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Estimation of sensitivity and specificity of five serological tests for the diagnosis of porcine brucellosis

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ABSTRACT

While serological tests are essential in surveillance and control programs of animal diseases, to date none of the common serological tests approved in the EU (complement fixation test or Rose-Bengal test) has been shown to be reliable in routine individual diagnosis of porcine brucellosis, and some more recent tests like ELISA have not been fully evaluated yet. In the absence of a gold standard, this study allowed the estimation of sensitivities and specificities of these tests with a Bayesian approach using Markov Chain Monte Carlo algorithms.

The pig population that was tested included 6422 animals from Metropolitan France. Serum samples were collected from a large population of pigs, representative of European swine population and tested with five brucellosis serological tests: Rose-Bengal test (RBT), fluorescence polarization assay (FPA), indirect ELISA (I-ELISA) and two competitive ELISAs (C-ELISA). The sensitivity and the specificity of each test were estimated. When doubtful results were excluded, the most sensitive and specific test was C-ELISA$_2$ (Se C-ELISA$_2$ = 0.964, [0.907; 0.994], 95% credibility interval (CrI); Sp C-ELISA$_2$ = 0.996, [0.982; 1.0], 95% CrI).

When doubtful results were considered as negative, C-ELISA$_2$ was still the most sensitive and specific test (Se C-ELISA$_2$ = 0.960, [0.896; 0.994], 95% CrI and Sp C-ELISA$_2$ = 0.994, [0.977; 0.999], 95% CrI). The same conclusions were reached when doubtful results were considered as positive (Se C-ELISA$_2$ = 0.963, [0.904, 0.994], 95% CrI and Sp C-ELISA$_2$ = 0.996, [0.986; 1.0], 95% CrI).

1. Introduction

In France, until the 1970s, porcine brucellosis was limited to small domestic farms. Nowadays, while the pig industry is free of the disease, outbreaks occur sporadically. From 1993 to 2009, 60 outbreaks, of which 54 were

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confirmed as due to *Brucella suis* biovar 2, were reported in 30 French districts. All of them concerned outdoor rearing farms and available data suggest that currently wild boars or hares are the main source of infection of domestic pigs (EFSA, 2009).

Diagnosis of porcine brucellosis is based on the observation of clinical signs (*i.e.* abortion, infertility, orchitis or more rarely arthritis), associated with positive serology and/or *Brucella* isolation. Currently, in France and the EU, serological surveillance of porcine brucellosis is required only for breeding hogs and intra-community trade. However, in France, as soon as a brucellosis outbreak is confirmed, the corresponding farm is depopulated.

No reference test (or "gold standard") is currently available and, to date, none of the available serological tests has been shown to be reliable in routine individual diagnosis to be used as an only test. Isolation of *B. suis* is a good positive standard, but no good negative standard has been identified. Commonly used serological tests, *e.g.* Rose-Bengal test (RBT) and complement fixation test (CFT) (OIE, 2009), have an acceptable sensitivity that allows the use of the test to classify herds (EFSA, 2009). However, tests available and prescribed by the OIE (World Organisation for Animal Health) suffer from a lack of specificity (OIE, 2008, 2009). ELISA tests could replace the RBT and CFT because of their high sensitivity and specificity but have not yet been fully evaluated and standardized for use in pigs. Some studies have been carried out to estimate the sensitivity and the specificity of these tests, but in specific contexts and different species (Gall et al., 1998, 2003; Silva Paulo et al., 2000; Nielsen, 2002; Gall and Nielsen, 2004; Muñoz et al., 2005; EFSA, 2009; Muma et al., 2009; Nielsen et al., 2008) and only a few studies dealt with screening in pigs (Silva Paulo et al., 2000; Muma et al., 2009; Nielsen et al., 2008).

This makes difficult the interpretation of positive results obtained from animals controlled for trade or artificial insemination purpose and hampers the implementation of surveillance programs with brucellosis-free status definition.

Our study aimed at estimating the sensitivity and the specificity of five serological tests for the diagnosis of porcine brucellosis. We studied a population of French pigs, with genetic characteristics and breeding conditions similar to those of most pigs from European countries, allowing an extrapolation to this general population.

To do so, we used a Bayesian approach implemented via Markov Chain Monte Carlo algorithms. This approach is frequently used in human and veterinary medicine (Meyer et al., 2009) to estimate sensitivities and specificities of tests in the absence of a gold standard (Branscum et al., 2005; Rutjes et al., 2007).

2. Materials and methods

2.1. Data collection

The study included sera from 6422 pigs in Metropolitan France. Pigs were randomly selected from various farms, considered as free or infected. 1799 pigs from 32 outdoor farms were included in the analysis. In these 32 farms, an outbreak of porcine brucellosis, confirmed by the isolation of *B. suis* biovar 2 on at least one animal, had occurred 6–12 months earlier. In these farms, all pigs were tested. The number of animals tested ranged from 1 to 181 animals (mean = 56 animals; median = 35 animals).

The remaining 4623 pigs (hogs) were selected primarily in officially brucellosis free artificial insemination (AI) units (96.6%; 4466 pigs) and the rest from industrial pig breeding farms (3.4%; 157 pigs). These 4623 pigs were randomly selected among the breeding animals tested by the National Laboratory for the Control of Breeding Animals. They did not present any clinical evidence of brucellosis and were reared without any contact with wild reservoirs of brucellosis. Most of them were Large White, French Landrace and Piétrain.

Each serum sample was subjected to five serological tests. Blood samples were collected from 1997 and 2005, specifically for this research project.

2.2. Diagnostic tests

We studied five serological tests: (i) Rose-Bengal test (RBT), Institut Pourquier (France); (ii) fluorescence polarization assay (FPA), *B. abortus* antibody test kit, Diachemix/Prionics (Switzerland); (iii) indirect enzyme immunoassay (I-ELISA), Chekit *B. suis*, Idexx-Bommeli (Switzerland); (iv) competitive enzyme immunoassay (C-ELISA$_1$ and C-ELISA$_2$), respectively SVANOVIR® *Brucella*-Ab C-ELISA, Svanova (Sweden) and Compelisa, VLA (United Kingdom). The general principles of these tests are described in OIE (2009). C-ELISA$_1$ and C-ELISA$_2$ are based on different immunological principles: (i) the anti-S-LPS (smooth lipopolysaccharide major outer-membrane antigen) monoclonal antibody, competing with the pig antibody for binding to the S-LPS coated on the plate during the first incubation step is different and (ii), while in C-ELISA$_1$ the amount of monoclonal antibody bounded to the antigen is measured thanks to an anti-mouse conjugate, in C-ELISA$_2$, the monoclonal antibody is itself conjugated to the enzyme. To our knowledge, the properties of these two C-ELISA had not been compared but were expected to be different.

The RBT antigen was standardized according to OIE and EU requirements (OIE, 2008; EU, 2008). Cut-offs were as recommended by the tests manufacturers for the other tests (FPA = 20; I-ELISA > 70%; C-ELISA$_1 \geq 30\%$; C-ELISA$_2 \leq 60\%$). With FPA and I-ELISA, results could be "negative", "positive" or "doubtful".

Serological analyses were performed by a unique laboratory, the EU/OIE/FAO Brucellosis Reference Laboratory (ANSES, Maisons-Alfort; France) in order to limit bias linked to the tests performance. Samples were identified by a code number and tests were performed without knowing the results to the other tests. Results to the five tests were compared after having been merged in a database.

Animals with doubtful results were first excluded in the main analysis. They were further included in the model, considering them as either (i) negative results or (ii) positive results, in order to study their influence on the estimation of the parameters of interest.

Table 1
Parameters of the beta (a, b) prior distributions.

Parameter of interest		Lower limit 95%	Mean	Beta (a, b)	
				a	b
RBT	Se	0.75	0.86	33.37	5.432
	Sp	0.76	0.88	24.93	3.400
FPA	Se	0.87	0.92	107.4	9.341
	Sp	0.91	0.97	30.39	0.940
I-ELISA	Se	0.94	0.98	47.04	0.960
	Sp	0.94	0.98	47.04	0.960
C-ELISA	Se	0.91	0.96	58.02	2.418
	Sp	0.76	0.92	9.660	0.840

2.3. Statistical methods

A Bayesian approach was used to estimate the sensitivity and the specificity of the five tests without gold standard.

The Bayesian approach is based on the introduction of prior information, from literature and experts' advice. Since all parameters (sensitivity of the five tests, specificity of the five tests and prevalence in the population) were probabilities, their prior distributions were modelled as beta distributions (Johnson and Gastwirth, 1991; Praet et al., 2006).

To construct a beta prior distribution, the most probable value of the parameter (or "best guess"; θ_0) and a "lower limit" (θ_L; *i.e.* a value for which the experimenter is 95% sure that the parameter will be larger) were determined (Enøe et al., 2000).

As sensitivities and specificities of screening tests in swine were not well known, diffuse prior distributions were introduced in the model. Means and lower limits were determined using literature data on brucellosis tests in various species (cattle, swine and small ruminants). Mean sensitivities ranged from 0.597 to 1 for RBT, 0.72 to 0.993 for FPA, 0.96 to 1 for I-ELISA and 0.905 to 0.988 for C-ELISA. Mean specificities ranged from 0.81 to 1 for RBT, 0.93 to 0.984 for FPA, 0.938 to 1 for I-ELISA and 0.60 to 1 for C-ELISA (Gall et al., 1998, 2003; Silva Paulo et al., 2000; Nielsen, 2002; Gall and Nielsen, 2004; Muñoz et al., 2005; EFSA, 2009; Muma et al., 2009; Nielsen et al., 2008).

Then, parameters a and b were calculated for each parameter (Enøe et al., 2000). In the case of beta (a, b) priors, the mode of the distribution is given by the formula $\theta_0 = (a-1)/(a+b-2)$ when $a > 1$ and zero otherwise. Solving this equation, $a = 1 + \theta_0\ (b-2)/(1-\theta_0)$. So for a given guess ($\theta_0$) and a given value of b, a is determined. Once a pair (a, b) has been obtained, software like R can be used to determine whether the appropriate percentile of the specified beta (a, b) distribution is θ_0. If this constraint is not satisfied, another b is selected and the appropriate a is calculated. The process is repeated until a beta (a, b) distribution that satisfies the constraints posed by the prior specification is identified. The distribution is then presented graphically to the subject matter experts for verification; if not satisfactory, the process is repeated with another type of distribution. If $a < 1$ is appropriate, then since the formula for the mode cannot be used, one can equate $\theta_0 = a/(a+b)$ (which is the mean of the beta distribution) and proceed as described above. Best guesses, upper limits and distribution parameters are provided in Table 1.

Prevalence in the population was unknown: beta (1, 1) uninformative distribution (which correspond to uniform distribution between 0 and 1) was used as prior for prevalence.

Posterior distributions of the parameters were obtained using Markov chain Monte Carlo (MCMC) techniques (Gelman et al., 1995), and summarized using the mean and 95% credible interval (0.25th and 0.95th percentiles) (Enøe et al., 2000; Branscum et al., 2005). The analysis was performed with program WinBUGS (Lunn et al., 2000).

The convergence of the MCMC algorithm was assessed by checking the stabilisation of the plots of iterate values of parameters, after a given number of samples and by running multiple chains from dispersed starting values. Early samples were discarded as a "burn-in" period (1000 samples among 51,000).

The model presumed that the tests were conditionally dependent and allowed to estimate the Se and Sp covariances of the tests (Gardner et al., 2000). It was an adaptation of the multiple tests model developed by Berkvens et al. (2006). The sensitivity and the specificity of the five tests were estimated (five test-one population model). The number of parameters of the model was 63 (prevalence, sensitivity and specificity of the first test, two conditional sensitivities and two conditional specificities for the second test, four conditional sensitivities and four conditional specificities for the third test, eight conditional sensitivities and eight conditional specificities for the fourth test, and 16 conditional sensitivities and 16 conditional specificities for the fifth test). This model was inestimable because the data (32 'classes' of test results) provide only 31 degrees of freedom. This issue was handled introducing informative priors in the model (Dendukuri and Joseph, 2001; Rutjes et al., 2007). The components of the vector, defined as conditional probabilities, are given in Table 2.

The multinomial probability of test-outcome combinations is given in Appendix 1. Parameters of interest, *i.e.* sensitivities (Se) of the five tests, specificities (Sp) of the five tests (Appendix 2) and sensitivity and specificity covariances (CovSe and CovSp, respectively) were defined as

Table 2
Conditional probabilities for a five test-model.

Prevalence = $P(D^+)$	*p1*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^+\cap T_3^+\cap T_4^-)$	*p33*
$Se_1 = P(T_1^+\|D^+)$	*p2*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^+\cap T_3^-\cap T_4^+)$	*p34*
$Sp_1 = P(T_1^-\|D^-)$	*p3*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^+\cap T_3^-\cap T_4^-)$	*p35*
$P(T_2^+\|D^+\cap T_1^+)$	*p4*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^-\cap T_3^-\cap T_4^+)$	*p36*
$P(T_2^+\|D^+\cap T_1^-)$	*p5*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^-\cap T_3^+\cap T_4^-)$	*p37*
$P(T_2^-\|D^-\cap T_1^-)$	*p6*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^-\cap T_3^-\cap T_4^+)$	*p38*
$P(T_2^-\|D^-\cap T_1^+)$	*p7*	$P(T_5^+\|D^+\cap T_1^+\cap T_2^-\cap T_3^-\cap T_4^-)$	*p39*
$P(T_3^-\|D^+\cap T_1^+\cap T_2^+)$	*p8*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^+\cap T_3^+\cap T_4^+)$	*p40*
$P(T_3^-\|D^+\cap T_1^-\cap T_2^-)$	*p9*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^+\cap T_3^+\cap T_4^-)$	*p41*
$P(T_3^-\|D^+\cap T_1^-\cap T_2^+)$	*p10*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^+\cap T_3^-\cap T_4^+)$	*p42*
$P(T_3^-\|D^+\cap T_1^-\cap T_2^-)$	*p11*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^+\cap T_3^-\cap T_4^-)$	*p43*
$P(T_3^-\|D^+\cap T_1^-\cap T_2^-)$	*p12*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^+\cap T_4^+)$	*p44*
$P(T_3^-\|D^+\cap T_1^-\cap T_2^+)$	*p13*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^+\cap T_4^-)$	*p45*
$P(T_3^-\|D^-\cap T_1^-\cap T_2^-)$	*p14*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^-\cap T_4^+)$	*p46*
$P(T_3^-\|D^-\cap T_1^+\cap T_2^+)$	*p15*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^-\cap T_4^-)$	*p47*
$P(T_4^+\|D^+\cap T_1^+\cap T_2^+\cap T_3^+)$	*p16*	$P(T_5^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^-\cap T_4^-)$	*p48*
$P(T_4^+\|D^+\cap T_1^+\cap T_2^+\cap T_3^-)$	*p17*	$P(T_5^-\|D^-\cap T_1^-\cap T_2^-\cap T_3^-\cap T_4^+)$	*p49*
$P(T_4^+\|D^+\cap T_1^+\cap T_2^-\cap T_3^+)$	*p18*	$P(T_5^-\|D^-\cap T_1^-\cap T_2^-\cap T_3^+\cap T_4^-)$	*p50*
$P(T_4^+\|D^+\cap T_1^+\cap T_2^-\cap T_3^-)$	*p19*	$P(T_5^-\|D^-\cap T_1^-\cap T_2^-\cap T_3^+\cap T_4^+)$	*p51*
$P(T_4^+\|D^+\cap T_1^-\cap T_2^+\cap T_3^+)$	*p20*	$P(T_5^-\|D^-\cap T_1^-\cap T_2^+\cap T_3^-\cap T_4^-)$	*p52*
$P(T_4^+\|D^+\cap T_1^-\cap T_2^+\cap T_3^-)$	*p21*	$P(T_5^-\|D^-\cap T_1^-\cap T^+_2\cap T_3^-\cap T_4^+)$	*p53*
$P(T_4^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^+)$	*p22*	$P(T_5^-\|D^-\cap T_1^-\cap T_2^+\cap T_3^+\cap T_4^-)$	*p54*
$P(T_4^+\|D^+\cap T_1^-\cap T_2^-\cap T_3^-)$	*p23*	$P(T_5^-\|D^-\cap T_1^-\cap T_2^+\cap T_3^+\cap T_4^+)$	*p55*
$P(T_4^-\|D^-\cap T_1^-\cap T_2^-\cap T_3^-)$	*p24*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^-\cap T_3^-\cap T_4^-)$	*p56*
$P(T_4^-\|D^-\cap T_1^-\cap T_2^-\cap T_3^+)$	*p25*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^-\cap T_3^-\cap T_4^+)$	*p57*
$P(T_4^-\|D^-\cap T_1^-\cap T_2^+\cap T_3^-)$	*p26*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^-\cap T_3^+\cap T_4^-)$	*p58*
$P(T_4^-\|D^-\cap T_1^-\cap T_2^+\cap T_3^+)$	*p27*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^-\cap T_3^+\cap T_4^+)$	*p59*
$P(T_4^-\|D^-\cap T_1^+\cap T_2^-\cap T_3^-)$	*p28*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^+\cap T_3^-\cap T_4^-)$	*p60*
$P(T_4^-\|D^-\cap T_1^+\cap T_2^-\cap T_3^+)$	*p29*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^+\cap T_3^-\cap T_4^+)$	*p61*
$P(T_4^-\|D^-\cap T_1^+\cap T_2^+\cap T_3^-)$	*p30*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^+\cap T_3^+\cap T_4^-)$	*p62*
$P(T_4^-\|D^-\cap T_1^+\cap T_2^+\cap T_3^+)$	*p31*	$P(T_5^-\|D^-\cap T_1^+\cap T_2^+\cap T_3^+\cap T_4^+)$	*p63*
$P(T_5^+\|D^-\cap T_1^+\cap T_2^+\cap T_3^+\cap T_4^+)$	*p32*		

functions of the model parameters. For detailed information on conditional dependence in this model, we refer readers to Berkvens et al. (2006) and Praet et al. (2006). The WinBUGS code is available in Appendix 3.

To compare the models, we used the deviance information criterion (DIC; Spiegelhalter et al., 2002). Essentially, the DIC is a diagnostic tool that balances the requirements of model fit and low complexity. Typically, as models get more complex by the addition of extra parameters, their fit improves. The DIC diagnosis therefore penalizes additional parameters so that a parsimonious model is chosen, and the smaller the DIC value, the better the compromise is. One advantage is that the DIC can be calculated directly in WinBUGS from the chains produced by an MCMC run and can be applied in a variety of contexts (Spiegelhalter et al., 2002).

The results were compared by examining their 95% credibility intervals (CrI). According to Enøe et al. (2000), a credibility interval can be interpreted to mean that one is 95% sure that the corresponding parameter is in this interval. If two credibility intervals do not overlap, the parameters can be considered as different.

3. Results

3.1. Description of tests results

In the "free" population, most of the pigs (84.1%) showed negative results with all the five tests. In the "infected" population, 56.9% of the pigs had a positive outcome in at least one test.

697 pigs (10.9%) of 6422 got doubtful results. Most of them belonged to presumed free population. The results of the 5725 pigs without doubtful results are summarized in Table 3.

3.2. Estimations of sensitivities and specificities when doubtful results were excluded

The results are displayed in Table 4. Estimated prevalence was 0.121 ([0.105; 0.134], 95% CrI). C-ELISA$_2$ was the most sensitive and specific test (Se C-ELISA$_2$ = 0.964, [0.907; 0.994], 95% CrI; Sp C-ELISA$_2$ = 0.996, [0.982; 1.0], 95% CrI). C-ELISA$_1$ was a little less sensitive and specific than C-ELISA$_2$ (Se C-ELISA$_1$ = 0.953, [0.906; 0.989], 95% CrI and Sp C-ELISA$_1$ = 0.956, [0.942; 0.966], 95% CrI).

I-ELISA had the worst sensitivity (Se I-ELISA = 0.663, [0.607; 0.710], 95% CrI) and FPA the worst specificity (Sp FPA = 0.930, [0.917; 0.941], 95% CrI).

Our analysis indicated low to moderate average sensitivity and specificity covariances, with only one negative covariance ($CovSe_{I\text{-}ELISA\text{-}RBT}$). The average estimates of CovSe and CovSp ranged from −0.0015 to 0.0362. C-ELISA$_1$ and RBT had the higher sensitivity covariance ($CovSe_{C\text{-}ELISA1\text{-}RBT}$ = 0.0210; [0.00319; 0.0470], 95% CrI) and I-ELISA and FPA had the higher specificity covariance ($CovSp_{I\text{-}ELISA\text{-}FPA}$ = 0.0362 [0.0198; 0.0329], 95% CrI). The 95% CrI of CovSe included 0 for five pairs of tests and the 95% CrI of CovSp included 0 for one pair of tests. The fourteen other intervals included strictly positive values. Results are detailed in Appendix 4.

Table 3
Cross-classified test results in 5725 pigs in two subpopulations (697 pigs with doubtful results in FPA and/or I-ELISA excluded).

Tests results					Subpopulation	
RBT	FPA	I-ELISA	C-ELISA$_1$	C-ELISA$_2$	"Infected"	"Free"
0	0	0	0	0	700	3889
1	0	0	0	0	29	26
0	1	0	0	0	47	43
1	1	0	0	0	17	12
0	0	1	0	0	3	1
1	0	1	0	0	0	0
0	1	1	0	0	9	33
1	1	1	0	0	1	6
0	0	0	1	0	7	20
1	0	0	1	0	8	0
0	1	0	1	0	7	9
1	1	0	1	0	64	8
0	0	1	1	0	1	0
1	0	1	1	0	4	0
0	1	1	1	0	20	4
1	1	1	1	0	70	0
0	0	0	0	1	0	10
1	0	0	0	1	0	0
0	1	0	0	1	1	0
1	1	0	0	1	3	3
0	0	1	0	1	0	0
1	0	1	0	1	0	0
0	1	1	0	1	2	4
1	1	1	0	1	3	2
0	0	0	1	1	0	0
1	0	0	1	1	3	1
0	1	0	1	1	7	5
1	1	0	1	1	167	24
0	0	1	1	1	3	0
1	0	1	1	1	12	0
0	1	1	1	1	46	2
1	1	1	1	1	373	16
			Total		1607	4118

0: negative result; 1: positive result.

Table 4
Estimates of the sensitivity and the specificity of the five tests, doubtful results excluded (95% credibility intervals (95% CrI) are also provided).

Parameter		Average estimate [95% CrI]
RBT	Se	0.876 [0.835; 0.913]
	Sp	0.951 [0.939; 0.959]
FPA	Se	0.937 [0.890; 0.970]
	Sp	0.930 [0.917; 0.941]
I-ELISA	Se	0.663 [0.607; 0.710]
	Sp	0.969 [0.958; 0.976]
C-ELISA$_1$	Se	0.953 [0.906; 0.989]
	Sp	0.956 [0.942; 0.966]
C-ELISA$_2$	Se	0.964 [0.907; 0.994]
	Sp	0.996 [0.982; 1.0]
Prevalence		0.121 [0.105; 0.134]

3.3. Estimations of sensitivities and specificities when doubtful results were taken into account

Introducing the doubtful results in the study did not change the results.

When doubtful results were considered as negative results, C-ELISA$_2$ remained the most sensitive and specific (Se C-ELISA$_2$ = 0.960, [0.896; 0.994], 95% CrI and Sp C-ELISA$_2$ = 0.994, [0.977; 0.999], 95% CrI). I-ELISA was still the less sensitive test (Se I-ELISA = 0.664 [0.612; 0.717], 95% CrI) and FPA the less specific one (Sp FPA = 0.929 [0.913; 0.940], 95% CrI).

When doubtful results were considered as positive results, the same conclusions were reached (Se C-ELISA$_2$ = 0.963, [0.904, 0.994], 95% CrI and Sp C-ELISA$_2$ = 0.996, [0.986; 1.0], 95% CrI; Se I-ELISA = 0.687 [0.639; 0.730], 95% CrI and Sp FPA = 0.830 [0.817; 0.841], 95% CrI).

Values of DIC were respectively 168, 173 and 174 for the three hypotheses (doubtful excluded, considered as negative or considered as positive).

3.4. Sensitivity analysis results

To analyse the sensitivity of the model, we repeated the analyses by making the prior distributions for the sensitivity and specificity parameters progressively more diffuse (Enøe et al., 2000). Average estimates of parameters were minimally affected and the most/least sensitive and specific tests were the same ones as in the main analysis. For instance, when sensitivities and specificities of the tests were modelled as beta (1, 1) distributions, C-ELISA$_2$ sensitivity was 0.867 (95% CrI: [0.447; 0.997]), and C-ELISA$_2$ specificity was 0.977 (95% CrI: [0.908; 1.0]).

We performed an analysis estimating the parameters for only three or four tests among five (respectively three test-one population and four test-one population models). Parameters estimates were not different from the results of the five test-one population model. For instance, average values of Se C-ELISA$_2$ varied from 0.963 to 0.964 and average values of Sp C-ELISA$_2$ varied from 0.995 to 0.997.

4. Discussion

The characteristics of the five studied tests were estimated through a Bayesian approach. This approach is frequently used in evaluation of diagnostic tests, in veterinary or human medicine (Meyer et al., 2009). It offers some advantages over frequentist methods.

Its main interest is the possibility to take into account prior information about tests accuracy or prevalence in the form of experts' opinion or literature data. Priors should be generated independently of the study. According to Enøe et al. (2000), the best solution would be to use information collected in similar studies. As explained below, only a few studies have dealt with porcine brucellosis diagnostic tests and they were performed in different contexts and areas. For each parameter, means and lower limits were determined on brucellosis tests in various species (cattle, swine and small ruminants) (Gall et al., 1998, 2003; Silva Paulo et al., 2000; Nielsen, 2002; Gall and Nielsen, 2004; Muñoz et al., 2005; EFSA, 2009; Muma et al., 2009; Nielsen et al., 2008), in order to take into account the large range of the values.

Another advantage of the Bayesian method is that it provides true probability intervals (credibility interval contains the true parameter with 95% certainty), whereas a 95% frequentist confidence interval is considered to contain the true parameter value in 95% of the times (Enøe et al.,

2000). The width of the credibility intervals also depends on the adaptation of the prior distributions to the data. The prior distributions used in this study might not be ideal, but reflect the central tendency and variation of the sensitivity and specificity of the respective tests in previous studies about detection of *B. suis* in pigs.

Bayesian analyses of diagnostic test accuracy require a sensitivity analysis and the assumption of accuracy among the population. If this hypothesis is not perfectly verified, estimates of sensitivity and specificity might be biased. Johnson et al. (2009) recommend separate analyses of tests to evaluate the variation of the parameters of interest. Additional analyses performed as sensitivity analysis tend to prove that the assumption of accuracy among population is verified in our study.

Tests were considered as conditionally dependent. Two tests are said to be independent if, given that an animal is diseased (or not), the probability of positive (or negative) outcome for the first test is the same whatever the outcome for the other test (Enøe et al., 2000). The five tests studied are based on the same biological process (Nielsen, 2002): they detect anti-*Brucella*-S-LPS antibodies and can therefore be expected to be conditionally dependent (Gardner et al., 2000). Taking into account this dependence was essential; otherwise the estimates of sensitivity and specificity might have been biased due to an underestimation of the classification errors (Vacek, 1985). Posterior estimates of covariances confirmed the hypothesis of conditional dependence between tests, with low to moderate values of covariances.

Tests results were evaluated in a blind fashion and only one sample was collected from each animal (without retesting), which avoids introducing an additional observer dependence. The study of tests on samples from several animals in a herd can create another type of dependence, because factors affecting sensitivity and specificity can cluster among herds (Donald et al., 1994). These clusters were not taken into account in our study. We chose to merge infected and non-infected subpopulations in order to avoid limitations due to very low prevalence situations (presumed free population) (Nielsen and Toft, 2002).

Few studies investigated the characteristics of porcine brucellosis serological tests in the absence of a gold standard (Silva Paulo et al., 2000; Muma et al., 2009; Nielsen et al., 2008). Most of them compared only two or three tests at a time. Furthermore, studies were carried out in very different contexts, populations, numbers of animals, geographical areas and studied tests (principle, provider, cut-offs and dealing with doubtful results). Our results were coherent with the results obtained in these studies. More specifically, we found that C-ELISA tests were the most sensitive and specific tests. According to EFSA (2009), the average sensitivity of C-ELISA test was $Se_{C\text{-}ELISA\_EFSA} = 1.000$ ([0.988; 1.000], 95% CI) and its average specificity was $Sp_{C\text{-}ELISA\_EFSA} = 0.979$ ([0.976; 0.982], 95% CI). Our estimation of the sensitivity of RBT was consistent with the estimation of the EFSA ($Se_{RBT\_EFSA} = 0.870$; [0.802; 0.922], 95% CI) but our estimation of its specificity was lower ($Sp_{RBT\_EFSA} = 0.998$; [0.997; 0.998], 95% CI). The specificity of FPA seemed slightly higher than in our study ($Sp_{FPA\_EFSA} = 0.952$; [0.945; 0.958], 95% CI). More information on the validation of veterinary diagnostic test is available in Greiner and Gardner (2000).

According to Branscum et al. (2005), the estimation of the parameters is more correct when the studied disease is persistent and when antibodies are detectable throughout the main part of the life course. Nevertheless, this persistence of antibodies is not strictly correlated to the persistence of the positive outcome to the test, particularly when the antibody titre decreases. Another issue is raised by infected animals in early stages of the infection, during seroconversion time. These animals are infected but cannot be detected by serological tests.

Many factors like biological ones can influence the apparent sensitivity (proportion of non-responders to a test, prevalence in the population, etc.) and specificity (cross-reacting pathogens, removal of test-positive animals during eradication programs, etc.). Test parameters also vary with breed and management conditions of animals.

The studied population of pigs was genetically homogeneous (most of them were Large White, Landrace and Piétrain pigs) and representative of the European pig population (local breeds excepted) (SanCristobal et al., 2006). Their breeding conditions (in industrial farms or in outdoor farms) were quite similar to those of other pigs in European countries. Serological analyses were performed by the EU/OIE/FAO Brucellosis Reference Laboratory (ANSES, Maisons-Alfort; France). The results of our study could thus probably be extrapolated to these pig populations.

The possibility of doubtful outcomes to FPA and I-ELISA test lead us to perform three studies: (i) doubtful results excluded, (ii) doubtful results considered as negative ones and (iii) doubtful results considered as positive ones. The comparison of average values of the parameters of interest and their 95% credibility intervals showed that these three hypotheses gave similar results. This could be explained by the low number of doubtful results compared to the large number of serum samples. It would be possible to perform an analysis considering doubtful results as negative or positive ones according to the results of the 3 other tests for each animal, but this study could not be practically used in screening programs, in which pigs are subjected to one test (or two tests when the first one gives a positive outcome). Another possibility would be to collect epidemiological information on animals and farms and use it to interpret the doubtful results. It would also be possible to change the cut-offs of the tests in order to reduce the number of doubtful results and to simplify the interpretation of the results.

To conclude, this study is an original survey. We evaluated new tests for the detection of *B. suis* (FPA, I-ELISA and two C-ELISA tests), and compared their characteristics with CFT. The serum samples were collected from a large population of pigs, representative of European swine population.

We showed that C-ELISA tests (more specifically C-ELISA$_2$) were the most sensitive and specific swine brucellosis diagnostic tests. Among the five serological tests, none was sensitive or specific enough to be used alone. In order to screen porcine brucellosis (particularly on a herd scale), pigs should be tested by more than one

test. This diagnostic sequence should be adapted according to the epidemiological context. For instance, in European countries like France, where brucellosis occurs sporadically, causes clinical signs and is thus easily detected, a test with a high specificity would be useful to avoid false positive outcomes in free pig farms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.prevetmed.2011.10.014.

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