United States Department of Agriculture

Potential Pathways of Exposure to ST251 Strains of Virulent *Aeromonas hydrophila* in Farmed Catfish

United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Strategy and Policy

Center for Epidemiology and Animal Health

September 2021

Key Points

- There are phylogenetic differences between ST251 strains of virulent *Aeromonas hydrophila* (vAh; hypervirulent Ah) and non-virulent *A. hydrophila* (non-vAh).
- Disease caused by vAh is similar to that caused by *A. hydrophila*; however, there are differences in the disease epidemiology, pathogenesis, and presentation.
    - It is important to distinguish the disease and associated impacts caused by vAh from those caused by *A. hydrophila.*
- Currently the known global distribution of ST251 strains of vAh includes:
    - Three U.S. States: Arkansas, Alabama, and Mississippi
    - Six provinces in China: Guangdong, Hainan, Hubei, Hunan, Jiangsu, and Zhejiang
    - It is unknown whether the lack of reports from other regions reflects the absence of the pathogen in other regions, a lack of surveillance and detection of the pathogen, or a lack of reporting.
- vAh is not a World Organisation of Animal Health (OIE) -listed aquatic animal pathogen.
- vAh is not listed as a notifiable disease on the USDA-APHIS National Veterinary Accreditation Program (NVAP) Notifiable Diseases and Conditions website or on the Voluntary 2021 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections, and Infestations List.
- There are no Federal import regulations specific to vAh.
- vAh appears to be endemically present in affected areas.
    - Research suggests vAh can exist commensally in aquaculture ponds until environmental conditions are optimal for growth and heightened virulence.
- Diagnosis and differentiation of vAh-caused disease vs. *A. hydrophila*-caused motile *Aeromonas* septicemia (MAS) is based on case history, clinical signs, gross necropsy findings, and isolation and full identification of the pathogen.
- Potential pathways of entry include:
    - Importation of live fish and germplasms
    - Importation of consumable fish products
- The most plausible pathways of exposure for domestic farmed catfish are:
    - Movement of domestically reared catfish or germplasms
    - Contaminated water
    - Wildlife and birds
    - Fomites
- The consequences associated with the introduction and spread of vAh in affected States have been significant, sustained, and clearly proven.
- Changes in farm management and biosecurity post-vAh emergence have decreased the occurrence and severity of vAh-caused disease.
- Data gaps and inconsistencies affect descriptions of pathogen taxonomy and vAh disease epidemiology. Future research would resolve limitations and knowledge gaps.

Background

The U.S. catfish industry asked the U.S. Department of Agriculture (USDA) Animal Plant Health Inspection Service's (APHIS) Veterinary Services (VS) branch to identify potential entry and exposure pathways of virulent *Aeromonas hydrophila* (vAh) into the United States. This request was

due to concerns about the risk of vAh introduction to catfish farms via imported live food fish, raw food fish and fish food products, or by-products. To conduct this assessment, VS referenced the risk analysis framework established by the World Organisation for Animal Health (OIE) [1, 2] and the international standards described in the OIE *Aquatic Animal Health Code* [2], based on the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)[3]. As an OIE member, the United States adheres to these standards.

Scope

This assessment focuses on the plausible entry and exposure pathways that may lead to introduction of ST251 strains of virulent *Aeromonas hydrophila* (vAh; hypervirulent Ah) into catfish aquaculture in the United States. This document does not provide a comprehensive review of the disease caused by non-ST251 strains of *A. hydrophila* (non-vAh). Currently there is overlap of terminology to describe the epidemiology associated with non-vAh and vAh in some published literature. Motile Aeromonas septicemia (MAS) is the term that describes disease caused by infection with non-virulent *A. hydrophila* and other aeromonad bacteria (*A. sobria, A. caviae*). This term has been used to describe disease by ST251 strains of vAh; however, the epidemiology, pathogenesis, and disease caused by vAh as reported in the literature are distinctly different. Assessing the plausible entry and exposure pathways for non-virulent *A. hydrophila* associated with MAS is not within the scope of this document.

This assessment includes a hazard identification and assessments of potential entry and exposure pathways as defined by the OIE. According to the OIE, an entry assessment describes the biological pathways necessary for an importation activity to introduce a hazard into a particular environment. The exposure assessment describes the biological pathways necessary for exposure of animals or humans in the importing country to the hazard[1]. Each section was constructed using available data, peer-reviewed literature, and other information. When applicable, requirements and regulations by USDA-APHIS and other Federal agencies were referenced.

This assessment is qualitative and descriptive. It is not an import risk assessment or analysis due to significant gaps in surveillance, research, and epidemiologic data specific to vAh that generate high levels of uncertainty and prevent reliable estimations of likelihood which are critical components of risk estimation and risk ranking.

Appendix 1 provides a brief overview of the epidemiology associated with non-vAh strains of *A. hydrophila*.

Hazard Identification

History

The first vAh pathotype, *A. hydrophila* J-1, sequence type 251 (ST251) was isolated in 1989 from epizootics of disease described as MAS in farmed crucian carp (*Carassius carassius*), blunt-snout bream (*Mygalobrama amblycephala*), and silver carp (*Hypothalmichthys molitrix*) in the Jiangsu Province of China [4]. In 2010, disease epidemics caused by a different sequence type ST251 vAh isolate (*A. hydrophila* NJ-35) occurred in the same province, and a related vAh isolate (*A. hydrophila* ZC1) was identified in diseased farmed grass carp (*Ctenopharyngodon idella*) in the Guangdong

province [4, 5].

The first reported case of disease in U.S. farmed channel catfish (*Ictalurus punctatus*)caused by the sequence type ST251 vAh (*A. hydrophila* S04-690) occurred in Mississippi in 2004 [6]. In 2009 and 2010, high mortality outbreaks of disease caused by vAh strains occurred in Alabama catfish operations, resulting in industry-wide losses of food-sized catfish [7, 8]. Spread of the disease within the catfish industries in Mississippi and Arkansas followed in 2010 [7, 8]. Disease and mortality caused by ST251 vAh continue to occur and appear to be regionally variable, indicating that other factors (e.g., geographic differences, environmental conditions, regional differences in management and production practices) may be involved in development of vAh-caused disease [5].

Comparative genomic and phylogenetic analyses indicate that ST251 strains of vAh associated with disease of farmed catfish in the United States and carp and blunt-snout bream in China form a coherent monophyletic lineage (clade) [4, 6, 8]. While these data do establish the ancestry of vAh and the potential source of origin, evidence of the source of introduction cannot be determined [6, 8]. Analyses of representative ST251 isolates show that vAh strains isolated from diseased catfish in Alabama from 2009 to 2015 were associated with a single clonal clade (U.S. catfish-affiliated clade). The same analysis demonstrated that vAh strains isolated in Mississippi from 2013 to 2015 were affiliated with two ST251-vAh clades (Asian carp-affiliated clade, U.S. catfish-affiliated clade) [4, 6, 9, 10].

As of 2019, the literature has described 37 ST251 strains of vAh (**Appendix 2: Table 2-1**). Continued research may identify more strains. All identified vAh strains share unique genetic loci not present in sequenced non-virulent *A. hydrophila* strains, which appear to contribute to increased virulence, different pathogenesis, and confirm their close genetic affiliation [4, 6, 8, 11]. The entire suite of vAh virulence factors has not been identified [12]; however, ST251 strains of vAh can be classified as vAh pathotypes (a group of organisms of the same species that have the same pathogenicity in a specified host) if strong phylogenetic evidence unique to ST251 vAh strains is present, and the following traits that collectively define and distinguish vAh pathotypes from non-vAh strains of *A. hydrophila* are present [4]:

- Strong evidence of core genome phylogeny
- Average nucleotide identity values greater than 99 percent
- An inducible prophage
- A suite of conserved virulence factors
- 26 conserved genetic loci putatively linked with virulence
- Ability to induce disease characteristically followed by rapid mortality
- Unique metabolic activities including: L-fucose metabolism, the ability to use myo-inositol as a sole carbon source, and sialic acid metabolism

vAh is not the only pathogen that causes significant losses of farmed catfish; a study by Peterman *et al.* (2019) reported that the most predominant diseases of catfish in eastern Mississippi in 2016 were caused by *Flavobacterium columnare* (40 percent), vAh (35 percent), and *Edwardsiella ictaluri* (12 percent)[6]. In 2020, the primary causes of disease loss on Alabama catfish farms were reported as *A. hydrophila*, *Columnaris* spp, and *Edwardsiella*; losses attributed specifically to vAh were described as “decreased”[13].

Susceptible Fish Species

Global reports of disease caused by vAh are predominantly limited to blunt-snout bream, crucian carp, grass carp, *Ictalurid* spp. catfish, and silver carp [4, 5]. In the United States, disease caused by vAh has been described exclusively in commercially reared catfish species [4-6, 8]. Review of the literature did not find published reports identifying disease attributed to vAh in other wild or cultured fish (e.g., food fish, ornamental fish). This may reflect the lack of detection, lack of reporting, or lack of occurrence of vAh-caused disease in fish in these sectors.

Global Distribution

Rasmussen-Ivey *et al.* (2018) reported that the global geographical distribution of ST251-affiliated strains of vAh included three U.S. States: Arkansas, Alabama, and Mississippi, and six provinces in China: Guangdong, Hainan, Hubei, Hunan, Jiangsu, and Zhejiang (**Appendix 2: Figure 2-1**) [5, 14]. However, it is unknown if the lack of published accounts of vAh in other regions reflects an absence of, a lack of surveillance and detection of, or a lack of reporting of the pathogen.

Public Health

There are no published reports of confirmed human disease specifically attributed to ST251-affiliated strains of vAh. There is a lack of data and published reports definitively confirming vAh presence in food products. This may be associated with lack of surveillance for vAh in food products, the testing limitations of human clinical laboratories, lack of reporting or awareness, or lack of vAh-caused disease in humans.

The U.S. Catfish Industry

In the United States, catfish aquaculture species include channel catfish, blue catfish (*Ictalurus furcatus*), and hybrid catfish (i.e., an F1 hybrid produced by crossing female channel catfish and male blue catfish that exhibits improved production traits including survival, growth, disease resistance, and edible yield)[15, 16]. The percentage of catfish farms stocking hybrid catfish increased from 4.9 percent to 17.6 percent from 2002 to 2009 [17, 18]. Channel catfish and hybrid catfish account for approximately 50 percent of all food-fish aquaculture in the U.S. [19]. Channel catfish production is the leading U.S. aquaculture industry, generating more than 27 percent of the value of total aquaculture production. Approximately 80 percent of production occurs in two States, Mississippi and Alabama [19]. Domestic production decreased by approximately 50 percent from 2005 to 2013 due to disease, high feed prices, high domestic production costs, a prolonged sluggish economy, and increased volumes of foreign catfish imports; this decline continued through 2018 [16, 20] (**Appendix 3: Tables 3-1 to 3-4**).

Broodstock and juvenile catfish are produced in hatcheries; there are no introductions of wild or imported stocks [16]. Hatcheries in Mississippi and Arkansas supply fingerlings to major producing States (Mississippi, Arkansas, and Alabama)[16]. Catfish are then reared through four phases: 1) broodfish are held in ponds where mating occurs; 2) fertilized eggs are transferred from broodfish ponds to hatcheries; 3) hatched fry are transferred from the hatchery to nursery ponds until they reach fingerling size (2 to 8 inches; approximately 6 months of age); 4) fingerlings are moved to food fish production ponds until they reach approximately 0.5 to 1.0 kg./1.0 to 2.0 lb.[16].

Commercial catfish production occurs in ponds. Embankment (levee) ponds that typically source water from wells represent approximately 76 percent of catfish ponds used for culture [16, 18]. The

remainder of ponds are watershed ponds that rely on rainwater, watershed runoff, streams or springs, and hybrid watershed-embankment ponds [16, 18]. Hybrid ponds are split into a fish rearing/holding area (20 percent of pond area) and an algal growth basin (80 percent of pond area) connected by culverts through an earthen levee. Hybrid catfish are typically stocked in hybrid ponds due to their increased disease resistance and aggressive feeding behavior [16, 21]. Catfish ponds are static systems; nursery ponds may be drained periodically (at harvest), while grow-out ponds may not be drained or exchange water for an average of 10 years [16, 18]. There is potential for unintended water introduction via rainfall, run-off, and seepage, and water may be unexpectedly discharged if overflow occurs [16]. The static nature of catfish ponds and use of failsafe devices reduce the risk of fish escape and disease transfer to wild fish populations [16]. Catfish ponds provide potential habitat for a wide range of wildlife (e.g., aquatic reptiles, amphibians, mammals, birds).

Movement of Catfish – International and Domestic

Exports from U.S. to Another Country

U.S. exports of catfish and catfish products from 2015 to 2019 are summarized in **Appendix 3: Table 3-4**. Total volumes of product increased from 2015 to 2017 (2,443,468 kg/5,375,639 lb. to 3,575,888 kg/7,866,953 lb.), dropped sharply in 2018 (1,716,898 kg/3,766,388 lb.), and then increased in 2019 (1,899,114 kg/4,178,050 lb.).

Imports into U.S. from Another Country

The U.S. does not import live catfish for use in catfish farming but does import a variety of "catfish" products annually. *Ictalurus* spp. fish products are imported predominantly from China [22]. *Pangasius* spp. fish products are primarily sourced from Vietnam. Vietnam and China are also principal exporters of *Siluriformes* spp. fish products [22](**Appendix 3: Tables 3-5 to 3-8**) Smaller volumes of these products are imported from multiple countries worldwide.

Regulations –

APHIS & OIE

vAh is not listed as a notifiable disease on the USDA-APHIS National Veterinary Accreditation Program (NVAP) Notifiable Diseases and Conditions website or on the Voluntary 2021 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections, and Infestations List. [2, 23, 24]

Importation of Live Fish or Fish Eggs into U.S.

There are no Federal import regulations regarding vAh. According to published information, catfish and catfish eggs are not imported for use in the U.S. catfish industry[16].

Domestic Movement of Catfish

Producers must follow all applicable State and Federal regulations governing the movement of animals, production, harvest, preparation, preservation, labeling, safety, and sale of aquaculture products. Catfish aquaculture management and regulation data are publicly available at State and

Federal levels by each agency on their respective websites. Permitting and regulations vary among States.

Fish Species Other than *Siluriformes* for Human and Animal Foods and Food Ingredients

The USDA does not require an import permit for materials such as blood, chondroitin, collagen, emulsions, extracts, feces, fluids, oils, or tissues from any fish species, but does inspect such materials at points of entry [28]. The Food and Drug Administration (FDA) regulates imported and domestic human and animal food products. The FDA Center for Veterinary Medicine regulates animal food and feed products [29].

The FDA Food Safety Modernization Act (FSMA; 2011) contains Current Good Manufacturing Practice regulations specifically addressing the manufacturing, processing, packing, and holding of human and animal foods and food ingredients by domestic and foreign facilities registered as "food facilities"[30]. The regulations require that food facilities provide and maintain a written food safety plan, conduct a hazard analysis, develop and monitor risk-based preventive controls, conduct verification activities to ensure that controls are effective, take appropriate corrective actions, and maintain records documenting those actions. The hazard evaluation must include an assessment of environmental pathogens when ready-to-eat food is exposed to the environment prior to packaging and will not receive a treatment to control the pathogen. In all other circumstances the regulation allows facilities to decide if their environmental monitoring is appropriate to the facilities, food, and preventative control. Examples of environmental pathogens described in the rule include *Salmonella* spp. and *Listeria monocytogenes.* Testing for microbial and other contaminants by accredited laboratories is required. Microbial pathogens typically tested for include *Salmonella* spp. and *L. monocytogenes* [31]. The FDA does not test or require testing for non-virulent *A. hydrophila* or vAh.

Compliance with the FDA FSMA rule on Foreign Supplier Verification Programs (FSVP) for Importers of Food for Humans and Animals began on May 30, 2017 [32]**.** Importers of human and animal food, including fish and fish products, must verify that their processors and suppliers have preventive controls in place that meet all applicable U.S. safety standards. The rule requires that foreign suppliers conduct a hazard analysis to identify known or reasonably foreseeable hazards with each food that require a control (21 CFR 1.504); evaluate the risk posed by each food (21 CFR 1.505(a)); use the evaluation to determine, conduct, and approve appropriate supplier verification activities (21 CFR 1.505(b))(21 CFR 1.506); take corrective actions if necessary (21 CFR 1.508); and maintain records of FSVP activities (21 CFR 1.510). The regulation applies to all importers and food imported or offered for import into the U.S. unless there is an exemption (21 CFR 1.501(a)). Very small importers and importers of food from certain small foreign suppliers are subject to modified requirements that exempt them from conducting hazard analyses or evaluation of the food and foreign supplier(s) (21 CFR 1.512). Certain seafood products (21 CFR 1.501(b)) made in foreign facilities in compliance with FDA's seafood Hazard Analysis Critical Control Point (HACCP) requirements of 21 CFR part 123 are exempt from FSVP regulation. This exemption also covers imports of seafood raw materials or other ingredients for seafood products under the seafood HACCP regulation. The FMSA directs FDA to inspect a minimum 600 foreign facilities and double those inspections every year for the next 5 years following enactment of the rule. To encourage compliance, the FDA uses an Accredited Third-Party Certification Program to authorize qualified

third-party auditors for the inspection of foreign food facilities.

Siluriformes Fish and Fish Products

The term "catfish" is used to describe fish in the Linnean order Siluriformes, which contains approximately 3,000 species of fish, including *Ictalurus* spp. and *Pangasius* spp. fish. In 2002, legislation was passed that only allows the labeling or advertising of *Ictalurus* spp. fish (the family of catfish reared in the U.S.) as "catfish," and prohibits the labeling of basa (*Pangasius bocourti*), swai (*P.pangasius*), and tra (*P. hyophthalamus*) as "catfish"[20]. In 2003, the International Trade Commission imposed antidumping duties, which were upheld in 2009 and 2014, on "certain frozen pangasius fillets from Vietnam" [20]. The 2008 Farm Bill (P.L. 110-246) transferred catfish inspection (including basa, swai, and tra) from the FDA to the USDA [20]. The 2014 Farm Bill (P.L. 113-79) confirmed this transfer and requires the inspection of all *Siluriformes* fish (including Ictalurid catfish, and basa, swai and tra) in compliance with USDA requirements, and review by the USDA Food Safety and Inspection Service (FSIS) of catfish processing by other nations to ensure they meet USDA standards [20]. USDA published the final regulations for imported catfish inspection in the Federal Register in 2015 [20]. Foreign countries must have laws or legal measures in place providing authority to regulate the growing and processing of *Siluriformes* fish for human consumption in compliance with FDA regulatory requirements in 21 CFR part 123, Fish and Fishery Products [33, 34]. FSIS implements an equivalence process to ensure that the U.S. meets its treaty obligations under the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) [35-37].

Foreign countries must demonstrate their procedures meet U.S. levels of protection (Title 9 CFR §557.2)[34, 36-38]. To determine if a country's food safety inspection system is equivalent, FSIS assesses information collected through a self-reporting tool (SRT)[39], and documentation by the country's competent authority (CCA), the national government authority responsible for ensuring food safety and truthful labeling on a) food safety and inspection laws and legislation; b) regulations, policies, standards, decisions, annexes, and decrees; c) inspection procedures, manuals and directives; d) control programs; e) inspection training programs; f) mechanisms for documenting compliance/noncompliance; and g) enforcement and compliance programs. The CCA must annually provide an updated a) list of all certified establishments eligible to export to the U.S.; b) government residue control programs; c) microbiological sampling and testing program including reactions to *Salmonella* spp. in raw *Siluriformes* fish, and *L. monocytogenes*, *Salmonella* spp., or other pathogens of public health concern in ready-to-eat *Siluriformes* fish products. Foreign country food safety inspection systems must provide standards equivalent to FSIS standards to ensure that they meet other non-food safety requirements (e.g., accurate labeling, assurance that *Siluriformes* products are not economically adulterated). *Siluriformes* fish or fish products exported to the U.S. from a foreign country must be accompanied by a foreign inspection certificate issued by an official of the foreign government agency responsible for the inspection and certification. All fish and fish products are re-inspected prior to entry into the U.S. As of September 1, 2017, FSIS collects and submits samples of raw *Siluriformes* for speciation, residue, and *Salmonella* testing. Ready-to-eat *Siluriformes* fish products are sampled and tested for *Salmonella* spp. and *L. monocytogenes.* FSIS does not test raw *Siluriformes* fish or ready-to-eat fish or fish products for non-virulent strains of A. hydrophila or vAh.

Following the U.S. publication of its final regulation, the Vietnam Ministry of Foreign Affairs stated that the new regulations could constitute a non-tariff trade barrier. In January 2018, Vietnam filed a request for consultations with the WTO Dispute Settlement Body on the anti-dumping duties and cash deposit requirements by the U.S. Department of Commerce on "certain frozen fish fillets." In February 2018, Vietnam filed a WTO complaint stating that the U.S. inspection program for catfish imports violates the WTO Agreement on Sanitary and Phytosanitary Measures. In March 2018, China requested to be included in the inspection complaint consultation. The case is pending [20].

Domestic Catfish Aquaculture Production

Domestic Aquaculture – Water

The Environmental Protection Agency (EPA) regulates aquaculture effluent discharges from point sources and non-point sources under the Federal Water Pollution Control Act or Clean Water Act (CWA); 33 U.S.C. §§ 1251–1387 and 40 C.F.R. Parts 104–424), National Pollutant Discharge Elimination System (NPDES; CWA Section 402) permitting [25, 26]. States are mandated under the CWA to designate specific uses of water bodies and assign site-specific water quality standards [16]. Channel catfish ponds are exempt from Concentrated Aquatic Animal Production regulations because they are closed systems that infrequently discharge water, and are considered at low risk of environmental impact [27].

Domestic Catfish Processing

FSIS inspects *Siluriformes* catfish under the Federal Meat Inspection Act [40]. Regulatory requirements for inspection of processing establishments; pre-harvest; transportation; sanitation and HACCP requirements; mandatory disposition; performance standards respecting physical, chemical or biological contaminants; handling and disposal of condemned or inedible materials; preparation of food products; labeling; and importation are described in the Code of Federal Regulations; Title 9 – Animals And Animal Products, Subchapter F – Mandatory Inspection of Fish of the Order Siluriformes and Products of Such Fish. Samples of raw *Siluriformes* fish are collected for speciation, residue, and *Salmonella* spp. testing [41]. Ready-to-eat *Siluriformes* fish and fish products are sampled and tested for *Salmonella* spp. and *L. monocytogenes*. FSIS does not test for non-virulent *A. hydrophila* or ST251 strains of vAh. The FSIS Food Defense Guidelines for *Siluriformes* Fish Production and Processing provide voluntary measures for producers to assist federally inspected *Siluriformes* fish facilities in preventing contamination of farm-raised *Siluriformes* fish products [40].

Processing Plant Waste Streams

Fish processing operations produce solid waste, sludge, and wastewater that contain contaminating organisms [42]. Solid wastes may be incorporated into wastewater treatment steps to produce sludge or be disposed of via solid waste streams (e.g., landfills, compost, burying). Sludge contains organic material removed from treated wastewater and solids and is typically disposed of by transportation to sludge treatment facilitates, anaerobic digestion, or land disposal (i.e., fertilizer). Wastewater discharges are subject to regulation under the CWA NPDES permitting process and the EPA Seafood Processing Effluent Guidelines and Standards (a.k.a., Canned and Preserved Seafood Category; 40 CFR 408) [25, 27, 43, 44]. 40 CFR, Subpart A – Farm Raised Catfish Processing Subcategory applies to discharges by existing catfish facilities processing over 1,352 kg (3000 lbs.)

of raw material per day on any day during a calendar year and all new sources [45]. A NPDES permit may or may not be required if effluents are discharged into municipal storm sewer systems. Such permitting is not required if effluent is discharged into a municipal sanitary sewer system [26, 44]. States with NPDES permitting programs may regulate processing facility's wastewater discharges. Wastewater disinfection and treatment must be sufficient to prevent contamination or damage to public water works or natural water bodies. Facilities are required to monitor and sample wastewater discharges and notify the EPA and State regulatory agencies of the results [44].

Markets and Restaurants

Siluriformes catfish products prepared at markets (e.g., farm, live, retail stores) and restaurants are exempt from FSIS inspection, but are regulated by State and local health authorities and subject to State and local requirements for operating a food business [34, 38, 46]. Solid and liquid waste disposal may not be subject to regulation; however, regulations vary by State and may be accessed at their respective websites.

Epidemiology

Risk Factors

Infectious disease is the result of complex interactions between a pathogen, a host, and the environment. ST251 strains of vAh are detectable in catfish aquaculture ponds when disease is absent [12], indicating that environmental and other factors are associated with disease development. Disease outbreaks are reported to be linked with alterations in the environmental quality of ponds that elicit changes in host susceptibility and conditions favoring proliferation and production of virulence factors by vAh [12, 14]. For example, research suggests that vAh can exist commensally in aquaculture ponds until environmental conditions (e.g., iron-limited conditions, proliferation of cyanobacteria, low dissolved oxygen) induce growth and heightened virulence [14, 47]. Development of disease also appears associated with the organic load in the water and may be related to high concentrations of phosphorus and nitrogen, intensive feeding of nutrient-rich feeds, the amount of chitin present, and the chitinolytic capability of vAh [47-49]. Experimentally, vAh was observed to propagate rapidly to high densities, which persisted for 48 hours, following nutrient enrichment of water in 50-liter tanks with Tryptic soy broth or commercial fish feeds [48]. The percentage of channel catfish mortality that occurred during these studies was generally proportional to the densities of vAh present in the water [48] supporting the hypothesis that development of disease and mortality is vAh dose- and time-dependent, rising as the number of vAh in the water column and the length of time fish are exposed to the pathogen increase [50].

Fish body condition and feeding status may be related to morbidity and mortality. Experimentally, low body condition catfish fingerlings appeared less susceptible to vAh infection compared to fingerlings with high body condition scores, and dominant (larger, more aggressive) fish were observed to succumb faster and at higher mortality rates than subordinate fish following vAh challenge [47]. Experimentally, decreased morbidity and mortality rates have also been reported in catfish fasted for 1 to 2 hours post-vAh challenge [47].

A positive association has been reported between increase in water temperature, the presence and density of vAh in aquaculture water, biofilms, and sediments, and upregulation of virulence factor expression [12]. Temperature is also an environmental stressor for fish when it approaches the

high, long-term tolerance level of the fish (27.2 °C/80°F), or when it rapidly increases by 3°C to 5°C/5.4°F to 9°F in a short period of time [51]. Catfish mortality rates are greatest (80 percent or greater) when water temperatures approach 30°C to 32°C/86 °F to 89.6°F and lowest (10 percent) at 17°C/62.6°F [5, 52]. Bebak *et al*. (2015) and others identified risk factors in the U.S. catfish industry that appeared related to the occurrence of vAh disease outbreaks including pond size, stocking densities, movement of personnel and equipment into and between ponds, seining frequency, use of commercial seining, the intensive farming practices in place at the time, water quality management, co-infection with other pathogens or parasites, use of artificial feeds, and fertilization of pond water [7, 12, 53].

Transmission

Natural routes of transmission are believed to be horizontal via oral, dermal, or gill routes [5, 7]. Asymptomatic carriers shed vAh from the gastrointestinal tract (GIT); infected fish shed vAh from the GIT and skin lesions [7, 12, 54]. Injury to cutaneous mucus, fins, and skin predispose fish to development of disease. Piscivorous birds appear capable of serving as mechanical and transmission vectors [55-57]. The roles of amphibians, aquatic reptiles, invertebrates, and wild mammals in transmission are not fully understood. People, vehicles, and equipment may function as fomites [7, 12, 53].

Clinical Signs

Disease caused by vAh typically occurs in catfish at or near market size. [6, 9, 50, 58]. Rapid mortality may be the only clinical sign in peracute cases of the disease. Acute disease is characterized by rapidly fatal hemorrhagic septicemia [5]. When present, clinical signs include abnormal swimming behavior; abdominal swelling (ascites); anorexia; dermal hyperemia, necrosis, and ulceration; eye abnormalities (e.g., exophthalmia, hemorrhages of the iris); necrosis of the fins and tail; pale gills; and petechial hemorrhages [5, 52]. Gross necropsy findings include blood-tinged fluid in the coelomic cavity (ascites); diffuse hemorrhages in connective tissues, the GIT, liver, pancreas, skeletal muscle, and visceral fat; and swollen, friable to necrotic kidneys, liver, and spleen [5, 52]. Subclinical carriers exist, and some fish survive disease. Mortality rates are variable (5 percent to 100 percent)[48]**.** In peracute disease outbreaks, mortality rates approaching 100 percent may occur within 48 hours of disease onset**.** Initial mortality rates of 20 percent to 30 percent may be seen during the first week of acute disease outbreaks, with cumulative mortality rates approaching 80 percent to 100 percent.

Diagnostics

The diagnosis and differentiation of vAh-caused disease from *A. hydrophila*-caused MAS is based on the case history, clinical signs, gross necropsy findings, and isolation and full identification of the pathogen. Culture methods include use of selective culture media providing myo-inositol as the sole carbon source [14, 54, 59]. Genomic DNA-based polymerase chain reaction (PCR) assays amplifying the 167-base pairs region of a unique predicted open reading frame specific to vAh strains should be used for confirmatory diagnosis [7]. Quantitative PCR detection can be performed using an internal oligonucleotide hydrolysis probe [4, 9, 14, 59]. Other diagnostic testing methods include fluorescent antibody, indirect fluorescent antibody, and enzyme-linked immunosorbent assay [54].

Disease Prevention and Control

Subsequent to the emergence of vAh, the U.S. catfish industry implemented changes in farm management and biosecurity practices that have been successful in decreasing the incidence of vAh disease outbreaks; these include management of stocking densities, minimization of handling stress factors, changes in pond management, maintaining high standards of water quality and temperature control, and disinfection of equipment [16, 60, 61]. Changes in biosecurity include use of farm-specific trucks, equipment, and seins and establishment of personal protective equipment and disinfection protocols [60]. Biosecurity recommendations for catfish aquaculture and processing establishments are outlined in "Food Defense Guidelines for Siluriformes Fish Production and Processing" [40].

Treatment typically includes use of antibiotics (via oral or immersion routes) and reducing or withholding feed [62, 63]. A commercial vaccine is not currently available. The literature describes use of experimental vaccine constructs, probiotics, plants, and plant extracts to prevent or treat *A. hydrophila* infection in multiple fish species [64-71]. There is lack of data on use of these experimental therapies to treat vAh-caused disease.

Summary of Potential Entry and Exposure Pathways

Potential Entry Pathways

Importation of Live Fish or Germplasm for Domestic Production

Live catfish and eggs are not imported for use in domestic aquaculture and are rarely imported for ornamental or aquarium purposes [16]; therefore, importation of live catfish or catfish germplasms is not a plausible pathway of vAh entry into the U.S. There are no Federal import regulations for vAh. It is plausible that ST251 strains of vAh may enter the U.S. via shipments of live fish other than catfish, if imported fish and germplasms are sourced from specific areas where vAh is known to occur (e.g., provinces in China.) Assessment of this potential entry pathway is constrained by lack of data regarding a) the commensal carrier capability and susceptibility of many fish species to vAh; b) the points of origin, volumes, and final disposition of imported live fish and germplasms; c) published reports or research describing the presence of ST251 strains of vAh in many fish species.

Importation of *Clarias* spp., *Ictaluris* spp., *Pangasius* spp. (CIP) Fish and Fish Products for Human Consumption

It is plausible that ST251 strains of vAh may enter the U.S. on or in imported fresh, chilled, or frozen whole CIPS fish and fish products (e.g, fillets, steaks). However, estimates of contamination are not possible due to lack of a) surveillance for vAh in imported CIPS products for human consumption; b) scientific research and/or data documenting presence of vAh in imported consumable CIPS products; c) international data confirming detections of vAh in CIPS reared in countries other than China; and d) published literature or data confirming the presence of vAh outside of the Hainan, Hunan, Hubei, Jiangsu, Guangdong, and Zhejiang provinces in China.

In China, major areas of CIPS production are in the south central provinces of Anhui, Guangdong, Hunan, Hubei, Jiangxi, Jiangsu, and Sichuan, in the Yangtze River basin, and the Pearl River basin [72-74], with approximately 70 percent of production occurring in Guangdong, Hunan, Hubei,

Jiangxi, and Sichuan provinces [73]. Outbreaks of ST251 strain vAh-caused disease have been reported in four of these provinces: Guangdong, Hunan, Hubei, and Jiangsu. Published scientific epidemiological data reporting is lacking on disease caused by vAh in farmed CIPS in China; however, the literature describes channel catfish virus, enteric septicemia of catfish (ESC), columnaris disease, parasitism, and infection with *Edwardsiella ictaluri*, *Stenotrophomonas maltophila*, *Streptococcus iniae*, and *A. veronii* [73]. Polyculture with black carp, grass carp, and silver carp is practiced on some catfish farms [72, 73], so it is plausible that CIPS reared in provinces where vAh has been detected may be exposed to and be carriers of vAh, and that consumable product exported from these provinces may be contaminated.

Importation of Fish and Fish Products Other than CIPS for Human Consumption

It is plausible that ST251 strains of vAh may enter the U.S. in imported fresh, chilled, or frozen fish and fish products prepared from fish species other than CIPS. Data gaps that affect the assessment of the potential risk of entry associated with this pathway include lack of a) surveillance for vAh in imported fish and fish products for human consumption; b) scientific research and/or data documenting presence of vAh in imported consumable fish or fish products; c) international data confirming detections of vAh in food fish species reared in countries other than China; d) published literature or data confirming presence of vAh outside of Hainan, Hunan, Hubei, Jiangsu, Guangdong, and Zhejiang provinces in China; and e) data on the locations in China where imported consumable food fish products are reared, processed, or from which they are procured. Consequently, it is unknown which global regions may be plausible entry pathways of contaminated fish and fish products for human consumption other than those provinces in China where vAh has been detected and reported.

Importation of Fish Meal and Other By-Products

The U.S. imports fishmeal from approximately 28 countries and the European Union. Countries exporting animal food and food ingredients to the U.S. must meet FDA regulations for processing. Fishmeal is typically produced from the bones and offal left over from fish processing, by-catch, and wild-caught marine fish species [75]**.** Production includes cooking, pressing, drying, and grinding steps; fish oil is extracted during the pressing step. The high temperatures used during the processing steps are likely sufficient to inactivate vAh. Given that marine fish species are typical used to produce fishmeal, it does not appear plausible that imported fish meal, fish oil, or by-product could provide pathways of entry for vAh; however, assessment of this pathway is limited by lack of a) importation requirements for testing fishmeal, oil, or byproducts for aquatic animal pathogens, including vAh, and b) published literature on research or surveillance substantiating the validity of this potential entry pathway.

Potential Exposure Pathways

Exposure of Farmed Catfish to ST251 Strains of vAh via Domestic Live Catfish and Eggs

The movement and introduction of live fish that are commensal carriers or are subclinically infected with aquatic animal pathogens are described as the most likely pathways of disease introduction into freshwater fish farms [76, 77]. The transmission of some pathogens via egg surface contamination is possible as well [77]**.** Consequently, the movement of live commensal carrier or subclinically infected catfish or catfish eggs may result in exposure of catfish to ST251

strains of vAh. Commercial catfish growers may purchase eyed eggs (eggs containing an embryo that has developed enough so the eyes are visible), yolk-sac larvae, stock-sized fingerlings, and broodfish [78]. The potential for vAh introduction via this pathway depends on the geographic location of the farms supplying or receiving catfish eggs or live catfish (i.e., in areas where vAh is present vs. absent) and the biosecurity, quarantine, and disease surveillance protocols used by farms supplying and receiving catfish or catfish eggs. The commensal presence of vAh in/on healthy appearing eyed eggs, yolk-sac larvae, and fingerling or larger catfish is not well described, and there is a lack of data regarding the efficacy of washing eggs to remove vAh.

Exposure of Farmed Catfish to ST251 Strains of vAh via Imported Live Fish or Germplasms

Live catfish and eggs are not imported for use in domestic aquaculture and are rarely imported for ornamental or aquarium purposes [16]; therefore, it does not appear plausible that domestic farmed catfish would be exposed to ST251 strains of vAh via this pathway. Polyculture is typically not practiced on U.S. catfish farms, so it appears implausible that vAh would be introduced via addition or comingling of imported fish of any species (or their germplasms) into domestic catfish ponds.

Exposure of Farmed Catfish to ST251 Strains of vAh via Imported Fish Products for Human Consumption

Clarias spp.*, Ictaluris* spp.*, Pangasius spp.(CIP) Fish Products*

Imported CIP and their products intended for human consumption are not fed to domestic farmed catfish; therefore, such imported products are not plausible pathways of direct exposure for domestically farmed catfish to ST251 strains of vAh. Potential exposure following the disposal of imported CIP products via waste disposal pathways is summarized below in "Exposure of Farmed Catfish to ST251 strains of vAh via Contaminated Water."

Imported non-CIP Fish Products

Imported fish and fish products from non-CIP species intended for human consumption are not fed to domestic farmed catfish; therefore, these imported fish or fish products do not constitute a plausible pathway of exposure for domestically farmed catfish to ST251 strains of vAh. The potential for exposure following disposal of imported fish and fish products via food waste disposal pathways is discussed below in "Exposure of Farmed Catfish to ST251 strains of vAh via Contaminated Water."

Exposure of Farmed Catfish to ST251 Strains of vAh via Imported or Domestic Fishmeal, Fish Oil, or Catfish Feed

The exposure of farmed catfish to ST251 strains of vAh via the use of imported or domestically sourced fish meal, fish oil, or catfish offal meal as feed ingredients in catfish feeds does not appear highly plausible. A large volume of catfish feed is fed to domestic catfish; however, a minimal amount of animal-based ingredients, including fish meal or fish oil, are added to feed formulations**.** Diets for food-sized catfish may contain only 1 percent to 3 percent animal- or fish-meal, or the only animal ingredient may be a fish oil topcoat [16, 79, 80]. The percentage of fish meal used in fry and fingerling diets is greater (45 percent to 60 percent and 15 percent, respectively) [79-81].

Imported and domestically sourced fishmeal is typically produced from the bones and offal left over from fish processing, by-catch, and wild-caught marine fish species [75]**.** Domestically, catfish offal meal, prepared from catfish processing waste, may be used as a feed ingredient depending on availability [16, 81]. The capability of non-virulent *A. hydrophila* to serve as a surrogate for ST251 strains of vAh has not been validated; however, if the inactivation temperatures for vAh are the same as those published for non-virulent *A. hydrophila* (48°C to 70°C/118.4°F to 158°F) [82], it appears implausible that vAh would survive the fishmeal processing temperatures (dryer temperature, 500°C/932°F; internal product temperature, 100°C/212°F)[83], or temperatures used to produce pelleted and extruded catfish feeds (71°C to 84.6°C/160°F to 184°F; 87.7°C to 149°C/190°F to 300°F, respectively) [80]. Domestic catfish feed mills must meet FDA regulations for processing, have quality-assurance programs to ensure the quality of feed ingredients, and may conduct analyses to monitor or detect the presence of toxins, pesticides, or heavy metals. The FDA does not perform surveillance sampling for vAh, and there is little information on surveillance for vAh by processing plants.

There are no published reports that catfish feed has resulted in exposure of catfish to vAh or other pathogens. Fully assessing this pathway is limited by data gaps including a) lack of surveillance testing for presence of vAh in feed ingredients or feeds; b) lack of published data indicating that feed mills perform such surveillance; c) lack of published scientific research testing catfish feed ingredients or feed for vAh presence; and d) lack of published data confirming the physical inactivation temperatures for vAh.

Exposure of Farmed Catfish to ST251 Strains of vAh via Contaminated Water

Water may be contaminated with ST251 strains of vAh by a variety of pathways, including those described below. Factors impacting the potential for exposure via water include a) the proximity of farms to contamination sources (e.g., processing plants, waste disposal pathways, other infected catfish farms); b) the frequency of exchange or addition of water to catfish ponds; c) the water sources used by catfish farms and whether or not that water is treated prior to use or discharge; d) the potential for catfish pond contamination by surface water run-off or seepage; and e) biosecurity protocols of catfish farms regarding treatment and disposal of transport water [77]. Assessment of these pathways is limited due to lack of published literature describing surveillance for or detection of vAh in water sources used by catfish farms, or water associated with the transport of catfish. Data are also lacking on efficacy of influent and effluent water treatment on inactivation of ST251 strains of vAh.

Fish Processing Waste Streams

Commercial processing plants, markets (e.g., retail, live, farmgate or farmers markets), and restaurants may process domestically reared fish (including catfish) and imported fish. The literature have reported an association between the risk of aquatic animal pathogen introduction and the proximity of processing plants to aquaculture farms, with the risk being greatest when fish are processed at an on-farm processing facility [76, 77]. There is no published literature reporting on detection of ST251 strains of vAh in fish processing plants. There are published scientific literature documenting detection of non-virulent *A. hydrophila* on processing plant equipment; however, the capability of non-virulent A. hydrophila to serve as a surrogate for vAh in this scenario has not been validated.

Factors affecting the potential for exposure via this pathway include a) whether or not processing plants process domestic catfish reared in areas where vAh is endemically present; b) whether or not imported fish sourced from regions with documented vAh presence are processed; c) the types of imported fish processed; d) the volume of fish (domestic and imported) processed; e) commercial processing plant compliance with Federal and State regulations on the treatment and disposition of fish processing waste streams (small markets and restaurants are exempt from some Federal regulations and are regulated by State and local health authorities); and f) geographical proximity of fish processing sites to catfish farms and water sources used by catfish farms.

The most plausible pathways of any aquatic pathogen release associated with fish processing are associated with inadequate disinfection, discharge, disposal, or storage of solid, sludge, or liquid wastes [77]**.** Wastewater associated with commercial fish processing is discharged to public water treatment works, municipal storm sewer systems, municipal sanitary sewers, or natural water bodies. Wastewater discharges are subject to Federal and State regulations requiring disinfection steps and monitoring for contamination, dependent upon the size of the processing facility (small processing plants and farms may be exempt from some regulations). Despite contamination monitoring requirements, violations have been reported**.** Water that enters public water treatment works or municipal sanitary sewer systems is treated prior to final discharge into natural water bodies; however, there is a lack of data on the efficacy of such treatments on vAh. Water entering storm sewer systems is not treated prior to discharge into natural water systems. No regulations require surveillance of wastewater for vAh.

Disposition pathways of solid and sludge wastes may result in contamination of water sources used in catfish farming. There is a lack of information on the volume of catfish or other fish processing waste discarding via these mechanisms. Information is not available on monitoring of compost, buried, or ground-applied solid or sludge wastes for groundwater contamination. Landfills are required to monitor groundwater for microbial contamination; however, it is unknown if the methods used correlate with the presence of aquatic animal pathogens, including vAh [84-86]. The literature has reported microbial contamination of water bodies and groundwater associated with landfills, composting, burial, and land application of processing plant waste slurry [84, 85, 87-89]; such research has not been conducted relative to aquatic animal pathogens, including vAh.

The potential risk of vAh transmission occurring via this pathway cannot be reliably estimated due to a lack of scientifically validated epidemiological data substantiating fish processing as a pathway of introduction for ST251 strains of vAh in domestic catfish farming. Data and knowledge gaps affecting assessment include, but are not limited to lack of a) data on the proximity of catfish farms to fish processing sites; b) information on catfish farms that process fish or fish products on-site and disposition of the associated waste streams; c) information on markets and restaurants that may be exempt from Federal and State regulations; d) data on regulatory compliance by facilities that process fish; e) of regulatory surveillance or scientific research monitoring presence of vAh on processing equipment; f) information on the volume of processing wastes generated by fish processing in areas where catfish farming occurs is generally unknown; g) information on the volume of waste distributed to the various disposition pathways (e.g., wastewater, landfill, composting, burial, land application); h) information on surveillance for vAh in the various waste disposition pathways; i) data on vAh presence in ground or surface water sources for catfish farming; j) disease and hydrology modelling assessments regarding this pathway; and k)

scientifically validated epidemiological research documenting exposure of catfish via this pathway.

Consumable Food Waste Pathways

There are knowledge gaps on the quantity of catfish produced for human consumption that is discarded by consumers via composting, burial, or other pathways (e.g., used as bait). Approximately 40 percent of commercial fish produced for human consumption is discarded as waste [90]. Landfills are the most common waste disposal pathways and monitor groundwater for microbial contamination [91]; however, it is unknown how the monitoring methods correlate to the presence of aquatic animal pathogens. If discarded consumable fish product can contaminate natural water systems or groundwater in areas near catfish farms, it is plausible farmed catfish may be exposed via this pathway.

Plausibility of this pathway is affected by the source(s) of the consumable catfish products. The risk of catfish being exposed to vAh via this pathway is plausibly greater in areas that process or sell consumable domestic catfish or imported susceptible fish products sourced from regions where vAh is endemically present. Knowledge gaps affecting the assessment of this pathway include those described above for processing wastes, as well as lack of data regarding the a) sources and total volume of consumable domestic catfish, imported CIP, and other fish and fish products discarded as waste; b) proportions of product that enter landfills, is composted, buried, or disposed of via other methods (e.g., directly into water); c) published data providing evidence that this is a plausible pathway of exposure of catfish to vAh or other pathogens or contaminants.

Exposure of Farmed Catfish ST251 strains of virulent *Aeromonas hydrophila* via Wild Animals and Birds

Cunningham *et al.* (2019) reported that piscivorous birds are demonstrated transmission vectors of "atypical *A. hyrodphila"* among catfish farms (the manuscript did not denote if "atypical *A. hydrophila"* was synonymous with ST251 strains of vAh) [55-57]. Exposure of catfish to vAh by movement of terrestrial wild and/or domestic (e.g., cats, dogs) animals among catfish ponds, farms and natural bodies may be plausible means of exposure; however, documentation of this pathway has not been published and research is lacking to validate/invalidate the potential for exposure to occur via these pathways. Wild animals and birds that forage on landfills, compost, or buried waste could plausibly function as fomites or transmission vectors. Documentation of exposure occurring via this pathway has not been published and research is lacking to confirm that these are plausible exposure pathways.

The overall plausibility that exposure may occur via this pathway is influenced by several variables, including a) the geographic location of catfish farms (i.e. in areas where vAh is present vs. absent); b) the level of accessibility of catfish ponds by wildlife and birds; d) the use and efficacy of biosecurity and mitigation methods by catfish farms to deter wildlife, domestic animals, and birds; e) the size of the home ranges and movement patterns of wildlife and birds that may access catfish ponds; f) lack of published studies performing surveillance for vAh in wildlife, birds, and domestic animals that access catfish ponds; g) knowledge gaps on the length of time that vAh may remain viably present on or in terrestrial wild and domestic animals and some birds; and h) the awareness of farm managers regarding the potential for domestic animals (cats, dogs) to be mechanical or transmission vectors for aquatic animal pathogens.

Exposure of Farmed Catfish ST251 Strains of vAh via Wild Aquatic Animals

Exposure of domestic catfish to ST251 strains of vAh via wild aquatic animals serving as fomites or transmission vectors is plausible; however, the risk of exposure cannot be quantified due to the lack of published data or reporting of vAh presence in indigenous wild aquatic animals. The biosecurity of catfish ponds is relatively high (i.e., static ponds and screened drains, no intentional water discharge); however, catfish ponds are accessible to, and often serve as habitats for, wild aquatic animals. Ponds at greatest risk are those using surface water or near natural water bodies. Factors that impact the risk of exposure occurring via this pathway include the a) geographic location of farms (in areas where vAh is present vs. not present); b) water source used by individual catfish farms; c) source water biosecurity and treatment measures used by individual farms; and d) biosecurity and mitigation measures used by individual catfish farms to manage invasive aquatic animals. Data gaps for this pathway include, but are not limited to, a) research or data regarding surveillance for or detection of vAh in aquatic reptiles, amphibians, crustaceans or other species present in catfish ponds or adjacent bodies of water; b) published reports demonstrating that vAh introductions into catfish farms have occurred via this pathway; and c) published research demonstrating transmission of vAh via this pathway.

Exposure of Farmed Catfish ST251 Strains of vAh via Fomites

Contaminated fomites may result in exposure of farmed catfish to vAh. Potential fomites include people such as farm staff, staff shared with other farms; visitors wearing contaminated shoes or clothing and/or not following appropriate biosecurity and personal protective equipment (PPE) protocols; and contaminated vehicles and farm equipment entering farms or ponds [77, 92]. Several factors influence the plausibility of this pathway, including the a) geographic location of catfish farms (in areas where vAh is present vs. absent); b) degree of movement of potential fomites between catfish farms or areas where contamination could occur (e.g., processing plants); c) degree of movement of potential fomites between catfish farms where vAh is present vs. absent; d) level of stringency and compliance with farm biosecurity and risk management plans; and e) mechanisms by which susceptible catfish are exposed to contaminated fomites.

Consequence Evaluation

Commercial catfish production is the leading U.S. aquaculture industry, accounting for approximately 50 percent of all U.S. food-fish aquaculture and generating over 27 percent of the value of total aquaculture production. In 2018, the States of Arkansas, Alabama, Mississippi, and Texas accounted for approximately 97 percent of total sales of food-sized catfish (approximately 5 percent, 30 percent, 57 percent and 4 percent, respectively) [93]. Domestic production declined in 2005 through 2013 due to high feed prices, high domestic production costs, a prolonged sluggish economy, and increased volumes of foreign catfish imports, and continued to show declines through 2018 (**Appendix 3: Tables 3-1 to 3-4**).

Total sales declined by 21 percent from 2005 to 2018 ($461 million USD to $366 million USD). This trend was affected by decreased sales of food-sized fish ($429 million USD to $341 million USD; a

20 percent decline); fingerlings and fry ($24 million USD to $16 million USD; a 34 percent decline); and broodfish ($1.9 million USD to $794,000 USD; a 59 percent decline). Sales of stocker-sized catfish increased 30 percent during this period ($5.9 million USD to $7.7 million USD). The number of food fish produced from 2005 to 2018 decreased 21 percent (396 million vs. 312 million animals). Declines also occurred in number of fingerling /fry (683 million vs. 208 million; 69 percent decline) and broodfish (503,000 vs. 201,000; a 60 percent decline) produced. The number of stocker-sized fish increased 74 percent (33 million to 58 million) during this period.

Impacts of the introductions of ST251 strains of vAh into affected catfish production areas were significant and continue to be, although farm management and biosecurity changes post-vAh emergence have decreased the occurrence and severity of vAh-caused disease. In 2009, disease caused by vAh in Alabama was documented on approximately 48 farms, resulting in estimated losses of 1,447,272 kg/3,184,000 lbs. of catfish [5]. In 2010, the disease recurred in Alabama, affecting approximately 60 farms and caused estimated losses of 1,090,090 kg/2,400,000 lbs. of catfish [5]. Annual losses of approximately 2 million fish per year have been reported in some States since 2009 [5]. As of 2014, direct and indirect monetary losses associated with vAh disease outbreaks were estimated at $12 million USD (approximately $4 million USD annually) [5, 6, 63].

Naïve U.S. catfish-rearing regions would be expected to be similarly affected if vAh introduction were to occur. The exposure risk will most likely be associated with the movement of domestic live catfish, eyed eggs, or yolk-sac larvae, contaminated water, or via piscivorous birds and aquatic or terrestrial mammals. The development of knowledge and data-based measures to identify, control, treat, mitigate, and prevent exposure via these pathways will be most beneficial to the industry. Filling current knowledge gaps will require development of surveillance tools for vAh detection in catfish farms and natural water systems adjacent to or that receive water from catfish farms; potential aquatic, avian, and terrestrial hosts; processing plants, associated waste streams and waste disposal pathways; potential contamination of processed catfish product; and continued research examining the epidemiology of vAh.

Limitations

Research has offered insights into the emergence and epidemiology of ST251 strains of vAh; however, due to the relatively recent emergence of this pathogen, data gaps and inconsistencies in pathogen taxonomy and disease description currently affect the understanding of the epidemiology of vAh and the associated risks to farmed catfish. Inconsistent use of taxonomic nomenclature and discrimination between non-virulent strains of *A. hydrophila* and ST251 strains of vAh and the diseases associated with them in some published literature leads to confusion, confounding, or bias when interpreting the data or information. For example, disease caused by vAh is often incorrectly referred to as MAS, and the terms vAh and *A. hydrophila* are interchangeably used in some published literature specifically discussing the epidemiology of vAh. Whether this reflects taxonomic mistakes, lack of laboratory capability to discriminate between non-virulent *A. hydrophila* and vAh, or lack of awareness of the epidemiological differences between non-virulent A. *hydrophila* and ST251 strains of vAh is unclear. Such discrepancies negatively affect the capability to accurately assess the epidemiology of vAh and evaluate the risks, consequences, and impacts of vAh to the catfish industry, domestic aquaculture in general, and public health.

Aeromonas hydrophila and other motile aeromonads are recognized as pathogenic agents responsible for MAS in many fish species and are described as secondary pathogens that elicit disease in hosts compromised by primary disease, co-morbidity, injury, and/or unfavorable environmental factors. Globally, *A. hydrophila* is ubiquitously present in virtually all waterbodies, has been associated with disease in all vertebrate animals, and is considered an emerging foodborne pathogen. Despite the acceptance of this microbe as a pathogen, most countries do not require disease reporting, which results in lack of published data or literature establishing the prevalence and incidence of disease in humans and animals. Aquatic animals are the exception, given the economic impact of MAS outbreaks in aquaculture.

Disease caused by ST251 strains of vAh resembles MAS; however, vAh strains appear capable of functioning as primary pathogens, and there are differences in disease case history, presentation, clinical signs, and pathology distinguishing vAh-caused disease from MAS. Currently, disease caused by vAh is described only in blunt-snout bream, crucian carp, grass carp, *Ictalurid* spp. catfish, and silver carp. There is a global lack of research, surveillance, and reporting of vAh-caused disease in other animal (including fish) species and humans. The ST251 strains of vAh associated with disease of farmed catfish differ biochemically, molecularly, morphologically, and in expression of virulence factors, from non-virulent *A. hydrophila.* Complex biochemical, morphological, and molecular analyses are required to identify ST251 strains of vAh associated with disease outbreaks in farmed catfish in the U.S.

Lack of consistent use and standardization of such assays has historically resulted in knowledge gaps and inconsistencies on the phylogeny and taxonomy of all *Aeromonas* spp. in general [4, 14, 59]. Some published research studies and laboratories perform comprehensive assays; however, others do not perform detections beyond species (e.g., *A. hydrophila, A. sobria, A. caviae*). This inconsistency is present in studies of *A. hydrophila* in human and animal (including fish) diseases, as well as food and water contamination studies. Similar inconsistencies are present in studies and reports regarding vAh. Consistent use of appropriate diagnostic assays is required to accurately perform surveillance for vAh, confirm disease etiology, resolve knowledge gaps associated with the epidemiology of vAh, and more thoroughly identify and assess risks to the catfish industry and public health.

Disease occurs at the interface of pathogen, host, and environmental factors. Environmental factors that facilitate growth of vAh and expression of virulence factors have been identified, as have environmental factors that affect the health, physiology, and susceptibility of catfish hosts. Changes in management strategies affecting the environmental conditions in catfish ponds have successfully decreased the occurrence and severity of vAh outbreaks and demonstrate the effect of the environment on vAh-caused disease. At present, while the geographic distribution of vAh appears limited to specific areas in the United States (Arkansas, Alabama, and Mississippi) and China (Guangdong, Hainan, Hubei, Hunan, Jiangsu, Zhejiang provinces), published ecological modeling studies and other research examining the influences of environmental or geographic factors specific to those areas on vAh presence are limited or lacking.

There are knowledge and data gaps associated with the emergence of vAh that prevent full assessment of the epidemiology of vAh and the risks of introduction of vAh to regions where detection has not occurred. Many gaps are associated with the lack of surveillance for vAh in other fish and aquatic animal species; natural and man-made aquatic ecosystems, including those in

proximity to processing plants and catfish farms; in domestic and imported fish products including and other than catfish and CIP; and in processing plants, processing plant waste streams and all potential downstream pathways associated with processing waste disposal. Knowledge gaps associated with the development of vAh-caused disease in susceptible fish are affected by lack of field studies and capability to reproduce natural infection under laboratory conditions. Studies using methods of infection that do not meet the requirements for natural infection defined by the OIE are useful but must be interpreted with caution.

Additional information gaps include lack of information regarding the potential for vAh to elicit disease or be commensally present in aquatic and terrestrial animals, including fish species other than those known to be susceptible to infection. There is a general lack of information regarding the public health significance of vAh. Ongoing research, field studies, and data from disease occurrences on catfish farms should resolve some of these information and knowledge gaps.

Appendix 1. Epidemiology of Non-virulent *Aeromonas hydrophila*

Aeromonas hydrophila is a ubiquitous bacterium found in a variety of aquatic environments worldwide, including natural and artificial water bodies, aquaculture, public water systems, and sewage [61, 94-97]. It is found in the water column; can be closely associated with algae, biofilms, organic matter, sediments, and zooplankton; and is a natural component of the dermal and gut flora of fish and some aquatic amphibians, reptiles, and invertebrates [12, 98]. It is recognized as an etiological agent of disease in all vertebrate animals, including humans, typically functioning as a secondary pathogen causing disease following a primary infection, parasitism, injury, or host stress associated with unfavorable environmental conditions [8, 58, 59, 96]. The bacterium exhibits optimal growth at 28°C/82.4°F [6, 99] but can grow within a wide thermal range (4°C to 37°C/39.2°F to 98.6°F) in aerobic and anaerobic environments [59, 100-102]. Motile Aeromonas septicemia (MAS) is the term used to describe disease caused by infection with *A. hydrophila* and other aeromonad bacteria (*A. sobria*, *A. caviae)* [7, 103, 104]. Globally, large economic losses have occurred in many aquaculture systems from chronic and epidemic MAS outbreaks.

All *A. hydrophila* strains, including ST251 strains of vAh, are known to express virulence factors that may be pathogenic in hosts. Published literature on A*. hydrophila* presents conflicting reports regarding the molecular determinants of virulence due to changes in classification and misidentification [59]. For example, many initial experimental studies identifying *Aeromonas* spp. virulence determinants were performed using *A. hydrophila* strain SSU, which was later recognized to be affiliated with *A. dhakensis* [59]. An array of biochemical, morphological, and molecular analyses are required to comparatively study *A. hydrophila* strains and identify the genetic regulation leading to situational expression of strain-specific virulence factors [6, 8, 14]. Globally as of 2016, there were not enough phylogenetically confirmed strains of *A. hydrophila* available with sufficient supporting data to facilitate comprehensive comparative studies [14, 59].

Knowledge gaps are present regarding the variable pathogenicity and virulence factors associated with many strains of *A. hydrophila* that cause illness in animals (including fish) and humans, including the vAh strains present in the United States that cause disease in domestic catfish. Virulence factors alone do not predict the capability of a particular *A. hydrophila* to induce illness [97]. In addition to the presence of microbial virulence factors, the susceptibility and immune

responses of the host influence the severity of infection [97, 105]. Some *A. hydrophila* strains and the suite of virulence factors expressed are pathogenic to specific hosts and relatively harmless to others [106]. Presently, identified *A. hydrophila* virulence factors include but are not limited to adhesins, cell adherence factors, cytotoxins, elastases, endotoxins, enterotoxins, hemolysins, lipases, metalloprotease, pili, proteases, the S-layer, serine protease, surface proteins, the type III secretion system, use of specific metabolic pathways, the ability to form biofilms, and the capability to modulate virulence factor expression via quorum sensing [59, 107-110]. Virulence factors reported as important in fish disease include enterotoxins, hemolysin, metalloprotease, pili, the S-layer, serine protease, and the type III secretion system [4, 9, 52].

Susceptible Fish Species

MAS has been reported in many ornamental, cultured, and indigenous fish species globally [96, 104, 107].

Global Distribution

Aeromonas hydrophila is ubiquitous; there are published reports describing outbreaks of MAS in ornamental, cultured and wild fish populations worldwide [107, 109, 111-117].

Public Health

Aeromonas hydrophila is a zoonotic pathogen. Worldwide, including in the U.S., the exact incidence rates of A*. hydrophila*-related illnesses are unknown because diseases of any type caused by *Aeromonas* spp. are not reportable [118, 119]. Clinical signs of illness in humans include cellulitis, diarrhea, endocarditis, erythema gangrenosum, flu-like symptoms (e.g., fever, muscle aches, headache, lethargy), gastroenteritis, meningitis, myonecrosis, necrotic fasciitis, ocular infections, otitis, peritonitis, septic arthritis, septicemia, urinary tract infections, and wound infections [61, 96, 97, 110, 120-124]. Disease may be seasonal, with more cases occurring in the summer and fall, and is more severe in children, elderly, and immune-compromised persons [61, 103, 110]. Treatment may be compromised by resistance to commonly prescribed antibiotics.

Relative to aquaculture and fish processing pathways, illness is considered an occupational zoonosis [124]. Primary routes for transmission include direct contact with mucus and tissues from infected or carrier fish, and contaminated water or equipment. In healthy individuals, the most common signs of infection associated with this exposure pathway are localized cellulitis and infected wounds [97, 103, 124]. Globally, *A. hydrophila* has been associated with cases of gastroenteritis (i.e., traveler's diarrhea), often in co-infection with other enteric pathogens (*Campylobacter* spp.*, Salmonella* spp.*, E. coli, Clostridium difficile*) and/or parasites (e.g., giardia, cryptosporidium) [97, 122, 125]. Reporting of *A. hydrophila*-associated traveler's diarrhea is typically based on retrospective, case, and case-control studies; there have been few to no prospective or population-based studies published [122]. Cases are typically attributed to ingestion of contaminated water and/or foods.

Foodborne Illness

Aeromonas hydrophila was identified as a foodborne pathogen by the FDA in 1984 and is listed on the EPA contaminant candidate list of emerging water-borne pathogens due to its capability to persist in chlorinated water [61]. Strains associated with foodborne illness are stable at -80°C/-112°F, capable of growth at refrigeration temperatures (1.7°C to 5°C/35°F to 40°F), and produce heat-stable enterotoxins (up to 100°C/212°F for 30 min) [100-102]. *Aeromonas hydrophila* has

been isolated from a wide range of foods, including finfish, seafood, meat, poultry, milk and dairy products, vegetables, and ready-to-eat food products [19, 20, 61, 110, 120, 126]. The likelihood that aquatic animal food products may be contaminated is especially high due to the ubiquity of *A. hydrophila* in the environments they live in. Contamination likely occurs during food processing, preparation, and storage steps. For example, in one U.S. study, A*. hydrophila* comprised 37.5 percent of gram-negative bacterial isolates present in 39 swab samples collected from catfish processing equipment in two plants [121]. There are global knowledge gaps regarding the frequency, prevalence, virulence, and strains of *A. hydrophila* present in food processing pathways due to lack of surveillance.

The literature review indicates that documented outbreaks of *A. hydrophila*-associated foodborne illness are sporadic and infrequent [98, 127-129]. In the U.S., the incidence and detection rates of foodborne *A. hydrophilia* gastroenteritis have remained low, based on 2009 to 2016 Foodborne Disease Outbreak Surveillance System data and prior published reports [128, 130]. Higher incidence and prevalence rates in some countries may be associated with disparities in food, water, and public health sanitation factors [61]. Knowledge and data gaps regarding the frequency, prevalence, severity, and strains of *A. hydrophila* associated with cases of human foodborne illness may be related to the self-limiting nature and underreporting of foodborne illness [128]. Testing for *Aeromonas* spp. is not routinely available or included in enteric pathogen isolation protocols in many diagnostic laboratories and, when included may not identify isolates beyond the phenotypic group level (e.g., *A. hydrophila, A. caviae, A. sobria*), because the laboratories may lack capability to perform the biochemical, morphological, and molecular analyses required for strain and virulence factor identification [97].

Presence in Consumable Fish Products

Published research studies report detection of *A. hydrophila* in the fresh, chilled, and thawed products of many fish species [19, 118, 119, 122-125, 131-140]. Variables affecting the prevalence and concentration of *A. hydrophila* included the type of fish product (e.g., fresh, frozen, thawed, whole fish, fillets, cutlets, ready-to-eat product); whether or not the raw product was damaged; length, time, and type of storage and packaging used; time of year processing occurred; and country in which sampling occurs [114, 118, 125, 131-134, 141]. Globally, positive detections occurred in 33 percent to 75 percent of sampled fish and fish products, with higher levels of contamination during the summer months. In the U.S., Allred et al. (2019) isolated *A. hydrophila* from 19.8 percent of "red," 18.6 percent of "punctured," and 2.44 percent of "acceptable" channel catfish fillets collected directly from processing plants [19]. In many of these studies, enrichment techniques were required to recover isolates (indicating that starting concentrations of *A. hydrophila* were low). Concentrations then increased during 7 to 12 days storage at refrigeration temperatures by 1 to 6 logs [118, 122, 125, 131, 134, 141].

Appendix 2. ST251 Strains of Virulent *Aeromonas hydrophila*

Table 2-1. ST251 strains of vAh currently reported in the literature

Strain	GenBank Reference Number	Isolation Source	Location	Reference
214-296	SAMN05292365	Channel catfish	AL, MS USA	[14]
214-458	SAMN05223364	Channel catfish	USA	[14]
Ahy_IDx71	SAMN05292361	Channel catfish	USA	[14]
AL09-71	NZ_C-007566.1	Channel catfish	AL, USA	[9]
AL09-71	NZ_CP007566.1	Channel catfish	USA	[9]
AL09-72		Channel catfish	AL, USA	[9]
AL09-73		Channel catfish	AL, USA	[9]
AL09-79	NZ_LRRV00000000.1	Channel catfish	AL, USA	[6, 8]
AL10-121		Channel catfish	AL, USA	[6, 8]
AL98-C1B			USA	[9]
ALG15-098	SAMN05223361	Channel catfish	USA	[14]
IPRS15-28	SAMN05223362	Channel catfish	USA	[14]
J-1	NZ-CP006883.1	Crucian carp	Jiangsu province, China	[14]
JBN2301	NZ_CP013178.1	Crucian carp	Hubei province, China	[142]
ML09-119	NC_021290.1	Channel catfish	AL, USA	[6, 8]
ML09-121	NZ_LRRX00000000.1	Channel catfish	AL, USA	[6, 8]
ML09-122	NZ_LRRX00000000	Channel catfish	AL, USA	[6, 8]
ML10-51K	SAMN05223363		USA	[14]
NJ-35	NZ_CP007576.1	Crucian carp	Jiangsu province, China	[14]
PB10-118	SAMN01085622	Channel catfish	AR, USA	[6, 8]
pc104A	NZ_CPOO7576.1	Soil	USA	[10]
S04-690	SAMN02404466	Channel catfish	MS, USA	[6]
S13-512		Channel catfish	AL, MS USA	[14]
S13-612	SAMN05292362	Channel catfish	USA	[14]
S13-700	SAMN05292363	Channel catfish	AL, MS USA	[14]
S14-296	SAMN05292365	Channel catfish	USA	[14]
S14-452	SAMN05256776	Channel catfish	MS, USA	[14]

S14-458	SAMN05223364	Channel catfish	MS, USA	[14]
S14-606	SAMN05292366	Channel catfish	AL, MS USA	[14]
S15-130	SAMN05223365	Channel catfish	USA	[14]
S15-130	SAMN05223364	Channel catfish	MS, USA	[14]
S15-242	SAMN05223366	Channel catfish	USA	[14]
S15-400	SAMN05223367	Channel catfish	MS, USA	[14]
S15-500		Channel catfish	MS, USA	[14]
S15-591	SAMN05223368	Channel catfish	USA	[14]
S15-591	SAMN05223368	Channel catfish	MS, USA	[14]
Z1C	SAMN02404465	Grass carp	Guangdong province, China	[6, 14]

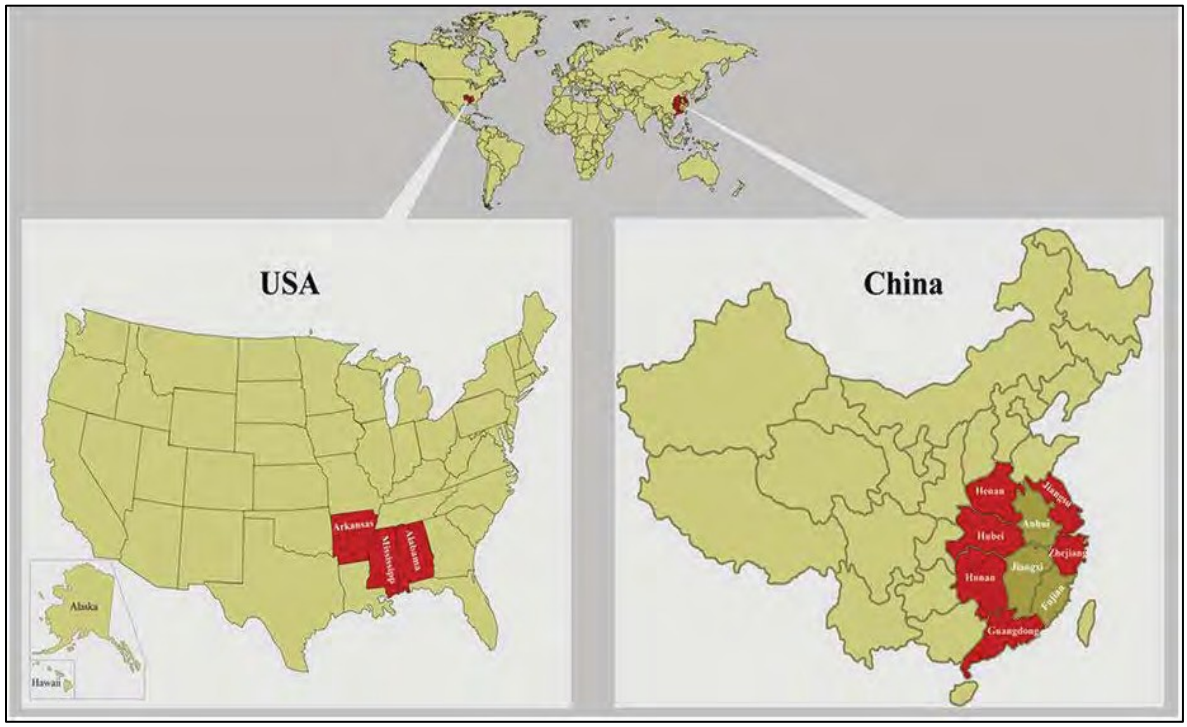

Figure 2-1. Geographical distribution of currently identified ST251 strains of virulent *Aeromonas hydrophila* [5, 14]

Appendix 3. Domestic Catfish Production and Trade Summary

Table 3-1. United States domestic catfish sales from 2005 through 2018 [16, 93, 143]

Year	Number of Farms	Total sales ($1,000)	Food fish sales ($1,000)	Stockersales ($1,000)	Fingerling/frysales ($1,000)	Broodfishsales ($1,000)
2005	1,160	461,855	429,245	5,983	24,697	1,958
2013	695	375,865	354,337	10,121	11,161	245
2018	531	366,843	341,915	7,752	16,382	794

Table 3-2. Volume of farmed catfish produced (live weight produced) in the United States from 2005 through 2018 [16, 93, 143]

Year	Number of Farms	Food fish (454 kg; 1,000 lb)	Stockers (454 kg; 1,000 lb)	Fingerling/fry (454 kg; 1,000 lb)	Broodfish (454 kg; 1,000 lb)
2005	1,160	276,332 kg 607,932 lb	3,127 kg 6,880 lb	x	1,377 kg 3,030 lb
2013	695	162,900 kg 358,380 lb	4,280 kg 9,418 lb	x	103 kg 227 lb
2018	531	159,756 kg 351,464 lb	2,681 kg 5,899 lb	x	398 kg 877 lb

Table 3-3. Number of United States produced farmed catfish fish sold from 2005 through 2018 [16, 93, 143]

Year	Number of Farms	Food fish (1,000)	Stockers (1,000)	Fingerling/fry (1,000)	Broodfish (1,000)
2005	1,160	396,533	33,636	683,111	503
2013	695	211,356	73,997	172,876	41
2018	531	312,692	58,640	208,801	201

Table 3-4. Volume of catfish products exported by the United States from 2015 to 2019 [144]

Product Type	2015 kg/lb	2016 kg/lb	2017 kg/lb	2018 kg/lb	2019 kg/lb
Fresh or chilled catfish excluding fillets, livers, and roes	82,862 kg 182,303 lb	83,940 kg 184,448 lb	227,886 kg 501,349 lb	186,050 kg 409,310 lb	211,622 kg 456,568 lb
Frozen catfish excluding livers, roes, fillets, and other fish meat	600,582 kg 1,321,280 lb	682,448 kg 1,501,385 lb	1,008,416 kg 2,218,515 lb	455,160 kg 1,001,352 lb	630,566 kg 1,387,245 lb
Catfish fillets and other meat, excluding fish steaks, fresh or chilled	569,522 kg 1,303,548 lb	513,506 kg 1,129,713 lb	530,888 kg 1,167,953 lb	274,988 kg 604,973 lb	174,318 kg 383,499 lb
Catfish fillets, frozen	1,190,502 kg 2,619,104 lb	1,641,196 kg 3,610,631 lb	1,808,698 kg 3,979,135 lb	800,700 kg 1,761,540 lb	882,608 kg 1,941,737 lb
Total	2,443,468 kg 5,375,629 lb	2,921,090 kg 6,426,398 lb	3,575,888 kg 7,866,953 lb	1,716,898 kg 3,766,388 lb	1,899,114 kg 4,178,050 lb

Table 3-5. *Ictalurus* spp. catfish products imported to the United States from 2015 to 2019

Imported Product	Country of Origin	2015 kg/lb	2016 kg/lb	2017 kg/lb	2018 kg/lb	2019 kg/lb
Fillet Fresh	China	36,049 kg 79,307 lb	74,842 kg 164,652 lb	17,010 kg 37,422 lb		
Fillet Frozen	China	4,760,415 kg 10,472,913 lb	4,769,304 kg 10,492,468 lb	5,460,767 kg 12,013,687 lb	5,530,168 kg 12,166,370 lb	4,489,985 kg 9,877,967 lb
Frozen Whole	China		8845 kg 19,459 lb	6,804 kg 14,968 lb	11,603 kg 25,526 lb	21,956 kg 48,303 lb
Meat Fresh	Iceland	1,612 kg 3,546 lb	1,269 kg 2,791 lb			
Total		4,798,076 kg 10,555,766 lb	4,854,260 kg 10,679,370 lb	5,484,581 kg 12,066,077 lb	5,541,771 kg 12,191,869 lb	4,511,941 kg 9,926,270 lb

Table 3-6. *Pangasius* spp. catfish products imported to the United States from 2015 to 2019

Imported Product	Country of Origin	2015 kg/lb	2016 kg/lb	2017 kg/lb	2018 kg/lb	2019 kg/lb
Fillet Fresh	China	76,239 kg 167,725 lb				
	Vietnam	116,392 kg 256,062 lb	45704 kg 100,548 lb	44,923 kg 16,495 lb	7,498 kg 16,495 lb	
Fillet Frozen	Burma	66,955 kg 147,301 lb				
	China				118,951 kg 261,692	618,588 kg 1,360,893
	Vietnam	107,673,371 kg 236,881,415 lb	130,851,125 kg 287,872,475 lb	104,409,664 kg 204,170,553 lb	92,804,797 kg 201,170,553 lb	52,795,427 kg 116,149,939 lb
Frozen Whole	Thailand		10,040 kg 2,2088 lb			
	Vietnam	433,987 kg 954,771 lb	355,981 kg 783,158 lb	465,859 kg 1,024,889 lb	190,691 kg 429,520 lb	598,416 kg 1,316,515 lb
Meat Fresh	China	68,888 kg 151,553 lb	9,900 kg 21,780 lb			
Total		108,435,832 kg 238,558,830 lb	131,272,750 kg 288,800,050 lb	104,920,446 kg 230,824,981 lb	93,121,937 kg 204,868,261 lb	54,012,431 kg 118,827,348 lb

Table 3-7. *Siluriformes* and *Clarias* spp. catfish products imported to the United States from 2015 to 2019

Imported Product	Country of Origin	2015 kg/lb	2016 kg/lb	2017 kg/lb	2018 kg/lb	2019 kg/lb
Catfish (*Silurus*, Other) Fillet Frozen	Bangladesh		1,448 kg 3,185 lb			
	China		16,452 kg 36,194 lb	46,004 kg 101,209 lb	19,800 kg 43,560 lb	19,731 kg 43,408 lb
	Thailand		5,852 12,874	1,060 2,332		
	Vietnam	70,479 kg 155,054 lb	121,908 kg 268,198 lb	1,7690 kg 38,918 lb	22,098,967 kg 48,647,727 lb	30,944,876 kg 68,078,727 lb
Catfish (*Silurus, Clarias spp.*) Fillet Fresh	China	118,199 kg 260,038 lb	306,055 kg 673,321 lb	290,857 kg 639,885 lb	138,965 kg 305,723 lb	60,591 kg 133,300 lb
	Guyana				10,614 kg 23,350 lb	
	Panama		521 kg 1,146 lb			
	Taiwan	1,905 kg 4,191 lb				33,256 kg 73,163
Total		190,583 kg 419,283 lb	452,236 kg 994,919 lb	356,611 kg 784,544 lb	22,268,346 kg 48,990,361 lb	31,058,454 kg 68,328,599 lb

Table 3-8. NSPF catfish products imported to the United States from 2015 to 2019

Imported Product	Country of Origin	2015 kg/lb	2016 kg/lb	2017 kg/lb	2018 kg/lb	2019 kg/lb
Catfish NSPF Fresh	Guyana	1,359 kg 2,989 lb	9,634 kg 21,195 lb	4298 kg 9455 lb		
	Vietnam					23,950 kg 52,690
Catfish NSPF Frozen	Bangladesh	3,258 kg 7,167 lb		9,958 kg 21,908		
	Brazil	41,707 kg 91,755 lb	560 kg 1,232 lb	5,040 kg 11,088		
	Burma	8,903 kg 19,586 lb	14,682 kg 32,300 lb	29,324 kg 64,513		
	China	38,981 kg 85,758 lb		676 kg 1,487	20,833 kg 45,833	
	Guyana	11,080 kg 24,376 lb	11,162 kg 24,556 lb	5,393 kg 11,865	6,105 kg 13,431	
	India	547 kg 1,203 lb		9,766 kg 21,485 lb		
	Pakistan	10,773 kg 23,700 lb				
	Thailand	2,600 kg 5,720 lb	36,370 kg 80,014 lb	7,175 kg 15,785 lb	3,408 kg 7,497 lb	4,182 kg 9200 lb
	Venezuela	1,313 kg 2,888 lb				
	Vietnam	22,133 kg 48,692 lb	63,190 kg 139,018 lb	142,585 kg 313,687 lb	44,475 kg 97,845 lb	22,970 kg 50,534 lb
Total		141,295 kg 310,845 lb	125,964 kg 277,120 lb	209,917 kg 461,818 lb	74,821 kg 164,606 lb	27,152 kg 59,734 lb

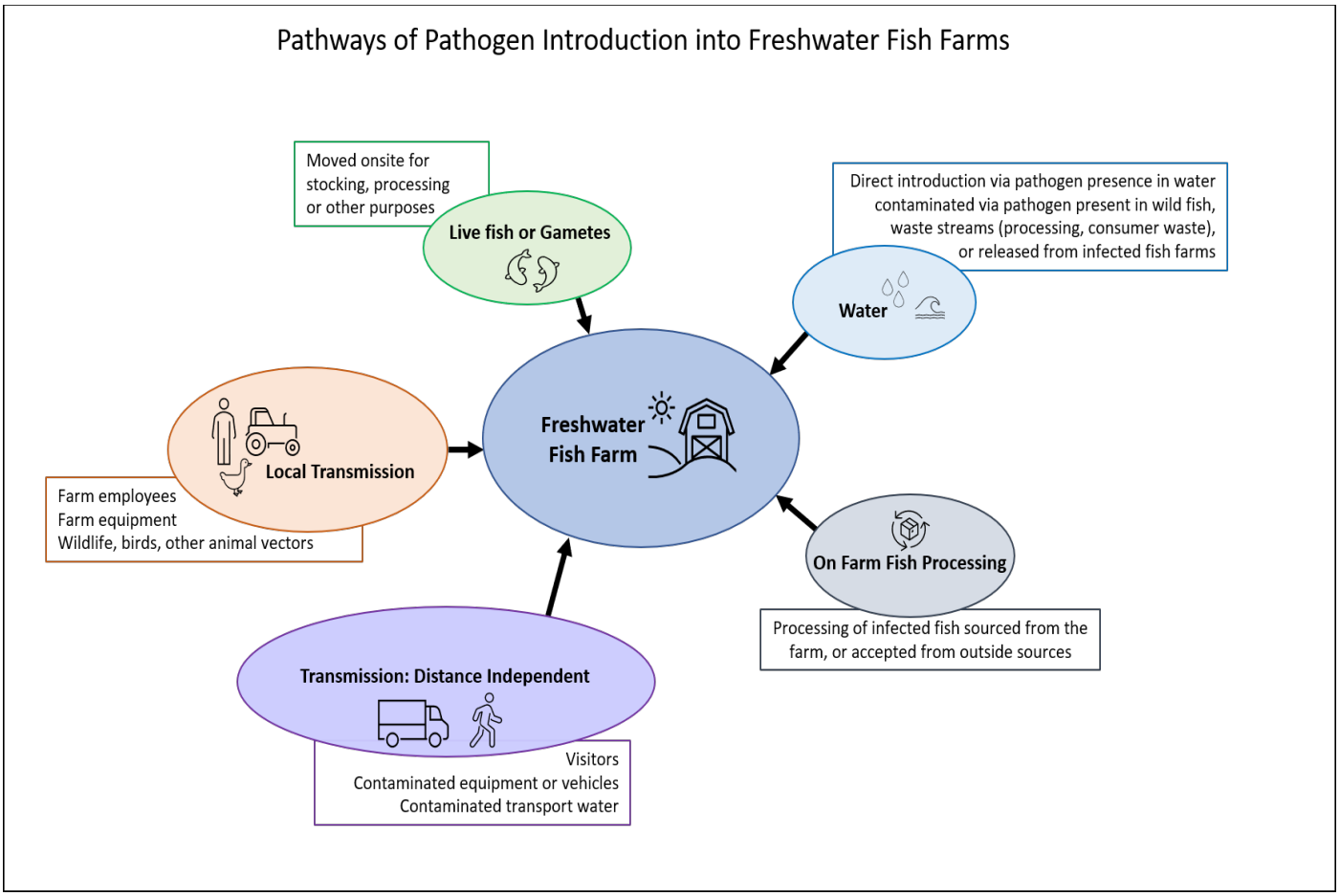

Figure 3-1. Potential pathways of introduction of pathogens onto freshwater fish farms. Exposure may occur via multiple routes.

References

1. World Organisation for Animal Health (OIE), *Handbook on Import Risk Analysis for Animals and Animal Products*. 2 ed. 2010.
2. World Organisation for Animal Health (OIE), *Aquatic Animal Health Code*. 22nd ed. 2019.
3. Agreement, W., *The WTO agreement on the application of Sanitary and Phytosanitary Measures (SPS Agreement)*. 1994.
4. Rasmussen-Ivey, C.R., et al., *Classification of a hypervirulent Aeromonas hydrophila pathotype responsible for epidemic outbreaks in warm-water fishes.* Frontiers in microbiology, 2016. **7**: p. 1615.
5. Baumgartner, W., et al., *Experience with Mortalities of Cultured Catfish Ictalurus punctatus (Rafinesque 1818) and I. punctatus X I. furcatus (Valenciennes 1840) caused by Highly Virulent Strains of Aeromonas hydrophila.* ASIAN FISHERIES SOCIETY, 2018. **31**: p. 59-75.
6. Hossain, M.J., et al., *An Asian origin of virulent Aeromonas hydrophila responsible for disease epidemics in United States-farmed catfish.* MBio, 2014. **5**(3).
7. Hanson, L., et al., *Motile aeromonas septicemia.* AFS-FHS (American Fisheries Society–Fish Health Section), FHS blue book: Suggested procedures for the detection and identification of

certain finfish and shellfish pathogens. Motile Aermonas Septicemia, 2014. **1**(9).
8. Hossain, M.J., et al., *Implication of lateral genetic transfer in the emergence of Aeromonas hydrophila isolates of epidemic outbreaks in channel catfish.* PLoS One, 2013. **8**(11).
9. Pridgeon, J.W. and P.H. Klesius, *Molecular identification and virulence of three Aeromonas hydrophila isolates cultured from infected channel catfish during a disease outbreak in west Alabama (USA) in 2009.* Diseases of aquatic organisms, 2011. **94**(3): p. 249-253.
10. Pridgeon, J.W., et al., *Identification, virulence, and mass spectrometry of toxic ECP fractions of West Alabama isolates of Aeromonas hydrophila obtained from a 2010 disease outbreak.* Veterinary microbiology, 2013. **164**(3-4): p. 336-343.
11. Pang, M., et al., *Novel insights into the pathogenicity of epidemic Aeromonas hydrophila ST251 clones from comparative genomics.* Scientific reports, 2015. **5**: p. 9833.
12. Cai, W., et al., *Biofilm and Sediment are Major Reservoirs of Virulent Aeromonas hydrophila (vA h) in Catfish Production Ponds.* Journal of aquatic animal health, 2019. **31**(1): p. 112-120.
13. Kelly, *The 2020 Impact of Diseases in West Alabama*, in *Extension Newsletter*. 2021, Alabama Fish Farming Center.
14. Rasmussen-Ivey, C., *Comparative Genomic Analysis and Evaluation of Control Strategies for Hypervirulent Aeromonas hydrophila in Channel Catfish (Ictalurus punctatus).* 2019.
15. Liu, Z., et al., *The channel catfish genome sequence provides insights into the evolution of scale formation in teleosts.* Nature communications, 2016. **7**: p. 11757.
16. Raver, *Channel Catfish*. 2017, Monterey Bay Aquarium Seafood Watch.
17. United States Department of Agrigulture, Animal Plant and Helath Inspection Serivce, Veteirnary Services (USDA-APHIS-VS)., *Use of Channel x Blue Hybrid in U.S. Catfish Operations*, USDA-VS-CEAH, Editor. 2011.
18. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veteirnary Services, National Animal Health Monitoring system (USDA-APHIS-VS-NAHMS) *Catfish 2010 Part II: Health and Production Practices for Foodsize Catfish in the United States, 2009.* 2010.
19. Allred, S., et al., *An assessment of red fillet prevalence in the catfish industry.* Aquaculture, 2019. **507**: p. 203-210.
20. Anonymous, *U.S. Catfish Industry and Foreign Trade: A Fact Sheet*, C.R. Service, Editor. 2015.
21. Dunham, R.A. and M.P. Masser, *Production of hybrid catfish*. Vol. 436. 2012: Southern Regional Aquaculture Center Stoneville, Mississippi, USA.
22. National Oceanic and Atmospheric Administration (NOAA), *Foreign Fishery Trade Data*. Available from: https://www.fisheries.noaa.gov/national/sustainable-fisheries/foreign-fishery-trade-data.
23. United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), *Voluntary 2021 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections, and Infestations List*. 2021; Available from:. 2021 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections, and Infestations List (usda.gov)
24. United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), Notifiable Diseases and Conditions 2021; Available from: www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Animal-Health-Emergency-Management/Notifiable-Diseases-and-Conditions.
25. United States Environmental Protection Agency (EPA) *Summary of the Clean Water Act*. 2020; Available from: https://www.epa.gov/laws-regulations/summary-clean-water-act.
26. United States Environmental Protection Agency (EPA) *National Pollutant Discharge Elimination System NPDES Aquaculture Permitting*. Available from: www.epa.gov/npdes/npdes-aquaculture-permitting.
27. United States Environmental Protection Agency (EPA) *Concentrated Aquatic Animal*

Production Effluent Guidelines. 2020; Available from: https://www.epa.gov/eg/concentrated-aquatic-animal-production-effluent-guidelines.
28. United States Department of Agriculture, Animal and Plant Inspection Service (USDA-APHIS) *Guidelines: Animal Products That Do Not Require an Import Permit*. 2020; Available from: www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/import-live-animals/no-import-permit-req/!ut/p/z1/lZLLcoMgFIafJQuXysGkxnSH1om2mnSS2Fg2HdNBZUbFIq0zffrSy6aXJJYNA_N9B84PiKIM0TZ_4WWuuGjzWq_vqfOwwv4S3BmOl8EVBhIuoptw7sAaO2j_AcTrmY-9Leg58IAEm3kSBJENeIro__y70NH-Lt2tXOxFC3ucD0cGgXH-CYCeLn997gCdoC0TPykR7XJVmbwtBMpaYfKmE1KZHZMNV6ZkT_ou9GQ1cvEFHIt7Y58B3t_jB_A78BEtl7U4fP4O0h6mru5NsoJJJq1nqbcrpbr-0gADhmGwSiHKmlmPojHgL6USvULZdxJ1TZqm2WtcbCN-2-zdnkwmb_M9syw!/#collapse0cf13ffc-9cc7-4efb-8f45-048a3a6c88f3.
29. United States Food and Drug Administration (FDA) website 2020; Available from: https://www.fda.gov/.
30. United States Food and Drug Administration (FDA), *Food Safety Modernization Act and Animal Food*. 2011; Available from: https://www.fda.gov/animal-veterinary/animal-food-feeds/food-safety-modernization-act-and-animal-food.
31. United States Food and Drug Administration (FDA), *Compliance program guidance manual.* FDA: Silver Spring, MD, USA, 2005. Available from: https://www.fda.gov/media/84887/download
32. United States Food and Drug Administration (FDA), *Food Safety Moderinzation Act (FSMA) Final Rule on Foreign Supplier Verification Programs (FSVP) for Importers of Food for Humans and Animals.* 2019. Available from: https://www.fda.gov/food/food-safety-modernization-act-fsma/fsma-final-rule-foreign-supplier-verification-programs-fsvp-importers-food-humans-and-animals
33. United States Food and Drug Administration (FDA), *Code of Federal Regulations (CFR) Title 21, Subchapter B - Food for Human Consumption; Part 123 Fish and Fishery Products*. 2019. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=123
34. United States Department of Agriculture, Food Safety and Inspection Service (FSIS), *FSIS Complicance Guideline for Establishments that Slaughter or Further Process Siluriformes Fish and Fish Products*. 2017. Available from: https://www.fsis.usda.gov/guidelines/2017-0003
35. United States Department of Agriculture, Food Safety and Inspection Service (FSIS) *Frequently Asked Questions-Siluriformes*. Available from: https://www.fsis.usda.gov/wps/wcm/connect/66f02fb0-f0fc-4b1e-8077-bbbb816867e0/FAQ-Siluriformes.pdf?MOD=AJPERES.
36. United States Department of Agriculture, Food Safety and Inspection Service (FSIS). *Siluriformes*. 2020; Available from: https://www.fsis.usda.gov/wps/portal/fsis/topics/inspection/siluriformes.
37. United States Department of Agriculture, Food Safety and Inspection Service (FSIS). *Inspection Program for Siluriformes Fish, Including Catfish*. Available from: https://www.fsis.usda.gov/wps/portal/fsis/topics/inspection/siluriformes.
38. United States Department of Agriculture, Food Safety and Inspection Service (FSIS). *Answers to Frequently Asked Questions - Siluriformes Fish and Fish Product*. Available from: https://www.fsis.usda.gov/wps/portal/fsis/topics/inspection/siluriformes/faq-siluriformes.
39. United States Department of Agriculture, Food Safety and Inspection Service (FSIS). *Siluriformes Self Reporting Tool*. Available from: ttps://www.fsis.usda.gov/sites/default/files/media_file/2021-04/SRT-Sample-2016-Fish-

V.5.pdf
40. United States Department of Agriculture, Food Safety and Inspection Service (FSIS), *Food Defense Guidelines for Siluriformes Fish Production and Processing*. 2017. Available from: https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/Food-Defense-Guidelines-Catfish.pdf
41. United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS), *Code of Federal Regulations Title 9- Animals and Animal Products*. Available from: https://www.ecfr.gov/current/title-9
42. Gonzalez, J., *Wastewater treatment in the fishery industry. Food and Agriculture Organization of the United Nations (FAO) Fisheries Technical Paper No 355/FAO, Rome (Italy).* Fisheries Dept, 1996.
43. United States Environmental Protection Agency (EPA). *Seafood Processing Effluent Guidelines*. Available from: https://www.epa.gov/eg/seafood-processing-effluent-guidelines.
44. United States Environmental Protection Agency (EPA). *National Pollutant Discharge Elimination System (NPDES)*. 2020; Available from: https://www.epa.gov/npdes.
45. Code of Federal Regulations. Title 40, Chapter I, Subchapter N, Part 408 *Canned and Preserved Seafood Processing Point Source Category*, 2020. Available from: https://www.ecfr.gov/current/title-40/chapter-I/subchapter-N/part-408
46. Center, N.S.G.L., *Legal Guide for Direct Marketing Aquaculture Products in Alabama*. 2014.
47. Peatman, E., et al., *Mechanisms of pathogen virulence and host susceptibility in virulent Aeromonas hydrophila infections of channel catfish (Ictalurus punctatus).* Aquaculture, 2018. **482**: p. 1-8.
48. Zhang, D., et al., *The severity of motile Aeromonas septicemia caused by virulent Aeromonas hydrophila in channel catfish is influenced by nutrients and microbes in water.* Aquaculture, 2020. **519**: p. 734898.
49. Zhang, D., et al., *Chitin degradation and utilization by virulent Aeromonas hydrophila strain ML10-51K.* Archives of microbiology, 2017. **199**(4): p. 573-579.
50. Zhang, D., D.-H. Xu, and C. Shoemaker, *Experimental induction of motile Aeromonas septicemia in channel catfish (Ictalurus punctatus) by waterborne challenge with virulent Aeromonas hydrophila.* Aquaculture Reports, 2016. **3**: p. 18-23.
51. Goharrizi, L., M. Zorriehzahra, and M. Adel, *The study on effect of temperature stress on occurrence of clinical signs caused by Aeromonas hydro-phila in Capoeta damascina in in vitro condition.* Adv. Anim. Vet. Sci, 2015. **3**(7): p. 406-412.
52. Baumgartner, W.A., L. Ford, and L. Hanson, *Lesions caused by virulent Aeromonas hydrophila in farmed catfish (Ictalurus punctatus and I. punctatus× I. furcatus) in Mississippi.* Journal of Veterinary Diagnostic Investigation, 2017. **29**(5): p. 747-751.
53. Bebak, J., et al., *Farm size, seining practices, and salt use: risk factors for Aeromonas hydrophila outbreaks in farm-raised catfish, Alabama, USA.* Preventive veterinary medicine, 2015. **118**(1): p. 161-168.
54. Thurlow, C.M., et al., *The gfc operon is involved in the formation of the O antigen capsule in Aeromonas hydrophila and contributes to virulence in channel catfish.* Aquaculture, 2019: p. 734334.
55. Cunningham, F.L., et al., *Environmental factor (s) and animal vector (s) associated with atypical Aeromonas hydrophila abundance and dissemination among channel catfish ponds.* Journal of the World Aquaculture Society, 2019.
56. Cunningham, F.L., et al., *Potential of Double-crested Cormorants (Phalacrocorax auritus), American White Pelicans (Pelecanus erythrorhynchos), and Wood Storks (Mycteria americana) to Transmit a Hypervirulent Strain of Aeromonas hydrophila between Channel Catfish Culture Ponds.* Journal of wildlife diseases, 2018. **54**(3): p. 548-552.

57. Jubirt, M.M., et al., *Potential for Great Egrets (Ardea alba) to transmit a virulent strain of Aeromonas hydrophila among channel catfish (Ictalurus punctatus) culture ponds.* Journal of wildlife diseases, 2015. **51**(3): p. 634-639.
58. Liles, M., et al. *Comparative genomics of Aeromonas hydrophila isolates from an epidemic in channel catfish*. in *American Society for Microbiology Meeting*. 2011.
59. Rasmussen-Ivey, C.R., et al., *Virulence factors of Aeromonas hydrophila: in the wake of reclassification.* Frontiers in Microbiology, 2016. **7**: p. 1337.
60. James, W. 2020.
61. Igbinosa, I.H., et al., *Emerging Aeromonas species infections and their significance in public health.* The Scientific World Journal, 2012. **2012**.
62. Zhang, D., D.-H. Xu, and B. Beck, *Analysis of agglutinants elicited by antiserum of channel catfish immunized with extracellular proteins of virulent Aeromonas hydrophila.* Fish & shellfish immunology, 2019. **86**: p. 223-229.
63. Peterman, M.A. and B.C. Posadas, *Direct Economic Impact of Fish Diseases on the East Mississippi Catfish Industry.* North American Journal of Aquaculture, 2019.
64. Hardi, D. and E. Handayani, *Utilization of several herbal plant extracts on Nile tilapia in preventing Aeromonas hydrophila and Pseudomonas sp. bacterial infection.* 2017.
65. Wang, E., et al., *Plant polysaccharides used as immunostimulants enhance innate immune response and disease resistance against Aeromonas hydrophila infection in fish.* Fish & shellfish immunology, 2016. **59**: p. 196-202.
66. Yun, S., et al., *Immunostimulation of Cyprinus carpio using phage lysate of Aeromonas hydrophila.* Fish & shellfish immunology, 2019. **86**: p. 680-687.
67. Yun, S., et al., *Efficacy of PLGA microparticle-encapsulated formalin-killed Aeromonas hydrophila cells as a single-shot vaccine against A. hydrophila infection.* Vaccine, 2017. **35**(32): p. 3959-3965.
68. Tamamdusturi, R. and M. Yuhana, *Administration of microencapsulated probiotic Bacillus sp. NP5 and prebiotic mannan oligosaccharide for prevention of Aeromonas hydrophila infection on Pangasianodon hypophthalmus.* Journal of Fisheries and Aquatic Science, 2016. **11**(1): p. 67.
69. Korni, F.M., F.I.A. El-Ela, and U.K. Moawad, *Role of Moringa oleifera leaves and aqueous extract in prevention of Motile Aeromonas Septicemia in common carp, Cyprinus carpio fingerlings with a reference to histopathological alterations.* Aquaculture International, 2020. **28**(1): p. 153-168.
70. Jiang, X., et al., *Immune effects of the vaccine of live attenuated Aeromonas hydrophila screened by rifampicin on common carp (Cyprinus carpio L).* Vaccine, 2016. **34**(27): p. 3087-3092.
71. Viswanathan, K. and J. Arockiaraj, *Immune protection role and disease prevention in common carp, Cyprinus carpio (Actinopterygii, Cypriniformes, Cyprinidae) against a heterotrophic Gram-negative bacteria, Aeromonas hydrophila due to spirulina, Arthrospira platensis supplement.* Aquaculture, Aquarium, Conservation & Legislation, 2019. **12**(3): p. 968-976.
72. Wang, Q., et al., *Freshwater aquaculture in PR C hina: trends and prospects.* Reviews in Aquaculture, 2015. **7**(4): p. 283-302.
73. Aquarium, M.B., *Channel Catfish (Ictalurus punctatus): China*. 2017.
74. Food and Agriculture Organization of the United Nations (FAO).*Fishery and Aquaculture Country Profiles. China*. 2017; Country Profile Fact Sheet]. Available from: http://www.fao.org/fishery/facp/CHN/en.
75. Miles, R. and F. Chapman, *The benefits of fish meal in aquaculture diets.* IFAS Extension, University of Florida, 2006.
76. Oidtmann, B., et al., *Ranking freshwater fish farms for the risk of pathogen introduction and spread.* Preventive veterinary medicine, 2011. **102**(4): p. 329-340.

77. Oidtmann, B., et al., *Risk-based methods for fish and terrestrial animal disease surveillance.* Preventive veterinary medicine, 2013. **112**(1-2): p. 13-26.
78. Chapman, F.A., *Farm-raised channel catfish*. 2000: University of Florida Cooperative Extension Service, Institute of Food and ….
79. Peatman, E. and B. Beck, *From floor sweepings to fish flesh–phytasesuperdosing in the US catfish industry.* Phytate destruction–consequences for precision animal nutrition, 2016: p. 237-250.
80. Robinson, E.H., M.H. Li, and B.E. Manning, *A practical guide to nutrition, feeds, and feeding of catfish*. Vol. 1113. 2001: Office of Agricultural Communications, Division of Agriculture, Forestry
81. Towers, L. *Feed ingredients and feeds for channel catfish*. 2013; Available from: https://thefishsite.com/articles/feed-ingredients-and-feeds-for-channel-catfish.
82. Feiner, G., *Meat products handbook: Practical science and technology*. 2006: Elsevier.
83. Windsor, M., *FISH MEAL. Department of Trade and Industry, Torry Research Station*. 2001, Torry advisory note.
84. United States Environmental Protection Agency (EPA). *Landfill Effluent Guidelines*. Available from: https://www.epa.gov/eg/landfills-effluent-guidelines.
85. United States Environmental Protection Agency (EPA). *Municipal Solid Waste Landfills*. 2018; Available from https://www.epa.gov/landfills/municipal-solid-waste-landfills
86. Office, G.P., *Code of Fegeral Regulations Title 40 Chapter I Subchapter N Part 445 Landfills Point Source Category*, E.P. Agency, Editor. 2020.
87. Gerba, C.P., et al., *Sources of microbial pathogens in municipal solid waste landfills in the United States of America.* Waste Management & Research, 2011. **29**(8): p. 781-790.
88. Grisey, E., et al., *Survival of pathogenic and indicator organisms in groundwater and landfill leachate through coupling bacterial enumeration with tracer tests.* Desalination, 2010. **261**(1-2): p. 162-168.
89. Xiang, R., et al., *Isolation distance between municipal solid waste landfills and drinking water wells for bacteria attenuation and safe drinking.* Scientific reports, 2019. **9**(1): p. 1-11.
90. Love, D.C., et al., *Wasted seafood in the United States: Quantifying loss from production to consumption and moving toward solutions.* Global Environmental Change, 2015. **35**: p. 116-124.
91. Gunders, D. and J. Bloom, *Wasted: How America is losing up to 40 percent of its food from farm to fork to landfill*. 2017: Natural Resources Defense Council New York.
92. Scarfe, A.D. and D. Palić, *Aquaculture biosecurity: Practical approach to prevent, control, and eradicate diseases*, in *Aquaculture Health Management*. 2020, Elsevier. p. 75-116.
93. United States Department of Agriculture National Agricultural Statistics Service (USDA-NASS), *2018 Census of Aquaculture*. 2019.
94. Araujo, R.M., R.M. Arribas, and R. Pares, *Distribution of Aeromonas species in waters with different levels of pollution.* Journal of Applied Bacteriology, 1991. **71**(2): p. 182-186.
95. Ünüvar, S., *Microbial foodborne diseases*, in *Foodborne Diseases*. 2018, Elsevier. p. 1-31.
96. Hayes, J., *Aeromonas hydrophila.* Oregon State University. http://hmsc. oregonstate. edu/classe s/MB492/hydrophilahayes, 2000.
97. J.G. Morris, A.H. *Aeromonas Infections*. 2020; Available from: www.uptodate.com/contents/aeromonas-infections?search=morris%202020%20aeromonas-infections.&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1.
98. Batra, P., P. Mathur, and M.C. Misra, *Aeromonas spp.: an emerging nosocomial pathogen.* Journal of laboratory physicians, 2016. **8**(1): p. 1.
99. Cortés-Sánchez, A.D.J., et al., *Foodborne diseases, fish and the case of Aeromonas spp.* African

Journal of Agricultural Research, 2019. **14**(11): p. 617-628.
100. Dodd, C., *Infrequent Microbial Infections*, in *Foodborne Diseases*. 2017, Elsevier. p. 277-288.
101. Tomás, J., *The main Aeromonas pathogenic factors.* ISRN microbiology, 2012. **2012**.
102. Merino, S., et al., *Emerging pathogens: Aeromonas spp.* International journal of food microbiology, 1995. **28**(2): p. 157-168.
103. Stratev, D. and O.A. Odeyemi, *An overview of motile Aeromonas septicaemia management.* Aquaculture international, 2017. **25**(3): p. 1095-1105.
104. Camus, A., et al., *Aeromonas bacterial infections-motile aeromonad septicemia*. Vol. 478. 1998: Southern Regional Aquaculture Center.
105. Galindo, C.L., et al., *Host immune responses to Aeromonas virulence factors.* Current Immunology Reviews, 2006. **2**(1): p. 13-26.
106. Zhao, X.l., et al., *Analysis of virulence and immunogenic factors in Aeromonas hydrophila: Towards the development of live vaccines.* Journal of Fish Diseases, 2020. **43**(7): p. 747-755.
107. Santiago TC, Aeromonas Hydrophila (SAH 93) in Experimentally Infected Tilapia, Oreochromis Mossambicus (L.). Journal of Aquaculture in the Tropics, 2001.
108. Zhu, D., et al., *Correlation between the distribution pattern of virulence genes and virulence of Aeromonas hydrophila strains.* Frontiers of Biology in China, 2007. **2**(2): p. 176-179.
109. Wu, C.-J., et al., *Clinical significance and distribution of putative virulence markers of 116 consecutive clinical Aeromonas isolates in southern Taiwan.* Journal of Infection, 2007. **54**(2): p. 151-158.
110. Shuang, M., et al., *Genetic Diversity, Antimicrobial Resistance, and Virulence Genes of Aeromonas Isolates from Clinical Patients, Tap Water Systems, and Food.* Biomedical and Environmental Sciences, 2020. **33**(6): p. 385-395.
111. Crumlish, M., et al., *Experimental challenge studies in Vietnamese catfish, Pangasianodon hypophthalmus (Sauvage), exposed to Edwardsiella ictaluri and Aeromonas hydrophila.* Journal of fish diseases, 2010. **33**(9): p. 717-722.
112. Sarkar, M. and M.M. Rashid, *Pathogenicity of the bacterial isolate Aeromonas hydrophila to catfishes, carps and perch.* Journal of the Bangladesh Agricultural University, 2012. **10**(452-2016-35552): p. 157-161.
113. Swann, L. and M.R. White, *Diagnosis and treatment of" Aeromonas Hydrophila" infection of fish*. 1991: Aquaculture Extension, Illinois-Indiana Sea Grant Program.
114. Ansary, A., et al., *Plasmids and antibiotic resistance in Aeromonas hydrophila isolated in Malaysia from healthy and diseased fish.* Journal of Fish Diseases, 1992. **15**(2): p. 191-196.
115. Sugita, H., et al., *Distribution of Aeromonas species in the intestinal tracts of river fish.* Applied and Environmental microbiology, 1995. **61**(11): p. 4128-4130.
116. Kozińska, A., *Dominant pathogenic species of mesophilic aeromonads isolated from diseased and healthy fish cultured in Poland.* Journal of fish diseases, 2007. **30**(5): p. 293-301.
117. Rey, A., et al., *Pathogenesis of Aeromonas hydrophila strain KJ99 infection and its extracellular products in two species of fish.* Veterinary Record, 2009. **164**(16): p. 493-499.
118. Isonhood, J.H. and M. Drake, *Aeromonas species in foods.* Journal of Food Protection, 2002. **65**(3): p. 575-582.
119. Daskalov, H., *The importance of Aeromonas hydrophila in food safety.* Food control, 2006. **17**(6): p. 474-483.
120. Pal, M., *Is Aeromonas hydrophila a potential pathogen of food safety concern.* J Food Microbiol 2018; 2 (1): 1-2. 2 J Food Microbiol 2018 Volume 2 Issue, 2018. **1**.
121. Praveen, P.K., et al., *Incidence of Aeromonas spp. infection in fish and chicken meat and its related public health hazards: A review.* Veterinary world, 2016. **9**(1): p. 6.
122. Ghenghesh, K.S., et al., *Aeromonas-associated infections in developing countries.* The Journal of Infection in Developing Countries, 2008. **2**(02): p. 081-098.
123. Gauthier, D.T., *Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages*

between fish and human infections. The Veterinary Journal, 2015. **203**(1): p. 27-35.
124. Lowry, T. and S.A. Smith, *Aquatic zoonoses associated with food, bait, ornamental, and tropical fish.* Journal of the American Veterinary Medical Association, 2007. **231**(6): p. 876-880.
125. Borchardt, M.A., M.E. Stemper, and J.H. Standridge, *Aeromonas isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis.* Emerging Infectious Diseases, 2003. **9**(2): p. 224.
126. Janda, J.M. and S.L. Abbott, *The genus Aeromonas: taxonomy, pathogenicity, and infection.* Clinical microbiology reviews, 2010. **23**(1): p. 35-73.
127. World Health Organization (WHO), *Foodborne disease outbreaks: Guidelines for investigation and control.* 2008. Available from: https://www.epa.gov/landfills/municipal-solid-waste-landfills
128. Dewey-Mattia, D., et al., *Surveillance for foodborne disease outbreaks—United States, 2009–2015.* MMWR Surveillance Summaries, 2018. **67**(10): p. 1.
129. Centers for Disease Control and Prevention (CDC), *Foodborne Disease Outbreak Surveillance System (FDOSS)*. Available from: https://www.cdc.gov/fdoss/index.html.
130. Chenais, E., et al., *Epidemiological considerations on African swine fever in Europe 2014–2018.* Porcine Health Management, 2019. **5**(1): p. 6.
131. Tsai, G.-J. and T.-H. Chen, *Incidence and toxigenicity of Aeromonas hydrophila in seafood.* International journal of food microbiology, 1996. **31**(1-3): p. 121-131.
132. Igbinosa, O., et al., *Incidence and prevalence of Aeromonas species from retail food: public health implications.* Science Focus, 2006. **12**(2): p. 19-22.
133. McCOY, E., et al., *Foodborne agents associated with the consumption of aquaculture catfish.* Journal of food protection, 2011. **74**(3): p. 500-516.
134. Stratev, D., I. Vashin, and V. Rusev, *Prevalence and survival of Aeromonas spp. in foods–a review.* Revue Med Vet, 2012. **163**: p. 486-494.
135. Liu, X., et al., *Characterization of the microbiota in lightly salted bighead carp (Aristichthys nobilis) fillets stored at 4° C.* Food microbiology, 2017. **62**: p. 106-111.
136. Simon, S.S., K. Lalitha, and T.C. Joseph, *Virulence properties of Aeromonas spp. from modified-atmosphere-and vacuum-packed milk fish (Chanos chanos Forsskal, 1775).* Annals of Microbiology, 2016. **66**(3): p. 1109-1115.
137. Costa, A.M., et al., *Aeromonas hydrophila and Campylobacter jejuni isolated in fresh tuna (Thunnus spp.) sold in São Paulo, Brazil.* Brazilian Journal of Veterinary Research and Animal Science, 2016. **53**(1): p. 48-54.
138. Ramadan, H., et al., *Aeromonas hydrophila from marketed mullet (Mugil cephalus) in Egypt: PCR characterization of β-lactam resistance and virulence genes.* Journal of applied microbiology, 2018. **124**(6): p. 1629-1637.
139. Hoel, S., O. Vadstein, and A.N. Jakobsen, *The significance of mesophilic Aeromonas spp. in minimally processed ready-to-eat seafood.* Microorganisms, 2019. **7**(3): p. 91.
140. Laly, S., et al., *Formation of biogenic amines and associated biochemical and microbial attributes of whole sutchi catfish (Pangasianodon hypophthalmus) during iced storage.* Journal of Aquatic Food Product Technology, 2019. **28**(2): p. 221-232.
141. Møretrø, T., et al., *Contamination of salmon fillets and processing plants with spoilage bacteria.* International Journal of Food Microbiology, 2016. **237**: p. 98-108.
142. Yang, W., et al., *Complete genome sequence of fish pathogen Aeromonas hydrophila JBN2301.* Genome announcements, 2016. **4**(1).
143. United States Department of Agriculture National Agricultural Statistics Service (USDA-NASS), *QuickStats*. Available from: https://quickstats.nass.usda.gov/.
144. United States International Trade Commission (USITC) *Dataweb*. Available from: www.dataweb.usitc.gov.