Prevalence of calves persistently infected with bovine viral diarrhea virus in beef cow-calf herds enrolled in a voluntary screening project

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Objective—To report the prevalence of bovine viral diarrhea virus (BVDV) in calves and calf groups (ie, calves from the same farm) in beef breeding herds and evaluate the ability of biosecurity risk assessment questionnaires to identify calf groups with positive results for BVDV.

Design—Nonrandom survey.

Animals—12,030 calves born in spring from 102 operations.

Procedures—Cow-calf producers that voluntarily enrolled in a screening project submitted ear notch specimens from calves and answered a 29-question survey instrument. Ear notch specimens were tested for BVDV with an antigen-capture ELISA (ACE), and ear notch specimens with positive ACE results for BVDV were immediately retested by performing immunohistochemistry (IHC). Follow-up testing, 3 to 4 weeks after initial positive ACE results, was done by use of a second IHC test and virus isolation on a subsequently submitted ear notch specimen from the same calves to identify those that were persistently infected (PI).

Results—102 producers submitted ear notch specimens for BVDV screening. Initially, 24 of 12,030 calves had positive ACE results for BVDV. A second ear notch specimen was submitted for 20 of these 24 calves. Of 20 retested calves, 12 had positive ICH results for BVDV, confirming PI status. The 12 PI calves came from 4 calf groups (3 singletons and 1 calf group with 9 PI calves).

Conclusions and Clinical Relevance—Prevalence of BVDV in calf groups was low, and questions designed to identify high-risk biosecurity behaviors had little value in identifying calf groups with positive results for BVDV. (*J Am Vet Med Assoc* 2007;230:1691–1696)

Bovine viral diarrhea virus is an important pathogen affecting cattle worldwide. Bovine viral diarrhea virus infection can have substantial negative impacts on cow-calf herds associated with reduced fertility, abortions, and reduced growth rate. In feedlot cattle, BVDV is primarily thought to cause immunosuppression and contribute to respiratory tract disease.[1-3]

Bovine viral diarrhea virus has the ability to create PI cattle as a consequence of in utero infection by a noncytopathic strain of BVDV between days 40 and 125 of gestation.[4] Persistently infected cattle are the most important source of BVDV transmission within a herd.[5] Spread of BVDV from postnatally (transiently) infected calves failed to occur in at least 1 experimental study.[6] Identifying PI cattle has thus been the primary objective for eradication and control programs.

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Abbreviations

BVDV	Bovine viral diarrhea virus
PI	Persistently infected
VIBSP	Voluntary Iowa Bovine Viral Diarrhea Virus Screening Program
ACE	Antigen-capture ELISA
S:P	Optical density of specimen-to-positive control
IHC1	First immunohistochemistry test
IHC2	Second immunohistochemistry test
VI	Virus isolation
CI	Confidence interval

Several European and Scandinavian countries have eradicated BVDV or instituted control programs.[7-11] In the United States, however, no state or federally sponsored BVDV control programs exist and there are only a handful of regional programs or biologic-company–sponsored programs.[12,13] In October 2005, the Iowa Veterinary Medical Association and the Iowa State University College of Veterinary Medicine launched a pilot project for cow-calf producers in Iowa, referred to as the VIBSP. The aim of the project was to screen groups of calves for BVDV to determine the prevalence of BVDV in a subset of cattle in Iowa, knowledge of which would aid in the future development of strategies for BVDV control in Iowa. The program did not offer official certification of herd BVDV status, as it was a pilot program

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and did not use a random sample of herds. On the basis of success in previous studies,[14,15] the project was designed to test calf groups (ie, all calves as a group that were born from January 2006 to July 2006 on a single farm) by use of ACE as a screening test for BVDV on ear notch specimens. Funding to test additional cattle such as nonpregnant cows, replacement heifers, or bulls was not provided. Calves that had positive ACE results for BVDV were confirmed PI via immunohistochemistry on an additional ear notch specimen. The purpose of the study reported here was to report the prevalence of BVDV in calf groups in Iowa beef breeding herds and evaluate the ability of biosecurity risk assessment questionnaires to identify calf groups with positive results for BVDV.

Methods and Materials

Recruitment—Recruitment efforts for the screening project were divided into 2 phases. The initial phase was aimed at informing veterinarians that the project was available for their clients. The second phase was aimed at informing producers. Contact with veterinarians began with a presentation at the Iowa Veterinary Medical Association (Ames, Iowa, September) and Eastern Iowa Veterinary Medical Association (Iowa City, Iowa, October) 2005 fall meetings. In October 2005, a letter describing the project and several enrollment brochures were sent to each veterinarian listed as a participant in the Iowa Veterinary Medical Association preconditioning program in 2004. Veterinarians were asked to distribute the information and enrollment brochures to interested producers. The brochure described BVDV briefly and the testing requirements for the project (ear notch specimens from all calves born in spring 2006). The brochure included an enrollment form that requested contact information for the producers and their veterinarians and a 29-item questionnaire. Eligibility was limited to cow-calf producers in Iowa; however, no herd size restrictions were imposed. As testing occurred only in the spring and summer of 2006, calves born in the fall were not eligible. Other mailings to veterinarians occurred in November 2005 and January 2006. Another presentation about the project was made at the Iowa Veterinary Medical Association (Ames, Iowa, February) 2006 spring meeting. A news item was presented in the February edition of the Iowa Cattlemen's Association newsletter,[16] and a presentation was made at the Iowa Cattlemen's Association (Des Moines, December) 2005 winter meeting. Iowa State University extension agents and faculty were also made aware of the project and encouraged to direct potential participants to the project directors (AOC and SDS).

Specimen collection and submission—Upon receipt of an application for enrollment, specimen collection tubes[a]; a brochure describing specimen collection, storage, and shipping; and submission forms were mailed either to the veterinarian or the producer (on the basis of indicated preference) prior to the expected onset of calving. Directions for collecting and shipping specimens were as follows: collect ear notches from all calves at or shortly after birth (including stillborn calves) and submit groups of ear notch specimens to the laboratory each week until calving is over or until specimens have been collected from most calves during spring and summer processing prior to breeding, while submitting specimens from all calves that die prior to processing throughout the calving season; use commercially available ear notchers[b] that will provide a specimen with a ≥ 2.54-cm-long cut edge; place specimens in prelabeled tubes that contain no fluid; on the submission sheet, record calf identification (ear tag) number next to the tube number; prior to shipping, store specimens in a refrigerator (if they will be shipped in < 48 hours) or freezer (if they will be held for > 48 hours); and on Monday, Tuesday, or Wednesday of each week (ie, do not ship specimens on Thursday or Friday), ship specimens with ice packs and the complete submission sheet by use of an overnight courier service to the laboratory.

Producers submitted specimens from January 2006 to July 2006. Producers who had not submitted specimens by May 2006 were contacted by phone to inquire whether specimens would be submitted before July 2006. Another follow-up letter was provided to producers and their veterinarian in July 2006.

Survey—To assess the value of currently available biosecurity risk assessment questionnaires as tools for screening for herds with BVDV, survey questions were obtained from the following 2 sources: the University of Nebraska Farm and Ranch Biosecurity Web site[12] and the Colorado State University Rocky Mountains Laboratory BVDV Screening Program.[13] The combined questionnaire contained 29 unique questions and is available from the authors upon request.

Specimen processing—Upon arrival at the laboratory, 1 mL of PBS solution[c] was added to the ear notch specimen, vortexed, and stored at −20°C for a minimum of 48 hours and a maximum of 7 days. Each Monday, specimens were thawed in the refrigerator and submitted to the Iowa State University Veterinary Diagnostic Laboratory. At this time, between 2 and 6 ear notch specimens that had known positive results for BVDV were inserted among the test samples at locations unknown to the personnel performing the ACE.

Each Tuesday, specimens (ear notches in PBS solution) were submitted to the Iowa State University Veterinary Diagnostic Laboratory for testing by use of a commercially available kit[d] that tests for the presence of the Erns protein of BVDV.[17,18] A calf with an initial ACE-determined S:P ratio of < 0.2 was considered to have a negative result for BVDV. Every calf with an initial S:P ratio of 0.2 to 0.4 was retested with a modified ELISA,[d] as described by the manufacturer. A calf with an initial ACE-determined S:P ratio of > 0.4 was considered to have a positive result for BVDV. If specimens were positive on the basis of ACE, the herd veterinarian was contacted and the client was requested to submit a second ear notch specimen as well as a blood sample, drawn into a tube containing EDTA, from the calf and its dam. Guidelines for resubmission as stated in a VIBSP mailing to all producers and veterinarians enrolled were as follows: retest calves with positive results 14 to 21 days after the initial test before making important management decisions (a shorter interval is

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not recommended); samples submitted for retesting should include an ear notch specimen fixed in formalin for immunohistochemistry and a whole blood sample containing EDTA; and while waiting for the results of the confirmatory test, it is prudent to isolate calves with positive results from the rest of the herd, especially from pregnant cows.

Initial specimens with positive results were also retested for BVDV by use of IHC1; however, IHC1 results were not incorporated into the decision of how to advise a client. For the follow-up samples, IHC2 and VI were requested. Calves that tested positive on the basis of IHC2 or VI were considered PI.

Analysis—All statistical analyses were conducted with a commercial software package[e] by use of an exact procedure suitable for rare-event analysis in the extension software.[19,20,f] Basic descriptive measures were reported as proportions with Fisher exact CIs.[e,f] Differences in the characteristics of producers who completed the program from those who did not were evaluated by use of exact binomial tests for proportions, as was the agreement between the ACE and IHC1 results.[g] To assess the predictive value of survey responses in identifying calf groups with positive ACE results for BVDV, a univariate exact logistic model[f] was used to identify responses with a value of $P < 0.25$; these responses were then included in a multivariate model.[f] In the multivariate model, responses with 95% CIs for the odds ratio that did not include 1 were considered significant. After identifying single items associated with disease status, 2-item combinations were evaluated and associated with calf group status by use of exact logistic regression. To assess the predictive value of survey responses in identifying calf groups PI with BVDV, the same approach was used; however, because data were sparse, only univariate models fit.

The predictive value of positive responses (positive predictive value) was derived from estimates of the comparative sensitivity and specificity from exact logistic regression by use of only calf groups with positive ACE results for BVDV.[21] The predictive value of negative responses was derived from exact logistic regression by use of calf groups with ACE negative results for BVDV by use of the same formula.[21] These values are only reflective of our study population and prevalence. A value of $P < 0.05$ was considered significant for all final comparisons.

Results

Enrollment was closed on March 15, 2006, and testing was completed on July 20, 2006. Although 131 producers in Iowa enrolled in VIBSP with 74 veterinari-

Table 1—Prevalence of BVDV-positive calves and calf groups that were born in the spring of 2006 and enrolled in VIBSP.

Animals	Variable	No. of calves	Prevalence of BVDV	
			Percentage	95% CI
Calf groups*	No. tested	102	NA	NA
	No. with positive ACE results	11	10.8	5.50–18.5
	Confirmed PI†	4	3.9	1.1–9.7
Calves	No. tested	12,030	NA	NA
	No. with positive ACE results	24	0.20	0.1–0.3
	Confirmed PI†	12	0.09	0.05–0.16

*All calves that were born from January 2006 to July 2006 on a single farm. †PI confirmed with IHC2 conducted 3 to 4 weeks after initial positive ACE results for BVDV.
NA = Not applicable.

Table 2—Frequency (No. [%]) of responses to survey instrument questions from 102 VIBSP-enrolled producers who submitted ear notch specimens of calves for BVDV testing, compared with 29 producers who withdrew from VIBSP.

Survey question*	Producers		*P* value
	Tested (n = 102)	Not tested (n = 29)	
Do employees have routine contact with cattle outside your herd?			
Yes	27 (21)	2 (1)	0.024
No	75 (57)	27 (21)	
Do you isolate, segregate, or restrict the movement of sick animals?			
Yes	77 (59)	28 (21)	0.015
No	25 (19)	1 (1)	
Do you necropsy animals to determine exact cause of death?			
Yes	72 (55)	27 (21)	0.013
No	30 (23)	2 (1)	
Last year, did < 5% of your calves die after weaning?			
Yes	86 (66)	19 (14)	0.035
No	16 (12)	10 (8)	

*Only questions with significantly ($P < 0.05$) different responses between the 2 groups of producers are included.

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ans, only 102 producers submitted ear notch specimens for testing. Only 11 of the 29 producers who withdrew could be contacted, and most indicated the only convenient time for specimen collection was during processing, which would occur after July 2006. From the 102 producers who submitted specimens, 12,030 specimens were tested with ACE and IHC1. The mean number of specimens per calf group was 131, and the median was 100. Forty-six producers shipped the calf group specimens as a single consignment; these specimens could have been collected either as 1 group and shipped immediately or collected intermittently, stored, then shipped as a single group. Although producers were directed to ship specimens in coolers with ice, some specimens arrived without any cooling devices. We did not keep records of the state that specimens were in upon arrival.

Twenty-four of 12,030 calves had positive ACE results for BVDV. These 24 calves came from 11 of the 102 calf groups (**Tables 1 and 2**). Ear notch specimens from the 24 calves with positive ACE results for BVDV were submitted for IHC1. Of the 24 calves, 13 (54%) had positive IHC1 results for BVDV, 8 (33%) had negative IHC1 results, and 3 (13%) had IHC1 results that were inconclusive. Follow-up samples (ie, ear notch specimen and a whole blood sample containing EDTA) were received for 20 calves. Four of the 24 calves were not retested (2 calves died before they could be retested, and 2 producers did not resubmit samples). Of the 20 retested calves, 12 were confirmed PI on the basis of positive IHC2 results for BVDV (**Figure 1**). Of the 20 retested calves, 5 had whole blood samples with positive VI results for BVDV. Follow-up samples were received for 21 dams of the 24 calves with positive ACE results for BVDV. None of the dams had positive immunohistochemistry results for BVDV; a whole blood sample from 1 dam was positive for BVDV on VI.

The frequency of responses to 4 questions in the questionnaire differed between the producers who submitted specimens for testing and those who did not (**Table 3**). None of these questions were subsequently found to be associated with the BVDV status of a calf group.

None of the survey questions were associated with high comparative sensitivity, and the positive predictive values were low. The question "Has your veterinarian ever diagnosed BVDV in your herd?" was most strongly associated with a calf group testing positive for BVDV (odds ratio, 6.32; 95% CI, 1.07 to 47.8). The sensitivity for this response was 55% (95% CI, 23% to 83%); the positive predictive value for this response was 30%, whereas all other predictive values for other questions were lower.

When responses to 2-question combinations were considered, 5 combinations were significantly associated with calf group status for BVDV (Table 3). Many single and combined responses were rare, and therefore, the associations had wide CIs. For the question "Has your veterinarian ever diagnosed BVDV in your herd?" the comparative specificity was 85% (95% CI, 76% to 91%). All other responses were not associated with calf group status, and therefore, the comparative specificity and negative predictive values are not reported.

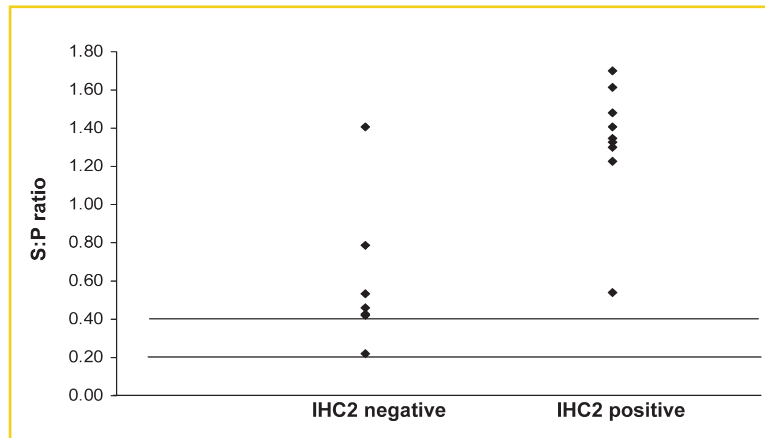

Figure 1—Graph of segregation of VIBSP-enrolled spring-born calves with initial positive ACE results for BVDV into confirmatory positive or negative results for BVDV on the basis of ICH2. Calves with an S:P ratio of < 0.2 had negative ACE results for BVDV; calves with an S:P ratio of 0.2 to 0.4 were retested with a modified ELISA (datum points between horizontal lines); and calves with S:P ratios of > 0.4 had positive ACE results (data points above top horizontal line). A significant ($P < 0.001$) difference in S:P ratios was found between calves with confirmatory positive or negative results on IHC2.

Table 3—Odds ratio and exact 95% CI for univariate logistic analysis of combined responses from producers with positive herd status for BVDV, compared with producers with negative herd status for BVDV.

Survey question combined with "Has your veterinarian ever diagnosed BVDV in your herd?"	Response	Herd BVDV status* Pos	Herd BVDV status* Neg	Odds ratio (95% CI)	Sensitivity (95% CI)†	PPV
Do you routinely use necropsy to determine cause of death?	Yes	6	9	10.49 (2.20–53.7)	0.55 (0.23–0.83)	0.40
	No	5	82			
Was your weaning rate < 90% last year?	Yes	7	2	23.58 (2.85–304)	0.36 (0.11–0.69)	0.66
	No	4	89			
Do you background calves‡ or run a feedlot?	Yes	5	8	8.33 (1.64–42.2)	0.46 (0.17–0.77)	0.39
	No	6	83			
Do any employees have contact with cattle outside the herd?	Yes	3	1	31.11 (2.2–1,790)	0.27 (0.06–0.61)	0.75
	No	8	90			

*Herd status determined on the basis of positive (≥ 1 calf) or negative (no calves) ACE results for BVDV. †Derived from exact logistic regression by use of herds with positive BVDV status. ‡Background calves defined as retaining ownership of calves after weaning for any period of time.
Pos = Positive. Neg = Negative. PPV = Positive predictive value.

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Discussion

In our study population, the individual-animal–level prevalence of PI cattle was lower (0.09%; 95% CI, 0.05% to 0.16%) than previously reported in US-based cattle. Other US-based studies[2,14,22] have reported point estimates of 0.4% and 0.3% for the prevalence of PI cattle at entry to a feedlot, and a 0.32% prevalence was reported for 2 stocker operations. At the herd level, a 5-state US-based study[5] reported that 3% of randomly selected cow-calf herds contained PI cattle while 1 additional herd had a suspected PI cattle that was lost to follow-up and never confirmed, which would have brought the prevalence to 4% if it had been confirmed. This closely resembles our finding of 4% of calf groups with PI calves. For calves that initially tested positive by use of ACE and had samples submitted for retesting, 12 of 20 were confirmed PI. This low level of agreement between the first and follow-up test has not been an observation in other studies. For example, a study[15] of 559 calves < 5 months old confirmed that 59 of 67 (88%) calves were PI with follow-up testing. Another study[5] testing calves < 4 months old reported that 33 of 43 (77%) calves that were initially positive for BVDV were confirmed PI by follow-up testing. Because of confounding, it is not possible to attribute the differences or similarities in prevalence or PI confirmation estimates across the study populations to characteristics such as test protocol, age, production system, breed, or location.

The S:P ratios of calves confirmed PI was higher than calves not confirmed PI. A common explanation for failure to reconfirm BVDV status is detection of transient infection with BVDV by the first test. Another explanation may be true false-positive results associated with the test characteristics. Several estimates of the ACE test have been reported, and generally, these have > 95% sensitivity and specificity.[14,15] However, even with a test with a theoretic specificity of 99.9%, we should reasonably expect 12 false-positive results among 12,000 tested ear notches given our apparent prevalence of 0.09%. The level of agreement between the initial and follow-up test results provide evidence for the common recommendation to isolate and retest cattle suspected of having BVDV in cow-calf herds prior to making management decisions such as culling, especially if the test is being used without clinical evidence for a diagnosis of BVDV.

The evaluation of our survey instrument revealed low utility for biosecurity risk assessment questions as a screening tool for identifying herds that might benefit from more individual BVDV testing. The question with the strongest association with positive BVDV results was, "Has your veterinarian ever diagnosed BVDV in your herd?" This question had a sensitivity of 55% and a specificity of 85%. With < 11% of the participants with calf groups initially testing positive for BVDV with ACE, a specificity of 85% is poor, as the specificity on the basis of random sampling is 89% (91/102). The positive predictive value was 30%; that is, of the 20 herds which answered yes, 6 had calf groups with positive test results. The question combination with the highest positive predictive value was "Has your veterinarian ever diagnosed BVDV in your herd?" and "Do any employees have contact with cattle outside the herd?"; however, only 4 participants responded yes, of which 3 had an operation with a positive herd status for BVDV. Biosecurity risk assessment questions may benefit producers by helping veterinarians identify important deficits in biosecurity programs but will likely have little role as a screening tool for herds that might benefit from BVDV testing. Our results also seemed to indicate that herds with BVDV either fail to control the disease after initial identification or continue practices that introduce the virus. In follow-up studies, the actions, if any, that producers should take to control BVDV after diagnosis warrant investigation.

There were many difficulties associated with the recruiting, sample collecting, and testing aspects of our study. Our experiences may be useful for others considering a BVDV control and eradication program. To maintain data integrity and ensure we knew the origin of each sample, we assigned a unique number to each tube in the program before it was mailed to the producer, which was labor intensive. Of the original 131 recruited producers, 102 actually submitted samples for testing before July 20, 2006, nearly a quarter of the producers dropping out as a result of the inconvenience of collecting ear notch specimens. Further, although not a surprise, despite our efforts at communication, many producers did not submit specimens as directed. We requested that each producer ship their samples in coolers with ice packs; however, we received many boxes without any insulation or cold packs. Also, to save money on shipping charges, producers stored their samples as they collected them, waiting until after calving was complete to send them. It is possible both factors may have allowed the specimens to deteriorate, causing false-negative test results. However, within-laboratory test results suggest that storage of BVDV-positive specimens at room temperature (approx 23°C) for 4 days (n = 30) did not interfere with detection of BVDV. We did not evaluate beyond 4 days. An alternative time for testing could be at weaning. Our rationale for testing calves born in the spring was to allow identification and removal of the PI cattle before breeding to enable the transmission cycle to be broken. Considering that this was a voluntary program with producers who seemingly were interested in testing their herds for BVDV, it is likely that the difficulties we encountered would be exacerbated in any mandatory eradication-control program.

a. CAT No. 055407, Fisher Scientific Co LLC, Hanover Park, Ill.
b. Prod No. C10635N or C07156N, Nasco, Fort Atkinson, Wis.
c. CAT No. 23-312-651, Fisher Scientific Co LLC, Hanover Park, Ill.
d. HerdChek BVDV Ag/Serum test kit, IDEXX Laboratories, Westbrook, Me.
e. SAS, version 9.1, SAS Institute Inc, Cary, NC.
f. Proc Logxact, Cytel Inc, Cambridge, Mass.
g. Proc Freq, SAS Institute Inc, Cary, NC.

References

1. Welsh MD, Adair BM, Foster JC. Effect of BVD virus infection on alveolar macrophage functions. *Vet Immunol Immunopathol* 1995;46:195–210.
2. Loneragan GH, Thomson DU, Montgomery DL, et al. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. *J Am Vet Med Assoc* 2005;226:595–601.

RUMINANTS

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3. Houe H. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Vet Microbiol* 1999;64:89–107.
4. Brock KV, Grooms KL, Givens MD. Reproductive disease and persistent infections. In: Goyal SM, Ridpath JF, eds. *Bovine viral diarrhea virus: diagnosis, management and control*. Ames, Iowa: Blackwell Publishing Professional, 2005;145–156.
5. Wittum TE, Grotelueschen DM, Brock KV, et al. Persistent bovine viral diarrhoea virus infection in US beef herds. *Prev Vet Med* 2001;49:83–94.
6. Niskanen R, Lindberg A, Traven M. Failure to spread bovine virus diarrhoea virus infection from primarily infected calves despite concurrent infection with bovine coronavirus. *Vet J* 2002; 163:251–259.
7. Moennig V, Houe H, Lindberg A. BVD control in Europe: current status and perspectives. *Anim Health Res Rev* 2005;6:63–74.
8. Ferrari G, Scicluna MT, Bonvicini D, et al. Bovine virus diarrhoea (BVD) control programme in an area in the Rome province (Italy). *Vet Microbiol* 1999;64:237–245.
9. Hult L, Lindberg A. Experiences from BVDV control in Sweden. *Prev Vet Med* 2005;72:143–148.
10. Joly A, Fourichon C, Beaudeau F. Description and first results of a BVDV control scheme in Brittany (western France). *Prev Vet Med* 2005;72:209–213.
11. Valle PS, Skjerve E, Martin SW, et al. Ten years of bovine virus diarrhoea virus (BVDV) control in Norway: a cost-benefit analysis. *Prev Vet Med* 2005;72:189–207.
12. University of Nebraska, Lincoln. Beef breeding herd biosecurity guide. Available at: www.farmandranchbiosecurity.com/Breeding_Herd_Insert.pdf. Accessed Jul 24, 2006.
13. Veterinary Diagnostic Laboratory, Colorado State University. Colorado Voluntary BVD Control Program questionnaire. Available at: tekki.cvmbs.colostate.edu/bvdcontrol/webform1.aspx. Accessed Jul 20, 2006.
14. Fulton RW, Hessman B, Johnson BJ, et al. Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot. *J Am Vet Med Assoc* 2006;228:578–584.
15. Cornish TE, van Olphen AL, Cavender JL, et al. Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. *J Vet Diagn Invest* 2005;17:110–117.
16. Kreidler M. Voluntary screening for BVDV. *Iowa Cattlemen* 2006;33:19.
17. Saliki JT, Fulton RW, Hull SR, et al. Microtiter virus isolation and enzyme immunoassays for detection of bovine viral diarrhea virus in cattle serum. *J Clin Microbiol* 1997;35:803–807.
18. Saliki JT, Huchzermeier R, Dubovi EJ. Evaluation of a new sandwich ELISA kit that uses serum for detection of cattle persistently infected with BVD virus. *Ann N Y Acad Sci* 2000;916:358–363.
19. King EN, Ryan TP. A preliminary investigation of maximum likelihood logistic regression versus exact logistic regression. *Am Stat* 2002;56:163–170.
20. Bull SB, Mak C, Greenwood CMT. A modified score function estimator for multinomial logistic regression in small samples. *Comput Stat Data Anal* 2002;39:57–74.
21. Dohoo IR, Martin W, Stryhn H. Screening and diagnostic tests. In: *Veterinary epidemiologic research*. Charlottetown, PE, Canada: AVC Inc, 2003;109–111.
22. Larson RL, Miller RB, Kleiboeker SB, et al. Economic costs associated with two testing strategies for screening feeder calves for persistent infection with bovine viral diarrhea virus. *J Am Vet Med Assoc* 2005;226:249–254.

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