

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

Immunization with Vaccine	Challenging Bacteria	Dead Fish			Cumulative Mortality (%)		
		I	II	III	I	II	III
<i>S. agalactiae</i>	<i>S. agalactiae</i>	1	1	2	5	5	10
<i>A. hydrophila</i>	<i>S. agalactiae</i>	7	5	6	35	25	30
Bivalent	<i>S. agalactiae</i>	2	1	2	10	5	10
<i>S. agalactiae</i>	<i>A. hydrophila</i>	4	5	5	20	25	25
<i>A. hydrophila</i>	<i>A. hydrophila</i>	1	1	1	5	5	5
Bivalent	<i>A. hydrophila</i>	1	1	1	5	5	5
<i>S. agalactiae</i>	Coinfection	7	7	7	35	35	35
<i>A. hydrophila</i>	Coinfection	7	7	7	35	35	35
Bivalent	Coinfection	3	2	2	15	10	10
Control	<i>S. agalactiae</i>	12	10	10	60	50	50
Control	<i>A. hydrophila</i>	10	11	9	50	55	45
Control	Coinfection	12	13	14	60	65	70

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.

Immunization with Vaccine	Challenging Bacteria	Dead Fish			Cumulative Mortality (%)		
		I	II	III	I	II	III
<i>S. agalactiae</i>	<i>S. agalactiae</i>	3	2	1	15	10	5
<i>A. hydrophila</i>	<i>S. agalactiae</i>	8	10	10	40	50	50
Bivalent	<i>S. agalactiae</i>	3	2	2	15	10	10
<i>S. agalactiae</i>	<i>A. hydrophila</i>	9	8	8	45	40	40
<i>A. hydrophila</i>	<i>A. hydrophila</i>	1	3	1	5	15	5
Bivalent	<i>A. hydrophila</i>	2	2	2	10	10	10
<i>S. agalactiae</i>	Coinfection	9	10	8	45	50	40
<i>A. hydrophila</i>	Coinfection	9	10	10	45	50	50
Bivalent	Coinfection	4	2	3	20	10	15
Control	<i>S. agalactiae</i>	14	13	14	70	65	70
Control	<i>A. hydrophila</i>	14	13	12	70	65	60

Control	Coinfection	14	14	16	70	70	80
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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.

Immunization with Vaccine	Challenging Bacteria	Dead Fish			Cumulative Mortality (%)		
		I	II	III	I	II	III
<i>S. agalactiae</i>	<i>S. agalactiae</i>	3	3	5	15	15	25
<i>A. hydrophila</i>	<i>S. agalactiae</i>	11	13	12	55	65	60
Bivalent	<i>S. agalactiae</i>	5	5	5	25	25	25
<i>S. agalactiae</i>	<i>A. hydrophila</i>	12	10	10	60	50	50
<i>A. hydrophila</i>	<i>A. hydrophila</i>	4	4	5	20	20	25
Bivalent	<i>A. hydrophila</i>	6	5	5	30	25	25
<i>S. agalactiae</i>	Coinfection	13	12	13	65	60	65
<i>A. hydrophila</i>	Coinfection	13	13	13	65	65	65
Bivalent	Coinfection	6	5	7	30	25	35
Control	<i>S. agalactiae</i>	17	15	16	85	75	80
Control	<i>A. hydrophila</i>	16	15	16	80	75	80
Control	Coinfection	17	18	15	85	90	75

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.

Immunization with Vaccine	Challenging Bacteria	Dead Fish			Cumulative Mortality (%)		
		I	II	III	I	II	III
<i>S. agalactiae</i>	<i>S. agalactiae</i>	4	6	5	20	30	25
<i>A. hydrophila</i>	<i>S. agalactiae</i>	11	12	14	55	60	70
Bivalent	<i>S. agalactiae</i>	5	6	7	25	30	35
<i>S. agalactiae</i>	<i>A. hydrophila</i>	11	12	12	55	60	60
<i>A. hydrophila</i>	<i>A. hydrophila</i>	8	6	6	40	30	30
Bivalent	<i>A. hydrophila</i>	7	6	7	35	30	35
<i>S. agalactiae</i>	Coinfection	15	16	14	75	80	70
<i>A. hydrophila</i>	Coinfection	14	16	15	70	80	75
Bivalent	Coinfection	9	7	7	45	35	35
Control	<i>S. agalactiae</i>	15	15	18	75	75	90
Control	<i>A. hydrophila</i>	17	15	16	85	75	80
Control	Coinfection	17	18	19	85	90	95

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

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 larvae immunization.

Group Immunization	Challenging Bacteria	Dead Fish			Cumulative Mortality (%)		
		I	II	III	I	II	III
<i>Pbs1</i>	<i>S. agalactiae</i>	4	4	5	40	40	50
<i>Biv1</i>	<i>S. agalactiae</i>	2	2	3	20	20	30
<i>Pbs1</i>	<i>A. hydrophila</i>	4	3	3	40	30	30
<i>Biv1</i>	<i>A. hydrophila</i>	2	2	1	20	20	10
<i>Pbs1</i>	Coinfection	5	5	6	50	50	60
<i>Biv1</i>	Coinfection	3	2	3	30	20	30
Control	<i>S. agalactiae</i>	8	7	9	80	70	90
Control	<i>A. hydrophila</i>	7	8	6	70	80	60
Control	Coinfection	9	7	10	90	70	100

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.

Group Immunization	Challenging Bacteria	Dead Fish			Cumulative Mortality (%)		
		I	II	III	I	II	III
<i>Pbs1</i>	<i>S. agalactiae</i>	4	4	5	40	40	50
<i>Biv1</i>	<i>S. agalactiae</i>	2	2	2	20	20	20
<i>Pbs1</i>	<i>A. hydrophila</i>	3	4	3	30	40	30
<i>Biv1</i>	<i>A. hydrophila</i>	1	2	2	10	20	20
<i>Pbs1</i>	Coinfection	5	6	6	50	60	60
<i>Biv1</i>	Coinfection	2	3	3	20	30	30
Control	<i>S. agalactiae</i>	8	7	9	80	70	90
Control	<i>A. hydrophila</i>	6	7	6	60	70	60
Control	Coinfection	9	10	9	90	100	90

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