



Bayesian estimation of diagnostic accuracy of a new bead-based antibody detection test to reveal *Toxoplasma gondii* infections in pig populations



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ABSTRACT

The success of a *Toxoplasma gondii* surveillance program in European pig production systems depends partly on the quality of the test to detect infection in the population. The test accuracy of a recently developed serological bead-based assay (BBA) was investigated earlier using sera from experimentally infected animals. In this study, the accuracy of the BBA was determined by the use of sera from animals from two field subpopulations. As no *T. gondii* infection information of these animals was available, test accuracy was determined through a Bayesian approach allowing for conditional dependency between BBA and an ELISA test. The priors for prevalence were based on available information from literature, whereas for specificity vague non-informative priors were used. Priors for sensitivity were based either on available information or specified as non-informative. Posterior estimates for BBA sensitivity and specificity were (mode) 0.855 (Bayesian 95% credibility interval (bCI) 0.702–0.960) and 0.913 (bCI 0.893–0.931), respectively. Comparing the results of BBA and ELISA, sensitivity was higher for the BBA while specificity was higher for ELISA. Alternative priors for the sensitivity affected posterior estimates for sensitivity of both BBA and ELISA, but not for specificity. Because the difference in prevalence between the two subpopulations is small, and the number of infected animals is small as well, the precision of the posterior estimates for sensitivity may be less accurate in comparison to the estimates for specificity. The estimated value for specificity of BBA is at least optimally defined for testing pigs from conventional and organic Dutch farms.

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1. Introduction

Toxoplasma gondii (*T. gondii*), a protozoan parasite, can be transmitted to humans through the consumption of infected meat (Tenter et al., 2000). Even though this transmission route was discovered in 1965, in Europe, control

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measures to prevent this route of infection were not implemented on farm or slaughterhouse level. Recently, publications have provided scientific evidence of the impact of the parasite on human health (Havelaar et al., 2007, 2012; Kortbeek et al., 2009), and indicate that *T. gondii* is one of the leading emerging zoonoses in the Netherlands (Havelaar et al., 2010).

In 2011, the European Food Safety Agency (EFSA) published an opinion in which *T. gondii* is recognized as a public health hazard which needs to be covered by providing food chain information at the abattoir level (Anon., 2011a). Another EFSA report recommends the implementation of three harmonized epidemiological indicators in the pig production chain, whereby two of these indicator points provide information on infection of tested animals through serological determination (Anon., 2011b). In a practical application, indirect tests like ELISA with failing sensitivity and specificity are preferred over direct tests like bioassays (Dubey et al., 1995; Dubey, 2005) and PCR-based analysis methods with (almost) perfect specificity. The use of a *T. gondii* bioassay, a reference test which uses cats and/or mice to indicate the presence of the parasite (Dubey et al., 1995; Dubey, 2005), is unsuitable for large scale detection purposes. Additionally, a recently developed Magnetic Capture PCR (Opsteegh et al., 2010) is less feasible for this purpose due to its laboriousness. For an efficient implementation of a serological test in e.g. EFSA recommended surveys (Anon., 2011b), the test accuracy should be well defined in order to e.g. estimate sample size (Cameron, 1998).

Recently, we developed a bead-based assay (BBA), a serological test which can concurrently detect *T. gondii* and *Trichinella spiralis* antibody responses in serum samples (Bokken et al., 2012). The flow cytometric analysis of specific antibodies by the use of antigen-coated beads is a relatively new application to demonstrate infections in animals. This test bears large similarities to the detection of antibodies by ELISA, and could be used as an alternative to these tests. An initial study showed that the values of test accuracy of the BBA were 0.86 and 0.96 for sensitivity (Se) and specificity (Sp), respectively (Bokken et al., 2012). However, these test accuracy values were determined using sera of animals which were infected with one dose of *T. gondii* in animals of the same race and age living under an experimentally conditioned environment. Animals from conventional pig populations, however, the population for which the test is intended.

In the current study, the sensitivity and specificity of BBA were determined in pigs from two subpopulations which originated from a conventional and an organic pig farm in the Netherlands. In absence of a gold standard, a Bayesian approach was used.

2. Materials and methods

2.1. Serum sets

Serum set 1 (SS1): a collection of 847 serum samples were compiled in 2007 from conventional Dutch finisher pigs at slaughter (Bokken et al., 2012).

Serum set 2 (SS2): a selection of 376 serum samples of pigs at slaughter originating from 6 organic farms, presented as farms G, H, W, X, AA and AC, were collected in 2004. ELISA results from the study of Meerburg and co-workers indicated that 43 of these 376 animals were infected with *T. gondii* (Meerburg et al., 2006).

2.2. Tests

Analysis of the infection status of the animals of SS1 was described in a previous study (Bokken et al., 2012). Determination of SS2 animal status was performed accordingly by bead-based assay (BBA) and ELISA (E).

2.2.1. Bead-based assay

The procedure of the BBA and the detection of antibody binding have been described (Bokken et al., 2012). Correction of nonspecific binding responses of each sample and additionally, calculation of normalized responses (%NR) were performed according to the procedure described (Bokken et al., 2012).

2.2.2. ELISA

The *T. gondii* infection status was analyzed by a *T. gondii* ELISA (ID Screen Toxoplasmosis Indirect, ID-VET, Montpellier, France). The test was run according to the protocol described by the producer. Sera were ten times diluted and the responses of the ELISA, measured at OD 450 nm, were normalized in %S/P values against a double implemented positive and negative control. The %S/P is expressed as the percentage of the ratio of negative control corrected sample response and negative control corrected positive control response.

2.2.3. Categorization of test results

The infection status of the animals of SS2 were categorized using a BBA cut-off value of %NR = 13.9, expressed as the percentage of normalized response, and an ELISA cut-off value of %S/P = 26.9, expressed as the percentage of sample to positive (Bokken et al., 2012). The result of a test was categorized as negative when the outcomes were below the cut-off value, or positive when the outcome was equal to or higher than the cut-off value. The animals of SS1 were previously categorized using the same definitions (Bokken et al., 2012). The respective combination of negative (designated as 1) and positive (designated as 2) reactive animals for BBA and ELISA for each population were scored in a 2×2 table.

2.3. Statistics

2.3.1. Prior distributions

The Bayesian model requires priors for a total of eight parameters: infection prevalence for two populations, sensitivity and specificity for two tests, and the correlation between test results in infected and non-infected animals, respectively. An overview of the prior distributions that were used, is provided in Tables 1 and 2.

We used Beta distributions to assign prior distributions for the prevalence parameters (Table 1). The prior for the prevalence in animals from conventional farms

Table 1

Estimated beta distribution of prevalence priors based on previously obtained parameter information.

Name	Prior information prevalence	Estimated 95th %	Beta distribution	Mean [mode, 2.5%, 97.5%]
Prev. SS1	Conventional farms ^a : 0.4%	<1.5%	dbeta (1, 250)	0.004 [0, 1×10^{-4} , 0.01]
Prev. SS2	Organic farms ^b : 11.7%	<35%	dbeta (3, 16)	0.16 [0.12, 0.036, 0.35]

^a Van der Giessen et al. (2007).^b Meerburg et al. (2006).**Table 2**

Two sets of estimated beta distribution priors of sensitivity and specificity.

Prior set	Parameter	Estimated mean	Estimated 5/95th %	Beta distribution	Mean [mode, 2.5%, 97.5%]
Set 1	Se _{BBA} and Se _E	85% ^a	>60%	dbeta (8.6, 1.4)	0.86 [0.95, 0.60, 0.99]
Sets 1 and 2	Sp _{BBA} and Sp _E	Non-informative		dbeta (2, 1)	0.67 [1.00, 0.16, 0.99]
Set 2	Se _{BBA} and Se _E				

^a Bokken et al. (2012).

(SS1) was based on the study of van der Giessen and co-workers (van der Giessen et al., 2007). The prior for the prevalence in animals from organic farms (SS2) was based on the results reported by the group of Meerburg (Meerburg et al., 2006). The beta-distributions for the priors for the sensitivity of the ELISA and BBA assays, described in set 1 (Table 2), were based on results from a small experimental study which indicated a value of 0.84 and 0.86, respectively (Bokken et al., 2012). The slightly lower and less precise values that were used in the modeling were chosen to reflect the fact that the populations in the present study were subject to natural infection. Furthermore, to assess the sensitivity of our results to the prior specification an alternative set of prior distributions was constructed in which vague uninformative priors were used for the sensitivity (set 2, Table 2). Because no information was available to allow specification of an informative prior for the specificity and conditional covariance of both tests, uninformative priors were used for these parameters. For the (conditional) covariance between test results we used an uninformative (flat) uniform prior over the positive range of possible correlations (Dendukuri, 2001; Georgiadis et al., 2003). The model assumes an equal sensitivity, specificity and conditional covariance in the two populations.

2.3.2. Estimation of diagnostic parameters

In the absence of a gold standard, *T. gondii* bead assay test accuracy was evaluated by fitting a Bayesian model to data from three populations and two tests. As both the BBA and ELISA rely on a similar immunological (antibody) response to the parasite, the model was adapted to allow for conditional dependence between test results following the approach advocated by Dendukuri and the group of Georgiadis for three different populations (Dendukuri, 2001; Georgiadis et al., 2003).

Our model involves a total of eight parameters, i.e. prevalence of two subpopulations, Se and Sp for both tests and both positive conditional dependency (ρ_p) and negative dependency (ρ_n) between the tests. Testing of two populations with two tests generates six degrees of freedom (Hui and Walter, 1980) and eight data points,

which does not generate enough information to estimate parameter values. This type of problem is known to be non-identifiable (i.e. there are more parameters to be estimated than there is information in the data) and one therefore has to rely on (weakly) informative priors for at least some of the parameters to obtain a well-defined posterior distribution. From information of a small experimental study and literature, informative priors for the prevalence of infection and the sensitivity of the tests were selected. The model was implemented using JAGS 3.3.0 (Plummer, 2003) and the results were analyzed in R 3.0.1 (R Core Team, 2013). Preliminary investigation of results suggested slow exploration of the full parameter space for models with clear multimodal posterior distributions and we therefore used a relatively large number of chains ($n = 100$) with over dispersed starting values to avoid sensitivity to initial values. Visual inspection of trace plots indicated that convergence was relatively quick and the posterior distributions were therefore based on a total of 2500 iterations for each chain after an initial burn-in of 3000 iterations. To assess model fit and evaluate potential discrepancy between prior distributions and test results we calculated the deviance information criterion (DIC), the p_D value and Bayesian p value (B_p) following the approach outlined by Spiegelhalter and Berkvens (Spiegelhalter et al., 2002; Berkvens et al., 2006). The values obtained for the two alternative sets of prior were compared and model fit was evaluated based on the most optimal DIC, p_D and B_p value. A good model fit is indicated by low positive values for all three indicators (Spiegelhalter et al., 2002; Berkvens et al., 2006; Rahman et al., 2013). Larger than three units of differences between DIC values can be considered significantly different (Spiegelhalter et al., 2002; Rahman et al., 2013). For our model a B_p of 1 would indicate a poor fit, while for a model with uninformative priors a B_p of 0.5 indicates satisfactory fit. Discrepancy between prior distribution and test results were further visually evaluated by plotting samples from the prior and posterior distributions for each chain. To summarize the posterior distribution we calculated the mean, standard deviation, mode and Bayesian credible interval (bCI) (Gelman et al., 2004). Estimation of the mode and bCI to summarize the posterior distribution

was done using the R packages *modeest* and *coda*, respectively.

3. Results

3.1. Test results

BBA- and ELISA-categorized results of the three serum sets are presented in Table 3. Overall, the BBA scored more positives in the serum sample populations than ELISA. The ratio between SS1/SS2 infection scored higher in ELISA than in BBA.

3.2. Model analysis

Results for the posterior distributions of the prevalence of the two subpopulations, and sensitivity and specificity for the ELISA and BBA assay under both prior sets are summarized in Tables 4 and 5, respectively. Visual comparison of the shape of the prior and posterior distributions showed that the modes of both distributions were generally not very different, but that the posterior distribution was more concentrated (graphs not presented). This indicated that there was no strong conflict between priors and posteriors, and that posterior estimates were at least partly based on information from the data itself. This finding was substantiated by the estimated values of the parameters and their bCI's. The posterior distributions of all parameters seemed to be compatible with the prior distributions used in the model. Conditional dependency analysis shows that within the infected and non-infected animals the ρ_p and ρ_n had modes of 0.107 and 0.151 for prior set 1 and 0.150 and 0.161 for prior set 2, respectively (Table 5). The estimated posterior parameter values from the models under conditional dependence and independence are shown in Tables 5 and 6, respectively.

The estimated parameter values calculated with the different sensitivity priors of sets 1 and 2 showed little difference in the posterior estimation of prevalence of SS1 and a slightly larger difference was observed in SS2 (Table 4). Posterior values of sensitivity of both tests demonstrate a larger difference between two prior sets, while the specificity shows little difference (Tables 5 and 6).

Analysis of model fit for the two prior sets showed that the values of DIC, p_D and Bp values estimated in the posterior means of the multinomial probability (Berkvens et al., 2006), were almost equal between the two prior sets (Table 7).

4. Discussion

By estimation of the DIC, p_D and Bp values (Table 7), the fitness of the model is satisfactory and both prior sets are equally acceptable as shown by the similar results for both sets of priors. However, based on slightly lower values of p_D , prior set 1 estimates may be favored slightly. The values for conditional dependency between tests (Table 5) do not rule out the possibility that the tests may be independent. Models that did not allow for dependency resulted in similar estimates for specificity, but a more narrow distribution for sensitivity (Tables 5 and 6). However, because in

both tests the same antigen is used, i.e. SAG-1 structures are present on tachyzoites (Ma et al., 2009), it is very likely that the tests are conditional dependent. We therefore report the posterior values based on conditional dependency in our models.

This study showed that based on prior set 1 the mean estimated sensitivity of BBA is 0.844 (mode 0.855, bCI 0.702–0.960) and mean specificity is 0.912 (mode 0.913, bCI 0.893–0.931) (Table 5). Comparatively, the sensitivity of BBA estimated with prior set 2 has a lower mean and wider bCI while the posterior specificity distributions resembles the estimates obtained with prior set 1 (Table 5). The same pattern is observed in the comparison of accuracy values of ELISA (Table 5).

Because the sensitivity was based on populations with low prevalence's, i.e. the highest prevalence probability was expected below 35% and the lowest was close to 0, the value of sensitivity is based on a limited amount of data information and more on the prior of this parameter. Theoretically these factors would affect the precision of the estimated sensitivity as the posterior values are more dependent on the prior information (Toft et al., 2005). The wider distributions of posterior sensitivity using prior set 2 which contains uninformative sensitivity priors is indicative of such prior effect (Table 5). However these distribution profiles have a more narrow distribution than the posterior distributions based on priors alone (graphs not shown) illustrating the use of information from the data. Therefore, the sensitivity estimates can be indicative of the test performance, but the precision of these values is challengeable. On the other hand, the posterior values for specificity depend on a larger number of animals and are therefore more precise. This is illustrated by the values in Table 5, which show narrow posterior specificity distributions although the specificity priors are uninformative.

The posterior values for sensitivity and specificity of the bead test estimated in this study are slightly lower than the values perceived by the previous estimation with experimental infection sera (Bokken et al., 2012). It should be noted that these previous sensitivity and specificity values fall within the corresponding, currently estimated 95% Bayesian credibility intervals.

The difference of sensitivity and prevalence of SS2 observed between the two prior sets (Tables 4 and 5) was caused by the two prior sensitivity distributions which are constituents of the two prior sets (Berkvens et al., 2006). The use of these priors had no noteworthy effect on the posterior values of specificity and prevalence of SS1. The higher estimates of sensitivity and lower estimates of specificity of BBA in comparison to their counterpart in ELISA can be explained by the antigens used in these two tests. BBA uses a *T. gondii* (RH strain) tachyzoite lysate as antigen which compared to the antigen in ELISA, a recombinant produced *T. gondii* zoites (tachyzoites, bradyzoites, sporozoites, and merozoites) surface protein SAG-1 (Dubey et al., 1998), contains a large variety of potential antibody binding sites. This higher variety in epitopes increases the probability of antibody binding, thus increases sensitivity. A similar sensitivity/specificity balance between tachyzoite and SAG-1 as antigen in an indirect test was observed in another study (Basso et al.,

Table 3Categorized datasets of the *Toxoplasma* serum sets. Animal statuses were determined by the bead-based assay (BBA) and ELISA (E).

Pop	<i>n</i> (BBA, E)				% Pos	
	(1, 1)	(1, 2)	(2, 1)	(2, 2)	BBA	E
SS1	769	6	65	7	8.5	1.5
SS2	286	9	35	46	21.4	13.5

Pop, serum sets of animal population; *n*, number of categorized animals; (BBA, E), status of animals as determined by BBA and E in which 1 reflects an uninfected status and 2 an infected status; % pos, the percentage of animals scoring positive in tests.

Table 4

Posterior values of prevalence in two populations.

Parameter	Prior set 1				Prior set 2			
	Mean	SD	Mode	bCI	Mean	SD	Mode	bCI
Prev. SS1	0.004	0.003	0.001	0.000–0.012	0.004	0.003	0.001	0.000–0.012
Prev. SS2	0.159	0.027	0.154	0.112–0.218	0.179	0.039	0.168	0.119–0.270

Prev., posterior prevalence; SS1 to SS2, serum sets of two pig populations; mean, averaged posterior value; SD, standard deviation; mode, the mode of the of posterior parameter distribution; bCI, HPD-based credibility interval of the posterior distribution.

Table 5

Posterior values of Se and Sp of BBA and ELISA based on a conditional dependence model.

Parameter	Prior set 1				Prior set 2			
	Mean	SD	Mode	bCI	Mean	SD	Mode	bCI
Se _{BBA}	0.844	0.066	0.855	0.702–0.960	0.784	0.101	0.819	0.548–0.947
Sp _{BBA}	0.912	0.010	0.913	0.893–0.931	0.914	0.010	0.914	0.894–0.933
Se _E	0.846	0.092	0.889	0.649–0.986	0.757	0.129	0.758	0.490–0.981
Sp _E	0.986	0.005	0.986	0.975–0.995	0.986	0.005	0.987	0.975–0.995
ρ_p	0.254	0.181	0.107	0.010–0.660	0.321	0.203	0.151	0.015–0.730
ρ_n	0.149	0.073	0.150	0.015–0.294	0.158	0.074	0.161	0.018–0.304

Se, posterior sensitivity; BBA, bead-based assay; Sp, posterior specificity; E, ELISA; ρ_p , conditional dependency value in infected animals, ρ_n , conditional dependency value in non-infected animals; mean, averaged posterior value; SD, standard deviation; mode, the mode of the of posterior parameter distribution; bCI, HPD-based credibility interval of the posterior distribution.

Table 6

Posterior values of Se and Sp of BBA and ELISA using a conditional independence model.

Parameter	Prior set 1				Prior set 2			
	Mean	SD	Mode	bCI	Mean	SD	Mode	bCI
Se _{BBA}	0.882	0.050	0.890	0.777–0.971	0.871	0.055	0.879	0.754–0.971
Sp _{BBA}	0.919	0.009	0.920	0.901–0.937	0.921	0.009	0.921	0.902–0.938
Se _E	0.863	0.075	0.880	0.703–0.984	0.832	0.088	0.840	0.654–0.985
Sp _E	0.991	0.004	0.992	0.983–0.997	0.991	0.004	0.992	0.983–0.998

Se, posterior sensitivity; BBA, bead-based assay; Sp, posterior specificity; E, ELISA; mean, averaged posterior value; SD, standard deviation; mode, the mode of the of posterior parameter distribution; bCI, HPD-based credibility interval of the posterior distribution.

2013). In comparison, the specificities of both tachyzoite (mean: 0.927, bCI 0.977–0.960) and SAG-1 (mean: 0.988, bCI 0.966–0.999) ELISA's (Basso et al., 2013) are comparable with the values of specificity of tachyzoite BBA and SAG-1 ELISA in the current study, while sensitivities in our study scored lower. This difference may be caused by failing test capacity to score infected animals, however, this is hard to conclude as several factors like differences in prior parameter settings, the prevalence of infection in the sampled populations and incorporation of covariance into the model played a role in the estimation of the posterior values.

The variability of biological factors in sera of different pig populations can be the cause of a variable test accuracy to determine infection (Greiner and Gardner, 2000). It is therefore evident that the sensitivity and specificity

is more accurately determined in the population in which the test eventually will be utilized e.g. to estimate prevalence or to determine the sample size for surveys. As such, the value for specificity determined in this study by the use of a population of naturally infected animals may be considered more accurate than its equivalent determined

Table 7

Multinomial model fit analysis under conditional dependency.

Parameter	Prior set 1	Prior set 2
Bp value	0.51	0.49
DIC	39.53	39.72
p_D	5.22	5.56

Bp, Bayesian *p* value; DIC, Deviance information criterion; p_D , effectively estimated number of parameters.

from experimentally infected animals in our previous study (Bokken et al., 2012).

5. Conclusion

This reports described the estimation of sensitivity and specificity in a conventional European pig population via Bayesian estimation methods. Comparatively, the sensitivity of BBA was higher while the specificity was lower than that of ELISA. However, the Bayesian estimation of posterior values of sensitivity is impaired by the rather low prevalence's in these populations. Therefore, the posterior estimated sensitivity values may be less reliable when compared to these for the specificity. Because the test accuracy of BBA was determined using pigs from conventional farms, at least the estimated value for specificity is optimally defined for testing this population.

Conflicts of interest

There are no conflicts of interest.

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