STATISTICAL METHODS FOR THE CALCULATION OF VACCINE PROTECTIVE DOSE 50

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INTRODUCTION

To ensure adequate animal health the supply of pure, safe, effective and potent vaccines is essential. Potency testing is an important component of final product control to guarantee satisfactory levels of protection. International authorities such us the World Animal health organisation (OIE) have developed and published requirements and methods for the potency testing of several vaccines. According to OIE, the potency of vaccines for Newcastle disease (NDV) must be evaluated by testing 3 vaccine doses (dilutions of a full dose) in groups of 20 animals each. Moreover, the OIE requires that a full dose contains 50 PD50 (50% protective dose) and that the lower confidence interval (CI) is not less that 35 PD50 per dose. Following the experimental infection of the vaccinated groups, the PD50 is calculated on the basis of the number of vaccinated and infected chickens that survive using the Reed-Muench and/or the Spearman-Kärber's formula. These methods were compared to probit, logit and cloglog models in order to investigate whether they allow for a more accurate calculation of the PD50 and its confidence interval.

METHODS

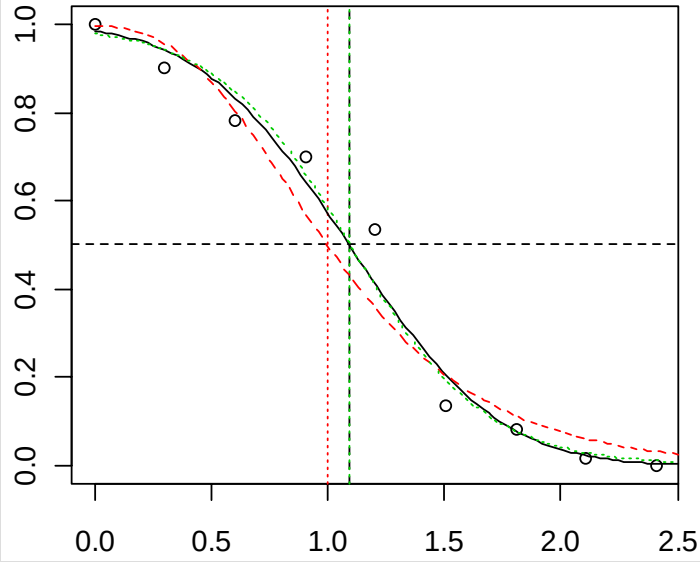
- Number of vaccine dilutions tested: 5 and 9, 4-fold and 2-fold respectively tested in groups of 10, 30 and 60 animals each;
- Evaluation of dose-response relationship and comparison of different methods of calculating PD50:
  - Reed-Muench and the Spearman- Kärber's formula;
  - *maximum likelihood estimation* (MLE) for probit, logit and cloglog models.
- Calculation of confidence interval (CI) asymptotically and with a bootstrap resampling.

The model supposes:

- a binomial distribution of survivors (r) per dose (x);
- the probability of a subject surviving (p) for a given dose is the ratio between r and tested subject numbers (n).

RESULTS

Figure 1: log PD50 calculated by the probit (dark continuous line), cloglog (red dotted line) and logit (green dashed line) dose-response models for 9 and 5 dilutions respectively



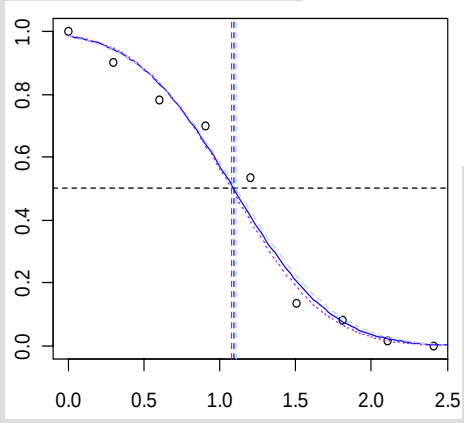


Figure 2: log PD50 calculated by the probit dose-response models for n=60 (blue line), n=30 (light-blue line), n=10 (purple line)

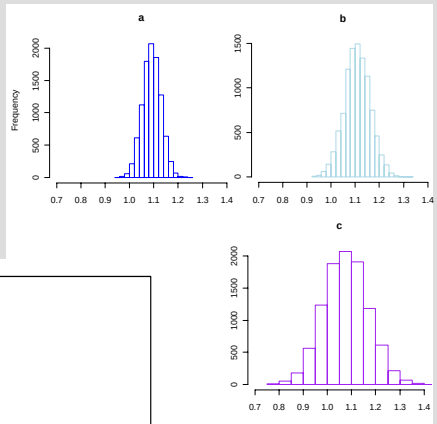


Figure 3: (a) log PD50 bootstrap estimate distribution (10000 resampling) for n=60 ; (b) n=30, (c) n=10

CONCLUSIONS

High numbers of animals and of vaccine doses allow accurate and similar estimates, and very small CI. The approximate normal distribution of estimates gives comparable CI. For a low "n" the log PD50, calculated according to different methods, is nevertheless similar but the CIs are larger. The accuracy of estimate and its CI decrease further if the number of vaccine doses decreases as well. This study demonstrates that it is possible to obtain acceptable results with a small number of animals per dose (i.e. 10), provided that a sufficient number of doses is tested. In order to calculate the lower limit of CI it is important to test a vaccine dose that protects less than 50 %of animals.

N° animals=10

N° dilutions= 9			N° dilutions= 5		
Reed- Muench	log PD_{50} = 1.01		Reed- Muench	log PD_{50} = 0.658	
Spearman- Käbel	log PD50 = 1.08 CI [0.895; 1.265]		Spearman- Käbel	log PD50 = 1.144 CI [0.866; 1.422]	
probit	cloglog	logit	probit	cloglog	logit
Log PD_{50} =1.077	Log PD_{50} =0.983	Log PD_{50} =1.082	Log PD_{50} = 1.141	Log PD_{50} = 1.045	Log PD_{50} = 1.143
Bias= -0.0013	Bias= 0.0045	Bias= 0.0011	Bias= 0.0011	Bias = 0.0097	Bias = 0.0012
IC[a] [0.899; 1.254]	IC[a] [0.800; 1.166]	IC[a] [0.902; 1.262]	IC[a] [0.885 ;1.398]	IC[a] [0.786; 1.303]	IC[a] [0.881; 1.406]
IC[b] [0.900; 1.256]	IC[b] [0.798; 1.159]	IC[b] [0.900; 1.262]	IC[b] [0.886; 1.394]	IC[b] [0.765; 1.305]	IC[b] [0.883; 1.402]
IC[c] [0.900; 1.254]	IC[c] [0.795, 1.154]	IC[c] [0.899; 1.263]	IC[c] [0.890; 1.386]	IC[c] [0.768; 1.286]	IC[c] [0.897; 1.395]
IC[d] [0.899; 1.253]	IC[d] [0.813; 1.172]	IC[d] [0.901; 1.265]	IC[d] [0.897; 1.393]	IC[d] [0.803; 1.321]	IC[d] [0.892; 1.390]

N° animals=60

N° dilutions= 9			N° dilutions= 5		
Reed&Muench	log PD_{50} = 0.9917		Reed&Muench	log PD_{50} = 0.641	
Spearman&Käbel	log PD50 = 1.095 CI [1.021; 1.169]		Spearman&Käbel	log PD50 = 1.144 CI [1.036; 1.252]	
Probit	cloglog	logit	Probit	cloglog	logit
Log PD_{50} =1.092	Log PD_{50}=0.997	Log PD_{50} =1.097	Log PD_{50} =1.1380	Log PD_{50} =1.042	Log PD_{50} =1.143
Bias=-0.0005	Bias=0.0006	Bias=0.0002	Bias =0.0003	Bias=0.0006	Bias = -0.0002
IC[a] [1.018; 1.166]	IC[a] [0.921; 1.074]	IC[a] [1.022; 1.172]	IC[a] [1.033; 1.242]	IC[a] [0.935; 1.148]	IC[a] [1.036; 1.251]
IC[b] [1.018; 1.166]	IC[b] [0.922; 1.072]	IC[b] [1.021; 1.172]	IC[b] [1.034; 1.242]	IC[b] [0.934; 1.148]	IC[b] [1.038; 1.249]
IC[c] [1.019; 1.166]	IC[c] [0.921; 1.072]	IC[c] [1.020; 1.172]	IC[c] [1.033; 1.243]	IC[c] [0.935; 1.146]	IC[c] [1.033; 1.253]
IC[d] [1.018, 1.164]	IC[d] [0.923; 1.074]	IC[d] [1.022; 1.173]	IC[d] [1.033; 1.242]	IC[d] [0.938; 1.149]	IC[d] [1.034; 1.254]

Table 1: log PD50 according to Reed- Muench, the Spearman- Kärber and for each model: the MLE of log PD50, the differences between *MLE and bootstrap estimate (bias)*, the confidence interval asintotical (CIa), the bootstrap interval using the normal approximation (CIb), using the basic bootstrap method (CIc) and using the bootstrap percentile method (CId).