

This is the peer reviewed version of the following article: Machimbirike, VI, Crumlish, M, Dong, HT, Santander, J, Khunrae, P, Rattanarojpong, T. *Edwardsiella ictaluri*: A systemic review and future perspectives on disease management. *Reviews in Aquaculture* 2022; 14: 1613-1636, which has been published in final form at <https://doi.org/10.1111/raq.12665>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.

1 ***Edwardsiella ictaluri*: a systemic review and future perspectives on disease** 2 **management**

3 Vimbai Irene Machimbirike^{a,d*}, Margaret Crumlish^b, Ha Thanh Dong^c, Javier Santander^d, Pongsak
4 Khunrae^a, Triwit Rattanarojpong^a

5 ^aDepartment of Microbiology, Faculty of Science, King Mongkut's University of Technology
6 Thonburi (KMUTT), Bangkok, 10140, Thailand

7 ^bInstitute of Aquaculture, University of Stirling, Stirling, UK

8 ^cSchool of Environment, Resources & Development, Asian Institute of Technology, Pathum
9 Thani, 12120, Thailand

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

12

13 *Corresponding author: V. I. Machimbirike

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

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

18 Tel: +66957745871

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

20 **Running title:** *Edwardsiella ictaluri* review

21

22 **Data Availability Statement**

23 The authors declare that they do not have any shared data available.

24

25 **Funding statement**

26 V. I. Machimbirike was supported by the Petchra Pra Jom Klao Ph.D. scholarship for international
27 students, King Mongkut's University of Technology Thonburi, Thailand (KMUTT).

28

29 **Conflict of interest disclosure**

30 The authors declare no conflict of interest.

31

32 **Permission to reproduce material from other sources**

- 33 • Images 1(A) and 1(B) reproduced with permission granted © 2010 The Authors.
34 Aquaculture Research © 2010 Blackwell Publishing Ltd. Image 1(C) reproduced with
35 permission granted © 2016 John Wiley & Sons Ltd. Images 1(D), 1(E), 1(F) reproduced
36 with permission granted © 2020 Wiley Periodicals LLC.
- 37 • Images 2(A) reproduced with permission granted © 2020 John Wiley & Sons Ltd.
38 Image 2(B) Reprinted from Aquaculture Volume 499/15, Dong et al., Natural occurrence
39 of edwardsiellosis caused by *Edwardsiella ictaluri* in farmed hybrid red tilapia
40 (*Oreochromis* sp.) in Southeast Asia, Pages 17-23, Copyright (2019), with permission from
41 Elsevier. Images 2(C), 2(D), 2(E), 2(F) reproduced with permission granted © 2020 Wiley
42 Periodicals LLC.
- 43 • Image 3(A) Reprinted from Fish and Shellfish Immunology Volume 72, Abdelhamed et
44 al., The virulence and immune protection of *Edwardsiella ictaluri* HemR mutants in
45 catfish, Pages 153-160, Copyright (2018), with permission from Elsevier. Image 3(B)
46 Reprinted from Aquaculture Volume 499/15, Dong et al., Natural occurrence of
47 edwardsiellosis caused by *Edwardsiella ictaluri* in farmed hybrid red tilapia
48 (*Oreochromis* sp.) in Southeast Asia, Pages 17-23, Copyright (2019), with permission from
49 Elsevier. Image 3(C) reproduced with permission granted © 2020 John Wiley & Sons Ltd.
50 Images 3(D) and 3(E) reproduced with permission granted © 2020 Wiley Periodicals LLC.
51 Images 4(A), 4(B) and 4(C) reproduced with permission granted from Dr. Javier
52 Santander.

53 **Abstract**

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

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

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

71

72 **Introduction**

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

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

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

99 **Pathogen discovery, susceptible hosts, geographical distribution, and epidemiology**

100 The first report on the isolation of *E. ictaluri* was by Hawke in 1979²⁰. *E. ictaluri* was identified
101 as the causative agent of enteric septicemia of catfish (ESC), primarily infecting fingerlings of
102 channel catfish (*Ictalurus punctatus*) in the United States of America (USA) aquaculture industry
103²⁰. However, it was later discovered that ESC was already present in Arkansas a decade before the
104 first official report using archived samples²¹. After the initial report in the USA in 1979 in channel
105 catfish, *E. ictaluri* has been identified in several continents, for instance in Asia it is the frequent
106 causative agent of bacillary necrosis of Pangasius (BNP)²² and red-head disease¹⁰ in striped and
107 yellow catfish, respectively.

108
109 *E. ictaluri* is a fish-host generalist infecting up to 44 fish species, of which 31 species are naturally
110 infected and 13 species were experimentally infected as shown in Table 1. A total of seven catfish
111 families have been described to be susceptible to *E. ictaluri*, including Ictaluridae, Bagridae,
112 Clariidae, Pangasiidae, Ariidae, Siluridae and Plotosidae. For non-catfish species, 10 fish families
113 are susceptible including Plecoglossidae, Sternopygidae, Cyprinidae, Cichlidae, Salmonidae,
114 Moronidae, Anguillidae, Percichthyidae, Balaenopteridae and Pleuronectidae. To date, there are
115 several documented isolations of *E. ictaluri* in several continents that include North America,
116 Caribbean, Asia, Australia, and Europe with mortalities reaching 100% (Table 1). A timeline of
117 *E. ictaluri* isolation in different host species and geographical locations is described in Table 2.
118 Even though up to 44 susceptible fish hosts have been reported, *E. ictaluri* predominantly affects
119 intensively reared channel catfish and striped catfish in USA and Vietnam^{8,23}, respectively, yellow
120 catfish (*Pelteobagrus fulvidraco*) in China^{10,24}, and riverine ayu (*Plecoglossus altivelis*) in Japan
121²⁵. Most of the available literature on *E. ictaluri* is related to these 4 hosts and only 3 articles
122 describe *E. ictaluri* infections in tilapia culture^{12,13,26}.

123

124 In the United States, epizootics in channel catfish are mainly experienced during late spring and
125 early fall whereby acute ESC is usually experienced when temperatures are between 22 °C and 28
126 °C and chronic ESC usually occur when temperatures are cooler in the range of 18°C-22 °C or
127 above 28 °C^{27,28}. On the other hand, epizootics in striped catfish and tilapia in Southeast Asia are
128 experienced during the rainy season when temperatures range from 23 to 30 °C where only an
129 acute form is exhibited^{12,14,15,29}. Studies have shown that the peak mortality of channel catfish
130 from *E. ictaluri* is experienced at 25 °C^{30,31} and hypoxia results in increased bacterial load in
131 channel catfish tissues³². Environmental persistence studies of *E. ictaluri* using specific
132 bacteriophages suggested that *E. ictaluri* can survive for up to 15 days in pond water and up to 95
133 days in pond sediments, implicating that water and mud could be *E. ictaluri* reservoirs^{33,34}. *E.*
134 *ictaluri* has also been shown experimentally to produce biofilms on common aquaculture material
135 which might be a reservoir for recurrent epizootics and contributes to disinfectant resistance³⁵. So
136 far *E. ictaluri* has not yet been implicated in zoonosis and this might be because *E. ictaluri* is not
137 capable of growth at 37 °C³⁶. Nevertheless, *E. ictaluri* was isolated from the mammal minke
138 whale (*Balaenoptera acutorostrata*) excrement³⁷. Although *E. ictaluri* infections can occur
139 independently of stressors and still cause high mortalities of up to 77%, stressors such as handling,
140 adverse environmental conditions and stocking density greatly enhance mortalities up to 97%³⁸⁻
141⁴⁰. A recent epidemiological survey on environmental factors that influence *E. ictaluri* infection in
142 riverine ayu was conducted in Japan over a five-year period. The survey revealed that *E. ictaluri*
143 related mortalities in ayu are exacerbated by adverse environmental conditions that include an
144 increase in diurnal water temperature range (DWTR), high water temperatures, higher than normal
145 air temperatures and lower levels of streamflows⁴¹.

146
147 Naturally, *E. ictaluri* is mainly transmitted horizontally from dead infected catfish to naïve
148 population due to infected fish cannibalization or *E. ictaluri* being shed from dead fish^{39,42} whereas
149 vertical transmission has not been reported yet⁴³ although presence of the bacteria in gonads may
150 imply possible vertical transmission^{44,45}. A high bacterial count was also found in the vicinity of
151 the dying fish which decreased with the removal of the dead fish whilst survivors of an epizootic
152 become carriers and pathogen reservoirs^{32,46,47}. Contrarily, bacterial shedding into water was not
153 observed for experimentally infected striped catfish⁴⁸. Fish eating birds such as Great blue heron
154 (*Ardea herodias*), Double-crested cormorants (*Phalacrocorax auritus*), Snowy egret (*Egretta*

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

159

160 ***E. ictaluri* general characteristics and genomic composition**

161 *E. ictaluri*, a Gram-negative Enterobacteriaceae family member, is a pleomorphic rod of varied
162 lengths and widths depending on host ^{13,14,55,56} that is peritrichous and was found to be weakly
163 motile at optimal growth temperature, but non-motile strains were also isolated ^{55,57,58} (Table 2).
164 *E. ictaluri* culture conditions in complex media are optimal temperature between 25-30 °C and pH
165 range of 7.0-7.5 ⁵⁹, respectively, and it reaches stationary phase in about 48 hours ^{14,55}. Generally,
166 strains of *E. ictaluri* is facultative anaerobic ^{44,60}. In terms of biochemical characteristics, *E. ictaluri*
167 strains isolated from different host species exhibit heterogeneity mainly in striped catfish, sea bass,
168 yellowhead catfish, hybrid catfish and tilapia strains with differences mainly in activities from
169 ornithine decarboxylase, cytochrome oxidase, H₂S production and production of gas and acid
170 from glucose (Table 2) ^{59,61-63}. Serologically, *E. ictaluri* is heterogenous and has antigenic
171 variations in the O antigens and immunogenic epitopes that are recognized by different isolates
172 ^{58,62,64-67}, however, a serotyping scheme is yet to be developed ⁶⁸. One of the most intriguing aspects
173 of *E. ictaluri* isolates from different hosts is the failure to cross-infect and failure of immunization
174 with one *E. ictaluri* isolate from catfish to cross-protect against heterologous isolates, suggesting
175 high genetic variations within the different isolates and genotypes ^{58,69,70}. All *E. ictaluri* isolates
176 from different hosts were generally susceptible to the antibiotics florfenicol, penicillins,
177 quinolones, fluoroquinolones, aminoglycosides, tetracyclines and resistant to macrolides whereas
178 tilapia and striped catfish strains were additionally resistant to sulphonamides ^{64,71,72}. Intrinsic
179 resistance to cationic antimicrobial peptides (CAMPs) such as colistin and polymyxin B of *E.*
180 *ictaluri* is well documented ^{71,73}.

181

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

186 (QBLD0000000.1) and S07-698 (QDAD0000000.1). Only 1 striped catfish isolate genome is
187 available namely T1-1 (CP054060). Two *E. ictaluri* genomes isolated from zebrafish (*Danio*
188 *rerio*) isolates are available, including LADL11-100 (LDWX0000000.1) and LADL11-194
189 (LEAL0000000.1). Two *E. ictaluri* genomes, isolated from Nile tilapia (*Oreochromis niloticus*)
190 and red hybrid tilapia (*Oreochromis* spp.), respectively, have been described including RUSVM-
191 1 (CP020466.1) and 2234 (CP053781). The *E. ictaluri* isolates have genomic sizes ranging from
192 3.6 to above 3.9 Mbp, with similar G+C contents (~57%) and between 3,235 to 3,641 protein
193 coding sequences.

194
195 Catfish isolates from the USA and Thailand were found to contain an intervening sequence (IVS)
196 located in helix-45 of the 23S rRNA gene that is absent in *E. tarda* and can provide a basis for
197 differentiating the two closely related species⁷⁴. Genetic variation of *E. ictaluri* isolates from
198 diverse hosts have been investigated using fingerprinting based on amplified-fragment length
199 polymorphism (AFLP) analysis, repetitive-sequence-mediated polymerase chain reaction (rep-
200 PCR) and phylogenetic analysis using the *gyrB* gene and have revealed that the species consists of
201 host-based genotypes^{13,64,75}. *E. ictaluri* genomes consists of Type I, III, V, and VI secretion
202 systems with variations in Type IV secretion system among genotypes^{70,76}. Comparative genomics
203 studies have shown variation in the O-antigen biosynthesis cluster and type IV secretion system
204 (T4SS) genes between channel catfish and zebrafish isolates⁷⁰, absence of T4SS-type G genes in
205 Nile tilapia isolate RUSVM-1⁷⁶ and presence of oxidative resistance stress gene (aconitate
206 hydratase B, *acnB*) in a virulent *E. ictaluri* isolated from ayu⁷⁷. Genes encoding for surface
207 structures such as cell wall, capsule and flagellar biosynthesis were found to be under positive
208 selection which might explain some adaptive traits in the species⁷⁸. Recently, our research group
209 carried out comparative genomics of the 11 *E. ictaluri* genomes mentioned above and the results
210 revealed that host specificity is brought about by intra-species evolution driven by gene gain and
211 loss driven by prophages and insertion sequences⁷⁹.

212

213 *E. ictaluri* plasmidome

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

217 Type III secretory system genes that are responsible for direct injection of effectors into host cells
218 and invasion⁸². Striped catfish *E. ictaluri* isolates were found to contain 3 different plasmids (~
219 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
220 *E. ictaluri* isolated from non-silurids such as zebrafish and tilapia were shown to contain 2
221 plasmids (pEI1 and pEI2 homologs), and a green knife fish isolate had 4 plasmids (3.1, 4.1, 5.7
222 and 6.0 kb)^{12,64,66}. From the data in the public database, the *E. ictaluri* plasmids have common
223 lengths ranging from 3kb to about 9kb as reported earlier⁸⁴ with the exception of 2 plasmids pEI-
224 MS-17-156-1 and pEI-2234-3 that have lengths above 100kb. Plasmid similarities within a host-
225 based genotype as well as differences among genotypes from different hosts were reported,
226 although most of the plasmids carried Type III secretion system proteins except plasmids from
227 zebrafish isolates, whilst a striped catfish isolate contained a unique plasmid^{58,64,82,84}. Recent
228 studies in comparative genomics revealed that 2 isolates, MS-17-156 from channel catfish (USA)
229 and 2234 from red hybrid tilapia (Vietnam) contain plasmids containing multi-drug resistant genes
230⁷⁹. *E. ictaluri* isolates also contain species specific bacteriophages (φeiAU, φeiDWF, φeiMSLS)
231 that are lytic, showing homogeneity despite isolation temporal and spatial divergence⁸⁵ as well as
232 a large number of insertion elements and genomic islands^{76,78}.

233 **Pathogenesis, pathology, clinical signs of disease and virulence**

234 Pathogenesis mechanism of *E. ictaluri* has been elucidated in channel catfish, striped catfish, and
235 Nile tilapia. Ports of *E. ictaluri* entry into susceptible hosts include the nares, oral-gastric route,
236 gills and skin (Figure 1A). For ESC in channel catfish, the acute form seems to occur when *E.*
237 *ictaluri* infects via the oral-gastric route, likely when channel catfish ingest infected carcasses,
238 contaminated water or food^{54,86,87}. Upon the bacterial attachment in the intestinal mucosa,
239 intestinal epithelial cells are rapidly invaded, and the bacteria is translocated and systemic
240 disseminated to the liver, spleen, and kidney, likely through infected macrophages^{52,54,86}. The gills
241 and skin are also primary sites for infection and systemic infection of lymphoid organs^{88,89}. It
242 seems that chronic infection happens when *E. ictaluri* infects channel catfish nares, colonizing the
243 brain via the olfactory bulb and olfactory nerve^{51,52,54}. After colonizing the brain, a systemic
244 infection could occur⁹⁰. The major clinical sign in channel catfish that appears 2-4 weeks post
245 infection is the classic 'hole in the head' lesion, which is related to cartilaginous skull cap digestion
246 caused by *E. ictaluri* chondroitinase activity^{54,91}. Experimentally challenged channel catfish were
247 shown to have reduced plasma components like erythrocyte, leucocyte counts, plasma glucose

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

252
253 Interestingly, in striped catfish, experimental immersion challenge with *E. ictaluri* revealed that
254 one of the ports of entry of *E. ictaluri* during pathogenesis are gills ⁴⁸. Another immersion
255 challenge of striped catfish with *E. ictaluri* showed that the gastrointestinal tract is also a port of
256 bacterial entry into the fish ⁹⁴. Edwardiellosis clinical signs in striped fish is different from channel
257 catfish. Typically, striped fish exhibit external clinical signs such as skin lesions, pale gills and
258 pale colour ^{8,57,94} but the classic ‘hole in the head’ lesion has not been reported. In experimentally
259 challenged striped catfish, behavioral changes (e.g., gasping for air, lethargy, lack of appetite,
260 erratic swimming) were observed as early as 4 hours post infection (hpi) whereas gross clinical
261 signs were seen 96 hpi ^{39,94,183}. *E. ictaluri* bacterial cells were notably absent in the brain of BNP
262 experimentally infected striped catfish although the bacteria were found in the other internal organs
263 including head kidney, trunk kidney, liver, spleen, gills, skin and muscle ^{48,94}. Intracellular
264 replication of *E. ictaluri* in macrophages was also elucidated in striped catfish and the pathogen
265 could persist in necrotic-participating phagocytic cells and in melano-macrophage centers up to 1
266 month ^{48,94}. From their findings, Pirarat et al., suggested that *E. ictaluri* damages the endothelial
267 cells leading to inflammation of the perivascular sheath and blood vessels and results in tissue
268 hypoxia and necrosis ⁴⁸.

269
270 Although *E. ictaluri* infection has been reported in tilapia, there are no reports of behavioral change
271 or external clinical signs, but increased fish morbidity and mortality was reported ^{12,13}.
272 Pathogenesis of *E. ictaluri* in tilapia was investigated by Soto et al., in 2013 ²⁶. As reported earlier,
273 the port of entry of *E. ictaluri* for colonization into Nile tilapia is via the oral-gastric route and
274 cutaneous routes. The bacteria are then disseminated hematogenously to organs such as gills,
275 brain, head kidney, heart, and spleen. The spleen and head kidney are the main targets of infection
276 and bacterial survival as shown by presence of high bacterial DNA levels and presence of clumps
277 of rod-shaped bacteria in the organs ^{12,26}. Bacterial systemic dissemination is facilitated by antigen-
278 presenting cells like macrophages ²⁶.

279
280 Channel catfish and striped catfish suffering from *E. ictaluri* infection have been reported to
281 display behavioral changes. Infected catfishes show erratic rapid circular swimming and spinning
282 caused by meningoencephalitis as well as lethargy, listless up-side down hanging or slow
283 swimming near pond edge^{20,23,57,95}. *E. ictaluri* infected fish such as catfishes (channel, striped and
284 yellow) and ayu, exhibit external gross clinical signs like skin haemorrhage and ulceration,
285 distended abdomen, discoloration, reddened anus, exophthalmos and meningio-encephalitis (red
286 head) (Figure 2)^{96,23,24,57}. The general internal clinical signs reported in the susceptible hosts that
287 include catfishes and tilapia and ayu are white nodules granulomas, abdomen ascites, pale gills,
288 enlarged gallbladder, reddened gonads and enlarged and haemorrhagic posterior kidney (Figure 3)
289^{12,23,39,96}. Both channel catfish and yellow catfish display classic ‘hole in the head’ lesion whilst
290 yellow catfish additionally display the ‘hole-under-the-jaw’ lesion^{24,97}. Histopathological
291 examinations in most susceptible hosts revealed similar results such as granulomatous
292 inflammatory reactions, necrosis, haemorrhage, pyknosis and karyorrhexis in internal organs,
293 epithelial lining hyperplasia in gills and observation of clumps of rod shaped bacteria in tissues
294 (Figure 4)^{12,39,96,98}. Electron transmission microscopy also revealed the intracellular localisation
295 of *E. ictaluri* in macrophage in infected zebrafish zebrafish head kidney tissue (Figure 5).

296
297 The molecular mechanisms of *E. ictaluri* pathogenesis were described in channel catfish and
298 zebrafish using epithelial cells, phagocytic cells and macrophages and a graphical illustration is
299 shown in Figure 1B. Pathogen attachment is facilitated by the recognition of *E. ictaluri* by host
300 cell receptors e.g. Toll-like receptor 5 (TLR5) and (NOD)-like receptor subfamily C (NLRC)⁹⁹
301 and the help of *E. ictaluri* proteins Hcp2¹⁰⁰, EseI and EseH¹⁰¹. For invasion, the plasmid encoded
302 protein, EseI plays a role¹⁰¹, and *E. ictaluri* enters into the target cells using Ca²⁺-dependent
303 receptor-mediated endocytosis and macropinocytosis^{102,103}. Endocytosis of *E. ictaluri* into the
304 epithelial cells is enabled when the polymerization of actin, manipulation of myosin components
305 and apical junction complex (AJC) components are dysregulated^{99,102,103}. This facilitates entry of
306 the bacteria enclosed in an *Edwardsiella*-containing-vacuole (ECV) thereby protecting the bacteria
307 from lysosomal degradation⁹⁹. The ECV is acidified immediately by host cell vacuolar ATPases
308¹⁰⁴. Consequently, intracellular survival of *E. ictaluri* is enabled by the upregulated expression of
309 T3SS by the two-component regulatory proteins EsrA and EsrB¹⁰⁵ and the activity of the Type VI

310 Secretion System (T6SS) effector, Hcp2¹⁰⁰. Also, using urea that would have been produced by
311 arginase enzyme from the host cell, the *E. ictaluri* acid-activated urease produces ammonia, which
312 neutralizes the ECV acidic environment to a pH level (>pH 5.0). This creates an environment
313 conducive for *E. ictaluri* replication and translocation of T3SS effectors (EseGHIJKLMNO)
314 directly into the host cytoplasm^{106,107}. These T3SS effectors interact with target host proteins to
315 disrupt host defense mechanisms^{105,106}. Conducive pH is then maintained by the prevention of
316 phagosomal/lysosomal fusion, nutrients for bacterial growth and ECV enrichment are supplied by
317 the Golgi and programmed cell death is suppressed¹⁰⁸. Lysosomal acid hydrolases and reactive
318 oxygen species production is downregulated by the T6SS effector, EvpP, indicating exploitation
319 of the endosomal machinery thereby enabling intra-phagosome survival^{99,100}. Lastly,
320 inflammatory and immune responses are modulated with the putative aid of the EseN protein, for
321 disease progression and then genes responsible for endocrine and growth are downregulated which
322 may contribute to faltering growth⁹⁹. It was also shown that *E. ictaluri* replicates intracellularly
323 in macrophages¹⁰⁹ and can survive in fish organs up 65 days post infection⁴⁵.

324

325 Virulence and pathogenesis of *E. ictaluri* is facilitated by type III, IV and VI secretion systems
326 that enable intracellular replication and survival in channel catfish^{76,106,110-112}. Several
327 investigations also demonstrate that *E. ictaluri* employs lipopolysaccharide (LPS), extracellular
328 capsular polysaccharide, outer membrane proteins, adhesins and fibrillar processes for attachment
329 to and survival in macrophages and host cells¹¹³⁻¹¹⁶. *E. ictaluri* requires flagella for motility¹¹⁷,
330 oligo-polysaccharide (O-PS) for modulation of host immune responses¹¹⁸ as well as hemolysins
331 and chondroitinase whose activities were mentioned earlier^{91,93}. Pathogenesis of *E. ictaluri* is
332 regulated by a number of mechanisms. Intracellular multiplication of *E. ictaluri* requires iron
333 uptake and heme synthesis systems both under the regulation of the ferric uptake regulator (Fur)
334¹¹⁹. TonB is an important virulent factor that is required by *E. ictaluri* for TonB-mediated active
335 transport of nutrients, especially iron, which is critical for survival of pathogenic bacteria during
336 infection¹²⁰. Urease activity is required for intracellular virulence and proliferation and is
337 facilitated by pH increase due to production of ammonia. This probably neutralizes the acidic
338 phagosome environment^{104,107}. Pathogenesis of *E. ictaluri* is also promoted by stress-related genes
339 that also enable survival of the pathogen in phagolysosomal conditions that are harsh¹²¹. Two

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

342

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

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

353

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

367

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

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

377
378 Complement related genes such as C1r, C3, C5, C7, C9, and C1-INH were identified in *E. ictaluri*-
379 infected darkbarbel catfish (*Pelteobagrus vachelli*) and are essential for linking innate to adaptive
380 immune responses ¹⁵³. Immune regulators such as chemokines, cytokines in channel catfish and
381 Cyclophilin A (CypA) in yellow catfish also play a role in inflammatory response and bridging
382 innate to adaptive immunity after *E. ictaluri* infections ^{150,154,155-158} with the mediation of Janus
383 kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathway proteins
384 ¹⁵⁹. Other innate immune response molecules produced channel catfish in response to *E. ictaluri*
385 infection are annexins ¹⁶⁰, Intelectins (IntL2) which probably plays an immune response
386 downstream role ¹⁶¹ and apolipoproteins that modulates inflammatory response to LPS ¹⁶². *E.*
387 *ictaluri* infected channel catfish also mounts antioxidant defense mechanisms using stress response
388 proteins like calreticulin and Hsp70 ^{163,164}.

389
390 Channel catfish infected with *E. ictaluri* are able to mount protective T and B cell-dependent
391 adaptive immunity ^{165,166}. IgM antibody is produced as humoral response to *E. ictaluri* in channel
392 catfish ¹²². Cell-mediated immune response was evidenced in resistant channel catfish family
393 whereby macrophages formed aggregations in the posterior kidney and spleen ^{165,167}. On the other
394 hand, channel catfish before 4 weeks old failed to mount a detectable immune response, even after
395 two exposures to the pathogen, probably due to poorly differentiated primary lymphoid organs and
396 tissues ¹⁶⁸. Leukocyte immune-type receptors (LITRs) were also found to play a role in cell-
397 mediated immunity of channel catfish ¹⁶⁹. Catfish also utilize the major histocompatibility complex
398 (MHC) class I as a cell-mediated defense mechanism against *E. ictaluri* in resistant blue catfish.
399 In a study by Peatman et al. two different MHC class I alpha chains and beta-2-microglobulin
400 (β_2m) were significantly upregulated in the *E. ictaluri* resistant blue catfish 3 days post *E. ictaluri*
401 infection but not in channel catfish ¹⁷⁰. Moreover, Recombination-activating gene 2 (*rag 2*) was

402 detected in high quantities in the thymus and head-kidney of yellow catfish indicating a role in
403 diversification of B and T cells via V(D)J (variable/diversity/joining) recombination¹⁷¹. Also, co-
404 upregulation with pro-inflammatory cytokines implicated Rag 2 involvement in yellow catfish
405 immune responses¹⁷¹.

406

407 **Disease diagnosis**

408 Laboratory diagnosis of diseases caused by *E. ictaluri* is typically by first isolating the bacterium
409 from the internal organs or brain tissue on culture media. Commonly used media include tryptic
410 soy agar (TSA) or brain heart infusion (BHI) agar supplemented with 5% blood and selective
411 morphology differentiating medium (*E. ictaluri* medium, EIM), that is inhibitive of most Gram-
412 negative and Gram-positive bacteria^{23,172}. A defined minimal medium was formulated that
413 contains only 8 essential components instead of 46 and can sustain growth of *E. ictaluri*¹⁷³.
414 Subsequently, bacterial isolation is usually followed by biochemical tests using kits like Crystal™
415 or the API 20E⁹⁷ which distinguishes between *E. ictaluri* and *E. tarda*. Histopathology is then
416 employed to diagnose based on microscopic cellular analysis^{90,95,98}. Invasive techniques for
417 identifying bacterial location in host tissues include *in situ* hybridization, immunohistochemistry
418 and radioisotope labeling^{15,114,89,48}. *In vivo* bioluminescence imaging (BLI) was introduced by
419 Karsi et al.,¹⁷⁴ for non-invasive identification of bacterial in host tissues. Identification of *E.*
420 *ictaluri* was also carried out using MALDI-TOF (matrix-assisted laser desorption ionization–time-
421 of-flight mass spectrometer)^{175,176}.

422

423 Confirmatory tests performed in identification of the bacterium are necessary for diagnosis of
424 diseases caused by *E. ictaluri* and these include serology tests and molecular detection¹⁷⁷.
425 Serology tests used to confirm *E. ictaluri* infection include enzyme-linked immunosorbent assay
426 (ELISA) such as a FAST-ELISA that rapidly detected antibodies to *E. ictaluri* exoantigen¹⁷⁸;
427 indirect ELISA using rabbit anti-catfish immunoglobulin and mouse anti-catfish immunoglobulin
428^{179,180}; and modified ELISA using detergent coupled with filtration¹⁸¹. Enzyme immunoassay
429 (EIA) to detect *E. ictaluri* in decomposing fish samples¹⁸² and indirect fluorescent antibody
430 technique (IFA) using either highly specific monoclonal antibodies¹⁸³ or antibody conjugated
431 fluorochromes¹⁸⁴ were also employed. For detection of *E. ictaluri* in yellow catfish, a dot-enzyme
432 linked immunosorbent assay (Dot-ELISA) and an indirect fluorescence antibody technique (IFAT)

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

436
437 The other confirmatory tests are based on molecular detection using Polymerase chain reaction
438 (PCR). Generally, *E. ictaluri* was confirmed as the causative agent using amplification and
439 sequencing of the 16S rRNA gene and the *gyrB* gene ^{12,187,188}, *E. ictaluri*-specific PCR targeting
440 upstream region of fimbrial gene ¹⁸⁹ and IVS /IRS PCR assay method using primers targeting
441 regions between the ribosomal DNA gene clusters, inter-ribosomal spacer (IRS) and 23S rRNA
442 gene intervening sequence (IVS) ¹⁹⁰. Rapid PCR and a real-time PCR assay which could detect
443 low levels of *E. ictaluri* in water, were also developed ^{188,191}. The molecular diagnostic loop-
444 mediated isothermal amplification method (LAMP) which recognizes the *eip18* gene was also used
445 for *E. ictaluri* confirmation ¹⁹². Application of OmniAmp DNA polymerase (Pol) in LAMP using
446 lateral flow strips to detect *E. ictaluri* amplification was demonstrated as sensitive, rapid, and easy-
447 to-use point-of-care (POC) method ¹⁹³. Recently, high-gradient immunomagnetic separation
448 (HGIMS) coupled with PCR was also evaluated as a diagnostic tool with a higher detection
449 sensitivity when compared with conventional PCR ¹⁹⁴.

450 451 **Disease management and limitations**

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

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

469
470 Vaccine formulations have been made using either bacterins or attenuated bacteria (Table 3). The
471 early bacterin vaccines that were developed for the channel catfish reported high relative percent
472 survival (RPS) values more than 90% under experimental laboratory trials but varying
473 effectiveness under field conditions and did not provide long term acquired immunity ^{205,206}. The
474 formalin killed vaccine also failed to protect the fish unless administered in Freund's complete
475 adjuvant (FCA) ²⁰⁷, probably due to failure of killed *E. ictaluri* to invade the fish ⁸⁹. For striped
476 catfish, two commercial inactivated vaccines, Alpha ject Panga 1 and Alpha Ject Panga 2 were
477 licensed in Vietnam for prevention of BNP ²⁰⁸. Alpha ject Panga 1 and 2 vaccines have reported
478 high efficacy, where the mortalities of vaccinated striped catfish were reduced to 0-4.7% ²⁰⁹. There
479 are two patented attenuated vaccines available in the USA namely live attenuated *E. ictaluri*
480 bacterium lacking the *evpB* gene (patent number US20170065695A1) ²¹⁰ and AQUAVAC-ESC®
481 (US Patent no. 6,019,981) that was attenuated by multiple passages in increased concentrations of
482 rifampicin resulting in a mutant that is missing part of the O-lipopolysaccharide ²¹¹. Other attempts
483 at producing high efficacy live attenuated vaccines (Relative percent survival, RPS \geq 66%) for
484 both channel and striped catfish included the construction of *E. ictaluri* mutants of *wzzE*, *purA*,
485 *fhuC*, *aroA*, *crp* and *asdA* genes and a novobiocin attenuated *E. ictaluri* ^{200,212-217}. Another vaccine
486 approach was the use of *E. ictaluri* bacterial ghosts (EIGs), generated by introduction of a plasmid
487 that encodes the phage PhiX174 lysis gene *E*, that had an RPS of 89.3% in channel catfish ²¹⁸.
488 Limited studies on subunit-based vaccines development and their efficacy against *E. ictaluri* have
489 been carried out. Attempts to produce a subunit vaccines with promising results (RPS 62.5-95%)
490 have been made using the *E. ictaluri* lipopolysaccharide in Freund's complete adjuvant ²⁰⁷ and *E.*
491 *ictaluri* outer membrane proteins (OMP_{N1-3}) ²¹⁹, while five different *E. ictaluri* proteins including
492 hypothetical protein (*yggE*), specific inhibitor of chromosomal initiation of replication (*iciA*),
493 ribose 5-phosphate isomerase (*rpiA*) and fructose 1,6-bisphosphate aldolase (*fda*) ²²⁰ provided
494 inclusive results. We recently constructed a multi-epitope chimeric subunit vaccine (EiCh) that

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

502
503 Selective-breeding programs that have been implemented for resistance against *E. ictaluri*
504 infections in aquaculture include a genetically improved channel catfish strain (NWAC103) that
505 is a non-transgenically purebred produced after breeding fish with desired traits whereby the traits
506 were identified using microsatellite loci identification method and DNA fingerprinting ²²⁴. Also,
507 selective genotyping and genome-wide association studies (GWAS) identified a microsatellite and
508 quantitative trait locus (QTL) using interspecific backcross progenies, respectively, that confer *E.*
509 *ictaluri* resistance in channel catfish ^{123,225,226} implying applicability of marker-assisted selection
510 for disease resistance selective breeding. Although genetic selection was shown to enhance
511 resistance against *E. ictaluri* ²²⁴, the method can also result in the genetically improved channel
512 catfish strain being more susceptible to other pathogens (e.g., ictalurid herpesvirus, CCV) ⁹⁷.

513
514 Dietary supplements such as vitamins, minerals, nutrients and glycans have been proven
515 experimentally to enhance immune response of channel catfish but did not conclusively alter
516 susceptibility to *E. ictaluri* infections ^{97,227-230}. In fact, Menhaden oil supplemented alone in fish
517 feed was reported to increase susceptibility of catfish to ESC infection ²³¹. On the other hand, β -
518 glucan enhanced protection of striped catfish from *E. ictaluri* infection ²³². The studies on the
519 application of probiotics for growth enhancement and ESC resistance indicated that commercial
520 probiotics supplemented in feed could neither enhance growth nor protect juvenile catfish ²³³,
521 however, *Vibrio parahaemolyticus* and *E. coli* could protect zebrafish larvae ²³⁴ and *Bacillus*
522 *pumilus* inhibited *E. ictaluri* in striped catfish ²³⁵. Studies of effects of commercial prebiotics
523 (mannan oligosaccharide, MOS) in channel catfish were encouraging as there was a significant
524 increase in survival rate ²³⁶. Essential oils in prevention of *E. ictaluri* infections also proved
525 efficacious ²³⁷.

526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556

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 heterogeneous^{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 pathogenicity²³⁸⁻²⁴³. Also, potential universal vaccine candidates and drug targets towards different genotypes can be developed using reverse vaccinology based on identified antigenic proteins²⁴⁴.

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 procedures²⁴⁵. Since training is usually costly, the participation at government and international level is greatly anticipated to help fund such programs²⁴⁶. 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*.

557 **Biosecurity Measures**

558 Movement of live fish for aquaculture usually contributes to movement of pathogens.
559 Transboundary importation of *E. ictaluri* was implicated in Trinidad and Tobago and Australia
560 where outbreaks occurred during quarantine of imported fish^{57,248}. This calls to attention the need
561 for policy makers to enforce stricter biosecurity at national and local levels. The biosecurity
562 measures should include disease surveillance using rapid, highly accurate diagnostic tools and self-
563 quarantine in closed system for imported animals at farm level. This will assist in preventing
564 pathogen spread and development of control strategies²⁴⁹. Moreover, it will be beneficial to use
565 genetically modified fish for *E. ictaluri* resistance coupled with strict biosecurity at the farms to
566 prevent and contain epizootics²⁴⁷. It is imperative to perform Import risk analysis (IRA) including
567 passive and active surveillance both for wild and farmed fish to prevent pathogen spread to new
568 hosts and geographical locations²⁵⁰.

569

570 **Alternatives to antibiotic and chemical use**

571 On top of the antibiotic alternatives already researched against *E. ictaluri* mentioned above such
572 as vaccines, prebiotics, probiotics, essential oils and feed supplements, there are yet other therapies
573 that remain unexplored. These include use of bacteria capable of disrupting quorum sensing
574 molecules and phage therapy²⁵¹. In aquaculture, bacteria such as *Bacillus*, *Halobacillus salinus*
575 and *Actinobacteria Streptomyces albus* have been identified as biocontrol agents due to their
576 ability to quench pathogen quorum sensing system for bacteria like *Vibrio sp.* and *Aeromonas*
577 *hydrophila* thereby increasing fish survival after challenge²⁵¹. Quorum sensing therapy can be
578 enhanced by using Biofloc technology whereby extra carbon is added to pond water resulting in
579 improved growth of biocontrol agents *in situ*²⁵². Bacteriophages are known for their therapeutic
580 properties by inhibiting pathogens in single doses without reported side effects²⁵³. For the control
581 and inhibition of *E. ictaluri* using phage therapy, a patent exists of 2 bacteriophages, ΦeiAU and
582 ΦeiDWF²⁵⁴ but extensive use and efficacy is still to be reported Hence, these alternative to
583 antibiotics therapies can help in the control of *E. ictaluri* to curb antimicrobial resistance (AMR).

584

585 An emerging ozone nanobubble technology has been reported to be effective in reducing pathogen
586 concentration in water and its safety in marine and aquaculture species was also exhibited. This
587 technique entails injection of ozone created nanobubbles (NB-O₃) into water with various salinity

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

596

597 **Application of genomics in disease control**

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

611

612 **Selective breeding for disease resistant traits**

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

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

629

630 **Trained Innate Immunity**

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

646

647 **Concluding remarks**

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

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

662

663 **Acknowledgments**

664 V. I. Machimbirike was supported by the Petchra Pra Jom Klao Ph.D. scholarship for international
665 students, King Mongkut's University of Technology Thonburi (KMUTT).

666 **References**

- 667 1. FAO. The State of World Fisheries and Aquaculture. Sustainability in action 2020.
668 doi:<https://doi.org/10.4060/ca9229en>. Accessed 14 February 2021.
- 669 2. Jantrakajorn S, Lukkana M, Wongtavatchai J. Serological and molecular characterization of
670 *Streptococcus iniae* in cultured Nile tilapia (*Oreochromis niloticus*) in Thailand. *The Thai Journal*
671 *of Veterinary Medicine*. 2014;44(1):49. doi:[https://he01.tci-](https://he01.tci-thaijo.org/index.php/tjvm/article/view/17314)
672 [thaijo.org/index.php/tjvm/article/view/17314](https://he01.tci-thaijo.org/index.php/tjvm/article/view/17314).
- 673 3. Subasinghe R. Disease control in aquaculture and the responsible use of veterinary drugs and
674 vaccines: the issues, prospects and challenges. In: C. R, B. B, eds. *The use of veterinary drugs and*
675 *vaccines in Mediterranean aquaculture*. Vol 86. Zaragoza : CIHEAM: Options Méditerranéennes;
676 2009:5-11.
- 677 4. Klesius P, Pridgeon J. Live attenuated bacterial vaccines in aquaculture. Paper presented at:
678 Proceedings of the 9th International Symposium on Tilapia in Aquaculture 2011
- 679 5. Machimbirike VI, Jansen MD, Senapin S, Khunrae P, Rattanarojpong T, Dong HT. Viral infections
680 in tilapines: More than just tilapia lake virus. *Aquaculture*. 2019;503:508-518.
681 doi:<https://doi.org/10.1016/j.aquaculture.2019.01.036>.
- 682 6. Plumb JA, Hanson LA. *Health maintenance and principal microbial diseases of cultured fishes*.
683 John Wiley & Sons; 2010.
- 684 7. Rodger HD. Fish disease causing economic impact in global aquaculture. In: *Fish vaccines*.
685 Springer; 2016:1-34.
- 686 8. Crumlish M, Thanh PC, Koesling J, Tung VT, Gravningen K. Experimental challenge studies in
687 Vietnamese catfish, *Pangasianodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri*

- 688 and *Aeromonas hydrophila*. *Journal of fish diseases*. 2010;33(9):717-722.
689 doi:https://doi.org/10.1111/j.1365-2761.2010.01173.x.
- 690 9. Declercq AM, Haesebrouck F, Van den Broeck W, Bossier P, Decostere A. Columnaris disease in
691 fish: a review with emphasis on bacterium-host interactions. *Veterinary research*. 2013;44(1):1-17.
692 doi:https://doi.org/10.1186/1297-9716-44-27.
- 693 10. Li R, Huang H, Zhang X, Ye S, Li Q. Monoclonal antibody based Dot-ELISA and indirect
694 fluorescence antibody technique for detecting *Edwardsiella ictaluri* infection in yellow catfish
695 (*Pelteobagrus fulvidraco*). *Aquaculture and Fisheries*. 2017;2(5):207-212.
696 doi:https://doi.org/10.1016/j.aaf.2017.10.003.
- 697 11. Thongkao K, Sudjaroen Y. Human pathogenic bacteria isolation from tilapia fishes (*Oreochromis*
698 *niloticus*), a possible reservoir for zoonotic transmission. *Annals of Tropical Medicine and Public*
699 *Health*. 2017;10(6). doi:http://dx.doi.org/10.4103/ATMPH.ATMPH_511_17.
- 700 12. Dong H, Senapin S, Jeamkunakorn C, et al. Natural occurrence of edwardsiellosis caused by
701 *Edwardsiella ictaluri* in farmed hybrid red tilapia (*Oreochromis* sp.) in Southeast Asia.
702 *Aquaculture*. 2019;499:17-23. doi:https://doi.org/10.1016/j.aquaculture.2018.09.007.
- 703 13. Soto E, Griffin M, Arauz M, Riofrio A, Martinez A, Cabrejos ME. *Edwardsiella ictaluri* as the
704 causative agent of mortality in cultured Nile tilapia. *Journal of Aquatic Animal Health*.
705 2012;24(2):81-90. doi:https://doi.org/10.1080/08997659.2012.675931.
- 706 14. Crumlish M, Dung T, Turnbull J, Ngoc N, Ferguson H. Identification of *Edwardsiella ictaluri* from
707 diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta,
708 Vietnam. *Journal of Fish Diseases*. 2002;25(12):733-736. doi:https://doi.org/10.1046/j.1365-
709 2761.2002.00412.x.
- 710 15. Dong HT, Nguyen VV, Phiwsaiya K, et al. Concurrent infections of *Flavobacterium columnare*
711 and *Edwardsiella ictaluri* in striped catfish, *Pangasianodon hypophthalmus* in Thailand.
712 *Aquaculture*. 2015;448:142-150. doi:https://doi.org/10.1016/j.aquaculture.2015.05.046.
- 713 16. Ye S, Li H, Qiao G, Li Z. First case of *Edwardsiella ictaluri* infection in China farmed yellow
714 catfish *Pelteobagrus fulvidraco*. *Aquaculture*. 2009;292(1/2):6-10.
715 doi:http://dx.doi.org/10.1016/j.aquaculture.2009.03.036.
- 716 17. Yuasa K, Kholidin EB, Panigoro N, Hatai K. First isolation of *Edwardsiella ictaluri* from cultured
717 striped catfish *Pangasius hypophthalmus* in Indonesia. *Fish Pathology*. 2003;38(4):181-183.
718 doi:https://doi.org/10.3147/jsfp.38.181.
- 719 18. Hanson TR, Shaik S, Coble KH, Edwards S, Miller JC. Identifying risk factors affecting weather-
720 and disease-related losses in the US farm-raised catfish industry. *Agricultural and Resource*
721 *Economics Review*. 2008;37(1):27-40. doi:http://dx.doi.org/10.1017/S1068280500002124.
- 722 19. Peterman MA, Posadas BC. Direct economic impact of fish diseases on the East Mississippi catfish
723 industry. *North American Journal of Aquaculture*. 2019;81(3):222-229.
724 doi:https://doi.org/10.1002/naaq.10090.
- 725 20. Hawke JP. A bacterium associated with disease of pond cultured channel catfish, *Ictalurus*
726 *punctatus*. *Journal of the Fisheries Board of Canada*. 1979;36(12):1508-1512.
727 doi:https://doi.org/10.1139/f79-219.sa
- 728 21. Mitchell AJ, Goodwin AE. Evidence that enteric septicemia of catfish (ESC) was present in
729 Arkansas by the late 1960s: New insights into the epidemiology of ESC. *Journal of Aquatic Animal*
730 *Health*. 1999;11(2):175-178. doi:https://doi.org/10.1577/1548-
731 8667(1999)011%3C0175:ETESOC%3E2.0.CO;2.
- 732 22. Ferguson H, Turnbull J, Shinn A, Thompson K, Dung TT, Crumlish M. Bacillary necrosis in
733 farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish*
734 *Diseases*. 2001;24(9):509-513. doi:https://doi.org/10.1046/j.1365-2761.2001.00308.x.
- 735 23. Hawke JP, Durborow R, Thune R, Camus A. Enteric septicemia of catfish. *SRAC publication*.
736 1998(477). doi:https://appliedecology.cals.ncsu.edu/wp-content/uploads/477.pdf.
- 737 24. Liu JY, Li AH, Zhou DR, Wen ZR, Ye XP. Isolation and characterization of *Edwardsiella ictaluri*
738 strains as pathogens from diseased yellow catfish *Pelteobagrus fulvidraco* (Richardson) cultured

- 739 in China. *Aquaculture Research*. 2010;41(12):1835-1844. doi:<https://doi.org/10.1111/j.1365-2109.2010.02571.x>.
- 740
- 741 25. Sakai T, Kamaishi T, Sano M, et al. Outbreaks of *Edwardsiella ictaluri* infection in ayu
742 *Plecoglossus altivelis* in Japanese rivers. *Fish Pathology*. 2008;43(4):152-157.
743 doi:<http://dx.doi.org/10.3147/jsfp.43.152>.
- 744 26. Soto E, Illanes O, Revan F, Griffin M, Riofrio A. Bacterial distribution and tissue targets following
745 experimental *Edwardsiella ictaluri* infection in Nile tilapia *Oreochromis niloticus*. *Diseases of*
746 *aquatic organisms*. 2013;104(2):105-112. doi:<https://doi.org/10.3354/dao02593>.
- 747 27. Francis-Floyd R, Beleau M, Waterstrat P, Bowser P. Effect of water temperature on the clinical
748 outcome of infection with *Edwardsiella ictaluri* in channel catfish. *Journal of the American*
749 *Veterinary Medical Association*. 1987;191(11):1413-1416.
- 750 28. Tucker CC, Robinson EH. *Channel catfish farming handbook*. Springer Science & Business Media;
751 1990.
- 752 29. Dung TT. *Edwardsiella ictaluri* in *Pangasianodon catfish*: antimicrobial resistance and the early
753 interactions with its host, Ghent University; 2010.
- 754 30. Baxa-Antonio D, Groff J, Hedrick R. Effect of water temperature on experimental *Edwardsiella*
755 *ictaluri* infections in immersion-exposed channel catfish. *Journal of aquatic animal health*.
756 1992;4(2):148-151. doi:[https://doi.org/10.1577/1548-8667\(1992\)004%3C0148:EOWTOE%3E2.3.CO;2](https://doi.org/10.1577/1548-8667(1992)004%3C0148:EOWTOE%3E2.3.CO;2).
- 757
- 758 31. Plumb JA, Shoemaker C. Effects of temperature and salt concentration on latent *Edwardsiella*
759 *ictaluri* infections in channel catfish. *Diseases of aquatic organisms*. 1995;21(3):171-175.
760 doi:<http://dx.doi.org/10.3354/dao021171>.
- 761 32. Mqolomba TN, Plumb J. Effect of temperature and dissolved oxygen concentration on
762 *Edwardsiella ictaluri* in experimentally infected channel catfish. *Journal of Aquatic Animal Health*.
763 1992;4(3):215-217. doi:[https://doi.org/10.1577/1548-8667\(1992\)004%3C0215:EOTADO%3E2.3.CO;2](https://doi.org/10.1577/1548-8667(1992)004%3C0215:EOTADO%3E2.3.CO;2).
- 764
- 765 33. Hassan ES, Mahmoud MM, Kawato Y, et al. Subclinical *Edwardsiella ictaluri* infection of wild
766 ayu *Plecoglossus altivelis*. *Fish Pathology*. 2012;47(2):64-73.
767 doi:<https://doi.org/10.3147/jsfp.47.64>.
- 768 34. Plumb J, Quinlan E. Survival of *Edwardsiella ictaluri* in pond water and bottom mud. *The*
769 *Progressive Fish-Culturist*. 1986;48(3):212-214. doi:[https://doi.org/10.1577/1548-8640\(1986\)48%3C212:SOEIP%3E2.0.CO;2](https://doi.org/10.1577/1548-8640(1986)48%3C212:SOEIP%3E2.0.CO;2).
- 770
- 771 35. Cai W, Arias CR. Biofilm formation on aquaculture substrates by selected bacterial fish pathogens.
772 *Journal of Aquatic Animal Health*. 2017;29(2):95-104.
773 doi:<https://doi.org/10.1080/08997659.2017.1290711>.
- 774 36. Janda J, Abbott S, Kroske-Bystrom S, et al. Pathogenic properties of *Edwardsiella* species. *Journal*
775 *of clinical microbiology*. 1991;29(9):1997-2001. doi:<https://doi.org/10.1128/jcm.29.9.1997-2001.1991>.
- 776
- 777 37. Ogawa G, Ishida M, Kato H, Fujise Y, Urano N. Identification of facultative anaerobic bacteria
778 isolated from the intestine of the minke whale *Balaenoptera acutorostrata* by 16S rRNA
779 sequencing analysis. *Fisheries Science*. 2010;76(2):177-181. doi:<https://doi.org/10.1007/s12562-009-0211-0>.
- 780
- 781 38. Ciembor P, Blazer V, Dawe D, Shotts E. Susceptibility of channel catfish to infection with
782 *Edwardsiella ictaluri*: effect of exposure method. *Journal of Aquatic Animal Health*.
783 1995;7(2):132-140. doi:[https://doi.org/10.1577/1548-8667\(1995\)007%3C0132:SOCCTI%3E2.3.CO;2](https://doi.org/10.1577/1548-8667(1995)007%3C0132:SOCCTI%3E2.3.CO;2).
- 784
- 785 39. Ngoc Phuoc N, Richards R, Crumlish M. Establishing bacterial infectivity models in striped *Catfish*
786 *Pangasianodon hypophthalmus* (Sauvage) with *Edwardsiella ictaluri*. *Journal of fish diseases*.
787 2020;43(3):371-378. doi:<https://doi.org/10.1111/jfd.13135>.

- 788 40. Wise DJ, Schwedler TE, Otis DL. Effects of stress on susceptibility of naive channel catfish in
789 immersion challenge with *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 1993;5(2):92-
790 97. doi:https://doi.org/10.1577/1548-8667(1993)005%3C0092:EOSOSO%3E2.3.CO;2.
- 791 41. Takeuchi H, Hiratsuka M, Hori K, et al. Environmental factors affecting *Edwardsiella ictaluri*-
792 induced mortality of riverine ayu, *Plecoglossus altivelis* (Temminck & Schlegel). *Journal of Fish*
793 *Diseases*. 2021. doi:https://doi.org/10.1111/jfd.13368.
- 794 42. Klesius P. Transmission of *Edwardsiella ictaluri* from infected, dead to noninfected channel
795 catfish. *Journal of aquatic animal health*. 1994;6(2):180-182. doi:https://doi.org/10.1577/1548-
796 8667(1994)006%3C0180:TOEIFI%3E2.3.CO;2.
- 797 43. Tucker CS, Hargreaves JA. *Biology and culture of channel catfish*. Elsevier; 2004.
- 798 44. Kalindamar S, Kordon AO, Abdelhamed H, Tan W, Pinchuk LM, Karsi A. *Edwardsiella ictaluri*
799 evpP is required for colonisation of channel catfish ovary cells and necrosis in anterior kidney
800 macrophages. *Cellular microbiology*. 2020;22(3):e13135. doi:https://doi.org/10.1111/cmi.13135.
- 801 45. Mqolomba TN, Plumb J. Longevity of *Edwardsiella ictaluri* in the organs of experimentally
802 infected channel catfish, *Ictalurus punctatus*. *Aquaculture*. 1992;101(1-2):1-6.
803 doi:https://doi.org/10.1016/0044-8486(92)90226-B.
- 804 46. Earlix DJ. *Host, pathogen, and environmental interactions of enteric septicemia of catfish*. Auburn,
805 Alabama, USA, Auburn University; 1996.
- 806 47. Klesius PH. Carrier state of channel catfish infected with *Edwardsiella ictaluri*. *Journal of Aquatic*
807 *Animal Health*. 1992;4(3):227-230. doi:https://doi.org/10.1577/1548-
808 8667(1992)004%3C0227:CSOCCI%3E2.3.CO;2.
- 809 48. Pirarat N, Ooi EL, Thompson KD, Thinh NH, Maita M, Katagiri T. Examination of entry portal
810 and pathogenesis of *Edwardsiella ictaluri* infection in striped catfish, *Pangasianodon*
811 *hypophthalmus*. *Aquaculture*. 2016;464:279-285.
812 doi:https://doi.org/10.1016/j.aquaculture.2016.06.043.
- 813 49. Taylor PW. Fish-eating birds as potential vectors of *Edwardsiella ictaluri*. *Journal of Aquatic*
814 *Animal Health*. 1992;4(4):240-243. doi:https://doi.org/10.1577/1548-
815 8667(1992)004%3C0240:FEBAPV%3E2.3.CO;2.
- 816 50. Waterstrat PR, Dorr B, Glahn a, James F, Tobin ME. Recovery and viability of *Edwardsiella*
817 *ictaluri* from great blue herons *Ardea herodias* fed *E. ictaluri*-infected channel catfish *Ictalurus*
818 *punctatus* fingerlings. *Journal of the World Aquaculture Society*. 1999;30(1):115-122.
819 doi:https://doi.org/10.1111/j.1749-7345.1999.tb00324.x.
- 820 51. Morrison EE, Plumb JA. Olfactory organ of channel catfish as a site of experimental *Edwardsiella*
821 *ictaluri* infection. *Journal of Aquatic Animal Health*. 1994;6(2):101-109.
822 doi:https://doi.org/10.1577/1548-8667(1994)006%3C0101:OOCCA%3E2.3.CO;2.
- 823 52. Newton J, Wolfe L, Grizzle J, Plumb J. Pathology of experimental enteric septicaemia in channel
824 catfish, *Ictalurus punctatus* (rafinesque), following immersion-exposure to *Edwardsiella ictaluri*.
825 *Journal of Fish Diseases*. 1989;12(4):335-347. doi:https://doi.org/10.1111/j.1365-
826 2761.1989.tb00322.x.
- 827 53. Plumb J, Sanchez D. Susceptibility of five species of fish to *Edwardsiella ictaluri*. *Journal of Fish*
828 *Diseases*. 1983;6(3):261-266. doi:https://doi.org/10.1111/j.1365-2761.1983.tb00075.x.
- 829 54. Shotts E, Blazer V, Waltman W. Pathogenesis of experimental *Edwardsiella ictaluri* infections in
830 channel catfish (*Ictalurus punctatus*). *Canadian Journal of Fisheries and Aquatic Sciences*.
831 1986;43(1):36-42. doi:http://dx.doi.org/10.1139/f86-005.
- 832 55. Hawke JP, Mcwhorter AC, Steigerwalt AG, Brenner DJ. *Edwardsiella ictaluri* sp. nov., the
833 causative agent of enteric septicemia of catfish. *International Journal of Systematic and*
834 *Evolutionary Microbiology*. 1981;31(4):396-400. doi:https://doi.org/10.1099/00207713-31-4-396.
- 835 56. Shotts E, Teska J. *Bacterial pathogens of aquatic vertebrates*. Vol 21. New York: John Wiley and
836 Sons; 1989.

- 837 57. Phillips A, Reichley S, Ware C, Griffin M. *Edwardsiella ictaluri* infection in *Pangasius catfish*
838 imported from West Bengal into the Southern Caribbean. *Journal of fish diseases*. 2017;40(6):743-
839 756. doi:https://doi.org/10.1111/jfd.12552.
- 840 58. Rogge ML, Dubytska L, Jung TS, et al. Comparison of Vietnamese and US isolates of *Edwardsiella*
841 *ictaluri*. *Diseases of aquatic organisms*. 2013;106(1):17-29. doi:https://doi.org/10.3354/dao02620.
- 842 59. Plumb JA, Vinitnantharat S. Biochemical, biophysical, and serological homogeneity of
843 *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 1989;1(1):51-56.
844 doi:https://doi.org/10.1577/1548-8667(1989)001%3C0051:BBASHO%3E2.3.CO;2.
- 845 60. Mitchell AJ, Goodwin AE. The isolation of *Edwardsiella ictaluri* with a limited tolerance for
846 aerobic growth from channel catfish. *Journal of Aquatic Animal Health*. 2000;12(4):297-300.
847 doi:http://dx.doi.org/10.1577/1548-8667(2000)012%3C0297:TIOEIW%3E2.0.CO;2.
- 848 61. Iwanowicz LR, Griffin AR, Cartwright DD, Blazer VS. Mortality and pathology in brown
849 bullheads *Amieurus nebulosus* associated with a spontaneous *Edwardsiella ictaluri* outbreak under
850 tank culture conditions. *Diseases of aquatic organisms*. 2006;70(3):219-225.
851 doi:https://doi.org/10.3354/dao070219.
- 852 62. Panangala VS, Shoemaker CA, McNulty ST, Arias CR, Klesius PH. Intra-and interspecific
853 phenotypic characteristics of fish-pathogenic *Edwardsiella ictaluri* and *E. tarda*. *Aquaculture*
854 *Research*. 2006;37(1):49-60. doi:https://doi.org/10.1111/j.1365-2109.2005.01394.x.
- 855 63. Waltman W, Shotts E, Hsu T. Biochemical characteristics of *Edwardsiella ictaluri*. *Applied and*
856 *Environmental Microbiology*. 1986;51(1):101-104. doi:https://doi.org/10.1128/aem.51.1.101-
857 104.1986.
- 858 64. Griffin M, Reichley S, Greenway T, et al. Comparison of *Edwardsiella ictaluri* isolates from
859 different hosts and geographic origins. *Journal of fish diseases*. 2016;39(8):947-969.
860 doi:https://doi.org/10.1111/jfd.12431.
- 861 65. Hassan ES, Mahmoud MM, Dung NH, Yuasa K, Nakai T. Serological characterization of
862 *Edwardsiella ictaluri* strains isolated from wild ayu *Plecoglossus altivelis*. *Fish Pathology*.
863 2010;45(1):43-46. doi:https://doi.org/10.3147/jsfp.45.43.
- 864 66. Lobb CJ, Ghaffari SH, Hayman JR, Thompson DT. Plasmid and serological differences between
865 *Edwardsiella ictaluri* strains. *Applied and Environmental Microbiology*. 1993;59(9):2830-2836.
866 doi:http://dx.doi.org/10.1128/AEM.59.9.2830-2836.1993.
- 867 67. Plumb J, Klesius P. An assessment of the antigenic homogeneity of *Edwardsiella ictaluri* using
868 monoclonal antibody. *Journal of Fish Diseases*. 1988;11(6):499-509.
869 doi:https://doi.org/10.1111/j.1365-2761.1988.tb00749.x.
- 870 68. Abbott SL, Janda JM. The genus *Edwardsiella*. In: Dworkin M. FS, Rosenberg E., Schleifer KH.,
871 Stackebrandt E. , ed. *Prokaryotes*. Vol 6. New York NY: Springer; 2006:72-89.
- 872 69. Klesius P, Shoemaker C. Heterologous isolates challenge of channel catfish, *Ictalurus punctatus*,
873 immune to *Edwardsiella ictaluri*. *Aquaculture*. 1997;157(1-2):147-155.
874 doi:https://doi.org/10.1016/S0044-8486(97)00076-8.
- 875 70. Wang R. *Comparative Genomic Studies of Catfish and Zebrafish Strains of Edwardsiella ictaluri*:
876 Pathobiological Sciences, Louisiana State University and Agricultural and Mechanical College;
877 2015.
- 878 71. Dung TT, Haesebrouck F, Tuan NA, Sorgeloos P, Baele M, Decostere A. Antimicrobial
879 susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis
880 of *Pangasianodon hypophthalmus* in Vietnam. *Microbial drug resistance*. 2008;14(4):311-316.
881 doi:https://doi.org/10.1089/mdr.2008.0848.
- 882 72. McGinnis A, Gaunt P, Santucci T, Simmons R, Endris R. In vitro evaluation of the susceptibility
883 of *Edwardsiella ictaluri*, etiological agent of enteric septicemia in channel catfish, *Ictalurus*
884 *punctatus* (Rafinesque), to florfenicol. *Journal of Veterinary Diagnostic Investigation*.
885 2003;15(6):576-579. doi:https://doi.org/10.1177/104063870301500612.
- 886 73. Santander J, Martin T, Loh A, Pohlenz C, Gatlin III DM, Curtiss III R. Mechanisms of intrinsic
887 resistance to antimicrobial peptides of *Edwardsiella ictaluri* and its influence on fish gut

- 888 inflammation and virulence. *Microbiology*. 2013;159(Pt 7):1471.
889 doi:https://doi.org/10.1099/mic.0.066639-0.
- 890 74. Zhang Y, Arias CR. Identification and characterization of an intervening sequence within the 23S
891 ribosomal RNA genes of *Edwardsiella ictaluri*. *Systematic and applied microbiology*.
892 2007;30(2):93-101. doi:https://doi.org/10.1016/j.syapm.2006.04.004.
- 893 75. Sakai T, Yuasa K, Ozaki A, et al. Genotyping of *Edwardsiella ictaluri* isolates in Japan using
894 amplified-fragment length polymorphism analysis. *Letters in applied microbiology*.
895 2009;49(4):443-449. doi:https://doi.org/10.1111/j.1472-765x.2009.02686.x.
- 896 76. Tekedar HC, Blom J, Kalindamar S, Nho S, Karsi A, Lawrence ML. Comparative genomics of the
897 fish pathogens *Edwardsiella ictaluri* 93-146 and *Edwardsiella piscicida* C07-087. *Microbial*
898 *genomics*. 2020;6(2). doi:https://doi.org/10.1099/mgen.0.000322.
- 899 77. Sakai T, Kuwada T, Muto Y, Takano T, Yuasa K, Oseko N. Comparative Proteomic Analysis
900 Between Virulent and Less Virulent Strains of *Edwardsiella ictaluri* Isolated from Ayu
901 *Plecoglossus altivelis*. *Fish Pathology*. 2018;53(1):29-35. doi:http://dx.doi.org/10.3147/jsfp.53.29.
- 902 78. Yang M, Lv Y, Xiao J, et al. *Edwardsiella* comparative phylogenomics reveal the new intra/inter-
903 species taxonomic relationships, virulence evolution and niche adaptation mechanisms. *PloS one*.
904 2012;7(5):e36987. doi:https://doi.org/10.1371/journal.pone.0036987.
- 905 79. Machimbirike VI, Uthapaisanwong P, Khunrae P, et al. Comparative genomics of *Edwardsiella*
906 *ictaluri* revealed four distinct host-specific genotypes and thirteen potential vaccine candidates.
907 *Genomics*. 2021;113(4):1976-1987. doi:https://doi.org/10.1016/j.ygeno.2021.04.016.
- 908 80. Newton JC, Bird R, Blevins W, Wilt G, Wolfe L. Isolation, characterization, and molecular cloning
909 of cryptic plasmids isolated from *Edwardsiella ictaluri*. *American journal of veterinary research*.
910 1988;49(11):1856-1860.
- 911 81. Speyerer P, Boyle J. The plasmid profile of *Edwardsiella ictaluri*. *Journal of Fish Diseases*.
912 1987;10(6):461-469. doi:http://dx.doi.org/10.1111/j.1365-2761.1987.tb01097.x.
- 913 82. Fernandez DH, Pittman-Cooley L, Thune RL. Sequencing and analysis of the *Edwardsiella ictaluri*
914 plasmids. *Plasmid*. 2001;45(1):52-56. doi:https://doi.org/10.1006/plas.2000.1499.
- 915 83. Bartie K, Austin FW, Diab A, et al. Intraspecific diversity of *Edwardsiella ictaluri* isolates from
916 diseased freshwater catfish, *Pangasianodon hypophthalmus* (Sauvage), cultured in the Mekong
917 Delta, Vietnam. *Journal of fish diseases*. 2012;35(9):671-682. doi:https://doi.org/10.1111/j.1365-
918 2761.2012.01376.x.
- 919 84. Reid WS, Boyle JA. Plasmid homologies in *Edwardsiella ictaluri*. *Applied and environmental*
920 *microbiology*. 1989;55(12):3253-3255. doi:https://doi.org/10.1128/aem.55.12.3253-3255.1989.
- 921 85. Carrias A, Welch TJ, Waldbieser GC, Mead DA, Terhune JS, Liles MR. Comparative genomic
922 analysis of bacteriophages specific to the channel catfish pathogen *Edwardsiella ictaluri*. *Virology*
923 *journal*. 2011;8(1):1-12. doi:https://doi.org/10.1186/1743-422X-8-6.
- 924 86. Baldwin TJ, Newton JC. Pathogenesis of enteric septicemia of channel catfish, caused by
925 *Edwardsiella ictaluri*: bacteriologic and light and electron microscopic findings. *Journal of aquatic*
926 *animal health*. 1993;5(3):189-198. doi:https://doi.org/10.1577/1548-
927 8667(1993)005%3C0189:POESOC%3E2.3.CO;2.
- 928 87. Wise DJ, Schwedler TE, Terhune JS. Uptake and clearance of *Edwardsiella ictaluri* in the
929 peripheral blood of channel catfish *Ictalurus punctatus* fingerlings during immersion challenge.
930 *Journal of the World Aquaculture Society*. 1997;28(1):45-51. doi:https://doi.org/10.1111/j.1749-
931 7345.1997.tb00960.x.
- 932 88. Menanteau-Ledouble S, Karsi A, Lawrence ML. Importance of skin abrasion as a primary site of
933 adhesion for *Edwardsiella ictaluri* and impact on invasion and systematic infection in channel
934 catfish *Ictalurus punctatus*. *Veterinary microbiology*. 2011;148(2-4):425-430.
935 doi:https://doi.org/10.1016/j.vetmic.2010.08.022.
- 936 89. Nusbaum K, Morrison E. Entry of 35S-labelled *Edwardsiella ictaluri* into channel catfish. *Journal*
937 *of Aquatic Animal Health*. 1996;8(2):146-149. doi:https://doi.org/10.1577/1548-
938 8667(1996)008%3C0146:CEOSLE%3E2.3.CO;2.

- 939 90. Miyazaki T, Plumb J. Histopathology of *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases*. 1985;8(4):389-392. doi:https://doi.org/10.1111/j.1365-2761.1985.tb00961.x.
- 942 91. Cooper RK, Shotts Jr EB, Nolan LK. Use of a mini-transposon to study chondroitinase activity associated with *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 1996;8(4):319-324. doi:https://doi.org/10.1577/1548-8667(1996)008%3C0319:UOAMTT%3E2.3.CO;2.
- 945 92. Areechon N, Plumb JA. Pathogenesis of *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus*. *Journal of the World Mariculture Society*. 1983;14(1-4):249-260. doi:https://doi.org/10.1111/j.1749-7345.1983.tb00081.x.
- 948 93. Williams M, Lawrence M. Identification and characterization of a two-component hemolysin from *Edwardsiella ictaluri*. *Veterinary microbiology*. 2005;108(3-4):281-289. doi:https://doi.org/10.1016/j.vetmic.2005.04.017.
- 951 94. Dung T, Chiers K, Tuan N, Sorgeloos P, Haesebrouck F, Decostere A. Early interactions of *Edwardsiella ictaluri*, with *Pangasianodon catfish* and its invasive ability in cell lines. *Veterinary research communications*. 2012;36(2):119-127. doi:https://doi.org/10.1007/s11259-012-9521-2.
- 954 95. Jarboe H, Bowser P, Robinette H. Pathology associated with a natural *Edwardsiella ictaluri* infection in channel catfish (*Ictalurus punctatus* Rafinesque). *Journal of Wildlife Diseases*. 1984;20(4):352-354. doi:https://doi.org/10.7589/0090-3558-20.4.352.
- 957 96. Gaafar AY, Hassan Abdullah ES, Mahmoud MM, Younes AM, Nakai T. Pathological and immunohistochemical studies following the experimental infection of ayu (*Plecoglossus altivelis*) by *Edwardsiella ictaluri*. *Microscopy Research and Technique*. 2021;84(3):460-470. doi:https://doi.org/10.1002/jemt.23602.
- 961 97. Hawke JP, Durborow R, Thune R, Camus A. Enteric septicemia of catfish. In. *SRAC publication*2015.
- 963 98. Blazer VS, Shotts E, Waltman W. Pathology associated with *Edwardsiella ictaluri* in catfish, *Ictalurus punctatus* Rafinesque, and *Danio devario* (Hamilton-Buchanan, 1822). *Journal of Fish Biology*. 1985;27(2):167-175. doi:https://doi.org/10.1111/j.1095-8649.1985.tb04018.x.
- 966 99. Li C, Zhang Y, Wang R, et al. RNA-seq analysis of mucosal immune responses reveals signatures of intestinal barrier disruption and pathogen entry following *Edwardsiella ictaluri* infection in channel catfish, *Ictalurus punctatus*. *Fish & shellfish immunology*. 2012;32(5):816-827. doi:https://doi.org/10.1016/j.fsi.2012.02.004.
- 970 100. Kalindamar S, Abdelhamed H, Kordon AO, Pinchuk LM, Karsi A. Hemolysin Co-regulated Family Proteins Hcp1 and Hcp2 Contribute to *Edwardsiella ictaluri* Pathogenesis. *Frontiers in Veterinary Science*. 2021;8. doi:https://doi.org/10.3389/fvets.2021.681609.
- 973 101. Zhao LJ, Lu JF, Nie P, Li AH, Xiong BX, Xie HX. Roles of plasmid-encoded proteins, EseH, EseI and EscD in invasion, replication and virulence of *Edwardsiella ictaluri*. *Veterinary microbiology*. 2013;166(1-2):233-241. doi:https://doi.org/10.1016/j.vetmic.2013.05.023.
- 976 102. Skirpstunas RT, Baldwin TJ. *Edwardsiella ictaluri* invasion of IEC-6, Henle 407, fathead minnow and channel catfish enteric epithelial cells. *Diseases of aquatic organisms*. 2002;51(3):161-167. doi:https://doi.org/10.3354/dao051161.
- 979 103. Hohn C, Lee S-R, Pinchuk LM, Petrie-Hanson L. Zebrafish kidney phagocytes utilize macropinocytosis and Ca²⁺-dependent endocytic mechanisms. *PLoS one*. 2009;4(2):e4314. doi:https://doi.org/10.1371/journal.pone.0004314.
- 982 104. Baumgartner WA, Dubytska L, Rogge ML, Mottram PJ, Thune RL. Modulation of vacuolar pH is required for replication of *Edwardsiella ictaluri* in channel catfish macrophages. *Infection and immunity*. 2014;82(6):2329-2336. doi:http://dx.doi.org/10.1128/IAI.01616-13.
- 985 105. Rogge ML, Thune RL. Regulation of the *Edwardsiella ictaluri* type III secretion system by pH and phosphate concentration through EsrA, EsrB, and EsrC. *Applied and environmental microbiology*. 2011;77(13):4293-4302. doi:https://dx.doi.org/10.1128%2FAEM.00195-11.

- 988 106. Dubytska LP, Rogge ML, Thune RL. Identification and characterization of putative translocated
989 effector proteins of the *Edwardsiella ictaluri* type III secretion system. *MSphere*.
990 2016;1(3):e00039-00016. doi:http://dx.doi.org/10.1128/mSphere.00039-16.
- 991 107. Booth NJ, Beekman JB, Thune RL. *Edwardsiella ictaluri* encodes an acid-activated urease that is
992 required for intracellular replication in channel catfish (*Ictalurus punctatus*) macrophages. *Applied*
993 *and environmental microbiology*. 2009;75(21):6712-6720. doi:https://doi.org/10.1128/aem.01670-
994 09.
- 995 108. Dubytska LP, Thune RL. Early Intracellular Trafficking and Subsequent Activity of Programmed
996 Cell Death in Channel Catfish Macrophages Infected with *Edwardsiella ictaluri*. *Microorganisms*.
997 2020;8(11):1649. doi:https://doi.org/10.3390/microorganisms8111649.
- 998 109. Booth NJ, Elkamel A, Thune RL. Intracellular replication of *Edwardsiella ictaluri* in channel
999 catfish macrophages. *Journal of aquatic animal health*. 2006;18(2):101-108.
1000 doi:https://doi.org/10.1577/H05-025.1.
- 1001 110. Abdelhamed H, Tekedar HC, Ozdemir O, et al. Complete genome sequence of multidrug-resistant
1002 *Edwardsiella ictaluri* strain MS-17-156. *Genome announcements*. 2018;6(22):e00477-00418.
1003 doi:https://doi.org/10.1128/genomea.00477-18.
- 1004 111. Thune RL, Fernandez DH, Benoit JL, et al. Signature-tagged mutagenesis of *Edwardsiella ictaluri*
1005 identifies virulence-related genes, including a *Salmonella* pathogenicity island 2 class of type III
1006 secretion systems. *Applied and environmental microbiology*. 2007;73(24):7934-7946.
1007 doi:https://doi.org/10.1128/aem.01115-07.
- 1008 112. Williams M, Gillaspay A, Dyer D, et al. Genome sequence of *Edwardsiella ictaluri* 93-146, a strain
1009 associated with a natural channel catfish outbreak of enteric septicemia of catfish. 2012.
1010 doi:https://dx.doi.org/10.1128%2FJJB.06522-11.
- 1011 113. Lawrence ML, Banes MM, Azadi P, Reeks BY. The *Edwardsiella ictaluri* O polysaccharide
1012 biosynthesis gene cluster and the role of O polysaccharide in resistance to normal catfish serum
1013 and catfish neutrophils. *Microbiology*. 2003;149(6):1409-1421.
1014 doi:https://doi.org/10.1099/mic.0.26138-0.
- 1015 114. Menanteau-Ledouble S, Lawrence ML. Use of bioluminescence mutant screening for identification
1016 of *Edwardsiella ictaluri* genes involved in channel catfish (*Ictalurus punctatus*) skin colonization.
1017 *Veterinary microbiology*. 2013;162(2-4):724-730. doi:https://doi.org/10.3791/2687.
- 1018 115. Stanley L, Hudson J, Schwedler T, Hayasaka S. Extracellular products associated with virulent and
1019 avirulent strains of *Edwardsiella ictaluri* from channel catfish. *Journal of Aquatic Animal Health*.
1020 1994;6(1):36-43. doi:https://doi.org/10.1577/1548-
1021 8667(1994)006%3C0036:EPAWVA%3E2.3.CO;2.
- 1022 116. Williams ML, Azadi P, Lawrence ML. Comparison of cellular and extracellular products expressed
1023 by virulent and attenuated strains of *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*.
1024 2003;15(4):264-273. doi:https://doi.org/10.1577/H03-051.1.
- 1025 117. Newton JC, Triche PL. Isolation and characterization of flagella from *Edwardsiella ictaluri*.
1026 *Journal of aquatic animal health*. 1993;5(1):16-22. doi:https://doi.org/10.1577/1548-
1027 8667(1993)005%3C0016:IACOFF%3E2.3.CO;2.
- 1028 118. Santander J, Kilbourne J, Park J-Y, et al. Inflammatory effects of *Edwardsiella ictaluri*
1029 lipopolysaccharide modifications in catfish gut. *Infection and immunity*. 2014;82(8):3394-3404.
1030 doi:https://dx.doi.org/10.1128%2FIAI.01697-14.
- 1031 119. Santander J, Golden G, Wanda S-Y, Curtiss III R. Fur-regulated iron uptake system of
1032 *Edwardsiella ictaluri* and its influence on pathogenesis and immunogenicity in the catfish host.
1033 *Infection and immunity*. 2012;80(8):2689-2703. doi:https://dx.doi.org/10.1128%2FIAI.00013-12.
- 1034 120. Abdelhamed H, Lawrence ML, Karsi A. The role of *TonB* gene in *Edwardsiella ictaluri* virulence.
1035 *Frontiers in physiology*. 2017;8:1066. doi:https://doi.org/10.3389/fphys.2017.01066.
- 1036 121. Akgul A, Akgul A, Lawrence ML, Karsi A. Stress-related genes promote *Edwardsiella ictaluri*
1037 pathogenesis. *Plos one*. 2018;13(3):e0194669. doi:https://doi.org/10.1371/journal.pone.0194669.

- 1038 122. Klesius P. Immune system of channel catfish: an overture on immunity to *Edwardsiella ictaluri*.
 1039 *Annual Review of Fish Diseases*. 1992;2:325-338. doi:https://doi.org/10.1016/0959-
 1040 8030(92)90069-A.
- 1041 123. Zhou T, Yuan Z, Tan S, et al. A review of molecular responses of catfish to bacterial diseases and
 1042 abiotic stresses. *Frontiers in physiology*. 2018;9:1113.
 1043 doi:https://doi.org/10.3389/fphys.2018.01113.
- 1044 124. Tan S, Wang W, Zhong X, et al. Increased alternative splicing as a host response to *Edwardsiella*
 1045 *ictaluri* infection in catfish. *Marine Biotechnology*. 2018;20(6):729-738.
 1046 doi:https://doi.org/10.1007/s10126-018-9844-2.
- 1047 125. Gao L, He C, Liu X, et al. The innate immune-related genes in catfish. *International Journal of*
 1048 *Molecular Sciences*. 2012;13(11):14172-14202. doi:https://doi.org/10.3390/ijms131114172.
- 1049 126. Peatman E, Baoprasertkul P, Terhune J, et al. Expression analysis of the acute phase response in
 1050 channel catfish (*Ictalurus punctatus*) after infection with a Gram-negative bacterium.
 1051 *Developmental & Comparative Immunology*. 2007;31(11):1183-1196.
 1052 doi:https://doi.org/10.1016/j.dci.2007.03.003.
- 1053 127. Baoprasertkul P, Peatman E, Somridhivej B, Liu Z. Toll-like receptor 3 and TICAM genes in
 1054 catfish: species-specific expression profiles following infection with *Edwardsiella ictaluri*.
 1055 *Immunogenetics*. 2006;58(10):817-830. doi:https://doi.org/10.1007/s00251-006-0144-z.
- 1056 128. Bilodeau AL, Waldbieser GC. Activation of TLR3 and TLR5 in channel catfish exposed to virulent
 1057 *Edwardsiella ictaluri*. *Developmental & Comparative Immunology*. 2005;29(8):713-721.
 1058 doi:https://doi.org/10.1016/j.dci.2004.12.002.
- 1059 129. Chen K, Zhao F, Ouyang G, et al. Molecular characterization and expression analysis of Tf_TLR4
 1060 and Tf_TRIL in yellow catfish *Tachysurus fulvidraco* responding to *Edwardsiella ictaluri*
 1061 challenge. *International Journal of Biological Macromolecules*. 2021;167:746-755.
 1062 doi:https://doi.org/10.1016/j.ijbiomac.2020.11.196.
- 1063 130. Schmitz M, Baekelandt S, Bequet S, Kestemont P. Chronic hyperosmotic stress inhibits renal Toll-
 1064 Like Receptors expression in striped catfish (*Pangasianodon hypophthalmus*, Sauvage) exposed or
 1065 not to bacterial infection. *Developmental & Comparative Immunology*. 2017;73:139-143.
 1066 doi:https://doi.org/10.1016/j.dci.2017.03.020.
- 1067 131. Zhang J, Liu S, Rajendran K, et al. Pathogen recognition receptors in channel catfish: III Phylogeny
 1068 and expression analysis of Toll-like receptors. *Developmental & Comparative Immunology*.
 1069 2013;40(2):185-194. doi:https://doi.org/10.1016/j.dci.2013.01.009.
- 1070 132. Sun L, Liu S, Wang R, Li C, Zhang J, Liu Z. Pathogen recognition receptors in channel catfish: IV.
 1071 Identification, phylogeny and expression analysis of peptidoglycan recognition proteins.
 1072 *Developmental & Comparative Immunology*. 2014;46(2):291-299.
 1073 doi:https://doi.org/10.1016/j.dci.2014.04.018.
- 1074 133. Sha Z, Abernathy JW, Wang S, et al. NOD-like subfamily of the nucleotide-binding domain and
 1075 leucine-rich repeat containing family receptors and their expression in channel catfish.
 1076 *Developmental & Comparative Immunology*. 2009;33(9):991-999.
 1077 doi:https://doi.org/10.1016/j.dci.2009.04.004.
- 1078 134. Rajendran K, Zhang J, Liu S, et al. Pathogen recognition receptors in channel catfish: II.
 1079 Identification, phylogeny and expression of retinoic acid-inducible gene I (RIG-I)-like receptors
 1080 (RLRs). *Developmental & Comparative Immunology*. 2012;37(3-4):381-389.
 1081 doi:https://doi.org/10.1016/j.dci.2012.02.004.
- 1082 135. Zhou S, Zhao H, Thongda W, et al. Galectins in channel catfish, *Ictalurus punctatus*:
 1083 characterization and expression profiling in mucosal tissues. *Fish & shellfish immunology*.
 1084 2016;49:324-335. doi:https://doi.org/10.1016/j.fsi.2016.01.005.
- 1085 136. Bao B, Peatman E, Li P, He C, Liu Z. Catfish hepcidin gene is expressed in a wide range of tissues
 1086 and exhibits tissue-specific upregulation after bacterial infection. *Developmental & Comparative*
 1087 *Immunology*. 2005;29(11):939-950. doi:https://doi.org/10.1016/j.dci.2005.03.006.

- 1088 137. Bao B, Peatman E, Xu P, et al. The catfish liver-expressed antimicrobial peptide 2 (LEAP-2) gene
1089 is expressed in a wide range of tissues and developmentally regulated. *Molecular Immunology*.
1090 2006;43(4):367-377. doi:https://doi.org/10.1016/j.molimm.2005.02.014.
- 1091 138. Xu P, Bao B, He Q, Peatman E, He C, Liu Z. Characterization and expression analysis of
1092 bactericidal permeability-increasing protein (BPI) antimicrobial peptide gene from channel catfish
1093 *Ictalurus punctatus*. *Developmental & Comparative Immunology*. 2005;29(10):865-878.
1094 doi:https://doi.org/10.1016/j.dci.2005.03.004.
- 1095 139. Zhu J, Wang H, Wang J, et al. Identification and characterization of a β -defensin gene involved in
1096 the immune defense response of channel catfish, *Ictalurus punctatus*. *Molecular immunology*.
1097 2017;85:256-264. doi:https://doi.org/10.1016/j.molimm.2017.03.009.
- 1098 140. Zhu R, Wu Y-S, Liu X-X, et al. Membrane disruptive antimicrobial potential of NK-lysin from
1099 yellow catfish (*Pelteobagrus fulvidraco*). *Fish & shellfish immunology*. 2020;97:571-580.
1100 doi:https://doi.org/10.1016/j.fsi.2019.10.046.
- 1101 141. Yeh H-Y, Klesius PH. Channel catfish, *Ictalurus punctatus*, cysteine proteinases: cloning,
1102 characterisation and expression of cathepsin H and L. *Fish & shellfish immunology*.
1103 2009;26(2):332-338. doi:https://doi.org/10.1016/j.fsi.2008.11.010.
- 1104 142. Feng T, Zhang H, Liu H, et al. Molecular characterization and expression analysis of the channel
1105 catfish cathepsin D genes. *Fish & shellfish immunology*. 2011;31(1):164-169.
1106 doi:https://doi.org/10.1016/j.fsi.2011.04.006.
- 1107 143. Wang R, Feng J, Li C, Liu S, Zhang Y, Liu Z. Four lysozymes (one c-type and three g-type) in
1108 catfish are drastically but differentially induced after bacterial infection. *Fish & shellfish*
1109 *immunology*. 2013;35(1):136-145. doi:https://doi.org/10.1016/j.fsi.2013.04.014.
- 1110 144. Yao J, Li C, Zhang J, et al. Expression of nitric oxide synthase (NOS) genes in channel catfish is
1111 highly regulated and time dependent after bacterial challenges. *Developmental & Comparative*
1112 *Immunology*. 2014;45(1):74-86. doi:https://doi.org/10.1016/j.dci.2014.02.005.
- 1113 145. Yeh H-Y, Klesius P. Changes of serum myeloperoxidase and nitric oxide in the early stage of
1114 *Edwardsiella ictaluri* infection in channel catfish, *Ictalurus punctatus* (Rafinesque). 2013.
1115 doi:https://doi.org/10.1111/jfd.12038.
- 1116 146. Gao L, Yuan Z, Zhou T, et al. FOXO genes in channel catfish and their response after bacterial
1117 infection. *Developmental & Comparative Immunology*. 2019;97:38-44.
1118 doi:https://doi.org/10.1016/j.dci.2019.03.010.
- 1119 147. Li Z, Yao J, Xie Y, Geng X, Liu Z. Phosphoinositide 3-kinase family in channel catfish and their
1120 regulated expression after bacterial infection. *Fish & shellfish immunology*. 2016;49:364-373.
1121 doi:https://doi.org/10.1016/j.fsi.2016.01.002.
- 1122 148. Liu H, Takano T, Abernathy J, et al. Structure and expression of transferrin gene of channel catfish,
1123 *Ictalurus punctatus*. *Fish & Shellfish Immunology*. 2010;28(1):159-166.
1124 doi:https://doi.org/10.1016/j.fsi.2009.10.014.
- 1125 149. Mu W, Yao J, Zhang J, et al. Expression of tumor suppressor genes in channel catfish after bacterial
1126 infections. *Developmental & Comparative Immunology*. 2015;48(1):171-177.
1127 doi:https://doi.org/10.1016/j.dci.2014.10.004.
- 1128 150. Chen H, Yuan G, Su J, Liu X. Hematological and immune genes responses in yellow catfish
1129 (*Pelteobagrus fulvidraco*) with septicemia induced by *Edwardsiella ictaluri*. *Fish & shellfish*
1130 *immunology*. 2020;97:531-539. doi:https://doi.org/10.1016/j.fsi.2019.11.071.
- 1131 151. Ainsworth A. Carbohydrate and lectin interactions with *Edwardsiella ictaluri* and channel catfish,
1132 *Ictalurus punctatus* (Rafinesque), anterior kidney leucocytes and hepatocytes. *Journal of Fish*
1133 *Diseases*. 1993;16(5):449-459. doi:https://doi.org/10.1111/j.1365-2761.1993.tb00878.x.
- 1134 152. Jenkins JA, Ourth DD. Opsonic effect of the alternative complement pathway on channel catfish
1135 peripheral blood phagocytes. *Veterinary Immunology and Immunopathology*. 1993;39(4):447-459.
1136 doi:https://doi.org/10.1016/0165-2427(93)90074-e.

- 1137 153. Li J, Zhang X, Xu J, et al. iTRAQ analysis of liver immune-related proteins from darkbarbel catfish
1138 (*Pelteobagrus vachelli*) infected with *Edwardsiella ictaluri*. *Fish & shellfish immunology*.
1139 2019;87:695-704. doi:https://doi.org/10.1016/j.fsi.2019.01.036.
- 1140 154. Dong X, Qin Z, Hu X, et al. Molecular cloning and functional characterization of cyclophilin A in
1141 yellow catfish (*Pelteobagrus fulvidraco*). *Fish & shellfish immunology*. 2015;45(2):422-430.
1142 doi:https://doi.org/10.1016/j.fsi.2015.04.002.
- 1143 155. Fu Q, Yang Y, Li C, et al. The chemokine superfamily: II. The 64 CC chemokines in channel
1144 catfish and their involvement in disease and hypoxia responses. *Developmental & Comparative*
1145 *Immunology*. 2017;73:97-108. doi:https://doi.org/10.1016/j.dci.2017.03.012.
- 1146 156. Fu Q, Zeng Q, Li Y, et al. The chemokine superfamily in channel catfish: I. CXC subfamily and
1147 their involvement in disease defense and hypoxia responses. *Fish & shellfish immunology*.
1148 2017;60:380-390. doi:https://doi.org/10.1016/j.fsi.2016.12.004.
- 1149 157. Jiang R, Zhang G-R, Zhu D-M, et al. Molecular characterization and expression analysis of IL-22
1150 and its two receptors genes in yellow catfish (*Pelteobagrus filivdraco*) in response to *Edwardsiella*
1151 *ictaluri* challenge. *Fish & shellfish immunology*. 2018;80:250-263.
1152 doi:https://doi.org/10.1016/j.fsi.2018.06.012.
- 1153 158. Yao J, Mu W, Liu S, Zhang J, Wen H, Liu Z. Identification, phylogeny and expression analysis of
1154 suppressors of cytokine signaling in channel catfish. *Molecular immunology*. 2015;64(2):276-284.
1155 doi:https://doi.org/10.1016/j.molimm.2014.12.003.
- 1156 159. Jin Y, Zhou T, Li N, et al. JAK and STAT members in channel catfish: identification, phylogenetic
1157 analysis and expression profiling after *Edwardsiella ictaluri* infection. *Developmental &*
1158 *Comparative Immunology*. 2018;81:334-341. doi:https://doi.org/10.1016/j.dci.2017.12.019.
- 1159 160. Yeh H-Y, Klesius PH. Identification, phylogenetic relationships, characterization and gene
1160 expression patterns of six different annexins of channel catfish (*Ictalurus punctatus* Rafinesque,
1161 1818). *Veterinary immunology and immunopathology*. 2010;136(1-2):176-183.
1162 doi:https://doi.org/10.1016/j.vetimm.2010.02.013.
- 1163 161. Takano T, Sha Z, Peatman E, et al. The two channel catfish intelectin genes exhibit highly
1164 differential patterns of tissue expression and regulation after infection with *Edwardsiella ictaluri*.
1165 *Developmental & Comparative Immunology*. 2008;32(6):693-705.
1166 doi:https://doi.org/10.1016/j.dci.2007.10.008.
- 1167 162. Yang Y, Fu Q, Zhou T, et al. Analysis of apolipoprotein genes and their involvement in disease
1168 response of channel catfish after bacterial infection. *Developmental & Comparative Immunology*.
1169 2017;67:464-470. doi:https://doi.org/10.1016/j.dci.2016.09.007.
- 1170 163. Liu H, Peatman E, Wang W, et al. Molecular responses of calreticulin genes to iron overload and
1171 bacterial challenge in channel catfish (*Ictalurus punctatus*). *Developmental & Comparative*
1172 *Immunology*. 2011;35(3):267-272. doi:https://doi.org/10.1016/j.dci.2010.11.009.
- 1173 164. Song L, Li C, Xie Y, et al. Genome-wide identification of Hsp70 genes in channel catfish and their
1174 regulated expression after bacterial infection. *Fish & shellfish immunology*. 2016;49:154-162.
1175 doi:https://doi.org/10.1016/j.fsi.2015.12.009.
- 1176 165. Camp KL, Wolters WR, Rice CD. Survivability and immune responses after challenge with
1177 *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish, *Ictalurus punctatus*.
1178 *Fish & shellfish immunology*. 2000;10(6):475-487. doi:https://doi.org/10.1006/fsim.2000.0261.
- 1179 166. Kordon AO, Abdelhamed H, Karsi A, Pinchuk LM. Adaptive immune responses in channel catfish
1180 exposed to *Edwardsiella ictaluri* live attenuated vaccine and wild type strains through the specific
1181 gene expression profiles. *Developmental & Comparative Immunology*. 2021;116:103950.
1182 doi:https://doi.org/10.1016/j.dci.2020.103950.
- 1183 167. Bilodeau A, Small B, Wise D, Wolters W. Pathogen levels, lysozyme, and cortisol response in
1184 channel catfish with susceptibility differences to *Edwardsiella ictaluri*. *Journal of Aquatic Animal*
1185 *Health*. 2005;17(2):138-146.

- 1186 168. Patrie-Hanson L, Ainsworth AJ. Humoral immune responses of channel catfish (*Ictalurus punctatus*) fry and fingerlings exposed to *Edwardsiella ictaluri*. *Fish & Shellfish Immunology*. 1999;9(8):579-589. doi:https://doi.org/10.1006/fsim.1999.0215.
- 1187
- 1188
- 1189 169. Blackmon LE, Quiniou SM, Wilson M, Bengtén E. Catfish lymphocytes expressing CC41-reactive leukocyte immune-type receptors (LITRs) proliferate in response to *Edwardsiella ictaluri* infection in vitro. *Developmental & Comparative Immunology*. 2020;106:103610. doi:https://doi.org/10.1016/j.dci.2020.103610.
- 1190
- 1191
- 1192
- 1193 170. Peatman E, Terhune J, Baoprasertkul P, et al. Microarray analysis of gene expression in the blue catfish liver reveals early activation of the MHC class I pathway after infection with *Edwardsiella ictaluri*. *Molecular immunology*. 2008;45(2):553-566. doi:https://doi.org/10.1016/j.molimm.2007.05.012.
- 1194
- 1195
- 1196
- 1197 171. Dawar FU, Babu S, Kou H, et al. The RAG2 gene of yellow catfish (*Tachysurus fulvidraco*) and its immune response against *Edwardsiella ictaluri* infection. *Developmental & Comparative Immunology*. 2019;98:65-75. doi:https://doi.org/10.1016/j.dci.2019.04.006.
- 1198
- 1199
- 1200 172. Shotts EB, Waltman WD. A medium for the selective isolation of *Edwardsiella ictaluri*. *Journal of Wildlife Diseases*. 1990;26(2):214-218. doi:https://doi.org/10.7589/0090-3558-26.2.214.
- 1201
- 1202 173. Collins LA, Thune RL. Development of a defined minimal medium for the growth of *Edwardsiella ictaluri*. *Applied and Environmental Microbiology*. 1996;62(3):848-852. doi:https://doi.org/10.1128/aem.62.3.848-852.1996.
- 1203
- 1204
- 1205 174. Karsi A, Menanteau-Ledouble S, Lawrence ML. Development of bioluminescent *Edwardsiella ictaluri* for noninvasive disease monitoring. *FEMS microbiology letters*. 2006;260(2):216-223. doi:https://doi.org/10.1111/j.1574-6968.2006.00310.x.
- 1206
- 1207
- 1208 175. Kelly E, Martin P, Gibson-Kueh S, et al. First detection of *Edwardsiella ictaluri* (Proteobacteria: Enterobacteriaceae) in wild Australian catfish. *Journal of fish diseases*. 2018;41(2):199-208. doi:https://doi.org/10.1111/jfd.12696.
- 1209
- 1210
- 1211 176. Nhu TQ, Park SB, Kim SW, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry based identification of *Edwardsiella ictaluri* isolated from Vietnamese striped catfish (*Pangasius hypophthalmus*). *Journal of veterinary science*. 2016;17(3):377-383. doi:https://doi.org/10.4142/jvs.2016.17.3.377.
- 1212
- 1213
- 1214
- 1215 177. OIE. Manual of diagnostic tests for aquatic animals. 2009. Published 6th. Accessed 18 September 2019.
- 1216
- 1217 178. Klesius PH. Rapid enzyme-linked immunosorbent tests for detecting antibodies to *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus*, using exoantigen. *Veterinary Immunology and Immunopathology*. 1993;36(4):359-368. doi:https://doi.org/10.1016/0165-2427(93)90031-x.
- 1218
- 1219
- 1220 179. Klesius P, Johnson K, Durborow R, Vinitnantharat S. Development and evaluation of an enzyme-linked immunosorbent assay for catfish serum antibody to *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 1991;3(2):94-99. doi:https://doi.org/10.1577/1548-8667(1991)003%3C0094:DAEOAE%3E2.3.CO;2.
- 1221
- 1222
- 1223
- 1224 180. Waterstrat P, Ainsworth J, Capley G. Use of an indirect enzyme-linked immunosorbent assay (elisa) in the detection of channel catfish, *Ictalurus punctatus* (Rafinesque), antibodies to *Edwardsiella ictaluri*. *Journal of Fish Diseases*. 1989;12(2):87-94. doi:https://doi.org/10.1111/j.1365-2761.1989.tb00280.x.
- 1225
- 1226
- 1227
- 1228 181. Earlix D, Plumb JA, Rogers W. Isolation of *Edwardsiella ictaluri* from channel catfish by tissue homogenization, filtration and enzyme linked immunosorbent assay. *Diseases of aquatic organisms*. 1996;27(1):19-24. doi:https://doi.org/10.3354/DAO027019.
- 1229
- 1230
- 1231 182. Hanson LA, Rogers WA. Enzyme immunoassay identification of *Edwardsiella ictaluri* in decomposing channel catfish. *Journal of the World Aquaculture Society*. 1989;20(4):279-280. doi:https://doi.org/10.1111/j.1749-7345.1989.tb01016.x.
- 1232
- 1233
- 1234 183. Ainsworth A, Capley G, Waterstreet P, Munson D. Use of monoclonal antibodies in the indirect fluorescent antibody technique (IFA) for the diagnosis of *Edwardsiella ictaluri*. *Journal of Fish Diseases*. 1986;9(5):439-444. doi:https://doi.org/10.1111/j.1365-2761.1986.tb01037.x.
- 1235
- 1236

- 1237 184. Panangala VS, Shelby RA, Shoemaker CA, Klesius PH, Mitra A, Morrison EE. Immunofluorescent
1238 test for simultaneous detection of *Edwardsiella ictaluri* and *Flavobacterium columnare*. *Diseases*
1239 *of aquatic organisms*. 2006;68(3):197-207. doi:https://doi.org/10.3354/dao068197.
- 1240 185. Bertolini JM, Cipriano RC, Pyle S, McLaughlin JJ. Serological investigation of the fish pathogen
1241 *Edwardsiella ictaluri*, cause of enteric septicemia of catfish. *Journal of Wildlife Diseases*.
1242 1990;26(2):246-252. doi:https://doi.org/10.7589/0090-3558-26.2.246.
- 1243 186. Saeed M, Plumb J. Serological detection of *Edwardsiella ictaluri* Hawke lipopolysaccharide
1244 antibody in serum of channel catfish, *Ictalurus punctatus* Rafinesque. *Journal of Fish Diseases*.
1245 1987;10(3):205-209. doi:https://doi.org/10.1111/j.1365-2761.1987.tb01062.x.
- 1246 187. Geng Y, Wang K, Li C, et al. Isolation and characterization of *Edwardsiella ictaluri* from southern
1247 catfish, *Silurus soldatovi meridionalis*, (Chen) cultured in China. *Journal of the World Aquaculture*
1248 *Society*. 2013;44(2):273-281. doi:http://dx.doi.org/10.1111/jwas.12025.
- 1249 188. Griffin MJ, Mauel MJ, Greenway TE, Khoo LH, Wise DJ. A real-time polymerase chain reaction
1250 assay for quantification of *Edwardsiella ictaluri* in catfish pond water and genetic homogeneity of
1251 diagnostic case isolates from Mississippi. *Journal of Aquatic Animal Health*. 2011;23(4):178-188.
1252 doi:https://doi.org/10.1080/08997659.2011.637006.
- 1253 189. Sakai T, Yuasa K, Sano M, Iida T. Identification of *Edwardsiella ictaluri* and *E. tarda* by species-
1254 specific polymerase chain reaction targeted to the upstream region of the fimbrial gene. *Journal of*
1255 *aquatic animal health*. 2009;21(2):124-132. doi:https://doi.org/10.1577/h08-061.1.
- 1256 190. Williams M, Lawrence M. Verification of an *Edwardsiella ictaluri*-specific diagnostic PCR.
1257 *Letters in applied microbiology*. 2010;50(2):153-157. doi:https://doi.org/10.1111/j.1472-
1258 765x.2009.02770.x.
- 1259 191. Sanchez-Martinez J, Perez-Castaneda R, Vazquez Saucedo M, Rabago Castro J, Aguirre-Guzman
1260 G. Rapid detection of *Edwardsiella ictaluri* from Channel catfish tissue, water samples by PCR
1261 amplification. *Journal of Animal and Veterinary Advances*. 2012;11(20):3823-3826.
1262 doi:http://dx.doi.org/10.3923/javaa.2012.3823.3826.
- 1263 192. Yeh H-Y, Shoemaker CA, Klesius PH. Evaluation of a loop-mediated isothermal amplification
1264 method for rapid detection of channel catfish *Ictalurus punctatus* important bacterial pathogen
1265 *Edwardsiella ictaluri*. *Journal of microbiological methods*. 2005;63(1):36-44.
1266 doi:https://doi.org/10.1016/j.mimet.2005.02.015.
- 1267 193. Chander Y, Koelbl J, Puckett J, et al. A novel thermostable polymerase for RNA and DNA loop-
1268 mediated isothermal amplification (LAMP). *Frontiers in Microbiology*. 2014;5:395.
1269 doi:https://doi.org/10.3389/fmicb.2014.00395.
- 1270 194. Wu H, Sugata K, Hirai M, Yoshiura Y, Hibi K, Endo H. Ultra highly sensitive method for detecting
1271 *Edwardsiella ictaluri* using high-gradient immunomagnetic separation with polymerase chain
1272 reaction. *Sensing and bio-sensing research*. 2017;16:68-73.
1273 doi:https://doi.org/10.1016/j.sbsr.2017.11.003.
- 1274 195. Wise DJ, Johnson MR. Effect of feeding frequency and Romet-medicated feed on survival,
1275 antibody response, and weight gain of fingerling channel catfish *Ictalurus punctatus* after natural
1276 exposure to *Edwardsiella ictaluri*. *Journal of the World Aquaculture Society*. 1998;29(2):169-175.
1277 doi:https://doi.org/10.1111/j.1749-7345.1998.tb00976.x.
- 1278 196. Gaunt P, Endris R, Khoo L, et al. Preliminary assessment of the tolerance and efficacy of florfenicol
1279 against *Edwardsiella ictaluri* administered in feed to channel catfish. *Journal of Aquatic Animal*
1280 *Health*. 2003;15(3):239-247. doi:https://doi.org/10.1577/H03-022.
- 1281 197. Phu TM, Phuong NT, Dung TT, et al. An evaluation of fish health-management practices and
1282 occupational health hazards associated with *Pangasius catfish* (*Pangasianodon hypophthalmus*)
1283 aquaculture in the Mekong Delta, Vietnam. *Aquaculture Research*. 2016;47(9):2778-2794.
1284 doi:https://doi.org/10.1111/are.12728.
- 1285 198. Plumb J, Maestrone G, Quinlan E. Use of a potentiated sulfonamide to control *Edwardsiella ictaluri*
1286 infection in channel catfish (*Ictalurus punctatus*). *Aquaculture*. 1987;62(3-4):187-194.
1287 doi:https://doi.org/10.1016/0044-8486(87)90165-7.

- 1288 199. Xu N, Li M, Ai X, Lin Z. Determination of Pharmacokinetic and Pharmacokinetic-
1289 Pharmacodynamic Parameters of Doxycycline against *Edwardsiella ictaluri* in Yellow Catfish
1290 (*Pelteobagrus fulvidraco*). *Antibiotics*. 2021;10(3):329. doi:<https://www.mdpi.com/2079-6382/10/3/329>.
1291
- 1292 200. Abdelhamed H, Lawrence ML, Karsi A. Development and characterization of a novel live
1293 attenuated vaccine against enteric septicemia of catfish. *Frontiers in microbiology*. 2018;9:1819.
1294 doi:<https://dx.doi.org/10.3389/fmicb.2018.01819>.
- 1295 201. Klesius PH, Shoemaker CA. Development and use of modified live *Edwardsiella ictaluri* vaccine
1296 against enteric septicemia of catfish. *Advances in veterinary medicine*. 1999;41:523-537.
1297 doi:[https://doi.org/10.1016/s0065-3519\(99\)80039-1](https://doi.org/10.1016/s0065-3519(99)80039-1).
- 1298 202. Starliper CE, Cooper RK, Shotts Jr EB, Taylor PW. Plasmid-mediated Romet resistance of
1299 *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 1993;5(1):1-8.
1300 doi:[https://doi.org/10.1577/1548-8667\(1993\)005%3C0001:PMRROE%3E2.3.CO;2](https://doi.org/10.1577/1548-8667(1993)005%3C0001:PMRROE%3E2.3.CO;2).
- 1301 203. Kim MK, Lovell RT. Effect of overwinter feeding regimen on body weight, body composition and
1302 resistance to *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus*. *Aquaculture*.
1303 1995;134(3-4):237-246. doi:[https://doi.org/10.1016/0044-8486\(95\)00045-4](https://doi.org/10.1016/0044-8486(95)00045-4).
- 1304 204. Mainous ME, Smith SA, Kuhn DD. Effect of common aquaculture chemicals against *Edwardsiella*
1305 *ictaluri* and *E. tarda*. *Journal of aquatic animal health*. 2010;22(4):224-228.
1306 doi:<https://doi.org/10.1577/H10-020.1>.
- 1307 205. Shoemaker C, Klesius P. Protective immunity against enteric septicaemia in channel catfish,
1308 *Ictalurus punctatus* (Rafinesque), following controlled exposure to *Edwardsiella ictaluri*. *Journal*
1309 *of Fish Diseases*. 1997;20(5):361-368. doi:<https://doi.org/10.1046/j.1365-2761.1997.00310.x>.
- 1310 206. Thune RL, Hawke JP, Johnson MC. Studies on vaccination of channel catfish, *Ictalurus punctatus*,
1311 against *Edwardsiella ictaluri*. *Journal of Applied Aquaculture*. 1994;3(1-2):11-24.
1312 doi:https://doi.org/10.1300/J028v03n01_02.
- 1313 207. Saeed M, Plumb J. Immune response of channel catfish to lipopolysaccharide and whole cell
1314 *Edwardsiella ictaluri* vaccines. *Diseases of Aquatic Organisms*. 1986;2(1):21-25.
1315 doi:<http://dx.doi.org/10.3354/dao002021>.
- 1316 208. Thanh PC, Berntsen JO. Towards sustainability: Vaccinated pangasius in Vietnam. In. *AQUA*
1317 *Culture Asia Pacific Magazine*2012:36-37.
- 1318 209. Kayansamruaj P, Areechon N, Unajak S. Development of fish vaccine in Southeast Asia: A
1319 challenge for the sustainability of SE Asia aquaculture. *Fish & shellfish immunology*. 2020;103:73-
1320 87. doi:<https://doi.org/10.1016/j.fsi.2020.04.031>.
- 1321 210. Karsi A, Lawrence ML, Abdelhamed H, Inventors; Mississippi State University assignee. Live
1322 attenuated *Edwardsiella ictaluri* vaccine and method of using the same. 2019.
1323 <https://patents.google.com/patent/US20170065695A1/en>.
- 1324 211. Klesius PH, Shoemaker CA, Inventors; The United States of America as represented by the
1325 Secretary of Agriculture, Washington, D.C., assignee. Modified live *Edwardsiella ictaluri* against
1326 enteric septicemia in channel catfish. US patent 6,019,9812000.
1327 <https://patents.google.com/patent/US6019981A/en>.
- 1328 212. Lawrence ML, Cooper RK, Thune RL. Attenuation, persistence, and vaccine potential of an
1329 *Edwardsiella ictaluri* purA mutant. *Infection and immunity*. 1997;65(11):4642-4651.
1330 doi:<http://dx.doi.org/10.1128/IAI.65.11.4642-4651.1997>.
- 1331 213. Pridgeon JW, Klesius PH. Development of a novobiocin-resistant *Edwardsiella ictaluri* as a novel
1332 vaccine in channel catfish (*Ictalurus punctatus*). *Vaccine*. 2011;29(34):5631-5637.
1333 doi:<https://doi.org/10.1016/j.vaccine.2011.06.016>.
- 1334 214. Santander J, Mitra A, Curtiss III R. Phenotype, virulence and immunogenicity of *Edwardsiella*
1335 *ictaluri* cyclic adenosine 3', 5'-monophosphate receptor protein (Crp) mutants in catfish host. *Fish*
1336 *& shellfish immunology*. 2011;31(6):1142-1153. doi:<https://doi.org/10.1016/j.fsi.2011.10.009>.

- 1337 215. Santander J, Xin W, Yang Z, Curtiss R. The aspartate-semialdehyde dehydrogenase of
1338 *Edwardsiella ictaluri* and its use as balanced-lethal system in fish vaccinology. *PLoS One*.
1339 2010;5(12):e15944. doi:https://doi.org/10.1371/journal.pone.0015944.
- 1340 216. Thune RL, Fernandez DH, Battista JR. An *aroA* mutant of *Edwardsiella ictaluri* is safe and
1341 efficacious as a live, attenuated vaccine. *Journal of Aquatic Animal Health*. 1999;11(4):358-372.
1342 doi:https://doi.org/10.1577/1548-8667(1999)011%3C0358:AAMOEI%3E2.0.CO;2.
- 1343 217. Triet TH, Tinh BT, Hau LV, Huong TV, Binh N-Q. Development and potential use of an
1344 *Edwardsiella ictaluri* wzz mutant as a live attenuated vaccine against enteric septicemia in
1345 *Pangasius hypophthalmus* (Tra catfish). *Fish & shellfish immunology*. 2019;87:87-95.
1346 doi:https://doi.org/10.1016/j.fsi.2019.01.005.
- 1347 218. Wang R-h, Xiao T-y, Zeng L-b, Liu X-y, Zhou Y, Ma J. Generation and use of *Edwardsiella*
1348 *ictaluri* ghosts as a vaccine against enteric septicemia of catfish (ESC). *Aquaculture*. 2016;456:9-
1349 15. doi:http://dx.doi.org/10.1016/j.aquaculture.2016.01.017.
- 1350 219. Yang Q, Pan Y-L, Wang K-Y, et al. OmpN, outer membrane proteins of *Edwardsiella ictaluri* are
1351 potential vaccine candidates for channel catfish (*Ictalurus punctatus*). *Molecular immunology*.
1352 2016;78:1-8. doi:https://doi.org/10.1016/j.molimm.2016.08.011.
- 1353 220. Moore MM, Fernandez DL, Thune RL. Cloning and characterization of *Edwardsiella ictaluri*
1354 proteins expressed and recognized by the channel catfish *Ictalurus punctatus* immune response
1355 during infection. *Diseases of aquatic organisms*. 2002;52(2):93-107.
1356 doi:https://doi.org/10.3354/dao052093.
- 1357 221. Machimbirike VI, Pornputtpong N, Senapin S, et al. A multi-epitope chimeric protein elicited a
1358 strong antibody response and partial protection against *Edwardsiella ictaluri* in Nile tilapia. *Journal*
1359 *of Fish Diseases*. 2021. doi:https://doi.org/10.1111/jfd.13525.
- 1360 222. Kumar G, Byars TS, Greenway TE, et al. Economic assessment of commercial-scale *Edwardsiella*
1361 *ictaluri* vaccine trials in US catfish industry. *Aquaculture Economics & Management*.
1362 2019;23(3):254-275. doi:https://doi.org/10.1080/13657305.2019.1632392.
- 1363 223. Bebak J, Wagner B. Use of vaccination against enteric septicemia of catfish and columnaris disease
1364 by the US catfish industry. *Journal of aquatic animal health*. 2012;24(1):30-36.
1365 doi:https://doi.org/10.1080/08997659.2012.667048.
- 1366 224. Wolters W, Waldbieser G, Bosworth B, Silverstein J, Robinson E, Inventors. Genetically distinct
1367 strain of channel catfish designated NWAC103, with improved growth performance. US patent US
1368 2004/0055029 A12004. https://www.freepatentsonline.com/y2004/0055029.html.
- 1369 225. Dunham RA, Liu Z. Gene mapping, isolation and genetic improvement in catfish. In: N. S, T. A, I.
1370 H, F. T, eds. *Aquatic Genomics*. Tokyo: Springer; 2003:45-60.
- 1371 226. Tan S, Zhou T, Wang W, et al. GWAS analysis using interspecific backcross progenies reveals
1372 superior blue catfish alleles responsible for strong resistance against enteric septicemia of catfish.
1373 *Molecular Genetics and Genomics*. 2018;293(5):1107-1120. doi:https://doi.org/10.1007/s00438-
1374 018-1443-4.
- 1375 227. Barros MM, Lim C, Klesius PH. Effect of soybean meal replacement by cottonseed meal and iron
1376 supplementation on growth, immune response and resistance of Channel Catfish (*Ictalurus*
1377 *punctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture*. 2002;207(3-4):263-279.
1378 doi:https://doi.org/10.1016/S0044-8486(01)00740-2.
- 1379 228. Buentello JA, Gatlin III DM. Effects of elevated dietary arginine on resistance of channel catfish
1380 to exposure to *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*. 2001;13(3):194-201.
1381 doi:https://doi.org/10.1577/1548-8667(2001)013%3C0194:EOEDAO%3E2.0.CO;2.
- 1382 229. Duncan PL, Klesius PH. Dietary immunostimulants enhance nonspecific immune responses in
1383 channel catfish but not resistance to *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*.
1384 1996;8(3):241-248.
- 1385 230. Fracalossi DM, Lovell RT. Dietary lipid sources influence responses of channel catfish (*Ictalurus*
1386 *punctatus*) to challenge with the pathogen *Edwardsiella ictaluri*. *Aquaculture*. 1994;119(2-3):287-
1387 298. doi:https://doi.org/10.1016/0044-8486(94)90183-X.

- 1388 231. Li MH, Wise DJ, Johnson MR, Robinson EH. Dietary menhaden oil reduced resistance of channel
1389 catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri*. *Aquaculture*. 1994;128(3-4):335-344.
1390 doi:https://doi.org/10.1016/0044-8486(94)90321-2.
- 1391 232. Sirimanapong W, Thompson KD, Ooi EL, et al. The effects of feeding β -glucan to *Pangasianodon*
1392 *hypophthalmus* on immune gene expression and resistance to *Edwardsiella ictaluri*. *Fish &*
1393 *shellfish immunology*. 2015;47(1):595-605. doi:https://doi.org/10.1016/j.fsi.2015.09.042.
- 1394 233. Shelby RA, Lim C, Yildirim-Aksoy M, Klesius PH. Effects of probiotic bacteria as dietary
1395 supplements on growth and disease resistance in young channel catfish, *Ictalurus punctatus*
1396 (Rafinesque). *Journal of applied aquaculture*. 2007;19(1):81-91.
1397 doi:https://doi.org/10.1300/J028v19n01_a.
- 1398 234. Rendueles O, Ferrières L, Frétau M, et al. A new zebrafish model of oro-intestinal pathogen
1399 colonization reveals a key role for adhesion in protection by probiotic bacteria. *PLoS pathogens*.
1400 2012;8(7):e1002815. doi:https://doi.org/10.1371/journal.ppat.1002815.
- 1401 235. Thy HTT, Thinh NH, Tri NN, et al. Identification and characterization of potential probiotic
1402 *Bacillus* spp. for application in striped catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]).
1403 *Journal of fisheries and environment*. 2017;41(2):20-36.
- 1404 236. Callaway TR, Ricke SC. *Direct-fed microbials and prebiotics for animals: science and mechanisms*
1405 *of action*. Springer Science & Business Media; 2011.
- 1406 237. Peterson BC, Peatman E, Ourth D, Waldbieser G. Phytogetic feed-additive effects on channel
1407 catfish rhamnose-binding lectin levels, and susceptibility to *Edwardsiella ictaluri*. *Diseases of*
1408 *aquatic organisms*. 2018;129(2):99-106. doi:https://doi.org/10.3354/dao03235.
- 1409 238. Kayansamruaj P, Soontara C, Unajak S, et al. Comparative genomics inferred two distinct
1410 populations of piscine pathogenic *Streptococcus agalactiae*, serotype Ia ST7 and serotype III
1411 ST283, in Thailand and Vietnam. *Genomics*. 2019;111(6):1657-1667.
1412 doi:https://doi.org/10.1016/j.ygeno.2018.11.016.
- 1413 239. Liu G, Zhang W, Lu C. Comparative genomics analysis of *Streptococcus agalactiae* reveals that
1414 isolates from cultured tilapia in China are closely related to the human strain A909. *BMC genomics*.
1415 2013;14(1):1-10. doi:https://doi.org/10.1186/1471-2164-14-775.
- 1416 240. Liu ZJ. *Bioinformatics in aquaculture: principles and methods*. In: John Wiley & Sons; 2017.
- 1417 241. Nakamura Y, Takano T, Yasuike M, Sakai T, Matsuyama T, Sano M. Comparative genomics
1418 reveals that a fish pathogenic bacterium *Edwardsiella tarda* has acquired the locus of enterocyte
1419 effacement (LEE) through horizontal gene transfer. *BMC genomics*. 2013;14(1):1-14.
1420 doi:https://doi.org/10.1186/1471-2164-14-642.
- 1421 242. Pang M, Jiang J, Xie X, et al. Novel insights into the pathogenicity of epidemic *Aeromonas*
1422 *hydrophila* ST251 clones from comparative genomics. *Scientific reports*. 2015;5(1):1-15.
1423 doi:https://doi.org/10.1038/srep09833.
- 1424 243. Raskin DM, Seshadri R, Pukatzki SU, Mekalanos JJ. Bacterial genomics and pathogen evolution.
1425 *Cell*. 2006;124(4):703-714. doi:https://doi.org/10.1016/j.cell.2006.02.002.
- 1426 244. Bambini S, Rappuoli R. The use of genomics in microbial vaccine development. *Drug discovery*
1427 *today*. 2009;14(5-6):252-260. doi:https://dx.doi.org/10.1016%2Fj.drudis.2008.12.007.
- 1428 245. Idowu T, Adedeji H, Sogbesan O. Fish disease and health management in aquaculture production.
1429 *International Journal Environmental & Agricultural Science*. 2017;1(1):2.
- 1430 246. Tonguthai K. Training and extension in aquaculture health management in the Asia-Pacific: present
1431 status and future requirements. In: Subasinghe RP, Arthur JR, Shariff M, eds. *Health Management*
1432 *in Asian Aquaculture. Proceedings of the Regional Expert Consultation on Aquaculture Health*
1433 *Management in Asia and the Pacific*. Vol 142. Rome: FAO Fisheries Technical Paper No. 360,
1434 FAO; 1996:60-74.
- 1435 247. Flegel TW. A future vision for disease control in shrimp aquaculture. *Journal of the World*
1436 *Aquaculture Society*. 2019;50(2):249-266. doi:http://dx.doi.org/10.1111/jwas.12589.

- 1437 248. Herbert B. *Edwardsiella ictaluri* – a significant pathogen of fish. In: Policy AH, ed. Emergency
1438 Animal Diseases Bulletin No 108.: Australian Government Department of Agriculture, Fisheries
1439 and Forestry; 2019.
- 1440 249. Assefa A, Abunna F. Maintenance of fish health in aquaculture: review of epidemiological
1441 approaches for prevention and control of infectious disease of fish. *Veterinary medicine*
1442 *international*. 2018;2018. doi:https://doi.org/10.1155/2018/5432497.
- 1443 250. Oidtmann B, Thrush M, Denham K, Peeler E. International and national biosecurity strategies in
1444 aquatic animal health. *Aquaculture*. 2011;320(1-2):22-33.
1445 doi:https://doi.org/10.1016/j.aquaculture.2011.07.032.
- 1446 251. Romero J, Feijoó CG, Navarrete P. Antibiotics in aquaculture–use, abuse and alternatives. *Health*
1447 *and environment in aquaculture*. 2012;159. doi:http://dx.doi.org/10.5772/28157.
- 1448 252. Crab R, Defoirdt T, Bossier P, Verstraete W. Biofloc technology in aquaculture: beneficial effects
1449 and future challenges. *Aquaculture*. 2012;356:351-356. doi:https://doi.org/10.1577/1548-
1450 8667(1996)008%3C0319:UOAMTT%3E2.3.CO;2.
- 1451 253. Mathur M, Vidhani S, Mehndiratta P, Bhalla P, Reddy B. Bacteriophage therapy: an alternative to
1452 conventional antibiotics. *Journal-Association of Physicians of India*. 2003;51:593-596.
- 1453 254. Liles MR, Walakira JK, Carrias AA, Terhune JS, Inventors; Auburn University, assignee.
1454 *Edwardsiella Ictaluri Bacteriophage and Uses Thereof*. 2010.
1455 https://patents.google.com/patent/US20100092431.
- 1456 255. Jhunkeaw C, Khongcharoen N, Rungrueng N, et al. Ozone nanobubble treatment in freshwater
1457 effectively reduced pathogenic fish bacteria and is safe for Nile tilapia (*Oreochromis niloticus*).
1458 *Aquaculture*. 2021;534:736286. doi:https://doi.org/10.1016/j.aquaculture.2020.736286.
- 1459 256. Kurita Y, Chiba I, Kijima A. Physical eradication of small planktonic crustaceans from aquaculture
1460 tanks with cavitation treatment. *Aquaculture International*. 2017;25(6):2127-2133.
1461 doi:https://doi.org/10.1007/s10499-017-0179-1.
- 1462 257. Linh NV, Panphut W, Thapinta A, et al. Ozone nanobubble modulates the innate defense system
1463 of Nile tilapia (*Oreochromis niloticus*) against *Streptococcus agalactiae*. *Fish & Shellfish*
1464 *Immunology*. 2021;112:64-73. doi:https://doi.org/10.1016/j.fsi.2021.02.015.
- 1465 258. Munang'andu HM, Galindo-Villegas J, David L. Teleosts genomics: Progress and prospects in
1466 disease prevention and control. *International journal of molecular sciences*. 2018;19(4):1083.
1467 doi:https://doi.org/10.3390/ijms19041083.
- 1468 259. Jiao X-d, Dang W, Hu Y-h, Sun L. Identification and immunoprotective analysis of an in vivo-
1469 induced *Edwardsiella tarda* antigen. *Fish & shellfish immunology*. 2009;27(5):633-638.
1470 doi:https://doi.org/10.1016/j.fsi.2009.08.006.
- 1471 260. Wang Y, Chen H, Guo Z, et al. Quantitative proteomic analysis of iron-regulated outer membrane
1472 proteins in *Aeromonas hydrophila* as potential vaccine candidates. *Fish & shellfish immunology*.
1473 2017;68:1-9. doi:https://doi.org/10.1016/j.fsi.2017.07.002.
- 1474 261. Elasad A, Dunham R. Disease reduction in aquaculture with genetic and genomic technology:
1475 current and future approaches. *Reviews in Aquaculture*. 2018;10(4):876-898.
1476 doi:https://doi.org/10.1111/raq.12205.
- 1477 262. Hosoya S, Kikuchi K, Nagashima H, et al. Genomic selection in aquaculture. The 43rd Scientific
1478 Symposium of UJNR Aquaculture Panel; 10-11 November 2015, 2017; Nagasaki Japan.
1479 https://www.fra.affrc.go.jp/bulletin/bull/bull45/45-0506.pdf.
- 1480 263. Khatkar MS. Genomic selection in aquaculture breeding programs. In: Z. L, ed. *Bioinformatics in*
1481 *Aquaculture: Principles and Methods*. John Wiley & Sons; 2017.
- 1482 264. Vallejo RL, Leeds TD, Fragomeni BO, et al. Evaluation of genome-enabled selection for bacterial
1483 cold water disease resistance using progeny performance data in rainbow trout: insights on
1484 genotyping methods and genomic prediction models. *Frontiers in genetics*. 2016;7:96.
1485 doi:https://doi.org/10.3389/fgene.2016.00096.

- 1486 265. Zhang Y, Liu Z, Li H. Genomic prediction of columnaris disease resistance in catfish. *Marine*
1487 *Biotechnology*. 2020;22(1):145-151. doi:https://link.springer.com/article/10.1007/s10126-019-
1488 09941-7.
- 1489 266. Sanz VA. Specific pathogen free (SPF), specific pathogen resistant (SPR) and specific pathogen
1490 tolerant (SPT) as part of the biosecurity strategy for whiteleg shrimp (*Penaeus vannamei* Boone
1491 1931). *Asian Fish Soc*. 2018;31:112-120. doi:http://dx.doi.org/10.33997/j.afs.2018.31.S1.008.
- 1492 267. Barman D, Kumar V, Roy S, Mandal SC. Specific pathogen free shrimps: Their scope in
1493 aquaculture. In. *World Aquaculture*. Vol 432012:67.
- 1494 268. Zhang Z, Chi H, Dalmo RA. Trained innate immunity of fish is a viable approach in larval
1495 aquaculture. *Frontiers in immunology*. 2019;10:42.
1496 doi:https://dx.doi.org/10.3389/fimmu.2019.00042.
- 1497 269. Netea MG, Joosten LA, Latz E, et al. Trained immunity: a program of innate immune memory in
1498 health and disease. *Science*. 2016;352(6284). doi:https://doi.org/10.1126/science.aaf1098.
- 1499 270. Petit J, Wiegertjes GF. Long-lived effects of administering β -glucans: indications for trained
1500 immunity in fish. *Developmental & Comparative Immunology*. 2016;64:93-102.
1501 doi:https://doi.org/10.1016/j.dci.2016.03.003.
- 1502 271. Chen D, Ainsworth A. Glucan administration potentiates immune defence mechanisms of channel
1503 catfish, *Ictalurus punctatus* Rafinesque. *Journal of Fish Diseases*. 1992;15(4):295-304.
1504 doi:https://doi.org/10.1111/j.1365-2761.1992.tb00667.x.
- 1505 272. Wolters WR, Johnson MR. Enteric septicemia resistance in blue catfish and three channel catfish
1506 strains. *Journal of Aquatic Animal Health*. 1994;6(4):329-334. doi:https://doi.org/10.1577/1548-
1507 8667(1994)006%3C0329:ESRIBC%3E2.3.CO;2.
- 1508 273. Klesius P, Lovy J, Evans J, Washuta E, Arias C. Isolation of *Edwardsiella ictaluri* from tadpole
1509 madtom in a southwestern New Jersey river. *Journal of Aquatic Animal Health*. 2003;15(4):295-
1510 301. doi:https://doi.org/10.1577/H03-026.1.
- 1511 274. Takeuchi H, Nakano D, Iwadare M, et al. Evaluation of pathogenicity of *Edwardsiella ictaluri* to
1512 riverine fish species using an immersion infection method. *Fish Pathology*. 2019;54(3):61-63.
1513 doi:https://doi.org/10.3147/jsfp.54.61.
- 1514 275. Kasornchandra J, Rogers W, Plumb J. *Edwardsiella ictaluri* from walking catfish, *Clarias*
1515 *batrachus* L., in Thailand. *Journal of Fish Diseases*. 1987;10(2):137-138.
1516 doi:https://doi.org/10.1111/j.1365-2761.1987.tb00729.x.
- 1517 276. Suanyuk N, Rogge M, Thune R, Watthanaphiromsakul M, Champhat N, Wiangkum W. Mortality
1518 and pathology of hybrid catfish, *Clarias macrocephalus* (Günther) \times *Clarias gariepinus* (Burchell),
1519 associated with *Edwardsiella ictaluri* infection in southern Thailand. *Journal of fish diseases*.
1520 2014;37(4):385-395. doi:https://doi.org/10.1111/jfd.12127.
- 1521 277. Mawardi M, Jaelani J, Zainun Z, Mundayana Y, Chilora BS, Hardi EH. Identification and
1522 characterization of *Edwardsiella ictaluri* from diseased *Pangasius pangasius*, cultured in Cirata
1523 Lake, Indonesia. *Biodiversitas*. 2018;19(3):816-822. doi:https://doi.org/10.13057/biodiv/d190309.
- 1524 278. Plumb J, Hilge V. Susceptibility of European catfish (*Silurus glanis*) to *Edwardsiella ictaluri*.
1525 *Journal of applied ichthyology*. 1987;3(1):45-48. doi:https://doi.org/10.1111/j.1439-
1526 0426.1987.tb00450.x.
- 1527 279. Rem P. *Effects of Water Temperature on Growth Performance and Health of Butter Catfish*
1528 (*Ompok bimaculatus*), Prince of Songkla University; 2019.
- 1529 280. Kent M, Lyons J. *Edwardsiella ictaluri* in the green knifefish, *Eigemannia virescens*. *Fish Health*
1530 *News*. 1982.
- 1531 281. Hawke JP, Kent M, Rogge M, et al. Edwardsiellosis caused by *Edwardsiella ictaluri* in laboratory
1532 populations of zebrafish *Danio rerio*. *Journal of aquatic animal health*. 2013;25(3):171-183.
1533 doi:https://doi.org/10.1080/08997659.2013.782226.
- 1534 282. Waltman W, Shotts E, Blazer V. Recovery of *Edwardsiella ictaluri* from danio (*Danio devario*).
1535 *Aquaculture*. 1985;46(1):63-66. doi:https://doi.org/10.1016/0044-8486(85)90176-0.

- 1536 283. Humphrey J, Lancaster C, Gudkovs N, McDonald W. Exotic bacterial pathogens *Edwardsiella*
1537 *tarda* and *Edwardsiella ictaluri* from imported ornamental fish *Betta splendens* and *Puntius*
1538 *conchonius*, respectively: isolation and quarantine significance. *Australian Veterinary Journal*.
1539 1986;63(11):369-371. doi:<https://doi.org/10.1111/j.1751-0813.1986.tb02900.x>.
- 1540 284. Takeuchi H, Hiratsuka M, Oinuma H, et al. Infection status of ayu and other wild fish with
1541 *Flavobacterium psychrophilum* and *Edwardsiella ictaluri* in the Tama River, Japan. *Fish*
1542 *Pathology*. 2016;51(4):184-193. doi:<https://doi.org/10.3147/jsfp.51.184>.
- 1543 285. Topić Popović N, Hacmanjek M, Teskeredžić E. Health status of rudd (*Scardinius*
1544 *erythrophthalmus hesperidicus* H.) in Lake Vrana on the Island of Cres, Croatia. *Journal of Applied*
1545 *Ichthyology*. 2001;17(1):43-45. doi:<http://dx.doi.org/10.1046/j.1439-0426.2001.00236.x>.
- 1546 286. Baxa D, Groff J, Wishkovsky A, Hedrick R. Susceptibility of nonictalurid fishes to experimental
1547 infection with *Edwardsiella ictaluri*. *Diseases of Aquatic Organisms*. 1990;8(2):113-117.
1548 doi:<http://dx.doi.org/10.3354/dao008113>.
- 1549 287. Keskin O, Secer S, Izgür M, Türkyilmaz S, Mkakosya RS. *Edwardsiella ictaluri* infection in
1550 rainbow trout (*Oncorhynchus mykiss*). *Turkish journal of veterinary and animal sciences*.
1551 2004;28(4):649-653.
- 1552 288. Pasnik DJ, Evans JJ, Klesius PH. Experimental *Edwardsiella ictaluri* infection causes mortality in
1553 white perch (*Morone americana*). *Journal of Animal and Veterinary Advances*. 2007.
1554 doi:<https://medwelljournals.com/abstract/?doi=javaa.2007.646.649>.
- 1555 289. Blanch A, Pinto R, Jofre J. Isolation and characterization of an *Edwardsiella* sp. strain, causative
1556 agent of mortalities in sea bass (*Dicentrarchus labrax*). *Aquaculture*. 1990;88(3-4):213-222.
1557 doi:[https://doi.org/10.1016/0044-8486\(90\)90149-H](https://doi.org/10.1016/0044-8486(90)90149-H).
- 1558 290. Tong Y, Sun X, Wang B, et al. Transcriptome of intraperitoneal organs of starry flounder
1559 *Platichthys stellatus* challenged by *Edwardsiella ictaluri* JCM1680. *Chinese journal of oceanology*
1560 *and limnology*. 2015;33(1):20-27. doi:<https://doi.org/10.1007/s00343-015-3307-7>.
- 1561 291. Australia. AH. Animal Health in Australia 2011. In. Vol 2021: Canberra, Australia; 2012.

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

Host family	Host species	Geographical location	Occurrence	Affected fish stage	Mortality	Reference
Ictaluridae	<i>Ictalurus punctatus</i>	USA	Natural infection	fingerling	100% (experimental challenge)	20
	<i>Ictalurus furcatus</i>	USA	Experimental infection	fingerling	0.7% (natural infection)	272
	<i>Ameiurus catus</i>	USA	Natural infection	Information not available	Information not available	55
	<i>Ameiurus nebulosus</i>	USA	Natural infection	mixed sizes	35 to 40% (natural infection)	61
	<i>Noturus gyrinus</i>	USA	Natural infection	juvenile	Not reported	273
Bagridae	<i>Pelteobagrus fulvidraco</i>	China	Natural infection	Juvenile-adult	50% (natural infection)	16
	<i>Pelteobagrus nudiceps</i>	Japan	Natural subclinical infections	Not specified	100% (experimental challenge)	33
	<i>Pelteobagrus vachelli</i>	China	Experimental infection	Juvenile	26-62% (natural infection)	153
	<i>Tachysurus tokiensis</i>	Japan	Experimental infection	Juveniles	100% (natural infection)	274
Clariidae	<i>Clarias batrachus</i>	Thailand	Natural infection	Not specified	Not reported	275
	<i>Clarias macrocephalus</i> x <i>Clarias gariepinus</i>	Thailand	Natural infection	Not specified	100% (experimental challenge)	276
Pangasiidae	<i>Pangasianodon hypophthalmus</i>	Thailand	Natural concurrent infection	juvenile	80% (experimental challenge)	15
	<i>Pangasianodon hypophthalmus</i>	West Indies	Natural infection	juvenile	approximately 2000 animals	57
	<i>Pangasius hypophthalmus</i> (Sauvage)	Vietnam	Natural infection	Not specified	Not reported	22
	<i>Pangasius hypophthalmus</i> (Sauvage)	Indonesia	Natural infection	fingerlings and immature fish	50 to 100% (natural infection)	17
	<i>Pangasius pangasius</i>	Indonesia	Natural infection	Young adults	95% (natural infection)	277
Plecoglossidae	<i>Plecoglossus altivelis</i>	Japan	Natural infection and experimental	Fingerlings-adult	100% (experimental challenge)	25
Siluridae	<i>Silurus asotus</i>	Japan	Experimental infection	fingerlings, juveniles	100% (natural infection)	33
	<i>Silurus soldatovi meridionalis</i>	China	Natural infection	Juveniles	60% (natural infection)	187
	<i>Silurus glanis</i>	USA	Experimental infection	Juveniles	80% (natural infection)	278
	<i>Ompok bimaculatus</i>	Thailand	Experimental infection	Fingerlings	2.5%-100% (natural infection)	279
Plotosidae	<i>Anodontiglanis dahli</i>	Australia	Natural infection	Not specified	Not reported	248
	<i>Neosilurus ater</i>	Australia	Natural infection	Not specified	Not reported	248
	<i>Tandanus tropicanus</i>	Australia	Natural infections	Not specified	Not reported	175
Ariidae	<i>Neoarius berneyi</i>	Australia	Natural infection	Not specified	Not reported	248
Sternopygidae	<i>Eigenmannia virescens</i>	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	<i>Danio rerio</i>	USA	Natural infection	adult	19% (natural infection)	281
	<i>Danio devario</i>	USA	Natural infection	Not specified	100% (experimental challenge)	282
	<i>Puntius conchonius</i>	Australia	Natural infection	Not specified	40% (natural infection)	283
	<i>Zacco platypus</i>	Japan	Experimental infection	Not specified	15% (experimental challenge)	33
	<i>Tribolodon hakonensis</i>	Japan	Experimental infection	Fingerlings	40% (natural infection)	274
	<i>Tribolodon brandtii maruta</i>	Japan	Natural infection	Not specified	Not reported	284
	<i>Candidia temminckii</i>	Japan	Natural infection	Not specified	Not reported	284
	<i>Hemibarbus barbus</i>	Japan	Natural infection	Not specified	Not reported	284
	<i>Rhynchocypris lagowskii</i>	Japan	Natural infection	Not specified	Not reported	284
<i>Scardinius erythrophthalmus hesperidicus</i> H.	Croatia	Natural infection	Juveniles	Not reported	285	
Cichlidae	<i>Sarotherodon aureus</i>	USA	Experimental infection	fingerlings	70% (experimental challenge)	53
	<i>Oreochromis niloticus</i>	West Indies	Natural infection	fry and fingerlings	100% (experimental challenge)	13
	<i>Oreochromis spp.</i>	Vietnam	Natural infection	juveniles	40–50% (natural infection)	12
Salmonidae	<i>Oncorhynchus tshawytscha</i>	USA	Experimental infection	Juveniles	75% (experimental challenge)	286
	<i>Oncorhynchus mykiss</i>	Turkey	Natural infection	juveniles	100% (experimental challenge)	287
Moronidae	<i>Morone americana</i>	USA	Experimental infection	Information not available	100% (experimental challenge)	288
	<i>Dicentrarchus labrax</i> †	Spain	Natural infection	fry	90% (experimental challenge)	289
Anguillidae	<i>Anguilla japonica</i>	Japan	Experimental infection	Fingerlings	10% (natural infection)	274
Percichthyidae	<i>Coreoperca kawamebari</i>	Japan	Natural infection	Not specified	Not reported	284
Balaenopteridae	<i>Balaenoptera acutorostrata</i>	Japan	Natural infection	Not specified	Not reported	37
Pleuronectidae	<i>Platichthys stellatus</i>	China	Experimental infection	Juveniles	Not reported	290

1563 †-*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 ₂ S 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 ₂ S 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

1566 ‡ represents publication year where year of isolation was not specified.

1567 V-variable

1568 NR-not reported.

1569 NA-data not available

1570

1571

1572

1573

1574

1575

1576

1577

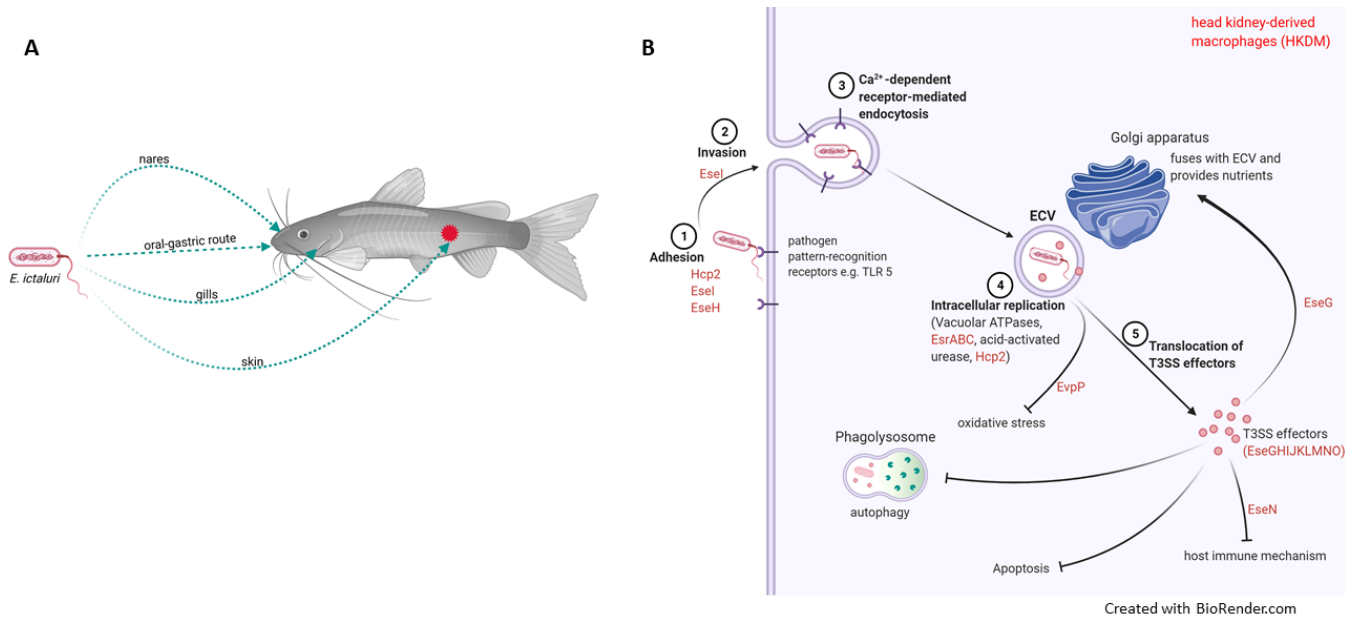
1578 Table 3. Summary of antibiotic and vaccines used in aquaculture against *E. ictaluri*.

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	<i>evpB</i> gene mutant (patent number US20170065695A1)	Immersion, injection, oral or combination	Channel catfish (Fry/fingerlings)	80.83%-92.58%	²¹⁰
	bacteria	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

1580 **Figures and legends**

1581



1582

1583 **Figure 1.** Pathogenesis of *E. ictaluri*. A) Ports of entry into the host fish used by *E. ictaluri* during
1584 infection. B) molecular mechanisms of *E. ictaluri* pathogenesis into host cells e.g. macrophage.

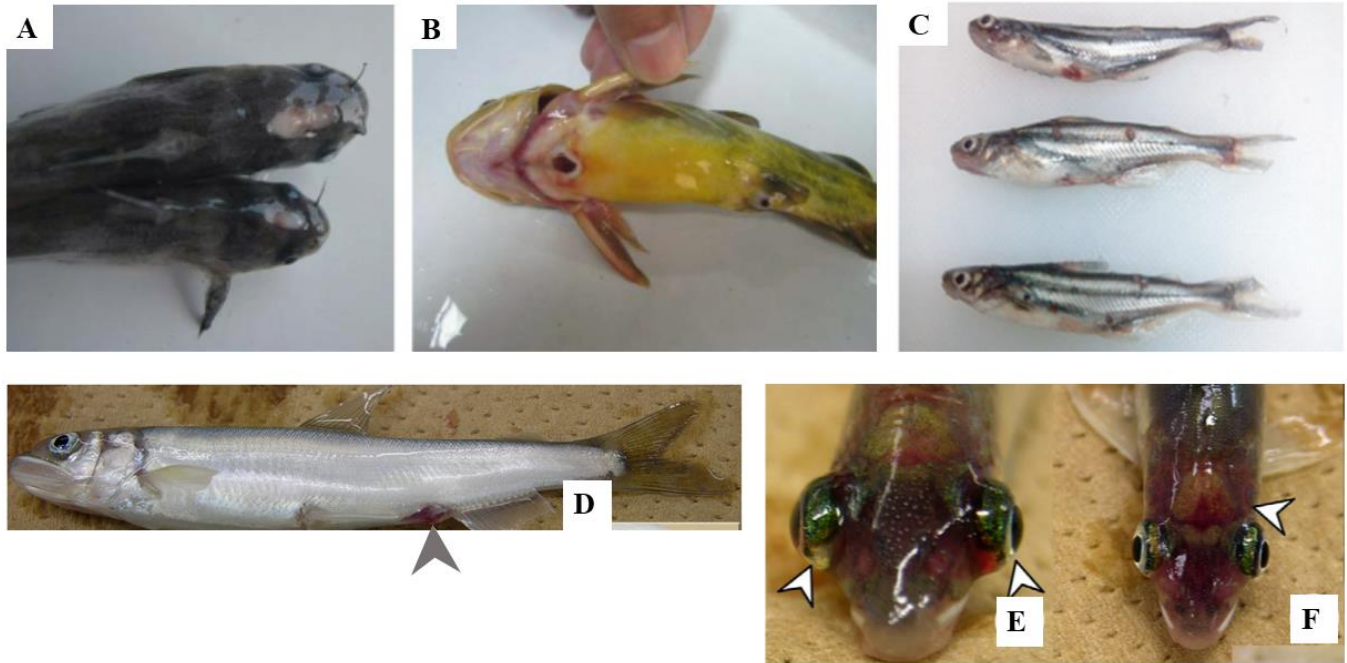
1585

1586

1587

1588

1589



1590

1591 **Figure 2.** Examples of gross external clinical signs of natural *E. ictaluri* infections in fish hosts. Channel
 1592 and yellow catfish exhibit ‘Hole in the head’ lesion (A). Yellow catfish also present ‘hole under the jaw’
 1593 lesion (B). Striped catfish exhibit haemorrhage and ulceration on the skin (C). Ayu exhibits distended
 1594 abdomen with reddened anus (D), exophthalmos (E) and meningio-encephalitis (red head) (F) shown by
 1595 arrowheads. Images A) and (B) reproduced with permission granted © 2010 The Authors. Aquaculture
 1596 Research © 2010 Blackwell Publishing Ltd. Image (C) reproduced with permission granted © 2016 John
 1597 Wiley & Sons Ltd. Images (D), (E), (F) reproduced with permission granted © 2020 Wiley Periodicals
 1598 LLC.

1599

1600

1601

1602

1603

1604

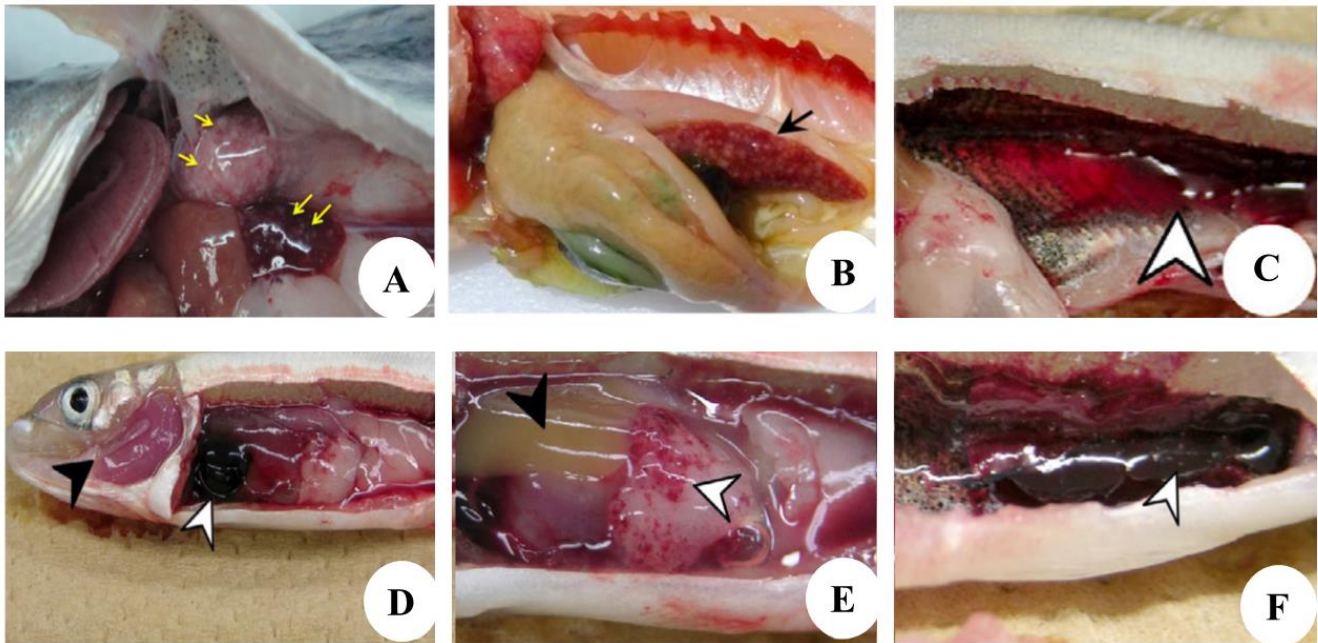
1605

1606

1607

1608

1609



1611

1612 **Figure 3.** Examples of gross internal clinical signs of natural *E. ictaluri* infections include mottled spleen
 1613 and anterior kidney indicated by yellow arrows in striped catfish (A), pale liver and mottled spleen and
 1614 kidney in tilapia indicated by black arrow (B), and in ayu; bloody ascites in peritoneal region (C), pale
 1615 gills and a gallbladder that is enlarged (D), reddened gonads (E) and posterior kidney that is enlarged and
 1616 haemorrhagic (F) all indicated by arrowheads. Images A) reproduced with permission granted © 2020
 1617 John Wiley & Sons Ltd. Image (B) Reprinted from Aquaculture Volume 499/15, Dong et al., Natural
 1618 occurrence of edwardsiellosis caused by *Edwardsiella ictaluri* in farmed hybrid red tilapia (*Oreochromis*
 1619 sp.) in Southeast Asia, Pages 17-23, Copyright (2019), with permission from Elsevier. Images (C), (D),
 1620 (E), (F) reproduced with permission granted © 2020 Wiley Periodicals LLC.

1621

1622

1623

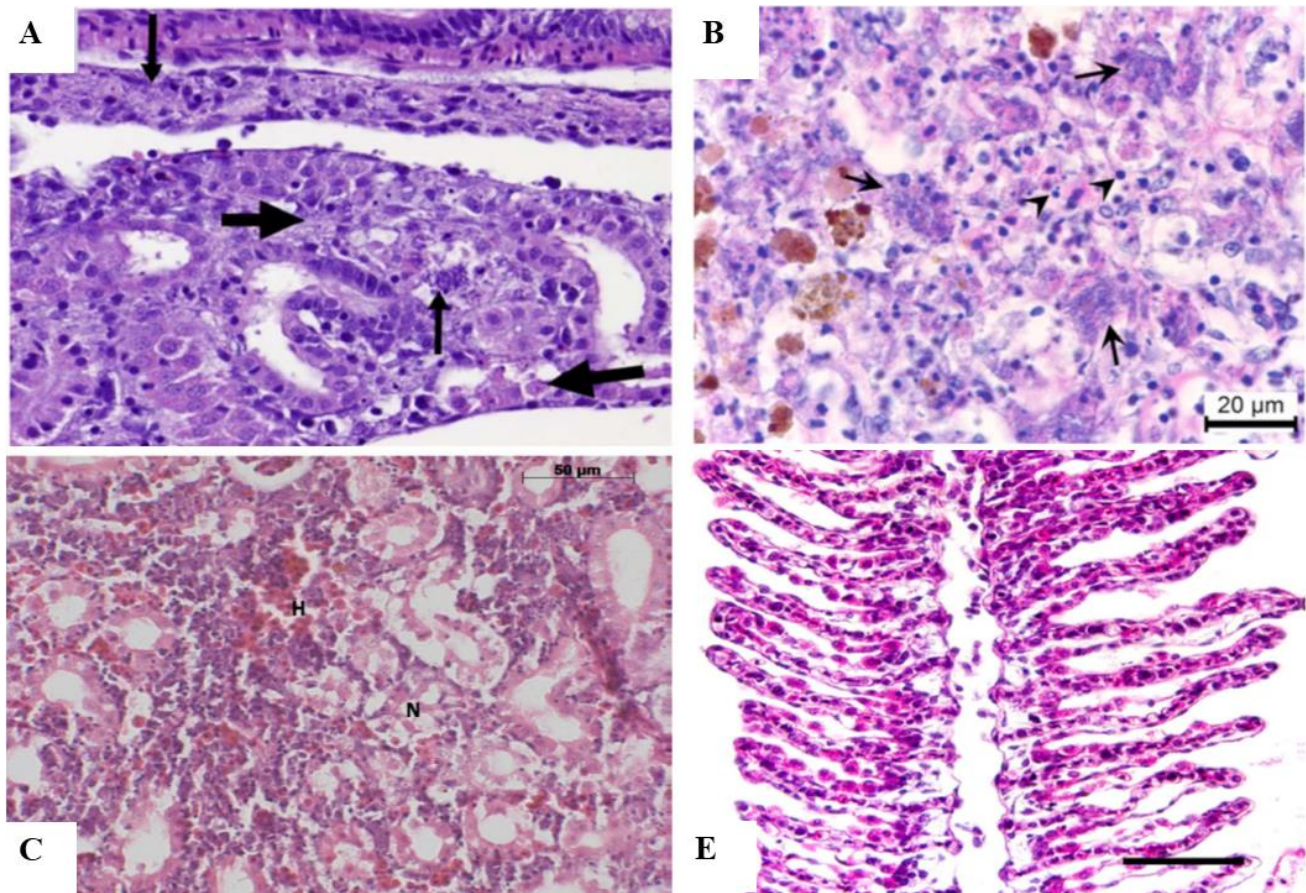
1624

1625

1626

1627

1628



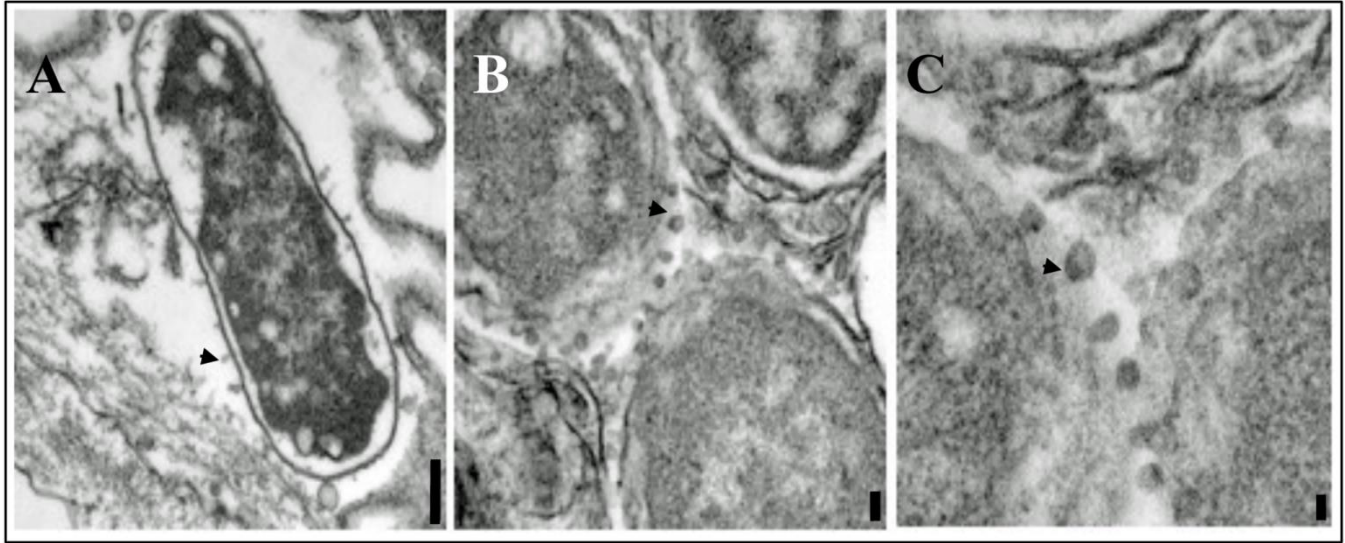
1630

1631 **Figure 4.** Typical histopathological findings. (A) in channel catfish fry, diffuse necrosis of the
 1632 hematopoietic tissues (arrows) was identified. (B) in red hybrid tilapia, there was spleen and cell pyknosis
 1633 and karyorrhexis (arrow heads). (C) in striped catfish kidney, histopathology showed necrosis (denoted
 1634 by N) and haemorrhagic areas (denoted by H). (D) in the gills of ayu, epithelial lining hyperplasia was
 1635 evident at base of secondary gill lamellae together with in-between separation of the underlying capillary
 1636 bed from the epithelial cell lining of secondary gill lamellae. Image (A) Reprinted from *Fish and Shellfish*
 1637 *Immunology* Volume 72, Abdelhamed et al., The virulence and immune protection of *Edwardsiella*
 1638 *ictaluri* HemR mutants in catfish, Pages 153-160, Copyright (2018), with permission from Elsevier. Image
 1639 (B) Reprinted from *Aquaculture* Volume 499/15, Dong et al., Natural occurrence of edwardsiellosis
 1640 caused by *Edwardsiella ictaluri* in farmed hybrid red tilapia (*Oreochromis* sp.) in Southeast Asia, Pages
 1641 17-23, Copyright (2019), with permission from Elsevier. Image (C) reproduced with permission granted
 1642 © 2020 John Wiley & Sons Ltd. Image (D) reproduced with permission granted © 2020 Wiley Periodicals
 1643 LLC.

1644

1645

1646



1647

1648 **Figure 5.** Transmission electron microscopy intracellular visualization of *E. ictaluri* of infected zebrafish
1649 head kidney. The tissue samples were taken 6 h post-infection (10^5 CFU dose⁻¹). (A) Intracellular *E.*
1650 *ictaluri* in infected zebrafish head kidney macrophage (Scale bar = 0.5 μ m). (B) Magnification of
1651 transverse sectioned intracellular *E. ictaluri* in infected zebrafish head kidney macrophage (Scale bar 0.5
1652 = μ m). (C) High magnification of cross-sectioned TEM images of intracellular *E. ictaluri* membrane (Scale
1653 bar = 0.2 μ m). Arrowheads indicate outer membrane vesicles-like (Images kindly provided by Dr.
1654 Santander).