



Collection and identification of the target antigen

From infected tilapia, more than 150 isolates of *Streptococcus agalactiae* were collected from North, Northeast, South and Central part of Thailand

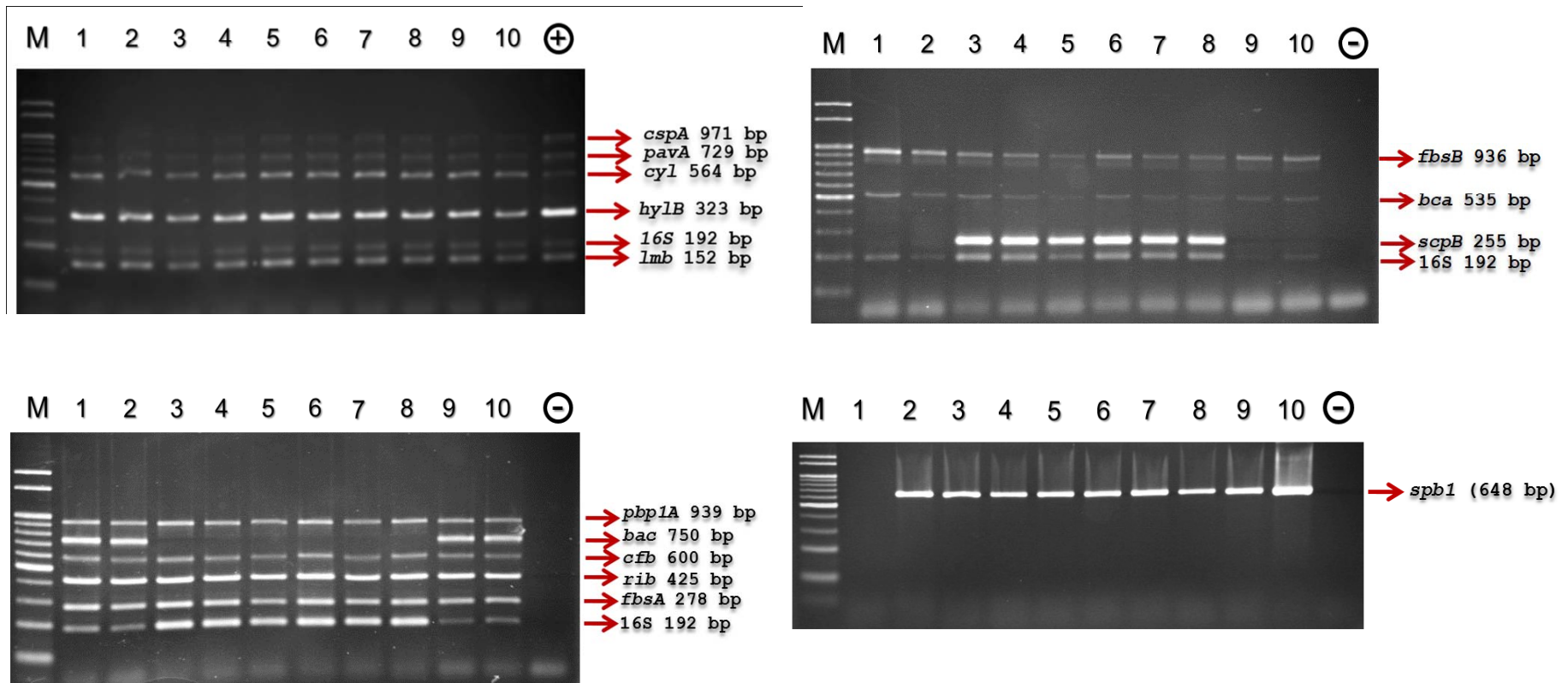


M 1 2 3 4 5 6 7 8 9
10 -

192 bp
(16s rRNA)

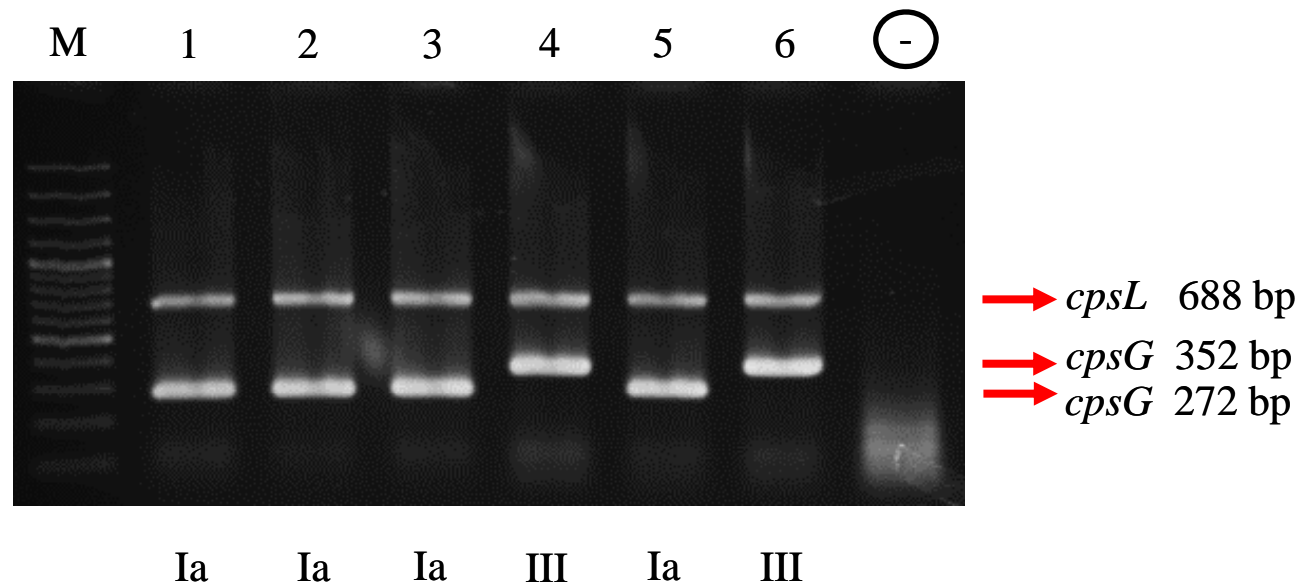
Identification of virulence and *cps* genes

Multiplex-PCR of 14 virulence genes of *S. agalactiae* isolates



Kannika *et al.* (2017) J. Appl. Microbiol. 122:1497 - 1507

Multiplex PCR of *cps* gene cluster



- ✓ Serotype Ia : *cpsL* (688) + *cpsG* (272)
- ✓ Serotype III : *cpsL* (688) + *cpsG* (352)

Serotyping based on the capsular polysaccharide (*cps*) gene profile
(Imperi *et al.*, 2010)

Virulence gene and *cps* gene profile

| <i>cps</i> cluster | Category of Virulence gene of <i>S. agalactiae</i> | | | | | | | | | | | | | |
|-----------------------|--|-------------|-------------|-------------|-------------|------------|------------|------------|------------|-------------|----------------|-------------|------------|-------------------|
| | Adhesins | | | | | Invasins | | | | | Immune evasion | | | |
| | <i>lmb</i> | <i>scpB</i> | <i>pavA</i> | <i>fbsA</i> | <i>fbsB</i> | <i>cyl</i> | <i>bca</i> | <i>rib</i> | <i>cfb</i> | <i>hylB</i> | <i>spb1</i> | <i>cspA</i> | <i>bac</i> | <i>pbp1A/ponA</i> |
| Serotype Ia | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Serotype III | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |

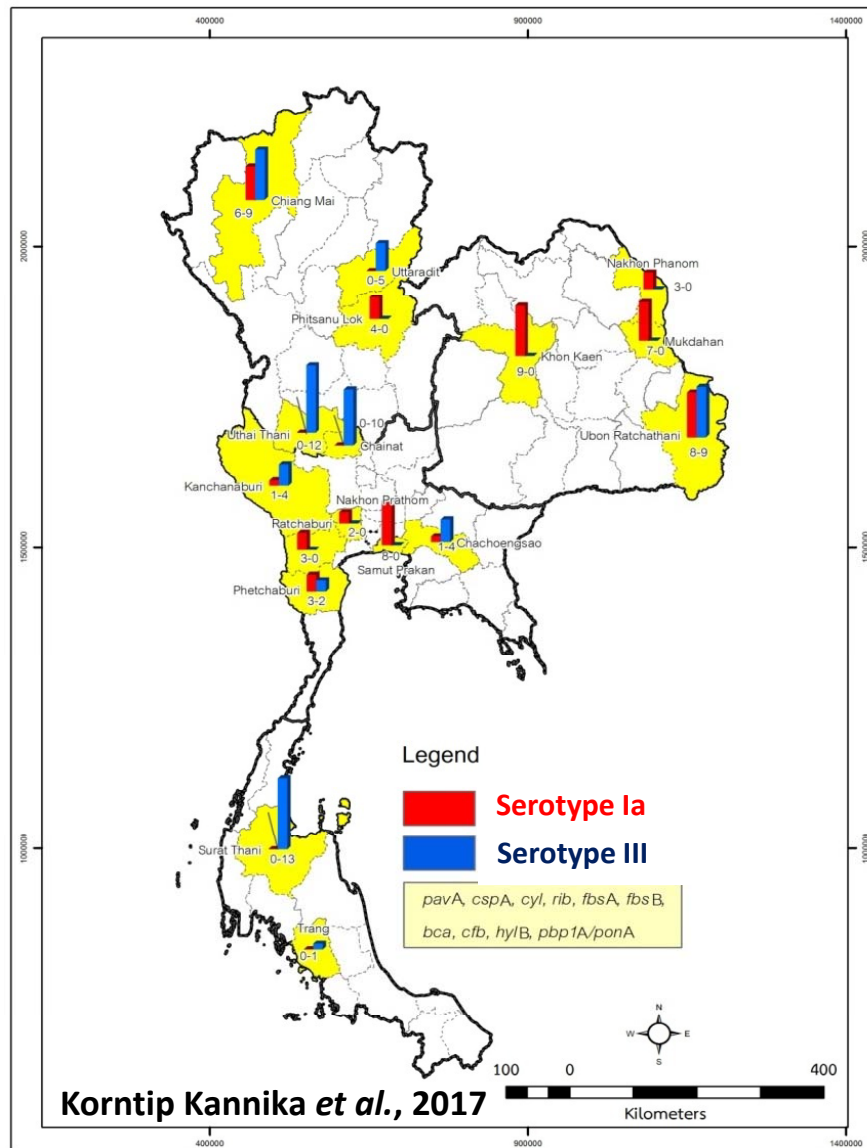
Serotype Ia: 11/14 virulence genes

fbsA, fbsB, pavA, cyl, cfb, hylB, rib, bca, cspA, pbp1A/ponA and *bac*

Serotype III: 13/14 virulence genes

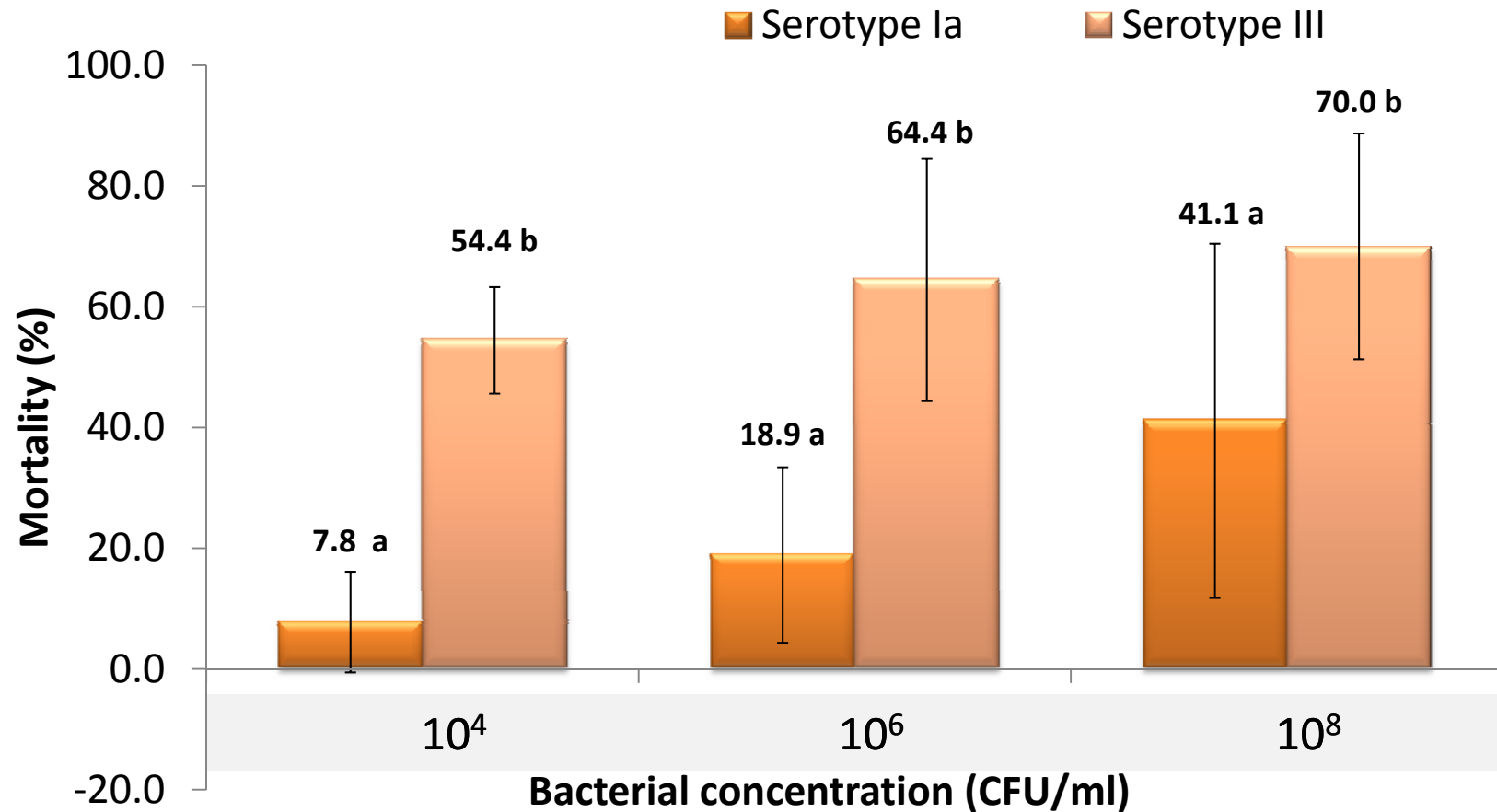
fbsA, fbsB, pavA, cyl, cfb, hylB, rib, bca, cspA, pbp1A/ponA, lmb, scpB and *spb1*

Geographical distribution of *S. agalactiae* isolated from tilapia farms in Thailand



| Province | Fish | Isolates | Serotype | |
|------------------|------------|------------|-----------|-----------|
| | | | Ia | III |
| Khon Kaen | 13 | 9 | 9 | 0 |
| Nakhon Phanom | 26 | 3 | 3 | 0 |
| Mukdahan | 26 | 7 | 7 | 0 |
| Ubon Ratchathani | 69 | 17 | 8 | 9 |
| Chiang Rai | 18 | 0 | 0 | 0 |
| Chiang Mai | 57 | 15 | 6 | 9 |
| Uttaradit | 70 | 5 | 0 | 5 |
| Phitsanulok | 25 | 4 | 4 | 0 |
| Phayao | 11 | 0 | 0 | 0 |
| Lampun | 5 | 0 | 0 | 0 |
| Samut Prakan | 8 | 8 | 8 | 0 |
| Chai Nat | 11 | 10 | 0 | 10 |
| Uthai Thani | 15 | 12 | 0 | 12 |
| Ratchaburi | 3 | 3 | 3 | 0 |
| Nakhon Pathom | 2 | 2 | 2 | 0 |
| Kanchanaburi | 12 | 5 | 1 | 4 |
| Chachoengsao | 5 | 5 | 1 | 4 |
| Phetchaburi | 5 | 5 | 3 | 2 |
| Surat Thani | 15 | 13 | 0 | 13 |
| Trang | 2 | 1 | 0 | 1 |
| Chumphon | 3 | 0 | 0 | 0 |
| Total | 401 | 124 | 55 | 69 |

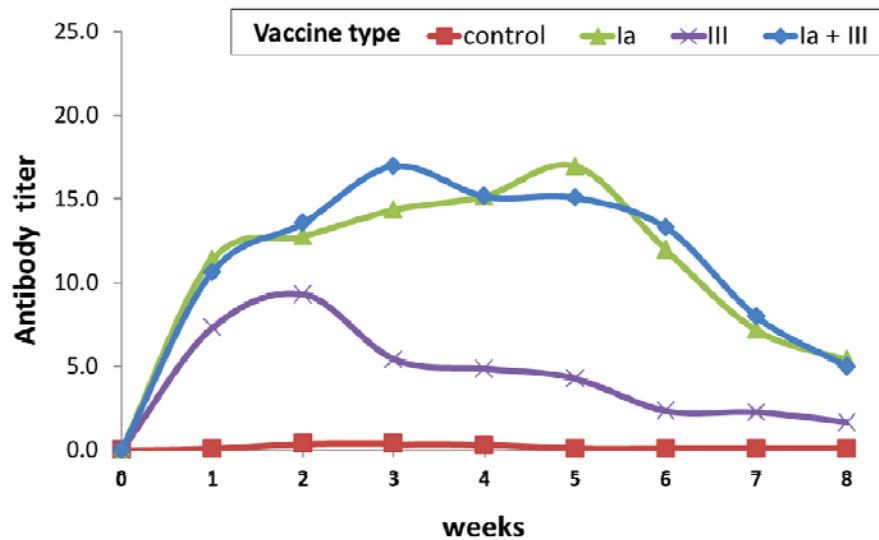
Pathogenicity (degree of virulence) of Serotype Ia and III



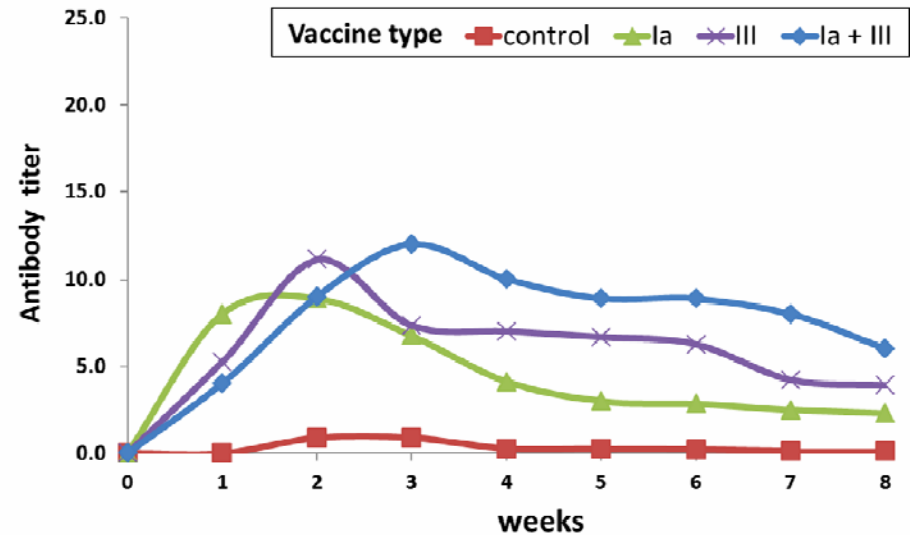
Serotype III is more virulent than serotype Ia

Antibody titer of Nile tilapia injected with Formalin-Killed vaccine

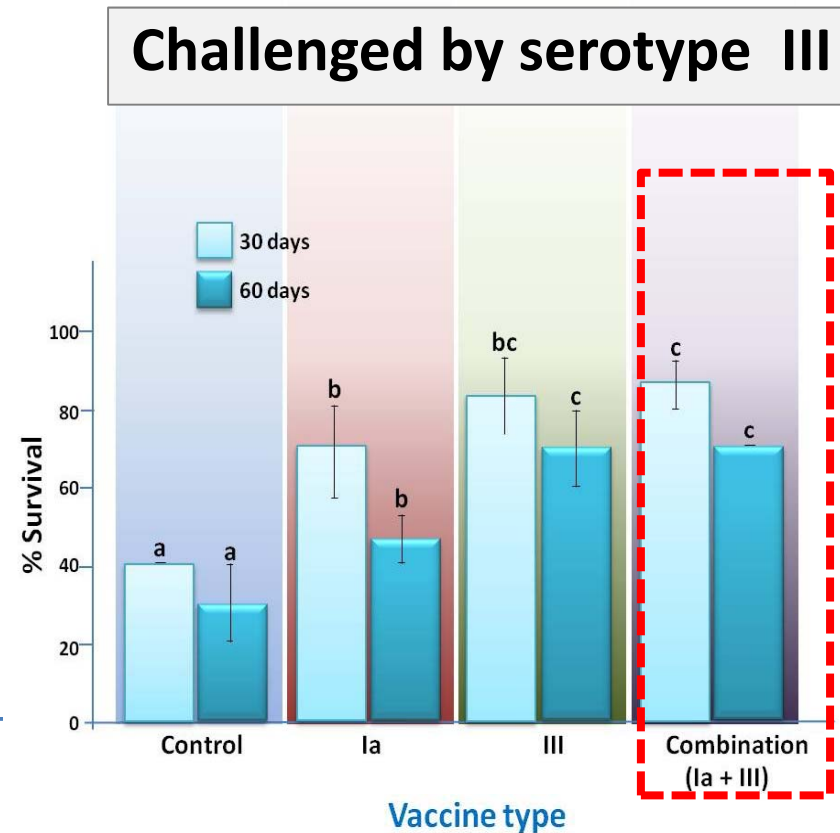
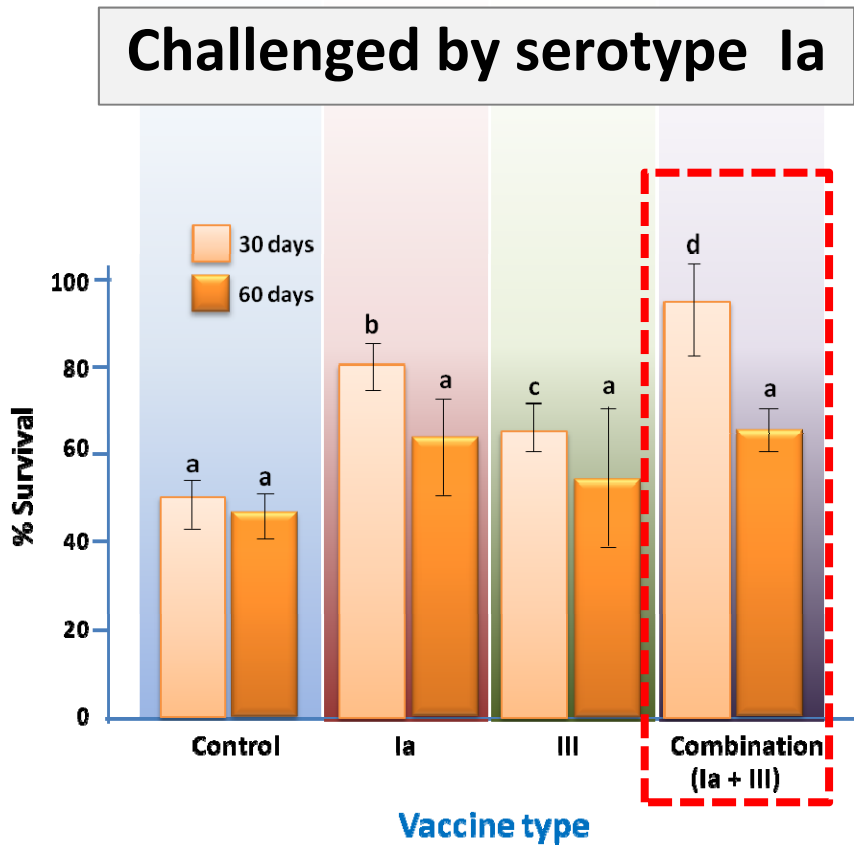
Detected by Ag serotype Ia



Detected by Ag serotype III



Survival rate of vaccinated Nile tilapia by injection method (Challenged with *S. agalactiae* serotype Ia and III)

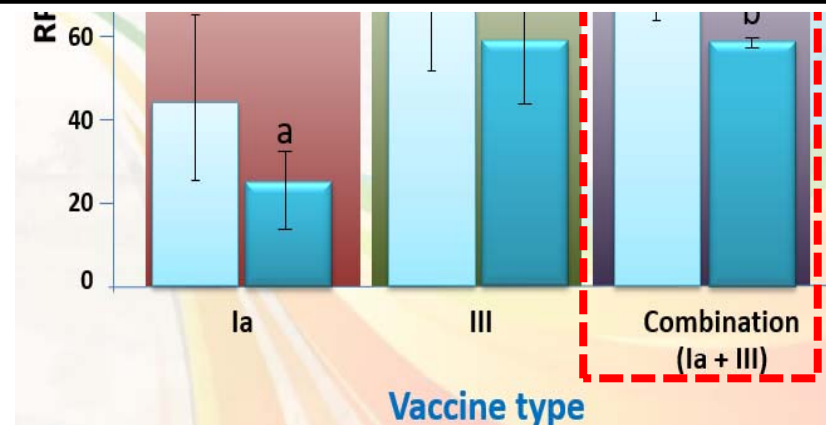
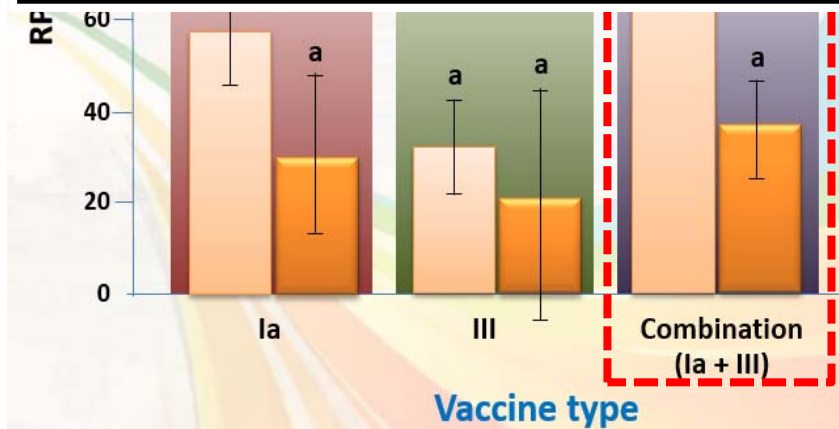


Relative percent survival (RPS) of vaccinated Nile tilapia by injection method (Challenged with serotype Ia and III)

Challenged by serotype Ia

Challenged by serotype III

1. Cross immunity was observed
2. Combination of FKC vaccine (Ia + III) showed higher and longer immune response





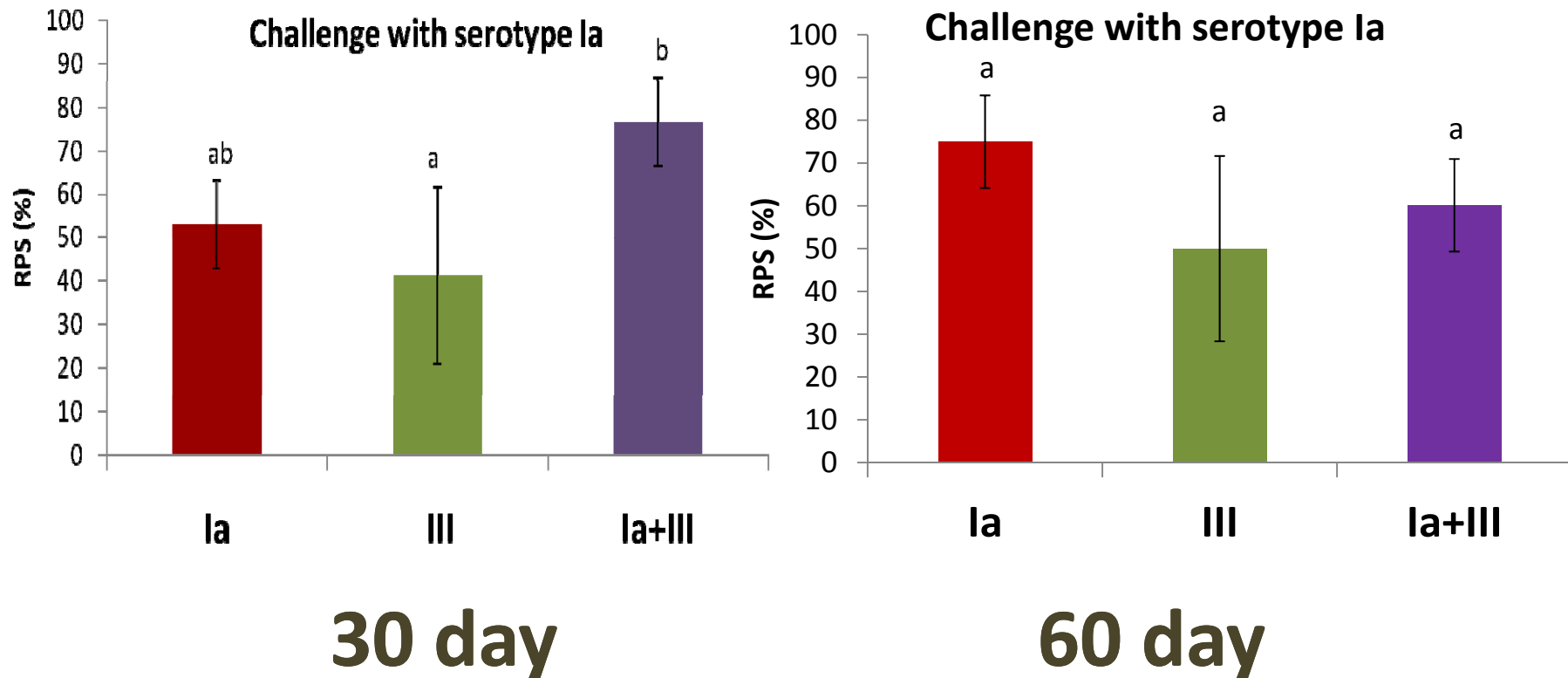
Immune response of Nile tilapia fry to *S. agalactiae* vaccine by immersion method

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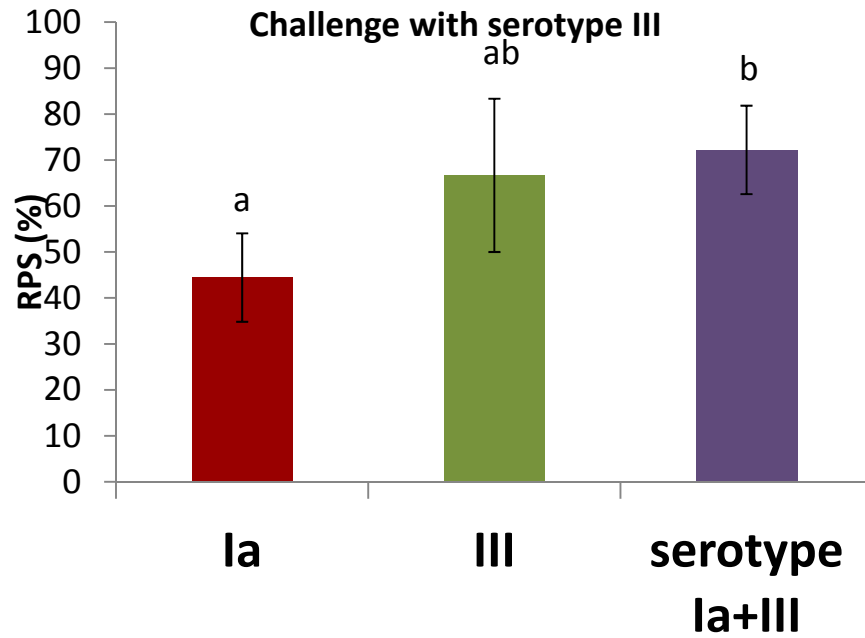
² Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

RPS of Nile tilapia challenged with *S. agalactiae* serotype Ia

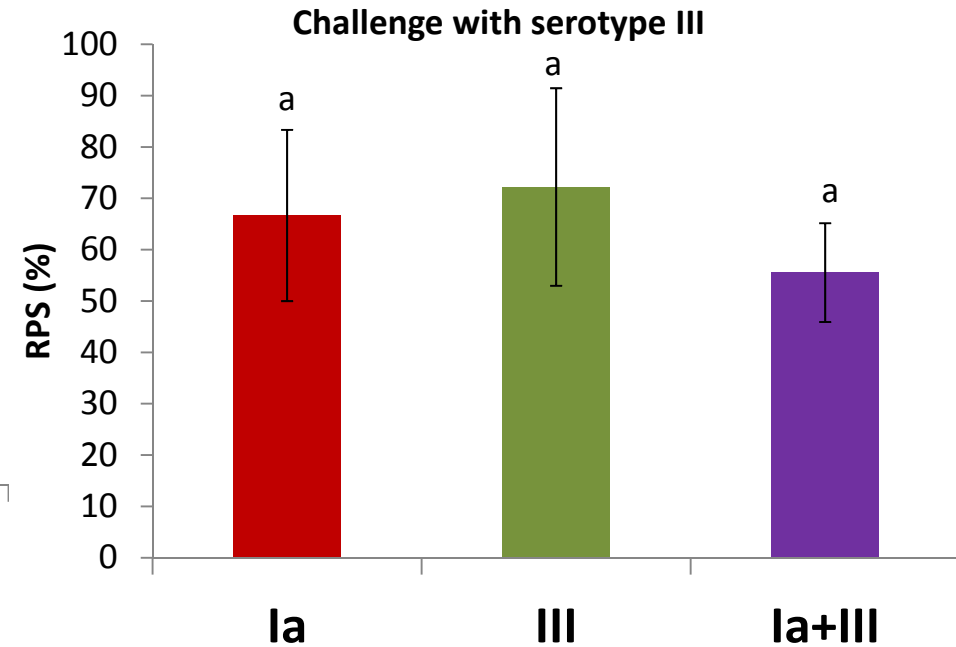


RPS = Relative percent survival

RPS of Nile tilapia challenged with *S. agalactiae* serotype III



30 day



60 day

Vaccine field trials





1

Strep-KU: TYPE 1



MG_1764.MOI

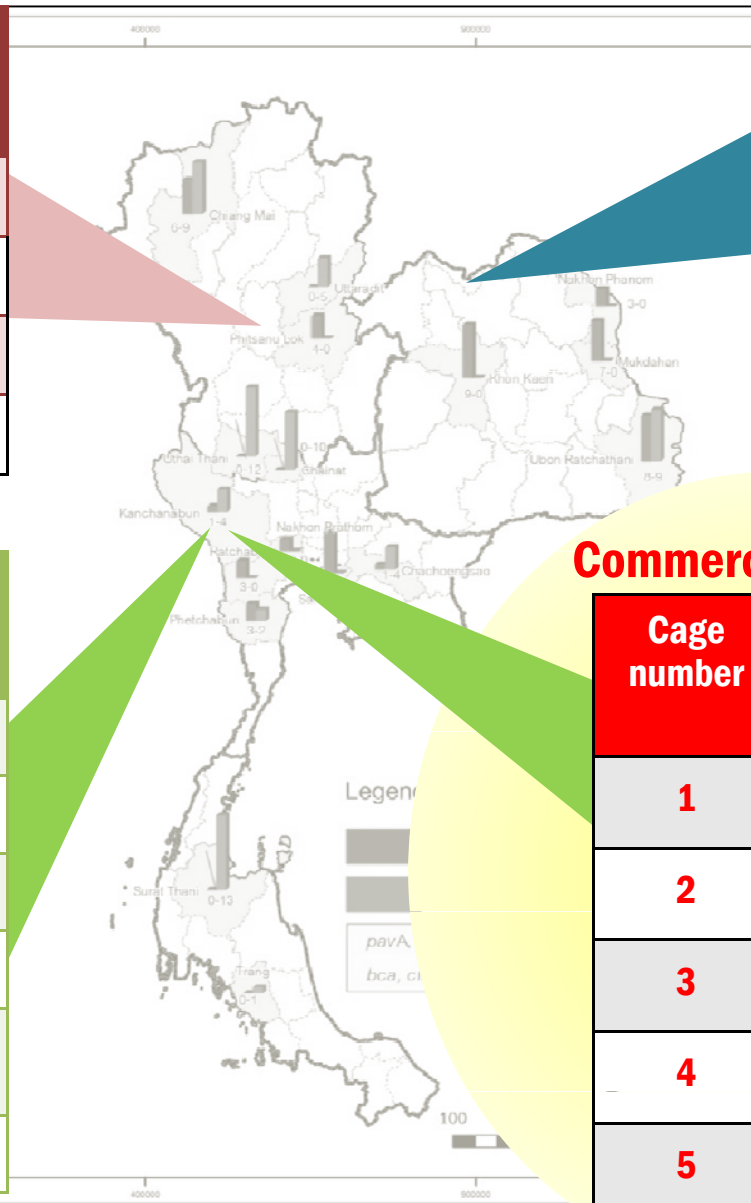




RESULT FROM FIELD STUDY

| Cage number | % Survival (#1) | % Survival (#2) |
|-------------|-----------------|-----------------|
| T1 (R1) | 70.1 | 90.5 |
| (R2) | 75.4 | 90.8 |
| T2 (R1) | 84.5 | 91.7 |
| (R2) | 80.1 | 94.2 |

| Cage number | % Survival (#1) | % Survival (#2) |
|-------------|-----------------|-----------------|
| T1 (R1) | 78.7 | 91.6 |
| (R2) | 42.6 | 80.3 |
| T2 (R1) | 86.1 | 75.9 |
| (R2) | 68.6 | 79 |
| Control | 48.8 | |
| | 57.1 | |



| Cage number | % Survival (#1) | % Survival (#2) |
|-------------|-----------------|-----------------|
| T1 (R1) | 88.4 | 81.2 |
| (R2) | 89.6 | 79.6 |
| T2 (R1) | 96.2 | 82.9 |
| (R2) | 98.0 | 83.7 |

Commercial vaccine

| Cage number | % Survival |
|-------------|------------|
| 1 | 63.6 |
| 2 | 58.2 |
| 3 | 60.0 |
| 4 | 62.8 |
| 5 | 56.5 |



2

Strep-KU: TYPE 2