Scientific Article

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Estimation of sensitivity and specificity of pregnancy diagnosis using transrectal ultrasonography and ELISA for pregnancy-associated glycoprotein in dairy cows using a Bayesian latent class model

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Abstract

AIMS: To determine the sensitivity (Se) and specificity (Sp) of pregnancy diagnosis using transrectal ultrasonography and an ELISA for pregnancy-associated glycoprotein (PAG) in milk, in lactating dairy cows in seasonally calving herds approximately 85–100 days after the start of the herd's breeding period.

METHODS: Paired results were used from pregnancy diagnosis using transrectal ultrasonography and ELISA for PAG in milk carried out approximately 85 and 100 days after the start of the breeding period, respectively, from 879 cows from four herds in Victoria, Australia. A Bayesian latent class model was used to estimate the proportion of cows pregnant, the Se and Sp of each test, and covariances between test results in pregnant and non-pregnant cows. Prior probability estimates were defined using beta distributions for the expected proportion of cows pregnant, Se and Sp for each test, and covariances between tests. Markov Chain Monte Carlo iterations identified posterior distributions for each of the unknown variables. Posterior distributions for each parameter were described using medians and 95% probability (i.e. credible) intervals (PrI). The posterior median estimates for Se and Sp for each test were used to estimate positive predictive and negative predictive values across a range of pregnancy proportions.

RESULTS: The estimate for proportion pregnant was 0.524 (95% PrI = 0.485–0.562). For pregnancy diagnosis using transrectal ultrasonography, Se and Sp were 0.939 (95% PrI = 0.890–0.974) and 0.943 (95% PrI = 0.885–0.984), respectively; for ELISA, Se and Sp were 0.963 (95% PrI = 0.919–0.990) and 0.870 (95% PrI = 0.806–0.931), respectively. The estimated covariance between test results was 0.033 (95% PrI = 0.008–0.046) and 0.035 (95% PrI = 0.018–0.078) for pregnant and non-pregnant cows, respectively. Pregnancy diagnosis results using transrectal ultrasonography had a higher positive predictive value but lower negative predictive value than results from the ELISA across the range of pregnancy proportions assessed.

CONCLUSIONS AND CLINICAL RELEVANCE: Pregnancy diagnosis using transrectal ultrasonography and ELISA for PAG in milk had similar Se but differed in predictive values. Pregnancy diagnosis in seasonally calving herds around 85–100 days after the start of the breeding period using the ELISA is expected to result in a higher negative predictive value but lower positive predictive value than pregnancy diagnosis using transrectal ultrasonography. Thus, with the ELISA, a higher proportion of the cows with negative results will be non-pregnant, relative to results from transrectal ultrasonography, but a lower proportion of cows with positive results will be pregnant.

KEY WORDS: *Dairy cow, pregnancy diagnosis, ultrasonography, pregnancy-associated glycoprotein, ELISA, latent class model, sensitivity, specificity, Bayesian estimation*

Introduction

Accurate diagnosis of pregnancy shortly after conception provides important management information for dairy farmers. Early pregnancy diagnosis can identify the date of conception and, as a result, the sire of pregnancy, the expected calving date, cows not pregnant and suitable for oestrous synchronisation or anoestrous cow treatments, non-pregnant cows for culling, and the future calving pattern for the herd. Examination of the uterus and its contents by an experienced operator using transrectal ultrasonography is currently the most common method for early pregnancy diagnosis in dairy herds.

The sensitivity (Se) and specificity (Sp) of a diagnostic test, such as pregnancy diagnosis, are usually considered relatively constant characteristics of the test, whereas the positive predictive value (PPV) and the negative predictive value (NPV) of diagnoses for individual cows depend upon the Se and Sp of the test and the prior probability that the cow is pregnant. If no prior information specific to the cow is considered, the prior probability that the cow is pregnant is the same as the true proportion of cows in

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MSD	Breeding start date
NPV	Negative predictive value
PAG	Pregnancy-associated glycoprotein
PPV	Positive predictive value
PrI	Probability (i.e. credible) interval
Se	Sensitivity
Sp	Specificity

the herd that are pregnant. Establishing the Se and Sp of early pregnancy diagnosis is challenging because there is no gold standard reference test, not all pregnancies are carried through to term or are of the same duration, and not all inseminations may be recorded. This makes the use of subsequent calving records too inaccurate to determine the true pregnancy status at the time of diagnosis.

Published test characteristics for pregnancy diagnosis obtained from studies that assume a reference test to be a gold standard have intrinsic biases because of inevitable classification errors made by the imperfect reference test. The Se and Sp of transrectal ultrasonography for pregnancy diagnosis were estimated to be 0.977 and 0.878, respectively, for cows examined between 26 and 33 days after insemination, in a study that used rectal palpation 50–90 days after insemination and calving date as the gold standard for pregnancy (Pieterse *et al.* 1990). This positive gold standard took account of pregnancy losses after transrectal ultrasonography and so the Sp estimate is likely to be negatively biased if the aim is to identify the status of the cow at time of testing rather than to predict which cows will calve. An Australian study estimated the Se and Sp of transrectal ultrasonography as 0.965 and 0.856, respectively, in cows examined 28–35 days after insemination which were not detected returning to oestrus within 24 days of insemination, using a gold standard of results of manual rectal pregnancy examination conducted between 70 and 91 days after insemination (Nation *et al.* 2003). Pregnancy diagnosis using transrectal ultrasonography performed between 35–91 days of gestation by experienced operators was demonstrated to provide a reliable estimation of calving date in >90% of cows in 116 herds in New Zealand (Brownlie *et al.* 2016). However pregnancy diagnosis using transrectal ultrasonography requires considerable operator expertise, expensive equipment and adequate animal restraint.

In seasonally calving herds, transrectal ultrasonography is usually first undertaken in all eligible cows on one day, therefore pregnant cows can be examined at varying stages of gestation, including <28 days. This is quite different from pregnancy diagnosis programmes commonly used in year-round calving herds that do not run bulls with lactating cows, where pregnant cows are mostly within a narrow range of stages of gestation when examined, and pregnancy diagnosis can be timed to ensure the stage of gestation is sufficiently advanced to ensure high Se for detection. The Se of transrectal ultrasonography increases with fetal age and can be <100% for detecting pregnancies before 29 days of gestation (Romano *et al.* 2006; Quintela *et al.* 2012). Thus, when performing pregnancy diagnosis in seasonally calving herds where fetal age could be <28 days, interventions for cows diagnosed non-pregnant that could result in embryonic loss are not appropriate. Instead, follow-up visits are usually used to re-examine cows that were not diagnosed pregnant at the first examination (Brownlie *et al.* 2016). Accordingly, there is little advantage in detecting pregnancies before day 35 of gestation. High negative predictive values for negative final pregnancy diagnoses performed ≥35 days after the end of the breeding period are much more important.

An ELISA that categorises cows as pregnant, not pregnant, or inconclusive through a semi-quantitative measurement of concentrations of pregnancy-associated glycoprotein (PAG) in milk is available through commercial laboratories. Although it cannot be used to determine fetal age, this test has advantages over transrectal ultrasonography; it is non-invasive to the cow, is cheaper when testing small numbers of cows, and does not require access to a skilled operator. This ELISA was found to have a Se of 0.992 and Sp of 0.955 when compared to a gold standard of veterinary pregnancy diagnosis using transrectal ultrasonography at >60 days of gestation in cows that were previously diagnosed pregnant (LeBlanc 2013).

As both ELISA for PAG and transrectal ultrasonography are imperfect tests for pregnancy diagnosis, neither is an ideal gold standard reference test to assess Se and Sp of the other. An alternative approach for determining test characteristics is use of Bayesian latent class models. When used with two diagnostic tests, such models relate the unknown variable e.g. the true pregnancy status of the cows, through the test Se and Sp, to the observed paired test results from the two tests e.g. transrectal ultrasonography and ELISA. The model estimates the likelihood of the observations from across a feasible range of unknown test characteristics and pregnancy proportions, based on defined prior distributions of the model parameters. The distribution of likelihoods then defines the parameter estimate for each of the unknown variables. Using latent class model methodology, the Se and Sp of both tests can be estimated (Kostoulas *et al.* 2017).

These methods have been used in some recent studies. In Canadian cows between 28–45 days after breeding, the Se and Sp of pregnancy diagnosis using transrectal ultrasonography were estimated as 0.96 and 0.99, respectively, and using an ELISA measuring PAG were estimated as 0.99 and 0.95, respectively (Dufour *et al.* 2017). For cows in South African herds examined using transrectal ultrasonography by five veterinarians between 28–35 days after insemination, estimated Se and Sp ranged between 0.78–0.91 and 0.95–1.00, respectively (Fosgate *et al.* 2017). In the same cows, the estimated Se and Sp of the ELISA for PAG in milk were 0.99 and 0.93, respectively.

The objective of the current study was to use Bayesian latent class models to determine the Se and Sp of pregnancy diagnosis using transrectal ultrasonography and ELISA for PAG in milk in lactating dairy cows from seasonally calving herds, carried out approximately 85–100 days after the start of the breeding period (MSD).

Materials and methods

Animals and herds

The current study was part of a longitudinal study investigating the time and risk factors for pregnancy loss in dairy cows conducted in the Macalister Irrigation District of Victoria, Australia, in 2015. Four split-calving herds, ranging in size between 488–718 cows were used for study. All herds had two distinct calving and breeding periods in each calendar year with the majority of calvings in spring, and were pasture-based with cows fed concentrates during milking on rotary dairy platforms. Cows were primarily Friesian or Friesian-Jersey crossbred and average milk production over the lactation was 1.60 kg milk solids per cow per day.

Cows were recruited if eligible for insemination during the spring breeding period. This included some cows that had calved before the spring calving period in 2015 and had not conceived since then. All cows were treated to synchronise ovulation, commencing 10 days before the MSD, using I/M 50 μg gonadorelin (Selenta-Gonasyn, Boehringer Ingelheim Pty Ltd., North Ryde, NSW, Australia) and an intravaginal progesterone releasing device containing 1 g progesterone (Selenta-DIB-V, Boehringer Ingelheim Pty Ltd). Seven days later, cows were injected I/M with 500 mg cloprostenol (Selenta-Cyclase, Boehringer Ingelheim Pty Ltd) and the progesterone device was removed. The following day all cows were injected I/M with 50 µg gonadorelin and any cow detected in oestrus was inseminated. All remaining cows were inseminated the following day, which was the MSD. Oestrus detection and artificial insemination were undertaken for the following 71–75 days in each herd.

Pregnancy diagnosis

All cows across all four herds were examined by a single experienced veterinarian using a portable ultrasound machine (BCF Easi Scan linear, BCF Technology, Bellshill, Scotland) around 35 days after the MSD, for pregnancy diagnosis and to estimate the age of the fetus in cows diagnosed pregnant. The same veterinarian re-examined all cows around 85 and 175 days after MSD. At each examination, each cow was diagnosed as either pregnant or non-pregnant. Diagnosis of pregnancy was based on detection of a fetus and the presence of fluid in a uterine horn. Diagnosis of non-pregnancy was based on both uterine horns being devoid of fluid and a fetus. Cows suspected to be pregnant, but with inconclusive confirmatory evidence, were classified as non-pregnant and included in analyses. This was the usual practice, and was done to ensure that these cows were re-examined at a later date.

Milk samples were collected from all cows during milk recording on three occasions; approximately 70, 100 and 150 days after MSD. Individual cow sub-samples were sent to a local laboratory (Herd Improvement Co-operative Australia Ltd, Maffra, VIC, Australia), where they were analysed within 3 days of sample collection using an ELISA for PAG in milk (Idexx Milk Pregnancy Test; IDEXX Laboratories Pty Ltd, Rydalmere, NSW, Australia). A technician performed all ELISA according to the manufacturer's instructions. There were three possible results for the ELISA; pregnant (positive), non-pregnant (negative) and inconclusive. Results were defined based on the difference in optical density between the test and negative control samples (S−N value). Based on the manufacturer's guidelines, samples with S−N <0.100 were classified as non-pregnant; samples from cows calved between 28 and 44 days at the time of testing, with S−N ≥0.100 and <0.250 were unable to be classified and were marked for retesting, and samples with S−N ≥0.250 were classified as pregnant. Samples from cows calved >44 days at the time of testing with S–N ≥0.100 were classified as pregnant. Cows with inconclusive ELISA results were excluded from analyses on the basis that such cows would usually be retested with the ELISA at a subsequent date and managed according to the result of that subsequent test.

Both the veterinarian performing the pregnancy diagnosis using transrectal ultrasonography, and the laboratory technician conducting the ELISA, were blinded to results from the alternative tests.

Selection of cows for analyses

Results for statistical analyses were from pregnancy diagnoses using transrectal ultrasonography at approximately 85 days after MSD, and testing of milking samples collected at approximately 100 days after MSD. Approximately half of the herd was expected to be pregnant at that time, and this is when early pregnancy diagnosis is typically undertaken in seasonally calving cows. Results were selected for all cows that were examined using transrectal ultrasonography and were subsequently sampled for ELISA no more than 15 days later. Cows that were <35 days of gestation when examined using transrectal ultrasonography were excluded from analyses, as well as those diagnosed non-pregnant at approximately 85 days after MSD, which when subsequently examined were pregnant with a fetus that would have been <35 days of age at 85 days after MSD.

Statistical analyses

A Bayesian latent class model was used to estimate the true, but unknown, pregnancy proportion, the unknown Se and Sp for pregnancy diagnosis using transrectal ultrasonography and the ELISA, and the covariance between both tests in each of pregnant and non-pregnant cows. The model used is presented in Supplementary Figure 1[1]. Data manipulation and analysis was carried out using R v3.4.0 (R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria). The Se and Sp for pregnancy diagnosis using transrectal ultrasonography and ELISA were constrained to lie within the range 0.5–1.0, and the covariance between test results in pregnant and non-pregnant cows were constrained to lie within the range 0.0–0.50.

Prior distributions for the proportion of cows pregnant were determined using as the mode the median 6-week in-calf proportion for Australian seasonally and split calving herds in 2009 of 0.50 (Morton 2011). The proportion pregnant for the study population was assumed to lie within the range 0.43–0.57 with 95% confidence (i.e. 2.5% of the prior distribution was <0.43 and 2.5% >0.57) and a beta distribution was built using these restrictions. Prior distributions for the Se and Sp of the tests were obtained by building beta distributions around published point estimates for each parameter, after allowing a potentially feasible range around the point estimate. Prior distributions for the covariances between test results within pregnant and non-pregnant cows were defined using theorised maxima, and included only positive covariance values. The α and β parameter values that described each beta distribution were obtained using the epi.betabuster function in R (www.rdocumentation.org/packages/epiR/versions/0.9-87/topics/epi.betabuster), based on the defined mode and the upper (or lower) 95% confidence limits. Prior distributions are presented in Table 1 and Figure 1.

The α and β parameters of the beta distribution that defined each prior probability density distribution were included in the latent class model. The sensitivity of the model to the priors used was examined by rerunning the model with less informative priors for Se and Sp of both tests; for these, the limits of the middle 95% were twice as far apart as those used to define the original prior.

Bayesian statistical inference was performed using Stan version 2.15.0 software (NumFocus, Austin, TX, USA). Stan uses a no-U turn variant of the Hamiltonian Markov Chain Monte Carlo algorithm (Neil 2011) for iteratively sampling the parameter space. This algorithm effectively and efficiently samples from across the parameter space whilst providing a robust and rapid convergence capability. Four chains replicated each simulation and the posterior distributions were obtained by summarising

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Table 1. Prior distributions (mode with middle 95% range), and α and β parameters for beta distributions for inclusion in Bayesian latent class models used to estimate the true proportion of cows pregnant, the sensitivity (Se) and specificity (Sp) of pregnancy diagnosis using transrectal ultrasonography and an ELISA for pregnancy associated glycoprotein in milk, and the covariance between test results within pregnant and non-pregnant cows.

			Beta distribution	
	Mode	95% range	α	β
Proportion pregnant	0.5	0.43–0.57	100	100
Transrectal ultrasonography				
Se	0.95	0.89–0.98	99.7	6.19
Sp	0.97	0.89–0.99	53.6	2.63
ELISA				
Se	0.98	0.91–0.99	80.5	3.04
Sp	0.93	0.81–0.97	42.3	4.35
Covariance	0.05	0.01–0.17	2.88	36.7

the aggregated post burn-in iterations of all four chains. Each chain simulation had 50,000 iterations with the first 10,000 iterations discarded (burn-in).

Models were examined for convergence by examining the distribution of the negative log of the posterior probability for the presence of a single maximum. Autocorrelations between iterations within each chain series were examined for evidence of patterns, and the effective sample size of the combined chains was compared to the expected number under the assumption of independence between chains.

Positive predictive values and NPV were calculated across the range of pregnancy proportions from 0.1 to 0.9 using standard formulae (Dohoo *et al.* 2009) and the posterior median estimates for Se and Sp for transrectal ultrasonography and the ELISA. The lower and upper 95% credible interval limit estimates for Se and Sp were used to calculate 95% probability (i.e. credible) intervals (PrI).

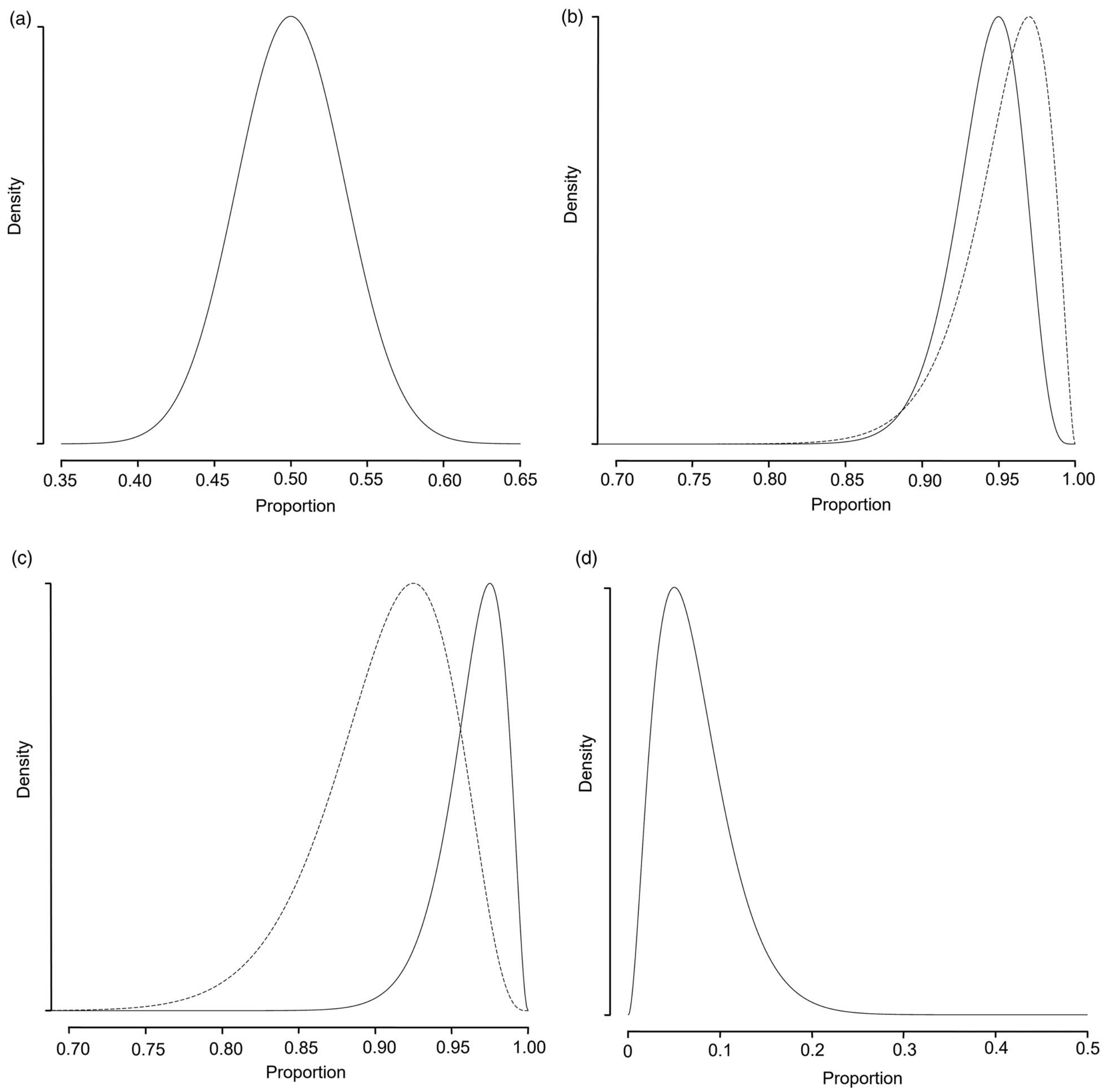

Figure 1. Prior probability distributions for inclusion in Bayesian latent class models used to estimate (a) the true proportion of cows pregnant, the sensitivity (—) and specificity (–) of pregnancy diagnosis using (b) transrectal ultrasonography and (c) an ELISA for pregnancy-associated glycoprotein in milk, and (d) the covariance between test results within pregnant and non-pregnant cows.

Results

Results were selected from the 923 cows that were examined using transrectal ultrasonography and were sampled for ELISA no more than 15 days apart. Of these, cows with inconclusive ELISA results (n=22) were excluded, as were cows with an estimated fetal age <35 days (n=10), and cows diagnosed as non-pregnant when examined 85 days after MSD which were subsequently diagnosed pregnant with a fetus that would have been <35 days of age at 85 days (n=22). The remaining 879 cows were used for analyses. For these cows, the median interval from MSD to pregnancy diagnosis using transrectal ultrasonography was 84 (min 83, max 85) days, and to milk sampling for the ELISA was 94 (min 88, max 100) days. In the four study herds, the number of cows included was 245, 119, 176, and 339, respectively. Of the 879 cows, 512 (58.2%) were diagnosed pregnant using transrectal ultrasonography, and 501 (57.0%) had positive results using the ELISA. The median stage of gestation in cows diagnosed pregnant using transrectal ultrasonography was 77 (min 49, max 86) days. The cross tabulation of results for pregnancy diagnosis using transrectal ultrasonography and ELISA is presented in Table 2.

For the whole study, 2,702 ELISA were carried out, of which 40 (1.48%) had inconclusive results. Of these 40, seven returned a positive result at the preceding test and a negative result at the following test, five returned a negative result at the preceding test and a positive result at the following test, eight returned both negative results, and 20 returned both positive results at the preceding and following tests.

Table 2. Number of cows that were classified as pregnant or non-pregnant following pregnancy diagnosis using transrectal ultrasonography (PD) or testing of milk samples using an ELISA for pregnancy associated glycoprotein, in four dairy herds in Victoria, Australia, approximately 100 days after the start of the herd's breeding period.

	ELISA pregnant	ELISA non-pregnant	Total
PD pregnant	452	6	458
PD non-pregnant	49	372	421
Total	501	378	879

All Markov Chain Monte Carlo models converged to a unique solution. Median estimates with 95% PrI from the model are presented in Table 3. The estimates for covariances between tests within each of pregnant and non-pregnant cows indicated low correlations between tests. Estimates from the model using less informative (more widely distributed) priors are also presented in Table 3. The maximum percentage change in the medians of the posterior distributions from the models using the original priors and the less informative priors was 2.2%.

Figure 2 shows the PPV and NPV, using medians of the posterior distributions for Se and Sp from the model using the original (more informative) prior distributions that included covariance terms, across the feasible range of prior pregnancy proportions (0.1 to 0.9) in tested cows.

Discussion

Results from this study using Bayesian latent class models demonstrated that the ELISA for PAG in milk was slightly more sensitive than transrectal ultrasonography for diagnosing pregnancy, whereas transrectal ultrasonography was slightly more specific than the ELISA, when pregnancy diagnosis was carried out in seasonally calving herds approximately 85–100 days after MSD. Our Se results are relevant for cows pregnant with a fetus ≥35 days of age. Given the test parameter estimates obtained from this study, a PPV of ≥95% can be expected when at least 55 or 76% of the herd is diagnosed pregnant using transrectal ultrasonography or ELISA, respectively, as shown in Figure 2. Conversely, a NPV of ≥95% can be expected when less than 43 or 55% of the herd is diagnosed pregnant using transrectal ultrasonography or ELISA, respectively.

The test parameter estimates obtained from this study were slightly lower than previous estimates obtained using Bayesian modelling. Dufour *et al.* (2017) reported estimated median Se and Sp for pregnancy diagnosis using transrectal ultrasonography of 0.96 (95% PrI = 0.91–1.00) and 0.99 (95% PrI = 0.97–1.00), respectively, and for ELISA for PAG in milk 0.99 (95% PrI =

Table 3. Posterior point estimates with 95% probability intervals (PrI) from Bayesian latent class models used to estimate the true proportion of cows pregnant, the sensitivity (Se) and specificity (Sp) of pregnancy diagnosis using transrectal ultrasonography and an ELISA for pregnancy-associated glycoprotein in milk, and covariances between test results within pregnant and non-pregnant cows.

	Model 1[a]		Model 2[b]	
	Median	95% PrI	Median	95% PrI
Proportion pregnant	0.524	0.485–0.562	0.524	0.460–0.581
Transrectal ultrasonography				
Se	0.939	0.890–0.974	0.927	0.842–0.982
Sp	0.943	0.885–0.984	0.926	0.826–0.953
ELISA				
Se	0.963	0.919–0.990	0.956	0.882–0.994
Sp	0.870	0.806–0.931	0.854	0.750–0.952
Covariance				
Pregnant cows	0.033	0.008–0.046	0.041	0.006–0.118
Non-pregnant cows	0.035	0.018–0.078	0.044	0.006–0.122

[a]Model used prior distributions shown in Table 1.
[b]Model used prior distributions with the middle 95% twice as wide as those used in Model 1.

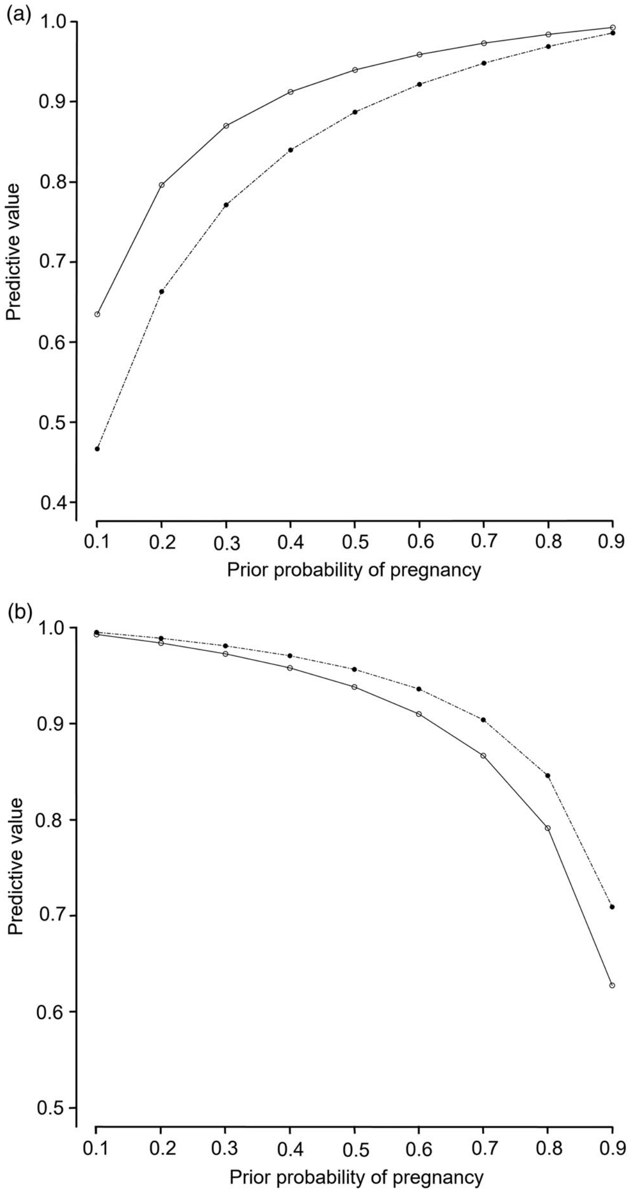

Figure 2. Estimated (a) positive predictive values and (b) negative predictive values for varying probabilities of pregnancy in dairy cows, based on the test characteristics of pregnancy diagnosis using transrectal ultrasonography (—○—), or ELISA for pregnancy-associated glycoprotein in milk (-●-), estimated from Bayesian latent class models.

0.98–1.00) and 0.95 (95% PrI = 0.89–1.00), respectively, for cows between 28–45 days post-breeding. Fosgate *et al.* (2017) reported median Se and Sp for pregnancy diagnosis using transrectal ultrasonography as between 0.78–0.91 and 0.95–1.00, respectively, across the five study veterinarians, for cows between 28–35 days post-breeding. The median Se and Sp for ELISA were reported as 0.99 (95% PrI = 0.98–1.00) and 0.93 (95% PrI = 0.90–0.96), respectively. The differences between studies may reflect the different pregnancy diagnosis scenario used in our study to those used in the previous studies. Cows in our study had a greater range of stages of gestation (49–86 days) than the other studies and many pregnant cows had more time at risk for them to lose their pregnancy before being examined for pregnancy diagnosis. We found that 7.8% of pregnancies were lost from day 35 to around day 140 of gestation, with 36 cows (3.1% of pregnancies) losing their pregnancy between approximately day 35 and 90 after mating (RW Shephard, JM Morton, L Reynolds, unpublished data). The lower Sp of the ELISA in the current and previous studies is likely to be due, in part, to positive ELISA results following pregnancy loss (Fricke *et al.* 2016), as concentrations of PAG are detectable for >9 days after induction of pregnancy loss (Giordano *et al.* 2012).

The studies reported by Dufour *et al.* (2017) and Fosgate *et al.* (2017) describe a testing regimen that is not easily applied within seasonally calving herds, where individual cows were examined between 28–45 days after insemination. In contrast, most pregnant cows in seasonally calving herds are examined at a whole-herd pregnancy test performed around 84 days after the MSD. This enables more accurate aging of the fetus, as accuracy was found to be greatest when cows were between 56–84 days of gestation (Brownlie *et al.* 2016). Therefore, in the current study, we used cows that were examined between 83 and 100 days after the MSD, which corresponds to the timing of a typical early pregnancy test for seasonally calving herds.

Ideally, both the pregnancy diagnosis using transrectal ultrasonography and the ELISA for PAG in milk would have been carried out on the same day, but this was not possible due to farm, herd testing and veterinary logistical constraints. Thus cows correctly identified as pregnant using transrectal ultrasonography may subsequently have lost their pregnancy before the ELISA test, resulting in discordant results where both tests yielded correct results for the cow's status at the time the test was performed. A total of 39 of the 879 study cows were identified as losing their pregnancy between their second and third pregnancy diagnoses using transrectal ultrasonography (RW Shephard, JM Morton, L Reynolds, unpublished data). The interval between these examinations was between 34–48 days across the four herds. It was not possible to identify the number of pregnancy losses that occurred between the pregnancy diagnosis at approximately 85 days after the MSD and the ELISA at approximately 100 days after MSD, but fewer than 39 losses would be expected because the time between tests within cows was no more than 15 days. However the ELISA can remain positive after pregnancy loss (Fricke *et al.* 2016), partly negating this bias due to delayed ELISA testing.

Estimates for covariance between the two tests indicated that there was some correlation between the two tests. The two methods of pregnancy diagnosis use very different mechanisms for diagnosing pregnancy but it is unlikely that they are completely independent from each other. Both the concentration PAG in milk and the size of the uterus and its contents, the focal sites for examination when performing pregnancy diagnosis using transrectal ultrasonography, increase as pregnancy advances. This implies that some correlation between test results by stage of pregnancy should be expected and that the model with covariance terms is most representative. Estimates obtained from the model using less informative priors resulted in very small decreases in the Se and Sp estimates compared with the model using the original priors, indicating only modest sensitivity of our results to choice of priors.

These methods used for pregnancy diagnosis have advantages and disadvantages. Transrectal ultrasonography can be used to age the fetus when carried out by an experienced operator at 35–91 days of gestation (Brownlie *et al.* 2016), whereas the ELISA can only be used to classify cows as pregnant or not. Cows that are ELISA-positive are typically assumed to have conceived to their last recorded insemination. This is likely to be valid in systems where individual cows are examined within a narrow time interval after each insemination, there are no inseminations after conception, inseminated non-returning cows are examined before any further inseminations are performed, and there are no unrecorded inseminations or bull services. However, all four of these conditions are rarely satisfied when cows are examined in seasonally-calving herds. There is potential for trauma to the uterus and its contents or to the ovaries following any transrectal examination of the reproductive tract. However, the risk of pregnancy loss was only 1.3% higher in cows and heifers that underwent manual transrectal pregnancy diagnosis compared to non-palpated cattle (Romano *et al.* 2007), and pregnancy losses as a result of pregnancy diagnosis using transrectal ultrasonography are expected to be less than this because it is a less manipulative procedure.

The half-life of the PAG detected by the ELISA is reported to be between 7–14 days in the maternal circulation (Fricke *et al.* 2016). One potential use of this persistence of PAG in maternal serum and milk is that it may provide an opportunity for retrospectively investigating low conception rates in herds when used in conjunction with pregnancy diagnosis using transrectal ultrasonography. Cows that have recently experienced pregnancy loss may return a positive ELISA result but a negative result when examined using transrectal ultrasonography. Herds with low pregnancy proportions due to high pregnancy loss may have high numbers of cows that are not pregnant when examined using transrectal ultrasonography but are positive when tested using ELISA. In contrast, herds with a low pregnancy proportions due to conception failure should have similar numbers of cows diagnosed not pregnant using both methods.

Both pregnancy diagnosis using transrectal ultrasonography and ELISA for PAG in milk had similar Se but had important differences in predictive values at both low and at high pregnancy proportions. The impact of a classification error depends in part on the proposed action to be taken following pregnancy diagnosis for animals diagnosed as either pregnant or not pregnant, and may vary with the test used as well as expected pregnancy proportion before diagnoses are performed. Early pregnancy diagnosis of seasonally calving herds around 77–91 days after the MSD using the ELISA is expected to result in a higher NPV but lower PPV than pregnancy diagnosis using transrectal ultrasonography. Thus, with the ELISA, a higher proportion of the cows with negative results will be non-pregnant, relative to results from transrectal ultrasonography, but a lower proportion of the cows with positive results will be pregnant.

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References

Brownlie TS, Morton JM, McDougall S. Accuracy of fetal age estimates using transrectal ultrasonography for predicting calving dates in dairy cows in seasonally calving herds in New Zealand. *New Zealand Veterinary Journal* 64, 324–9, 2016

*__Dohoo I, Martin W, Stryhn H.__ Veterinary Epidemiologic Research, 2nd Edtn. VER Inc., Charlottetown, Canada, 2009

Dufour S, Durocher J, Dubuc J, Dendukuri N, Hassan S, Buczinski S. Bayesian estimation of sensitivity and specificity of a milk pregnancy-associated glycoprotein-based ELISA and of transrectal ultrasonographic exam for diagnosis of pregnancy at 28–45 days following breeding in dairy cows. *Preventive Veterinary Medicine* 140, 122–33, 2017

Fosgate GT, Motimele B, Ganswindt A, Irons PC. A Bayesian latent class model to estimate the accuracy of pregnancy diagnosis by transrectal ultrasonography and laboratory detection of pregnancy-associated glycoproteins in dairy cows. *Preventive Veterinary Medicine* 145, 100–9, 2017

Fricke PM, Ricci A, Giordano JO, Carvalho PD. Methods for and implementation of pregnancy diagnosis in dairy cows. *Veterinary Clinics of North America: Food Animal Practice* 32, 165–80, 2016

Giordano JO, Guenther JN, Lopes Jr. G, Fricke PM. Changes in serum pregnancy-associated glycoprotein, pregnancy specific protein B, and progesterone concentrations before and after induction of pregnancy loss in lactating dairy cows. *Journal of Dairy Science* 95, 683–7, 2012

Kostoulas P, Nielsen SS, Branscum AJ, Johnson WO, Dendukuri N, Dhand NK, Toft N, Gardner IA. STARD-BLCM: standards for the reporting of diagnostic accuracy studies that use Bayesian latent class models. *Preventive Veterinary Medicine* 138, 37–7, 2017

LeBlanc S. Field evaluation of a pregnancy confirmation test using milk samples in dairy cows. *Journal of Dairy Science* 96, 2345–8, 2013

*__Morton, JM.__ *Dairy Australia Fertility Data Analysis* 2010 https://www.dairyaustralia.com.au/farm/animal-management/fertility/incalf-books (accessed 24 September 2017). Dairy Australia, Melbourne, VIC, Australia, 2011

Nation DP, Malmo J, Davis GM, Macmillan KLM. Accuracy of bovine pregnancy detection using transrectal ultrasonography at 28–35 days after insemination. *Australian Veterinary Journal* 81, 63–65, 2003

*__Neil RM.__ MCMC using Hamiltonian dynamics. In: Brooks S, Gelman A, Jones GL, Meng X-L (eds). *Handbook of Markov Chain Monte Carlo*. Pp 113–62, Chapman & Hall/CRC, Boca Raton, FL, USA, 2011

Pieterse MC, Szenci O, Willemse AH, Bajcsy CS, Dieleman SJ, Taverne MA. Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology* 33, 697–707, 1990

Quintela LA, Barrio M, Peña AI, Becerra JJ, Cainzos J, Herradón PG, Díaz C. Use of ultrasound in the reproductive management of dairy cattle. *Reproduction in Domestic Animals* 47 (Suppl. 3), 34–44, 2012

Romano JE, Thompson JA, Forrest DW, Westhusin ME, Tomaszweski MA, Kraemer DC. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. *Theriogenology* 66, 1034–41, 2006

Romano JE, Thompson JA, Kraemer DC, Westhusin ME, Forrest DW, Tomaszweski MA. Early pregnancy diagnosis by palpation per rectum: influence on embryo/fetal viability in dairy cattle. *Theriogenology* 67, 486–93, 2007

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