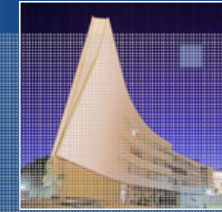


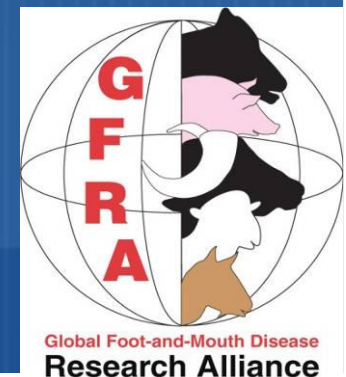
A naive approach to r_1 -value calculation: Variability, friend or Foe?



GFRA Workshop
17-19 April 2002, Hazyview, South Africa

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Warning!



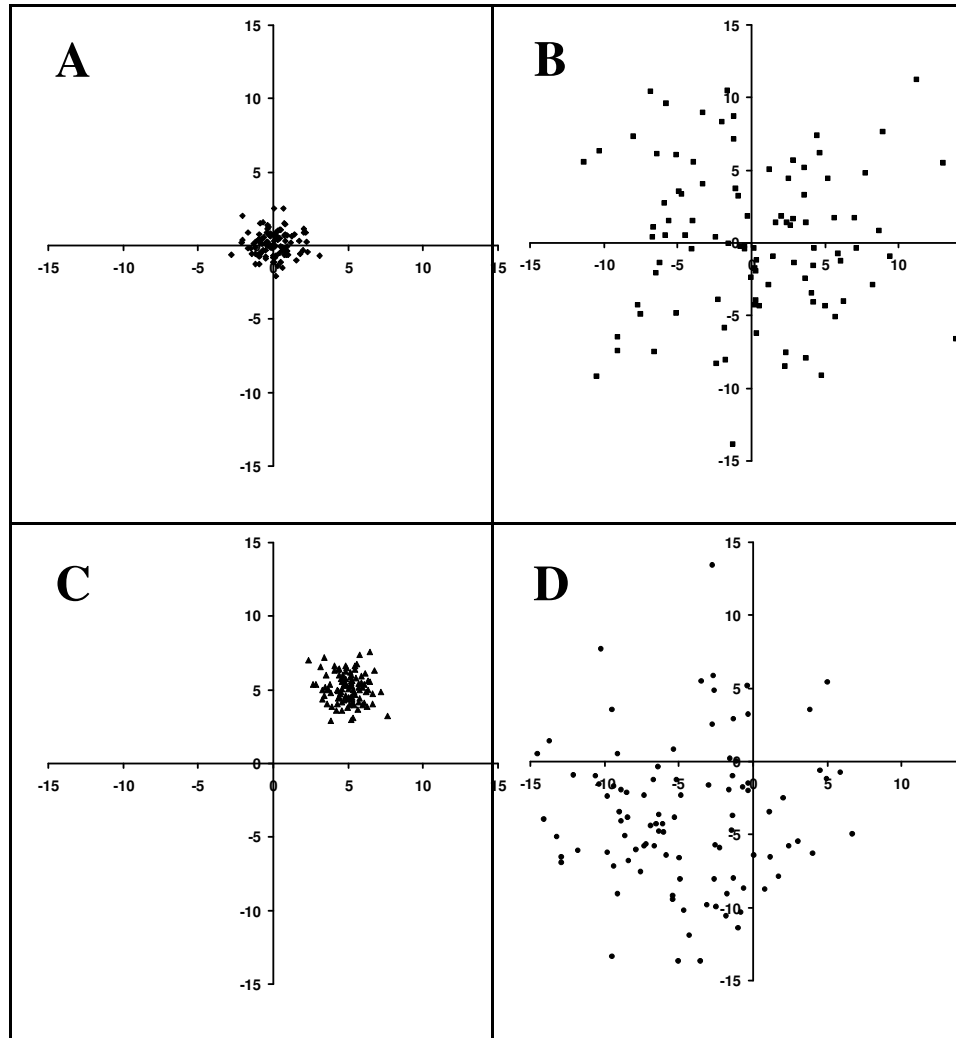
Simulations can be dangerous in the absence biological understanding

Outline

- Introduction to bias, precision and variability
- Study design and FMD titer determination
- Questions
 - What variables contribute to the variability in virus neutralization titers?
 - How much variability should be expected when estimating r_1 -values?
 - Will pooled sera from 5 animals give similar r_1 -values as the average of the 5 individual sera?
 - How many animals are necessary to reliably estimate r_1 -values?
- Conclusions

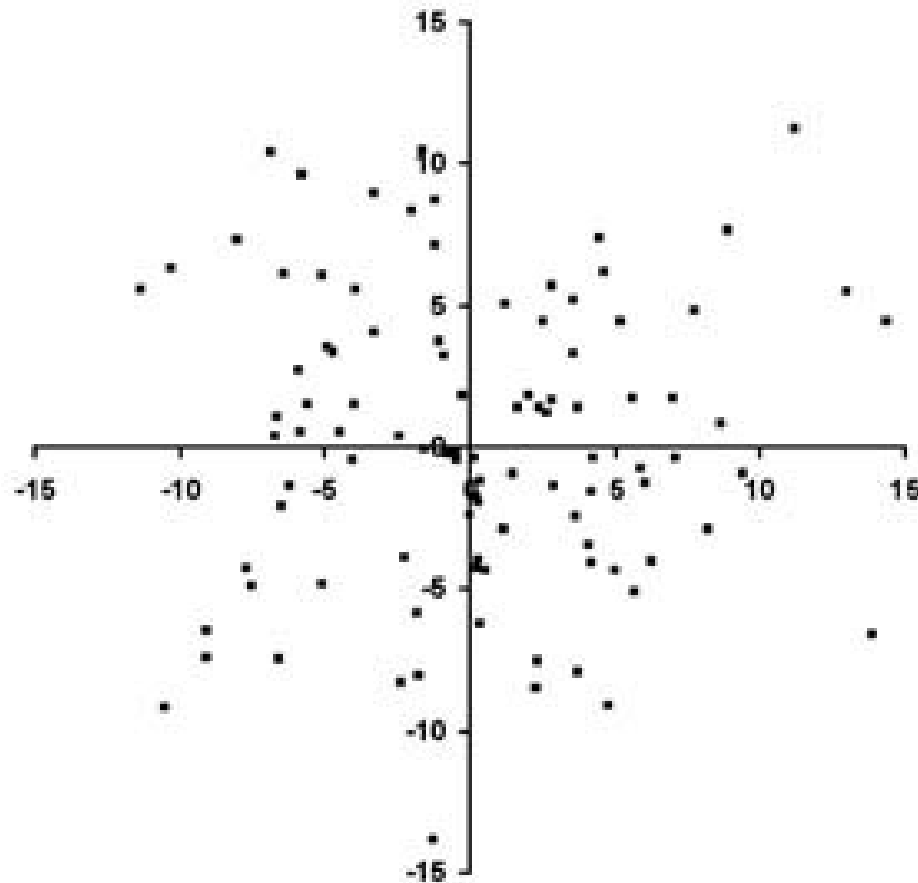


Precision and bias



- The effect of bias and precision on epidemiological measurements. The origin (0,0) is considered to be the true value. Values simulated from a precise and valid (unbiased) system (A), an imprecise and valid system (B), a precise and invalid (biased) system (C), and an imprecise and biased system (D).

Precision and bias



- The preferential removal or retesting of the large positive values will result in a more precise but potentially biased distribution
- Titers often do not look “too small” – especially since expected to follow a log-normal distribution



Random variation

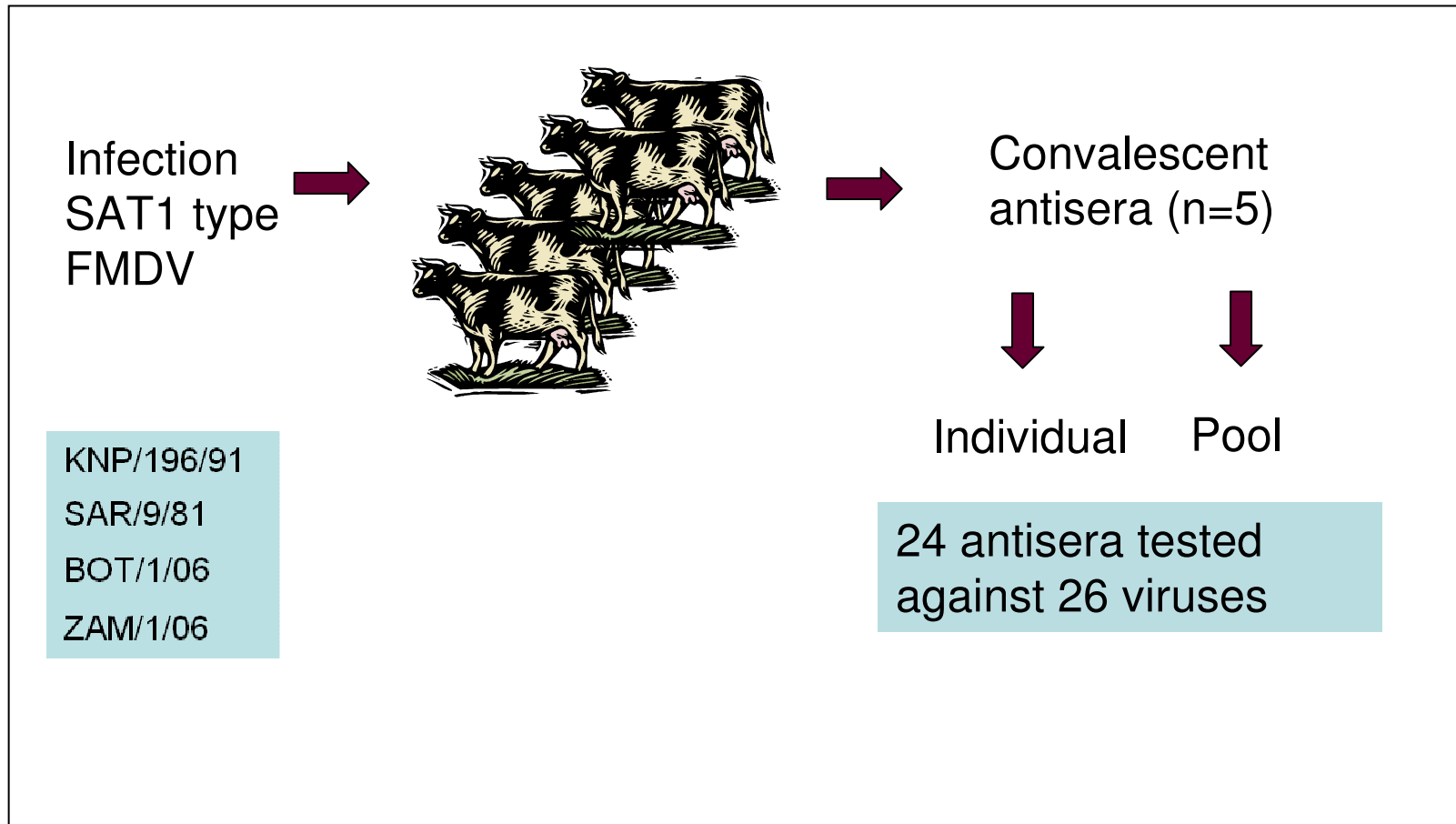
- **Epidemiology operates under the assumption that random variation only appears random because we have not yet identified the causes for its existence**
- **Sources of variability**
 - **Biological – fluctuations of the true values within an animal that are cyclical (diurnal variations)**
 - **Temporal – trends over time related to changes within the animal**
 - **Analytical – fluctuations in measured values due to imprecision in the method of measurement**
- **Removal of variation could introduce bias**
- **Research should embrace the inherent variation as if it is excluded then it will not be possible to identify the causes of the variation**
- **Diagnostic and research approaches to variation might be different**



Data collection

- **4 SAT1 reference strains:**
 - BOT/1/06/1, KNP/196/91/1, SAR/9/81/1, ZAM/1/06/1
- **5 cattle infected with each reference strain (20 cattle total)**
- **26 SAT1 test viruses for VNT**
 - **22 field viruses**
 - BOT/2/98/1, KNP/10/03/1, KNP/11/03/1, KNP/3/03/1, KNP/7/03/1, MAL/1/85/1, MOZ/1/02/1, NAM/1/10/1, NAM/272/98/1, NAM/308/98/1, SAR/2/09/1, SAR/2/10/1, SAR/33/00/1, SAR/7/03/1, SAR/8/02/1, SAR/9/03/1, TAN/2/99/1, ZAM/2/93/1, ZIM/11/03/1, ZIM/14/98/1, ZIM/3/03/1, ZIM/3/95/1
 - **4 reference viruses**
- **1860 total VNT tests performed**
- **3 operators**
 - **Operator 1 – 700 tests**
 - **Operator 2 – 440 tests**
 - **Operator 3 – 720 tests**

Design overview



Q1: Titer variability

- *What variables contribute to the variability in virus neutralization titers?*
- VNT titers \log_{10} transformed
- Variance components analysis performed
 - Random effects using restricted maximum likelihood
 - Main effects only model
 - Evaluated variables – operator, animal, reference sera, test virus, virus topotype, (day, serum and virus controls)
- Coefficient of variation calculated – $sd / mean$
 - Independently per operator
 - Calculated only for those combinations where test virus, reference sera, and animal were repeated (variability due to day and operator)

Q1: Titer variability

Variable	Variance estimate	Variance percentage
Test virus	0.051	23.9%
Topotype	0.010	4.7%
Animal	0.009	4.2%
Reference sera	0.008	3.8%
Operator	0.003	1.4%
Error	0.132	62.0%

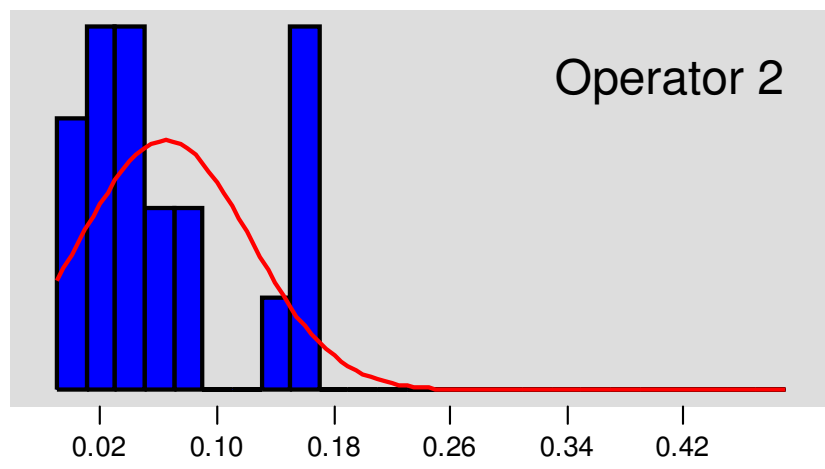
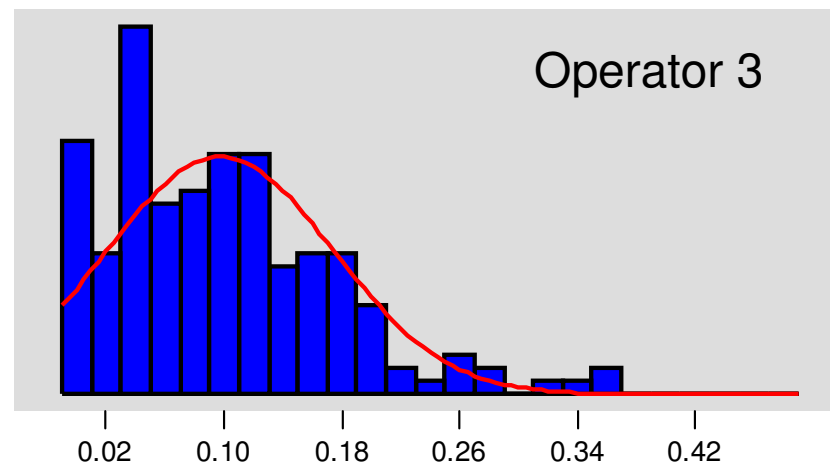
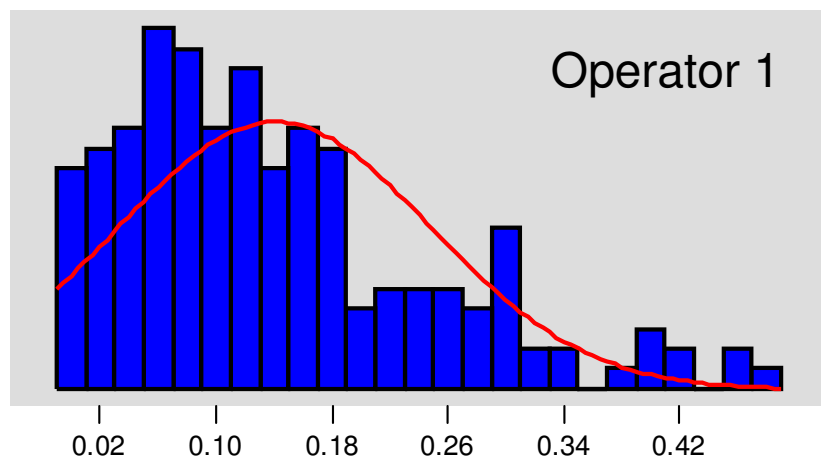
- Design did not allow for sufficient evaluation of day-to-day variation
 - Day (testing sequence) did not account for any variability
 - Virus and serum controls did not explain overall variability but accounted for all of the operator-associated variability

Q1: Titer variability

Operator	n	Mean (sd) %	Median (range)
1	180	14.1 (10.8)	11.7 (0 – 47.1)
2	20	6.5 (5.8)	3.6 (0.3 – 16.4)
3	180	9.8 (7.6)	8.8 (0 – 36.5)
Overall	380	11.6 (9.5)	9.6 (0 – 47.1)

- 20-30% coefficient of variation typically considered acceptable for a serological test

Q1: Titer variability



- Operator 1 results might suggest too much variability
- Operator 2 had a small sample size for evaluation

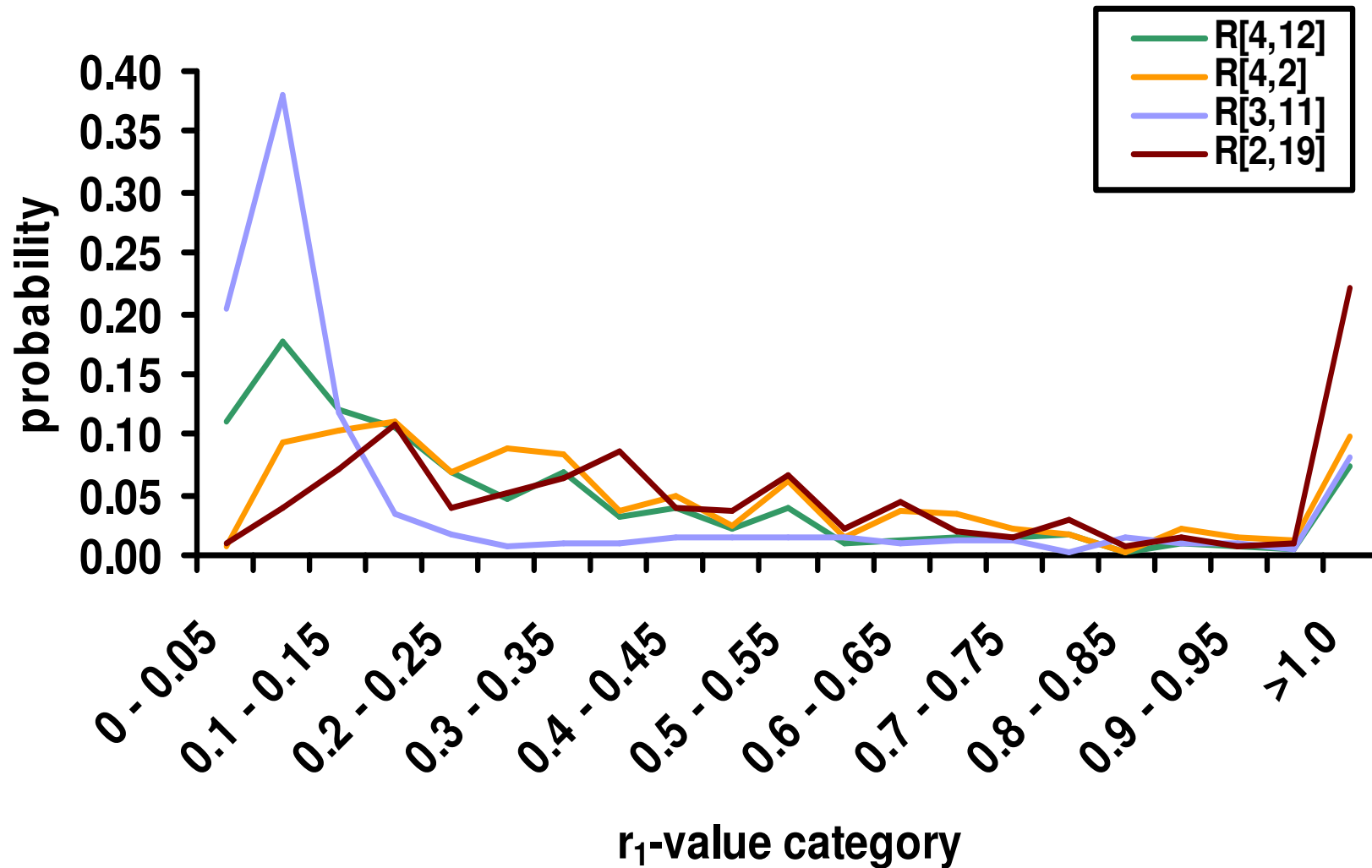
Q2: r_1 -value variability

- *How much variability should be expected when estimating r_1 -values?*
- Monte Carlo (MC) simulation procedure – sample of 100,000 iterations
- Randomly selected titer values
 - Reference titer (homologous) (1-4)
 - Test virus (heterologous) (1-26; included homologous)
- Individual r_1 -value calculated at each iteration
- Description of r_1 -values
 - Point estimate – median of MC sample
 - 95% CI – percentiles of MC sample
 - Probability function
 - 1 – cumulative probability

Q2:r₁-variability results

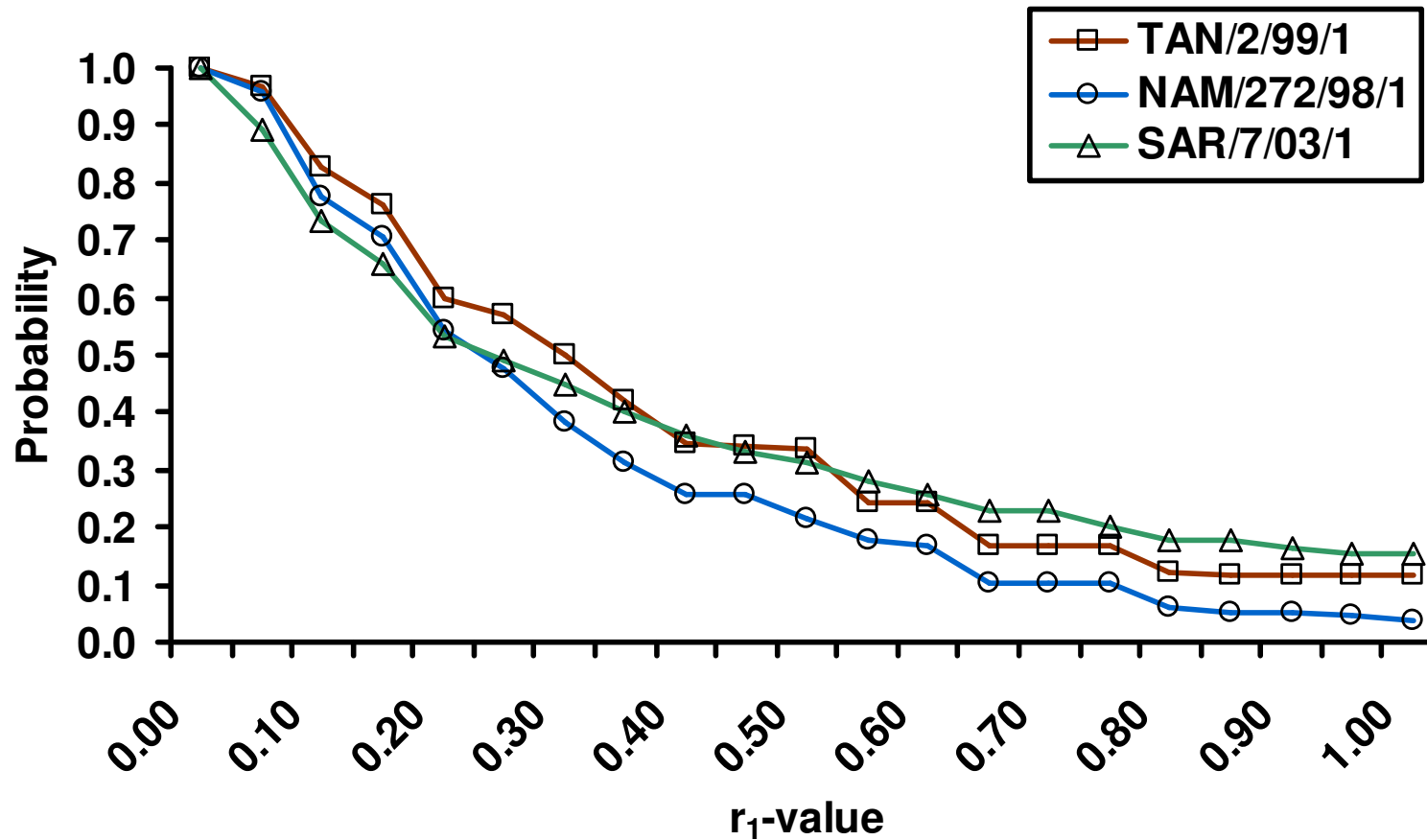
Reference serum	Test virus	Median [95% CI]	Pr >0.3	Pr >0.5	Pr >0.7
ZAM/1/06	SAR/9/03/1	0.25 [0.01, 5.49]	0.429	0.306	0.233
BOT/1/06	ZAM/1/06/1	0.25 [0.02, 3.02]	0.475	0.329	0.227
BOT/1/06	SAR/7/03/1	0.25 [0.02, 4.47]	0.448	0.315	0.231
BOT/1/06	NAM/272/98/1	0.25 [0.04, 1.26]	0.385	0.216	0.103
SAR/9/81	KNP/10/03/1	0.25 [0.04, 1.95]	0.452	0.283	0.191
SAR/9/81	BOT/1/06/1	0.25 [0.05, 1.35]	0.412	0.214	0.101
BOT/1/06	TAN/2/99/1	0.25 [0.05, 1.62]	0.499	0.334	0.170
KNP/196/91	BOT/1/06/1	0.25 [0.05, 3.16]	0.469	0.304	0.216
ZAM/1/06	KNP/196/91/1	0.25 [0.05, 5.89]	0.467	0.345	0.265

Q2: r_1 -variability results



Q2:r₁-variability results

BOT/1/06 Reference sera

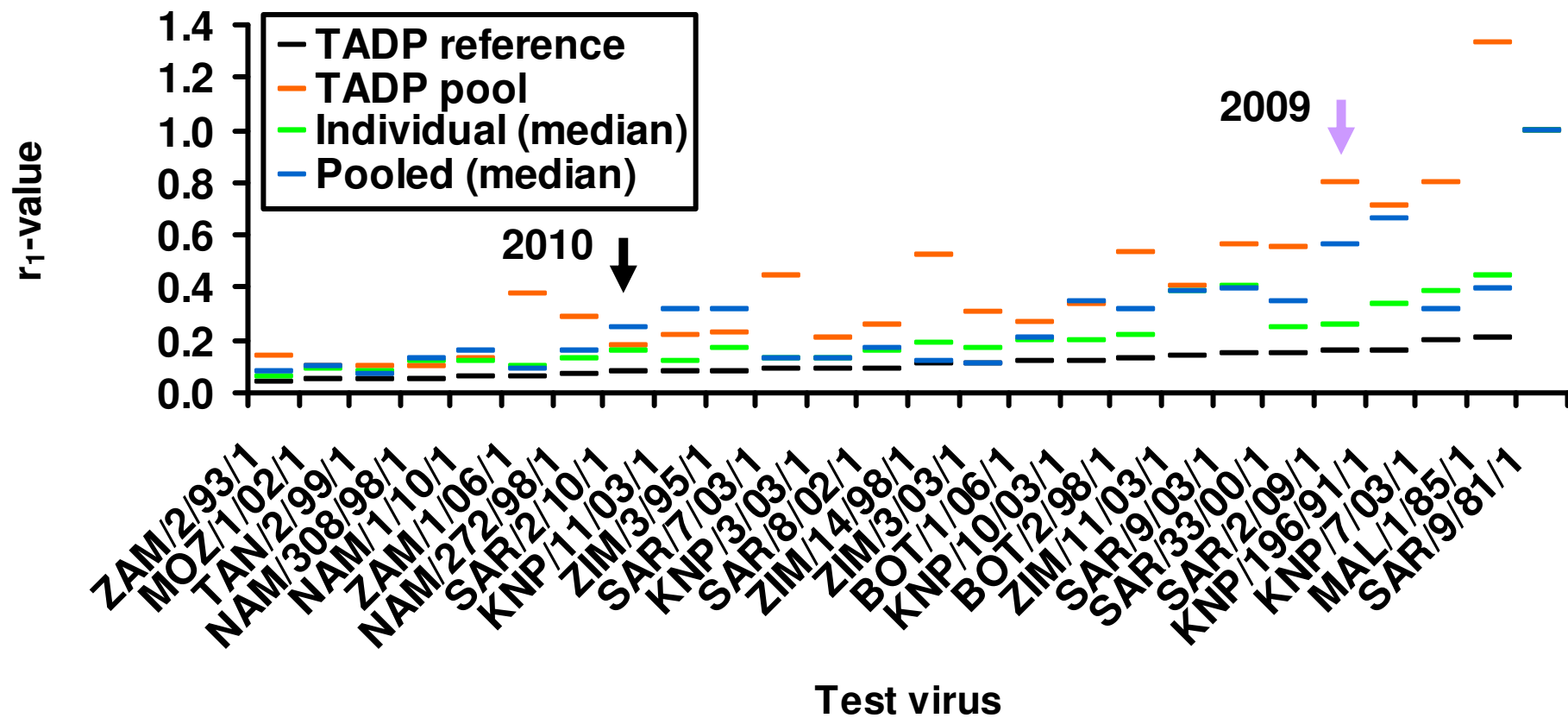


Q3: Pooled vs individual

- Will pooled sera from 5 animals give similar r_1 -values as the average of the 5 individual sera?
- Monte Carlo simulation procedure – sample of 100,000 iterations
- Randomly selected titer values
 - Reference titer (homologous) (1-4)
 - Test virus (heterologous) (1-26; included homologous)
- Individual r_1 -values calculated
- Pooled r_1 -values calculated from pooled titers corresponding to the randomly selected individual titers
- Descriptive evaluation for differences in r_1 -values
 - Point estimate – median of MC sample
 - Compared to individual and pool r_1 -values calculated by an experienced diagnostician

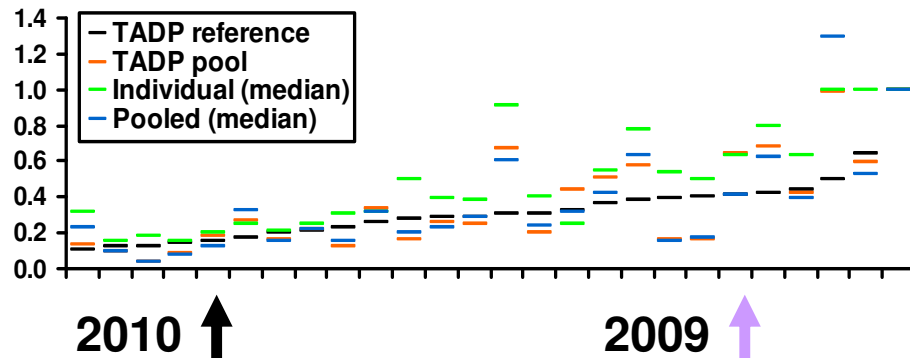
Q3:Pooled results

Reference sera SAR/9/81

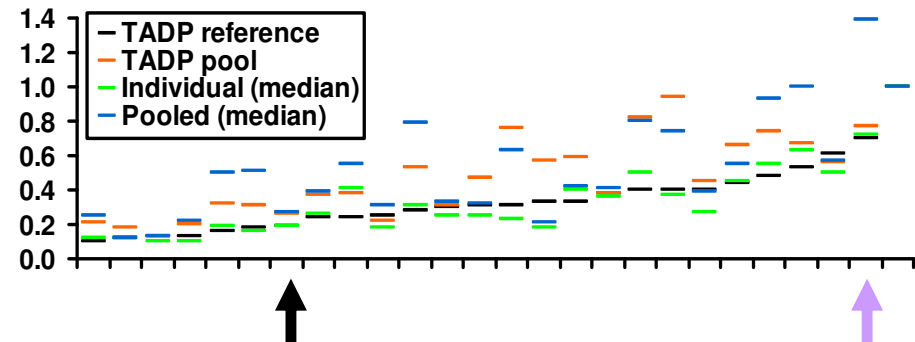


Q3:Pooled results

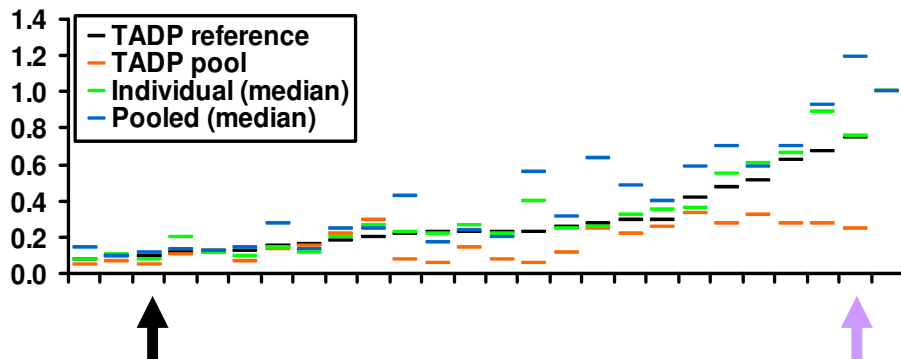
Reference sera BOT/1/06



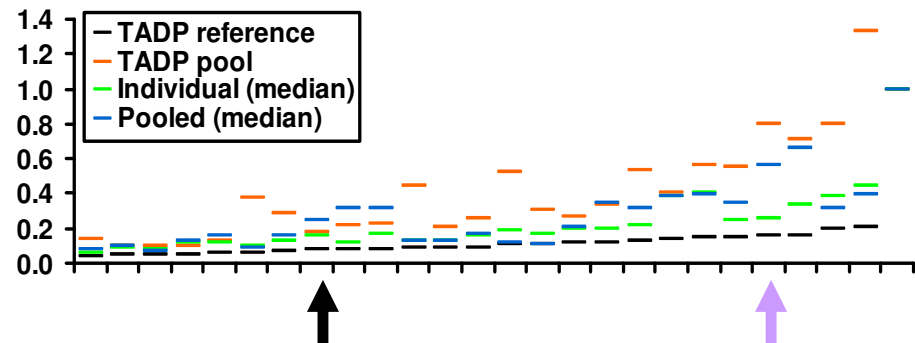
Reference sera KNP/196/91



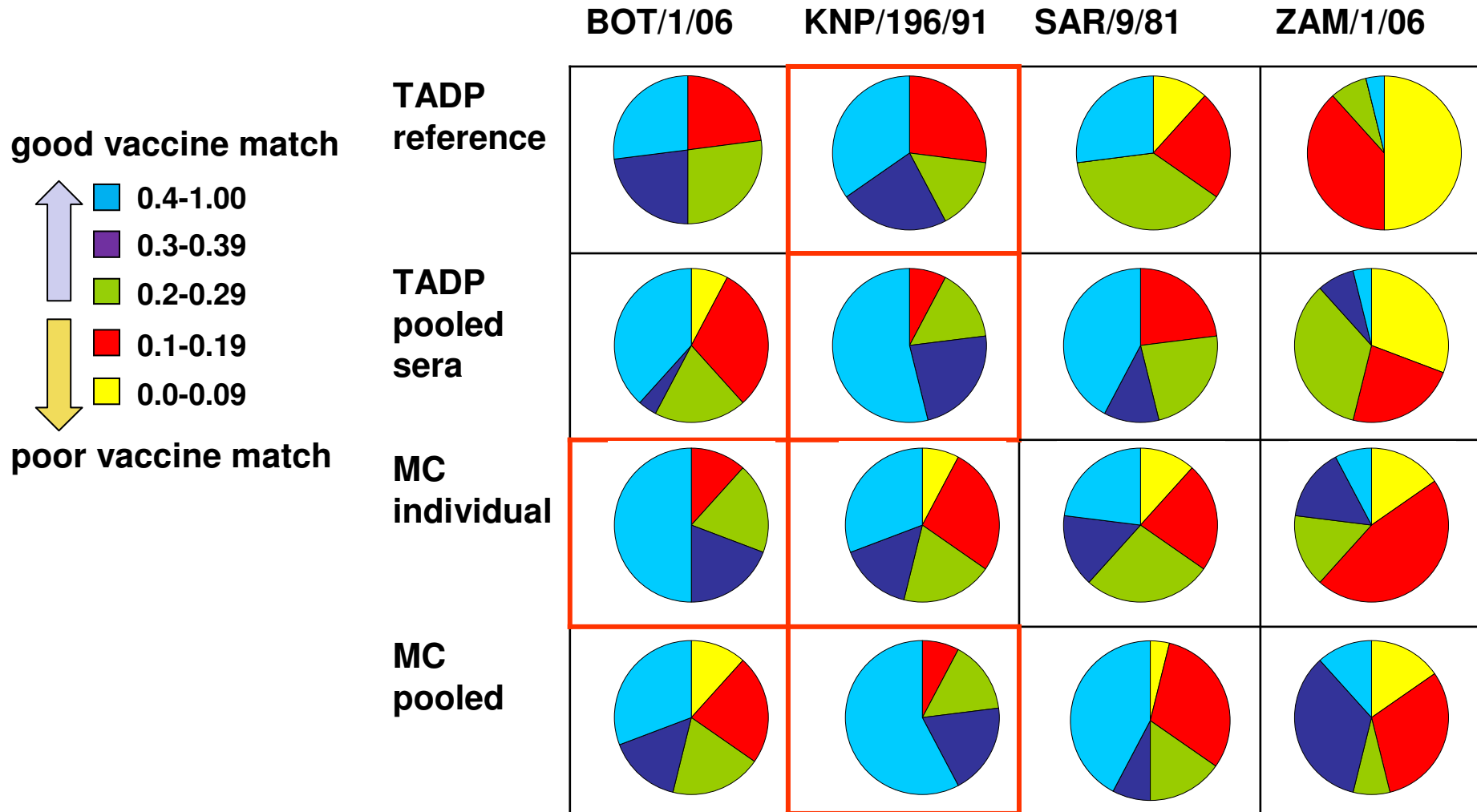
Reference sera ZAM/1/06



Reference sera SAR/9/81



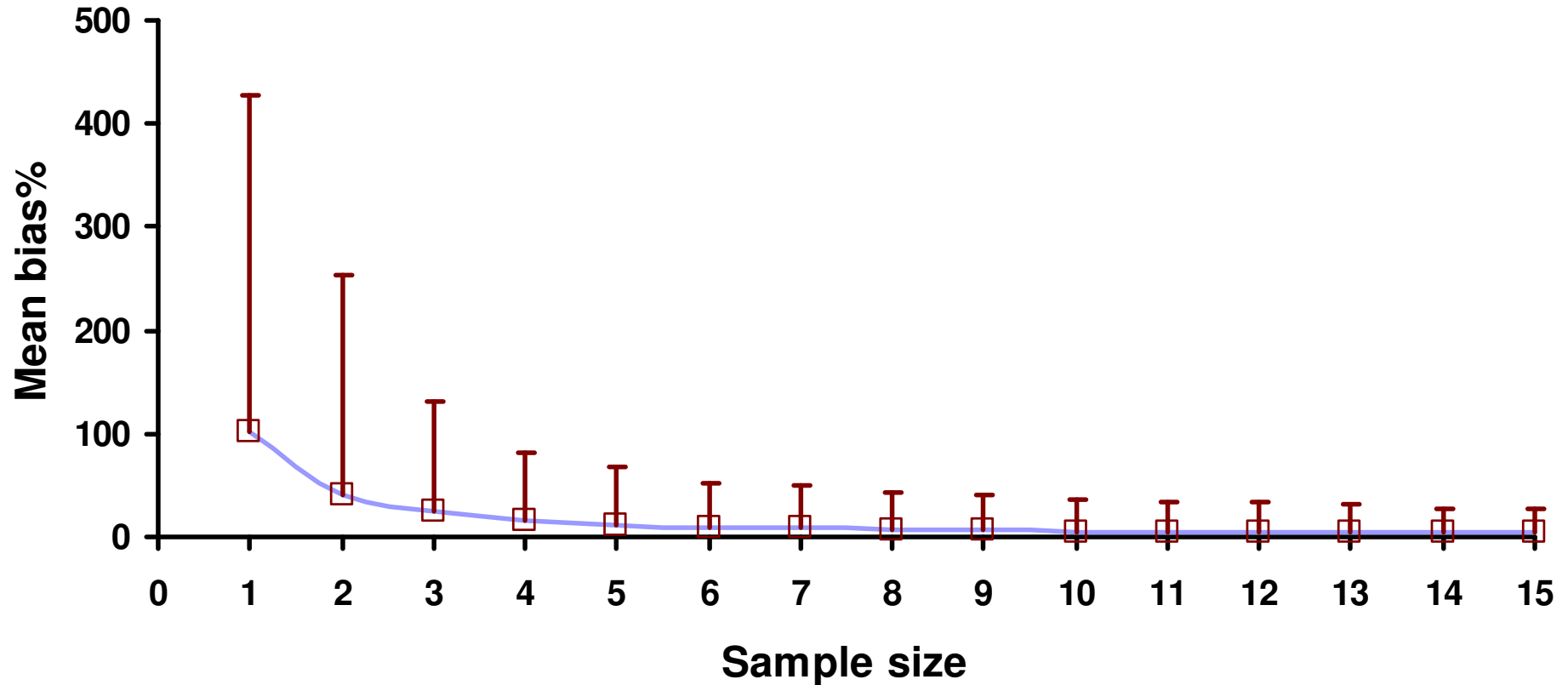
Q3: Vaccine match(?)



Q4: Number of animals

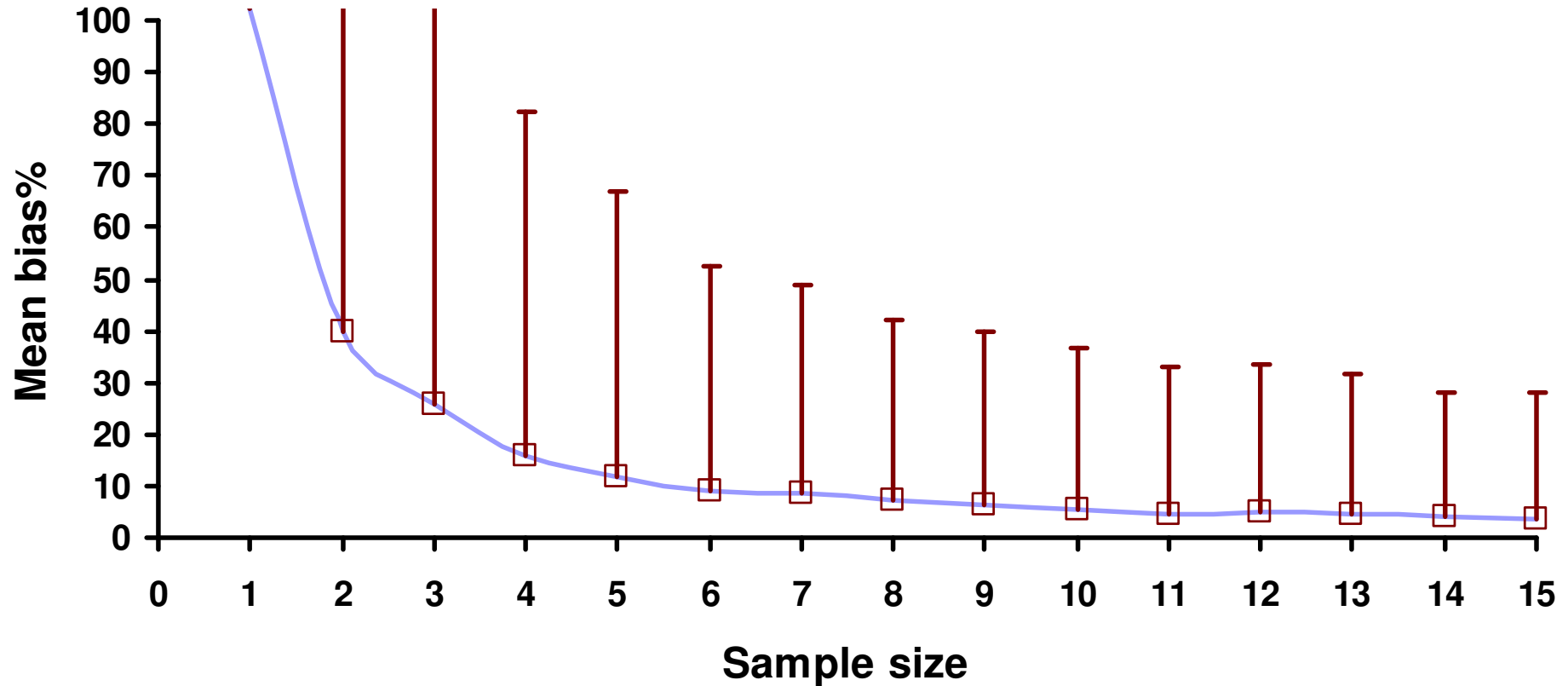
- **How many animals are necessary to reliably estimate r_1 -values?**
- **r_1 -values calculated from the entire dataset using the mean values over all operators and animals were considered the true r_1 -values**
- **A sample of 20,000 MC iterations were performed**
- **Possible samples sizes from 1-15 evaluated**
- **Reference sera titers randomly selected**
- **Test virus titers randomly selected**
- **r_1 -value calculated as Mean (test virus) / Mean (ref sera) titers**
- **Bias calculated as Sample r_1 -value – GS r_1 -value**
- **Bias% calculated as Bias / GS r_1 -value * 100**
- **Means used as point estimates with variability evaluated by percentiles of MC iterations**

Q4: Number results



- Mean percentage and 97.5% upper limit for MC bias

Q4: Number results



- Mean percentage and 97.5% upper limit for MC bias

Conclusions

- Preferential re-testing of high positive titers could introduce bias in r_1 -value determination
- VNT repeatability is acceptable and operator accounts for minimal variability
- There is substantial variability due to virus within topotypes
- A single r_1 -value point estimate might be sufficient without a measure of variability
- r_1 -value calculation method could affect determination of the best vaccine match
- Sample size of 5-6 is reasonable for r_1 -value estimation

