Probability of introduction of exotic strains of bluetongue virus into the US and into California through importation of infected cattle

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Abstract

Strategies designed to minimize the probability of bluetongue virus (BTV) introduction to new areas should be based on a quantitative assessment of the probability of actually establishing the virus once it is introduced. The risk of introducing a new strain of bluetongue virus into a region depends on the number of viremic animals that enter and the competency of local vectors to transmit the virus. We used Monte Carlo simulation to model the probability of introducing BTV into California, USA, and the US through importation of cattle. Records of cattle and calf imports into California and the US were obtained, as was seroprevalence information from the exporting countries. A simulation model was constructed to evaluate the probability of importing either a viremic PCR-negative animal after 14-day quarantine, a c-ELISA BTV-antibody-negative animal after 28-day quarantine, or an untested viremic animal after 100-day quarantine into California and into the US. We found that for animals imported to the US, the simulated (best to worst scenarios) median percentage that tested positive for BTV-antibody ranged from 5.4 to 7.2%, while for the subset imported to California, the simulated median percentage that tested positive for BTV-antibody ranged from 20.9 to 78.9%. Using PCR, for animals imported to the US these values were 71.8–85.3%, and for those imported to California, the simulated median that test positive ranged from 74.3 to 92.4%. The probability that an imported animal was BTV-viremic is very low regardless of the scenario selected.
1. Introduction

Bluetongue (BT) is an infectious, noncontagious, insect-borne viral disease of sheep and other domestic and wild ruminants (Sellers, 1980). BT is one of 15 diseases considered by the Office International des Epizooties (OIE) to be “transmissible diseases that have the potential for very serious and rapid spread, and that are of major importance in the international trade of animals and animal products” (http://www.oie.int/eng/maladies/en_classification.htm), and is, therefore, a List A disease. The outcome of infection ranges from inapparent (in the vast majority of infected animals) to fatal in a variable percent (between 0 and 90, depending on species) of infected sheep, deer and wild ruminants (Jessup, 1985; Osburn, 1994). The causative agent of BT is bluetongue virus (BTV), considered to be the prototype virus of the genus Orbivirus within the family Reoviridae (Roy, 2001). Insect vectors in the genus Culicoides transmit this virus. 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 BT epidemiology. Because cattle rarely express clinical disease but can harbor the virus asymptomatically in their blood for up to 2 months after infection (MacLachlan, 1994), infected cattle can serve as reservoirs from which competent vectors become infected. BTV can be recovered from ovine skin biopsies for more than 9 weeks post-infection, although the viremic phase had ended after only 3–4 weeks (Takamatsu et al., 2003). The likely source of the virus is γδ T-cells that have become persistently infected. This may represent a previously unrecognized potential source of infection for competent vectors (Takamatsu et al., 2003).

Disease distribution is influenced by seasonal considerations, environmental factors, the presence of competent vectors, and the presence of pathogenic serotypes of the virus. In general, BTV infects livestock in all countries located in the tropics and subtropics. Virus serotypes and competent vectors can differ between and within countries. Within large countries spanning different latitudes, there can be areas where virus activity is absent (Gibbs and Greiner, 1994).

The major impact of BTV infection for many countries is its effect on international trade and movement of ruminant livestock and germplasm rather than direct losses from BT disease (Roberts et al., 1993). Rational trade policies pertaining to BTV infection must be based on consideration of the risk posed by ruminants previously exposed to the virus. Most (if not all) of the current restrictions on trade of animals from regions where BTV occurs are based on qualitative assessments of the risks of moving BTV into regions that are currently free of the virus (Gibbs and Greiner, 1994; Osburn, 1994). Many of the risks of moving
BTV when importing live animals into the United Kingdom have been outlined, and the conclusion was that a benefit–cost analysis should be performed prior to formulating importation guidelines (Roberts et al., 1993). However, we are not aware of any quantitative assessments of the probability of moving BTV published in the scientific literature. Therefore, strategies designed to minimize the risk of BTV introduction to new areas have not been based on a quantitative assessment of the probability of actually moving and transmitting the virus.

In California, USA, 4 of the 24 recognized serotypes of BTV (10, 11, 13, and 17) have been isolated from cattle (Stott et al., 1985). Neither clinical disease nor reproductive problems associated with BTV in cattle were observed in one study (Stott et al., 1985). However, another study (Osburn et al., 1986) estimated an annual cost of US$ 9.6 million (consumer price index—adjusted to 2004) to the dairy industry of the San Joaquin Valley of California; costs were associated with infertility and/or early embryonic mortality. Statewide serosurveys have found from 41% (Stott et al., 1985) to 47% (Metcalf et al., 1981) of cattle seropositive by agar-gel immunodiffusion (AGID).

Our objective was to apply quantitative modeling to the movement of BTV specifically related to the international importation of BTV into the US and into California through BTV-infected cattle. This scenario involves the probability that a BTV-infected animal escaped detection by the exporting country, and was shipped to and eventually released from quarantine into the US and into California. The probability that this animal was actually viremic after importation was estimated, which is the most-relevant endpoint to measure in terms of risk of disease introduction. The probability of subsequent transmission to other species following virus introduction was not considered, nor were the consequences of establishment and/or spread of the introduced virus.

2. Methods

Records of cattle and calf imports into the US for 1989–2000 and into California for the period 1996–2001 were obtained from US Department of Agriculture/Animal and Plant Health Inspection Service/Veterinary Services. Cattle imported for immediate slaughter were excluded from the analysis, because they were not considered a risk for BTV transmission.

Seroprevalence information from foreign countries exporting cattle and calves to the US and California was obtained from the OIE (http://www.oie.int). Data were categorical; therefore, countries were classified according to seroprevalence as either low (0–0.02) (given that specificity of the available diagnostic tests are <100%, no country can achieve a seroprevalence of zero) or high (0.201–0.80). Within each of these seroprevalence categories, the minimum, midpoint, and maximum values for prevalence were used in the analysis to reflect low, moderate, and high seroprevalence estimates.

Of countries that exported to the US during the time period under consideration, the OIE considers Belgium, Canada, Gabon, Germany, Ireland and the United Kingdom to be BTV negative. Mexico had serologic evidence and/or isolation of the causative agent (but no clinical signs of disease), and the disease was limited to specific zones during each of the
years 1997–2000. Australia, France, Italy, Japan and Saudi Arabia have similar BTV disease status to Mexico. Therefore, Belgium, Canada, Gabon, Germany, Ireland and the United Kingdom were placed in the low-seroprevalence category and the remaining countries in the high category.

The OIE recommends that veterinary administrations accepting importation should require one of three options when importing from a country or zone with serologic evidence of BTV (http://www.oie.int/eng/normes/mcode/A_00038.htm). The first option is to keep animals in Culicoides-free isolation for at least 100 days before export. This option relies on the finding that the maximal duration of viremia is <2 months (MacLachlan, 1994); therefore, a 100-day isolation period will assure that virtually no animals will be viremic upon leaving quarantine. The second option is to keep animals in Culicoides-free isolation for a minimum of 28 days and have negative results to two serological tests (either AGID or c-ELISA) performed 7 days apart with the first test being performed ≥21 days after entry into quarantine. Based on the fact that the results of the two tests are not independent and that the second serologic test is performed in an attempt to detect animals with negative results on the first test that might have seroconverted in the interval between tests, we chose to examine results from only the second test. The OIE handbook recommends use of the c-ELISA for detection of anti-BTV antibodies (Office International des Epizooties, 2000); therefore, we considered only the c-ELISA. The third option is 14-day isolation and with negative results to two BTV-isolation tests or PCR tests, with an interval of ≥7 days between tests and the first test ≥7 days after entering the quarantine station. We analyzed results from PCR testing at 14 days post-isolation, because results obtained from PCR can be used to not only identify viral nucleic acid, but also to provide information on serotype of virus and because results are available in a few days (compared to a few weeks when virus isolation is attempted) (Office International des Epizooties, 2000).

The current standard BTV-antibody test is the competitive enzyme-linked immunosorbent assay (c-ELISA). Two distributions were specified to describe the sensitivity (Se) of this test: one for the initial 50-day period following infection when an antibody response is developing, and one for all subsequent days post-infection. Within the first week post-infection, Se is low, and increases rapidly to close to 100% (Reddington et al., 1991; Gustafson et al., 1992; Afshar et al., 1992; Afshar et al., 1993; Zhou et al., 2001). Cumulative distribution functions (CDFs) estimating Se relative to days post-infection were determined based on the data presented in the original papers. Data from the studies were weighted based on the number of animals used to determine the antibody response. A function was chosen to represent all the data based on statistical and biological criteria (Carpenter et al., 1998). The statistical criterion was that the selected distribution was not significantly different ($P > 0.05$) from the empirical data based on chi-square-test statistics. The biologic criterion was that the statistical distribution fit our biologic knowledge of the immunologic process of infection (Carpenter et al., 1998).

After 50-days post-infection (when, from the CDF fitted above, it is evident that test Se has reached its maximum), Se of the c-ELISA was estimated using the beta distribution. This is a two-parameter distribution bounded by 0 and 1, and often is used to provide a suitable model for Se and specificity (Sp) (Greiner and Gardner, 2000). We asked a
BT expert (N.J. MacLachlan, University of California, Davis) for his opinion of the most-likely value for Se, as well as the fifth percentile of the possible values (e.g. 95% certain that the parameter exceeds this value). Answers to these questions were used to obtain the appropriate parameters for the beta distribution. Specificity of the c-ELISA also was estimated using a beta distribution based on the opinion of the expert.

Polymerase chain-reaction (PCR) is another test that is prescribed by the OIE for international trade. Estimates for Se and Sp of the PCR test similarly were obtained based on the opinion of the same expert (N.J. MacLachlan, University of California, Davis), using a beta distribution for each parameter.

A simulation model was constructed using a spreadsheet program (EXCEL, Microsoft Corp., Redmond, WA) to evaluate the probability of importing either a BTV-viremic PCR-negative animal after 14-day quarantine, a BTV-viremic c-ELISA antibody-negative animal after 28-day quarantine or an untested BTV-viremic animal after 100-day quarantine into the US and into California. A simulated animal was exported from a randomly selected country, which was assigned a value for seroprevalence. To determine the expected number of cattle that develop antibodies within a specified period, the incidence for each exporting country was determined by dividing the seroprevalence of BTV for that country by the presumed duration of infection.

\[
\text{Incidence} = \frac{\text{prevalence}}{\text{duration}}
\]  

A study from Queensland, Australia, found that a simple probabilistic estimate for duration of immunity of 33 months was consistent with observed prevalence estimates indicating that about one-third (12/33) of infected cattle in the herd become susceptible to re-infection in each year (Ward and Carpenter, 1997). We used this value to represent a most-likely duration of immunity. Because the duration of immunity is nevertheless uncertain, we used a triangular distribution with 33 months as the most-likely value, and 1 month and 60 months to represent the minimum and maximum (representing a range of period of immunity to cover plausible durations of immunity which could be expected in the field) (Ward and Carpenter, 1997).

Using a uniform distribution, a random number of days exposed prior to entry into quarantine was generated. To this was added the number of days in quarantine (either 14 days representing time before the final PCR test, 28 days representing time before the final serologic test, or 100 days for the option where no serologic testing was performed). For the 14- and 28-day quarantine periods, PCR or c-ELISA was performed on the sample, and the probability of the animal testing positive by either of these tests was calculated using the equation:

\[
P(T+) = (\text{prevalence} \times \text{Se}) + (1-\text{prevalence}) \times (1-\text{Sp})
\]  

Duration of viremia was estimated using a gamma distribution (with parameters \(\alpha = 7.27\) and \(\beta = 3.43\), where the mean = \(\alpha\beta\)) as described by Singer et al., 2001. The probability that an imported animal was viremic was calculated, based on the number of days post-infection. Using this value, the probability of importing at least one test-negative
and viremic animal into the US or California in a year was calculated using the binomial expression:

\[ P(\#\text{viremic} \geq 1 | \text{test - ve}) = 1 - \left( 1 - \left( \frac{P_1(1 - Se)}{P_1(1 - Se) + (1 - P_1)Sp \times P_2} \right) \right)^n \]  

(3)

where \( P_1 \) is probability of an animal being infected (incidence); \( P_2 \) is probability of animal being viremic given a random number of days infected prior to quarantine +14 or 28 days; \( n \) is number of animals imported per year (into California or US depending on the scenario). This value was selected randomly from a discrete distribution using the actual number of animals imported in a given year.

For the 100-day quarantine without testing, this becomes:

\[ P(\#\text{viremic} \geq 1 | \text{test - ve}) = 1 - (1 - (P_1 \times P_2))^n \]  

(4)

where \( P_2 \) is probability of animal being viremic given a random number of days infected prior to quarantine +100 days.

The importation of one or more viremic animal per “x” years was calculated using the inverse of these probabilities. It is assumed that the probability of the event occurring is constant for each year, and that importations remain at current levels.

A total of 900,000 iterations (100,000 for each of nine simulations; low, moderate, and high seroprevalence by 14-, 28- or 100-day quarantine) using Monte Carlo sampling with the computer program @RISK (Palisade Corp., Newfield, NY) was performed. Results were tabulated and reported as mean, median, 5th and 95th percentiles, as appropriate.

3. Results

A total of 25,334,700 cattle and calves were imported into the US between 1989 and 2000. Of these, 13,131,041 (51.8%) came from Canada and 12,201,683 (48.2%) from Mexico. The remaining imports (1976 animals, 0.00008%) during this 12-year period were from several countries, including Australia, Belgium, France, Gabon, Germany, Ireland, Italy, Japan, Saudi Arabia, and the United Kingdom.

Most international imports of cattle into California come from Mexico. From 1996 to 2001, 498,331 (94.0%) cattle and calves were imported from Mexico. Another 6.0% were from Canada, with 50 cattle from Australia and one from Japan.

Estimates for the time-varying Se of c-ELISA (i.e., during the first 50 days post-infection) were generated using data from several studies where animals were infected experimentally with BTV and subsequently tested for antibody (Afshar et al., 1989; Reddington et al., 1991; Afshar et al., 1992; Gustafson et al., 1992; Zhou et al., 2001). Using a probability distribution-fitting software program (Best Fit, Palisade Corp., Newfield, NY), a log–normal distribution with parameters \( \mu = 1.82 \) and \( \sigma = 1.14 \) was determined to provide good fit (chi-square \( P \) from 0.96 to >0.99, depending on data set) to the data (Fig. 1). Following the initial 50-days after infection, a beta distribution \((\alpha = 88.28, \beta = 1.88)\) was used to model Se of the c-ELISA based on expert opinion. Sp was modeled with the same beta distribution \((\alpha = 88.28, \beta = 1.88)\). This distribution has a median value of 0.98.
For the PCR, a beta distribution \((\alpha = 212.12, \beta = 3.14; \text{median} = 0.99)\) for Se and a beta distribution \((\alpha = 6.28, \beta = 13.32; \text{median} = 0.34)\) for Sp were used, based on expert opinion.

Using c-ELISA, for animals imported to the US, the simulated (best to worst scenarios) median percentage that tested positive (in import quarantine) for BTV ranged from 5.4 to 7.2\%, while for the subset imported to California, the simulated median percentage that tested positive for BTV ranged from 20.9 to 78.9\% (Table 1). Using PCR, for animals imported to the US, these values were 71.8–85.3\%, and for those imported to California, the simulated median that test positive ranged from 74.3 to 92.4\% (Table 2). The probability that an imported animal is test-negative (either PCR or c-ELISA) and BTV-viremic or BTV-viremic with no testing is very low regardless of the scenario selected (Table 3). The median probability was 0.0\% for all scenarios. Changing from a 14- or 28-day to a 100-day quarantine reduced the probability of viremia by a factor of \(\sim 10^6\) for all prevalence and duration of immunity scenarios (Table 3).

The probability of importing \(\geq 1\) BTV-viremic animals into the US and California in any given year was low, regardless of destination or scenario (Table 4). The expected number of

![Graph](image1.png)

Fig. 1. Proportion of samples testing positive for BTV-antibody by days post-infection using c-ELISA.

### Table 1

<table>
<thead>
<tr>
<th>US</th>
<th>California</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best</td>
<td>Midpoint</td>
</tr>
<tr>
<td>5th percentile</td>
<td>0.5</td>
</tr>
<tr>
<td>Median</td>
<td>5.4</td>
</tr>
<tr>
<td>95th percentile</td>
<td>22.8</td>
</tr>
</tbody>
</table>

*a For exporting countries with low BTV prevalence, prevalence (proportion) scenarios were 0.001 (best), 0.01005 (midpoint) and 0.02 (worst). For countries with high BTV prevalence, prevalence scenarios were 0.201 (best), 0.5005 (midpoint) and 0.80 (worst).*
years per importation of ≥1 BTV-viremic animals into the US under the worst-case scenario ranged from 25 years (14-day quarantine and PCR) to 22,000 years (100-day quarantine, no test), while for California, these values were 37 years (14-day quarantine and PCR) to 290,000 years (100-day quarantine, no test) (Table 5).

Table 2
Simulated percent animals identified for importation into the USA and California, USA, testing positive for BTV using PCR for three prevalence scenarios

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>California</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Best</td>
<td>Midpoint</td>
</tr>
<tr>
<td>5th percentile</td>
<td>53.2</td>
<td>54.3</td>
</tr>
<tr>
<td>Median</td>
<td>71.8</td>
<td>77.9</td>
</tr>
<tr>
<td>95th percentile</td>
<td>85.6</td>
<td>89.8</td>
</tr>
</tbody>
</table>

* For exporting countries with low BTV prevalence, prevalence (proportion) scenarios were 0.001 (best), 0.01005 (midpoint) and 0.02 (worst). For countries with high BTV prevalence, prevalence scenarios were 0.201 (best), 0.5005 (midpoint) and 0.80 (worst).

Table 3
Simulated mean (median) probability of BTV viremia for cattle imported into the USA and California, USA

<table>
<thead>
<tr>
<th>Quarantine (days)</th>
<th>USA</th>
<th>California</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Best</td>
<td>Midpoint</td>
</tr>
<tr>
<td>14 and negative (PCR)</td>
<td>3.7 × 10⁻⁶ (0.0)</td>
<td>9.4 × 10⁻⁶ (0.0)</td>
</tr>
<tr>
<td>28 and negative (c-ELISA)</td>
<td>8.2 × 10⁻⁷ (0.0)</td>
<td>2.1 × 10⁻⁶ (0.0)</td>
</tr>
<tr>
<td>100</td>
<td>5.2 × 10⁻¹³ (0.0)</td>
<td>1.3 × 10⁻¹² (0.0)</td>
</tr>
</tbody>
</table>

Table 4
Simulated mean (median) probability of importing ≥1 BTV-viremic animals into the US and California, in any given year

<table>
<thead>
<tr>
<th>Quarantine (days)</th>
<th>USA</th>
<th>California</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Best</td>
<td>Midpoint</td>
</tr>
<tr>
<td>14 and negative (PCR)</td>
<td>2.2 × 10⁻² (0.0)</td>
<td>3.5 × 10⁻² (0.0)</td>
</tr>
<tr>
<td>28 and negative (c-ELISA)</td>
<td>1.3 × 10⁻² (0.0)</td>
<td>1.9 × 10⁻² (0.0)</td>
</tr>
<tr>
<td>100</td>
<td>1.1 × 10⁻⁶ (0.0)</td>
<td>2.9 × 10⁻⁶ (0.0)</td>
</tr>
</tbody>
</table>

For exporting countries with low BTV prevalence, prevalence (proportion) scenarios were 0.001 (best), 0.01005 (midpoint) and 0.02 (worst). For countries with high BTV prevalence, prevalence scenarios were 0.201 (best), 0.5005 (midpoint) and 0.80 (worst).
4. Discussion

As presented, the model predicts that selecting either PCR-positive or BTV antibody-positive animals for importation into the US or California is likely (Tables 1 and 2). In a previous study, we showed that animal groups selected for export from the US had a high likelihood of being BTV antibody-positive (Hoar et al., 2003). However, from a risk-management standpoint, it is more important to consider whether the animals selected for international trade are viremic and thus a risk to ruminants in the importing country, and if so, whether the current testing and/or quarantine requirements adequately reduce the probability of releasing a viremic animal into naive ruminant populations to an acceptable level.

The proportion of cattle imported into the US and California positive for BTV using either PCR or c-ELISA had a large degree of uncertainty, depending on the prevalence scenario considered. Many countries do not have accurate estimates of national BTV prevalence; therefore, we used three separate estimates of prevalence to account for this lack of knowledge. Proportions of cattle that were test-positive for California are considerably higher than for the US because >90% of cattle and calves imported into California come from Mexico (a high-prevalence country) while approximately half of cattle imported into the US come from Canada (a BTV-free country).

Singer et al., 2001 estimated that there was a >99% probability of detectable BTV-viremia by virus isolation ceasing by 63 days post-infection. In contrast, viral nucleic acid has been detected by reverse transcriptase polymerase chain-reaction (RT-PCR) in blood cells for 16–24 weeks after infection (Bonneau et al., 2002; MacLachlan et al., 1994). However, calf blood containing viral RNA (as determined by PCR) but not infectious virus (as determined by virus isolation), was not infectious for sheep or Culicoides insects (MacLachlan et al., 1994). The maximal duration of viremia that was infectious to Culicoides sonorensis was 21 days post-infection (Bonneau et al., 2002), and with the exception of one sheep, only ruminants whose blood contained BTV (as determined by virus isolation) were able to infect C. sonorensis after oral feeding. Thus, PCR is a very conservative test for the screening of cattle and sheep for the presence of BTV (Bonneau et al., 2002). Knowledge of the duration of viremia can be used to determine appropriate quarantine periods prior to movement of animals from BTV-endemic to BTV-free regions.

The c-ELISA distinguishes between antibodies to viruses in the BT and EHD serogroups, and c-ELISAs specifically to detect anti-BTV antibodies are now available and

<table>
<thead>
<tr>
<th>Quarantine scenario (days)</th>
<th>USA Best</th>
<th>USA Midpoint</th>
<th>USA Worst</th>
<th>California, USA Best</th>
<th>California, USA Midpoint</th>
<th>California, USA Worst</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 and negative (PCR)</td>
<td>45</td>
<td>29</td>
<td>25</td>
<td>71</td>
<td>45</td>
<td>37</td>
</tr>
<tr>
<td>28 and negative (c-ELISA)</td>
<td>77</td>
<td>52</td>
<td>45</td>
<td>164</td>
<td>91</td>
<td>71</td>
</tr>
<tr>
<td>100</td>
<td>$9.1 \times 10^5$</td>
<td>$3.4 \times 10^5$</td>
<td>$2.2 \times 10^5$</td>
<td>$1.1 \times 10^7$</td>
<td>$4.5 \times 10^6$</td>
<td>$2.9 \times 10^6$</td>
</tr>
</tbody>
</table>
recommended (Gustafson et al., 1992). Following infection with BTV, an animal will not be viremic until \( \geq 4 \) days post-infection, and antibodies to the virus usually are not detectable until \( \geq 7 \) days post-infection (Barrett-Boyes and MacLachlan, 1995). Consequently, an animal infected and tested within 7 days of infection with one of the major antibody-based tests typically will be negative. For the next 2–3 weeks (3–4 weeks post-infection), as antibody concentrations increase, the Se of the test will increase; thus, test Se usually will increase during the course of infection. An individual might not develop high antibody concentrations; therefore, the Se of the test on this subpopulation would remain low. This has been demonstrated with the c-ELISA (Reddington et al., 1991; Afshar et al., 1992; Afshar et al., 1993). Although Fig. 1 represents a fitted distribution, sensitivity was estimated to have reached approximately 54% by day 7 post-infection. Only one of the studies evaluated tested animals \((n = 5)\) on day 7 for presence of antibody (and found none) (Reddington et al., 1991), making this result appear erroneous. However, this contradiction does not deleteriously affect the model because a value of 28 or 100 days is added to represent the minimum quarantine period, as recommended by the OIE. By implementing the 28-day minimum quarantine before the final serological sample is obtained, the sensitivity of the c-ELISA has reached approximately 91% for cattle infected on the day they entered quarantine.

To our knowledge, no estimates derived from field studies of the duration of immunity to re-infection have been published and experimental studies have been unable to provide reliable estimates of the duration of immunity to homologous bluetongue virus serotype re-infection in cattle (Ward and Carpenter, 1997). Consequently, we used a triangular distribution with values of 1 and 60 months as extreme values with a most-likely value of 33 months for duration of immunity to re-infection. The value of 1 month might be an unrealistically short estimate of duration of immunity; thus, these results likely overestimate probabilities. Although use of a triangular distribution might overestimate the probabilities at the tails of the distribution, we believe that until more information is available, a conservative approach is warranted.

The probability that an animal selected for import was also viremic was close to zero. The long presumed duration of immunity (most-likely value of 33 months) (Ward and Carpenter, 1997), combined with the finite maximal duration of viremia (Singer et al., 2001), resulted in estimates of very-low probability of viremia. This translated into a very-low mean probability of importing \( \geq 1 \) viremic animal in any given year. In all scenarios, the median probability was 0.0%; in the worst-prevalence scenario, \( \geq 1 \) PCR-negative viremic animal would be imported into California on average every 37 years. Under the same assumptions, \( \geq 1 \) PCR-negative viremic animal would be imported into the US every 25 years. As modeled, c-ELISA performed better than PCR, nearly doubling the mean number of years per event. The probability of introducing an exotic strain of BTV into California or the US by importing infected cattle was remote, and the current recommendation of a final PCR test performed 14 days after entry into quarantine or c-ELISA performed 28 days after entry into quarantine is likely adequate. Lengthening the quarantine period to 100 days and removing the testing requirement reduced the simulated mean probability of an imported animal being viremic by \( > 1 \) million-fold. A reduction of the 100-day quarantine period might be appropriate, if an acceptable probability can be established by regulatory bodies.
The Terrestrial Animal Health Code 2003 of the Office International des Epizooties (2003) allows a country to define geographical areas of different animal health status within its territory for the purpose of international trade, depending on the epidemiology of the disease, environmental factors, and surveillance and control measures applicable. Creation of BTV-free zones will result in a stratification of risk within a country. Unfortunately, data available to us did not differentiate region of origin of cattle shipments; therefore, we were not able to incorporate any regional differences that might exist within countries. In the future, disease zoning and regional distribution of disease should be incorporated into models assessing the probability of disease importation from various countries (Hoar et al., 2003).

There were some limitations of the model. For each prevalence scenario, we applied a single prevalence value to all cattle from a country, which is unlikely to be accurate. Within a country there will be different climatic regions (resulting in different vector activity and disease incidence within these climates). Additionally, animal type and use was not considered in this model. Beef cattle and dairy cattle will have different probabilities of infection, based on the management systems used. However, by applying three different prevalence scenarios, we believe we adequately have captured the uncertainty that these simplifying assumptions add. In converting from prevalence to incidence, we assumed that the duration of immunity is equivalent to the duration of protective antibodies. To our knowledge, there is no published scientific evidence to either support or refute this assumption. If antibody levels dropped to undetectable levels before animals became susceptible to re-infection, then our model overestimates incidence of infection and overestimates the probability of importing a viremic animal. However, if animals become susceptible to re-infection while antibodies are still present, then our model underestimates incidence and the probability of importing a viremic animal. We feel comfortable that we accounted for this lack of knowledge by using a triangular distribution with relatively extreme minimum and maximum values.

In summary, we present a model that demonstrates the very-low probability of importing a variant strain of BTV by importing infected cattle. The current OIE recommendations for testing and quarantine are likely adequate to protect cattle in the US and California, from an exotic strain of BTV. It must be emphasized that the importation of a viremic animal does not imply that secondary transmission is likely to occur. There still must be competent vectors that are capable of transmitting this imported strain of virus. Current research as well as epidemiological data suggests that vectors and viruses have co-evolved over time such that it would be unlikely for a local vector to be competent for exotic strains of BTV (Gibbs and Greiner, 1994).

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