Journal of Zoonotic Diseases
https://jzd.tabrizu.ac.ir/
Published by the University of Tabriz
Online ISSN: 2717-2910

Transmission of zoonotic infections (bacteria, parasites, viruses, and fungi) from aquaculture to humans and molecular methods for organism identification

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Article type:
Review article

Keywords:
Zoonosis
Fishes
Human
Aquaculture
Fungus
Parasites

Article history:
Received:
May 30, 2024
Revised:
July 1, 2024
Accepted:
July 2, 2024
Available online:
August 10, 2024

Abstract

This review investigates the different zoonotic infections transmitted from fish to humans, focusing on microorganisms such as bacteria, parasites, viruses, and fungi. The study aims to elucidate the pathways through which these infections occur, the severity of the diseases in humans, and the clinical manifestations in fish and humans. The primary transmission methods include ingesting undercooked fish, contact with contaminated water, and exposure through open wounds. Notably, zoonotic bacteria, such as *Vibrio*, *Mycobacterium*, *Streptococcus*, and *Aeromonas*, present significant health risks and economic impacts on aquaculture. Parasitic infections, such as those caused by *Anisakis* and liver flukes, often result from consuming raw or undercooked fish, leading to gastrointestinal and systemic diseases. Viral pathogens like noroviruses and hepatitis A virus are primarily transmitted via contaminated seafood. Though less common, fungal infections can occur through direct contact with infected fish or the environment. This review emphasizes the importance of molecular diagnostic techniques for detecting zoonotic agents and highlights preventive measures to mitigate the risks. Effective management strategies include educating those in fish handling, ensuring proper cooking and handling practices, and implementing robust monitoring and quality control systems within aquaculture settings.

Introduction

An infectious illness called zoonosis is transferred from animals to humans. (1). Numerous organisms that cause illnesses, including parasites, viruses, fungi, and bacteria, can spread from animals to people by a variety of means, such as animal bites, ingestion, damaged or irritated skin, and vectors (insects), as well as interactions between animals and human i.e. Breathing in respiratory particles or touching the skin or mucous membranes) (2). The severity of aquatic zoonotic infections largely depends on the immune system. But there are

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https://doi.org/10.22034/jzd.2024.18311
https://jzd.tabrizu.ac.ir/article_18311.html
Cite this article: Alsulivany BSA, Abdolrahman PMS, Ahmed DV, Naif RO and Omer EA. Transmission of zoonotic infections (bacteria, parasites, viruses, and fungi) from aquaculture to humans and molecular methods for organism identification. Journal of Zoonotic Diseases, 2024, 8 (4): 580-591

primarily two ways that these illnesses might affect humans. First, they eat undercooked fish and drink water or other liquids tainted with fish excrement or mucous. Second, if you have open wounds or skin abrasions and come into touch with an infectious agent (3). 46% of the zoonotic illnesses from fish are spread orally, and 15% are spread through several channels. Skin contacts when handling fish contributes to 19% of transmission, whereas drinking water contaminated by infected organisms accounts for 24%. Two main families of bacteria are the predominant zoonotic agents discovered in fish. However, a tiny percentage of these bacteria are Gram-positive, whereas others are Gram-negative (4). The clinical signs and symptoms observed in infected fish, because so many distinct bacterial species can cause the disease and various host species are affected (5)**.** Viruses are a dangerous type of foodborne illness that can cause outbreaks and individual instances. In addition to being parasites of humans, animals, and plants, parasites frequently exist as saprophytes in soil and decaying organic matter. Fungi often found in the environment can frequently lead to fungal illnesses. When a naturally occurring zoonotic fungus spreads from animals to people, it can seriously harm public health (6). People who work with fish or are involved in fish-related activities need to know about zoonotic diseases and how to prevent them. Since it is challenging to restrict all interactions with water and fish inside aquaculture systems, reducing the risk of zoonotic infections is crucial (7). Numerous parasite species, many transmissible to humans, may be found in several kinds of edible fish. Many genetic techniques have been used to quickly identify zoonotic pathogens and reduce the likelihood of people becoming infected. Molecular approaches provide diagnostic strategies that are both quicker and more accurate than conventional treatments (6)**.**

Table. 1 Common organisms and their route of transmission to the host

No	Outbreak name	Pathogen	Host	References
1	Eating smoked fish has been connected to two listeriosis outbreaks.	*Listeria monocytogenes*	Smoked fish	(7)
2	Eating raw freshwater fish was connected to a 2015 outbreak of severe Streptococcus agalactiae sequencing type 283 infections in Singapore.	*Streptococcus agalactiae*	Asian bighead carp (*Hypophthalmichthys nobilis*) and snakehead fish (*Channa* species)	(7)
3	There was a tularemia outbreak connected to crayfish fishing	*Francisella tularensis*	Crayfish	(6)
4	Following the ingestion of fish, two outbreaks of botulism illnesses were documented in Germany and Norway.	*Clostridium botulinum*	Rak fish	(6)
5	The Netherlands saw a significant Salmonella Thompson epidemic connected to smoked salmon	*Salmonella enterica*	Smoked salmon	(5)
6	Gnathostomiasis suddenly sprang out in a fishing village in Sinaloa, Mexico.	*Gnathostoma binucleated*	Spotted sleeper perch (*Eleotrispicta)*	(5)

Bacteria Cause Zoonotic Infections

The primary zoonotic agents found in fish are bacteria, categorized into two main groups. Most of these bacteria are Gram-negative, with only a small proportion being Gram-positive. Bacterial infections can be present in fish that appear healthy, especially in their intestines and kidneys (7). Certain bacteria are only harmful to humans, which means they might cause common illnesses with minimal symptoms. Of these bacteria, *Escherichia coli* and *Vibrio cholerae* are only dangerous to humans and do not show symptoms in fish (8). It is well acknowledged that infections with *Vibrio* and *Mycobacterium* species are the main source of financial losses and are occasionally portrayed as the factor restricting fish productivity (9). Mycobacteriosis is a disease that is common in freshwater, brackish water, and marine fish. It is known to be a major cause of fish population death in both farmed and wild fish (10). Because so many different bacterial species are implicated and so many different host species are impacted, the clinical signs and symptoms seen in infected fish vary (7). Fish with Mycobacterium species infections usually show particular symptoms, such as enlarged kidney, spleen, and liver, along with internal organ nodules. Lethargy, changes in pigmentation, distention of the abdomen, bulging eyes, exophthalmia, skin lesions, and death are possible further symptoms (6). Affected fish may experience both acute and chronic disease as a result of these illnesses (7).

Streptococcaceae are known to be newly discovered zoonotic agents that may infect humans, especially when they come into touch with sick fish. (7). Direct contact with sick or dead fish and indirect contact with tainted water are the two ways that the sickness can spread to people. (11). The illness's clinical symptoms differ according to the kind of fish affected, but frequent ones include exophthalmia (protruding eyes), stomach distention, disorientation, erratic swimming, anorexia, opacity in the eyes, darkening, and hemorrhagic skin, and finally demise. According to studies on Streptococcus pathogenicity, tilapia's gastrointestinal system is where *S. agalactiae* primarily enters the fish, having the bacteria able to penetrate intestinal and mucosal layers. (12). The virulence factors of Streptococcus include secreted compounds, polysaccharides in capsules, and surface proteins. Human fibrinogen can attach to the surface proteins of bacteria. Thus, preventing the functioning of phagocytes. Furthermore, these surface proteins can attach to immunoglobulins (13). Complement component C5A and the interleukin-8 chemokine are broken down by the bacterial peptidase C5 and protease, respectively. This interferes with chemotactic signals and prevents phagocyte recruitment. The bacteria's streptolysin kills neutrophils, erythrocytes, and lymphocytes. Furthermore, increased adhesion and tolerance to harmful chemicals are facilitated by the creation of extracellular exopolysaccharides and polysaccharides surrounding the cell. Bacterial fibrin clots are broken down by the α-enolase enzyme, which promotes bacterial spread.

Fish zoonoses are associated with the Gram-positive bacteria *Erysipelothrix*. It is connected to marine animals and can cause acute sepsis or skin conditions (14). Both humans and animals can contract *Erysipelothrix*, a disease that mostly affects the skin, connective tissues, and vascular walls. Cellulitis, myositis, and necrotizing dermatitis are possible clinical symptoms (15). When fresh or dead fish come into direct touch with human mucus, which carries the germs, humans may contract the bacterial illness (14).

Aquaculture experts and users of aquatic goods are in danger of contracting vibriosis from gram-negative bacteria known as Vibrio species (16). Both freshwater and brackish settings are home to a variety of Vibrio species, which may infect humans through skin sores and contaminated seafood consumption. Three bacteria, *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* cause illnesses in humans. Fish with Vibrio infections may exhibit

nonspecific clinical symptoms such as lethargy, skin lesions, exophthalmia, and even death (6). Additionally, other symptoms have been documented, including a bloated spleen, intestinal inflammation, abdominal dropsy, epidermal hemorrhage, scale shedding, pop-eye, and tail rot. The three primary phases of vibriosis are usually as follows: the bacterium enters through the skin, fins, gills, and anus; tissue and host cell damage follow; and finally, the bacteria exit the host. These phases may have a detrimental effect on the host and even result in death. Siderophores, extracellular products (ECPs), hydrolytic enzymes, and toxins are a few of Vibrio's virulence factors. The interaction of the virus, host, and environment determines resistance to vibriosis, yet instances of almost 100% fish death with some Vibrio species exist (17). Vibrio species transmission from fish to humans can cause erythema, septicemia, lesions, and tissue necrosis, among other illnesses (18). *V. vulnificus* is a significant zoonotic pathogen of public health concern. It is known to cause primary septicemia in humans following the ingestion of raw shellfish when raw shellfish is consumed by humans, it is known to induce primary septicemia. Furthermore, exposing wounds to seawater might result in subsequent septicemia (19).

Another Gram-negative bacterium that affects fish is Aeromonas, and unless environmental stress and weakening happen, infections with this pathogen usually show no symptoms. (20). Aeromonas is mostly transmitted to people through fish. (20). Fish with Aeromonas infection may exhibit petechiae in the skin and fins, skin ulcers, arrhythmias, anorexia, exophthalmia, and stomach enlargement as clinical signs (21). Although these illnesses are uncommon, individuals can get Aeromonas species by ingestion or ulceration. Individuals may experience septicemia, cellulitis, and muscular necrosis as clinical outcomes. (22). Edema and swelling at the injection site are examples of clinical indications of the illness in humans. (14) Aeromonas can also cause diarrhea, bacteremia, gastroenteritis, lung infections, sepsis, and urinary tract infections in people. (23).

Food poisoning can also be caused by the opportunistic gram-negative pseudomonas. (6) Unusual bleeding on the skin's surface, exophthalmia, cloudiness in the eyes, separation of the scales, skin darkening, clogged gills, ulceration, distention of the abdomen, and ascites are among the clinical symptoms seen (24). Numerous animals' gastrointestinal tracts contain the ubiquitous bacteria Campylobacter, which is regarded as a zoonotic agent. (25). It is unusual for eating fish products to cause campylobacteriosis. On the other hand, drinking untreated water and coming into interaction with polluted work surfaces or the hands of a food handler might potentially expose one to *Campylobacter jejuni* infection (26). The bacteria that cause pneumonia, or legionnaires' disease, is called *Legionella pneumophila*, and it is spread via aerosols and water. Additionally, a patient who worked at a seafood shop has been isolated from it (27). The fish microbiome contains members of the Enterobacteriaceae family, which may be responsible for human illness. Species belonging to this family are accountable for several human illnesses. (6). These gram-negative bacilli are frequently discovered in fish digestive systems and aquaculture. (14).

Parasites Cause Zoonotic Diseases

The parasite was first discovered in fish roundworms (*Anisakis spp*.), flukes (*Metagonimus Yokogawa*), and tapeworms (*Dibothriocephalus Tatum*). The main way that people get these parasites is by eating raw or undercooked fish or fish products (28). Several species of edible fish are home to a variety of parasites, many of which are transmissible to humans (5). At least 680 million individuals are in danger of catching freshwater fish liver flukes, of which an estimated 45 million people currently have the infection (29). When it comes to parasites that affect seafood, helminthic species are of great concern because of their diversity and prevalence in tropical aquatic

environments. As a result, their transfer to fish happens often (30).

Fish parasites belonging to more than 40 species have the potential to infect humans. Some are rare, but others have the potential to be extremely pathogenic and seriously endanger public health (31). The severity of parasitic infections originating from fish might vary, presenting as allergic responses or gastrointestinal problems such as dyspepsia, and diarrhea. In extreme situations, they might result in harmful illnesses including cancer, hemiparesis, and cerebral bleeding**.** Fish-derived zoonoses are caused by many genera of trematodes (flukes) that are members of the *Opisthorchiidae* and *Heterophyidae* families (32). Chronic infections and high liver fluke loads can cause inflammation and damage to the bile duct's epithelium, which can result in liver damage and gastrointestinal problems (33). May result in serious clinical issues such as pancreatitis, choledocholithiasis, cholangiocarcinoma, and cholangitis (34).

The lung fluke illness, known as paragonimiasis, affects people who eat freshwater crab or crayfish transmitting the fluke's metacercariae (35). Infections with nematodes usually arise after consuming raw fish or shellfish in freshwater environments (31). Risk factors for humans and other animals may include trematode infections and the spread of pollutants in the environment (36). Cestodes, or tapeworms, are another widespread class of fish parasites. They may reach significant lengths of up to 20 meters, unlike trematodes. Diphyllobothriosis is a disease that may result from this. Diphyllobothriosis is usually not life-threatening and is a minor condition. Although most infected people don't show any symptoms, some may, including diarrhea, stomach discomfort, anemia, weight loss, and vitamin B12 insufficiency (37). It is believed that diphyllobothriosis affects up to 20 million individuals worldwide (38).

Worldwide records of human illnesses resulting from nematodes originating from fish exist, although only a small number of these illnesses seem likely to emerge in the future (39). When squid or fish is eaten raw or incorrectly prepared, it can infect humans. The diseases this seafood can cause can be fatal, especially for those who are afflicted (40). During their infectious larval stages, nematodes show no regard for their host. Even in the fish's digestive tract, the fish nematode larvae can pass through the mucosa of the gastrointestinal tract and enter the viscera and surrounding muscle tissues (41). Indeed, these nematodes can still be dangerous to human health long after the fish has perished (42).

The word "anisakiasis" or "anisakiasis" refers to the parasitic illness that nematodes of the genus Anisakis induce in humans (43). Anisakiasis refers exclusively to the sickness produced by members of the genus Anisakis, whereas anisakidosis refers to the disease caused by any member of the family *Anisakidae*. Anisakis simplex sensu lato members are frequently the cause of anisakidosis (40). Eating raw or undercooked fish and fish products can increase the risk of spreading zoonotic fish parasites (44).The location of the parasite in people and the histology of the related lesions might affect the symptoms of anisakiasis (45). Individual differences exist in the duration of symptoms, which can range from days to months. Usually, when the parasite is excreted or regurgitated spontaneously from the body, the symptoms go away. (46) or extracted by surgery (47). Another extremely serious nematode infection is Gnathostomatidae, which may be contracted by eating raw or undercooked foods like sushi or ceviche that contain fish species from both fresh and brackish waters, as well as other freshwater creatures like eels and frogs. Gnathostomiasis is a disease caused by parasites that is contracted by consuming infective larvae (48). Usually appearing 24-48 hours after transmission, the clinical signs of gnathostomiasis include nausea, vomiting, and stomach discomfort (49).

Viruses Cause Zoonotic Diseases

There is a growing recognition that acute gastroenteritis, caused by Noroviruses (Nov), is a serious foodborne infection that can produce both isolated cases and outbreaks. One frequent method of transmission is the ingestion of shellfish and ready-to-eat fisheries products tainted with feces. This problem poses a serious threat to public health and has resulted in significant global financial losses (50). Additional classification of noroviruses is done into seven genogroups, GI through GVII (51). Gastroenteritis mediated by NoV can cause vomiting, nausea, watery diarrhea, and abdominal discomfort as clinical symptoms. Lethargy, weakness, headaches, pains in the muscles, low-grade fevers, and loss of taste are possible additional symptoms. The onset of symptoms usually occurs 12- 48 hours after eating the tainted meal. The illness is often self-limited, meaning it goes away on its own (51). On the other hand, those who are immunocompromised may contract the virus over an extended period, which might result in enteropathy and malabsorption. Hepatitis, which is characterized by inflammation of the liver, can be contracted by consuming fresh and frozen foods, such as fish, bivalves, and water infected with the hepatitis A virus (HAV). Fatigue, nausea, vomiting, diarrhea, jaundice (yellowing of the skin and eyes), dark urine, fever, arthralgias (joint pain), and myalgias (muscle pain) are among possible manifestations of this. The duration of the symptoms might be many weeks or months. Nonetheless, rare instances may result in liver failure or even death, especially in the elderly and those suffering from chronic liver disease (6).

Fungus Causes Zoonotic Diseases

Fungi are categorized as non-photosynthetic microorganisms. They are parasites of people, animals, and plants. Of the 1.5 million fungal species, only 300 are known to be harmful to humans. Common fungi present in the environment can often cause fungal diseases. A naturally occurring zoonotic fungus can create major public health problems by spreading from animals to humans (6). An uncommon fungal infection called Basidiobolomycosis is brought on by the fungus *Basidiobolus ranarum*. This causal agent is often found in the environment as a saprophyte that is widely distributed and separated from decomposing plant matter, food, soil, fruits, and deciduous tree leaves (52). Usually, the illness manifests as a gastrointestinal and subcutaneous infection (53). This fungus causes spores to enter the body through skin scratches and grow slowly, resulting in the formation of an enlarged, hard node beneath the skin, often in the arms and legs (54). Another way, these zoonotic fungi spread is by the ingestion of infected food or soil (52). If treatment is not received, the infection may spread to deeper tissues and affect vital organs including the brain, which might result in the patient's death (6).The fungus *Sporothrix schenckii* is the cause of the fungal illness known as Sporotrichosis (55). Typically, exposure to soil, organic materials, and plants tainted with fungi causes infection. The fungus can spread through activities including farming, fishing, hunting, gardening, and other similar pastimes (55). Insect bites and scratches from animals including squirrels, dogs, cats, horses, rats, and birds can potentially spread the fungus (56).

Molecular Techniques for Identifying Agents that Cause Zoonotic Diseases

Many genetic methods have been used to quickly detect zoonotic bacteria and reduce the risk of human infection. When compared to traditional procedures, molecular techniques provide faster and more accurate diagnostic methodologies. For the majority of pathogenic zoonoses, polymerase chain reaction (PCR) is frequently the principal molecular method employed for detection. Additional PCR-based methods, including droplet digital PCR, multiplex-PCR, and real-time (RT)-PCR, are used for the quantitative, comprehensive, and simultaneous detection of various zoonotic bacteria. Aside from restriction fragment length polymorphism (RFLP) and random amplification of polymorphic DNA (RAPD), additional methods have been used to identify genetic diversity and

differentiate microorganisms. Without specialized equipment, isothermal approaches have been used to detect bacteria in isothermal environments. These methods include rolling circle amplification (RCA), nucleic acid sequence-based amplification (NASBA), recombinase polymerase amplification (RPA), and small molecule accurate recognition technology (SMART). These methods allow for the sensitive and quick detection of almost two-thirds of zoonotic bacteria. Among molecular approaches, some biosensors have been used as the best, most economical, and fastest detection methods. A few of these biosensors have several applications, which increases their adaptability. These biosensors are useful for identifying zoonotic bacteria and include quartz crystal microbalance (QCM)-based biosensors, fiber optic microchannel biosensors coupled with photolithography, and electrochemical immunosensors (8).

Management and Control of Zoonotic Diseases

Individuals engaged in fish-related activities must be knowledgeable about zoonotic illnesses and how to avoid them. As limiting all interaction with water and fish inside aquaculture systems is difficult, preventing zoonotic illnesses is essential for minimizing hazards (6). Microbial agents present in fish can pose significant public health concerns. Therefore, it is crucial to educate the public about microorganisms and the risks associated with consuming raw or undercooked fish Quality control measures and routine monitoring of consumed fish are essential is important to implement quality control methods and regularly check fish consumption. This provides important information for the prevention and treatment of aquatic zoonotic agents and enables quick and effective disease control (6). Since fish are raised in systems where natural environmental variables have an impact on their production, controlling fish zoonotic pathogens presents issues. Fish health is significantly influenced by environmental variables since the degradation of the aquatic environment is a major cause of many fish illnesses. In reality, the environment is a major component of fish health (57).

Certain nematode species can have their life cycle disrupted by cleaning and sterilizing ponds, which effectively lowers the population of intermediate hosts. Ponds that have not been cleaned and sterilized before being refilled are more likely to have significant numbers of intermediate hosts (36). Fish-derived illnesses in communities can be caused by a wide range of factors, such as geographic location, availability of fresh seafood, sanitary standards, methods for handling fish, and dietary patterns. The frequency of illnesses resulting from fish is also significantly influenced by societal and personal habits (58). Antibiotic therapy is frequently used for bacterial zoonotic diseases. Antibiotics function as a means of controlling certain zoonotic factors (59).

Humans can become infected with multidrug-resistant animal diseases by eating contaminated food in cases of foodborne zoonoses. Monitoring multidrug-resistant bacteria in humans and animals is essential to addressing this basic problem and ensuring that community actions are coordinated. Furthermore, doctors, veterinarians, and environmental specialists must work closely together (60). Important precautions include wearing disposable gloves and protecting your skin to prevent direct contact with fish mucous. But regular handwashing is the most effective approach, especially after coming into direct contact with fish and water. It's also important to avoid eating or drinking anything just before washing your hands. In addition to contamination from inanimate things, direct or indirect contact with vectors, such as insects, can result in the spread of zoonotic illnesses. Additional possible modes of transmission include ingestion and inhalation (14). Fish can be cooked at 62°C for 15 seconds to eradicate parasites, however, some bacterial toxins may not be sufficiently detoxified at this time (6).

The most efficient means of lowering danger is by freezing or heat inactivation (61). When they have open sores or abrasions, veterinarians and fish

workers should always take steps to protect themselves by limiting their exposure to water. Fish workers need disposable gloves to protect themselves when doing a variety of tasks, such as handling fish feces or tissue or mucus. Gels, tissue glue, and topical ointments, such as triple antibiotic and silver sulfadiazine, can be administered to surface wounds for further protection in circumstances when water contact is unavoidable. Deep penetration injuries should be cleaned as quickly as possible after they occur, preferably with salty water or regular water. After that, the wound has to be properly cleaned with substances like alcohol, hydrogen peroxide, betadine, or chlorhexidine. Severe bruising should be treated right away since it provides a higher risk. It is crucial to warn clients about zoonoses while working with fish without overstating the risks. A doctor can receive a thorough medical history from informed patients if a possible fish-derived zoonosis is identified. Veterinarians must interact with patients, staff members, and healthcare institutions to inform and support the management of fish-derived zoonoses (62). Despite the increasing attention to this neglected disease, several traditional challenges persist limited treatment options, inadequate diagnostics, and insufficient community awareness (63).

Conclusion

In conclusion, zoonotic diseases originating from aquatic environments pose significant public health challenges. The transmission of these diseases occurs through various pathways, including ingestion, skin contact, and exposure to contaminated water. Effective management and prevention require a multidisciplinary approach, involving education, proper hygiene practices, and stringent quality control measures. Awareness and understanding of zoonotic pathogens are crucial for individuals involved in fish-related activities. Additionally, molecular techniques provide rapid and accurate detection, aiding in the control and treatment of infections. Future efforts should focus on enhancing diagnostic methods, promoting community awareness, and developing effective treatment options to mitigate the impact of zoonotic diseases from fish. To reduce the effects of zoonotic diseases, several actions can be suggested. First, public education campaigns should be launched to raise awareness about the effects of consuming raw or undercooked fish and the importance of maintaining proper hygiene. Second, regular monitoring and quality control of fish products are crucial to detect and prevent the spread of zoonotic pathogens. Third, enhancing diagnostic capabilities with advanced molecular techniques can enable faster identification and treatment of infections. Finally, the collaboration between veterinarians, healthcare providers, and environmental specialists is essential for the effective management and prevention of zoonotic diseases.

Acknowledgments

Not Applicable.

Conflict of Interest

The authors reported no potential conflict of interest.

Ethical Approval

Not Applicable.

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