

EXPERIMENTAL DOUBLE INFECTION OF ATLANTIC SALMON (*SALMO SALAR* L.) WITH DIFFERENT *A. SALMONICIDA* STRAINS

BY D. W. SUTHERLAND¹ & VALERIE INGLIS²

Study of antibacterial sensitivity of *A. salmonicida* by sampling isolates from one or more points on the same fish has revealed more than one pattern existing within individual fish. (Inglis, *et al*, 1991a, Sutherland & Inglis, in prep). Michel & Faivre (1991) showed that the bacterial strain carried by a fish has an advantage over subsequent challenging strains and may even confer some protection against such challenge. As well as this effect, the presence of numerous variants in one fish, has implications for the chemotherapy of furunculosis. This manuscript describes an experimental model of a double infection, which can be used to investigate re-isolation of variants and demonstrate the effect of differential chemotherapy.

Method & Materials

One hundred Atlantic salmon (*Salmo salar* L.) parr were randomly distributed in groups of 20 between five static freshwater tanks equipped with airlines and internal filters. They were maintained at 18°C during acclimatisation and the ten day experimental period and fed at 0.8% body weight daily to encourage uptake of all feed. Water and filters were changed every other day.

These fish were exposed to a bath challenge (McCarthy, 1983; Adams *et al*, 1987) by two virulent strains of *Aeromonas salmonicida*, which had been isolated from Atlantic salmon in Scotland. These were FCS (Marine Harvest) and 90130E1(1) (Institute of Aquaculture, Stirling) and differed in that

the first was sensitive to oxolinic acid and the second resistant. The challenge procedure described by Inglis *et al*. (1991b) was modified in that three groups were challenged simultaneously with cultures of both strains, one group with the oxolinic acid-resistant strain only and one was left unchallenged as control.

One of the groups of fish exposed to the double challenge was not provided with medicated feed, one was given a feed medicated with florfenicol at 20 mg kg⁻¹ day⁻¹, which was effective against both bacterial strains (Inglis *et al*, 1991b) and one group was supplied with medicated feed effective against only one strain i.e. oxolinic acid at 10 mg⁻¹ kg⁻¹ day⁻¹. The group exposed to the single challenge with the oxolinic acid resistant strain were treated only with oxolinic acid and the fish in the control tank were not treated.

The fish were inspected daily for signs of ill health; mortalities were removed and kidneys and lesions sampled for bacteriological examination. Rectal smears were taken and plated out on TSA containing 0.01% Coomassie Brilliant Blue, to facilitate easy identification of *A. salmonicida* (Markwardt *et al*, 1989). Ten well separated presumptive *A. salmonicida* colonies were taken from every isolation plate and tested for oxolinic acid sensitivity, to differentiate the two challenge strains. At the end of ten days, any survivors were culled and treated in the same way.

Results

In the untreated double infection all fish died within 8 days and *A. salmonicida* was isolated from every kidney and rectal swab

as well as from surface lesions on 8 of the 10 fish where these showed. All samples yielded the oxolinic sensitive isolate (FCS). The oxolinic acid resistant isolate was recovered from two fish only; from a kidney and rectal swab respectively. In both these cases FCS was also isolated. Fish exposed to the double infection and treated with florfenicol all survived and, on sacrifice, no *A. salmonicida* were detected.

In the double infection treated with oxolinic acid, only one fish died and this was on the fifth day. Both strains were recovered from this mortality but neither from any of the survivors on termination. Similarly, only one fish died in the group exposed only to the oxolinic acid resistant strain and treated with oxolinic acid, and only this isolate was recovered. All the control fish survived and no abnormalities were found on sacrifice.

Discussion

In the double infection where both strains were allowed to compete without antibacterial chemotherapeutic intervention, one (FCS) predominated despite approximately equal cell numbers being used in the challenge. The resistant strain, 90130E1(1) was isolated from only two fish and it cannot be determined whether one or both was responsible for the mortalities. However when this strain was the sole challenger and subjected to oxolinic acid treatment (to which it was resistant), this strain was lethal. In the double infection treated with oxolinic acid the otherwise predominant strain was severely restricted but not completely eliminated. It may have survived because the fish had been compromised by the resistant strain although it is not clear whether one or both contributed to the mortality. This supports the conclusion reached by Michel & Faivre (1991) that in the case of mixed infections, the two strains compete and the most aggressive emerges in overt

furunculosis. Concurrent infections may provide conditions which allow sensitive strains to survive what should be effective therapy. Concurrent infections therefore, could possibly also fail to respond to treatment unless this is effective against all the pathogens involved in the outbreak as demonstrated here when the double infection was treated with oxolinic acid, and one fish still died. There is evidence from examination of samples taken from the field that a single fish may be infected with two strains of *A. salmonicida* at the same (Sutherland & Inglis, 1992), although it has been suggested (Michel & Faivre, 1991) that in a latent infection only one strain can be harboured at a time. It is not uncommon for two or more antibiotic sensitivity patterns to be detected in a single outbreak of furunculosis (Inglis *et al.*, 1991a). This experimental study therefore confirms these field observations and underlines that care should be taken to detect all strains of *A. salmonicida* in a disease outbreak.

Summary

When Atlantic salmon (*S. salar* L.) parr were challenged with two strains of *A. salmonicida*, both could be isolated from mortalities, although one predominated. Treatment effective against one strain still resulted in *A. salmonicida* specific mortality due to the resistant strain.

Authors addresses

- 1) Shetland Aqua Lab, Kergord, Weisdale, Shetland, ZE2 9LW,
- 2) Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland.

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