

**USE OF POLY(DL-LACTIDE-CO-GLYCOLIDE)  
MICROPARTICLES AS ADJUVANTS IN ATLANTIC SALMON**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

Vaccination of atlantic salmon in the Scottish salmon farming industry generally involves intraperitoneal injection of individual fish using vaccines containing oil adjuvants. This method is thought to confer good protection for considerable lengths of time. However, this method is labour intensive and causes a considerable amount of stress to the fish, since the fish have to be anaesthetized, handled, injected and returned to clean water to recover. The size of the fish also has to be considered since injecting fish weighing less than 15g is difficult. Other potential problems with injection immunisation are adhesion formation, the use of potentially toxic adjuvants, temporary reduced feeding, potential puncture of intestines and wound creation (Ellis, 1988). It is therefore desirable to use safer adjuvants and also to develop immunisation strategies which are less stressful and labour intensive. Over the past thirty years in human medicine there has been an interest in the use of biodegradable polymers as adjuvants and vaccine delivery systems both for oral and non-oral routes (O'Hagan *et al*, 1993, Jones *et al*, 1996). One of the most thoroughly researched polymers is that of poly(DL-lactide-co-glycolide) (PLG), which has a safe record of use within medical and veterinary uses (Visscher *et al*, 1987, Vert *et al*, 1991). Used as an adjuvant it is thought to act by effective presentation of the vaccine to antigen processing cells and as a depot for prolonged release. Our

work concentrates on the use of PLG as an adjuvant and the potential of PLG as a carrier for oral vaccine delivery in atlantic salmon.

PLG microparticles were prepared using a commercially available lactide/glycolide polymer (Medisorb, USA) incorporating a commercially available furunculosis vaccine (AVL, UK). Three different grades of PLG microparticles were prepared incorporating vaccine and were subsequently injected or orally intubated into atlantic salmon (weighing 10-15g, 80 per group) at three doses of PLG (100µg, 10µg or 1µg PLG per fish). Control groups were ip injected or orally intubated with blank PLG microspheres (containing PBS), PBS or 100µl of oil adjuvanted furunculosis vaccine (AVL, UK). Fifty fish per group were challenged at week 13 post immunisation with a virulent strain of *Aeromonas salmonicida* MT1326D, which had previously been passaged through atlantic salmon to increase its virulence. 30 fish per group were bled at week 22 post immunisation and serum antibody titres determined by a bacterial agglutination test.

In both the ip injected and orally intubated groups no significant protection was conferred by any of the PLG constructs upon experimental challenge. Significant protection was achieved using the oil adjuvanted vaccine. RPS values are as shown in Table 1. Upon testing sera from the ip injected groups by bacterial agglutination, titres recorded from the oil adjuvanted ip injected group were consistently high, whereas the sera from all bar three fish in the PLG construct groups failed to produce recordable agglutinating titres.

Our initial studies have shown that we were unable to record agglutinating antibody titres in fish immunised either by ip injection and oral intubation of the PLG/vaccine and that these groups failed to produce significant protection against experimental challenge with a virulent strain of *Aeromonas salmonicida*. Future work will concentrate on improving encapsulation and optimising release of the vaccine.

Table 1: RPS values for experimental groups upon experimental challenge with *Areomonas salmonicida*

<b>Group</b>	<b>RPS ip injection</b>	<b>RPS Oral intubation</b>
PLG Blanks	-9.4	-13.6
PLG Low IV 100µg/fish	12.5	-59.1
PLG Low IV 10µg/fish	12.5	-36.4
PLG Low IV 1µg/fish	-37.5	-50
PLG 2A 100µg/fish	28.1	-54.5
PLG 2A 10µg/fish	9.4	-40.9
PLG 2A 1µg/fish	-25	-40.9
PLG 4A 100µg/fish	-12.5	-13.6
PLG 4A 10µg/fish	-28.1	4.5
PLG 4A 1µg/fish	-9.4	4.5
Furovac oil adjuvant	37.5	81.8

## References

- Ellis, A.E. (1988). General principles of fish vaccination. In Fish Vaccination (A.E. Ellis *ed.*). London, Academic Press. pp.1-19
- Jones, D.H., B.W. McBride, & G.H. Farrar (1996). Poly(lactide\_co\_glycolide) microencapsulation of vaccine antigens. *Journal of Biotechnology* 44: 29\_36
- O'Hagan, D.T., J.P. McGee, J. Holmgren, A.MCI. Mowat, A.M. Donachie, K.H.G. Mills, W. Gaisford, D. Rahman & S.J. Challacombe (1993). Biodegradable microparticles for oral immunisation. *Vaccine* 11(2): 149\_154
- Vert, M., S. Li & H. Gerreau (1991). More about the degradation of LA/GA\_derived matrices in aqueous media. *Journal of Controlled Release* 16: 15\_26

Visscher, G.E., R.L. Robison & G.J. Argentero (1987). Tissue response to biodegradable injectable microcapsules. *Journal of Biomaterials Applications* 2(July): 118\_131

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