CHICKEN EMBRYO LETHALITY ASSAY FOR DETERMINING THE VIRULENCE OF *ENTEROCCUS FAECALIS*

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Ph.D. Genetic approaches to reduce the vulnerability of amyloidosis in laying hens.

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INTRODUCTION

- **AA Amilodosis** = Amiloid Artropathy in chickens:
  - Affects ≈ 20 – 30 % of all European chicken flocks (Landman *et al.,* 1998).

IBERTEC

Landman *et al*. (1999)

- ***Enterococcus faecalis:***
  - Commensal bacterium inhabiting the gastrointestinal tract.
  - Resistance
    - Intrinsic → antimicrobial agents (i.e. vancomycin).
    - Acquired → virulence factors (genes).

INTRODUCTION

- **Embryo Lethality Assay (ELA).**

  Determine the degree of virulence of bacteria:

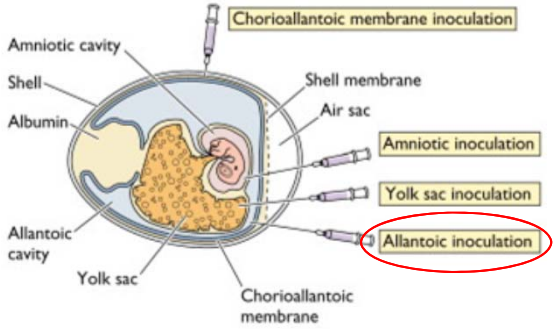
  - *Escherichia coli.*
  - *Yersinia enterocolitica.*
  - *Campylobacter jejuni.*
  - *Enterococcus cecorum.*
  - *Francisella tularensis*.
  - (...)

OBJECTIVE

- **Evaluate the ELA in avian *E. faecalis* strains:**
  - → Pathogenic avian strain K923/96 ≈ (AA) (Petersen *et al.,* 2009).
  - → It will be used as reference strain in further analysis.

MATERIAL & METHODS

- **Embryo Lethality Assay (ELA):**
  - Inoculate embryo chickens
    - First quality hatching eggs.
    - 10-day old embryos.
    - Allantoic cavity.
    - Inoculation volume: 0.2 ml/egg.
  - Embryonic mortality → Candling daily over a period of 7 days.
  - Evaluation of the macroscopic lesions.
  - Re-isolation of *E. faecalis p.i.*.



MATERIAL & METHODS

➢ Overall scope:

3,443 white eggs

Trial	I	II	III	IV	Viable count (cfu/ml)
Avian *E. faecalis* strain	K923/96	K923/96	K923/96	K923/96	-
*** No. eggs/control**	60	57	108	148	-
No. eggs/dose	100	290	300	300	-
**** Infectious dose (cfu/ml)**	2,500	-	-	-	> 1,000
	250	-	-	-	> 250
	25	25	25	25	85 - 258
	-	5	5	5	15 - 60
	2.5	2.5	2.5	2.5	10 – 35

Inoculum verification

*Control group → Sterile phosphate-buffered saline.

**Rudolph (2004) → The number of counted colonies may vary between 500 and 5,000 cfu/ml.

MATERIAL & METHODS

- **Statistical Analysis (SAS/STAT 9.4):**
  - Using flowing generalised linear model with SAS GLIMMIX Proc.:

$$\text{Logit}\ (\pi_{rs}) = \eta_{rs} = \log\ (\pi_{rs}/1-\pi_{rs}) = \varphi + \alpha_r + \beta\ (\alpha)_{rs}$$

η_{rs} = Linear predictor.
π_{rs} = Probability to survive.
φ = Overall mean effect.
α_r = Fixed effect of concentration level.
β_s = Random effect of trial.

MATERIAL & METHODS

- **Statistical Analysis (SAS/STAT 9.4):**
  - Generalised linear model:

$$\text{Logit}\ (\pi_{rs}) = \eta_{rs} = \log\ (\pi_{rs}/1-\pi_{rs}) = \varphi + \alpha_r + \beta\ (\alpha\beta)_{rs}$$

→ Estimated parameters on the logit scale were back-transformed using the inverse link function:

$$\pi = \frac{\exp(x\beta)}{1 + \exp(x\beta)}$$

MATERIAL & METHODS

- **Statistical Analysis (SAS/STAT 9.4):**
  - Using survival analysis with SAS LIFETEST Proc.:

    (Kaplan-Meier method)

$$\hat{S}(t) = \prod_{j:t_j \geq t} \left[1 - \frac{d_j}{n_j}\right] ; \; for \; t_1 \leq t \leq t_k$$

$\hat{S}(t)$ = Survival function.
n_j = Individuals at risk.
d_j = Number of individual die at time t_j.

RESULTS & DISCUSSION

➢ Embryonic mortality rate (%):

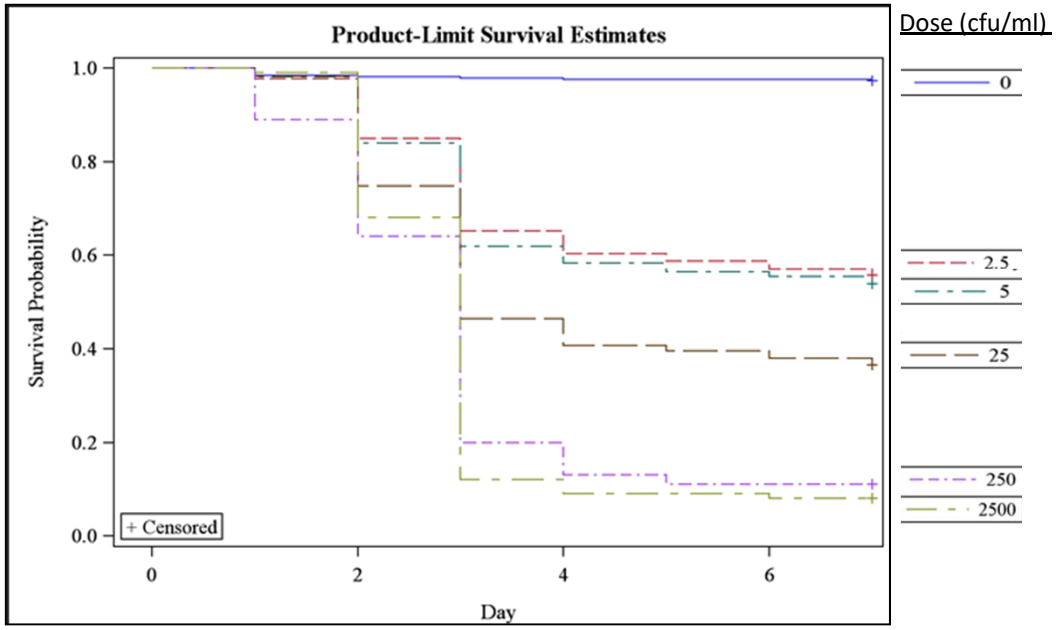
Infectious dose (cfu/ml)*	Trials				Average mortality rate (%)
	I	II	III	IV	
2,500	92.0	-	-	-	92[a]
250	89.0	-	-	-	89[a]
25	71.0	48.0	85.0	56.0	66[ab]
5	-	50.0	53.0	38.0	46[b]
2.5	25.0	45.2	68.0	30.0	41[b]
Control	3.3	0	3.7	2.0	2.6[c]

* By an inoculation volume of 0.2 ml/egg, 500, 50, 5, 1 and 0.5 cfu/egg were inoculated respectively.

RESULTS & DISCUSSION

➢ Embryo survival expressed as Kaplan-Meier curve:

Survival probability over the experimental time period (7 days).



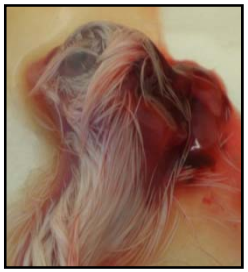
The higher the infectious dose:

- the greater the embryo mortality rate.
- the lower the embryonic survival time.

RESULTS & DISCUSSION

➢ Macroscopic lesions:

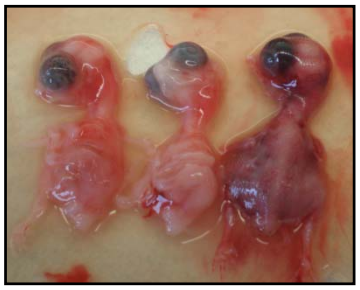
Cranial & skin haemorrhages & plumage loss.



Malformations & underdevelopment.

➢ Re-isolation of *E. faecalis* p.i.:

- Infected embryos: always positive.
- Control group: always negative.



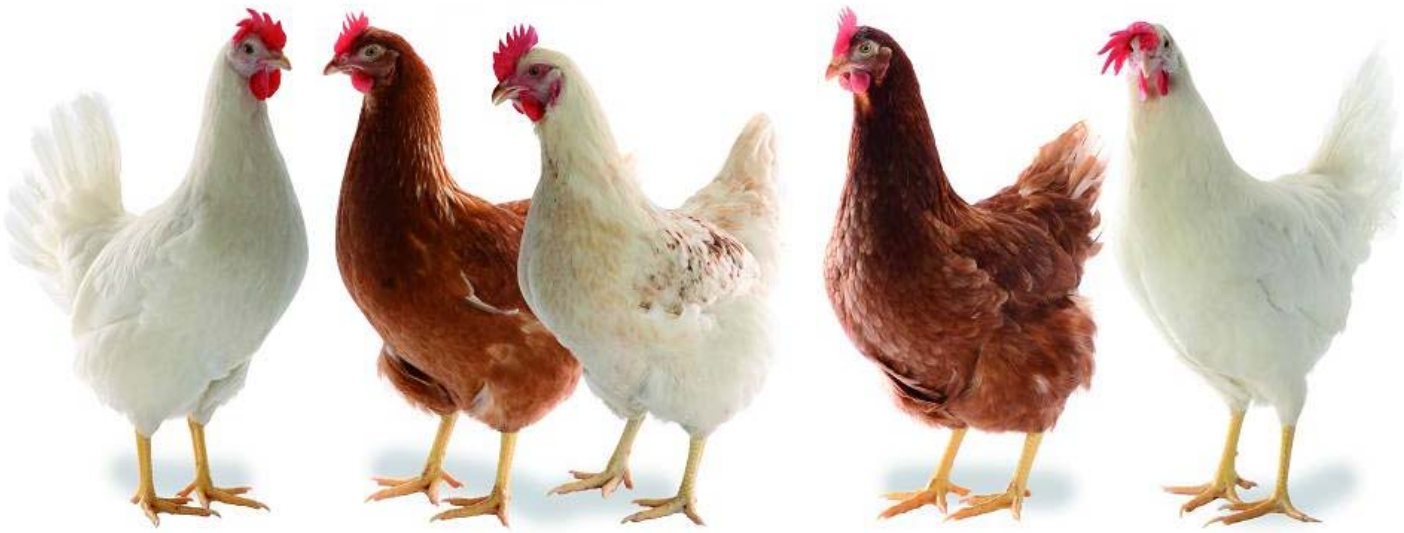
CONCLUSION

- **ELA:**
  - Can be used as an *in vivo* model → Dependent on the dose.
  - Determine the virulence of *E. faecalis* strains.

RESEARCH NEEDS

- More ELAs with different *E. faecalis* strains are required:
  - To confirm its capacity for discriminating strains.

THANK YOU FOR YOUR ATTENTION



QUESTIONS?