

**FIGURE 7** ELISA antibody reactivities in groups of fish sampled in vaccination Trial-1 (n=10, 50 dpv) and vaccination Trial 2 (n=6, 48 dpv). Antigens included sonicated *A. salmonicida* cells, purified VapA-SpyCatcher, and purified *A. salmonicida* LPS.

**Antibody reactivities in ELISA (absorbance at A405 with 1/100 serum dilution, average of two replicate wells) in sera from fish in vaccination Trial-1 (50dpv)**

Antigen: *A. salmonicida*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 0.1 | 1 | 0.6 | 0.6 | 3.8 | 4 | 0 | 0 |
| 2 | 0.3 | 0.7 | 0.3 | 0.9 | 3.7 | 3.9 | 0 | 2.3 |
| 3 | 0.4 | 0.59 | 0.1 | 1.2 | 4 | 3.8 | 0 | 0.9 |
| 4 | 0.1 | 1.8 | 0 | 0.6 | 4 | 4 | 0 | 0 |
| 5 | 0.1 | 0.82 | 0.2 | 0.4 | 4 | 4 | 0.1 | 0.1 |
| 6 | 0.2 | 0.7 | 0.3 | 2.6 | 4 | 4 | 0.1 | 0.5 |
| 7 | 0.1 | 2.6 | 0.1 | 1.3 | 4 | 4 | 0.1 | 0.4 |
| 8 | 0.2 | 0.6 | 0.9 | 1.6 | 4 | 4 | 0.1 | 0.2 |
| 9 | 0.1 | 0.6 | 0.2 | 0.4 | 2.7 | 4 | 0 | 2 |
| 10 | 0.1 | 0.6 | 0 | 1.6 | 4 | 4 | 0 | 0.2 |

Antigen: VapA-SpyCatcher

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 0.2 | 4 | 3.3 | 4 | 0.3 | 4 | 0.1 | 0 |
| 2 | 0.8 | 4 | 2.8 | 4 | 0.1 | 1.4 | 0 | 0.2 |
| 3 | 1.7 | 4 | 0.4 | 4 | 0 | 4 | 0 | 0 |
| 4 | 0.2 | 4 | 0 | 4 | 0.1 | 1 | 0 | 0 |
| 5 | 0.6 | 4 | 1.5 | 4 | 0 | 4 | 0 | 0 |
| 6 | 0.6 | 4 | 1.6 | 4 | 0 | 4 | 0 | 0 |
| 7 | 0.7 | 2.5 | 0.6 | 4 | 0.2 | 4 | 0 | 0.2 |
| 8 | 0.8 | 4 | 4 | 4 | 0 | 4 | 0 | 0.1 |
| 9 | 0.4 | 4 | 1.4 | 4 | 0.1 | 0.2 | 0 | 3.2 |
| 10 | 0.4 | 4 | 0.5 | 4 | 0.2 | 0.1 | 0 | 0.1 |

Antigen: LPS of A. salmonicida

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | -0.02 | 0 | 0.03 | -0.04 | 1.09 | 3.69 | 0.05 | 0.02 |
| 2 | 0.08 | 0 | 0.07 | -0.01 | 0.47 | 1.88 | 0.13 | 0.56 |
| 3 | 0.04 | 0 | 0.07 | -0.01 | 3.01 | 2.54 | 0.09 | 1.16 |
| 4 | 0.08 | 0 | 0.48 | -0.01 | 1.54 | 2 | 0.05 | 0.02 |
| 5 | 0.2 | 0 | 0.06 | -0.01 | 1.8 | 2.61 | 0.03 | 0.05 |
| 6 | 0.05 | 0 | 0.06 | -0.07 | 1.75 | 2.84 | 0.02 | 0.23 |
| 7 | 0.08 | 0.92 | 0.1 | 0.02 | 1.17 | 2.63 | 0 | 0.2 |
| 8 | 0.2 | 0 | 0 | 0 | 1.54 | 3.26 | 0.07 | 0.07 |
| 9 | 0.05 | 0 | 0.03 | 0.01 | 0.59 | 3.81 | 0.01 | 0.01 |
| 10 | 0.05 | 0 | 0.07 | 0.02 | 1.53 | 2.67 | 0.03 | 0.05 |

**Antibody reactivities in ELISA (absorbance at A405 with 1/100 serum dilution, average of two replicate wells) in sera from fish in accination Trial-1 (69dpv)**

Antigen: *A. salmonicida*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 0.83 | 0.12 | 0.82 | 2.19 | 3.24 | 4 | 0.09 | 0.3 |
| 2 | 0.14 | 3.16 | 0.14 | 2.7 | 2.48 | 4 | 0.18 | 0.23 |
| 3 | 0.38 | 2.16 | 0.1 | 2.32 | 3.22 | 4 | 0.15 | 1.39 |
| 4 | 0.18 | 1.72 | 0.3 | 2.31 | 1.89 | 4 | 0.09 | 2.49 |
| 5 | 0.09 | 4.1 | 0.42 | 2.15 | 1.45 | 4 | 0.06 | 0.55 |
| 6 | 0.31 | 2.62 | 0.09 | 2.45 | 4 | 3.73 | 0.06 | 0.44 |
| 7 | 0.15 | 1.76 | 0.44 | 3.09 | 3.98 | 3.33 | 0.05 | 0.8 |
| 8 | 0.16 | 1.26 | 0.56 | 3.23 | 4 | 4 | 0.07 | 0.21 |
| 9 | 0.31 | 3.18 | 0.82 | 2.85 | 3.47 | 4 | 0.02 | 0.18 |
| 10 | 0.07 | 2.31 | 0.09 | 0.37 | 2.51 | 4 | 0.1 | 0.1 |
| 11 | 0.22 | 1.47 | 1.32 | 2.56 | 4 | 4 | 0.03 | 0.09 |
| 12 | 0.22 | 2 | 0.2 | 2.73 | 2.15 | 4 | 0.09 | 2.43 |

Antigen: VapA-SpyCatcher

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 0.74 | 0.54 | 1.13 | 4 | 2.01 | 3.95 | 0.05 | 0.18 |
| 2 | 0.14 | 4.1 | 0.39 | 4 | 2 | 4 | 0.1 | 0.04 |
| 3 | 1.16 | 4.1 | 0.16 | 4 | 2.05 | 4 | 0.07 | 0.03 |
| 4 | 0.37 | 4.1 | 0.5 | 4 | 1.65 | 4 | 0.06 | 3.41 |
| 5 | 0.29 | 4.1 | 1.27 | 4 | 0.03 | 3.18 | 0.04 | 0.1 |
| 6 | 0.35 | 4.1 | 0.03 | 4 | 2.06 | 4 | 0.05 | 0.21 |
| 7 | 0.11 | 4.1 | 1.35 | 3.95 | 0.24 | 4 | 0.03 | 0.02 |
| 8 | 0.28 | 4.1 | 1.52 | 3.92 | 0.06 | 0.45 | 0.09 | 0.18 |
| 9 | 0.61 | 3.99 | 0.92 | 4 | 0.08 | 2.82 | 0.02 | 0.06 |
| 10 | 0.09 | 4.1 | 0.16 | 3.64 | 0.06 | 1.39 | 0 | 0.03 |
| 11 | 1.18 | 4.1 | 4 | 4 | 0.04 | 4 | 0 | 0.08 |
| 12 | 0.79 | 4.1 | 0.75 | 4 | 0.13 | 4 | 0 | 0 |

Antigen: LPS of A. salmonicida

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 0.1 | 0 | 0 | 0.05 | 1.47 | 2.28 | 0.05 | 0.06 |
| 2 | 0.1 | 0 | 0 | 0.03 | 0.79 | 1.79 | 0.13 | 0.12 |
| 3 | 0.1 | 0 | 0 | 0.08 | 1.32 | 2.92 | 0.09 | 0.46 |
| 4 | 0 | 0 | 0 | 0.01 | 0.66 | 2.26 | 0.05 | 0.06 |
| 5 | 0 | 0 | 0 | 0.08 | 0.62 | 2.18 | 0.03 | 0.23 |
| 6 | 0 | 0 | 0 | 0.01 | 3.79 | 3.55 | 0.02 | 0.08 |
| 7 | 0 | 0 | 0 | 0.03 | 1.82 | 1.76 | 0 | 0.6 |
| 8 | 0 | 0 | 0.1 | 0.01 | 2.94 | 4 | 0.07 | 0.16 |
| 9 | 0.1 | 0 | 0.5 | 0.34 | 1.73 | 3.56 | 0.01 | 0.1 |
| 10 | 0 | 0 | 0.1 | 0.01 | 1.09 | 3.57 | 0.06 | 0.06 |
| 11 | 0 | 0 | 0.1 | 0.06 | 2.57 | 3.61 | -0.01 | 0.04 |
| 12 | 0 | 0 | 0.1 | 0.13 | 0.95 | 2.75 | 0.02 | 1.58 |

**Antibody reactivities in ELISA (absorbance at A405 with 1/100 serum dilution, average of two replicate wells) in sera from fish in vaccination Trial-2 (48dpv)**

Antigen: *A. salmonicida*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | RGNNV VLP+VapA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | AP205 VLP+VapA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 1.26 | 1.87 | 0.3 | 1.82 | 0.95 | 0.33 | 4.1 | 3.76 | 0.31 | 0.23 |
| 2 | 1.93 | 1.69 | 0.79 | 2.77 | 2.07 | 0.33 | 4.1 | 4.1 | 0.2 | 0.61 |
| 3 | 0.97 | 1.21 | 0.58 | 2.3 | 1.14 | 0.37 | 4.1 | 4.1 | 0.13 | 0.35 |
| 4 | 1.13 | 2.25 | 0.6 | 2.17 | 1.82 | 0.71 | 4.1 | 4.1 | 0.12 | 0.38 |
| 5 | 0.81 | 0.61 | 0.46 | 2.02 | 0.81 | 0.4 | 4.1 | 4.1 | 0.15 | 0.55 |
| 6 | 2.12 | 2.12 | 0.81 | 1.64 | 2.26 | 0.13 | 4.1 | 4.1 | 0.22 | 0.17 |

Antigen: VapA-SpyCatcher

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | RGNNV VLP+VapA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | AP205 VLP+VapA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 3.95 | 2.77 | 1.1 | 4.1 | 4.1 | 0.5 | 0.19 | 2.6 | 0.09 | 0.06 |
| 2 | 3.96 | 4.1 | 0.7 | 4.1 | 4.1 | 0.46 | 0.23 | 0.87 | 0.11 | 0.15 |
| 3 | 2.05 | 4.1 | 0.47 | 4.1 | 4.1 | 0.5 | 2.03 | 2.87 | 0.03 | 0.09 |
| 4 | 2.85 | 4.1 | 0.63 | 4.1 | 4.1 | 1.11 | 0.42 | 0.39 | 0.06 | 0.06 |
| 5 | 2.32 | 4.1 | 0.74 | 4.1 | 3.34 | 0.59 | 0.21 | 0.34 | 0.15 | 0.06 |
| 6 | 4.1 | 4.1 | 1.42 | 4.1 | 4.1 | 0.15 | 0.09 | 0.28 | 0.08 | 0.05 |

Antigen: LPS of *A. salmonicida*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vaccine  Individual | RGNNV VLP-VapA | RGNNV VLP-VapA+FIA | RGNNV VLP+VapA | AP205 VLP-VapA | AP205 VLP-VapA+FIA | AP205 VLP+VapA | FKC | FKC+FIA | Buffer | Buffer + FIA |
| 1 | 0 | 0.04 | 0.02 | 0.1 | 0.02 | 0.03 | 2.66 | 2.97 | 0.01 | 0.03 |
| 2 | 0.08 | 0 | 0.02 | 0.06 | 0 | 0.04 | 2.46 | 3.45 | 0.02 | 0.04 |
| 3 | 0.04 | 0 | 0.01 | 0.05 | 0 | 0.03 | 3.04 | 3.47 | 0.04 | 0.03 |
| 4 | 0.08 | 0.02 | 0 | 0 | 0.05 | 0.03 | 2.72 | 3.57 | 0.01 | 0.03 |
| 5 | 0.2 | 0 | 0.04 | 0.01 | 0 | 0.01 | 3.48 | 3.11 | 0.01 | 0.03 |
| 6 | 0.05 | 0.04 | 0.02 | 0.02 | 0 | 0.02 | 3.54 | 2.12 | 0.01 | 0.04 |