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Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in the United States: Rapid Risk Assessment

Key Points

- Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is globally distributed in wild and cultured shrimp populations. Some affected countries are not World Organization for Animal Health (WOAH) affiliates, which may affect disease reporting.
- IHHNV is present in wild shrimp populations in the coastal waters of South, Central, and North America and in the Caribbean Ocean.
- Potentially susceptible wild and introduced penaeid shrimp, other crustaceans, bivalves, and fish are present in U.S. coastal waters. Susceptibility studies have not been conducted for many species.
- Infected shrimp may appear clinically healthy and remain infected for life.
- There are no Federal regulations requiring documentation of the health of shrimp prior to importation. Some States do require health certification prior to entry.
- The United States has reported previous detections of IHHNV to WOAH. These detections resulted in serious consequences domestically and relative to international trade. If a large-scale outbreak occurred, it is plausible that significant economic and export trade consequences could occur.
- There is no national surveillance plan for IHHNV. Some States may require testing prior to entry.
- Plausible pathways of entry for IHHNV include:
  - Ballast water and ship fouling
  - Movement of IHHNV in water
  - Importation of live shrimp
  - Importation of commodity shrimp products
  - Importation of bait shrimp
- Plausible pathways of exposure for wild and farmed shrimp include:
  - Documented domestic exposure pathways
    - Introduction of imported IHHNV-infected shrimp into aquaculture facilities
    - Transfer of IHHNV-infected shrimp between aquaculture facilities
  - Commodity shrimp processing and waste disposition systems
  - Use of commodity shrimp as bait or food for aquatic animals
  - Use of imported bait shrimp for fishing
  - Movement or presence of IHHNV in water
- Data, surveillance, and resource gaps diminish capability to fully assess the plausibility of all identified pathways.
  - These gaps will affect capability to develop additional risk assessments or risk analyses products.
  - Development of a domestic response plan may be affected.

Background

History

Infectious hypodermal and hematopoietic necrosis (IHHNV) is a viral disease that has affected wild and cultured shrimp and prawn (collectively referred to in this document as “shrimp”) globally for approximately 40 years.[1] The virus was first identified following a high mortality disease outbreak in farmed *Penaeus stylirostris* (Pacific blue shrimp) in Hawaii in 1981. Diagnostic examination of *P.*

monodon (black tiger shrimp) and *P. vannamei* (whiteleg shrimp) from the same aquaculture system identified subclinical infection. From January 2019 through January 2020, IHHNV was among the diseases with the highest number of immediate notifications submitted to the World Organisation for Animal Health (WOAH) worldwide.[2]

Distribution

IHHNV has been identified in cultured shrimp populations worldwide including, but not limited to, Argentina, Australia, Brazil, Cambodia, China, Columbia, Costa Rica, Ecuador, Egypt, El Salvador, French Polynesia, Guam, Guatemala, Honduras, India, Indonesia, Japan, Madagascar, Malaysia, Mexico, Mozambique, New Caledonia, Nicaragua, Papua New Guinea, Panama, Peru, the Philippines, Singapore, South Korea, Sri Lanka, Taiwan, Tanzania, Thailand, the United States, Venezuela, and Vietnam.[1, 3-5] Countries with WOAH status include Australia, Brazil, Cambodia, China, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, India, Indonesia, Madagascar, Mexico, Nicaragua, Papua New Guinea, Panama, Peru, Philippines, Sri Lanka, and Thailand (**Table 1**).[6, 7]

Table 1. Countries with an WOAH IHHNV status [6]

WOAH Status	Country
Disease suspected	Cambodia El Salvador Madagascar Peru
Infection present (no clinical disease)	Papua New Guinea
Infection/infestation limited to one or more zones	Australia China Thailand
Disease restricted to certain zones/regions	Ecuador India Mexico
Clinical disease demonstrated	Brazil Costa Rica Guatemala Honduras Nicaragua Indonesia Panama Philippines Sri Lanka

Infection has been reported in wild penaeid shrimp populations in Australia, the Indo-Pacific region, and in the coastal waters of Mexico, Central America, and South America (**Table 2**).[3-5] In 1986 IHHNV was documented in wild shrimp populations in the Gulf of California (GoC), Mexico. From 2004 to 2005, IHHVN was detected at Bahia Magdalena on the Pacific coast of the Baja Peninsula (e.g., California Baja Sur; approximately 389 kilometers/640 miles south of the U.S. border) [5, 8-12]**.** IHHNV is reported as widely distributed among wild shrimp populations on the Pacific (including El Salvador, Guatemala, Nicaragua, and Panama) and Caribbean coasts of Central America (Panama and Honduras).[12-16] The virus has also been reported present in wild shrimp populations in coastal waters of South America. On the Pacific side of the continent, positive detections have been reported along the coasts of Peru and Ecuador. [12, 15, 17-19]**.** Affected countries on the Atlantic side include Argentina, Brazil, and Columbia.[20, 21] IHHNV has been reported as widely distributed in cultured shrimp in the Caribbean.[16] Review of the literature did not identify any publications reporting presence of this virus in wild Caribbean shrimp populations.

Table 2. North, Central and South American countries with reported IHHNV presence in farmed and wild shrimp populations [3-5, 8-19]

Continent	Country	Coastal Area
North America	Mexico	Gulf of California Gulf of Mexico Pacific Ocean
Central America	El Salvador Guatemala Nicaragua Panama	Pacific Ocean
	Honduras Panama	Caribbean Sea
South America	Ecuador Peru	Pacific Ocean
	Argentina Brazil Columbia	Atlantic Ocean

Prior to 2019, IHHNV had not been reported in U.S. farmed shrimp since 1993.[22] From January 1, 2019, through January 8, 2020, the United States, Canada, and the United Kingdom reported IHHNV infections in cultured shrimp to WOAH via immediate notifications. The United States reported two disease events. The first event in March 2019 was a recurrence of IHHNV infection in farmed *P. vannamei* in New Mexico followed by transfer of infected shrimp to a facility in California.[2, 23] The second event, in May 2019, was detected via routine surveillance sampling and was the first reported occurrence of IHHNV in Texas and Florida aquaculture facilities.[2, 23] In both events, control measures included quarantine of affected facilities and depopulation of affected tanks. In June 2019, Canada reported a first occurrence in four of the country’s five premises that culture *P. vannamei*. These facilities became infected via post-larval shrimp imported from a common source.[2, 23] The third disease event, involving two sites, was reported by the United Kingdom in July 2019. The first site, an experimental facility, imported live animals that developed high mortality after import. The second site, an aquaculture facility, imported post-larval shrimp designated as specific pathogen free. These shrimp exhibited no clinical signs of disease, but the United Kingdom Competent Authority opened a disease investigation after it was reported that one of the exporting suppliers had become positive for IHHNV.[2, 23]

Current Regulations

IHHNV has been a WOAH listed notifiable pathogen since 1995.[7]

IHHNV is listed as a reportable Foreign Animal Disease (FAD) on the United States National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections, and Infestations List and the USDA-APHIS National Veterinary Accreditation Program (NVAP) Notifiable Diseases and Conditions website [24]. Suspect cases must be reported to State animal health officials. There are no Federal regulations requiring documentation of the health of shrimp prior to importation into the United States. Imported animals are not quarantined at ports of entry prior to distribution within the United States. There is currently no national surveillance plan for IHHNV. Some States require health certification prior to entry for imported and domestic crustacean species.

Management of ballast water discharge via ballast water treatment and exchange (BWTE) is regulated federally by the U.S. Environmental Protection Agency (EPA), the U.S. Coast Guard (USCG), and at the State level. Ballast water discharges are required to meet International Maritime Organization (IMO) ballast water management convention D-2 standards for allowable concentrations of living organisms and microorganisms considered "indicators problematic for ballast water."[25-28] Such microorganisms include human enteric pathogens; however, aquatic animal pathogens are not included. The efficacy of BWTE is estimated to range from 50 percent to 90 percent and may not treat microorganisms found in biofilms or sediments in ballast tanks.[29-31] Data on the efficacy of BWTE on viruses are lacking. Published literature suggests that a) testing for viruses may not occur; and b) using BWTE may decrease the concentration of viruses present in ballast water below the detection limits of available assays currently used to evaluate treatment methods.[29-31] Some vessels are not required to install a Ballast Water Treatment System (BWTS), including those that do not discharge ballast water, or discharge only to shoreside facilities or to water that presents little threat (e.g., public drinking water). Vessels operating in only one Captain of the Port (COPT) Zone are exempted from ballast water exchange reporting and recordkeeping requirements.[26, 27, 29, 31] COPT zones are administrative, are not established using ecological or biological bases, and may not be appropriate boundaries for addressing invasive species. [31]

Processing plant effluent and wastewater discharges are regulated under the Clean Water Act (CWA) and the EPA Seafood Processing Effluent Guidelines and Standards (a.k.a., Canned and Preserved Seafood Category; 40 CFR 408).[32-35] Some States require a National Pollutant Discharge Elimination System (NPDES) permit if effluents are discharged into municipal storm sewer systems. However, such permitting is not required if effluent is discharged into a municipal sanitary sewer system.[34, 36] Processing effluents and wastewater are discharged to public water treatment works, municipal storm sewer systems, municipal sanitary sewers, or natural water bodies. Wastewater and effluents that enter public water treatment works or municipal sanitary sewer systems are treated prior to final discharge into natural water bodies. Wastewater and effluents that enter storm sewer systems are not treated prior to discharge into natural water systems. Wastewater disinfection and treatment requirements state that treatment must be sufficient to prevent contamination or damage to public water works or natural water bodies. However, requirements depend on the size of the processing facility (i.e., small processing plants and farms may be exempt from some regulations). Processing facilities are required to monitor and sample wastewater discharges and notify the EPA and State regulatory agencies of the results. However, regular monitoring may not occur and violations have been reported.[34]

The U.S. aquaculture industry is regulated by Federal, State, and local laws. Types of permits typically issued for aquaculture include siting and discharge permits. Siting permits are governed by Section 404 of the CWA and control the siting, number, and size of shrimp farms; establish requirements that must be met; regulate discharge of dredged or fill material into the nation's waters; and prevent the degradation of national waters and wetlands. The United States Army Corps of Engineers administers Section 404 under the overview of the EPA, often cooperatively with State coastal zone management programs. [37] The United States Fish and Wildlife Service (USFWS) investigates potential fish and wildlife impacts. [38]

The EPA is responsible for enforcing the CWA, which authorizes the NPDES permit program.[33, 36, 38] States administer the NPDES permit program, which regulates concentrated aquatic animal production (CAAP) facilities (e.g., fish farm, hatchery, production, or other facility) that discharge pollutants into Federal waters.[34, 36] Permits and requirements are structured for each farm based on the characteristics of the water body that the farm is discharging effluent to; farm production; and levels of ammonia, dissolved oxygen, and total suspended solids present in the effluents.[38]

CAAP facilities must a) meet permit requirements for ongoing monitoring metrics; b) keep records on animal numbers and weights, quantity of feeds, and frequency of cleaning, inspections, maintenance, and repairs; c) report failures or damage to containment systems; d) report use of experimental animal drugs and drugs not used according to label requirements; e) minimize discharges of excess feed; f) prevent discharge of drugs and pesticides that have been spilled; g) regularly maintain production and wastewater treatment systems; g) minimize solid waste discharges (e.g., uneaten feed, settled solids, animal carcasses) if they are facilities with flow-through and recirculating discharge systems; h) adequately train staff to properly operate and maintain production and wastewater treatment systems and to prevent and respond to spills; and i) develop, maintain, and certify a Best Management Practice (BMP) plan.[38] A BMP describes how the aquaculture production facilities will meet the set requirements and guidelines. Some States may develop or enact State-specific BMPs.[38-40] Aquatic animal production facilities producing less than 45,359 kg/100,000 lbs annually are not subject to CAAP effluent guidelines but may still be required to have NPDES permits.

States are mandated under the CWA to designate specific uses of water bodies and assign site-specific water quality standards.[37, 38] State aquaculture regulations are not standardized; regulations may vary by location (e.g., coastal, inland, wetland) and type of operation, and may include oversight of aquaculture facility design, control measures to prevent escape of all shrimp life stages at all water/effluent discharge points, effluent treatment and discharge, species certification relative to wildlife management and disease freedom status, and water use.[38, 41] States may conduct unannounced BMP inspections annually or as needed, and variably regulate and monitor biosecurity and disease prevention measures to prevent potential release of aquatic pathogens into approximate natural water bodies.[38] State coastal management guidelines must follow or may be more restrictive than those described in the Coastal Zone Management Act.[42]

States variably require written authorization, an import permit, or a certificate of health from the State Veterinarian prior to interstate import of shrimp. Health requirements for shrimp prior to international import or export also vary by state. Best Management Practice guidelines that summarize federal and state regulations relative to shrimp culture, containment, water treatment, health and biosecurity may be available in some States.

Hazard Identification

A hazard, as defined by WOAH, is a chemical, physical, or biological agent, or condition of an animal or animal product, with potential to cause an adverse health event. [43]

IHHNV is a linear, single-stranded, non-enveloped DNA parvovirus also known as *Penaeus stylirostris densovirus* (PstDNV).[1] In July 2019, the International Committee on Taxonomy of Viruses (ICTV) proposed classification of IHHNV as decapod penstyldensovirus 1 (*Parvoviridae*; *Penstyldensovirus*, *Hamaparvovirinae)*.[1, 44] Genbank contains approximately 50 IHHNV genomes, many of which are incomplete.[45] Lack of complete genomic information limits capability to fully assess phylogenetic relatedness and virus transmission characteristics of the virus.[46] To date, genetic analyses have identified high rates of genetic variation, nucleotide substitutions and clustering among identified strains.[46-51]

Three infectious IHHNV lineages (Types I, II and III) and two non-infectious types (A and B) have been described in the literature.[46, 47, 51, 52] Type I and II have a global distribution. Type I IHHNV isolates are known to infect *Peneaus monodon* and *P. vannamei* shrimp.[46] Type II isolates have been identified in infected *P. monodon*, *P. stylirostris*, and *P. vannamei*.[46, 51] Type II strains detected in the United States (Florida and Texas) in 2019 and 2020 were phylogenetically related to a Type II strain found in South America (Ecuador).[46] Type III strains infect (enters, survives and multiplies in the host) and become integrated into the genome of *P. monodon*, but are not infectious (transmitted to other hosts).[46] Non-infectious Type A and B IHHNV are inherited, noninfectious, endogenous viral elements (EVE) present in the genomes of *P. monodon* and *P. vannamei*. These EVE sequences contain incomplete IHHNV genome sequences and are randomly inserted into the chromosomal DNA of affected shrimp.[7, 46, 50, 51, 53] Shrimp may carry one or both types.[47, 53] EVE sequences are present in the germline, and are passed from adults to offspring.

The function of EVE sequences is not fully understood. It is hypothesized that EVE sequences evolved in response to chronic or persistent IHHNV infection.[48, 50, 51] Some authors state that EVE sequences appear to lower susceptibility to IHHNV infection via viral accommodation and tolerance.[51, 54] Other authors suggest that some EVE sequences may not be fully protective, and that co-presence of EVE sequences and IHHNV virus in shrimp could result in recombination events leading to return to virulence or evolution of new IHHNV strains.[51, 54]

Viral accommodation is described as a naturally occurring autonomous genetic modification process. It is hypothesized to be an evolutionary strategy for heritable immunity via the EVE sequences present in adult shrimp and passed on in germ cells.[50] The resultant immunity is thought to occur by interfering with infectious IHHNV virus replication. Affected shrimp persistently harbor the IHHNV virus but do not develop clinical signs of disease.[51] Experimental studies have demonstrated that presence of EVE sequences results in significant reduction of IHHNV viral replication. Shrimp maintained viral loads of varying intensity but remained free of clinical signs.[50] It should be noted that these studies did not meet WOAH criteria for natural infection.[55]

Tolerance (resistance), which is described as a diminished response to IHHNV infection, has been described in some shrimp species. For example, a genetic line of *P. stylirostris* ("Super Shrimp") developed in the 1990s was reported to be IHHNV resistant at the conclusion of a 32-day experimental challenge study, and *P. vannamei* farmed in Latin America and Asia are reportedly tolerant to IHHNV.[7, 46] Farmed IHHNV tolerant shrimp are described as exhibiting no clinical signs of disease despite detectable presence of virulent IHHNV in individual animals.[7, 46] In field studies in IHHNV-endemic regions of Ecuador, in-pond prevalence rates of IHHNV in apparently healthy IHHNV tolerant *P. monodon*, *P. stylirostris* and *P. vannamei* ranged from 3.3 to 100 percent.[7] Shrimp exhibited no clinical signs of disease despite viral load (virus copies/ng DNA) ranging from low (zero) to high (2.9 x 10^5) in individual animals.[7] Some published literature state that viral load does not affect individual shrimp weight or other production parameters. Other reports state that histological evidence of infection and decreased body weight can occur when viral loads are high.[7, 56] In several experimental challenge studies, IHHNV tolerant/resistant specific pathogen free (SPF) *P. monodon*, *P. stylirostris* and *P. vannamei* had high detectable viral loads post-challenge, but did not exhibit clinical signs of disease. Species differences in viral load (*P. vannamei* > *P. monodon* > *P. stylirostris*) were noted in one study.[46] Mortality rates were low ($\leq$ 3 percent) among challenged treatment groups. The challenge method used in these studies did not meet WOAH criteria for natural exposure.[55]

Many aspects related to the epidemiology of IHHNV are still unclear due to lack of research on the pathogenicity, genetics and other factors associated with the virus.[50] Recently published research suggests that prevalence and virus detection rates, and presence of clinical signs, morbidity, and mortality rates are highly variable and may be influenced by shrimp species, age, genetics and health status; virus genetics; the presence of EVE sequences; virus genetics; laboratory diagnostic testing modalities; farm husbandry and biosecurity practices; and unknown factors.[7]

Transmission

Horizontal transmission appears to occur via direct contact with contaminated water and infected shrimp, and orally via ingestion of infected shrimp tissues and organic matter. Shrimp tissues containing homologous EVE sequences do not appear to be infectious to *P. vannamei* and *P. monodon*. Vertical transmission occurs in infected females, leading to production of eggs with poor hatchability, leading to poor development and survival of larvae and post-larvae. While data are limited on stability of the virus outside of the host in natural environments, reports of transmission via organic matter and contaminated water support the plausibility of environmental persistence. Vector transmission is unknown.

Clinical Signs and Pathogenicity

IHHNV infection manifests as acute or chronic disease. Response to infection is variable and is affected by multiple host characteristics (e.g., age, body size, gender, genetics, nutritional status, species) and external conditions (e.g., environment, population density, stress). Chronically infected animals retain viable virus in tissues for life.

Acute disease is typically seen in *P. stylirostris*, although there are published reports of acute disease occurrence in *Machrobrachium rosenbergii* as well.[50, 57] Clinical signs are most often observed in post-larvae and juvenile shrimp and include noticeable decrease in food consumption; lethargic swimming or motionlessness at the water surface followed by rolling over and sinking; motionlessness at the bottom of the tank/pond prior to mortality; pale musculature; bluish color; and white to buff-colored mottling on the cuticular epidermis, especially at the junction of the tergal plates. Mortality rates may be greatest (up to 90 percent) in small shrimp (5.0 mg – 3.7 gm body weight). Chronic infection commonly occurs in *P. vannamei* and *P. monodon* but has been reported in *P. stylirostris* and *M. rosenbergii*.[50, 57] Clinical signs include deformed rostrum (bent 45º to 90º left or right); sixth abdominal segment deformity; wrinkled antennae; cuticular roughness; "bubble head" appearance; runting; and disparate growth rates (Runt Deformity Syndrome; RDS). Cuticular deformities are considered pathognomonic when present in some shrimp species.

Disease in *P. stylirostris*: Clinical disease is affected by the transmission pathway and the age, body weight, and size of affected shrimp. Vertically infected larvae and post-larvae often appear healthy until approximately 35 days of age when clinical signs appear, followed approximately 14 days later by high mortality (50 percent to 90 percent). Clinical disease in horizontally infected shrimp is age and body size dependent. Acute disease with clinical signs and high mortality occurs in juvenile and sub-adult shrimp, while adult shrimp and individuals of greater body weight exhibit less severe disease. Shrimp surviving disease remain infected and are transmission capable. RDS has also been documented.

Disease in *P. vannamei*: This species shows relative resistance to IHHNV infection; however, asymptomatic infection has been detected in all life stages including eggs.[7] Vertical transmission results in reduced egg production, reduced egg hatchability, and high prevalence of infection in nauplii (first larval stage). Juvenile shrimp develop RDS. Horizontally infected juvenile shrimp may develop RDS, while adult shrimp rarely demonstrate clinical signs. Juvenile shrimp surviving to adulthood and infected adults remain infected for life. *P. vannamei* containing EVE sequences have been reported, and do not appear capable of producing infectious virions or of horizontal IHHNV transmission to susceptible hosts.[7]

Disease in *P. monodon*: IHHNV infection rarely causes disease in *P. monodon*; however, RDS has been reported.[46, 56] Shrimp remain infected for life. Sellars et al., 2019 reported reduced performance (growth, general health, and survival) in asymptomatically infected *P. monodon* in farm ponds and experimentally simulated farm environments when high viral loads were present in affected animals.[56] *P. monodon* containing integrated IHHNV EVE sequences have been reported. Such shrimp do not appear capable of producing infectious IHHNV virions or of horizontal IHHNV transmission to susceptible hosts.[7]

Asymptomatic carriers of IHHNV: Asymptomatic IHHNV has been reported in *M. rosenbergii, P. monodon and P. vannamei*.[1] Mechanisms by which these species function as asymptomatic carriers may include presence of EVE sequences, accommodation and tolerance as described above. Asymptomatic carriers exhibit no clinical signs but may be capable of transmitting virus to susceptible shrimp.[50] A negative trend between IHHNV viral load present in ponds containing asymptomatic shrimp and performance (growth, general health, and survival) has been reported in the literature.[7, 46, 56]

Affected Species

Table 3 summarizes the shrimp species meeting WOAH criteria for listing as susceptible to IHHNV (e.g., *P. californiensis, P. monodon, P. setiferus, P. stylirostris, P. vannamei*) and other crustacean, bi-valve, and fish species in which IHHNV presence has been detected or infection experimentally induced. The epidemiological significance of detection and/or experimental infection in non-shrimp species has not yet been determined.

Table 3. Aquatic animal species meeting WOAH criteria for listing as susceptible to IHHNV, or with reported, confirmed, or suspected IHHNV detection based on review of the literature.[1, 14, 16, 20, 57-66]

Group	Genus species	Common name
Shrimp/Crayfish	*Artemesia longinaris* [c]	Argentine stiletto shrimp
	Farfantepenaeus duorarum	Northern pink shrimp
	Palaemon macrodactylus	Oriental shrimp
	Macrobrachium rosenbergii [c]	Giant river prawn
	Penaeus aztecus [b]	Northern brown shrimp
	Penaeus californiensis [a]	Yellowleg shrimp
	Penaeus chinensis (P. orientalis)	Chinese fleshy prawn
	Penaeus duorarum [c]	Northern pink shrimp
	Penaeus indicus	Indian prawn
	Penaeus japonicus [c]	Kuruma prawn; Japanese tiger prawn
	Penaeus latisulcatus	Western king prawn
	Penaeus merguiensis	Banana shrimp
	Penaeus monodon [a]	Black tiger prawn
	Penaeus monodon x Penaeus esculentus hybrid	Tiger prawn hybrid
	Penaeus occidentalis [c]	Western white shrimp
	Penaeus schmitti	Southern white shrimp
	Penaeus semisulcatus [c]	Green tiger prawn
	Penaeus setiferus [a]	Atlantic white shrimp
	Penaeus stylirostris [a]	Western blue shrimp
	Penaeus subtilis (Farfantepenaeus subtilis)	Brown shrimp
	Penaeus vannamei [a]	Whiteleg shrimp
	Procambarus clarkii	Louisiana red crayfish
Crab	*Callinectes arcuatus* [c]	Arched swimming crab
	Chionoecetes angulatus	Triangle tanner crab
	Hemigraspus penicillatus [c]	Hairy-clawed shore crab
	Neohelice granulate	Tropical crab
Bi-valve shellfish	*Mactra chinesis*	Chinese trough clam, Sunray surf clam
	Mactra veneriformis	Intertidal clam

Group	Genus species	Common name
	Meretrix meretrix	Asiatic hard clam
	Mytilus edulis	Atlantic blue mussel
	Ruditapes philippinarum	Manila clam, Japanese littleneck clam
	Sinonvacula constricta	Chinese razor clam
	Tegillarca granosa	Ark clam, blood cockle, blood clam
Fish	*Achirus mazatlanus* [c]	Mazatlan sole
	Gerres cinereus [c]	Yellowfin mojarra
	Oreochromis spp. [c]	Tilapia spp.
	Lile stolifera [c]	Pacific piquitinga
	Centropomus medius [c]	Blackfin snook

[a]Fulfills the criteria for listing as susceptible to infection according to the WOAH *Aquatic Animal Health Code, Chapter 1.5*

[b]Species for which there is incomplete evidence to fulfill the criteria for listing as susceptible

[c]Species for which there is no evidence for infection, but have had positive PCR results

Farmed shrimp reared in the United States that are susceptible to IHHNV include *P. vannamei*, *P. monodon,* and *P. stylirostris*.[38] Potentially susceptible indigenous wild and introduced penaeid shrimp populations and other indigenous and wild crustaceans, bi-valves, and fish species are found along the Atlantic, Gulf, and Pacific coasts of the continental United States, Hawaii, Puerto Rico, and U.S. Virgin Islands (**Table 4)**.

Table 4. Indigenous and introduced aquatic animals that are susceptible or potentially susceptible to IHHNV infection, based on literature review.[67-88]

Genus species	Common name	Location	Indigenous/Introduced
Farfantepenaeus duorarum	Northern pink shrimp	East Coast: Maryland to Florida; Gulf of Mexico: Florida to Texas	Indigenous
Macrobrachium rosenbergii	Giant river prawn	Gulf of Mexico: possible established population in Mississippi	Introduced
Palaemon macrodactylus	Oriental shrimp	East Coast: Northwest Atlantic Ocean; West Coast: Oregon to California	Introduced
Penaeus aztecus	Northern brown shrimp	East Coast: Massachusetts to Florida; Gulf of Mexico: Florida to Texas	Indigenous
Penaeus californiensis	Yellowleg shrimp *(Farfantepenaeus californiensis)*	West Coast: San Francisco Bay, California to Chile	Indigenous
Penaeus duorarum	Northern pink shrimp *(Farfantepenaeus duorarum)*	East Coast: Chesapeake Bay, Maryland to the Florida Keys; Gulf of Mexico: Florida to Texas	Indigenous

Genus species	Common name	Location	Indigenous/Introduced
Penaeus monodon	Black tiger prawn	East Coast: North Carolina to Florida; Gulf of Mexico: Florida to Texas	Introduced
Penaeus schmitti		Western Atlantic Ocean: Greater Antilles, Cuba, Virgin Islands	Indigenous
Penaeus setiferus	Atlantic white shrimp	East Coast: New York to Florida; Gulf of Mexico: present throughout	Indigenous
Penaeus vannamei	Pacific whiteleg shrimp	Accidental aquaculture releases in Hawaii, Texas, South Carolina Puerto Rico; no evidence of established populations	Introduced
Procambarus clarkii	Louisiana red crayfish	Gulf coastal plain from the Florida panhandle to Mexico; southern Mississippi River drainage to Illinois	Indigenous
Callinectes arcuatus	Arched swimming crab	West Coast: Los Angeles, California to Peru and northern Chile	Indigenous
Chionoecetes angulatus	Triangle tanner crab	West Coast: Aleutian Islands, Bering Sea, British Columbia to the continental slope of California	Indigenous
Mytilus edulis	Atlantic blue mussel	East Coast: Canada to North Carolina; West Coast: California	Indigenous, Introduced
Ruditapes philippinarum	Manila clam, Japanese littleneck clam	West Coast: British Columbia to California; Hawaii	Introduced
Gerres cinereus	Yellowfin mojarra	Gulf of Mexico and Caribbean Ocean	Indigenous
Oreochromis spp.	Tilapia spp.	East Coast: North Carolina to Florida; Gulf of Mexico: Florida, Alabama, Texas; Puerto Rico	Introduced

Diagnostic Testing

IHHNV has affinity for tissues of ectodermal and mesodermal origin (gills, cuticular epithelium, hypodermis, connective tissues, hematopoietic tissues, lymphoid organs, antennal glands, and neural tissues) and may have affinity for the hepatopancreas of *M. rosenbergii*. Tissues suitable for diagnostic testing include pooled small life stage shrimp (post-larvae or animals $\leq$ 0.5 gm), individual large shrimp, or target tissues harvested from large shrimp (e.g., connective tissue, gills, hematopoietic nodules, hemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, lymphoid organ parenchymal cells, hemolymph). Tissues that may be submitted for non-lethal surveillance testing include hemolymph and pleopods. Spawned eggs and larval shrimp are not suitable for disease testing or IHHNV disease freedom certification because viral loads may be below detection limits.[89]

Recommendations regarding diagnostic test suitability for surveillance and diagnosis are described in the WOAH *Manual of Diagnostic Tests for Aquatic Animals*.[89] Polymerase chain reaction (PCR) is recommended for diagnosis of acute and chronic infections. Conventional PCR and TaqMan rtPCR assays can be used to detect and differentiate IHHVN virus from EVE sequences. Unexpected PCR results (positive or negative) should be confirmed with a different PCR primer set or another diagnostic test (e.g., genetic sequencing, histopathology, *in situ* hybridization assay of histological sections, molecular diagnostics, recombinase polymerase amplification assay, trans-electron microscopy). The WOAH *Manual of Diagnostic Tests* states that geographical IHHNV variants may not be detectable by all diagnostic modalities.[6] Attempts to culture IHHNV *in vitro* have not been successful. There are no serological assays.

Histology is often used to detect IHHNV-specific Cowdry inclusion bodies, which are considered pathognomonic (indicative of a specific disease or condition) for IHHNV infection. Experimentally, histopathologic changes may be more prevalent in tissues collected from larger shrimp. Caro et al., 2022 demonstrated that while Cowdry bodies were detectable in experimentally challenged *P. monodon* and *P. vannamei*, they were not reliably present in similarly challenged *P. stylirostris*.[7] The authors also noted that the presence of Cowdry bodies appeared to be influenced by the level of viral load in infected individuals (less likely to be present with low viral loads).[7]

Molecular assays and genome sequencing should be used to confirm virulent IHHNV presence/absence and rule-out presence of known or novel EVE sequences in *P. monodon* and *P. vannamei*.[46, 51, 53] According to the literature, presence of EVE sequences in the genome of some shrimp species may present challenges to molecular assays used for IHHNV surveillance and diagnostic testing. If diagnostic assays designed to detect virulent IHHNV genomic material also detect EVE sequences, non-IHHNV infected shrimp with EVE sequences in their genomes will test positive (false-positives).[51] At present, some diagnostic assays recommended by WOAH for detection of infectious IHHNV may also detect EVE sequences present shrimp.[6, 51]

Molecular assays designed to detect known EVE sequences are available. If these assays are used and no additional testing performed to detect virulent IHHNV genomic material, shrimp with EVE sequences may be identified as falsely positive for infectious IHHNV virus.[51] It is also plausible that assays designed to detect known EVE sequences could return false-negative results if shrimp have EVE sequences containing inverted, missing, reordered, repeated, or unique IHHNV genome fragments.[48, 53, 54, 58, 90] It may be advantageous for laboratories to utilize molecular assays capable of detecting both viral IHHNV and EVE sequence DNA. Other assays that could present options for distinguishing between infectious IHHNV and EVE sequences include clustered interspaced short palindromic repeats (CRISPER), digital droplet PCR, next-generation sequencing assays, protein modeling, and real-time PCR (rtPCR) employing fluorescent probes or SYBER Green dye amplicon detection methods.[46, 51, 53] This has been previously achieved using a series of seven overlapping primer sets to verify the presence of the whole IHHNV genome.[48] Taengchaivaphum et al., 2022 reported that using a “long-amp” PCR method covering 90 percent of the IHHNV genome (approximately 4000 base pairs) was effective in confirming the absence of IHHNV in a sample that had returned a positive PCR test result with the WOAH recommended testing method.[51]

Treatment

There is no treatment or vaccine available for IHHNV. Use of RNA interference gene silencing has been used experimentally; however, a delivery technology suitable for aquaculture use is not available. Eggs and larvae may be disinfected to reduce IHHNV contamination; however, this does not prevent vertical transmission.

Disease Prevention and Control Measures

Appropriate biosecurity practices and husbandry to prevent IHHNV introduction and spread should include a) international and domestic procurement of live shrimp and germplasm from IHHNV-free sources; b) quarantine of all new shrimp stocks upon arrival; c) appropriate disinfection and disposal of transport water; d) health and mortality monitoring of shrimp present on site; e) pre- and post-treatment of farm water sources; f) minimizing or eliminating water exchanges between populations; and g) appropriate sanitization of equipment and tanks.[2] The virus is temperature stable (-20 °C/-4 °F x 5 years) and will survive freeze/thaw cycles in shrimp tissues, clarified suspensions, and 50 percent glycerin. In experimentally infected live *P. vannamei*, increased viral titers ($\log10^2$) were noted at lower temperatures (24 °C/75.2 °F, compared to 32 °C/ 89.6 °F).[6]

Public Health

IHHNV is not a zoonotic disease.

Potential Entry Pathways

The WOAH defines an entry pathway as any pathway that allows movement of a hazard from a point of origin (e.g., foreign country) to domestic points of entry.[43]

Pathway 1: Ballast water, ship fouling

Ballast water and fouling present on the hulls of ships contain a variety of biological materials including animals (e.g., crustaceans, echinoderms, fish, mollusks, plankton), microorganisms (e.g., bacteria, viruses), and plants. Crustacean species can compose up to 50 percent of taxa fouling commercial and recreational ships and boats in fresh and marine water. Both ballast water and ship fouling have been associated with invasive aquatic animal and pathogen introductions in coastal and freshwater systems globally and in the United States.[29-31, 91, 92]

Ballast water is to be treated and exchanged in accordance with International, Federal, and State regulations and standards; however, BWTE reduces with variable efficacy, but does not eliminate, living organisms and microorganisms, and the efficacy of BWTE on viruses is generally unknown. Some ships are exempt from BWTE and recordkeeping requirements, or are allowed to discharge ballast water in COPT zones. COPT zones are administrative and do not consider ecological or biological factors that may permit movement of invasive microorganisms, organisms or plants out of the zone. Regulatory non-compliance does occur, and has been reported.[93]

Current regulations and standards do not list conditions for ballast water treatment, monitoring, or testing for aquatic pathogens, including IHHNV. A literature review did not identify surveillance data or studies monitoring ballast water or ship hull fouling for IHHNV presence/absence. The literature did not identify any reports definitively linking ballast water or ship fouling to introduction of IHHNV at a port of entry, coastal waterway, or aquaculture facility. This lack of data or reporting does not preclude the potential for ballast water or ship fouling to function as transboundary pathways of IHHNV introduction. Given the above information and previous documentation of invasive species and pathogen introductions, ballast water and ship fouling appear to be plausible transboundary pathways of entry for IHHNV.

Pathway 2: Movement of infected shrimp, eggs, or IHHNV in water

A comprehensive evaluation of IHHNV dispersal and movement in ocean currents is beyond the scope of this document. Briefly, movement of aquatic pathogens in ocean currents has been demonstrated and epidemiologically linked to disease introductions and outbreaks [94-96], and dispersals of invasive aquatic organisms and microorganisms from shipping ports (after introduction via Pathway 1) have been documented via ocean tides and currents.[94, 97] Oceanographic current systems in the GoC are considered factors affecting IHHVN distribution and prevalence among resident wild shrimp populations. Prevalence is highest in the northern zone, which is a semi-isolated oceanographic cell affected by a gyre (rotary pattern) that limits, but does not prevent, water-borne out-migration of infected shrimp and IHHNV.[10, 98] Lower prevalence rates in the central and southern zones appear related to ocean currents that reduce contact rates between infected shrimp and the concentration of IHHNV in the water column, allow water exchanges between the zones and the open ocean, and may have provided transmission routes to wild shrimp located along the west coast of the Baja California Sur (specifically Bahia Magdalena) and the south coast of Mexico.[9, 12, 99]

It appears highly plausible that ocean currents may provide a transboundary pathway of entry for IHHNV or IHHNV infected shrimp or eggs into coastal waters along the continental United States, given a) documented evidence of aquatic pathogen introductions occurring via movement in water and ocean currents; and b) published literature linking the spread of IHHNV infection in wild shrimp populations in South, Central, and North America to movement of IHHNV virus or infected shrimp in ocean currents and/or water exchanges (**Figures 1 - 4)**.

Figure 1. Literature reported locations of IHHNV-infected wild shrimp populations in North, Central, and South American coastal waters. [4, 5, 8-19, 62, 89, 100]. USDA figure.

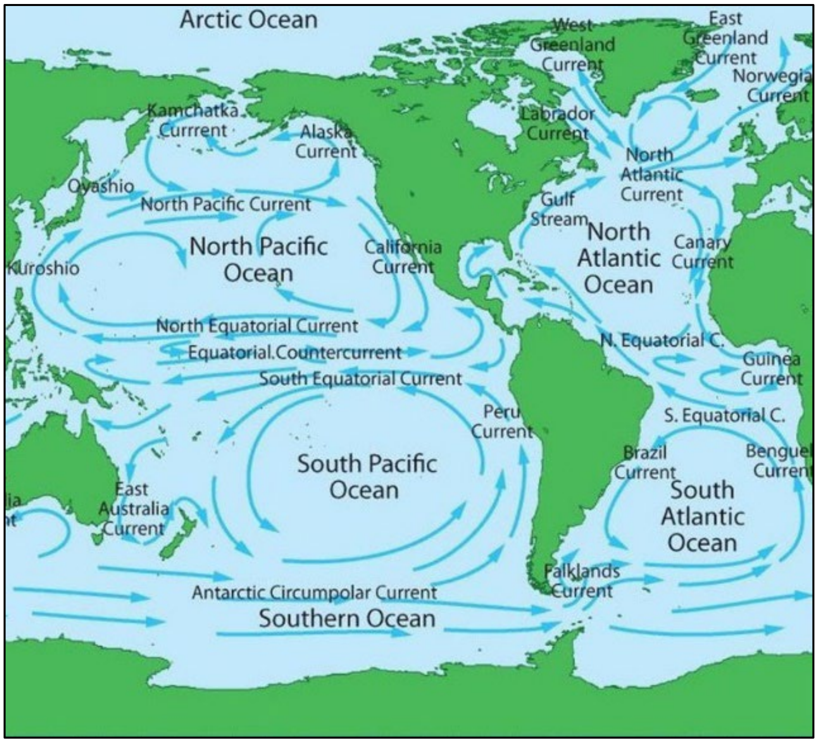

Figure 2. Brief schematic overview of global ocean currents. All ocean currents, seasonal upwellings, and gyres are not represented. [100-109]

Figure 3. Brief schematic overview of ocean currents on the Pacific coast of the Baja Peninsula. All ocean currents, seasonal upwellings, and gyres are not represented.[100, 101, 103, 107] USDA figure.

Figure 4. Brief schematic overview of ocean currents in the Gulf of Mexico. All ocean currents, seasonal upwellings, and gyres are not represented.[100, 101, 105, 106, 108-111]

Pathway 3: Nauplii, post-larvae, or broodstock imported for aquaculture purposes

The literature identifies imported nauplii and juvenile and broodstock shrimp as potential and known sources of pathogen introduction in shrimp aquaculture.[2, 112] Non-native shrimp from foreign hatcheries are imported to the United States for aquaculture purposes, which may result in transboundary introduction of IHHNV.[113] As examples, introductions of IHHNV into domestic shrimp aquaculture facilities were associated with importation of live shrimp from IHHNV-affected countries in 2013, 2018, and 2019.[2, 23] In general, there are no import regulations or requirements for Certificates of Veterinary Inspection to certify that imported live shrimp are healthy or disease-free, and there is a lack of capability to quarantine live shrimp prior to import release. It is highly plausible that live shrimp imported for aquaculture purposes will serve as transboundary pathways of IHHNV introduction, given the above and a) the pathogenesis and epidemiology of IHHNV in some shrimp species; b) the rapid transportation time associated with air freight; c) lack of data reporting the sources, volumes, and final disposition pathways of shrimp imported for aquaculture; d) lack of a national standardized domestic surveillance program for IHHNV; and e) lack of standardized biosecurity and disease surveillance measures among domestic shrimp aquaculture facilities.

Pathway 4: Imported shrimp for human consumption

IHHNV is viable in chilled and frozen-thawed shrimp tissues. Since the 1990s, the literature has reported presence of IHHNV and other pathogenic shrimp viruses including Taura syndrome virus (TSV), white spot syndrome virus (WSSV), and yellowhead virus (YHV) in imported frozen commodity shrimp.[112, 114, 115]

The United States imports shrimp seafood commodities from countries that a) are WOAH -listed; b) have reported an IHHNV outbreak to WOAH in the last 2 years; and c) have literature-reported presence in cultured or wild shrimp populations (**Table 5**). It is possible the United States may import seafood commodities from non- WOAH member countries where IHHNV is present, but surveillance and reporting are lacking.

Table 5. Edible shrimp products[1] exported to the United States by countries that are a) WOAH listed; b) reported to have IHHNV in aquaculture; c) reported to have IHHNV in wild shrimp populations; or d) have reported an outbreak to WOAH from 2019 to 2021.[2, 3, 62, 66, 115-117]

Country	Kilograms of Product Imported				
	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019
Argentina[b,c]	4,041,212	9,710,213	13,841,636	13,117,648	12,747,562
Australia[a,b,c]	1,890	14,400	36,000	30,099	90,976
Brazil[a,b,c]	1,659	0	0	0	5,488
Canada[d]	4,309,067	247,544	74,219	35,927	14,017
China[a,b]	10,113,426	14,224,726	24,081,767	24,014,187	1,168,243
Colombia[a,b,c]	80,000	88,728	124,016	50,254	8,250
Costa Rica[a,b]	0	0	0	0	0
Ecuador[a,b,c]	56,823,987	46,890,510	46,800,791	51,393,612	60,971,175
El Salvador[a,b,c]	115,019	61,611	84,673	13,561	23,366
Guatemala[a,b]	2,256,746	1,671,898	1,078,964	775,842	529,602
Honduras[a,b,c]	1,210,888	736,388	1,105,644	2,373,608	1,730,132
India[a,b,c]	136,330,285	175,988,486	25,8331,199	314,515,241	361,992,508
Indonesia[a,b,c]	110,054,483	102,388,074	102,954,430	109498472	99,725,618
Madagascar[a,b]	0	0	0	4,644	14,760
Malaysia[b]	1,498,8008	190,936	142,731	68,149	34,350
Mexico[a,b,c]	1,473,296	2,480,682	1,459,743	1,061,690	1,571,671
Mozambique[b]	0	0	0	0	0
New Caledonia[b]	0	0	6,012	0	0
Nicaragua[a,b,c]	79,3940	885,002	396,554	320,624	421,719
Panama[a,b,c]	1,035,289	851,254	604,222	586,100	163,336
Peru[a,b,c]	9,513,336	7,809,602	7,342,220	9,481,584	6,314,514
Philippines[a,b,c]	994,549	700,274	1,077,130	476,027	383,300
Singapore[b]	447	2,307	0	0	5,000
South Korea[b]	215,246	236,952	110,373	105,512	150,972
Sri Lanka[a,b,c]	6,440	55,732	83,882	164,028	33,682
Taiwan[b]	254,598	246,123	107,086	149,630	19,354
Thailand[a,b,c]	41,718,491	47,166,512	41,792,577	26,333,449	18,671,196
Venezuela[b]	556,492	672,664	1,305,656	3,172,056	3,507,740

[a]WOAH listed
[b]Literature reported presence in cultured aquaculture
[c]Literature reported presence in wild shrimp populations in the Americas
[d]WOAH reported outbreak in the last 2 years

[1] Edible shrimp product includes fresh or chilled peeled, frozen peeled, frozen not peeled, frozen headless shell-on, not frozen shell-on, and live fresh or chilled shell-on)

Only healthy-appearing shrimp are to be processed for human consumption and imported products must be prepared in processing facilities regulated and inspected by the country of origin's government and meet USDA and Food and Drug Administration processing requirements. However, IHHNV-infected shrimp can appear healthy, and some IHHNV outbreak control measures may include harvest of infected shrimp for human commodity pathways.[2, 115, 118] Inspection rates by U.S. regulatory agencies or contracted third-party inspectors are typically low at foreign processing facilities compared to the number of facilities that produce seafood. In addition, country-of-origin government oversight may be unknown to U.S. regulatory agencies. Currently, there are no U.S. Federal regulations requiring imported frozen shrimp destined for human consumption to be screened for aquatic pathogens.[114] Entry of IHHNV into the United States via this pathway has not been documented; however, given this information, it seems highly plausible that imported shrimp seafood commodities may pose a transboundary pathway of entry for IHHNV.

Pathway 5: Imported bait shrimp

The United States imports bait shrimp for use in inland freshwater and marine sport fishing.[114] A simple internet search shows that imported bait shrimp may be purchased online and in bait shops throughout the United States.

The literature has identified imported bait shrimp as a potential source of aquatic pathogen introduction [112, 114], and studies by Prior et al. (2001) and Hasson et al. (2006) demonstrated the presence of this pathway in the United States via identification of TSV and WSSV in imported frozen bait shrimp purchased at bait and grocery stores.[114, 119] IHHNV was not detected in either published study, and a literature search did not identify subsequent studies performing surveillance for IHHNV in imported bait shrimp.

The volume of bait shrimp imported to the United States annually is not known, because bait shrimp are imported under the Harmonized Tariff Schedule of the United States, subheading 0511.91.0090 "Products of Fish or Crustacean, Molluscs, or Other Aquatic Invertebrates; Dead Animals of Chapter 3, Unfit for Human Consumption, NESOI."[120] From 2015 to 2020, the United States imported an average 76,909,345 kg/169,556,081 lb (range 64,343,192 to 97,492,406 kg/141,852,456 to 214,933,964 lb) of such product, including from countries with a WOAH listing for IHHNV, that have reported IHHNV detections in the literature, or that experienced a recent outbreak or occurrence of IHHNV that was reported to WOAH (**Table 6)**. The percentage of bait shrimp in these shipments is unknown.

Table 6. Quantity of dead fish, crustaceans, mollusks, or other aquatic invertebrates unfit for human consumption exported to the United States between 2015 and 2020 from countries with IHHNV presence in cultured or wild shrimp populations as determined by WOAH or as reported in the literature.[3-5, 8-19, 120, 121]

Country	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019	Year 2020
Argentina[b,c]	1,942,102	2,626,144	2,294,126	1,285,622	641,424	911,610
Australia[a,b,c]	3,350	1,040	6,630	2,000	1,052	468
Canada[d]	43,769,276	58,584,020	51,351,640	43,871,560	51,180,538	47,799,388
China[a,b]	1,982,636	5,533,350	3,687,750	1,846,862	1,442,630	2,103,800
Colombia[a,b,c]	6,758	0	0	0	0	0
Costa Rica[a,b]	0	0	0	0	0	590
Ecuador[a,b,c]	2,583,980	2,342,336	2,924,522	4,103,810	93,720	2,131,740
Guatemala[a,b]	0	0	0	0	0	0
Honduras[a,b,c]	0	0	0	0	0	1,000
India[a,b,c]	47,992	62,690	60,562	181,098	49,744	5,526
Indonesia[a,b,c]	131,636	255,822	202,194	145,382	153,366	664,058
Japan[b]	205,418	855,868	781,942	520,390	1,035,364	1,639,290
Mexico[a,b,c]	5,639,276	9,128,838	15,132,120	2,524,924	1,845,292	3,518,322
Nicaragua[a,b,c]	55,002	43,924	44,686	34,058	103,858	39,608
Panama[a,b,c]	36,000	39,374	39,600	90,874	77,162	94,352
Peru[a,b,c]	466,462	1,641,486	341,848	92,356	1,077,084	221,300
Philippines[a,b,c]	219,336	224,614	130,252	151,064	95,546	78,708
Singapore[b]	0	0	0	0	0	0
South Korea[b]	6,380,930	5,424,456	4,297,974	2,369,874	294,000	157,642
Sri Lanka[a,b,c]	0	0	0	0	0	0
Taiwan[b]	7,587,192	10,079,116	8,622,556	5,691,488	6,392,058	5,139,018
Thailand[a,b,c]	60,840	24,256	68,296	328,298	77,266	610,878
United Kingdom[d]	1,065,272	47,132	76,940	448,762	415,718	3,967,848
Venezuela[b]	0	0	0	0	41662	63140
Vietnam[b]	280,326	577,940	775,048	654,770	1,273,290	878,942
Total	72,463,784	97,492,406	90,838,686	64,343,192	66,290,774	70,027,228

[a]WOAH listed
[b]Literature reported presence in cultured aquaculture
[c]Literature reported presence in wild shrimp populations in the Americas
[d]WOAH reported outbreak in the last 2 years

It is highly plausible that imported bait shrimp may provide a transboundary pathway of entry for IHHNV given the volume and sources of dead fish, crustaceans, mollusks, or other aquatic invertebrates unfit for human consumption exported to the United States, and the documented presence of aquatic pathogens present in imported bait shrimp. Lack of detection of IHHNV in the limited number of studies that have explored this entry pathway does not negate the potential for IHHNV introduction to occur.

Potential Exposure Pathways

An entry pathway, as defined by WOAH, is a pathway from the point of entry that leads to exposure of vulnerable animal or human populations to a hazard.[43]

Pathway 1: Introduction of IHHNV-infected shrimp into aquaculture facilities

As stated in the Entry Assessment, a) non-native shrimp from foreign hatcheries are imported to the United States for aquaculture purposes [113]; and b) IHHNV introductions in domestic shrimp aquaculture facilities have been epidemiologically linked to imported IHHNV-infected live shrimp in 2013, 2018, and 2019.[2, 117]. These occurrences, and the transfer of IHHNV-infected animals between domestic facilities in 2019, demonstrate that a) shrimp are imported to stock aquaculture facilities; b) some of the imported shrimp included IHHNV-infected animals; and c) this is a documented exposure pathway of IHHNV introduction. These introductions have occurred despite availability of selectively bred specific pathogen-free (SPF) shrimp [122], regulation or permitting guidelines for non-native shrimp culture in some States [123, 124], and guidelines and standards for importation and transit of aquatic animal products and disease control and prevention outlined in the WOAH *Aquatic Animal Health Code*.[2, 55]

Pathway 2: Shrimp imported for human consumption

The presence of this exposure pathway has been documented via epidemiological investigations of aquaculture disease outbreaks (e.g., WSSV in Australia), and research studies that have a) detected aquaculture pathogens, including IHHNV, in imported shrimp commodities sold in grocery stores and marketplaces; and b) demonstrated transmission of WOAH-listed shrimp pathogens from imported shrimp seafoods to susceptible animals.[112, 114, 115, 119, 121, 125, 126] Methods by which this exposure pathway may occur include the following:

Processing plants

An unknown volume of imported commodity shrimp products is reprocessed in domestic plants located throughout the United States, including those on coastal bays and estuaries where native penaeid shrimp are present. Some plants may be located near shrimp aquaculture facilities.[112, 115] Processors of fish and fishery products are subject to Seafood HACCP Regulation (21 CFR Part 123 and other regulation [i.e., Current Good Manufacturing Practice regulation, 21 CFR Parts 113 and 114]).[127] However, such regulations are designed to ensure human public health; monitoring seafood processing for aquaculture safety is not under the purview of such regulations. Surveillance for human pathogens of concern is conducted as part of normal regulatory inspection, but such surveillance for aquaculture pathogens is not performed.

Primary routes of pathogen release from processing plants occur via inadequate disinfection, discharge, disposal, or storage of solid or liquid wastes.[128] Proximity between processing plants and susceptible wild or farmed shrimp populations increases the risk of disease introduction, with on-farm processing presenting the greatest level of risk, especially if the plant processes shrimp from outside sources.[128, 129] Data are not readily accessible on the proximity of plants that process imported commodity shrimp to susceptible farmed or indigenous shrimp populations.

While processing plants are required to monitor waste streams to prevent inadvertent release of contaminants, a) the level of oversight at all plants is unknown; b) violations occur; c) there are no regulations requiring surveillance of processing waste for aquatic pathogens; and d) the efficacy of effluent, wastewater, and solid waste treatments on inactivation of IHHNV is not known. Discharge of effluent or wastewater containing viable IHHNV into sewer systems or coastal or inland waterways could provide a plausible pathway of exposure to susceptible wild and cultured shrimp populations.[112, 130, 131] Solid seafood processing wastes may be incorporated into wastewater treatment steps to produce sludge or disposed of via solid waste streams (e.g., landfills, compost, burying) or land application (as fertilizer). Landfill operators are required to monitor groundwater for microbial contamination but it is unknown a) how methods used may correlate with the presence of aquatic animal pathogens; and b) whether or not groundwater is monitored for presence of aquatic animal pathogens.[132-134] The literature has reported microbial contamination of groundwater associated with landfills, composting, burial, and land application of processing plant waste slurry [132, 133]; such research has not been conducted relative to aquaculture pathogens, including IHHNV. Lack of documentation does not mean that such events may not occur. Wildlife and birds that forage on landfills and composted or buried waste could consume IHHNV-infected tissues and serve as fomites or transmission vectors, transporting the virus to shrimp farms or natural water bodies. Given the above, it does appear plausible that waste streams associated with processing of imported shrimp could provide a plausible IHHNV exposure pathway.

Disposal of imported shrimp seafood as waste

Approximately 40 percent of commercial seafood produced is discarded as waste [135] with landfill disposal listed as the most common waste disposal pathway. Seafood waste may also be disposed of via composting and burial, and consumers may dispose of shrimp heads and other tissues into native waters.[123] The total volume of imported commodity shrimp discarded as waste is not known, and there are no published studies assessing a) landfilled, composted, or buried shrimp seafood waste, or adjacent ground or surface waters; and b) the transmission potential of wildlife and birds. However, it is highly plausible that disposal of commodity shrimp wastes as described may provide a pathway of IHHNV exposure to susceptible crustaceans given a) the viability of IHHNV in frozen-thawed shrimp tissues; b) documented presence of IHHNV in imported commodity shrimp products; c) the potential water contamination pathways associated with landfill, composted, and buried wastes as described in the section above, and d) the potential for direct disposition of imported shrimp wastes into water bodies.[136] [137] [138-140]

Use of imported shrimp seafood as bait

Imported shrimp products intended for human consumption may be used as bait and have been associated with the introduction of aquatic FADs. For example, use of imported frozen uncooked shrimp for bait by recreational fishermen was considered the most likely pathway of WSSV introduction into Moreton Bay, South East Queensland, Australia in 1992.[141, 142] In 2019, a survey indicated that approximately 27 percent of recreational fishermen in Australia still purchase raw imported shrimp from supermarkets for use as bait, and that hundreds of tons of frozen imported shrimp were entering Australian waterways annually due to this activity. The frequency of use and volume of imported human food quality shrimp used as bait for sportfishing in the United States is not known. However, is reasonable to assume that a) this activity occurs; and b) the volume of such products used may be large. An internet search identified several recreational fishing sites and chat boards recommending use of frozen shrimp purchased from grocery stores or supermarkets; such product was described on some sites as "superior to commercial bait shrimp." IHHNV introductions via this pathway have not been reported in the United States; however, given the epidemiology of IHHNV, persistence of viable IHHNV in frozen shrimp tissues, and apparent use of commodity shrimp for bait in the United States, exposure of susceptible wild or farmed shrimp via this pathway appears plausible.

Use of imported shrimp seafood as food for aquatic animals

Imported human food quality shrimp may be used as food for aquatic animals. Zoos, aquariums, and similar facilities incorporate seafood into the diets of aquatic animals (e.g., cephalopods, crustaceans, fish, marine mammals).[143-145] People may also incorporate frozen shrimp seafood into diets for aquatic pets. An internet search identified multiple websites a) recommending feeding of freeze-dried and/or thawed frozen fresh- and salt-water shrimp to a variety of aquatic pets; and b) advertising imported pet foods containing or composed of krill and/or freeze-dried or frozen fresh- and saltwater shrimp products for sale. The validity of this exposure pathway was demonstrated in the 1990s when introduction of WSSV into a population of freshwater crayfish housed at a public institution (the National Zoo) in the United States was traced back to the feeding of imported shrimp seafood.[115, 146] In the United States, transmission of IHHNV to susceptible wild or farmed shrimp populations via this practice has not been documented. However, it appears plausible that exposure of susceptible animals may occur via this exposure pathway, given that a) FAD introduction has occurred via this practice; b) oral IHHNV transmission can occur; c) the stability of IHHNV in thawed frozen shrimp tissues; d) current lack of surveillance of shrimp seafood for diseases of concern to aquaculture; and e) recommended incorporation of shrimp in aquatic animal diets.

Pathway 3: Imported bait shrimp

Bait shrimp are used for recreational freshwater and marine fishing. The volume of bait shrimp exported to the United States is unknown; however, an internet search identified a) imported bait shrimp (e.g., freshwater and saltwater) for sale in the United States; and b) chat boards discussing use of bait shrimp. There are currently no Federal regulations requiring screening of imported bait shrimp for aquatic pathogens of concern. Some States (e.g., Texas) do have regulations in place regarding use of any type of imported shrimp (e.g., live, dead, whole or in pieces) from a non-Gulf of Mexico State or different country as bait.[147] Rates of enforcement of and compliance with such regulations by fishermen are unknown. Given the accessibility of such product and recommendations for use on various internet sites, it appears that bait shrimp may be used for fishing with some regularity. Currently, there are no published reports describing detection of IHHNV in imported bait shrimp or exposure of susceptible shrimp populations to IHHNV via use of bait shrimp. Lack of data or confirmation of an IHHNV introduction occurring via this pathway does not preclude the potential for exposure of susceptible wild or farmed shrimp to occur via use of bait shrimp for fishing.

Pathway 4: Imported shrimp by-product

Unknown quantities of imported shrimp meal, cuticle meal, and other byproducts are used as feed ingredients in aquaculture. The high temperatures used to prepare meal-type byproducts is likely sufficient to inactivate IHHNV, and there are no studies or field reports showing that IHHNV transmission has occurred via this pathway. Given this information, it does not appear plausible that imported shrimp byproducts incorporated into aquatic animal feeds would provide a pathway of IHHNV exposure to susceptible farmed shrimp.

Pathway 5: Exposure via water used for aquaculture

The literature reports that movement of aquatic pathogens, including IHHNV, via organic matter, plankton, and shrimp at all life stages suspended in ocean currents (e.g., from endemically affected areas, shipping ports, processing plants, aquaculture facilities) is a plausible disease exposure pathway for susceptible farmed and wild shrimp populations. For example, genetic analysis suggests that IHHNV was introduced into wild shrimp populations in the GoC via intensive penaeid shrimp aquaculture, and introduction of other shrimp pathogens (WSSV) from local wild populations into aquaculture facilities has been reported.[10, 12, 62, 112, 148, 149] Shrimp farms that are not highly biosecure, are located on coastlines, have open ponds, and use seawater may be more prone to disease introduction via this exposure pathway.[9, 150, 151] Inland facilities and farms with good biosecurity measures are less likely to incur an IHHNV introduction via this pathway. Biosecurity measures include using deep sea water wells or underground fresh water aquifers as water sources, treating/filtering influent, covering rearing structures and ponds, ensuring that ponds are dried overwinter, and using low exchange or recirculating water systems.[150, 152] Close proximity of shrimp farms to other shrimp-rearing facilities, shrimp-processing plants, shipping ports, and susceptible wild shrimp populations, increases the risk of disease introduction as well.[5, 10, 14]

Federal and State effluent regulations are typically specific to water quality (e.g., levels of ammonia, dissolved oxygen, total suspended solids) and do not mandate testing for presence of aquatic pathogens.[38] Some facility-specific and/or State mandated BMP biosecurity measures include effluent water treatment guidelines designed to prevent releases of aquatic pathogens and animals.[38, 114] Aquaculture facilities typically treat influent water and use screens or filters at ingress points to optimize water quality and prevent introduction of aquatic pathogens or animals. Despite these control measures, the literature describes aquatic animal and pathogen introductions via inadequately treated or contaminated influent water and/or accidental intake or migration of infected crustaceans. The literature also describes aquatic pathogen introductions from aquaculture facilities to wild populations associated with release of inadequately treated effluent water and/or accidental release or migration of infected animals.[14, 112, 150] Unexpected water control failures (e.g., water treatment failures, accidental releases, weather events, overtopping of ponds, flooding) may lead to accidental exposure events as well. Given the above, it does appear IHHNV present in water used or discharged by shrimp aquaculture facilities can pose a plausible pathway of virus exposure to susceptible shrimp populations.

Pathway 6: Introduction of IHHNV-infected wild shrimp into aquaculture facilities (or vice versa)

Published literature describes escape of farmed shrimp from, and introduction of wild shrimp into, aquaculture facilities as potential mechanisms for transfer of IHHNV and other aquaculture pathogens (e.g., WSSV, YHV, TSV) into susceptible cultured or wild shrimp populations.[112, 153] Escapes or accidental releases of cultured shrimp (e.g., *P. vannamei, P. monodon, M. rosenbergii*) have been documented in continental U.S. coastal waters, Puerto Rico, and Hawaii, as have introductions of wild shrimp and other animals into aquaculture facilities.[77, 79, 82, 153, 154] Because most shrimp species cannot walk on land (except *M. dienbienphuense*) such introductions likely occurred via water as described in previous pathway. Exposure of susceptible wild or cultured shrimp to pathogens, including IHHNV, has not been confirmed via this pathway, but lack of data does not preclude the plausibility that this pathway could occur.

Pathway 7: Intentional, accidental, or malicious release

There are no national or international reports of intentional, accidental, or malicious releases of shrimp or other aquaculture species a) into shrimp aquaculture facilities; or b) resulting in introduction of an aquatic pathogen, including IHHNV. Intentional releases may include release of pet shrimp or aquatic animals, or transfer of wild aquatic animals, into aquaculture facilities.[155] Accidental releases may include release of farmed shrimp into natural water bodies or introduction of wild shrimp into aquaculture via unexpected failure of biosecurity measures, equipment, or rearing structures as described above. Malicious releases would include acts of sabotage or agricultural terrorism.[156, 157]

Shrimp farms that are not highly biosecure (e.g., lack perimeter fencing, have open ponds, are near urban areas or roadways) may be more susceptible to release of animals into shrimp-rearing structures. Farms with good biosecurity measures (e.g., perimeter fencing, limited public access, or covered or contained rearing structures) would be less susceptible to introduction of aquatic animals or an aquatic pathogen. Introduction of IHHNV or other aquatic pathogens via this exposure pathway has not been reported; however, lack of reporting does not preclude the plausibility that diseases may be introduced into an aquaculture facility via these mechanisms.

Pathways Summary

Given available data and reference materials, the most plausible pathways of entry include a) live shrimp imported for use in aquaculture; b) imported commodity and bait shrimp; and c) movement of IHHNV in water from areas of presence to locations where naïve susceptible shrimp are present. There is lack of published literature definitively linking ballast water, ship fouling, or imported commodity and bait shrimp to IHHNV entry. However, given that these are known modes of aquatic pathogen introduction, it is plausible that these pathways may lead to IHHNV introduction. Introduction of IHHNV from areas of known presence (wild and farmed shrimp) to areas where naïve shrimp populations are present has been documented, indicating that this is a plausible entry pathway. Live shrimp imported for use in aquaculture has been demonstrated to be a pathway of entry, including into the United States.

The most plausible pathway of exposure for domestic aquaculture is introduction of IHHNV-infected shrimp into facilities, given that past IHHNV introductions have occurred via imported shrimp and shrimp transferred among facilities. Presence of IHHNV in water and introductions of IHHNV-infected wild shrimp present in water are less plausible, given that aquaculture facility influent water is likely filtered and treated prior to use. However, inadvertent or accidental introduction may occur via these mechanisms due to poor biosecurity an unexpected water quality management failure. Exposures via commodity shrimp, bait shrimp, shrimp by-product, and accidental, malicious, or intentional release appear least plausible. Plausible pathways of exposure for indigenous wild penaeid shrimp include the various pathways associated with commodity shrimp imported for human consumption, imported bait shrimp, presence of IHHNV in water, and accidental release of cultured shrimp.

Potential Impacts

IHHNV is an economically relevant viral pathogen of shrimp aquaculture. Sellars et al. 2019 reported that shrimp farms stocked with high viral load IHHNV-infected post-larvae shrimp resulted in reduced performance, lower survivability, higher food conversion ratios, and production losses approximating $67,000 per hectare/2.47 acres of farm gate value compared to farms stocked with post-larvae carrying a low to zero IHHNV viral load.[7, 56] Primary shrimp-producing States include Alabama, Arkansas, California, Florida, Georgia, Hawaii, Illinois, Indiana, Kentucky, Louisiana, Mississippi, Ohio, South Carolina, Tennessee, and Texas.[120] Cultivated susceptible crustaceans reared in the United States include whiteleg shrimp (*P. vannamei*), which are a principal focus of production in U.S. aquaculture. Most broodstock is produced in Texas, Hawaii, and Florida. Texas is the leading shrimp-producing State, producing 1.68 million kg/3.7 million lb. of product in 2009. In 2007, *P. vannamei* was considered the fourth most valuable aquaculture species in Texas, following catfish, red drum, and hybrid striped bass. In 2016, the United States produced 1.8 million kg/4 million lb. of shrimp, valued at $10 million USD. In 2019, the United States exported over $123 million USD worth of shrimp products (e.g., fresh, $50 million USD; frozen, $40 million USD; and prepared, $33 million USD). The top receiving countries were India ($20 million USD), Canada ($20 million USD), Vietnam ($14 million USD), and Denmark ($12 million USD) (DATAWEB).

The impact that introduction of IHHNV might have on U.S. shrimp aquaculture depends on a) where the introduction(s) occur; b) time to detection and reporting of the introductions; c) the number and type of aquaculture facilities affected; d) the type of control measures implemented; and f) surveillance and biosecurity measures used by the industry as preventative and outbreak response measures. The United States reported two disease events between January 2019 and January 2020. Both events were controlled with quarantine and voluntary depopulation measures. These IHHNV detections had serious consequences on domestic reputations and international trade, including effectively closing the shrimp export market to Canada since 2019. If a large-scale outbreak occurred within the industry, it is plausible that significant economic and export trade consequences may occur.

Lack of capability to perform parallel or series surveillance or diagnostic testing for IHHNV and EVE genomic material could lead to transboundary or domestic a) movement of apparently healthy IHHNV-infected farmed shrimp; b) culling of healthy non-IHHNV infected shrimp with EVE sequences. Both potential outcomes could cause local primary economic impacts to shrimp producers, secondary impacts to associated industries and could result in international import/export consequences.

Assessment of the effect of IHHNV introduction into native penaeid shrimp populations was not within the scope of this document. The effect that such introduction may have is not known due to lack of data on species susceptibility to IHHNV and other factors. If native penaeid shrimp are susceptible to IHHNV and if environmental conditions are favorable to sustaining an outbreak, local and regional consequences may be impactful. Environmental consequences may be direct to the affected shrimp populations and could indirectly disrupt food webs and the overall ecology of affected areas. Local fisheries that harvest wild shrimp would be economically impacted.

Limitations

There are data gaps present for every pathway identified in this document that limited capability to fully assess their individual potential for transboundary entry or animal exposure. Unless resolved, these data gaps will affect capability to develop future transboundary or domestic risk assessments or risk analyses and to develop a domestic response plan.

Data and resource gaps include, but are not limited to:

- Lack of research relative to the mechanisms of virus infection and virulence in crustacean species
- Lack of research and development of diagnostic modalities relative to EVE sequences
- Lack of data reporting on IHHNV surveillance in domestic aquaculture
- Lack of data reporting on IHHNV surveillance in indigenous penaeid shrimp populations
- Lack of published research exploring IHHNV accommodation and tolerance in affected shrimp species
- Incomplete understanding of the effect of vial loads and performance parameters in shrimp that exhibit viral accommodation or tolerance
- Lack of experimental challenge studies meeting WOAH criteria for natural exposure evaluating the susceptibility of farmed and native shrimp to IHHNV
- Lack of data reporting on the volume and disposition of live shrimp imported for aquaculture purposes
- Lack of data reporting on the domestic transit of live shrimp for aquaculture purposes
- Lack of data on surveillance for IHHNV in ballast water and fouling present on ship hulls
- Lack of data and hydrological modelling studies evaluating the potential for movement of IHHNV from a) potential points of entry to susceptible wild and cultured shrimp population; b) known areas of IHHNV presence into the coastal waters of the United States
- Lack of data reporting IHHNV presence in imported commodity and bait shrimp
- Lack of data on disposition pathways for commodity shrimp, including waste streams
- Lack of data on the volume and disposition pathways for imported bait shrimp
- Lack of data on the volume of imported commodity, bait shrimp, or shrimp by-product used as food for aquatic animals
- Lack of data regarding aquaculture facilities' BMPs, biosecurity measures, disease surveillance practices, and other factors
- Lack of studies modelling potential distribution of IHHNV-susceptible penaeid shrimp species and the IHHV virus

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