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

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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, although it has more recently been detected at 45° N in 1 study and 50° N in eastern and southern Europe as well as northern Africa. Disease typically develops in summer and fall. Clinical signs are evident mainly in sheep, but infection has regularly been documented in cattle, deer, and other ruminants. 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. In addition, transmission may depend on environmental conditions. 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.

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. Culicoides sonorensis, the documented primary vector of BTV, is generally found in the southwest, south, and southeastern United States, whereas the nonvector species Culicoides varipennis is found in the northeast and north-central United States. Accordingly, exposure to BTV is low in the northern and northeastern United States, where there is only C. varipennis. Of 19,758 serum samples obtained from cattle across the United States and tested by use of an

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The Blue Tongue 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-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 and after 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).

Alitude 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. *Culicoides* vectors captured during trapping activities were speciated.

**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 VN tests. 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 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. 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 categoric 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, which 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 \( \kappa \) values for each operation 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 \( \geq 0.5 \) 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. 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 on operation, and size of operation. The results of the logistic regression analysis are presented in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categories</th>
<th>( P )</th>
<th>( \kappa )</th>
<th>( S )</th>
<th>( N )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported cattle from selected states* [125]</td>
<td>Yes</td>
<td>0.01</td>
<td>0.01</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>No or unknown†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of cattle born on operation [117]</td>
<td>&lt; 80%</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
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<tr>
<td></td>
<td>80% to 100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>or unknown‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle born outside 160-km radius from operation [117]</td>
<td>Yes</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>No or unknown§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Commingling with cattle from other herds [117]</td>
<td>Yes</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>No or unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer or antelope on operation [117]</td>
<td>Yes</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>No or unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of operation [122]</td>
<td>1 to 99</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>100 to ( \geq 300 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd type [116]</td>
<td>Predominantly beef</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Dairy or mixed</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sheep on operation [117]</td>
<td>Yes</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>No or unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graze on nonprivate lands [117]</td>
<td>Yes</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of negative herds</th>
<th>Mean ± SE</th>
<th>No. of positive herds</th>
<th>Mean ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of cattle (y)</td>
<td>55</td>
<td>4.1 ± 0.3</td>
<td>51</td>
<td>4.4 ± 0.3</td>
<td>0.49</td>
</tr>
<tr>
<td>Blood collection interval (d)</td>
<td>67</td>
<td>283.5 ± 10.8</td>
<td>52</td>
<td>282.8 ± 10.3</td>
<td>0.96</td>
</tr>
<tr>
<td>Latitude (° N)</td>
<td>64</td>
<td>45.99 ± 0.23</td>
<td>48</td>
<td>42.95 ± 0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Longitude (° W)</td>
<td>64</td>
<td>-99.95 ± 0.27</td>
<td>48</td>
<td>-100.24 ± 0.30</td>
<td>0.48</td>
</tr>
<tr>
<td>Altitude (m above sea level)</td>
<td>63</td>
<td>566.8 ± 20.0</td>
<td>50</td>
<td>721.4 ± 34.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Import cattle from selected states*</td>
<td>Yes</td>
<td>1.51±0.23</td>
<td>95% CI = 1.09 ± 0.29</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latitude†</td>
<td>NA</td>
<td>0.52</td>
<td>95% CI = 0.40 ± 0.68</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Altitude†</td>
<td>NA</td>
<td>1.10</td>
<td>95% CI = 1.05 ± 1.14</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

*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. NA=Not applicable. See Table 1 for remainder of key.

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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categories</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Import cattle from selected states*</td>
<td>Yes</td>
<td>14.30</td>
<td>1.51–135.73</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>1.00</td>
<td>NA</td>
</tr>
<tr>
<td>Latitude†</td>
<td>NA</td>
<td>0.52</td>
<td>0.40–0.68</td>
</tr>
<tr>
<td>Altitude†</td>
<td>NA</td>
<td>1.10</td>
<td>1.05–1.14</td>
</tr>
</tbody>
</table>

*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. NA=Not applicable. See Table 1 for remainder of key.

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 for all vector herds (range, 40°03160’N to 48°62560’N), each change of 1° N was associated with a decrease in the 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. 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...
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, 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.\textsuperscript{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 $\kappa$ value for observed and predicted status did not change ($x$, 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,\textsuperscript{22} contributing to greater vector capacity.

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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 333 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 ecological and altitude-related factors that affect C sonorensis populations as well as BTV exposure.


References

viremia infections to Culicoides sonorensis in bluetongue virus-infect-


