AQUAVAC® Strep *Sa*
technical bulletin

Issue 2

Development of Novel *Streptococcus agalactiae* Biotype 2 Vaccine – Laboratory Challenge Trials

MSD Animal Health

Introduction

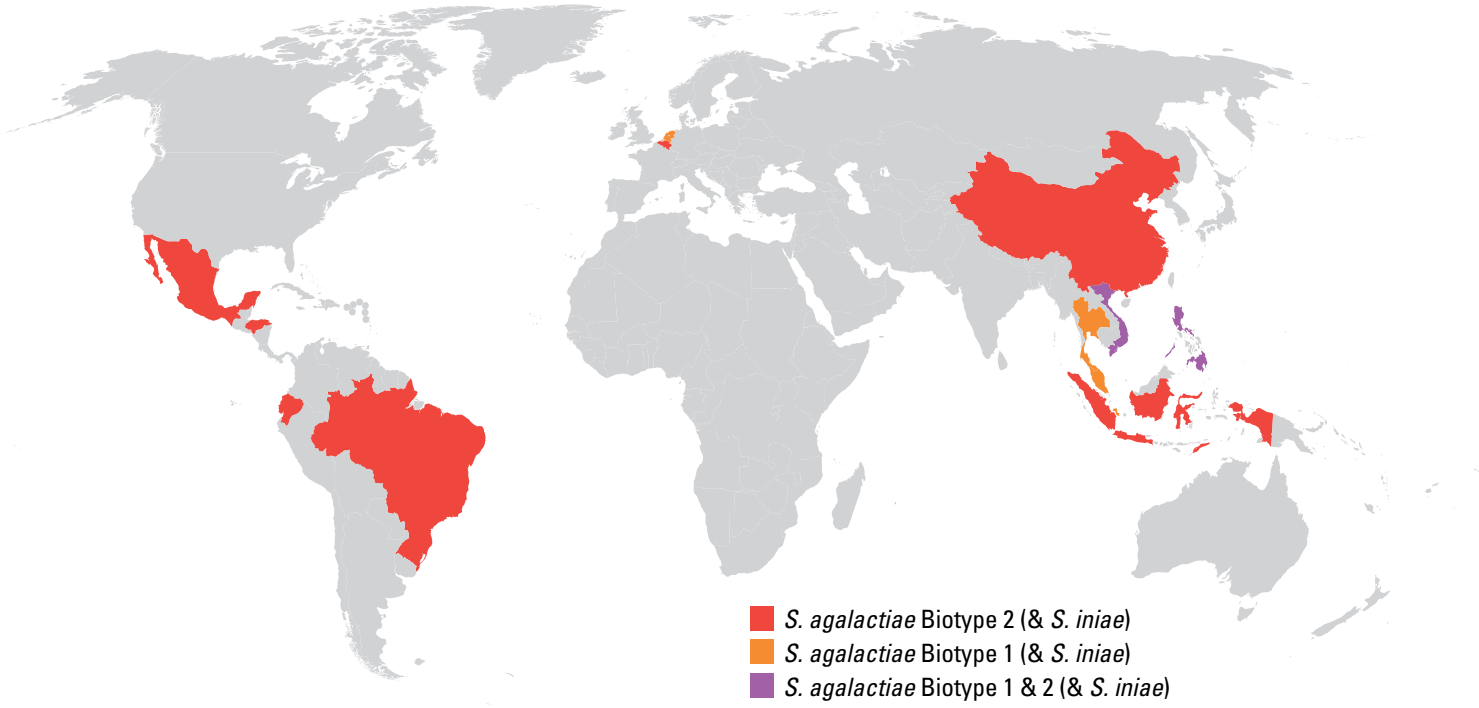
Streptococcus agalactiae infections in farmed tilapia are responsible for significant morbidity, mortality and economic loss. Infections with *S. agalactiae* result in septicemia and colonization of various internal organs, particularly the brain, leading to tell-tale disease signs. Clinical signs of *S. agalactiae* infection include abnormal swimming, 'C'-shaped body posturing, and inappetance. *S. agalactiae* is prevalent throughout temperate and tropical regions and MSD Animal Health scientists have recovered it from diseased tilapia in Europe, Central and Latin America, and throughout Asia.

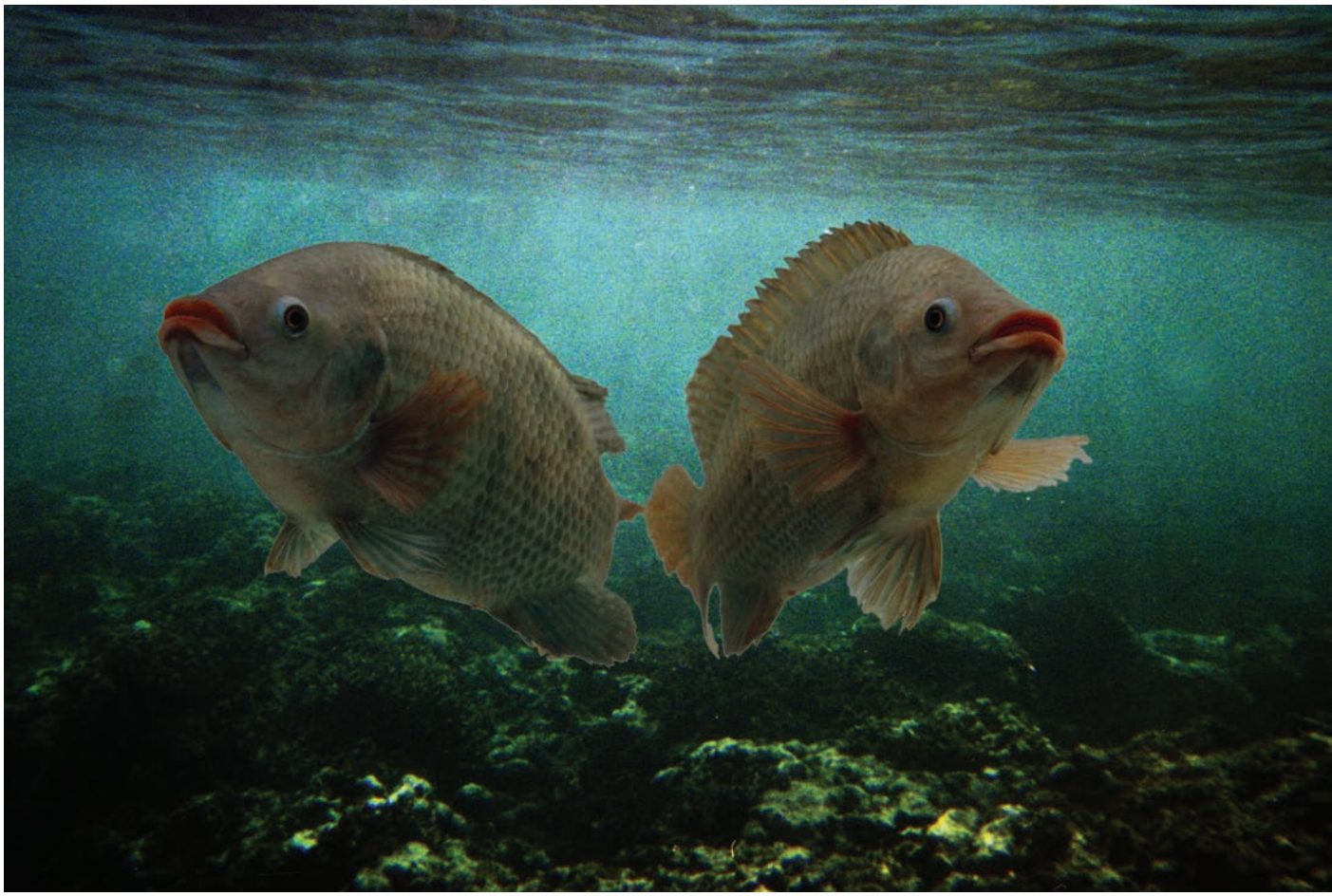
Amongst the *S. agalactiae* isolates collected from tilapia we differentiate between typically ß-haemolytic 'classical' *S. agalactiae* (hereafter referred to as *S. agalactiae* Biotype 1) and typically non-ß-haemolytic *S. agalactiae* (hereafter referred to as *S. agalactiae* Biotype 2). These latter strains were previously classified as *S. difficile*[1,2] but have subsequently been reclassified as non-haemolytic variants of *S. agalactiae*.[3] MSD Animal Health has conducted extensive epidemiological surveys, and to date, with hundreds of streptococcal isolates from many countries, the majority of all streptococcal isolates were found to be *S. agalactiae* Biotype 2. The company has identified *S. agalactiae* Biotype 2 in diseased fish from most of the major tilapia-producing countries including Indonesia, China, Vietnam, Philippines, Ecuador, Honduras, Mexico and Brazil.

In general terms, across the epidemiological work conducted by MSD Animal Health, it has been seen that the two *S. agalactiae* Biotypes cause subtly distinct disease syndromes, with Biotype 1 infecting fish throughout the production cycle from juvenile to grow-out, while Biotype 2 causes disease predominantly in larger fish. Moreover, and most significantly from a health management perspective, MSD Animal Health has demonstrated that immunity is biotype-specific *("MSD Animal Health Technical Bulletin, Streptococcus in the Tilapia Environment")*.

Vaccination has proven to be an effective strategy to prevent or reduce the impact of infectious diseases in aquaculture. Although there are several reports in the literature describing the development of vaccines against *S. agalactiae*, none of these are commercialized internationally. This technical bulletin describes the laboratory phase of the development of AQUAVAC Strep *Sa*, the first commercially available vaccine to combat *S. agalactiae* Biotype 2 infections in tilapia.

Figure 1: Regional Prevalence of *S. agalactiae* Biotypes





Trial

Materials and methods

AQUAVAC Strep *Sa* is a water-in-oil emulsion vaccine containing inactivated *Streptococcus agalactiae* Biotype 2 bacterial antigens and metabolizable non-mineral oil adjuvant. It is applied as a single dose of 0.05ml by intra-peritoneal injection in fish of 15g or more.

The efficacy of AQUAVAC Strep *Sa* was evaluated in laboratory trials following intra-peritoneal vaccination of 15g tilapia with a full dose (0.05ml) of the vaccine.

Age-matched unvaccinated fish from the same cohort and origin were maintained as controls. At various time points from three to thirty weeks post-vaccination, vaccinated and control fish were challenged by intra-peritoneal injection with a virulent heterologous *S. agalactiae* Biotype 2 strain. Fish were observed for 14 days post challenge and mortality recorded daily. Post-mortem recovery of the challenge organism was performed on all fish that died during the observation period and on all surviving fish at the end of the observation period after challenge.

Results

Vaccination with AQUAVAC Strep *Sa* protected against mortality caused by *S. agalactiae* Biotype 2. In laboratory challenge models, vaccinated fish were protected against lethal *S. agalactiae* Biotype 2 challenge with no significant decrease in protection observed from 3 weeks post-vaccination (the first time point tested) to thirty weeks post-vaccination (the last time point tested).

In general, mortality in the unvaccinated control tilapia began on the second day post-challenge and reached a maximum some 10 days later (Figure 2 and 3). Vaccination with AQUAVAC Strep *Sa* resulted in a significant reduction in mortality at all time points tested (3, 12, 24 and 30 weeks post-vaccination period. Prentesis Fisher Exact, P = 0.0002, P = 0.046, P = 0.0002 and P = 0.0002, respectively).

In addition to reducing mortality, results (Figure 4) demonstrate that vaccination with AQUAVAC Strep *Sa* significantly reduced the development of *S. agalactiae* Biotype 2-infected carrier fish.

Figure 2: Laboratory efficacy of AQUAVAC Strep *Sa* 3 weeks post-vaccination, challenged with a virulent heterologous *S. agalactiae* Biotype 2 strain

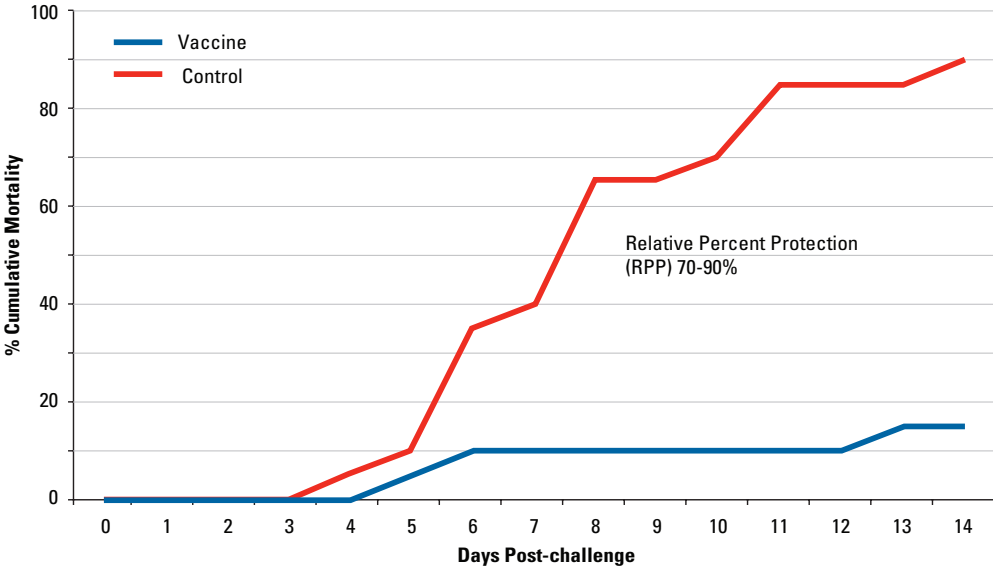
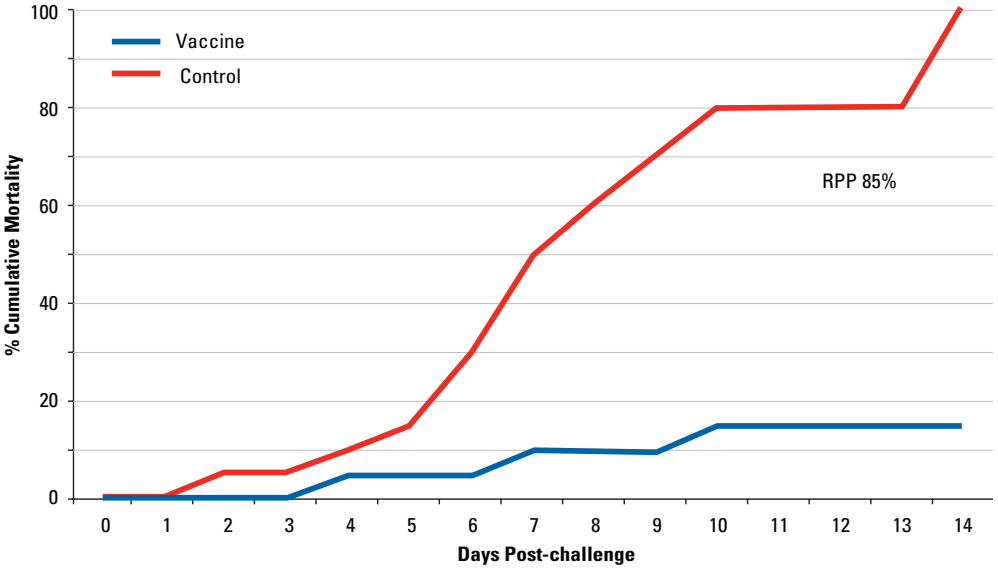


Figure 3: Laboratory efficacy of AQUAVAC Strep *Sa* 30 weeks post-vaccination, challenged with a virulent heterologous *S. agalactiae* Biotype 2 strain



At the end of the two week post-challenge observation period, the challenge bacteria could be recovered from the internal organs of one out of three surviving unvaccinated control fish from the week 3 challenge and from all of the surviving unvaccinated control fish from the week 30 challenge. In contrast, *S. agalactiae* Biotype 2 was not recovered from any of the 14 (week 3) or 16 (week 30) surviving vaccinated fish.

Similarly, the challenge bacteria could be recovered from the internal organs of twelve out of thirteen surviving unvaccinated control fish from the week 12 challenge. In contrast, the challenge bacteria could only be recovered from two out of nineteen surviving, vaccinated fish from the same challenge.

All of the unvaccinated control fish from the week 24 challenge died as a result of the experimental infection whereas the challenge organism could be recovered from only three out of eleven vaccinated fish. Although the numbers of fish available for this analysis at the end of the observation period were relatively low, the trend is overwhelmingly in favor of the vaccinated group and the differences were statistically significant at all times (Figure 4).

Figure 4: *S. agalactiae* Biotype 2 isolated from surviving vaccinate and control fish at various time points post-vaccination

Time Post-vaccination Treatment Groups	3 weeks		12 weeks		24 weeks		30 weeks	
	V	C	V	C	V	C	V	C
Survivors (No. fish)	13	4	19	13	11	0	16	4
Sa2 re-isolation (No. fish)	0	2	2	12	3	na	0	4
Fisher Exact	P = 0.044		P = 0.0001		NA†		P = 0.0002	

† Fisher exact value was not calculated as there were no controls left to re-isolate *S. agalactiae* and therefore no possibility to establish statistical significance.

Conclusions

MSD Animal Health has developed AQUAVAC Strep *Sa*, a safe and effective vaccine against streptococcal disease caused by *S. agalactiae* Biotype 2.

A single application of AQUAVAC Strep *Sa* conferred protection against laboratory challenge with virulent heterologous *S. agalactiae* Biotype 2 isolates for at least 30 weeks post-vaccination.

In addition to reducing mortality, our results demonstrate that vaccination with AQUAVAC Strep *Sa* significantly reduced the development of *S. agalactiae* Biotype 2-infected carrier fish. This reduction in numbers of infected fish would indicate that the potential for long-term control of the disease in sites where all fish are vaccinated is very good.

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Following this work, field trials were conducted to demonstrate efficacy of the development of AQUAVAC Strep *Sa* under commercial field conditions. This is discussed in MSD Animal Health technical bulletin *Development of Novel Streptococcus agalactiae Biotype 2 Vaccine - Field Trials.*

References

1. Eldar A, *et al.* Experimental streptococcal meningo-encephlitis in cultured fish. *Veterinary Microbiology* 1995; 45: 33-40.

2. Vandamme P, *et al. Streptococcus difficile* is a non hemolytic Group B, Type 1b Streptococcus. *International Journal of Systemic Bacteriology* 1997; 24: 81-85.

3. Kawamura Y *et al.* High genetic similarity of *Streptococcus difficilis: S. difficilis* Eldar *et al.* 1995 is a later synonym of *S. agalactiae*. Lehmann and Neumann 1896 (approved lists 1980) International *Journal of Systemic and Evolutionary Microbiology* 2005; 55: 961-96.

STATEMENT OF THE ACTIVE SUBSTANCE(S)
The vaccine contains inactivated *Streptococcus agalactiae* TI 513 cells with oil adjuvant.

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TARGET SPECIES
Fish.

INDICATION(S)
Aid in protection against *Streptococcus agalactiae* infections in susceptible fish species (see also below under special warnings).

DOSAGE FOR EACH SPECIES
Each animal should receive one dose of vaccine.

ADVICE ON CORRECT ADMINISTRATION
Inject 0.05 ml intraperitoneally in fish of minimum 15 gram. Ensure the needle penetrates through the muscle wall and ideally deposits the vaccine in the peritoneal cavity where the visceral fat is located. For example, in barramundi (*Lates calcarifer*) and tilapia (*Oreochromis spp*) of 15 gram this is between and just before the tip of the pelvic fins. This is to ensure the needle does not penetrate important internal organs such as the liver, stomach and spleen.
Shake the vaccine well before use. Sterile injection equipment should be used. For injection, fish should be anaesthetised.
Food should be withheld for a period of 1 day prior to vaccination. Avoid stress in the period prior to and after vaccination.

CONTRA-INDICATIONS
None known.

WITHDRAWAL PERIOD
Zero days.

SPECIAL STORAGE CONDITIONS
Store at 2 - 8°C. Protect from light. Do not freeze.

SPECIAL WARNINGS
Personal protective equipment consisting of e.g. needle protector should be used when handling the product. In case of accidental self-injection, seek medical advice immediately and show the package insert or label to the physician.
To the user:
This product contains oil. Accidental injection/self injection may result in severe pain and swelling, particularly if injected into a joint or finger, and in rare cases could result in the loss of the affected finger if prompt medical attention is not given.
If you are accidentally injected with this product, seek prompt medical advice even if only a very small amount is injected and take the package leaflet with you.
If pain persists for more than 12 hours after medical examination, seek medical advice again.
To the physician:
This product contains oil. Even if small amounts have been injected, accidental injection with this product can cause intense swelling, which may, for example, result in ischaemic necrosis and even the loss of a digit. Expert, PROMPT, surgical attention is required and may necessitate early incision and irrigation of the injected area, especially where there is involvement of finger pulp or tendon.

The vaccine has been tested for safety and efficacy in Tilapia *(Oreochromis sp.)* as a representative species. The vaccine may be used in other fish species. However, if so, its use should be undertaken with care and it is advisable to test the vaccine on a small number of fish prior to mass vaccination.

OTHER INFORMATION
For animal treatment only.

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