Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

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KEYNOTES

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

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Work to improve the quality of diagnostic methods for Q fever is being pursued in France, by the National Reference Laboratory (NRL), together with producers of available commercial kits, analytical laboratories, and with scientific collaborators. Standardization and calibration of methods are a prerequisite for the production of reliable and usable data for a network of laboratories, involved in surveillance programs as well as epidemiological studies, case diagnostics and confirmation or investigations linked to human outbreaks. The role of the NRL is also to ensure that the performance of methods by analytical laboratories, and, their harmonization across a network of laboratories, are properly maintained.

Serological analyzes are carried out in France using three ELISA commercial kits, which use antigens obtained from different strains of *Coxiella burnetii*. Discordant results between kits are observed. Moreover, no reference method exists, and there is no collection of true positive and true negative sera, representative of the diversity of the epidemiological situations encountered for the three main target species (sheep, goat and bovine) bred in French regions. First, to overcome these difficulties, a reference material (RM), provided by the NRL, was included into the manufacturers' quality control. This allows estimating the variability of the measurements around the positivity threshold, which corresponds to the critical zone, and defining calibration criteria for each kit batches. Second, a comparative study was undertaken using a probabilistic modeling approach to better characterize the diagnostic performances of the kits in clinical or epidemiological contexts (PhD in progress). The results are expected to assess the kits' specificities and sensitivities. Based on these characterizations, a common standard serum for all kits, or even a common reference serological antigen, could be developed to be available to kit producers.

Real-time PCR methods, based on commercial kits, were validated in compliance with the U47-600 standard provided by the French normalization body (AFNOR), and, harmonized within the framework of a network of laboratories. Because these methods are used for the etiological diagnosis of abortion to Q fever, a bacterial load threshold was suggested. Then in order to reduce the financial costs associated to quantitative PCR (qPCR), a principle of PCR relating to this clinical interpretation threshold (relative PCR, rPCR) has been proposed. A list of validated 23 qPCR and rPCR methods has thus been established and recommended in France for the clinical diagnosis of laboratories. Adoption assays were performed, in laboratories conditions, to confirm initial performance of a specific method before routine analysis. Instructions on how maintaining this performance were provided, in particular on the basis of a bacterial RM and a control chart. Beyond this global harmonization work, additional studies must also be carried out to consolidate or change the definition of the threshold.

Kontakt:
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DIAGNOSTIC METHODS FOR Q FEVER IN RUMINANTS:

CONTRIBUTIONS TO THE VALIDATION OF PERFORMANCES AND TO THEIR HARMONIZATION

MEETING OF THE NATIONAL REFERENCE LABORATORY OF CHLAMYDIOSES, PARATUBERCULOSIS (PTB), BOVINE TUBERCULOSIS (BT) AND Q FEVER (QF)

FLI, GERMANY, WEB CONFERENCES, 2021 21-23

Missions of the French RNL for Q fever

Reference laboratory at:

> national level * (mandated by the French Ministry of Agriculture)

> international level since 2013 (OIE)

Contributions to research projects / reference activities / expertises for:

- Methods for diagnosis and epidemiology
- Measures for sanitary management (protection of animal and public health)
- Investigations linked to clustered human cases (health crisis, risks of exposure)
- Epidemiology (understanding the infection, contribution to monitoring)

*List of RNL in France at ANSES : https://www.anses.fr/en/content/reference-mandates

Sanitary situation of Q fever in ruminants in France

First large survey in ten departments

(2012-2015 / 10 labs)

Gache *et al*, Epidemiol. Infect. 2017

No mandatory monitoring in France

(E category within new Animal Health Law in Europe => to prepare)

Data at herd level

Species	Total (episodes)	% Pos	Min-Max (department)
Cattle	3 324	**2,7**	0 – 5.1
Sheep	776	**6,2**	0 – 17.9
Goats	114	**15,8**	0 – 36.4

Q fever abortive episodes

OSCAR

(27 departments)

Species	Total (herds)	% Pos	Min-Max (department)
Cattle	731	**36**	6.4 – 75.5
Sheep	522	**56**	11.4 – 84.4
Goats	349	**61**	25.0 – 82.6

***C. burnetii* infection serological survey**

Reference missions on available tests for current Q fever diagnosis

In France, the tests routinely used in veterinary laboratories are

(10 mandated laboratories, 27 OSCAR volunteer departments, 50-80 participating labs in ILPTs)

Serological methods :
Several indirect ELISA commercial kits

PCR methods (DNA extraction + PCR run) :
Commercial kits and homemade methods

Aim = to ensure the quality of the results / the reliability of the methods

- **Standardization + validation** => Defined and maintained performances of a fixed SOP
- **Harmonization** => Comparable results from several methods performed by a network of labs

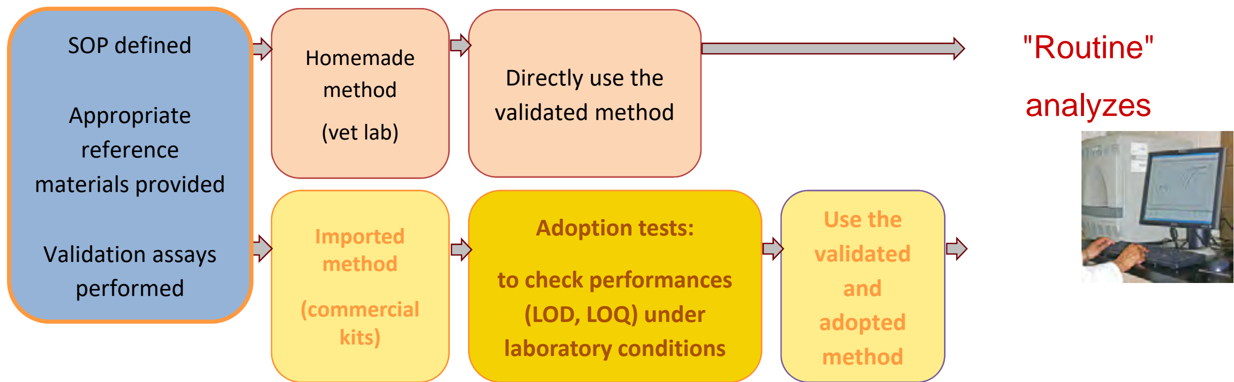
1 — Contributions of the QF-RNL to the real-time PCR tests

Validations carried out in collaboration with PCR kits manufacturers

- Priority on methods required for the **diagnosis of abortion series**
- Validation in accordance with:
  - the French ***AFNOR U47-600*** **standard** (first published in 2011)
  - the **QF-NRL requirements** and **conformity criteria**

- **Biological matrices targeted**: vaginal or endocervical mucus and placental cotyledons
- **Two thresholds**: 10^4 bact / mL (individual) and 10^3 bact / mL (pool of 3 animals)
- **Quantification**
  - including a maximum of 10^6 bact / mL and the thresholds (LOQ < 10^3 bact / mL)
  - a 5-point range
  - accuracy maximal limits of ± 0.70 log10 bact / mL on the entire quantification domain

Conformity criteria for assay validation / each Performance characteristic


anses
Adoption by labs for the implementation of a new or a modified method
SOP defined
Appropriate reference materials provided
Validation assays performed
Homemade method
(vet lab)
Directly use the validated method
"Routine" analyzes
Imported method
(commercial kits)
Adoption tests:
to check performances (LOD, LOQ) under laboratory conditions
Use the validated and adopted method
Rousset et al, Euroreference 2012
https://pro.anses.fr/euroreference/Documents/ER08-Meth-FievreQAvortEN.pdf
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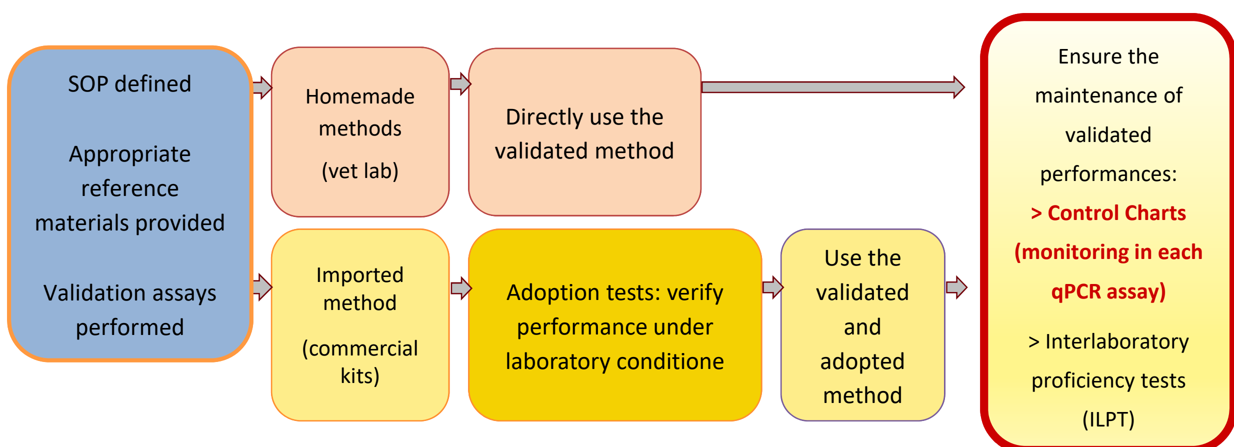
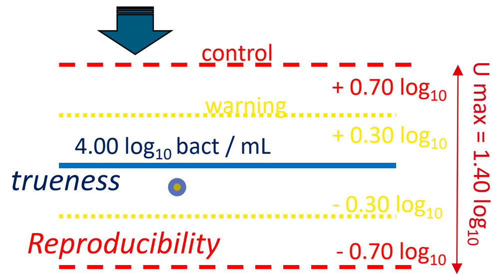

Continuous verification of routine analyzes (internal control chart)
anses
SOP defined
Appropriate reference materials provided
Validation assays performed
Homemade methods
(vet lab)
Directly use the validated method
Imported method
(commercial kits)
Adoption tests: verify performance under laboratory conditione
Use the validated and adopted method
Ensure the maintenance of validated performances:
> Control Charts (monitoring in each qPCR assay)
> Interlaboratory proficiency tests (ILPT)
Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

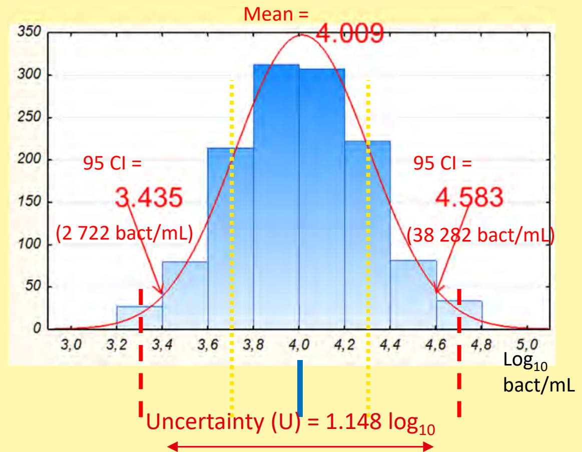
Control chart (CC) data from a laboratory network

Bacterial CC tracer (prepared from the QF-RNL Reference Material) **included in each tested series**



Verification of the value obtained in comparison with the expected value and the maximum authorized limits at 0.70 $\log_{10}$

Distribution of 1,274 tracer data obtained by 10 networked laboratories over 3 years



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Inter-laboratory proficiency tests (ILPT) for Q fever PCR in 2018

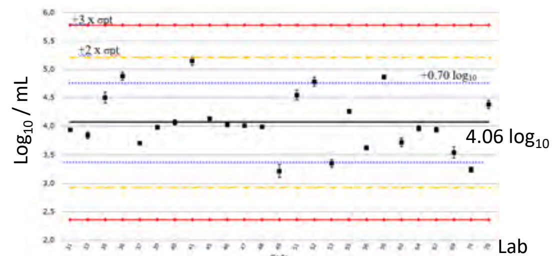
Data obtained by 25 participating laboratories

- Precision and trueness obtained by each lab
- Global mean at 4.06 $\log_{10}$
- Global standard deviation at 0.56 $\log_{10}$

=> **Measurement uncertainty U =** 2 x 0.56= **1,12 $\log_{10}$;** in this **ILPT network labs,** for results close to the “"the threshold currently considered to attribute abortions to Q fever“ at 4 $\log_{10}$ bact / mL (individual)

S4 sample

Results of the measurements in repeatability conditions (three repetitions per test)



Consensus reference quantitative values

Sample ID	Value (in $\log_{10}$ bacteria/ml)	Measurement range
S3	3.33	2.85 – 3.81 (σ = 0.48)
S4	4.06	3.50 – 4.61 (σ = 0.56)
S5	5.24	4.66 – 5.82 (σ = 0.58)

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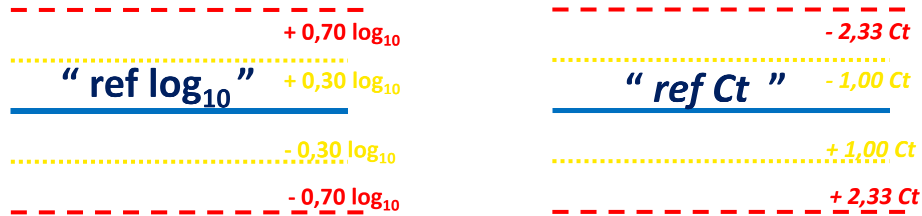
Relative (or semi-quantitative) PCR for abortive diagnosis : rPCR

In the rPCR mode, the tracer is calibrated to set at the interpretation threshold



The maximal limits on the control chart are:

+/- 0.70 log10 for the tracer using qPCR

+/- 2.33 Ct for the tracer at interpretation threshold using rPCR

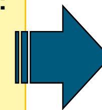
↳more affordable cost than qPCR

2 — Contributions of the QF-RNL to the serological ELISA tests

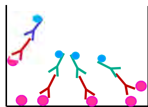
Available serological methods

No reference method (no collection of samples, no validation of methods):

- ✓ ELISA and IFI generally shown to be more sensitive than CF
- ✓ IFI less robust than ELISA (operator dependent IFI reading)
- ✓ CF is no longer prescribed by the OIE for international trade



3 semi-quantitative ELISA kits



Indirect ELISA kit	IDEXX	PrioCHECK	ID Screen
Antigen (*C. burnetii* strain)	Nine Mile (reference)	Ovine Cb (French isolate)	Bovine Cb (French isolate)
Conjuguate (HRP) binding	To ruminant IgG	To multi-species IgG (protein G)	
Thresholds set by manufacturers (anti-Cb antibody rates in %OD, Optical Density)	Negatif < 30 OD% 30 ≤ Doubful < 40 Positive ≥ 40	Negatif ≤ 40 OD% 40 < Positive + ≤ 100 100 < + + ≤ 200 200 < + + + ≤ 300 Positive ++++ > 300	Negatif ≤ 40 OD% 40 < Doubful ≤ 50 50 < Positive ≤ 80 Strongly positive > 80

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

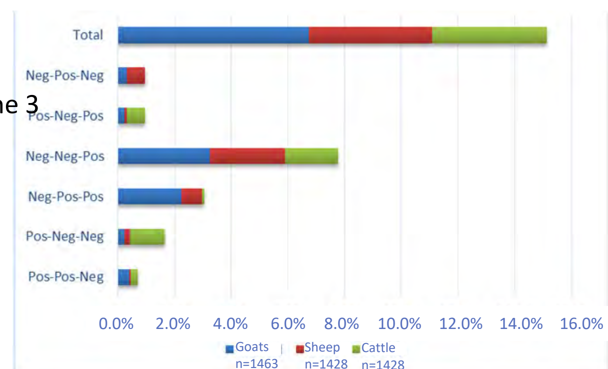
Discordant results between available ELISA tests

Example

4319 sera analyzed under the same conditions with the 3 kits

⇨ 15% of discordant results !

(other than Neg-Neg-Neg and Pos-Pos-Pos)



Performances for harmonization ?

- Difference in specificity ? *Serological Ag involved ?*
- Difference in sensitivity ? *Threshold not set correctly ?*
- Bad reproducibility (precision) and trueness ? *Variability between kit batches ?*

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Calibration of kit batches

Reference Material-calibrating (RNL)

Preparation:
2 levels around the threshold of each tested kit

Idexx->1:1 & 1:2
PrioCHECK->1:2 & 1:4
ID Screen->1:4 & 1:8

RM Certificate

Experimental plan

Tested batch :

- ✓ 3 independant assays (1 to 3 operators),
- ✓ 20 repetitions minimum (for each level)

Calculation of variability parameters (RNL file) **:**

- mean (trueness)
- repeatability
- inter-series SD
- reproducibility (limits)
- coefficient of variation

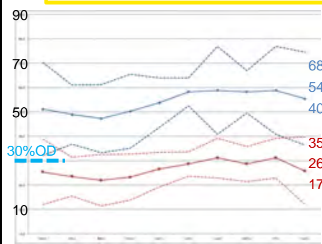
Kit batch certificate

Batch of External Reference Serum (French MRE) : SCE1/2011-12
Lot du MRE (français) utilisé

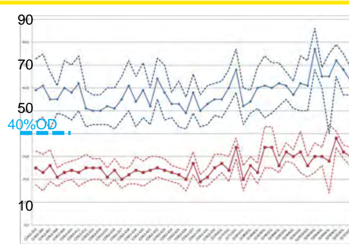
MRE dilution *Niveaux du MRE calibrant FQ*	Titre *Titre*	Low limit of 2 Standard Deviation *Limite basse de 2 écart-types*	High limit of 2 Standard Deviation *Limite haute de 2 écart-types*	Repeatability *Répétabilité (%CV)*	Reproducibility *CV (%) de fidélité intermédiaire (FI)*
1/2	54	43	65	8.3	10.3
1/4	24	18	30	8.4	13.0

Calibration of kit batches

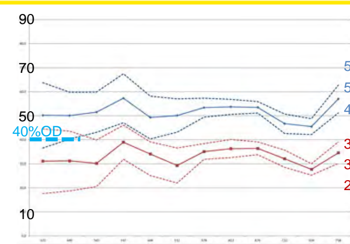
Monitoring of between-batch standardization : data reported on batch certificates (since 2012)



ELISA 1 (27 batches*)
Threshold at 30%OD
RM at 1:1 & 1:2
U = 14.0 & 9.1
CV% = 11.4 & 10.4



ELISA 2 (45 batches)
Threshold at 40%OD
RM at 1:2 & 1:4
U = 9.0 & 5.2
CV% = 7.6 & 6.2

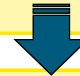


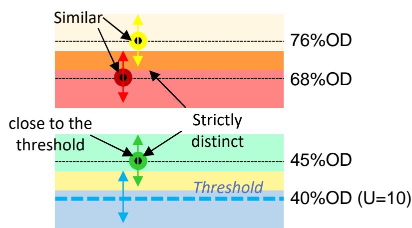
ELISA 3 (12 batches)
Threshold at 40%OD
RM at 1:4 & 1:8
U = 6.7 & 6.3
CV% = 7.2 & 10.8

Define the maximal limits and the expected values

Importance of measurement calibration for semi-quantitative ELISA

- **Acceptance criteria of precision (limits) and trueness (expected value) at the positivity threshold**
  - data to help with batch acceptance by the laboratory user
  - a single control chart monitored for successive batches





- **A standardization over time for each ELISA kit =** Threshold and uncertainty at threshold are controlled
  - Similar and strictly distinct results / **Comparisons or evolutions (statistical differences)**
  - the "Doubtful" in the diagnosis / **"Positive or negative close to the threshold"**

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

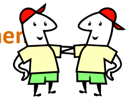
Conclusions

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

Take home messages

- The **tools** (reference materials, standardization, validations, adoptions, bilateral tests, control charts, inter-laboratory tests) contribute to the **reliability of methods**
  - within each laboratory's environment
  - within a network of laboratories

  Thus, to determine whether the results could be gathered at national level and used for ***infection control, epidemiological investigation or monitoring*** *(e.g. for new AHL)*
- As NRL, we encourage the presentation of **results with their level of uncertainty**, inherent in any measurement method. This is also a performance characteristic.
- The exchanges* with diagnostic laboratories and kit producers provide means for **proactive improvemen**



**Rousset et al, Euroreference 2017:* https://euroreference.anses.fr/sites/default/files/17_12_ED_ER_03-1_ROUSSET.PDF

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

Work in progress

PCR (real time) :

Progress has been rapid

Reference Material
- ☒ validation (bacteria and gDNA)
- ☒ clinical treshold *(in test)*

Validation (AFNOR standard)
- ☒ methods based on kits (list)
- *☐☒ other matrices (in progress)*

Vigilance on performances
- ☒ adoptions
- ☒ control charts (qPCRq et rPCR)
- ☒ ILPT (since 2017)

ELISA (indirect) :

A delay in standardization

Reference Material
- ☒ for batch calibration (around set thresholds)
- ☐ at detectability (threshold harmonization)

Validation (scientific publications)
- ☐ comparative evaluation in progress
- ☐ towards reference tools ?

Vigilance on performances
- ☒ bilateral tests (using qualified ILPT panels)
- *☐☒ batches std (control chart to improve)*
- ☒ ILPT (since >30 years, ELISA since 2001)

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

Thanks for your attention

VetAgro Sup

Available Q fever diagnostic methods and objectives of application

Table 1. Test methods available for the diagnosis of Q fever and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
PCR	+++	n/a	+++	+++	++	+[1]
Culture	+	n/a	+	–	+	–
Staining	+	n/a	+	+	+	–
Genotyping	n/a	n/a	n/a	n/a	++	n/a
Detection of immune response						
ELISA	+++	n/a	+++	++	+++	+++
IFA	++	n/a	++	++	++	++
CFT	–	n/a	–	++	+	+

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = not applicable.

From the Chapter "Q fever" of the OIE manual https://www.oie.int/en/standard-setting/terrestrial-manual/access-online/

(Manual of Diagnostic Tests and Vaccines for Terrestrial Animals)

Diagnosis is realized at group level.

(expert reports: French ACERSA 2007, EU EFSA 2010)

⇨ No test prescribed for individual diagnosis

In France, official purposes are :

- ⇨ **Differential diagnosis of abortions *(OSCAR)***
- ⇨ **Investigations linked to human clusters *(State Note)***
- ⇨ **Transversal epidemiological survey**

For other purposes, we have to:

-define methods to be used, sampling and results interpretations (sheme for free status, movement and introduction, trade)

-develop other tests (early test, DIVA / vaccine).

Summary of main diagnostic schemes in France

Purpose	Samples	Targeted animals (Unit sample = herd or kidding group)	Used tests	Basis for interpretation	References
Abortive diagnosis	2 vaginal swabs, 6 sera from cattle 2 vaginal swabs, 10 sera from sheep or goats	max of females having aborted (for < 8 days -> PCR)	qPCR or rPCR ELISA	Clinical threshold (10ᵉ4 or 10ᵉ3 bact/swab if individual or pool of 3) If one PCR-results is positive, check a 50% seroprevalence	ACERSA (2007) NS DGAL (2010-8262) EFSA (2010) OSCAR (2017->)
Investigation of shedding herds *(clustered human cases)*	20 sera (stratified by 3 ages classes) 15 vaginal swabs *± Environmental samples **	max of females having kidding or aborted for < 1 month (10 primiparous and 5 females 2 to 4 years old, 5 over 4 years old) max of females having kidding or aborted for < 1 month (10 primiparous and 5 multiparous if possible) *a dust sample on a cloth per building and per group of female having kidding during the exposure period*	ELISA qPCR *qPCR*	Analyze sera first Semi- and quantitative data If one ELISA-result is positive (threshold at 10e4) per herd *or if one dust result is positive* *Threshold at 10e4 bact/swab* *Analyze dust first* *Quantitative data*	NS DGAL (2011-8124) *in revision*

**No threshold in terms of transmission risk has yet been established (the use of dust as a risk indicator is at the research stage).*

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

Sanitary situation of Q fever in ruminants in France, *rapid overview*

No monitoring in France *(cat E within new AHL: in preparation)*

First large survey in ten departments (2012-2015 / 10 labs)

Q fever abortive episodes

Species	Total (episodes)	% Pos	Min-Max (department)
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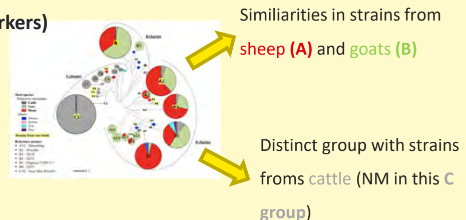
Gache *et al*, Epidemiol. Infect. 2017

Database of > 300 *C. burnetii* genotypes

(samples from abortions / 2006-2015 / 9 labs)

3 main MLVA genogroups (17 markers)



Similiarities in strains from sheep **(A)** and goats **(B)**

Distinct group with strains froms cattle (NM in this C group)

Joulié *et al*, Infect Genet Evol. 2017

Diagnostic methods for Q fever in ruminants: contribution to the validation of performances and to their harmonization

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

21. bis 22. April 2021

Online-Tagung veranstaltet vom
Friedrich-Loeffler-Institut, Naumburger Straße 96 a, Jena

Organisatoren:

Dr. Christiane Schnee, NRL Chlamydiose
Dr. Katja Mertens-Scholz, NRL Q-Fieber
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

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RESEARCH ARTICLE — Open Access

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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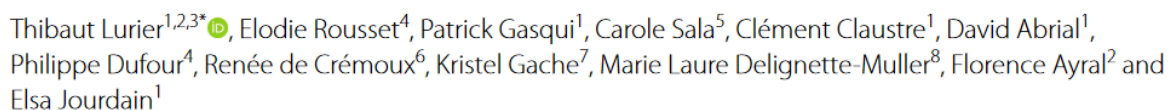


Serological diagnosis of *Coxiella burnetii* infection

- **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)

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

$\Rightarrow$ **6 DF for 6 parameters to assess** $(P_1\ P_2\ Se_1\ Sp_1\ Se_2\ Sp_2)$

$\Rightarrow$ Then an analytical solution exists to assess all 6 parameters.

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.

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

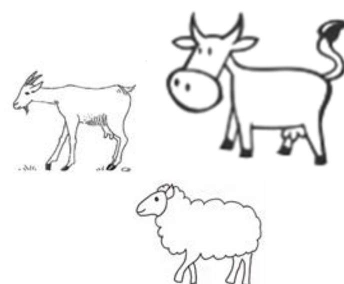
Limits of previous LCM studies

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

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

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**



Study sample

Epidemiol. Infect. (2017), **145**, 3131–3142. © Cambridge University Press 2017
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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

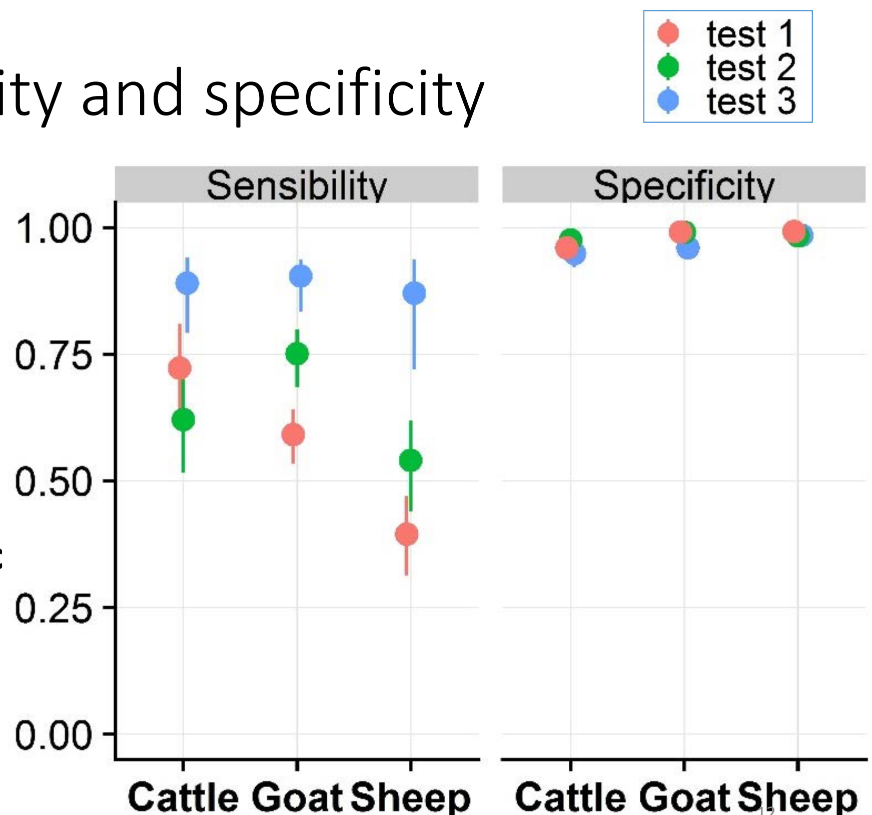
species	Number of	A	B	C	D	E	F	G	H	I	J
		Department									
cattle	herds	10	12	11	13	12	12	10	12	13	1
	animals	143	157	150	181	155	161	155	150	152	9
goat	herds	11	11	12	12	11	9	11	1	12	13
	animals	154	161	201	175	152	134	146	11	153	187
sheep	herds	11	11	10	10	11	11	11	10	11	3
	animals	165	162	149	145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

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**

Results : test sensitivity and specificity



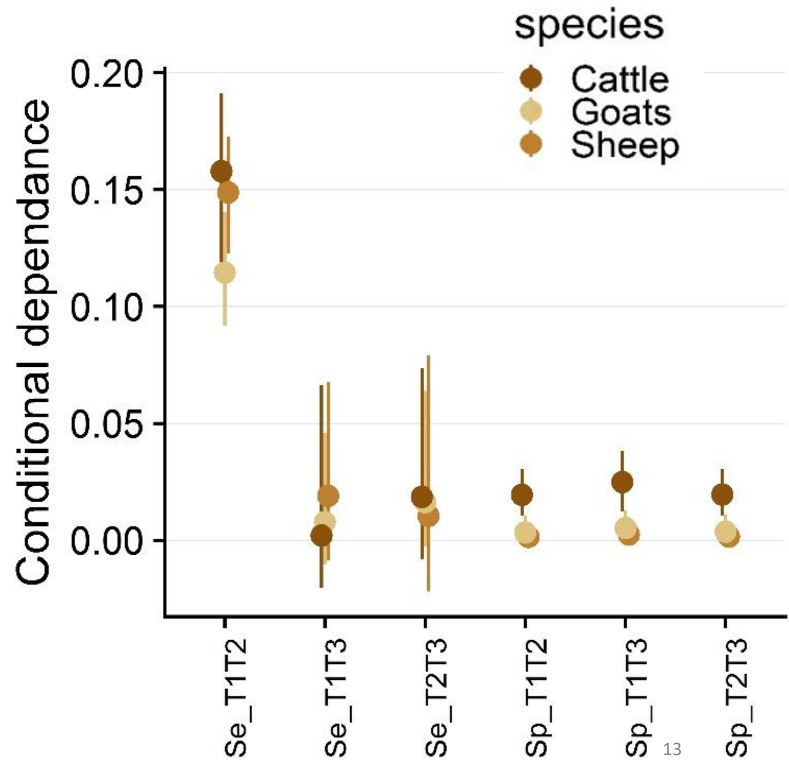
- **Sensitivity** values are **weak** (particularly in sheep)
- **All tests** seem **highly specific**
  - Slightly lower in cattle
- *Test 3* is the **most sensitive** in all species but also the **least specific**
- All tests are not identical in each ruminant species

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)

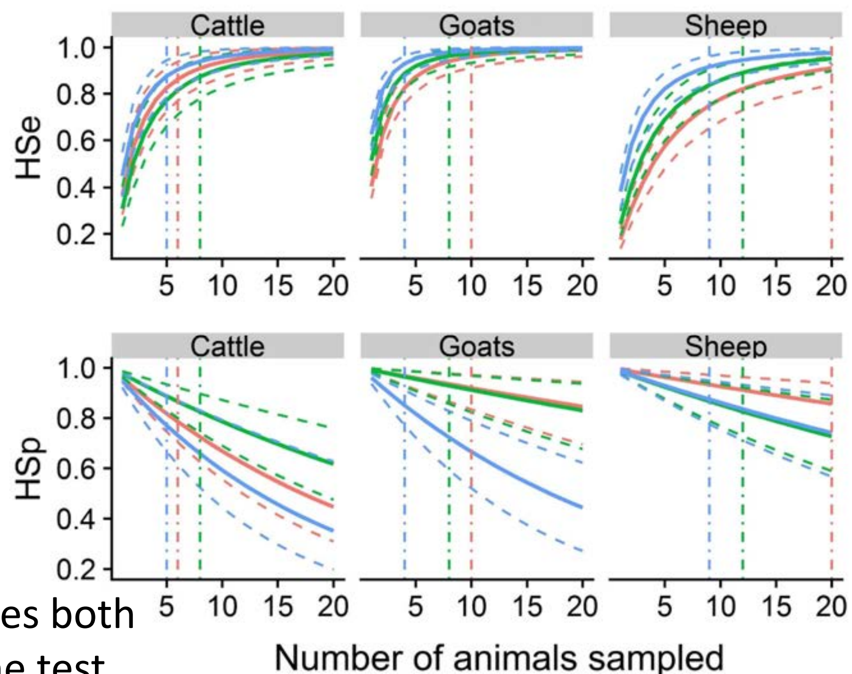
⇒ Diagnostic errors in truly seropositive animals are likely to **occur simultaneously for T1 and T2**

⇒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

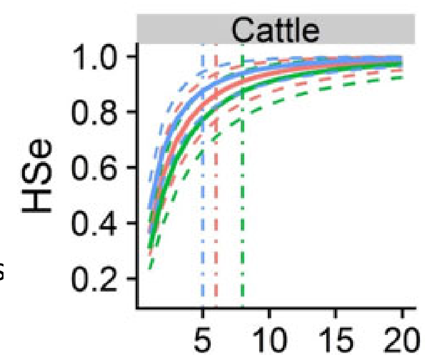
Perspective : Mandatory surveillance (Cat E)

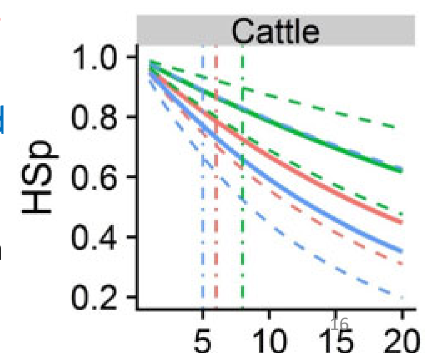
- If we want to detect seropositive herds
- Which test should we use?
  - Not the same in every species…
- How many animals should we sample?
  - If we sample many animals –> HSp decreases

  ⇒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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