CURRICULUM VITAE

Lloyd VAUGHAN

Nationalities: Switzerland and New Zealand

Private address: Ibergweg 5, 8483 Kollbrunn, Switzerland

Expertise: Fish biology, intracellular bacterial pathogens, fish pathogens, fish models, zebrafish, chlamydial biology, including chlamydial infections of terrestrial animals and fish. Molecular and cell biology, biochemistry, genomics, microbiology, proteomics, microscopy. Earlier research areas include clinical biochemistry, extracellular matrix, signal transduction and cell surface signaling receptors.

Academic qualifications:

BSc (Hons) **1975** Department of Biochemistry, Victoria University of Wellington, New Zealand

PhD (Biochemistry) **1982** Christchurch Clinical School and Department of Biochemistry, Canterbury University, New Zealand

Habilitation in Biochemistry and Cell Biology, **1997,** ETH-Zürich, Switzerland

Habilitation in Molecular and Cellular Biology, **2004** Vetsuisse Faculty, University of Zürich, Switzerland

Professor (titular) for Molecular and Cellular Biology, **2011** Vetsuisse Faculty, University of Zürich, Switzerland

Working Experience.

1982-1987 Assistant, Laboratorium für Biochemie I, ETH-Zürich

1987-2001 Oberassistant, Dozent and Group leader Laboratorium für Biochemie ETH-Zürich

2001-2016 Head of Molecular and Cellular Biology,

Veterinary Pathology, Vetsuisse Faculty, University of Zürich

2016- Director, PathoVet AG, Tagelswangen, Switzerland

COST activities

2005-2008 Member of COST 855, Animal chlamydiosis

2007-2011 Management committee (Switzerland) of COST 867, Welfare of fish in European aquaculture

Research grants in period 2005-2016

2005-2008 Chronic chlamydial infections in herds (SBF, C05.0093)

2005-2006 Chlamydial infections in herds: A unique phenotype escaping detection? (Vetsuisse)

2006-2008 Waddlia chondrophila: role of surface proteins in adhesion, internalization and intracellular trafficking of this abortigenic agent in macrophages (SBF, C06.0100)

2007-2009 Characterization of chlamydial epithelial agents from cultured fish (SBF, C07.0125)

2009-2012 Pathobiology of chlamydial epitheliocystis agents: zebrafish as a model of infection (SBF C09.0039)

2011-2015 Zebrafish embryos as a model host for the (real time) analysis of infection with the opportunistic pathogen *Cronobacter* spp. (SNF, 310030_138533/1)

2012-2015 Epitheliocystis in larvae (Aquaexcel 01/05/0004/B)

2013-2015 ChlaFish EU Marie Curie IEF grant number 332058 to HSS (LV co-applicant)

2013-2015 Swiss Government Scholarship to MSG (LV co-applicant)

Other competitive grants in period 2004-2010

2006-2008 The Swiss Virtual Veterinary Pathologist (Swiss Virtual Campus Projects, 4th Call 2005)

Research

Brief resumé of research experience.

Bacterial intracellular pathogens of marine and fresh water environments. (PathoVet AG, Tagelswangen, Switzerland). Together with Drs. Maja Ruetten and Nicolas Kuehn, we offer and are developing, state of the art and rapid diagnostic tools for veterinary applications, including fish, wild and zoo animals, as well as domestic and farmed animals. Iron availability as a key element of intracellular bacteria infections is the focus of research by Dr. Ruetten, together with the Institute of Veterinary Physiology, University of Zurich, Swizerland (Prof. Max Gassmann). Epithelioycstis research, along with pathological diagnoses of fish diseases, is a continuing program, together with Dr. Pantelis Katharios, HCMR, Crete and Prof. Gilbert Greub, Head of Clinical Microbiology, CHUV, Lausanne, Switzerland.

Currently available in German and French, the website http://www.pathovet.ch/ will soon be available also in Italian and English. Diagnostic reports are offered in all four languages.

Epitheliocystis: A disease of pathogenic intracellular bacteria, affecting both wild and farmed fish populations. (*Institute of Veterinary Pathology, University of Zurich*)

The role of *Chlamydia* and *Chlamydia*-like organisms as fish pathogens (Polkinghorne 2010), and the value of fish as model organisms for investigation is yet to be fully appreciated. To further this, Prof Segner, FIWI, Bern and myself initiated Swiss membership of COST Action 867: Fish Welfare in European Aquaculture and for which we formed the management committee for Switzerland. This led a review with collaborators from COST 867 (Segner et al., 2012) where we made the case for fish health as a cornerstone of welfare. This review has >50 citations. Together with Dr Heike Schmidt-

Posthaus, FIWI, University Bern, we begun a collaboration which is still continuing, examining the role of Chlamydia in Swiss salmonids. Brown trout (*Salmo trutta*) are close relatives to Atlantic salmon (*Salmo salar*) and share many of the diseases. Situated at the headwaters of several major European rivers, Switzerland offers the chance of investigating salmonids which are well separated from the nearest marine environment, and as such will be missing potential intermediate or alternative hosts present around the coasts of Ireland and Norway. We could show that individual trout could be co-infected with chlamydial agents, *Ca*. Piscichlamydia salmonis and *Ca*. Clavichlamydia salmonicola and that infections were river-site specific (Schmidt-Posthaus et al., 2012). Extensive investigations of Brown trout from both the Rohn and the Rhine showed that infection levels with the two agents were similarly high in both river systems, although the Rhone drains into the Mediterranean and the Rhine into the Atlantic and that both farmed and wild fish were infected, with no evidence for farms as reservoirs for the wild fish population (Guevara Soto et al., 2016a, b). A few cases of *Ca.* Similichlamydia were also observed. Infections were confirmed to be site-specific and were seasonal, peaking late Summer. No cases were found late Spring/early Summer, indicative of an unknown Winter reservoir (Guevara Soto et al., 2016c).

The breakthrough in epitheliocystis research came through support from the EU FP7 and H2020 programs (Aquaexcell and Marie Curie), which brought together Pantelis Katharios (HCMR) and Helena Seth-Smith (Cambridge University and the Sanger Institute) and two groups at the University of Zurich, Ralph Schlapbach (Functional Genomics Center UZH and ETHZ) and my own to work on the genomics and environmental reservoir of these intracellular bacterial pathogens. We developed the first environmental epitheliocystis infection system for fish larvae (Katharios et al., 2015) and and discovered a new pathogenic member of the Endozoicomonas genus, which is otherwise know for its wide host distribution in shell fish, sponges and corals as a beneficial symbiont. New methods were also developed to successfully sequence and describe the genome and characterise the morphology with high resolution 3D light and electron microscopy from this uncultivated bacteria. Similar genomics and microscopy approaches were applied to wide spread epitheliocystis infections of Sparus aurata cultured fish in Greece, leading to the discovery and description of two species from a novel family of intracellular pathogenic bacteria which we named *Ichthyocystis helenicum* and *Ichthyocystis creta* (Seth-Smith et al., 2016a; Qi et al., 2016), described for a wider aquaculture audience in GAA (Seth-Smith et al., 2016b).

A genetically tractable fish model for Chlamydia and other intracellular bacteria was an essential aspect we also wished to provide for examining mechanisms of infection and as a test-bed for treatment strategies and this we achievded with the first zebrafish models for the food-born pathogen *Cronobacter* sp. (Fehr et al., 2015) and for water born Waddlia chondrophila, pathogen of animals and humans and member of a chlamydial family common in the marine environment (Fehr et al., 2016).

Chlamydia research (*Institute of Veterinary Pathology, University of Zurich*)

Progress in combating outbreaks of disease is dependent on the one hand on tools which permit precise monitoring of pathogen distributions, including the pathology of infected individuals and, on the other, of improving our knowledge of the molecular mechanisms by which *Chlamydia* infect the cell and also establish persistence.

Our studies have extended these investigations to include the role of *Chlamydia* in conjunctivitis in cats (von Bomhard, 2003) and in reproductive problems in pigs (Camenische, 2004, Becker, 2007). In both cases, the application of new molecular biological analyses (see also Schiller, 2003; this work received the prize for the best paper of the year in this journal) revealed not only the presence of members of the *Chlamydiaceae*, but also the involvement of novel strains of *Chlamydia*-like organisms (Lutz-Wolgroth 2006, Blumer 2007). Another major project concerns the role of signal transduction proteins in chlamydial cell cycle regulation and in persistence, and although this has taken some time to develop the tools within the Institute of Veterinary Pathology, this has now come to fruition with the first in a series of publications addressing this topic (Polkinghorne, 2006). This work includes the roles of critical regulatory proteins, the prokaryotic GTPases, and chlamydial membrane proteins, in adhesion, trafficking and persistence (Polkinghorne, 2008, 2009). The potential role of amoeba as vectors and hosts for *Chlamydia* (Wirz, 2008) and the recognition through studies of ourselves and others of the importance of *Chlamydia*-like organisms as a major group of emerging pathogens, has shifted the emphasis of our research to also include genomic analysis (Bertelli, 2010).

Proteomics and cell signal transduction research (*Clinical Biochemistry, Christchurch and Institute for Biochemistry, ETH Zurich*): Following a solid grounding in proteomics investigating glycosylation of the serum glycoprotein alpha-1-antitrypsin during my PhD in Clinical Biochemistry, Christchurch New Zealand (reviewed in Carrell 1982), I moved to the ETH Zürich to establish a group investigating collagen proteoglycan structures (Vaughan 1985).

A thorough carbohydrate and protein chemical characterization of a glycosaminoglycan attachment site on type IX collagen (Huber 1986, 1988) raised the question of how this unique hybrid might contribute to collagen fibril formation. The breakthrough came through structural resolution in the transmission electron microscope, made possible by rotary shadowing combined with monoclonal antibodies as markers (Vaughan 1988; Mendler 1989). This work remains a bench mark (see for instance the section on extracellular matrix in Molecular Biology of the Cell, 5th edition, (Alberts 2008).

The emphasis now shifted to how, in multi-cellular organisms, signals arising from the extra-cellular matrix (ecm) are presented to the cell, and then transposed across the plasma membrane to regulate cellular behaviour. This included the tenascin family of ecm molecules, which impressed through their striking structure (Vaughan 1987), their regulation of embyo development (Kaplony 1991; Lochter 1991), and their range of cell receptors (Zisch 1992; Vaughan 1994, 1996). Receptor binding sites on tenascin and its receptor were mapped using a combination of molecular and cellular biological techniques (fusion proteins, cell transfectants) together with monoclonal and polyclonal antibody technology. We confirmed the contactin binding site (Zisch 1995; Weber 1996) and identified and characterised a new heparin binding site on tenascin (Weber 1995). This included generating fusion proteins corresponding to the putative heparan sulfate binding binding site in native form, established by their structural characterisation after crystallization (Bisig 1999) and measuring their binding with the BiaCore plasmon resonance technology (Weber 1996). During this time, we used similar approaches to identify carbohydrate binding sites on Laminin (Hall, 1997)

Cell signalling via GPI-anchored cell surface receptors has been somewhat of an enigma; anchored in the outer lipid leaflet of the membrane how could they transmit a signal to the cell? A clue came from our observations that the GPI-receptor contactin can activate a protein tyrosine kinase pathway involving the intracellular kinase Fyn (Zisch 1995). To identify members of this pathway, a combined proteomics and cell biology approach was applied. This involved isolating GPI-receptor signalling complexes from brain and analyzing their individual components by 2D gel electrophoresis, coupled to protein sequencing to identify the genes involved. Candidate members of the complex were tested for interactions with contactin using phosphorylation and pull-down assays from native complexes as well as from in vitro experiments with transfected cells. Through this process we identified a receptor protein phosphatase (RPTPα) and showed it associates with the GPI-receptor contactin (Zeng 1999).

PUBLICATION LIST

Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland (2001-2016)

Fehr AGJ, Ruetten M, Seth-Smith HMB, Nufer L, Voegtlin A, Lehner A, Greub G, Crosier PS, Neuhauss SCF and Vaughan L (2016) A Zebrafish Model for *Chlamydia* Infection with the Obligate Intracellular Pathogen *Waddlia chondrophila*. **Front Microbiol.** 7, 1829. doi: 10.3389/fmicb.2016.01829

Qi W, Vaughan L, Katharios P, Schlapbach R, Seth-Smith HM. (2016) Host-Associated Genomic Features of the Novel Uncultured Intracellular Pathogen Ca. Ichthyocystis Revealed by Direct Sequencing of Epitheliocysts. **Genome Biol Evol.** 8, 1672-89. doi: 10.1093/gbe/evw111

Guevara Soto M, Vidondo B, Vaughan L, Rubin JF, Segner H, Samartin S, Schmidt-Posthaus H.(2016**c**) Investigations into the temporal development of epitheliocystis infections in brown trout: a histological study. **J Fish Dis**. Sep 27. doi: 10.1111/jfd.12562

Seth-Smith HM, Dourala N, Fehr A, Qi W, Katharios P, Ruetten M, Mateos JM, Nufer L, Weilenmann R, Ziegler U, Thomson NR, Schlapbach R, Vaughan L. (2016b) Emerging pathogens of gilthead seabream: characterisation and genomic analysis of novel intracellular β-proteobacteria. **ISME J.** 10, 1791-803. doi: 10.1038/ismej.2015.223.

Seth-Smith HM, Katharios P, Vaughan L. (2016a) Emerging epitheliocystis disease in Mediterranean sparids caused by novel bacteria. **Global Aquaculture Advocate**, Feb 12 (Review) (http://advocate.gaalliance.org/emerging-epitheliocystis-disease-in-mediterranean-sparids-caused-by-novel-bacteria/)

Guevara Soto M, Vaughan L, Segner H, Wahli T, Vidondo B, Schmidt-Posthaus H. (2016b) Epitheliocystis Distribution and Characterization in Brown Trout (Salmo trutta) from the Headwaters of Two Major European Rivers, the Rhine and Rhone. **Front Physiol**. Apr 18;7, 131. doi: 10.3389/fphys.2016.00131.

Guevara Soto M, Vidondo B, Vaughan L, Seth-Smith HM, Nufer L, Segner H, Rubin JF, Schmidt-Posthaus H. (2016a) The emergence of epitheliocystis in the upper Rhone region: evidence for Chlamydiae in wild and farmed salmonid populations. **Arch Microbiol.** 198, 315-24. doi: 10.1007/s00203-016-1192-x

Katharios P, Seth-Smith HM, Fehr A, Mateos JM, Qi W, Richter D, Nufer L, Ruetten M, Guevara Soto M, Ziegler U, Thomson NR, Schlapbach R, Vaughan L. (2015) Environmental marine pathogen isolation using mesocosm culture of sharpsnout seabream: striking genomic and morphological features of novel Endozoicomonas sp. **Nat Sci Rep.** Dec 7;5, 17609. doi: 10.1038/srep17609.

Fehr A, Eshwar AK, Neuhauss SC, Ruetten M, Lehner A, Vaughan L. (2015) Evaluation of zebrafish as a model to study the pathogenesis of the opportunistic pathogen Cronobacter turicensis. **Emerg Microbes Infect**. 201;4(5), e29. doi: 10.1038/emi.2015.29.

Taylor-Brown A, Vaughan L, Greub G, Timms P, Polkinghorne A. **(**2015**)** Twenty years of research into Chlamydia-like organisms: a revolution in our understanding of the biology and pathogenicity of members of the phylum Chlamydiae. **Pathog Dis**. 73, 1-15. doi: 10.1093/femspd/ftu009. Review.

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Fehr A, Walther E, Schmidt-Posthaus H, Nufer L, Wilson A, Svercel M, Richter D, Segner H, Pospischil A, Vaughan L. (2013) Candidatus Syngnamydia venezia, a novel member of the phylum Chlamydiae from the broad nosed pipefish, Syngnathus typhle. **PLoS One**. Aug 12;8(8), e70853. doi: 10.1371/journal.pone.0070853.

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Polkinghorne A, Schmidt-Posthaus H, Meijer A, Lehner A, Vaughan L. (2010) Characterization of a *Chlamydia*-like bacterium associated with epitheliocystis in a Leopard Shark (*Triakis semifasciata*). **Dis. Aquat. Organ**. 91, 75-81

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Pospischil A, Rütten M, Djamei V, Mathys M, Sydler T, Vaughan L. (2009) Swiss Virtual Animal Pathology: www.animalpatho.org The 7th International Conference on Education and Information Systems, Technologies and Applications: **EISTA** 2009

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Wirz M, Polkinghorne A, Dumrese C, Ziegler U, Greub G, Pospischil A, Vaughan L. (2008) Predator or prey? Chlamydophila abortus infections of a free-living amoebae, Acanthamoeba castellani 9GU **Microbes Infect.** 10, 591-597

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Pospischil A, Djamei V, Rütten M, Sydler T, Vaughan L. (2007) Introduction to the Swiss way of teaching veterinary pathology in the twenty-first century: application of e-learning modules. **J Vet Med Assoc**. 34, 445-449.

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Laboratorium for Biochemistry I, Dept. Biology, ETH- Zurich, Switzerland (1982-2000)

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Dept. Pathology, Christchurch Clinical School of Medicine and Dept. Biochemistry, Lincoln College, Christchurch, New Zealand (1977-1982)

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