Preventive Veterinary Medicine 154 (2018) 1–8

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

Evaluation of a multiplex immunoassay for bovine respiratory syncytial virus and bovine coronavirus antibodies in bulk tank milk against two indirect ELISAs using latent class analysis

Ingrid Toftaker[a,*], Nils Toft[b], Maria Stokstad[a], Liv Sølverød[c], Gordon Harkiss[d], Neil Watt[d], Amanda O' Brien[e], Ane Nødtvedt[a]

[a] Norwegian University of Life Sciences, Department of Production Animal Clinical Sciences, P.O. Box 8146 Dep, Oslo, Norway
[b] Technical University of Denmark, National Veterinary Institute, Kemitorvet Building 204, DK-2800 Lyngby, Denmark
[c] Mastitis Laboratory, TINE SA, P.O. Box 2038, 6402 Molde, Norway
[d] MV Diagnostics Ltd., Roslin Innovation Centre, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, UK
[e] Enfer Scientific, Unit T, M7 Business Park, Newhall, Naas, Co. Kildare, Ireland

ARTICLE INFO

Keywords:
BRSV
Bayesian analysis
BCV
Diagnostic test evaluation
Sensitivity
Specificity

ABSTRACT

Bovine respiratory syncytial virus (BRSV) and bovine coronavirus (BCV) are responsible for respiratory disease and diarrhea in cattle worldwide. The Norwegian control program against these infections is based on herd-level diagnosis using a new multiplex immunoassay. The objective of this study was to estimate sensitivity and specificity across different cut-off values for the MVD-Enferplex BCV/BRSV multiplex, by comparing them to a commercially available ELISA, the SVANOVIR® BCV-Ab and SVANOVIR® BRSV-Ab, respectively. We analyzed bulk tank milk samples from 360 herds in a low- and 360 herds in a high-prevalence area. As none of the tests were considered perfect, estimation of test characteristics was performed using Bayesian latent class models. At the manufacturers' recommended cut-off values, the median sensitivity for the BRSV multiplex and the BRSV ELISA was 94.4 [89.8–98.7 95% Posterior Credibility Interval (PCI)] and 99.8 [98.7–100 95% PCI], respectively. The median specificity for the BRSV multiplex was 90.6 [85.5–94.4 95% PCI], but only 57.4 [50.5–64.4 95% PCI] for the BRSV ELISA. However, increasing the cut-off of the BRSV ELISA increased specificity without compromising sensitivity. For the BCV multiplex we found that by using only one of the three antigens included in the test, the specificity increased, without concurrent loss in sensitivity. At the recommended cut-off this resulted in a sensitivity of 99.9 [99.3–100 95% PCI] and specificity of 93.7 [88.8–97.8 95% PCI] for the multiplex and a sensitivity of 99.5 [98.1–100 95% PCI] and a specificity of 99.6 [97.6–100 95% PCI] for the BCV ELISA.

1. Introduction

Bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) are commonly occurring agents among cattle worldwide (Valarcher and Taylor, 2007; Boileau and Kapil, 2010). They are endemic and prevalent also in the Norwegian dairy herd (Gulliksen et al., 2009; Klem et al., 2014a). BCV causes respiratory disease, calf diarrhea and winter dysentery (contagious diarrhea in adult cattle) (Boileau and Kapil, 2010). BRSV causes respiratory disease mostly in young animals but can affect animals of all ages, and is a common cause of respiratory outbreaks in Norway (Larsen, 2000; Klem et al., 2014a). Consequences of these infections are herd health problems, reduced animal welfare and increased use of antibiotics due to secondary bacterial infections (Larsen, 2000; Valarcher and Taylor, 2007; Boileau and Kapil, 2010). Therapy costs and reduced production entails considerable financial loss for the farmer, and contributes to a present focus in Nordic countries on how to limit the spread of these viruses in the cattle population.

In 2016, a national control program against BRSV and BCV infections was launched in Norway as a joint initiative between the producer organizations. This prompted the need for an easy and cost-effective way to screen dairy herds for a herd level diagnosis for BRSV and BCV. The initial screening in the control program was conducted using bulk tank milk samples (BTM). There are already commercially available indirect enzyme-linked immunosorbent assays (ELISAs) widely used in routine diagnostics and research in the Nordic countries (SVANOVIR® BRSV-Ab and SVANOVIR® BCV-Ab) (Tråvén et al., 1999; Klem et al.,

* Corresponding author.
E-mail address: ingrid.toftaker@nmbu.no (I. Toftaker).

https://doi.org/10.1016/j.prevetmed.2018.03.008
Received 17 November 2017; Received in revised form 12 March 2018; Accepted 12 March 2018

2014b; Toftaker et al., 2016). However, in order to optimize cost-effectiveness of the control program, the development of a new multiplex antibody ELISA was initiated (MVD-Enferplex BCV/BRSV multiplex). The new test allowed screening for both viruses by the use of a single test.

The performance of a diagnostic test is characterized by the test's sensitivity (Se) and specificity (Sp), where Se is the proportion of true positives correctly classified as positive by the test, and the Sp is the proportion of true negative subjects correctly classified as negative. The true antibody status of each test subject can be determined in two ways: By use of a perfect reference test, or based on populations with known status. However, a perfect reference test (often termed a "gold standard") is rarely available and for endemic diseases, which is the case for BRSV and BCV in Norway, no reference population with complete certainty regarding disease or disease freedom exists. Consequently, the underlying true infection status for test subjects remains unknown. Test validation studies (erroneously) assuming perfect reference tests are common, even though this has been shown to introduce bias in the estimation of accuracy parameters (Valenstein, 1990; Lijmer et al., 1999). Latent class analysis (LCA) allows for the estimation of test parameters in populations where the underlying true infection status cannot be determined (Hui and Walter, 1980). In LCA the true infection status is treated as an existing, but unknown (latent), variable and test accuracy and prevalence are parameterized according to this latent variable.

As the BRSV/BCV multiplex is a new test, it needs to be validated. Test characteristics are different when a test is used as a herd test, compared to when it is used on individual samples (Christensen and Gardner, 2000) and validation for the relevant application is therefore important. BTM testing is a key component of the Norwegian BRSV/BCV control-program, it is therefore of interest to estimate test accuracy, at different cut-off values, for this application.

The aim of this study was to estimate the test sensitivity and specificity of the newly developed MVD-Enferplex BCV/BRSV multiplex across different cut-off values, for detection of antibodies in BTM. The BCV part of the multiplex was compared to the commercially available SVANOVIR® BCV-Ab, and the BRSV part of the multiplex was compared to the SVANOVIR® BRSV-Ab. As neither test could be considered perfect, the evaluation was done using LCA.

2. Materials and methods

2.1. Study population and sample material

A cross-sectional sampling design was used for the present study. Herds were eligible for inclusion if they delivered milk to the largest dairy company in Norway (TINE SA), and provided a BTM sample during the study period (March 2016). Herds from two counties with an expected difference in true prevalence (TP) were selected in order to meet the model assumptions, described in the LCA section. Using a random numbers generator, 360 samples were randomly chosen from herds in "Oppland" (Pop 1) and 360 from herds in "Sogn og Fjordane" (Pop 2) counties. "Sogn og Fjordane" is located in western Norway, and was assumed to have a relatively low prevalence, based on results from a previous study (Toftaker et al., 2016). Oppland county, located in eastern Norway, was thought to have higher prevalence based on known patterns of animal movements and a history of previous outbreaks of disease (Toftaker et al., 2017).

BTM samples were collected from nearly all Norwegian dairy herds delivering milk to the largest dairy cooperation (TINE SA) during March 2016. The samples were collected as part of the national control program against BRSV and BCV. The milk truck driver collected samples at ordinary milk shipment using standard procedures for BTM sampling. The milk was then stored at 4 °C until received at the laboratory (TINE Mastitis Laboratory, Molde, Norway) where samples were frozen and shipped over-night to the Enfer laboratory in Ireland (Enfer Scientific, Naas, Ireland). Samples were kept frozen until the time of laboratory analysis.

2.2. Diagnostic tests

2.2.1. ELISA

The SVANOVIR® BRSV-Ab, hereafter designated the BRSV ELISA, and SVANOVIR® BCV-Ab, hereafter designated the BCV ELISA, were used on all 720 samples, following the manufacturer's instructions. The optical density (OD) reading of 450 nm was corrected by the subtraction of OD for the negative control antigen, and percent positivity (PP-value) was calculated as (corrected OD/positive control corrected OD) × 100. According to the test manuals, the recommended cut-off values of sample positive > 10 PP for both tests were used as a starting point for these tests (Svanova; Svanova). For the BRSV ELISA the Se and Sp provided by the manufacturer were 94% and 100%, respectively. These parameters are calculated from serum samples, and parameters specific for BTM samples have not been reported (Elvander et al., 1995). For the BCV ELISA the test parameters provided by the manufacturer were Se of 84.6% and Sp of 100%, and as for BRSV the calculations are based on serum samples (Alenius et al., 1991).

2.2.2. Multiplex

All 720 samples were analyzed using the MVD-Enferplex BCV/BRSV multiplex, hereafter referred to as the BRSV/BCV multiplex (Enfer Scientific, Naas, Ireland). A panel of three BCV recombinant proteins (BCV A-C), along with a panel of two recombinant proteins and two synthetic peptides for BRSV (BRSV A-D) were used as antigens. Briefly, the antigens were deposited in a multiplex planar array as individual spots into wells of 96 well microtiter plates to produce arrays of antigens. Samples were diluted 1:3 into sample dilution buffer and mixed before added to the well and incubated at 37 °C for 60 min with agitation. After washing procedures, the detection antibody diluted in conjugate buffer was added and plates were incubated (37 °C for 60 min with agitation) before new washing. Finally, the chemiluminescent substrate was added. Relative light units (RLU) were captured (45 s exposure) immediately, using Quansys biosciences imaging system, and data was extracted using Quansys Q view software (v 1.5.4.7). Antigens were combined in a parallel reading, i.e. the test was considered positive when the RLU-value of at least one antigen was above the applied cut-off. Laboratory personnel were not formally blinded to test results, but due to the large volume of samples they were considered blinded for any practical purposes.

2.3. Data management and descriptive statistics

Because the multiplex consisted of several antigens each giving a separate response, a separate cut-off value was needed for each antigen. We calculated the proportion of herds that had a positive response to each of the individual antigens within the test-positive group (at manufacturers recommended cut-off values), and defined the antigen with the highest proportion of positive responses as the most influential. This was done for both viruses. When later choosing which cut-off values to assess, changing the cut-off for the most influential antigen for each virus was prioritized. We used an explorative approach to selecting cut-off values, and several different cut-off values were tried for the most influential antigen (Fig. 1). Furthermore, we evaluated test performance when including only the single most important antigen. Data preparation and descriptive analysis were performed in Stata (Stata SE/14; Stata Corp., College Station, TX).

2.4. Latent class analysis

In the present study, we used guidelines for reporting of diagnostic accuracy in studies that use Bayesian LCA (Kostoulas et al., 2017).

The target condition was herds with one or more animals producing