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Review

Chlamydial infections of fish: Diverse pathogens and emerging causes of disease in aquaculture species

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ABSTRACT

Chlamydial infections of fish are emerging as an important cause of disease in new and established aquaculture industries. To date, epitheliocystis, a skin and gill disease associated with infection by these obligate intracellular pathogens, has been described in over 90 fish species, including hosts from marine and fresh water environments. Aided by advances in molecular detection and typing, recent years have seen an explosion in the description of these epitheliocystis-related chlamydial pathogens of fish, significantly broadening our knowledge of the genetic diversity of the order *Chlamydiales*. Remarkably, in most cases, it seems that each new piscine host studied has revealed the presence of a phylogenetically unique and novel chlamydial pathogen, providing researchers with a fascinating opportunity to understand the origin, evolution and adaptation of their traditional terrestrial chlamydial relatives. Despite the advances in this area, much still needs to be learnt about the epidemiology of chlamydial infections in fish if these pathogens are to be controlled in farmed environments. The lack of *in vitro* methods for culturing of chlamydial pathogens of fish is a major hindrance to this field. This review provides an update on our current knowledge of the taxonomy and diversity of chlamydial pathogens of fish, discusses the impact of these infections on the health, and highlights further areas of research required to understand the biology and epidemiology of this important emerging group of fish pathogens of aquaculture species.

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First reported as 'Mucophilosis' in the common carp (*Cyprinus carpio*) in 1920 (Plehn, 1920), the term 'Epitheliocystis' was coined after cyst-like inclusions within the epithelial cells of the gills were observed in the freshwater species, bluegill (*Lepomis macrochirus*) (Hoffman et al., 1969). Since this initial diagnosis, epitheliocystis has been reported from over 90 species of fish globally (Appendix S1) (Nowak and LaPatra, 2006; Stride et al., 2013a), including fishes from marine and freshwater sources as well as from wild and farmed environments. Taxonomically, the host range for this disease is diverse, including but not limited to; [a] Acipenseridae: white sturgeon (*Acipenser trasmonanus*) (Groff et al., 1996), [b] Cyprinidae: carp (*C. carpio*) (Plehn, 1920), [c] Salmonidae: coho salmon (*Oncorhynchus kisutch*) (Carvajal et al., 1990), Atlantic salmon (*Salmo salar*) (Carvajal et al., 1990), Arctic charr (*Salvelinus aplinus*) (Draghi et al., 2007) and brown trout (*Salmo trutta*) (Karlsen et al., 2008), [d] Carangidae: amberjack (*Seriola dumerili*) (Crespo et al., 1990), yellowtail kingfish (*S. lalandi*) (Venizelos and Benetti, 1996) and yellowtail (*S. mazatlana*) (Venizelos and Benetti, 1996), [e] Latidae: barramundi (*Lates calcarifer*) (Meijer et al., 2006), [f] Muglidae: golden grey mullet (*Liza aurata*) (Paperna and Sabnai, 1980), thinlip grey mullet (*L. ramada*) (Paperna, 1977), greenback mullet (*L. subviridis*) (Paperna and Sabnai, 1980) and striped mullet (*Mugil cephalus*) (Paperna and Sabnai, 1980), [g] Terapontidae: silver perch (*Bidyanus bidyanus*) (Meijer et al., 2006), and [h] Centrachidae: bluegill (*Lepomis macrochirus*) (Hoffman et al., 1969) and largemouth bass (*Micropterus salmoides*) (Goodwin et al., 2005) (Appendix S1).

Only a limited number of the affected species are cultured for commercial purposes. In these fish species, epitheliocystis is usually considered insignificant. However, there have been cases in aquaculture, such as in Atlantic salmon farmed in Norway, where epitheliocystis has been linked to high mortalities (Draghi et al., 2004; Mitchell and Rodger, 2011). While its exact effect on the fish is still not fully understood, aquaculture species are more at risk of being infected due to the higher stocking densities and greater stresses placed upon the fish (Grau and Crespo, 1991; Nowak and LaPatra, 2006). Consequently, there has been an increase in the general awareness of the impact and prevalence of this disease (Nowak and LaPatra, 2006).

Initially, it was believed that the same aetiological agent caused epitheliocystis in all fish species. However, as early as 1977 it was recognised that the epitheliocystis forms were a distinct taxonomic entity that demonstrated a high degree of host specificity (Paperna, 1977). In addition, it could not be determined whether epitheliocystis was due to *Rickettsia*-like organisms (RLO) or *Chlamydia*-like organisms (CLO), due to both bacterial groups being defined as 'gram-negative, obligate and intracellular' (Zachary and Paperna, 1977; Bradley et al., 1988; Venizelos and Benetti, 1996). The identification of the actual causative agent(s) of epitheliocystis as belonging to the *Chlamydiales* was only a recent advance, primarily aided through the use of molecular techniques (Everett et al., 1999; Draghi et al., 2004).

Chlamydiales pathogens exhibit a two-stage developmental cycle of replication (Everett et al., 1999; Corsaro and Greub, 2006). This developmental cycle rotates between (i) an extracellular infectious elementary body (EB), which is endocytosed by eukaryotic cells and resides within a cytoplasmic inclusion and; (ii) an intracellular vegetative reticulate body (RB), which replicates by binary fission (Everett et al., 1999; Corsaro and Greub, 2006). The unique developmental cycle has hampered efforts to culture the bacteria *in vitro*. Many attempts have been made to culture the aetiological agent(s) *in vitro* as a means of proving causation and fulfilling Koch's postulates, however all have been unsuccessful to date (Hoffman et al., 1969; Bradley et al., 1988; Valdron et al., 1994; Venizelos and Benetti, 1996; Kumar et al., 2012).

In the absence of an *in vitro* culture system for this group of CLOs, researchers have focussed on fulfilling Fredricks and Relman's molecular postulates to establish a role for these organisms in epitheliocystis (Fredricks and Relman, 1996). Using these methods, the first CLO agent characterised in fish was *Candidatus* Piscichlamydia salmonis, which was described in Atlantic salmon in 2004 (Draghi et al., 2004). Since the description of this new chlamydial species, seven new *Candidatus* species have been described with many more partially sequenced from epitheliocystis cases in different host species. Although other agents have been associated with epitheliocystis (Toenshoff et al., 2012; Mitchell et al., 2013), the majority of evidence points to a role for *Chlamydia*-related bacteria, the obligate intracellular parasites of animals and humans. Much more, however, still needs to be known about the impact of these infections on fish, their epidemiology and methods to control the disease. In this review we provide an update on the current taxonomy and diversity of *Chlamydia*-like epitheliocystis agents, discuss the impact of chlamydial infections on fish health and review the potential source and dissemination of these infections in aquaculture.

An update on the taxonomy, genetic diversity and geographical distribution of *Chlamydia*-like agents of epitheliocystis

Bacteria belonging to the order *Chlamydiales* are an extremely important and diverse group of pathogens of vertebrates, which include respiratory diseases of fishes (Corsaro and Greub, 2006). Since the epitheliocystis review of 2006 (Nowak and LaPatra, 2006), there have been numerous reports of epitheliocystis (Table 1). Many of these studies have focussed on molecular characterisation of the aetiological agent(s) involved and this has resulted in several new *Candidatus* species being described. It is important to note that all members of the order *Chlamydiales*, not just those associated with epitheliocystis in fish underwent a significant review in 1999, which substantially altered the taxonomy of the order (Everett et al., 1999). The taxonomic guidelines were updated to reflect the increasing use and reliance of molecular techniques and DNA sequences. Consequently, for any future bacterial isolate or sequence to be included within the order *Chlamydiales* it required a $\geq$ 80% sequence similarity of the 16S rRNA gene to the known and accepted members already within the order. The review went