Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 122 (2007) 1–15
veterinary microbiology
www.elsevier.com/locate/vetmic

Review

Streptococcus iniae: An aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination

Wendy Agnew [a], Andrew C. Barnes [b,*]

[a] *School of Veterinary Science, University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia*
[b] *Aquatic Animal Health Laboratory, Centre for Marine Studies, University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia*

Received 27 November 2006; received in revised form 5 February 2007; accepted 8 March 2007

Abstract

Streptococcus iniae has become one the most serious aquatic pathogens in the last decade causing high losses in farmed marine and freshwater finfish in warmer regions. Although first identified in 1976 from a captive Amazon freshwater dolphin, from which it derives its name, disease outbreaks had most likely been occurring for several decades in marine aquaculture in Japan. *S. iniae* is globally distributed throughout warm water finfish aquaculture. In common with other encapsulated beta-haemolytic streptococci and in direct contradiction to the phenomenal success story of bacterial vaccines in finfish aquaculture, control of *S. iniae* by vaccination has met with limited success. Thus, antibiotic usage is the current practice for reducing mortality and consequent economic loss. Vaccine failure appears to result in part from serotypic variation and, whilst 2 serotypes have been named, variation would appear to be more complex. *S. iniae* also has zoonotic potential, with human infections identified in the USA, Canada, and throughout Asia. In humans, infection is clearly opportunistic with all cases to date associated with direct infection of puncture wounds during preparation of contaminated fish, and generally in elderly or immunocompromised individuals. Significant progress has been made in terms of research into pathogenic mechanisms of *S. iniae*, with recent research elucidating the role of capsule in virulence for fish through antiopsonic activity. In light of this recent coverage in the literature, the present review centres on areas of direct veterinary interest including identification, epidemiology, therapy and prevention in farmed finfish. Clearly as the prevalence of *S. iniae* and associated economic losses continue to increase, further work towards developing a reliable vaccine is essential. This would appear to require a much better understanding of cell-surface variability amongst *S. iniae* isolates.
© 2007 Elsevier B.V. All rights reserved.

Keywords: *Streptococcus iniae*; Vaccines; Aquaculture; Zoonoses

* Corresponding author. Tel.: +61 7 3346 9416; fax: +61 7 3365 4755.
E-mail address: a.barnes@uq.edu.au (A.C. Barnes).

0378-1135/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2007.03.002

Contents

1. Introduction

Streptococcus iniae has emerged as an important fish pathogen over the past few decades. Although originally isolated from freshwater dolphins (Pier and Madin, 1976), its major significance is as an aetiological agent of streptococcosis in farmed finfish. More recently, it has also been identified as a potential zoonotic pathogen, with at least 25 cases of human infection by *S. iniae* confirmed to date (Facklam et al., 2005; Lau et al., 2006, 2003). In 1997 the estimated annual impact of infection by this bacterium on the aquaculture industry in the USA alone was US$ 10 million and estimated globally to be US$ 100 million (Shoemaker et al., 2001).

The first confirmed streptococcal infection in cultured fish was reported in 1958 in rainbow trout (*Oncorhynchus mykiss*) in Japan (Hoshina et al., 1958). Over the next 2 decades, more streptococcal infections were identified as causes of mortality in farmed and wild species, the majority of which were reported from Japan and the USA (Kitao et al., 1981; Kusuda et al., 1979; Minami et al., 1979; Ohnishi and Jo, 1981; Robinson and Meyer, 1966). During this time, new species of streptococci were also being recognised. In 1976 a novel streptococcus was identified as the causative agent of ''golf ball disease'', a disorder characterised by multiple subcutaneous abscesses in a captive Amazon freshwater dolphin (*Inia geoffrensis*) in San Francisco and was named *Streptococcus iniae* (Pier and Madin, 1976). A second isolate was obtained from skin lesions in another freshwater dolphin housed at the Niagara Falls Aquarium in New York in 1978 (Pier et al., 1978). A third dolphin isolate was also recovered in Ohio in 1987, although the findings were not published until 2003 (Bonar and Wagner, 2003).

The first descriptions of infection in fish by *S. iniae* occurred in the 1980s; epizootics were described in Japan in 1981 and 1983, and Singapore in 1985, although they were not recognised as *S. iniae* until years later (Foo et al., 1985; Inglis et al., 1993; Kitao et al., 1981; Nakatsugawa, 1983; Nguyen et al., 2002; Stoffregen et al., 1996). Infections also occurred in Israel and Taiwan in 1986, with the aetiological agent initially thought to be a new species and named *Streptococcus shiloi* (Eldar et al., 1994). Later analysis found the bacterial cause of the 1986 infections to be identical to the first *S. iniae* type strain (isolated from the San Francisco dolphin in 1976) and the name was later corrected to *S. iniae* (Eldar et al., 1994, 1995b). In Israel, infection spread rapidly throughout the country, causing considerable mortalities to tilapia and rainbow trout stocks with significant economic effects (Eldar et al., 1994). To combat these losses, the first *S. iniae* vaccination program was initiated in Israel (Eldar et al., 1997a). Vaccination was successfully used in rainbow trout farms from 1995 to 1997, resulting in *S. iniae*-related mortalities decreasing from 50% to less than 5% (Bachrach et al., 2001). However, massive new outbreaks were recorded shortly thereafter, with isolates showing slight differences in biochemical properties, and new pathological changes in infected fish (Bachrach et al., 2001; Barnes et al., 2003b).

Confirmation of infections caused by *S. iniae* continued in various other locations around the world. Australia has suffered losses of cultured barramundi in marine cages every summer since 1992 (Bromage and Owens, 2002; Bromage et al., 1999). In the USA, infections in cultured fish were first reported in Texas in 1994 (Perera et al., 1994) and Massachusetts in 1996 (Stoffregen et al., 1996). *S. iniae* has also infected fish in other parts of Asia, the Middle East,

and the Caribbean (Ferguson et al., 2000; Kitao et al., 1981; Nakatsugawa, 1983; Nguyen et al., 2002; Shen et al., 2005; Yuasa et al., 1999).

More recently, it has also been shown that *S. iniae* is able to infect and cause disease in humans. In the winter of 1995–1996 four cases of invasive *S. iniae* infection were documented in people in the Toronto area that had recently handled fresh, whole farmed fish from wholesale markets (Weinstein et al., 1996). Subsequent studies have identified at least 25 cases in Canada, the USA, Hong Kong and Singapore (Facklam et al., 2005; Koh et al., 2004; Lau et al., 2003; Weinstein et al., 1997).

2. Distribution

To date, *S. iniae* has been identified in three main regions: North America (Canada, USA, and the Caribbean), Middle East (Bahrain and Israel), and Asia-Pacific (Australia, China, Japan, Singapore, and Taiwan) (Table 1).

It is currently unknown whether this bacterium could have originated from a single source and been distributed via ocean currents or movement of fish stocks. Eldar et al. (1994) postulated that since the pathogen was first identified in the USA nearly a decade earlier than the first Israeli infections, that the bacteria may have travelled to Israel from fish eggs imported from the USA in the early 1980s. But the first documented evidence of *S. iniae* causing infections in farmed fish in the USA occurred after the first Israeli infections, which later led to speculation that the disease may have been imported along with Israeli tilapia (Eldar et al., 1995b). However, an epidemiological link between *S. iniae* infections in these countries has since been rejected based on ribotype differences between each country's strains (Eldar et al., 1997b). It seems possible that improvements in the identification of this bacterial species have simply led to the identification of isolates which may have been present in various locations for years.

It is also unknown if the bacterium has always been present in wild fish, or if the amplification of pathogens in culture situations has led to disease in neighbouring wild fish communities. It seems likely that the relatively limited monitoring of wild populations compared with cultured ones may have biased the reporting of infections towards farmed fish. At present, there are few reports of *S. iniae* infections

Table 1
Distribution of *S. iniae* infections

Host	Location	Year first isolated	Year reported	Refs.
Dolphin	San Francisco CA, USA	1976	1976	Pier and Madin (1976)
Dolphin	Niagara Falls NY, USA	1978	1978	Pier et al. (1978)
Fish	Japan	1979	1981	Inglis et al. (1993), Kitao et al. (1981), Nguyen et al. (2002)
Fish	Singapore	1985	1985	Foo et al. (1985), Stoffregen et al. (1996)
Fish	Israel	1986	1994	Eldar et al. (1994)
Fish	Taiwan	1986	1994	Eldar et al. (1994)
Dolphin	Ohio, USA	1987	2003	Bonar and Wagner (2003)
Human	Texas, USA	1991	1997	Weinstein et al. (1997)
Fish	Texas, USA	1992	1994	Perera et al. (1994)
Fish	Massachusetts, USA	1993	1996	Stoffregen et al. (1996)
Human	Ontario, Canada	1994	1995	Weinstein et al. (1996, 1997)
Fish	Bahrain	1995	1999	Yuasa et al. (1999)
Fish	North Queensland, Australia	1995	2002	Bromage and Owens (2002), Yuniarti (2005)
Human	Vancouver, BC, Canada	1997	1998	Goh et al. (1998)
Fish	South-east Caribbean basin	1999	2000	Ferguson et al. (2000)
Human	California, USA	2000	2005	Facklam et al. (2005)
Fish	Western Australia, Australia	2001	2005	Bromage and Owens (2002), Yuniarti (2005)
Human	Hong Kong, China	2001	2003	Lau et al. (2006, 2003)
Fish	Zhejiang Province, China	2001	2005	Shen et al. (2005)
Human	Singapore	2003	2004	Koh et al. (2004)
Human	Pennsylvania, USA	2003	2005	Facklam et al. (2005)

in wild fish. In Israel, wild specimens were found to be infected with the same strains as nearby cultured fish in two locations: one on the Gulf of Eilat in the Red Sea, and the other from the Mediterranean coast (Colorni et al., 2002; Zlotkin et al., 1998). A study in Australia also isolated *S. iniae* from fish found co-habiting in barramundi enclosures (Bromage and Owens, 2002). However, not all wild fish infections are associated with intensive fish rearing. A large reef fish kill due to infection by *S. iniae* occurred in the south-east Caribbean basin with no known association with any aquaculture facilities (Ferguson et al., 2000). More epidemiological studies are needed to elucidate the primary sites and species of amplification of this bacterium as well as the full extent of its natural distribution in order to control its future spread.

3. Host range

At least 27 species of fish have been documented to have been infected by *S. iniae* (see Table 2).

Table 2
Species reported to have been infected by *S. iniae*

Common name	Scientific name	Location(s)	Refs.
Amago salmon	*Oncorhynchus rhodurus* var. *macrostomus*	Japan	Inglis et al. (1993), Ohnishi and Jo (1981)
Ayu	*Plecoglossus altivelius*	Japan	Inglis et al. (1993), Kitao et al. (1981), Ohnishi and Jo (1981)
Barramundi	*Lates calcarifer*	Australia	Bromage and Owens (2002), Bromage et al. (1999)
Barramundi cod	*Cromileptes altivelis*	Australia	Bromage and Owens (2002)
Black margate	*Anisotremus* spp.	The Grenadines	Ferguson et al. (2000)
Chubb	*Scaridae* spp.	Barbados	Ferguson et al. (2000)
Coho salmon	*Oncorhynchus kisutch*	Israel	Eldar et al. (1995a)
European seabass	*Dicentrarachus labrax*	Israel	Kvitt and Colorni (2004), Zlotkin et al. (1998)
Gilthead sea bream	*Sparus aurata*	Israel	Zlotkin et al. (1998)
Gold spot cod	*Epinephalis tauvina*	Australia	Bromage and Owens (2002)
Grey mullet	*Mugus cephalus*	Israel	Eldar et al. (1995a)
Grunt	*Haemulidae* spp.	Barbados	Ferguson et al. (2000)
Hybrid nile × blue tilapias	*Tilapia nilotica* × *T. aurea*	USA (Texas)	Perera et al. (1994)
Hybrid striped bass (Sunshine bass)	*Morone chrysops* × *M. saxatilis*	USA	Shoemaker et al. (2001), Stoffregen et al. (1996)
Japanese flounder	*Paralichthys olivaceus*	Japan	Nakatsugawa (1983), Nguyen et al. (2002)
Lizard fish	*Synodus variegates*	Israel	Colorni et al. (2002), Kvitt and Colorni (2004)
Lyretail grouper	*Variola louti*	Israel	Kvitt and Colorni (2004)
Parrot fish	*Sparisoma aurofrenatum* and *S. viridae*	Barbados and The Grenadines	Ferguson et al. (2000)
Puffer fish	*Arothron hispidus*	Australia	Bromage and Owens (2002)
Rabbit fish	*Siganus* spp.	Singapore	Foo et al. (1985), Stoffregen et al. (1996)
		Israel	Zlotkin et al. (1998)
		Bahrain	Yuasa et al. (1999)
		Australia	Bromage and Owens (2002)
Rainbow trout	*Oncorynchus mykiss*	Israel (upper Galilee)	Eldar et al. (1995a), Eldar and Ghittino (1999), Lahav et al. (2004)
		Japan	Kitao et al. (1981)
Red drum	*Sciaenops occellatus*	Israel	Zlotkin et al. (1998)
		China	Shen et al. (2005)
Silver bream	*Acanthopagrus australis*	Australia	Bromage and Owens (2002)
Snapper	*Ocyurus chrysurus*	Barbados	Ferguson et al. (2000)

Table 2 (*Continued*)

Common name	Scientific name	Location(s)	Refs.
Striped piggy	*Pomadasys stridens*	Israel	Colorni et al. (2002), Kvitt and Colorni (2004)
Tilapia	*Oreochromis* spp.	USA	Bowser et al. (1998), Shoemaker et al. (2001)
		Taiwan	Eldar et al. (1994)
		Israel	Eldar et al. (1994, 1995a,b), Kvitt and Colorni (2004)
		Japan	Kitao et al. (1981)
Yellowtail	*Seriola quinqueradiata*	Japan	Inglis et al. (1993), Kaige et al. (1984), Minami et al. (1979)
Amazon freshwater dolphin	*Inia geoffrensis*	USA (San Francisco, New York, and Ohio)	Bonar and Wagner (2003), Pier and Madin (1976), Pier et al. (1978)
Flying fox	*Pteropus alecto*	Australia	Yuniarti (2005)
Human	*Homo sapiens*	Canada	Weinstein et al. (1996, 1997)
		USA	Facklam et al. (2005), Weinstein et al. (1996, 1997)
		China (Hong Kong)	Lau et al. (2006, 2003)
		Singapore	Koh et al. (2004)

Freshwater, marine, and euryhaline species are represented, as well as both cultured and wild populations. There has also been documentation of species that have been resistant to infection in the face of challenge. These species include the common carp (*Cyprinus carpus*), when reared in community with infected tilapia (Eldar et al., 1995a), and market-sized channel catfish (*Ictalurus punctatus*) from commercial fish farms in the USA (Shoemaker et al., 2001). Channel catfish and goldfish (*Carassius auratus*) were also found to be resistant to infection even after intraperitoneal injection of one of the first streptococcal fish pathogens identified in the USA (Robinson and Meyer, 1966). Humans, Amazon freshwater dolphins, and flying foxes are the only mammals known to have been infected naturally (Lau et al., 2003; Pier and Madin, 1976; Pier et al., 1978; Weinstein et al., 1996, 1997; Yuniarti, 2005).

4. Identification and typing

The initial morphological and biochemical characteristics of this species were based on the type strain isolated from the Amazon freshwater dolphin in 1976 which was deposited in the American Type Culture Collection under the Accession number 29178 (Pier and Madin, 1976). *S. iniae* is a Gram-positive, encapsulated coccus that most often occurs in long chains in broth culture. On solid blood agar media most strains form a small (up to 1 mm diameter) white, umbonate colony that is beta-haemolytic surrounded by a diffuse outer ring of alpha-haemolysis. There is some variation in colony morphology, with strains isolated from human patients in Asia tending to be larger and more mucoid (Lau et al., 2006). Extent and type of haemolysis produced may also vary, and seems to be dependent on the type of blood used in the agar with greater reactions noted with sheep than with human or bovine blood (Eldar et al., 1995b; Pier et al., 1978). The greatest amount of beta-haemolysis has been noted when grown on freshwater dolphin blood (Pier et al., 1978). Beta-haemolysis is most reliably observed in anaerobically inoculated cultures (Facklam et al., 2005). This species is aerobic and facultatively anaerobic, and does not react with any Lancefield group.

S. iniae is not currently listed in the databases of the most commonly used rapid or automated identification systems, including the RAPID Strep strip, the Vitek system, API 20 STREP system, or the ATB Expression system (Facklam et al., 2005; Lau et al., 2006). Use of one of these systems in isolation may result in misidentification or a reading of ‘‘unidentified’’ (Facklam et al., 2005; Lau et al., 2006; Weinstein et al., 1997). However, the individual biochemical reactions read from these systems can still be utilised and compared to known standards. A list of biochemical reactions and growth properties averaged from selected isolates can be found in Table 3.

Table 3
Summary of biochemical results of *S. iniae* found by various authors (Barnes and Ellis, 2003; Bromage et al., 1999; Colorni et al., 2002; Creeper and Buller, 2006; Eldar et al., 1994, 1995b; Facklam et al., 2005; Foo et al., 1985; Kaige et al., 1984; Kitao et al., 1981; Lau et al., 2006; Minami et al., 1979; Nakatsugawa, 1983; Nguyen and Kanai, 1999; Ohnishi and Jo, 1981; Perera et al., 1994; Pier and Madin, 1976; Pier et al., 1978; Stoffregen et al., 1996; Weinstein et al., 1997; Yuasa et al., 1999)

Test (*n*)	Result (% positive)	Bergey's (Holt et al., 1994)	Test (*n*)	Result (% positive)	Bergey's (Holt et al., 1994)	Test (*n*)	Result (% positive)	Bergey's (Holt et al., 1994)
Lancefield grouping (10)	None	Uncertain	Melezitose (6)	+(100)		0.1% Tetrazolium (3)	+(100)	
Adonitol (6)	−(0)		Melibiose (8)	−(1)		Bile-esculin media (5)	V(20)	
Amygdalin (4)	V(25)		α-Methyl-D-glucoside (3)	−(0)		Nitrate reduction (9)	−(0)	
Arabinose (20)	−(0)		α-Methyl-D-mannoside (3)	V(33)		H_2S production (5)	−(0)	
Arabitol (5)	−(0)		*N*-Acetylglucosamine (3)	+(100)		Methylene blue milk 0.1% (9)	V(22)	
Arbutin (4)	+(100)		Raffinose (20)	−(0)		Growth at 10 °C (14)	V(43)	+
β-Gentiobiose (3)	+(100)		Rhamnose (8)	−(0)		Growth at 45 °C (14)	−(7)	−
Cellobiose (6)	+(100)		Ribose* (11)	+(100)	+	Growth at pH 9.6 (10)	V(20)	−
Dextran (3)	+(100)		Salicin (16)	V(88)	+	Voges-Proskauer (17)	−(0)	
Dulcitol (8)	−(0)		Sorbitol (20)	−(3)	−	α-Galactosidase (10)	−(6)	
Erythritol (3)	−(0)		Starch (7)	+(100)		β-Galactosidase (11)	V(16)	
Esculin (3)	+(100)		L-Sorbose (3)	−(0)		β-Glucuronidase (9)	+(100)	
Fructose (9)	+(100)		Sucrose (17)	+(97)		PYRA (12)	+(100)	−
Fucose (3)	−(0)		Tagatose (5)	−(0)		Alkaline phosphatase (10)	+(100)	
Galactose (9)	V(56)		Trehalose (20)	+(95)	+	Leucine arylamidase (9)	+(100)	
Gluconate (3)	−(0)		Turanose (3)	−(0)		Indole (7)	−(0)	
2-Keto-gluconate (3)	−(0)		Xylose (15)	−(0)		Oxidase (8)	−(0)	
5-Keto-gluconate (3)	−(0)		Xylitol (3)	−(0)		Catalase (15)	−(0)	
Glucose (15)	+(100)		Sodium hippurate hydrolysis (21)	−(0)	−	Citrate (4)	−(0)	
Glycerol (12)	V(33)		Gelatin hydrolysis (10)	−(0)		Urease (7)	−(0)	
Glycogen (10)	+(85)		Starch hydrolysis (15)	+(100)		O-F test (7)	Fermentative (100)	
Inositol (9)	−(0)		Esculin hydrolysis (19)	+(92)	+	Heat tolerance 60° for 30 min (5)	−(0)	
Inulin (16)	−(0)	−	Arginine dehydrolase (16)	+(97)		Lysine decarboxylation (6)	−(0)	
Lactose (20)	V(11)	−	Growth in 6.5% NaCl (16)	V(13)	−	Ornithine decarboxylation (6)	−(0)	
Maltose (16)	+(100)		Growth with 10% bile (8)	V(38)		Arginine decarboxylation (4)	V(75)	
Mannitol (20)	+(100)	+	Growth with 40% bile (12)	−(0)	−	Methyl red (6)	V(63)	
Mannose (9)	+(100)		Growth in 0.04% tellurite (3)	−(0)		Litmus milk (5)	V(60)	

+90% or more strains are positive, −90% or more strains are negative and V 11–89% of strains are positive.
* = varies with serotype.

Identification characteristics as listed in the 9th edition of Bergey's Manual of Determinative Bacteriology are also listed for reference (Holt et al., 1994). It should be noted that isolates often differ in a few areas, but the overall characteristics of beta-haemolytic streptococci ungroupable by Lancefield grouping and positive for pyrrolidonyl arylamidase (PYRA) are retained. Confusingly, Bergey's lists *S. iniae* as being PYRA negative, but all reports of isolates reviewed in this paper list the test as being positive. *Streptococcus pyogenes* and *Streptococcus porcinus* may be differentiated from *S. iniae* by conventional tests such as the CAMP and Voges-Proskauer reactions (Facklam et al., 2005; Lau et al., 2006).

Several types of selective agar have been suggested for the isolation of *S. iniae*. Nguyen and Kanai (1999) found that heart infusion agar combined with either thallium acetate-oxolinic acid or colistin sulphate-oxolinic acid was effective when the sample contained a substantial concentration of bacteria, such as found with samples of heart, brain or intestinal contents (Nguyen and Kanai, 1999). A selective enrichment broth, containing 0.5 g thallium acetate, 10 mg colistin sulphate and 5 mg oxolinic acid in 1 litre of Todd-Hewitt broth was found to be a useful first step when detecting *S. iniae* in samples which may have a low concentration of bacteria, such as water samples (Nguyen et al., 2002).

Molecular techniques have been developed in recent years in an attempt to provide an alternative to culture-based identification. This has been especially useful in the identification of carrier fish, as culture techniques alone have been shown to be insufficient in asymptomatic tilapia and hybrid striped bass, in particular (Zlotkin et al., 1998). Molecular tools being used include sequencing the intergenic spacer region between the 16S and 23S ribosomal genes, 16S ribosomal gene or DNA hybridization of the chaperonin 60 gene (Berridge et al., 1998; Goh et al., 1998; Lau et al., 2003). A PCR assay based on the lactate oxidase gene has also been developed which has been reported to be able to detect *S. iniae* with greater specificity than when the 16S rRNA gene is used, and within 1 day of receiving a sample (as opposed to 2–3 days required for traditional isolation and identification by biochemical testing) (Mata et al., 2004).

Recently a monoclonal antibody-based IFAT has been shown to be more sensitive, specific and reliable than traditional plate culture techniques (Klesius et al., 2006). Non-lethal sensitive sampling of infected tilapia was achieved by swabbing the nares followed by IFAT using a commercial monoclonal antibody kit (Klesius et al., 2006). Although a number of *S. iniae* isolates were tested in the trial, it was not indicated whether different serotypes were used and it would be interesting to determine whether the monoclonal antibody is equally capable of binding different capsular serotypes.

Two distinct serotypes based on reaction with rainbow trout antibodies have been recognized which differ biochemically in their ability to react with arginine dihydrolase (ADH) and ribose. Serotype I, the group into which most original isolates fall, is ADH and ribose positive (Bachrach et al., 2001; Barnes et al., 2003b; Zlotkin et al., 1998). Serotype II, which was first detected after the Israeli vaccination program, is negative for both of these tests. These serotypes can also be distinguished using the rapid amplified polymorphic DNA (RAPD) technique. Evidence indicates that the differences between these two serotypes are mainly conferred by changes to the capsule, with the serotype II ADH negative isolates having capsules that provide more complete coverage of the bacterial cell surface which allows them better evasion of immune responses and therefore to be more invasive (Zlotkin et al., 2003). It must be indicated here, however, that the arginine dihydrolase reaction variability appears to an artefact of certain test kits used, as a simple tube based biochemical test for arginine dihydrolase activity in lysates is always positive (Barnes and Ellis, 2003). Thus, the ADH reaction should not be routinely used as a reliable indication of serotype or virulence (Barnes and Ellis, 2003).

Diagnosis of streptococcosis in fish due to infection by *S. iniae* should be based on the combination of clinical signs, pathology findings, and aetiological isolation. Bacteria are most commonly isolated from the brains and kidneys of infected fish, although other organs and water samples may also yield positive cultures (Bromage et al., 1999; Nguyen and Kanai, 1999; Perera et al., 1994; Stoffregen et al., 1996).

5. Pathophysiology

S. iniae tends to cause different disease states depending on the type of hosts it infects. In dolphins it

has only been shown to cause multifocal subcutaneous abscesses (Bonar and Wagner, 2003; Pier and Madin, 1976; Pier et al., 1978). Humans tend to develop a bacteraemic cellulitis, with occasional localisation in other organs or joints (Facklam et al., 2005; Lau et al., 2003; Weinstein et al., 1997). In fish, the disease state caused by infection with *S. iniae* generally results in a meningitis and panophthalmitis, and results in high levels of morbidity and mortality (Bromage and Owens, 2002).

Disease progression in fish is somewhat variable and has been shown to be dependent on the virulence of the isolate, the host species affected, route of infection, fish age and other environmental and water quality factors. However, most infections and outbreaks share a number of features and are generally similar to that of other streptococcal infections. Clinical signs may include any or all of the following: exophthalmia (which may be bilateral), corneal opacity, melanosis, lethargy, loss of orientation, swimming erratically, dorsal rigidity, vertebral deformity, tachypnoea, anorexia, emaciation, or sudden death with few accompanying signs (Bromage and Owens, 2002; Bromage et al., 1999; Eldar et al., 1994, 1995a, 1997a; Eldar and Ghittino, 1999; Kaige et al., 1984). Grossly, the most consistent finding is of intracranial oedema and ocular alterations resulting from a severe haemorrhagic panopthalmitis (Bromage and Owens, 2002; Eldar and Ghittino, 1999). Other findings may include ascites, mild peritonitis, scattered haemorrhages in the body cavity, petechiae around the anal zone, and gill rot (Bercovier et al., 1996; Bromage and Owens, 2002; Colorni et al., 2002; Eldar et al., 1995a; Eldar and Ghittino, 1999). The main histopathological finding is a severe acute suppurative meningitis where the meningeal surface and Virchoff's spaces may be covered by an exudate which contains colonies of bacteria (Bercovier et al., 1996; Bromage and Owens, 2002; Eldar and Ghittino, 1999). Subarachnoid haemorrhages, parenchymal monoculear infiltrates, posterior cerebritis with abscess formation, ventriculitis, fibrin deposition in the spleen and brain, mononuclear invasion of heart tissues, granulomas in the hepatic capsule, cavitation of gill capillaries, and renal tubular infiltration with hyaline droplet degeneration may also be observed (Bromage and Owens, 2002; Eldar et al., 1995a; Eldar and Ghittino, 1999; Kaige et al., 1984). In general, serotype I isolates tend to be restricted mainly to neural lesions, and infections with serotype II isolates tend to also show signs of a generalised septic disease, including multisystem organ involvement and diffuse internal haemorrhages (Bachrach et al., 2001).

Fish have been shown to be susceptible to infection by *S. iniae* through a variety of routes. Oral, bath exposure with or without the presence of abrasions, and intra-peritoneal injection routes have all been shown to be effective experimentally (Bromage and Owens, 2002; Shoemaker et al., 2000). It has also been suggested that infection may occur through the olfactory route, through direct contact in crowded conditions, or through cannibalism of moribund or dead infected fish (Bromage and Owens, 2002; Shoemaker et al., 2000). The median lethal dose (LD_{50}) varies for different host species, route of infection and isolates, but has been shown to range from 3×10^4 to 10^8 CFU/fish for trout, barramundi and tilapia (Bercovier et al., 1996; Bromage et al., 1999; Eldar et al., 1995a; Eldar and Ghittino, 1999). Exposure routes have been shown to have effects on the course of disease, with bath exposure causing an acute disease resulting in high levels of mortality observed within 48 h, and oral exposure causing a more prolonged disease course in barramundi (Bromage and Owens, 2002). This led Bromage and Owens (2002) to propose that epizootics begin with fish initially becoming infected by carrier fish through cannibalism or the faecal-oral route, with the resultant sub-acute infections allowing sufficient amplification of the pathogen to lead to acute disease through waterborne exposure. In a study in hybrid striped bass (*Morone chrysops* × *M. saxatilis*), infection was successfully established through the gills (McNulty et al., 2003), but lower doses were required to establish infection when striped bass were inoculated through the nares (Evans et al., 2000, 2001).

Many predisposing or environmental factors have been identified which enhance morbidity and mortality. High virulence levels and increased numbers of bacteria are often associated with an increase in water temperature, generally greater than 17 °C (Bercovier et al., 1996; Inglis et al., 1993). Deteriorating water quality, especially increased levels of ammonia, nitrate and nitrite, and dissolved oxygen levels less than 4 mg/ml also promote rapid spread and mortality (Eldar et al., 1995a). Increased fish density and other

stresses are also contributing factors (Eldar et al., 1995a; Shoemaker et al., 2000, 2001). Similar to other streptococcoses, it has been shown that adult and subadult fish are the groups most susceptible to infection (Bercovier et al., 1996), although recent incidents at farm sites in Australia suggest juveniles may also become susceptible. The presence of susceptible wild fish in or around pens may also contribute to development of an outbreak if they are able to act as a source or amplifier of the bacteria.

Several virulence factors have been identified in this species. First is the presence of a capsule, which is found in all isolates, but with serotype II capsules providing more coverage of surface antigens, thereby conferring additional antiphagocytic properties (Barnes et al., 2003b; Buchanan et al., 2005). A cell surface Fc binding factor has also been identified which is able to block the region of Ig molecules responsible for the binding and activation of complement and opsonisation (Barnes et al., 2003a). A phosphoglucomutase gene has also been identified which plays a role in normal cell wall morphology, surface capsule expression, and resistance to innate immune clearance mechanisms (Buchanan et al., 2005). Most recently, capsule has been shown to play an important role in resistance to phagocytic clearance by fish macrophages (Locke et al., 2007).

The pathogenesis of disease caused by *S. iniae* is not yet fully understood, but Zlotkin et al. (2003) suggest that it is the result of a multistep process. It is likely that infection may begin with colonisation of external or gastrointestinal tissues followed by local spread and subsequent invasion of the blood stream. Failure of initial phagocytosis and killing of the bacteria will allow establishment of disease. The ability of serotype II isolates to more effectively evade the immune response confers an advantage in the maintenance of a generalised bacteraemia. To enter the central nervous system (CNS) from the bloodstream, free bacteria may make their way through the blood brain barrier or be carried in association with monocytes or phagocytes. Incidence of CNS infection has been shown to be directly correlated to the concentration of the pathogen in the blood and length of time bacteraemia is maintained (Zlotkin et al., 2003). Thus, the ability of serotype II isolates to survive within phagocytes also confers an advantage in the establishment of meningitis. The ability to promote apoptosis of infected cells is also considered an advantage to the establishment of disease, as it causes cell death without the release of cellular components, thereby reducing or suppressing inflammation (Zlotkin et al., 2003). Thus, a credible model for establishment of infection may be that the fish become infected through gut, gills or nares, but *S. iniae* rapidly establishes in the blood where is phagocytosed by peripheral blood leucocytes, including macrophages (Zlotkin et al., 2003). *S. iniae* is reportedly able to withstand the bactericidal activity of the macrophages and can trigger apoptosis to facilitate its exit (Zlotkin et al., 2003). It is hypothesised that this hijacking of migrating macrophages may be the major means that *S. iniae* finds its way into the brain from the blood system, so causing fatal meningitis (Zlotkin et al., 2003). The present authors remain open minded about phagocytic survival of *S. iniae* in fish macrophages. Recent work by Locke et al. (2007) showed that virulent isolates expressing complete polysaccharide capsules were more resistant to opsonophagocytosis than isogenic knockout mutants. As capsulated forms are more virulent in fish (Buchanan et al., 2005), this suggests that phagocyte colonisation and survival are not perhaps the foremost strategy for establishing infection in fish. Further work in this area is clearly required to clarify this apparent contradiction.

Not all fish infected with *S. iniae* show signs of disease. The bacterium has been cultured from the brains of healthy barramundi, evidence that they are able to carry the pathogen asymptomatically (Bromage et al., 1999). Other studies have also noted the existence of carrier fish in infected populations (Bromage and Owens, 2002; Eldar et al., 1995a; Zlotkin et al., 1998). The bacterium is often isolated from fish that have survived an outbreak, which may serve as a pathogen reservoir for future infections.

6. Prevention and therapy

Dermal infections in freshwater dolphins have been successfully treated with antibiotics. The first animal responded to a 10-day course of penicillin and tylocin (Pier and Madin, 1976; Pier et al., 1978). The third was eventually successfully treated with carbenicillin followed by erythromycin, but the course was

continued for 13 weeks and recovery took over 18 months (Bonar and Wagner, 2003).

All known human infections have also been treated successfully with antimicrobial therapy. Most were treated with penicillin, although erythromycin, cloxacillin, ampicillin and cephalexin have also been used either alone or in combination (Koh et al., 2004; Lau et al., 2006; Weinstein et al., 1997).

Overall, most strains of *S. iniae* have been shown to be susceptible to β-lactams, macrolides, quinolones, and vancomycin, with penicillin being the drug of choice for treating *S. iniae* infections when antimicrobial therapy is required in mammals (Facklam et al., 2005).

Antibiotics have also shown to be effective in treating some fish infections. Although oxytetracycline (Terramycin for fish dosed 82.7 mg/kg fish SID × 10d) was unsuccessful, Stoffregen et al. (1996) showed increased survival in infected fish treated with enrofloxacin (at 5 and 10 mg/kg fish SID × 10d). Amoxicillin was also found to improve survival in blue tilapia and sunshine bass when given orally as medicated feed after experimentally induced infection (Darwish and Ismaiel, 2003; Darwish and Hobbs, 2005). In barramundi, oxytetracycline, furazolidone and amoxicillin would appear to be most widely used in Australia, but erythromycin is considered most effective (Creeper and Buller, 2006).

The use of antimicrobials in culture situations does have some limitations and concerns. The first is selection for resistance amongst dense populations. Stoffregen et al. (1996) noted evidence of selection for an enrofloxacin-resistant isolate with the treatment regimes given above. Development of resistance may also be increased when bacteria are not fully eliminated from fish or their environment. The ability of streptococci to survive in macrophages (Zlotkin et al., 2003) and the failure of sick fish to eat sufficient quantities of medicated feed may contribute to the development of resistant strains or carrier fish (Shoemaker et al., 2001).

Drug residues are also of concern in farmed fish destined for human consumption. Elimination of therapeutic compounds is highly dependent on species, initial dose rate, route of administration, and water temperature (which affects fish metabolic rates) (Stoffregen et al., 1996). Often, research to determine the withholding periods for specific drug and species combinations have not been undertaken. There are currently no antibiotics registered for use in aquaculture in Australia (Owusu, 2006).

A second area of disease control is through prevention of new disease by vaccination. A program was successfully initiated in farmed rainbow trout in Israel from 1995 to 1997 using autogenous vaccines consisting of whole-cell formalin inactivated *S. iniae* injected intra-peritoneally (Bercovier et al., 1996; Eldar et al., 1997a). Fish vaccinated at 50 g were protected for over 4 months, covering the majority of the short trout production cycle in Israel (Bercovier et al., 1996). Large-scale vaccination programs in Upper Galilee reduced mortalities due to *S. iniae* from 50% to less than 5% annually (Eldar et al., 1997a). Evidence suggested that the basic mechanism of protection was antibody mediated, probably generated in response to heat-stable, protein based antigenic determinants (Bercovier et al., 1996). Evidence for the role of antibody in conferring protection was supported by Shelby et al. (2002), who showed that passive immunization of tilapia with anti-*S. iniae* sera was also protective.

However, the success of the vaccination program was short-lived. In 1997 massive new outbreaks occurred due to a new variant of the bacterium. Unlike the previous isolates, this variant was arginine dihydrolase and ribose negative, and seemed to have shifted its capsular composition (Bachrach et al., 2001; Zlotkin et al., 1998). The vaccination program in Israel was shown to have allowed some pathogen to remain in fish or the environment and provided enough selective pressure for a distinctly different serotype to become dominant (Bachrach et al., 2001).

More recently, two new vaccines have become available in parts of Asia to protect against *S. iniae* infection. Intervet is marketing a monovalent inactivated vaccine (Norvax® Strep *Si*) in Indonesia that can be used as an immersion or an injectable (http://aqua.intervet.com/products/127_18752_2/productdetails_127_112315.asp). Schering-Plough has developed AquaVac™ Garvetil™, which combines protection against *S. iniae* and *Lactococcus garvieae*, and can be given either as an immersion or orally in feed (http://www.spaquaculture.com/default.aspx?pageid=631). Neither vaccine is currently available for use in Australia. It is unknown at this stage whether these vaccines will have more longevity and usefulness than those used in the Israeli program.

Autogenous vaccines (formalin killed bacterins based on a strain or strains isolated from the affected farm site, and used in subsequent production runs on that site) have also been used in Australia (Creeper and Buller, 2006) and elsewhere. Once again, these have met with limited success, with re-emergence of infection occurring in most cases, often within weeks of vaccination. The reasons behind this lack of efficacy are not yet clear although serological diversity is evident in some cases. Re-emergence of infection may also result from rapid immune kinetics associated with water-based bacterins particularly at high water temperatures, where primary antibody titres subside within 30–40 days. Moreover, the regulatory framework associated with use of autogenous vaccines predisposes them to failure as they may only be used at sites where infection with that particular strain is prevalent resulting in high likelihood of vaccinating fish that already carry *S. iniae*.

Other sources of improved vaccines are also being considered for future use. A phosphoglucomutase mutant strain has been identified that is able to reproduce and disseminate widely but is able to be eliminated within 24 h without any organ damage (Buchanan et al., 2005). It has been shown to stimulate a protective immune response and may have value as a live attenuated vaccine, theoretically proving more effective than killed, bacterin based vaccines (Buchanan et al., 2005). However, how this will overcome the problem of serological diversity amongst strains is not apparent. Moreover, the question of reversion to virulence of attenuated fish pathogens under selective pressure in the environment has not yet been adequately addressed. *S. iniae* is capable of surviving in water and sediments (Nguyen et al., 2002), infecting wild fish (Colorni et al., 2002), and has been recovered from mammals thereby presenting numerous opportunities maintenance, propagation and potential mutation. The consequences of reversion to virulence of a zoonotic pathogen that can also spread to wild fish would clearly be significant and potentially severely adverse.

Killed bacterins supplemented with extracellular products (ECPs) have been reported to be effective in tilapia (Klesius et al., 2000) when delivered by either intramuscular or intraperitoneal routes. The study identified the importance of considering serological variation of the isolates in formulating vaccines and indicated that a bivalent formulation containing two serologically distinct isolates was able to cross-protect against both isolates where monovalent preparations were not effective (Klesius et al., 2000). More recently, the ECP-enriched vaccine has been demonstrated effective when delivered orally using a commercial oral adjuvant (Oralject) against homologous challenge (Shoemaker et al., 2006). Further support of the efficacy of ECP-enriched vaccines was reported in a recent study in olive flounder (*Paralichthys olivaceus*) in which a proteomics approach revealed high antigenicity of ECP proteins in 2D-electrophoresis (Shin et al., 2007). A subsequent vaccine prepared using bacterins enriched with ECP proved effective at preventing infection by the homologous isolate. To date, all reports on efficacy of the ECP-enriched *S. iniae* vaccine have centred on laboratory challenges against predominantly homologous isolates. It will be interesting to note how these vaccines perform under field conditions where reservoirs of *S. iniae* may have opportunity to adapt giving rise to novel capsular variants under the selective pressure of vaccination.

The most widely used control measures have focused on improving water quality/environmental conditions to help keep the challenge to cultured fish stocks as low as possible. This includes reducing fish density, minimising feral fish inside sea cages through the use of more effective barrier netting, diligent removal of moribund fish, and adequate quarantine protocols for new additions (Bromage and Owens, 2002; Shoemaker et al., 2000, 2001). The increased susceptibility of some wild fish to *S. iniae* may be useful to monitor for outbreaks (Zlotkin et al., 1998). Sudden death in wild fish near a farm or cages can be used as a sign of increased risk and that measures should be taken promptly to limit loss.

7. Zoonotic potential

There is substantial evidence that *S. iniae* is able to be transferred to and infect humans. As of 2005, there have been at least 25 confirmed cases of *S. iniae* infections in humans reported in the literature (Facklam et al., 2005; Lau et al., 2006, 2003). The true number of human infections is likely to be much higher due to problems with identification. Its reported

similarities to *Streptococcus pyogenes* and the viridans group of streptococci may lead to misidentification, especially when rapid phenotypic systems or automated devices, which are based on traditional strains of human relevance, are used (Facklam et al., 2005). The most common manifestation of infection is a bacteraemic cellulitis developing 16–24 h after a percutaneous injury while preparing fish for consumption (Facklam et al., 2005; Lau et al., 2003; Weinstein et al., 1997).

Weinstein et al. (1996) were the first to report bacteraemic illnesses due to *S. iniae* in four patients over a 15-month period in an Ontario hospital. All four patients had a history of handling fresh, whole, cultured fish. In a subsequent prospective and retrospective surveillance based study, Weinstein et al. (1997) identified a total of 11 patients with invasive *S. iniae* infections. The earliest occurred in Texas in 1991, with all other reported cases occurring in Canada from 1994 to 1996. All but the two earliest patients were of Asian descent, with a median age of 69 years, and all patients recalled handling whole, raw fish. Eight patients recalled puncturing the skin of their hands during preparation, and six were able to identify the fish they were preparing as tilapia. No fish remained for possible sampling, but *S. iniae* was cultured from fish from Vancouver retail stores, Toronto suppliers, and from fish farms in Texas and Virginia (Weinstein et al., 1997). When analysed by pulsed-field gel electrophoresis (PFGE), some similarities between isolates were noted from the retailers, suppliers, farms and patients, and it was suggested that two closely related genotypes were responsible for human infections (Weinstein et al., 1997). However, subsequent PFGE-based studies indicate that multiple genotypes were capable of infecting humans (Facklam et al., 2005; Lau et al., 2006). Increased vigilance for *S. iniae* infections led to the identification of two more human patients in Vancouver by 1998 (Goh et al., 1998). In the USA, the Centers for Disease Control (CDC) in Atlanta, Georgia identified seven more clinical cases from humans in California and Pennsylvania between 2001 and 2004 (Facklam et al., 2005). All 12 samples of *S. iniae* isolated from humans, fish and dolphins that were tested by the CDC were also shown to have some virulence to humans using a phagocytosis assay (based on survival and multiplication in human blood) (Facklam et al., 2005).

Human infections have also been reported in Asia. In 2003, two cases of humans infected with *S. iniae* were reported in Hong Kong. Both patients recalled handling fresh fish in the period leading up to their bacteraemic illnesses. In 2004, a patient in Singapore was reported to have an infection in her lumbar spine caused by *S. iniae* (Koh et al., 2004). Upon sequencing of the 16S rRNA gene it was found to be identical to the first dolphin isolate of 1976. In 2006, it was reported that two more elderly patients in Hong Kong were confirmed to have been infected with *S. iniae* in 2004 (Lau et al., 2006).

Almost all persons shown to have been infected with *S. iniae* have been elderly and of Asian descent. The youngest known age is 40, with the rest ranging from 58 to 88 years (Facklam et al., 2005; Lau et al., 2003; Weinstein et al., 1996, 1997). Many patients interviewed by Weinstein et al. (1997) and Lau et al. (2003) had one or more underlying medical conditions at the time of infection. Thus, the greatest zoonotic risk appears to be associated with older or immunocompromised people who incur a puncture wound while handling or preparing fresh, whole fish for cooking (Shoemaker et al., 2001). Shoemaker et al. (2001) dismissed claims of *S. iniae* being a serious public health threat by demonstrating that not all US farms grew fish that tested positive for *S. iniae*, and that the overall prevalence was 4.34% for tilapia and hybrid striped bass in 2001. Further, the authors could not find any evidence of infection amongst workers on the farms studied. However, they did suggest that methods of housing fish before distribution to retailers may favour contamination of fish as prevalence was 18–50 times higher amongst fish sampled from retailers than from the farms (Shoemaker et al., 2001).

Not all *S. iniae* isolates show the same ability to colonise humans, as shown by the limited growth and survivability of the two original dolphin isolates in human blood (Facklam et al., 2005). There are also some differences in the banding patterns of isolates studied using PFGE, indicating that not all human isolates are clonal (Facklam et al., 2005; Lau et al., 2003; Weinstein et al., 1997), and are unlikely to be derived from a single point source. Weinstein et al. (1997) found 21 different PFGE patterns from tilapia in Canada and the USA, some of which matched the bands produced by the human isolates they studied,

but could not determine if the invasive clones were restricted to one or more farms. Although most healthy people have good natural immunity to fish pathogens (Lehane and Rawlin, 2000), and it is likely that only certain clones of *S. iniae* are responsible for zoonotic infections in humans, it would be prudent to raise public awareness of safe handling procedures for fresh whole fish and of good food hygiene practices in general. Notably, there has been no evidence of human infection through the ingestion of contaminated fish (WHO, 1998).

8. Conclusion

The list of susceptible species as well as the distribution of this bacterium highlight the importance of *S. iniae* not only as a pathogen to the aquaculture industry, but one which also has the potential to adversely affect human health. Although much work has been done on this species to date, more research is needed to further reduce the effects of this bacterium worldwide. Aquaculture is one of the fastest growing industries in the world. As global wild fish stocks continue to be depleted, it will be even more important to provide safe, efficient production of food for human consumption. The implementation of effective, long-term vaccination programs is the most likely target for future control of the potentially industry-limiting disease caused by *S. iniae*. Whilst there are a number of promising vaccine candidates, not least the ECP-enriched formulations, the key issue remains effective protection against multiple serotypes. Further research is needed to fully understand this pathogen, particularly its serological diversity and epidemiology before vaccination programs can become successful on a worldwide scale. With the current USDA-funded *S. iniae* genome project nearing completion (http://www.hgsc.bcm.tmc.edu/projects/microbial/microbial-detail.xsp?project_id=157), rapid advances may soon be made in our understanding of this organism at the molecular level.

References

Bachrach, G., Zlotkin, A., Hurvitz, A., Evans, D.L., Eldar, A., 2001. Recovery of *Streptococcus iniae* from diseased fish previously vaccinated with a streptococcus vaccine. Appl. Environ. Microbiol. 67, 3756–3758.

Barnes, A.C., Ellis, A.E., 2003. Variation in arginine dihydrolase activity in *Streptococcus iniae* may be an artefact of the assay. Bull. Eur. Assoc. Fish Pathol. 23, 163–166.

Barnes, A.C., Horne, M.T., Ellis, A.E., 2003a. *Streptococcus iniae* expresses a cell surface non-immune trout immunoglobulin-binding factor when grown in normal trout serum. Fish Shellfish Immunol. 15, 425–431.

Barnes, A.C., Young, F.M., Horne, M.T., Ellis, A.E., 2003b. *Streptococcus iniae*: serological differences, presence of capsule and resistance to immune serum killing. Dis. Aquat. Org. 53, 241–247.

Bercovier, H., Ghittino, C., Eldar, A., 1996. Immunization with bacterial antigens: infections with streptococci and related organisms. In: Second International Symposium on Progress in Fish Vaccinology, Soria Moria Hotel, Oslo, Norway, June 5–7, pp. 153–160.

Berridge, B.R., Fuller, J.D., de Azavedo, J., Low, D.E., Bercovier, H., Frelier, F., 1998. Development of a specific nested oligonucleotide PCR primers for the *Streptococcus iniae* 16S-23S ribosomal DNA intergenic spacer. J. Clin. Microbiol. 36, 2778–2781.

Bonar, C.J., Wagner, R.A., 2003. A third report of ‘‘golf ball disease’’ in an Amazon River dolphin (*Inia geoffrensis*) associated with *Streptococcus iniae*. J. Zoo Wildlife Med. 34, 296–301.

Bowser, P.R., Wooster, G.A., Getchell, R.G., Timmons, M.B., 1998. *Streptococcus iniae* infection of tilapia *Oreochromis niloticus* in a recirculation production facility. J. World Aquacult. Soc. 29, 335–339.

Bromage, E.S., Thomas, A., Owens, L., 1999. *Streptococcus iniae*, a bacterial infection in barramundi *Lates calcarifer*. Dis. Aquat. Org. 36, 177–181.

Bromage, E.S., Owens, L., 2002. Infection of barramundi *Lates calcarifer* with *Streptococcus iniae*: effects of different routes of exposure. Dis. Aquat. Org. 52, 199–205.

Buchanan, J.T., Stannard, J.A., Lauth, X., Ostland, V.E., Powell, H.C., Westerman, M.E., Nizet, V., 2005. *Streptococcus iniae* phosphoglucomutase is a virulence factor and a target for vaccine development. Infect. Immun. 73, 6935–6944.

Colorni, A., Diamant, A., Eldar, A., Kvitt, H., Zlotkin, A., 2002. *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. Dis. Aquat. Org. 49, 165–170.

Creeper, J., Buller, N., 2006. An outbreak of *Streptococcus iniae* in barramundi (*Lates calcarifera*) in freshwater cage culture. Aust. Vet. J. 84, 408–411.

Darwish, A.M., Ismaiel, A.A., 2003. Laboratory efficacy of amoxicillin for the control of *Streptococcus iniae* infection in sunshine bass. J. Aquat. Anim. Health 15, 209–214.

Darwish, A.M., Hobbs, M.S., 2005. Laboratory efficacy of amoxicillin for the control of *Streptococcus iniae* infection in blue tilapia. J. Aquat. Anim. Health 17, 197–202.

Eldar, A., Bejerano, Y., Bercovier, H., 1994. *Streptococcus shiloi* and *Streptococcus difficile*: two new streptococcal species causing a meningoencephalitis in fish. Curr. Microbiol. 28, 139–143.

Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A., Bercovier, H., 1995a. Experimental streptococcal meningo-encephalitis in cultured fish. Vet. Microbiol. 43, 33–40.

Eldar, A., Frelier, P.F., Assenta, L., Varner, P.W., Lawhon, S., Bercovier, H., 1995b. *Steptococcus shiloi*, the name for an agent causing septicaemic infection in fish, is a junior synonym of *Streptococcus iniae*. Int. J. Syst. Bacteriol. 45, 840–842.

Eldar, A., Ghittino, C., 1999. *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. Dis. Aquat. Org. 36, 227–231.

Eldar, A., Horovitcz, A., Bercovier, H., 1997a. Development and efficacy of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. Vet. Immunol. Immunopathol. 56, 175–183.

Eldar, A., Lawhon, S.F., Frelier, P., Assenta, L., Simpson, B.R., Varner, P.W., Bercovier, H., 1997b. Restriction fragment length polymorphisms of 16S rDNA and of whole rRNA genes (ribotyping) of *Streptococcus iniae* strains from the United States and Israel. FEMS Microbiol. Lett. 151, 155–162.

Evans, J.J., Shoemaker, C.A., Kelsius, P.H., 2000. Experimental *Streptococcus iniae* infection of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) and tilapia (*Oreochromis niloticus*) by nares inoculation. Aquaculture 198, 197–210.

Evans, J.J., Shoemaker, C.A., Kelsius, P.H., 2001. Distribution of *Streptococcus iniae* in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) following by nare inoculation. Aquaculture 194, 233–243.

Facklam, R., Elliott, J., Shewmaker, L., Reingold, A., 2005. Identification and characterization of sporadic isolates of *Streptococcus iniae* isolated from humans. J. Clin. Microbiol. 43, 933–937.

Ferguson, H., St John, V., Roach, C., Willoughby, S., Parker, C., Ryan, R., 2000. Caribbean reef fish mortality associated with *Streptococcus iniae*. Vet. Rec. 147, 662–664.

Foo, J., Ho, B., Lam, T., 1985. Mass mortality in *Siganus canaliculatus* due to streptococcal infection. Aquaculture 49, 185–195.

Goh, S.H., Driedger, D., Gillett, S., Low, D.E., Hemmingsen, S.M., Amos, M., Chan, D., Lovgren, M., Willey, B.M., Shaw, C., Smith, J.A., 1998. *Streptococcus iniae*, a human and animal pathogen: specific identification by the chaperonin 60 gene identification method. J. Clin. Microbiol. 36, 2164–2166.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. Bergey's Manual of Determinative Bacteriology. Williams & Wilkins, Baltimore.

Hoshina, T., Sano, T., Morimoto, Y., 1958. A streptococcus pathogenic to fish. J. Tokyo Univ. Fish. 44, 57–68.

Inglis, V., Roberts, R.J., Bromage, N.R., 1993. Bacterial Diseases of Fish. The University Press, Cambridge.

Kaige, N., Miyazaki, T., Kubota, S.S., 1984. The pathogen and histopathology of vertebral deformity in cultured yellowtail. Fish Pathol. 19, 173–179.

Kitao, T., Aoki, T., Sakoh, R., 1981. Epizootic caused by beta-haemolytic *Streptococcus* species in cultured freshwater fish. Fish Pathol. 15, 301–307.

Klesius, P.H., Shoemaker, C.A., Evans, J.J., 2000. Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). Aquaculture 188, 237–246.

Klesius, P., Evans, J., Shoemaker, C., Yeh, H., Goodwin, A.E., Adams, A., Thompson, K., 2006. Rapid detection and identification of *Streptococcus iniae* using a monoclonal antibody-based indirect fluorescent antibody technique. Aquaculture 258, 180–186.

Koh, T.H., Kurup, A., Chen, J., 2004. *Streptococcus iniae* discitis in Singapore. Emerg. Infect. Dis. 10, 1694–1696.

Kusuda, R., Kawai, K., Toyoshima, T., Komatsu, I., 1979. A new pathogenic bacterium belonging to the genus *Streptococcus*, isolated from a epizootic of cultured yellowtail. Nihon Suisan Gakkai Shi 42, 1345–1352.

Kvitt, H., Colorni, A., 2004. Strain variation and geographic endemism in *Streptococcus iniae*. Dis. Aquat. Org. 61, 67–73.

Lahav, D., Eyngor, M., Hurvitz, A., Ghittino, C., Lublin, A., Eldar, A., 2004. *Streptococcus iniae* type II infections in rainbow trout *Oncorhynchus mykiss*. Dis. Aquat. Organ. 62, 177–180.

Lau, S.K.P., Woo, P.C.Y., Luk, W.-k., Fung, A.M.Y., Hui, W.-t., Fong, A.H.C., Chow, C.-w., Wong, S.S.Y., Yuen, K.-y., 2006. Clinical isolates of *Streptococcus iniae* from Asia are more mucoid and B-hemolytic than those from North America. Diagnostic Microbiol. Infect. Dis. 54, 177–181.

Lau, S.K.P., Woo, P.C.Y., Tse, H., Leung, K.-W., Wong, S.S.Y., Yuen, K.-Y., 2003. Invasive *Streptococcus iniae* infections outside North America. J. Clin. Microbiol. 41, 1004–1009.

Lehane, L., Rawlin, G.T., 2000. Topically acquired bacterial zoonoses from fish: a review. Med. J. Aust. 173, 256–259.

Locke, J.B., Colvin, K.M., Datta, A.K., Patel, S.K., Naidu, N.N., Neely, M.N., Nizet, V., Buchanan, J.T., 2007. *Streptococcus iniae* capsule impairs phagocytic clearance and contributes to virulence in fish. J. Bacteriol. 189, 1279–1287.

Mata, A.I., Blanco, M.M., Dominguez, L., Fernandez-Garayzabal, J.F., Gibello, A., 2004. Development of a PCR assay for *Streptococcus iniae* based on the lactase oxidase (*lctO*) gene with potential diagnostic value. Vet. Microbiol. 101, 109–116.

McNulty, S.T., Klesius, P.H., Shoemaker, C.A., Evans, J.J., 2003. *Streptococcus iniae* infection and tissue distribution in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) following inoculation of the gills. Aquaculture 220, 165–173.

Minami, T., Nakamura, M., Ikeda, Y., Ozaki, H., 1979. A beta-hemolytic *Streptococcus* isolated from cultured yellowtail. Fish Pathol. 14, 33–38.

Nakatsugawa, T., 1983. A streptococcal disease of cultured flounder. Fish Pathol. 17, 281–285.

Nguyen, H.T., Kanai, K., 1999. Selective agars for the isolation of *Streptococcus iniae* from Japanese flounder, *Paralichthys olivaceus*, and its cultural environment. J. Appl. Microbiol. 86, 769–776.

Nguyen, H.T., Kanai, K., Yoshikoshi, K., 2002. Ecological investigation of *Streptococcus iniae* in cultured Japanese flounder (*Paralichthys olivaceus*) using selective isolation procedures. Aquaculture 205, 7–17.

Ohnishi, K., Jo, Y., 1981. Studies on streptococcal infection in pond-cultured fishes—I Characteristics of a beta-hemolytic Streptococcus isolated from cultured Ayu and Amago in 1977–1978. Fish Pathol. 16, 63–67.

Owusu, J., 2006. Email to author (August 4. Australian Pesticide and Veterinary Medicine Authority).

Perera, R.P., Johnson, S.K., Collins, M.D., Lewis, D.H., 1994. *Streptococcus iniae* associated with mortality of *Tilapia nilotica* × *T. aurea* hybrids. J. Aquat. Anim. Health 6, 335–340.

Pier, G.B., Madin, S.H., 1976. *Streptococcus iniae* sp. nov., a beta-hemolytic *Streptococcus* isolated from an Amazon freshwater dolphin, *Inia geoffrensis*. Int. J. Syst. Bacteriol. 26, 545–553.

Pier, G.B., Madin, S.H., Al-Nakeeb, S., 1978. Isolation and characterization of a second isolate of *Streptococcus iniae*. Int. J. Syst. Bacteriol. 28, 311–314.

Robinson, J.A., Meyer, F.P., 1966. Streptococcal fish pathogen. J. Bacteriol. 92, 512.

Shelby, R.A., Klesius, P.H., Shoemaker, C.A., Evans, J.J., 2002. Passive immunization of tilapia, *Oreochromis niloticus* (L.), with anti-*Streptococcus iniae* whole sera. J. Fish Dis. 25, 1–6.

Shen, Z.-H., Qian, D., Xu, W.-J., Gu, J.-H., Shao, J.-Z., 2005. Isolation, identification and pathogenicity of *Streptococcus iniae* isolated from red drum *Sciaenops ocellatus*. Acta Hydrobiol. Sinica 29, 678–683.

Shin, G.W., Palaksha, K.J., Kim, Y.R., Nho, S.W., Kim, S., Heo, G.J., Park, S.C., Jung, T.S., 2007. Application of immunoproteomics in developing *Streptococcus iniae* vaccine for olive flounder (*Paralichthys olivaceus*). J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 849, 315–322.

Shoemaker, C.A., Evans, J.J., Klesius, P.H., 2000. Density and dose: factors affecting mortality of *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). Aquaculture 188, 229–235.

Shoemaker, C.A., Klesius, P.H., Evans, J.J., 2001. Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. Am. J. Vet. Res. 62, 174–177.

Shoemaker, C.A., Vandenberg, G.W., Désormeaux, A., Klesius, P.H., Evans, J.J., 2006. Efficacy of a *Streptococcus iniae* modified bacterin delivered using Oralject technology in Nile tilapia (*Oreochromis niloticus*). Aquaculture 255, 151–156.

Stoffregen, D., Backman, S., Perham, R., Bowser, P., Babish, J., 1996. Initial disease report of *Streptococcus iniae* infection in hybrid striped (sunshine) bass and successful therapeutic intervention with the fluoroquinolone antibacterial enrofloxacin. J. World Aquacult. Soc. 27, 420–434.

Weinstein, M., Low, D.E., McGeer, A., Willey, B., Rose, D., Coulter, M., Wyper, P., Borczyk, A., Lovgren, M., 1996. Invasive infection due to *Streptococcus iniae*—Ontario. In: In Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention) 1995–1996. pp. 650–653.

Weinstein, M.R., Litt, M., Kertesz, D.A., Wyper, P., Rose, D., Coulter, M., McGeer, A., Facklam, R., Ostach, C., Willey, B.M., Borczyk, A., Low, D.E., 1997. Invasive infections due to a fish pathogen *Streptococcus iniae*. New England J. Med. 337, 589–594.

WHO, 1998. WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe No. 57. World Health Organisation.

Yuasa, K., Kitancharoen, N., Kataoka, Y., Al-Murbaty, F.A., 1999. *Streptococcus iniae*, the causative agent of mass mortality in rabbitfish *Siganus canaliculatus* in Bahrain. J. Aquat. Anim. Health 11, 87–93.

Yuniarti, A., 2005. Serological Characterization of *Streptococcus iniae* Isolates from Different Parts of Australia. Masters. University of Queensland, St. Lucia, Queensland.

Zlotkin, A., Hershko, H., Eldar, A., 1998. Possible transmission of *Streptococcus iniae* from wild fish to cultured marine fish. Appl. Environ. Microbiol. 64, 4065–4067.

Zlotkin, A., Chilmonczyk, S., Eyngor, M., Hurvitz, A., Ghittino, C., Eldar, A., 2003. Trojan horse effect: phagocyte-mediated *Streptococcus iniae* infection of fish. Infect. Immun. 71, 2318–2325.