IV Congresso Nazionale di Ecopatologia della Fauna
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When zero is a result: how to demonstrate the absence of an infection

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Can you trust your zeroes?

Negative results are important: certifying freedom from a disease can be fundamental for sanitary, veterinary or conservation reasons

Problem: demonstrating the absence of an infection would theoretically require testing the whole population with a perfect diagnostic test, but..

a perfect test is a dream

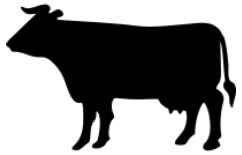
testing the whole population is often unfeasible

HOW CAN WE TRUST A NEGATIVE RESULT?

Confidence of Freedom

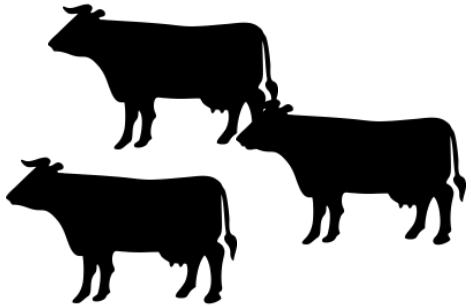
Confidence of Freedom = **Herd-level Negative Predictive Value**

Negative Predictive Value = probability that a test-negative individual is truly negative



it depends on prevalence, specificity and sensitivity

Herd-level Negative Predictive Value = probability that a test-negative herd is truly negative



it depends on prevalence, specificity, sensitivity and **sample size**

Confidence of Freedom

(Martin et al. 1992; Christensen & Gardner 2000; Humphry et al. 2004)

HERD-LEVEL SPECIFICITY

$$HNPV = \frac{(1 - eP) \times HSp}{(1 - eP) \times HSp + eP \times (1 - HSe)}$$

EXPECTED PREVALENCE

HERD-LEVEL SENSITIVITY

$$HSe = 1 - [(1 - (eP \times Se) + (1 - eP) \times (1 - Sp)]^{N}$$

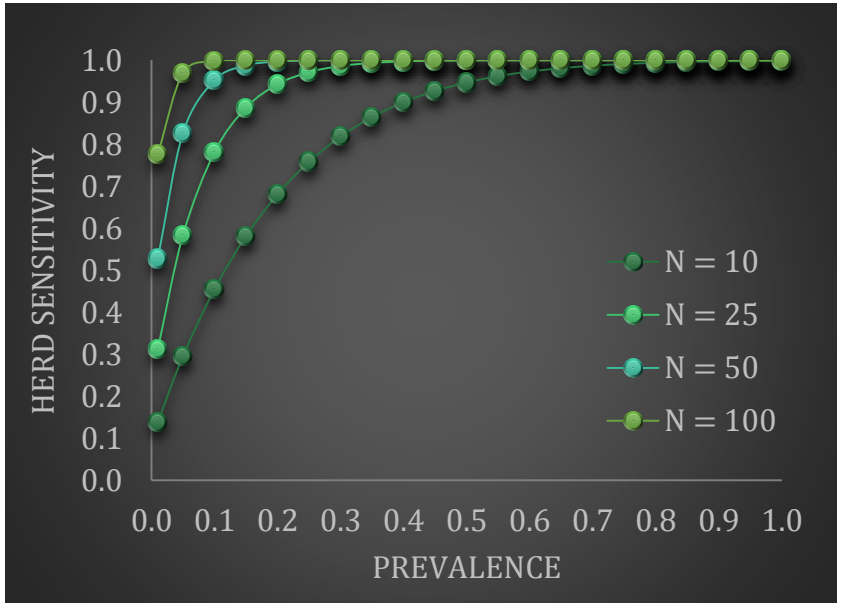
$$HSp = Sp^{N}$$

SAMPLE SIZE

Confidence of Freedom

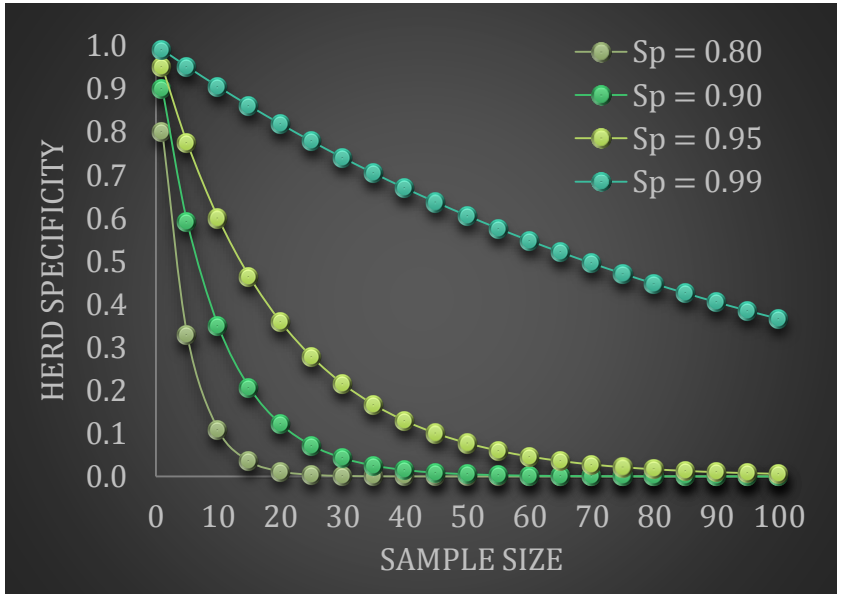
Herd-sensitivity: the probability that an infected herd will give a positive result to a defined testing protocol

it depends on both **sample size** & **prevalence**



Herd-specificity: the probability that an uninfected herd will give a negative result to a defined testing protocol

it depends only on **sample size**



Alien hosts & introduced parasites

ALIEN HOST INTRODUCTION → POTENTIAL ALIEN PARASITE INTRODUCTION → SANITARY, VETERINARY & CONSERVATION ISSUES

Problems:

- No epidemiological "history" about either the host or the pathogen in the new range
- Ad hoc sampling might not be feasible

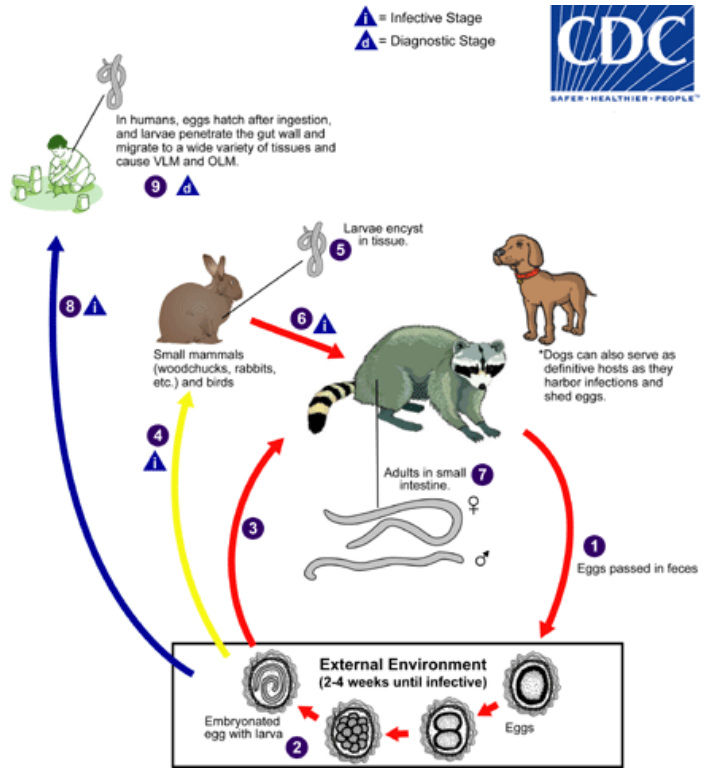
Confidence of Freedom can be useful

CASE STUDIES

Case I - Raccoons & *Baylisascaris*

Case II – Grey squirrels & SQPV

Case I - Raccoons & *Baylisascaris*



- *B. procyonis* is a gastro-intestinal helminth of **raccoons**
- **Dogs** may act as alternative definitive hosts
- Other **mammals are dead-end** hosts
- Infection in dead-end hosts may cause ocular, visceral or neural **LM syndrome**

Case I - Raccoons & *Baylisascaris*

(Beltràn-Beck et al. 2012)

- Introduced in several European countries
- Present along **Adda river** since 2004 (N>200)
- *B. procyonis* has been found in Germany, Denmark and Poland

WHAT ABOUT **ITALY**?

end of 2016: Survey through **gastro-intestinal content examination** on raccoons culled within a control program started by **Adda Nord Regional Park**

Results - Raccoons & *Baylisascaris*

Preliminary results:
no *B. procyonis* detected

SCENARIO 1

Input data

N = 17
Se = 0.99
Sp = 0.99
eP = **0.60**

→

HSe = 0.99

HSp = 0.84

→

HNPV = 100%

SCENARIO 2

Input data

N = 17
Se = 0.99
Sp = 0.99
eP = **0.10**

→

HSe = 0.87

HSp = 0.84

→

HNPV = 98%

Case II - Grey squirrels & SQPV

- Introduced in UK, Ireland and Italy
- Causes native red squirrels' extinction through trophic competition

- In the UK & Ireland also **apparent competition** mediated by **SQ**uirrel**P**ox**V**irus
- SQPV infection is **subclinical in grey** and **lethal for red squirrels**
- Replacement is accelerated up to 25 times

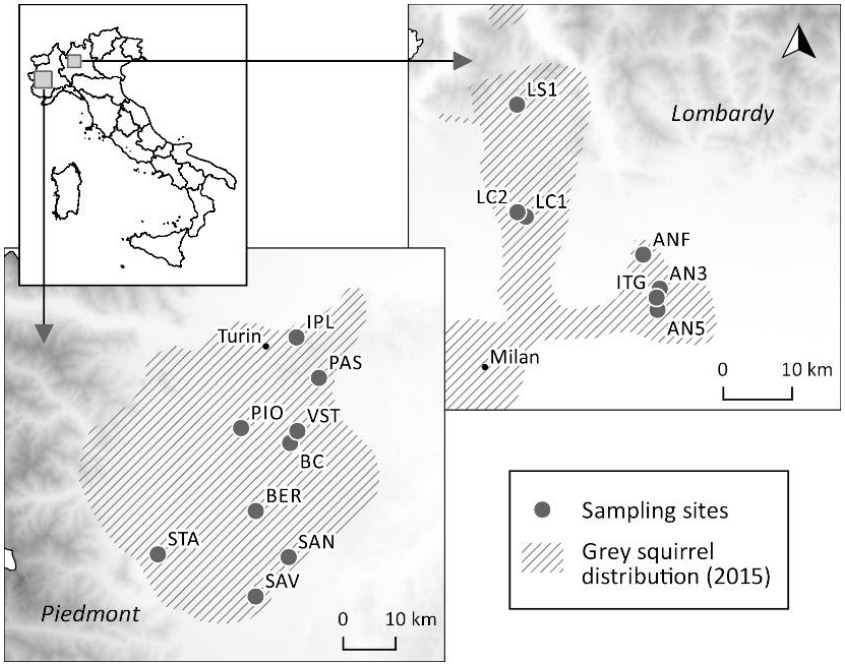
WHAT ABOUT **ITALY**?

Case II - Grey squirrels & SQPV

No evidence of diseased red squirrels in Italy..but this is not a proof of SQPV absence!

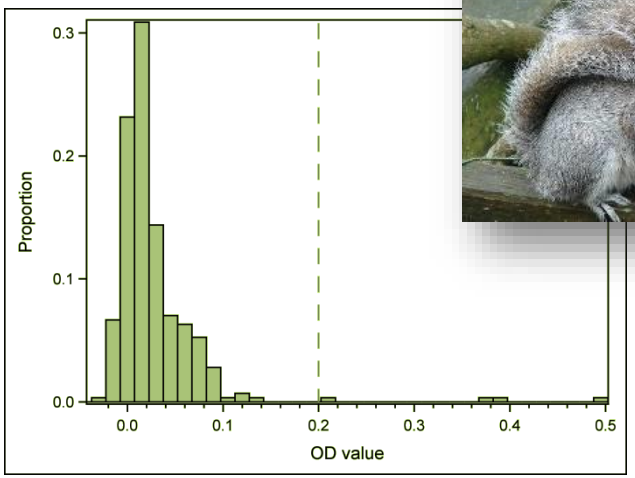
In Ireland there was a 14-year gap between the detection of seropositive grey squirrels and the recovery of the first diseased red squirrel

2011-2014: Survey through **serological** and **molecular** method on grey squirrels culled within **LIFE EC-SQUARE**



Results – Squirrels & SQPV

ELISA test:
4 positive reactors
(cross-reactions?)



SCENARIO 1

Input data

N = 285
Se = 0.98
Sp = 0.95
eP = **0.50**

HSe = 1

HSp = 0.0012

HNPV = 99.9%

SCENARIO 2

Input data

N = 285
Sp = 0.98
Se = 0.95
eP = **0.25**

HSe = 1

HSp = 0.0012

HNPV = 99.9%

Results – Squirrels & SQPV

Multiplex RT-PCR:
no reactors

SCENARIO 1

Input data

N = 66
Se = 0.99
Sp = 0.99
eP = **0.50**

→

HSe = 1

HSp = 0.51

→

HNPV = 100%

SCENARIO 2

Input data

N = 66
Sp = 0.99
Se = 0.99
eP = **0.25**

→

HSe = 1

HSp = 0.51

→

HNPV = 100%

Conclusions

- HNPV is a useful tool to achieve a greater confidence in negative results

- HNPV is in most cases easy to calculate (an excel sheet is enough)

- For more complicated cases, free tools are available on-line (e.g. epitools.ausvet.com.au)

Confidence of Freedom estimation can be a useful approach when alien hosts are suspected of a disease introduction

- Although sanitary investigations on **raccoons** are still ongoing, absence of *B. procyonis* is good news for public health

- Absence of SQPV in Italian **grey squirrels** is good news for the conservation of the whole continental red squirrel population

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Thanks for your attention!