



Figure S1. Experimental design. 1. *Avibacterium paragallinarum* strains were isolated from clinical cases of infectious coryza. These strains were identified by biochemical tests and PCR and kept frozen until usage. 2. Infection trials were performed to evaluate their capacity to cause clinical signs, horizontal infection as well as cause septicemia and death in experimentally inoculated chickens. 3. The most pathogenic strains were selected to formulate an inactivated vaccine that was tested for its capacity to protect chickens against experimental infection with *Avibacterium paragallinarum* of serogroups A, B, Bvar, and C.

Table S1 – List of *Avibacterium paragallinarum* strains isolated from infectious coryza outbreaks in Peru.

| Strain N° | Province (City) | Chicken type | Year of isolation | Serogoup <sup>1</sup> |
|-----------|-----------------|--------------|-------------------|-----------------------|
| Q1        | Lima            | Breeders     | 1999              | A                     |
| Q2        | Lima            | Laying hens  | 2006              | A                     |
| Q3        | Lima            | Laying hens  | 2012              | A                     |
| Q4        | Lima            | Laying hens  | 2008              | A                     |
| Q5        | Ica             | Laying hens  | 2016              | A                     |
| Q6        | Lima            | Broilers     | 2015              | A                     |
| Q7        | Lima            | Broilers     | 2010              | A/B <sup>2</sup>      |
| Q8        | Lima            | Laying hens  | 2004              | B                     |
| Q9        | Loreto          | Broilers     | 2005              | B                     |
| Q10       | Lima            | Broilers     | 2006              | B                     |
| Q11       | Lima            | Laying hens  | 2007              | B                     |
| Q12       | Lima            | Broilers     | 2009              | B                     |
| Q13       | Lima            | Broilers     | 2010              | B                     |
| Q14       | La Libertad     | Laying hens  | 1998              | B <sup>3</sup>        |
| Q15       | Ica             | Laying hens  | 1999              | B <sup>3</sup>        |
| Q16       | La Libertad     | Laying hens  | 1999              | B <sup>3</sup>        |
| Q17       | Arequipa        | Broilers     | 1999              | B <sup>3</sup>        |
| Q18       | San Martín      | Broilers     | 2013              | C                     |
| Q19       | Ica             | Breeders     | 2007              | C                     |
| Q20       | Lima            | Broilers     | 2008              | C                     |
| Q21       | Lima            | Broilers     | 2008              | C                     |
| Q22       | Lima            | Laying hens  | 2009              | C                     |
| Q23       | La Libertad     | Laying hens  | 2016              | C <sup>4</sup>        |

<sup>1</sup> All strains were serotyped according to the Page scheme [2], modified by Blackall et al. [4]

<sup>2</sup> Serotyping by inhibition of the haemagglutination test, using antisera of serogroups A and B, both of the international reference strains as well as the Argentinean strains could not discriminate between serogroup A or B.

<sup>3</sup> Variants of serogroup B [5].

<sup>4</sup> NAD independent.

Table S2 – Trial 1. Pathogenicity. Chickens were inoculated into the left infra-orbital sinus with 0.2 mL of each strain (n=20). Clinical signs score for all birds were observed on days 2 and 5 post-inoculation. Each bird was given between 0 and 4 points for each facial side. On day 5 post-inoculation, all birds were euthanized and both infra-orbital sinuses were cultured for the presence of *Avibacterium paragallinarum*. The total score for each strain is the sum of the clinical signs scores and the number of positive infra-orbital sinuses. No deaths were registered.

| Strain    | Inoculation Dose (CFU/mL) | Clinical signs scores* |    |    |   | Sum of Clinical Signs | Presence of <i>Av. paragallinarum</i> * |    | Total score |
|-----------|---------------------------|------------------------|----|----|---|-----------------------|---|----|-------------|
|           |                           | Days post-inoculation  |    |    |   |                       | L                                       | R  |             |
|           |                           | 2                      |    | 5  |   |                       |   |    |             |
| Q1(A)     | 3x10 <sup>7</sup>         | 10                     | 3  | 2  | 5 | 20                    | 19                                      | 19 | 58          |
| Q2(A)     | 2x10 <sup>7</sup>         | 32                     | 15 | 0  | 3 | 50                    | 20                                      | 18 | 88          |
| Q3(A)     | 1x10 <sup>8</sup>         | 27                     | 4  | 4  | 1 | 36                    | 20                                      | 18 | 74          |
| Q4(A)     | 6x10 <sup>7</sup>         | 2                      | 0  | 1  | 0 | 3                     | 20                                      | 20 | 43          |
| Q5(A)     | 3x10 <sup>7</sup>         | 4                      | 5  | 0  | 1 | 10                    | 20                                      | 19 | 49          |
| Q6(A)     | 8x10 <sup>7</sup>         | 2                      | 0  | 0  | 0 | 2                     | 20                                      | 20 | 42          |
| Q7(A/B)   | 2x10 <sup>7</sup>         | 28                     | 15 | 0  | 6 | 49                    | 20                                      | 20 | 89          |
| Q8(B)     | 1x10 <sup>7</sup>         | 34                     | 4  | 14 | 1 | 53                    | 20                                      | 20 | 93          |
| Q9(B)     | 3x10 <sup>7</sup>         | 28                     | 1  | 4  | 0 | 33                    | 17                                      | 20 | 70          |
| Q10(B)    | 2x10 <sup>7</sup>         | 39                     | 3  | 11 | 6 | 59                    | 18                                      | 19 | 96          |
| Q11(B)    | 9x10 <sup>6</sup>         | 30                     | 0  | 10 | 1 | 41                    | 18                                      | 20 | 79          |
| Q12(B)    | 1x10 <sup>7</sup>         | 35                     | 0  | 7  | 1 | 43                    | 20                                      | 20 | 83          |
| Q13(B)    | 7x10 <sup>6</sup>         | 45                     | 11 | 8  | 5 | 69                    | 20                                      | 20 | 109         |
| Q14(Bvar) | 2x10 <sup>7</sup>         | 46                     | 10 | 5  | 0 | 61                    | 19                                      | 20 | 100         |
| Q15(Bvar) | 1x10 <sup>7</sup>         | 38                     | 14 | 12 | 3 | 67                    | 20                                      | 20 | 107         |
| Q16(Bvar) | 1x10 <sup>7</sup>         | 41                     | 15 | 5  | 0 | 61                    | 19                                      | 20 | 100         |
| Q17(Bvar) | 5x10 <sup>7</sup>         | 42                     | 12 | 8  | 6 | 68                    | 20                                      | 20 | 108         |
| Q18(C)    | 1x10 <sup>8</sup>         | 55                     | 2  | 23 | 3 | 83                    | 20                                      | 17 | 120         |
| Q19(C)    | 4x10 <sup>7</sup>         | 21                     | 1  | 9  | 5 | 36                    | 20                                      | 20 | 76          |
| Q20(C)    | 1x10 <sup>8</sup>         | 42                     | 2  | 13 | 1 | 58                    | 20                                      | 19 | 97          |
| Q21(C)    | 1x10 <sup>8</sup>         | 67                     | 3  | 20 | 6 | 96                    | 18                                      | 18 | 132         |
| Q22(C)    | 6x10 <sup>7</sup>         | 47                     | 2  | 10 | 1 | 60                    | 20                                      | 20 | 100         |
| Q23(C)    | 9x10 <sup>7</sup>         | 26                     | 3  | 2  | 0 | 31                    | 20                                      | 19 | 70          |
| Q24(C)    | 1x10 <sup>7</sup>         | 44                     | 3  | 7  | 5 | 59                    | 20                                      | 19 | 98          |

\* Left (L) or Right (R) infraorbital sinus.

CFU – Colony Forming Units

Table S3 – Trial 2. Horizontal infection. Part 1 – Serogroups A and B. Chickens were inoculated into the left infra-orbital sinus with 0.2 mL of each strain (n=8) and were housed in the same isolator with another 12 birds that were not inoculated. Clinical signs score for all birds were observed on days 2, 5, and 7 post-inoculation. Each bird was given between 0 and 4 points for each facial side. On day 7 post-inoculation, all birds were euthanized and both infra-orbital sinuses were cultured for the presence of *Avibacterium paragallinarum*. The total score for each strain is the sum of the clinical signs scores and the number of positive infra-orbital sinuses.

| Strain<br>(Serogroup)/          |    | Clinical Signs Score<br>(Day post-inoculation) |    |   |    |   |    |   |       | Presence of <i>Av.</i><br><i>paragallinarum</i> |    | Total<br>Score |
|---------------------------------|----|--|----|---|----|---|----|---|-------|---|----|----------------|
| Inoculation<br>Dose<br>(CFU/mL) | n= | Inoculation                                    | 2  |   | 5  |   | 7  |   | Total | L   | R  |                |
|                                 |    |  | L  | R | L  | R | L  | R |       |   |    |                |
| Q1 (A)                          | 8  | Yes  | 17 | 0 | 3  | 3 | 5  | 2 | 30    | 8   | 8  | 59             |
| 1,6x10 <sup>7</sup>             | 12 | No   | 0  | 0 | 9  | 7 | 7  | 6 | 29    | 10  | 10 |                |
| Q2 (A)                          | 8  | Yes  | 29 | 6 | 13 | 5 | 10 | 3 | 66    | 6   | 6  | 87             |
| 4,2x10 <sup>7</sup>             | 12 | No   | 0  | 0 | 8  | 3 | 5  | 5 | 21    | 8   | 8  |                |
| Q3 (A)                          | 8  | Yes  | 27 | 0 | 11 | 2 | 9  | 1 | 50    | 8   | 8  | 75             |
| 5x10 <sup>7</sup>               | 12 | No   | 0  | 0 | 13 | 4 | 5  | 3 | 25    | 8   | 8  |                |
| Q4 (A)                          | 8  | Yes  | 24 | 0 | 9  | 4 | 12 | 3 | 52    | 8   | 8  | 74             |
| 6,7x10 <sup>7</sup>             | 12 | No   | 0  | 0 | 7  | 5 | 7  | 3 | 22    | 10  | 10 |                |
| Q5 (A)                          | 8  | Yes  | 16 | 0 | 7  | 5 | 4  | 1 | 33    | 6   | 6  | 53             |
| 1,6x10 <sup>8</sup>             | 12 | No   | 2  | 1 | 6  | 5 | 5  | 1 | 20    | 11  | 10 |                |
| Q6 (A)                          | 8  | Yes  | 16 | 0 | 4  | 0 | 8  | 1 | 29    | 6   | 6  | 39             |
| 8x10 <sup>8</sup>               | 12 | No   | 0  | 0 | 5  | 2 | 3  | 0 | 10    | 10  | 6  |                |
| Q7 (A/B)                        | 8  | Yes  | 25 | 0 | 7  | 1 | 6  | 0 | 39    | 6   | 6  | 68             |
| 5x10 <sup>7</sup>               | 12 | No   | 0  | 0 | 15 | 4 | 8  | 2 | 29    | 10  | 9  |                |
| Q8 (B)                          | 8  | Yes  | 7  | 1 | 4  | 0 | 3  | 0 | 15    | 5   | 5  | 17             |
| 2,7x10 <sup>8</sup>             | 12 | No   | 1  | 0 | 1  | 0 | 0  | 0 | 2     | 2   | 2  |                |
| Q9 (B)                          | 8  | Yes  | 12 | 1 | 6  | 2 | 3  | 1 | 25    | 6   | 6  | 36             |
| 4x10 <sup>8</sup>               | 12 | No   | 0  | 0 | 3  | 1 | 5  | 2 | 11    | 7   | 7  |                |
| Q10 (B)                         | 8  | Yes  | 17 | 1 | 11 | 2 | 6  | 3 | 40    | 5   | 5  | 45             |
| 5x10 <sup>8</sup>               | 12 | No   | 1  | 0 | 2  | 1 | 1  | 0 | 5     | 6   | 5  |                |
| Q11 (B)                         | 8  | Yes  | 13 | 4 | 5  | 1 | 0  | 2 | 25    | 5   | 5  | 41             |
| 1,2x10 <sup>8</sup>             | 12 | No   | 2  | 2 | 2  | 4 | 4  | 2 | 16    | 7   | 6  |                |
| Q12 (B)                         | 8  | Yes  | 9  | 4 | 1  | 0 | 0  | 1 | 15    | 3   | 3  | 19             |
| 2,5x10 <sup>8</sup>             | 12 | No   | 1  | 1 | 1  | 1 | 0  | 0 | 4     | 2   | 0  |                |
| Q13 (B)                         | 8  | Yes  | 26 | 9 | 9  | 4 | 3  | 0 | 51    | 8   | 8  | 59             |
| 2x10 <sup>8</sup>               | 12 | No   | 2  | 0 | 2  | 0 | 3  | 1 | 8     | 9   | 9  |                |

\* Left (L) or Right (R) infraorbital sinus.

CFU – Colony Forming Units

Table S4 – Trial 2. Horizontal infection. Part 2 – Serogroups Bvar and C. Chickens were inoculated into the left infra-orbital sinus with 0.2 mL of each strain (n=8) and were housed in the same isolator with another 12 birds that were not inoculated. Clinical signs score for all birds were observed on days 2, 5, and 7 post-inoculation. Each bird was given between 0 and 4 points for each facial side. On day 7 post-inoculation, all birds were euthanized and both infra-orbital sinuses were cultured for the presence of *Avibacterium*

*paragallinarum*. The total score for each strain is the sum of the clinical signs scores and the number of positive infra-orbital sinuses.

| Strain<br>(Serogroup)/<br>Inoculation<br>Dose<br>(CFU/mL) | n= | Inoculation | Clinical Signs Score<br>(Day post-inoculation) |   |    |    |   |   | Total | Presence of <i>Av.<br/>paragallinarum</i> |    | Total<br>Score |
|---|----|-------------|--|---|----|----|---|---|-------|---|----|----------------|
|   |    |             | 2  |   | 5  |    | 7 |   |       | L   | R  |                |
|   |    |             | L  | R | L  | R  | L | R |       |   |    |                |
| Q14 (Bvar)  | 8  | Yes         | 19   | 0 | 2  | 0  | 0 | 0 | 21    | 6   | 7  | 42             |
| 1,9x10 <sup>8</sup>                                       | 12 | No          | 1  | 0 | 7  | 8  | 3 | 2 | 21    | 12  | 12 |                |
| Q15 (Bvar)  | 8  | Yes         | 17   | 2 | 7  | 4  | 4 | 4 | 38    | 7   | 7  | 66             |
| 2,4x10 <sup>8</sup>                                       | 12 | No          | 1  | 0 | 7  | 8  | 5 | 7 | 28    | 12  | 9  |                |
| Q16 (Bvar)  | 8  | Yes         | 25   | 1 | 10 | 2  | 5 | 0 | 43    | 7   | 7  | 75             |
| 4,2x10 <sup>8</sup>                                       | 12 | No          | 0  | 0 | 10 | 10 | 6 | 6 | 32    | 12  | 12 |                |
| Q17 (Bvar)  | 8  | Yes         | 25   | 0 | 9  | 2  | 2 | 0 | 38    | 6   | 7  | 57             |
| 1,4x10 <sup>8</sup>                                       | 12 | No          | 1  | 0 | 4  | 9  | 3 | 2 | 19    | 10  | 9  |                |
| Q18 (C)   | 8  | Yes         | 16   | 0 | 6  | 0  | 6 | 0 | 28    | 8   | 8  | 29             |
| 1,1x10 <sup>8</sup>                                       | 12 | No          | 0  | 1 | 0  | 0  | 0 | 0 | 1     | 10  | 10 |                |
| Q19 (C)   | 8  | Yes         | 12   | 0 | 3  | 0  | 1 | 1 | 17    | 8   | 8  | 18             |
| 1,2x10 <sup>8</sup>                                       | 12 | No          | 0  | 0 | 1  | 0  | 0 | 0 | 1     | 10  | 9  |                |
| Q20 (C)   | 8  | Yes         | 8  | 0 | 3  | 0  | 2 | 0 | 13    | 3   | 3  | 13             |
| 3,5x10 <sup>8</sup>                                       | 12 | No          | 0  | 0 | 0  | 0  | 0 | 0 | 0     | 6   | 5  |                |
| Q21 (C)   | 8  | Yes         | 11   | 0 | 2  | 0  | 3 | 0 | 16    | 7   | 6  | 21             |
| 1,1x10 <sup>8</sup>                                       | 12 | No          | 1  | 0 | 3  | 1  | 0 | 0 | 5     | 11  | 11 |                |
| Q22 (C)   | 8  | Yes         | 7  | 0 | 3  | 1  | 0 | 0 | 11    | 5   | 5  | 11             |
| 2x10 <sup>8</sup>   | 12 | No          | 0  | 0 | 0  | 0  | 0 | 0 | 0     | 5   | 5  |                |
| Q23 (C)   | 8  | Yes         | 27   | 0 | 8  | 3  | 3 | 1 | 42    | 7   | 7  | 64             |
| 2,2x10 <sup>8</sup>                                       | 12 | No          | 0  | 0 | 2  | 8  | 4 | 8 | 22    | 12  | 11 |                |
| Q24 (C)   | 8  | Yes         | 13   | 0 | 7  | 0  | 3 | 0 | 23    | 6   | 6  | 23             |
| 2,2x10 <sup>8</sup>                                       | 12 | No          | 0  | 0 | 0  | 0  | 0 | 0 | 0     | 3   | 3  |                |

\* Left (L) or Right (R) infraorbital sinus.

CFU – Colony Forming Units

Table S5 - Trial 3. Septicaemia and death. Chickens were inoculated intraperitoneally with 0.5 mL of each strain (n=20). Mortality was registered until day 3 post-inoculation when all surviving birds were euthanized. Livers from dead or euthanized birds were cultured for the presence of *Avibacterium paragallinarum*.

| Serogroup | Strain | Dose CFU/mL         | Dead Birds*     | Positive Livers* |
|-----------|--------|---------------------|-----------------|------------------|
| A         | Q02    | 1.7x10 <sup>8</sup> | 7               | 18 <sup>a</sup>  |
|           | Q03    | 7.8x10 <sup>7</sup> | 2               | 8 <sup>b</sup>   |
|           | Q07    | 3.5x10 <sup>7</sup> | 4               | 18 <sup>a</sup>  |
| B         | Q10    | 5.9x10 <sup>8</sup> | 0               | 2                |
|           | Q13    | 2.3x10 <sup>8</sup> | 0               | 3                |
| Bvar      | Q14    | 2.3x10 <sup>8</sup> | 3 <sup>ab</sup> | 6 <sup>a</sup>   |
|           | Q15    | 1.2x10 <sup>8</sup> | 1 <sup>b</sup>  | 1 <sup>b</sup>   |
|           | Q16    | 2.1x10 <sup>8</sup> | 1 <sup>b</sup>  | 7 <sup>a</sup>   |
|           | Q17    | 2.8x10 <sup>8</sup> | 6 <sup>a</sup>  | 11 <sup>a</sup>  |
| C         | Q18    | 2.1x10 <sup>7</sup> | 4 <sup>ab</sup> | 18 <sup>a</sup>  |

|     |                     |                |                 |
|-----|---------------------|----------------|-----------------|
| Q21 | 6.3x10 <sup>7</sup> | 5 <sup>a</sup> | 12 <sup>a</sup> |
| Q24 | 7.5x10 <sup>7</sup> | 0 <sup>b</sup> | 4 <sup>b</sup>  |

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\* For each serogroup, rates in the same column with different superscripts are significantly different by the chi-square test (P < 0.05)