

Comparison of the safety of 0.05 ml and 0.1ml dose of inactivated vaccines in seabass

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Introduction

Existing research has pointed out that increasing fish size at the time of vaccination may mitigate intrabdominal lesions (Bakopoulos et al., 2003, Berg et al., 2007) , with a recommended size of 15 grams for seabass vaccination (Brudeseth et al., 2013). However, the demands of aquaculture production systems often necessitate the vaccination of smaller fish, prompting a need for comprehensive investigations into the associated side-effects. The primary objective of the current study is to macro and microscopically evaluate the side-effects from 2 different vaccines, associated with fish size, dosage, and adjuvant utilization.

Materials and methods

European seabass (*Dicentrarchus labrax*) were separated based on weight in fish of 10 g and 15 g, and then distributed into 2 groups of 44 fish each for each category, a total of 176. Group A (15 g) was injected with PBS at a dose of 0.1 ml per fish; group B (10 g) was vaccinated with ICTHIOVAC® VR/PD at a dose of 0.1 ml per fish; group C (10 g) was vaccinated with a registered inactivated vaccine at a dose of 0.05 ml per fish; group D (15 g) was vaccinated with ICTHIOVAC® VR/PD at a dose of 0.1 ml per fish. All treatments were administered by intraperitoneal route. The health status of fish was monitored during 59 days after vaccination. Body weight (total weight, TW), length (Fork length, FL), macroscopic and microscopic intraperitoneal lesions were evaluated periodically. For this purpose, 10 fish per groups were euthanised by an overdose of MS-222. Adhesions were scored by using the Spielberg's methods. Visceral organs were fixed in 10 % buffered formalin and stained with haematoxylin and eosin. The statistical comparisons between groups were performed by Kruskal-Wallis with Holm correction test implemented in R Studio program. The significance level was set at 5 %.

Results

There was not mortality in any of the groups after vaccination. Moreover, no statistically significant differences (p<0.05) were observed in terms of body weight and length among fish within the same category of weight at vaccination (A vs D and B vs C).

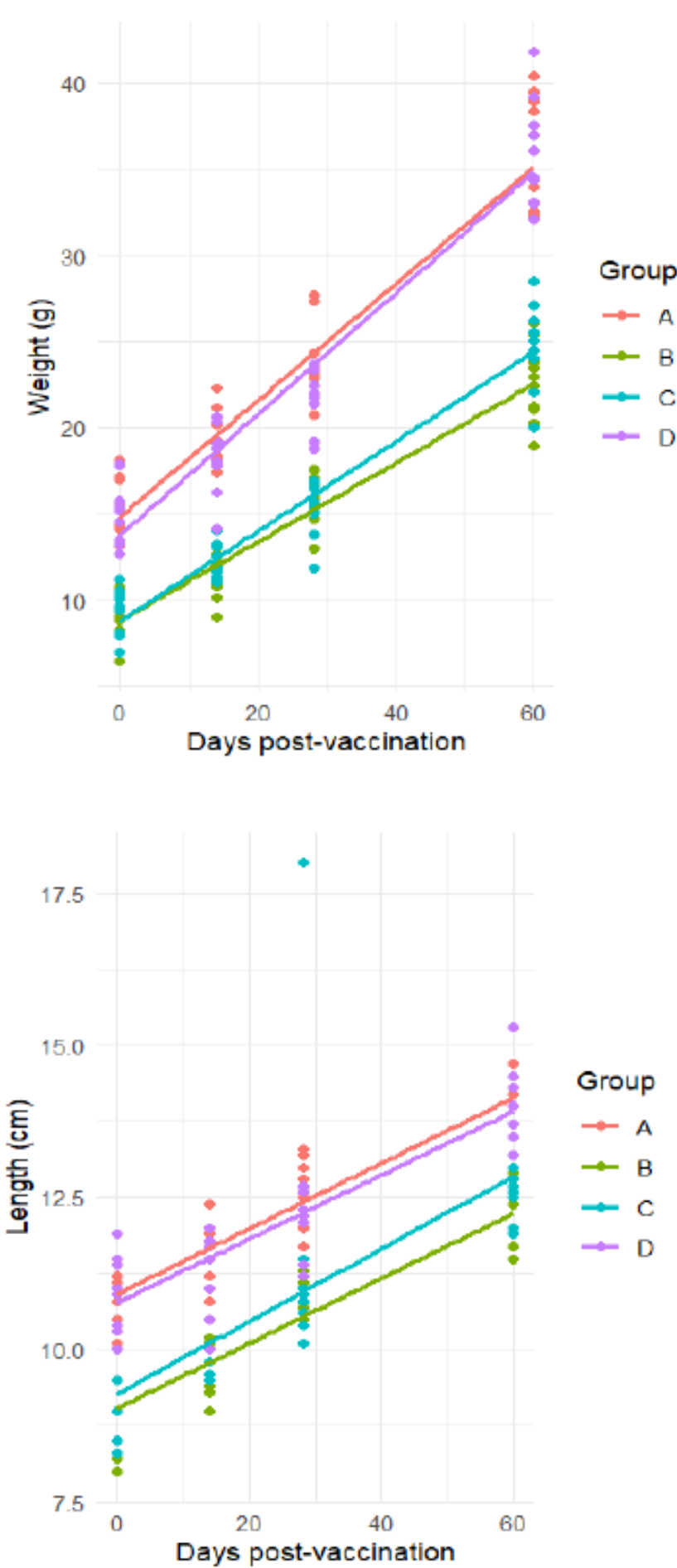


Fig 1. Progression of the TW and FL over the different samplings. Control group A (n=10 per sampling) received no-treatment, group B (n=10 per sampling) and D (n=10 per sampling) were vaccinated with Vaccine A and group C (n=10 per sampling) with Vaccine B.

Post-mortem inspection of the peritoneal cavity revealed the formation of mild or very mild adhesions and the presence of deposits of vaccines in form of vesicles. No statistically significant differences (p>0.05) were observed among the average Spielberg's score of the groups.

	Day 0	Day 14	Day 28	Day 60
A	0	0	0	0
B	0	1,1	1,8	1,6
C	0	1,5	1,7	1,7
D	0	1,7	1,5	1

Table 1. Progression of local reactions. Mean Spielberg scores are shown per day and group.

The main alteration observed by the histological inspection were the formation of temporal vesicles containing vaccine residues and granulomas in the peritoneal adipose tissue which were accompanied by inflammatory infiltration.

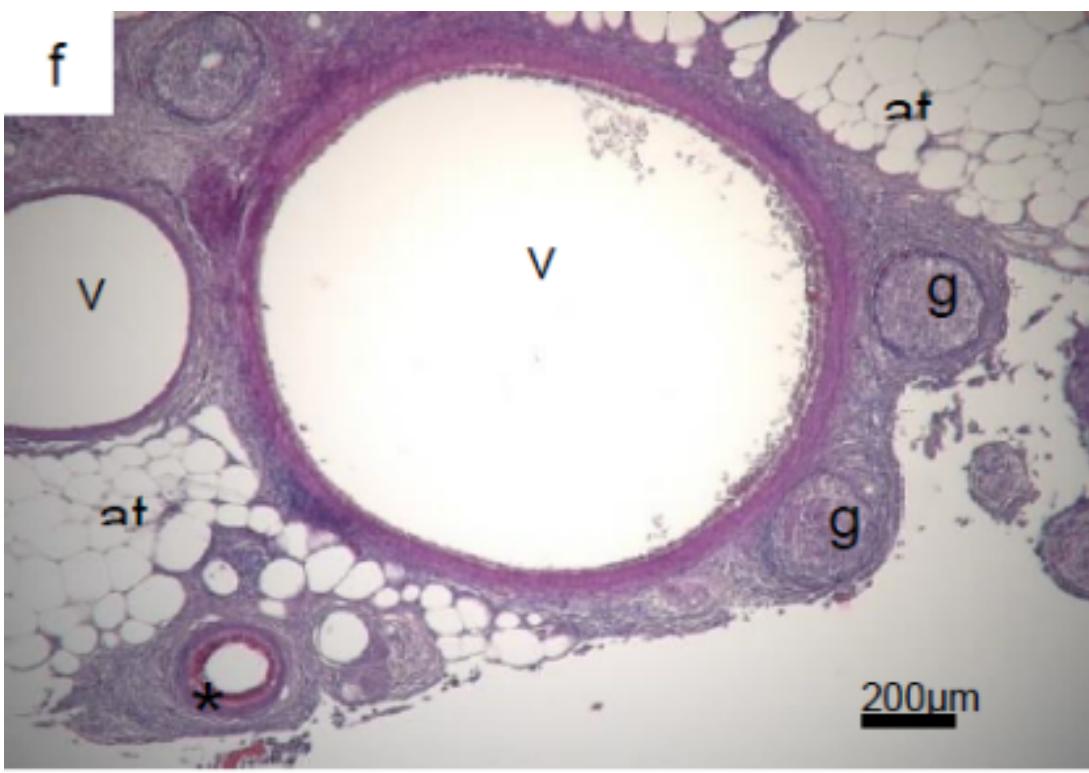


Fig 2. Adipose tissue (at) with vaccine droplets (v) and granulomas (g) surrounded by concentric layers of inflammatory reaction. Notice the eosinophilic layer in one of the VD (*)

After 14 days all the vaccinated groups exhibit the development of granulomas within the adipose tissue. These granulomas proved to be temporary and gradually diminished as time progressed, with a notable decrease observed by the 28th day post-vaccination for group C and later, by day 60th for groups B and D. The main difference observed among groups was that the formation of these transient granulomas in group B and D were more abundant and lasted longer compared to group C.

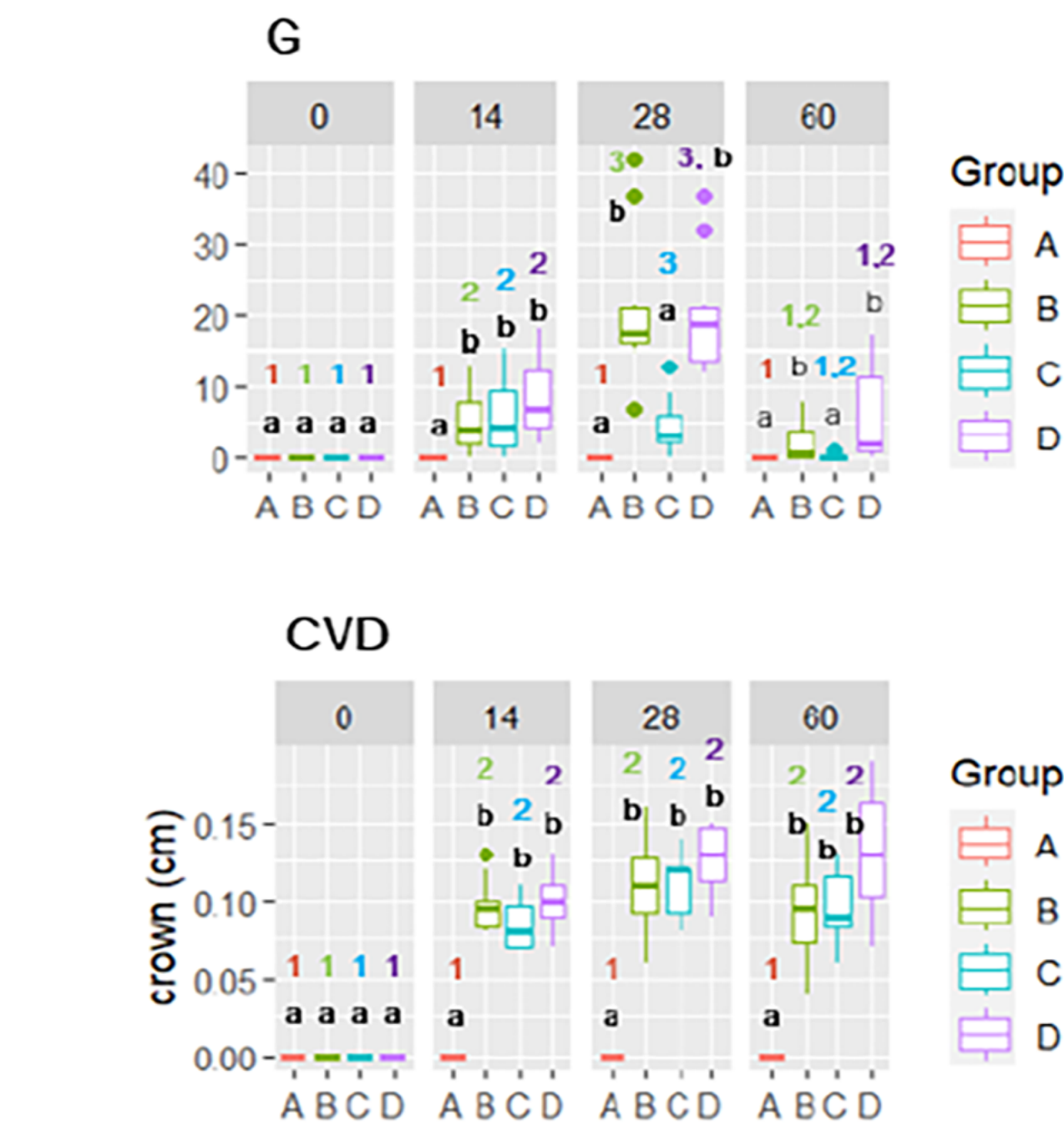
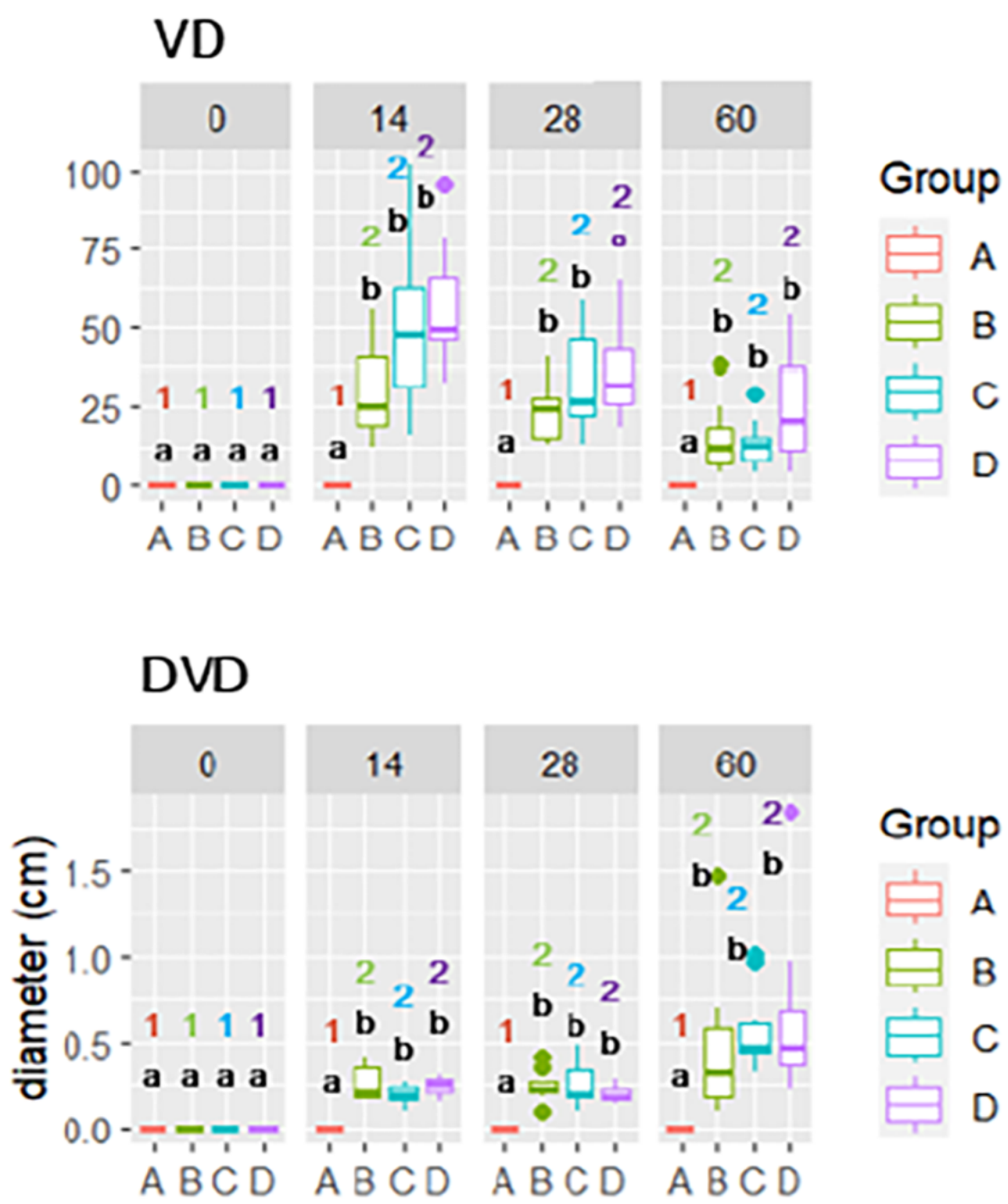


Fig 3. Evolution of inflammation. Boxplots representing the VD (vaccine droplets) G (granulomas), DVD (diameter of vaccine droplets) and CVD (inflammatory crown of vaccine droplets). Different letters indicate statistically significant differences among groups in the same sampling. Different numbers indicate a statistically significant difference among sampling time points.

Discussion and conclusions

Group B, which received ICTHIOVAC® VR/PD at a volume of 0.1 ml containing a non-mineral oil adjuvant—double the volume administered to Group C with 0.05 ml of mineral oil adjuvant—did not show any noticeable reaction at a macroscopic level. The performance of the two groups throughout the study was similar.

The main findings at a microscopic level induced by the vaccines were vesicles and granulomas located in the adipose tissue. The ICTHIOVAC® VR/PD vaccinated groups produced more granulomas, however, the observed reactions were temporal and did not compromise the health of the animals, nor their growth performance. Given this information and considering the inflammation linked to the microscopic changes, it is crucial to delve deeper into understanding their contribution to the production of the immune response induced by the vaccines.

In conclusion, this study demonstrated that the tested mineral oil adjuvanted vaccine administered at 0.05ml showed no significant differences in terms of safety nor growth performance compared with ICTHIOVAC® VR/PD, whether administered to 10 or 15 g seabass at 0.1 ml.

These findings suggest the need for further research into the role of granulomas and adipose tissue in the immune response.

References

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