DEVELOPMENT OF GALTS AND ROLE OF M CELLS TOWARDS FEED-BASED VACCINE AGAINST Streptococcus iniae INFECTION IN RED HYBRID TILAPIA (Oreochromis sp.)

MOHAMMAD HAYAT

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By

MOHAMMAD HAYAT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

November 2019

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DEDICATION

To my family who made my professional progress possible;
To my instructors, teachers, mentors who made it a reality;
And to the fellow researchers who may be able to use the results of this research, I dedicate my work to all with
Love and gratitude

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Veterinary Science

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Chairman : Associate Professor Md Sabri bin Mohd Yusoff, DVM, PhD
Faculty : Veterinary Medicine

This study was conducted to determine the IgM antibodies, development of gut-associated lymphoid tissues (GALTs) and M cell's role due to streptococcosis in red hybrid tilapia following oral administration of feed based formalin killed vaccine (FKV) of *S. iniae* and challenged with live *S. iniae*. Three hundred red hybrid tilapia fish of 80 ± 10 g were divided into 6 major groups (1A, 1B, 2A, 2B, 3A, 3B). Each group consisted of 50 Red hybrid tilapia fish, kept in duplicate 2000 L glass aquaria. At week 1, all fish from the groups 1A,1B, 2A, 2B were started to feed with the feed-based FKV of *S. iniae* for different weeks and boosters dose were administered to group 2B only on day 14 and 21. Groups 3A, 3B were kept as negative (unchallenged) and positive (challenged) control groups respectively without vaccination and fed with normal commercial pellet (Cargill) throughout the experiment. At week 4, all the remaining tilapia from each groups except 3A were challenged intraperitoneally (i.p.) by injecting 0.5mL of the inoculums containing 1×10^6 CFU/mL of live *S. iniae*. Fish from each groups (except 3A) were sacrificed on a weekly basis for the entire 6 weeks for the collection of serum, body surface mucus and gut lavage fluid for the evaluation of antibody responses by indirect ELISA. Upon i.p. challenge, clinical signs were observed in the vaccinated groups 1A,1B, 2A, 2B and positive control group 3B. The clinical signs included exophthalmia, body discoloration, lethargy, erratic swimming, and haemorrhagic eye. The groups 2A, 2B had the lowest mortality rate ranging from 45-30% compared to groups 1A, 1B with 80-55% and group 3B 100% respectively. Necropsy findings included nephritis, splenomegaly and haemorrhagic brain. In all vaccinated groups Red hybrid tilapia hindgut showed the presence of GALTs. The diameter of GALTs in groups 1A,1B were significantly lower ($p > 0.05$) compared to groups 2A, 2B whereas there was no observation of GALTs in group 3B. Results for humoral response, the groups 1A,1B, 2A and 2B had significant ($p < 0.05$) increasing levels of antibody as early as week 1, but absent in groups 3B. The serum IgM levels of groups 1A,1B showed similar pattern as those of groups 2A, 2B but remained lower

than the groups 2B. Group 2B was significantly higher ($p < 0.05$). Meanwhile, group 3B did not show any significant ($p > 0.05$) changes throughout the experiment and all fish died at week 5 after challenge. Similar findings were recorded for the mucus and gut lavage IgM antibody levels between the treatment and 3B group. For the IHC staining, group 3B recorded the highest antigen intensity, followed by groups 1A,1B with moderate intensity and lowest in groups 2A, 2B at 12 h, 24 h, 24 h, 48h and 72 h respectively. SEM results revealed in groups 1A,1B, intestinal M cells were relatively depressed and had an irregular apical surface, with darker, short, and uneven microvilli. In groups 2A, 2B, M cells increased in size, number and vertically projected as a result of frequent and booster feeding of FKV. There were inflammatory indications in the group 3B. Nevertheless, group 3A showed slightly upward projection of intestinal M cells with normal dome epithelium. In conclusion, the FKV had a better protection rate by stimulating the production of GALTs within the lamina propria of Red hybrid tilapia hindgut, as well eliciting humoral response following oral vaccination. The IHC and SEM findings showed the existence of antigens and M cells in the villous epithelium. GALTs and villous M cells in red hybrid tilapia are important biological component of the mucosal immunity and promotes the resistance to streptococcal pathogens.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PEMBENTUKAN TISU LIMFOID BERKAIT USUS (GALT) DAN PERANAN SEL M TERHADAP VAKSIN BERASASKAN MAKANAN KE ATAS JANGKITAN *Streptococcus iniae* PADA IKAN TILAPIA HIBRID MERAH (*Oreochromis* sp.)

Oleh

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Pengerusi : Profesor Madya Md Sabri Mohd Yusoff, DVM, PhD
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Kajian ini dijalankan untuk menentukan antibodi IgM, pembentukan "*gut-associated lymphoid tissues"* (GALTs) dan peranan sel M disebabkan oleh "*streptococcosis"* tilapia hibrid merah berikutan pemberian *formalin-killed vaccine* (FKV) berasaskan makanan melalui kaedah oral terhadap *S. iniae* dan dijangkitkan dengan *S. iniae* hidup. Tiga ratus ikan tilapia hibrid merah seberat 80 ± 10 g dibahagikan kepada 6 kumpulan utama (1A, 1B, 2A, 2B, 3A, 3B). Setiap kumpulan mengandungi 50 ikan tilapia Hibrid Merah, yang disimpan dalam 2000 L akuarium kaca secara duplikasi. Pada minggu 1, semua ikan dari kumpulan 1A, 1B, 2A dan 2B mula diberi makan dengan FKV berasaskan makanan oleh *S. iniae* untuk minggu yang berlainan dan dos penggalak hanya diberikan kepada kumpulan 2B pada hari ke-14 dan 21. Kumpulan 3A dan 3B disimpan sebagai kumpulan kawalan negatif (tidak dijangkit) dan positif (dijangkit) masing-masing tanpa vaksinasi dan diberi makan pelet komersil biasa (Cargill) sepanjang eksperimen. Pada minggu ke-4, semua baki tilapia dari setiap kumpulan kecuali kumpulan 3A dijangkitkan secara intraperitoneal (i.p.) dengan menyuntikkan 0.5mL inokulum yang mengandungi 1×10^6 CFU/mL daripada *S. iniae* hidup. Ikan dari setiap kumpulan (kecuali 3A) telah dikorbankan secara mingguan selama 6 minggu untuk pengumpulan serum, mukus permukaan badan dan air usus untuk penilaian respon antibodi melalui kaedah ELISA tidak langsung. Selepas i.p, tanda-tanda klinikal diperhatikan terhadap kumpulan-kumpulan yang di vaksin iaitu 1A, 1B, 2A, 2B dan kumpulan kawalan positif 3B. Tanda-tanda klinikal termasuk *exophthalmia*, perubahan warna tubuh, kelesuan, berenang tidak menentu dan perdarahan mata. Kumpulan 2A dan 2B mempunyai kadar kematian paling rendah antara 45-30% berbanding kumpulan 1A dan 1B dengan 80-55% dan kumpulan 3B 100% masing-masing. Antara penemuan nekropsi termasuklah nefritis, splenomegali dan perdarahan otak. Di dalam semua kumpulan yang di vaksinasi, *hindgut* tilapia hibrid merah menunjukkan kehadiran GALTs. Diameter GALT dalam kumpulan 1A

dan 1B adalah jauh lebih rendah ($p > 0.05$) berbanding kumpulan 2A dan 2B sedangkan tidak ada pembentukan GALT dalam kumpulan 3B. Keputusan untuk tindak balas humoral, kumpulan 1A, 1B, 2A dan 2B adalah signifikan ($p < 0.05$) terhadap peningkatan tahap antibodi seawal minggu 1, tetapi tidak dalam kumpulan 3B. Aras IgM serum kumpulan 1A dan 1B menunjukkan corak yang sama seperti kumpulan 2A dan 2B tetapi kekal rendah berbanding kumpulan 2B. Kumpulan 2B lebih tinggi ($p < 0.05$). Sementara itu, kumpulan 3B tidak menunjukkan sebarang perubahan ($p > 0.05$) yang signifikan sepanjang eksperimen dan semua ikan mati pada minggu 5 selepas diberi jangkitan. Penemuan yang sama direkodkan untuk antibodi IgM pada mucus dan air usus antara rawatan dan kumpulan 3B. Untuk pewarnaan IHC, kumpulan 3B mencatatkan intensiti antigen tertinggi, diikuti oleh kumpulan 1A dan 1B dengan intensiti sederhana dan intensiti terendah adalah kumpulan 2A dan 2B pada 12 jam, 24 jam, 24 jam, 48 jam dan 72 jam masing-masing. Keputusan SEM dalam kumpulan 1A dan 1B, menunjukkan sel M usus mengalami tekanan (*depressed*) dan mempunyai permukaan apikal yang tidak teratur, dengan mikrovilli yang lebih gelap, pendek, dan tidak rata. Dalam kumpulan 2A dan 2B, saiz dan bilangan sel M meningkat, akibat kekerapan pemberian dos penggalak FKV. Terdapat tanda-tanda keradangan dalam kumpulan 3B. Walau bagaimanapun, kumpulan 3A memperlihatkan sedikit peningkatan sel M pada usus dengan epitelium kubah (*dome epithelium*) biasa. Kesimpulannya, FKV memberi kadar perlindungan yang lebih baik dengan merangsang pengeluaran GALT dalam lamina propria dari hindgut tilapia hibrid Merah, serta menggalak tindak balas humoral selepas vaksinasi oral. Penemuan IHC dan SEM menunjukkan kewujudan antigen dan sel M dalam epitelium villus. GALT dan villus M dalam tilapia hibrid merah adalah komponen biologi yang penting dalam imuniti mukosa dan menggalakkan penentangan terhadap patogen *streptococcus.*

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science . The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

CFSPH	Center for Food Security and Public Health
Fc	Fragment Crystallizable
MS-222	Tricaine Methanesulfonate
AICD	Activation-Induced Cytidine Deaminase
FAV	Feed-based Adjuvant Vaccine
ASC	Antibody-Secreting Cells
PBS	Phosphate Buffered Saline
UEA	Ulex Europaeus Agglutinin
WGA	Wheat Germ Agglutinin
IPNV	Intubation of Pancreas Necrosis Virus
GP2	Glycoprotein 2
ELISA	Enzyme-Linked Immunosorbent Assay
FKV	Feed-based Killed Vaccine
Cps	Capsular Polysaccharide Phosphotransferase
bp	Base Pair
ng	Nano Gram
Pg	Picogram
RBC	Red Blood Cells
CFU	Colony Forming Units
Igs	Immunoglobulins
BHIB	Brain Heart Infusion Broth
RAPD	Random Amplified Polymorphic DNA
LFTS	Lateral Flow Test Strip
Rpm	Round Per Minute
PBST	Phosphate Buffered Saline + Tween 20

DMSO	Dimethyl Sulphoxide
OCT	Optimum Cutting Temperature
BSA	Bovine Serum Albumin
HRP	Horseradish Peroxidase
DPX	Dibutylphthalate Polystyrene Xylene
CPD	Critical Point Dryer
IHC	Immunohistochemistry
Ag	Antigen
SIS	Small Intestinal Samples
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of Variance
PP	Peyer's Patches
Srt A	Sortase A
Glutamine	GLn
GAS	Group A Streptococcus
MALTs	Mucosal Associated Lymphoid Tissues
GALT	Gut-Associated Lymphoid Tissue
SALT	Skin-Associated Lymphoid Tissue
GIALT	Gill-Associated Lymphoid Tissue
NALT	Nasopharynx-Associated Lymphoid Tissue
ASC	Antibody Secreting Cells
Igs	Immunoglobullins
IEL	Intestinal Epithelial Lymphoid Cells
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
SLS	Streptolysin S

Kb	Kilobyte
KDa	Kilodaltons
rSrr	Recombinant protein Serine-Rich Repeat
GST	Glutathione-S-Transferase
IL8	Interleukin 8
MHC	Major Histocompatibility Complex
AICD	Activation Induced Cytidine Deaminase
IPNV	Intubation of Pancreas Necrosis Virus
CPS	Capsular Polysaccharide
LD 50	Lethal Dose 50
PCR	Polymerase Chain Reaction
REP-PCR	Repetitive Extragenic Palindromic Polymerase Chain Reaction
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
HSP70	Heat Shock Protein 70

CHAPTER 1

INTRODUCTION

1.1 Background of study

With the expanding fish aquaculture in Southeast Asia, Tilapia, *Oreochromis* sp. has become an important fish cultured in the region (Hassan et al., 2013). Tilapia characterized by certain features that make it important to the aquaculture industry. This includes the capacity to grow under captive environment and varying water condition such as alkaline pH (5-9), low dissolved oxygen and ammonia levels of <2mg/L and 50 mg/L, respectively (Morrison et al., 2006).

About 90% of the total tilapia produced in Malaysia is the Red hybrid tilapia, which is mostly cultured under pen system, ponds, tanks, and cages (Hamzah et al., 2007). However, despite the hardy nature of red hybrid tilapia, there are several bacterial diseases could induce septicaemia in the species, especially in the immunocompromised state (Hassan et al., 2013).

Streptococcus iniae is an important pathogen in aquaculture causing the disease; streptococcosis (Suanyuk et al., 2010). The bacteria pathogen; *S. iniae*, is a sphere-shaped, Gram positive cocci, a catalase-negative and haemolysin positive bacterium (Rattanachaikunsopon and Phumkhachorn, 2010). Streptococcosis induces vast economic loss in the aquaculture industry, affecting many marine and freshwater fish species such as hybrid striped bass (Shoemaker et al., 2001), European sea bass (Kvitt et al., 2004), rainbow trout (Lahav et al., 2004), channel catfish (Shoemaker et al., 2001), rabbitfish (Yuasa et al., 1999), and Japanese flounder (Nguyen et al., 2002). Additionally, the bacterium is of public health significance and recently described as an emerging zoonotic pathogen (Pretto-Giordano, 2015).

Streptococcal infection and its clinical signs differ from one fish species to another. For instance, diseased Red hybrid tilapia shows signs of exophthalmia, haemorrhage, ascites, abdominal swelling, and lesions of the internal organs (Austin and Austin, 2008). High mortalities often result from meningoencephalitis and generalized septicaemia, which are responsible for the massive financial victims worldwide (Facklam et al., 2005). Generally, the effective ways of controlling *S. iniae* infection is by immunisation and use of antimicrobial agents (Rahmatullah et al., 2017).

In fish, mucosa-associated lymphoid tissues (MALTs) are the first line of defence against diseases and vital for vaccine development (Salinas et al., 2011). There have been improvements in the formulation of fish vaccines in recent times; nevertheless, there is a need to design novel vaccines for stimulating both systemic and mucosal immune responses. Among the various vaccination routes, oral vaccines are relatively

more cost-effective, practical, less stressful to fish, and require the least of manpower and facilities (Holten-Andersen et al., 2012).

To assess the success of immune response following vaccination, analysis of gene expression, post-challenge mortality or pathogen load size, and measure of specific antibodies against pathogens in systemic circulation are key indicators. However, only a few research has been conducted to study the efficacies of various vaccines formulations against streptococcal infections in fish, as well as the specific antibody responses (Shoemaker et al. 2006). Also, gut-associated lymphoid tissue (GALT) is an integral portion of mucosal-associated lymphoid tissue (MALT) and the first line of defence in the protection of the gut epithelium against offending agents (Vazquez-Juarez et al., 2003). In fish, various authors have observed the role of GALT in the stimulation of mucosal immunity (Firdaus et al., 2011) and humoral response associated with oral vaccines (Nur-Nazifah et al.,2014).

Furthermore, the mechanisms involved in antigen uptake following oral vaccines have been well reported in other mammals. As described Lokka and Koppang (2016), the uptake and transportation of various antigens from the gut to lymphoid tissues are predominantly carried out by specialized cells in the follicle associated epithelium of intestinal Peyer's patches known as intestinal microfold cells (M cells). (Corr, Gahan, and Hill 2018). Fish were recognized to lack secretory IgA, tonsils and Peyer's patches, which are a vital component of mucosal immune response (Kaattari and Piganelli, 1996). Nevertheless, recent findings indicated the presence of specific cells adjacent to enterocytes with similar morphology to immature mammalian M cells in the hindgut of trout (Fuglem et al., 2010). Therefore, such cells might be important in the uptake of antigens present in vaccines aimed at eliciting a strong mucosal immune response (Rombout et al., 2014). Research in these areas is pertinent for the developing effective mucosal vaccines for the protection against streptococcosis in fish.

Both local and systemic immune response could be triggered if antigen eventually reaches the end gut segment insufficient amount (Lokka et al., 2014). For a better understanding of the mechanisms of action of oral vaccines, it is pertinent to investigate antigen uptake, delivery and how they bring about immune responses in fish (Rombout et al., 2014). Specifically, M cells are specific cells in the follicle associated epithelium (FAE) of intestinal Peyer's patches and are responsible for the transportation of antigens (Ag) from the gut to lymphoid tissues (Lokka and Koppang, 2016). Evidence from available literature shows that intestinal epithelial cells, M cells could be involved in antigen uptake and activation of mucosal immunity in fish (Rombout et al., 2011). For pathogens such as *S. iniae* to penetrate the intestinal epithelium and establish infection in fish, mucosal defences need to be evaded. However, oral vaccines containing the antigen could enhance the local and systemic immune response if taken up and transported to the hindgut or mid-segment (Rombout et al., 2014).

1.2 Problems statement

The effects of FKV vaccination against *S. iniae* infections for the development of GALTs, M cells stimulation and humoral immune responses is still unknown. The actual role of M cells is conflicting in Red hybrid tilapia. M cells detection in microbial challenge within Red hybrid tilapia needs special techniques due to lack of well-defined ultrastructural and histochemical markers. In solving and filling out this mysterious markers, we need vaccination as an antigenic substance to drive and boost up the humoral response, GALTs and role of M cells within the dome epithelium (FAE). However, development of GALTs and M cells formation raises question. How can aggregation of lymphoid cells have modified into M cells by FKV. GALTs phenotypically remains a matter of speculations in Red hybrid tilapia against streptococcosis. Only the humoral immune response is not possible to live against streptococcus infections to which mostly fish species are being exposed on daily basis. Because of such problems it would be demand of time to boost up specific immunity at the site of streptococcus infections. For a potential immunological system, a feed based FKV vaccine mechanism would provide valuable information for the development of GALTs and role of M cells in the recognition or trans-epithelial transport of antigens.

1.3 Justification of the study

Streptococcosis is the most important bacterial pathogen in Red hybrid tilapia culture. However, the interaction of *S. iniae* with the Red hybrid tilapia host is still not fully understood. In humans, streptococcus is thought to evade tissue by handling of infected fish and of eating other food animals. This emphasizes the need for a comparative investigation of the *S. iniae* in the Red hybrid tilapia whose defence system has also completely evolved with substituents of both adaptive and innate immunity. In addition, the persistence of streptococcosis in Red hybrid tilapia has been observed in aquaculture and the *S. iniae* bacteria may be involved.

1.4 Objectives of the study

The specific objectives set for this study were:

1. to investigate the effects of feed-based formalin killed oral vaccine of *S. iniae* on stimulation of antibody level and gut-associated lymphoid tissues (GALTs) in Red hybrid tilapia.
2. to evaluate the efficacy of M cell's role due to streptococcosis and feed-based formalin killed oral vaccines of *S. iniae* in Red hybrid tilapia.

1.5 Hypothesis

1. **Null:** Feed based formalin-killed oral vaccine of *S. iniae* do not stimulate the antibody level, gut-associated lymphoid tissues (GALTs) and M cells immune response in the intestine of Red hybrid tilapia towards the streptococcosis.
2. **Alternate:** Feed based formalin-killed oral vaccine of *S. iniae* stimulate the antibody level, gut-associated lymphoid tissues (GALTs) and M cells immune response in the intestine of Red hybrid tilapia towards the streptococcosis.

1.6 Significance of the study

The findings of this study will explain the pathogenesis of *S. iniae* infection in Red hybrid tilapia. Consequently, it provides an additional basis for testing the most effective strategy for prevention and adoption of better measures in the control of disease by *S. iniae* in aquaculture.

REFERENCES

Aamri F El, Padilla D, Acosta F, Caballero MJ, Roo J, et al. (2010) First report of Streptococcus iniae in red porgy (Pagrus pagrus, L.) J Fish Dis 33: 901-905

Abdelsalam, M., Elgendy, M. Y., Shaalan, M., Moustafa, M., & Fujino, M. (2017). Rapid identification of pathogenic streptococci isolated from moribund red tilapia (Oreochromis spp.). Acta Vet Hung, 65(1), 50-59. doi: 10.1556/004.2017.005

Abdullah, Syuhaidah et al. 2013. *Clinicopathological Features and Immunohistochemical Detection of Antigens in Acute Experimental Streptococcus Agalactiae Infection in Red Tilapia (Oreochromis Spp .)*

Adel, Milad et al. 2015. "Experimental Study to Evaluate the Pathogenicity of Streptococcus Iniae in Guppy (Poecilia Reticulata)." *International Journal of Aquatic Biology Journal homepage: www.NPAJournals.com* 3(2): 68–71.

Adomako M., Y. Zheng, J. Eley, R. D. Marcum, W. Sealey, and B. C. Donahower, "Oral DNA vaccination of rainbow trout , Oncorhynchus mykiss (Walbaum), against infectious haematopoietic necrosis virus using PLGA [Poly (D , L-Lactic-Co-Glycolic Acid)] nanoparticles," pp. 203–214, 2012.

Agricultural Research Service (ARS), United States Department of Agriculture (USDA) (2010). "New Streptococcal Vaccine. research/serialnum"10807575.

Agnew W, Barnes AC. Streptococcus iniae: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. Vet Microbiol 2007; 122:1–15

Ahmed N, Garnett S T. 2011. Integrated rice-fish farming in Bangladesh: Meeting the challenges of food security. Food Sec, 3(1): 81–92.

Ahmad N, Zander K K, Garnett S T. 2011. Socioeconomic aspects of rice-fish farming in Bangladesh: Opportunities, challenges and production efficiency. Austr J Agric Res Econ, 55: 199–219.

Al-Harbi AH (2011) Molecular characterization of Streptococcus iniae isolated from hybrid tilapia (Oreochromis niloticus × Ore- ochromis aureus). Aquaculture 312:15–18.

A. H. A. T and N. Uddin, "Bacterial diversity of tilapia (Oreochromis niloticus) cultured in brackish water in Saudi Arabia," vol. 250, pp. 566–572, 2005.

Amal, M. N. A., M. Zamri-Saad, A. Siti-Zahrah, R. Zulkafli, S. Misri, M. Nur-Nazifah, and H. Shahidan. 2010. Prevalence of Streptococcus aga- lactiae in tilapia kept in different water bodies. Online Journal Veterinary Researcher [online serial] 11:153–162.

Anon. (2004). Electronic Newsletter, Fish Health Section/Asian Fisheries Society Issue No. 3, January/February 2004 (pp. 1-3).

Anshary, H., R. A. Kurniawan, S. Sriwulan, R. Ramli, and D. V. Baxa. 2014. Isolation and molecular identification of the etiological agents of streptococcosis in Nile Tilapia (Oreochromis niloticus) cultured in net cages in Lake Sentani, Papua, Indonesia. Springerplus 3:627.

Austin B., Austin D.A. (2008). Bacterial fish pathogens, diseases of farmed and wild fish. 3nd. Chichester, UK, Springer Praxis Publishing. 648 p.

Aviles, F., Zhang, M. M., Chan, J., Delamare-Deboutteville, J., Green, T. J., Dang, C., & Barnes, A. C. (2013). The conserved surface M-protein SiMA of Streptococcus iniae is not effective as a cross-protective vaccine against differing capsular serotypes in farmed fish. Vet Microbiol, 162(1), 151-159. doi: 10.1016/j.vetmic.2012.08.018.

Azad, I. S., Shankar, K. M., Mohan, C. V., & Kalita, B. (2000). Uptake and processing of biofilm and free-cell vaccines of Aeromonas hydrophila in indian major carps and common carp following oral vaccination--antigen localization by a monoclonal antibody. Dis Aquat Organ, 43(2), 103-108. doi: 10.3354/dao043103.

Baiano, J. C. F., and A. C. Barnes. 2009. Towards control of Streptococcus iniae. Emerging Infectious Diseases 15:1891–1896.

Baiano, J. C., Tumbol, R. A., Umapathy, A. & Barnes, A. C. (2008). Identification and molecular characterisation of a fibrinogen binding protein from Streptococcus iniae. BMC Microbiol 8, 67.

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

Bockman, D.E., Cooper, M.D., 1971. Fine structural analysis of pinocytosis in lymphoid follicle-associated epithelium in chick bursa and rabbit appendix. Fed. Proc. Fed. Am. Soc. Exp. Biol. 30, 511.

Bolotin, S., Fuller, J. D., Bast, D. J., & de Azavedo, J. C. (2007). The two-component system sivS/R regulates virulence in Streptococcus iniae. FEMS Immunol Med Microbiol, 51(3), 547-554.

Bolotin, S., Fuller, J. D., Bast, D. J., Beveridge, T. J., & de Azavedo, J. C. (2017). Capsule expression regulated by a two-component signal transduction system in Streptococcus iniae. FEMS Immunol Med Microbiol, 50(3), 366-374. doi: FIM261.

Borghesi, C., Taussig, M.J., Nicoletti, C. (1999). Rapid appearance of M cells after microbial challenge is restricted at the periphery of the follicle-associated epithelium of Peyer's patch. Lab. Investig. 79, 1393e1401.

Brayden, D. J., Jepson, M. A. & Baird, A.W (2005). Keynote review: intestinal Peyer's patch M cells and oral vaccine targeting. Drug Discov. Today, 10(17):1145-57.

Buchanan, J. T., Colvin, K. M., Vicknair, M. R., Patel, S. K., Timmer, A. M., & Nizet, V. (2008). Strain-associated virulence factors of Streptococcus iniae in hybrid-striped bass. Vet Microbiol, 131(1-2), 145-153.

Buchanan, J."Drug policy under New Labour 1997-2010 : Prolonging the war on drugs Introduction : The context for change," vol. 57, no. 3, pp. 250–262, 2010.

Carvalho, Plpf, Yamamoto, F. Y., Barros, M. M., & Gatlin Iii, D. M. (2018). l-glutamine in vitro supplementation enhances Nile tilapia Oreochromis niloticus (Linnaeus, 1758) leukocyte function. Fish Shellfish Immunol, 80, 592-599. doi: S1050-4648(18)30384-X.

Casteleyn, Christophe et al. 2013. "M Cell Specific Markers in Man and Domestic Animals: Valuable Tools in Vaccine Development." *Comparative Immunology, Microbiology and Infectious Diseases* 36(4): 353–64. http://dx.doi.org/10.1016/j.cimid.2013.03.002.

CFSPH (2005). Streptococcosis, disease data sheet. Ames, IA: Center for Food Security and Public Health. http://www.cfsph.iastate.edu/ Factsheets/pdfs/streptococcosis.

Cheng, S., Zhang, W. W., Zhang, M., & Sun, L. (2010). Evaluation of the vaccine potential of a cytotoxic protease and a protective immunogen from a pathogenic Vibrio harveyi strain. Vaccine, 28(4), 1041-1047.

Cheng, K. A. Demirci, J.M. Catchmark, Advances in biofilm reactors for pro- duction of value-added products, Appl. Microbiol. Biotechnol. 87 (2010) 445e456.

Chen, J. W.H. Garza, M. Choquette, J. Hawkins, A. Hoeper, D.I. Bernstein, M.B. Cohen, Safety and immunogenicity of escalating dosages of a single oral administration of Peru-15 pCTB, a candidate live, attenuated vaccine against enterotoxigenic Escherichia coli and Vibrio cholerae, Clin. Vaccine Immunol. 22 (1) (2015) 129–135.

Chettri, J. K. *et al.* Comparative evaluation of administration methods for a vaccine protecting rainbow trout against Yersinia ruckeri O1 biotype 2 infections. *Vet. Immunol. Immunopathol.* 154, 42–47 (2013).

Chettri, J. K. *et al.* Comparative evaluation of infection methods and environmental factors on challenge success: Aeromonas salmonicida infection in vaccinated rainbow trout. *Fish Shellfish Immunol.* 44, 485–495 (2015).

Corr SC, Hill C & Gahan CGM (2006) An in vitro cell-culture model demonstrates internalin- and hemolysin-independent translocation of Listeria monocytogenes across M-cells. Microb Pathog 41: 241–250.

Corr, C.S., Cormac C.G.M. Gahan, & Colin Hill. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol Med Microbiol 52 (2008) 2–1

Corr, Sinead C, Cormac C G M Gahan, and Colin Hill. 2018. "M-Cells : Origin , Morphology and Role in Mucosal Immunity and Microbial Pathogenesis." 52(July): 2–12.

Corr S. C.,. Gahan C. C. G. M, and Hill, C. "M-cells : origin , morphology and role in mucosal immunity and microbial pathogenesis," vol. 52, no. July, pp. 2–12, 2018.

Costa-Pierce, B.A., 2003. Rapid evolution of an established feral tilapia (Oreochromis spp.): the need to incorporate invasion science into regulatory structures. Biol. Invasions 5 (1–2), 71–84.

Debard, N., Sierro, F., Browning, J., & Kraehenbuhl, J. P. (2001). Effect of mature lymphocytes and lymphotoxin on the development of the follicle-associated epithelium and M cells in mouse Peyer's patches. Gastroenterology, 120(5), 1173-1182. doi: 10.1053/gast.2001.22476.

DesRieux A, Ragnarsson EG, Gulberg E, Preat V, Schneider YJ & Artursson P (2005) Transport of nanoparticles across an in vitro model of the human intestinal follicle associated epithelium. Eur J Pharm Sci 25: 455–465.

Dickerson, Harry Wilson. 2018. "Cutaneous Antibody-Secreting Cells and B Cells in a Teleost Fish." (February 2008).

Dodson, S., Maurer, J., and Shotts, E. 1999. Biochemical and molecular typing of Streptococcus iniae isolated from fish and human cases. J. Fish Dis. 22, 331–336.

D. Parra, F. E. Reyes-lopez, and L. Tort, "Mucosal immunity and B cells in teleosts : effect of vaccination," vol. 6, no. July, pp. 1–12, 2015.

Eldridge J.H., C.J. Hammond, J.A. Meulbroek, J.K. Staas, R.M. Gilley, T.R. Tice, Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches, J. Contr. Release 11 (1990) 205–214.

El-Sayed, A.F.M. (2006). Tilapia Culture. Cambridge, USA: CABI Publishing.

Embregts, Carmen W E, and Maria Forlenza. 2016. "Oral Vaccination of Fish: Successes, Challenges and Future Perspectives." *Developmental and Comparative Immunology*. http://dx.doi.org/10.1016/j.dci.2016.03.024.

Evans, J. J., Klesius, P. H., & Shoemaker, C. A. (2006). An overview of Streptococcus in warmwater fish. Aquaculture Health International, 7, 10–14.

Evans J.J., Klesius P.H., Gilbert P.M., Shoemaker C.A., Al Sarawi M.A., Landsberg J., Duremdez R., Al Markouk A. & Al Kenzi S. (2002) Characterization of b-haemo- lytic group B Streptococcus agalactiae in cultured sea bream, Sparus auratus L., and wild mullet, Liza klunzin- geri (Day), in Kuwait. Journal of Fish Diseases 25, 505– 513.

Facklam, R., Elliott, J., Shewmaker, L., Reingold, A., 2005. Identi- fication and characterization of sporadic isolates of Streptococ- cus iniae isolated from humans. J. Clin. Microbiol. 43, 933–937.

F. Biology, V. Science, and A. Veterinary, "Elimination of foreign material by epidermal malpighian cells during wound healing in fish skin," no. 953, pp. 953–966, 2001.

Facklam, C. Ostach, B.M.Willey, A. Borczyk, and D.E. Low. 1997. "Invasive infections due to a fish pathogen, Streptococcus iniae." The New England Journal of Medicine, August 28, 337(9): 589–94.

Fawzy, N. M., Osman, K., Ibrahim, M. E.-D. E.-S., Naguib, M., Ali, M., & Abd-Elrahman, S. (2014). Streptococcosis in tilapia: Clinico-pathological picture of experimentally infected tilapia. Life Science Journal, 11, 1005–112.

Fan, W et al. 2017. "Performance of the Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry System for Rapid Identification of Streptococci : A Review." (99).

Fan W., Qin T., R. Bi, H. Kang, P. Ma, and B. Gu, "Performance of the matrix-assisted laser desorption ionization time-of-flight mass spectrometry system for rapid identification of streptococci : a review," no. 99, 2017.

Faria AMC, Weiner HL. Oral tolerance. Immunol Rev 2005; 206:232-59.

Figueiredo HCP, Carneiro DO, Faria FC, Costa GM (2006) Streptococcus agalactie associated to meningoencefalitis and systemic infection from tilapia (Oreochromis niloticus) in Brazil. Arquivo Brasileiro Medicina Veterinária e Zootecnia 58: 1678-1680.

Firdaus-Nawi, M., Noraini, O., Sabri, M. Y., SitiZahrah, A., Zamri-Saad, M., & Latifah, H. (2011). The effects of oral vaccination of Streptococcus agalactiae on stimulating gutassociated lymphoid tissues (galts) in tilapia (Oreochromis spp.). Pertanika Journal of Tropical Agricultural Science, 34(1), 137–143.

Firdaus-Nawi, M., Yusoff, S. M., Yusof, H., Abdullah, S. Z. & Zamri-Saad, M. Efficacy of feed-based adjuvant vaccine against Streptococcus agalactiae in Oreochromis spp. in Malaysia. *Aquac. Res.* 45, 87–96 (2013).

Flajnik, M. All GOD's creatures got dedicated mucosal immunity. Nat. Immunol. 2010, 11, 777–779.

Fletcher T.C. & White A. (1973) Antibody production in the plaice (Pleuronectes platessa L.) after oral and parenteral immunization with Vibrio anguillarum antigens. Aquaculture 1, 417–428.

Fuglem, B., Jirillo, E., Bjerkas, I., Kiyono, H., Nochi, T., Yuki, Y., . . . Koppang, E. O. (2010). Antigen-sampling cells in the salmonid intestinal epithelium. Dev Comp Immunol, 34(7), 768-774. doi: 10.1016/j.dci.2010.02.007.

Fuller, J. D., Camus, A. C., Duncan, C. L., Nizet, V., Bast, D. J., Thune, R. L., . . . De Azavedo, J. C. (2002). Identification of a streptolysin S-associated gene cluster and its role in the pathogenesis of Streptococcus iniae disease. Infect Immun, 70(10), 5730-5739. doi: 10.1128/iai.70.10.5730-5739.2002.

Fuglem, B., Jirillo, E., Bjerkas, I., Kiyono, H., Nochi, T., Yuki, Y., & Koppang, E. O. (2010). Antigen-sampling cells in the salmonid intestinal epithelium. Dev Comp Immunol, 34(7), 768-774. doi: 10.1016/j.dci.2010.02.007.

Gaiping, Zhang et al. 2016. "Morphological and Immunohistochemical Identification of Villous M Cells in the Small Intestine of Newborn Piglets." *International Journal of Morphology* 33(4): 1261–68.

Garcia J.C., Klesius P.H., Evans J.J. & Shoemaker C.A. (2008) Non-infectivity of cattle Streptococcus agalactiae in Nile tilapia, Oreochromis niloticus and channel cat- fish, Ictalurus punctatus. Aquaculture 281, 151–154. Grabowski.

Garvrieli Y, Sherman Y & Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labelling of nuclear DNA fragmentation. J Cell Biol 119: 493.

Gebert, A., Steinmetz, I., Fassbender, S., & Wendlandt, K. H. (2004). Antigen transport into Peyer's patches: increased uptake by constant numbers of M cells. Am J Pathol, 164(1), 65-72. doi: S0002-9440(10)63097-0

Gicheva, Nadezhda et al. 2016. "Biochemical and Biophysical Research Communications Siglec-F Is a Novel Intestinal M Cell Marker." *Biochemical and Biophysical Research Communications* 479(1): 1–4. http://dx.doi.org/10.1016/j.bbrc.2016.08.055.

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. Journal of Clinical Microbiology. 36(7): 2164-2166.

Goitsuka R, Chen CL, Benyon L, Asano Y, Kitamura D & Cooper MD (2007) Chicken cathelicidin-B1, an antimicrobial guardian at the mucosal M cell gateway. Proc Natl Acad Sci USA 104: 15063–15068.

Gomez, D., Sunyer, J. O., & Salinas, I. (2013). The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol, 35(6), 1729-1739. doi: 10.1016/j.fsi.2013.09.032.

Gonzalez-Hernandez, T. Liu, H.C. Payne, J.E. Stencel-Baerenwald, M. Ikizler, H. Yagita, et al., (2014) "Efficient norovirus and reovirus replication in the mouse intestine requires microfold (M) cells". J. Virol. 886934–6943.

Grove, S., Johansen, R., Reitan, L.J. and Press, C.M., 2006. Immune- and enzyme histochemical characterisation of leucocyte populations within lymphoid and mucosal tissues of Atlantic halibut (Hipoglossus hipoglossus). Fish Shellfish Immunol. 20: 693-708.

Grabowski, L. D., LaPatra, S. E. & Cain, K. D. Systemic and mucosal antibody response in tilapia, Oreochromis niloticus (L.), following immunization with Flavobacterium columnare. *J. Fish Dis.* 27, 573–581 (2004).

Gupta PN, Vyas SP. Investigation of lectinized liposomes as M-cell targeted carrier-adjuvant for mucosal immunization. Colloid Surface B 2011; 82:118-25. 63.

Guo, H., Miao, Q., Zhu, J., Yang, Z., & Liu, G. (2018). Isolation and molecular characterization of a virulent systemic feline calicivirus isolated in China. Infect Genet Evol, 65, 425-429. doi: S1567-1348(18)30669-5.

Hassan,B. M. El-salhia, A. Khalifa, H. Assem, A. Al Basomy, and M. El-sayed, "Environmental isotonicity improves cold tolerance of Nile tilapia , Oreochromis niloticus , in Egypt," *Egypt. J. Aquat. Res.*, vol. 39, no. 1, pp. 59–65, 2013.

Heath, C., Gillen, C. M., Chrysanthopoulos, P., Walker, M. J., & Barnes, A. C. (2016). Streptococcus iniae cpsG alters capsular carbohydrate composition and is a cause of serotype switching in vaccinated fish. Vet Microbiol, 193, 116-124. doi: 10.1016/j.vetmic.2016.08.011.

Heath JP. Epithelial cell migration in the intestine. Cell Biol Int 1996;20:139–46.

Herbomel P., B. Thisse, and C. Thisse, "Mesenchyme and Developing Brain , Retina , and Epidermis through a M-CSF Receptor-Dependent Invasive Process," vol. 288, pp. 274–288, 2001,

Holten-Andersen, Lars, Inger Dalsgaard, Jørgen Nylén, Niels Lorenzen, and Kurt Buchmann. 2012. "Determining Vaccination Frequency in Farmed Rainbow Trout Using Vibrio Anguillarum O1 Specific Serum Antibody Measurements." *PLoS ONE* 7(11):1–6.

Hopkins, S. A., Niedergang, F., Corthesy-Theulaz, I. E. & Kraehenbuhl, J. P. (2000) Cell. Microbiol. 2, 59–68.

Howe. P.H. Schiffer, J. Zielinski, T. Wiehe, G.K. Laird, J.C. Marioni, O. Soylemez, F. Kondrashov, M. Leptin Structure and evolutionary history of a large family of NLR proteins in the zebrafish.

Huang, Ting et al. 2019. "Spatiotemporal Distribution of Streptococcus Agalactiae Attenuated Vaccine Strain YM001 in the Intestinal Tract of Tilapia and Its Effect on Mucosal Associated Immune Cells." *Fish and Shellfish Immunology* 87(January): 714–20. https://doi.org/10.1016/j.fsi.2019.02.003.

Huang, Hsing Yen et al. 2014. "Efficacy of a Formalin-Inactivated Vaccine against Streptococcus Iniae Infection in the Farmed Grouper Epinephelus Coioides by Intraperitoneal Immunization." *Vaccine* 32(51): 7014–20. http://dx.doi.org/10.1016/j.vaccine.2014.08.039.

Iger, Y. & Abraham, M. (1990). The process of skin healing in experimentally wounded carp. Journal of Fish Biology 36, 421–437.

Inami, M., Taverne-Thiele, A.J., Schrøder, M.B., et al., 2009. Immunological differences in intestine and rec- tum of Atlantic cod (Gadus morhua L.). Fish Shellfish Immunol 26: 751–9.

Islam, M. A. *et al*. Biomaterials M cell targeting engineered biomaterials for e ff ective vaccination. *Biomaterials* 192, 75–94 (2019).

Ismail, M. S., Syafiq, M. R. M. & Amal, M. N. A. Feed-based vaccination regime against streptococcosis in red tilapia , Oreochromis niloticus x Oreochromis mossambicus. *BMC Vet. Res.* 1–6 (2016). doi:10.1186/s12917-016-0834-1.

Jang, M. H., Kweon, M. N., Iwatani, K., Yamamoto, M., Terahara, K., Sasakawa, C. Kiyono, H. (2004). Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc Natl Acad Sci U S A, 101(16), 6110-6115. doi: 10.1073/pnas.0400969101.

Jensen VB, Harty JT & Jones BD (1998) Interaction of the invasive pathogens Salmonella typhimurium, Listeria monocytogenes, and Shigella flexneri with M-cells and murine Peyer's patches. Infect Immun 66: 3758–3766.

Kaattari, S., & Piganelli, J. D. (1996). The specific immune system: humoral defense. Fish Physiology, 15, 207-254

Kahieshesfandiari, M., Sabri, M. Y., Hassan, M. D. & Noraini, O. Streptococcosis in Oreochromis sp .: is feed-based biofilm vaccine of Streptococcus agalactiae effective ? 817–832 (2019).

Kamaruzzaman, B.N Subha, J. B. Maung, and B. Maung, "PERFORMANCE AND SURVIVAL OF THREE RED TILAPIA STRAINS (OREOCHROMIS SPP) IN POND ENVIRONMENT IN KEDAH STATE , MALAYSIA," pp. 199–211, 2008.

Kanaya T, Aso H, Miyazawa K et al. (2007) Staining patterns for actin and villin distinguish M-cells in bovine follicle- associated epithelium. Res Vet Sci 82: 141–149.

Kayansamruaj, P., H. T. Dong, V. V. Nguyen, H. D. Le, N. Pirarat, and C. Rodkhum. 2017. Susceptibility of freshwater rearing Asian Seabass (Lates calcarifer) to pathogenic Streptococcus iniae. Aquaculture Research 48:711–718.

Kerneis S, Bogdonova A, Kraehenbuhl JP & Pringault E (1997) Conversion by Peyer's patch lymphocytes ofhuman enterocytes into M-cells that transport bacteria. Science 277: 949–952.

Khimmakthong, U., Deshmukh, S., Chettri, J.K., Bojesen, A.M., Kania, P.W., Dalsgaard, I., Buchmann, K., 2013. Tissue specific uptake of inactivated and live Yersinia ruckeri in rainbow trout (Oncorhynchus mykiss): visualization by immunohistochemistry and in situ hybridization. Microb. Pathog. 59–60, 33–41.

Kim, C.H. Ra, Y.J.S.J. Park, C.W. Jeong, Y.K. Nam, K.H. (2009) "Evaluation of optimal culture conditions for recombinant ghost bacteria vaccine production with the antigen of Streptococcus iniae GAPDH," J. Microbiol. Biotechnol. 19 982e986.

Kim, S-H. et al. (2011) M cells expressing the complement C5a receptor are efficient targets for mucosal vaccine delivery. Eur. J. Immunol. 41, 3219–3229

Kim, S-H. et al. (2010) The M cell-targeting ligand promotes antigen delivery and induces antigen-specific immune responses in mucosal vaccination. J. Immunol. 185, 5787–5795

Kim, S.H.; Lee, K.Y.; Jang, Y.S. Mucosal immune system and M cell-targeting strategies for oral mucosal vaccination. Immune Netw. 2012, 12, 165–175.

Kim, Si Won et al. 2017. "Rapid MALDI Biotyper-Based Identification and Cluster Analysis of Streptococcus Iniae §.

Klesius, P. H., C. A. Shoemaker, and J. J. Evans. 2008. Streptococcus: a worldwide fish health problem. Pages 83–107 in Proceedings of the 8th International Symposium on Tilapia in Aquaculture. Central Laboratory for Aquaculture Research, Tall Al Kabir, Ismailia Governorate, Egypt.

Klesius, Phillip H, Craig A Shoemaker, and Joyce J Evans. 2000. "Efficacy of Single and Combined Streptococcus Iniae Isolate Vaccine Administered by Intraperitoneal and Intramuscular Routes in Tilapia ž Oreochromis Niloticus /." : 237–46.

Knoop, K. A.; Kumar, N.; Butler, B. R.; Sakthivel, S. K.; Taylor, R. T.; Nochi, T.; Akiba, H.; Yagita, H.; Kiyono, H. & Williams, I. R. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. J. Immunol., 183(9):5738-47, 2009

Koppang . *et al.*, "Journal of," pp. 728–739, 2010

Kraehenbuhl JP &Neutra MR (2000) Molecular and cellular basis ofimmune protection of mucosal surfaces. Physiol Rev 72: 853–879.

Kruger, By Dina, and Mariët Van Der Werf. 2018. "Ohly Application Note Benefits of Nucleotide Supplementation in Aquaculture : Fish." : 1–4.

Kumar, Jiwan et al. 2015. "Fish & Shell Fish Immunology Comparative Evaluation of Infection Methods and Environmental Factors on Challenge Success : Aeromonas

Kumar, J. S. Deshmukh, and L. Holten-andersen, "Comparative evaluation of administration methods for a vaccine protecting rainbow trout against Yersinia ruckeri O1 biotype 2 infections," *Vet. Immunol. Immunopathol.*, pp. 1–6, 2013.

Kucharzik, T, N Lügering, K Rautenberg, and A Lügering. 2011 "Role of M Cells in Intestinal Barrier Function." : 171–83

Kuolee, R., & Chen, W. (2008). M cell-targeted delivery of vaccines and therapeutics. Expert Opin Drug Deliv, 5(6), 693-702. doi: 10.1517/17425247.5.6.693

Kvitt, H., & Colorni, A. (2004). Strain variation and geographic endemism in Streptococcus iniae. Dis Aquat Organ, 61(1-2), 67-73. doi: 10.3354/dao061067

Kyd, J.M., Cripps, A.W., 2008. Functional differences between M cells and enterocytes in sampling luminal antigens. Vaccine 26, 6221e6224

Koh, C.-B., Romano, N., Zahrah, A.S. & Ng, W.-K. (2016) Effects of a dietary organic acids blend and oxytetracycline on the growth, nutrient utilization and total cultivable gut microbiota of the red hybrid tilapia, Oreochromis sp., and resistance to Streptococcus agalactiae. Aquaculture Research, 47, 357- 369

Lahav, D., Eyngor, M., Hurvitz, A., Ghittino, C., Lublin, A. and Eldar, A. 2004. Streptococcus iniae type II infections in rainbow trout (Oncorhynchus mykiss). Dis Aquat Organ. 62(1-2): 177-180.

Liang E, Kabcenell AK, Coleman JR, Robson J, Ruffles R & Yazdanian M (2001) Permeability measurement of macromolecules and assessment of mucosal antigen sampling using in vitro converted M cells. J Pharmacol Toxicol Methods 46: 93–101.

Liu S., M. Hu, Z. Wu, B. Wehner, A. Wiedensohler, Y. Cheng Aerosol number size distribution and new particle formation at a rural/coastal site in Pearl River Delta (PRD) of China ,Atmos. Environ., 42 (2008), pp. 6275-6283

Liu,T.
Lin,Hung, T.J. Liu, Chen, Y.C. Huang, T.H. Tsai, H.H. Wang, D.W. Wang,J.K .Wang, Y.LWang, C.H. LinA high speed detection platform based on surface-enhanced Raman scattering for monitoring antibiotic-induced chemical changes in bacteria cell wall PLoS One, 4 (2009), p. e5470

Liu, Haitian et al. 2017. "Fish & Shell Fi Sh Immunology Dietary Administration of Bacillus Subtilis HAINUP40 Enhances Growth , Digestive Enzyme Activities , Innate Immune Responses and Disease Resistance of Tilapia , Oreochromis Niloticus." *Fish and Shellfish Immunology* 60: 326–33. http://dx.doi.org/10.1016/j.fsi.2016.12.003.

Liu, H. X. Wu, X. Chang, Y. Tang, Q. Liu, Y. Zhang, Notable mucosal immune responses induced in the intestine of zebrafish (Danio rerio) bath-vaccinated with a live attenuated Vibrio anguillarum vaccine, Fish Shellfish Immunol. 40 (1) (2014) 99–108.

Lorenzen N. and Buchmann K., "Determining Vaccination Frequency in Farmed Rainbow Trout Using Vibrio anguillarum O1 Specific Serum Antibody Measurements," vol. 7, no. 11, pp. 1–6, 2012.

Locke, J. B., Aziz, R. K., Vicknair, M. R., Nizet, V., & Buchanan, J. T. (2008). Streptococcus iniae M-like protein contributes to virulence in fish and is a target for live attenuated vaccine development. PLoS One, 3(7), e2824. doi: 10.1371/journal.pone.0002824

Locke, J. B., Colvin, K. M., Varki, N., Vicknair, M. R., Nizet, V., & Buchanan, J. T. (2007). Streptococcus iniae beta-hemolysin streptolysin S is a virulence factor in fish infection. Dis Aquat Organ, 76(1), 17-26. doi: 10.3354/dao076017

Lorenzen, Niels and Kurt Buchmann. 2012. "Determining Vaccination Frequency in Farmed Rainbow Trout Using Vibrio Anguillarum O1 Specific Serum Antibody Measurements." 7(11):1–6.

Lokka, G., & Koppang, E. O. (2016). Antigen sampling in the fish intestine. Dev Comp Immunol, 64, 138-149. doi: 10.1016/j.dci.2016.02.014S0145-305X(16)30038-6

Lokka, G., Falk, K., Austbo, L., & Koppang, E. O. (2014a). Uptake of yeast cells in the Atlantic salmon (Salmo salar L.) intestine. Dev Comp Immunol, 47(1), 77-80. doi: 10.1016/j.dci.2014.07.005

Lokka, Guro, and Erling Olaf Koppang. 2016. "Antigen Sampling in the Fi Sh Intestine." 64.

Lokka, G., Austbø, L., Falk, K., Bromage, E., Fjelldal, P.G., Hansen, T., Hordvik, I., Koppang, E.O.,(2014b). Immune parameters in the intestine of wild and reared unvaccinated and vaccinated Atlantic salmon (Salmo salar L.). Dev. Comp. Immunol. 47, 6e16.

Lowe, Beth A et al. 2007. "Analysis of the Polysaccharide Capsule of the Systemic Pathogen Streptococcus Iniae and Its Implications in Virulence □." 75(3): 1255–64.

Low DE, Liu E, Fuller J, McGeer A. Streptococcus iniae: an emerging pathogen in the aquaculture industry. In: Scheld WM, Craig WA, Armstrong D, Hughes JM, editors. Emerging infections, vol. 3. Washington, DC: ASM Press; 1999. p. 53–65

Luo L., "Effects of dietary chitosan oligosaccharides and Bacillus coagulans on the growth , innate immunity and resistance of koi (Cyprinus carpio koi)," no. April 2012, 2017.

Mach, Julie et al. 2005. "Development of Intestinal M Cells." *Immunological Reviews* 206: 177–89.

Makesh, M.; Ponnerassery, S.S.; Cain, K.D. Systemic and mucosal immune response of rainbow trout to immunization with an attenuated Flavobacterium psychrophilum vaccine strain by different routes. Fish Shellfish Immunol. 2015, 44, 156–163

Mata AI, Gibello A, Casamayor A, Blanco MM, Dominguez L, Fernandez-Garayzabal JF (2004). Multiplex PCR assay for detection of bacterial pathogens associated with warm-water streptococcosis in fish. Appl. Environ. Microbial. 70:3183-3187

Menard, S. et al. (2010) Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol. 3, 247–259

Meynell, Helen M et al. 2018. "Up-Regulation of Microsphere Transport across the Follicle-Associated Epithelium of Peyer ' s Patch by Exposure to Streptococcus Pneumoniae R36a." : 611–19.

Miao Wang et al. 2014. "Roles of M Cells in Infection and Mucosal Vaccines." *Human Vaccines and Immunotherapeutics* 10(12): 3544–51

Miller, H.; Zhang, J.; Kuolee, R.; Patel, G. B. & Chen, W (2007). Intestinal M cells: the fallible sentinels? World J. Gastroenterol., 13(10):1477-86, 2007

Mishra, Anshuman et al. 2017. "Comparative Evaluation of 16S RRNA Gene in World-Wide Strains of Streptococcus Iniae and Streptococcus Parauberis for Early Diagnostic Marker." *Genes & Genomics* 39(7): 779–91.

Morrison, C.M., Fitzsimmons, K. and Wright J.R.Jr. 2006. Atlas of Tilapia Histology. World Aquaculture Society, 102 pp.

Mulero I, Sepulcre MP, Meseguer J, Garcia- Ayala A, Mulero V (2007) Histamine is stored in mast cells of most evolutionarily advanced fish and regulates the fish inflammatory response. *Proceedings of the National Academy of Sciences of the United States of America* 104(49): 19434– 19439.

Myoung, Ho et al. 2004. "Intestinal Villous M Cells : An Antigen Entry Site in the Mucosal Epithelium."

Nayak, Sukanta K. 2010. "Role of Gastrointestinal Microbiota in Fish." (Horsley 1973): 1553–73.

Neutra, M.R., Mantis, N.J., Kraehenbuhl, J.P., 2001. Collaboration of epithelial cells with organized mucosal lymphoid tissues. Nat. Immunol. 2, 1004e1009

Nicoletti C (2000) Unsolved mysteries of intestinal M-cells. Gut 47: 735–739

Nur-nazifah M., M. Y. Sabri, and A. Siti-zahrah, "Fish & Shell fi sh Immunology Development and ef fi cacy of feed-based recombinant vaccine encoding the cell wall surface anchor family protein of Streptococcus agalactiae against streptococcosis in Oreochromis sp .," *Fish Shellfish Immunol.*, vol. 37, no. 1, pp. 193–200, 2014.

Nguyen, H. T., Kanai, K., & Yoshikoshi, K. (2002). Ecological investigation of Streptococcus iniae isolated in cultured Japanese flounder, Paralicthys olivaceus using selective isolation procedure. Aquaculture, 205, 7-17.

Nizet, V., Beall, B., Bast, D.J., Datta, V., Kilburn, L., Low, D.E., and De Azavedo, J.C. (2000) Genetic locus for streptolysin S production by group A *Streptococcus*. *Infect Immun* 68: 4245–4254.

Noraini, O. *et al.* The effect of heat stress on clinicopathological changes and immunolocalization of antigens in experimental Streptococcus agalactiae infection in Red hybrid tilapia (Oreochromis spp .). 6, 997–1003 (2013).

Ohno, H., & Hase, K. (2010). Glycoprotein 2 (GP2): grabbing the FimH bacteria into M cells for mucosal immunity. Gut Microbes, 1(6), 407-410. doi: 10.4161/gmic.1.6.14078

Ohno, Hiroshi. 2016. "JB Special Review — Crosstalk between the Intestinal Immune System and Gut Commensal Microbiota Intestinal M Cells." 159(2): 151–60.

Ohtani M, Villumsen KR, Koppang EO, Raida MK. Global 3D imaging of Yersinia ruckeri bacterin uptake in rainbow trout fry. *PLoS ONE* (2015) 10:e0117263. doi: 10.1371/journal.pone.0117263

Ortega, C., Garcia, I., Irgang, R., Fajardo, R., Tapia-Cammas, D., Acosta, J., & Avendano-Herrera, R. (2018). First identification and characterization of Streptococcus iniae obtained from tilapia (Oreochromis aureus) farmed in Mexico. J Fish Dis, 41(5), 773-782. doi: 10.1111/jfd.12775

Owen, R.L., Jones, A.L., 1974. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology 66, 189e203.

Owen R. L., "Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer ' s patches a personal and historical perspective," vol. 11, 1999.

Owen, R.L., Nemanic, P., 1978. Antigen processing structures of the mammalian intestinal tract: a SEM study of lymphoepithelial organs. Scanning Electron Microsc. 2, 367e377.

Palareti, G. et al. 2016. "Comparison between Different D-Dimer Cutoff Values to Assess the Individual Risk of Recurrent Venous Thromboembolism: Analysis of Results Obtained in the DULCIS Study." *International Journal of Laboratory Hematology* 38(1): 42–49.

Park, S. B., Kwon, K., Cha, I. S., Jang, H. B., Nho, S. W., Fagutao, F. F.,Jung, T. S. (2014). Development of a multiplex PCR assay to detect Edwardsiella tarda, Streptococcus parauberis, and Streptococcus iniae in olive flounder (Paralichthys olivaceus). J Vet Sci, 15(1), 163-166. doi: jvs.2013.091

Parra, David, Felipe E Reyes-lopez, and Lluis Tort. 2015. "Mucosal Immunity and B Cells in Teleosts : Effect of Vaccination." 6(July): 1–12.

Pappo J & Mahlman RT (1993) Follicle epithelial M-cells are a source of interleukin-1 in Peyer's patches. Immunology 78: 505–507.

Pereira, U. de P. *et al.* Complete genome sequence of Streptococcus agalactiae strain SA20-06, A fish pathogen associated to meningoencephalitis outbreaks. *Stand. Genomic Sci.* 8, 188–197 (2013)

Petrie, A. G., & Ellis, A. E. (2006). Evidence of particulate uptake by the gut of Atlantic salmon (Salmo salar L.). Fish Shellfish Immunol, 20(4), 660-664. doi: S1050-4648(05)00143-9

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

Phuong, N.M. and Hoa, T.T.T., 2016. Rapid detection of Streptococcus iniae in red tilapia tissue (Oreochromis sp.) by polymerase chain reaction. Can Tho University Journal of Science. Vol 2: 84-89.

Pontius, H. & Ambrosius, H. (1972). Beitrage zur Immunobiologie poikilothermer Wirbeltiere. IX Untersuchungen zur zellularen Grundlage humoraler Immunoreak- tionen der Knochenfische am Beispiel des Flussbarsches (PercaJluviatilis L.). Acta Biol. Med. Germ. 29,3 19-339.

Pretto-giordano L.G., J. A. Scarpassa, A. R. Barbosa, C. S. Altrão, and C. G. Gumiero, "Streptococcus iniae : An Unusual Important Pathogen Fish in Brazil," vol. 6, no. 9, pp. 9–11, 2015

Rahmatullah, M. Ariff, M. Kahieshesfandiari, H. M. Daud, M. Zamri-Saad, M. Y. Sabri, M. N. A. Amal & M. Y. Ina-Salwany (2017) Isolation and Pathogenicity of Streptococcus iniae in Cultured Red Hybrid Tilapia in Malaysia, Journal of Aquatic Animal Health, 29:4, 208-213,

Raida, M.K.; Buchmann, K. Bath vaccination of rainbow trout (Oncorhynchus mykiss Walbaum) against Yersinia ruckeri: Effects of temperature on protection and gene expression. Vaccine 2008, 26, 1050–1062

Rattanachaikunsopon, P. and Phumkhachorn, P. 2009. Prophylactic effect of Andrographis paniculata extracts against Streptococcus agalactiae infection in Nile tilapia (Oreochromis niloticus). Journal of Bioscience and Bioengineering 107 (5), 579–582.

Rattanachaikunsopon, P. and Phumkhachorn, P. 2010. Effect of Cratoxylum formosum on innate immune response and disease resistance against Streptococcus agalactiae in tilapia Oreochromis niloticus. Fisheries Science 76, 653-659.

Renfeng, L.; Xiangqin, T.; Songlin, Q.; Yanyan, Y.; Enmin, Z. & Gaiping, Z. Morphological and immunohistochemical identification of villous M cells in the small intestine of newborn piglets. Int. J. Morphol., 33(4):1261-1268, 2015.

Ringo E, Holzapfel W. Identification and characterization of carnobacteria associated with the gills of Atlantic salmon (Salmo salar L.). Systematic and Applied Microbiology. 2000; 23:523–527

Ringo, E., Olsen, R.E., Vecino, J.L., Wadsworth, S. and Song, S.K. (2012) Use of immunostimulants and nucleotides in aquaculture: a review. J marine Sci Res Development 1:104

Rodkhum, C., Pattanapon, K., Nopadon, P., Janenuj, W., 2012. Duplex PCR for similtaneous and unambiguous detection of Streptococcus iniae and *Stretptococcus agalactiae* associated with Streptococcocis of cultured tilapia in Thailand. Thai Journal of Veterinary Medicine. 42(2): 153-158

Roggenbuck D, Reinhold D, Schierack P, Bogdanos DP, Conrad K, Laass MW. Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges. Clinical chemistry and laboratory medicine :CCLM / FESCC 2014;52:483-94.

Rombout, J.H.W.M., Abelli, L., Picchietti, S., et al., 2011. Teleost intestinal immunology. Fish Shellfish Immunol 31: 616–26.

Rombout, Jan H.W.M., and Viswanath Kiron. 2014. "Mucosal Vaccination of Fish." *Fish Vaccination* 9780470674: 56–67.

Rombout J.H.W.M. & Van den Berg A.A. (1989) Immunological importance of the second gut segment of carp: I. Uptake and processing of antigens by epithelial cells and macrophages. Journal of Fish Biology 35, 13–22.

Rombout, J.H.W.M., Taverne. N., van de Kamp, M. and Taverne-Thiele, A.J. 1993. Differences in mucus and serum immunoglobulin of carp (Cyprinus carpio L.). Dev Comp Immunol 17: 309–17

Rombout, J. H., Yang, G., & Kiron, V. (2014). Adaptive immune responses at mucosal surfaces of teleost fish. Fish Shellfish Immunol, 40(2), 634-643. doi: 10.1016/j.fsi.2014.08.020

Salah, A. S., El Nahas, A. F., & Mahmoud, S. (2017). Modulatory effect of different doses of beta-1,3/1,6-glucan on the expression of antioxidant, inflammatory, stress and immune-related genes of *Oreochromis niloticus* challenged with Streptococcus iniae. Fish Shellfish Immunol, 70, 204-213. doi: S1050-4648(17)30519-3

Salinas, I., Zhang, Y. A., & Sunyer, J. O. (2011). Mucosal immunoglobulins and B cells of teleost fish. Developmental and Comparative Immunology, 35(12), 1346–1365.

Salinas, I. (2015). The Mucosal Immune System of Teleost Fish. Biology (Basel), 4(3), 525-539. doi: 10.3390/biology4030525

Salinas, I., Moseguer, J. and Esteban, M.A. (2007). Assessment of different protocols for the isolation and purification of gut associated lymphoid cells from the gilthead seabream (Sparus aurata L.). Biological Procedures Online, 9, 43-55.

Salmonicida Infection in Vaccinated Rainbow Trout." *Fish and Shellfish Immunology* 44(2): 485–95. http://dx.doi.org/10.1016/j.fsi.2015.03.003

Sansonetti, P.J., 2004. War and peace at mucosal surfaces. Nat. Rev. Immunol. 4, 953e964.

Sansonetti PJ & Phalipon A (1999) M-cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. Semin Immunol 11: 193–20

Schulz, Olga, and Oliver Pabst. 2013. "Antigen Sampling in the Small Intestine." *Trends in Immunology* 34(4): 155–61. http://dx.doi.org/10.1016/j.it.2012.09.006.

Shephard KL. 1994. Functions for fish mucus, in Reviews in Fish Biology and Fisheries, vol. 4, pp. 401-429.

Sepulcre M. P., A. García-ayala, and V. Mulero, "Histamine is stored in mast cells of most evolutionarily advanced fish and regulates the fish inflammatory response," pp. 1–6, 2007.

Sheng, X., Gao, J., Liu, H., Tang, X., Xing, J., & Zhan, W. (2018). Recombinant phosphoglucomutase and CAMP factor as potential subunit vaccine antigens induced high protection against *Streptococcus iniae* infection in flounder (Paralichthys olivaceus). J Appl Microbiol, 125(4), 997-1007. doi: 10.1111/jam.13948

Shelby, Richard A, Craig A Shoemaker, J Joyce, and Phillip H Klesius. 2009. "Development of an Indirect ELISA to Detect Humoral Response to Strep to Coc Cus Iniae Infection of Nile Tilapia , Oreochromis Niloticus." (December 2014): 37–41

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. Journal of Fish Diseases 26,1–6.

Shoemaker, C. A., D. Xu, J. J. Evans, and P. H. Klesius. 2006. Parasites and diseases. Pages 561–582 in C. Lim and C. D. Webster, editors. Tilapia biology, culture and nutrition. Haworth Press, New York.

Shoemaker CA, Vandenberg GW, Désormeaux A, Klesius PH, Evans JJ. Efficacy of a Streptococcus iniae modified bacterin delivered using OraljectTM technology in Nile tilapia (*Oreochromis niloticus*). Aquaculture 2006; 255:151–6

Shoemaker, C. A., P. H. Klesius, and J. J. Evans. 2001. Prevalence of *Streptococcus iniae* in tilapia, hybrid Striped Bass, and Channel Catfish on commercial fish farms in the United States. American Journal Veterinary Researcher 62:174–177

Sierro F, Pringault E, Assman PS, Kraehenbuhl JP & Debard N (2000) Transient expression of M-cell phenotype by enterocyte-like cells of the follicle-associated epithelium of mouse Peyer's patches. Gastroenterology 119: 734–743.

Sitjà-Bobadilla, A., Peña-Llopis, S., Gómez-Requeni, P., Médale, F., Kaushik, S. & Pérez-Sánchez, J. (2006) Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (Sparus aurata). Aquaculture, 249, 387-400

Siti-Zahrah A, Padilah B, Azila A, Rimatulhana R, Shahidan H. Multiple Streptococcal species infection in cage-cultured red tilapia, but showing similar clinical signs. In: Bondad-Reantaso Melba G, Mohan CV, Crumlish Margaret, Subasinghe Rohana P, editors. Proceedings of the sixth symposium on diseases in Asian aquaculture, 25e28th Oct 2005, Colombo, Sri Lanka; 2005. pp. 332e9.

Siti-Zahrah A., Misri S., Padilah B., Zulkafli R., Kua B.C., Azila A. & Rimatulhana R. (2004) Pre-disposing fac- tors associated with outbreak of Streptococcal infection in floating cage-cultured red tilapia in reservoirs. Abstracts of the 7th Asian Fisheries Forum 04, The Triennial Meeting of The Asian Fisheries Society 30th Nov–4th Dec 2004, Penang, Malaysia. pp. 129

Slack, J. M. (2000). Stem cells in epithelial tissues. *Science, 287*(5457), 1431-1433

Smith, P.D., MacDonald, T.T., Blumberg, R.R.S., 2013. Immune Function of Epithelial Cells, Principals of Mucosal Immunlogy, first ed. Garland Science, New York, pp. 55e68.

Suanyuk, N., Sukkasame, N., Tanmark, N., Yoshida, T., Itami, T., Thune, R.L., Tantikitti, C. and Supamattaya, K., "Streptococcus iniae infection in cultured Asian sea bass (Lates calcarifer) and red tilapia (Oreochromis sp.) in southern Thailand. Songklanakarin". Journal of Science and Technology. 2010, 32, 341-348

Sun, M. S. Hyuk, E. Hye, Y. Kwon, S. Koo, and K. Hong, "α -enolase , a plasminogen binding and cell wall associating protein from a fish pathogenic Streptococcus iniae strain," vol. 265, pp. 55–60, 2007.

Sun Y, Liu CS, Sun L (2011) A multivalent killed whole-cell vaccine induces effective protection against Edwardsiella tarda and Vibrio anguillarum. Fish Shellfish Immunol 31: 595–599.

Sun, Min et al. 2007. "α -Enolase , a Plasmin (Ogen) Binding and Cell Wall Associating Protein from a Fish Pathogenic Streptococcus Iniae Strain." 265: 55–60.

Sun Y.,Yang H., Ling Z., Chang J. & Ye J. (2009) Gut microbio- ta of fast and slow growing grouper Epinephelus coioides. AfricanJournal ofMicrobiology Research 3,713^720.

Swan, C. M., Lindstrom, N. M., & Cain, K. D. (2008). Identification of a localized mucosal immune response in rainbow trout, Oncorhynchus mykiss (Walbaum), following immunization with a protein-hapten antigen. J Fish Dis, 31(5), 383-393. doi: 10.1111/j.1365-2761.2008. 00918.x

Tabata Y, Inoue Y, Ikada Y. Size effect on systemic and mucosal immune responses induced by oral administration of biodegradable microspheres. Vaccine 1996 ; 14 (17-18): 1677 -85

Tacchi .L. *et al.*, "immune system of vertebrates," *Nat. Commun.*, no. May, 2014.

Tacchi, L. Musharrafieh, R.; Larragoite, E.T.; Crossey, K.; Erhardt, E.B.; Martin, S.A.M.; LaPatra, S.E.; Salinas, I. Nasal immunity is an ancient arm of the mucosal immune system of vertebrates. Nat. Commun. 2014, doi:10.1038/ncomms6205

Tafalla C, Bogwald J, Dalmo RA (2013) Adjuvants and immunostimulants in fish vaccines: current knowledge and future perspectives. Fish Shellfish Immunol 35(6):1740–1750

Taylor, Publisher, Rich A Shelby, Joyce J Ev, and Phillip H Klesius. 2009. "De Vel Op Ment of an In Di Rect ELISA to De Tect Humoral Re Sponse to Strep to Coc Cus Iniae In Fec Tion of Nile Tilapia , Oreochromis Niloticus." *Health (San Francisco)* (781191585): 37–41.

Taylor P., "Ac ce ed us cr t," no. January 2015, pp. 37–41, 2014.

Tomonaga, S., Kobayashi, K., Hagiwara, K., Yamaguchi, K. & Awaya, K. (1986). Gut associated lymphoid tissue in elasmobranchs. Zool. Science 3,453458.

Tran Ngọc Thien Kim, Johan A.J. Prof. dr. Verreth, Johan W. Dr. co-promotor Schrama, and Arjen J. Dr. co-promotor Roem. 2017. *Feeds, Water Quality, Gut Morphology and Digestion in Nile Tilapia (Oreochromis Niloticus)*. http://library.wur.nl/WebQuery/clc/2210744.

Turner, J.R., 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9, 799e809.

Tyrer, P. C., Ruth Foxwell, A., Kyd, J. M., Otczyk, D. C., & Cripps, A. W. (2007). Receptor mediated targeting of M-cells. Vaccine, 25(16), 3204-3209. doi: S0264-410X(07)00058-8

Valpotić, H. et al. 2010. "Increased Number of Intestinal Villous M Cells in Levamisole - Pretreated Weaned Pigs Experimentally Infected with F4ac+ Enterotoxigenic Escherichia Coli Strain." *European Journal of Histochemistry* 54(2): 18.

Vallon-Eberhard, A. et al. (2006) Transepithelial pathogen uptake into the small intestinal lamina propria. J. Immunol. 176, 2465–2469

Valdenegro-Vega, V. A., Crosbie, P., Vincent, B., Cain, K. D., & Nowak, B. F. (2013). Effect of immunization route on mucosal and systemic immune response in Atlantic salmon (Salmo salar). Vet Immunol Immunopathol, 151(1-2), 113-123. doi: 10.1016/j.vetimm.2012.10.010

Vandenberg G.W. (2004) Oral vaccines for finfish: academic theory or commercial reality? Animal Health Research Reviews 5, 301–304.

Vásquez- Machado, Gersson, Paola Barato- Gómez, and Carlos Iregui- Castro. 2019. "Morphological Characterization of the Adherence and Invasion of *Streptococcus Agalactiae* to the Intestinal Mucosa of Tilapia *Oreochromis* Sp.: An in Vitro Model." *Journal of Fish Diseases* 552(January): jfd.13042. https://onlinelibrary.wiley.com/doi/abs/10.1111/jfd.13042.

Vazquez-Juarez RC, Barrera-Saldana HA, Hernandez-Saavedra NY, GomezChiarri M, Ascencio F. Molecular cloning, sequencing and characterization of omp48, the gene encoding for an antigenic outer membrane protein from *Aeromonas veronii*. J Appl Microbiol 2003; 94:908–18.

Velazquez, J., Acosta, J., Lugo, J. M., Reyes, E., Herrera, F., Gonzalez, O., . . . Estrada, M. P. (2018). Discovery of immunoglobulin T in Nile tilapia (*Oreochromis niloticus*): A potential molecular marker to understand mucosal immunity in this species. Dev Comp Immunol, 88, 124-136. doi: 10.1016/j.dci.2018.07.013

Vervarcke, S., Ollevier, F., Kinget, R., & Michoel, A. (2005). Mucosal response in African catfish after administration of *Vibrio anguillarum* O2 antigens via different routes. Fish Shellfish Immunol, 18(2), 125-133. doi: S1050-4648(04)00068-3

Villumsen, K. R., Neumann, L., Ohtani, M., Strom, H. K., & Raida, M. K. (2014). Oral and anal vaccination confers full protection against enteric redmouth disease (ERM) in rainbow trout. PLoS One, 9(4), e93845. doi: 10.1371/journal.pone.0093845

Wang, Shuai, Philip A Thacker, Malcolm Watford, and Shiyan Qiao. 2015. "Functions of Antimicrobial Peptides in Gut Homeostasis Functions of Antimicrobial Peptides in Gut Homeostasis." (December).

Wang, E., Wang, X., Wang, K., He, J., Zhu, L., He, Y., . . . Lai, W. (2018). Preparation, characterization and evaluation of the immune effect of alginate/chitosan composite microspheres encapsulating recombinant protein of *Streptococcus iniae* designed for fish oral vaccination. Fish Shellfish Immunol, 73, 262-271. doi: S1050-4648(17)30775-1

Wang, J., Zou, L. L., & Li, A. X. (2014). Construction of a *Streptococcus iniae* sortase A mutant and evaluation of its potential as an attenuated modified live vaccine in Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol, 40(2), 392-398

Wang, Jing, Veronica Gusti, Andrea Saraswati, and D David. 2018. "Convergent and Divergent Development among M Cell Lineages in Mouse Mucosal Epithelium."

Wang, X., Xu, L., Wang, J., Wang, S., & Cao, H. (2014). Isolation, identification and drug sensitivity of *Streptococcus iniae* from hybrid sturgeons (Huso dauricus female x Acipenser schrencki male). Wei Sheng Wu Xue Bao, 54(4), 442-448.

Weinstein, M.R., M. Litt, D.A. Kertesz, P. Wyper, D.Rose, M.Coulter, A. McGeer, R. Hansen, J.D., Landis, E.D. and Phillips, R.B. 2005. Discovery of a unique Ig heavy chain isotype (IgT) in rainbow trout: implications for a distinct B cell developmental pathway in teleost fish. Proc Natl Acad Sci USA 102: 6919–24.

Yan, Y., Gong, P., Jin, W., Xu, J., Wu, X., Xu, T., Hang, Q., Fu, H., Kei, K & Gao Y (2012). The cell-specific upregulation of bone morphogenetic protein-10 (BMP-10) in a model of rat cortical brain injury. J Mol Histol, 43:543–552. doi: 10.1007/s10735-012-9431-1.

Yamamoto, F. J. Sutili, M. Hume, and D. M. Gatlin, "The effect of β - 1 , 3 - glucan derived from Euglena gracilis (Algamune ™) on the innate immunological responses of Nile tilapia (Oreochromis niloticus L .)," no. April, pp. 1579–1588, 2018.

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.

Zamri-Saad, M., M. N. A. Amal, A. Siti-Zahrah, and A. R. Zulkafli. 2014. Control and prevention of streptococcosis in cultured tilapia in Malaysia: a review. Pertanika Journal Tropical Agricultural Sciences 37:389–410.

Zeng, Y., He, Y., Wang, K. Y., Wang, J., Zeng, Y. K., Chen, Y. X., . . . OuYang, P. (2016). cpsJ gene of *Streptococcus iniae* is involved in capsular polysaccharide synthesis and virulence. Antonie Van Leeuwenhoek, 109(11), 1483-1492. doi: 10.1007/s10482-016-0750-1

Zhang, Y., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z. … Sunyer, O. J. (2010). IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nature Immunology, 11(9), 827–835.

Zhang, Z., Yu, A., Lan, J., Zhang, H., Hu, M., Cheng, J., . . . Wei, S. (2017). GapA, a potential vaccine candidate antigen against *Streptococcus agalactiae* in Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol, 63, 255-260. doi: S1050-4648(17)30091-8

Zhou, Chao. 2018. "Intelligent Feeding Control Methods in Aquaculture with an Emphasis on Fish : A Review." : 975–93.

Zlotkin A, Eldar A, Ghittino C, Bercovier H. Identification of Lactococcus garvieae by PCR. J Clin Microbiol 1998, 36, 983-985

Zhang, Y., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z. … Sunyer, O. J. (2010). IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nature Immunology, 11(9), 827–835

Zhou, Y., Xiao, J., Ma, X., Wang, Q., & Zhang, Y. (2018). An effective established biosensor of bifunctional probes-labeled AuNPs combined with LAMP for detection of fish pathogen *Streptococcus iniae*. Appl Microbiol Biotechnol, 102(12), 5299-5308. doi: 10.1007/s00253-018-90163

Zhou, S. M., Fan, Y., Zhu, X. Q., Xie, M. Q., & Li, A. X. (2011). Rapid identification of Streptococcus iniae by specific PCR assay utilizing genetic markers in ITS rDNA. J Fish Dis, 34(4), 265-271. doi: 10.1111/j.1365-2761.2010. 01233.x

Z. Zhang *et al.*, "GapA, a potential vaccine candidate antigen against Streptococcus agalactiae in Nile tilapia (Oreochromis niloticus)," *Fish Shellfish Immunol.*, vol. 63, pp. 255–260, 2017.

Z. Zhang *et al.*, "The pathogenic and antimicrobial characteristics of an emerging Streptococcus agalactiae serotype IX in Tilapia," *Microb. Pathog.*, 2018.