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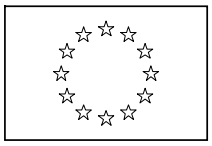

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

Estimations of the Infective Period for Bluetongue in Cattle

Report of the
Scientific Committee on Animal Health and Animal Welfare

Adopted 8 December 1999

SCIENTIFIC COMMITTEE ON ANIMAL HEALTH AND ANIMAL WELFARE

Estimations of the Infective Period for Bluetongue in Cattle

Contents

1. Request for opinion

In the light of recent analysis and draft proposal from the OIE, the Scientific Committee on Animal Health and Animal Welfare is requested to consider if the proposal to allow movement of untested bovines from an infected region to a free region following a quarantine of 60 days (or the infective period) are in line with the advice of the Committee of 21 October 1998[1] .If not, the Committee is invited to consider if these proposed measures provide a sound basis for the importation, with minimal risk, of live animals into the European Union.

1 This report is available at http://europa.eu.int/comm/dg24/health/sc/scah/out15_en.html

2. Background

2.1 Previous Opinion of the Committee

The Scientific Committee on Animal Health and Animal Welfare adopted the following reports[1] (XXIV/B3/AH/R05/1998 final) on 21 October 1998;

1. Suggested Protocol for the Importation of Live Animals from Bluetongue Virus (BTV) and Epizootic Haemorrhagic Disease Virus (EHDV) Endemic Areas, and
2. Criteria for Definition of Geographical Areas in Australia which can be considered as Low Risk Areas as regards importation of Species susceptible to Bluetongue Virus (BTV) and Epizootic Haemorrhagic disease Virus (EHDV) into the European Union

In these reports, the Committee suggested sanitary protocols for the importation of animals from bluetongue infected regions. These protocols are set out below. However, for a full explanation, the complete text of that opinion should be consulted.

"Seronegative Animals

These animals should be kept in quarantine under vector-free conditions for at least 40 days , be sampled and tested at the beginning of the quarantine period and at least 28 days later and have been shown to be negative for antibodies against BTV and EHDV. If a positive animal is identified, it should be removed and all other animals re-quarantined and sampled and tested 28 days later with negative results.

Seropositive Animals

If seropositive, virus negative, animals are to be considered for importation into the EU the following protocol may be useful. However, it should be noted, that such seropositive animals, may later cause potential problems in establishing convincing evidence for absence of infection by routine serosurveys or during re-export. Such animals may represent an additional risk of introducing the infection.

However, a suitable protocol could provide an acceptable safeguard and is as follows:

The animal is kept in quarantine under vector-free conditions until completion of the protocol (with a minimum period of 40 days.) During this period at least 40 ml of blood shall be collected from the animal on each of two separate occasions at least 7 days apart, the first collection being taken in the first week of quarantine. The blood samples shall be heparinised and shall be kept under sterile conditions at $4^{o}C$ for a maximum of 10 days before inoculation into sheep for the purposes of virus detection. The two samples from the animal are pooled and half of the total volume (40 ml) is inoculated subcutaneously into each of two sheep (previously shown to be negative for antibodies against BTV and EHDV). Both sheep must remain BTV and EHDV seronegative when sampled and tested at 28 days, with a second sample taken and tested at least 35 days after the inoculation."

2.2 New Analysis

A recent meta-analysis was carried out on the infective period for Bluetongue virus in cattle (Singer and Mac Lachlan, maximal Predicted Duration of Viraemia in Bluetongue Virus infected Cattle). This document analysed three data sets

Dataset 1. 477 cattle naturally infected with Australian strains of BTV from two studies

Dataset 2. 32 cattle experimentally infected with Australian strains of BTV from two studies

Dataset 3. 30 cattle experimentally infected with US strains of BTV from six studies

Probability distributions were then fitted to the observed data. The paper is attached in Annex.

The study produced statistical calculations for the length of the infective period. The following

table gives the viraemic period calculated in weeks (99% cut off), the study involved, and the statistical distribution assumed.

	Dataset 1	**Dataset 2**	**Dataset 3**
Gamma	7.21	5.23	10.95
Weibull	7.11	4.44	10.25
Lognormal	7.91	5.43	12.33

Table: Viraemic period calculated in weeks (99% cut off), for the three datasets using three statistical distributions.

2.3 Proposal from the OIE

The OIE have proposed the following health conditions apply to live animals coming from a bluetongue infected region moving to a free region (the principle is repeated for other movements) ;

'Article 2.1.9.6.

When importing from BTV infected countries or zones, *Veterinary Administrations* should require:

for ruminants and other BTV susceptible herbivores

the presentation of an *international animal health certificate* attesting that the animals:

1) were kept in a *Culicoides*-proof *quarantine station* for a period at least [60 days (under study)] equivalent to the infective period defined in Article 2.1.9.1. prior to shipment; or

2) were kept in a *Culicoides*-proof *quarantine station* for at least 28 days prior to shipment, and were subjected during that period to a serological test to detect antibody to the BTV group, such as the BT competition ELISA or the BT AGID test, with negative results on two occasions, with an interval of not less than 7 days between each test, the first test being carried out at least 21 days after introduction into the *quarantine station*; or

3) were kept in a *Culicoides*-proof *quarantine station* for at least 14 days prior to shipment, and were subjected during that period to a BTV isolation test or nucleic acid detection test, with negative results, on blood samples taken on two occasions, with an interval of not less than 7 days

between each test, the first test being carried out at least 7 days after introduction into the *quarantine station*;

AND

4) were protected from *Culicoides* attack during transportation from the *quarantine station* to the *place of shipment*.'

Thus two options are currently under consideration, trade on the basis of a 60 day infective period and trade based on a period which is not defined but determined to be the infective period with a 99% level of confidence.

3. Discussion

After having reviewed the report from the Scientific Committee on Animal Health and Animal Welfare of 21 October 1998 and the study "Maximal predicted duration of Viraemia in bluetongue virus infected cattle" by Randall S. Singer and N. James MacLachlan, the Committee can make the following comments:

The protocols for importation of live animals as suggested in the report of 21 October 1998 are still valid and will give an acceptably low risk of importing the infection. This is based on the fact, that the animals with the lowest risk will be seronegative animals, that go through a 40 day quarantine period including serological tests at the beginning and towards the end of the quarantine period. If seropositive animals should be included, the Committee still support the original report's indication, that such animals may cause problems for routine serosurveys or during re-export, and furthermore, that such animals should go through a 40 day quarantine including a test to detect virus in blood samples taken at two occasions. Testing could be done using the PCR for BT viral RNA, which has just been accepted by the OIE Standards Commission as a prescribed test for international trade testing (see 2.3 above). However, more details on this PCR test are needed.

Based on the study by Singer and MacLachlan, it is suggested to extend the quarantine period to 60 days, or the infective period as defined above. Under such conditions, the testing requirements (for either antibody or virus) could be dispensed with. The sole barrier to the introduction of infection into a free area would depend on viraemic animals becoming non infective by the end of the quarantine period. The Committee has not carried out a thorough statistical analysis of the study of Singer and MacLachlan. However, the estimated percentage of infected animals which could take longer than 60 days to become non infective seems to vary from less than 0.001% (study 2, weibull distribution assumed) to 8.2% (study 3, lognormal distribution) and could clearly pose an unacceptable risk. This risk must be considered in the context of these conditions being applied to potentially large number of animals in the future.

The alternative suggestion of using the infective period defined with 99% confidence is similarly flawed. Indeed, instead of containing a safety margin, this method contains a built in failure estimate of 1%!.

Moreover, the methods used for detection of viraemia in some of the studies included in the Singer and MacLachlan analysis (BHK) are of limited sensitivity as compared to sheep or embryonating hen's egg inoculation. In the report of the Scientific Committee on Animal Health and Animal Welfare of 21 October 1998 a double testing system is recommended which was estimated to remove 99% or more of potentially INFECTED animals. The testing also acted as a safeguard against infection either during the quarantine period or transportation to the quarantine station. Both these latter risks must, in reality, be a cause for concern due to the difficulty in delineating vector-free or virus-free zones.

In the opinion of the Committee the available evidence does not support the case that trading in untested animals from infected regions following a 60 day (or infective period as defined) quarantine period is safe, particularly when large numbers of animals are traded. In order to support such a decision, more data would be required as follows;

a. More accurate estimates of number of animals being viraemic after either 40 days or 60 days quarantine and especially, accurate estimates of LEVELS of viraemia between 40 days and 60, and after 60 days quarantine.

b. The above mentioned values should be evaluated in relation to the sensitivity of the methods used, especially in relation to the amounts of virus needed to infect vectors.

c. The influence of age, breed, immune status, virus serotype etc. on the levels and duration of viraemia.

d. Detailed data are also needed for EHDV which is not covered at all in the Singer and MacLachlan analysis but which is dealt with in the Scientific Committee report.

If an alternative, safe, non-testing procedure should be envisioned based on the current level of knowledge, it would have to include a significant safety margin. On the basis of the maximum duration of viraemia in BTV infected animals, linked to the lifespan of virus-bearing erythrocytes, an initial suggestion for such a period of quarantine in a vector free environment without testing could be set, for cattle, at 120 days, this being the approximate life span of cattle erythrocytes. However, since the approximate limit of detection by RT-PCR of viral RNA in the blood of an infected animal is 180 days after infection, the quarantine period should, for safety reasons, be extended at 180 days. It should be emphasised that such a suggestion is tentative and more experimental data, as listed above, are required.

4. Conclusion

The estimated percentage of infected animals which could take longer than 60 days to become non infective seems to be variable and could clearly pose an unacceptable risk. This risk must be considered in the context of these conditions being applied to potentially large number of animals in the future. The alternative suggestion of using the infective period defined with 99% confidence is similarly flawed. Indeed, instead of containing a safety margin, this method contains a built in failure estimate of 1%!.

Neither method would provide a sound basis for the importation, with minimal risk, of live animals into the European Union

5. References

Scientific Committee on Animal Health and Animal Welfare (1998) Suggested Protocol for the Importation of Live Animals from Bluetongue Virus (BTV) and Epizootic Haemorrhagic Disease Virus (EHDV) Endemic Areas. European Commission 21 October 1998 http://europa.eu.int/comm/dg24/health/sc/scah/out15_en.html

Scientific Committee on Animal Health and Animal Welfare (1998) Criteria for Definition of Geographical Areas in Australia which can be considered as Low Risk Areas as regards importation of Species susceptible to Bluetongue Virus (BTV) and Epizootic Haemorrhagic disease Virus (EHDV) into the European Union. European Commission 21 October 1998 http://europa.eu.int/comm/dg24/health/sc/scah/out14_en.html

6. Acknowledgements

The Scientific Committee on Animal Health and Animal Welfare requested Prof. Soren Alexandersen, who was chairman for the working group which prepared the 1998 report, to produce a response. In preparing this document Prof. Alexandersen worked in close consultation with Dr. John Anderson, Dr. Philip Mellor and Dr. Alex Donaldson of the OIE Reference Laboratory for Bluetongue at Pirbright, UK.

7. Annex Maximal Predicted Duration of Viraemia

MAXIMAL PREDICTED DURATION OF VIREMIA IN

BLUETONGUE VIRUS INFECTED CATTLE

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Bluetongue is an insect transmitted, noncontagious viral disease of domestic and wild ruminants that is caused by bluetongue virus (BTV). 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, which are of serious socioeconomic or public health consequence, and which are of major importance to the international trade of livestock and livestock products. The major adverse economic impact of BTV infection in many regions of the world is on international trade and movement of ruminant livestock and germplasm, and not disease associated with bluetongue disease of ruminants.

Central to the development of more 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. Virus is highly cell-associated prolonged viremia as well as infection of the hematophagous vector insects that transmit infection. While it is clear that duration of viremia in BTV-infected cattle is related to the lifespan of the bovine erythrocyte, 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-infected to BTV-free regions. The goal of this study was, therefore, to better predict the duration of viremia in BTV-infected cattle using statistical analysis of existing data.

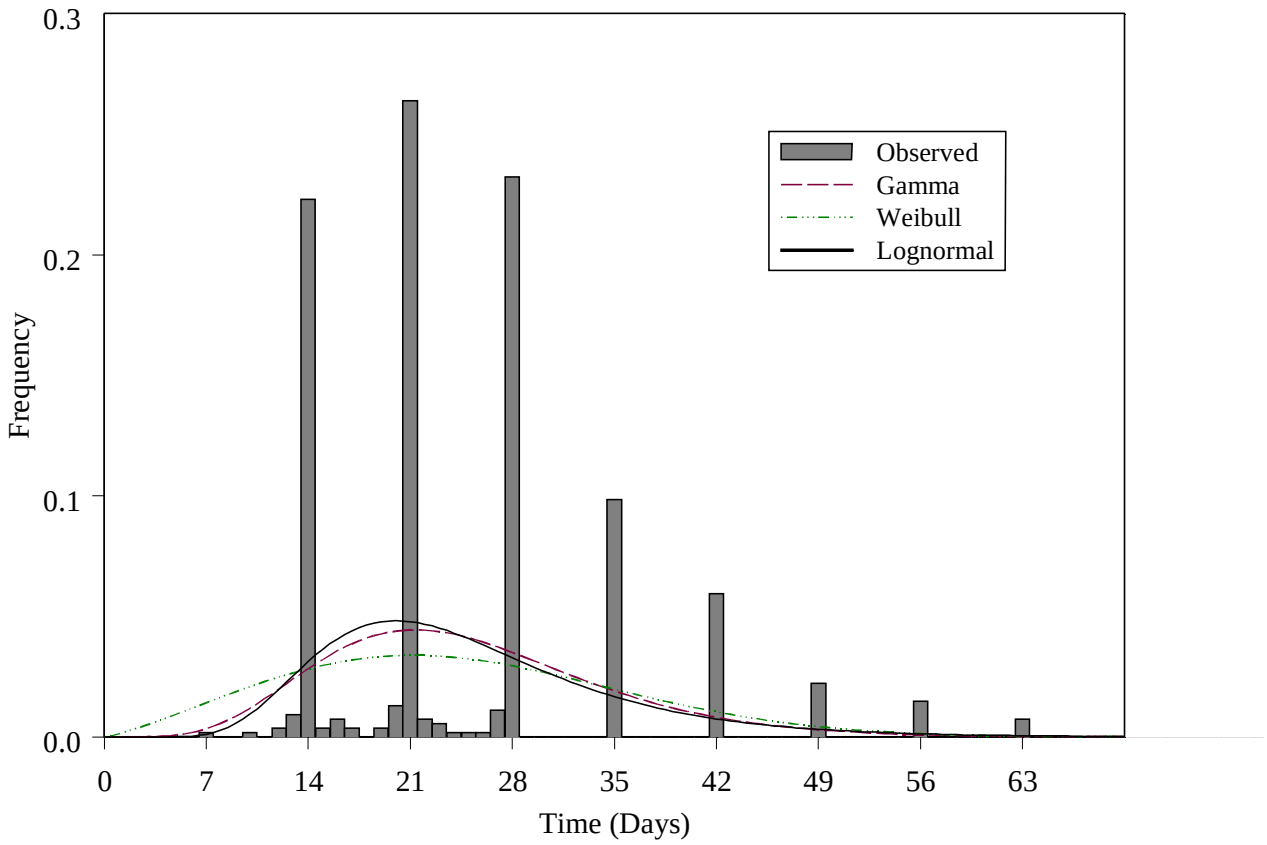
Three different datasets were compiled, including (1) 477 cattle that were naturally infected with Australian strains of BTV, (2) 32 cattle that had been experimentally infected with Australian serotypes of BTV, and (3) 30 cattle that had been experimentally infected with U.S. serotypes of BTV. Data was included only from studies in which the possibility of sequential infection of individual animals with different serotypes and laboratory contamination of samples had been adequately addressed. The data used in this study are listed in Table 1. Probability distributions were then fitted to the observed data using the software BestFit® (Palisade Corporation).

Table 1. Studies detailing duration of viremia in BTV-infected cattle

	Reference	Samp. Size	Inoc. Route	Isolation Meth.	Viremia (Range;
Natural (Aus)	Gard (1988) Melville	476	Natural	ECE	14-63*
Inoculation (Aus)	Melville (1992)	23	Not provided	ECE-BHK	7-27
	Parsonson (1987)	9	ID and SC	BHK	12-28
Inoculation (US)	Barratt-Boyes (1994)	2	SC	BHK	42-49
	Richards (1988)	3	IV	BHK	49-63
	MacLachlan (1986)	4	IV	BHK	42-63
	MacLachlan (1990)	3	IV	Cell culture	42-56
	Luedke (1969)	10	IV (7) Fly bite	ECE	19-27
	Parsonson (1994)	8	ID and SC	ECE	12-28

* 1 bull that was infected with more than 1 BTV serotype was excluded, because it was not obvious what the duration of viremia was with each BTV serotype

Duration of viremia in Australian cattle naturally infected with BTV



Duration of predicted viremia (in weeks) using different probability distributions and cutoffs

Distribution	50%	75%	90%	95%	99%
Gamma	3.34	4.27	5.24	5.88	7.21
Weibull	3.48	4.52	5.47	6.05	7.11
Lognormal	3.32	4.27	5.36	6.13	7.91

Probability of predicted viremia extending beyond specified number of weeks

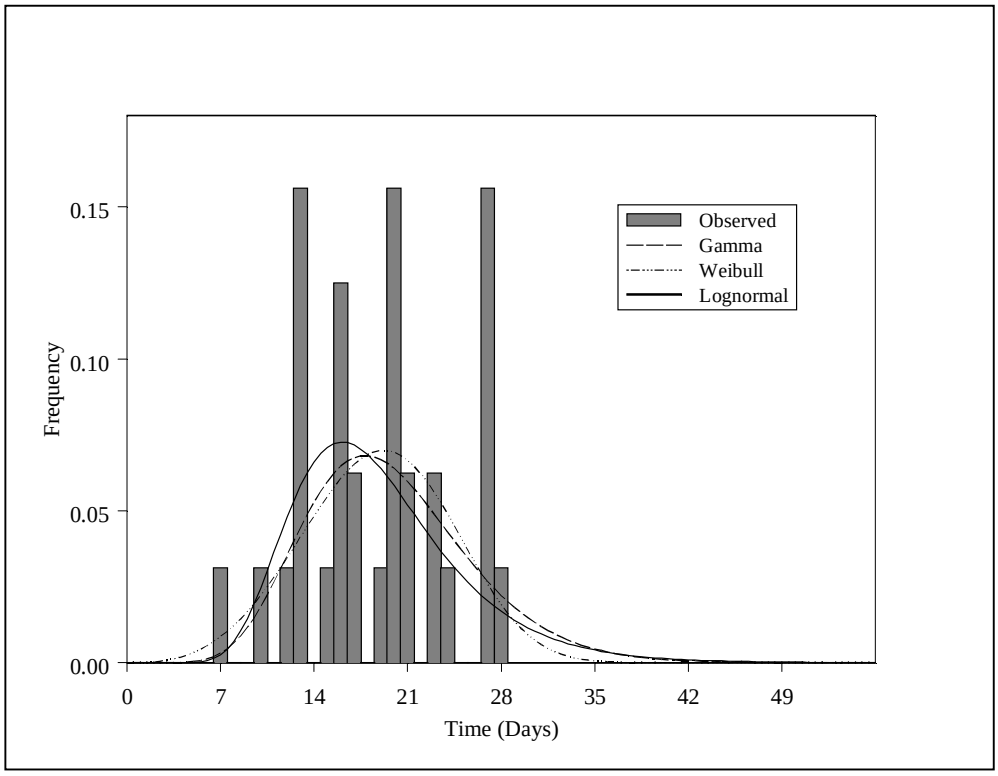
Distribution	Week 4	Week 6	Week 8	Week 9	Week 10
Gamma	0.31259	0.04347	0.00352	0.00088	0.00020
Weibull	0.36631	0.05303	0.00186	0.00019	0.00001
Lognormal	0.30884	0.05634	0.00919	0.00375	0.00156

- There is a 99% probability that cattle will clear the virus in 7.11 to 7.91 weeks, depending on the probability distribution used.

- There is between 0.19 and 0.92% probability of an animal being viremic after 8 weeks, again depending on distribution used.

- These are conservative estimates for at least two important reasons. First, the end point of viremia was considered to be the first week that a negative result was obtained, whereas viremia actually could have terminated at any time after the final positive sampling (up to 7 days prior to the interval reported). Second, the time from infection to detectable viremia was assumed to be 1 week, thus 1 week was added to the duration of viremia for each animal. These estimates, therefore, likely overestimate viremia by as much as 14 days, and on average 7 days per individual animal.

Duration of viremia in cattle experimentally infected with Australian serotypes of BTV



Duration of predicted viremia (in weeks) using different probability distributions and cutoffs

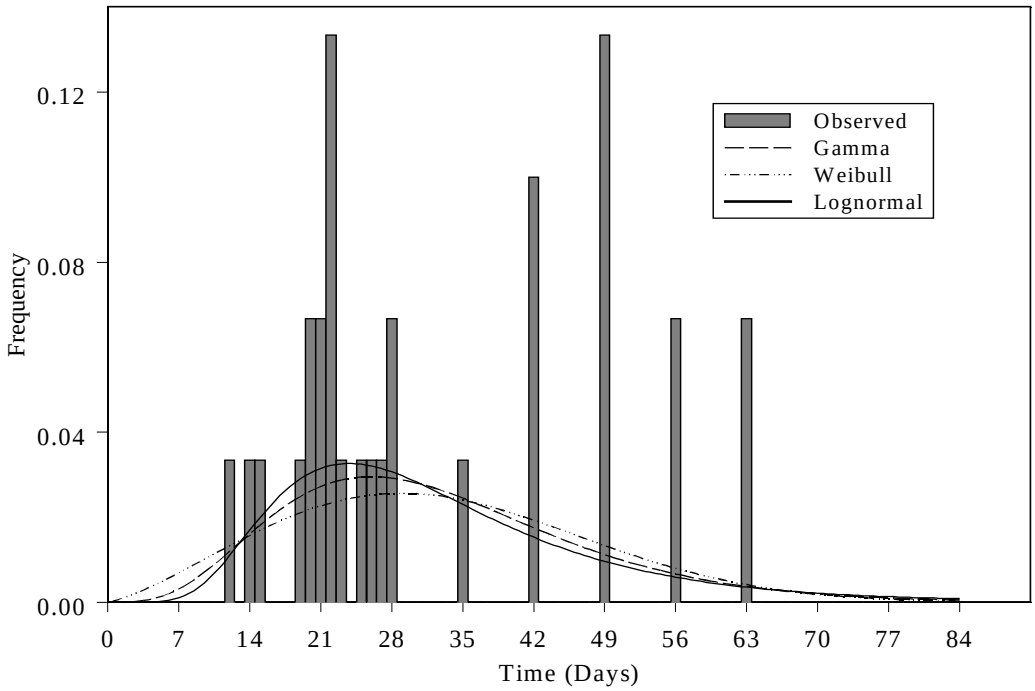
Distribution	50%	75%	90%	95%	99%
Gamma	2.73	3.35	3.98	4.39	5.23
Weibull	2.70	3.24	3.70	3.97	4.44
Lognormal	2.56	3.18	3.87	4.35	5.43

Probability of predicted viremia extending beyond specified number of weeks

Distribution	Week 4	Week 6	Week 8	Week 9	Week 10
Gamma	0.09615	0.00192	0.00002	< 0.00001	< 0.00001
Weibull	0.04541	< 0.00001	< 0.00001	< 0.00001	< 0.00001
Lognormal	0.08313	0.00418	0.00021	0.00005	0.00001

- There is a 99% probability that cattle will clear the virus in 4.44 to 5.43 weeks, depending on the probability distribution used.
- There is between 0.0001 and 0.021% probability of an animal being viremic after 8 weeks, again depending on distribution used.
- These are more precise estimates. Animals were sampled on almost a daily basis, and therefore, the endpoint of viremia was determined over consecutive days.

Duration of viremia in cattle and calves inoculated with U.S. serotypes of BTV



Duration of predicted viremia (in weeks) using different probability distributions and cutoffs

Distribution	50%	75%	90%	95%	99%
Gamma	4.36	6.00	7.51	8.62	10.95
Weibull	4.51	6.09	7.59	8.51	10.25
Lognormal	4.21	5.75	7.61	9.00	12.33

Probability of predicted viremia extending beyond specified number of weeks

Distribution	Week 4	Week 6	Week 8	Week 9	Week 10
Gamma	0.57234	0.23502	0.07415	0.03887	0.01967
Weibull	0.59162	0.26246	0.07443	0.03307	0.01294
Lognormal	0.54297	0.22107	0.08215	0.04999	0.03055

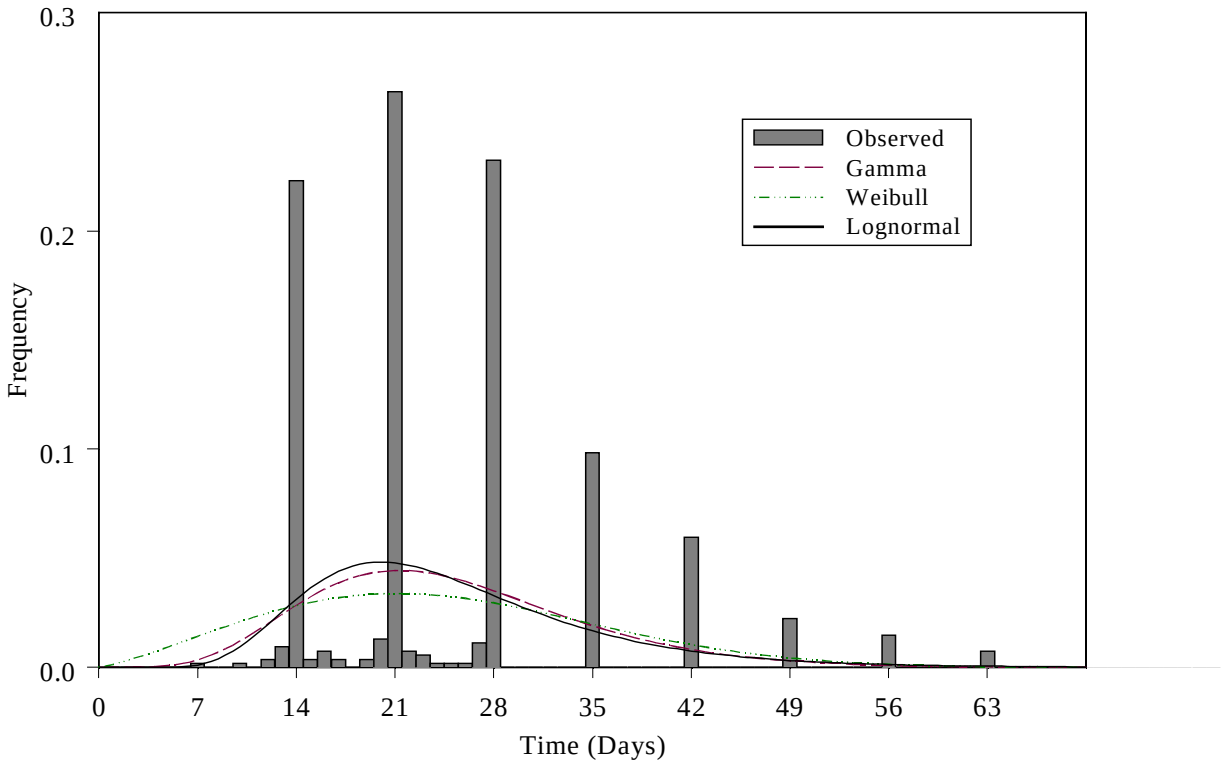
- There is a 99% probability that cattle will clear the virus in 10.25 to 12.33 weeks, depending on the probability distribution used.

- There is between 7.42 and 8.22% probability of an animal being viremic after 8 weeks, again depending on distribution used.

- These are conservative estimates. The week that a negative result was obtained was used as the endpoint of viremia. In reality, viremia could have ended at any time during the week following the final positive sampling (7 days previously).

- Newborn, colostrum-deprived calves were used instead of adult cattle in several of these studies, and these animals consistently had longer viremias than did adult animals. The protracted viremias identified in this group, therefore, likely are somewhat of an artifact associated with the experimental approach used in these studies.

- Data and conclusions from this study are consistent with those obtained by Pearson *et al.* (1973) and Roeder *et al.*, (1991), who also infected cattle with U.S. serotypes of BTV. Roeder reported that viremia was last found at 38 days after inoculation of 20 pregnant cattle with U.S. BTV serotype 11. Pearson *et al*. reported that only one of 18 calves still was viremic at 60 days after inoculation with one of 3 different U.S. serotypes of BTV. Unfortunately, regular (daily or weekly) virus isolation results were not reported in these studies, thus they were not included in the current analysis.



Duration of viremia in cattle and calves inoculated with BTV: Pooled data

Duration of predicted viremia (in weeks) using different probability distributions and cutoffs

Distribution	50%	75%	90%	95%	99%
Gamma	3.40	4.38	5.40	6.08	7.50
Weibull	3.34	4.54	5.69	6.40	7.75
Lognormal	3.31	4.29	5.42	6.23	8.10

Probability of predicted viremia extending beyond specified number of weeks

Distribution	Week 4	Week 6	Week 8	Week 9	Week 10
Gamma	0.3347	0.0545	0.0054	0.0015	0.0004
Weibull	0.3530	0.0749	0.0071	0.0016	0.0003
Lognormal	0.3121	0.0610	0.0109	0.0046	0.0020

Using the results from the pooled data:

- There is a 99% probability that cattle will clear the virus in 7.50 to 8.10 weeks, depending on the probability distribution used.
- There is between 0.54 and 1.09% probability of an animal being viremic after 8 weeks, again depending on distribution used.

CONCLUSIONS

- Viremia persisted up to 9 weeks in cattle naturally and experimentally infected with Australian and U.S. serotypes of BTV, but was considerably shorter in the great majority of animals.

- PCR, which can detect viral nucleic acid up to 180 days after BTV infection of cattle, provides an even more conservative method for detection of BTV infection of cattle than does virus isolation. Animals that are confirmed negative by PCR assay could not pose a risk for movement.

- The longer viremia identified in cattle that were experimentally infected with U.S. serotypes of BTV, as compared to the other study groups, likely reflects the use of calves in several of the studies with U.S. serotypes of BTV. Adult cattle inoculated with U.S. serotypes of BTV had a viremia of comparable duration to that of cattle inoculated with Australian serotypes of the virus.