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FIELD OBSERVATION OF CLINICAL FURUNCULOSIS IN ATLANTIC SALMON SMOLTS VACCINATED WITH AN OIL-BASED FURUNCULOSIS VACCINE

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Abstract
This note presents field data on the observation of clinical furunculosis in Atlantic salmon in freshwater, two months after vaccination with an oil-adjuvanted furunculosis vaccine. The stressful event that precipitated this outbreak is postulated to be early smolting of these fish. From epizootiological evidence the most likely source of infection was covertly infected wild salmonids in the water body on which the hatchery was situated. The observations reported demonstrate that vaccination cannot be considered a barrier treatment for the movement of covertly infected fish and are of particular significance for freshwater restocking programmes.

Introduction
Vaccination has become the management tool of choice in the Irish Aquaculture industry for reduction in the incidence of clinical furunculosis, associated with the gram negative bacterium *Aeromonas salmonicida* (Bernoth, 1997). As a result of this widespread vaccination policy, furunculosis is no longer seen as a threat to the industry. Epizootics do, however, continue to occur in freshwater hatcheries including restocking hatcheries. In a previous paper Hiney (1995) reported the induction of clinical furunculosis in Atlantic salmon pre-smolts that had been vaccinated with an oil-adjuvant vaccine, following the application of the standard Stress Test of McCarthy (1977). Hiney (1995) raised the possibility that the extent of protection provided by vaccination could be insufficient to prevent clinical disease following of a stressful event. It could be argued that the level of stress produced by the standard Stress Test is excessive and would not be encountered by natural populations. However, this note presents field observations that support the concerns expressed by Hiney (1995).

Methods used in this study
The kidneys of Atlantic salmon mortalities were examined for the presence of *A. salmonicida* by culture on tryptone soya agar (TSA), following aseptic exposure through an incision behind the dorsal fin. TSA plates were incubated for 48-72 h at 22°C and examined daily for the presence of small pigmenting colonies. Putative *A. salmonicida* colonies were confirmed biochemically by the method of Bernoth (1997) and by the application of an *A. salmonicida* specific latex agglutination test (Drinan, 1985).
Antibiotic susceptibility profiles of isolated *A. salmonicida* strains were assessed by a disc diffusion assay using Mastring-S antibiogram discs (UCG4 and M26) (Mast Diagnostics Ltd., Merseyside, UK), with the exception of amoxycillin (2 µg) which was obtained from Oxoid Ltd, UK. The disk diffusion assay was performed essentially by the Kirby-Bauer method (Bauer *et al.*, 1966) with the modification that plates were incubated for 48 h at 22°C. The diameters of zones of inhibition for each antibiotic were measured in mm using a Vernier callipers.
Salinity tolerance testing was carried out essentially by the method of Clark and Blackburn (1977) with the modification that levels of plasma Cl^- rather than Na^+ was assayed. In two consecutive weeks, groups of 12 fish were transferred to 33‰ and 34.8‰ salinity water, respectively, and exposed for a period of 24 h. Following exposure 0.5 ml blood was drawn from the caudal vein of each fish and dispensed into heparinised tubes. Blood samples were also collected from 12 control fish from the same tank which had not been exposed to sea water. Blood samples were transported to the laboratory on ice and examined within 6 hours. Samples were centrifuged at

10,000rpm (Jouan A14 Microfuge) and the plasma removed to fresh, non-heparinised tubes for analysis. Plasma Cl^- values (mmol l^{-1}) were assayed by silver chloride titration using a Jenway PCLM3 chloride analyser (Essex, UK).

Site studied

The hatchery studied here was a freshwater smolt unit. Atlantic salmon parr were taken into the site in early August and transferred to sea as smolts during March of the following year (approximately 8 month cycle). The hatchery was situated on the shore of a freshwater lake from which it obtained its water supply. This lake was periodically stocked with salmonids.

Disease history of pre-vaccinated fish stocks

An outbreak of furunculosis was diagnosed in salmon parr in the three weeks following their introduction to the site in August of year 0. Antibiotic susceptibility tests of the infecting *A. salmonicida* strain indicated the presence of two distinct subtypes, at least with respect to antibiotic susceptibility profile (Table 1 - 1st outbreak). A 10 day oral therapy with Amoxycillin, to which both subtypes were susceptible, was initiated and effectively controlled the outbreak. No further clinical furunculosis was noted in the 5 months following this outbreak.

Disease history of post-vaccinated fish stocks

In November of year 0 all fish at the site were vaccinated with an oil-adjuvanted anti-furunculosis vaccine at a water temperature of 10 - 11.5°C. Vaccination was accompanied by a prophylactic treatment with amoxycillin, administered as an IP injection, at 100 mg/kg body weight. No clinical furunculosis was precipitated by vaccination.

In January of year 1 premature smolting was observed in fish in one tank (Tank A), ave. wt. 150 g. Smolting was not observed in fish from any other tank at the hatchery at this time. Smolting of Tank A fish was manifested as loss of parr markings and silvering and was confirmed by the performance of

Table 1 Antibiotic susceptibility profile of *A. salmonicida* isolates for clinically diseased fish 3 months prior to (1st outbreak) and 2 months after (2nd outbreak) vaccination with an oil-based furunculosis vaccine.

		1st outbreak Mean zone diameter (mm)		**2nd outbreak** Mean zone diameter (mm)	
Antibiotic	**µg/disc**	**Subtype A**	**Subtype B**	**Subtype A**	**Subtype B**
Tetracycline	10	33	13	26	8
	100	38	11	41	12
Streptomycin	25	14	≤6	16	≤6
Cotrimoxazole	100	46	≤6	40	≤6
Oxolinic acid	2	11	16	18	17
Flumequine	2	12	19	16	18
	30	29	36	-	-
Furazolidone	25	34	34	31	33
Chloramphenicol	25	41	48	32	34
Amoxycillin	2	26	26	29	29
Ampicillin	25	39	40	36	35
Nitrofurantin	50	32	34	33	28
Kanamycin	30	28	28	28	25
Naladixic acid	30	≤6	≤6	≤6	≤6

two salinity tolerance tests (Clark and Blackburn, 1977). These were carried out by 24 h exposure of Tank A fish to saltwater at 33ppt and 34.8ppt NaCl. The plasma Cl^- values of saltwater exposed Tank A fish were 113% and 111%, respectively, of the plasma Cl^- obtained from control groups which had not been exposed to salt water. Experience in our laboratory has indicated that, in fish exposed to saltwater at concentrations of 32.5ppt or above, a plasma Cl^- value at or below 115% of that obtained from a freshwater control group correlates strongly with a fully smolted state.

In the week following the second salinity tolerance test (2 months post-vaccination), *A. salmonicida* subsp. *salmonicida* was isolated from the kidney of 4 mortalities from Tank A. The total percentage mortalities incurred in Tank A was 3.2%. *A. salmonicida* was not isolated from fish in any other tank at the hatchery. The antibiotic susceptibility profile of the infecting *A. salmonicida* strain was assessed and is presented in Table 1 (2nd outbreak).

Discussion

A number of observations can be made about the field situation reported here. Fish at the hatchery experienced two distinct outbreaks of furunculosis during a five month period in freshwater. The first, and more widespread outbreak, was experienced by fish in all tanks at the hatchery and occurred three weeks after their introduction to the site as parr. Three months after the first outbreak all fish at the hatchery were vaccinated with an oil-adjuvanted furunculosis vaccine. Two months post vaccination a second outbreak of furunculosis was experienced by fish in a single tank (Tank A).

A number of sources of *A. salmonicida* infection could be postulated for both the first and second furunculosis outbreak. These included: an underlying covert infection in hatchery stock acquired prior to their introduction to the site; transmission of infection by wild fish in the supplying waters of the hatchery; and, in the case of the second outbreak, failure of some component of the vaccine.

The similarity of the antibiotic susceptibility profiles of the *A. salmonicida* strains isolated during the first and second outbreaks would strongly suggest that the source of infection was the same for both outbreaks (Table 1), thus eliminating vaccine components as a probable source of infection. The available data is not adequate to distinguish definitively between covert infection acquired prior to or following introduction of fish to the site, although some epizootiological evidence is available that would strongly favour a post-introduction infection.

After the first outbreak in the hatchery, investigations were made concerning the presence of covert infections in the two farms which supplied the parr. Fish at each farm, neither of which has a history of either clinical or covert furunculosis, were submitted to the standard Stress Test. No covert infections were detected in either population. The wild salmonids living upstream of the hatchery water supply were not tested for covert infections. These feral populations had, however, been periodically supplemented by restocking from a third farm. This third farm had a history of both clinical and covert furunculosis. In more recent years fish at this third hatchery had been vaccinated against furunculosis prior to release but Hiney (1995) reported the precipitation of clinical furunculosis in vaccinated fish from this third hatchery following a standard stress test. Therefore, the epizootiological evidence would favour a model of the outbreaks in which wild salmonids restocked into the lake from the third farm were the source of the infections.

In the second outbreak at the hatchery studied here, *A. salmonicida* was isolated from a single tank of fish that were smolting but was not isolated from non-smolting fish at the same hatchery. The period of smolting is associated with major physiological changes including chronic cortisol elevation which can bring about severe depression of the fishes defence system and increase susceptibility to bacterial infections (Pickering,

1997). Farm management could identify no stressful event that had been experienced by only the smolting fish. It would, therefore, seem reasonable to postulate that early smolting of the fish in Tank A was the stressor that precipitated the second epizootic.

Hiney (1995) has demonstrated that application of the standard Stress Test was sufficient to precipitate clinical disease in fish which had been vaccinated using an oil-adjuvanted vaccine. A possible criticism of the relevance of this observation might be that the stress experienced during this laboratory-based test would far exceed any stress that would be naturally experienced by fish in a hatchery or in the wild. However, the field observations reported here demonstrate that, even in vaccinated fish, a natural stress such as that experienced during smolting is adequate to precipitate clinical furunculosis in covertly infected fish.

Although a natural incidence of clinical furunculosis in vaccinated salmonids has not, to my knowledge, been previously reported in the scientific literature, anecdotal evidence from the Irish and Scottish aquaculture industries would suggest that this is not an isolated event. For commercial aquaculture, where low level losses to furunculosis are often tolerable, the significance of the observations made here may be slight. However, for restocking programmes these observations may have major epizootiological significance. The introduction of covertly infected fish into disease-free water bodies is one of the major ways by which furunculosis can be transmitted (Smith, 1997). This note suggests that movement of such fish may still represent a significant risk even if they have been vaccinated prior to transport. Vaccination, even with oil-adjuvanted vaccines, cannot be considered a barrier treatment where the movement of covertly infected fish between freshwater sites is contemplated.

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