Edwardsiella ictaluri: a systemic review and future perspectives on disease management

Vimbai Irene Machimbirike[a,d*], Margaret Crumlish[b], Ha Thanh Dong[c], Javier Santander[d], Pongsak Khunrae[a], Triwit Rattanarojpong[a]

[a]Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, 10140, Thailand

[b]Institute of Aquaculture, University of Stirling, Stirling, UK

[c]School of Environment, Resources & Development, Asian Institute of Technology, Pathum Thani, 12120, Thailand

[d] Marine Microbial Pathogenesis and Vaccinology Laboratory, Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL, Canada

*Corresponding author: V. I. Machimbirike

Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi (KMUTT), 126 Pracha Uthit Rd, Bang Mot, Thung Khru, Bangkok 10140, Thailand

Marine Microbial Pathogenesis and Vaccinology Laboratory, Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL, Canada

Tel: +66957745871

E-mail: vimbai.matafi@kmutt.ac.th/imachimbirik@mun.ca

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Abstract

Edwardsiella ictaluri, a non-zoonotic Gram-negative bacterium, has been known to science for more than 4 decades. It was reported for the first time in 1979 in *Ictalurus punctatus* in the USA, and later in *Pangasianodon hypophthalmus* and *Pelteobagrus fulvidraco* in Asia. Even though catfish species are more susceptible to *E. ictaluri*, other fish species are also affected, and up to 44 fish species in 4 continents are known to be susceptible. The diseases caused by *E. ictaluri* are known as enteric septicaemia of catfish (ESC) in channel catfish, bacillary necrosis of pangasius (BNP) in striped catfish, red-head disease in yellow catfish and edwardsiellosis in tilapia. Outbreaks caused by *E. ictaluri* can cause up to 100% mortality resulting in substantive economic losses to the industry, threatening food security and undermining sustainability. Although efforts

have been made to prevent and control this pathogen using vaccines, antibiotics, disease resistance selective breeding, functional feed ingredients, prebiotics and probiotics, and biosecurity measures, *E. ictaluri* is still causing health issues in different countries. Here, we provided with a comprehensive review that addressed the current knowledge of *E. ictaluri* bacteriological characteristics, epidemiology, pathogenesis, diagnosis, control and management. Furthermore, we also provided the future perspectives based on advanced technologies and biosecurity management in aquaculture to assist pathogen control and/or eradication.

Keywords: *Edwardsiella ictaluri*, fish, pathogenesis, control strategies

Introduction

Aquaculture is an important sector of the food industry, which had a total value of USD 263.6 billion in 2018, employs 59.5 million people globally, and provides approximately 17% of the animal protein consumed, as well as essential nutrients such as Omega-3 fatty acids, iodine, vitamin D, trace minerals like iron, calcium, and zinc [1]. However, despite the positive contribution of aquaculture, it is an intensive farming practice and there are health management issues that impede both economic and socio-economic expansion of the sector [2,3]. The primary constraint to the culture of many aquaculture species is the emergence of infectious diseases caused by pathogens such as bacteria, viruses, fungi, and infestations caused by parasites [4-7]. The most prevalent bacterial infections in channel, yellow, and striped catfishes are caused by *Edwardsiella ictaluri* followed by *Flavobacterium columnare*, and *Aeromonas hydrophila* [8-10]. In tilapia culture, substantial losses are experienced from infections caused by the bacteria *Aeromonas* spp., *Francisella* spp., *F. columnare*, *Streptococcus agalactiae* and *Streptococcus iniae* [11], and recently due to *E. ictaluri* infections [12,13].

E. ictaluri is a freshwater fish host generalist pathogen that causes mortalities up to 50% and 100% in naturally infected tilapia, and yellow and striped catfishes in Asia, respectively [10,14-17]. Also, *E. ictaluri* causes losses of up to 50.5% to catfish operations in the USA [18]. A channel catfish study on the direct impacts of fish diseases carried out in East Mississippi Catfish Industry identified a total loss of USD $16.9 million in 2016 [19]. Of the pathogens studied, *E. ictaluri* contributed a loss of 1.2 million fish and USD 0.7 million farm-gate value [19]. Thus, *E. ictaluri* is an economically important pathogen in aquaculture and extensive research has been carried out to study the

pathogen. Even though there is a lot of literature available related to *E. ictaluri* infections in aquatic animals, a comprehensive updated review could contribute to potential disease control and management. Based on the economic importance of *E. ictaluri* and the need to explore potential ways to manage the pathogen, the present study is conducted to provide a systemic review on current state of knowledge on *E. ictaluri* infections in aquaculture and future perspectives on combating the pathogen.

Pathogen discovery, susceptible hosts, geographical distribution, and epidemiology

The first report on the isolation of *E. ictaluri* was by Hawke in 1979 [20]. *E. ictaluri* was identified as the causative agent of enteric septicemia of catfish (ESC), primarily infecting fingerlings of channel catfish (*Ictalurus punctatus*) in the United States of America (USA) aquaculture industry [20]. However, it was later discovered that ESC was already present in Arkansas a decade before the first official report using archived samples [21]. After the initial report in the USA in 1979 in channel catfish, *E. ictaluri* has been identified in several continents, for instance in Asia it is the frequent causative agent of bacillary necrosis of Pangasius (BNP) [22] and red-head disease [10] in striped and yellow catfish, respectively.

E. ictaluri is a fish-host generalist infecting up to 44 fish species, of which 31 species are naturally infected and 13 species were experimentally infected as shown in Table 1. A total of seven catfish families have been described to be susceptible to *E. ictaluri*, including Ictaluridae, Bagridae, Clariidae, Pangasiidae, Ariidae, Siluridae and Plotosidae. For non-catfish species, 10 fish families are susceptible including Plecoglossidae, Sternopygidae, Cyprinidae, Cichlidae, Salmonidae, Moronidae, Anguillidae, Percichthyidae, Balaenopteridae and Pleuronectidae. To date, there are several documented isolations of *E. ictaluri* in several continents that include North America, Caribbean, Asia, Australia, and Europe with mortalities reaching 100% (Table 1). A timeline of *E. ictaluri* isolation in different host species and geographical locations is described in Table 2. Even though up to 44 susceptible fish hosts have been reported, *E. ictaluri* predominantly affects intensively reared channel catfish and striped catfish in USA and Vietnam [8,23], respectively, yellow catfish (*Pelteobagrus fulvidraco*) in China [10,24], and riverine ayu (*Plecoglossus altivelis*) in Japan [25]. Most of the available literature on *E. ictaluri* is related to these 4 hosts and only 3 articles describe *E. ictaluri* infections in tilapia culture [12,13,26].

In the United States, epizootics in channel catfish are mainly experienced during late spring and early fall whereby acute ESC is usually experienced when temperatures are between 22 °C and 28 °C and chronic ESC usually occur when temperatures are cooler in the range of 18°C-22 °C or above 28 °C [27,28]. On the other hand, epizootics in striped catfish and tilapia in Southeast Asia are experienced during the rainy season when temperatures range from 23 to 30 °C where only an acute form is exhibited [12,14,15,29]. Studies have shown that the peak mortality of channel catfish from *E. ictaluri* is experienced at 25 °C [30,31] and hypoxia results in increased bacterial load in channel catfish tissues [32]. Environmental persistence studies of *E. ictaluri* using specific bacteriophages suggested that *E. ictaluri* can survive for up to 15 days in pond water and up to 95 days in pond sediments, implicating that water and mud could be *E. ictaluri* reservoirs [33,34]. *E. ictaluri* has also been shown experimentally to produce biofilms on common aquaculture material which might be a reservoir for recurrent epizootics and contributes to disinfectant resistance [35]. So far *E. ictaluri* has not yet been implicated in zoonosis and this might be because *E. ictaluri* is not capable of growth at 37 ºC [36]. Nevertheless, *E. ictaluri* was isolated from the mammal minke whale (*Balaenoptera acutorostrata*) excrement [37]. Although *E. ictaluri* infections can occur independently of stressors and still cause high mortalities of up to 77%, stressors such as handling, adverse environmental conditions and stocking density greatly enhance mortalities up to 97% [38-40]. A recent epidemiological survey on environmental factors that influence *E. ictaluri* infection in riverine ayu was conducted in Japan over a five-year period. The survey revealed that *E. ictaluri* related mortalities in ayu are exacerbated by adverse environmental conditions that include an increase in diurnal water temperature range (DWTR), high water temperatures, higher than normal air temperatures and lower levels of streamflows [41].

Naturally, *E. ictaluri* is mainly transmitted horizontally from dead infected catfish to naïve population due to infected fish cannibalization or *E. ictaluri* being shed from dead fish [39,42] whereas vertical transmission has not been reported yet [43] although presence of the bacteria in gonads may imply possible vertical transmission [44,45]. A high bacterial count was also found in the vicinity of the dying fish which decreased with the removal of the dead fish whilst survivors of an epizootic become carriers and pathogen reservoirs [32,46,47]. Contrarily, bacterial shedding into water was not observed for experimentally infected striped catfish [48]. Fish eating birds such as Great blue heron (*Ardea herodias*), Double-crested cormorants (*Phalacrocorax auritus*), Snowy egret (*Egretta*

thula) and Great egret (*Casmerodius albus*) have also been implicated in the spread of *E. ictaluri* between ponds [49,50]. *E. ictaluri* can be experimentally transmitted via exposure to pathogen in water, injection both intramuscular and intraperitoneal, intubation of the intestines and infection via the nares [8,51-54].

E. ictaluri general characteristics and genomic composition

E. ictaluri, a Gram-negative Enterobacteriaceae family member, is a pleomorphic rod of varied lengths and widths depending on host [13,14,55,56] that is peritrichous and was found to be weakly motile at optimal growth temperature, but non-motile strains were also isolated [55,57,58] (Table 2). *E. ictaluri* culture conditions in complex media are optimal temperature between 25-30 ºC and pH range of 7.0-7.5 [59], respectively, and it reaches stationary phase in about 48 hours [14,55]. Generally, strains of *E. ictaluri* is facultative anaerobic [44,60]. In terms of biochemical characteristics, *E. ictaluri* strains isolated from different host species exhibit heterogeneity mainly in striped catfish, sea bass, yellowhead catfish, hybrid catfish and tilapia strains with differences mainly in activities from ornithine decarboxylase, cytochrome oxidase, H_2S production and production of gas and acid from glucose (Table 2) [59,61-63]. Serologically, *E. ictaluri* is heterogenous and has antigenic variations in the O antigens and immunogenic epitopes that are recognized by different isolates [58,62,64-67], however, a serotyping scheme is yet to be developed [68]. One of the most intriguing aspects of *E. ictaluri* isolates from different hosts is the failure to cross-infect and failure of immunization with one *E. ictaluri* isolate from catfish to cross-protect against heterologous isolates, suggesting high genetic variations within the different isolates and genotypes [58,69,70]. All *E. ictaluri* isolates from different hosts were generally susceptible to the antibiotics florfenicol, penicillins, quinolones, fluoroquinolones, aminoglycosides, tetracyclines and resistant to macrolides whereas tilapia and striped catfish strains were additionally resistant to sulphonamides [64,71,72]. Intrinsic resistance to cationic antimicrobial peptides (CAMPs) such as colistin and polymyxin B of *E. ictaluri* is well documented [71,73].

A total of 11 whole genome sequenced *E. ictaluri* isolates obtained from the USA and Southeast Asia are publicly available in the National Center for Biotechnology Information (NCBI). Genomes from channel catfish isolates include 93-146 (**CP001600.2**), MS-17-156 (**CP028813.1**), NCTC12122 (**UFXT00000000.1**), ATCC 33202 (**AFJI00000000.1**), S97-773

(**QBLD00000000.1**) and S07-698 (**QDAD00000000.1**). Only 1 striped catfish isolate genome is available namely T1-1 (**CP054060**). Two *E. ictaluri* genomes isolated from zebrafish (*Danio rerio*) isolates are available, including LADL11-100 (**LDWX00000000.1**) and LADL11-194 (**LEAL00000000.1**). Two *E. ictaluri* genomes, isolated from Nile tilapia (*Oreochromis niloticus*) and red hybrid tilapia (*Oreochromis* spp.), respectively, have been described including RUSVM-1 (**CP020466.1**) and 2234 (**CP053781**). The *E. ictaluri* isolates have genomic sizes ranging from 3.6 to above 3.9 Mbp, with similar G+C contents (~57%) and between 3,235 to 3,641 protein coding sequences.

Catfish isolates from the USA and Thailand were found to contain an intervening sequence (IVS) located in helix-45 of the 23S rRNA gene that is absent in *E. tarda* and can provide a basis for differentiating the two closely related species [74]. Genetic variation of *E. ictaluri* isolates from diverse hosts have been investigated using fingerprinting based on amplified-fragment length polymorphism (AFLP) analysis, repetitive-sequence-mediated polymerase chain reaction (rep-PCR) and phylogenetic analysis using the *gyrB* gene and have revealed that the species consists of host-based genotypes [13,64,75]. *E. ictaluri* genomes consists of Type I, III, V, and VI secretion systems with variations in Type IV secretion system among genotypes [70,76]. Comparative genomics studies have shown variation in the O-antigen biosynthesis cluster and type IV secretion system (T4SS) genes between channel catfish and zebrafish isolates [70], absence of T4SS-type G genes in Nile tilapia isolate RUSVM-1 [76] and presence of oxidative resistance stress gene (aconitate hydratase B, *acn*B) in a virulent *E. ictaluri* isolated from ayu [77]. Genes encoding for surface structures such as cell wall, capsule and flagellar biosynthesis were found to be under positive selection which might explain some adaptive traits in the species [78]. Recently, our research group carried out comparative genomics of the 11 *E. ictaluri* genomes mentioned above and the results revealed that host specificity is brought about by intra-species evolution driven by gene gain and loss driven by prophages and insertion sequences [79].

E. ictaluri plasmidome

E. ictaluri genomes contain different number of plasmids. Generally, channel catfish isolates were found to contain between 1 to 3 plasmids, with most of them containing the plasmids pEI1 (4,807 kb) and pEI2 (5,643 kb) [64,80,81]. Plasmids pEI1 and pEI2 are involved in virulence as they contain

Type III secretory system genes that are responsible for direct injection of effectors into host cells and invasion [82]. Striped catfish *E. ictaluri* isolates were found to contain 3 different plasmids (~ 4.0 kb, 5.7 kb, 10 kb) and yellow catfish contains 2 plasmids (~ 4.1 kb and 5.6 kb) [24,58,83] whereas *E. ictaluri* isolated from non-silurids such as zebrafish and tilapia were shown to contain 2 plasmids (pEI1 and pEI2 homologs), and a green knife fish isolate had 4 plasmids (3.1, 4.1, 5.7 and 6.0 kb) [12,64,66]. From the data in the public database, the *E. ictaluri* plasmids have common lengths ranging from 3kb to about 9kb as reported earlier [84] with the exception of 2 plasmids pEI-MS-17-156-1 and pEI-2234-3 that have lengths above 100kb. Plasmid similarities within a host-based genotype as well as differences among genotypes from different hosts were reported, although most of the plasmids carried Type III secretion system proteins except plasmids from zebrafish isolates, whilst a striped catfish isolate contained a unique plasmid [58,64,82,84]. Recent studies in comparative genomics revealed that 2 isolates, MS-17-156 from channel catfish (USA) and 2234 from red hybrid tilapia (Vietnam) contain plasmids containing multi-drug resistant genes [79]. *E. ictaluri* isolates also contain species specific bacteriophages (φeiAU, φeiDWF, φeiMSLS) that are lytic, showing homogeneity despite isolation temporal and spatial divergence [85] as well as a large number of insertion elements and genomic islands [76,78].

Pathogenesis, pathology, clinical signs of disease and virulence

Pathogenesis mechanism of *E. ictaluri* has been elucidated in channel catfish, striped catfish, and Nile tilapia. Ports of E. ictaluri entry into susceptible hosts include the nares, oral-gastric route, gills and skin (Figure 1A). For ESC in channel catfish, the acute form seems to occur when *E. ictaluri* infects via the oral-gastric route, likely when channel catfish ingest infected carcasses, contaminated water or food [54,86,87]. Upon the bacterial attachment in the intestinal mucosa, intestinal epithelial cells are rapidly invaded, and the bacteria is translocated and systemic disseminated to the liver, spleen, and kidney, likely through infected macrophages [52,54,86]. The gills and skin are also primary sites for infection and systemic infection of lymphoid organs [88,89]. It seems that chronic infection happens when *E. ictaluri* infects channel catfish nares, colonizing the brain via the olfactory bulb and olfactory nerve [51,52,54]. After colonizing the brain, a systemic infection could occur [90]. The major clinical sign in channel catfish that appears 2-4 weeks post infection is the classic 'hole in the head' lesion, which is related to cartilaginous skull cap digestion caused by *E. ictaluri* chondroitinase activity [54,91]. Experimentally challenged channel catfish were shown to have reduced plasma components like erythrocyte, leucocyte counts, plasma glucose

levels [92]. Also, whole-blood components like hematocrit counts and hemoglobin concentration are reduced after *E. ictaluri* infection, mostly due to hemolysin activity [92,93]. It also has been reported the *E. ictaluri* persist in the posterior kidney, brain, and blood of surviving infected channel catfish fingerlings [87], suggesting that some individual might be able to resist the acute infection.

Interestingly, in striped catfish, experimental immersion challenge with *E. ictaluri* revealed that one of the ports of entry of *E. ictaluri* during pathogenesis are gills [48]. Another immersion challenge of striped catfish with *E. ictaluri* showed that the gastrointestinal tract is also a port of bacterial entry into the fish [94]. Edwardiellosis clinical signs in striped fish is different from channel catfish. Typically, striped fish exhibit external clinical signs such as skin lesions, pale gills and pale colour [8,57,94] but the classic 'hole in the head' lesion has not been reported. In experimentally challenged striped catfish, behavioral changes (e.g., gasping for air, lethargy, lack of appetite, erratic swimming) were observed as early as 4 hours post infection (hpi) whereas gross clinical signs were seen 96 hpi [39,94,183]. *E. ictaluri* bacterial cells were notably absent in the brain of BNP experimentally infected striped catfish although the bacteria were found in the other internal organs including head kidney, trunk kidney, liver, spleen, gills, skin and muscle [48,94]. Intracellular replication of *E. ictaluri* in macrophages was also elucidated in striped catfish and the pathogen could persist in necrotic-participating phagocytic cells and in melano-macrophage centers up to 1 month [48,94]. From their findings, Pirarat et al., suggested that *E. ictaluri* damages the endothelial cells leading to inflammation of the perivascular sheath and blood vessels and results in tissue hypoxia and necrosis [48].

Although *E. ictaluri* infection has been reported in tilapia, there are no reports of behavioral change or external clinical signs, but increased fish morbidity and mortality was reported [12,13]. Pathogenesis of *E. ictaluri* in tilapia was investigated by Soto et al., in 2013 [26]. As reported earlier, the port of entry of *E. ictaluri* for colonization into Nile tilapia is via the oral-gastric route and cutaneous routes. The bacteria are then disseminated hematogenously to organs such as gills, brain, head kidney, heart, and spleen. The spleen and head kidney are the main targets of infection and bacterial survival as shown by presence of high bacterial DNA levels and presence of clumps of rod-shaped bacteria in the organs [12,26]. Bacterial systemic dissemination is facilitated by antigen-presenting cells like macrophages [26].

Channel catfish and striped catfish suffering from *E. ictaluri* infection have been reported to display behavioral changes. Infected catfishes show erratic rapid circular swimming and spinning caused by meningoencephalitis as well as lethargy, listless up-side down hanging or slow swimming near pond edge [20,23,57,95]. *E. ictaluri* infected fish such as catfishes (channel, striped and yellow) and ayu, exhibit external gross clinical signs like skin haemorrhage and ulceration, distended abdomen, discoloration, reddened anus, exophthalmos and meningio-encephalitis (red head) (Figure 2) [96,23,24,57]. The general internal clinical signs reported in the susceptible hosts that include catfishes and tilapia and ayu are white nodules granulomas, abdomen ascites, pale gills, enlarged gallbladder, reddened gonads and enlarged and haemorrhagic posterior kidney (Figure 3) [12,23,39,96]. Both channel catfish and yellow catfish display classic 'hole in the head' lesion whilst yellow catfish additionally display the 'hole-under-the-jaw' lesion [24,97]. Histopathological examinations in most susceptible hosts revealed similar results such as granulomatous inflammatory reactions, necrosis, haemorrhage, pyknosis and karyorrhexis in internal organs, epithelial lining hyperplasia in gills and observation of clumps of rod shaped bacteria in tissues (Figure 4) [12,39,96,98]. Electron transmission microscopy also revealed the intracellular localisation of *E. ictaluri* in macrophage in infected zebrafish zebrafish head kidney tissue (Figure 5).

The molecular mechanisms of *E. ictaluri* pathogenesis were described in channel catfish and zebrafish using epithelial cells, phagocytic cells and macrophages and a graphical illustration is shown in Figure 1B. Pathogen attachment is facilitated by the recognition of *E. ictaluri* by host cell receptors e.g. Toll-like receptor 5 (TLR5) and (NOD)-like receptor subfamily C (NLRC) [99] and the help of *E. ictaluri* proteins Hcp2 [100], EseI and EseH [101]. For invasion, the plasmid encoded protein, EseI plays a role [101], and *E. ictaluri* enters into the target cells using Ca^{2+}-dependent receptor-mediated endocytosis and macropinocytosis [102,103]. Endocytosis of *E. ictaluri* into the epithelial cells is enabled when the polymerization of actin, manipulation of myosin components and apical junction complex (AJC) components are dysregulated [99,102,103]. This facilitates entry of the bacteria enclosed in an *Edwardsiella*-containing-vacuole (ECV) thereby protecting the bacteria from lysosomal degradation [99]. The ECV is acidified immediately by host cell vacuolar ATPases [104]. Consequently, intracellular survival of *E. ictaluri* is enabled by the upregulated expression of T3SS by the two-component regulatory proteins EsrA and EsrB [105] and the activity of the Type VI

Secretion System (T6SS) effector, Hcp2 [100]. Also, using urea that would have been produced by arginase enzyme from the host cell, the *E. ictaluri* acid-activated urease produces ammonia, which neutralizes the ECV acidic environment to a pH level (>pH 5.0). This creates an environment conducive for *E. ictaluri* replication and translocation of T3SS effectors (EseGHIJKLMNO) directly into the host cytoplasm [106,107]. These T3SS effectors interact with target host proteins to disrupt host defense mechanisms [105,106]. Conducive pH is then maintained by the prevention of phagosomal/lysosomal fusion, nutrients for bacterial growth and ECV enrichment are supplied by the Golgi and programmed cell death is suppressed [108]. Lysosomal acid hydrolases and reactive oxygen species production is downregulated by the T6SS effector, EvpP, indicating exploitation of the endosomal machinery thereby enabling intra-phagosome survival [99,100]. Lastly, inflammatory and immune responses are modulated with the putative aid of the EseN protein, for disease progression and then genes responsible for endocrine and growth are downregulated which may contribute to faltering growth [99]. It was also shown that *E. ictaluri* replicates intracellularly in macrophages [109] and can survive in fish organs up 65 days post infection [45].

Virulence and pathogenesis of *E. ictaluri* is facilitated by type III, IV and VI secretion systems that enable intracellular replication and survival in channel catfish [76,106,110-112]. Several investigations also demonstrate that *E. ictaluri* employs lipopolysaccharide (LPS), extracellular capsular polysaccharide, outer membrane proteins, adhesins and fibrillar processes for attachment to and survival in macrophages and host cells [113-116]. *E. ictaluri* requires flagella for motility [117], oligo-polysaccharide (O-PS) for modulation of host immune responses [118] as well as hemolysins and chondroitinase whose activities were mentioned earlier [91,93]. Pathogenesis of *E. ictaluri* is regulated by a number of mechanisms. Intracellular multiplication of *E. ictaluri* requires iron uptake and heme synthesis systems both under the regulation of the ferric uptake regulator (Fur) [119]. TonB is an important virulent factor that is required by *E. ictaluri* for TonB-mediated active transport of nutrients, especially iron, which is critical for survival of pathogenic bacteria during infection [120]. Urease activity is required for intracellular virulence and proliferation and is facilitated by pH increase due to production of ammonia. This probably neutralizes the acidic phagosome environment [104,107]. Pathogenesis of *E. ictaluri* is also promoted by stress-related genes that also enable survival of the pathogen in phagolysosomal conditions that are harsh [121]. Two

component regulatory system RstA/B and putative regulatory ribonuclease were shown to be important for regulation of invasion and adhesion, respectively [114].

Immune response to *E. ictaluri* experimental infections in catfish

Immune responses to *E. ictaluri* infections have only been documented in catfishes. An earlier review on the immune response of channel catfish to *E. ictaluri* infections stated that *E. ictaluri* triggers innate immune response, specific antibody-based humoral response and cell-mediated immunity [122]. Also, transcriptome analysis of differentially expressed immune response genes induced by *E. ictaluri* infections in channel catfish was carried out by numerous investigators and these are listed in a review by Zhou et al. [123]. On top of inducing immune responses in catfish, *E. ictaluri* was found to also increase alternative splicing of catfish genes. This facilitates the regulation of host gene expression with a subsequent increase in proteomic complexity, resulting in enhanced immune regulatory networks [124].

Numerous molecules related to the innate immune response of catfish infected with *E. ictaluri* were reported and are described in a review by Gao et., al [125]. *E. ictaluri*-infected channel catfish initially undergoes rapid physiological and metabolic responses known as acute phase response (APR) in the liver triggered by recognition of pathogen-associated molecular patterns (PAMPs) by Pattern recognition receptors (PRRs) [126]. These PRRs include Toll-like receptors such as TLR3, TLR5 and TLR21, that recognise flagellin, and LPS as well as activate systemic immunity [127-131]. The other PRRs involved in *E. ictaluri* infection are Peptidoglycan recognition proteins (PGRPs) that recognize bacterial cell wall and function in direct bacterial killing, and multiple signalling pathways regulation [132]. NOD-like receptors (NLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) were also identified which play a role in the recognition of cytosolic microbial components and trigger inflammatory responses [133,134]. Galectins that recognize surface exposed glycans and play key roles in inflammatory responses and apoptosis were also identified in channel catfish after *E. ictaluri* exposure [135].

Innate immune response molecules involved in antigen degradation that were found in *E. ictaluri*-infected channel catfish were antimicrobial peptides (AMPs) [136-140], cathepsins [141,142], Lysozymes [143], nitric oxide (NOS) [144], myeloperoxidase [145] and FOXO proteins that regulate the expression of

antimicrobial peptides [146]. The proteins phosphoinositide-3-kinase (PI3Ks) [147], transferrin, an acute response protein responsible for iron storage [148] and expression of tumour suppressor genes like PTEN that can induce elevated cytokines production in response to TLR agonists [149] were also upregulated in channel catfish in response to *E. ictaluri* infection. Phagocytosis of *E. ictaluri* can be enhanced by increased monocytes and neutrophils [150], septins [35] and lectins [151] while the alternative complement pathway plays a role in bacterial opsonophagocytosis [152].

Complement related genes such as C1r, C3, C5, C7, C9, and C1-INH were identified in *E. ictaluri*-infected darkbarbel catfish (*Pelteobagrus vachelli*) and are essential for linking innate to adaptive immune responses [153]. Immune regulators such as chemokines, cytokines in channel catfish and Cyclophilin A (CypA) in yellow catfish also play a role in inflammatory response and bridging innate to adaptive immunity after *E. ictaluri* infections [150,154,155-158] with the mediation of Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathway proteins [159]. Other innate immune response molecules produced channel catfish in response to *E. ictaluri* infection are annexins [160], Intelectins (IntL2) which probably plays an immune response downstream role [161]and apolipoproteins that modulates inflammatory response to LPS [162]. *E. ictaluri* infected channel catfish also mounts antioxidant defense mechanisms using stress response proteins like calreticulin and Hsp70 [163,164].

Channel catfish infected with *E. ictaluri* are able to mount protective T and B cell-dependent adaptive immunity [165,166]. IgM antibody is produced as humoral response to *E. ictaluri* in channel catfish [122]. Cell-mediated immune response was evidenced in resistant channel catfish family whereby macrophages formed aggregations in the posterior kidney and spleen [165,167]. On the other hand, channel catfish before 4 weeks old failed to mount a detectable immune response, even after two exposures to the pathogen, probably due to poorly differentiated primary lymphoid organs and tissues [168]. Leukocyte immune-type receptors (LITRs) were also found to play a role in cell-mediated immunity of channel catfish [169]. Catfish also utilize the major histocompatibility complex (MHC) class I as a cell-mediated defense mechanism against *E. ictaluri* in resistant blue catfish. In a study by Peatman et al. two different MHC class I alpha chains and beta-2-microglobulin (β_2m) were significantly upregulated in the *E. ictaluri* resistant blue catfish 3 days post *E. ictaluri* infection but not in channel catfish [170]. Moreover, Recombination-activating gene 2 (*rag 2*) was

detected in high quantities in the thymus and head-kidney of yellow catfish indicating a role in diversification of B and T cells via V(D)J (variable/diversity/joining) recombination [171]. Also, co-upregulation with pro-inflammatory cytokines implicated Rag 2 involvement in yellow catfish immune responses [171].

Disease diagnosis

Laboratory diagnosis of diseases caused by *E. ictaluri* is typically by first isolating the bacterium from the internal organs or brain tissue on culture media. Commonly used media include tryptic soy agar (TSA) or brain heart infusion (BHI) agar supplemented with 5% blood and selective morphology differentiating medium (*E. ictaluri* medium, EIM), that is inhibitive of most Gram-negative and Gram-positive bacteria [23,172]. A defined minimal medium was formulated that contains only 8 essential components instead of 46 and can sustain growth of *E. ictaluri* [173]. Subsequently, bacterial isolation is usually followed by biochemical tests using kits like Crystal™ or the API 20E [97] which distinguishes between *E. ictaluri* and *E. tarda*. Histopathology is then employed to diagnose based on microscopic cellular analysis [90,95,98]. Invasive techniques for identifying bacterial location in host tissues include i*n situ* hybridization, immunohistochemistry and radioisotope labeling [15,114,89,48]. *In vivo* bioluminescence imaging (BLI) was introduced by Karsi et al.,[174] for non-invasive identification of bacterial in host tissues. Identification of *E. ictaluri* was also carried out using MALDI-TOF (matrix-assisted laser desorption ionization–time-of-flight mass spectrometer) [175,176].

Confirmatory tests performed in identification of the bacterium are necessary for diagnosis of diseases caused by *E. ictaluri* and these include serology tests and molecular detection [177]. Serology tests used to confirm *E. ictaluri* infection include enzyme-linked immunosorbent assay (ELISA) such as a FAST-ELISA that rapidly detected antibodies to *E. ictaluri* exoantigen [178]; indirect ELISA using rabbit anti-catfish immunoglobulin and mouse anti-catfish immunoglobulin [179,180]; and modified ELISA using detergent coupled with filtration [181]. Enzyme immunoassay (EIA) to detect *E. ictaluri* in decomposing fish samples [182] and indirect fluorescent antibody technique (IFA) using either highly specific monoclonal antibodies [183] or antibody conjugated fluorochromes [184] were also employed. For detection of *E. ictaluri* in yellow catfish, a dot-enzyme linked immunosorbent assay (Dot-ELISA) and an indirect fluorescence antibody technique (IFAT)

with high specificity and sensitivity were designed [10]. Additional serology tests used in *E. ictaluri* diagnosis are passive hemagglutination, bacterial agglutination, microagglutination, complement-dependent passive hemolysis, agar gel immunodiffusion and indirect immunofluorescence [185,186].

The other confirmatory tests are based on molecular detection using Polymerase chain reaction (PCR). Generally, *E. ictaluri* was confirmed as the causative agent using amplification and sequencing of the 16S rRNA gene and the *gyrB* gene [12,187,188], *E. ictaluri*-specific PCR targeting upstream region of fimbrial gene [189] and IVS/IRS PCR assay method using primers targeting regions between the ribosomal DNA gene clusters, inter-ribosomal spacer (IRS) and 23S rRNA gene intervening sequence (IVS) [190]. Rapid PCR and a real-time PCR assay which could detect low levels of *E. ictaluri* in water, were also developed [188,191]. The molecular diagnostic loop-mediated isothermal amplification method (LAMP) which recognizes the *eip*18 gene was also used for *E. ictaluri* confirmation [192]. Application of OmniAmp DNA polymerase (Pol) in LAMP using lateral flow strips to detect *E. ictaluri* amplification was demonstrated as sensitive, rapid, and easy-to-use point-of-care (POC) method [193]. Recently, high-gradient immunomagnetic separation (HGIMS) coupled with PCR was also evaluated as a diagnostic tool with a higher detection sensitivity when compared with conventional PCR [194].

Disease management and limitations

Of importance to note is the fact that despite extensive research on *E. ictaluri* in aquaculture for a period spanning over 4 decades, the pathogen continues to be problematic in spite of efforts to prevent outbreaks. The widely adopted treatment strategies of ESC in channel catfish aquaculture include restricted feeding, administration of medicated feed and water chemical treatment [97,195]. However, the pitfalls of restricted feeding that can lead to production loss are that growth of fish can be reduced and careful monitoring of the water temperature is required [97]. Approved antibiotics for treatment of *E. ictaluri* infections are Romet ® (a 5:1 mixture of sulfadimethoxine and ormetoprim) and Aquaflor® (florfenicol) in the USA, enrofloxacin and florfenicol in Vietnam and doxycycline (DC) in China (Table 3) [29,196-199]. The constraints of using feed medicated with antibiotics are that the cost of antibiotics can be prohibitive to small scale farmers, also, there is emergence of antimicrobial resistant strains and the inefficient drug delivery via medicated feed because of loss of appetite in sick fish [29,200-202]. On the other hand, prevention of *E. ictaluri*

infection can be aided by avoiding stress in fish, use of chemicals, winter overfeeding, production of disease resistant hybrids and use of specific pathogen free (SPF) fish [40,97,203,204]. The limitation of stocking SPF fish in ponds where they can encounter *E. ictaluri* carriers is that very high mortalities occur in the naïve SPF fish therefore it is preferable to stock survivors from a previous outbreak that would have acquired immunity [97].

Vaccine formulations have been made using either bacterins or attenuated bacteria (Table 3). The early bacterin vaccines that were developed for the channel catfish reported high relative percent survival (RPS) values more than 90% under experimental laboratory trials but varying effectiveness under field conditions and did not provide long term acquired immunity [205,206]. The formalin killed vaccine also failed to protect the fish unless administered in Freund's complete adjuvant (FCA) [207], probably due to failure of killed *E. ictaluri* to invade the fish [89]. For striped catfish, two commercial inactivated vaccines, Alpha ject Panga 1 and Alpha Ject Panga 2 were licensed in Vietnam for prevention of BNP [208]. Alpha ject Panga 1 and 2 vaccines have reported high efficacy, where the mortalities of vaccinated striped catfish were reduced to 0-4.7% [209]. There are two patented attenuated vaccines available in the USA namely live attenuated *E. ictaluri* bacterium lacking the *evp*B gene (patent number US20170065695A1) [210] and AQUAVAC-ESC® (US Patent no. 6,019,981) that was attenuated by multiple passages in increased concentrations of rifampicin resulting in a mutant that is missing part of the O-lipopolysaccharide [211]. Other attempts at producing high efficacy live attenuated vaccines (Relative percent survival, RPS $\geq$ 66%) for both channel and striped catfish included the construction of *E. ictaluri* mutants of *wzz*E, *pur*A, *fhuC, aroA, crp* and *asdA* genes and a novobiocin attenuated *E. ictaluri* [200,212-217]. Another vaccine approach was the use of *E. ictaluri* bacterial ghosts (EIGs), generated by introduction of a plasmid that encodes the phage PhiX174 lysis gene *E*, that had an RPS of 89.3% in channel catfish [218]. Limited studies on subunit-based vaccines development and their efficacy against *E. ictaluri* have been carried out. Attempts to produce a subunit vaccines with promising results (RPS 62.5-95%) have been made using the *E. ictaluri* lipopolysaccharide in Freund's complete adjuvant [207] and *E. ictaluri* outer membrane proteins (OMPN1-3) [219], while five different *E. ictaluri* proteins including hypothetical protein (*ygg*E), specific inhibitor of chromosomal initiation of replication (*ici*A), ribose 5-phosphate isomerase (*rpi*A) and fructose 1,6-bisphosphate aldolase (*fda*) [220] provided inclusive results. We recently constructed a multi-epitope chimeric subunit vaccine (EiCh) that

provided partial protection in Nile tilapia with an RPS of 42% [221] Economic assessment of vaccination in catfish aquaculture in the US depicted that the practice could result in significant profits for the farmer around $71,758 to $133,887/400-ha per farm [222]. However, efficacy of the *E. ictaluri* vaccines under field conditions has not been entirely elucidated due to prohibitive costs and varied field efficacies with 41.9% of farmers the farmers reporting improved survival rates after vaccination and 37.5% of the farmers being unsure of vaccination efficacy thus posing a limitation in vaccine use [123,223].

Selective-breeding programs that have been implemented for resistance against *E. ictaluri* infections in aquaculture include a genetically improved channel catfish strain (NWAC103) that is a non-transgenically purebred produced after breeding fish with desired traits whereby the traits were identified using microsatellite loci identification method and DNA fingerprinting [224]. Also, selective genotyping and genome-wide association studies (GWAS) identified a microsatellite and quantitative trait locus (QTL) using interspecific backcross progenies, respectively, that confer *E. ictaluri* resistance in channel catfish [123,225,226] implying applicability of marker-assisted selection for disease resistance selective breeding. Although genetic selection was shown to enhance resistance against *E. ictaluri* [224], the method can also result in the genetically improved channel catfish strain being more susceptible to other pathogens (e.g., ictalurid herpesvirus, CCV) [97].

Dietary supplements such as vitamins, minerals, nutrients and glycans have been proven experimentally to enhance immune response of channel catfish but did not conclusively alter susceptibility to *E. ictaluri* infections [97,227-230]. In fact, Menhaden oil supplemented alone in fish feed was reported to increase susceptibility of catfish to ESC infection [231]. On the other hand, β-glucan enhanced protection of striped catfish from *E. ictaluri* infection [232]. The studies on the application of probiotics for growth enhancement and ESC resistance indicated that commercial probiotics supplemented in feed could neither enhance growth nor protect juvenile catfish [233], however, *Vibrio parahaemolyticus* and *E. coli* could protect zebrafish larvae [234] and *Bacillus pumilus* inhibited *E. ictaluri* in striped catfish [235]. Studies of effects of commercial prebiotics (mannan oligosaccharide, MOS) in channel catfish were encouraging as there was a significant increase in survival rate [236]. Essential oils in prevention of *E. ictaluri* infections also proved efficacious [237].

FUTURE PERSPECTIVES

Urgent need for more *E. ictaluri* sequenced genomes

Despite the knowledge that *E. ictaluri* has been isolated from 44 diverse hosts, only 11 sequenced genomes exist in public database. From literature, we already know that the species is composed of host specific genotypes and members of the species are biochemically, antigenic, and serological heterogenous [62,64,67,79]. This implies genomic variations among the isolates and a deeper understanding can only be achieved by sequencing more host specific isolates and conducting comparative genomic studies. Most of the groundwork in aquaculture disease studies are being accomplished with whole genome sequencing and comparative genomics. This provides valuable information on host-pathogen relationships, pathogen evolution, niche adaptation and pathogenicity [238-243]. Also, potential universal vaccine candidates and drug targets towards different genotypes can be developed using reverse vaccinology based on identified antigenic proteins [244].

Grassroot capacity building

The primary tool in combating spread of *E. ictaluri* that need to be implemented sooner rather than later is capacity building at grassroot level of mainly the farmers as well as technical personnel. These key players should be educated in proactive programs like awareness on *E. ictaluri* infections in aquaculture, good aquaculture practices, preventative measures, and management of fish health. Also, they should be educated in reactive strategies like remedial action, timeous reporting in epizootics and performing simple diagnostic procedures [245]. Since training is usually costly, the participation at government and international level is greatly anticipated to help fund such programs [246]. To address the need for timeous pathogen identification, early forecast of disease outbreak and disease diagnosis, the concept of point-of-care (POC) methods was suggested whereby simple diagnostic methods can be carried out at farm-level using portable devices like real-time polymerase chain reaction (PCR) device, MinIon devices for DNA/RNA sequencing (Oxford Nanopore Technologies, Oxford, UK) and lateral flow strips [193,247]. These approaches can facilitate bio-surveillance but however, need to be coupled with remedial strategies for effective and efficient control of *E. ictaluri*.

Biosecurity Measures

Movement of live fish for aquaculture usually contributes to movement of pathogens. Transboundary importation of *E. ictaluri* was implicated in Trinidad and Tobago and Australia where outbreaks occurred during quarantine of imported fish [57,248]. This calls to attention the need for policy makers to enforce stricter biosecurity at national and local levels. The biosecurity measures should include disease surveillance using rapid, highly accurate diagnostic tools and self-quarantine in closed system for imported animals at farm level. This will assist in preventing pathogen spread and development of control strategies [249]. Moreover, it will be beneficial to use genetically modified fish for *E. ictaluri* resistance coupled with strict biosecurity at the farms to prevent and contain epizootics [247]. It is imperative to perform Import risk analysis (IRA) including passive and active surveillance both for wild and farmed fish to prevent pathogen spread to new hosts and geographical locations [250].

Alternatives to antibiotic and chemical use

On top of the antibiotic alternatives already researched against *E. ictaluri* mentioned above such as vaccines, prebiotics, probiotics, essential oils and feed supplements, there are yet other therapies that remain unexplored. These include use of bacteria capable of disrupting quorum sensing molecules and phage therapy [251]. In aquaculture, bacteria such as *Bacillus, Halobacillus salinus* and *Actinobacteria Streptomyces albus* have been identified as biocontrol agents due to their ability to quench pathogen quorum sensing system for bacteria like *Vibrio sp.* and *Aeromonas hydrophila* thereby increasing fish survival after challenge [251]. Quorum sensing therapy can be enhanced by using Biofloc technology whereby extra carbon is added to pond water resulting in improved growth of biocontrol agents *in situ* [252]. Bacteriophages are known for their therapeutic properties by inhibiting pathogens in single doses without reported side effects [253]. For the control and inhibition of *E. ictaluri* using phage therapy, a patent exists of 2 bacteriophages, ΦeiAU and ΦeiDWF [254] but extensive use and efficacy is still to be reported Hence, these alternative to antibiotics therapies can help in the control of *E. ictaluri* to curb antimicrobial resistance (AMR).

An emerging ozone nanobubble technology has been reported to be effective in reducing pathogen concentration in water and its safety in marine and aquaculture species was also exhibited. This technique entails injection of ozone created nanobubbles (NB-O_3) into water with various salinity

and results in up to 99.27 % concentration reduction of pathogen such as *Streptococcus agalactiae* or *Aeromonas veronii* after 3 treatments of fish-cultured water [255]. Safety was established for Nile tilapia (*Oreochromis niloticus*), sea urchins *(Strongylocentrotus intermedius*) and sea cucumbers (*Apostichopus japonicas*) [255,256]. Ozone nanobubble treatment also modulates the fish immune system to fight infection more effectively [257]. Application of ozone nanobubble technology in disinfection against *E. ictaluri* might be a feasible approach that could contribute to reducing chemical disinfectants and antibiotics use thereby reducing AMR and negative impact to the environment.

Application of genomics in disease control

Improved disease resistance in aquaculture production has been greatly enhanced by application of genome-based biotechnologies which can also help in managing and controlling *E. ictaluri* infections. Metagenomic analysis have been employed to study microbiomes to monitor fish health indices, aquatic environments safety and susceptibility of skin invasion by microbes [258]. By applying whole genome sequencing coupled with *in vivo* induced antigen technology (IVIAT) and tandem mass tag (TMT) labelling-based quantitative proteomics, immunogenic proteins have been identified for vaccine production [259,260]. The other important application of genomics to *E. ictaluri* control would be the editing of host species genomes by manipulating disease-resistance genes with techniques such as zinc finger nucleases (ZFNs), clustered regularly interspaced short palindromic repeats (CRISPRs)-CRISPR-associated protein 9 (Cas9) and transcription activator-like effector nucleases (TALENs) [261]. Most of these techniques have already been applied to channel catfish but application on other susceptible hosts and investigations of the consequences from the induced mutations are yet to be carried out.

Selective breeding for disease resistant traits

Although a number of trials in selective breeding and even a patented selectively bred strain of channel catfish strain (NWAC103) was reported, there are technological advances that has been made that can be applied not only to channel catfish but to all susceptible hosts. One such advance is Genomic Selection (GS) whereby genomic estimated breeding values (GEBV) are calculated based on marker-assisted selection such as single-nucleotide polymorphisms (SNPs) using a genotyped and phenotyped 'training population' that will provide the next generation parents with

desirable traits such as disease resistance [262]. Furthermore, the introgression technique can be applied to introduce and transfer disease resistance genes to a population through backcrossing and marker-assisted selection repeatedly [263]. The technique has been applied in Rainbow trout (*Onchorhynchus mykiss*) for inferring resistance against bacterial cold water disease (BCWD) [264], and columnaris resistance in channel catfish [265]. Control of *E. ictaluri* can also be achieved by identification of *E. ictaluri* resistance traits in host species and production of specific pathogen resistant (SPR) fish species. SPR is a qualitative trait where the fish is resistance to a particular pathogen [266] and in aquaculture the application of SPR species has been implimented in shrimp culture where in the USA, commercial SPR *Litopenaeus vannamei* with resistance to Taura syndrome virus (TSV) are available [267].

Trained Innate Immunity

It is crucial to stimulate protective immunity in fish before they reach the susceptible stages mainly fry, fingerlings and juvenile stages (Table 1). The stimulation of defenses of the innate immunity resulting in enhanced non-specific resistance against pathogens is what is termed trained innate immunity and can be transferred vertically from brood stock or used to prime fish at the larval stage [268]. The innate immune cells e.g., macrophages, natural killer cells and monocytes undergo epigenetic reprogramming when they encounter a pathogen thereby acquiring immunological memory resulting in enhanced clearance of the pathogen upon a subsequent encounter [269]. Pattern recognition receptors (Toll-like receptors, C-type lectin receptors, RIG-1-like receptors, NOD-like receptors (NLRs) and scavenger receptors) are stimulated by ligands such as flagellin, ß-glucan, CpG containing oligodeoxynucleotides and muramyl dipeptide resulting in trained innate immunity [268]. Evidence of trained innate immunity of fish by administration of ß-glucan was reviewed by Petit &Wiegertjes [270] and when channel catfish were injected with ß-glucans, phagocytic and bactericidal abilities were enhanced as well as reduced mortality [271]. This evidence proves the potential of priming the trained innate immunity of fish especially catfish to fight against *E. ictaluri*.

Concluding remarks

Despite efforts that have been made to control or manage infections, new susceptible hosts and evidence of spread in new geographical locations keep on being reported. Research on this

pathogen is lacking in areas that include available whole genomes, serotyping scheme and bio-surveillance programmes, universal vaccines against all genotypes and selective breeding for resistant host species. It is important to prioritise research on whole genome sequencing of all genotypes from all host species as this will enable a deeper understanding of the pathogen which is instrumental in understanding host-pathogen interactions, bacterial evolution vaccine development via reverse vaccinology. Implementation of increased biosecurity measures, use of genetically modified and selective bred fish species can help avoid spread of the *E. ictaluri* into new territories and facilitate pathogen management and control. To counter antimicrobial resistance, there is need for new alternative to antibiotics through the use biocontrol agents and technologies such as Biofloc technology and ozone nanobubble. This review provided comprehensive current knowledge of *E. ictaluri* infection in aquatic animals with special reference to aquaculture susceptible hosts and future perspective on disease management.

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1562 Table 1. *E. ictaluri* hosts, distribution, and occurrence

Host family	Host species	Geographical location	Occurrence	Affected fish stage	Mortality	Reference
Ictaluridae	*Ictalurus punctatus*	USA	Natural infection	fingerling	100% (experimental challenge)	20
	Ictalurus furcatus	USA	Experimental infection	fingerling	0.7% (natural infection)	272
	Ameiurus catus	USA	Natural infection	Information not available	Information not available	55
	Amieurus nebulosus	USA	Natural infection	mixed sizes	35 to 40% (natural infection)	61
	Noturus gyrinus	USA	Natural infection	juvenile	Not reported	273
Bagridae	*Pelteobagrus fulvidraco*	China	Natural infection	Juvenile-adult	50% (natural infection)	16
	Pelteobagrus nudiceps	Japan	Natural subclinical infections	Not specified	100% (experimental challenge)	33
	Pelteobagrus vachelli	China	Experimental infection	Juvenile	26-62% (natural infection)	153
	Tachysurus tokiensis	Japan	Experimental infection	Juveniles	100% (natural infection)	274
Clariidae	*Clarias batrachus*	Thailand	Natural infection	Not specified	Not reported	275
	Clarias macrocephalus x Clarias gariepinus	Thailand	Natural infection	Not specified	100% (experimental challenge)	276
Pangasiidae	*Pangasianodon hypophthalmus*	Thailand	Natural concurrent infection	juvenile	80% (experimental challenge)	15
	Pangasianodon hypophthalmus	West Indies	Natural infection	juvenile	approximately 2000 animals	57
	Pangasius hypophthalmus (Sauvage)	Vietnam	Natural infection	Not specified	Not reported	22
	Pangasius hypophthalmus (Sauvage)	Indonesia	Natural infection	fingerlings and immature fish	50 to 100% (natural infection)	17
	Pangasius pangasius	Indonesia	Natural infection	Young adults	95% (natural infection)	277
Plecoglossidae	*Plecoglossus altivelis*	Japan	Natural infection and experimental	Fingerlings-adult	100% (experimental challenge)	25
Siluridae	*Silurus asotus*	Japan	Experimental infection	fingerlings, juveniles	100% (natural infection)	33
	Silurus soldatovi meridionalis	China	Natural infection	Juveniles	60% (natural infection)	187
	Silurus glanis	USA	Experimental infection	Juveniles	80% (natural infection)	278
	Ompok bimaculatus	Thailand	Experimental infection	Fingerlings	2.5%-100% (natural infection)	279
Plotosidae	*Anodontiglanis dahli*	Australia	Natural infection	Not specified	Not reported	248
	Neosilurus ater	Australia	Natural infection	Not specified	Not reported	248
	Tandanus tropicanus	Australia	Natural infections	Not specified	Not reported	175
Ariidae	*Neoarius berneyi*	Australia	Natural infection	Not specified	Not reported	248
Sternopygidae	*Eigenmannia virescens*	USA	Information not available	Information not available	Information not available	55,280

Host family	Host species	Geographical location	Occurrence	Affected fish stage	Mortality	Reference
Cyprinidae	*Danio rerio*	USA	Natural infection	adult	19% (natural infection)	281
	Danio devario	USA	Natural infection	Not specified	100% (experimental challenge)	282
	Puntius conchonius	Australia	Natural infection	Not specified	40% (natural infection)	283
	Zacco platypus	Japan	Experimental infection	Not specified	15% (experimental challenge)	33
	Tribilodon hakonensis	Japan	Experimental infection	Fingerlings	40% (natural infection)	274
	Tribolodon brandtii maruta	Japan	Natural infection	Not specified	Not reported	284
	Candidia temminckii	Japan	Natural infection	Not specified	Not reported	284
	Hemibarbus barbus	Japan	Natural infection	Not specified	Not reported	284
	Rhynchocypris lagowskii	Japan	Natural infection	Not specified	Not reported	284
	Scardinius erythrophthalmus hesperidicus H.	Croatia	Natural infection	Juveniles	Not reported	285
Cichlidae	*Sarotherodon aureus*	USA	Experimental infection	fingerlings	70% (experimental challenge)	53
	Oreochromis niloticus	West Indies	Natural infection	fry and fingerlings	100% (experimental challenge)	13
	Oreochromis spp.	Vietnam	Natural infection	juveniles	40–50% (natural infection)	12
Salmonidae	*Oncorhynchus tshawytscha*	USA	Experimental infection	Juveniles	75% (experimental challenge)	286
	Oncorhynchus mykiss	Turkey	Natural infection	juveniles	100% (experimental challenge)	287
Moronidae	*Morone americana*	USA	Experimental infection	Information not available	100% (experimental challenge)	288
	Dicentrarchus labrax†	Spain	Natural infection	fry	90% (experimental challenge)	289
Anguillidae	*Anguilla japonica*	Japan	Experimental infection	Fingerlings	10% (natural infection)	274
Percichthyidae	*Coreoperca kawamebari*	Japan	Natural infection	Not specified	Not reported	284
Balaenopteridae	*Balaenoptera acutorostrata*	Japan	Natural infection	Not specified	Not reported	37
Pleuronectidae	*Platichthys stellatus*	China	Experimental infection	Juveniles	Not reported	290

†-*Edwardsiella ictaluri*-like infection

1564 Table 2. Timeline of *Edwardsiella ictaluri* isolations from natural infected fish including the biochemical characteristics.

Year of isolation	**Country**	**Fish host**	**Biochemical characteristics**											**Reference**
			Motility	Nitrate reductase	Catalase	Ornithine decarboxylase	Lysine decarboxylase	Cytochrome oxidase	NaCl >1.5%	Gas/acid from glucose	Methyl Red test	H_2S production	Urease	Citrate	
1979	USA	channel catfish	+	+	N/R	+	+	-	NR	+/NR	+	-	-	-	20
1981‡	USA	white bullhead	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	55
1982‡	USA	green knife fish	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	280
1983	USA	danio	+	+	NR	+	+	-	NR	+/NR	+	-	-	-	282
1985 ‡	Thailand	walking catfish	+	NR	NR	+	+	-	NR	NR/+	NR	-	-	+	275
1985	Australia	rosy barb	-	NR	+	+	+	-	NR	+/+	-	-	-	-	283
1989	Spain	sea bass	+	+	+	-	+	-	+	+	-	-	-	-	289
2001 ‡	Vietnam	striped catfish	+	-	-	-	-	+	NR	-/-	NR	+	-	NR	22
2002	USA	tadpole madtom	NR	NR	NR	NR	+	-	NR	+	NR	NR	NR	NR	273
2004 ‡	Turkey	rainbow trout	-	+	+	+	+	-	NR	NR/+	+	-	-	-	287
2004	USA	brown bullhead	+	+	+	+	+	-	NR	-/NR	-	NR	NR	-	61
2006	China	yellow catfish	+	+	+	-	+	-	-	+/+	-	-	-	-	16
2007	Japan	ayu	+	NR	+	+	+	-	-	+/NR	+	+	NR	-	25
2008-2010	Japan	Forktail bullhead	+	+	+	+	+	-	-	NR/+	+	+	-	-	33
2010-2011	West Indies	Nile tilapia	NR	NR	NR	-	+	-	NR	+	NR	-	-	-	13
2011	China	southern catfish	+	+	+	+	+	-	NR	+/+	-	-	+	NR	187
2011	USA	zebrafish	+	NR	NR	NR	NR	-	NR	+	NR	-	NR	+	281

1565

Year of isolation	Country	Fish host	Biochemical characteristics												Reference
			Motility	Nitrate reductase	Catalase	Ornithine decarboxylase	Lysine decarboxylase	Cytochrome oxidase	NaCl >1.5%	Gas/acid from glucose	Methyl Red test	H_2S production	Urease	citrate	
2011	Australia	toothless catfish narrowfront tandan Berney's catfish	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	[291]
2011-2012	Japan	Pacific redfin dark chub Japanese barbel Amur minnow Japanese aucha perch	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	[284]
2014 ‡	Thailand	hybrid catfish	+	NR	+	-	+	-	NR	NR/+	NR	-	-	+	[276]
2016 ‡	Australia	eeltail catfish/ tandan	+	+	NR	+	+	-	+	V/NR	+	NR	-	-	[175]
2016	Vietnam	red hybrid tilapia	NR	NR	+	-	+	-	NR	NR/+	NR	-	-	V	[12]

‡ represents publication year where year of isolation was not specified.

V-variable

NR-not reported.

NA-data not available

1578 Table 3. Summary of antibiotic and vaccines used in aquaculture against *E. ictaluri.*

Method	Type	Description	Delivery route	Fish species (age)	Efficacy (survival rate)	Reference
Antibiotics	Sulfonamide	Romet-30™	Oral	Channel catfish (fingerlings)	Up to 89.1%	[198]
	florfenicol	Aquaflor®	Oral	Channel and striped catfish (fingerlings)	Up to 100%	[196]
	Enrofloxacin		Oral	Striped catfish (fingerlings)	Not reported	[197]
	Doxycycline		Oral	Yellow catfish (fingerlings)	Not reported	[199]
Vaccines	Live attenuated bacteria	*evp*B gene mutant (patent number US20170065695A1)	Immersion, injection, oral or combination	Channel catfish (Fry/fingerlings)	80.83%-92.58%	[210]
		AQUAVAC-ESC® (US Patent no. 6,019,981)	immersion	Channel catfish (Fry/fingerlings)	Up to 94.7%	[211]
	Inactivated bacteria	Alpha ject Panga 1 and 2	injection	Striped catfish (fingerlings)	95.3-100%	PHARMAQ Vietnam (https://www.pharmaq.no/sfiles/1/58/4/file/pharmaq-vn-handout_2013-2-_lighter-version.pdf)

1579

Figures and legends

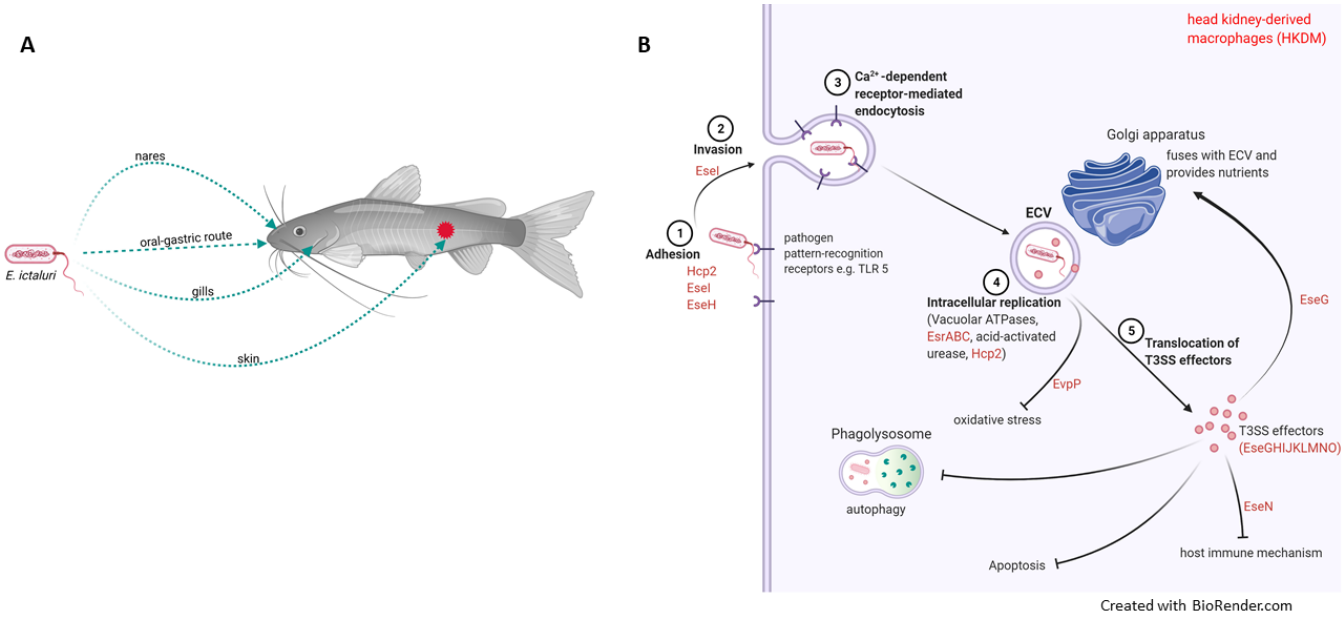

Figure 1. Pathogenesis of *E. ictaluri*. A) Ports of entry into the host fish used by *E. ictaluri* during infection. B) molecular mechanisms of *E. ictaluri* pathogenesis into host cells e.g. macrophage.

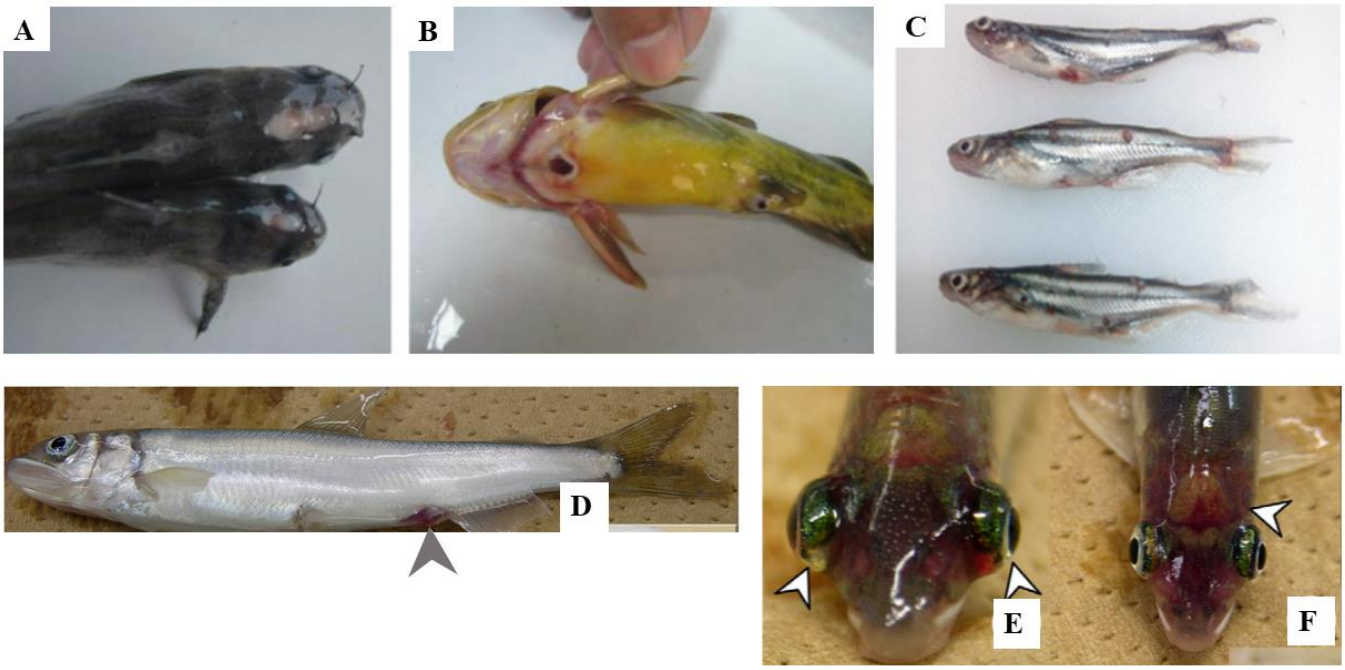

Figure 2. Examples of gross external clinical signs of natural *E. ictaluri* infections in fish hosts. Channel and yellow catfish exhibit 'Hole in the head' lesion (A). Yellow catfish also present 'hole under the jaw' lesion (B). Striped catfish exhibit haemorrhage and ulceration on the skin (C). Ayu exhibits distended abdomen with reddened anus (D), exophthalmos (E) and meningio-encephalitis (red head) (F) shown by arrowheads. Images A) and (B) reproduced with permission granted © 2010 The Authors. Aquaculture Research © 2010 Blackwell Publishing Ltd. Image (C) reproduced with permission granted © 2016 John Wiley & Sons Ltd. Images (D), (E), (F) reproduced with permission granted © 2020 Wiley Periodicals LLC.

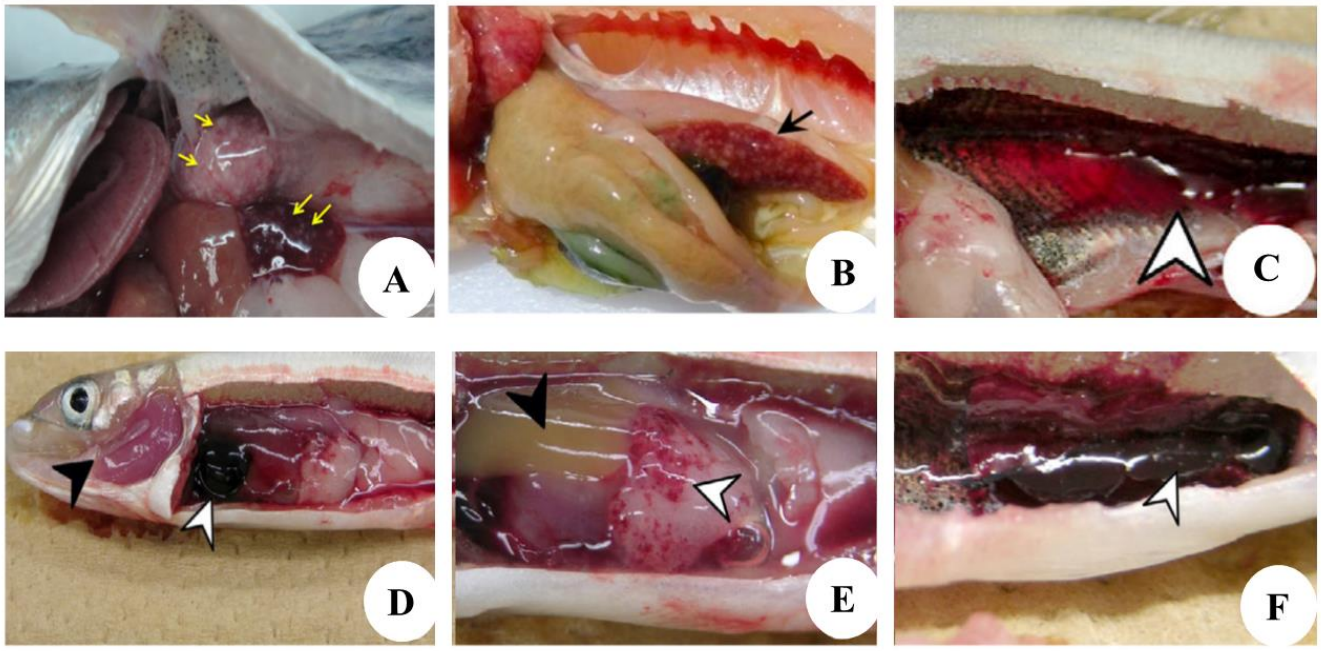

Figure 3. Examples of gross internal clinical signs of natural *E. ictaluri* infections include mottled spleen and anterior kidney indicated by yellow arrows in striped catfish (A), pale liver and mottled spleen and kidney in tilapia indicated by black arrow (B), and in ayu; bloody ascites in peritoneal region (C), pale gills and a gallbladder that is enlarged (D), reddened gonads (E) and posterior kidney that is enlarged and haemorrhagic (F) all indicated by arrowheads. Images A) reproduced with permission granted © 2020 John Wiley & Sons Ltd. Image (B) Reprinted from Aquaculture Volume 499/15, Dong et al., Natural occurrence of edwardsiellosis caused by *Edwardsiella ictaluri* in farmed hybrid red tilapia (*Oreochromis* sp.) in Southeast Asia, Pages 17-23, Copyright (2019), with permission from Elsevier. Images (C), (D), (E), (F) reproduced with permission granted © 2020 Wiley Periodicals LLC.

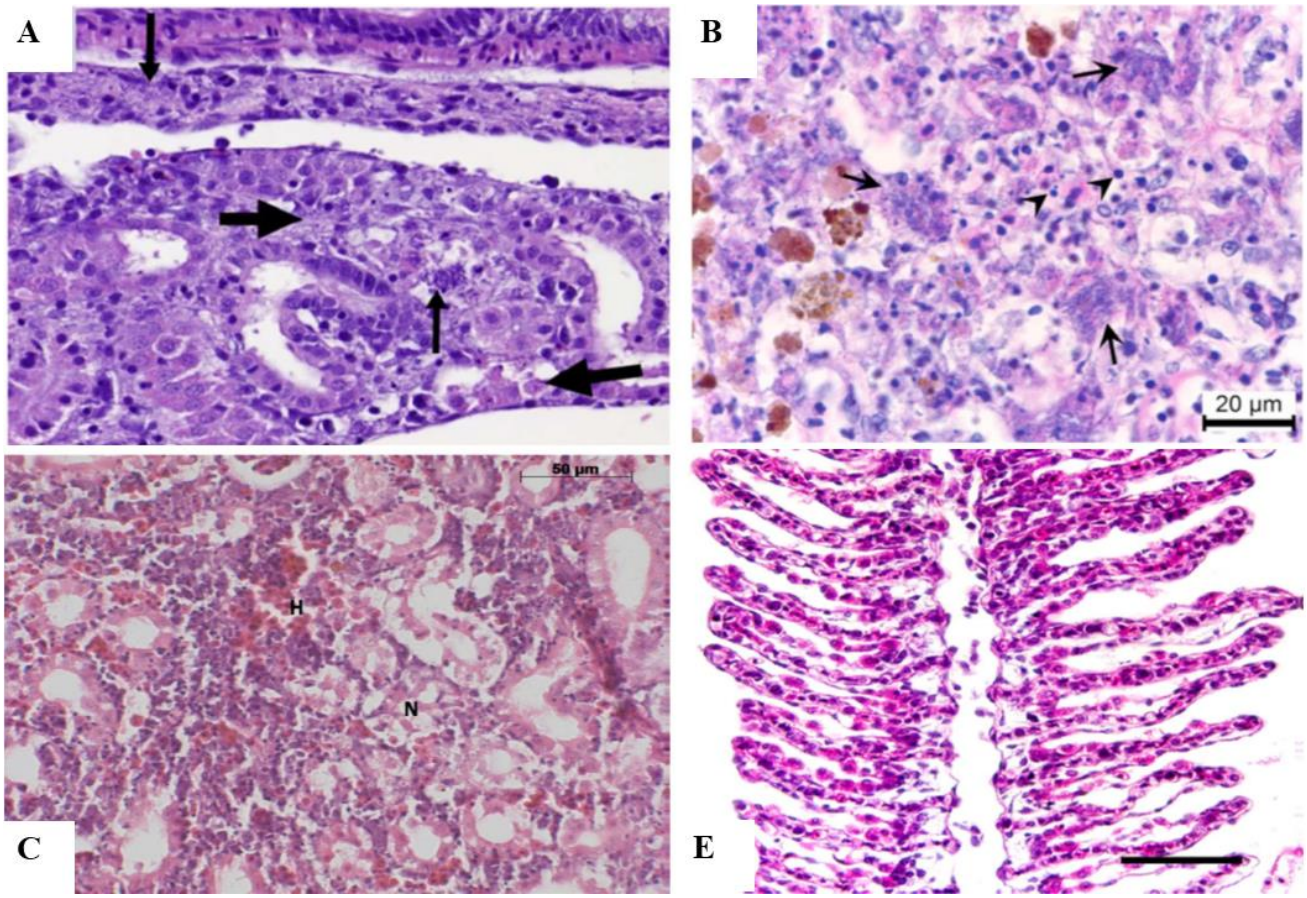

Figure 4. Typical histopathological findings. **(A)** in channel catfish fry, diffuse necrosis of the hematopoietic tissues (arrows) was identified. **(B)** in red hybrid tilapia, there was spleen and cell pyknosis and karyorrhexis (arrow heads). **(C)** in striped catfish kidney, histopathology showed necrosis (denoted by N) and haemorrhagic areas (denoted by H). **(D)** in the gills of ayu, epithelial lining hyperplasia was evident at base of secondary gill lamellae together with in-between separation of the underlying capillary bed from the epithelial cell lining of secondary gill lamellae. Image (A) Reprinted from Fish and Shellfish Immunology Volume 72, Abdelhamed et al., The virulence and immune protection of *Edwardsiella ictaluri* HemR mutants in catfish, Pages 153-160, Copyright (2018), with permission from Elsevier. Image (B) Reprinted from Aquaculture Volume 499/15, Dong et al., Natural occurrence of edwardsiellosis caused by *Edwardsiella ictaluri* in farmed hybrid red tilapia (*Oreochromis* sp.) in Southeast Asia, Pages 17-23, Copyright (2019), with permission from Elsevier. Image (C) reproduced with permission granted © 2020 John Wiley & Sons Ltd. Image (D) reproduced with permission granted © 2020 Wiley Periodicals LLC.

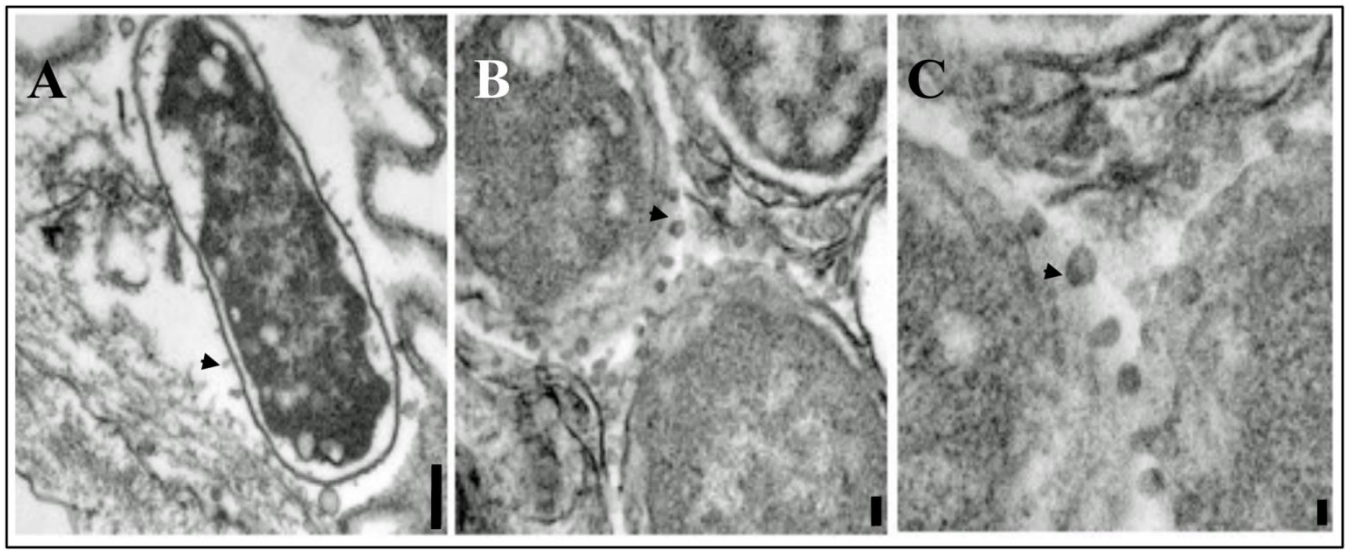

Figure 5. Transmission electron microscopy intracellular visualization of E. ictaluri of infected zebrafish head kidney. The tissue samples were taken 6 h post-infection (105 CFU dose^{-1}). (A) Intracellular E. ictaluri in infected zebrafish head kidney macrophage (Scale bar = 0.5 μm). (B) Magnification of transverse sectioned intracellular E. ictaluri in infected zebrafish head kidney macrophage (Scale bar 0.5 = μm). (C) High magnification of cross-sectioned TEM images of intracellular E. ictaluri membrane (Scale bar = 0.2 μm). Arrowheads indicate outer membrane vesicles-like (Images kindly provided by Dr. Santander).