# Regional risk of exporting cattle seropositive for bluetongue virus from the United States

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**Objective**—To create a stochastic model to quantify the risk that shipments of cattle from regions within the United States would contain animals seropositive for bluetongue virus and to determine shipment-level accuracy of serologic testing by use of a competitive ELISA (c-ELISA).

**Sample Population**—19,216 shipments containing 528,918 cattle and calves.

**Procedure**—Data were obtained on number of animals and state of origin of cattle in export shipments originating within the United States between January 1994 and March 2002. Probability distributions for size of export shipments were determined for all states within the United States, and distributions for agar gel immunodiffusion and c-ELISA accuracy (sensitivity and specificity) were determined from expert opinion and review of the literature. The model simulated selection of a shipment and then determined the probability that a threshold number or percentage of cattle within that shipment would have a positive c-ELISA result. Shipment-level sensitivity, specificity, positive-predictive value, and negative-predictive value were calculated.

**Results**—Substantial differences were evident in the regional probability of a shipment being declared positive, with shipments from northeastern states having the lowest probability and shipments from southwestern states having the highest probability. The c-ELISA had variable predictive values at the shipment level, depending on the threshold used and the prevalence of antibody-positive cattle within the region.

**Conclusions and Clinical Relevance**—Results from this study will aid importers in making scientifically based decisions regarding risk of importing antibody-positive cattle. (*Am J Vet Res* 2003;64:520–529)

Historically, major restrictions have been placed on the international movement of live animals and germ plasm from countries that contain **bluetongue virus (BTV)**-infected animals because of the potential of transmitting the virus to BTV-free regions of the world or of introducing exotic serotypes or genotypes of BTV into new areas. Bluetongue viruses are distributed throughout tropical and temperate regions of the world (approx between the latitudes of 40°N and 35°S).[1] **Bluetongue (BT)** is among the most economically important arthropod-borne animal diseases in the United States.[1] For example, the United States exported live nonavian animals worth more than $727 million in 2000. Of this total, $271 million (37%) was exports of cattle and calves.[2] However, the United States livestock industry has an annual loss of approximately $144 million (adjusted on the basis of the consumer price index) because of an inability to trade with BTV-free countries, especially those in the European Union.[1] In 1998, 66 countries imposed 159 BT-based import measures on US ruminants and their products.[3] Of these, there were 106 protocols for live animals, 27 for semen from ruminants, and 26 for embryos or ova from ruminants. Most import protocols require serologic tests to document lack of recent exposure to BTV.[3]

Current proposals for disease regionalization within a country[4] indicate that animals from regions of low or zero prevalence of BTV would not have to be tested for exposure to the agent prior to export. A region-based estimation of the distribution and prevalence of BTV would allow the regional risk of BTV to be quantified. Regions within the United States with low prevalence of BTV infection would potentially pose little risk for export of BTV-infected shipments of animals; consequently, these regions would be able to export animals without testing for BTV. Thus, the economic burden of BTV restrictions on the livestock industry in these regions would be dramatically reduced.

The objective of the study reported here was to determine the regional risk of exporting shipments of cattle sero-positive for BTV from the United States. To accomplish this, we simulated results of serologic testing for BTV at the shipment level to characterize cattle shipments exported from various regions within the United States. When determining the BTV status of a shipment, serologic status of each animal was only 1 component of the serologic status of that shipment. At the shipment level, test **sensitivity (SE)** and **specificity (SP)**, number of animals tested, prevalence of infection, and number or percentage of positive results used to classify the group as positive (ie, cutoff value) determined interpretation of the test results.[5] We assumed that the cattle were tested at the import destination and returned to the exporter when at least a minimum predetermined cutoff number or percentage of animals in a shipment had detectable antibodies to BTV. If regions of low antibody prevalence can provide reasonable assurance to importing countries of the negative status of cattle shipments, export requirements may be eased, thereby reducing expense to exporters. It should be

Received October 18, 2002.
Accepted January 20, 2003.
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Supported by the USDA National Research Initiative Competitive Grants Program (grants Nos. 97-35204-4772 and 2001-35204-10153).
The authors thank Dr. N. James MacLachlan for technical assistance.
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mentioned that detection of antibodies is not the same as detection of virus and that importing seropositive animals does not equate to importing viremic animals. Consequently, importing antibody-positive animals that are not viremic will not pose a biological risk of importing BTV; viremia is essential for transmission of BTV, and duration of viremia in an animal species has a direct relation to the importance of that species in the epidemiologic characterization of BT.

## Materials and Methods

**Sample population**—Records of cattle and calves exported from the United States during the period of January 1994 to March 2002 were obtained from the USDA. Cattle exported for immediate slaughter were excluded from the analysis, because they would have had minimal contact with animals and insects in the importing country.

**Record analysis**—Records were analyzed to derive distributions for the number of animals per shipment exported from each state. **Cumulative distribution functions (CDFs)** were fitted to the data by use of a probability distribution-fitting software package.[a] Parameters were derived by use of maximum-likelihood estimation, and fit of the data was optimized by use of the Levenberg-Marquardt method.[6] Fitted distributions were evaluated quantitatively by use of $\chi^2$, Kolmogorov-Smirnov, and Anderson-Darling goodness-of-fit test statistics. Fit of the distributions was also evaluated by visual inspection. A distribution for shipment size was obtained separately for each state.

**Evaluation of serologic tests**—Two diagnostic tests were considered in the study: **agar-gel immunodiffusion (AGID)** and the **competitive ELISA (c-ELISA)**. The AGID test was extensively used until the mid-1990s; hence, national estimates of BTV-antibody prevalence obtained from the literature[7,8] were based on this method. By use of the number of test-positive animals and the total number of animals tested in those studies, **apparent prevalence (AP)** within each state was modeled as a binomial distribution.

To convert the observed AP for each state to an estimate of **true prevalence (TP)**, estimates for the SE (defined as the conditional probability of a positive test result given that there were antibodies [ie, true-positive test result]) and SP (defined as the conditional probability of a negative test result given that there was a lack of antibodies [ie, true-negative test result]) of the AGID test were required. The beta distribution, bounded by 0 and 1, is a 2-parameter distribution that is often used to provide a suitable model for SE and SP.[9] We asked an expert[b] to provide us with an opinion about the most likely value for SE and SP (which was equated to the mode of the beta [a, b] distribution), as well as the 5th percentile of the possible values (eg, 95% certain that the parameter exceeded this value). Answers to these questions were used to obtain the appropriate beta (a, b) distribution.[10] We also searched the literature for reported point estimates and 5th-percentile values for SE and SP.[11] Beta distributions chosen were as follows: SE (a = 99.70, b = 6.19) and SP (a = 80.47, b = 3.04). These distributions have mean values (determined by use of the equation a/[a + b]) of 94.2 and 96.4%, respectively. Probability of a positive test result (ie, AP) can be calculated by use of the following equation:

$$AP = (SE \times TP) + [1 - SP] \times [1 - TP]$$

Solving this formula for TP and sampling from distributions for SE and SP (**Fig 1**), we converted observed AP to estimated TP as determined by use of the following equation[12]:

$$TP = (AP + [SP - 1])/(SE + [SP - 1])$$

The current standard BTV antibody test is the c-ELISA. A beta distribution with parameters a = 88.28 and b = 1.88 was used for SE and SP, as determined on the basis of expert opinion[b] and results from a literature search[13] (Fig 1). Mean value of the distribution was 97.9%.

**Possible shipment testing results**—A shipment of cattle was considered truly positive when it contained 1 or more antibody-positive animals. Shipments were declared positive when the total number of true-positive and false-positive test results exceeded a cutoff value. A shipment of cattle was con-

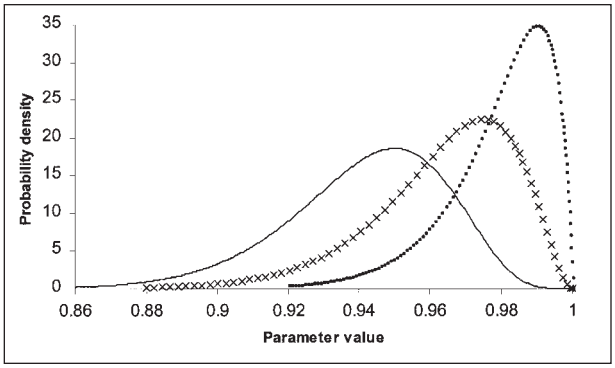


Figure 1—Probability distributions of an agar gel immunodiffusion (AGID) test and a competitive ELISA (c-ELISA) for the detection of antibodies against bluetongue virus in cattle. For the AGID test, distribution for sensitivity was (beta [99.7, 6.2])(solid line) and specificity was (beta [80.5, 3.0])(crosses), whereas for the c-ELISA, distributions for sensitivity and specificity were each (beta [88.3, 1.9])(dots).

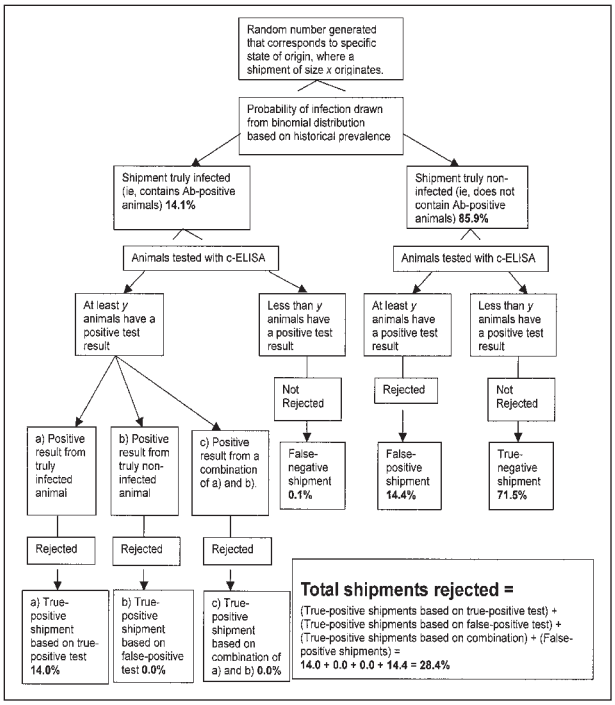


Figure 2—Flow chart of the possible test results for detection of bluetongue virus in cattle in export shipments from the United States. Percentages in bold are results for cattle from low-prevalence states and use of a cutoff value of 1 antibody-positive animal/shipment. Ab = Antibody. y = Cutoff value (ie, required number of cattle with positive test results [1 antibody-positive animal/shipment, 2 antibody-positive animals/shipment, or 2% of cattle in shipment were seropositive] necessary for the shipment to declared positive.

sidered truly negative when it contained no antibody-positive animals. Shipments were declared negative when less than the cutoff number or percentage of cattle had positive test results, regardless of whether the shipment did or did not contain the cutoff number or percentage of antibody-positive cattle. Possible test results were determined (**Fig 2**).

**Initial designation of US regions**—The United States was initially categorized into 2 regions on the basis of the current USDA classification for prevalence of BT. High-prevalence states were Alabama, Arizona, Arkansas, California, Florida, Georgia, Illinois, Kentucky, Louisiana, Mississippi, Missouri, Nevada, New Mexico, North Carolina, Oklahoma, Oregon, South Carolina, Tennessee, Texas, and Virginia, and low-prevalence states were Colorado, Connecticut, Delaware, Idaho, Indiana, Iowa, Kansas, Maine, Maryland, Massachusetts, Michigan, Minnesota, Montana, Nebraska, New Hampshire, New Jersey, New York, North Dakota, Ohio, Pennsylvania, Rhode Island, South Dakota, Utah, Vermont, Washington, West Virginia, Wisconsin, and Wyoming. Two states (Alaska and Hawaii) were considered by the USDA to be free of BT; therefore, they were not included for this model. The model was designed to evaluate scenarios involving export of cattle from the United States to other countries. Separate models were generated for high- and low-prevalence regions.

**Use of the model**—A uniformly distributed random number was generated, and this number corresponded to the state of origin of the shipment, based on the proportion of cattle exported by the state from within the region. Number of cattle in the shipment was determined by sampling from the distribution for shipment size, and a value for AP for the shipment was obtained by sampling from the appropriate binomial distribution. Shipment size and AP distributions were specific for each state. The SE and SP of the AGID diagnostic test for the shipment were determined by sampling from their respective beta distributions, and these values were combined with AP to estimate shipment TP by use of the following equation[12]:

$$TP = (AP + [SP - 1])/(SE + [SP - 1])$$

The estimated number of truly seropositive cattle in the population (true positives) from which the sample was drawn was calculated by multiplying population size (for convenience, a population of 10,000 cattle was used for this calculation) by estimated TP. The hypergeometric CDF was

Table 1—Exports of cattle and calves, best-fitting distributions used to describe shipment sizes, and apparent bluetongue virus antibody prevalence in shipments from the north-central region of the United States, January 1994 to March 2002

| State | Shipments | | Cattle | | Shipment distribution | Apparent prevalence | Sample size* |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | |
| Illinois | 344 | 7.6 | 8,147 | 7.5 | Lognormal (5.42, 10.46) | 0.075 | 612 |
| Iowa | 678 | 15.1 | 16,104 | 14.9 | Lognormal (26.01, 109.12) | 0.077 | 1,217 |
| Minnesota | 844 | 18.8 | 23,160 | 21.4 | Weibull (0.59, 17.0) | 0.058 | 5,731 |
| Nebraska | 233 | 5.2 | 3,402 | 3.1 | Lognormal (14.45, 39.78) | 0.504 | 552 |
| North Dakota | 636 | 14.1 | 5,895 | 5.4 | Lognormal (5.62, 11.30) | 0.017 | 4,655 |
| South Dakota | 439 | 9.8 | 5,872 | 5.4 | Lognormal (12.03, 32.86) | 0.128 | 920 |
| Wisconsin | 1,323 | 29.4 | 45,803 | 42.3 | Weibull (0.56, 19.19) | 0.006 | 6,933 |

*Number of cattle from which samples were obtained for use in calculating apparent prevalence in other studies.[7,8]

Table 2—Results of model simulations for the percentage of export shipments from the United States (classified into high- and low-prevalence regions) that would contain cattle seropositive for bluetongue virus and the percentage of shipments rejected by the importing country on the basis of 3 cutoff values

| Variable | Low-prevalence region | High-prevalence region |
|---|---|---|
| Shipments with ≥ 1 antibody-positive animal (%) | 14.1 (12.6, 15.8) | 82.8 (80.8, 84.7) |
| Shipments rejected on the basis of a cutoff value of 1 antibody-positive animal/shipment (%) | 28.4 (26.3, 30.5) | 83.6 (81.7, 85.5) |
| Shipments rejected on the basis of a cutoff value of 2 antibody-positive animals/shipment (%) | 14.3 (12.7, 16.0) | 68.1 (65.6, 70.5) |
| Shipments rejected on the basis of a cutoff value of 2% of cattle in shipment were seropositive (%) | 26.1 (24.1, 28.1) | 83.6 (81.6, 85.4) |
| Shipments rejected because of true test-positive results and a cutoff value of 1 antibody-positive animal/shipment (%) | 14.0 (12.3, 15.7) | 82.5 (80.5, 84.4) |
| Shipments rejected because of false-positive results and a cutoff value of 1 antibody-positive animal/shipment (%) | 14.4 (12.8, 16.0) | 1.1 (0.6, 1.7) |
| False-negative shipments on the basis of a cutoff value of 1 antibody-positive animal/shipment (%) | 0.1 (0.0, 0.2) | 0.3 (0.1, 0.6) |
| Simulated median shipment size (number of cattle) | 5 (4, 5) | 10 (9, 11) |
| Simulated mean shipment size (number of cattle) | 21.4 (17.8, 26.5) | 24.1 (22.0, 26.3) |
| Simulated mean within shipment TP (%) | 4.4 (3.8, 5.1) | 35.4 (34.1, 36.7) |
| Simulated mean within shipment AP (%) | 6.3 (5.6, 7.1) | 36.0 (34.7, 37.3) |
| Regional AP determined from other reports[7,8] (%) | 5.9 | 35.8 |

Values in parentheses are 95% prediction intervals. TP = True prevalence. AP = Apparent prevalence.

calculated for the given variables of shipment size, population size, and estimated number of truly seropositive cattle in the population. A randomly selected value from the hypergeometric CDF was used to provide the number of truly seropositive cattle in the shipment. Number of truly seropositive cattle that had a positive test result (ie, true test-positive cattle) in the shipment was simulated by sampling a binomial distribution from the truly seropositive cattle as determined on the basis of the distribution of SE values for the c-ELISA. The difference between the number of truly seropositive cattle and the number of true test-positive cattle (false test-negative results) was then calculated. Number of truly seronegative cattle that had a positive test result (false test-positive cattle) in the shipment was generated on the basis of a random binomial sample from the truly seronegative cattle and the false-positive proportion (ie, 1 – SP). Number of true test-negative cattle was the difference between the total number of truly seronegative cattle and the number of false test-positive cattle. Number of true test-positive reactors was combined with the number of false test-positive reactors to determine the apparent prevalence of seropositivity within the shipment, whereas the number of true seropositive cattle was used to calculate the true prevalence of seropositivity within the shipment.

**Classification of shipments**—A shipment was declared positive by use of 3 cutoff values (1 animal/shipment, 2 animals/shipment, or 2% of the cattle in a shipment). Traditionally, 2% of the cattle in a shipment has often been used as the cutoff value, because SP of the AGID test is < 100%, and a 2% proportion for false-positive results has been considered acceptable. The proportion of shipments declared positive was graphed as a function of shipment size for the 1-animal and 2-animal cutoff values. A fitted regression line was generated by use of the **natural logarithm (ln)** of shipment size and $(\ln \text{shipment size})^2$ as the predictor variables.

We also determined whether a shipment was correctly identified as positive (true positive), falsely identified as positive (false positive), correctly identified as negative (true negative), or falsely identified as negative (false negative). For the true-positive rejected shipments, we determined whether they were rejected because of truly seropositive cattle that had positive test results (reflecting test SE), seronegative cattle in a true-positive shipment that falsely had positive results (reflecting lack of test SP), or a combination of the 2 (Fig 2). These data were summarized by calculating the SE and SP at the shipment level. Shipment SE was defined as the probability that a shipment was declared positive, given there were at least the cutoff value of truly seropositive cattle in the shipment. Shipment SP was defined as the probability that a shipment was declared negative, given that there were less than the cutoff value of truly seropositive cattle in the shipment. **Positive-predictive value (PPV)**, defined as the probability that a shipment that was declared positive was truly positive, was calculated by dividing the proportion of true positive shipments that were declared positive by the total proportion of shipments that were declared positive. **Negative-predictive value (NPV)**, defined as the probability that a shipment that was declared negative was truly negative, was calculated by dividing the proportion of true negative shipments by the total proportion of shipments that were declared negative. Number of iterations per simulation performed was equal to the average number of shipments from a region in a year. We ran 1,000 simulations for the high-prevalence region and 1,000 simulations for the low-prevalence region by use of Monte-Carlo sampling in a computer program.[c] Results were summarized and graphed.

**Subsequent designation of US regions**—After we analyzed the data by use of the preceding models, we then categorized the United States into 6 smaller regions on the basis of geography. These categories roughly paralleled those of the USDA in that low-prevalence states are generally in the north, whereas high-prevalence states are generally in the south. Categorizing the country into smaller regions allowed for greater delineation of risk among regions. The regions were designated as northeast (Connecticut, Delaware, Indiana, Maine, Maryland, Massachusetts, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Vermont, Virginia, and West Virginia), north-central (Illinois, Iowa, Minnesota, Nebraska, North Dakota, South Dakota, and Wisconsin), northwest (Idaho, Montana, Oregon, Washington, and Wyoming), southeast (Alabama, Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, and Tennessee), south-central (Arkansas, Colorado, Kansas, Louisiana, Missouri, New Mexico, Oklahoma, and Texas), and southwest (Arizona, California, Nevada, and Utah). A total of 1,000 simulations, with each simulation comprising a number of iterations equal to the average

Table 3—Shipment sensitivity, specificity, positive-predictive value (PPV), and negative- predictive value (NPV) determined by the use of 3 cutoff values to identify a shipment from the United States (classified into high- and low-prevalence regions) as containing cattle seropositive for bluetongue virus

| Cutoff value | Test attribute | Low-prevalence region | High-prevalence region |
|---|---|---|---|
| 1 antibody-positive animal/shipment | Sensitivity | 0.99 (0.98, 1.00) | 1.00 (0.99, 1.00) |
| | Specificity | 0.83 (0.81, 0.85) | 0.94 (0.90, 0.96) |
| | PPV | 0.49 (0.45, 0.54) | 0.99 (0.98, 0.99) |
| | NPV | 1.00 (1.00, 1.00) | 0.98 (0.96, 0.99) |
| 2 antibody-positive animals/shipment | Sensitivity | 0.66 (0.60, 0.72) | 0.82 (0.80, 0.84) |
| | Specificity | 0.94 (0.93, 0.95) | 0.99 (0.98, 1.00) |
| | PPV | 0.65 (0.59, 0.70) | 1.00 (1.00, 1.00) |
| | NPV | 0.94 (0.93, 0.96) | 0.54 (0.49, 0.58) |
| 2% of cattle in shipment were seropositive | Sensitivity | 0.98 (0.95, 0.99) | 1.00 (0.99, 1.00) |
| | Specificity | 0.86 (0.84, 0.87) | 0.94 (0.91, 0.97) |
| | PPV | 0.53 (0.48, 0.57) | 0.99 (0.98, 0.99) |
| | NPV | 1.00 (0.99, 1.00) | 0.98 (0.96 0.99) |

Values in parentheses are 95% prediction intervals.

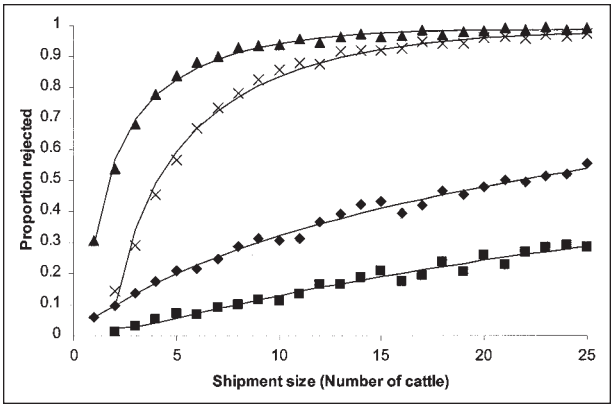


Figure 3—Simulation results and fitted regression lines of the proportion of shipments rejected because of bluetongue-infected cattle on the basis of the number of cattle in the shipment (shipment size) and cutoff value (1 or 2 antibody-positive animals/shipment) for high- and low-prevalence regions of the United States. The 4 scenarios depicted are high-prevalence region and a cutoff value of 1 antibody-positive animal/shipment (triangle), high-prevalence region and a cutoff value of 2 antibody-positive animals/shipment (cross), low-prevalence region and a cutoff value of 1 antibody-positive animal/shipment (diamond), and low-prevalence region and a cutoff value of 2 antibody-positive animals/shipment (square).

number of shipments in each region per year, were run separately for each region, and results were summarized and graphed as for the preceding 2-region simulations.

## Results

A total of 19,216 shipments containing 528,918 cattle (mean shipment size, 27.5 cattle; median, 4 cattle) were sent to 79 countries during the 8.25-year period of the study. Mean number of shipments per year was 2,329, and mean number of cattle exported per year was 64,111. Mexico and Canada received the most shipments (42.5 and 41.7%, respectively) and cattle (51.1 and 31.5%, respectively). Colombia (2.6%) and Brazil (2.1%) were the only other countries that received more than 2% of total shipments. Texas was the leading exporter, accounting for 21.7% of all shipments and 11.9% of all cattle, followed by Montana (7.7% of shipments, 8.0% of cattle), Arizona (7.5% of shipments, 11.2% of cattle), and Wisconsin (6.9% of shipments, 8.7% of cattle). None of the other states accounted for more than 5% of shipments or 7% of cattle.

The AP of antibodies for each state used in the models ranged from 0.5% (Massachusetts) to 81% (Nevada).[7,8] Data on total number of cattle exported per state were used to generate distributions for shipment sizes (**Table 1**).

Low-prevalence states accounted for 10,697 shipments (1,297 shipments/y), whereas high-prevalence states had 8,500 shipments (1,030 shipments/y) during this time period. Data for Alaska and Hawaii were not included in these totals.

Results of the model revealed that 14.1% of simulated shipments from low-prevalence states had at least 1 antibody-positive animal, whereas 82.8% of shipments from high-prevalence states had at least 1 antibody-positive animal (**Table 2**). This yielded 28.4 and 83.6% of shipments being rejected, respectively, on the basis of a cutoff value of 1 animal with positive results/shipment. Few shipments had false-negative results at a cutoff value of 1 animal/shipment (0.1 and 0.3% for low- and high-prevalence states, respectively). Mean within-shipment APs were slightly higher than regional estimates for apparent antibody preva-

Table 4—Results of model simulations for the percentage of export shipments from the United States (classified into 6 geographic regions) that would contain cattle seropositive for bluetongue virus and the percentage of shipments rejected by the importing country on the basis of 3 cutoff values

| | Northeast | North-central | Northwest | Southeast | South-central | Southwest |
|---|---|---|---|---|---|---|
| Shipments with ≥ 1 antibody-positive animal (%) | 2.4 (1.3, 3.6) | 15.7 (13.2, 18.2) | 20.3 (16.7, 23.9) | 77.0 (70.6, 83.3) | 84.8 (82.4, 87.0) | 96.4 (94.6, 98.1) |
| Shipments rejected on the basis of a cutoff value of 1 antibody-positive animal/shipment (%) | 15.5 (12.8, 18.3) | 32.4 (29.2, 35.6) | 33.1 (28.8, 37.3) | 78.7 (72.5, 85.3) | 85.1 (82.8, 87.5) | 96.4 (94.6, 98.1) |
| Shipments rejected on the basis of a cutoff value of 2 antibody-positive animals/shipment (%) | 5.0 (3.4, 6.6) | 16.3 (13.8, 18.9) | 17.6 (14.1, 21.2) | 61.6 (53.9, 69.6) | 65.3 (62.1, 68.5) | 91.6 (88.8, 94.2) |
| Shipments rejected on the basis of a cutoff value of 2% of cattle in shipment were seropositive (%) | 14.0 (11.5, 16.8) | 29.4 (26.2, 32.8) | 30.5 (26.5, 34.6) | 78.6 (72.5, 85.3) | 85.1 (82.8, 87.5) | 96.4 (94.6, 98.1) |
| Shipments rejected because of true test-positive results and a cutoff value of 1 antibody-positive animal/shipment (%) | 2.4 (1.3, 3.6) | 15.6 (13.0, 18.0) | 20.1 (16.3, 23.9) | 76.7 (69.6, 83.3) | 84.3 (82.0, 86.7) | 96.3 (94.6, 98.1) |
| Shipments rejected because of false-positive results and a cutoff value of 1 antibody-positive animal/shipment (%) | 13.00 (10.4, 15.5) | 16.8 (14.3, 19.4) | 13.0 (9.8, 16.3) | 1.9 (0.0, 3.9) | 0.8 (0.3, 1.4) | 0.1 (0.0, 0.4) |
| False-negative shipments on the basis of a cutoff value of 1 antibody-positive animal/shipment (%) | 0.02 (0.0, 0.2) | 0.13 (0.0, 0.37) | 0.16 (0.0, 0.65) | 0.32 (0.0, 0.98) | 0.41 (0.0, 0.93) | 0.11 (0.0, 0.39) |
| Simulated median shipment size (number of cattle) | 3 (3, 4) | 6 (5, 7) | 6 (5, 7) | 13 (9, 18) | 7 (6, 8) | 35 (31, 38) |
| Simulated mean shipment size (number of cattle) | 11.4 (9.3, 13.9) | 21.9 (18.2, 26.0) | 42.4 (23.2, 71.1) | 33.4 (25.5, 43.0) | 16.1 (14.2, 18.4) | 43.4 (38.8, 49.2) |
| Simulated mean within shipment TP (%) | 0.3 (0.1, 0.6) | 4.7 (3.6, 5.8) | 3.9 (2.7, 5.1) | 20.2 (16.8, 23.7) | 38.8 (37.3, 40.5) | 43.4 (41.8, 45.0) |
| Simulated mean within shipment AP (%) | 2.4 (1.6, 3.1) | 6.5 (5.4, 7.8) | 5.8 (4.4, 7.3) | 21.4 (18.0, 25.1) | 39.3 (37.7, 41.0) | 43.7 (42.0, 45.3) |
| Regional AP determined from other reports[7,8] (%) | 1.4 | 6.1 | 6.7 | 22.0 | 38.8 | 42.9 |

Values in parentheses are 95% prediction intervals.

lence calculated from other reports[7,8] (6.3 vs 5.9% for low-prevalence states and 36.0 vs. 35.8% for high-prevalence states); however, the 95% prediction interval in our study included the values reported in those studies. For low-prevalence states, simulated TP values (4.4%) were significantly lower than simulated AP values (6.3%). For high-prevalence states, simulated TP values (35.4%) were not significantly different from simulated AP values (36.0%). Simulated median and mean shipment sizes were smaller for low-prevalence states.

Shipment SE at a cutoff value of 1 animal with positive results/shipment or at 2% of cattle in the shipment that had positive results was high for low-prevalence and high-prevalence states (**Table 3**). When a cutoff value of 2 animals with positive results/shipment was used, the decrease in shipment SE was more dramatic for low-prevalence states. Shipment SP was higher for high-prevalence states for all cutoff values. The PPV was relatively low for low-prevalence states and high for high-prevalence states for all cutoff values. The NPV was high except for high-prevalence states when using a cutoff value of 2 animals with positive test results/shipment.

The proportion of shipments rejected for shipments of up to 25 cattle, based on 1 or 2 animals with positive results/shipment as the cutoff value for classifying a shipment as positive, was graphed, and a regression line was fitted (**Fig 3**). The $R^2$ values for the regression equations ranged from 0.97 to 0.99. Using results of the regression, shipments of 4 (overall median number of cattle per shipment) and 10 cattle, the predicted probabilities of 1 or more test-positive cattle in a shipment were 77.5 and 94.1% for high-prevalence states and 16.9 and 32.1% for low-prevalence states, respectively. When the threshold for declaring a shipment positive was raised to 2 positive test results, these probabilities decreased to 49.0 and 83.5% for high-prevalence states and 4.2 and 12.9% for low-prevalence states, respectively. Shipments consisting of 1 animal from a high- or low-prevalence state were predicted to have a probability of 29.0 and 6.0%, respectively, of being test positive on the basis of the 2-category designation. The curve for the cutoff value of 2% of the cattle with positive results in a shipment was identical to the cutoff value for 1 animal with positive results/shipment for shipments of < 25 cattle.

In the model in which the United States was divided into 6 regions, the south-central region accounted for 5,338 shipments (647 shipments/y), followed by the north-central (4,497 shipments; 545 shipments/y), northeast (3,877 shipments; 470 shipments/y), northwest (2,528 shipments; 306 shipments/y), southwest (2,132 shipments; 258 shipments/y), and southeast (844 shipments; 102 shipments/y). Only 2.4% of shipments from northeastern states had 1 or more antibody-positive cattle, whereas 96.4% of shipments from southwestern states had 1 or more antibody-positive cattle (**Table 4**). Other regions had intermediate values; however, northern regions were always less likely to have shipments that contained antibody-positive cattle than were southern regions. Regional AP, as calcu-

Table 5—Shipment sensitivity, specificity, PPV, and NPV determined by the use of 3 cutoff values to identify a shipment from the United States (classified into 6 geographic regions) as containing cattle seropositive for bluetongue virus

| Cutoff value | Test attribute | Northeast | North-central | Northwest | Southeast | South-central | Southwest |
|---|---|---|---|---|---|---|---|
| 1 antibody-positive animal/shipment | Sensitivity | 0.99 (0.92, 1.00) | 0.99 (0.98, 1.00) | 0.99 (0.97, 1.00) | 1.00 (0.99, 1.00) | 1.00 (0.99, 1.00) | 1.00 (1.00, 1.00) |
| | Specificity | 0.87 (0.84, 0.89) | 0.80 (0.77, 0.83) | 0.84 (0.80, 0.88) | 0.91 (0.81, 1.00) | 0.91 (0.81, 1.00) | 0.97 (0.83, 1.00) |
| | PPV | 0.16 (0.90, 0.23) | 0.48 (0.42, 0.54) | 0.61 (0.52, 0.69) | 0.97 (0.94, 1.00) | 0.97 (0.94, 1.00) | 1.00 (1.00, 1.00) |
| | NPV | 1.00 (1.00, 1.00) | 1.00 (0.99, 1.00) | 1.00 (0.99, 1.00) | 0.99 (0.94, 1.00) | 0.99 (0.94, 1.00) | 0.97 (0.83, 1.00) |
| 2 antibody-positive animal/shipment | Sensitivity | 0.54 (0.29, 0.82) | 0.61 (0.53, 0.70) | 0.65 (0.54, 0.75) | 0.80 (0.72, 0.87) | 0.80 (0.72, 0.87) | 0.95 (0.93, 0.97) |
| | Specificity | 0.96 (0.95, 0.98) | 0.92 (0.90, 0.94) | 0.94 (0.92, 0.97) | 0.99 (0.94, 1.00) | 0.99 (0.94, 1.00) | 1.00 (1.00, 1.00) |
| | PPV | 0.27 (0.11, 0.44) | 0.59 (0.50, 0.68) | 0.74 (0.64, 0.84) | 1.00 (0.98, 1.00) | 1.00 (0.98, 1.00) | 1.00 (1.00, 1.00) |
| | NPV | 0.99 (0.98, 1.00) | 0.93 (0.91, 0.95) | 0.91 (0.88, 0.94) | 0.60 (0.46, 0.73) | 0.60 (0.46, 0.73) | 0.43 (0.26, 0.61) |
| 2% of cattle in shipment were seropositive | Sensitivity | 0.95 (0.93, 1.00) | 0.99 (0.96, 1.00) | 0.95 (0.91, 1.00) | 0.99 (0.98, 1.00) | 0.99 (0.98, 1.00) | 1.00 (1.00, 1.00) |
| | Specificity | 0.88 (0.86, 0.90) | 0.84 (0.81, 0.86) | 0.86 (0.82, 0.90) | 0.91 (0.82, 1.00) | 0.91 (0.82, 1.00) | 0.97 (0.83, 1.00) |
| | PPV | 0.16 (0.09, 0.24) | 0.53 (0.46, 0.59) | 0.63 (0.55, 0.71) | 0.97 (0.95, 1.00) | 0.97 (0.95, 1.00) | 1.00 (1.00, 1.00) |
| | NPV | 1.00 (1.00, 1.00) | 1.00 (0.99, 1.00) | 0.99 (0.97, 1.00) | 0.98 (0.92, 1.00) | 0.98 (0.92, 1.00) | 0.97 (0.83, 1.00) |

Values in parentheses are 95% prediction intervals.

lated from data reported elsewhere,[7,8] was in general agreement with the simulated mean within-shipment AP. For all regions, values reported elsewhere were similar to the simulated APs, and values reported elsewhere were within the 95% prediction intervals determined here, except for values for Nebraska. Simulated TP was lower than simulated AP for several states (Nebraska, 87.5%; North Carolina, 27.7%; and South Carolina, 1.3%) and several regions (northwest, 32.8%; southeast, 5.6%; and southwest, 0.7%). Mean shipment sizes were larger in western states, compared with values for central or eastern regions in both the north and south sections of the country. Shipments from the northeast contained the fewest cattle.

Performance characteristics for the model using 6 US regions had high shipment SE at a cutoff value of 1 animal with positive results/shipment or 2% of cattle in the shipment that had positive results (**Table 5**). When a cutoff value of 2 animals with positive results/shipment was used, the decrease in shipment SE was more dramatic for northern regions. Shipment SP was higher for southern regions for all cutoff values. The PPV was relatively low for all northern regions and high for all southern regions for all cutoff values. The NPV was high except for southern regions when we used a cutoff value of 2 animals with positive test results/shipment.

The predicted proportion of shipments rejected, determined for shipment size and based on 1 animal with positive results/shipment as the cutoff value for classifying a shipment as positive, for the data set in which the United States was divided into 6 regions was calculated (**Fig 4**). The $R^2$ values for the regression equations ranged from 0.97 to 1.0. Using a cutoff value of 1 animal with positive test results/shipment to cause rejection, shipments of 4 and 10 cattle had a predicted probability of rejection ranging from 8.0 and 20.8%, respectively, for the northeast to 88.3 and 99.7%, respectively, for the southwest. Shipments consisting of 1 animal had predicted probabilities of having a positive test result that ranged from 1.5% for the northeast to 48.0% for the southwest.

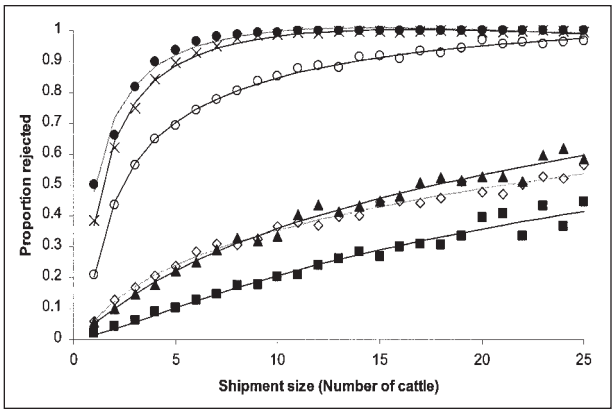


Figure 4—Simulation results and fitted regression lines of the proportion of shipments rejected because of bluetongue-infected cattle on the basis of the number of cattle in the shipment (shipment size) and a cutoff value of 1 antibody-positive animal/shipment for 6 geographic regions of the United States. The 6 regions depicted are southwest (solid circle), south-central (cross), southeast (open circle), northwest (solid triangle), north-central (open diamond), and northeast (solid square).

## Discussion

Exports of cattle are a major contributor to the total value of live animals shipped from the United States, and if restrictions were eased, there would undoubtedly be an increase in export of live cattle. Not surprisingly, Mexico and Canada received the majority of shipments, and states that border Mexico and Canada contributed the most to exports. If BT-based export restrictions to European countries were eased, it is likely that other states would contribute more shipments. Therefore, it is important to quantify the regional risk of BTV antibody-positive cattle in shipments intended for export.

By using probability distributions to represent input parameters, variability and uncertainty about parameter estimates (such as prevalence, test SE, and test SP) can be accounted for. Monte-Carlo simulations can then propagate variability and uncertainty through the model so that it is represented in model output.[14] Also, we believe that converting AP estimates to TP estimates makes data from various populations and from the same population over time more comparable and that performing 1,000 simulations of yearly exports (based on current export activity) for each region provided a realistic representation of possible outcomes.

The models used in the study reported here quantified the probability of exporting cattle seropositive for BTV in shipments from the United States. Our results confirmed the validity of the current USDA division of low- and high-prevalence states. There were almost 6 times more shipments containing 1 or more antibody-positive cattle from high-prevalence states, compared with the shipments from low-prevalence states. Given the high probability of at least 1 animal having a positive test result in a shipment of any size, exporting from high-prevalence states without pre-export testing would not be prudent if the policy of the importing country were to be to return any shipment containing test-positive cattle to the exporter.

Furthermore, dividing the United States along geographic boundaries revealed that there were extreme differences in the regional risk of exporting antibody-positive cattle in shipments. The northeast region had the lowest probability of having a shipment that contained antibody-positive cattle (2.4%), followed by other northern regions. Exporting from northern regions without pre-export testing would be practical, especially for small shipments from states in the northeastern region. Southern regions all had a considerably higher probability of a shipment that contained antibody-positive cattle, with the southwest region having the greatest probability (96.4%). The proportion of shipments that contained cattle with false-positive results, determined by use of a cutoff value of 1 animal with positive results/shipment, was greatest for the north-central region (13.0%) and smallest for the southwest (0.1%).

Shipments with false-negative cattle were infrequent, ranging from 0.02% for the northeast to 0.4%

for the South-central shipments at a cutoff value of 1 animal with positive results/shipment. Thus, importers can be assured that shipments are unlikely to be incorrectly declared negative. Raising the cutoff value increased the proportion of shipments with false-negative cattle. This was documented by the change in NPV in high-prevalence states when the cutoff was raised from 1 seropositive animal/shipment (NPV = 0.98) to 2 seropositive animals/shipment (NPV = 0.54) that had positive results (Table 3), indicating that almost half of shipments declared negative at a cutoff of 2 animals/shipment will actually contain antibody-positive cattle. Therefore, other cutoff values were not considered, because it is unlikely that an importing country would consider relaxing such a requirement.

Shipment size had a distribution that was skewed to the right. Most export shipments were small, and mean size of shipment (27.5 cattle) was much larger than the median number of cattle per shipment (4 cattle). If restrictions were changed, it is likely that any increase in exports would continue to consist of small shipments. It is clearly in an exporter's best interests to have small shipments, if the understanding is that shipments that contain antibody-positive cattle will be returned to the exporter. Even from the region with the lowest risk (ie, northeast), 21% of shipments that consisted of 10 cattle were predicted to have 1 or more antibody-positive cattle.

Given that most shipments were of only a few cattle, the 2% cutoff value for determining a positive shipment was not generally applicable. Using this cutoff value, only shipments of > 50 cattle will have results potentially different from those obtained by use of a cutoff value of 1 antibody-positive animal/shipment. Because the probability of false-positive reactions increases as the number of cattle in a shipment increases, it is unlikely that shipments of ≥ 50 cattle would be considered for export without pre-export serologic testing.

Simulated AP values were always larger than simulated TP values, because SP of the test was imperfect. The difference between simulated AP and simulated TP was proportionately greater for regions with lower prevalence. Relative magnitude of false-positive reactions was greater when prevalence was lower.

An examination of calculated predictive values revealed the value of serologic testing. A high PPV provides assurance that shipments declared positive truly contain seropositive cattle. This attribute would be important to potential exporters. The PPV will, on average, be higher from regions with higher prevalence, because the relative proportion of false-positive test results will be smaller when prevalence is higher. A comparison of the northeast and southwest regions provides the most dramatic illustration of this relationship. At a cutoff value of 1 antibody-positive animal/shipment, only 16% of shipments from the northeast that are declared positive will truly contain seropositive cattle, whereas all shipments from the southwest that are declared positive will truly contain seropositive cattle. Conversely, a high NPV indicates that shipments declared negative have a high probability of being free of seropositive cattle. At cutoff values of 1 antibody-positive animal/shipment or 2% seropositive animals in a shipment, NPV was high (≥ 98%) for shipments from high- and low-prevalence regions. At a cutoff value of 2 antibody-positive animals/shipment, NPV was low (54%) for shipments from southern regions and relatively high (94%) for northern regions. On the basis of these results, we conclude that serologic testing with a cutoff of 1 antibody-positive animal/shipment or 2% seropositive animals in a shipment will be extremely accurate at identifying positive and negative shipments from southern regions, but that shipments declared positive from northern states are not likely to contain any seropositive cattle (ie, shipments that contain cattle with false-positive results).

A population size of 10,000 cattle was chosen by convenience for determining the number of seropositive cattle by use of the hypergeometric CDF. Although the true population of cattle within any given state is much larger, the model was insensitive to this assumption, particularly when number of cattle in a shipment was small (data not shown).

The effect of shipment size on probability of containing antibody-positive cattle was illustrated by comparing the northwest and north-central regions. Although the simulated mean AP was similar for the northwest and north-central regions (5.8 and 6.5%, respectively), the proportion of shipments with ≥ 1 antibody-positive animal was much greater for shipments from the northwest (20.3% vs 15.7%). This was attributed to the fact that the mean shipment size for the northwest region was nearly twice that of the north-central region. Thus, the larger shipments from the northwest region were more likely to contain antibody-positive cattle.

The proportion of shipments from northeastern states that was rejected was approximately one-half to one-fourth that for other northern states, depending on the cutoff value used. For this reason, we believe it is logical to create more than the current 2 divisions within the United States when considering BT export risk. It may be useful to consider smaller regions than those used in the study reported, given that prevalence among states within a specific region varied considerably. The fact that Alaska and Hawaii have been declared free of BT indicates that some states can be recognized as having a status other than low or high prevalence.

The model we used revealed that a test with high SP is required to make it feasible to export animals without the need for pre-export testing. Even though expert opinion for the mode for SP was 0.99, there were substantial numbers of shipments that contained cattle with false-positive results. For example, although the model simulated that only 14.1% of shipments from low-prevalence states contained antibody-positive cattle, 28.4% were declared positive at a cutoff value of 1 antibody-positive animal/shipment. Based on the assumption that 1 reactor is needed for the entire shipment to be rejected, it is obvious that large groups of cattle shipped in a single shipment will have a high probability of being returned to the exporter, irrespective of the true prevalence of antibody-positive cattle in the region from which they originated.

Because SP of currently available tests is not perfect, false-positive results will still cause misclassification of shipments, even in cattle from regions or states with a low prevalence of antibody-positive cattle. Import restrictions based on seropositivity of cattle in shipments will continue to be a hindrance to export of live cattle from the United States.

Although we found that many export shipments may contain antibody-positive cattle, the risk of transmitting virus to another country through export is low irrespective of serologic status, provided an adequate quarantine period is observed. The duration of viremia in cattle with BTV is finite; the probability is > 99% that viremia detectable by use of virus isolation testing will cease by 63 days after infection.[15] Although viral nucleic acid has been detected by use of a reverse transcriptase **polymerase chain reaction (PCR)** assay in blood cells for 16 to 24 weeks after infection,[16,17] calf blood samples that contained viral RNA (as determined by use of a PCR assay), but not infectious virus (as determined by use of virus isolation testing), was not infectious to sheep or *Culicoides* insects.[16] Maximal duration of viremia that was infectious to *C sonorensis* was 21 days after infection,[17] and, except for 1 sheep, only ruminants whose blood contained BTV, as determined by use of virus isolation testing, were able to infect *C sonorensis* after oral feeding. Knowledge of the duration of viremia can be used to determine appropriate quarantine periods prior to movement of animals from regions endemic for BTV to regions free of BTV. However, even when viruses are introduced into another ecosystem, they appear to die out for lack of an efficient vector.[18]

The model used in our study had some limitations. Prevalence data we used were at least 10 years old and based on surveys of slaughter cattle. Although a BT survey is regularly performed by the USDA Centers for Epidemiology and Animal Health (primarily in northeastern and north-central states), and antibody prevalence generally has been < 2% in these states,[19] to our knowledge, a national survey that used the c-ELISA technique has not been published. For some high-prevalence states, estimates were based on relatively small, nonrandom sample sizes. Prevalence of antibody-positive cattle in states may have systematically changed during the past decade, so these estimates are subject to scrutiny. We applied 1 prevalence estimate to all cattle from a state, which is unlikely to always be appropriate. Estimates of the prevalence of antibody-positive cattle were derived from surveys of slaughter cattle,[7,8] which may not reflect the prevalence of infection in beef or dairy cattle retained in herds. Management systems for beef and dairy cattle are likely to result in a difference in prevalence of infection, yet a single prevalence value was used. Despite these deficiencies, we believe that our model captures variability and uncertainty in prevalence estimates by sampling from a distribution for AP and then correcting to TP by sampling from distributions for SE and SP. Additional contemporary data would enable us to improve the accuracy and precision of our risk estimates. For some large states with varied geography (eg, California) and states in areas of transition between presence or absence of a vector, prevalence of infection will vary considerably by location. Rather than categorizing the United States on the basis of political boundaries (ie, states), a more valid approach may be to create divisions based on ecologic habitat and presence and competence of a vector. If regulations on importation of cattle and calves from the United States were to be revised, it is likely that the makeup of exporting states (and, therefore, the risk of exporting shipments that contain antibody-positive cattle) would change, so the models would need to be updated to reflect the proportional differences in state representation in terms of export shipments.

The model we used did not account for trade of cattle among regions. If a shipment of cattle were raised in a high-prevalence region and then sold to someone in a region with a lower prevalence before being exported, the model would potentially underestimate the probability that the shipment would be declared positive. Conversely, shipments of cattle raised in regions with lower prevalence and then sold to someone in a high-prevalence region prior to export could cause the model to potentially overestimate the probability of the shipment being declared positive. This would depend on the amount of time that the cattle lived in the high- and low-prevalence regions. Exporters should take into account the region of origin when considering whether to conduct pre-export tests on cattle.

Although the model reported here has been applied specifically to the probability that export shipments of cattle contain 1 or more cattle seropositive for BTV, by use of appropriate knowledge of disease prevalence and characteristics of diagnostic tests, it could easily be adapted to address a number of other import-export situations. For example, a similar approach was taken to determine the risk of importing horses infected with equine infectious anemia virus into California.[20]

In the study reported here, we used a model to document that further classification of additional regions with respect to BTV-antibody status beyond the current low- and high-prevalence designation of states is desirable for US cattle exporters. This should allow for reduced unnecessary animal-health regulations and increased opportunities for international trade.

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