Economic costs associated with two testing strategies for screening feeder calves for persistent infection with bovine viral diarrhea virus

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Objective—To develop partial budgets of the economic costs of 2 test strategies for screening cattle for persistent infection with bovine viral diarrhea virus (BVDV).

Design—Partial budget analysis.

Animals—938 calves arriving at 2 stocker operations.

Procedure—Calves were tested to determine prevalence of persistent BVDV infection. Test strategies that were evaluated included a single-test strategy consisting of immunohistochemical staining of skin biopsy specimens from all animals and a 2-test strategy consisting of polymerase chain reaction (PCR) assaying of pooled blood samples followed by immunohistochemical staining of skin biopsy specimens from animals in pools for which assay results were positive. Break-even costs (ie, cost of persistent BVDV infection per animal necessary to justify whole-herd diagnostic testing) associated with each test strategy were calculated as a function of disease prevalence and test cost.

Results—Apparent prevalence of persistent BVDV infection was 0.32%. Sensitivity and specificity of the PCR assay for pooled samples were 100% and 89.7%, respectively. Regardless of the prevalence of persistent BVDV infection, the break-even cost for the 2-test strategy was lower than the break-even cost for the single-test strategy. However, the economic advantage was greatest when prevalence was low.

Conclusions and Clinical Relevance—Results suggest that using a 2-test strategy to screen cattle for persistent BVDV infection, whereby the first test involves PCR assaying of pooled samples and the second involves immunohistochemical testing only of those animals represented in pooled samples with positive assay results, will reduce the cost of screening incoming feedlot cattle, compared with immunohistochemical testing of all animals. (J Am Vet Med Assoc 2005;226:249–254)

Infection with bovine viral diarrhea virus (BVDV) contributes to a variety of economically important disease syndromes in beef cattle, including bovine respiratory tract disease and immunosuppression in stocker and feedlot cattle.1,2 The primary source of BVDV exposure in stocker and feedlot cattle is cattle with persistent infection (PI). Cattle acquire PI as a result of exposure in utero to noncytopathic BVDV prior to development of a competent immune system, which occurs by about 125 days of gestation.3,4 Exposed fetuses that survive to term become immunotolerant to the virus; are viremic throughout their lives5–7; and shed the virus in virtually all secretions and excretions, including nasal discharges, saliva, semen, urine, tears, milk, and, to a lesser extent, feces.8–11 Various methods have been developed to identify PI with BVDV in cattle, including virus isolation from serum, blood, and other tissues; immunohistochemical (IHC) staining of skin biopsy specimens for viral antigen; antigen-capture ELISAs; and reverse-transcriptase polymerase chain reaction (RT-PCR) assays.12 Viral isolation from buffy coat or serum samples and RT-PCR assays detect viremia but are not able to differentiate between transient infection and PI. Thus, for cattle with positive test results, a second sample must be obtained and tested 3 to 4 weeks later to differentiate transient infection from PI. In contrast, IHC staining of skin biopsy specimens can differentiate transient infection from PI,13 in that transiently infected animals have no staining or staining confined to the epidermal keratinocytes and follicular ostia, whereas cattle with PI have staining of cells in all layers of the epidermis, all levels of the hair follicles, and the hair bulb.

Collection of skin biopsy specimens for IHC staining is simple, specimens are stable during transport and handling, and the test is sensitive and specific for PI with BVDV in cattle. In addition, recent use of modified-live BVDV vaccines does not cause false-positive results.16 However, the test can only be performed on individual samples, and pooled samples cannot be used. In contrast, RT-PCR assays can be performed on pooled samples of blood, serum, or milk in surveillance programs. These assays can, for instance, detect viral RNA from a single animal with PI in pooled blood samples representing up to 250 uninfected animals.17 However, these assays do not differentiate between transient infection and PI, and the transient viremia that occurs 3 to 10 days after vaccination with modified-live BVDV vaccines can cause false-positive results.18

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Screening cattle for PI with BVDV prior to introduction to a herd can be accomplished through testing of individual skin biopsy specimens by means of IHC staining or through testing of pooled blood or serum samples with an RT-PCR assay. The use of pooled samples minimizes the expense of screening cattle from herds with a low prevalence of PI, in that all cattle represented in pooled samples for which assay results are negative are themselves considered to be negative for BVDV infection. However, pooled samples for which results are positive come from a mixture of cattle with PI, transiently infected cattle, and uninfected cattle, and confirmatory tests, such as IHC staining, must be performed on animals represented in the pooled sample to identify animals with PI. A simulation model for determining the economically optimum sample size in populations with various prevalences of PI has been developed.\textsuperscript{17} As prevalence decreases, the least-cost initial pool size increases.

The purpose of this study was to develop partial budgets to compare the economic costs of 2 test strategies for screening cattle for PI with BVDV. The test strategies that were evaluated included a single-test strategy, which consisted of IHC staining of skin biopsy specimens from all animals, and a 2-test strategy, which consisted of RT-PCR assaying of pooled blood samples followed by IHC staining of skin biopsy specimens from animals in pools for which assay results were positive. Generating defensible estimates of the prevalence of PI with BVDV among feeder cattle and the sensitivity and specificity of RT-PCR assays performed on pooled samples was necessary for development of these partial budgets.

**Materials and Methods**

**Sample collection**—Blood samples and skin biopsy specimens were collected over a 3-week period from 938 feeder calves at the time of arrival at 2 stocker operations. Blood samples were collected in tubes containing EDTA; skin biopsy specimens were collected from the pinna with ear-notching pliers and placed in neutral-buffered 10% formalin. All samples were shipped to the University of Missouri Veterinary Medical Diagnostic Laboratory within 1 day after collection.

**IHC staining**—Immunohistochemical staining of skin biopsy specimens was considered the gold standard method for determining the economically optimum sample size in populations with various prevalences of PI has been developed.\textsuperscript{17} As prevalence decreases, the least-cost initial pool size increases.

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The cost of the disease and was calculated with the following formula: cost of false-negative test results = (prevalence × (1 – test sensitivity)) × (cost of PI with BVDV per animal + test cost).

To justify a testing strategy, the cost of true-positive test results must exceed the combined cost of true-negative, false-positive, and false negative test results. The cost of true-positive test results is largely influenced by the negative cost of feeder calves with PI for a feedlot or stocker operation and was calculated with the following formula: cost of true-positive test results = (prevalence × test sensitivity) × (cost of PI with BVDV per animal – test cost).

The cost of PI with BVDV represents a variety of costs, including costs associated with medication and treatment, loss in average daily gain, and death of noninfected animals, and could not be directly calculated in the present study. Therefore, the cost of whole-herd diagnostic testing could also not be directly calculated. For the partial budget analyses, therefore, the cost of persistent BVDV infection in a single feeder calf necessary to justify testing with either a single-test or 2-test strategy was calculated by forcing the cost of the whole-herd diagnostic testing strategy to be equal to the cost of true-positive test results. The cost of PI with BVDV per animal was then designated as the break-even cost, as it represented the value at which a particular testing strategy was justified.

For partial budget analysis of the single-test strategy (ie, IHC staining of skin biopsy specimens from all animals), the input prevalence of PI with BVDV was used as the prevalence in all formulas. For partial budget analysis of the 2-test strategy (ie, RT-PCR assaying of pooled blood samples followed by IHC staining of specimens from animals in pools for which assay results were positive), sensitivity and specificity of the RT-PCR assay were assumed to be 99.9% and 87%, respectively, and sensitivity and specificity of follow-up IHC staining were assumed to be 97% and 99.9%, respectively. Prevalence of PI with BVDV was assumed to be 0.1%; the number of cattle samples pooled for RT-PCR assay was assumed to be 30; the cost of the RT-PCR assay was assumed to be $1.50/animal; the cost of a single follow-up IHC test was assumed to be $6; the profit per calf was assumed to be $25; and the cost of a feeder calf was assumed to be $600. Under this scenario, the break-even cost (ie, the cost of PI with BVDV per animal) was calculated to be $7,047 (Figure 1). The break-even cost increased progressively as the assumed cost of a single IHC test was increased but decreased as the assumed prevalence of PI with BVDV was increased.

Partial budget analysis of a 2-test strategy—For the initial partial budget analysis of the 2-test strategy (ie, RT-PCR assaying of pooled blood samples followed by IHC staining of specimens from animals in pools for which assay results were positive), sensitivity and specificity of the RT-PCR assay were assumed to be 99.9% and 87%, respectively, and sensitivity and specificity of follow-up IHC staining were assumed to be 97% and 99.9%, respectively. Prevalence of PI with BVDV was assumed to be 0.1%; the number of cattle samples pooled for RT-PCR assay was assumed to be 30; the cost of the RT-PCR assay was assumed to be $1.50/animal; the cost of a single follow-up IHC test was assumed to be $6; the profit per calf was assumed to be $25; and the cost of a feeder calf was assumed to be $600. Under this scenario, the break-even cost was calculated to be $2,522 (Figure 2). As with the

Results

Results of IHC staining were positive for 3 of the 938 (0.32%) calves. True prevalence, therefore, was calculated to be 0.33% (95% CI, 0.299% to 0.360%).

Results of the RT-PCR assay were positive for 5 of the 31 (16%) pooled blood samples. One of the pooled blood samples for which results of the RT-PCR assay were positive contained a single calf with PI, and a second contained 2 calves with PI. Because results of the RT-PCR assay were positive for the 2 pooled samples that contained blood from the 3 calves determined, on the basis of IHC staining, to have PI, sensitivity of the RT-PCR assay to identify calves with PI in pooled blood samples was 100% for the pool sizes used, with IHC staining as the gold standard.

The remaining 3 pooled samples for which results of the RT-PCR assay were positive did not contain any calves with PI, as determined on the basis of results of IHC staining. Thus, specificity of the RT-PCR assay was 89.7%.

Partial budget analysis of a single-test strategy—For the initial partial budget analysis of the single-test strategy (ie, IHC staining of skin biopsy specimens from all animals), sensitivity and specificity of IHC staining were assumed to be 97% and 99.9%, respectively; prevalence of PI with BVDV was assumed to be 0.1%; the cost of a single IHC test was assumed to be $6; the...
single-test strategy, the break-even cost increased progressively as the assumed cost of a single IHC test was increased, although the increase was less dramatic, and decreased as the assumed prevalence of PI with BVDV was increased. The break-even cost also progressively increased as the assumed cost of the RT-PCR assay was increased (Figures 3 and 4).

Comparison of test strategies—Regardless of the prevalence of PI with BVDV, the break-even cost for the 2-test strategy was lower than the break-even cost for the single-test strategy (Figure 5). However, the economic advantage of the 2-test strategy was greatest when prevalence was assumed to be low. For example, when the prevalence of PI with BVDV was assumed to be 0.3%, the cost of a single IHC test was assumed to be $8, and the cost of the RT-PCR assay was assumed to be $1.50/animal, the break-even cost for the single-test strategy was $3,058, whereas the break-even cost for the 2-test strategy was only $939. Even when the cost of the RT-PCR assay was assumed to be higher (ie, $2.50 and $3.50/animal), the break-even cost for the 2-test strategy was lower ($1,294 and $1,649, respectively) than the break-even cost of the single-test strategy.

Discussion
An important question commonly confronted by veterinary practitioners is whether to use currently available diagnostic tests to screen cattle for a particular disease. Information needed to arrive at a logical conclusion includes epidemiologic data about the disease, the sensitivity and specificity of the available tests, and the economic costs of the disease and its treatment. Evaluating all of these factors in a meaningful way can be difficult, however. As a result, partial budget analysis has been developed as a method to determine the cost of testing for certain diseases in stocker or feedlot operations.21 Partial budget analysis assists in quantifying the value of diagnostic test strategies by evaluating the economic effects of making single adjustments in the underlying assumptions. This analysis method is based on the principle that a change in the organization of a business will increase some costs, while decreasing others, and increase some returns, while decreasing others. The net effect will be the sum of positive economic effects minus the sum of negative economic effects.21

When screening large numbers of animals for a particular disease, many of the positive test results will be falsely positive if prevalence of the disease is low or if the diagnostic test that is used has poor specificity. In contrast, many of the negative test results will be falsely negative if prevalence of the disease is high or if the diagnostic test that is used has poor sensitivity. The prevalence of PI with BVDV among cattle in the present study was low (0.33%), which was consistent with prevalence reported previously for cattle in the United States.21

Other researchers have reported that feeder cattle with PI are a primary source of BVDV transmission to in-contact susceptible cattle during marketing and trucking, while in feeding pens and pastures, and that feeder cattle with PI have a negative impact on the health and growth of susceptible cattle in the same and adjacent pens. Persistent BVDV infection is therefore suspected of causing measurable economic losses. In the present study, we could not directly measure the economic cost of having an animal with PI in a group of feeder cattle. However, by forcing the difference between the cost and benefit of whole-herd diagnostic testing to be $0, we were able to calculate the cost of PI with BVDV necessary to economically justify whole-herd diagnostic testing of feeder cattle.

Even though IHC testing was assumed to be highly sensitive and specific for detection of cattle with PI,
the positive predictive value was low because the prevalence was low and test specificity was < 100%. In other words, under these conditions, a substantial proportion of positive test results were falsely positive. Because we assumed that the most likely response to a positive test result would be immediate euthanasia, these false-positive test results were associated with high costs.

In the present study, we found that if the prevalence of disease was low, the cost of PI with BVDV would need to be quite high when using the single-test strategy, even if the test cost (ie, costs associated with animal handling, sample collection and submission, and laboratory testing and interpretation) was low ($6/test). As prevalence increased, the economic cost of PI with BVDV necessary to justify this testing strategy decreased because the costs of screening were spread over more true-positive cattle. When using a 2-test strategy for screening a population for a condition with a low prevalence, the positive predictive value of the initial test becomes the prevalence used for calculating the cost of the confirmatory test. Because a confirmatory test is performed on all animals for which results of the initial test are positive, the positive predictive value for the 2-test strategy is much closer to 100% than the positive predictive value for a single-test strategy, meaning that there are fewer animals with false-positive test results, with the result that the overall cost of false-positive test results is lower. Because some animals have 2 diagnostic tests performed instead of 1, the cost of true-negative test results is higher with a 2-test strategy than with a single-test strategy.

Because the RT-PCR assay can reliably detect BVDV in pooled blood or serum samples from multiple animals, the use of a 2-test strategy to screen cattle for PI with BVDV, whereby the first test involves RT-PCR assaying of pooled samples from 30 animals and the second involves IHC testing only of those animals represented in pooled samples with positive RT-PCR assay results, will reduce the number of IHC tests necessary to screen incoming feeder cattle. Although RT-PCR assaying of pooled samples does not allow identification of viremic individuals or differentiation of transient viremia and persistent infection, the cost of the RT-PCR assay per animal, when used on pooled samples from 30 animals, is lower than the cost of IHC staining per animal.21 Thus, if RT-PCR assaying of pooled samples is adequately specific for BVDV viremia and the percentage of cattle transiently infected with BVDV is relatively low, the break-even cost for the 2-test strategy will always be lower than the break-even cost for the single-test strategy.

Because finding cattle represented in pools for which RT-PCR assay results are positive and removing them from their pen so that skin biopsy specimens can be collected is not practical in commercial feedlots, a possible strategy would be to collect both blood and skin biopsy specimens when animals first enter the feedlot. Blood samples could then be pooled, and if results of the RT-PCR assay were negative, the skin biopsy specimens could be discarded. Because results of the RT-PCR assay can be positive following administration of modified-live BVDV vaccines, blood samples should be collected prior to or at least 14 days after administration of modified-live virus vaccination.18

A negative aspect of the 2-test strategy described in the present report is the time delay between initial sample collection and identification of animals with PI for removal from the population. During this delay, animals with PI would be in contact with other cattle and, presumably, causing negative biologic and economic effects. Before widespread adoption of any testing strategy can be recommended, more information about the cost of cattle with PI to stocker and feedlot operations and the accuracy of currently available diagnostic tests is needed. In particular, information is needed on the effects of having a single animal versus multiple animals with PI in a single pen of feeder cattle and on the roles that group size and animal density play in the negative biologic and economic effects of having leeder calves with PI. As more information becomes available, the economic return of various testing strategies can be evaluated by use of the same techniques described in the present report.

References

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