Risk-based surveillance for bluetongue virus in cattle on the south coast of England in 2017 and 2018

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Abstract

Background Bluetongue (BT) is a viral disease of ruminants and camelids which can have a significant impact on animal health and welfare and cause severe economic loss. The UK has been officially free of bluetongue virus (BTV) since 2011. In 2015, BTV-8 re-emerged in France and since then BTV has been spreading throughout Europe. In response to this outbreak, risk-based active surveillance was carried out at the end of the vector seasons in 2017 and 2018 to assess the risk of incursion of BTV into Great Britain.

Method Atmospheric dispersion modelling identified counties on the south coast of England at higher risk of an incursion. Blood samples were collected from cattle in five counties based on a sample size designed to detect at least one positive if the prevalence was 5 per cent or greater, with 95 per cent confidence.

Results No virus was detected in the 478 samples collected from 32 farms at the end of the 2017 vector season or in the 646 samples collected from 43 farms at the end of the 2018 vector season, when tested by RT-qPCR.

Conclusion The negative results from this risk-based survey provided evidence to support the continuation of the UK's official BTV-free status.

Introduction

Bluetongue (BT) is a viral disease of ruminants and camelids transmitted between hosts by certain species of *Culicoides* biting midges. In susceptible animals, clinical signs of BT infection include fever, haemorrhage, ulceration of oral and nasal mucosa, excessive salivation, facial oedema, coronitis, lameness, abortion, reduction in fertility, weight loss, disrupted wool growth and death.[1] The severity of clinical signs varies and ranges from inapparent infection to mortality depending on the strain of the virus, the species infected and individual host factors such as breed, age or immune status.[2] BT is a concern for animal health and welfare and can result in large economic losses,[3] consequently it is listed as a notifiable disease by the World Organisation for Animal Health (OIE) and requirements for the surveillance and control of bluetongue in relation to the trade of live ruminants, camelids or germplasm are stated in the Terrestrial Animal Health Code.[4] Under the Animal Health Law,[5] BTV serotypes 1–24 are subject to control within the EU and the specific requirements for the surveillance and control of bluetongue in susceptible species in Member States are laid out in Commission Regulation (EC) No 1266/2007.[6]

BT was not reported in northwest Europe until 2006, when a serotype 8 strain of virus (BTV-8) was discovered in the Netherlands. In subsequent years, cases of BT were reported in France, Spain, Italy, Portugal, Germany, Luxembourg, Switzerland, Denmark and the Czech Republic,[7] resulting in the most costly outbreak of the virus in recorded history.[8] The virus was reported in the UK for the first time in September 2007.[9] The

disease was controlled through a combination of vaccination, movement restrictions, vector control and surveillance. A total of 137 affected holdings were reported in southern England in 2007.[10] In accordance with the legislative requirements at the time, active and passive surveillance to demonstrate disease freedom continued after the outbreak for two years. No cases of BTV infection were detected in 2008 and it was possible to demonstrate with 95 per cent confidence that BTV was not circulating at or above a prevalence of 2 per cent in either 2009 or 2010. The data were submitted to the EC and the UK was able to regain BTV-free status, which it has since maintained.

The requirements for implementing Commission Regulation No 1266/2007 were updated in 2012 and are detailed in Commission Implementing Regulation 456/2012.[11] Under this legislation, Member States (including those which are free of BTV) are required to carry out surveillance to detect any possible incursions of BTV. The surveillance should be composed of passive and active surveillance.

For the passive surveillance, there must be a formal and documented system under which owners and veterinarians must promptly report any suspicion of the disease. In the UK, animal health and welfare policy is devolved and in England, Scotland & Wales, operational delivery of passive and active surveillance is undertaken by the Animal and Plant Health Agency (APHA). Suspicion of BTV (and other notifiable diseases) must be reported to APHA. Farm visits and sampling to test for the disease will be conducted where BTV is suspected or cannot be ruled out. In addition, submissions to the APHA diagnostic service will be raised as a suspect case, prompting investigation, where BTV is considered as a differential diagnosis for the clinical and pathological presentation.

For the active surveillance, the legislation states that annual laboratory-based studies may be carried out based on risk assessment. In Great Britain (GB), the outputs of multiple surveillance components are analysed to provide an assessment of the risk of BTV incursion and spread and this is used to target surveillance, when and where it is required. The surveillance components consist of international disease monitoring for cases of BTV reported in neighbouring countries, post import testing of livestock imported from high-risk areas, including BTV restricted zones, atmospheric dispersion modelling to estimate the likelihood that *Culicoides* could be carried from other countries to GB in wind plumes,[12] *Culicoides* trapping to detect the level of midge activity within GB[13 14] and meteorological assessment to determine the likelihood that midges could survive and be competent to transmit the virus if introduced. The outputs of these different surveillance components are combined to provide an overall assessment of conditions that would facilitate the introduction and onward circulation of BTV in GB livestock.

BTV-8 re-emerged in 2015 in France, following an absence of detection of over five years in this region, and spread throughout the country.[15] In May 2017, French authorities reported a case of BTV-8 in the Seine Maritime department close to the north coast.[16] Considering the BTV situation in Europe, targeted, risk-based surveillance was carried out in cattle in the south of England, designed to detect a possible incursion of BTV during the vector seasons of 2017 and 2018. This paper provides a summary of that active surveillance.

Methods

A risk-based, cross-sectional study was carried out in cattle in five counties on the southern coast of England in 2017. In 2018, the study was repeated using the same methodology, with increased sampling in the larger counties of Kent and Hampshire.

In 2017, it was predicted that the most likely period in which transmission of BTV could occur in the GB would be from May to October taking into account both high rates of seasonal vector activity[14] and transmission of BTV in Europe[17]: BTV RNA is detectable by RT-qPCR in blood for five months after infection,[18] therefore if blood samples were collected at the end of the season, RT-qPCR could detect evidence of infection during the highest risk period of the expected season.

Counties were used as the geographical unit of reference for the purposes of BTV monitoring and surveillance, as these are similar in size to the 45×45 km (2000 km^2) units specified in Commission Implementing Decision 456/2012, and are well known units which facilitated the implementation of the sampling plans. International disease monitoring and simulations of midge movement from neighbouring countries indicated that incursions of BTV were most likely to occur along the southern coast of England. Therefore, farms along the coast of Kent, East Sussex, West Sussex, Hampshire and Dorset were the focus for sampling.

Surveillance was focused on cattle as their larger body size results in a greater range of attraction to *Culicoides* than sheep, and they are therefore more likely to be involved in virus transmission.[19 20] Additionally, cattle holdings are likely to be attractive to midges as they tend to contain a range of habitats suitable for *Culicoides* larval development, including dung heaps. A list of all registered premises with cattle was obtained from the APHA customer data base (SAM), Arc GIS (ESRI Arc GIS V.10.2) was used to identify cattle farms within 5–10 miles of the coast in each of the five counties of interest. This distance was chosen on the basis that *Culicoides* would be likely to land immediately following crossing large water bodies and that local dispersal over land is limited to relatively short flights.[21]

Large farms in areas of high cattle density were considered for inclusion in the study if they had over 20 cattle aged between six months and four years, which

had never been vaccinated against BT, had been resident on the farm for more than six months and had access to pasture at dawn and dusk (as this increased the likelihood they would have been exposed to *Culicoides* due to their crepuscular adult activity profile). Farms on which cattle were home bred were prioritised for inclusion in the study as this would increase that likelihood that they would have been located in the geographic area of interest throughout the vector season and there would be complete knowledge of their vaccination history.

As there had not been an outbreak of BTV in the UK since 2008, a design prevalence of five per cent was selected in order to balance the expected low prevalence with the desire to limit the number of farms and animals that would need to be sampled as part of this study. The confidence in detection was set at 95 per cent in accordance with Commission Implementing Decision 456/2012.

The sample size calculations were designed to accommodate a degree of clustering within herds, the overall design prevalence (P) was divided between herd level (P_h) and within-herd level (P_w). In the outbreak in the south east of England in 2007–2008, the mean, within herd prevalence was reported as 11 per cent and the between herd prevalence ranged from 5 per cent to 83 per cent depending on herd size and location.[22] For this study, it was assumed that an overall prevalence of 5 per cent could be distributed as 10 per cent of animals infected within 50 per cent of herds.

The sensitivity of the RT-qPCR assay on pooled, whole blood from infected and viraemic cattle is high (>99 per cent)[23] and so test sensitivity was not specifically included in the calculation. The specificity was assumed to be 100 per cent.

The equation below was used to calculate the sample size based on simple random sampling using a spreadsheet (Microsoft, Microsoft Excel 2013), where P_h was 0.5 and P_w was 0.1, the nW and nH were adjusted until the equation could be balanced to achieve a confidence of 95 per cent or greater. Hypergeometric sampling was not accounted for in these calculations as the sample size was small compared with the population size and the effect on the probability of detection as a result of removing sampled animals from the population was considered to be small.[24] The required sample was calculated as 15 animals being sampled on 6 farms in each county:

$$Se = 1 - \{1 - P_h \times [1 - (1 - P_w)^{nW}]\}^{nH}$$
$$= 1 - \{1 - 0.5 \times [1 - (1 - 0.1)^{15}]\}^6$$
$$= 0.952$$

In order to mitigate against possible dropout from the survey, the aim was to recruit at least eight farms in each county. For the 2018 survey, it was determined that twice as many farms would be sampled in Kent and Hampshire (including the Isle of Wight) as these counties were relatively larger than the other counties included in the survey.

The APHA Customer Service Centre did initial screening to identify potentially suitable farms which were willing to take part in the survey. The contact details for each farm that had agreed to take part in the survey were provided to the APHA Veterinary Delivery Partners (VDPs) (veterinarians contracted to undertake sampling and investigation on behalf of APHA) and they contacted the farmers to arrange the date and time of sampling. The VDPs were provided with the selection criteria for the animals to be sampled and were instructed to collect one blood sample stored in EDTA tubes from 15 eligible cattle on the farm. As the survey was voluntary, the VDPs were advised that sampling could be based on convenience in order to cause the least disruption to the farmers that had volunteered, therefore animals were not randomly sampled. Samples were sent to the Non-vesicular Reference Laboratory at the Pirbright Institute for testing.

The EDTA blood samples were tested in pools of five as described previously,[23] where 100 µl of each sample was used to create the pool. BTV RNA was extracted from 100 µl of the pooled EDTA blood and eluted into 80 µl buffer using the KingFisher Flex automated extraction platform and the MagVet Universal nucleic acid extraction kit (ThermoFisher Scientific, Paisley, UK). Ten microlitres of sample RNA was analysed as per the assay described by Hofman and others[25] using the Express One-Step qRT-PCR kit (ThermoFisher) on an Applied Biosystems 7500 Fast instrument (ThermoFisher).

Results
2017 survey
In total, 478 blood samples were collected from 32 farms in the study, it was only possible to sample 5 farms in West Sussex whereas additional farms were available for Kent and Hampshire, where 7 and 8 farms were sampled, respectively. The required 15 samples were collected from all farms, apart from one on which only 13 samples were collected. All farms sampled were within five miles of the coast (figure 1). See table 1 for the timing of sampling.

Of the 32 farms sampled, 21 (65 per cent) were beef farms. This was an over-representation when compared with the distribution of farm type in the five counties, for which 45.8 per cent of 1525 herds were registered as beef, 51.3 per cent were registered as dairy and 2.9 per cent were registered as mixed.

The largest herd sampled was a mixed dairy and beef enterprise which had 650 cattle in total, the smallest was a beef herd with 31 cattle. The mean herd size was 213 cattle and the median herd size was 175 cattle. The mean herd size for the five counties was 134 cattle and therefore the average herd size in the sample was larger than the average herd size in the wider population.

The cattle sampled were aged between 6 and 48 months, apart from three, one of which was 54 months,
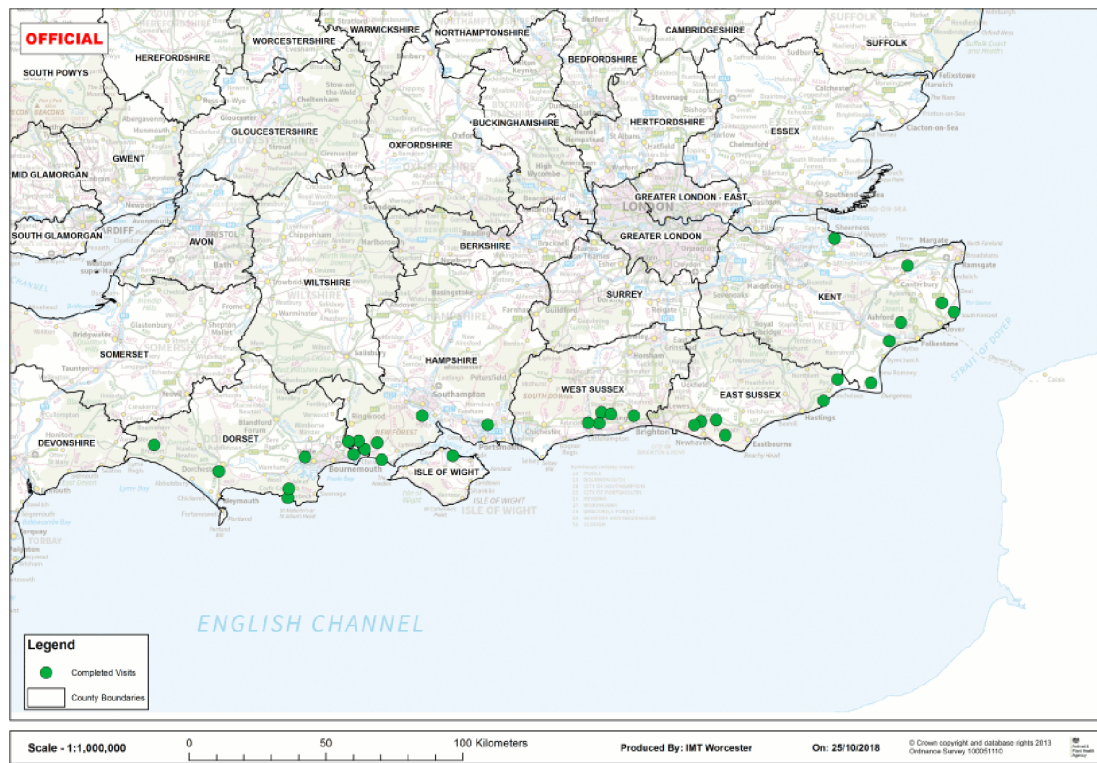
Figure 1 Geographical distribution of farms sampled in Kent, East Sussex, West Sussex, Hampshire, Isle of Wight and Dorset as part of the 2017 survey.

one was 79 and one was 101 months old. The mean age was 19 months and median age was 21 months. The veterinarians that carried out the sampling reported that all of the animals had had access to pasture during the summer months, and that none had been vaccinated against BTV.

All samples were tested negative (no virus detected) using real-time RT-qPCR.

2018 survey

In total, 646 samples were collected from 43 farms in the five counties. The number of required farms (six) was sampled in every county and in East Sussex an additional farm was sampled. The requirement to sample 15 cattle was met on every farm and on one farm in Hampshire one additional animal was sampled (ie, 16 cattle in total) as one sample was presumed to have clotted. All farms were within in 10 miles of the coast with the majority being situated within five miles of the coast (figure 2) . See table 1 for the timing of sampling.

Of the 43 herds sampled 28 (65 per cent) were beef farms. This was an over-representation when compared with the distribution of farm type in the five counties, for which 48.5 per cent of 1431 herds were registered

as beef, 48.6 per cent were registered as dairy and 2.8 per cent were registered as mixed. The largest herd was a dairy holding which had 1200 cattle in total, the smallest was a beef suckler herd with 38 cattle. The mean herd size was 244 cattle and the median herd size was 151 cattle. The mean herd size for the five counties was 140 cattle.

A total of 27 cattle on six farms in total exceeded the age criteria, the mean age was 23 months and median age was 19 months.

The veterinarians who carried out the sampling reported that all of the sampled animals had access to pasture during the grazing period except for one farm, and that none of the sampled cattle had ever been vaccinated against BTV. All of the sampled animals were either homebred or had been present on the farms for over six months.

All samples were tested negative (no virus detected) using real-time RT-qPCR.

Discussion

In both years, the required sample size was met or exceeded in each county with the exception of West Sussex in 2017. This gave 95 per cent confidence in detecting infection, if BTV was present at five per cent prevalence or greater. Where the target sample size was not achieved in West Sussex in 2017, the equation mentioned earlier was used to estimate the design prevalence that could be used where 15 animals were sampled on five holdings to achieve 95 per cent confidence in detection, this was calculated as 5.7 per cent, assuming a 10 per cent within-herd prevalence. The results of the sampling provides evidence in accordance with the requirements of the EC

Table 1 Number of farms sampled per month in the 2017 and 2018 surveys

	2017 (%)	2018 (%)
November	29 (91)	0
December	0	14 (33)
January	2 (6)	21 (49)
February	1 (3)	6 (14)
March	0	2 (4)
Total	32	43

implementing regulation 456/2012[11] that BTV did not occur above a prevalence of 5 per cent (or 5.7 per cent in West Sussex in 2017) in the highest risk area in the high-risk periods of the 2017's or 2018's vector seasons.

Beef herds were over-represented in the samples compared with the proportion of beef farms in the sampled counties. However, it is unlikely that the risk of incursion would be increased in one production type compared with the other[26] and therefore this is not considered to be a limitation. The average herd size in the samples was larger than the average herd size for the five counties. During the recruitment process, larger farms were targeted to ensure there would be enough animals available for sampling, so this result was expected. The larger herd size is not likely to have had a negative impact and in fact may have increased the probability of detection in the sample as a greater abundance of *Culicoides* are likely to reside in areas where there are abundant sources of blood meals and semi-aquatic organic matter.[22] [27]

The real-time RT-qPCR assay can detect BTV RNA present in the blood for five months, therefore in this study infection could be detected if it occurred in the five months preceding sampling. From table 1 it can be seen that in 2017, 91 per cent of the farms were sampled in November, whereas in 2018 all of the sampling took place between December and March. As stated earlier, it was predicted that transmission could occur between May and October, the earliest possible infection that this study could detect would have been in June. It is a limitation that infections that occurred earlier would not have been detected. However, due to a number of factors including midge abundance,[17] cumulative time that the temperature exceeds the threshold for BTV replication in the insect host[28] and increasing numbers of infected ruminant hosts as the outbreak progresses, the highest risk period for transmission and incursion of BTV is likely to be at the end of the expected period of transmission and this study would have been able to detect cases that occurred in the highest risk period.

In order to ensure that all animals sampled were susceptible to BTV during the vector seasons under investigation, the inclusion criteria stipulated that the sampled cattle had to be unvaccinated and over the age of six months (to mitigate the risk of calves having passive immunity from vaccinated or infected dams). The veterinarians carrying out the sampling confirmed that all animals tested met these criteria and therefore the scope for animals to test negative as a result of pre-existing immunity is considered to be minimal.

In order to ease the burdens on farms which had volunteered to take part, the animals tested on each farm did not have to be randomly selected. It is possible this may have introduced bias to the sample, for instance, all 15 animals may have been sampled from one group, which may have been located in an area of the farm with higher or lower exposure to *Culicoides* than another group. It is not possible to ascertain the level of bias that may have been introduced, but it was confirmed that all animals (apart from one farm) had access to pasture during the vector season and therefore the risk of exposure to infection was comparable for all those included in the study.

A risk-based approach was used to select farms in areas where incursion of infected *Culicoides* was most likely to occur, this had the benefit of refining
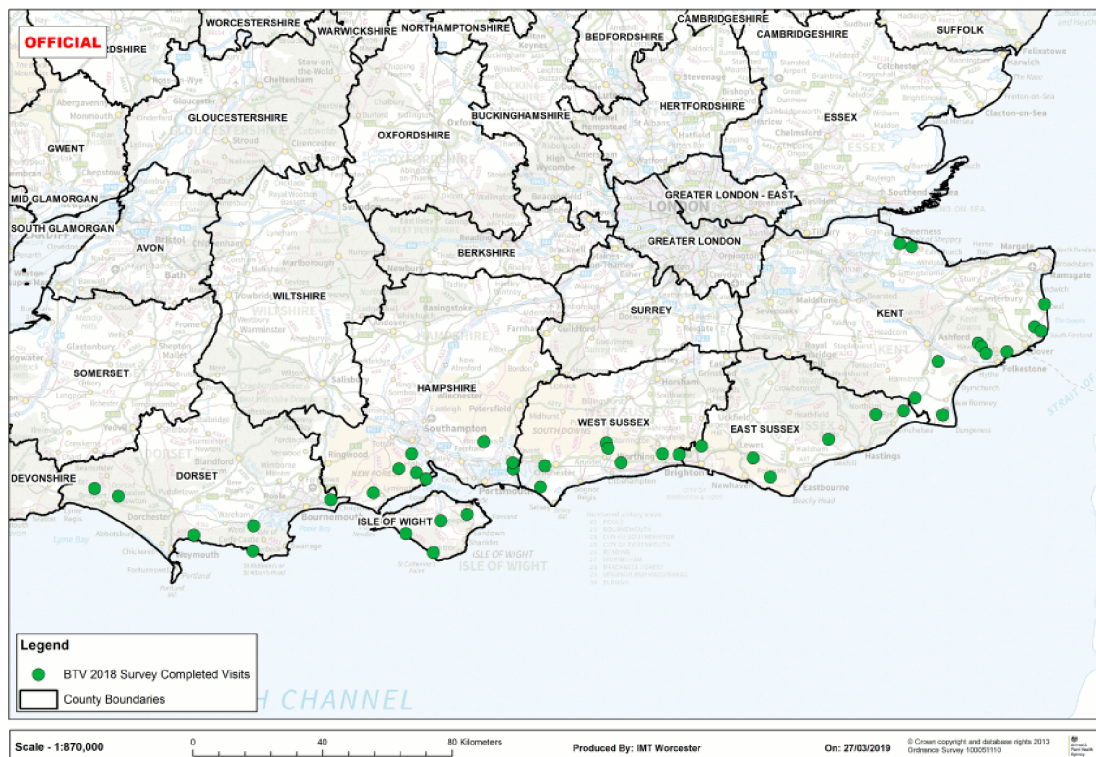
Figure 2 Geographical distribution of farms sampled in Kent, East Sussex, West Sussex, Hampshire, Isle of Wight and Dorset as part of the 2018 survey.

the sampling so that the number of animals that were required to be sampled was reduced compared with random sampling across the whole population. The counties at highest risk were identified by atmospheric dispersion modelling (NAME),[12] which provided a qualitative assessment of risk. The sample size calculations were based on simple random sampling and did not take into account the increased probability in detection as a result collecting samples from an area and species at higher risk, therefore the confidence in detection may be greater than has been calculated. From the surveillance carried out it can be concluded with a high level of confidence that infection would have been detected were it present at a prevalence of five per cent or greater, in accordance with the requirements of Commission Implementing regulation 456/2012.[11]

The approach taken was able to provide assurance that an incursion had not occurred in the highest risk counties during the period of highest expected risk, while minimising the number of animals and farms that needed to be sampled. The surveillance has tested a risk-based approach which proved practical to implement and a similar approach could be adopted again. The voluntary participation of a small number of cattle farmers in a high-risk area of the UK has been able to provide additional evidence which can be viewed alongside other components of the BTV surveillance programme to support the continued BTV-free status of the UK, facilitating trade for ruminant farmers.

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Competing interests None declared.

Patient consent for publication Not required.

Data availability statement Data are available on reasonable request. Further information on the approach to this study may be requested but information on participating farms will not be made available. Requests may be made to Katherine.grace@apha.gov.uk.

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