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EMERGING BACTERIAL FISH PATHOGENS

B. AUSTIN
Department of Biological Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, Scotland

Introduction

It is pertinent to enquire what is meant by an emerging fish pathogen? Some apparently "new" pathogens may reflect developments in other areas of science such as taxonomy. Here, a new pathogen may represent improvements in taxonomy, e.g. the old established *Streptomyces* (= *Streptoverticillium) salmonis* became re-classified as *Nocardia salmonicida* (Isik *et al.* 1999). Perhaps, an organism may have been misidentified previously, for example there is some evidence that isolates of *Hafnia alvei* had been labelled previously as *Yersinia ruckeri* (Austin, unpublished information). Also, there may be situations whereby symptoms are common to more than disease and therefore the culprit had been wrongly assigned to other taxa. Alternatively, there may be changes in the severity or incidence of a disease, resulting in increased attention by scientists. Maybe, the incidence of a specific disease had been too low previously to attract scientific scrutiny.

What are the reasons for emerging diseases?

- A change in the environment favouring a "new" organism – such as attributable to pollution
- Introduction of an exotic [fish] species, with the disease transferring from indigenous fish stocks [or *vice versa*]
- Change in the health index of the host – perhaps reflecting the presence of external stressors, e.g. due to overcrowding/poor hygiene
- Natural selection by which a change selects for a "new" pathogen – such as use of antibiotics selecting for antibiotic-resistant taxa.

Then, there is the issue concerning the time-scale for an "emerging" disease? Notwithstanding, many names of apparently "new" fish pathogens have appeared in the scientific literature sometime within a decade (Table 1). These new pathogens will be discussed, below:

Gram-positive bacterial fish pathogens

Bacillus spp.

The initial outbreaks of disease in 1989-1991 led to mortalities of 10-15% of the fish in earthern ponds in Nigeria (Oladosu *et al.,* 1994). Diseased fish showed weakness, lethargy, emaciation and generalised necrotising dermatitis, with death occurring in a few days. Blood tinged fluid was present in the peritoneal cavity, and petechia and focal necrosis were evident in the liver and kidney. The spleen was enlarged, soft and friable; the myocardium was described as soft and flabby, and the stomach was hyperaemic (Oladosu *et al.,* 1994). Gram-positive rods of 1-4 µm in length were observed, and linked to *Bacillus*. Generally, there was insufficient information to achieve a proper identification. Similar constraints did not prevent the labelling of organisms as *Bacillus cereus,* which was associated with branchionecrosis in common carp (Pychynski *et al.,* 1981) and striped bass (Baya *et al.,* 1992). Also, some reservations must be expressed regarding the association of *Bacillus mycoides* with an epizootic in channel catfish from Alabama during 1992 (Goodwin *et al.,* 1994). The fish were darker in colour, inappetant, displayed pale areas or ulcers on the dorsal surface, focal necrosis of the epaxial muscle, and opaque muscle, with histopathological examination revealing the presence of chains of Gram-positive rods (Goodwin *et al.,* 1994).

Mycobacterium spp.

M. abscessus became associated with 2-27 month old Japanese Meduka *(Oryzias latipes),* which had been cultured in the USA for aquatic toxicology testing (Teska *et al.,* 1997). During a routine examination, granulomas, notably in the buccal cavity and vent, and a few acid-fast bacteria were noted in <1% of the otherwise healthy fish. On clinically diseased fish, the disease signs would include listlessness, inappetance, swollen abdomen and visible granulomas (Teska *et al.,* 1997). A limited range of phenotypic tests was used to equate the pathogen with *M. abscessus* (Teska *et al.,* 1997). Rather more appropriate procedures, i.e. the high performance liquid chromatography of cell wall mycolic acids, served to recognise *M. poriferae,* which was recovered from cultured snakehead with nodular lesions (Tortoli *et al.,* 1996).

Streptococcus spp.

S. difficilis was named as a result of an outbreak of disease in St. Peter's fish *(Tilapia)* and rainbow trout within Israel during 1986 (Eldar *et al.,* 1994). Disease signs in *Tilapia* included lethargy, erratic swimming and dorsal rigidity. In rainbow trout, there was septicaemia and brain damage (Eldar *et al.,* 1994). The initial bacteriology resulted in the recognition of two groups of streptococci, the separation of which was made by use of API 50 CH and API 20 STREP, and by growth and haemolysis characteristics (Eldar *et al.,* 1994). The fairly unreactive non-haemolytic mannitol negative group was labelled as *S. difficile* (Eldar *et al.,* 1994), and the specific epithet corrected to *difficilis,* i.e. *S. difficilis* (Euzéby, 1998), whereas a second more reactive α-haemolytic, mannitol positive group became known as *S. shiloi,* and later equated with *S. iniae* (Teixeira *et al.,* 1996). *S. difficilis* was considered to belong to a separate and distinct DNA homology group, with DNA relatedness between members of 89-100% (Eldar *et al.,* 1994). Whole-cell protein electrophoresis has revealed that the type strain of *S. difficilis* is indistinguishable to *S. agalactiae* (Vandamme *et al.,* 1997).

S. iniae was initially recovered from an Amazon freshwater dolphin, *Inia geoffrensis* (Pier and Madin, 1976). The association with fish diseases came when it was described as a cause of mortality in tilapia hybrids *(Tilapia nilotica x T. aurea)* (Perera *et al.,* 1994) and later in dusky spinefoot *(Siganus fuscenscens)* (Sugita, 1996) and hybrid striped bass (Stoffregen *et al.,* 1996). The organism has been transmitted from wild to cultured fish (Zlotkin *et al.,* 1998), On the basis of DNA:DNA hybridisation, i.e. 77-100% DNA homology, *S. iniae* was found to be synonymous with *S. shiloi,* with the change in taxonomy being confirmed by others (e.g. Teixeira *et al.,* 1996).

Table 1 New bacterial pathogens of finfish.

Taxon	Fish species affected	Geographical distribution
Gram-positive bacteria		
Bacillus spp.	various freshwater fish	Nigeria
B. cereus	common carp, striped bass	USA
B. mycoides	channel catfish	USA
Mycobacterium abscessus	Japanese meduka	USA
M. poriferae	snakehead	Italy
Streptococcus difficilis	rainbow trout, St. Peters fish	Israel
S. iniae	dusky spinefoot, hydrid striped bass, rainbow trout, St. Peters fish	Israel, USA
S. parauberis	turbot	Spain
Gram-negative bacteria		
Aeromonas caviae	Atlantic salmon, rainbow trout	Kenya, Turkey
A. jandaei	eel	Spain
Aquaspirillum sp.	catfish, snakehead	Thailand
Escherichia vulneris	balloon moly, Caucasian Carp, silver moly	Turkey
Moritella marina	Atlantic salmon	Iceland
Vibrio furnissii	eel	Spain
V. ichthyoenteri	Japanese flounder	Japan
V. logei	Atlantic salmon	Iceland
V. parahaemolyticus	Iberian toothcarp	Spain
V. pelagius	turbot	Spain
V. trachuri	Japanese horse mackerel	Japan
V. viscosus	Atlantic salmon	Iceland, Norway, Scotland
Yersinia intermedia	Atlantic salmon	Tasmania

Streptococcosis, attributed to *S. parauberis,* was originally recognised in farmed turbot (weight: 0.8 - 2 kg) from 5 sites in northern Spain during 1993 and 1994 (Doménech *et al.,* 1996). Disease signs included weight loss, haemorrhaging on the anal and pectoral fins, petechial haemorrhages on the abdomen, bilateral exophthalmia, haemorrhaging and pus in the eyes, pale liver, congested kidney and spleen, ascites, and mucohaemorrhagic enteritis (Doménech *et al.,* 1996). Isolates were identified by phenotypic (Rapid ID32 and API 50CH systems) and genotypic data (16S rRNA sequencing) as *S. parauberis*; an organism known previously as *S. uberis* genotype II. There was a 100% sequence homology between the fish isolates and *S. parauberis* (Doménech *et al.,* 1996).

Gram-negative bacterial fish pathogens

Aeromonas spp.

In 1991, a septicaemic condition was diagnosed on four Atlantic salmon farms located on the Black Sea in Turkey (Candan *et al.,* 1995). Diseased fish displayed signs of haemorrhagic septicaemia, namely haemorrhages on the body, intestine filled with bloody exudate, enlarged liver and spleen, and liquefying kidney. The causal agent, identified as *A. caviae,* has also been associated with eye disease and haemorrhagic septicaemia in farmed rainbow trout from Kenya (Ogara *et al.,* 1998).

A. jandaei has been reported as pathogenic to eel in Spain (Esteve *et al.,* 1993; 1994; Esteve, 1995). Initially, 8 isolates were recovered during 1987 and 1988. Whereas initially, the method of identification was not stated (Esteve *et al.,* 1994), a subsequent numerical taxonomy study equated isolates with *A. jandaei* (Esteve, 1995).

Aquaspirillum sp.

There has been one report of putative *Aquaspirilllum* sp. being associated with a disease, termed epizootic ulcerative syndrome, in snakeheads and catfish obtained from two fish farms in Thailand (Lio-Po *et al.,* 1998). However, the evidence for the involvement of *Aquaspirillum* is not convincing.

Escherichia vulneris

An organism, subsequently identified as *E. vulneris,* was first isolated in 1994 from naturally infected balloon moly *(Poecilia* sp.), silver moly *(Poecilia* sp.) and Caucasian carp *(Carassius carassius)* from Turkey (Aydin *et al.,* 1997). Clinical signs included haemorrhagic lesions on the skin, pale gills, digestive tract full of bloody exudate, haemorrhaging in the gonads, and yellow liver with hyperaemic areas (Aydin *et al.,* 1997).

Moritella marina

Nineteen Icelandic and one Norwegian isolate from shallow skin lesions on Atlantic salmon, and the type strain of *Vibrio marinus* NCIMB 1144 were identified as *V. marinus* after an examination of phenotypic data and analyses by numerical taxonomy (Benediktsdóttir *et al.,* 1998). On the basis of 16S rRNA sequencing, the taxon was transferred to the newly established genus, as *Moritella marina* (Urakawa *et al.,* 1998).

Vibrio spp.

There has been an indication that *V. furnissii* may be associated with eel disease in Spain (Esteve, 1995). However, isolates were recovered from water rather than diseased eels.

Since, 1971, opaque intestines and intestinal necrosis accompanied by high mortalities have been reported in Japanese hatcheries rearing Japanese flounder (Ishimaru *et al.,* 1996). *V. ichthyoenteri* was described as a result of an examination of 7 isolates from flounder larvae (Ishimaru *et al.,* 1996).

An organism, with similarities to *V. logei,* was associated with shallow skin lesions of Atlantic salmon farmed in Iceland at low temperatures, i.e. ~10°C (Benediktsdóttir *et al.,* 1998). Fifteen Icelandic and one Norwegian isolates from shallow skin lesions in Atlantic salmon were considered to be similar to *V. logei* (Benediktsdóttir *et al.,* 1998).

There has been some dispute about whether or not *V. parahaemolyticus* constitutes a *bona fide* fish pathogen. However, one article has reported the recovery of isolates with the diagnostic features of *V. parahaemolyticus* from laboratory-cultured Iberian toothcarp, *Aphanius iberus,* which displayed external haemorrhages and tail rot. Isolates were found to infect other species, such as eels (Alcaide *et al.,* 1999).

An epizootic of juvenile farmed turbot in Northwest Spain occurred during January and February 1991 when the water temperature was 12-15°C, with fish displaying eroded dorsal fins and tail, haemorrhages at the base of the fins, haemorrhages in the internal organs, and intestines full of mucus liquid (Angulo *et al.,* 1992). The total losses amounted to 3% of the turbot population. Four isolates were obtained in pure culture, and identified as *V. pelagius* (Angulo *et al.,* 1992). Infectivity experiments with rainbow trout (10 g) and turbot (5 g in size) confirmed virulence, and an LD_{50} of 1.9×10^5 cells/fish and 9.5×10^4 cells/fish, respectively (Angulo *et al.,* 1992).

A disease, resembling vibriosis, has been long associated with Japanese horse mackerel *(Trachurus japonicus)* especially during summer when the seawater temperature exceeds 25°C (Iwamoto *et al.,* 1995). Infected fish displayed erratic swimming, darkened in colour, developed pronounced bilateral exophthalmia, and developed haemorrhages on the internal organs (Iwamoto *et al.,* 1995). An organism was recovered, and named as a new species, as *V. trachuri.* However, comparatively high doses were required to cause disease in Japanese horse mackerel. Using 36.8 g fish at a water temperature of 26°C, 1.1×10^8 cells/fish caused 100% mortalities within 24 h of i.p. injection. A dose of 1.1×10^7 cells/ml led to 50% mortalities within 4 days. By immersion in 3.6×10^7 cells/ml for 2 min, 100% mortalities ensued within 3 days (Iwamoto *et al.,* 1995). The disease signs mimicked those on naturally infected fish.

Ulcers, of indeterminate cause, have been appearing on the flanks of Atlantic salmon in seawater during winter, principally in Iceland and Norway (Salte *et al.,* 1994; Lunder *et al.,* 1995; Benediktsdóttir *et al.,* 1998), and more recently in Scotland (Bruno *et al.*, 1998). Since its first recognition, a view has emerged that a new *Vibrio,* coined *V. viscosus,* may be responsible.

Yersinia intermedia

Affected Atlantic salmon were of 40-50 g in weight, and were held at a water temperature of 5°C. Disease signs included lazy movement with the fish congregating at the surface of the water, darkening of the body pigment, tail erosion, haemorrhaging on the flank and abdominal inflammation (Carson and Schmidtke, 1993).

Conclusions

Certainly, an increasing range of Gram-positive and Gram-negative bacteria have become associated with diseases of fresh water and marine fish, of which just under half of the taxa appeared in only two countries, i.e. Spain and USA. The apparent increase in the range of streptococci may well represent improvements in taxonomy. Yet, with the vibrios, there appears to be a dramatic increase in the number of fish-pathogenic taxa. Other as yet unsubstantiated reports suggest that there may well be a further increase in those numbers in the forseeable future. Certainly, diagnosticians need to be aware of these organisms. The significance of these emerging pathogens to aquaculture and, indeed, wild fish stocks will only become apparent in the future.

References

Alcaide, E., Amaro, C., Todolí, R., Oltra, R. (1999). Isolation and characterization of *Vibrio parahaemolyticus* causing infection in Iberian toothcarp *Aphanius iberus. Dis. Aquat. Organ.* **35**, 77-80.

Angulo, L., Lopez, J.E., Lema, C., Vicente, J.A. (1992). *Vibrio pelagius* associated with mortalities in farmed turbot, *Scophthalmus maximus. Thalassas* **10**, 129-133.

Aydin, S., Çelebi, S., Akyurt, I. (1997). Clinical, haematological and pathological investigations of *Escherichia vulneris* in rainbow trout *(Oncorhynchus mykiss). Fish Pathol.* **32**, 29-34.

Baya, A.M., I, T., Lupiani, B., Hetrick, F. (1992). *Bacillus cereus,* a pathogen for striped bass. Eastern Fish Health and American Fisheries Society Fish Health Section Workshop, 16-19 June 1992, Auburn University, Auburn, p. 67.

Benediktsdóttir, E., Helgason, S., Sigurjónsdóttir, H. (1998). *Vibrio* spp. isolated from salmonids with shallow skin lesions and reared at low tempterature. *J. Fish Dis.* **21**, 19-28.

Bruno, D.W., Griffiths, J., Petrie, J., Hastings, T.S. (1998). *Vibrio viscosus* in farmed Atlantic salmon *Salmo salar* in Scotland: field and experimental observations. *Dis. Aquat. Organ.* **34**, 161-166.

Candan, A., Küçüker, M.Ang, Karatas, S. (1995). Motile aeromonad septicaemia in *Salmo salar* cultured in the Black Sea in Turkey. *Bull. Eur. Ass. Fish Pathol.* **15**, 195-196.

Carson, J., Schmidtke, L.M. (1993). Opportunistic infection by psychrotrophic bacteria of cold-compromised Atlantic salmon. *Bull. Eur. Ass. Fish Pathol.* **13**, 49-52.

Doménech, A., Fernández-Garayzábal, J.F., Pascual, C., Garcia, J.A., Cutuli, M.T., Moreno, M.A., Collins, M.D., Dominguez, L. (1996). Streptococcosis in cultured turbot, *Scophthalmus maximus* (L.), associated with *Streptococcus parauberis. J. Fish Dis.* **19**, 33-38.

Eldar, A., Bejerano, Y., Bercovier, H. (1994). *Streptococcus shiloi* and *Streptococcus difficile:* two new streptococcal species causing a meningoencephalitis in fish. *Curr. Microbiol.* **28**, 139-143.

Esteve, C. (1995). Numerical taxonomy of *Aeromonadaceae* and *Vibrionaceae* associated with reared fish and surrounding fresh and brackish water. *System. Appl. Microbiol.* **18**, 391-402.

Esteve, C., Biosca, E.G., Amaro, C. (1993). Virulence of *Aeromonas hydrophila* and some other bacteria isolated from European eels *Anguilla anguilla* reared in fresh water. *Dis. Aquat. Organ.* **16**, 15-20.

Esteve, C., Amaro, C., Toranzo, A.E. (1994). O-serotyping and surface components of *Aeromonas hydrophila* and *Aeromonas jandaei* pathogenic for eels. *FEMS Microbiol. Lett.* **117**, 85-90.

Euzéby, J.P. (1998). Taxonomic note: necessary correction of specific and subspecific epithets according to Rules 12c and 13b of the International Code of Nomenclature of Bacteria (1990 Revision). *Int. J. System. Bacteriol.* **48**, 1073-1075.

Goodwin, A.E., Spencer Roy, Jr, J., Grizzle, J.M., Terrell Goldsby Jr, M. (1994). *Bacillus mycoides:* a bacterial pathogen of channel catfish. *Dis. Aquat. Organ.* **18**, 173-179.

Ishimaru, K., Akagawa-Matsushita, M., Muroga, K. (1996). *Vibrio ichthyoenteri* sp. nov., a pathogen of Japanese flounder *(Paralichthys olivaceus). Int. J. System. Bacteriol.* **46**, 155-159.

Isik, K., Chun, J., Hah, Y.C., Goodfellow, M. (1999). *Nocardia salmonicida* nom. Rev., a fish pathogen. *Int. J. System. Bacteriol.* **49**, 833-837.

Iwamoto, Y., Suzuki, Y., Kurita, A., Watanabe, Y., Shimizu, T., Ohgami, H., Yanagihara, Y. (1995). *Vibrio trachuri* sp. nov., a new species isolated from diseased Japanese horse mackerel. *Microbiol. Immunol.* **39**, 831-837.

Lio-Po, G.D., Albright, L.J., Michel, C., Leaño, E.M. (1998). Experimental induction of lesions in snakeheads *(Ophiocephalus striatus)* and catfish *(Clarias batrachus)* with *Aeromonas hydrophila, Aquaspirillum* sp., *Pseudomonas* sp. and *Streptococcus* sp. *J. Appl. Ichthyol.* **14**, 75-79.

Lunder, T., Evensen, Ø., Holstad, G., Håstein, T. (1995). 'Winter ulcer' in the Atlantic salmon *Salmo salar.* Pathological and bacteriological investigations and transmission experiments. *Dis. Aquat. Organ.* **23**, 39-49.

Ogara, W.O., Mbuthia, P.G., Kaburia, H.F.A., Sørum, H., Kagunya, D.K., Nduthu, D.I., Colquhoun, D. (1998). Motile aeromonads associated with rainbow trout *(Oncorhynchus mykiss)* mortality in Kenya. *Bull. Eur. Ass. Fish Pathol.* **18**, 7-9.

Oladosu, G.A., Ayinla, O.A., Ajiboye, M.O. (1994). Isolation and pathogenicity of a *Bacillus* sp. associated with a septicaemic condition in some tropical freshwater fish species. *J. Appl. Ichthyol.* **10**, 69-72.

Perera, R.P., Johnson, S.K., Collins, M.D., Lewis, D.H. (1994). *Streptococcus iniae* associated with mortality in *Tilapia nilotica* x *T. aurea* hybrids. *J. Aquat. An. Health* **6**, 335-340.

Pier, G.B., Madin, S.H. (1976). *Streptococcus iniae* sp. nov., a beta hemolytic streptococcus from an Amazon freshwater dolphon, *Inia geoffrensis. Int. J. System. Bacteriol.* **26**, 545-553.

Pychynski, T., Malanowska, T., Kozlowski, M. (1981). Bacterial flora in branchionecrosis of carp (particularly *Bacillus cereus* and *Bacillus subtilis). Med. Weter.* **37**, 742-743.

Salte, R., Rørvik, K.-A., Reed, E., Norberg, K. (1994). Winter ulcers of the skin in Atlantic salmon, *Salmo salar* L.: pathogenesis and possible aetiology. *J. Fish Dis.* **17**, 661-665.

Stoffregen, D.A., Backman, S.C., Perham, R.E., Bowser, P.R., Babish, J.G. (1996). Initial disease report of *Streptococcus iniae* infection in hybrid striped (sunshine) bass and successful therapeutic intervention with the fluoroquinolone antibacterial enrofloxacin. *J. Wd. Aqua. Soc.* **27**, 420-434.

Sugita, A. (1996) A case of streptococcicosis in dusky spinefoot. *Fish Pathol.* **31**, 47-48.

Teska, J.D., Twerdok, L.E., Beaman, J., Curry, M., Finch, R.A. (1997). Isolation of *Mycobacterium abscessus* from Japanese Meduka. *J. Aquat. An. Health* **9**, 234-238.

Teixeira, L.M., Merquior, V.L.C., Vianni, M. da C.E., Carvalho, M. da G.S., Fracalanzza, S.E.L., Steigerwalt, A.G., Brenner, D.J., Facklam, R.R. (1996). Phenotypic and genotypic characterization of atypical *Lactococcus garvieae* strains isolated from water buffalos with subclinical mastitis and confirmation of *L. garvieae* as the senior subjective synonym of *Enterococcus seriolicida*. *Int. J. System. Bacteriol.* **46**, 664-668.

Tortoli, E., Bartoloni, A., Bozzetta, E., Burrini, C., Lacchini, C., Mantella, A., Penati, V., Simonetti, M.T., Ghittino, C. (1996). Identification of the newly described *Mycobacterium* poriferae from tuberculous lesions of snakehead fish *(Channa straitus)*. *Comp. Immunol. Microbiol. Infect. Dis.* **19**, 25-29.

Urakawa, H., Kita-Tsukamoto, K., Stevens, S.E., Ohwada, K., Colwell, R.R. (1998). A proposal to transfer *Vibrio marinus* (Russell 1891) to a new genus *Moritella* gen. nov. as *Moritella marina* comb. nov. *FEMS Microbiol. Lett.* **165**, 373-378.

Vandamme, P., Devriese, L.A., Kersters, B.P.K., Melin, P. (1997). *Streptococcus difficile* is a nonhemolytic group B, type Ib streptococcus. *Int. J. System. Bacteriol.* **47**, 81-85.

Zlotkin, A., Hershko, H., Eldar, A. (1998). Possible transmission of *Streptococcus iniae* from wild fish to cultured marine fish. *Appl. Environ. Microbiol.* **64**, 4065-4067.