Evidence for transplacental and contact transmission of bluetongue virus in cattle

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This paper presents evidence that a field strain of bluetongue virus serotype 8 (BTV-8) was transmitted transplacentally and that it was also spread by a direct contact route. Twenty pregnant heifers were imported from the Netherlands into Northern Ireland during the midge-free season. Tests before and after the animals were imported showed that eight of them had antibodies to bluetongue virus, but no viral RNA was detected in any of them by reverse transcriptase-PCR (RT-PCR). Two of the seropositive heifers gave birth to three calves that showed evidence of bluetongue virus infection (RT-PCR-positive), and one of the calves was viraemic. Two further viraemic animals (one newly calved Dutch heifer, and one milking cow originally from Scotland) were also found to have been infected with BTV-8 and evidence is presented that these two animals may have been infected by direct contact, possibly through the ingestion of placentas infected with BTV-8.

BLUETONGUE is an economically important viral disease of ruminants, particularly sheep, which can cause high levels of mortality and abortions in susceptible flocks. There are 24 known serotypes of bluetongue virus (BTV), which belongs to the genus *Orbivirus* within the family Reoviridae (Mellor and Wittmann 2002). Until recently, BTV was confined mainly to subtropical and tropical areas of the world, including Africa, with only a few sporadic incursions into the Iberian Peninsula (between 1956 and 1960); however, outbreaks have become much more frequent in southern Europe since 1998 (Purse and others 2005, Saegerman and others 2008). The virus is thought to be transmitted by specific species of *Culicoides* midges, for example, *Culicoides imicola* in Africa and *Culicoides sonorensis* in North America, and this vector dependency was thought to limit the geographical distribution of the disease. For the disease to be transmitted, the ambient temperature must be adequate to allow for vector activity and for BTV to replicate within the vector, that is, more than 12°C (Mellor and Wittmann 2002; S. Carpenter, personal communication). The mechanism or mechanisms that have enabled BTV to survive over the winter period in regions with more temperate climates, such as northern Europe, are poorly understood. Viraemia in the ruminant host is considered to last for only up to 60 days (Luedke and others 1977, Nunamaker and others 1990, Mellor 1996, World Organisation for Animal Health [OIE] 2007), and although transovarial transmission of BTV in the vector has been suggested as a possible overwintering mechanism, infectious virus has so far not been shown to be transmitted by this route (White and others 2005). The persistent infection of certain host cells (T lymphocytes) has also been suggested, but this mechanism has not been confirmed in the field (Takamatsu and others 2003). Another possibility is the survival of small numbers of adult midges in cowsheds during the winter, which has been recorded in Belgium, although there was no evidence that these midges were infected with bluetongue (Losson and others 2007).

In August 2006, BTV serotype 8 (BTV-8) was found to be circulating in the Netherlands, Belgium, Germany and, to a smaller extent, Luxembourg and France, with the epicentre of the infection being in the area around Maastricht in the Netherlands (Elbers and others 2007, Méroc and others 2008). Infections with BTV-8 reappeared during the summer of 2007, with higher morbidity and mortality rates in sheep and cattle than had been observed in the previous year. The area affected by the disease expanded to include Great Britain, through the windborne spread of infected *Culicoides* midges to south-east England (Gloster and others 2008). The successful overwintering and re-emergence of BTV-8 in 2007 was observed in many regions of northern mainland Europe (Saegerman and others 2008).

In September 2007, the introduction of amended legislation within the European Union allowed for the export of live susceptible animals from bluetongue-affected areas to disease-free areas during periods when competent vectors were shown to be inactive, termed vector-free periods (Anon 2007). Susceptible ruminants that were shown to be free of BTV more than 14 days after the start of a vector-free period could be exported (Anon 2007).

In Northern Ireland, the veterinary authorities considered that susceptible animals imported from countries with bluetongue restriction zones carried an unacceptable risk of being infected with BTV-8. To minimise this risk, all susceptible animals imported into the country were tested seven to 10 days after their importation for evidence of BTV and antibodies to BTV by reverse transcriptase-PCR (RT-PCR) and competitive ELISA (cELISA).

At the time of writing, the island of Ireland is considered free of BTV. However, entomological and serological surveillance programmes were initiated in Northern Ireland during October 2007 in response to the introduction of BTV-8 into England.

This paper describes evidence for the transplacental transmission of a field strain of BTV and for the possibility that BTV may be spread by a direct route, possibly by the ingestion of placenta infected with the virus.

MATERIALS AND METHODS

Farm premises

The farm business on which the bluetongue incident occurred was mainly a sheep and beef cattle enterprise, but a dairy unit was in the process of being established. The business operated three farms, with one holding only sheep (over 700 animals), a second holding only beef cattle (63 animals) and a third (herd 6) holding 171 beef cattle and 51 adult dairy cattle and their calves. The dairy herd was the epidemiological unit that experienced the bluetongue incident; it consisted of 21 animals (20 pregnant heifers and one bull) imported from The Netherlands on January 11, 2008 and their calves, and 30 cows that all originated from one herd (herd 5) in Scotland (imported in three batches on January 28, and February 7 and 14, 2008).

The dairy herd was housed in a new section of a cubicle house adjacent to a newly constructed milking parlour (Fig 1). The cubicle house also accommodated 110 beef cattle

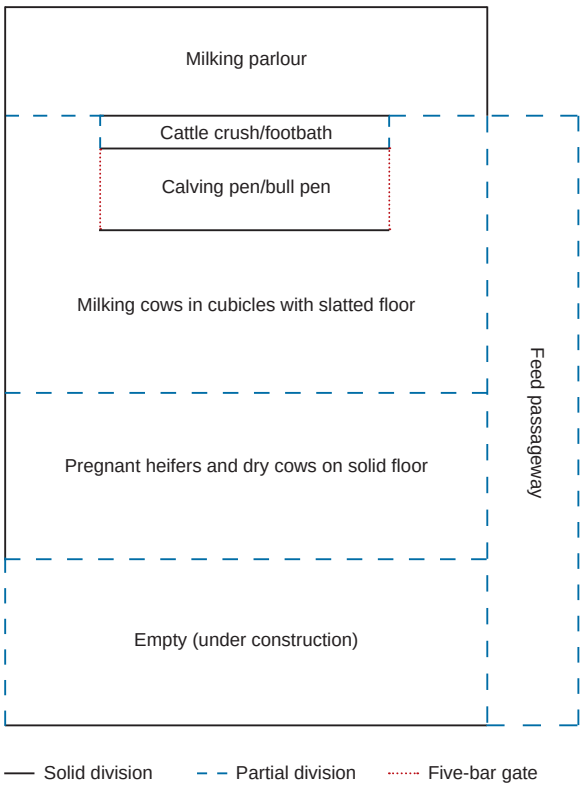

FIG 1: Diagram of the section of the cattle house that accommodated the dairy herd

separated from the dairy herd by a feed passage. The calves were all born in a calving pen in the same cubicle house, and they were moved shortly after calving to another building where they were placed in individual calf pens.

Herd investigations

The epidemiological investigations involved a number of interviews with the herd owner by several interviewers. This approach elicited more information, particularly relating to livestock management, and made it possible to cross-check and validate information previously supplied. Animal movement licences were used to validate the transfer of animals entering and leaving the farming enterprise. Data on the herds of origin of the imported animals were also obtained.

The 21 animals imported from the Netherlands were subjected to postimportation bluetongue tests, 11 and 30 days after their arrival on the farm (Fig 2). These animals had all tested negative for BTV viral RNA by RT-PCR on January 4, before they were exported from the Netherlands, and the same samples were again negative when they were tested after the disclosure of the outbreak by the Institute for Animal Health, Pirbright. Owing to the results of the postimportation tests, a further herd and flock test was carried out on all the cattle and sheep owned by the farming enterprise on February 15; the blood samples from the cattle were tested by RT-PCR and cELISA and the samples from the sheep by cELISA only. Samples of spleen and lymph nodes were taken postmortem from animals that tested RT-PCR-positive for BTV and from a subset of animals within the same cohort. Samples of fetal blood and fetal spleen samples were also obtained on February 18 from seven of the pregnant heifers for testing by RT-PCR, together with spleen samples from the heifers. All the samples found to be RT-PCR-positive were subjected to virus isolation procedures. Blood samples from the five calves and their dams were cross-checked to authenticate their parentage by use of a microsatellite DNA test (StockMarks for bovine parentage typing kit; Applied Biosystems).

Laboratory tests

RNA extraction and RT-PCR The RNA was extracted from 200 µl EDTA blood samples lysed in 300 µl lysis buffer (Roche) by using the MagNA Pure LC automated extraction method (Roche). The NA/External lysis protocol (Total) was used to give a final elution volume of 50 µl. The RT-PCR was carried out as described by Shaw and others (2007), except that a single redundant probe was used to amplify the cDNA (C. Batten, personal observation), on a Mx3005p (Stratagene), and analysed using the manufacturer's software.

cELISA Serum samples were tested for the presence of BTV antibodies using a commercial ELISA kit (Bluetongue Competitive ELISA; Institut Pourquier) according to the manufacturer's instructions.

Virus isolation Blood samples positive for BTV RNA by RT-PCR were washed three times with phosphate-buffered saline to remove antibodies. The washed blood samples were sonicated to lyse the red blood cells and release BTV. Washed, lysed BTV-positive blood (200 µl) was diluted in 1·8 ml Schneider's insect medium (Sigma) supplemented with 10 per cent fetal calf serum (Sigma), 1 per cent 100 iu/mg/ml penicillin/streptomycin (Sigma) and 1 per cent 2·1 mg/ml amphotericin B (Sigma). The media was removed from 75 cm^3 flasks of confluent KC cells (*Culicoides variipennis* larvae cell line) and the cell monolayer was inoculated with 2 ml inoculum for 30 minutes at room temperature to allow the virus to absorb. The flask was then topped up with approximately 10 ml of supplemented Schneider's medium and incubated overnight at 25 to 26°C. The following day, the media and inoculum were removed and replaced with 20 ml supplemented Schneider's medium, the cells were then incubated for five to seven days at 25 to 26°C. Virus isolation was determined by BTV segment 1 real-time RT-PCR.

Entomological surveillance

Twelve Onderstepoort-type suction light traps had been installed at locations across Northern Ireland, each being operated for one night a week from October 15, 2007. Each of the 240 V down draught light traps was equipped with a 28 cm, 8 W blacklight tube (F8W/BLB; General Electric) and the insect collection methods used were similar to those described by Venter and Meiswinkel (1994) and Goffredo and Meiswinkel (2004).

After the positive serological tests on January 22, one Onderstepoort-type suction light trap (Advanced Pest Solutions; University of Edinburgh) was installed, on January 23, in the cattle shed that housed the animals that had been imported from the Netherlands on January 11. Two further light traps were installed in two more of the farm buildings on February 15. These three traps were new, operated continuously, and were examined and re-set at 48-hour intervals.

Adult midges were identified under x 100 magnification using the taxonomic keys of Rawlings (1996) and Glukhova (2005).

Meteorological data

Meteorological data were extracted from the Met Office database, to estimate the likelihood of viral replication in the midge population and estimate the level of midge activity. Hourly observations of temperature, precipitation and wind speed were obtained for the period from January 10 to February 25 from an automatic station situated less than 15 km from the infected premises.

Wittmann and others (2002) investigated the extrinsic incubation period of midges as a function of temperature.

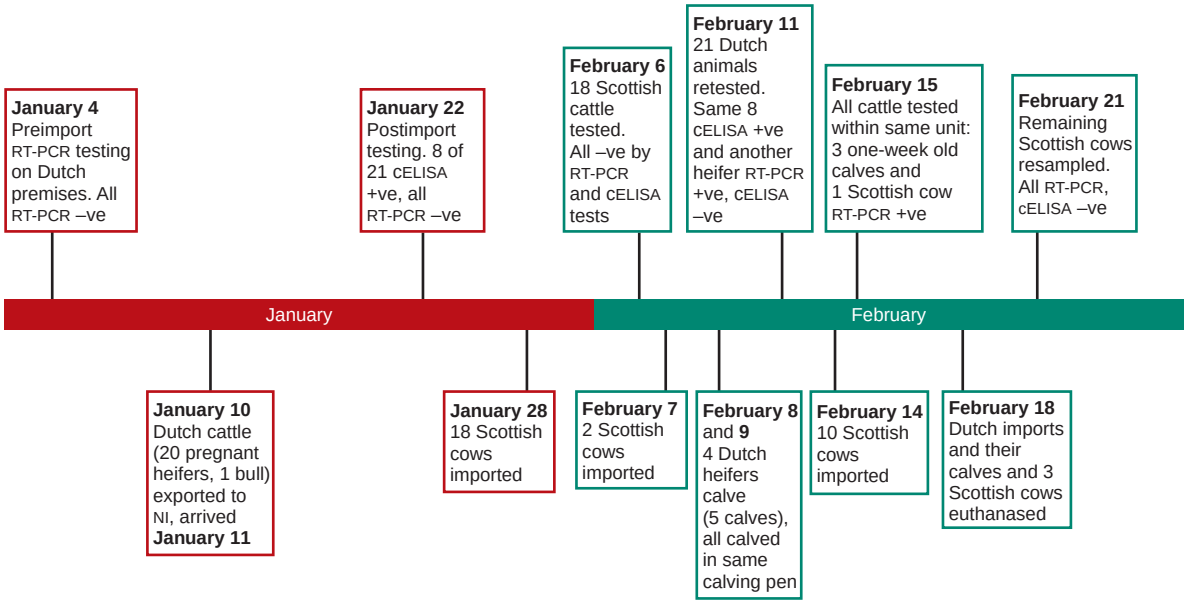

FIG 2: Time line of animal movements, sampling dates and test results. RT-PCR Reverse transcriptase-PCR, +ve Positive, NI Northern Ireland, cELISA competitive ELISA, –ve Negative

They concluded that at 15°C, 16 days were required, but that at 30°C, only two days were required; temperatures below 12°C are predicted to inhibit virus replication.

Midge activity is also related to temperature, and although midge activity cannot be excluded completely below 10°C its level is likely to be low. Hendry (1996) found that wind speeds of more than 3 m/s reduced midge activity, and Gloster and others (2008) considered that precipitation would be likely to reduce flight activity.

To assess the level of viral replication and midge activity, the numbers of hourly observations when the temperature was more than 10°C, more than 12°C and more than 15°C were totalled. For each hour during the dusk to early evening period that the temperature exceeded these limits, the wind speed and the presence or absence of precipitation were investigated. The dusk to early evening period was assumed to be from 15.00 to 21.00. If the wind speed was more than 3 m/s (6 knots) and/or if rainfall was recorded, the likelihood of midge flight was discounted.

RESULTS

Dairy herd management

A time-line describing the animal movements on to the farm and the sampling dates and test results is shown in Fig 2. The 21 animals from the Netherlands were the first dairy animals to arrive on the farm, on January 11, 2008. The 20 pregnant heifers originated from four herds (herds 1 to 4) and were placed in a section of the cattle house with a solid floor; the bull was placed in a separate pen (Fig 1).

The milking herd was initiated by the importation of 18 cows from Scotland on January 28, followed by two further consignments of two cows on February 7 and 10 cows on February 14. The milking cohort was placed in a section of the cattle house next to the milking parlour (Fig 1) that surrounded the calving pen, in which four of the pregnant heifers gave birth on February 8 and 9.

Parenteral drug administration

One of the Dutch heifers received parenteral antibiotics for lameness shortly after its arrival. Heifer 1 received oxytocin injections to assist with milk let-down at the first three milkings after it calved on February 8. Heifer 2 received parenteral and intramammary antibiotics for three days after calving, on February 8 to 10. Twin calves born on February 9 received parenteral antibiotics for diarrhoea the day before they were euthanased on February 18. No other injections were administered to any of the dairy herd and the syringes and needles used were different for each animal, apart from the twin calves, for which the same equipment was used to administer the antibiotics.

Calving details

Four of the pregnant heifers (three of which were cELISA-positive) calved on the premises on February 8 and 9. The births were observed and all four heifers calved unassisted in the one calving pen (Fig 1). They all successfully completed the third stage of labour in the calving pen without any intervention and joined the milking herd after being released from the calving pen. The placentas were not removed by the herd keeper and the only action taken between the births was the addition of straw for extra bedding in the calving pen. All the calves were born healthy and received their first two feeds of colostrum from an oesophageal feeder, which was washed with warm water between uses. Parentage testing confirmed that the calves and dams were correctly matched by the herd owner.

Evidence for transplacental transmission of BTV-8

The 21 heifers imported from the Netherlands were tested before they were imported (RT-PCR only), and again 11 days after they were imported into Northern Ireland. On both occasions, all 21 tested negative by RT-PCR and eight of them tested positive by cELISA on postimportation testing (Table 1, Fig 3). One of the cELISA-positive, RT-PCR-negative heifers (heifer 1) calved at 06.00 on February 8 and was with its calf (calf 1) for at least two hours before joining the milking herd. A second cELISA-positive heifer (heifer 3) calved at 13.00 on February 9, after being moved into the calving pen that morning. This heifer produced identical twin calves (calves 2 and 3). Blood samples were taken from the three calves on February 15. Calf 1 tested RT-PCR-positive with a cycle threshold (C_t) of 22, indicating a high level of viral RNA consistent with that expected at the peak of infection, and BTV was isolated from the blood sample. Calves 2 and 3 were also positive by RT-PCR, giving C_ts of 27 and 28, respectively, but no virus was isolated from the blood of these calves.

Evidence for contact transmission of BTV-8

One of the RT-PCR-negative and cELISA-negative heifers imported from the Netherlands (heifer 2) was moved into the calving pen soon after heifer 1 had calved, and gave birth

TABLE 1: Sampling dates and results of reverse transcriptase-PCR (RT-PCR) and competitive ELISA (cELISA) for bluetongue virus for positive animals in a herd in Northern Ireland, 2008

Animal	Description	Herd of origin	Test	January 4	January 22	February 6	February 11	February 15	February 18
Heifer 2	PCR +ve Dutch heifer: dam of calf 4	2	RT-PCR (C_t)	–	–	NT	+ (25, 22)*	NT	NT
			cELISA	NT	–	NT	–	NT	NT
Calf 1	PCR +ve Dutch calf	6	RT-PCR (C_t)	NT	NT	NT	NT	+ (22)†	+ (23)
			cELISA	NT	NT	NT	NT	+	+
Calf 2	PCR +ve Dutch calf	6	RT-PCR (C_t)	NT	NT	NT	NT	+ (27)†	+ (28)
			cELISA	NT	NT	NT	NT	+	+
Calf 3	PCR +ve Dutch calf	6	RT-PCR (C_t)	NT	NT	NT	NT	+ (27)†	+ (29)
			cELISA	NT	NT	NT	NT	+	+
Calf 4	Dutch calf 4: PCR +ve Dutch dam	6	RT-PCR	NT	NT	NT	NT	–‡	NT
			cELISA	NT	NT	NT	NT	+‡	NT
Calf 5	Dutch calf 5	6	RT-PCR	NT	NT	NT	NT	–	–
			cELISA	NT	NT	NT	NT	+	+
Heifer 1	Dam of calf 1	1	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 3	Dam of calves 2 and 3	1	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 4	Dam of calf 5	1	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 5	ELISA +ve heifer	1	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 6	ELISA +ve heifer	1	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 7	ELISA +ve heifer	1	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 8	ELISA +ve heifer	3	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Heifer 9	ELISA +ve heifer	4	RT-PCR	–	–	NT	–	–	–
			cELISA	NT	+	NT	+	+	+
Cow 1	PCR +ve Scottish cow	5	RT-PCR (C_t)	NT	NT	–	NT	+ (29)	+ (27)
			cELISA	NT	NT	–	NT	–	–

* Cycle threshold (C_t) 25 sampled on February 11, C_t 22 sampled on February 14
† Sampled on February 16
‡ Sampled on February 14 and 15
– Negative test, NT Not tested, + Positive test

at 15.00 on February 8 to calf 4. Heifer 1 and calf 1 had been moved out of the calving pen a few hours previously. However, the placenta from heifer 1 had not been removed, and the only action taken between the births was the addition of extra straw bedding. Heifer 2 would therefore have had free access to the placenta produced by heifer 1, which remained in the calving pen and was likely to have contained high titres of BTV. Three days later, on February 11, heifer 2 was tested and found to be RT-PCR positive, with a C_t of 25, and cELISA-negative. The heifer was tested three days later at slaughter and was again RT-PCR-positive, with a C_t of 22, and cELISA-negative. These results indicated that the heifer was in the early stage of infection (three to six days after infection), suggesting that it was likely to have been infected with BTV at calving, when it had contact with the placenta from heifer 1. Samples of spleen and lymph node from the heifer also tested RT-PCR-positive, and BTV was isolated from a blood sample. Samples taken from this heifer's calf (calf 4) revealed that it was cELISA-positive and RT-PCR-negative. This was expected, because calf 4 was fed colostrum from heifer 1 (which was antibody-positive) because its dam, heifer 2, had mastitis.

Further testing of all the in-contact animals carried out on February 15 revealed that another dairy cow (cow 1) brought on to the farm on January 28 was RT-PCR positive, with a C_t of 29, and cELISA negative. Cow 1 was resampled three days later on February 18, when it had a higher viral RNA titre (C_t of 27) but remained cELISA negative. Bluetongue virus was isolated from its blood. These results indicated that cow 1 was in the early stage of infection. Cow 1 was in a section of the cattle house next to the milking parlour (Fig 1) that surrounded the calving pen, so that it would potentially have had access to the infected calves and their calving products. Cow 1 had also been tested on February 6, 10 days after its arrival on the farm, when it was RT-PCR and cELISA-negative, indicating that it had been infected while on the farm.

All the remaining animals imported from the Netherlands and their offspring were slaughtered on February 18, and samples were obtained from seven of the heifers (Fig 3). In three of the four heifers whose blood samples were RT-PCR-negative but cELISA-positive, the spleen samples were RT-PCR-positive with high C_ts indicating that low levels of BTV RNA were persisting for longer in the spleen than in the blood. The spleen samples from a further three heifers whose blood samples were RT-PCR-negative and cELISA-negative were RT-PCR-negative. The blood and spleen samples obtained from the fetuses of all seven heifers were all RT-PCR-negative.

These results provide strong evidence for the transplacental and contact (possibly oral) transmission of BTV-8. The most probable sequence of events relating to the transmission of BTV during this outbreak is shown in Fig 4.

Herds of origin

The eight RT-PCR-negative, cELISA-positive Dutch heifers originated from three herds (herds 1, 3 and 4) and six of them came from herd 1 (Table 1). The three RT-PCR-negative, cELISA-positive heifers that calved had been artificially inseminated between May 1 and 7, 2007, and all came from herd 1. Herd 1 was first reported as being BTV-8 infected on September 10, 2007 and BTV-8 had first been identified as being in the region of herd 1 on August 4. The RT-PCR-positive, cELISA-negative heifer (heifer 2) originated from herd 2, which has not been recorded as being infected with BTV-8. The herd of origin of the 30 Scottish cows was in a country that was free of BTV-8 infection and all 30 cows initially tested negative by RT-PCR and cELISA tests carried out 10 days after they had been imported (Fig 2).

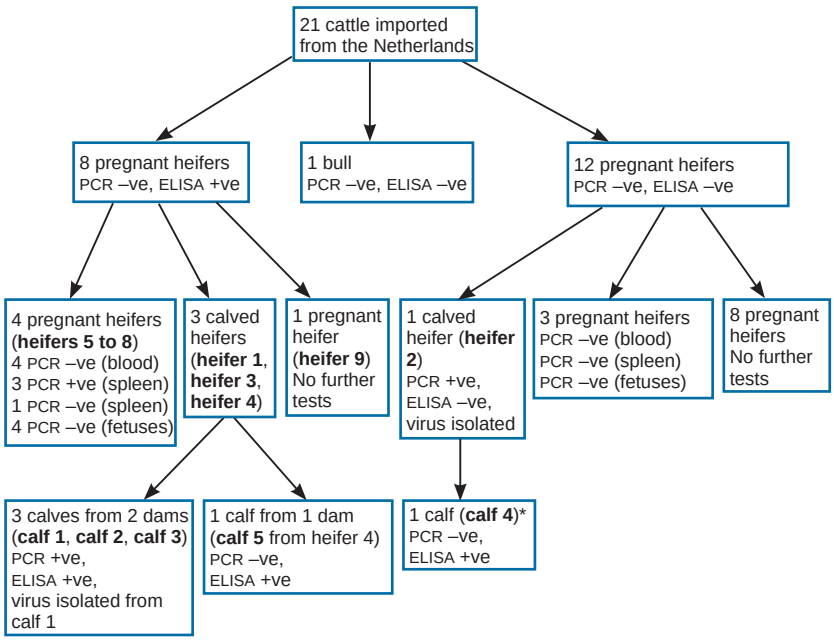

FIG 3: Diagrammatic representation of the laboratory results in relation to the heifers and their offspring. * Calf 4 was ELISA-positive because it had received colostrum from heifer 1. –ve Negative, +ve Positive, PCR Reverse transcriptase-PCR, ELISA Competitive ELISA

Entomological and meteorological data

The main route of transmission of BTV is through the host being bitten by infected *Culicoides* midges. However, the 12 vector traps located throughout Northern Ireland had not captured any parous *Culicoides* since late December 2007. Furthermore, the vector traps placed inside the buildings on the affected premises failed to capture any parous *Culicoides*. One nulliparous female *Culicoides obsoletus/scoticus* was found in samples collected from the suction light trap in the calving shed on February 25, seven days after the removal of the RT-PCR positive calves and cow.

The meteorological analysis showed that viral replication was unlikely to have been possible in the midge population (Fig 5). At no time during the period did the ambient temperature reach 15°C (a maximum temperature of 14·2°C was recorded on February 13) and there were only 17 hours out of a possible 1128 that were above 12°C. If a lower limit of 10°C is adopted, there were only 96 hours above this threshold (equivalent to four days), well short of the 16 days at 15°C established by laboratory measurements as the required incubation period.

Out of the 96 hours when the temperature was more than 10°C, only 27 observations were made in the dusk period, and of these there were only nine hours when there was no precipitation and the wind speed was less than 3 m/s. It is therefore likely that midge activity was either minimal or nonexistent, and this conclusion is consistent with the trapping data.

DISCUSSION

This paper describes a field outbreak of bluetongue in imported cattle and their calves in Northern Ireland (a BTV-free country) during a vector-free period. This outbreak provided the opportunity for a detailed epidemiological study of the transmission of BTV-8 in the absence of the *Culicoides* vector. The *Culicoides* surveillance programme that started in October 2007, along with the *Culicoides* monitoring inside the affected cattle house and the temperature monitoring at a nearby meteorological station, all provide good evidence that Northern Ireland was in a vector-free period during the period of risk for the transmission of BTV during this outbreak. It is unlikely that midges were overwintering in the cattle shed, as has been suggested has happened in Belgium (Losson and others 2007), because no midges were caught in new Onderstepoort-type light traps placed in the shed during the period when infection was possible.

The failure to detect BTV viral RNA in the blood samples analysed and confirmed by different laboratories in the Netherlands, Northern Ireland and England, taken 18 days apart from the 20 pregnant heifers and one bull before and after they were imported from the Netherlands, indicates that they were not viraemic when they were imported or shortly afterwards. The results of the RT-PCR testing of the animals from the Scottish herd after they were imported, together with the fact that Scotland is free of BTV, indicate that these animals were not the source of the virus for the animals imported from the Netherlands.

The detection of eight cELISA-positive, RT-PCR-negative pregnant heifers (six from herd 1) indicated that these animals had been exposed to the infection several months before they were imported into Northern Ireland. The time when the two cELISA-positive heifers that gave birth to transplacentally infected calves had been infected with BTV was estimated to be between 90 and 150 days of gestation, because they had been inseminated in early May 2007 and BTV-8 was first detected in the region in early August 2007. Furthermore, all the heifers were RT-PCR-negative in early January 2008, and in the majority of cases viral RNA is thought to persist for at least 100 days after infection. The immune system of cattle does not become fully competent until approximately 150 days' gestation, so that it was possible that the calves could have been immunotolerant to BTV if the virus had crossed the placenta before the calf's immune system was fully

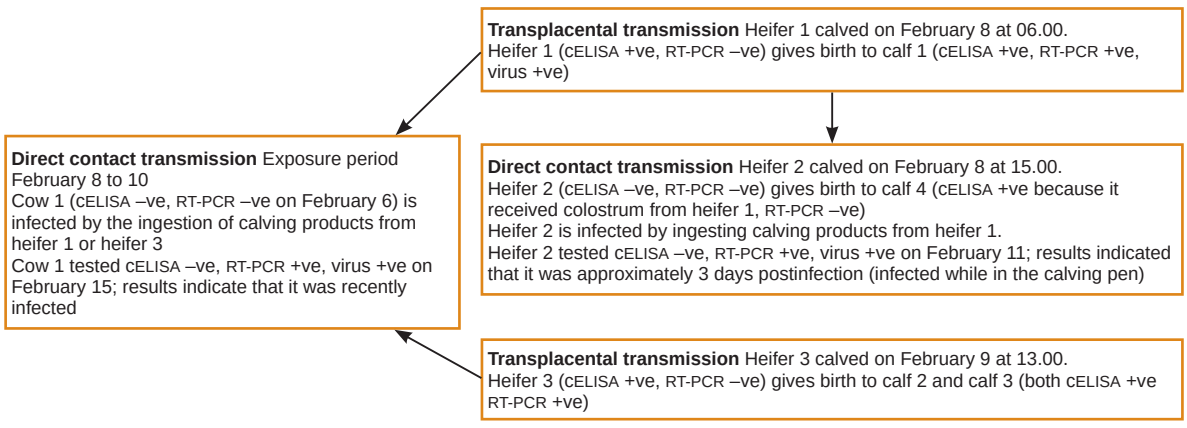

FIG 4: Most probable routes by which three calves were infected with bluetongue virus transplacentally, and two adult cattle were infected by direct contact with infected calving products. RT-PCR Reverse transcriptase-PCR, cELISA Competitive ELISA, +ve Positive, –ve Negative

competent (Brown and others 1979). Sheep and cattle have been infected transplacentally by laboratory-adapted, tissue culture-passaged strains of the virus, although the results of such experiments have been inconsistent (Bowne and others 1968, Luedke and others 1970, 1977, Gibbs and others 1979, Luedke and Walton 1981, Richardson and others 1985, Roeder and others 1991, Parsonson and others 1994). In the present outbreak, strong evidence was obtained that transplacental infection was occurring with the field strain of BTV-8 currently circulating in northern Europe. The three one-week-old RT-PCR-positive healthy calves, one of which (calf 1) was confirmed as viraemic, born to two cELISA-positive, RT-PCR-negative dams provide evidence of the transplacental transmission of a field strain of BTV, because there was no evidence for its transmission by the natural vector or by iatrogenic or mechanical means.

The infection of heifer 2 and cow 1, which were both found to be viraemic and in the early stages of infection, is more difficult to explain. Heifer 2 occupied the same calving pen only hours after heifer 1 had given birth to the viraemic calf 1, and the products of that calving had not been removed from the pen. These calving products, including the placenta, are likely to have been contaminated with BTV-8. It is normal behaviour for a periparturient cow or heifer to ingest placenta, and this is postulated as the most probable route of infection of heifer 1, in the absence of any evidence of iatrogenic or vector spread of BTV-8. Such behaviour may explain why heifer 2 became infected but its calf (calf 4) did not. Oral transmission of BTV has never been documented in ruminants, although there is circumstantial evidence of oral transmission in wild and domestic carnivores through the assumed ingestion of prey infected with BTV (Alexander and others 1994). Additionally, African horse sickness virus, an orbivirus that is closely related to BTV, can be transmitted by the oral route (Van Rensburg and others 1981).

The detection of BTV RNA by RT-PCR in the blood of cow 1, which was kept within the milking group, provides further evidence that BTV-8 can be spread by direct contact or ingestion. This cow had the potential to come into direct contact with all three RT-PCR-positive calves and the products of their dams' calvings. Although the same opportunity for infection was present for both adult animals, the lower level of viraemia detected in cow 1 than heifer 2 may be accounted for by a lower infective dose, because cow 1 did not spend time in the calving pen.

The possibility that heifer 2 or cow 1 might have been the source of the outbreak and that they infected calves 1 to 3 by direct contact transmission is remote. Both of these adult cattle were negative by RT-PCR and cELISA 10 to 12 days after their arrival on the farm (Fig 2), indicating that they were not infected when they were imported into Northern Ireland; it is therefore very unlikely that they could have been the source of the outbreak. Furthermore, heifer 2 had no direct contact with calf 1 and there was no opportunity for the iatrogenic spread of BTV-8 from heifer 2 to calf 1. However, cow 1 did have the potential for limited contact with all three RT-PCR-positive calves, but this animal had considerably more contact with the rest of the milking herd, which remained uninfected. It is therefore considered very unlikely that BTV could have spread by direct contact from either heifer 2 or cow 1 to the three calves.

BTV viral RNA persisted in the spleen samples from three of the pregnant heifers that were cELISA-positive for longer than in their blood samples. This observation requires further experimental investigation to determine whether it is important in the maintenance and transmission of BTV-8 within susceptible populations. BTV has previously been isolated from the internal organs of a cow, infected under field conditions, more than 100 days after virus was isolated from its blood (Bowne and others 1968).

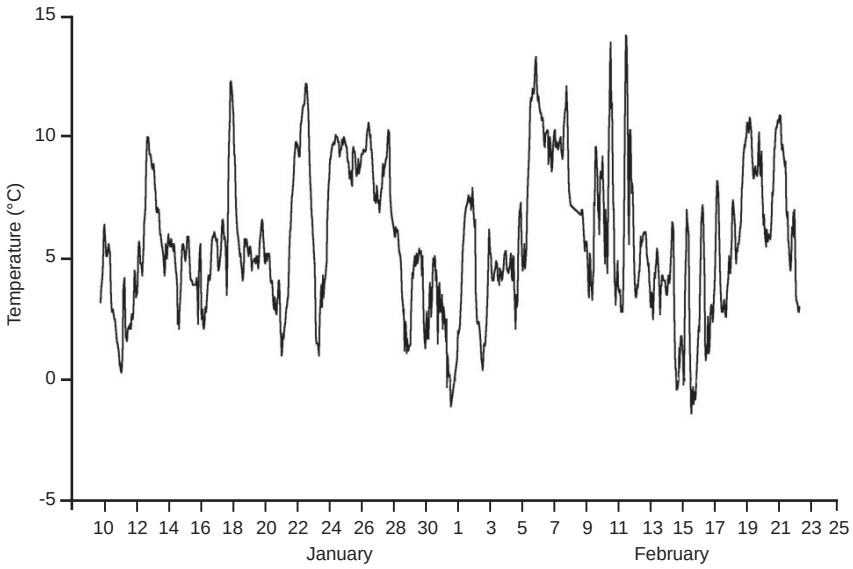

FIG 5: Hourly air temperature readings from a meteorological station located less than 15 km from the affected premises between January 10 and February 25, 2008

This field outbreak of BTV-8 provides compelling evidence for the transplacental transmission of the virus, and circumstantial evidence for its transmission by direct contact, most probably by the ingestion of infected placenta. With the overwintering mechanisms of BTV still unknown, transplacental transmission would provide a means by which BTV-8 could survive the extended vector-free period that can occur in temperate climates. The outbreak also emphasises the need for caution when BTV antibody-positive, RT-PCR-negative pregnant animals are moved into countries or regions that are free from the disease.

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