter.

vaccines is substantial and has been used to great effect in human medicine³⁰. A similar approach is ongoing for aquatic isolates, and surface expressed proteins unique to aquatic isolates but conserved across the ST260 and ST261 sequence types have been identified and tested for efficacy in preliminary trials in tilapia.

Whilst large-scale whole genome sequencing is identifying antigens conserved across the most important serotypes, there are a number of further problems that must be resolved for viable streptococcal vaccines for aquaculture. First, fish are a low value commodity and even in salmon, which fetch a relatively high wholesale price, margin per dose of vaccine is low relative to other animal vaccines, except poultry. Consider that the farm gate price of most warm water species is substantially lower than salmon, with tilapia valued at less than one third of the lowest salmon price, and one can envisage that the cost per dose of vaccine has to be very low indeed. Whilst there

is some margin in simple formalin-killed bacterins, recombinant protein vaccines are not economically viable in this market. Therefore, maximising expression of conserved antigens, identified through genomics, in culture for improvement of killed bacterins makes more commercial sense. The second problem relates to adjuvants. The success of vaccination in cool water salmonid aquaculture was founded upon oil emulsions that enable a single vaccination to protect for the complete farm lifecycle. This duration of immunity in excess of two years necessitates very slow antigen release from the emulsion. This works against warm water species that are farmed for maybe 9–12 months in the case of tilapias, particularly for streptococcal vaccines, where achieving an effective antigen dose against non-carbohydrate antigens is already challenging due to very low growth densities of aquatic streptococci. This will necessitate clever formulation of vaccines to enable initial fast antigen release, but also sustained protection for several months,

and all at a price of a few cents per dose. This represents a substantial challenge for the industry and the science.

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Biographies

Andy Barnes is Associate Professor in Aquatic Animal Health at The University of Queensland, with research activities focusing on preventative healthcare in commercially important aquatic animals including finfish, penaeid and freshwater prawns and oysters. After completing a PhD in medical microbiology at The University of Edinburgh Medical School in 1992, Andy joined Aqua Health Limited, market leader in aquaculture vaccines, in research and development of vaccines for fish before its takeover by Novartis Animal Health in 2000. Andy joined The University of Queensland from Novartis in October 2003. Recent research projects include tailoring generic vaccines against Streptococcosis for the global market, and specific vaccines for the specialist Australian market. He recently completed an evolutionary history of *Yersinia ruckeri* in Tasmania and New Zealand by whole genome analysis. His interests lie in evolution of bacterial pathogens of aquatic animals and adaptation to the immune host.

Oleksandra Rudenko is a PhD student at The University of Queensland, primarily interested in microbial evolution and interactions with a host. After she graduated as MSc in Biology at Uzhhorod National University, department of Genetics, Plant Physiology and Microbiology in 2008, she worked in bacteriological laboratory at Uzhhorod Central City Hospital up until her UQ studies commenced in 2014. Her project focuses on the role of hypermutable strains in microbial adaptation to the host and immune escape after vaccination.