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REGULAR ARTICLES

Evaluation of five serological tests for the diagnosis of porcine brucellosis in French Polynesia

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Abstract Porcine brucellosis due to *Brucella suis* biovar 1 raises important issues for pig breeders in French Polynesia. In this region, the disease is enzootic, spreads silently and engenders economic losses in infected farms as well as sporadic human cases. While serological tests are essential in surveillance and control programmes of animal diseases, to date none of the available tests have been shown to be reliable enough to be used as a gold standard in routine individual diagnosis of porcine brucellosis. Few studies about the estimation of the sensitivity and the specificity of porcine brucellosis screening tests have been published, none of them dealing with French Polynesia. The studied population included 1,595 pigs from French Polynesia. Five tests were evaluated: Rose Bengal test, fluorescence polarisation assay, indirect ELISA, and two competitive ELISAs (C-ELISA). The sensitivity and the specificity of each test were estimated. C-ELISA$_2$ was the most sensitive test (Se C-ELISA$_2$=0.954 [0.889; 0.992] 95 % credibility interval (CrI)) while both C-ELISA and Rose Bengal test (RBT) were the most specific ones (Sp C-ELISA$_1$=0.856 [0.806; 0.915] 95 % CrI; Sp C-ELISA$_2$=0.849 [0.817; 0.879] 95 % CrI; Sp RBT=0.853 [0.812; 0.898] 95 % CrI).

Keywords Bayesian theory · *Brucella suis* · Diagnostic tests · Sensitivity · Specificity · French Polynesia

Porcine brucellosis is a bacterial disease, usually due to *Brucella suis*, that induces economic losses due to infertility and other reproductive issues. In French Polynesia (FP), brucellosis is enzootic and is due to the zoonotic biovar 1 of *B. suis* (Antras and Garin-Bastuji 2011).

Diagnosis of porcine brucellosis is mainly based on the observation of clinical signs (i.e. abortion, infertility, orchitis and more rarely arthritis). It can be associated with *Brucella* isolation or positive serology, but to date, no reference test is available (OIE 2011). Few studies about the estimation of sensitivity and specificity of porcine brucellosis screening

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tests have been published (Muma et al. 2007; Nielsen et al. 2008; Praud et al. 2012), none of them dealing with the FP context. Our study aimed at estimating the sensitivity and the specificity of five serological tests for the diagnosis of porcine brucellosis in FP through a Bayesian approach implemented via Markov chain Monte Carlo algorithms (Branscum et al. 2005).

Materials and methods

Data collection

The study included 1,595 sera collected from pigs bred in FP. Two groups were randomly selected: (1) 694 pigs bred in six farms, where *B. suis* biovar 1 had been recently isolated and (2) 901 pigs from 11 farms without any sign or risk factor of brucellosis identified by the local veterinary office (i.e. no brucellosis outbreak in the last few years, no clinical signs of *B. suis* infection and no epidemiological link with domestic or wild infected animals).

Diagnostic tests

Each sample was subjected to five serological tests : (1) Rose Bengal test (RBT), Institut Pourquier, France; (2) fluorescence polarisation assay (FPA), *Brucella abortus* antibody test kit, Diachemix/Prionics, USA/Switzerland; (3) indirect ELISA (I-ELISA), Chekit *B. suis*, Idexx-Bommeli, Switzerland; (4) competitive ELISA (C-ELISA$_1$), SVANOVIR® Brucella-Ab C-ELISA, Svanova, Sweden and C-ELISA$_2$, Compelisa, VLA, UK (two- and one-step format, respectively). The general principles of these tests are described in OIE (2011). The RBT antigen was standardised according to OIE and EU requirements (EU 2008; OIE 2011). Cutoffs were as recommended by the tests manufacturers for the other tests (FPA=20; I-ELISA >70 %; C-ELISA$_1$ ≥30 %; C-ELISA$_2$ ≤60 %).

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.

Statistical methods

A Bayesian approach was used to estimate the sensitivity and the specificity of the five tests without gold standard (Enøe et al. 2000; Berkvens et al. 2006). The five test-one population model presumed that the tests were conditionally dependent and allowed to estimate the sensitivity and the specificity of the five tests and their covariances (Berkvens et al. 2006; Praud et al. 2012).

Table 1 Parameters of informative prior distributions

Parameter of interest		Lower limit (95 %)	Mean	Beta (a, b)	
				a	b
EAT	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

Se sensitivity, *Sp* specificity

Informative priors were introduced for sensitivity and specificity (Table 1). Since no information was available concerning covariances, they were modelled as Beta(1,1) non informative distributions.

The statistical analysis was performed with the WinBUGS program (Lunn et al. 2000). Early samples (1,000 out of 50,000) were discarded as a "burn-in" period. A sensitivity analysis was also performed by making the prior distributions more diffuse in order to check that the parameter estimates were little affected by these variations.

Results

In the presumed free population, 86.5 % of the animals showed negative results in all tests. In the infected herds, 67.3 % of pigs had a positive outcome in at least one test.

The parameters estimates are displayed in Table 2. Estimated prevalence in the sample was 0.210 ([0.136; 0.293], 95 % credibility interval (CrI)). C-ELISA$_2$ was the most sensitive test (Se C-ELISA$_2$=0.954 [0.889; 0.992]) while both

Table 2 Estimates of the sensitivity and the specificity of the five tests

Parameter of interest		Mean estimate [95 % CrI]
RBT	Sensitivity	0.712 [0.489; 0.921]
	Specificity	0.853 [0.812; 0.898]
FPA	Sensitivity	0.751 [0.554; 0.939]
	Specificity	0.819 [0.779; 0.858]
I-ELISA	Sensitivity	0.850 [0.708; 0.978]
	Specificity	0.824 [0.787; 0.862]
C-ELISA$_1$	Sensitivity	0.644 [0.356; 0.973]
	Specificity	0.856 [0.806; 0.915]
C-ELISA$_2$	Sensitivity	0.954 [0.889; 0.992]
	Specificity	0.849 [0.817; 0.879]

95% CrI 95 % credibility intervals

C-ELISAs were the most specific ones (Sp C-ELISA$_1$=0.856 [0.806; 0.915]; Sp C-ELISA$_2$=0.849 [0.817; 0.879]).

Discussion

Few studies investigated the characteristics of porcine brucellosis serological tests in the absence of a gold standard (Muma et al. 2007; Nielsen et al. 2008; Praud et al. 2012). Most of them compared only two or three tests at a time. Furthermore, these studies were carried out in very different contexts, populations, numbers of animals, geographical areas and studied tests (principle, provider, cutoffs and dealing with doubtful results). Our results were coherent with the results obtained in these studies. C-ELISA$_2$ was the most sensitive test (Se C-ELISA$_2$=0.954 [0.889; 0.992] 95 % CrI) while both C-ELISA and RBT were the most specific ones (Sp C-ELISA$_1$=0.856 [0.806; 0.915] 95 % CrI; Sp C-ELISA$_2$= 0.849 [0.817; 0.879] 95 % CrI; Sp RBT=0.853 [0.812; 0.898] 95 % CrI). The lack of specificity of the tests could be explained by their own limitations or by the existence of cross reactions due to *Yersinia enterocolitica O*:*9*.

A previous study (Praud et al. 2012) evaluated the characteristics of the five same tests (RBT, FPA, I-ELISA, C-ELISA$_1$ and C-ELISA$_2$) in Metropolitan France (MF), with the same statistical approach. No significant difference was observed between the sensitivities of the tests in the two areas, except in the case of the I-ELISA for which sensitivity resulted higher in FP. The most sensitive test was C-ELISA$_2$ in both populations. The five tests were more specific in MF and the C-ELISAs were the most specific in both populations.

Since in these two studies the testing as well as the statistical methods were identical, these peculiarities could be explained by differences of epidemiological contexts and tested populations. Greiner and Gardner (2000) have shown that test sensitivity and specificity could vary according to many biological factors. Porcine brucellosis is enzootic in FP, while MF is free of the disease, with sporadic occurrences due to a wildlife reservoir. In metropolitan farms, brucellosis often discloses an acute form with obvious symptoms, whereas in Polynesian farms, the disease spreads silently and is often detected later than in MF. In all reported outbreaks, *B*. *suis* biovar 2 has been isolated in MF, whereas biovar 1 is the one historically isolated in pigs and humans in FP as in the rest of the Pacific. To our knowledge, no study suggested that tests might react differently according to the biovar of *B*. *suis* in cause, but this cannot be excluded.

The differences between the specificities of the five tests could probably be explained by the differences of management of the animals from one area to the other. The metropolitan "free subpopulation" was reared in officially brucellosis-free artificial insemination units, without any contact with other animals and with less cross-reacting pathogens in the environment, while in FP, farms are less bio-secured and the presumed free status is based only on the absence of clinical signs and known risk factors.

To conclude, this study confirmed that competitive ELISA tests are highly sensitive and specific tests for the diagnosis of swine brucellosis. The sensitivity and the specificity could however vary greatly according to the C-ELISA format used as well as the studied population. This should be taken into account for designing surveillance and eradication schemes.

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