Regional seroprevalence of bluetongue virus in cattle in Illinois and western Indiana

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Objective—To estimate seroprevalence of bluetongue virus (BTV) and the geographic distribution of seropositive cattle herds in Illinois and western Indiana.

Sample Population—10,585 serum samples obtained from cattle in 60 herds during 3 transmission seasons (2000 through 2002).

Procedures—In a longitudinal study, serum samples were tested for BTV antibodies by use of a competitive ELISA. Four geographic zones were created by use of mean minimum January temperature. A multivariable mixed-effects logistic regression model with a random effect for herd was used to estimate seropositive risk for zone, age of cattle, herd type, and transmission season.

Results—Overall, BTV antibodies were detected in 156 (1.5%) samples. Estimated seroprevalence in 2000, 2001, and 2002 was 1.49%, 0.97%, and 2.18%, respectively. Risk of being seropositive for BTV was associated with geographic zone and age. Seroprevalence increased progressively from northern to southern zones, with no evidence of BTV infection in the northernmost zone. In the southernmost zone, annual seroprevalence ranged from 8.65% to 11.00%. Adult cattle were 2.35 times as likely as juvenile cattle to be seropositive.

Conclusions and Clinical Relevance—Overall seroprevalence was lower than has been reported for Illinois cattle. Bluetongue virus antibodies were distributed heterogeneously in this region. Only in the southernmost zone was seroprevalence consistently > 2%. Regionalization of BTV risk based on state borders does not account for such variability. Serologic data could be combined with landscape, climate, and vector data to develop predictive models of BTV risk within transitional regions of the United States. (*Am J Vet Res* 2007;68:1212–1219)

Bluetongue virus is a double-stranded RNA virus in the family Reoviridae, genus Orbivirus. The virus infects wild and domestic ruminants and is transmitted between infectious and susceptible animals via the bite of midges in the genus *Culicoides*.[1] Infection with BTV can lead to bluetongue disease, which is characterized by fever, ulcers in the mucous membranes, laminitis, and facial edema. Bluetongue disease can be severe and lead to death, particularly in naïve sheep populations. Infected cattle often do not have clinical signs of disease and have been considered to be reservoir hosts because of their extended duration of viremia[2]; therefore, they play an important role in the transmission cycle of BTV.[3]

Abbreviations

BTV	Bluetongue virus
OIE	World Organization for Animal Health
cELISA	Competitive ELISA
CI	Confidence interval
OR	Odds ratio
AGID	Agar gel immunodiffusion
EHDV	Epizootic hemorrhagic disease virus

Bluetongue disease is listed by the OIE as a notifiable disease because it can cause substantial morbidity and fatalities and has the potential for rapid international spread.[4] Inclusion on the OIE list allows countries with BTV-free status to impose nontariff trade restrictions on cattle imported from BTV-endemic countries. Bluetongue virus is the only OIE-notifiable disease that is endemic in a substantial portion of the United States. A 1998 review of the USDA APHIS International Regulations Retrieval System revealed that, at that time, 66 countries imposed 159 BTV-based import measures on US ruminants and their products.[5] Such restrictions were estimated to cause annual losses

Received March 26, 2007.
Accepted May 9, 2007.
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Supported in part by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (grant No. 2001-35204-10153) and by a grant from the Council for Food and Agricultural Research, Illinois Department of Agriculture. Dr. Singer is a University of Minnesota McKnight Land-Grant Professor.
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of $125 million for US livestock producers.[6] Inclusion of BTV on the OIE list was partly in response to a series of outbreaks around the world that led many to conclude the movement of ruminants was facilitating the spread of BTV from its perceived origin in southern Africa. In addition, evidence that cattle were capable of being persistently infected by BTV contributed to the creation of import barriers by BTV-free countries.[7]

During the past 30 years, the understanding of epidemiologic aspects of BTV has improved. For example, cattle are no longer considered to be persistent carriers of infection.[2,8] The OIE now considers that the infective period for cattle is 60 days. Although BTV has a worldwide distribution between latitude 35°S and 50°N, specific serotypes exist in distinct regions and are confined within the geographic range of *Culicoides* spp that serve as competent vectors for each serotype.[9,10] Therefore, the movement of ruminants is unlikely to be important in most situations. A possible exception was in northern Europe, where a large outbreak was apparently caused by an African strain (BTV-8) not previously detected in that region.[11] It is not clear how that strain was introduced and spread. Vector distribution is influenced by climatic and environmental conditions. Thus, BTV transmission is spatially and temporally heterogeneous, particularly in temperate zones.[12,13] This new information on the epidemiologic factors of BTV has influenced some countries to modify their trade policies with regard to BTV, which has allowed regionalization of BTV-infection status.[14,15]

Regionalization is an attempt to partition the risk of infection over spatial and temporal scales, which should minimize the risk of spreading infectious agents when animals and animal products are moved among regions. Regionalization recognizes the variability in the spatial and temporal distribution of infectious agents in animal populations. Therefore, differing levels of risk can be assigned to specified populations. These levels of risk can also vary among seasons. Efforts to support and implement regionalization within the United States have focused on state borders as convenient regional boundaries.[8,16,17] However, the use of state borders for regionalization does not allow recognition of the spatial gradients of disease risk that exist within the United States.

Bluetongue virus is predominantly distributed in the southern United States. The prevalence of infection is lower in Midwestern and eastern states and is probably extremely low, or the infection may be totally nonexistent, in northern and New England states.[16–18] This geographic distribution is similar to the range of *Culicoides sonorensis*, the primary vector of BTV in the United States.[6,19] The lower Midwest (including Illinois and Indiana) is a transition zone between endemic areas with high BTV transmission and areas in which there is little or no BTV transmission. The most difficult areas of the United States in which to develop surveillance strategies and to assess the risk of BTV infection are within such transition zones. In these areas, infection is likely to be highly variable from year to year and is spatially heterogeneous. Thus, these are areas in which it is particularly difficult to use regionalization but where regionalization is most needed.

The USDA APHIS conducted serosurveys of slaughter cattle annually in selected states from 1977 to 1996; since 1996, serosurveys have been conducted biennially.[16–18] Estimated BTV seroprevalence for cattle herds in Indiana has been highly variable and fluctuated between 0% and 8%. Cattle from Illinois were included in the initial 1977 serosurvey for BTV, but then they were only included in 1985, 1992, and 2002. In those 4 serosurveys, estimated seroprevalence ranged from 6% to 16%. Unfortunately, we are not aware of any studies that provide a confident estimate of BTV seroprevalence within the lower Midwestern United States. Therefore, the objectives of the study reported here were to estimate BTV seroprevalence and the geographic distribution of seropositive cattle herds located in Illinois and western Indiana. We hypothesized that seroprevalence would be higher in the southern regions of the study area. A serologic survey of cattle was conducted with a sampling strategy that was designed to test this hypothesis.

Materials and Methods

Study population—A longitudinal study was conducted for 3 BTV transmission seasons (2000 through 2002) on the cattle population of Illinois and western Indiana. The study was conducted for the 3-year period because of anticipated year-to-year variability in BTV transmission. Herds were selected to represent anticipated zones of BTV risk. The study area was categorized into 4 geographic zones on the basis of the mean minimum temperature during the month of January (zone 1, < –10°C; zone 2, –10° to –8°C; zone 3, –7° to –6°C; and zone 4, –6° to –3°C; **Figure 1**).[20] Zone 1 was the northernmost zone, and zone 4 was the southernmost zone. Minimum temperature was used as an indicator of vector distribution and activity. In other studies conducted within temperate zones, temperature had a strong influence on *Culicoides* distribution and activity[21,22] as well as on BTV amplification within the vector.[23]

Herds were selected on the basis of geographic location and willingness of owners to participate in the study. A minimum of 6 herds were selected in each zone, with more herds added in the zones with the lowest expected prevalence of BTV exposure to increase precision of seroprevalence estimates. To estimate a seroprevalence of 1% in a zone with 1% precision and 95% confidence, the target sample size was 375 cattle/zone. This sample size calculation assumed perfect sensitivity and specificity of the diagnostic test, a reasonable assumption for the ELISA used in the study.[24]

The precise geographic location of each herd was determined by use of a global positioning system.[a] Coordinates for location of each dairy herd were recorded at the milking parlor, and location of each beef herd was recorded at the primary corral of each operation. In total, 60 beef and dairy herds (52 in Illinois and 8 in Indiana) were recruited for the study (Figure 1). Selected herds were located in 34 counties in Illinois and Indiana. During the study period, there were approximately 500,000 cattle on 7,059 farms in these counties.[25]

Sample collection and testing—Samples were obtained from cattle during the winter and early spring months (typically November through March), when it was assumed there would be no BTV transmission. Thus, seropositive results represented transmission events during the preceding transmission season or seasons. A convenience sample of cattle was selected from each operation. Cattle included in the study were at least 6 months old at the time of the preceding vector season (June through October) to ensure that remaining maternal antibodies had not prevented seroconversion after natural exposure. Juvenile cattle (7 to 18 months old) were targeted to represent 25% of the sample group from each recruited operation. The remaining 75% of the sample group comprised adult cattle (> 18 months old). The target of 25% was established to estimate incidence among cattle that had lived through only a single BTV transmission season and were also at risk of seroconversion. Each animal was identified so that it could be retested the subsequent year.

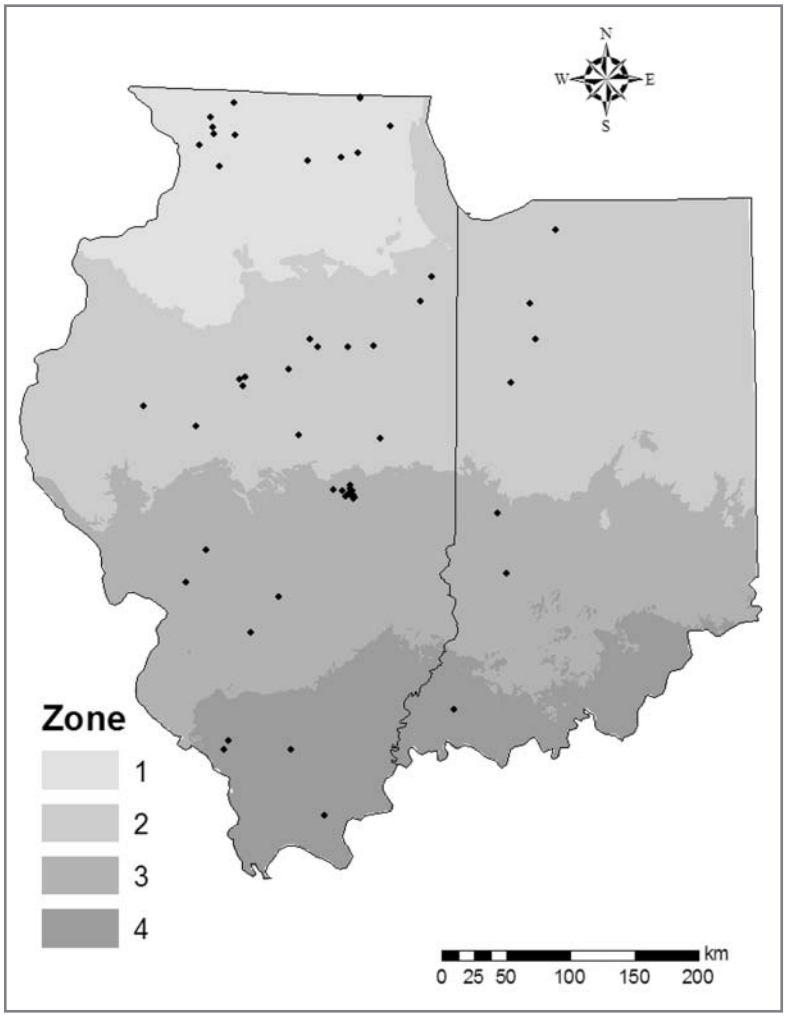

Figure 1—Map illustrating 4 geographic zones and the distribution of herds from which samples were obtained for testing to detect antibodies against BTV following the transmission seasons of 2000 through 2002. Zones were defined on the basis of the mean minimum temperature during the month of January (zone 1, < –10°C; zone 2, –10° to –8°C; zone 3, –7° to –6°C; and zone 4, –6° to –3°C). Each dot represents 1 beef or dairy herd from which samples were obtained during at least 1 year of the study.

Blood samples were stored at 4°C until processing, which was accomplished within 24 hours after collection. Collection tubes were centrifuged at 3,000 × *g* for 5 minutes at 4°C. Serum was removed with a sterile transfer pipet and stored at –20°C until testing. Each serum sample was tested for serogroup antibodies against BTV by use of a commercially available cELISA.[b] The assay was performed and results interpreted as per the manufacturer's instructions, and a cutoff inhibition value of 50% was used to determine a seropositive result.

Data analysis—Seroprevalence was defined as the proportion of cattle with positive cELISA results. Unadjusted seroprevalence was calculated for each zone and the entire study region. Comparisons of seroprevalence among zones and years were made by use of 95% CIs, with the assumption of perfect test sensitivity and specificity.

Multivariable mixed-effects logistic regression was used to assess differences among zones and years in the likelihood that a specific animal would be seropositive. The binary test result for each animal served as the outcome variable. Predictor variables included zone (4 levels), year (3 levels), herd type (dairy or beef), and age of animal (juvenile [< 18 months at time of sample collection] or adult), and all were modeled as fixed effects. A random effect for herd was used to account for interherd variability for which we did not have data. Initially, all main fixed effects were forced into the model. The significance of each variable and the likelihood ratio test were used to determine those covariates that should remain in the final model. Effect modification was assessed by including all first-order interaction terms for the main effects in the model. Finally, the random effect for herd was added to the model, and fixed-effect covariates and first-order interaction terms with values of $P > 0.05$ were excluded from the final model. All statistical analyses were performed with standard statistical software.[c,d] For all analyses, parameter estimates with a value of $P < 0.05$ were considered significant.

Results

During the 3 years of the study, 14,745 serum samples were obtained from 60 herds (**Table 1**). Of these, 10,585 samples were tested for BTV antibodies and included in the analysis. The remaining samples came from cattle younger than 6 months or were redundant samples (ie, large herds were sampled over multiple days, resulting in blood samples being collected from some cattle more than once during the same year). Also, some serum samples were excluded because they were collected during the spring and summer months and did not represent exposure over an entire transmission season. Mean

number of cattle tested for each herd was 72 (range, 13 to 281). The target sample size per zone was 375 cattle. This target was met in all zones, except for zone 4 in which the target was not met during the second year (samples were obtained from only 232 cattle).

Thirty-five herds participated in the study during all 3 years, 18 herds participated for 2 years, and 7 participated for 1 year. In 2000, 56 herds participated in the study. In 2001, 9 of the original 56 herds were lost to follow-up and 4 new herds were added to the study to yield a total of 51 herds that year. In 2002, 14 herds included in the previous year were lost to follow-up. No new herds were added in 2002, but 4 of the herds lost in 2001 rejoined the study. Thus, 41 herds were used in 2002.

Overall mean prevalence of antibodies against BTV in all herds in transmission seasons 2000, 2001, and 2002 was 1.49%, 0.97%, and 2.18%, respectively (**Table 2**). Overall seroprevalence in 2001 was significantly lower than in the other 2 years of the study. The difference in seroprevalence between 2001 and 2000 (ie, 0.52%) was significant (95% CI, 0.02% to 1.01%), as was the difference of 1.21% between 2001 and 2002 (95% CI, 0.60% to 1.83%) and the difference of 0.69% between 2000 and 2002 (95% CI, 0.03% to 1.36%).

Estimated BTV seroprevalence in each zone increased in a north-to-south gradient. There was no evidence of BTV exposure in the northernmost zone (zone 1) throughout the entire study period. Annual seroprevalence in zones 2 and 3 varied between 0.45% and 1.20%, whereas annual seroprevalence in the southernmost zone (zone 4) ranged from 8.65% to 11.00%. In each year, seroprevalence was significantly higher in zone 4 than in all other zones. Seroprevalence for zones 2 and 3 was significantly higher than for zone 1 in all 3 years of the study.

All 4 zones had herds that were lost to follow-up each year. The proportion of herds lost from each zone in any given year ranged from 7% to 33%. Seroprevalence of cattle from herds that were lost to follow-up was compared with that of cattle from herds that were retained. In zone 2, 6 of 18 herds were lost to follow-up in 2002. Cattle from those 6 herds had a seroprevalence that was significantly higher (1.04% higher; 95% CI, 0.03% to 2.05%) than the seroprevalence for cattle from the 12 herds that were retained. In zone 3, 2 of 17 herds were lost to follow-up after 2000. Cattle from those 2 herds had a seroprevalence that was significantly higher (5.22% higher; 95% CI, 1.36% to 9.08%) than the seroprevalence for cattle from the 15 herds that were retained. There were no differences between cattle lost to follow-up and cattle retained in the study from herds in zones 1 and 4.

The final multivariable logistic regression model that was used to assess the risk of being seropositive for BTV included zone and age factors (**Table 3**). A random effect for herd resulted in a better fit of the model to the data and was included in the final mixed model. None of the first-order interaction terms was significant. Herds located in the northernmost zone (zone 1; 2,950

Table 1—Number of herds and cattle tested for antibodies against BTV by use of a cELISA.

Variable	2000	2001	2002
No. of herds	56	51	41
No. of cattle	3,626	4,120	2,839
Mean No. of cattle (range) tested/herd	65 (18–132)	81 (20–281)	69 (13–223)

Table 2—Results for cattle tested by use of a cELISA to detect antibodies against BTV, on the basis of geographic zone and year.

Year	Zone*	No. of cattle tested	No. of cattle seropositive	Seroprevalence (%)	95% CI
2000	1	1,016	0	0	0.00–0.36
	2	1,261	12	0.95	0.49–1.66
	3	956	8	0.84	0.36–1.64
	4	393	34	8.65	6.07–11.88
	Total	**3,626**	**54**	**1.49**	**1.12–1.94**
2001	1	1,130	0	0	0.00–0.33
	2	1,651	13	0.79	0.42–1.34
	3	1,107	5	0.45	0.15–1.05
	4	232	22	9.48	6.04–14.00
	Total	**4,120**	**40**	**0.97**	**0.69–1.32**
2002	1	804	0	0	0.00–0.46
	2	1,035	9	0.87	0.39–1.64
	3	582	7	1.20	0.48–2.46
	4	418	46	11.00	8.17–14.41
	Total	**2,839**	**62**	**2.18**	**1.68–2.79**

*Geographic zones were created on the basis of the mean minimum temperature during the month of January (zone 1, < –10°C; zone 2, –10° to –8°C; zone 3, –7° to –6°C; and zone 4, –6° to –3°C); zone 1 was the northernmost zone, and zone 4 was the southernmost zone.

Table 3—Results of multivariable mixed-effects logistic regression* for risk of being seropositive for BTV.

Factor	Categories	Estimate	SE	OR	95% CI	*P* value
Intercept	NA	−4.949	0.407	NA	NA	< 0.001
Zone†	2	Referent	NA	NA	NA	NA
	3	−1.404	0.709	0.25	0.06–0.99	0.047
	4	2.784	0.696	16.18	4.14–63.32	< 0.001
Age‡	Juvenile	Referent	NA	NA	NA	NA
	Adult	0.855	0.413	2.35	1.05–5.28	0.038

*A random-effects term for herd, which explained 31.2% of the variance in the risk of being seropositive for BTV, was included in the model. †Seroprevalence for zone 1 was 0%; thus, data for zone 1 were excluded from this analysis. ‡Juvenile cattle were 7 to 18 months old and adult cattle > 18 months old at the time samples were obtained.
NA = Not applicable.

samples) were excluded from this model because of sparse data: no seropositive cattle were detected in this zone during the entire study period. The model would not converge when data for zone 1 were included. Therefore, zone 2 served as the referent category for this model. In addition, 1,366 cattle were excluded because of missing data for age. Thus, the final model was based on data from 6,269 cattle. Cattle in the southernmost zone (zone 4) had a significantly ($P < 0.001$) higher likelihood of being seropositive (OR, 16.18; 95% CI, 4.14 to 63.32), compared with the likelihood for cattle in zone 2, whereas cattle in zone 3 had a significantly lower likelihood of being seropositive (OR, 0.25; 95% CI, 0.06 to 0.99), compared with the likelihood for cattle in zone 2. Adult cattle had a significantly ($P = 0.04$) higher likelihood of being seropositive (OR, 2.35; 95% CI, 1.05 to 5.28) than did juvenile cattle. Herd, which was considered a random effect in this model, explained 31.2% of the total variance in the risk of being seropositive for BTV.

Characteristics of the 1,366 cattle that were excluded from the final model were assessed. In total, 677 cattle from zone 2, 442 cattle from zone 3, and 214 cattle from zone 4 were excluded because of a lack of age information. These represented 17%, 17%, and 21% of the total number of cattle from which samples were obtained in zones 2, 3, and 4, respectively. Number of seropositive cattle excluded because of a lack of age information was 3, 9, and 21 for zones 2, 3, and 4, respectively, which represented 9%, 45%, and 10% of the total number of seropositive cattle from zones 2, 3, and 4, respectively. Had these cattle been included in the mixed-effects logistic regression model, with the variable for animal age removed from the model, the cattle of zone 3 would no longer have had a significantly lower likelihood of being seropositive, compared with the likelihood for cattle from zone 2 (OR, 0.43; 95% CI, 0.13 to 1.38). For this model, cattle in the southernmost zone (zone 4) again would have had a significantly higher likelihood of being seropositive (OR, 19.30; 95% CI, 5.47 to 68.03), compared with the likelihood for cattle in zone 2.

A potential bias was introduced through repeated sample collection from individual cattle during the second and third years of the study. The 10,585 samples tested in this study represented 7,368 cattle. There were 5,019 cattle tested once, 1,873 cattle tested twice, and 476 cattle 3 times during the 3 years of the study. In addition, 392 samples could not be traced to specific cattle. Among cattle that were tested multiple times, 23 were seropositive on the first test, and 18 of these remained seropositive on subsequent tests. There were 13 cattle tested multiple times that seroconverted during the study. Because a small proportion of cattle with repeated samples were seropositive, the data were considered to be an independent cross-sectional sample each year with nearly all cattle at risk of exposure and seroconversion to BTV.

Discussion

The OIE recognizes a country or region as free of BTV when a surveillance program that uses internationally accepted diagnostic tests does not detect evidence of BTV in the livestock population for at least 2 years.[4] For many years, the AGID test was the test most widely used to detect BTV exposure. Because of less-than-optimal specificity of the AGID test, a seroprevalence of < 2% has been accepted as meeting the BTV-free requirement for the purpose of exporting live animals from the United States to Canada.[16] The cELISA has much higher specificity than the AGID test and is now the preferred method for BTV testing.[24,26,27] Although national BTV serosurveys conducted by the USDA APHIS currently use the cELISA as the accepted diagnostic test, the 2% seroprevalence rate is still used as a reference point for categorizing the BTV status of states included in the surveys. In the study reported here, the 2% seroprevalence threshold was exceeded consistently only in the southernmost zone (zone 4). We estimated (with 95% confidence) a seroprevalence of < 2% in the remaining 3 zones during all 3 years, except for zone 3 in 2002. This variability was also evident in the logistic regression model in which the likelihood of exposure to BTV was much higher in zone 4 than in zones 2 and 3. No seropositive cattle were found in the northernmost zone (zone 1), which suggested that there was no BTV transmission in the northern part of the study region during the 3 years of this study. Furthermore, although the duration of seropositivity in cattle exposed to BTV has not been

definitively established, it may last for an extended period.[28,29] Therefore, the lack of a single seropositive animal in zone 1 suggested that it was likely there was no BTV transmission in cattle in northern Illinois and northwest Indiana during the years immediately preceding our study.

European countries have not imported live cattle from the United States since 1980, largely because of BTV restrictions. In 2003, the United States and European Union reached an agreement to resume trade in live cattle.[14,30] The BTV agreement allows the United States to export cattle from 18 states in the Northeast and Midwest during the seasonally BTV-free period (mid-October to late March). Illinois is not included on this list, but Indiana is included. Both of these states are situated within the same approximate latitudes (37°N to 43°N) and have similar topography and climates. In the baseline 50-state national BTV serosurvey conducted during 1977 and 1978, 18 low-seroprevalence (< 1%) states were identified, including Indiana.[17] Those are the same 18 states included in the 2003 agreement with the European Union. Illinois was not included in the original list of low-seroprevalence states and subsequently has been included in the national serosurvey only 3 times since the baseline survey. In all 3 of those surveys, the estimated seroprevalence exceeded 2%; thus, Illinois has been excluded from being able to trade live cattle to the European Union.

Analysis of results of the study reported here suggested that the high seroprevalence in Illinois reported in the national serosurveys is not representative of the high variability that exists among herds located throughout the state. In national serosurveys, substantial variability in BTV seroprevalence has been observed in those states that were included annually for multiple years, presumably as a result of variability in annual BTV transmission. Thus, the difference in seroprevalence estimated in the study reported here, compared with estimates for the national serosurveys, could be explained by this variability in BTV transmission.

The discrepancy may also be explained by differences in sampling methods. The national serosurveys are convenience samples of slaughter cattle with relatively small sample sizes. Although the number of cattle in Illinois from which samples are obtained for the national surveys has not been reported, the general target is > 600 cattle/state/y, and sample size rarely exceeds 1,000 cattle for any given state.[17] A smaller sample size provides a less precise estimate. Also, the location in the state from which the sampled cattle originated was not reported. Assuming a disproportionate number of the cattle came from herds in southern Illinois, the state seroprevalence could have been overestimated. Additionally, slaughter cattle may not be representative of all cattle in the state. For example, many cull dairy cows are transported to Wisconsin or Michigan for slaughter. If the serosurveys did not account for state of origin, those data may be inaccurate.

Although the cELISA has become the preferred method of testing for BTV antibodies,[27] some countries still require the use of the AGID test for export certification.[15] The AGID test has the disadvantage of being cross-reactive for antibodies against other orbiviruses, including EHDV. Similar to BTV, EHDV is an orbivirus that is transmitted by *Culicoides* spp, infects domestic and wild ruminants, and causes clinical signs similar to those caused by BTV.[31] In the national BTV serosurveys, some AGID-positive results in low-incidence states were subsequently found by use of serum neutralization tests to have negative results for BTV and positive results for EHDV.[17] Notably, a large number of samples obtained from cattle in Indiana subsequently had positive results for EHDV. Epizootic hemorrhagic disease virus also exists in Illinois and could have influenced results of prior serosurveys. Cross-reactivity in the AGID test is the primary reason that the seroprevalence threshold of 2% was adopted for BTV. Even though the cELISA is more specific than the AGID for BTV, the epidemiologic aspects of EHDV need to be understood and accounted for to provide evidence that regions within states are free from BTV.

In the case of transition states such as Illinois and Indiana, classification of BTV status on the basis of state boundaries is not ideal. Analysis of the results reported here suggested that BTV transmission could be described by 3 geographic zones on the basis of estimated seroprevalence: a zone of no transmission, with a southern limit at approximately 42°N; a zone of low to moderate transmission (overall seroprevalence < 2%, but that includes a small proportion of herds with seroprevalence > 2%) located between 38°N and 42°N; and a zone of high transmission (seroprevalence in all herds is consistently > 2%) located south of 38°N. Although the logistic regression model suggested that risk of exposure to BTV was lower in zone 3 than in zone 2, this was likely influenced by a cluster of 11 herds in zone 3 (located adjacent to zone 2) in which there was no evidence of BTV exposure in any study year, with the exception of 1 herd in 2002. Seropositive herds in zone 3 generally had a higher seroprevalence than the seropositive herds in zone 2, and zone 3 could be considered a transition zone between the high-risk zone 4 and the lower-risk zone 2. Results of the study reported here corroborate those of a study[32] for 3 other states that span the BTV transition zone (ie, Nebraska, South Dakota, and North Dakota) in which the risk of exposure to BTV varied among specific regions, with risk increasing from north to south.

To build a prediction model of BTV transmission that can be used for regionalization, risk zones should be defined on the basis of more variables than just minimum temperature in the month of January. In Europe, minimum temperature during the coldest month, maximum temperature during the warmest month, and number of months with a mean temperature > 12.5°C best predicted the distribution of the principal BTV vector, *Culicoides imicola*.[22,23] Additional variables, such as rainfall, soil moisture, land cover, and other spatial and environmental risk factors, can be evaluated for their use in assigning predictive zones of risk. Also, a continuous gradient of risk, perhaps along latitude or longitude coordinates, would be more useful than discreet arbitrarily designated zones. Isopleth risk maps could be developed by use of geostatistic interpolation procedures, such as kriging.

A country (or zone) can also achieve BTV-free status when there is no evidence that competent BTV vectors are active.[4] Investigators have qualitatively described the distribution of *C sonorensis* within the United States.[19]

The described range of *C sonorensis* extends through southern Illinois. Results of the study reported here are consistent with that determination. However, more data on the distribution and abundance of *Culicoides* spp are needed to understand epidemiologic aspects of BTV in the lower Midwest and to develop an adequate prediction model. Also, an understanding of the temporal distribution of *Culicoides* activity would help define limits of the transmission season for the purpose of establishing a seasonally BTV-free zone.

The US National Animal Identification System was introduced in 2004 by the USDA. This program is currently voluntary and consists of 3 main components (premises identification, individual animal identification, and an animal-tracking system). Premises identification ties a livestock facility to a set of global positioning system coordinates. As of January 2007, < 350,000 US livestock premises had been registered.[33] The number of voluntary registrations has been disappointing. Currently, individual animal identification is voluntary. Mandatory individual identification is scheduled to begin in January 2009. Much of the reluctance by livestock producers to enroll in the voluntary components of this program may be attributable to a perceived lack of value from a production perspective. Regionalization of regulation and marketing of livestock diseases (such as BTV) could be coupled with the National Animal Identification System to determine risk potential. Cattle producers who could document, through the identification system, that their cattle originated and were raised in a BTV-free zone would have an advantage if this disease were considered on a regionalized basis rather than on the basis of arbitrary state borders. Providing livestock producers enrolled in the National Animal Identification System with a marketing advantage may encourage increased voluntary participation in the program.

The study reported here provided an estimate of BTV seroprevalence and described the geographic and temporal distribution of BTV in cattle in Illinois and western Indiana in greater detail than has been reported for this region. The random effect (herd) that was included in the logistic regression model explained 31.2% of the variation in BTV seropositivity, which implied that almost one third of the variability could be explained by interherd differences that were not explicitly included in the model. Because of this fact, the use of this model to predict the risk of seroprevalence in individual cattle is likely to be limited. Because the primary objective of the study was to develop a gradient for BTV risk, the model is useful in delineating differences in BTV risk among the zones. Use of only herd location and 1 climatic variable (mean minimum temperature in January) resulted in a zone with a seroprevalence of 0% within the study area. These data provide the starting point for regionalization of BTV transmission in this transition zone of Illinois and western Indiana. However, additional studies are needed to allow development of adequate risk models for use in developing rational trade policies. Future research should include analysis of climatic and environmental risk factors, vector distribution, and EHDV epidemiologic factors.

a. Trimble GeoExplorer, Trimble Navigation Ltd, Sunnyvale, Calif.
b. Bluetongue virus antibody test kit, cELISA, VMRD Inc, Pullman, Wash.
c. Stata, version 8.0, StataCorp LP, College Station, Tex.
d. SPSS, version 13.0 for Windows, SPSS Inc, Chicago, Ill.

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