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Detection of *Yersinia ruckeri* in naturally infected rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from trout farms in Anatolia, Turkey

Detección de *Yersinia ruckeri* en truchas arcoíris (*Oncorhynchus mykiss* Walbaum, 1792) infectadas naturalmente en criaderos de truchas en Anatolia, Turquía

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ABSTRACT

The present study aimed to detect *Yersinia ruckeri* bacteria in fish samples collected from the rainbow trout *(Oncorhynchus mykiss*, Walbaum, 1792) farms in the South Eastern Anatolia Region of Turkey through bacteriological culturing and polymerase chain reaction. Separate visits were made to trout farming facilities in Diyarbakır, Adıyaman, Şanlıurfa, and Batman in January and February 2021, and 30 fish with an average live weight of 200–250 g were collected from each facility. A total of 1,200 samples were taken from 40 trout farms. Liver, spleen, kidney, and tissue specimens were collected from rainbow trout. *Yersinia ruckeri* was isolated from the kidney, liver and muscle tissue of fish on McConkey Agar for bacteriological examinations. Isolated strains were identified by MALDI–TOF and PCR. It was detected the bacteria in the liver, kidney, and muscle tissue samples of fish from 23 out of 40 farms. In conclusion, these bacteria were detected in local farms. These farms must implement vaccination, minimize stress factors affecting the fish, and avoid overstocking. The best methods to protect against infection include avoiding overstocking, low oxygen levels, and underqualified labor force.

Key words: MALDI–TOF; *Oncorhynchus mykiss*; PCR; *Yersinia ruckeri*

RESUMEN

El presente estudio tuvo como objetivo detectar la bacteria *Yersinia ruckeri* en muestras de pescado recolectadas de las granjas de trucha arco iris (*Oncorhynchus mykiss,* Walbaum, 1792) en la región de Anatolia del sudeste de Turquía a través del cultivo bacteriológico y la reacción en cadena de la polimerasa. Además, también tenía como objetivo determinar la susceptibilidad antibiótica de la bacteria. Se realizaron visitas separadas a las instalaciones de cultivo de truchas en Diyarbakır, Adıyaman, Şanlıurfa y Batman en enero y febrero de 2021, y se recolectaron 30 peces con un peso vivo promedio de 200 a 250 g en cada instalación. Se tomaron un total de 1.200 muestras de 40 criaderos de truchas. Se recolectaron especímenes de hígado, bazo, riñón y tejido de trucha arcoíris. Se aisló *Yersinia ruckeri* del riñón, el hígado y el tejido de pescado en Agar McConkey para realizar exámenes bacteriológicos. Las cepas aisladas fueron identificadas por MALDI–TOF y PCR. Se detectó el agente bacteriano en muestras de hígado, riñón y músculo tejido de peces en 23 de 40 granjas. En conclusión, estas bacterias fueron detectadas en granjas locales. Estas granjas deben implementar la vacunación, minimizar los factores de estrés que afectan a los peces y evitar el exceso de la sobre población. Los mejores métodos para protegerse contra la infección incluyen evitar la alta densidad los bajos niveles de oxígeno y la mano de obra poco calificada.

Palabras clave: MALDI–TOF; *Oncorhynchus mykiss*; PCR; *Yersinia ruckeri*

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INTRODUCTION

Currently, one of the greatest challenges faced by the developing World is nutrition from a balanced, healthy, and adequate diet. A major cause for this challenge is the population boom [1]. Feeding the rapidly increasing World population is contingent upon increasing the agricultural output and ensuring its equitable distribution. Considering the prevalent rise in population and aggravated nutritional problems, these challenges are expected to persist in the future as well [2]. Therefore, the demand for seafood, a rich source of protein, increases by the day. It is necessary to ensure the continuity of production by protecting the scarce resources to benefit from the fishery stocks in an economic and sustainable way [3]. Accordingly, intensive efforts are in place to progressively increase the current fishery output through aquaculture production [4]. The fishery output in Turkey was 785,811 tons in 2020, including the aquaculture production of 421,411 tons where fishing accounted for 364,400 tons [5]. Aquaculture production increased by 12.9% in 2020. Trout was the most important fish species with an output of 127,905 tons [5] Rainbow trout (*Oncorhynchus mykiss*) is one of the most widespread cold water fish species farmed throughout the World. Production of rainbow trout in Turkey accounts for approximately 50% of the total aquaculture production [6] Infectious diseases and associated economic losses are the leading factors that have an adverse effect on aquaculture. Karabulut and Kurtoğlu [7], suggested that feed costs and diseases were the primary development associated with rainbow trout farming. The disease-associated annual economic losses increased with the rapid expansion of aquaculture and increased fish production levels, accurating for billions of dollars Worldwide [8]. Bacteria, fungi, viruses, and parasites were reported as the cause of the most prominent diseases jeopardizing the aquaculture industry [9] There are a number of agents associated with diseases in aquatic organisms.

Microorganisms typically occurring in the normal microflora of the aquatic environment and fish can be transmitted by broodstock fish to the roes as well as to fish during each stage of production. Vaccines, immunostimulants, prebiotics and probiotics have been shown to confer some protection against *Y. ruckeri* [10]. But the immune system is developed only in trout fish with ≥2 g of body weight; therefore, sufficient protection cannot be provided to smaller fish by means of vaccination. Accordingly, intensive antimicrobial use is inevitable for controlling the diseases [11].

Studies have demonstrated that aquatic and fish microflora have similar characteristics and that many bacteria that induce diseases in fish are present in aquatic resources. In particular, the causative factors associated with *Y. ruckeri*, which induces the systemic disease enteric red mouth disease, also known as yersiniosis in trout [12], ranked as the top disease-causing factor in rainbow trout farms.

Yersiniosis was first detected in the Hagerman Valley in Idaho State, United States, during the early 1950s; it has now proliferated in other Countries located in distant geographical Regions across Europe, South America, West Africa, and Australia, affecting trout farms almost all over the World [1]. Yersiniosis is associated with fry mortality in trout production. Mortality due to yersiniosis was reported in the rainbow trout species that generally populate fresh water and farmed in cages in the Black Sea region of Turkey [13]. In aquaculture, the acute epizootic infected formations are associated with a mortality rate of up to 70% [14], for yersiniosis, which occurs as a result of stress in fish farming and follows an acute or chronic course. The disease presents with symptoms such as darkening of the skin, hemorrhage at the inner and outer mouth, operculum, outer surface of the body and base of the fins, abdominal bloating, fluid accumulation, and unilateral or bilateral exophthalmos. Furthermore, lysis around anus, fins, and skin and hemorrhages in the orbit and iris may also occur. Hemorrhage in the internal organs and a yellowish fluid accumulated inside the abdominal cavity and stomach may be observed on postmortem examination [15].

MATERIAL AND METHODS

Trout farming facilities in Diyarbakır, Adıyaman, Şanlıurfa, and Batman Provinces were contacted and visited separately during January and February, 2021. All cages and ponds were individually examined and 30 fish showing signs of disease with an average live weight of 200–250 g were collected from each facility. All collected fish were insensitive to feed, immobile, floating on the water surface, and showing signs of disease such as color darkening. Overall, 40 trout farms were visited, and 1,200 samples were collected, which were transferred to Dicle University, Faculty of Veterinary Medicine, Department of Fisheries and Diseases Laboratory, on ice. There, the samples were first macroscopically examined and then dissected to examine the internal organs. The liver, kidneys, and muscle tissues were collected from the dissected samples and inoculated to bacteriological culture media to detect the presence of bacteria. Blood agar (BA) and McConkey agar (McA) plates were used for bacterial isolation by incubation for 24 h at 28°C (NÜVE-NC40M, Turkey) The dominant uniform bacterial colonies were purified by streaking onto the BA and McA plates three times (FIG. 1). Then, the causative agents were identified using MALDI-TOF mass spectrometry by MALDI Biotyper (Bruker, Daltonics, Germany) based on Gram staining and culture growth profiles (FIG. 1).

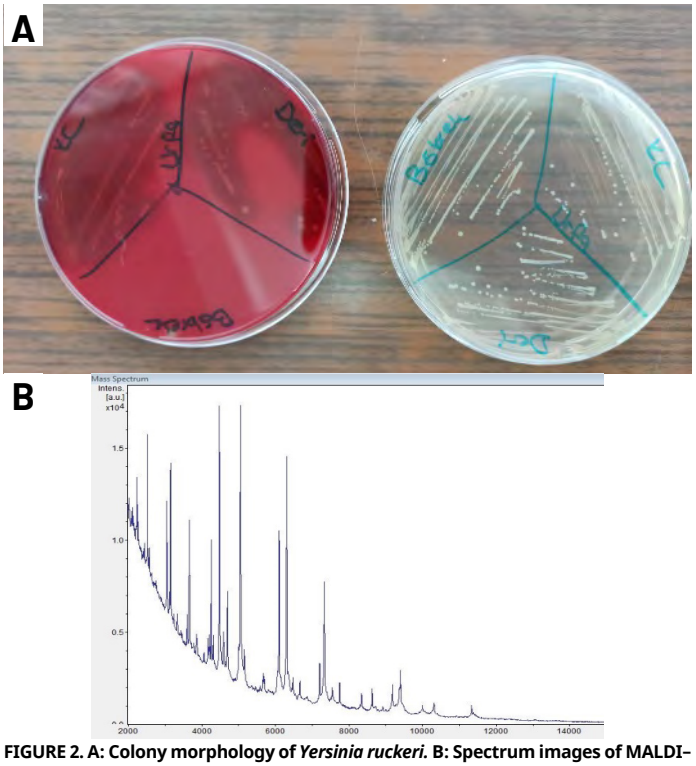

FIGURE 2. A: Colony morphology of *Yersinia ruckeri.* B: Spectrum images of MALDI-TOF *Yersinia ruckeri*

The disease usually has an acute course and is associated with high mortality rates. Therefore, there was a need for accurate and rapid diagnostic methods to combat it. PCR is the most widely used method for this purpose as it provides fast and accurate results [16]. Nyztech *Y. ruckeri* Real Time PCR Kit (Catalogue number: MD0317) was used for identification.

RESULTS AND DISCUSSION

The present study investigated 1,200 samples and *Y. ruckeri* was detected in the liver, kidneys, and tissues of the fish collected from 23 out of the 40 farms. The trout farms in the SouthEastern Anatolia Region were contaminated by *Y. ruckeri* at a rate of 0 –100% and different levels of resistance to antimicrobial agents were observed. *Y. ruckeri* is responsible for causing yersiniosis, one of the most important bacterial diseases in both freshwater and marine salmonids. Outbreak of this disease can lead to heavy economic losses [17]. The course of the disease is characterized by acute, subacute, and chronic forms. Although the disease spontaneously occurs due to sudden changes in water temperatures, lysis was reported at the base of the fins, around the anus, and inside the operculum [18]. Hemorrhage can be observed on the skin and at other parts of the body. Moreover, erythema and petechial hemorrhages may also occur within the internal organs, peritoneum, body fat, gonads, and mesenterium. Intestinal organs are typically erythemal and filled with bloody mucus. Additionally, the spleen and kidneys are swollen and the liver is pale. Furthermore, petechiae in the muscles have been reported [1]. Most of these abovementioned symptoms were also observed in the present study (FIG. 2).

Symptoms mostly observed in the study; bleeding in the operculum, at the base of the fins; and swelling and fluid accumulation in the abdomen the outer surface of the body.

Consistent with the results of the current study, Kumar *et al.* [18] reported exophthalmos, intestinal fluid accumulation, and darkening skin color in their study on salmon in 2015. In another study, Mohammed Saeed [19] reported clinical symptoms of difficulty in swimming; swimming closer to the water surface or near the ground; hemorrhage in the gills, around the eyes, and in the mouth and oral cavity; dark pigmentation; and petechial hemorrhages on the inner surface; these are consistent with the clinical symptoms observed in the current study. Most of the samples in this study had hemorrhages on the skin and various parts of the body and lysis in the operculum and fins. *Y. ruckeri* was detected in the tissue, liver, and kidney samples of the fish. Yersiniosis is a disease characterized by the occurrence of hemorrhagic zones in various tissues and organs, and general signs of hemorrhagic septicemia are manifested in course of the disease (FIG. 2). Yersiniosis due to *Y. ruckeri* is a disease that may induce high mortality rates and heavy economic losses in rainbow trout farming facilities [20]. This may be because adequate quarantine measures were not in place in the farms with the infected fish. Furthermore, the health specifications issued during fish transportation lacked adequate controls in terms of the carrier fish might have accounted for the spread of the infection throughout the entire region. In addition, the condition of fish scoops and negligence during feeding suggested that the pools might not have been disinfected. The occurrence of the disease was associated with organic pollution of the environment, heat, dense stocking, direct contact, and oxygen deficiency, all of which contributed in the stress factor [21].

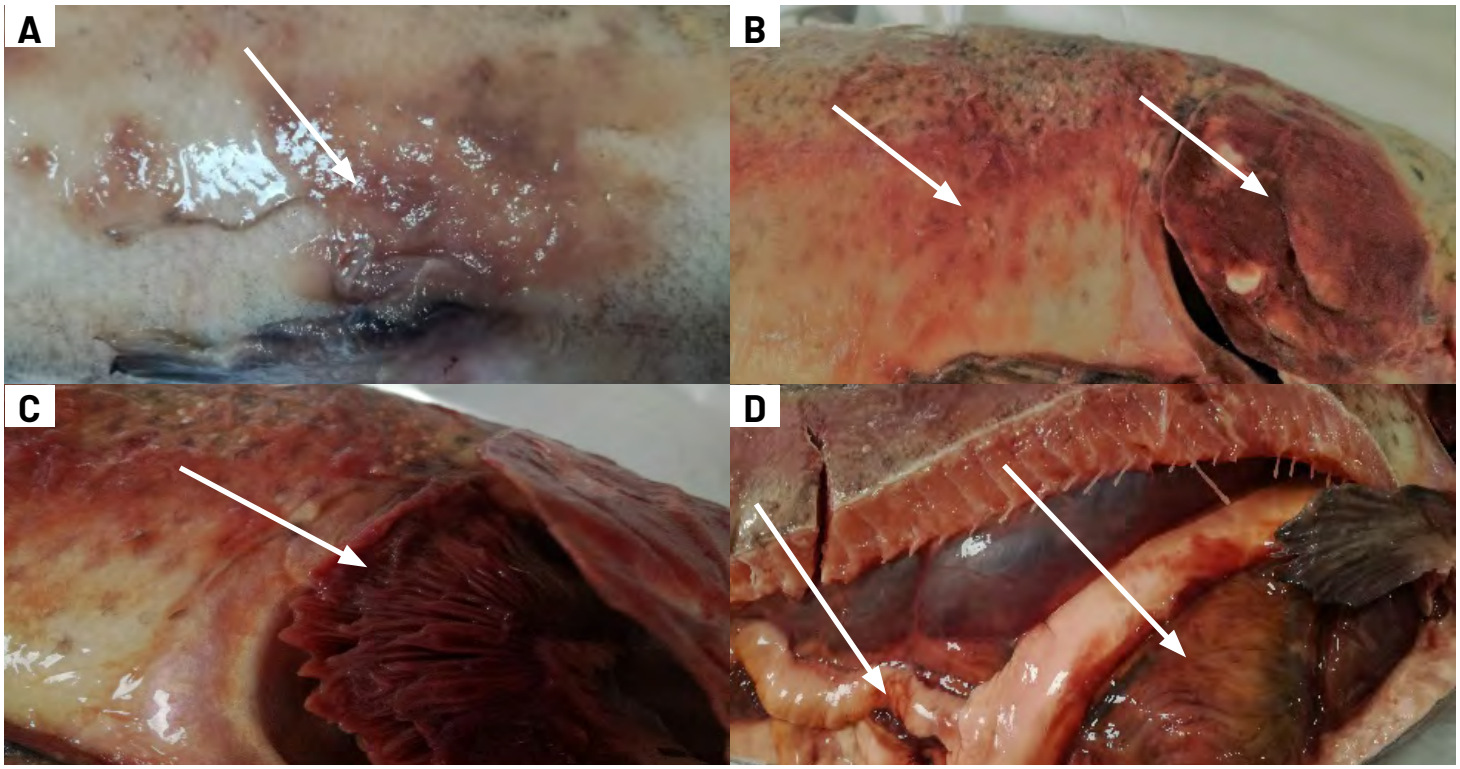

FIGURE 2. Postmortem examination revealing bleeding in the internal organs; at the base of the fins; and swelling and fluid accumulation in the abdomen (A); the outer surface of the body (B); bleeding in the operculum (C); yellowish fluid accumulated inside the abdominal cavity and stomach (D)

In this study, *Y. ruckeri* was isolated at a higher rate in the fish farms with excessively turbid water, higher water temperature, and overstocking. Tinsley *et al.* [22] reported that although the bacterial agents could also be isolated in the normal microflora of water, the incidence of disease increased in the presence of stress-inducing factors, including sudden seasonal changes in the water temperature, hygienic negligence, overpopulated production areas, and lack of clean barriers. Generally, bacterial infection occurs when fish are under physiological stress and hygiene conditions are below adequate levels. Avoiding overstocking, low oxygen, and underqualified labor force were reported as the best ways to avoid infection [23]. A number of similar reports indicated that *Y. ruckeri* had a wider host range and geographic distribution and can cause both epizootic and zoonotic diseases [17]. Therefore, further studies must be conducted in fish farms. Moreover, it was understood that regular vaccination was in place in certain farms.

CONCLUSIONS

Pathogens were detected in 23 farms. This is the first research to study yersiniosis in the SouthEastern Anatolia Region; the obtained results contribute to scientific knowledge regarding this disease. Although adequate care is essential for the treatment and control of bacterial diseases in aquaculture, vaccination and immunostimulant drugs can also be used. In general, diseases can be prevented and controlled by good administration and management practices and vaccination. Considering that clinical yerniosis is defined as a stress-related disease, the severity of the disease should be mitigated by reducing stress, controlling water quality, maintaining proper nutrition, and implementing good health practices across the regions where the disease is already prevalent. Appropriate care and feeding conditions, improved water quality, reduced stock intensity, and the removal of dead fish can reduce disease emergence and result in faster treatment results.

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Conflict of interest

The authors have no declaration of competing interests.

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