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Maximal predicted duration of viremia in bluetongue virus–infected cattle

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Abstract. Central to the development of rational trade policies pertaining to bluetongue virus (BTV) infection is determination of the risk posed by ruminants previously exposed to the virus. Precise determination of the maximal duration of infectious viremia is essential to the development of an appropriate quarantine period prior to movement of animals from BTV-endemic to BTV-free regions. The objective of this study was to predict the duration of detectable viremia in BTV-infected cattle using a probabilistic modeling analysis of existing data. Data on the duration of detectable viremia in cattle were obtained from previously published studies. Data sets were created from a large field study of naturally infected cattle in Australia and from experimental infections of cattle with Australian and US serotypes of BTV. Probability distributions were fitted to the pooled empirical data, and the 3 probability distributions that provided the best fit to the data were the gamma, Weibull, and lognormal probability distributions. These asymmetric probability distributions are often well suited for decay processes, such as the time to termination of detectable viremia. The analyses indicated a $>$ 99% probability of detectable BTV viremia ceasing after $\leq$ 9 weeks of infection in adult cattle and after a slightly longer interval in BTV-infected, colostrum-deprived newborn calves.

Bluetongue is an insect-transmitted, noncontagious viral disease of domestic and wild ruminants that is caused by bluetongue virus (BTV).[11,16,26] It is 1 of only 16 diseases included in List A by the Office International des Epizooties (OIE). Diseases included in OIE List A are defined as communicable diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socioeconomic or public health consequence, and that are of major importance to the international trade of livestock and livestock products.[1] The major adverse economic impact of BTV infection in many regions of the world is its effect on international trade and movement of ruminant livestock and germplasm and not direct losses from bluetongue disease.[16,22,25]

Central to the development of rational trade policies pertaining to BTV infection is determination of the risk posed by ruminants previously exposed to the virus. BTV infection of cattle is characterized by prolonged but not persistent viremia.[11] Virus is highly cell associated during viremia, and intimate association of BTV with erythrocytes is responsible for both prolonged viremia as well as infection of the hematophagous vector insects that transmit the virus.[4] Duration of viremia in BTV-infected cattle is related to the lifespan of the bovine erythrocyte, and precise determination of the maximal duration of infectious viremia is essential to the development of an appropriate quarantine period prior to movement of animals from BTV-endemic to BTV-free regions.

Many experimental inoculation studies have been conducted to characterize the pathogenesis of BTV infection and to determine the maximal duration of detectable viremia in cattle.[2,3,10,12,13,14,17,18,21] These studies have included a variety of serotypes of BTV, cattle of different ages, and different methods of virus isolation. The sample size in most studies was small, making it difficult to estimate precisely the maximal duration of detectable viremia. In a long-term study of cattle naturally infected with BTV in Australia, an estimate of the duration of detectable viremia was obtained for each animal that was infected.[14] However, because individual animals were sampled at weekly intervals, the time of infection and the termination of detectable viremia could not be precisely defined. Thus, the duration of detectable viremia for each infected animal was estimated with uncertainty.

The process of pooling data from studies that are similar in design can increase the accuracy and precision of predictions, in this case an estimate of the duration of detectable BTV viremia. For regulatory purposes, it would be highly desirable to assign a probability of detectable viremia terminating within a specified time period, and the pooled data could provide more precise estimates of these probabilities. This scenario is ideally suited to the technique of probabilistic modeling, in which probability distributions are fitted to observed data.[5,9,28] The desired probability estimates

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Table 1. Studies used for the analysis of BTV viremia duration in cattle.

Infection	Reference no.	Sample size	Inoculation route*	Isolation method†	Viremia (days)‡
Natural (Australia)	15	476	natural	ECE	14–63
Experimental (Australia)	14	23	not provided	ECE, BHK_{21}	7–27
	17	9	ID, SC	BKH_{21}	12–28
Experimental (USA)	2	2	SC	BHK_{21}	42–49
	21	3	IV	BHK_{21}	49–63
	12	4	IV	BHK_{21}	42–63
	13	3	IV	cell culture	42–56
	10	10	IV (7), fly bite (3)	ECE	19–27
	18	8	ID, SC	ECE	12–28

* ID = intradermal; SC = subcutaneous; IV = intravenous.
† ECE = embryonating chicken eggs; BHK_{21} = baby hamster kidney cells.
‡ Range of number of days postinfection.

can then be calculated using these distributions. The objective of this study was to predict the duration of detectable viremia in BTV-infected cattle using a statistical analysis of existing data. In this study, the duration of viremia is defined as the interval from the time of detectable infection to the termination of detectable viremia, at which time the animal may still be capable of transmitting the virus but available diagnostic assays would not detect the presence of virus.

Materials and methods

Data on duration of detectable viremia. Data on the duration of detectable viremia in cattle were gathered from the literature. The studies that were utilized in this analysis were then divided into 3 distinct data sets. These data sets consisted of natural infection data from a large field study in Australia[15] and experimental inoculation studies of cattle infected with Australian[14,17] and US[2,10,12,13,18,21] serotypes of BTV. All analyses were independently conducted on each data set.

For each study, a decision had to be made about the end point of detectable viremia. For example, if cattle were sampled on a weekly basis, detectable viremia could have terminated at any time during a 1-wk period. Some risk analyses will utilize the midpoint of the interval as the end point of detectable viremia. However, to ensure that the most conservative estimates of the duration of detectable viremia for each individual were obtained, the end point of detectable viremia was considered to be the first time at which drawn blood was negative by virus isolation. In addition, the animal had to remain negative for the remainder of the study; the end point of detectable viremia was extended to the next negative isolation attempt if the animal reverted to being positive by virus isolation. Because in many of the studies detectable viremia was assessed on a weekly basis, the choice of end point potentially added 7 days to the estimated duration of detectable viremia.

Cattle (n = 477) were monitored on a weekly basis for natural BTV infection in the Northern Territory of Australia between 1985 and 1997 (Table 1).[15] Isolation of virus was performed by intravenous inoculation into embryonating chicken eggs (ECE) followed by passage in baby hamster kidney (BHK_{21}) cell culture.[6] For this analysis, data from 476 of the cattle were used; 1 individual was considered to have become sequentially infected with 2 different serotypes of BTV and so would not provide accurate information about the duration of detectable viremia following infection.[15] Because the animals in this study were naturally infected, the estimation of duration of detectable viremia had the additional complication that the natural delay between infection and detectable viremia (latent period) prevents precise determination of the timing of infection. To provide a conservative estimate of the duration of detectable viremia, it was assumed that all exposures occurred 1 wk prior to the first positive virus isolation.[11,18]

Two different studies were combined to obtain the data on experimental infections of cattle with Australian serotypes of BTV.[14,17] Thirty-two cattle that were ≥1 yr of age when infected with any of BTV serotypes 1, 20, and 23 were included in these studies (Table 1). Viremia usually was monitored on a daily basis through inoculation of ECE and BHK_{21} cells. These studies provided accurate estimates of detectable viremia duration because both the exposure time and clearance time were determined to the precise day. Three animals in 1 trial died prior to virus clearance[14] and were excluded from the analysis.

Results of 6 different studies were combined to produce the data set on experimental infection of cattle with US serotypes of BTV.[2,10,12,13,18,21] In total, 30 animals were used, most of which were <1 yr of age at the time of infection (Table 1). Serotypes 10 and 11 were used in these studies. Viremia was measured daily in some studies and weekly in others. All data were converted into days as the time frame for the analyses. Two animals were necropsied before the end of viremia in 1 study, but it was predicted from the results of the other animals that the 2 necropsied cattle would have become virus isolation negative the following day. For this analysis, 3 days were added to the duration of detectable viremia in these 2 animals.

Data analysis. A separate analysis was performed for each of the 3 data sets. First, cumulative histograms of the pooled observed data were generated. Then, using a probability distribution–fitting software package,[a] continuous cumulative distribution functions were fitted to the observed data. This step involves overlaying various probability dis-

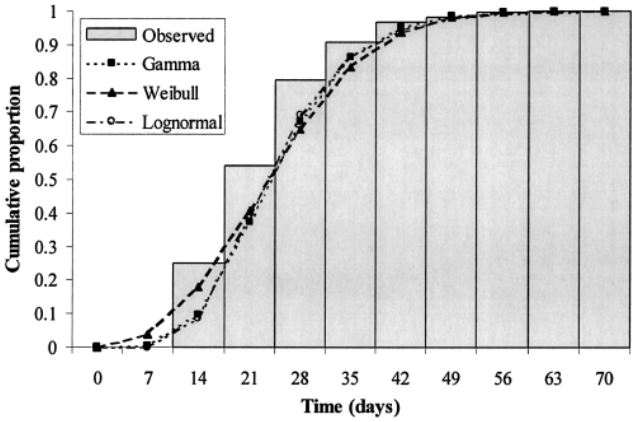

Figure 1. Histogram of observed BTV viremia data from the Australian cattle natural infection data set. Superimposed are the fitted gamma, Weibull, and lognormal probability distributions.

tributions onto the observed data and then assessing which of these distributions provides the best fit to the data. The parameters of each distribution are manipulated so that the fit is optimized. The distributions that provide the best fit to the observed data are then selected. The distributions that were assessed included the beta, chi-square, exponential, extreme value, gamma, logistic, lognormal, normal, and Weibull.

The fitted distribution functions were quantitatively evaluated with the Kolmogorov–Smirnov (K-S) and the Anderson–Darling (A-D) goodness-of-fit statistics. The K-S test is useful for determining the overall fit of the probability distribution to the observed data, especially around the mode of the distribution. The A-D test places more emphasis on the tail values and, therefore, is more effective than the K-S test for examining the fit in the extremities of the distribution. The parameters for each distribution function were initially obtained using maximum likelihood estimators, and the fit was then optimized using the Levenberg–Marquardt method.[20] This method uses an iterative approach to find the parameters of each probability distribution that minimize the goodness-of-fit statistic. The fit of the distributions to the data were also evaluated by visual inspection.[24,29] Minor adjustments were made to the distribution parameters to improve the visual representation of the peak and tails of the distribution and the observed data.

Using software packages,[a,b] the 50, 75, 90, 95, and 99 percentiles of each distribution function were calculated. The percentiles thus represented the probability that detectable viremia would cease within the estimated time interval. The probability that detectable viremia would extend beyond 28, 42, 56, 63, and 70 days postinfection was calculated using each distribution function.

Results

Detectable viremia ranged from 14 to 63 days in the 476 cattle that were naturally infected with BTV in Australia. The cumulative frequency of the observed data is shown in Fig. 1. Three of the probability distributions that provided the best fit to the data were the gamma, Weibull, and lognormal probability distributions. For this data set, the specific distributions were gamma ($\alpha = 7.27$, $\beta = 3.43$, where the mean = $\alpha\beta$), Weibull ($\alpha = 2.40$, $\beta = 27.49$), and the Lognormal ($\ln\mu = 3.15$, $\ln\sigma = 0.37$) and are plotted on Fig. 1. All of the goodness-of-fit tests for these distributions were significant ($P < 0.01$; Table 2), implying that the cumulative distribution functions were significantly different from the observed histogram. Thus, the distribution functions were visually inspected to finalize the selection. The probability distributions were used to predict the maximal duration of detectable viremia from these natural BTV infection data. There was a 90% probability that detectable viremia would cease within 37–39 days, depending on the probability distribution used, and a 99% probability that detectable viremia would cease within 51–55 days (Table 3). The estimated probability of detectable viremia extending beyond 63 days was 0.07–0.38%, depending on the probability distribution used (Table 4). These probabilities indicate, based on this data set, that there is an

Table 2. Goodness-of-fit test statistics* and associated *P* value for the different probability distributions in each data set of BTV-infected cattle.

		A-D test		K–S test	
Data set	Distribution	Statistic	*P*	Statistic	*P*
Natural	gamma	15.6	<0.01	0.17	<0.01
(Australia)	Weibull	17.5	<0.01	0.18	<0.01
	lognormal	16.5	<0.01	0.16	<0.01
Experimental	gamma	0.68	>0.15	0.13	>0.15
(Australia)	Weibull	0.54	>0.10	0.12	>0.10
	lognormal	0.55	>0.15	0.13	>0.15
Experimental	gamma	0.98	>0.15	0.17	>0.15
(USA)	Weibull	1.05	<0.01	0.19	<0.01
	lognormal	0.83	>0.15	0.15	>0.15

* A–D test = Anderson–Darling Test; K–S test = Kolmogorov–Smirnov test.

Table 3. The time (in days) and associated probability within which BTV viremia in cattle is predicted to terminate, using different probability distributions.

		Probability				
Data set	Distribution	50%	75%	90%	95%	99%
Natural	gamma	23.8	30.4	37.3	41.9	51.3
(Australia)	Weibull	23.6	31.5	38.9	43.4	52.0
	lognormal	23.3	29.9	37.5	42.9	55.3
Experimental	gamma	19.1	23.4	27.8	30.7	36.6
(Australia)	Weibull	18.9	22.7	25.9	27.8	31.1
	lognormal	17.9	22.3	27.1	30.5	38.0
Experimental	gamma	30.5	41.2	52.6	60.3	76.7
(USA)	Weibull	31.6	42.7	53.2	59.6	71.8
	lognormal	29.4	40.2	53.3	63.0	86.3

approximately 1:250 to 1:1,000 chance of detectable viremia extending beyond 63 days.

Detectable viremia ranged from 7 to 28 days in the 32 cattle that were experimentally infected with Australian strains of BTV (Fig. 2). Because the gamma, Weibull, and lognormal probability distributions were used for the natural infection data and have the appropriate right-skewed shape for modeling the termination of detectable viremia, all 3 distributions were used for the Australian experimental inoculation data set. For this data set, the specific distributions were gamma ($\alpha = 10.44$, $\beta = 1.89$), Weibull ($\alpha = 3.80$, $\beta = 20.81$), and lognormal ($\ln\mu = 2.88$, $\ln\sigma = 0.32$) and are plotted in Fig. 2. All of the goodness-of-fit tests for these distributions were nonsignificant ($P > 0.15$, Table 2), implying that the probability distributions were not significantly different from the observed histogram. Using the cumulative distribution functions fitted to the Australian experimental inoculation data, there was a 90% probability that detectable viremia would cease within 26–28 days, depending on the probability distribution used, and a 99% probability that detectable viremia would cease within 31–38 days (Table 3). The probability of detectable viremia extending beyond 63 days was < 0.001–0.005%, depending on the probability distribution used (Table 4). These probabilities indicate, based on this data set, that there is an approximately 1:20,000 to 1:100,000 chance of detectable viremia extending beyond 63 days.

Detectable viremia ranged from 12 to 63 days in the 30 cattle that were experimentally infected with serotypes of BTV from the USA (Fig. 3). The gamma, Weibull, and lognormal probability distributions were used to predict the maximal duration of detectable viremia. For this data set, the specific distributions were gamma ($\alpha = 4.87$, $\beta = 6.72$), Weibull ($\alpha = 2.31$, $\beta = 37.02$), and lognormal ($\ln\mu = 3.38$, $\ln\sigma = 0.46$) and are plotted in Fig. 3. The gamma and lognormal goodness-of-fit tests were nonsignificant ($P > 0.15$, Table 2), implying that these probability distributions were not significantly different from the observed histogram. The Weibull goodness-of-fit tests, however, were significant ($P < 0.01$, Table 2). Using the cumulative distribution functions fitted to these data, there was a 90% probability that detectable viremia would cease within 72–86 days (Table 3). The probability of detectable viremia extending beyond 63 days was estimated to be $<$ 3.3–5.0%, depending on the distribution used. These probabilities indicate, based on this data set, that there is an approximately 1:20 to

Table 4. Probability (%) of predicted BTV viremia in cattle extending beyond the specified number of days, using different probability distributions.

		Time				
Data set	Distrubtion	Day 28	Day 42	Day 56	Day 63	Day 70
Natural	gamma	32.97	4.88	0.42	0.11	0.03
(Australia)	Weibull	35.18	6.31	0.40	0.07	0.01
	lognormal	30.88	5.63	0.92	0.38	0.16
Experimental	gamma	9.62	0.91	0.002	<0.001	<0.001
(Australia)	Weibull	4.54	<0.001	<0.001	<0.001	<0.001
	lognormal	8.31	0.42	0.02	0.005	<0.001
Experimental	gamma	57.23	23.50	7.42	3.89	1.97
(USA)	Weibull	59.16	26.25	7.44	3.31	1.29
	lognormal	54.30	22.11	8.22	5.00	3.06

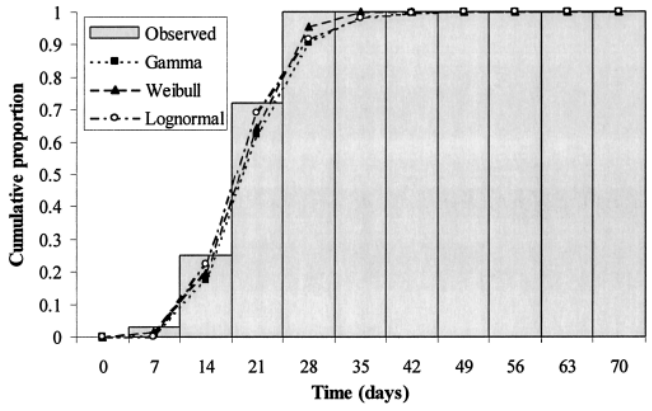

Figure 2. Histogram of observed BTV viremia data from the Australian cattle experimental inoculation data set. Superimposed are the fitted gamma, Weibull, and lognormal probability distributions.

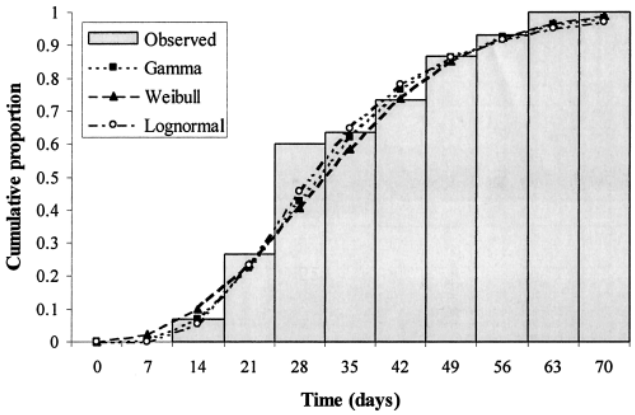

Figure 3. Histogram of observed BTV viremia data from the US cattle experimental inoculation data set. Superimposed are the fitted gamma, Weibull, and lognormal probability distributions.

1:30 chance of detectable viremia extending beyond 63 days.

Discussion

One purpose of a risk analysis is to assign probabilities to specific occurrences to quantify the risk of specific outcomes. When data are collected, the sample size is often not large enough to assign probabilities to rare events. The observed data have a finite range, and therefore it is difficult to assign probabilities of an occurrence outside that range using standard methods. The technique of probability distribution fitting can be used to predict the probability of occurrences that are outside the range of observed data. In this study, probabilistic modeling was used to assign probabilities to the risk of detectable BTV viremia extending beyond a specified time period. This type of analysis would then facilitate the development of trade policies pertaining to BTV infection that are based on the quantified risk of an animal being viremic beyond a certain time interval and not solely on subjective criteria.

To perform this analysis, data on BTV viremia were pooled into data sets. These data were comprised of infections caused by different BTV serotypes. We tested whether different serotypes had significantly different durations of detectable viremia. For the Australian natural infection data set, serotype 16 had a median duration of detectable viremia that was significantly longer than that of the other serotypes (data not shown) but only by an additional 3 days. For these data, detectable viremia was always measured on a weekly basis, so the finding of 3 days difference is not biologically significant. In fact, the pooling of serotype 16 with the other serotypes provides more conservative estimates of detectable viremia duration; therefore, all analyses were based on the entire pooled data set.

Detectable viremia persisted up to 63 days in cattle naturally and experimentally infected with Australian and US serotypes of BTV, although the duration of detectable viremia was considerably shorter in the great majority of animals. The results of the probabilistic modeling showed that there is a low probability of detectable BTV viremia extending beyond 63 days, especially when the Australian data were used ($<$ 0.001%–0.38%). Estimates of the duration of detectable viremia were similar for cattle naturally and experimentally infected with Australian BTV serotypes, and the slightly increased predicted duration of detectable viremia in the natural infection data set could be attributed to the uncertainty about the timing of infection and the end point of detectable viremia. Because the inoculation studies involved virus isolation methods that differed from those used in the natural infection study, differences in sensitivity and specificity could influence the duration of detectable viremia.

Cattle experimentally infected with US serotypes of BTV had a more protracted viremia than those infected with Australian strains of BTV (95% probability of clearance in $<$60 days vs. $<$43 days). This difference could be due to the extensive use of calves, newborn colostrum-deprived calves in particular, in the studies with US serotypes of BTV. Adult cattle inoculated with US serotypes of BTV had a detectable viremia of comparable duration to that of cattle inoculated with Australian serotypes of the virus. However, the varying sensitivity and specificity of the different isolation assays could also have contributed to the observed difference in detectable duration of viremia.

At least 2 additional inoculation studies conducted with US serotypes of BTV were excluded from this analysis.[19,23] In 1 of these studies,[19] viremia was measured at irregular intervals (30, 60, and 113 days postinoculation), but only 1 of the 18 animals that were inoculated with 1 of 3 BTV serotypes was still viremic at day 60. Because the next sampling was conducted

53 days later, it is impossible to determine when virus was cleared. In the other study,[23] the maximum length of detectable viremia for 20 pregnant cattle infected with US BTV serotype 11 was 38 days. No other information was provided. The data and conclusions from these studies are consistent with those in this analysis.

The process of selecting a probability distribution that fits the observed data involves some degree of subjectivity; therefore, several different probability distributions that have slightly different shapes were used. Some of these functions will better reflect the mode of the observed histogram, and other distributions will better represent the tails of the histogram. After the distributions are fitted to the observed data, goodness-of-fit statistics are commonly used to quantitatively assess the fit. Unfortunately, these statistics are highly dependent on sample size, and as the sample size increases, it becomes more difficult to find a fit that is not rejected as significantly different. A total of 476 animals were included in the Australian natural infection data set. This large sample size resulted in significant goodness-of-fit statistics, thus implying that the cumulative distribution functions were a poor fit to the observed data. By combining a visual inspection of the data with the goodness-of-fit statistics, the fits of the probability distributions to the Australian natural infection data were deemed appropriate. The gamma, Weibull, and lognormal distributions were used for all 3 data sets. These distributions not only demonstrated a good overall fit to the observed data, but because they are asymmetric probability distributions, they are also well suited for decay processes such as the time to termination of viremia.[7]

Detectable viremia as determined by virus isolation persisted up to 63 days in cattle naturally and experimentally infected with Australian and US serotypes of BTV. The probability distributions that were fitted to these data estimated a low probability of detectable viremia extending beyond 63 days. This information can then be incorporated into trade policy related to BTV. For example, the polymerase chain reaction (PCR) can detect viral nucleic acid up to 180 days after BTV infection of cattle and thus provides a more sensitive method for detection of BTV infection of cattle than does virus isolation.[8] Assuming the PCR assay has a high sensitivity, animals that are confirmed negative by the PCR assay should not pose a risk for movement.

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Sources and manufacturers

a. BestFit®, Palisade Corp., Newfield, NY.
b. S-plus 2000, Mathsoft, Inc., Seattle, WA.

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