

PROGRAM, ABSTRACTS AND REPORT OF

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(European Association of Fish Pathologists)

ZOONOTIC INFECTIONS FROM FISH AND SHELLFISH

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The worldwide development of aquaculture and the worldwide transport of live aquaculture products has, apart from many advantages, also worrisome consequences. Among them is the occurrence and increased recognition of zoonotic disease agents causing epidemics and carrier states in cultured fish and shellfish and their transmission to humans resulting in mild to severe disease. Diagnosis of zoonotic infections in humans by clinicians is often hampered by a poor knowledge of zoonotic disease agents derived from aquatic species and associated clinical signs.

The zoonotic infections can be divided into:

- 1) **topically acquired (contact) zoonosis:** risk groups: aquaculture professionals, fish culturists/processors/handlers & consumers
- 2) **food borne (ingestion) zoonosis:** risk group: consumers

We organize this workshop based on the increasing cases of zoonotic agent detection that have resulted in both aquaculture and human diseases.. In this workshop we strive to present an overview of the prevalence and incidence of zoonotic infections and discuss the best management practices, including preventive measures, for aquaculture professionals, fish processors, and the consumers. Our focus is on principal zoonotic pathogens: *Vibrio vulnificus*, *Streptococcus iniae*, *S. agalactiae*, *Edwardsiella tarda*, *Mycobacterium* spp. and noroviruses (Norwalk-like viruses).

PROGRAM:

1. J.J. Evans*, P.H. Klesius, O. Haenen, and C.A. Shoemaker : **Overview of zoonotic infections from fish and shellfish**
2. O. Haenen*, I. Roozenburg-Hengst, M. Voorbergen-Laarman, M. Engelsma, J. Evans: **Emerging problems with zoonotic bacteria in intensive warm water fish culture: facts and risks**
3. M. Longshaw*: **Parasitic diseases acquired from fish**
4. Franck Berthe* Ernesto Liebana-Criado, Ana Afonso: **Overview of the BIOHAZ opinions on food safety aspects at EFSA**
5. M. Dolores Furones*, A. Roque, C.L. Joven, B.Lacuesta, L. Elandaloussi: ***Vibrio vulnificus* isolated from bivalves in the Spanish Mediterranean.**
6. A. Manfrin et al. : **Mycobacteriosis in sea bass, rainbow trout, striped bass and Siberian sturgeon in Italy**
7. Joyce Evans supplied a hand out: G. Richards*, G. Meade and D. Kingsley: **Method to extract viral RNA from shellfish (training video)**

DISCUSSION POINTS

- What are the main risks for the aquaculture
- What are the main risks for professionals/consumers?
- We need better diagnosis
- What can we do about it:
 - prevention, how?
 - communication leaflets/publications?
 - disinfection/control?
- Need for consolidation of fragmented literature relative to human infection from fish by bacterial species, How to consolidate? Published literature, surveys to health practitioners, regional epidemiologists, EFSA? To result in literature review
- Should diseases be made notifiable? OIE/EU? Define the reasons....
- Standardized reporting mechanisms from health practitioners?
- What about antibiotic resistance studies?
- Central repository of records?
- General conclusions?

ABSTRACTS:

1. OVERVIEW OF ZONOTIC INFECTIONS FROM FISH AND SHELLFISH

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As aquaculture production and consumption of aquacultural products increases, the possibility of zoonotic infection from either handling or ingestion of these products also increases. The principal pathogens acquired topically from fish or shellfish through spine/pincer puncture or open wounds are *Aeromonas hydrophilia*, *Edwardsiella tarda*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus* and *V. damsela*. All of these indigenous pathogens have also been associated with disease outbreaks in food fish. Outbreaks are often related to management factors such as quality and quantity of nutrients and water and stocking density which increase bacterial loads on the external surface of the fish and risk of disease. As a result, diseased fish are more likely to transmit infection to humans. Although most fish-associated wound infections are self-limiting, more serious infections tend to be associated with immune impairment, highly virulent strains, a large inoculum, deep penetration of the skin, or a combination of these factors. Infections may often be mixed. Food-borne illnesses acquired through consumption of mishandled seafood are a result of indigenous pathogens such as *Vibrio* spp., *Edwardsiella tarda*, and noroviruses (Norwalk-like viruses) and by extraneous pathogens including *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococcus*, *Acinetobacter*, and *Escherichia coli*. This presentation will provide a literature review of worldwide human cases of zoonosis including clinical characteristics from the principal fish and shellfish zoonotic pathogens and potential zoonotic pathogens, *Streptococcus agalactiae* (group B streptococcus) and *Lactococcus garvieae*.

2. EMERGING PROBLEMS WITH ZONOTIC BACTERIA IN INTENSIVE WARM WATER FISH CULTURE: FACTS AND RISKS

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With the development of warm water aquaculture in intensive indoor recirculation systems in Europe and the United States new bacterial disease problems occur, especially in the cultured fish, and in humans. These so called zoonotic bacteria are more frequently isolated from severe disease outbreaks with high mortalities in fish, cultured at water temperatures of 24-28°C. In The Netherlands, in the last decennia several cases of *Vibrio vulnificus*, *Edwardsiella tarda*, *Streptococcus agalactiae* and *Mycobacterium marinum* a.o. have been diagnosed in eels, African catfish, tilapia, and barramundi, a.o., some of which were related to mild to more severe disease in the fish culturist or fish processor after contact of the infected fish with injured skin (hand or finger). Most zoonotic infections were related to *Vibrio vulnificus* and *Mycobacterium marinum*. During an investigation of the presence of zoonotic bacteria (*Edwardsiella tarda*, *Vibrio vulnificus*, *Streptococcus iniae*, *Streptococcus agalactiae* and *Mycobacterium* spp.) in healthy looking eels, African catfish and tilapia from Dutch warm water fish farms in 2008, 1 of 8 African catfish farms was found positive for *Streptococcus agalactiae*, 1 of 8 for *Edwardsiella tarda*, 3 of 7 tilapia portions were found positive for *Mycobacterium fortuitum*, and none of 6 eel farms were found positive for any of these bacteria. Moreover, in interviews with the consulted fish farmers and fish processors, most of them indicated they get regularly injuries from fin spines and from knives at fish slaughter, after which various inflammations of the fingers or hand may result. As hospitals culture bacteria at $\geq 37^{\circ}\text{C}$ only, a diagnosis of fish tuberculosis may be missed.

In this presentation, an overview of the fish clinical cases with the contact- zoonotic bacteria is given, with examples of zoonosis in humans. The consequences of the recent findings are discussed, both in fish culture, veterinary, as in human health perspective, and recommendations for prevention will be proposed.

3. PARASITIC DISEASES ACQUIRED FROM FISH

Matt Longshaw, Cefas Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, U.K.

It has long been recognised that raw or undercooked fish products have the potential to transmit parasitic infections to humans. In addition, some parasites, such as those causing “swimmers itch”, can be transmitted to humans via the water. This presentation will provide an overview of the main groups of parasites previously implicated in causing problems for humans through the consumption of fish. A particular focus will be on trematodes, nematodes and cestodes. These include clonorchiasis due to *Clonorchis* sp., anisakiasis due to *Anisakis*, *Pseudoterranova* and *Contracaecum* larvae and diphyllbothriasis due to *Diphyllbothrium* sp. Typically, these parasites are ones that will develop into adult forms in non-humans – accidental infections in humans result in the parasite attempting to develop which in turn can lead to a wide range of responses. These can include ulcer development, gastric problems and in some extreme cases, hypersensitivity and anaphylactic shock. Occasional reports of infections such as *Argulus* infections of the eye and the presence of myxozoans in stool samples which have lead practitioners to consider the possibility of abuse will be reported. Suggested future developments in the area of zoonotic parasites will be mentioned.

4. FOOD SAFETY ASPECTS OF FISH WELFARE DURING PRE-HARVEST AND HARVEST: A REVIEW OF EFSA OPINIONS

Franck C.J. Berthe¹, Ernesto Liebana-Criado² & Ana Afonso¹

EFSA, European Food Safety Authority, Parma, Italy

1. AHAW - Animal Health and Welfare Panel
2. BIOHAZ – Panel on Biological Hazards

The Council Directive 98/58/EC lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish. The European Commission asked the European Food Safety Authority (EFSA) to consider the food safety aspects of animal welfare during pre-harvest and harvest for several species of fish.

The Panel on biological hazards considered that farm location, the species being farmed, husbandry practices and environmental conditions, stunning and killing practices are all factors that influence the food safety risk associated with aquaculture products. Risk is also influenced by post-harvest processing, food preparation, and consumption. A major advantage of aquaculture in regard to food safety is that control can be exerted over the quality and safety of the product, and many of the hazards at the production level can be controlled by good aquaculture practices and safety management systems.

Specific information on the effect of welfare-related pre-harvest and harvest practices that may affect fish safety is limited and knowledge gaps abound. Therefore the scientific opinions provide general considerations of relationships or effects identified are given, based on general principles of hygiene and safety. Measures intended to preserve fish welfare by avoiding stress and improving environmental conditions, are expected to have a positive impact on the safety of the food product.

As far as location is concerned, European farms are located in a variety of habitats, with generally low levels of pathogenic micro-organisms and parasites as compared to other latitudes. The European aquaculture industry commonly implements fish health management and quality and safety control measures during pre-harvest/harvest stages, contributing to a significant reduction of risks associated with biological hazards and, at the same time, achieving a high degree of control over the production process.

Production procedures based on good aquaculture practices that result in provision of optimal animal welfare increase fish resistance to infections and therefore may lead to a reduction of the food safety risks associated with the resulting end products. Environmental and hygienic conditions related to water quality and practices at pre-harvest level could increase the prevalence of certain biological hazards at farm level, and may also have an effect on fish welfare and physiological condition (stress). Both these aspects impact on fish health, and subsequently may influence the safety of the end product. Some aquaculture practices and conditions inherent to specific production systems (intensive or extensive) may influence the safety of the food product.

After slaughtering the biochemistry of the muscle *post-mortem* is influenced by the method used in pre-slaughter handling and stunning/killing of fish and this may have an influence on its microflora. It is also established that slaughter-related operation that involve penetration of the skin carry a risk of introducing pathogenic bacteria from the skin onto/into edible parts of the animal directly or via blood circulation.

A holistic approach on animal health and welfare studies to include food safety aspects should be encouraged in order to improve the desired synergism.

5. VIBRIO VULNIFICUS ISOLATED FROM BIVALVES IN THE SPANISH MEDITERRANEAN

M.Dolores Furones; Carmen L. Joven; Beatriz Lacuesta; Laurence Elandaloussi and Ana Roque

Vibrio vulnificus is a gram-negative, halophilic bacterium that is typically isolated from estuarine environments throughout the world and it is responsible for 95% of all seafood-borne fatalities in the United States. *Vibrio vulnificus* can induce various syndromes depending on the mode of transmission, and mortality rates differ depending on the route of infection. Currently there is a lack of data on the incidence of this species and there is a general concern that it may cause more disease cases since there is a increment in international trade and in the consumption of raw food. The present study describes de the isolation of *Vibrio vulnificus* from four species of bivalves cultured in both bays of

the delta of the river Ebro, Western Mediterranean. Isolates were acquired by plating out bivalve homogenate enriched for 6h in alkalyine peptone water onto Chromagar Vibrio and incubating overnight at 37°C. Torquoise colonies were then purified and total bacterial DNA extracted from them. A one step PCR was then performed with the following primers: Forward- 5' C GCC GCT CAC TGG GGC AGT GGC TG 3' and Reverse 5' GCG GGT GGT TCG GTT AAC GGC TGG 3', These primers are homologous to regions flanking a portion of the 1,416-bp cytolyisin-hemolysin gene that is unique to *V vulnificus* but common to biotypes 1 and 2. PCR conditions were as follows, initial denaturation at 95°C for 8 min followed by 40 cycles of 1 min denaturation at 94°C, annealing at 55°C for 1 min, and elongation at 72° for 1 min and a final extension of 10 min at 72°C. 254 presumptive *Vibrio vulnificus*, of which 88 confirmed the identity by PCR, 12 came from the clam *Ruditapes decussatus*, 9 from the clam *Ruditapes phillipinarum*, 30 from the oyster *Crassostrea gigas* and 37 from the mussel *Mytilus galloprovincialis*. Most isolates were obtained in the summer. These isolates have been characterised further genotypically and phenotypically. Unfortunately, there is a lack of clinical data available in order to link the presented data with a risk estimation associated to shellfish consumption.

6. MYCOBACTERIOSIS IN SEA BASS, RAINBOW TROUT, STRIPED BASS AND SIBERIAN STURGEON IN ITALY

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During the last three years we had the opportunity to observe some outbreaks of Mycobacteriosis in different species reared in Italy. European sea bass (*Dicentrarchus labrax*), 350-650 g., reared in concrete tanks, water temperature 24 -26.5° C, normal salinity (30 ‰) showed drop mortality (20% total loss) during 2 months. Skin erosions and haemorrhages, typical white granulomatous lesions in the spleen, less evident in liver, kidney and hearth were observed.. Acid-fast bacteria (Ziehl –Neelsen positive) in all organs, included gonads, with necrotic and granulomatous lesions were reported. *Mycobacterium* was isolated after 2 weeks in Lowenstein-Jensen and Middlebrook 7H10 media and typed as *M. marinum* by biochemical tests and RFLP-PCR.

In 2006, in a small fish farming, some rainbow (*O. mykiss*) and brown trouts (*S. trutta fario*) broodstocks started to die (drop mortality) showing anorexia, slow growing, darkening and skin erosions. *Mycobacterium* sp. was isolated on Löwestein-Jensen e Stonebrink media from liver, kidney and spleen and then typed as *M. marinum*.

In May 2007 forty striped bass hybrides (*M. chrysops* x *M. saxatilis*) showed many granulomas and acid fast bacteria in gills, spleen, liver and kidney after histopathological examination..

The affected fish showed slow growing and drop mortality and *Mycobacterium marinum*, isolated in L.J. and Stonebrink media, was the aethiological agent.

The last outbreak affected siberian sturgeons (*Acipenser baeri*, 8 months old) imported from France. Skin erosions and haemorrhages, ascitis, pale gills and organs were the most relevant lesions. *Mycobacterium* sp. was isolated from frozen samples and then identified by RFLP-PCR as *M. marinum*. All the sturgeons were killed and destroyed in order to avoid the spreading of infection to *A. transmontanus* broodstoks (high economic value).

No infection in humans or other animal have been reported in each outbreak, all the fish were destroyed and the tanks fallowed and disinfected.

7. **METHOD TO EXTRACT VIRAL RNA FROM SHELLFISH (TRAINING VIDEO)** by G. Richards, G. Meade and D. Kingsley. It was distributed on CD, copies can be requested from Dr. Joyce Evans.

8. **DISCUSSION**

- The first point raised was by B. Austin who urged the community to use the term “zoonosis” in the right sense: an animal disease that can be transmitted to humans. However, the source of a human disease could be from diseased animals or waterborne for example. Risks are mainly associated to contact with 1) diseased fish, or 2) at slaughter, or 3) water contamination (e.g. *Aeromonas hydrophila*, *Mycobacterium marinum*). *Vibrio vulnificus* is responsible for a systemic infection and hence would be a real zoonosis. *V.parahaemolyticus* acts via the intestine, and may not be considered as a true zoonotic agent. Human diseases caused by fish parasites are “true zoonoses”.
- Another point raised in the discussion was the perception of zoonosis. For example, *Vibrio harveyi* was not mentioned by the speakers as an actual risk. Perceived risks go much broader, like *Vibrio cholerae*. Wounds or topical infections are mostly traceable to the host. Evidence for zoonotic infections may be difficult to gather.
- The newest review publication of Austin on zoonotic *Vibrio* infections (Austin, B., Vibrios as causal agents of zoonoses. Vet. Microbiol. (2009); doi:10.1016/j.vetmic.2009.03.015) was recommended as a must read.
- On the question of to be or not to be a real zoonosis, F. Berthe stressed that zoonoses and diseases transmissible to humans through food, whether or not animals are affected by such diseases should be considered here.
- In general it was mentioned, that prevalence of zoonotic cases are low. Matt Longshaw declared that if medical doctors would know the clinical signs, much more zoonotic cases would be traced than up to now. The audience confirmed the need to have better contact with the medical doctors community, without causing panic in the public. Olga Haenen recommended to avoid the mass media, and to prefer targeted means of communication (e.g. specialized flyers).
- The question was raised whether zoonotic infections should be made notifiable? Olga Haenen related that the medical doctors involved in the Dutch *V. vulnificus* case (see abstract) were surprised it was not notifiable: This case was found more severe than average *Streptococcus A*-infection in humans. Members of the audience agreed it should be made notifiable, but this would be a task for the medical doctors in the first place.
- It was discussed and agreed that raising awareness is also necessary for farmers, veterinarians, diagnostic laboratories, public health agencies, etc.
- With regards to the previous points, Joy Evans indicated a paper by Aubry and coll. (Aubry et al., 2002. *Archives of Internal Medicine* **162**, 1746) on *Mycobacterium marinum* as a type of reports needed. Although, as the audience stressed, some cases were found retrospectively and then difficult to publish because of possible missing information and lack of access to source material.
- Concerning monitoring, D. Furones raised the issue of cost. A pragmatic approach is to link to already ongoing monitoring programs to get more data. However, Joy Evans stressed that there is also a need to collect data that could be used in a risk assessment (e.g. number of outbreaks).

CONCLUSIONS

- There is a need to establish contact with target groups (medical doctors, fish farmers, processors, government) and health practitioners (medics, vets) through active communication (flyers, lectures at conferences, publications), to raise awareness of the issue, not to causing panic.
- It is recommended that the magnitude of the problem be investigated, retrospectively through hospital archives, and possibly also based on monitoring of cases in humans.
- Next EAFP should give the opportunity for a full session on this topic, including the issue of antimicrobial resistance.
- An article to be published in the EAFP Bulletin about this workshop was recommended.