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### Diagnostic performances study of three ELISA tests commercialized for Q fever diagnosis in domestic ruminants using latent class models

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Q fever is a worldwide zoonotic disease mainly responsible for reproductive disorder such as abortion in domestic ruminants. The serological diagnosis in domestic ruminants is mainly performed using ELISA tests. In France, there are three ELISA tests that are commercialized with little information about their sensitivities and specificities.

#### Objectives

This study focused on the three commercial ELISA tests with the following objectives (1) assess their sensitivity and specificity in sheep, goats and cattle, (2) assess the between- and within-herd Q fever seroprevalence distribution in these species, accounting for diagnostic error, and (3) estimate optimal sample

sizes considering sensitivity and specificity at herd level.

#### Materials and methods

The study sample was a sub-sample of a larger epidemiologic study, which assesses the Q fever seroprevalence in ten "Département" of France in cattle, goat and sheep herds. An aliquot of the first 150 sera in each species and in each department were sent to The National reference laboratory for Q fever in France which performs the three ELISA tests on a total of 1413, 1474 and 1432 sera from 106, 103 and 99 different herds (respectively from cattle, goats and sheep). All results were considered as positive and negative according to the manufacturer positivity threshold. Given that none of the test could be considered as a Gold Standard, we assessed sensitivities and specificities of the three ELISA tests by analyzing the crossed-test results with a hierarchical zero-inflated beta-binomial latent class model considering each herd as a population and conditional dependence as a fixed effect.

#### Results

Conditional dependence for truly seropositive animals was high in all species for two tests and conditional dependence for truly seronegative cattle was low but significantly above 0. Specificity estimates were high, ranging from 94.8 % [92.1;97.8] to 99.2 % [98.5;99.7] for all test in each species (except for the test 1 in one "département"), whereas sensitivity estimates were generally low, ranging from 39.3 % [30.7;47.0] to 72.0 % [61.8; 80.8] for test 1, between 53.8 % [43.3;61.8] and 75.2 % [68.4;79.9] for test 2 and between 86.9 % [71.2;93.6] and 90.5 % [83.3;93.8] for test 3 depending on the species. Between herd prevalence estimates were very variable in each "département" and species. Distributions of the within herd prevalence were wide but within herd prevalence in seropositive goat herds seemed to be higher than in the other species. At the

herd level, herd sensitivities, herd specificities were very variable depending on the sample size and interpretation rules of the series of tests. The optimal sample size maximizing both herd sensitivity and herd specificity varied from 3 to at least 20 animals depending on the test and ruminant species.

#### Conclusion

This study provides new insight about sensitivities, specificities and interpretations of three commonly used ELISA tests for detecting Q fever antibodies in domestic ruminants.

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Herzlich willkommen zur

## Gemeinsamen Arbeitstagung der Nationalen Referenzlabore Chlamydiose, Q-Fieber, Paratuberkulose und Tuberkulose der Rinder

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Online-Tagung veranstaltet vom  
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#### Organisatoren:

Dr. Christiane Schnee  
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Dr. Katja Mertens-Scholz  
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Dr. Heike Köhler  
NRL Paratuberkulose

Dr. Stefanie Barth  
NRL Tuberkulose der Rinder

# Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of *Coxiella burnetii* infection in domestic ruminants.

**Gemeinsame Arbeitstagung der NRLs Chlamydiose,  
Q-Fieber, Paratuberkulose und Tuberkulose der Rinder  
2021/04/21**

Thibaut Lurier, Elodie Rousset, Patrick Gasqui, Carole Sala, Eric Morignat, Clément Claustre, David Abrial, Philippe Dufour, Renée de Crémoux, Kristel Gache, Marie-Laure Delignette-Muller, Florence Ayrat, Elsa Jourdain



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RESEARCH ARTICLE

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## Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of *Coxiella burnetii* infection in domestic ruminants



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# Serological diagnosis of *Coxiella burnetii* infection

- **ELISA methods are recommended by the OIE**
  - **Three commercialized ELISA tests**
    - Test 1 , commercialized by Idexx
    - Test 2, commercialized by ThermoFisher Scientific
    - Test 3 : commercialized by Innovative Diagnostics Vet
  - **Only few studies about their sensitivity and specificity**
    - Depending on the test, study and species, estimates vary
      - *sensitivity* from 70 to 100%
      - *specificity* from 90 to 100%
    - **Not for all tests and/or species**
    - **Sometimes with an important bias**
- ⇒ **No Gold Standard** (reference test with 100% Se and Sp) <sup>3</sup>

## How assessing diagnostic performances in the absence of a Gold Standard?

Gold standard = reference test with 100% Se and Sp

- **Using serum samples of « known status » ?**

**Extreme values** → lack of « intermediate » values

Or Use of infection animal models with high infection doses and previously defined infection - sampling time period

- Tests performed on samples that are **far from the one on the field**
  - **Diagnostic performances are overestimated** (Quadas-2 : Whiting et al. 2011)

- **In comparison with another imperfect « reference » test**

Assessment of this « relative » Se and Sp is even more **biased** when:

- Se and Sp of the « reference » are poor
- both tests are **conditionally dependent** (Quadas-2 : Whiting et al. 2011)

# Latent class models

- Modeling the **crossed classified test results** from **multiple tests**
  - **Simultaneous assessment** of the diagnostic performances of multiple tests ( $Se$  and  $Sp$ ) and the prevalence of the populations ( $P$ ) corresponding to a non-directly observed **latent status**.

	Test 2 positive	Test 2 negative
Test 1 positive	$p_{11} = Se_1 \times Se_2 \times P + (1 - Sp_1) \times (1 - Sp_2) \times (1 - P)$	$p_{10} = Se_1 \times (1 - Se_2) \times P + (1 - Sp_1) \times Sp_2 \times (1 - P)$
Test 1 negative	$p_{01} = (1 - Se_1) \times Se_2 \times P + Sp_1 \times (1 - Sp_2) \times (1 - P)$	$p_{00} = (1 - Se_1) \times (1 - Se_2) \times P + Sp_1 \times Sp_2 \times (1 - P)$

- 3 degrees of freedom (DF) for 5 parameters ( $P$   $Se_1$   $Sp_1$   $Se_2$   $Sp_2$ )
- If we analyze results obtained in two different populations then
  - ⇒ **6 DF for 6 parameters to assess** ( $P_1$   $P_2$   $Se_1$   $Sp_1$   $Se_2$   $Sp_2$ )
  - ⇒ Then **an analytical solution** exists to assess all 6 parameters.

5

## Conditional dependence between tests

### **Are the tests frequently wrong/correct at the same time?**

**False negative/true positive results**, more likely to occur simultaneously

- When the level of antibodies is low/high and difficult/easy to detect with any test
- When the tests target closely related antigens of *C burnetii*

**False positive results**, less likely to occur simultaneously

- Observed for bacteria that have antigens closely related to the ones of *C. burnetii* (e.g., *Coxiella*-like tick symbionts) → **cross reactions**

Otherwise, errors are expected to be independent between tests

**In our case, conditional dependence between tests are expected**

**→ has to be taken into account to assess the tests diagnostic performances.**

6

# Accounting for conditional dependence in latent class models

→ Addition of **corrective terms** to model the lack or excess of probability related to concordant or discordant results

	Test 2 positive	Test 2 negative
Test 1 positive	$p_{11} = (Se_1 \times Se_2 + \gamma_{se}) \times P + ((1 - Sp_1) \times (1 - Sp_2) + \gamma_{sp}) \times (1 - P)$	$p_{10} = (Se_1 \times (1 - Se_2) - \gamma_{se}) \times P + ((1 - Sp_1) \times Sp_2 - \gamma_{sp}) \times (1 - P)$
Test 1 negative	$p_{01} = ((1 - Se_1) \times Se_2 - \gamma_{se}) \times P + (Sp_1 \times (1 - Sp_2) - \gamma_{sp}) \times (1 - P)$	$p_{00} = ((1 - Se_1) \times (1 - Se_2) + \gamma_{se}) \times P + (Sp_1 \times Sp_2 + \gamma_{sp}) \times (1 - P)$

- More parameters → **models are less easily identifiable**
- Models are potentially **non-identifiable** depending on the level of conditional dependence and the **modeled latent status**

7

## Limits of previous LCM studies

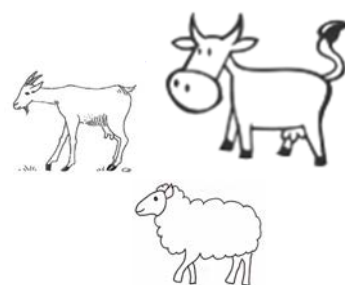
- Five studies which applied LCM with some of the three ELISA tests

Study	Tests included	Se/Sp of test 1	CONDITIONAL DEPENDENCE		Prior used	Comment
			Assesment	Results		
Horigan et al. 2011	Tests 1, test 2 et test 3, CFT	Se=87%, Sp=99%	<b>No</b>	NA	NA	High risk of bias with probable <b>overestimation of Sensitivities</b>
Paul et al. 2013	Test 1 (Blood/Milk)	Se=84% Sp=99%	Yes	“Not significant”	Non Informative	<b>Only test 1</b> => Latent status might be « is the animal positive with test 1 » $\neq$ « is the animal truly seropositive »
Lucchese et al. 2016	Test 1 et test 2, CFT	Se=97%, Sp=92%	Yes	Low (almost null)	Non Informative and Informative	Very High Se and Sp estimate, <b>potential bias if test are conditionally dependent</b>
Muleme et al. 2016	Test 1, CFT, Elisa mod, IFA	Se=70% Sp=96%	Yes	Not shown (but low)	Informative (from <b>Horrigan or human studies</b> )	<b>Prior information from potentially highly biased study</b> (Horrigan et al.)
Wood et al. 2019	Test 1, IFA	Se =88% Sp= 98%	Yes	Not shown (but low)	Informative (from <b>Muleme and Horrigan</b> )	Little information about conditional dependence between tests

8

# Objectives of this study

- Estimate the **sensitivity and specificity** values of the three ELISA tests commercialized for Q fever serodiagnosis in ruminants
  - From serum **samples of unknown status** originating from from cattle, sheep and goat herds in France
  - With **latent class models** considering the **cross-classified test results** of **the three tests**
  - Accounting for the likely **conditional dependence** between tests
- Assess **within/between-herd seroprevalence** accounting for diagnostic errors
- Calculate **herd sensitivity** and **herd specificity** values for **various sample sizes**



9

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## Study sample

Estimation of the frequency of Q fever in sheep, goat and cattle herds in France: results of a 3-year study of the seroprevalence of Q fever and excretion level of *Coxiella burnetii* in abortive episodes

- Sub-sample of a larger epidemiologic study (*Gache et al. 2017*) of 23,000 animals sampled from 1,500 randomly selected herds with no history of Q fever vaccination
- Inclusion of 150 animals from 10 herds in each *department*
  - 1,413 cows from 106 herds
  - 1,474 goats from 103 herds
  - 1,432 ewes from 99 herds
- Samples collected and analyzed in 2014 with the three ELISA tests at the NRL for Q fever

species	Number of	Department									
		A	B	C	D	E	F	G	H	I	J
cattle	herds	10	12	11	13	12	12	10	12	13	1
	animals	143	157	150	181	155	161	155	150	152	9
goat	herds	11	11	12	12	11	9	11	1	12	13
	animals	154	161	201	175	152	134	146	11	153	187
sheep	herds	11	11	10	10	11	11	11	10	11	3
	animals	165	162	149	145	155	157	161	146	156	36

10

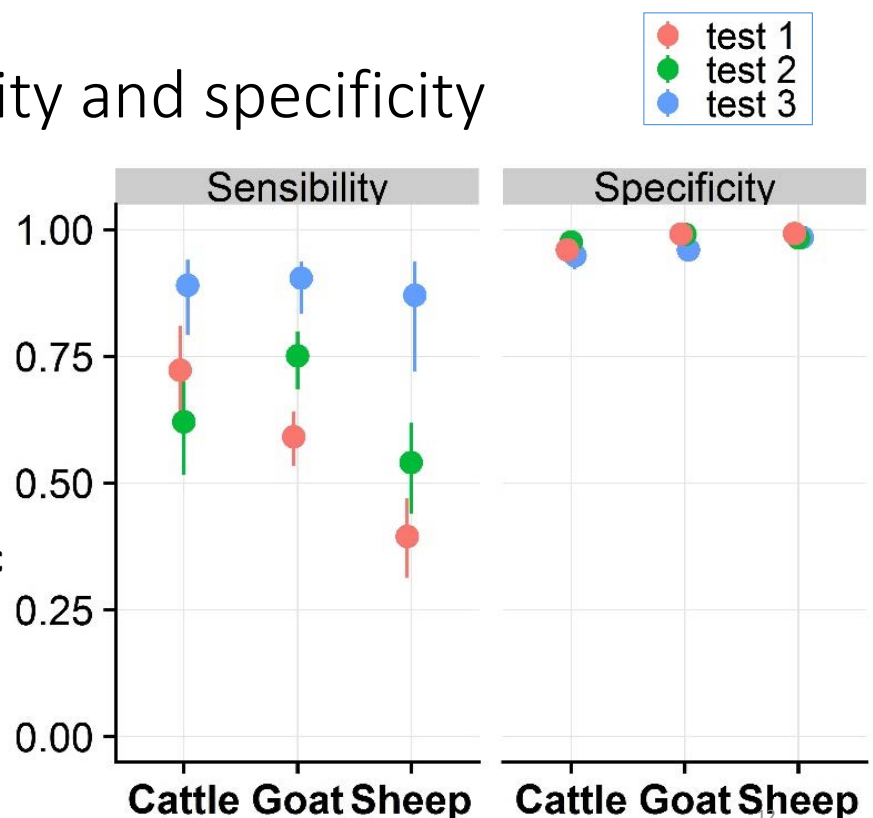
# Latent class analysis

- **One model by ruminant species**
- Conditional dependence between the three tests modeled with a **fixed effect model** (Wang et al. 2017)
- Each herd is considered as a population
- Modeling of the **within-herd seroprevalence distribution** across all seropositive herds
  - (zero inflated hierarchical beta-binomial distribution)  
⇒ Some herd could be free of *C.burnetii*
- **Between-herd seroprevalence** assessed in each department
- Use of **the least informative prior distributions**

11

## Results : test sensitivity and specificity

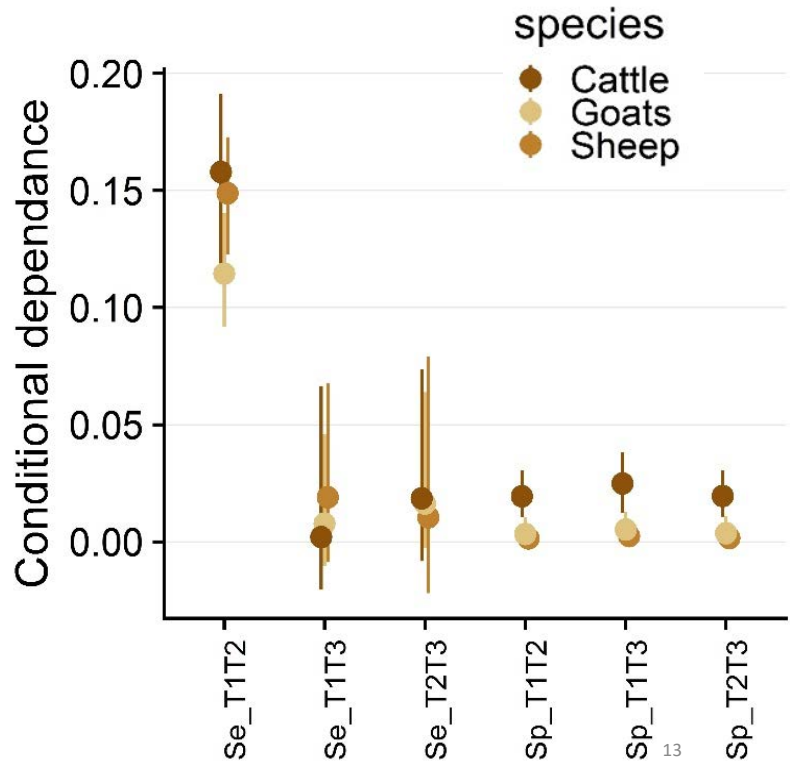
- **Sensitivity values are weak** (particularly in sheep)
- **All tests seem highly specific**
  - Slightly lower in cattle
- **Test 3 is the most sensitive** in all species but also the **least specific**
- All tests are **not identical in each ruminant species**





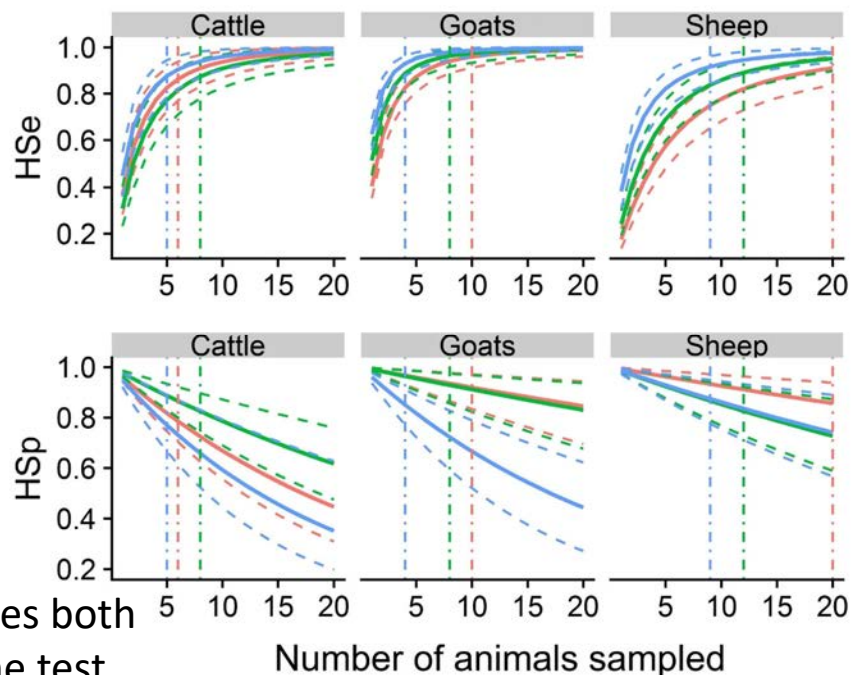
# Conditional dependence

- **High between T1 and T2** for **truly seropositive** animals
  - lower between **T3 and T1/T2**
  - **Negligible for truly seronegative animals** (except in cattle)
- ⇒ Diagnostic errors in truly seropositive animals are likely to **occur simultaneously for T1 and T2**
- ⇒ Diagnostic errors in truly seronegative animals are rare and random (**except in cattle**)



# Results : herd sensitivity (HSe) and specificity (HSp)

- HSe = Probability that **at least one** animal sampled is positive to the test in a **positive herd**
- HSp = Probability that **none** of the animal sampled is positive to the test in a **negative herd**
- Calculated with a sample size varying from 1 to 20 animals
- Test 3 has the worst HSp



**The best sample size** (which maximizes both Hse and HSp) **varies** depending on the test and species

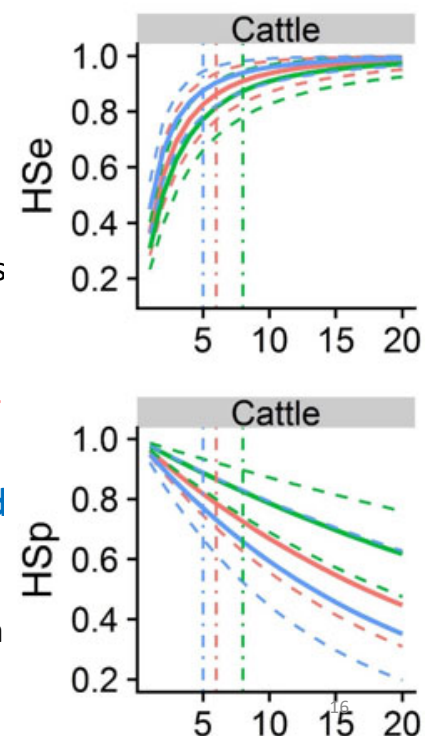
# Discussion

- **Unbiased estimation of test Se and Sp** which does not rely on an imperfect gold standard
- Comparison with other studies
  - **Similar specificity estimates**
  - **Lower sensitivity estimates**
- ⇒ **More relevant modeling of the conditional dependence in truly seropositive animals**
- High conditional dependence between tests 1 and 2
  - Potentially related to the relatively higher positivity cut-off of these two tests
- Important differences between ruminants species
  - Importance of the assessment of diagnostic performances in every species

15

## Perspective : Mandatory surveillance (Cat E)

- If we want to detect seropositive herds
  - **Which test** should we use?
    - Not the same in every species...
  - **How many animals** should we sample?
    - If we sample many animals → HSp decreases
    - ⇒ **Risk** to wrongly consider positive many truly “seronegative” herds
    - If we sample few animals → HSe decreases
    - ⇒ **Risk** to miss some truly “seropositive herds
  - **Which minimal number of seropositive animals to consider herd as “seropositive”?**
- ⇒ This study allows to find **the best combination of HSe and HSp** considering :
- The cost of the surveillance program
  - The consequences and cost of rightly/wrongly identifying a herd a seropositive or seronegative



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