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Presence of *Salmonella sp.* In Tilapia and Catfish from Cages in Pahandut Seberang Along the Kahayan River Flow

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Abstract

Salmonellosis is a *zoonosis,* which means that it may be spread from animals to people through fish meat. It is a sickness brought on by an infection with the *salmonella* bacteria that infect humans and animals and invade the digestive tract. According to data from the Institute for Health Metrics and Evaluation, the global rate of *salmonellosis* in 2019 was 4.22 cases per 100,000 people, with a 1.72 fatality rate. Indonesia had a mortality rate of 2.42 and 5.82 per 100,000 people 2019. The objective of this study is to identify *Salmonella* sp. bacteria in 14 samples of river water, catfish (*Clariidae*), and tilapia (*Oreochromis niloticus*) raised in rivers. This study includes two water samples, three catfish samples, and nine tilapia samples from the cage of the Pahandut Seberang River in Palangka Raya City. It employs a descriptive research design using an experimental technique. Among the metrics noted is the detection of *Salmonella sp.* The study found that all water samples, tilapia, and catfish tested positive for *Salmonella sp* bacteria in the selective media Salmonella Shigella Agar (SSA), that 14 samples (100%) in the gram staining test displayed the traits of *Salmonella sp* bacteria, and that 5 samples (B N1, C N2-3 samples in tilapia, B L1 samples in catfish, and water sample 2 showed the type of Salmonella paratyphoid b bacteria) and 9 samples, namely samples *Salmonella tyhpimarium* bacteria were found in samples A L1, C L1 in catfish, B N2-3, C N1 in tilapia, and water 1. As a result, every sample surpassed the SNI 7388:2009 maximum level of microbial contamination, which is positive/25g. According to the Indonesian National Standardization Agency's SNI 7388:2009 guidelines for the upper limit of microbial contamination in food, the tilapia meat, catfish, and river water in the river cage of Kahayan Pahandut Seberang Palangka Raya City do not satisfy the requirements.

Keywords: Catfish; River Water; *Salmonella* sp; SNI; Tilapia.

Abbreviations: *Salmonella Shigella* Agar (SSA); Simmon Citrate Agar (SCA); Sulfide Indol Motility (SIM); Triple Sugar Iron Agar (TSIA); Standar Nasional Indonesia (SNI), Foodborne Disease (FBD); Water Borne Disease (WBD).

INTRODUCTION

Salmonellosis is a disease that affects the stomach, small intestine, and large intestine and is caused by a *Salmonella* bacterial infection in humans and animals. Salmonellosis is a zoonotic disease, meaning that humans can contract this disease from animals (Rima et al., 2017). Humans can acquire Salmonella by consuming various products from infected animals. The spread of this disease is caused by animal and human feces contaminating the environment. Although there have been many reported cases of salmonellosis in wealthy countries, the percentage of reported cases remains low compared to the actual outbreaks. Worldwide, *Salmonella sp*. infections and contamination are widespread. According to data from the Institute for Health Metrics and Evaluation, the mortality rate for salmonellosis was 1.72, and its prevalence rate was 4.22 per 100,000 people globally in 2019. In 2019, Indonesia's mortality rate was 2.42, and its prevalence rate was 5.82 per 100,000 people. No documented cases of salmonellosis have been reported in Palangka Raya City or Central Kalimantan Province (Central Statistics Agency, 2022).

The area of 133,564.5 km² and public water area of 134,791.08 hectares, Central Kalimantan, whose capital is Palangka Raya, is one of the regions with significant fisheries potential due to its abundant public waters. In 2022, fish capture in the Palangka Raya river waters reached 1,544.7 tons and has been utilized. The river basin areas are used by communities around the rivers of Central Kalimantan, particularly Pahandut Seberang, for various purposes, including household activities, industry, irrigation, agriculture, and fisheries. Additionally, the river basin also serves as an ecosystem for living organisms (Central Statistics Agency, 2022). Fish, aquatic plants, benthos, plankton, crustaceans, gastropods, and periphyton are among the organisms that inhabit the river benthos (Aini, 2018). The waste in rivers can be caused by various human activities, including those from the household, industrial, and agricultural sectors (Estining & Solichim, 2018).

The continuous disposal of waste into rivers contributes to the decline in river quality. Both organic and inorganic waste from various activities around the river can act as pollutants. Plant debris, animal waste, food scraps, agricultural waste, and human excrement are all considered forms of organic waste. Meanwhile, inorganic waste may originate from factories, tin smelting industries, plastic use, diesel, chemicals such as detergents, and other sources carried by river currents. The biological, physical, and chemical characteristics of the river can be disrupted and altered by this waste pollution, leading to contamination. The accumulation of waste from various activities along the river, from upstream to downstream, including the river flow, can cause a decline in the water quality in the Pahandut Seberang subdistrict. Contaminated waste in sediments and aquatic biota can contribute to the development of bacteria or other microorganisms (Fauzia, 2021). One such microorganism, known as *Salmonella sp*., is present in large numbers and is used as an indicator of waste pollution (Fauzia, 2021).

Because Salmonella sp. bacteria are pathogenic and harmful to human health, particularly for those who consume fish contaminated with *Salmonella sp* (Imamah & Efendy, 2021). Their presence in fishery products is undesirable. The community's well-being has significantly improved due to the fish in the rivers of Central Kalimantan, particularly for those who catch fish in the Pahandut Seberang River. Several fish species, such as catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*), are abundant in the waters of the Pahandut Seberang River and hold significant economic value as food and savings (Mailoa et al., 2019). The development of bacteria in fishery products can lead to unfavorable physical and chemical changes, making the product unfit for human consumption.

According to SNI 01-2332.2-2006, food safety quality requirements for microbial contamination of Salmonella sp. must be negative. *Salmonella sp*. is an indicator of food safety and can be a sign of microbial contamination, particularly Salmonella sp. bacteria found in fish in aquatic environments. *Salmonella sp*. bacteria are very dangerous to human health. Worldwide, foodborne diseases are largely caused by the *Salmonella* genus (Purlianto, 2015). Salmonella sp. infections in humans are a significant public health issue, causing 93.8 million cases and 59,100 deaths annually worldwide genus (Purlianto, 2015). *Salmonella sp*. is commonly found in river water through waste, making the water where fish are cultured highly susceptible to this infection (Purlianto, 2015). Based on the above definition, fish is one of the most widely consumed and needed food sources, so its safety must be maintained appropriatelly. Food safety refers to the actions and measures taken to ensure that food is free from biological, chemical, or other pollutants that could contaminate and pose health risks (Putri & Kurnia, 2018).

To prevent food poisoning or foodborne diseases (FBD), which are caused by food contamination with harmful bacteria or microorganisms, it is crucial to assess the safety and quality of fresh fish using microbiological testing.To ensure the water quality of the Pahandut River against the risk of Waterborne Diseases (WBD) in fish farming and—fish survival, research on air quality assessment in catfish and tilapia farming areas along the river against microbiological parameters is essential. *Salmonella sp.* should also be identified in tilapia and catfish species raised in the Pahandut Seberang River to determine whether the fish are contaminated with microorganisms and to ensure their safety for human consumption. Researchers are encouraged to study the presence of Salmonella sp. bacteria in tilapia and catfish species raised in the Pahandut Seberang River, as there is currently a lack of information on this topic.

MATERIALS AND METHODS

Study area

The study occurred in the Faculty of Medicine Palangka Raya University between June and Desember 2024. Samples of tilapia and catfish were tested in the laboratory at Palangka Raya University to determine bacteriological results. The population in this study consists of river water, Nile tilapia, and catfish cultivated in the Pahandut Seberang River. The samples used in this study include live Nile tilapia, catfish, and river water from the cultivation site in Pahandut Seberang.

Procedures

Salmonella sp Testing on SSA Media

Sterilize the inoculating loop and cool it down. Collect a fish sample using the inoculating loop, then sterilize the SSA media. Open the press and streak the inoculating loop across the SSA media. Place the SSA media in an incubator for 24 hours. Observe the colonies growing on the SSA media.

Gram Staining

Sterilize the inoculating loop and SSA medium. Take a small amount of colony and spread it gently on a glass slide. Air-dry the smear and fix it by passing it over a flame 2–3 times. Place the glass slide on a staining rack and flood it with crystal violet for 1 minute. Rinse with distilled water, then flood with iodine for 1 minute and rinse again with distilled water. Flood with 95% ethanol for 30 seconds, rinse with distilled water, and finally flood with safranin for 20 seconds. Rinse with distilled water and examine.

Triple Sugar Iron Agar (TSIA) Test
The TSIA test is used to identify gram-negative bacteria based on their ability to ferment glucose, sucrose, and lactose, as well as their capacity to produce hydrogen sulfide (H2S). The media used includes slant and stab media. Specific responses for *Salmonella sp* in the TSIA test include a red slant indicating an alkaline reaction and a yellow butt indicating an acidic condition. Additionally, black precipitate formation suggests the production of hydrogen sulfide (H_2S).

Sulfide Indole Motility (SIM) Test
The SIM test is used to determine bacterial motility. For the indole test, a negative result is observed such as forming a green ring, indicating no bacterial isolates producing indole. This shows the bacteria cannot produce indole due to the absence of tryptophan as an energy source. The indole test is significant because only certain bacterial varieties can produce indole, and this product can be studied for identification purposes.

Simmon Citrate Agar (SCA) Test
A positive result in the Simmon Citrate Agar (SCA) test is indicated by a color change in the media from green to blue, showing that the bacteria can grow using citrate as the sole carbon source. Generally, *Salmonella sp* produces a positive result in the citrate test. However, *Salmonella typhi* does not use citrate as a carbon source.

Data analysis
The steps of data analysis are explained as follows:
1. The results on the selective medium Salmonella Shigella Agar (SSA) were observed for the presence of bacterial colony growth of *Salmonella* sp. The results were then presented in tabular form and described descriptively according to the provisions of SNI 01.2332.2-2006, which outlines the method for testing the determination of *Salmonella* sp. bacteria in fishery products.
2. The results of Gram staining were used to identify the characteristics of *Salmonella* sp. bacteria, while biochemical testing was performed to determine the specific type of *Salmonella* bacteria found.

RESULTS AND DISCUSSION

Results of Selective Medium Salmonella Shigella Agar (SSA) Test
An evaluation of the maximum microbial contamination limits was conducted by SNI 7388:2009, based on research carried out on tilapia, catfish, and river water samples taken from floating net cages in the Kahayan River at Pahandut Seberang, Palangka Raya City. Using Selective Salmonella Shigella Agar (SSA) media, *Salmonella sp*. bacteria were specifically found to be positive in samples of 25g, as shown in Table 1 below.

Table 1. Results of Selective Medium Salmonella Shigella Agar (SSA) Test.

Location	Fish Type	Sample	Result
Fish Cages in Pahandut Seberang River	Tilapia	A 1	Positive
		A 2	Positive
		A 3	Positive
		B 1	Positive
		B 2	Positive
		B 3	Positive
		C 1	Positive
		C 2	Positive
		C 3	Positive
	Catfish	A 1	Positive
		B 1	Positive
		C 1	Positive
	River Water	Water 1 (Upstream)	Positive
		Water 2 (Downstream)	Positive

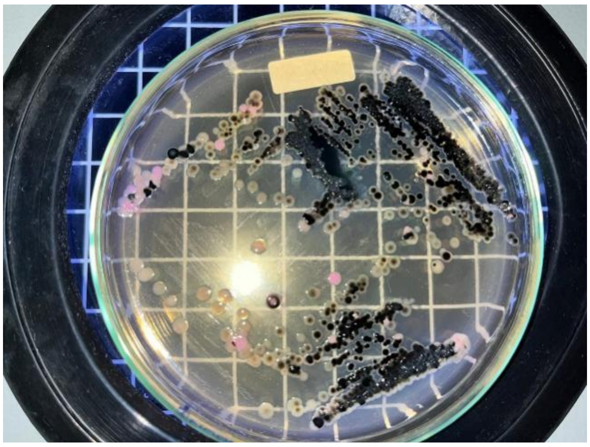

Figure 1. Results of Selective Medium Salmonella Shigella Agar (SSA) Test.

Salmonella bacteria contamination was found in all samples based on testing using Selective Salmonella Shigella Agar (SSA) media on tilapia, catfish, and water samples with varying dilutions. For tilapia and catfish, the dilution was 10^{-5}, while for water, the dilution was 10^{-2}. The bacteria were observed to grow on the culture media, with growth characteristics matching the colony traits of *Salmonella sp*., which has a black center and a transparent or white appearance. The presence of Salmonella sp. in the fish and water samples indicates that they do not meet food safety standards as outlined in SNI 7388:2009.

Result of Gram Staining Test

Table 2. Results of Gram Staining Test.

Location	Fish Type	Sample	Gram Staining	Result	
				Color	Shape
Fish Cages Pahandut Seberang	Tilapia	A 1	Negative	Red	Bacillus
		A 2	Negative	Red	Bacillus
		A 3	Negative	Red	Bacillus
		B 1	Negative	Red	Bacillus
		B 2	Negative	Red	Bacillus
		B 3	Negative	Red	Bacillus
		C 1	Negative	Red	Bacillus
		C 2	Negative	Red	Bacillus
		C 3	Negative	Red	Bacillus
	Catfish	A 1	Negative	Red	Bacillus
		B 1	Negative	Red	Bacillus
		C 1	Negative	Red	Bacillus
	River Water	Water 1 (Upstream))	Negative	Red	Bacillus
		Water 2 (Downstream)	Negative	Red	Bacillus

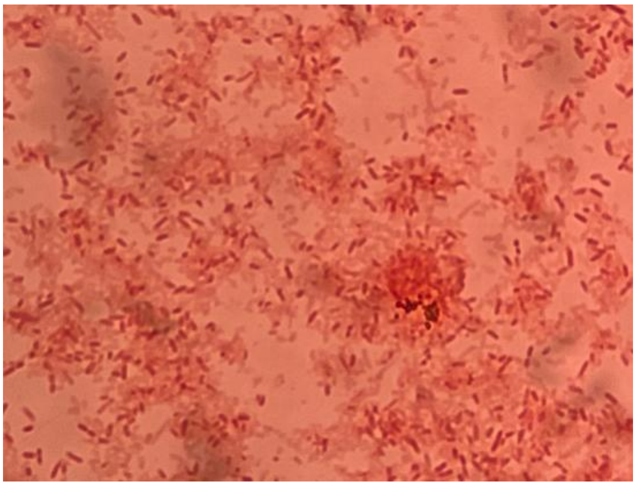

Figure 2. Results of Microscope Observation at 1000x Magnification.

The second test, Gram staining, was conducted using Salmonella Shigella Agar (SSA) media on tilapia, catfish, and river water samples collected from floating net cages in the Kahayan River at Pahandut, Palangka Raya City. Based on observations, 14 samples (100%) exhibited characteristics of Salmonella bacteria, appearing red and rod-shaped or basil-like.

Result of Gram Staining Test

Based on research using Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), and Simmon Citrate Agar (SCA) media on tilapia, catfish, and river water samples collected from floating net cages (KJA) in the Kahayan River at Pahandut, Palangka Raya City, the following results were obtained.

Table 3. Results of Biochemical Testing using *Simmon Citrate Agar* (SCA), *Sulfide Indol Motility* (SIM), and *Triple Sugar Iron Agar* (TSIA).

Sample	SCA	Sulfide	Indole	Motility	Gas	TSIA	Bacteria
A N1	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
A N2	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
A N3	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
B N1	+	-	-	+	+	Slant: Red, Butt: Yellow	*Salmonella Paratyphi B*
B N2	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
B N3	+	+	-	+	+	Slant: Yellow, Butt: Yellow	*Salmonella typhimurium*
C N1	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
C N2	+	-	-	+	+	Slant: Red, Butt: Yellow	*Salmonella Paratyphi B*
C N3	+	-	-	+	+	Slant: Red, Butt: Yellow	*Salmonella Paratyphi B*
A L1	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
B L1	+	-	-	+	+	Slant: Red, Butt: Yellow	*Salmonella Paratyphi B*
C L1	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
Air 1 (Hulu)	+	+	-	+	+	Slant: Red, Butt: Yellow	*Salmonella typhimurium*
Air 2 (Hilir)	+	-	-	+	+	Slant: Red, Butt: Yellow	*Salmonella Paratyphi B*

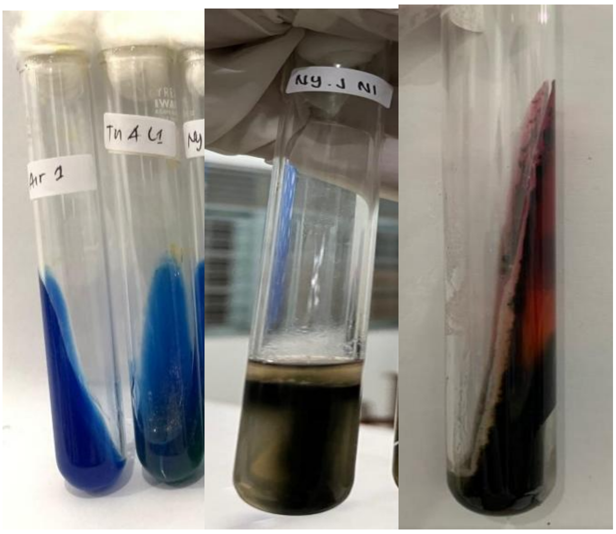

Figure 3. Results of Biochemical (A)Testing using *Simmon Citrate Agar* (SCA), (B) *Sulfide Indol Motility* (SIM), and (C) *Triple Sugar Iron Agar* (TSIA).

The biochemical test results using Simmon Citrate Agar (SCA) media on water sample 1 and sample A L1 showed a color change from green to blue. The biochemical test results using Sulfide Indole Motility (SIM) media on sample C N1 showed a color change to black, negative indole as no red ring formed at the top after adding Kovac's reagent, and positive motility as spreading was observed away from the inoculation line. The biochemical test results using Triple Sugar Iron Agar (TSIA) media on water sample 1 showed a color change on the slant to red/alkaline and on the butt to yellow/acid, with gas formation at the bottom. For 14 samples tested with TSIA, SIM, and SCA media, all gas, motility, and citrate tests showed positive results; all indole tests showed negative results; sulfide tests showed nine positive and five negative results; and all TSIA tests changed from red to acid-base, indicating that the bacteria detected in all tests were nine samples of *Salmonella typhimurium* and five samples of *Salmonella Paratyphi B*.

Discussion

The survival of all living beings, including humans, depends on water. One of the factors contributing to the decline in river quality is the continuous disposal of waste into rivers. Organic and inorganic waste from various activities around rivers can become contaminants. Organic waste includes plant residues, animal waste, food scraps, agricultural waste, and human excrement. Meanwhile, inorganic waste can originate from factories, tin smelting sectors, the use of plastics, diesel fuel, chemicals such as detergents, and other sources carried by river currents. The biological, physical, and chemical aspects of rivers can be disrupted and altered by this waste pollution, leading to contamination (Estining & Solichim, 2018). The proliferation of infection-causing microorganisms is influenced by the declining quality of river water due to pollution from organic and inorganic waste. Gram staining and culture results on Selective Salmonella Shigella Agar (SSA) media showed that both water samples tested positive for *Salmonella sp.* bacteria. Under a microscope, red, rod-shaped gram-negative bacteria were observed, while the cross-sectional view revealed colonies of *Salmonella sp.* bacteria appearing white or transparent with black centers. Furthermore, biochemical test results indicated that five samples contained *Salmonella Paratyphi B* species, and nine samples contained Salmonella typhimurium species. Rivers are essential for life, and human activities in domestic and industrial environments significantly impact their quality. Environmental pollution, particularly in river streams, can result from rapid population growth and environmental apathy in community activities. River water quality is affected by various factors, including activities conducted by communities in Watershed (DAS) areas. Human health and water quality are closely interconnected, and drinking water that is contaminated or contains harmful substances can pose significant risks to human health.

Contaminated water can harbor harmful microbes, including bacteria, viruses, and parasites. These microbes can infect humans and cause gastrointestinal diseases such as cholera, typhoid, and diarrhea when consumed or used for routine cooking and bathing. Heavy metals (such as lead and mercury) and industrial chemicals that contaminate water are examples of chemical pollutants that can be hazardous. Frequent exposure to these pollutants can increase the risk of cancer, poison humans, and damage vital organs such as the kidneys and liver. Diseases caused by consuming contaminated water can have various effects and are often quite severe. For instance, diarrhea is a common illness that can be fatal, especially for young people living in unsanitary conditions. Cholera, transmitted through water contaminated with *Vibrio cholerae* bacteria, causes severe diarrhea and dehydration. *Salmonella typhi* is the bacterium responsible for typhoid, which can lead to high fever and other severe symptoms. Fluorosis, caused by chemical pollutants such as fluoride, can damage teeth and bones. Serious health risks also stem from hepatitis A, which is associated with water contaminated by human waste.

The *Salmonella sp.* bacteria are rod-shaped, gram-negative, non-spore-forming, motile with peritrichous flagella, and have dimensions of 2-4 μm x 0.5-0.8 μm. Foodborne diseases are often caused by *Salmonella sp.* Many illnesses, including typhoid fever, gastroenteritis, diarrhea, salmonellosis, bacteremia (sepsis), and other localized infections, can be caused by *Salmonella sp.* These bacteria are undesirable in fishery products because they are pathogens that pose health risks to individuals consuming contaminated fish (Purlianto, 2015). Fish is a highly essential food product for humans as it contains nutrients the body requires, such as proteins, carbohydrates, fats, vitamins, and mineral salts. Due to its high nutritional protein content, fish may serve as a favorable substrate for bacterial growth.This is particularly true for tilapia and catfish, which have high water activity, making it easy for bacteria to grow and multiply. This contamination can occur due to pollution from organic and inorganic sources, as well as naturally occurring bacteria in the species. According to data from the Institute for Health Metrics and Evaluation (2019), the global prevalence of salmonellosis is 4.22 per 100,000 people, with a mortality rate 1.72. In Indonesia, the 2019 mortality rate was 2.42, and the prevalence rate was 5.82 per 100,000 people. According to the Centers for Disease Control and Prevention (CDC), contaminated food is the primary source of *Salmonella* infection in the United States (US), estimated to cause 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths annually. Rivers contaminated with industrial or domestic waste containing *Salmonella* are the initial sources of bacterial contamination. When fish live in polluted water, the bacteria adhere to their gills and skin. If humans consume *Salmonella*-contaminated fish that are not adequately cooked, the bacteria enter the body through the digestive tract and multiply. The hypothalamus then reacts by increasing body temperature, resulting in typhoid fever, which manifests as high fever, headache, and digestive issues.

Salmonella enterica, the pathogen causing gastroenteritis, can be transmitted from animals to humans through food. *Salmonella* adheres to enterocytes in the small intestine, causing the host cells to shrink. This triggers bacterial endocytosis from the apex to the basolateral membrane, leading to the disease. Furthermore, if the germs penetrate deeper, they multiply and cause inflammation, resulting in apoptosis. The primary causes of diarrhea include bacterial toxins, apoptosis, and the inflammatory response. Maintaining proper food sanitation and hygiene is the first step in preventing foodborne diseases. One can prevent infection by practicing good hand hygiene, such as washing hands before eating and after defecating. Salmonellosis is also influenced by nail conditions; therefore, thoroughly washing hands with soap and running water is crucial because finger and nail contact can prevent bacterial growth. Additionally, ensure food is cooked thoroughly, maintain kitchen cleanliness by regularly washing hands with soap and water, store food properly, and only purchase fresh produce. The *Salmonella sp.* bacteria were detected using Selective Salmonella Shigella Agar Media Testing (SSA) during a 24-hour incubation period at 37°C. Based on research findings, *Salmonella sp.* infections were found in all water samples, tilapia samples, and catfish samples. The bacteria were observed to grow on culture media, with colony characteristics matching *Salmonella sp.*—black with a clear or white appearance. *Salmonella sp.* in fish and water samples failed to meet food safety standards according to SNI 7388:2009. After performing SSA testing, Gram staining was conducted using safranin, Lugol's solution, 96% alcohol, and crystal violet solution. The color and morphology of *Salmonella sp.* bacteria were examined under a microscope. All 14 samples (100%) exhibited characteristics of *Salmonella* bacteria, which were gram-negative, red, and rod-shaped or bacilli. The species of *Salmonella* bacteria were identified, and SC, motility, indole, H2S, gas, and TSIA were evaluated using Triple Sugar Iron Agar (TSIA), Simmon Citrate Agar (SCA), and Sulfide Indole Motility (SIM) media. After 24-48 hours of incubation at 37°C, the results revealed that all 14 samples tested positive for citrate, motility, and gas, while the indole test yielded negative results. The H2S test revealed seven positive and seven negative samples, and the TSIA test showed color changes from red to acid and base, indicating that among the identified bacteria, five samples contained *Salmonella Paratyphi B*, while nine samples contained *Salmonella typhimurium*. The research findings align with Liantika's (2022) study on the identification of *Salmonella sp.* contamination in fresh milkfish at a fish auction site in Gersik, Gadukan

Lumpur. The study results revealed that *Salmonella sp.* contamination in milkfish samples exceeded the maximum microbiological contamination limit set by Indonesian National Standards (SNI) 7388:2009. Another study by Wanda (2021) detected *Salmonella sp.* contamination in tuna at traditional and modern markets in Semarang City. The results showed that all six samples were infected with *Salmonella sp.* and exceeded the microbiological contamination limit using Indonesian National Standards (SNI) 7388:2009 methodology.

CONCLUSIONS

Based on the research findings regarding the presence of *Salmonella sp.* in tilapia and catfish cages in Pahandut Seberang, which is traversed by the Kahayan River Basin, *Salmonella* bacteria—characterized by colonies appearing white or translucent with black centers—were identified in water samples, tilapia samples, and catfish samples using Selective Salmonella Shigella Agar (SSA) media; the BMI of tilapia and catfish samples exceeded the SNI 7388:2009 standard (positive/25g); the bacteria, identified as gram-negative, red, rod-shaped bacilli, were confirmed by Gram staining; the water samples from cage areas were contaminated with *Salmonella* bacteria, specifically *Salmonella Paratyphi B* and *Salmonella typhimurium*; biochemical tests revealed positive results for citrate, motility, and gas, negative results for indole, varied results for H2S, and changes in TSIA for all samples.

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