

Risk factors associated with herd-level exposure of cattle in Nebraska, North Dakota, and South Dakota to bluetongue virus

Alice L. Green, MS, DVM; David A. Dargatz, DVM, PhD; Edward T. Schmidtman, PhD; Marco V. Herrero, PhD; Ann H. Seitzinger, PhD; Eileen N. Ostlund, DVM, PhD; Bruce A. Wagner, PhD; Kathryn M. Moser, MS; Nora E. Wineland, DVM, MS; Thomas E. Walton, DVM, PhD, ScD

Objective—To evaluate herd-level risk factors for seropositive status of cattle to 1 or more bluetongue viruses.

Animals—110 herds of cattle in Nebraska, North Dakota, and South Dakota.

Procedure—Blood samples were collected before and after the vector season. Samples were tested for antibodies against bluetongue virus by use of a commercially available competitive ELISA. Factors evaluated included descriptors of geographic location and management practices. Trapping of insect vectors was conducted to evaluate vector status on a subset of 57 operations. A multivariable logistic regression model was constructed to evaluate associations.

Results—For the full data set, altitude and latitude were associated with risk of having seropositive cattle (an increase in altitude was associated with an increase in risk, and a more northerly location was associated with a decrease in risk of a premise having seropositive cattle). Import of cattle from selected states was associated with an increase in risk of having seropositive cattle. From the subset of herds with data on vector trapping, altitude and latitude were associated with risk of having seropositive cattle, similar to that for the full model. However, commingling with cattle from other herds was associated with a decrease in risk of seropositivity.

Conclusions and Clinical Relevance—Findings reported here may be useful in generating additional hypotheses regarding the ecologic characteristics of bluetongue viruses and other vector-borne diseases of livestock. Sentinel surveillance programs are useful for documenting regionalization zones for diseases, which can be beneficial when securing international markets for animals and animal products. (*Am J Vet Res* 2005;66:853–860)

Infection with bluetongue virus (BTV) is classified as a list A disease by the Office Internationale des Epizooties. As such, BTV has had an adverse impact on worldwide trade as countries take steps to protect themselves from virus introduction. In many cases, prearrival testing for serum antibodies is required to

document lack of exposure of livestock to BTV. Finding serum antibodies against BTV influences international and interstate movement of live animals and germ plasm.

Infection with BTV is common throughout the world in latitudes ranging from 40° N to 35° S,^{1,2} although it has more recently been detected at 45° N in 1 study^a and 50° N in eastern and southern Europe as well as northern Africa.^{b,c} Disease typically develops in summer and fall.³ Clinical signs are evident mainly in sheep, but infection has regularly been documented in cattle, deer, and other ruminants.⁴ Transient fever is often one of the first clinical signs. Vasculitis may result in a number of additional clinical signs that can include facial edema and hyperemia of the oral mucosa as well as excessive salivation and profuse serous nasal discharge. Pulmonary edema may also be evident. In later stages of infection, erosions and ulcers may develop in the oral mucosa, and lameness and cardiac problems may develop as a result of myopathic effects.

Bluetongue virus is an arthropod-borne agent dependent on insects of the *Culicoides* genus for transmission. The capacity to transmit BTV is affected by the species of *Culicoides* and the serotype of BTV.^{5,6} In addition, transmission may depend on environmental conditions.⁷ The distribution of *Culicoides* spp appears to be environmentally controlled by factors such as climatic conditions and possibly soil and water chemical characteristics at breeding sites.^{7,9}

In the United States, 2 species of *Culicoides* warrant primary concern relative to transmission of BTV. The differences in vector competence and variation in geographic distribution serve as a plausible explanation for the regionalized nature of BTV infection in the United States.¹⁰ *Culicoides sonorensis*, the documented primary vector of BTV, is generally found in the southwest, south, and southeast United States,¹¹ whereas the nonvector species *Culicoides variipennis* is found in the northeast and north-central United States.^{5,11} Accordingly, exposure to BTV is low in the northern and northeastern United States^{6,12} where there is only *C. variipennis*.⁵ Of 19,758 serum samples obtained from cattle across the United States and tested by use of an

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From the USDA:Animal and Plant Health Inspection Service (APHIS):Veterinary Services (VS), Centers for Epidemiology and Animal Health, 2150 Centre Ave, Bldg B, Mail Stop 2E7, Fort Collins, CO 80526-8117 (Green, Dargatz, Herrero, Seitzinger, Wagner, Wineland, Walton); the USDA Agricultural Research Service, 1000 E University Ave, Department 3354, Laramie, WY 82071 (Schmidtman); and the USDA:APHIS:VS, National Veterinary Services Laboratories, 1800 Dayton Ave, Ames, IA 50010 (Ostlund, Moser). Dr. Herrero's present address is the Tropical Disease Research Program, School of Veterinary Medicine, Universidad Nacional, Apartado 304-3000, Heredia, Costa Rica.
Address correspondence to Dr. Green.

agar-gel immunodiffusion (AGID) assay during 1977 and 1978 and 21,117 serum samples obtained from cattle and tested by use of an AGID assay during 1983 to 1985, $\leq 1.0\%$ from 18 northern and northeastern states (Connecticut, Delaware, Indiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, North Dakota, Ohio, Pennsylvania, Rhode Island, Vermont, West Virginia, and Wisconsin) and 2 other states (Alaska and Hawaii) had positive results for BTV antibody. In 1977 and 1978, 18.0% to 53.2% of the samples from 9 southwestern states (Arizona, California, Colorado, Kansas, Nebraska, Nevada, New Mexico, Oklahoma, and Texas) had positive results, 6.3% to 39.3% of the samples from 13 southeastern states (Alabama, Arkansas, Florida, Georgia, Illinois, Iowa, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, and Tennessee) had positive results, and 80.2% of the samples from Puerto Rico had positive results.^{12,13}

The study reported here (part of the Bluetongue Surveillance Pilot Project) was initiated to estimate the prevalence of herds seropositive for BTV in a population of cattle that spans the presumed limits of distribution for *C. sonorensis* in the north-central part of the United States. The study was also designed to evaluate potential herd- and animal-level risk factors for operations that had seropositive cattle.

Materials and Methods

Sample population—The initial study population consisted of a convenience sample of beef and dairy cattle herds in Nebraska, North Dakota, and South Dakota. All states were stratified on the basis of county, and target numbers of participating producers for each county were sent to field contacts in each state. Generally, all counties in each state were included once, although several large counties in all 3 states had target numbers of 2 or 3 operations. Criteria for inclusion required that each operation was a dairy, beef cow-calf, or mixed-cattle operation; provided at least 80% of its own replacement cattle; and uniquely identified all cattle.

Operations were selected on the basis of geographic location and willingness to participate in the study. In Nebraska and South Dakota, federal and state field veterinarians made initial contacts with cattle owners and enrolled suitable operations in the study. In North Dakota, operations were selected from a database established at North Dakota State University, and state veterinarians then contacted cattle owners to identify and enroll suitable participants. Initially, 149 operations were enrolled in the study. There were 128 producers who completed questionnaires and allowed collection of samples during the periods before and after vector seasons.

Data and sample collection—Participating producers completed a single questionnaire on herd-level data for various husbandry practices and vector exposure-related factors. Producer questionnaires were completed during the period from June 18, 2001, to June 1, 2002. Animal-level questionnaire data were gathered by federal or state veterinary medical officers who completed 2 forms that accompanied blood samples that were obtained from individually identified cattle during 2 time periods; the blood samples were obtained before (December 2, 2000 to May 9, 2001 in Nebraska; November 18, 2000 to June 15, 2001 in North Dakota; and November 14, 2000 to June 15, 2001 in South Dakota) and after (October 22, 2001 to April 5, 2002 in Nebraska;

November 19, 2001 to April 25, 2002 in North Dakota; and October 22, 2001 to May 12, 2002 in South Dakota) the vector season for the summer of 2001. Blood samples were collected from the same cattle during both time periods, other than those cattle that were culled or could not be located in the fall (after the vector season).

Altitude of the operations ranged from 324 to 1,260 m above sea level. Latitude of the operations ranged from 40.03160° N to 48.75411° N. Longitude ranged from -95.92060° W to -103.98643° W.

A subset of 61 producers agreed to allow trapping of *Culicoides* spp on their property near aquatic habitats that could potentially contain larvae. Miniature blacklight suction traps were placed near 1 or 2 potential aquatic larval habitats in pasture areas in which the test herd grazed. Traps were operated for 2 consecutive nights during the first week and for 2 additional nights during the subsequent week. Insects were captured in catch jars containing ethylene glycol that served as a preservative so that speciation could be performed.

Testing of samples—Blood samples were tested by use of a commercially available competitive ELISA (cELISA)[†] for BTV antibodies; the cELISA was conducted in accordance with the manufacturer's instructions. When only 1 sample for an operation had positive results for the cELISA, the serum was evaluated by use of a virus neutralization (VN) test against BTV serotypes 2, 10, 11, 13, and 17 (ie, the 5 BTV serotypes identified in Canada, northern Mexico, and the United States), as has been described elsewhere.¹⁴ *Culicoides* vectors captured during trapping activities were speciated.¹⁵

Data analysis—Data were entered into an electronic database and checked for entry errors. Each operation was categorized as positive or negative on the basis of a case definition involving serologic findings from samples obtained before and after the vector season. For each blood collection period, operations were classified as positive when 2 or more samples had positive results for the cELISA or 1 sample had positive results for the cELISA and also had positive results for the VN test. Operations were classified as suspect when 1 sample had positive results for the cELISA, but that sample could not be tested by use of the VN tests (eg, insufficient sample or toxic reactions to the cells). All other operations were classified as negative.

Operations were assigned a final serologic herd-level status on the basis of serologic categorization results for samples obtained before and after the vector season; those that had positive results during either period were classified as positive. Operations that had negative results during both periods were classified as negative. Three operations were negative in 1 period and had a single cELISA-positive sample that could not be confirmed by VN testing during the other period; therefore, these 3 operations were excluded from the analysis as they did not meet a case definition for positive or negative.

Each potential risk factor was screened for a significant association with the final serologic herd-level status of the operation by use of a χ^2 or Fisher exact test. Continuous variables related to mean age of cattle within herds as well as bleeding intervals and herd location were evaluated by use of a *t* test.

Any variables associated with the outcome ($P < 0.25$) were eligible for inclusion in the multivariable logistic regression model. In some situations, categories were collapsed because of sparse data. Given that the data were too sparsely distributed to analyze the effects of importing cattle from a specific state, a dichotomous variable was created for the import of cattle from 6 selected states (ie, Colorado, Iowa, Kansas, Nebraska, Oklahoma, and Wyoming). Risk levels for

states of origin were conservatively classified on the basis of published^{12,13} estimates of prevalence; the 6 states included in the import category all had estimates of prevalence of > 9%. Operations in Nebraska that did not have imported cattle from states with a historically increased serologic prevalence for BTV were categorized in the nonimport category. Operations in North Dakota or South Dakota that imported cattle from Nebraska were grouped in the import category.

Herd-level logistic regression modeling was performed by use of a statistical program.⁸ Fourteen variables were analyzed for potential inclusion in an all-herds model. Variables were removed from the model by use of a backward-elimination algorithm until all remaining variables had a value of $P < 0.05$. A final model was constructed by use of all herds that had data for the variables remaining in the model ($n = 110$), which included 2 premises that were not used in the model-building procedure.

Fifteen variables were analyzed for potential inclusion in a vector-herds model. These included potential risk factors examined for the all-herds model, with the addition of a categorical variable for on-site detection of *C sonorensis*. Variables were removed from this model by use of a backward-elimination algorithm until all remaining variables had a value of $P < 0.05$, except the variable for on-site *C sonorensis* vector detection, that was forced into the model. A final vector-herds model was constructed by use of all herds that had data for the variables remaining in the model ($n = 57$).

To assess the fit of each model, the amount of agreement between the observed and predicted status of κ values for each operation¹⁶ was calculated. In addition, sensitivity and

specificity of the model-predicted outcomes were assessed by treating the observed status as the criterion-referenced standard. A probability cut-point of ≥ 0.5 was used for predicting outcome; operations with a $\geq 50\%$ predicted probability of seropositivity were classified as positive.

Results

All-herds model—Descriptive results from the study have been reported elsewhere.^{h,i} Briefly, serum samples were obtained for testing from cattle in 125 herds (Nebraska, 36 herds [29%]; North Dakota, 42 herds [34%]; and South Dakota, 47 herds [38%]) for both sample collection periods, and those herds were classified as positive or negative on the basis of the aforementioned case definition. Fifty-four (43%) herds were classified as positive, and 71 (57%) were classified as negative. Fifteen operations were excluded from the final all-herds model because of lack of data on 1 or more of the 3 risk factors that remained in the final model. Of the 110 operations included in the final all-herds model, 48 (44%) were classified as positive and 62 (56%) were classified as negative.

Of the 14 variables that were considered as potential risk factors for herd-level BTV exposure, 7 met the criteria for entry into the initial model (Tables 1 and 2). These included latitude and altitude of herds, import of cattle from selected states (yes or no), deer or antelope

Table 1—Environmental and husbandry-related categorical variables for the all-herds analysis of herd-level risk for seropositive status for bluetongue virus (BTV) in cattle herds in Nebraska, North Dakota, and South Dakota.

Variable	Categories	P	Frequency	
			Negative herds	Positive herds
Imported cattle from selected states* [125]	Yes	0.01	2/71 (3)	10/54 (19)
	No or unknown†		69/71 (97)	44/54 (81)
Percentage of cattle born on operation [117]	< 80%	0.95	16/64 (25)	13/53 (25)
	80% to 100% or unknown‡		48/64 (75)	40/53 (75)
Cattle born outside 160-km radius from operation [117]	Yes	0.26	5/64 (8)	3/53 (6)
	No or unknown§		59/64 (92)	50/53 (94)
Commingling with cattle from other herds [117]	Yes	0.51	14/64 (22)	9/53 (17)
	No or unknown		50/64 (78)	44/53 (83)
Deer or antelope on operation [117]	Yes	0.15	54/64 (84)	39/53 (74)
	No or unknown		10/64 (16)	14/53 (26)
Size of operation [122]	1 to 99	0.30	24/68 (35)	24/54 (44)
	100 to ≥ 300		44/68 (65)	30/54 (56)
Herd type [116]	Predominantly beef	0.17	57/64 (89)	49/52 (94)
	Dairy or mixed		7/64 (11)	3/52 (6)
Sheep on operation [117]	Yes	0.14	9/64 (14)	3/53 (6)
	No or unknown		55/64 (86)	50/53 (94)
Graze on nonprivate lands [117]	Yes	0.15	9/64 (14)	13/53 (25)
	No		55/64 (86)	40/53 (75)

Frequency values are reported as number of herds with that result (ie, category) divided by number of negative or positive herds; values in parentheses are the percentages. Numbers in brackets are the number of operations.
 *Selected states were Colorado, Iowa, Kansas, Nebraska, Oklahoma, and Wyoming. †Includes 13 farms without data. ‡Includes 3 farms without data. §Includes 5 farms without data. ||Includes 1 farm without data.

Table 2—Environmental and husbandry-related continuous variables for the all-herds analysis of herd-level risk for seropositive status for BTV in cattle herds in Nebraska, North Dakota, and South Dakota.

Variable	No. of negative herds	Mean ± SE	No. of positive herds	Mean ± SE	P
Age of cattle (y)	55	4.1 ± 0.3	51	4.4 ± 0.3	0.49
Blood collection interval (d)	67	283.5 ± 10.8	52	282.8 ± 10.3	0.96
Latitude (° N)	64	45.89 ± 0.23	48	42.95 ± 0.31	0.01
Longitude (° W)	64	-99.95 ± 0.27	48	-100.24 ± 0.30	0.48
Altitude (m above sea level)	63	586.8 ± 20.0	50	721.4 ± 34.7	0.01

Table 3—Final multivariable all-herds analysis of herd-level risk for seropositive status for BTV in cattle herds in Nebraska, North Dakota, and South Dakota.

Variable	Categories	Odds ratio	95% CI
Imported cattle from selected states*	Yes	14.30	1.51–135.73
	No	1.00	NA
Latitude†	NA	0.52	0.40–0.68
Altitude‡	NA	1.10	1.05–1.14

†For each degree further north within the range 40.03160° N to 48.75411° N. ‡For each 30-m increase in altitude within the range of 324 to 1,260 m above sea level.
 95% CI = 95% Confidence interval. NA = Not applicable.
 See Table 1 for remainder of key.

on operation (yes or no), sheep on operation (yes or no), type of operation (predominantly beef cattle, dairy cattle, or a mixed-cattle operation), and grazing on nonprivate lands (yes or no). Longitude of sample sites was also of interest as a potential interaction variable with latitude; however, altitude and longitude were significantly ($P = 0.01$) correlated, and longitude did not have a significant ($P = 0.48$) correlation, so altitude was chosen for inclusion in the model. Linearity was checked for the continuous variables altitude and latitude by fitting the model to approximate quartiles with the lowest groups serving as referents. Estimated coefficients were plotted against midpoints of the groups, and a visual assessment of linearity was performed.¹⁷

Three variables from the final all-herds model were significantly associated with risk of outcome (Table 3). These variables were import of cattle from selected states (odds ratio [OR], 14.30; 95% confidence interval [CI], 1.51 to 135.73), latitude, and altitude. For altitude of all herds (range, 324 to 1,260 m), a 30-m increase in altitude was associated with an increase in risk (OR, 1.10; 95% CI, 1.05 to 1.14). For the latitude of all herds (range, 40.03160° N to 48.75411° N), each change of 1° N was associated with a decrease in the risk of having seropositive cattle (OR, 0.52; 95% CI, 0.40 to 0.68). In a comparison of model results with herd outcome data, the κ statistic was 0.68, indicating substantial agreement between predicted and actual herd-level seropositivity beyond chance.¹⁶ Sensitivity and specificity for the model were 77.1% and 90.3%, respectively.

Vector-herds model—Sixty-one of the 125 herds from which serum samples were obtained also were included in vector trapping. Four of the 61 operations were excluded from the model because of lack of a response on 1 or more survey questionnaires. Of the 57 operations included in the final vector-herds model, 27 (47%) were classified as negative and 30 (53%) were classified as positive.

Of the 15 variables considered as potential risk factors for herd-level BTV exposure, 7 met the criteria for entry into the initial model (Tables 4 and 5). These variables included *C sonorensis* (vector variable); latitude, longitude, and altitude of herds; cattle born outside a 160-km radius from the farm; deer or antelope on the operation; and commingling with cattle from other herds.

The vector variable initially was not included in the model ($P = 0.74$), but it was forced in so that the effects of operation-level vector detection could be evaluated. For the forced-vector model, 3 variables were significantly associated with risk of outcome (Table 6). An increase in altitude was associated with an increase in risk of herd-level seropositivity. For the altitude for all vector herds (range, 335 to 1,221 m), each 30-m increase in altitude was associated with an increase in risk (OR, 1.19; 95% CI, 1.09 to 1.29). A change in latitude (ie, more northerly) was associated with a decrease in risk of herd-level seropositivity. For the latitude of all vector herds (range, 40.03160° N to 48.62560° N), each change of 1° N was associated with a decrease in risk of having seropositive cattle (OR, 0.57; 95% CI, 0.47 to 0.69). Commingling with cattle from other herds was associated with a decrease in risk of having seropositive cattle (OR, 0.07; 95% CI, 0.01 to 0.62). Two-level interaction variables could not be included in the vector model because of overspecification of the model. The forced variable for *C sonorensis* caused a slight increase in the risk of having seropositive cattle (OR, 1.16; 95% CI, 0.23 to 5.80), although these results were not significant after accounting for the other variables in the model.

In a comparison of model results with herd outcome data, the κ value was 0.61, indicating substantial agreement between predicted and actual herd-level seropositivity.¹⁶ Sensitivity and specificity for the model were 86.7% and 74.1%, respectively. Within this population, the positive predictive value for the model was approximately 78.8% and the negative predictive value

Table 4—Environmental and husbandry-related categoric variables for vector-herd analysis of herd-level risk for seropositive status for BTV in cattle herds in Nebraska, North Dakota, and South Dakota.

Variable	Categories	P	Frequency	
			Negative herds	Positive herds
Imported cattle from selected states*[60]	Yes	1.00	1/29 (3)	2/31 (6)
	No or unknown†		28/29 (97)	29/31 (94)
<i>Culicoides sonorensis</i> on operation [61]	Yes	0.01	8/30 (27)	21/31 (68)
	No		22/30 (73)	10/31 (32)
Percentage of cattle born on operation [59]	< 80%	0.60	6/28 (21)	5/31 (16)
	80% to 100%		22/28 (79)	26/31 (84)
	or unknown‡			
Cattle born outside 160-km radius from farm [59]	Yes	0.01	20/28 (71)	12/31 (39)
	No or unknown‡		8/28 (29)	19/31 (61)
Commingling with cattle from other herds [59]	Yes	0.02	10/28 (36)	3/31 (10)
	No		18/28 (64)	28/31 (90)
Deer or antelope on operation [59]	Yes	0.01	27/28 (96)	21/31 (68)
	No		1/28 (4)	10/31 (32)
Size of operation [60]	1 to 99	0.88	12/30 (40)	13/31 (42)
	100 to ≥ 300		18/30 (60)	18/31 (58)
Herd type [58]	Predominantly beef	0.40	27/28 (96)	28/30 (93)
	Dairy or mixed		1/28 (4)	2/30 (7)
Sheep on operation [59]	Yes	0.42	4/28 (14)	2/30 (7)
	No		24/28 (86)	28/30 (93)
Graze on nonprivate lands [59]	Yes	0.46	5/28 (18)	3/31 (10)
	No		23/28 (82)	28/31 (90)

Frequency values are reported as number of herds with that result (ie, category) divided by number of negative or positive herds; values in parentheses are the percentages. Numbers in brackets are the number of operations.
 *Selected states were Kansas, Nebraska, Oklahoma, and Wyoming. †Includes 3 farms with cattle of unknown origin. ‡Includes 4 farms with cattle of unknown origin.

Table 5—Environmental and husbandry-related continuous variables for vector-herd analysis of herd-level risk for seropositive status for BTV in cattle herds in Nebraska, North Dakota, and South Dakota.

Variable	No. of negative herds	Mean ± SE	No. of positive herds	Mean ± SE	P
Age of cattle (y)	24	4.1 ± 0.4	30	4.0 ± 0.4	0.78
Blood collection interval (d)	29	287.1 ± 18.3	30	290.6 ± 14.7	0.88
Latitude (° N)	27	45.794 ± 0.363	30	42.971 ± 0.401	0.01
Longitude (° W)	27	-99.51 ± 0.39	30	-100.30 ± 0.55	0.18
Altitude (m above sea level)	28	553.0 ± 22.6	31	740.6 ± 47.0	0.01

Table 6—Final multivariable vector-herds analysis of risk factors associated with herd-level risk for seropositive status for BTV in cattle herds in Nebraska, North Dakota, and South Dakota.

Variable	Categories	Odds ratio	95% CI
Commingling with cattle from other herds	Yes	0.07	0.01–0.62
	No	1.0	NA
<i>C sonorensis</i> on operation	Yes	1.16	0.23–5.80
	No	1.0	NA
Latitude*	NA	0.57	0.47–0.69
Altitude†	NA	1.19	1.09–1.29

*For each degree further north within the range 40.03160° N to 48.62560° N. †For each 30-m increase in altitude within the range of 335 to 1,221 m above sea level.
 See Table 3 for remainder of key.

was approximately 83.3%. Positive predictive value within younger populations of cattle was likely to be decreased; however, an increase in negative predictive value would also be expected.

Discussion

For the all-herds model, operations that imported cattle from selected states were more likely to be classified as positive than operations that did not import cattle from those states. However, a positive serologic response does not necessarily indicate current viremia or recent viral exposure. Although viremia associated with BTV generally lasts for approximately 3 weeks in

cattle,¹⁸ cattle may remain seropositive for an extended period. The duration of seropositivity has not been established, but it may be lifelong in cattle with a strong immune response to the virus.¹⁹⁻²¹ Some cattle imported from selected states may have been seropositive at the time of arrival on operations at which samples were collected for testing in the study reported here, rather than having been exposed to BTV on these operations. When operations that imported cattle from selected states were excluded from the final model (ie, 100 herds remaining in the model), the κ value for observed and predicted status did not change (κ , 0.68) and sensitivity and specificity were similar (75.0% and 91.7%, respectively) to values for the original all-herds model.

Herd location in southerly latitudes was associated with an increase in risk of herd-level seropositivity. This relationship between latitude and seropositivity was most plausibly a climate- and vector-related phenomenon. Populations of *C sonorensis*, the primary proven vector of BTV in the United States, may be more dense and prevalent at lower latitudes, and warm temperatures promote more rapid virus replication,²² contributing to greater vector capacity.⁷

Herds at higher altitudes in the study reported here were more likely to be classified as seropositive than those at lower altitudes. Additionally, mean \pm SE altitude of positive operations was 721 ± 35 m above sea level, whereas the mean altitude of negative operations was 587 ± 20 m. Climatic and other ecologic factors associated with areas of higher altitude within the study area may have represented a more favorable environment for *C sonorensis* populations. Interactions among altitude, relative humidity, and temperature are likely to play a role in *C sonorensis* numbers. Atmospheric moisture content and temperature typically decrease with an increase in altitude. Evidence from a controlled study⁷ revealed that low humidity is detrimental for survival of *C sonorensis* at low temperatures, whereas high humidity is detrimental for survival at high temperatures. However, these findings should only be interpreted within the altitude range for the study; sites at altitudes of $\geq 2,134$ m above sea level are not favorable for survival of *C sonorensis*, and BTV is not transmitted to native or resident livestock at those altitudes.²³

Interestingly, a study²⁴ of the distribution of *Culicoides imicola*, a vector for BTV in Africa, Asia, and parts of Europe, revealed that the mean altitude of sites without *C imicola* was approximately 27 m lower than for sites with *C imicola* (56 ± 18 m vs 84 ± 19 m). Additional studies on ecologic variables of vector habitat and viral transmission would be useful in establishing a scientific basis for risks associated with livestock movement. Recommendations from an international symposium on BTV²⁵ suggested a movement toward consideration of BTV distribution based on ecologic zones rather than latitudes.

Neither sheep nor wild ungulates (deer or antelope or both) on an operation was a significant risk factor after controlling for other factors. Various domestic and wild ungulates may be susceptible to infection with BTV, although finding such species on an opera-

tion would not necessarily increase the risk of BTV exposure to cattle at that site.

Grazing on nonprivate lands did not remain in the model. The use of nonprivate lands may provide opportunities for commingling with seropositive cattle. However, such commingling does not necessarily increase the likelihood that a herd will come into contact with BTV-positive, competent vector populations that are required for disease transmission. Whereas populations may center around aquatic sites with larval populations and cattle that serve as hosts, *C sonorensis* can disperse up to 2 km during an 8-day period.²⁶

Herd type was not a significant risk factor in this analysis. Husbandry and genetic factors, such as housing method, diet, and breed, are likely to vary between herds of beef and dairy cattle. However, because of the variation in housing and other husbandry methods within as well as between herd types, it is likely that the study reported here did not have sufficient power to detect the effect of herd type on herd-level serologic status.

Failure of the backward-elimination model to include *C sonorensis* as a risk factor for the vector-herds model may have been attributable to several factors. Most apparent was the prevalence of operations positive for BTV antibodies in cattle at which *C sonorensis* was not detected. Despite trapping vectors for 4 nights during a 2-week period, inclement weather, particularly thunderstorms, may have limited host-seeking flight and insect capture. Conversely, *C sonorensis* was captured at some operations on which cattle with anti-BTV antibodies were not detected. Little is known about factors that maintain BTV in nature, and as such, there may have been vectors despite a failure to identify the virus.

Because *C sonorensis* is the primary proven vector for transmission of BTV in the United States, the variable for *C sonorensis* was forced into the final model. The method of vector trapping was similar on all operations; however, the overall sensitivity of vector detection by use of our trapping protocol is unknown. Additionally, in the study reported here, herd seropositivity in the period before or after vector season was used to classify a herd as having a positive status. The association of *C sonorensis* with herd-level outcome may have been influenced by the inclusion of the herds that were seropositive before the vector season but not after the vector season ($n = 3$) because detection of the vector during a potential disease season would be expected to predict seropositive status after the vector season. Moreover, the vector variable did not account for population or BTV infection rates of *C sonorensis*, factors that also play a role in herd-level disease risk. However, it is interesting that sensitivity for the vector-herds model (86.7%) was higher than that for the all-herds model (77.1%).

Similar to the all-herds data for altitude range, operations located at higher altitudes were more likely to be classified as positive than those at lower altitudes. Presumably, the increased herd-level seropositivity at higher altitudes reflects climatic and other ecologic conditions favorable to *C sonorensis* that are not found

at lower altitudes. However, as mentioned previously, this finding should only be interpreted within the altitude range of the study.

For the vector-herds portion of the study, commingling with cattle from other herds was associated with a decrease in risk of seropositivity. This apparent protective factor may have been spurious because it was not significant in the all-herds analysis. This practice was more common in the northern states (28% and 27% of the herds in North Dakota and South Dakota, respectively, commingled their cattle) than in Nebraska (only 11% of herds commingled cattle).

One limitation of the herd-level study reported here was that it focused on seropositivity rather than seroconversion as an outcome. Notably, there were only 4 herds in the study that converted from seronegative to seropositive status. Although seroconversion is a more accurate measure of seasonal disease risk to specific cattle, seroprevalence is more closely aligned with requirements for export of cattle to Canada. Additionally, a study of seroconversion in mature cows would be cost-prohibitive in high-risk areas, particularly at the herd level. Therefore, positive or negative outcome data at the herd level may be more useful as a basis for discussions of international import and testing requirements. The predictive value of the model may be limited by inclusion of herds in which cattle were seropositive during the period before the vector season but not during the period after the vector season because risk factors may have changed between these periods. Additional data are needed to establish whether the model has sufficient predictive value for use in the BTV policy for testing of livestock for international transport, but results are consistent with current beliefs on BTV distribution based on ecologic zones.

In the study reported here, we examined potential herd-level risk factors for BTV exposure. Environmental and management factors associated with an increase in risk of herd-level seropositivity included import of cattle from selected states, more southern herd location, and an increase in altitude of herd location within the range of 335 to 1,250 m above sea level. These factors are supportive of the following 2 hypotheses. First, exposure of cattle to BTV typically is seen in specific regions that are favorable to the vector species. Second, exposure may induce a long-lived serologic response in a proportion of exposed animals, although the duration of viremia is generally only a few weeks (21 days) in cattle. Areas for additional research should include investigation of ecologic and altitude-related factors that affect *C. sonorensis* populations as well as BTV exposure.

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