Yersiniosis outbreak in rainbow trout at fish farm in Oromia Regional State, Ethiopia

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Abstract

This study presents the results of an investigation conducted on an outbreak of Yersiniosis (Enteric red mouth disease) caused by *Yersinia ruckeri* at a rainbow trout farm situated at Adaba, Oromia Regional State, Ethiopia. Seven diseased rainbow trout fish having average weight 80 - 100 grams and aged 9 months, were brought to the National Animal Health Diagnostic and Investigation Center (NAHDIC) for further examination and laboratory testing. The young sick fish showed clinical signs of darkening of the skin, loss of appetite and gasping at the surface of the water before death. The fish were sacrificed and examined thoroughly externally for the presence of visible lesions. Scrapings were collected from the skin especially from areas around the fins and observed under the stereomicroscope and also under the low power objective of the compound microscope. Bacteriological tests were carried out on samples from the kidney, liver and spleen. It was concluded that the fish were affected by *Yersinia ruckeri* based on colony morphology during growth on Tryptose Soy Agar (yellow colonies, gram negative and rod-shaped) and distinctive biochemical characteristics. *Y. ruckeri* is identified from sick fish for the first time in Ethiopia. The protozoan parasites *Trichodina* species were also recovered in large colonies from the skin scrapings and histopathological sections of the gills. The pathological lesions recorded included high degree anaemia of oral and branchial mucosa, congested gills, kidney and spleen and pale liver, congestion, extensive necrosis in the kidney and spleen, and infiltration with inflammatory cells. Antibiogram test conducted on the bacteria showed that the *Y. ruckeri* strain were susceptible to Oxytetracycline, Furazolidone, Trimethoprim and Streptomycin. This study showed the importance of stress induced by higher temperature and poor water quality associated with infestations by *Trichodina* species as predisposing factors to bacterial diseases in intensive fish farming practices.

Key words: bacterial culture, histopathology, rainbow trout, *Yersinia ruckeri*, *Trichodina* species

Introduction

Both rainbow trout (*Oncorhynchus mykiss*) and brown trout or sea trout (*Salmo trutta*) originating from Kenya were introduced in Bale Ethiopia by UNESCO in 1967 to Denka and Webb Rivers (Balarin, 1986). Later rainbow trout were introduced into the rivers Sibilo, Chacha, Beressa, and Mugar and into Lake Wonchi in 1973 – 1974 (Meskal, 1984 Cited by Balarin, 1986). Recently, intense overfishing using destructive fishing methods like Birbirra has resulted in significant decline both in individual size of the catch and total population in the rivers of Bale. Ethiopia's physical environment and availability of agricultural residues which could be used as composting material for the fertilization of fish ponds, makes the country favorable for fish farming (fish like tilapia, catfish and others). Trout Fish Farmers Private Limited Company has established a commercial trout fish farm in 2009 for the first time in Ethiopian highlands in Adaba District, West Arsi Zone, Oromia Region of Ethiopia. The historical introduction and well survival of rainbow trout fish in the area was the major impetus for the start of this rainbow trout farm that was established in the area after leasing land, preparation of earthen ponds and stocking of eyed rainbow trout eggs brought from France.

Recently, there was an outbreak of a disease that resulted in the death of fish from time to time after they exhibit clinical signs of poor appetite, darkening of the body, gasping at the surface of the water and finally death. When freshwater fishes are cultured more intensively, it is natural that they develop various diseases more frequently and diseases become more prevalent. Therefore, it is necessary to conduct studies to isolate and identify the pathogens causing these diseases and establish preventive and therapeutic methods against them. During this study, parasitological, bacteriological and histopathological techniques were employed to reach to a specific diagnosis.

Bacteriological culture of swabs taken from of liver, kidney and spleen samples revealed the presence of *Yersinia ruckeri* from these organs indicating the bacteria to be the major cause of disease and mortality of fish in the farm. The protozoan parasite *Trichodina* species was also recovered from the skin scrapings and histopathological sections of the gills which further exacerbate the stress on the fish thus increasing their susceptibility to bacterial infections. Enteric redmouth disease (ERM) or Yersiniosis, caused by *Yersinia ruckeri*, is a serious infectious disease in the rainbow trout farming industry that causes economic problems in many countries (Austin and Austin, 1993). ERM caused a potential economic

loss of £20 million each year to the European trout industry (AquaVac ERM, 2009). This study revealed the significance of *Y. ruckeri* in the farm and based on the findings recommendations were forwarded for the control and prevention of the disease in this farm as well as on others to be established in the future.

Materials and Methods

Study Site

Trout Fish Farmers Private Limited Company has established a commercial trout fish farm in 2009 situated at 2,500 meters above sea level (masl) on the Meribo River in the district of Adaba located in West Arsi Administrative Zone of Oromia Region at 350 km from the capital Addis Ababa in South west of Ethiopia. The trout fish farm, of small capacity, is currently using the water of the Meribo River, a permanent river flowing from the Berinda Ridge 3,700 (masl) of Bale Mountains range. The water is channeled through an irrigation canal shared with a local farmers' cooperative and the water intake is located 1.5 km above the farm. The minimal water flow during dry season in the canal is 20 liters per second at the fish farm.

The fish farm is composed of hatchery facilities including; 1 hatchery trough (with a capacity to hatch 50,000 eggs) and 2 fingerlings ponds of 3 m^3 each, and of growing ponds: one big stony pond (divided into 2 parts) of 75 m^3 located on the enlarged part of the irrigation canal, and 2 serial earthen ponds of 17.5 m^3 each using water diverted from the canal at the flow rate of 5 liters/sec. The expected production per year with the current facilities is above 1,000 kg of rainbow trout of table size for the domestic market in Addis Ababa.

Fish samples

Seven rainbow trout fish of nine months old having an average weight of 80 – 100gm were harvested from the fish farm at 7 am and transported alive in plastic bag with the addition of oxygen in cold water (ice box) to NAHDIC at Sebeta on the 21st of October 2011. The fish were supplied with oxygen that was placed in cooler box to keep them cool as much as possible and slow down their metabolic activity so that the fish stay alive until they reach the laboratory. The fish reached to the laboratory after five hours of drive directly from Adaba to Sebeta. At arrival, six of the fish were alive while one of them was dead. Initial ante mortem examination was later followed by post mortem examination after sacrificing each one of them. The fish were kept wet and cool as much

as possible until the end of the procedures. Standard fish examination procedures (Roberts, 1989) were utilized to examine the fish both externally and internally.

Examination of fish for the presence of parasites

Fish were examined visually on their external body to check for the presence of visible lesions and macroscopic parasites. Then skin scrapings were taken from the surface of the skin by giving particular emphasis to the areas around the fin bases. The scrapings were spread on a microscope slide with a drop of water and examined under the low power objective of the microscope with cover slip on it. The gills were observed in situ and a piece was cut and put on a slide crushed between slides, covered with cover slip and observed under the microscope for the presence of microscopic parasites. The abdominal cavity was opened and the different organs were closely observed in situ for the presence of visible macroscopic parasites and lesions. Then the different abdominal organs like the liver, spleen, kidney and heart were carefully removed and examined for the presence of parasites and samples were also collected.

Bacteriological examinations

Bacteriological samples were collected from the liver, spleen and kidney which were cut aseptically and inoculated on to a Tryptose Soy Agar (TSA) using sterile wire loop. Sample collection and culturing was conducted following standard bacteriological techniques (Frerichs, 1984). The cultures were incubated at a temperature of between 28 – 30^0C and observed for the growth of any microorganism after 24 hours. The bacterial isolates were further tested for their sensitivity to different antimicrobial agents that included Oxytetracycline (OT-30 µg), Furazolidone (FR-100µg) Trimethoprim (W-5µg) and Streptomycin (S-10µg) by Kirby-Bauer disc diffusion method (Bauer *et al.,*1966) and interpreted using NCCLS Document M2 - A4 (1990) standards.

Histopathology

Following necropsy gills, liver, spleen and kidney were fixed in 10% buffered formalin for 48 hours. Tissue were trimmed to the thickness of 0.5cm in size and the block were subsequently dehydrated in a series of alcohol, clean with xylene and embedded in paraffin wax using an automated tissue processor. The tissues were sectioned at about 4-5µm on microtome and mounted on glass slides, de-

waxed and stained with haematoxylin and eosin (HE) Bancroft *et al.,* (1996) and examined under light microscope.

Results

The young sick fish showed clinical signs of darkening of the skin, loss of appetite and gasping at the surface of the water before death (Figure 1). Grossly the liver in one of the sick fish was pale and swollen and kidney and spleen were also congested.

Figure 1. Darkening of the skin of the sick rainbow trout

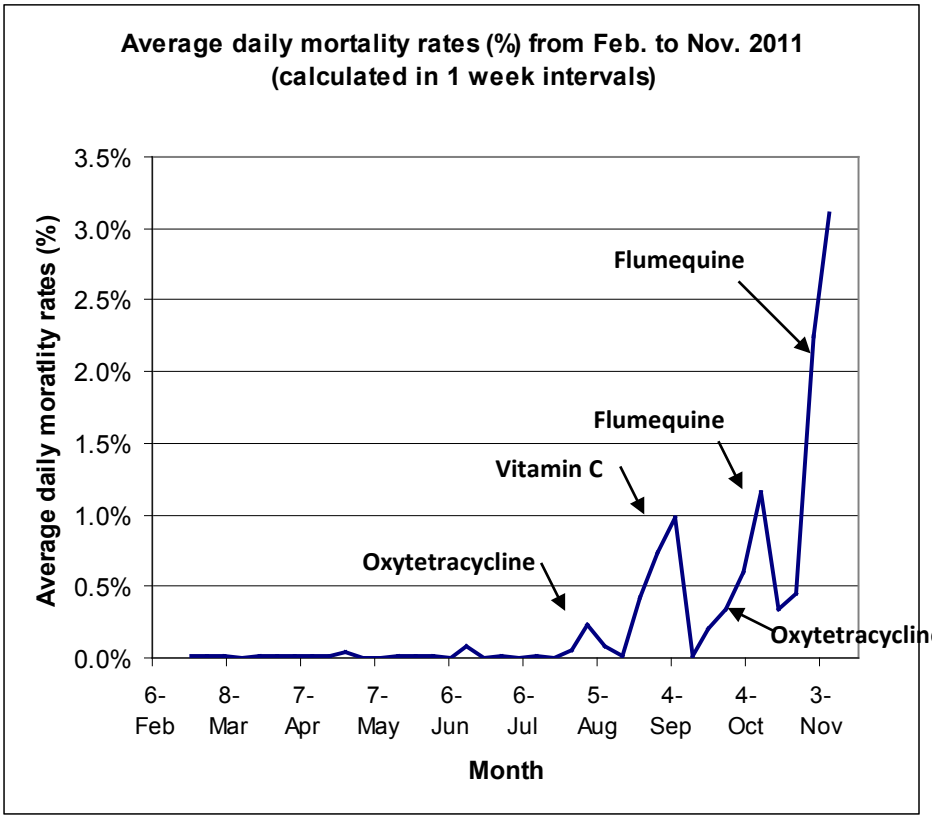


Figure 2. Average daily fish mortality recorded during the months of February to November 2011.

Fish parasites

The skin of some of the fish was very dark and *Trichodina* species were the major parasites recovered both from the skin and gills of the fish. The parasites were recovered from the skin scrapings collected from the dorsal surface and under the fins base of the fish. The gills were very much congested in some of the fish. The histopathological examination of sections of the gills showed extensive areas of the gills covered with the parasite *Trichodina* species. Up to five or six parasites were observed packed together over a very small area on the gills. Extensive necrosis of the gill epithelium with infiltration by inflammatory cells was also observed.

Bacterial isolation and identification

Following the 24 hours of incubation of the TSA agar inoculated with swabs from the liver, spleen and kidney tissues, the media were checked for the growth of bacteria and the following results were obtained. There was colony of bacterial growth in all the culture media inoculated with the samples from the different organs. The colonies were pure with the following characteristics; *Colony morphology,* yellow smooth colonies of bacteria that are circular with approximate diameter of 2-3 mm was seen. Pure colonies of the bacteria were observed without any other type of colony of bacteria (Figure 3). *Grams Reaction* showed that the bacteria were Gram negative short rods and also found to be Oxidase Negative and Fermentative with the following biochemical reactions (Table 1).

Table 1. Biochemical characteristics of *Y. ruckeri*

Biochemical Characteristics	
Indole	(-)
Methyl red	(+)
Voges-Proskauer	(-)
Citrate as C source	(+)
Gluconate oxidation	(-)
Malonate	(-)
H2S production	(-)
Arginine dehydrolase	(-)
Lysine decarboxylase	(+)
Ornithine decarboxylase	(+)
Urease	(-)
ß-galactosidase (ONPG)	(+)
Hydrolysis of gelatin	(+)
Gas from glucose	(-)
Acid production from	
adonitol	(-)
arabinise	(-)
dulcitol	(-)
maltose	(+)
mannitol	(+)
Salicin	(-)
Sorbitol	(-)
Sucrose	(+) Should have been (-)

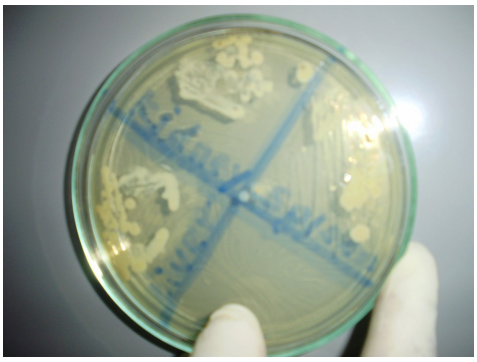


Figure 3. Colonies of *Y. ruckeri* on Tryptose Soy Agar.

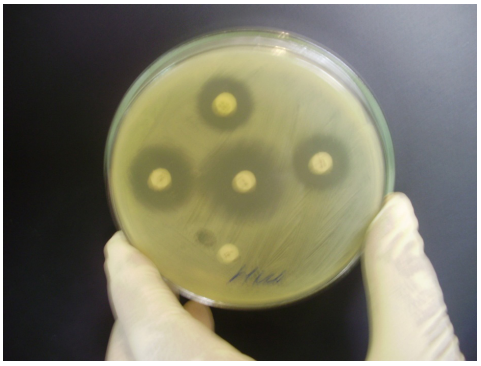


Figure 4. Antimicrobial sensitivity test of *Y. ruckeri* to Oxytetracycline. (OT-30 µg), Furazolidone (FR-100µg) Trimethoprim (W-5µg) and Streptomycin (S-10µg) following Kirby-Bauer disc diffusion method (NCCLS).

The bacterial isolates were found to be sensitive to all the antimicrobial agents used and most sensitive to Trimethoprim based on the diameter of zone of inhibition (Figure 4).

Pathological findings

Grossly darkening of the skin, pale gills, swollen and pale liver with multifocal necrosis was observed on the fish samples. Microscopically hemorrhages, degeneration and necrosis of primary and secondary lamellae were observed in the gills with infiltration by inflammatory cells (Figures 5-8).The Secondary lamellae were congested, edematous, with epithelial lifting, sloughing, desquamation, hypertrophy, hyperplasia and fusion with degeneration and necrosis and hyperplasia and hypertrophy of the goblet cells (Figure 10). Colonies of *Trichodina* species were also recorded in histological sections of the gills (Figure 9). The liver was congested and infiltrated with inflammatory cells with extensive areas of necrosis (Figure 11).

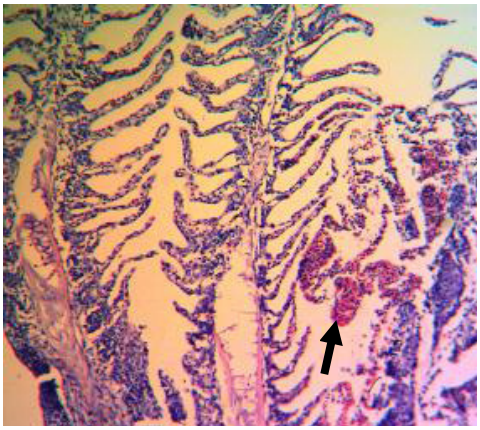


Figure 5. Histopathological sections of gills (x10). Hemorrhage (Arrow), degeneration and necrosis of primary lamellae and secondary lamellae with infiltration by inflammatory cells.

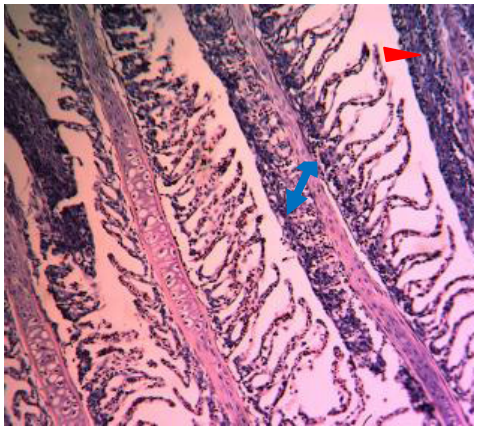


Figure 6. Sections of the gills (x10). Lifting (Arrow) and hypertrophy of epithelium, congestion, fusion (double arrow) and necrosis of the secondary lamellae with infiltration by inflammatory cells. (Arrow head).

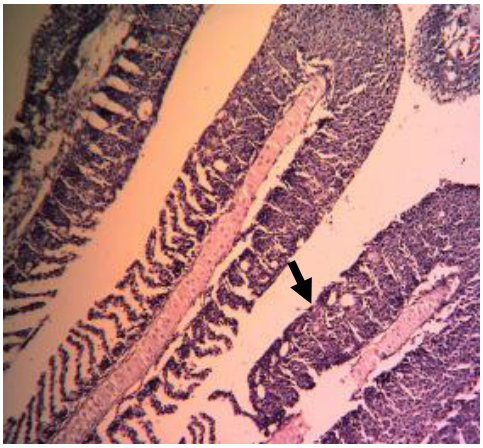


Figure 7. Sections of gills. Hypertrophy, hyperplasia and fusion of the secondary lamellae

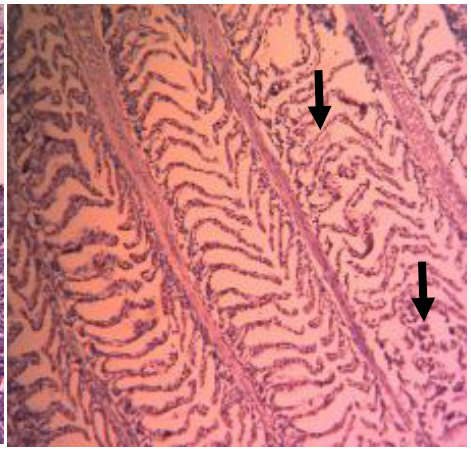


Figure 8. Sections of gills showing congestion, edema, degeneration and necrosis of secondary lamellae (Arrow)

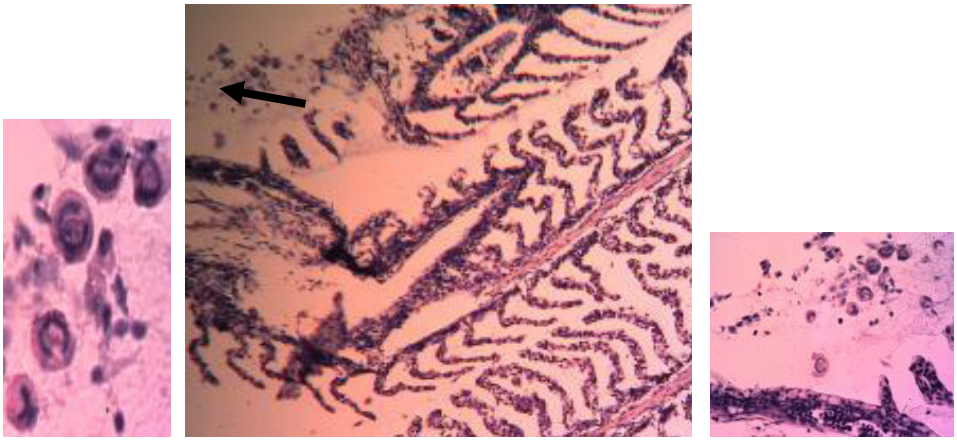


Figure 9. Sections of gills infested with *Trichodina* species. Degeneration and necrosis of primary lamellae, alteration of the structure of secondary lamellae due to infestation with *Trichodina* species (Arrow).

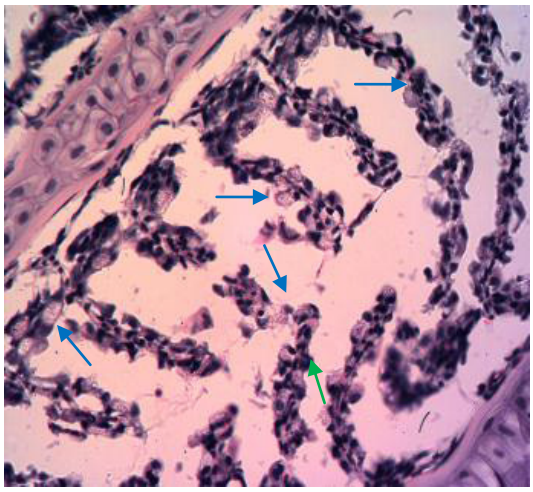
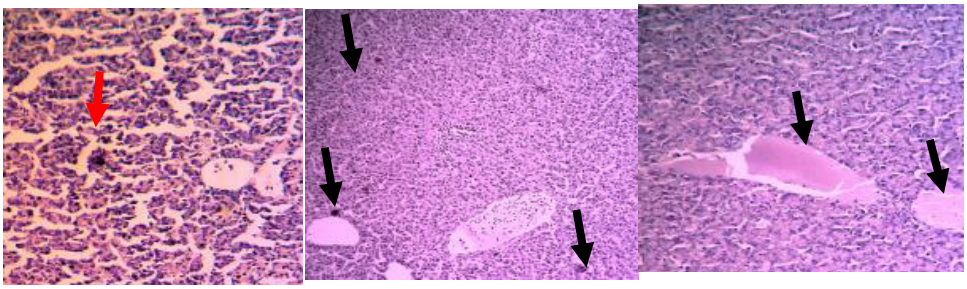


Figure 10. Sections of gills showing hypertrophy and hyperplasia of gill goblet (mucus) cells (x40).



A(x40) **B (x10)** **C (x40)**

Figure 11. Liver of rainbow trout (A) Degeneration and necrosis with infiltration of inflammatory cells and bacterial colonies. (B) Exudates, congestion, bacterial colonies (arrow) and infiltration of inflammatory cells. (C) Degeneration of hepatocytes, exudates (arrow head) and bacterial colonies (Arrow)

Discussion

Yersiniosis (Enteric red mouth disease) is a septicemic, haemorrhagic contagious disease of salmonids, induced by *Yersinia ruckeri*, and young rainbow trout are most susceptible to infection (Frerichs, 1993). This important and highly pathogenic bacterium was originally isolated from rainbow trout in Idaho, USA, by Rucker in 1950 Bullock *et al*, (1971) Cited by Roberts (1989), and initially it was also called Enteric red mouth disease. Yersiniosis or enteric red mouth disease leads to significant economic losses in salmonid aquaculture worldwide by infection that may result in a septicaemic condition with haemorrhages on the body surface and in the internal organs (Tobback, 2009). Like other members of the Enterobacteriaceae family, *Y. ruckeri* is glucose fermentative, oxidase-negative, and nitrate-reductive (Cipriano *et al*., 1986). *Y. ruckeri* is present in many parts of the world but its habitat is not known with certainty. Although the organism has been isolated from the aquatic environment, the main source of infection is probably carrier fish and the bacteria is known to survive in pond muds for up to two months (Roberts, 1989). The bacteria survives in water with low salt (survival of at least 4 months in water containing 0 to 20 per thousand salt, survival for more than 245 days in water containing 5 per 1000 of salt, but survival limited to 32 days in water containing salt 35 per 1000) in sediments and would be a constituent of the flora of fresh water.

In this study, *Y. ruckeri* is isolated for the first time from sick fish from a rainbow trout farm located at the highlands in south west of Ethiopia. During the acute stage of the disease, oral antibiotic treatments have been given with feed: oxytetracycline at 120 mg/kg of fish biomass per day during 8 days, and flumequine at 12 mg/kg during 4 to 5 days. Both were relatively successful at first attempt with significant decrease in mortality, but symptoms and mortality re-appeared a couple of weeks later, and treatments were not successful at second attempt (Figure 2). However, the isolates were sensitive to all the three anitimicrobial agents used during the *in vitro* antimicrobial sensitivity testing. In addition, Vitamin C was also given at 60 mg/kg of fish biomass during 5 days with success although it was not sustainable. In both conditions, treatment failure could be attributed to the occurrence of huge parasitic burden with *Trichodina* species on the gills. The mass parasitic burden was associated with degeneration, necrosis of primary and secondary lamellae, lifting and hypertrophy of epithelium, congestion, fusion and infiltration by inflammatory cells. This is commonly linked directly to reduced efficiency of the gills resulting in poor oxygen absorption from the water caused by these major pathological lesions and reduced immune status of the fish to defend itself against the invading pathogens. Yersiniosis outbreaks are often associated with excessive stocking densities, poor water quality, and the occurrence of environmental stressors (Seong Joon Joh *et al*., 2010). *Y. ruckeri* can also persist in asymptomatic carrier state, where infection through carrier fish is especially important under stress conditions (Tobback, 2009).

The diagnosis of Yersiniosis was made based on clinical, bacteriological and pathological findings of darkening of the skin, high degree anaemia of oral and branchial mucosa, congested gills, kidney and spleen with extensive necrosis; paleness, congestion, exudation and occurrence of bacterial colonies in the liver and infiltration with inflammatory cells. The histological findings of degeneration and necrosis of hepatocytes, exudates, congestion, infiltration by inflammatory cells and bacterial colonies were similar to the findings of Karatas *et al*, (2004).

The disease is commonly associated with temperatures above 10^0C and directly related to stressed carrier fish and it most commonly affects younger rainbow trout (Roberts, 1989). Fish having a size of about 7.5 cm are most severely affected, whereas in older animals, the disease progresses in a chronic form. This is consistent to the findings of this study whereby nine months old rain-

bow trout having a size of about 10 cm were the most commonly affected fish in the farm. Similar to the findings of Mahjoor and Akhlaghi (2012) in Iran, fish mortality due to the disease in this study occurred at temperatures above 10^0C, and fish had swollen gills and gasping at pond's water inlet during the hottest hours of the day (evening) and in fish that are in a state of stress associated with the excessive parasitic burden on their gills.

A study conducted on the effect of Levamisole on rainbow trout experimentally infected with *Yersinia ruckeri* in Turkey showed that an average 82.2% protection level was obtained at three different dose rates (Ispir, 2009). These results suggest that the application of levamisole in fish farms could increase resistance of fish to infection and offer economic benefits. This may be considered as an option for fish protection in the farm in the future. *Yersinia enterocolitica and Edwardsiella tarda* were isolated from apparently healthy fish at Lake Ziway, Eshetu Yimer (2000) and *Y. ruckeri* was isolated from the kidney and intestine of apparently healthy *Oreochromis niloticus* and *Barbus* species at Lake Tana, Ethiopia (Anwar Nuru *et al*., 2012). However, this is the first isolation of *Y. ruckeri* from sick rainbow trout in a fish farm in Ethiopia. This would serve as a source of basic information to prospective fish farmers in the country to take all precautionary measures to prevent the introduction and establishment of economically important fish bacterial pathogens in their farms.

Factors such as stress, handling, overcrowding, a decrease in the oxygen content of water, an overload of organic matter and a change in water temperature are predisposing factors. The water temperature is particularly important parameter and peak of infection occurs when the temperature is between 15 and 18°C. By cons, at a temperature below 10°C, the cases are fewer. Stress also plays an important role in the transmission because the healthy carriers do not transmit the disease to the extent they are subject to adverse environmental conditions and particularly when the water temperature is higher. Control may be achieved with sanitary measures, provided care is taken to remove dead and dying fish, and removing the original stressors, but antibiotic therapy is also usually necessary. Highly effective vaccines are now available, and, if used properly reduce losses, provided there is no inter-current immunosuppression due to environmental stress (Roberts, 1989).

Conclusions and recommendations

Yersinia ruckeri is a major fish bacterial pathogen causing heavy losses in trout farms in most parts of the world and its occurrence in this farm provides

a good lesson on how to prevent its occurrence and spread in the future. The occurrence of parasites like *Trichodina* species in large numbers is often associated with poor water quality as these parasites are most often referred to as debility parasites. Therefore, improving the water quality in the farm by increasing the water flow and minimizing excess organic load could minimize the occurrence of these and other related parasites that significantly stress fish. The water intake of the farm should be improved as it is coming through a 1.5km canal. Hence, it is much preferable to take water directly from the river so that contaminants are minimized. The farm design should also be improved, taking this into consideration the farm has recently built ponds (circular, plastic (geomembrane) with drainage at the pond bottom centre which helps to improves water flow. Vaccination is also recommended as there is an effective vaccine for Yersiniosis given to fingerlings at 2 – 5gm size by the immersion method. During outbreaks of Yersiniosis appropriate antimicrobial agents should be utilized after checking their sensitivity to the locally available antimicrobial agents and the isolates of the bacteria needs to be further characterized.

Intensive trout fish farming is being practiced for the first time in this country and this is the first outbreak of Yersiniosis recorded in a fish farm. The practical experience that could be obtained from this farm could assist future investors to be engaged in this type of venture to take all precautions in order to avoid the introduction and occurrence of diseases like this one and others. Since Ethiopia has a suitable climatic and environmental conditions for intensive fish farming practices, it is strongly recommended that private investors should be encouraged and supported to get involved in fish farming practices in the country.

Acknowledgments

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