

75% protection is too low as criterion for serology

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Outline

- Why 75% protection and how can producers make sure they pass the test
- Standard tests OIE
 - PD₅₀ determination (Europe)
 - PGP test (South-America)
- Statistical analysis
 - Which statistical method should be preferred
 - Spearman Kaerber
 - Probit or logit analysis
 - Combined probit
 - Which experiment provides the highest confidence
- Serological testing has benefits for producer



Why 75% protection

- Based on historical discussions
 - Should be sufficient to block transmission between farms
- Confirmed by recent outbreak analysis
 - Average number of new infections per farm 3 – 4 (R_0)
 - When 75% of the farms are not susceptible one infected farm can only infect 1 or less than 1 other farm → outbreak will fade out



Standard OIE test

■ PD₅₀ experiment

- Vaccinated 3 x 5 cattle with a 1/1, 1/4 and 1/16th dose
- Challenge 3 – 4 weeks later with homologous FMD virus
- 3 PD₅₀ of oil emulsion vaccine correlates with 71% protection <0.65, 0.76>

■ PGP test

- Vaccinate 16 cattle with a full dose
- Challenge 4 or more weeks later with homologous FMD virus
- At least 12 cattle should be protected



Analysis of the results

Probit analysis or Spearman Kaerber

- Based on results of the experiment

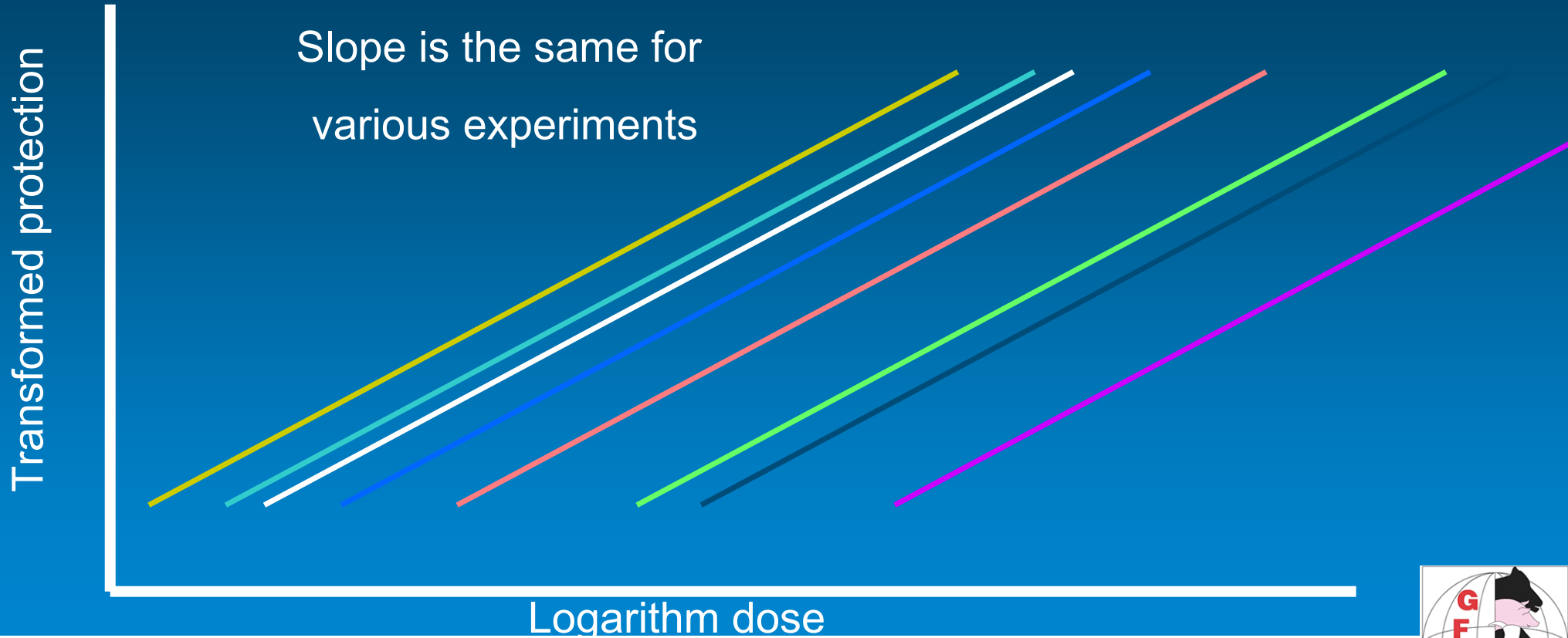
Combined probit

- Results of the experiment (batch 0) added to previous experiments (462 cattle in 22 exp.)

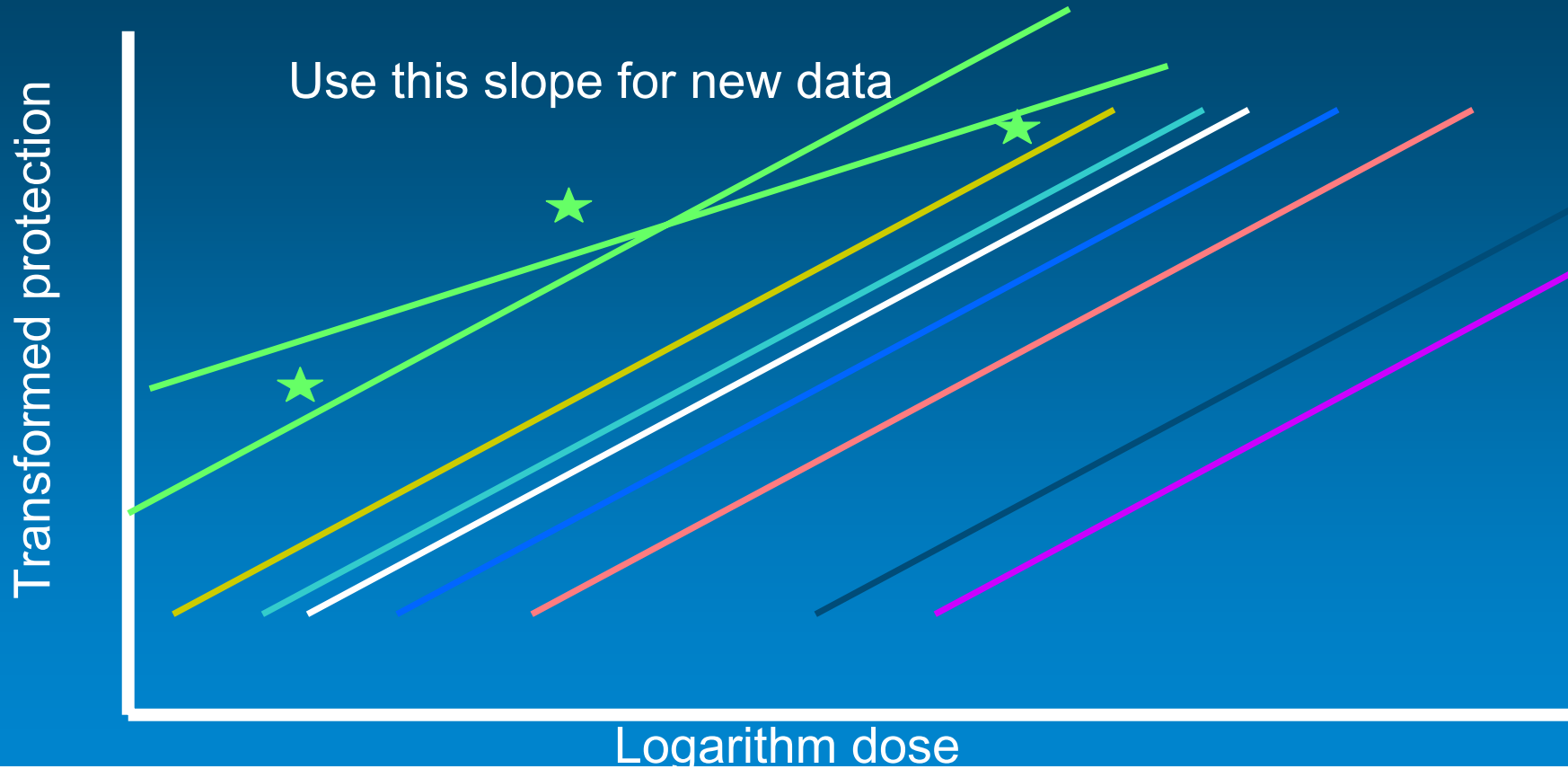
$$\text{Inv.norm}(p) = \beta_0 + \beta_1 \times \log(\text{dosis}) + \beta_2 \times \text{batch}$$



Generalised linear model

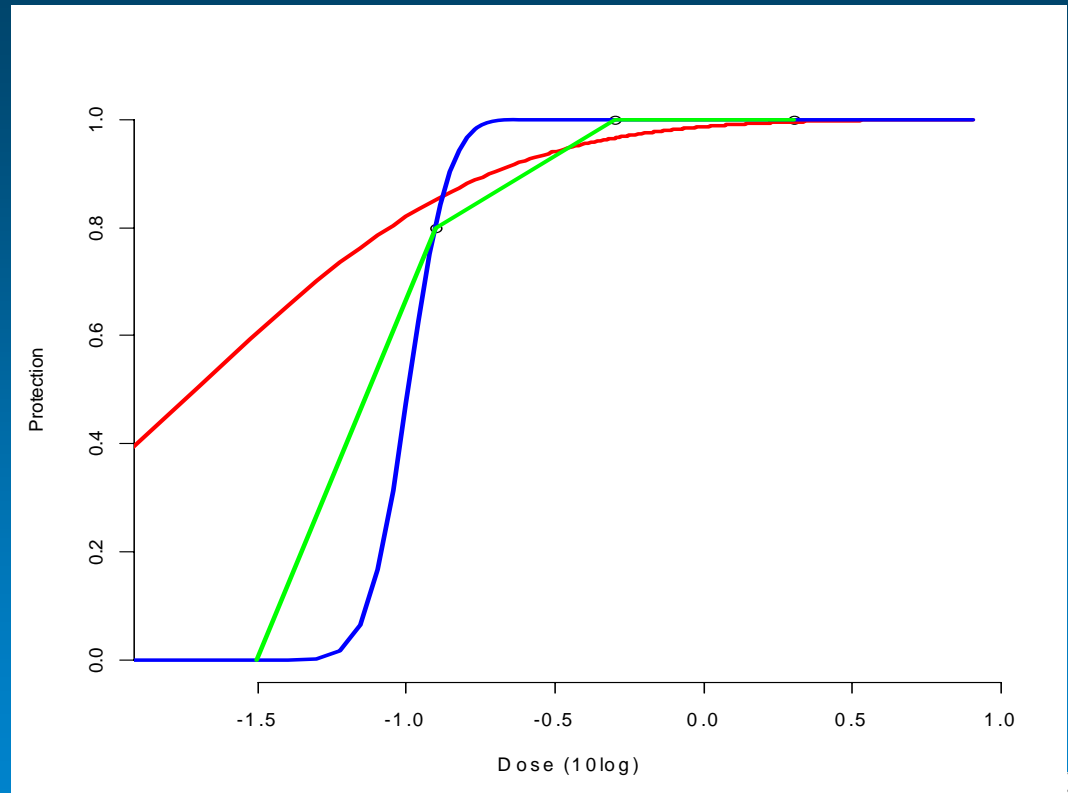


$$\text{Inv.norm}(p) = \beta_0 + \beta_1 \times \log(\text{dosis}) + \beta_2 \times \text{batch}$$



Variation in method of analysis

- Example 5, 5, 4 protected
- SK in green
 - 24.25 PD₅₀/dose
- Probit analysis in blue
 - 19.7 PD₅₀/dose
- Combined probit in red
 - 102 PD₅₀/dose



Design of the PD₅₀ experiment

- Standard 3 x 5 cattle
 - Dose 1/1, 1/4 and 1/16
 - $6^3 = 216$ possible outcomes
- Alternative 5 x 3 cattle
 - Dose 1/1, 1/2, 1/4, 1/8, 1/16
 - $4^5 = 1024$ possible outcomes
- Probability for each outcome can be calculated



For each possible outcome

Potency	probability	outcome	Probit	Comb. probit	SK
15.7	0.002196	5, 1, 2	5.006364	5.038689	4.594793
15.7	0.002159	5, 1, 3	8.211167	7.2575	6.062866
15.7	0.001062	5, 1, 4	30.94765	10.45189	8
15.7	0.000209	5, 1, 5	0	15.25257	10.55606
15.7	0.001582	5, 2, 0	3.799323	3.326107	3.482202
15.7	0.007776	5, 2, 1	4.835712	4.930463	4.594793
15.7	0.015293	5, 2, 2	6.807966	7.181545	6.062866
15.7	0.015037	5, 2, 3	12.0793	10.44558	8
15.7	0.007393	5, 2, 4	55.47092	15.39873	10.55606
15.7	0.001454	5, 2, 5	0	23.42981	13.92881
15.7	0.005508	5, 3, 0	4.211276	4.810428	4.594793
15.7	0.02708	5, 3, 1	6.259174	7.099942	6.062866



Table 1a: Percentage of outcomes that pass the criterion "at least 3 PD50"

		True potency of the vaccine (PD ₅₀ per dose)		
		3	6	15.7
3 groups of 5 cattle	Spearman Kärber	54.6%	89.4%	99.8%
	Probit	52.6%	84.3%	91.4%
	Combined probit	54.6%	89.4%	99.8%
5 groups of 3 cattle	Spearman Kärber	53.6%	89.2%	99.8%
	Probit	49.5%	82.1%	89.6%
	Combined probit	54.6%	89.4%	99.8%

Similar results



Table 1c: Percentage of outcomes that pass the criterion 12 or more protected

	True potency of the vaccine (PD ₅₀ per dose)		
	3	6	15.7
12 out of 16 cattle protected	55.7%	90.3%	99.7%

Based on the calculated probability of protection at a full dose and the binomial distribution

Table 2: Weighted average of the estimated PD_{50} per dose (excluding all animals protected)

		True potency of the vaccine (PD_{50} per dose)		
		3	6	15.7
3 groups of 5 cattle	Spearman Kärber	3.6	6.1	11.6
	Probit	4.0	9.1	22.3
	Combined probit	3.7	7.7	22.1
5 groups of 3 cattle	Spearman Kärber	3.6	5.7	9.9
	Probit	5.9	13.9	45.2
	Combined probit	3.6	7.7	21.9

Relation between serology and PD₅₀/dose

$$\text{logtitre}(XPD_{50}) - \text{logtitre}((aX)PD_{50}) = \frac{\beta_1 \ln(a)}{\beta_{1s}}$$

$\text{logtitre}(XPD_{50})$ = Log antibody titre of a vaccine containing X PD₅₀

β_1 = slope of the relation between vaccine dose and protection

β_{1s} = slope of the relation between antibody titre and protection



Table 4: Percentage outcomes that will pass the criterion equal to 3 PD₅₀ per dose by serology
 (when group average should be above titre found in a 3 PD₅₀ vaccine)

	True potency of the vaccine (PD ₅₀ per dose)		
	3	6	15.7
5 cattle per vaccine	50.0%	84.8%	99.3%
6 cattle per vaccine	50.0%	87.0%	99.6%
7 cattle per vaccine	50.0%	88.8%	99.8%
8 cattle per vaccine	50.0%	90.3%	99.9%
9 cattle per vaccine	50.0%	91.6%	99.9%
10 cattle per vaccine	50.0%	92.7%	100.0%

$$\beta_1 = 0.9531$$

$$\beta_{1s} = 4.0681$$

$$\text{SD serology} = 0.35$$



Conclusions

- Spearman Kärber and combined probit produce similar results when the outcome lies within the test range
- No difference between 3 times 5 or 5 times 3 animals
- PD_{50} experiments, when using combined results, give more information on vaccine potency than a PGP test
- When using serology producers can test their batches with a lower number of animals



Further thoughts

- Producers have to produce better vaccines to have confidence in passing the potency test
 - Replacement of potency test by serology will produce smaller confidence limits, therefore vaccines will more easily pass the test
 - New pass mark should be defined for serological tests (e.g. at least 75% protection with 95% confidence)
- Vaccination coverage in countries often low, so is 75% enough
- Vaccine is tested fresh
 - Goris et al. showed degradation in South-America

