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Review

IMPORTANT BACTERIAL DISEASES AND THEIR CONTROL IN RAINBOW TROUT IN SERBIAN AQUACULTURE

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Abstract

Global freshwater fish production in aquaculture has grown rapidly in recent decades. This constant growth, involving novel forms of intensive aquaculture, has increased global movements of fish and boosted various anthropogenic stresses to aquatic ecosystems, so rainbow trout aquaculture has encountered the emergence and outbreaks of many bacterial diseases. Due to the need to effectively prevent and control disease outbreaks, vaccines have become an important technology in intensive trout aquaculture. In this review, the applications of specific vaccines against important bacterial diseases of rainbow trout in Serbian aquaculture are summarized.

Key words: bacterial diseases, rainbow trout, autogenous vaccines

INTRODUCTION

Aquaculture is highly dynamic and characterized by high diversity in both the range of farmed species and in the production systems (Kibenge et al., 2016). Aquaculture has grown significantly since 1970, at average rate 8.9% per year (Huang et al., 2019; Subasinghe et al., 2005). Also, the diversity of global aquaculture production is now significantly higher than in the past, with a plethora of new aquatic animals, plants, and algal species produced in a variety of marine, brackish, and freshwater systems

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globally (Hou et al., 2022; Metian et al., 2021). As a consequence of this intensification and diversification of aquaculture, various infectious diseases occur, causing the death of almost 10% of all aquatic aquaculture animals, and resulting in losses of more than $US 10 billion per year globally (FAO, 2018). A significant part of those losses is caused by bacterial pathogens. Almost 50% of fish in aquaculture is lost due to bacterial infections (Algammal et al., 2020).

In Serbia, the main products of aquaculture are cyprinid fish species, primarily carp (*Cyprinus carpio*), followed by the salmonids, mostly rainbow trout (*Oncorhynchus mykiss*). The intensification and expansion of rainbow trout production in intensive systems provides conditions for easy disease transmission. The intensification of aquaculture and the fact that the local fish trade and movement of live fish between local aquaculture facilities are often not overseen by adequate fish health controls lead to a higher risk of fish health/disease issues.

MAJOR BACTERIAL DISEASES AFFECTING RAINBOW TROUT

Many bacterial disease agents have been detected in aquaculture fish in Serbia. Since common carp and rainbow trout are the predominant fish cultured in Serbia, with 149 registered fishponds, of which 77 are for carp farming and 68 are for trout farming (Relic and Markovic, 2021), most of the disease agents have been isolated from these species. The most noteworthy bacterial diseases in rainbow trout aquaculture in Serbia are motile aeromonad septicemia (MAS), *Pseudomonas* septicemia, furunculosis, yersiniosis, lactococcosis, flavobacteriosis (including columnaris disease), and bacterial kidney disease (BKD).

Motile aeromonad septicemia (MAS)

MAS caused by *Aeromonas hydrophila* and other aeromonads, such as *A. sobria, A. veronii*, and *A. caviae*, is one of the most important opportunistic infections in freshwater fish aquaculture (Pekala-Safinska, 2018; Shulz et al., 2020). Motile aeromonads like *A. hydrophila* can put an economic burden on aquaculture production, with outbreaks in fish farms causing high mortality rates (Zhang et al., 2020; Pridgeon et al., 2011). *A. hydrophila* is a common cause of septicemia in rainbow trout and other fish species in Serbian aquaculture (Jeremic et al., 2013, Radosavljevic et al., 2014).

MAS is usually considered as a secondary infection, but *A. hydrophila* can be a primary pathogen of the disease (Zhang et al., 2020; Pridgeon et al., 2011). Various environmental factors (high water temperature, low oxygen concentrations, sudden water pH changes, high levels of ammonia and nitrite, etc.) could predispose aquaculture fish to the disease (Jeremic et al., 2005). *A. hydrophila* is biochemically and serologically very heterogeneous (Đorđević et al., 2012), and this limits development of an effective commercial vaccine (Nayak 2020), even though a vaccine that could offer protection to fishes in intensive aquaculture is urgently needed. Development of commercial *A. hydrophila* vaccines is limited due to strain diversity (Mzula et al., 2019).

***Pseudomonas* septicemia.** Pseudomonads are opportunistic fish pathogens, mostly causing secondary infection, but also acting as primary pathogens. In many fish species, pseudomonads cause a generalized infection with ulcerative syndrome, hemorrhagic septicemia and high mortality rate (Algammal et al., 2020). Various *Pseudomonas* are known fish pathogens, with *P. fluorescens* and *P. putida* causing severe losses in rainbow trout aquaculture (Oh et al., 2019). Pseudomonads are common disease agents in different aquatic animals, including rainbow trout in Serbia (Jeremic et al., 2005, Jeremic et al., 2011, Radosavljevic et al., 2013).

Due to the facts that most pseudomonads are naturally resistant to several antibiotics (De Kievit et al., 2001), and common therapy for the pseudomonas infection in aquaculture is antibiotic treatment, problems with antimicrobial resistance are often present. Various experimental vaccines have been used for prevention of the diseases caused by pseudomonads (Romalde et al., 2005). Formalin inactivated whole cell vaccines have been developed for *P. anguilliseptica* and *P. plecoglossicida* (Austin and Austin, 2016), but currently, no commercial vaccine is available for aquaculture (Duman et al., 2021).

Furunculosis. Furunculosis, an important disease of cultured salmonids caused by *Aeromonas salmonicida subsp. salmonicida* (sometimes called typical *A. salmonicida*) is one of the oldest known fish diseases (Austin and Austin, 2016). The disease caused by different strains of *A. salmonicida* has been reported in more than 50 different fish species worldwide (Austin and Austin, 2016). *A. salmonicida subsp. salmonicida* is often detected in rainbow trout farms in Serbia, and the outbreaks result in high mortalities of infected trout (Jeremic et al., 2005, Jeremic et al., 2011, Radosavljevic et al., 2013). However, the incidence of furunculosis has been reduced in food fish using vaccines (Braden et al., 2019).

Yersiniosis. Yersiniosis, also known in fish as enteric red mouth disease, is caused by *Yersinia ruckeri*, and is one of the most important diseases in rainbow trout, causing significant economic pressure in aquaculture (Austin and Austin, 2016). It was detected in the USA in the 1950s, and it is now present in aquaculture all over the world (Plumb and Hanson, 2011). In Serbia, the disease was first diagnosed in 1987 (Ocvirk et al., 1987), but is now widespread and has become one of the most prevalent trout pathogens in Serbia (Jeremic et al., 2005; Radosavljevic et al., 2013). In the study by Jeremic et al., (2011), yersiniosis was present on the majority of examined trout farms in one-year-old rainbow trout. Vaccination has been shown to be the most successful strategy, providing protection against this disease in fish (Skov et al., 2018).

Lactococcosis. Lactococcosis is systemic disease of rainbow trout, caused by *Lactococcus garvieae,* and is one of the major threats during the warm period (Austin & Austin 2016). Surviving fish can suffer from chronic or persistent infections. It has been a cause of economic losses in rainbow trout aquaculture, especially in many

South European countries (Radosavljević et al., 2020; Vendrell et al., 2006). The disease is present in rainbow trout farms in Serbia and results in high mortalities of infected fish (Radosavljević et al., 2020). Vaccination seems the best approach to control this disease (Tanrikul, 2012).

Flavobacteriosis. Three important diseases of rainbow trout are caused by *Flavobacterium*: columnaris disease (caused by *F. columnare*), bacterial cold-water disease (caused by *F. psychrophilum*) and bacterial gill disease (caused by *F. branchiophilum*) (Starliper, 2011). Other *Flavobacterium* species (*F. scophtalmum*, *F. balustinum*, *F. hydatis*, *F. johnsoniae* and *F. oncorhynchi*) can also cause disease in fish (Austin and Austin, 2016). In Serbia, *F. columnare F. psychrophilum* and *F. branchiophilum* were identified in diseased rainbow trout (Jeremic et al., 2005, Jeremic et al., 2011, Radosavljevic et al., 2013). In order to prevent and control the diseases caused by *Flavobacterium* in aquaculture, various preventive and curative measures have been implemented, including vaccination (Declercq et al., 2013). Many attempts have been made to produce commercial vaccines for preventing diseases caused by flavobacteria (mostly *F. psychrophilum*) (Hoare et al., 2017; Madetoja et al., 2006). Despite the important advances that have been made, new strategies and initiatives for development of commercial vaccine are still needed (Gomez et al., 2014).

Bacterial Kidney Disease (BKD). This disease is caused by *Renibacterium salmoninarum*, a Gram-negative bacterium that targets the kidney of infected fish, causing creamy-white granulomatous lesions (Austin and Austin, 2016). It is a chronic disease with high mortality rates, particularly in salmonids (Delghandi et al., 2020; Zrncic & Radosavljevic 2017). Since there are no available therapeutic procedures or any commercial vaccine for complete eradication of the causative agents, BKD is hard to control (Delghandi et al., 2020). Rainbow trout are affected at all stages of life, and the disease has a serious impact on the aquaculture (Johansen et al., 2011). Various factors in the production system (food, good practice, size and age of fish, etc.) can contribute to the development of BKD (Jónsdóttir et al., 1998). The disease is present in rainbow trout farms in Serbia, resulting in a high mortality rate in infected fish (Radosavljević et al., 2012, Radosavljević et al., 2015).

CONTROL OF BACTERIAL DISEASES AFFECTING RAINBOW TROUT AQUACULTURE

Pathogenic viruses, bacteria, and parasites are key causes of mortality and economic losses in fish aquaculture (Naylor et al., 2021; Miccoli et al., 2021; Yildiz et al., 2019). In China, as the world's largest aquaculture producer, 15 to 20 % loss in production occurs per year due to diseases caused by bacteria (Khan et al., 2011). Disease prevention and control are challenges for aquaculture, and diseases are treated mostly by various chemicals and antibiotics. However, the overuse of antimicrobials in aquaculture has made the emergence of antimicrobial resistant bacteria more prominent (Cabello et al., 2013, Ljubojević et al., 2016). Antimicrobial resistance also presents a serious threat to human populations worldwide (Hendriksen et al., 2019). Although, there are strict regulations and recommendations regarding the use of antimicrobials in aquaculture, there is still extensive usage in many countries, including Serbia. The only antimicrobial drugs approved for use in fish aquaculture in the USA are oxytetracycline, florfenicol, and sulfadimethoxine/ormetoprim (Love et al., 2020).

Besides the other measures in disease control, vaccination is cost-effective, practical and environmentally friendly (Ma et al., 2019). In salmonid aquaculture, vaccines have been used for more than 40 years, and that practice led to greater productivity and fewer losses caused by infectious diseases. As a consequence of vaccination, the use of antibiotics in aquaculture dropped to a mere fraction of its original level (Sommerset et al., 2005). Future control of bacterial diseases will likely be based on vaccination (Gravningen et al., 2019). Therefore, the development and implementation of effective vaccines will be a key requirement for future aquatic production, in combination with other measures, such as genetics, immunostimulants, functional feeds and probiotics (Gravningen et al., 2019).

BACTERIAL VACCINES IN SERBIAN RAINBOW TROUT AQUACULTURE

Currently, no commercial vaccine for trout bacterial diseases is licensed for use in Serbia, although vaccines are commercially available for many fish species significant for aquaculture. Challenges in using commercial vaccines still exist for various reasons, including the route of administration, since injection, intramuscularly or intraperitoneally, is currently the most widely applied method for delivering commercial vaccines (Ma et al., 2019). The licensing costs and costs of injecting fish are also unaffordable for many countries, including Serbia. Commercial vaccines are affordable for high value species in aquaculture, but not for low value species, or for fish with a long-lasting production cycle or small sized fish.

However, autogenous vaccines can provide an adequate solution when a commercial vaccine is not available or affordable (Holm et al., 2014). Autogenous vaccines have been produced for preventing some trout diseases in Serbia, the first one being produced in 2000. The first autogenous vaccine was prepared on the basis of formalin-inactivated whole cells of *Yersinia ruckeri*. The strains of *Y. ruckeri* were isolated from parenchymatous organs of rainbow trout suffering from yersiniosis (Jeremic and Andjelic, 2000). Recently, autogenous, inactivated, whole-cell vaccines against three pathogens (*A. salmonicida* subsp. *salmonicida*, *L. garvieae* and *A. hydrophila*) were produced and implemented with success through a government-funded scheme (the Innovation Fund of the Republic of Serbia – Innovation Vouchers Funding Scheme, Project Contract ID 731, Project Contract ID 757). The pathogenic bacteria for autogenous vaccines were isolated from infected fish at aquaculture facilities. These autogenous vaccines effectively prevented outbreaks of MAS, furunculosis and lactococcosis in vaccinated rainbow trout populations. As a result of these activities, losses due to the diseases were significantly reduced compared to the situation where aquaculture trout were not vaccinated, as were costs for antibiotic therapy. In fact, the production of autogenous vaccine for an aquaculture facility with a specific fish disease was delivered in significantly shorter time and was more affordable than a commercial vaccine.

CONCLUSION

A preventive approach is the best course of action to overcome bacterial disease outbreaks in aquaculture. Vaccination for prevention of bacterial diseases in aquaculture, instead of antibiotic therapy, has proven effective and beneficial. Many different vaccines have been developed in order to protect aquaculture fish against various diseases, but efficient and affordable vaccines against important bacterial diseases are still needed. Thus, autogenous vaccines, prepared from pathogens present in a particular facility, could be an adequate, alternative strategy for reducing disease outbreaks in intensive rainbow trout aquaculture.

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Authors' contributions

VR and NZ made contributions in conceiving the study, collecting data and drafting the manuscript. OR and LJV revised the manuscript critically and together with VR prepared the final draft of the manuscript. All the authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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VAŽNE BAKTERIJSKE BOLESTI KALIFORNIJSKE PASTRMKE U AKVAKULTURI SRBIJE I NJIHOVO SUZBIJANJE

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Kratak sadržaj

Globalna proizvodnja slatkovodnih riba je u proteklim decenijama zabeležila značajan rast. Istovremeno, s pojavom novih vidova intenzivne akvakulture, povećanim prometom vodenih životinja i različitim oblicima antropogenog uticaja na vodene ekosisteme, slatkovodna akvakultura se susrela se sa pojavom mnogih bolesti. Zahvaljujući potencijalu da bezbedno i efikasno spreče pojavu bolesti u akvakulturi, vakcine su postale značajan alat za prevenciju i kontrolu bolesti riba u svetu. U radu su opisane značajne bakterijske bolesti kalifornijske pastrmke u akvakulturi Srbije i razvoj specifičnih vakcina za kontrolu ovih bolesti.

Ključne reči: bakterijske bolesti, kalifornijska pastrmka, autogene vakcine