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Estimation of Paratuberculosis Prevalence in Dairy Cattle in a Province of Korea using an Enzyme-linked Immunosorbent Assay: Application of Bayesian Approach

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Abstract

To draw inferences about the sensitivity and specificity of the newly developed ELISA test for bovine paratuberculosis (PTB) diagnosis and posterior distribution on the prevalence of PTB in a province of Korea, we applied Bayesian approach with Gibbs sampler to the data extracted from the prevalence study in 1999. The data were from a single test results without a designated gold test.

The prevalence estimates for PTB in study population ranged 3.2 5.3% for conservative and 6.7 7.1% for liberal, depending on the priors used. The simulated specificities of the ELISA close to one another, ranging 84.7 90.6%, whereas the sensitivity was somewhat spread out depending largely on the priors with a range of 46.4 88.2%. Our findings indicate that the ELISA method appeared useful as a screening tool at a minimum level in comparison to other diagnostic tests available for this disease in terms of sensitivity. However, this advantage comes at a cost of having low specificity of the test.

Key words: *Mycobacterium paratuberculosis*, ELISA, Bayesian, Gibbs sampling

Introduction

Paratuberculosis (PTB) caused by *Mycobacterium avium* subsp. *paratuberculosis* has been reported in Korea for several decades and affects a large proportion of dairy cattle throughout the country. Few studies so far have been reported on the prevalence at individual animal and at herd level in Korea, although PTB had been designated as a notifiable disease since 1961. Based on the reports from other studies [20, 21] the prevalence ranged 1.7-13.4%, but remains largely unknown.

For PTB diagnosis, bacterial identification in bovine feces has been considered the gold standard [29]. This method, however, is of limited use [6, 27, 32]. The enzyme-linked immunosorbent assay (ELISA) is the most commonly used serologic test because of its superior sensitivity relative to other serologic testing methods. The sensitivity of the commercially available kits has been reported to range from 15 to 87% depending on clinical stage of disease [8] and specificity was reported ranging from 99 to 99.7% [7].

Estimating the accuracy of a diagnostic test is straightforward in situations when gold standard methods with no errors are available. In many clinical settings, however, there is no gold standard to determine dichotomously an animal has the disease under study. When an imperfect test with less than 100% of sensitivity and specificity is used to determine disease status, biases are introduced into both measurements of test performance, and led to over-or under-estimates of a tests true capabilities [24, 31]. This makes it impossible to determine the sensitivity and specificity of a single diagnostic test with a traditional approach. The use of an alternative approach, therefore, has been proposed to deal with the situation when gold standard does not exist [9, 10, 18]. Approaches to assess diagnostic accuracy of a test in the absence of a gold standard have been reviewed in both human and veterinary medicine [1, 2, 3, 11, 12, 14, 15, 17, 24].

One of co-authors (DK) of the present paper developed a new ELISA method using the recombinant 34kDa protein, which is species-specific epitope of *M. paratuberculosis* [22]. This test is designed as a screening tool to detect antibodies to native protein in sera from PTB infected cattle and was applied to field samples as a single test to estimate the prevalence of PTB in study population. We applied Bayesian approach to the results of their study to draw inferences about the prevalence of the PTB in the target population along with the posterior distribution on the performance of the test.

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Materials and Methods

Study population and sampling scheme

In 1999, a total of 305,513 dairy cattle of at least 2 years of age or more were recorded in the Korean nationwide government statistics (Ministry of Agriculture and Fisheries, Korea, 1999). Kangwon province, the study area consisted of 25,532 or 8.4% of the total cattle population. Based on an estimated PTB prevalence of 10-15%, herds ranging 138-196 are needed to obtain 5% desired accuracy with 95% confidence [5]. Due to financial restriction and incompliance of the farm owners for participation 162 herds with 2,261 cattle (8.9% of total population in study area) were finally selected. Blood samples were collected from cattle that were greater than 2 years old during the period March through April 1999. Veterinary officials of the local laboratories in the province collected all samples. The detail procedures on the preparation of antigen for ELISA are described previously [22].

Assumptions for the parameters

Within Bayesian inference framework, some of the unknown parameters typically required to be assumed known in order to draw meaningful posterior distributions about the remaining parameters of interest [18]. To put this perspective to work in our current analysis, we used both informative and non-informative approaches to define prior distribution of the parameters. Prior information was basically assumed in the form of a beta density, $B(\alpha, \beta)$, as suggested by many authors [12, 15, 17, 23]. The prior density for each test parameter was selected with the mean of the beta distribution given by $\alpha/\alpha+\beta$, and the standard deviation, $[\alpha\beta/(\alpha+\beta)^2(\alpha+\beta+1)]^{0.5}$, and was formed covering 4 standard deviation (SD) of probable range.

Modeling scenario in Bayesian analysis and assumptions for the priors

Since the present ELISA is newly developed and employed once without gold standard the prior information on the sensitivity and specificity, denoted by η and θ, respectively, was primarily formed using the information obtained from the results against standard sera.

For prevalence (π), we considered several sets of beta priors from the previously reported studies [20, 21]. A summary of the estimates of prevalence and the corresponding beta priors using these rates were summarized in Table 1. The SD was not reported in the original papers so that we assumed them 0.01 for each study. This value was formed to cover 4 SD of most likely range of prevalence, 6-10%. As an alternative way of increasing the precision of the estimate we combined all these results (932 positive out of 10,289 samples), yielding a prior of B(74.5,748.2). By using the ELISA, of 2,261 serum samples screened 372 were positive, of which 75 samples were confirmed by the Western Blot test. This result was considered as the likelihood ratio in the calculation of the posterior using the formula: Posterior $\propto$ Prior x Likelihood. We therefore combined the observed data with the priors so that six posteriors were constructed for $\pi\sim$ B(127.0,2826.8), B(156.6,2963.2), B(230.4,3190.1), B(116.8,2768.3), B(77.8,2349.3), and B(149.5,2934.2). Of these priors we presented results from three priors because of similar outputs between them.

When using the standard positive and negative sera the ELISA showed 96.7% (29 of 30) of θ and 83.3% (25 of 30) of η. Thus, the prior for specificity was considered as a $\theta\sim$B(30,2) assuming a uniform prior, which was intended to avoid minimize the effect of priors on posteriors. As an alternative we assumed the specificity of the test as at least 95% and less than 99%. Based on this assumption we constructed parameters of 281.3 and 8.7 for beta distribution, and these values were updated using the observed data, yielding a posterior of a $\theta\sim$B(310.3,9.7).

For sensitivity, as a non-informative approach the posterior distribution was considered as a B(26,6) based on the result against standard sera. We also used information derived from the literatures [8, 25, 30, 32], which was intended to see the impact of priors for prevalence. These priors are based on the assumption that the sensitivity of the ELISA may similar at least to those of other commonly used ELISA. We thus considered five priors of sensitivity: $\eta\sim$B(696.5,366.7), B(210.7.145.1), B(186.1,215.1), B(49.0,29.1), and B(422.5,490.8) and combined the resulting beta prior with the observed data to elicit posteriors. SD of the sensitivity was calculated from the point estimates described in each paper, using the normal approximation to

Table 1. Prevalence estimates of PTB for various tests and beta priors

Study	Estimates (No. positives/No. tested)	Beta priors	Diagnostic test used
Kim *et al*. (1994)	205 / 2,719	B (52.0, 640.8)	Agar gel immunodiffusion
	245 / 2,641	B (81.6, 777.2)	Complement fixation
	363 / 2,719	B (155.4, 582.3)	ELISA
	109 / 1,633	B (41.8, 582.3)	Intradermal skin test
Kim *et al*. (1997)	10 / 577	B (2.8, 163.3)	Absorbed ELISA
Total	932 / 10,289	B (74.5, 748.2)	—

the binomial distribution in terms of the sampling distribution [28]. The combined estimate of sensitivity has a median of 54%, providing a posterior distribution of a η~B(70.5,43.7). In summary, we considered main scenario as B(149.5,2934.2), B(310.3,9.7), and B(70.5,43.7) for , π, η, and θ, respectively. Other scenarios can be considered as sensitivity analyses.

For parameter estimation S-plus (Mathsoft, Inc.) programs for the Gibbs sampler [16, 23] were used. We run for 20000 cycles, the first 1000 to assess convergence and the remaining cycle for inference.

Results

The median and 95% credible interval of PTB prevalence from the simulated values for sensitivity and specificity were summarized in Table 2. Among the three priors evaluated the prior, π~B(230.4,3190.1) yielded estimates ranging from 6.7 to 7.1% in every combinations of sensitivity and specificity. In contrast, the other two priors showed similar results with no great difference in posteriors between them.

Posterior medians and 95% credible intervals of the sensitivity and specificity by three different priors of prevalence (one for main prior and two for extreme prior) were given in Table 3 and 4. Sensitivity ranged 46.4-88.2%, with a great variation depending on the priors used: 46.4-47.7% for B(186.1,215.1), 62-65.9% for B(70.5,43.7) and 81.9-88.2% for B(26,6). For specificity two posteriors yielded similar estimates in every combination of sensitivity and prevalence, ranging 84.7-90.6%.

Discussion

Bayesian methods for estimating the prevalence have been utilized by many researchers [13, 15, 16, 17, 18, 19,

Table 2. Median (95% credible interval) of PTB prevalence (π) from the simulated values after burn-in phase using two specificities (θ), B(30,2) and B(310.3,9.7), by various prior of sensitivity (η)

Prior of π	θ	Posterior distributions for π	
		η~B (26, 6)	η~B (696.5, 366.7)
B (149.5,2934.2)	B (30, 2)	0.049 (0.042 - 0.056)	0.048 (0.041 - 0.057)
	B (310.3, 9.7)	0.052 (0.044 - 0.060)	0.051 (0.044 - 0.060)
B (230.4,3190.1)	B (30, 2)	0.067 (0.060 - 0.077)	0.067 (0.059 - 0.078)
	B (310.3, 9.7)	0.071 (0.060 - 0.081)	0.070 (0.061 - 0.080)
B (77.8,2349.3)	B (30, 2)	0.032 (0.026 - 0.040)	0.032 (0.026 - 0.040)
	B (310.3, 9.7)	0.035 (0.026 - 0.043)	0.034 (0.027 - 0.042)
		η~B (210.7, 145.1)	η~B (186.1, 215.1)
B (149.5,2934.2)	B (30, 2)	0.049 (0.041 - 0.057)	0.048 (0.041 - 0.056)
	B (310.3, 9.7)	0.051 (0.044 - 0.060)	0.050 (0.042 - 0.058)
B (230.4,3190.1)	B (30, 2)	0.068 (0.060 - 0.077)	0.067 (0.060 - 0.076)
	B (310.3, 9.7)	0.070 (0.062 - 0.079)	0.069 (0.061 - 0.078)
B (77.8,2349.3)	B (30, 2)	0.032 (0.025 - 0.040)	0.032 (0.025 - 0.039)
	B (310.3, 9.7)	0.034 (0.027 - 0.042)	0.033 (0.027 - 0.041)
		η~B (49.0, 29.1)	η~B (422.5, 490.8)
B (149.5,2934.2)	B (30, 2)	0.048 (0.041 - 0.055)	0.049 (0.042 - 0.058)
	B (310.3, 9.7)	0.051 (0.043 - 0.060)	0.050 (0.043 - 0.058)
B (230.4,3190.1)	B (30, 2)	0.068 (0.060 - 0.077)	0.068 (0.060 - 0.076)
	B (310.3, 9.7)	0.071 (0.062 - 0.080)	0.069 (0.062 - 0.079)
B (77.8,2349.3)	B (30, 2)	0.032 (0.025 - 0.039)	0.032 (0.025 - 0.039)
	B (310.3, 9.7)	0.034 (0.027 - 0.042)	0.033 (0.027 - 0.040)
		η~B (70.5, 43.7)	
B (149.5,2934.2)	B (30, 2)	0.048 (0.041 - 0.057)	
	B (310.3, 9.7)	0.050 (0.044 - 0.059)	
B (230.4,3190.1)	B (30, 2)	0.068 (0.060 - 0.076)	
	B (310.3, 9.7)	0.070 (0.062 - 0.079)	
B (77.8,2349.3)	B (30, 2)	0.032 (0.026 - 0.039)	
	B (310.3, 9.7)	0.034 (0.027 - 0.041)	

Table 3. Posterior means and 95% credible intervals of the sensitivity (η) by three different priors of prevalence, one for main prior and two for extreme priors, using two prior for specificity, θ~B(310.3,9.7) and θ~B(30,2)

Prior of π	Posteriors of η for:		
	B (70.5, 43.7)	B (186.1, 215.1)	B (26, 6)
	θ~B (310.3, 9.7)		
B (77.8, 2349.3)	0.639 (0.547 0.725)	0.469 (0.417 0.518)	0.861 (0.757 0.940)
B (149.5, 2934.2)	0.649 (0.559 0.729)	0.476 (0.427 0.520)	0.868 (0.743 0.946)
B (230.4, 3190.1)	0.659 (0.568 0.740)	0.477 (0.427 0.525)	0.882 (0.769 0.946)
	θ~B (30, 2)		
B (77.8, 2349.3)	0.623 (0.535 0.715)	0.464 (0.419 0.512)	0.819 (0.664 0.926)
B (149.5, 2934.2)	0.623 (0.535 0.709)	0.464 (0.415 0.513)	0.833 (0.687 0.938)
B (230.4, 3190.1)	0.620 (0.522 0.704)	0.465 (0.417 0.513)	0.829 (0.669 0.927)

Table 4. Posterior means and 95% credible intervals of the specificity (θ) by three different priors of prevalence (π), one for main prior and two for extreme priors, using two prior for sensitivity (η), B(186.1,215.1) and B(70.5,43.7)

Prior of π	Posteriors of θ for:	
	B (30, 2)	B (310.3, 9.7
	η~B (186.1, 215.1)	
B (77.8, 2349.3)	0.847 (0.830 0.862)	0.863 (0.849 0.877)
B (149.5, 2934.2)	0.852 (0.836 0.867)	0.869 (0.855 0.885)
B (230.4, 3190.1)	0.859 (0.841 0.876)	0.877 (0.861 0.892)
	η~B (70.5, 43.7)	
B (77.8, 2349.3)	0.852 (0.835 0.868)	0.869 (0.854 0.885)
B (149.5, 2934.2)	0.860 (0.844 0.876)	0.878 (0.862 0.893)
B (230.4, 3190.1)	0.870 (0.852 0.888)	0.890 (0.874 0.906)
	η~B (26, 6)	
B (77.8, 2349.3)	0.859 (0.842 0.876)	0.878 (0.861 0.893)
B (149.5, 2934.2)	0.871 (0.852 0.890)	0.878 (0.862 0.893)
B (230.4, 3190.1)	0.885 (0.865 0.902)	0.906 (0.888 0.923)

24]. In the context of Bayesian analysis, in particular, for a single diagnostic test with small sample size relative to the number of parameters to be estimated, at least two of the three parameters need to have good priors to obtain reasonable posteriors. In other words, in cases where there are relatively few data per parameter, drawing useful inferences require substantive prior information. Among the three priors tested for prevalence, π~B(230.4,3190.1) yielded 6.7-7.1% and the others yielded estimates of 3.2-5.3% in prevalence. We obtained information on prevalence from two previous studies conducted at the regional level. These results, however, have some limitations for its use in producing priors for prevalence because the results showed great variation depending on the diagnostic test employed, the study population such as age of the tested animals, different clinical stages of the tested animals, study region, and the study design including sample size. In addition these results are based only on the diagnostic test without employing confirmatory test.

Without comprehensive information available for prevalence we could not certain which estimate is more reasonable for the study population. However, we believe that both are a bit underestimated values. The information used for constructing priors in the current study was from the results conducted in several other provinces with different diagnostic tests, leading to different posteriors in prevalence. Large proportion of animals with stage of infection not detectable with ELISA may account for this. Short period of sampling for 2 months may not provide the real situation of population dynamics. Another possibility is that survey sampling error such as bias attributed by participation of farm owners with well-managed may be responsible for low prevalence.

The specificities produced by two priors seemed to be fairly stable with no great variations in posteriors, ranging 84.7-90.6%, which are a bit lower than previously reported [8, 26]. This result may be related to the prior for prevalence, in that expected specificity vary with disease

prevalence, as noted by Brenner and Gefeller [4]. For sensitivity the resulting posteriors of 46.4-88.2% were too wide enough to be certain. This may due partly to selection of improper priors for sensitivity. Bayesian inference is often criticized because it depends largely on the prior distribution. For example, beta prior for sensitivity derived from the results performed outside of Korea showed higher estimates (62.0-65.9%) than did those obtained from domestic studies (46.4-47.7%). Whereas uniform prior showed higher estimate, ranging 81.9-88.2%. The uniform prior was obtained from the results against standard serum sample size of 30. We think this prior has at least two problems. First, the data set to elicit prior for sensitivity was clearly too small so that the prior may yield biased or rough estimate for parameters of interest. Second, the features of standard sera may different from those obtained from field sample consisted of animals having a variety of clinical stage of infection. These results illustrated the importance of prior selection for the parameters.

We noted that Bayesian approach is useful alternative means to draw better inferences about the performance of a new diagnostic test in case when either gold test is not available or not employed, although it is evident this method is depend largely on the prior distributions of the parameter of interest, as in this study.

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