# Flock sensitivity and specificity of pooled fecal qPCR and pooled serum ELISA for screening ovine paratuberculosis

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# Introduction

- Challenges with paratuberculosis surveillance in small ruminants

**Sheep**
- Low individual economic value
- Low predictive values of individual diagnostic tests

**Flocks**
- Large size
- Collective management

Individual testing too costly, imperfect, useless
Rarely implemented

Need to develop less costly and more effective diagnostic approaches at the flock level

# Study objectives

- Evaluate the performance of screening strategies based on pooled fecal or serum samples

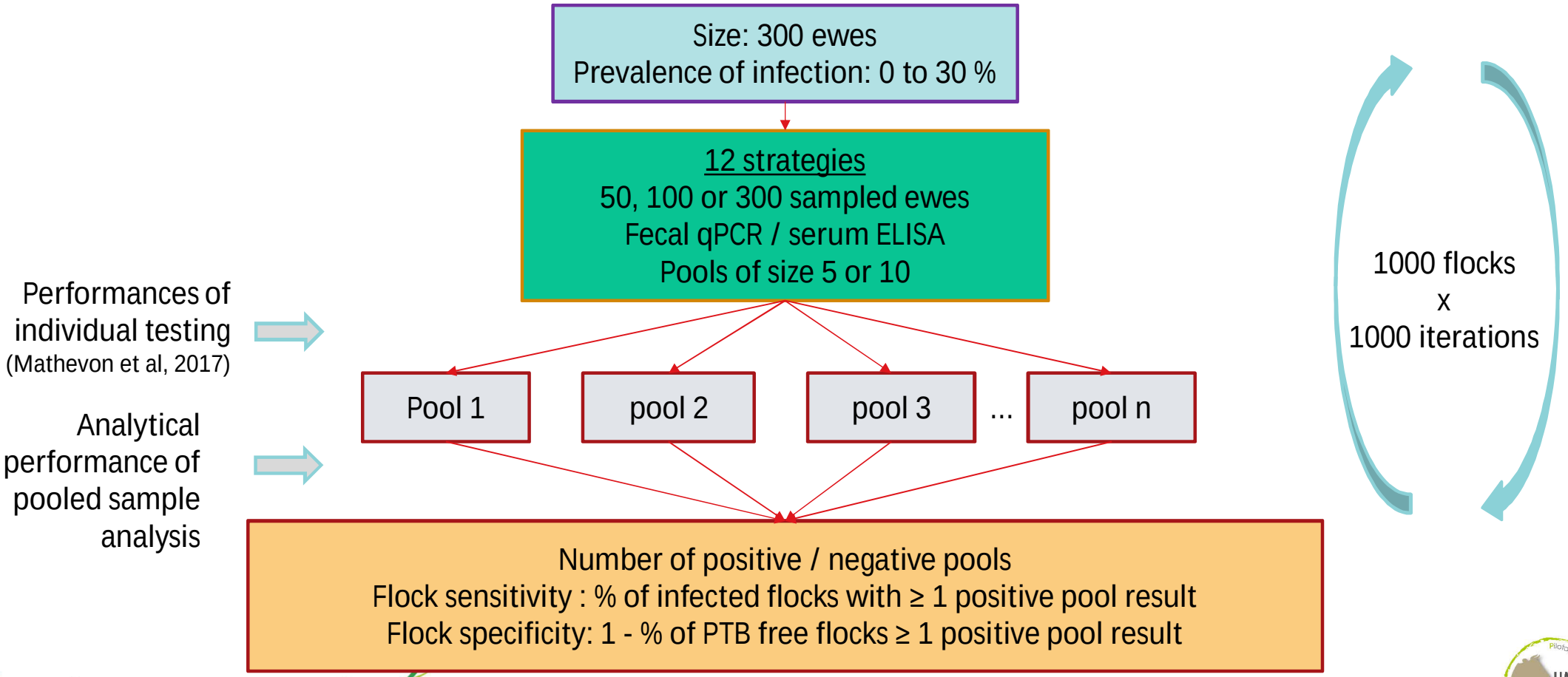
  – Experimental study: analytical performance of serum ELISA and qPCR applied to pooled-samples

  – Simulation study: epidemiological performance of screening strategies based on pooled-sample analysis
    - Sensitivity and specificity at the flock level
    - Infection prevalence estimation based on results from pooled-samples
    - Use in long-term flock monitoring

# Material and methods : experimental study

| | Pooled serum samples | Pooled fecal samples |
|---|---|---|
| Numbers | 362 pools<br>of 5 or 10 individual samples | 595 pools<br>of 5 or 10 individual samples |
| Composition | All negative<br>1 ou 2 weekly positive (low S/P value)<br>1 strongly positive (high S/P value) | All negative<br>1 weekly positive (high Ct)<br>1 strongly positive (low Ct) |
| Analysis & interpretation | Serum ELISA : Idexx ParaTB screening<br>New (lowered) positive threshold | qPCR : Adiavet ParaTB real time<br>Package qpcR |
| Judging criteria | Analytical sensitivity: proportion of 'positive' pools with positive result<br>Analytical specificity: proportion of 'negative' pools with negative result | |

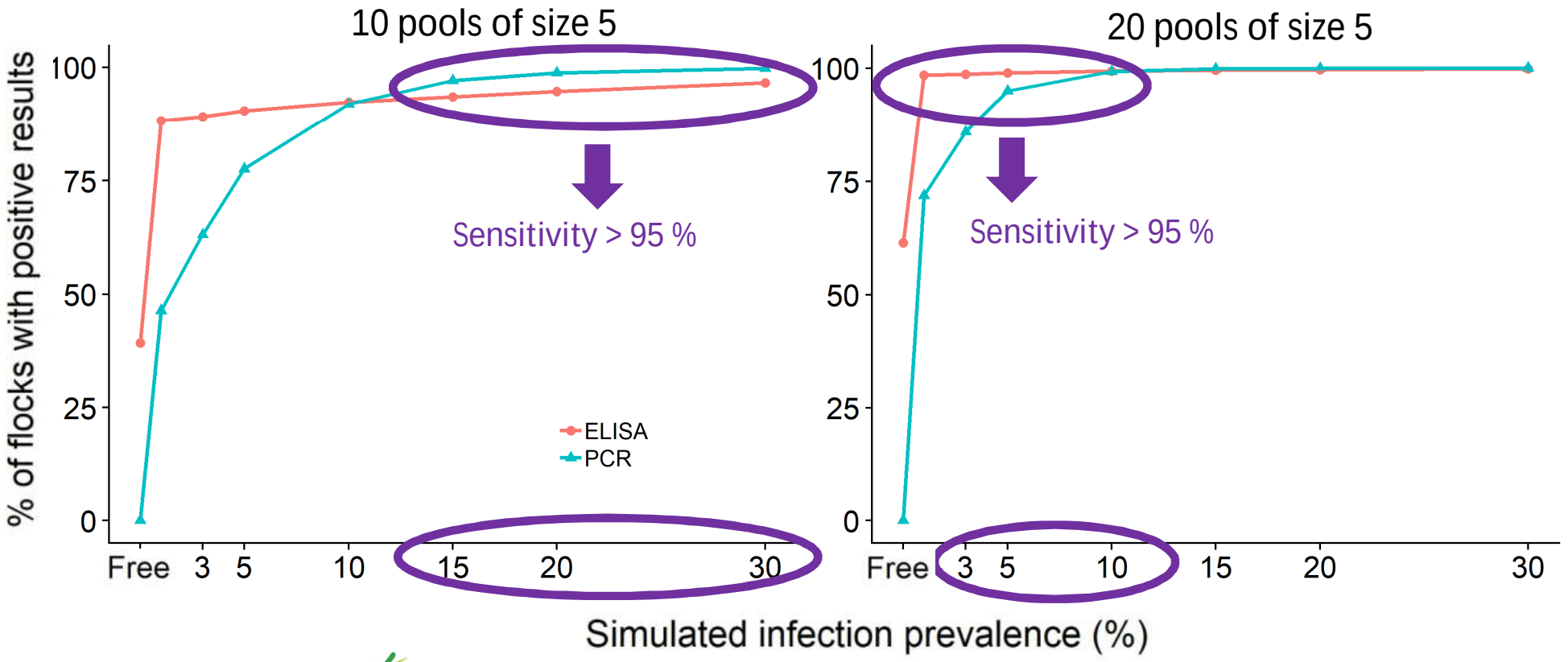
# Material and methods : simulation study



# Results: analytical performance of pooled-sample analysis

| Technique | Pool composition | Number of pools | % of pools detected positive | |
|---|---|---|---|---|
| | | | Pools of size 5 | Pools of size 10 |
| Serum ELISA | Negative | 102 + 102 | 0 | 0 |
| | 1 lowly positive | 37 + 37 | 62.2 | 62.2 |
| | 2 lowly positive | 21 + 21 | 100.0 | 100.0 |
| | 1 strongly positive | 21 + 21 | 100.0 | 100.0 |
| Fecal qPCR | Negative | 40 + 40 | 0 | 0 |
| | 1 lowly positive | 73 + 83 | 89.0 | 68.2 |
| | 1 strongly positive | 122 + 125 | 99.2 | 100.0 |

# Results : flock level performance of screening strategies



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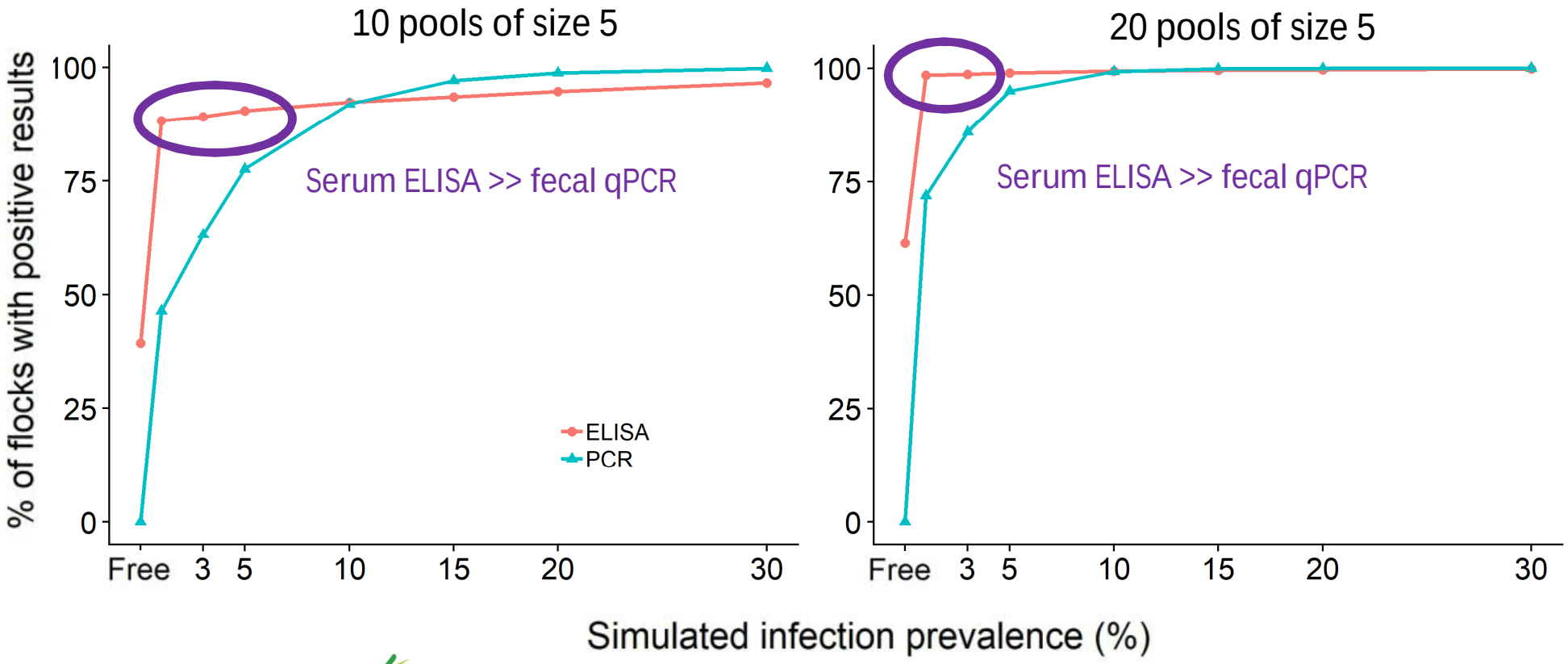

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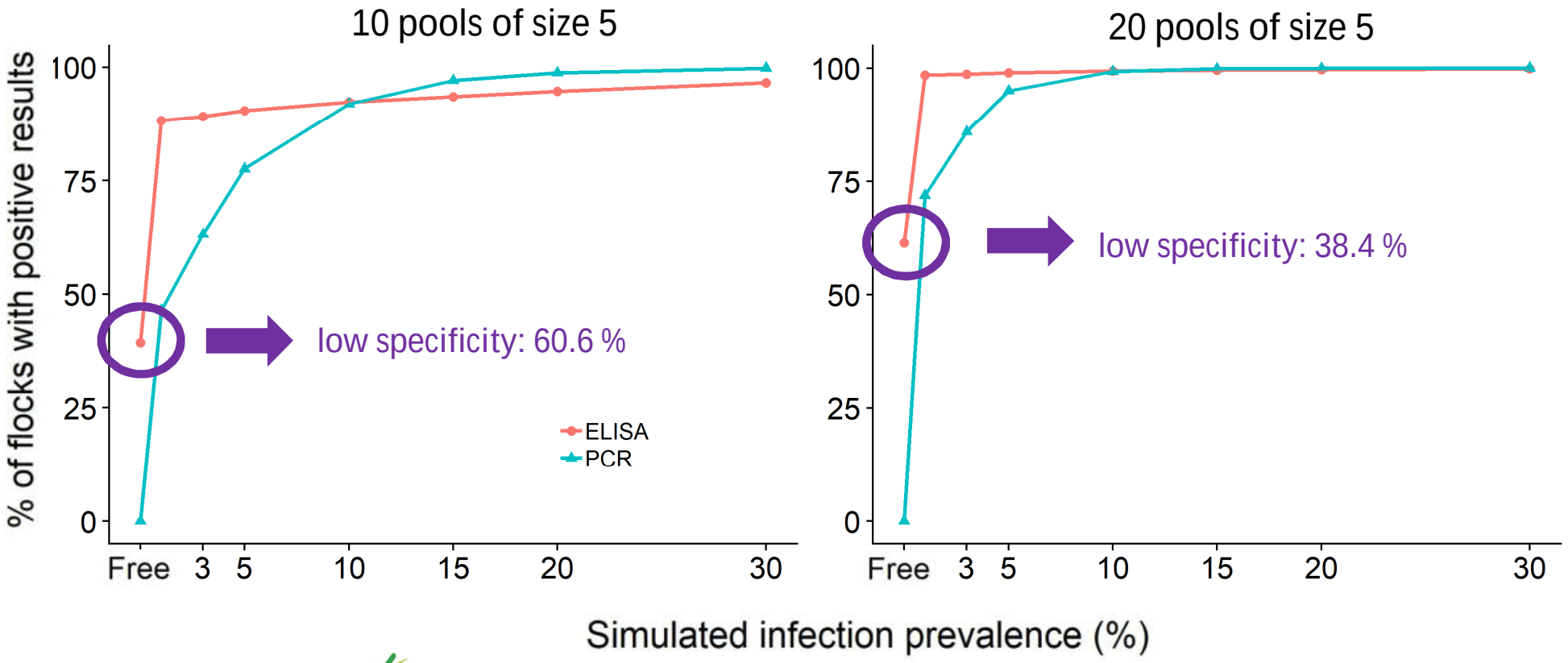
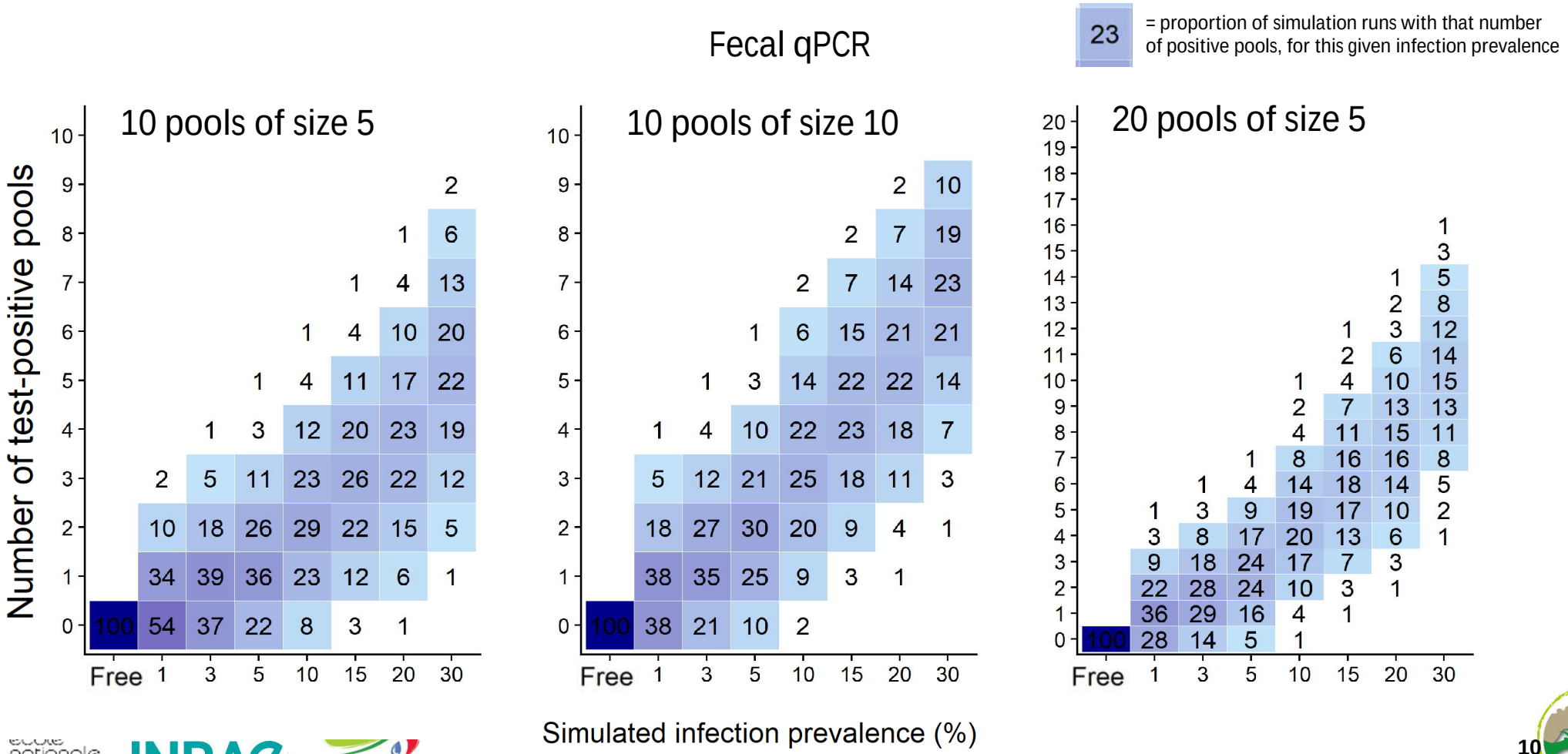


# Results : estimated prevalence of infection based on pooled-sample analysis



# Results : usefulness in long term flock surveillance

| | Strategy 1 | Strategy 2 | Strategy 3 |
|---|---|---|---|
| Number of pools / year | 10 | 10 | 10 |
| Pool size | 5 | 5 | 10 |
| Number of years | 3 | 5 | 5 |
| Estimated prevalence of infection (upper 95%CI) | | | |
| 0 positive pool | < 5 % | < 2.5 % | < 1% |
| 1 ou 2 positive pool(s) | < 10 % | < 5 % | < 2.5 % |

# Discussion and conclusion

- Analytical performance of pooled-sample analysis
  - Depends on the composition of the pools
    - Good to excellent for pools up to 10 or 20
    - Even if only one low shedder per pool

- Epidemiological performance of screening strategies
  - Based on pooled-serum samples: major lack of specificity
    - Related to a imperfect specificity at the individual level : 94.0 % (95%PCI : 92.2 – 95.7) (Mathevon et al, 2017)
  - Based on qPCR of pooled-fecal samples
    - High sensitivity for infection prevalence > 5 à 10 %
    - May help defining flocks as at “low risk of infection” if applied several years

# Interested in more details ?

PLOS ONE

RESEARCH ARTICLE

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Thank you for your attention.

Any question ?

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