

Timisoara, 25-26 May

HAFNIA ALVEI – OPPORTUNISTIC PATHOGEN INVOLVED IN SEPTICEMIA IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

**RÎMBU C.M.^{1,2}, HORHOGEA C.E.¹, VULPE V.¹, GRECU M.¹, ANIȚA D.^{1,2},
BALBARĂU A.¹, MIRON L.D.¹**

¹Faculty of Veterinary Medicine, Iasi University of Life Sciences Ion Ionescu de la Brad

²ROVETEMERG (Regional Center Of Advanced Research For Emerging Diseases, Zoonoses And Food Safety

8, Mihail Sadoveanu Alley, 700489, Iasi, Romania

crimbu@uaiasi.ro

Abstract: In veterinary medicine, *Hafnia alvei* is associated with various infections, including enteritis, septicemia, and respiratory problems in pigs and septicemia and gastroenteritis in fish. This bacterium is part of the normal gut microbiota in many animals, but it has been associated with a certain pathology in humans, including urinary tract infections, wound infections, and bacteremia. These microorganisms survive in fresh and saltwater and are transmitted from fish to fish by direct contact or through contaminated food and water. *Hafnia alvei* infections are more common in fish that are stressed, have a weakened immune system, or live in poor environmental conditions. In a fish farm, increased mortality was observed in rainbow trout (*Oncorhynchus mykiss*) of different ages (2-16 months) without being clinically apparent. After microbiological examination Gram-negative, oxidase-negative, catalase-positive and lactose-negative bacterial strains were isolated. Using MALDI-TOF mass spectrometry (Biotyper® Sirius One, Bruker), all isolated bacterial strains were identified and assigned to the species *Hafnia alvei*.

• Introduction

- ***Hafnia alvei*** is one of the opportunistic bacteria that survives in various natural environments, but especially in the aquatic one. The natural niche of these microorganisms is the digestive tract of many mammals, fish, birds and bees species. It is Gram negative aerobic/facultatively anaerobic bacillus *Hafniaceae* family, *Enterobacteriales* order.
- ***Hafnia alvei* is relevant to both veterinary and human medicine.** In humans, it has been implicated in a wide range of infections: septicemia, pneumonia, meningitis, urinary tract infections, peritonitis, ophthalmic infections, joint and bone infections, gastrointestinal infections, nosocomial infections. In veterinary medicine, necrotizing hepatitis and splenitis in chickens and ducklings, abortions in mares, pneumonia in sheep, septicemia in bees, and **hemorrhagic septicemia in fish, including rainbow trout** have been reported.

• Material and method

- During December 2022, **10 rainbow trout of different ages** from three hatcheries V1 (6-8 months), V2 (8-12 months), V3 (>16 months) of a trout farm from Moldova (Romania) region were analyzed.
- The microbiological examination consisted of cultivation on usual and special media, followed by assessment of cultural phenotypic traits and cell morphology. Identification of the bacterial species was based on the metabolic profile (API20E) and the **confirmation by MALDI-TOF mass spectrometry** (Bruker MALDI Biotyper® Sirius) from ROVETEMERG.
- The susceptibility of *Hafnia alvei* strains was tested by the disc diffusion technique against amoxicillin (AMO, 30µg), enrofloxacin (ENR, 30µg), doxycycline (DOX, 30µg), tetracycline (Te, 30µg) florfenicol (FFC, 30µg) chloramphenicol (CHL, 30µg) neomycin (Ne, 30µg), trimethoprim + sulfamethoxazole (STX, 20µg), flumequine (UBN, 30µg).

• Results and discussions

- In the natural environment, the trout fed and swam horizontally;
- Increased mortality;
- **External examination** of the trout: no lesions specific to infectious diseases were identified, except **pallor of the gills** (Fig. 2a) **and of the oral mucosa** observed in most of the trout from V2 and V3;
- **Necropsy:** most of the trout from the V2 and V3 showed **pallor of the liver** (Fig. 2b) or **hepatosis with miliary hemorrhages** (Fig. 2c). No external or internal lesions were identified in younger trout (V1).

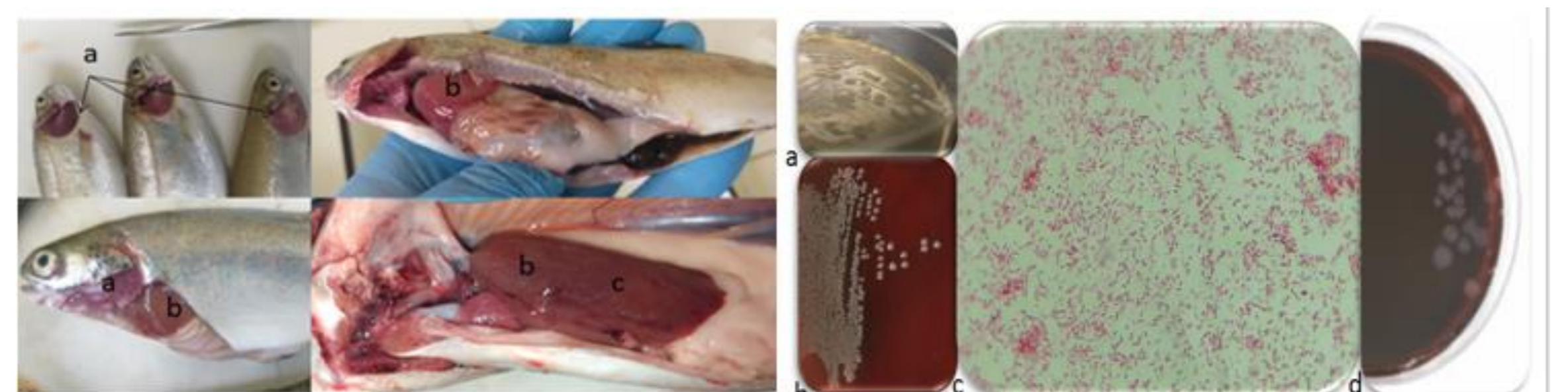


Fig. 2 Lesional aspect of rainbow trout

Fig. 3 *Hafnia alvei* - cultural aspects on culture media: TSA (a), Mueller Hinton Agar+blood (b), EMB Agar (d) and cell morphology (bacilli, Gram-negative) (c)

- *Hafnia alvei* possesses several **virulence factors** that contribute to its ability to cause damage to mammals, fish and birds. In vitro studies have revealed pathogenicity factors and mechanisms that can be compared with clinical manifestations similar to those produced by enteropathogenic *Escherichia coli* (EPEC) strains.
- ***Hafnia alvei* is known to be intrinsically resistant to ampicillin, amoxicillin ± clavulanic acid, ampicillin - sulbactam, cefazoline, cephalothin, cefoxitin, cefotetan** (CLSI-M100, 2017a)
- **The most effective antibiotics** were **florfenicol, chloramphenicol, neomycin** (3/n=3), followed by **tetracycline, trimethoprim + sulfamethoxazole** (2/n=3) and **doxycycline** (1/n=3).
- **Resistance** (3/n=3) to **flumequine** (quinolone).

• Conclusions

The importance of *Hafnia alvei* infection in fish can vary depending on several factors, including the affected fish species, environmental conditions, stress, and the presence of other predisposing factors. In aquaculture, controlling *Hafnia alvei* infection can be a target to minimize economic losses and ensure fish welfare.

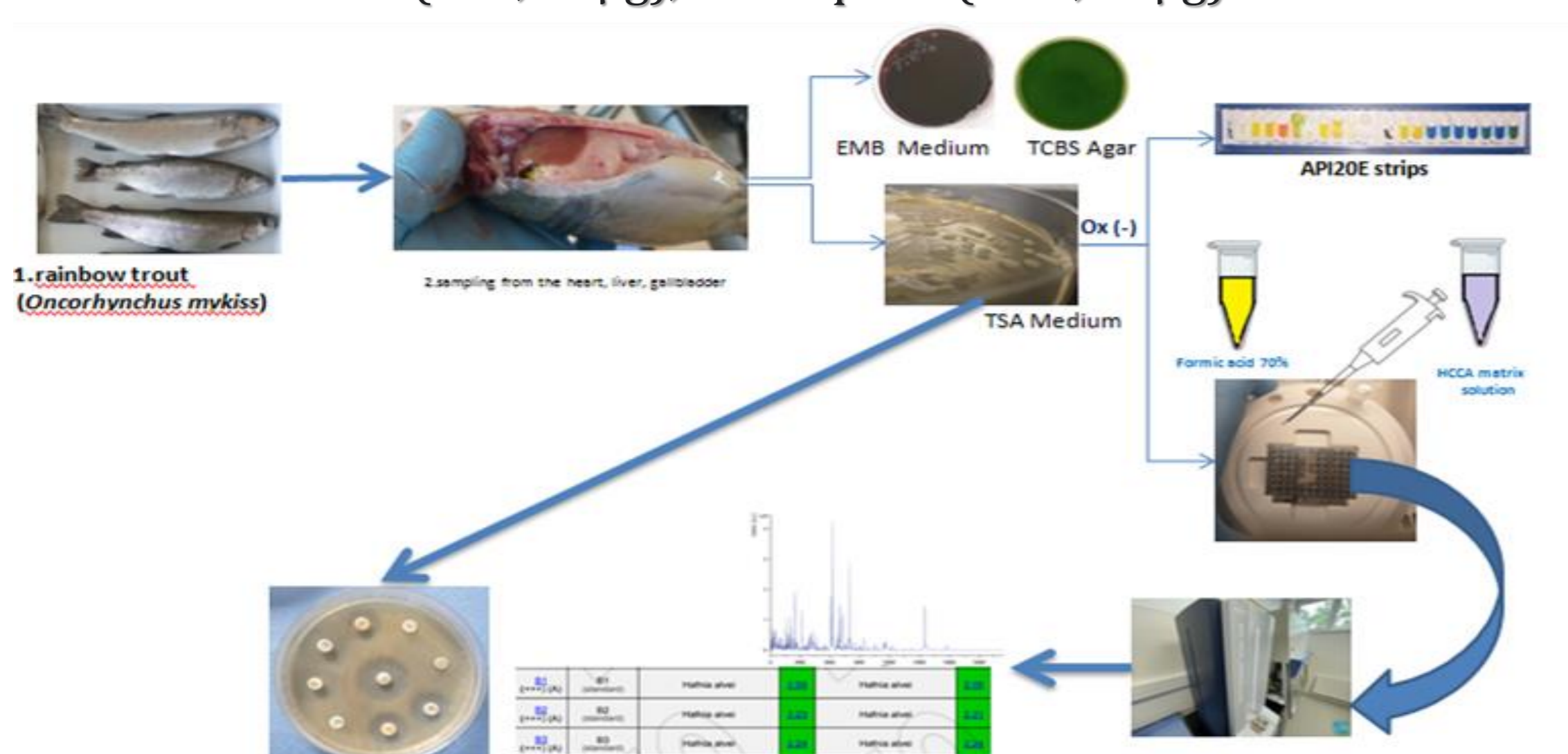


Fig. 1 Stages of *Hafnia alvei* species identification

