Supplementary file: The efficacy of Nile tilapia broodstock and larval immunization against *Streptococcus agalactiae* and *Aeromonas hydrophila*

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Supplementary Materials: Cumulative mortality of larvae produced from broodstock immunization after challenged with *S. agalactiae*, *A. hydrophila*, and coinfection at 5, 10, 15, and 20 days after hatch. In the first stage, the number of larvae challenged each replication was 20 fish (Tables S1A–S1D). Cumulative mortality larvae from larval immunization after challenged with *S. agalactiae*, *A. hydrophila*, and coinfection at 14 and 21 days after larvae immunization. In the second stage, the number of larvae challenged in each replication was 10 fish (Table S2A, S2B).

**Table S1A.** The cumulative mortalities from the Sa, Ah, and Biv groups after being challenged with *S. agalactiae*, *A. hydrophila*, and coinfection at larvae aged 5 days after hatch

<table>
<thead>
<tr>
<th>Immunization with Vaccine</th>
<th>Challenging Bacteria</th>
<th>Dead Fish</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I II III</td>
<td>I II III</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>S. agalactiae</em></td>
<td>3 2 1</td>
<td>15</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>S. agalactiae</em></td>
<td>8 10 10</td>
<td>40</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>S. agalactiae</em></td>
<td>3 2 2</td>
<td>15</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>A. hydrophila</em></td>
<td>9 8 8</td>
<td>45</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>A. hydrophila</em></td>
<td>1 3 1</td>
<td>5</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>A. hydrophila</em></td>
<td>2 2 2</td>
<td>10</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>Coinfection</td>
<td>9 10 8</td>
<td>45</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>Coinfection</td>
<td>9 10 10</td>
<td>45</td>
</tr>
<tr>
<td>Bivalent</td>
<td>Coinfection</td>
<td>4 2 3</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td><em>S. agalactiae</em></td>
<td>14 13 14</td>
<td>70</td>
</tr>
<tr>
<td>Control</td>
<td><em>A. hydrophila</em></td>
<td>14 13 12</td>
<td>70</td>
</tr>
</tbody>
</table>

**Table S1B.** The cumulative mortalities from Sa, Ah and Biv group after challenged with *S. agalactiae*, *A. hydrophila* and coinfection at larvae aged 10 days after hatch.

<table>
<thead>
<tr>
<th>Immunization with Vaccine</th>
<th>Challenging Bacteria</th>
<th>Dead Fish</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I II III</td>
<td>I II III</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>S. agalactiae</em></td>
<td>3 2 1</td>
<td>15</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>S. agalactiae</em></td>
<td>8 10 10</td>
<td>40</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>S. agalactiae</em></td>
<td>3 2 2</td>
<td>15</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>A. hydrophila</em></td>
<td>9 8 8</td>
<td>45</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>A. hydrophila</em></td>
<td>1 3 1</td>
<td>5</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>A. hydrophila</em></td>
<td>2 2 2</td>
<td>10</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>Coinfection</td>
<td>9 10 8</td>
<td>45</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>Coinfection</td>
<td>9 10 10</td>
<td>45</td>
</tr>
<tr>
<td>Bivalent</td>
<td>Coinfection</td>
<td>4 2 3</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td><em>S. agalactiae</em></td>
<td>14 13 14</td>
<td>70</td>
</tr>
<tr>
<td>Control</td>
<td><em>A. hydrophila</em></td>
<td>14 13 12</td>
<td>70</td>
</tr>
</tbody>
</table>

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Table S1C. The cumulative mortalities from Sa, Ah and Biv group after challenged with *S. agalactiae*, *A. hydrophila* and coinfection at larvae aged 15 days after hatch.

<table>
<thead>
<tr>
<th>Immunization with Vaccine</th>
<th>Challenging Bacteria</th>
<th>Dead Fish</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>S. agalactiae</em></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>S. agalactiae</em></td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>S. agalactiae</em></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>A. hydrophila</em></td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>A. hydrophila</em></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>A. hydrophila</em></td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>Coinfection</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>Coinfection</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Bivalent</td>
<td>Coinfection</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td><em>S. agalactiae</em></td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td><em>A. hydrophila</em></td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>Coinfection</td>
<td>17</td>
<td>18</td>
</tr>
</tbody>
</table>

*I, II, and III represent the dead fish % cumulative mortality of triplicate immunizations.

Table S1D. The cumulative mortalities from Sa, Ah and Biv group after challenged with *S. agalactiae*, *A. hydrophila* and coinfection at larvae aged 20 days after hatch.

<table>
<thead>
<tr>
<th>Immunization with Vaccine</th>
<th>Challenging Bacteria</th>
<th>Dead Fish</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>S. agalactiae</em></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>S. agalactiae</em></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>S. agalactiae</em></td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>A. hydrophila</em></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>A. hydrophila</em></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Bivalent</td>
<td><em>A. hydrophila</em></td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>Coinfection</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>Coinfection</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Bivalent</td>
<td>Coinfection</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td><em>S. agalactiae</em></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td><em>A. hydrophila</em></td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>Coinfection</td>
<td>17</td>
<td>18</td>
</tr>
</tbody>
</table>

Table S2A. The dead fish and cumulative mortalities of tilapia larvae Biv1 and Pbs1 were challenged *S. agalactiae*, *A. hydrophila* and coinfection at larvae 14 days after larva immunization.

<table>
<thead>
<tr>
<th>Group Immunization</th>
<th>Challenging Bacteria</th>
<th>Dead Fish</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td><em>Pbs1</em></td>
<td><em>S. agalactiae</em></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><em>Biv1</em></td>
<td><em>S. agalactiae</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Pbs1</em></td>
<td><em>A. hydrophila</em></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><em>Biv1</em></td>
<td><em>A. hydrophila</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Pbs1</em></td>
<td>Coinfection</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Biv1</em></td>
<td>Coinfection</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td><em>S. agalactiae</em></td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td><em>A. hydrophila</em></td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>Coinfection</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>
Table S2B. The dead fish and cumulative mortalities of tilapia larvae Biv1 and Pbs1 were challenged *S. agalactiae, A. hydrophila* and coinfection at larvae 21 days after larvae immunization.

<table>
<thead>
<tr>
<th>Group Immunization</th>
<th>Challenging Bacteria</th>
<th>Dead Fish</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Pbs1</td>
<td><em>S. agalactiae</em></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Biv1</td>
<td><em>S. agalactiae</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pbs1</td>
<td><em>A. hydrophila</em></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Biv1</td>
<td><em>A. hydrophila</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pbs1</td>
<td>Coinfection</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Biv1</td>
<td>Coinfection</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td><em>S. agalactiae</em></td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td><em>A. hydrophila</em></td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>Coinfection</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

*I, II, and III represent the dead fish % cumulative mortality of triplicate immunizations.