

Isolation and Identification of *Edwardsiella Tarda* from Fish in Haramaya Lake, East Hararghe Zone, Oromia Regional State, Ethiopia

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Abstract

Background: *Edwardsiellosis* is a serious systemic bacterial disease of *Edwardsiella tarda* which is known for causing diseases in humans, reptiles, amphibians, marine mammals and other warm-blooded animals. *E. tarda* is the most important diseases causing bacteria that lead to severe economic losses in fish farms of many countries due to its admirable effects on a variety of fish taxa including carp, tilapia, eel, catfish, mullet, salmon, trout and flounder. This study aimed on isolation and identification of *E. tarda* from the fishes (Catfish and Tilapia) of Lake Haramaya.

Methods: A cross-sectional study design was conducted from December 2021 to May 2022 to estimate the occurrence and distribution of *Edwardsiella tarda* from the kidney, spleen, liver and intestine of apparently healthy fish in the Lake Haramaya, Eastern Ethiopia. From a total 384 of swab and tissue samples were randomly taken from the kidney, liver, spleen and intestine of 96 apparently healthy fish (*Clarias gariepinus* and *Oreochromis niloticus*) originating from Lake Haramaya, 18 showing similar colony and biochemical characteristics to *E. tarda* were isolated and identified.

Results: Distribution of *E. tarda* infection among the four organs examined indicated that *E. tarda* was isolated most frequently from intestine 10 (10.4%) followed by liver 4 (4.2%) then kidney 2 (2.1%) and spleen 2 (2.1%) with statistically significant difference ($P < 0.05$) among organs. *E. tarda* was isolated more frequently from males 12 (26.7%) than females 6 (11.8%) and differences in the occurrence of *E. tarda* infection with respect to sex was not significant ($P > 0.05$) indicating that both sexes are equally susceptible. Concerning fish species *E. tarda* is more frequently isolated from Catfish than tilapia with no significant difference ($P > 0.05$).

Conclusion: The recovery of *E. tarda* from Lake Haramaya, which is potentially pathogenic to humans, from the organs and alimentary tracts of fish suggests that fish either improperly handled, undercooked or consumed raw may cause *Edwardsiellosis* in susceptible individuals and potential threat to both fishery sector/aquaculture. There is limited knowledge of *E. tarda* infection in fish and humans in the area and hence further awareness to have information on the agent is forwarded.

Keywords: Catfish; *Edwardsiella Tarda*; Ethiopia; Haramaya; Nile Tilapia.

Introduction

Fish farming has been practiced in various parts of the world. It has been in practice since the ancient civilization of Egypt and China, thus fisheries sector persists as a significant source of food, nutrition, income, and livelihoods for hundreds of millions of people around the world [1]. Fish and their products are an important source of easily digested and delicious animal protein with high biological value which compromise many essential amino acids, vitamins D and B, essential minerals (calcium, phosphorus, iodine, zinc, iron, and selenium) as well as amounts of trace elements which have beneficial effects for adult health and child cognitive [1,2]. Also fish has the role in the improvement of human bodily functions and reduction in susceptibil-

ity to cardiovascular diseases and cancer, which arrived from polyunsaturated fatty acids particularly those from the omega-3 family [3].

Artisanal freshwater fishery is fishery sector and the most valuable economic activities in Ethiopia, which contribute in poverty alleviation in the country [4]. It has been suggested that, the country has an estimated annual total exploitable fish potential of 51,481 tons, which can meet only 79% of the current actual demand at a time, 55% of the projected demand in 2010 and 44% of the projected demand in 2015, based solely on population size whereas the Lake Tana, Ashenge, Hayk, Koka, Ziway, Langano, Awassa, Abaya and Chamo are among the potential

fish rich lakes found in Ethiopia [5].

Unlike in all animal production systems, in fish subjected to intensive culture practices disease outbreaks are one of the main and high significant limiting factors [2]. especially that result from bacterial infections are one of the major problems hampering production, development and expansion of the aquaculture industry and difficult to control [6].

Among the most important bacterial diseases causing severe economic losses in fish farms of many countries is Edwardsiellosis, which are infectious diseases often associated with poor water quality and stress [6]. It is a serious systemic bacterial disease, which affects a variety of fish taxa include carp, tilapia, eel, catfish, mullet, salmon, trout and flounder, caused by *Edwardsiella tarda*, a Gram-negative bacilli that belongs to the *Enterobacteriaceae* family, which has many traits that are characteristic of many enterobacteria. These characteristics include it being a facultative anaerobe, motile, short, rod-shaped bacterium (1 µm in diameter and 2–3 µm long) [7]. *E. tarda* is known for causing diseases in both humans and fish also may infect reptiles, amphibians, marine mammals and other warm-blooded animals [8].

E. tarda is commonly found in the normal gut flora of fish and humans, and can be an opportunistic pathogen in human with the risk factors of being vulnerable to aquatic environment, pre-existing liver diseases, iron over load and raw sea food ingestion [9,10]. Many potential virulence factors have been identified and include siderophores, the ability to invade epithelial cells and internal fish tissues, resistance to serum and phagocyte-mediated killing and the production of toxins/exoenzymes, including dermatotoxins, catalase and haemolysins [11].

In fish *E. tarda* may affect internal organs such as liver, kidney, spleen and muscle and exhibit signs of distention of abdomen and swollen anus due to the accumulation of ascitic fluid, pigment loss, enlarged kidney, and abscesses on internal organs and also a variety of clinical syndromes, such as bacteremia or septicemia, enteric fever, gastroenteritis, an asymptomatic carrier state, and localized infections with extensive skin lesions that can develop into necrotic abscesses [12,13]. The affected fish shows the loss of scales from some the body areas, congestion, exophthalmia and as well as protruded and congested vent, ulcer and hemorrhages all over the fish body especially at the base of the pectoral fin, anal opening, and tail rot while their postmortem examination revealed variety of lesions as well as distention of gall bladder and abdomen with ascetic fluids, hepato-pancreas and kidney enlargement [2]. It has been reported in humans as the cause of gastroenteritis, minimal diarrhea, nausea and generalized infections mainly among individuals with impaired immune systems [14].

E. tarda can be isolated on Edwardsiella Isolation Media (EIM), Brain Heart Infusion (BHI), Tryptic Soya Agar (TSA), Xylose Lysine Deoxycholate (XLD) and MacConkey Agar with characteristics of small, circular, raised, whitish with black center on XLD and pale on MacConkey agar, grow best at a range of

25°C-37°C temperature and 7-8 PH being positive on catalase, indole, lysine and glucose fermentation, while being negative on cytochrome oxidase, lactose fermentation, citrate, mannitol, dulcitol, sorbitol, inositol, xylose, rhamnose, alkaline slant and acid butt on Triple sugar iron Agar [15]. It does not produce urease and similar to *Salmonella* in that it is able to generate hydrogen sulfide on laboratory media [6].

The practice of consuming uncooked fish meals, manual handling of fish and unhygienic practice during filleting in Ethiopia implies that the public is at higher risk of contracting the disease from infected fish meat. Therefore, the disease which cause with this bacteria or edwardsiellosis requires to give attention due to its potential threat to future aquaculture industry and public health. In Ethiopia, the bacterium has been isolated from apparently healthy fish of Lake Zeway and Langano, Lake Tana, crater lake around Bishoftu and Lake Hawasa and Lake Hayike [6,16-18]. Even though, the lake was re-emerged recently and using as source of income from fish meat for surrounding people, there is no work done in investigation of this bacterium on Lake Haramaya.

Therefore, this study was conducted with the objective of isolating and identification of *E. tarda* from the fishes (Catfish and Tilapia) of Lake Haramaya.

- To elucidate safety of fish products with respect to *E. tarda* contamination.
- To estimate the occurrence and distribution of *E. tarda* in the lake Haramaya from intestine, kidney, liver and spleen of Catfish and Tilapia.

Materials and Methods

Study Area

The Watershed of Lake Haramaya is located in Haramaya district, eastern Hararghe zone, Oromia National Region State, East Ethiopia. It is located at the upstream part of Wabishabele Drainage Basin. The Haramaya District which the Lake Haramaya catchment found in is situated 505 km away from Addis Ababa to East, about 14 km far from Harar city, and 38 km far from Dire Dawa city. The Watershed lies 9°23' 18" to 9° 26' 48" North of latitude and 41° 58' 30" to 42° 05' 30" East of longitude. The total area of the catchment is 5032 ha of which the area of the Lake Haramaya is 2.26 km². Lake Haramaya catchment encompasses a small part of Haramaya Town, the Haramaya University Campus, Bate town, three peasant associations; Damota, Ifa-Bate, and Tuji-Gebissa fully, and another two Ifa- Oromia (around 90%) and Gobe-Selama (around 10%) partially. Based on the agro-climatologically classification, Haramaya woreda has Woina Dega (wet and cool, 70%) and Kolla (dry and hot 30%) areas. The annual rainfall distribution record indicates that the area receives a bimodal rainfall type with the mean annual precipitation of 751 mm. The maximum and minimum mean annual temperatures for the area are 23.8°C and 9.60C respectively [19].

Study Design and Sampling Method

A cross-sectional study design was conducted from December 2021 to May 2022 at Haramaya Lake to isolate and identify *E. tarda* from kidney, liver, spleen and intestine of apparently

healthy fish. A simple random sampling technique was used to select the fish samples after it was drawn from different part of the lake based on approximately 100m distance between the harvested fish by different fisherman to transport them to the laboratory.

Study Animals

A total of 96 live fishes which comprise Nile Tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus*) were obtained using multi-mesh multifilament survey gillnet. From those species of fish a total of 384 organ samples (96 intestines, 96 kidneys, 96 spleens and 96 livers) were taken for the purpose of samples. The fish which was used for the samples may be apparently healthy and harvested for the human consumption.

Animal handling and sample collections of study animal were conducted according to standard guideline for animal research ethics, Haramaya University, College of Veterinary Medicine, 2021. Additionally, the animal was euthanized and become unconscious during sample collection in the laboratory for the welfare of the animals being studied. Experimental Protocols for bacteria isolation and identification from animal sample were conducted according to standard guideline for animal research ethics, Haramaya University, College of Veterinary Medicine, 2021 and those published in a book entitled as "Veterinary Microbiology and Microbial Disease," Second Edition by Quinn et al., [15]. All methods are reported in accordance with ARRIVE guideline (<https://arriveguidelines.org>) for the reporting of animal experiments.

Sample Size Determinations

The sample size was determined according to Thrusfield (2018) as follows:

$n = (1.96^2 \times P_{exp}(1 - P_{exp}) / d^2)$, where: n = required sample size;

P_{exp} = expected prevalence;

d = desired absolute precision.

Therefore, a total of 384 samples were taken to keep the precision at 50% expected prevalence or occurrence, 95% confidence interval and 5% desired absolute precision.

Sample Collection and Processing

The fish which harvested from different part of Lake Haramaya was transported directly in the ice box to the laboratory of College of Veterinary Medicine, Haramaya University where they were processed for bacterial isolation and identification within 30 minutes. In the laboratory the surfaces of the incision was disinfected with 70% alcohol. Then the ventral approach to kidney was employed and the fish sample was cut aseptically along the midline of the abdomen starting from the anus up to the mouth using sterile dissecting scissor followed by another dissection from the anus to the lateral line and further along the lateral line up to the gills cover to remove the lateral side of the abdominal wall and expose the internal organs using sterile scalpel blade and forceps. The peritoneal cavity was opened aseptically and with care not to puncture any part of the intestinal tract. Swab samples were then taken from liver, kidney, intestine and

spleen aseptically using sterile swab. All examination and tissue sampling procedures were carried out aseptically.

Isolation of *Edwardsiella Tarda*

The swab samples from intestine, spleen, liver and kidney were added to buffered peptone water then incubated at 37°C for 24 hours. The samples were then taken by sterile loop and streaked on MacConkey agar plates and then incubated at 37°C for 24 hours. All lactose non-fermenting colonies (pale colonies) was selected from MacConkey agar plates and sub-cultured on Xylose Lysine Deoxycholate agar plate and then incubated at 37°C for 24 hours. For further identification colonies showing or resembling with morphological characteristics of *E. tarda* with formation of grayish/black center on XLD agar plate were further sub-cultured on Nutrient agar plates for isolation and culture of single pure colony and incubated at 37°C for 24 hours.

Identification of *Edwardsiella Tarda*

Primary identification of pure culture of the isolates was done based on gram reaction, catalase and oxidase tests according to the procedures described previously by Quinn *et al.*, [20].

Gram Staining was done according to the procedure described by Rowland *et al.*, accordingly, colonies that will gram negative, short rods will be considered for further tests [21].

Catalase Test detects whether the bacterium has the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen. The test was carried out on pure fresh colony from Nutrient agar plates (Annex C). Since *E. tarda* is catalase positive, colony showing an elaborated bubble formation will be considered positive and taken for further tests.

Oxidase Test detects the presence of cytochrome oxidase enzyme in a bacterial cell and characterized by purple colour formation within 10 seconds when the bacterial sample is made in contact with 1 percent aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride (Kovac's oxidase reagent) (Annex C). Then the colonies was added to filter paper method for each bacterial isolates and *E. tarda* will not change the colour.

Biochemical Tests Secondary biochemical identification of bacterial isolates was conducted employing conventional biochemical tests according to the standard procedures described previously [22].

Triple Sugar Iron Agar (TSI) test shows hydrogen sulfide production, gas production, and fermentation of lactose, sucrose and glucose. *E. tarda* is expected to show red slant and yellow butt with hydrogen sulfide production. In this work, TSI test was carried out by inoculating (by stabbing the butt and streaking the slant) of the test tube of TSI agar slant using straight inoculating wire after which the inoculated tube was loosely capped and the findings recorded after 24 hours of incubation at 37°C (Annex C).

Indole Production Tests The indole test screens for the ability

of an organism to degrade the amino acid tryptophan and produce indole. Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 35°C for 24 to 48 hours. To test for indole production, add 5 drops of Kovács reagent directly to the tube. *E. tarda* is expected to show positive indole test, it indicated by the formation of a pink to red color ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent (Annex C).

Methyl Red (MR) Test Detects the production of sufficient acid during the fermentation of glucose and The pH indicator methyl red has been found to be suitable to measure the concentration of hydrogen ions between pH 4.4 (red) and 6.0 (yellow) [23]. Thus *E. tarda* is expected to bring positive result turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose.

Voges Proskauer (VP) Test is used to determine if an organism produces acetylmethyl carbinol (acetoin) from glucose fermentation. In the presence of KOH the intermediate acetoin is oxidized to diacetyl, a reaction which is catalyzed by a-naphthol. Diacetyl reacts with the guanidine group associated with molecules contributed by peptone in the medium, to form a pinkish-red colored product (Annex C). *E. tarda* expected to form pinkish- red colored product.

Simmon's Citrate Test Simmon's citrate slants in test tubes were stab inoculated and incubated at 37°C for a week after which the findings were recorded (Annex C). The test detects the ability of the bacterium to utilize citrate as the only carbon source which imparts blue in case of positive cases.

Mannitol Fermentation Tests Conventional biochemical tests comprising four alcohols (dulcitol, mannitol, inositol and sorbitol) and two sugars (rhaminose and xylose) were used for adequate presumptive identification of *E. tarda*. Phenol red manni-

tol broth in Durham tubes containing 1% mannitol was prepared and inoculated with the isolates. The in-oculates were then incubated at 37°C for 24 hours after which the results were recorded (Annex C). *E. tarda* does not ferment the sugar.

Data Analysis

Microsoft excel was employed for raw data entry and IBM SPSS Statistics 20 was used for descriptive statistics. Chi-square test of independence was employed in comparing the occurrence of *E. tarda* infection with respect to sex, fish species and organ of isolation from live fish samples which were collected at Lake Haramaya using SPSS Statistics 20. A confidence interval of 95% was used to interpret the statistical association and significance was considered when P-value is less than 0.05.

Results

Results of Characteristics of Isolated *E. Tarda*

From a total 96 apparently healthy live fishes of Nile tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*), a total of 384 organ samples (96 intestines, 96 kidneys, 96 livers and 96 spleen) were collected and sampled for this study and 18 results were isolated with consistent characteristics to *E. tarda* based on primary and secondary identification criteria and thus were presumptively identified as *E. tarda*. The isolates were showed small yellow-white colonies on MacConkey agar after incubation of 24 hours at 37°C and small grayish-white with red background colonies on Xylose Lysine Deoxycholate (XLD) agar after 24 hours of incubation at 37°C. In this study all isolates were showed typical characteristics of *E. tarda* isolated elsewhere which were gram negative short rods, positive for catalase, and oxidase negative. In biochemical tests, these typical isolates were positive for indole and methyl red, negative for Voges-Proskauer, H₂S and gas production on TSI agar and unable to utilize Simmon's citrate and the diferent sugars used in this study such as mannitol and sorbitol (table 1).

Table 1: Characteristics of isolated *E. tarda* on primary identification and biochemical test

Tests	Characteristics
Cultural characteristics on MAC agar	yellow-white colonies
Cultural characteristics on XLD agar	grayish-white with red background colonies
Morphological characteristics	gram negative, short rods
Catalase	Positive
Oxidase	Negative
H ₂ S production on TSI agar	Positive
Gas production on TSI agar	Production of gas
Indole	Positive
Methyl Red	Positive
Voges-Proskauer	Negative
Simmon's citrate	Negative
Mannitol	Negative
Sorbitol	Negative

Results Based on Organs Samples

Out of total 96 fish, distribution of *E. tarda* infection among the four organs examined indicated that *E. tarda* was isolated from 18 (18.8%), among those organs most frequently from intestine

10 (10.4%) followed by liver 4 (4.2%) then kidney 2 (2.1%) and spleen 2 (2.1%) with statistical significant difference ($P < 0.05$) among organs as described in (table 2).

Table 2: Distribution of *E. tarda* isolates by organ of isolation.

		Results		Total
		Negative (percentage)	Positive (percentage)	
Organs	Intestine	86 (89.6%)	10 (10.4%)	96
	Kidney	94 (97.9%)	2 (2.1%)	96
	Liver	92 (95.8%)	4 (4.2%)	96
	Spleen	94 (97.9%)	2 (2.1%)	96
Total		366 (81.2%)	18 (18.8%)	384

($X^2=8.287$, $df=3$, $P=0.018$). The value of X^2 used was taken from Fisher's Exact Test

Results Based on Fish Species

Even though, *E. tarda* was isolated more from Catfish (*Clarias gariepinus*) 10 (20.8%) than Nile Tilapia (*Oreochromis niloticus*) 8 (16.7%), the differences in the occurrence of *E. tarda*

infection with respect to species were not significant ($P > 0.05$) (table 3), indicating that both fish species are equally susceptible to the infection.

Table 3: Occurrence and distribution of *E. tarda* with respect to fish species

		Results		Total
		Negative (percentage)	Positive (percentage)	
Species	Cat fish	38 (79.2%)	10 (20.8%)	48
	Tilapia	40 (83.3%)	8 (16.7%)	48
Total		78(81.2%)	18 (18.8%)	96

($X^2=0.274^a$, $df=1$, $P=0.601$). X^2 used was Pearson chi-square

Results Based on Sex

The distribution of the isolates of *E. tarda* with respect to sex were 12 (26.7%) of male and 6 (11.8%) of female. However, it

was insignificant ($P > 0.05$) indicating that both sexes are equally susceptible as in (Table 4).

Table 4: Distribution of *E. tarda* isolates with respect to sex of fishes

		Result		Total
		Negative (percentage)	Positive (percentage)	
Sex	Male	33 (73.3%)	12 (26.7%)	45
	Female	45 (88.2%)	6 (11.8%)	51
Total		78 (81.2%)	18 (18.8%)	96

($X^2=3.485^a$, $df=1$, $P=0.062$). X^2 used was Pearson chi-square

Discussion

E. tarda is considered as one of the major species of *Edwardsiella* that infects fish and other animals including human being and causes severe economic losses due to morbidity and mortality [2,6]. Previously the organism has been isolated from different sources such as from humans faeces with sporadic cases of diarrhea and from dressed fish samples and from spleen [6,24]. In this work, *E. tarda* were isolated from liver, spleen, intestine and kidney of apparently healthy fish indicating that the bacterium is a potential threat to aquaculture as well as to public health.

In this study morphological and conventional biochemical characteristics of *E. tarda* isolates, that showed typical characteristics of gram negative short rod, the results were agreed with those reported previously by Roberts, [25]. Also all isolates were

positive for indole and methyl red, thus agreed with the study conducted at Malaysia and at Lake Hawassa and crater lakes around Bishoftu [17,26]. However, in contrary with present study, one isolate was negative for indole and else another one isolate positive for citrate was reported in the study conducted at Lake Zeway and Langano [6]. In this occurrence there were no isolates of *E. tarda* that show fermentation of sugar such as mannitol and sorbitol which agrees with the study of [17,27]. However, according to reports among *E. tarda* isolates there were both occurrence of fermenter and non-fermenter of the mannitol, due to the absence or presence of plasmid that control metabolic activities of bacteria [6].

In present study the overall occurrence of *E. tarda* was 18.8%, but higher distribution infection of *E. tarda* was recorded when

compared with other study conducted at Lake Zeway and Langan, Southern Oromia, Ethiopia, Lake Hawassa and crater lakes around Bishoftu, Ethiopia, Lake Hayiq, Ethiopia conducted on survey of Gram-Negative Bacterial Pathogens, at Southern Gulf of Lake Tana [6,16,17,28]. This is due to presence of higher water pollution in Lake Haramaya from disposal of waste products, plastics and waste drainage from surrounding areas which directed to the lake, which may increase stress to the aquacultures (fish), this will lead infection to the fish in the lakes. Edwardsiella infection in fish usually occurs under imbalanced environmental conditions such as high water temperature, poor water quality, and high organic content [18]. However, in contrary there was no report of the occurrence of *E. tarda* according to study conducted at selected Lake Adelle and Tinike of Haramaya District, Ethiopia in the study of helminthiasis and gram negative enteric bacteria in freshwater fish [29].

In this study the occurrence of the *E. tarda* with respect to organs were 18.8% out of this 10.4% in intestine, 4.2% in liver, 2.1% in kidney and 2.1% in spleen with significant difference ($P < 0.05$). There is an agreement with several works with presence of significant difference ($P < 0.05$) in which the most frequent occurrence of *E. tarda* from intestine compared to other organs with the study conducted at Lake Hawassa and crater lakes around Bishoftu and also at Southern Gulf of Lake Tana [16,28]. It was suggested that is due to the existence of *E. tarda*, as part of the normal intestinal micro biota of aquatic animals or non-pathogenic strains which may later acquire virulence and become a source of infection under certain circumstances such as when the host is stressed [17].

In contrary to this study higher frequency of *E. tarda* from liver was reported in the study conducted at Lake Zeway and Langan [6]. The isolation of *E. tarda* from liver and kidney samples indicates subclinical infection which may eventually develop to disease depending on stress factors that compromise the immune system and due to the metabolic activities of the liver. This indicates that each organ have a chance to exposed for septicemic infection of pathogenic *E. tarda* which may be a threat to future aquaculture practices. According to Tesfaye et al., the organism (*E. tarda*) was not recovered from liver and kidney. In present study *E. tarda* was also recovered from spleen which has an agreement with the study at china [17,18,24]. The severity of *E. tarda* is due to suppressing the host immune which proved by lymphoid depletion induced in spleen [6]. The absence of significant differences ($P > 0.05$) in the occurrence of *E. tarda* between males and females indicates that both sexes are equally susceptible to the bacterium. This has an agreement with several works which conducted at Southern Gulf of Lake Tana and at Lake Zeway and Langan [6, 28].

The present study displayed that *Clarias gariepinus* (Cat fish) species was the most infected species with bacterial isolates and *Oreochromis niloticus* (Nile tilapia) was the lowest infected species with the absence of significant difference ($P > 0.05$) and has no agreement with other study in which occurrence is insignificant, but higher in Tilapia than Catfish conducted at Lake Zeway and Langan, at Southern Gulf of Lake Tana and at Lake Hayik

[6, 18, 28]. However, according to Nemo et al., the frequency of *E. tarda* isolates with regard to fish species were significant ($P < 0.05$) and higher in Tilapia than Cat fish, due to that *Oreochromis niloticus* species were genetically suitable to be infected by investigated bacterial isolates [17]. *Clarias gariepinus* species may be more immunologically protected from that infection.

Conclusion and Recommendations

Edwardsiellosis is the most important food borne and zoonotic bacterial disease causing severe economic loss and mortality in aquaculture farms. *E. tarda* has also economic significance in fishery industry and decrease annual income from fish products. In the present study the *E. tarda* was isolated from the kidney, spleen, liver and intestine of fish. The findings have shown that the intestine of fish have been found to be harboring a large number of *E. tarda*. Furthermore, the recovery of *E. tarda* from Lake Haramaya, which are potentially pathogenic to humans, from the organs and alimentary tracts of fish suggest that fish either improperly handled, undercooked or consumed raw may cause Edwardsiellosis in susceptible individuals and potential threat for both fishery sector/aquaculture. Generally, there is water pollution, less management and other stress factors that enhancing the occurrence, distribution and severity of *E. tarda* in fish of Lake Haramaya. There is no awareness and enough of knowledge on the infection of *E. tarda* in fish and humans in the area. Moreover there are is a limitation of further investigations on food borne of microorganisms from fish and environment of the lake. The finding of this study indicates that there is a need for strict sanitary measurement of hygienic practices during the processing and handling of fish and fish products around Lake Haramaya that should be taken by the concerned authorities and organization to prevent the spread of pathogenic bacteria to both the public and the fish. Besides, public health risk awareness should be created among fish consumers and those who have contact with fish, fish products. Further study should be conducted on epidemiology and molecular characteristics of *E. tarda* pathogens of fish for further understand their ecology and possible health hazards to human either through contact or ingestion of fish and fish products.

Declarations.

Acronyms and Abbreviations

BHI	Brain Heart Infusion
DVM	Doctor of Veterinary Medicine
EIM	Edwardsiella Isolation Media
gyrB	gyrase B subunit
IBM	International Business Machine
MAC	MacConkey Agar
MR	Methyl Red
NY	New York
PCR	Polymerase Chain Reaction
SPSS	Statistical Package for the Social
Sciences	
TSA	Tryptic Soya Agar
TSI	Triple sugar iron agar
VP	Voges-Proskauer
XLD	Xylose Lysine Deoxycholate

Ethics Approval and Consent to Participate

Animal handling and sample collections of the study animal were conducted according to standard guideline for animal research ethics, Haramaya University, College of Veterinary Medicine, 2021. The study protocol was approved by Animal Research Ethics Committee of Haramaya University, College of Veterinary Medicine, 2021. Additionally, the animals were euthanized and become unconscious during sample collection in the laboratory for the welfares of the animals being studied. The euthanasia was performed according to “American Veterinary Medical Association Guideline for Euthanasia” published in 2020. Around 0.4ml of clove oil per liter of aquarium water was used. Clove oil was mixed with a little warm water then slowly added to the aquarium water containing the fish then the exposor was made the fish to lose consciousness and die from hypoxia.

Authors' Contributions

Nureidin A: data curation, investigation, methodology, resources, writing – original draft, writing – review and editing

Balisa Y: conceptualization, data curation, formal analysis, investigation, methodology, resources, supervision, visualization, writing – original draft, writing – review and editing

Leykun L: project administration, writing – review and editing, resources, supervision, approval. All authors have read and approved the final manuscript.

Human and Animal Rights

Animal handling and sample collections of study animal were conducted according to standard guideline for animal research ethics, Haramaya University, College of Veterinary Medicine, 2021. Additionally, the animal was euthanized and become unconscious during sample collection in the laboratory for the welfares of the animals being studied.

Statement for Experimental Protocols

The experimental protocols for euthanasia was performed according to “American Veterinary Medical Association Guideline for Euthanasia” published in 2020. Around 0.4ml of clove oil per liter of aquarium water was used. Clove oil was mixed with a little warm water then slowly added to the aquarium water containing the fish then the exposor was made the fish to lose consciousness and die from hypoxia. Experimental Protocols for bacteria isolation and identification from animal sample were conducted according to standard guideline for animal research ethics, Haramaya University, College of Veterinary Medicine, 2021 and Veterinary Microbiology and Microbial Disease, Second Edition. P.J. Quinn, B.K. Markey, F.C. Leonard, E.S. FitzPatrick, S. Fanning, P.J. Hartigan. © 2011 P.J. Quinn, B.K. Markey, F.C. Leonard, E.S. FitzPatrick, S. Fanning and P.J. Hartigan. Published 2011 by Blackwell Publishing Ltd. All methods are reported in accordance with ARRIVE guideline (<https://arriveguidelines.org>) for the reporting of animal experiments.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated during the current study are available

from the corresponding author on reasonable request.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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