Preventive Veterinary Medicine 145 (2017) 100–109

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

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

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ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form 27 June 2017
Accepted 4 July 2017

Keywords:
Dairy cattle
Pregnancy
Sensitivity
Specificity
Bayesian

ABSTRACT

Accurate diagnosis of pregnancy is an essential component of an effective reproductive management plan for dairy cattle. Indirect methods of pregnancy detection can be performed soon after breeding and offer an advantage over traditional direct methods in not requiring an experienced veterinarian and having potential for automation. The objective of this study was to estimate the sensitivity and specificity of pregnancy-associated glycoprotein (PAG) detection ELISA and transrectal ultrasound (TRUS) in dairy cows of South Africa using a Bayesian latent class approach. Commercial dairy cattle from the five important dairy regions in South Africa were enrolled in a short-term prospective cohort study. Cattle were examined at 28–35 days after artificial insemination (AI) and then followed up 14 days later. At both sampling times, TRUS was performed to detect pregnancy and commercially available PAG detection ELISAs were performed on collected serum and milk. A total of 1236 cows were sampled and 1006 had complete test information for use in the Bayesian latent class model. The estimated sensitivity (95% probability interval) and specificity for PAG detection serum ELISA were 99.4% (98.5, 99.9) and 97.4% (94.7, 99.2), respectively. The estimated sensitivity and specificity for PAG detection milk ELISA were 99.2% (98.2, 99.8) and 93.4% (89.7, 96.1), respectively. Sensitivity of veterinarian performed TRUS at 28–35 days post-AI varied between 77.8% and 90.5% and specificity varied between 94.7% and 99.8%. In summary, indirect detection of pregnancy using PAG ELISA is an accurate method for use in dairy cattle. The method is descriptively more sensitive than veterinarian-performed TRUS and therefore could be an economically viable addition to a reproductive management plan.

1. Introduction

Global milk production was estimated to be 466 metric tons in 2013 (Lagrange et al., 2015). Population growth in combination with continued urbanization is expected to continue to drive strong demand for increasing production. The average dairy herd size has increased over the past decades but the number of herds has decreased or remained relatively stable. Consequently, dairy cows are being managed in a smaller number of larger herds (Barkema et al., 2015). Increasing farm size is driven by "economies of scale" and supported by improved technology (von Keyserlingk et al., 2013). Increases in global production are largely due to increasing production per cow, rather than increasing cow numbers. Optimal reproductive programs maximize individual cow production by ensuring regular calvings during the cow's lifetime thus limiting the time spent in the tail of the lactation curve, when daily production is lower.

Sound reproductive management depends on establishing and maintaining pregnancy as soon as possible after the end of the voluntary waiting period after calving, or after the onset of the breeding season in the case of seasonal systems. This is achieved by ensuring normal cyclicity, breeding the cows at the optimal time in the estrus cycle and accurate pregnancy determination as soon as possible after breeding. Follow-up confirmation for the mainte-

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http://dx.doi.org/10.1016/j.prevetmed.2017.07.004
0167-5877/

nance of pregnancy is also required. The prompt detection of open cows is desirable to enable treatments to be applied that initiate estrus and allow repeated breeding attempts when a pregnancy is not present, thus reducing days open. The economic advantage of earlier detection of open cows is manifested through shorter inter-calving intervals (Oltenacu et al., 1990). The optimal situation would be a highly accurate test able to detect pregnancy before the first expected estrus following breeding, but no such tests have been developed.

Whereas the recurrence of estrus at the expected interval of 18–24 days is an obvious early indication of non-pregnancy, the weak expression of estrus behavior and the impracticality of maintaining consistent estrus detection in large dairy herds make this method unreliable. Diagnostic tests for pregnancy have therefore become indispensable to support ongoing increases in reproductive efficiency.

Pregnancy detection can be performed using both direct and indirect methods. Direct methods include transrectal palpation and transrectal ultrasonography (TRUS). Indirect methods utilize chemical markers of pregnancy including milk or plasma progestagen concentrations, or pregnancy-associated protein and pregnancy-associated glycoprotein (PAG) measurements (Fricke et al., 2016). Bovine PAGs are produced by the binucleate trophoblastic cells of the placenta early after implantation (Wooding et al., 2005). PAGs can be detected in the maternal circulation from 22 days of pregnancy (Zoli et al., 1992) until 2–3 months after parturition (Humblot, 2001; Whitlock and Maxwell, 2008).

The major advantages of indirect methods of pregnancy diagnosis are that a skilled, and thus comparatively costly, examiner is not required on the farm and they can be performed sooner after breeding compared to direct detection methods. Direct palpation of the conceptus early in gestation has also been associated with pregnancy loss (Franco et al., 1987; Thurmond and Picanso, 1993). The growing trend of larger herd sizes in conjunction with a trend towards fewer veterinarians entering rural veterinary practice (Villarroel et al., 2010) further support the development of indirect methods that will not require a highly-skilled operator to be present at the time of testing. The potential for the use of indirect pregnancy detection methods in automated systems incorporated into the milking parlor provides additional motivation for their development. PAG detection ELISAs are amenable to the design of automated systems since they have been validated for use in serum (Romano and Larson, 2010; Karen et al., 2015) and milk (Leblanc, 2013).

Accurate pregnancy diagnosis is critical to adoption in the field. High sensitivity of the diagnostic method is very important when considering the economic benefit of pregnancy detection using these modalities (Giordano et al., 2013). The economic losses associated with inducing estrus in pregnant cows (false-negative on the assay) are significant (Fricke et al., 2016). The specificity of the indirect method relative to traditional direct methods is also an important consideration in the selection of the best diagnostic method as improved specificity reduces the losses associated with the late detection of non-pregnancy and the resulting reduced opportunity cost. The cost of the diagnostic tests is also an important consideration.

Previously performed diagnostic evaluations estimated the sensitivity and specificity of PAG detection relative to direct pregnancy detection as the reference standard. This analytic approach is incapable of determining whether or not PAG detection is more accurate than the traditional pregnancy detection methods, and is not possible during early gestation when the true pregnancy status of the animals cannot be accurately determined. The objective of this study was to estimate the sensitivity and specificity of PAG detection ELISA and transrectal ultrasound (TRUS) in dairy cows of South Africa using a Bayesian latent class approach. The hypothesis was that the laboratory detection of PAGs would be a more sensitive but less specific method for the detection of pregnancies compared to veterinarian-performed TRUS.

2. Materials and methods

2.1. Study design

A short-duration prospective cohort study (July–September 2014) was implemented on commercial dairy farms within the five important dairy regions of South Africa. Selected regions were KwaZulu Natal Province, Mpumalanga Province, the west coast region of the Western Cape Province, the western coastal region of the Eastern Cape Province, and the interior of the Eastern Cape. One veterinary practice from each location was conveniently selected for participation in the study. Veterinary practitioners received training concerning study procedures and objectives at a workshop that preceded study commencement. These practitioners performed all veterinary activities on a single client herd that was conveniently selected after receiving informed consent from the herd owner. The sample size for each study location was estimated based on the desire to have 97 pregnant cows available for estimation of sensitivity and 97 additional cows available for estimation of specificity. This was based on assumed sensitivity and specificity of 95% and the desire to estimate these values +/− 0.05 at the 95% level of confidence using exact binomial methodology (Fosgate, 2005, 2009). The pregnancy proportion in sampled cows was unknown at the time of enrolment and assumed to be 50% for the sample size calculation. The sample size per study location was increased by 20% to account for losses during follow-up and errors including mislabeling of collected specimen tubes. The sample size was therefore estimated as 233 cows per location for a total 1165 cows. The study was reviewed and approved by the Animal Ethics Committee at the Faculty of Veterinary Science, University of Pretoria (Protocol No. V043-14).

2.2. Study animals and determination of reproductive status

Cows in commercial dairy herds that were presented to one of the participating veterinarians for pregnancy diagnosis were utilized for this study. Participating veterinarians were requested to enroll all eligible cows from the selected study farm until the necessary sample size was obtained. Cows were eligible to be enrolled in the study at 28–35 days after breeding by artificial insemination (AI) if no estrus activity had been detected post-breeding. Veterinarians excluded cows when the stage of pregnancy appeared inconsistent with the reported breeding to reduce the possibility of enrolling cows that were not at the correct stage of pregnancy (28–35 days post-AI). Cows that were diagnosed with any uterine or ovarian pathology by the veterinarian at the time of enrollment were excluded if treatment was required since treatment would likely cause fetal loss in pregnant cows. Veterinary diagnosis of pregnancy was performed using transrectal ultrasound (TRUS) and pregnancy was diagnosed by visualization of the embryonic/fetal membranes or by direct observation of the fetus. Participating veterinarians did not receive specific training in TRUS as part of the study and the previous experience level ranged from 1 to 8 years. Veterinarians performed TRUS blinded to the results of previous veterinary examinations and were unmasked after making their pregnancy diagnosis. Additional data that were collected at the time of examination included breed, lactation number, days in milk (DIM), and days post-AI.