Belgian Federal agency
for the safety of
the food chain

Report on the occurrence of a BTV11 strain in Belgium

Summary

In January 2009, the occurrence of BTV11 was diagnosed by the Community Reference Laboratory (CRL) in Pirbright in a cow in the northern part of Belgium. Since, in total, 14 holdings with 1 to 3 animals that have been in contact with the virus have been found in the framework of the various monitoring schemes in Belgium.

The laboratory analysis of the samples, both by the Belgian National Reference Laboratory (NRL) VAR and the CRL, the absence of relevant epidemiological links between the holdings and the geographical spread of the holdings suggest limited spread of virus by culicoides during the previous vector season. The absence of clinical signs, the very low prevalence both on herd level and on animal level, as well as the limited genetic information that is available all suggests that the virus involved is most likely a vaccine strain and not a field strain of BTV11.

As a precautionary measure awaiting further results, the Belgian authorities have delimited a temporary control zone around the holdings concerned. Since 17 February 2009, all ruminants for breeding and fattening that are kept in this control zone or that, at one time after 1 November 2008, were kept in this zone, have been sampled and virologically tested before dispatch to other Member states.

In view of the information currently available, it is now appropriate to lift this control zone.

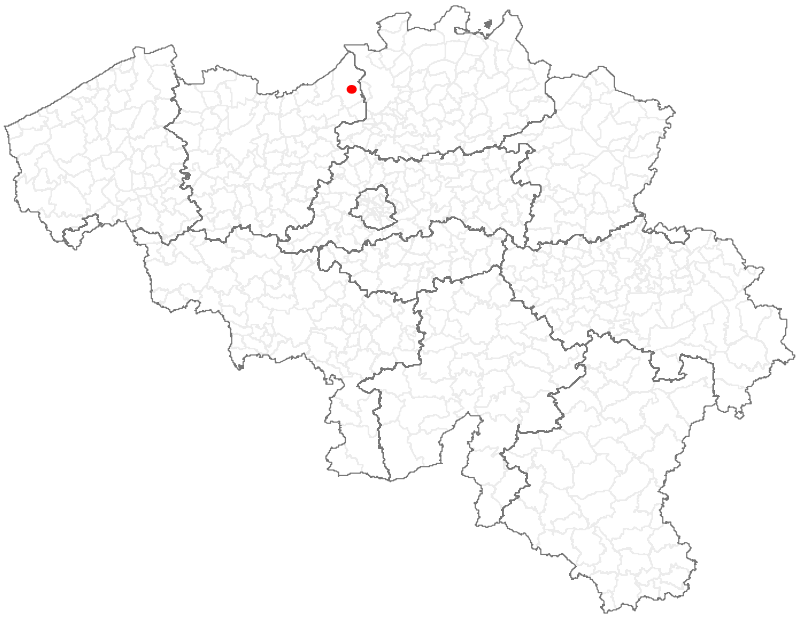
Initial findings regarding BTV11

On 20 November 2008, the Belgian NRL VAR notified an unusual laboratory result to the Belgian Federal Agency for the Safety of the Food Chain (FASFC): a sample taken in the framework of the Belgian passive surveillance programme tested positive in the generic PCR without showing the presence of RNA in the serotype specific PCR for serotypes 1, 6 and 8. BTV8 – being present in Belgium since 2006 – and BTV1 and BTV6 – both present or circulating in neighbouring countries and thus being the most likely serotypes to be introduced into the Belgian herd – are routinely included in all virological analysis that is conducted once the generic PCR that detects the presence of all 24 serotypes indicates the presence of a BTV.

The sample had been taken earlier the same month on a cow that had given early birth to a weak calf that died soon after delivery. Since the beginning of 2008 and the occurrence of congenital problems due to the transplacental transmission of BTV8, samples (blood, serum,

foetal material) of cows that have aborted or given early birth to weak calves are routinely tested for BTV. The holding in question, a mixed dairy farm, is situated in the province of East Flanders, close to the Dutch border and in the vicinity of the city of Antwerp (see figure 1).

Figure 1. Location of the first detected holding with BTV11 (red dot)



In compliance with the policy adopted when confronted with the possible introduction of another BTV than BTV8, FASFC staff visited the premise and an inquiry was launched. The investigation did reveal neither clinical signs nor history of clinical signs. Tracing of movements on the farm did not show any contact with the risk areas where other BTV than BTV8 are circulating. The suspected cow and 49 other animals present in the farm were sampled (blood and serum) for a serological and virological analysis.

The initial analysis of these samples was concluded in the first week of December. The result of the initial virological analysis of the suspected animal was confirmed; none of the 49 other animals sampled showed the presence of a BTV. As shown in table 1, the results of the serum neutralization test (SNT) of the serum of the suspected cow revealed low but consistent titres for BTV1 both in the initial and the second sample. An attempt to isolate the virus was not successful. Additional testing by the NRL with other primers gave the same result. Testing for BTV2 that is present in the Mediterranean basin also turned out to be negative.

On 20 December 2008, samples were sent to the CRL in Pirbright. The initial analysis by Pirbright fully confirmed the results of the NRL (see table 1).

On 15 January 2009, after further analysis by PCR, the CRL indicated the presence of a BTV11. This finding was confirmed on 21 January 2009, when Pirbright succeeded in showing significant SNT titres against this virus in the serum. As was the case at the VAR, the virus involved could not be isolated, thus restricting the genetic analysis.

BTV11 had never before been diagnosed in Europe. The virus is only present in Africa, Southeast Asia and Central and Northern America.

Table 1. Average Ct values and SNT titres found by the NRL and the CRL in the suspected animal

sample	PCR					SNT			
	BTV	BTV1	BTV6	BTV8	BTV11	BTV1	BTV6	BTV8	BTV11
NRL sample 1	28,84	neg	neg	neg	na	1:80	1:30	<1:20	na
NRL sample 2	30,75	neg	neg	neg	na	1:120	1:20	<1:20	na
CRL sample 2	31,61	neg	neg	neg	pos	na	na	na	1:320
NRL sample 3	33,00	neg	neg	neg	pos	na	na	na	na

na = result not available; neg = negative result; pos = positive result
date of sampling - sample 1: 05.11.2008; sample 2: 21.11.2008; sample 3: 19.01.2009

Initial measures and actions taken after confirmation by the CRL

Following the confirmation of BTV11 by PCR, the FASFC immediately informed the European Commission (EC) of the results and the actions undertaken till that point.

In the days following, the Belgian NRL VAR developed a PCR for BTV11 thus including this serotype into the standard virological testing procedure for BTV.

In the days and weeks following the detection, several attempts were made by the CRL and the VAR to isolate the virus: neither succeeded. Thus far, the genetic sequencing has therefore been performed on RNA material extracted from the blood and has been limited to segment 2, the segment that controls the virus serotype. The analysis by Pirbright showed a 99,73% similarity between the Belgian virus and the South African reference strain of BTV11. The strain differed markedly from a USA field strain (84% similarity) and from a Zimbabwean field strain (result intermediate between the reference strain and the USA strain). Further attempts are currently undertaken to expand the genetic analysis and obtain more information from this field.

FASFC staff conducted a new inspection on the farm involved. Again the investigation did not reveal any indication for the presence of BTV, nor any plausible route of introduction other than transmission by culicoides. All 206 ruminants on the farm were sampled (blood and serum). Analysis at the VAR only confirmed the BTV11 positive cow, but revealed no other infected animals on the farm.

In the last week of January 2009 and the first week of February 2009, in order to rule out or confirm spread of the virus and to get a clearer view of the situation:

- the sampling of all ruminants present in a 1 km radius around the farm involved was conducted;
- the analysis of the samples taken in the framework of the winter monitoring was speeded up (see below);

- all BTV-positive samples of 2008 were analysed again with the BTV11 specific PCR to rule out any previously missed infections.

Results of the monitoring and surveillance

Investigations in the 1 km area

The 20 farms present in a 1 km radius around the initial premise were all sampled by the farm veterinarians: 16 cattle holdings keeping 841 animals and 4 sheep holdings keeping in total 66 sheep.

Table 2 summarizes the results of the laboratory analysis of these animals: 9 animals on 5 holdings were revealed positive for BTV11 with the PCR; most animals showed Ct values near the threshold of 40.

Table 2. Results of the laboratory analysis of all ruminants in the 1 km area around the initial farm (including that farm)

farm ID	species	number of animals sampled	number of BTV11 positive animals	average Ct values of the PCR	prevalence
initial farm	cattle	206	1	30,9	0,5 %
farm 2	cattle	24	0	-	0,0 %
farm 3	cattle	1	0	-	0,0 %
farm 4	cattle	89	1	37,9	1,1 %
farm 5	cattle	125	3	38,7 - 39,4 - 39,5	2,4 %
farm 6	cattle	147	0	-	0,0 %
farm 7	cattle	3	0	-	0,0 %
farm 8	cattle	9	0	-	0,0 %
farm 9	cattle	30	2	33,1 - 35,8	6,7 %
farm 10	cattle	85	0	-	0,0 %
farm 11	cattle	24	0	-	0,0 %
farm 12	cattle	82	0	-	0,0 %
farm 13	cattle	6	0	-	0,0 %
farm 14	cattle	73	0	-	0,0 %
farm 15	cattle	96	2	33,9 - 39,0	2,1 %
farm 16	cattle	7	0	-	0,0 %
farm 17	cattle	40	1	37,2	2,5 %
farm 18	sheep	2	0	-	0,0 %
farm 19	sheep	2	0	-	0,0 %
farm 20	sheep	4	0	-	0,0 %
farm 21	sheep	58	0	-	0,0 %
	total	**1.113**	**10**	**average**	**0,9%**

Results of the winter monitoring

Since 2006, every year the FASFC organizes a cross-sectional monitoring once the vector free period has started to look into the virus spread and developments of the previous vector season. This winter, this monitoring consisted in the sampling of maximum 40 cattle in 208 dairy farms spread over the whole territory. The sampling was stratified in the sense that maximum 10 animals of each of the age categories “born in 2008”, “born in 2007”, “born in 2006” and “born before 2006” were taken. The sampling included a blood and a serum sample of each animal. In total, almost 7.300 animals were sampled and tested.

Table 3 summarizes the results of the laboratory analysis of the BTV11 positive farms: in total 12 animals on 8 holdings were revealed positive for BTV11 with the PCR. All ruminants present on the 8 holdings involved were (re)sampled to substantiate the epidemiological inquiry.

All holdings are situated in a limited area in the northern part of the country, in the provinces of Antwerpen, Limburg, Oost-Vlaanderen and Vlaams-Brabant. They are at the widest some 70 km apart from one another (see figure 2).

The prevalence both on herd and animal level are very low: only 12 out of 7.294 sampled animals and 8 out of 208 sampled farms are positive for BTV11, giving an overall prevalence of 0,2 % on animal level and 3,8 % on herd level.

Figure 2. Sampled farms (green dots) and BTV11 positive dairy farms (red dots) of the winter monitoring 2008-2009

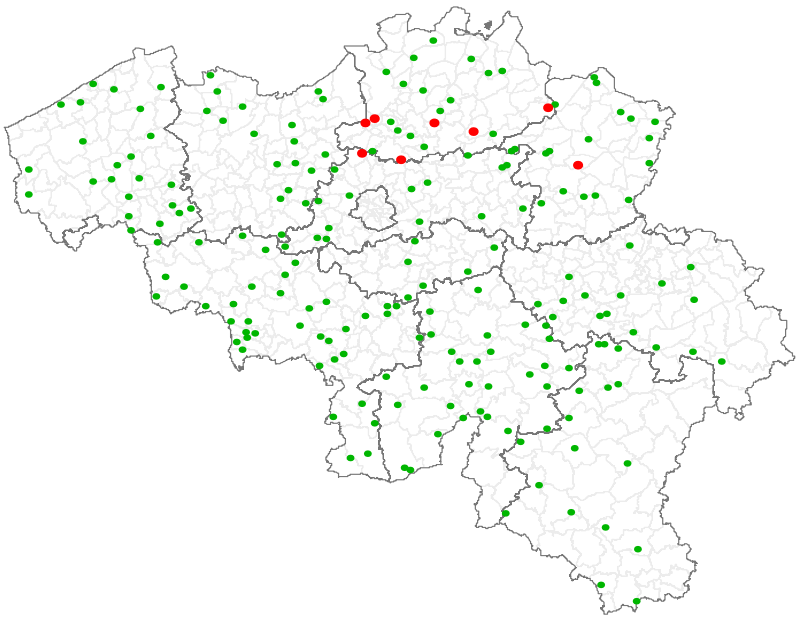


Table 3. Results of the analysis on BTV11 positive dairy farms of the winter monitoring 2008-2009

farm ID	number of animals sampled	number of BTV11 positive animals	average Ct values	SNT titre	prevalence
farm 1	90	2	31,5 - 35,7	1:120 - 1:120	2,2%
farm 2	20	1	35,3	1:120	5,0%
farm 3	177	2	28,6 - 35,7	1:160 - 1:240	1,1%
farm 4	117	1	31,6	1:120	0,9%
farm 5	74	2	33,6 - 33,7	na	2,7%
farm 6	22	1	29,35	na	4,5%
farm 7	118	2	30,2 - 36,5	na	1,7%
farm 8	23	1	32,7	na	4,3%
total	**641**	**12**		**average**	**1,9%**

na = result not available

Results of other surveillance

In 2008, during the vector season, on average some 50 clinical suspicions for BT were notified to the FASFC every week. Since the start of the vector free period, this number has dropped to 20 suspicions a week. Every dossier consist in one or 2 animals showing clinical signs resembling BT. As mentioned above, abortion, stillbirth and early birth of weak calves are also routinely included.

This passive surveillance has revealed most of the 21 BTV8 cases detected in 2008. In view of the detection of BTV11, all BTV positive samples were retested in the PCR with BTV11 specific primers. None showed the presence of this virus.

In addition, all samples of ruminants exchanged to Belgium from high-risk areas for BT and that turned out to be either BTV1 or BTV8 positive in PCR, were also retested. Again, none showed the presence of BTV11.

Outcome of the epidemiological investigation

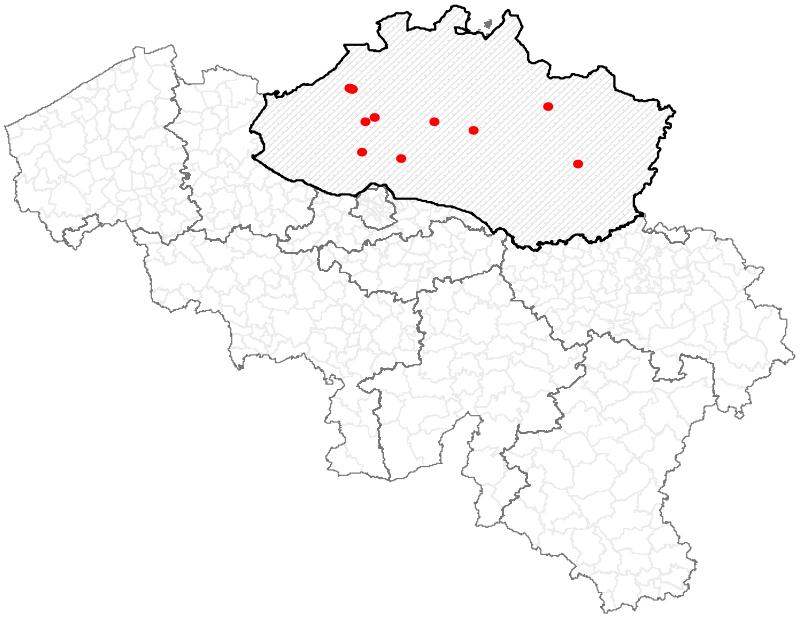
An epidemiological investigation was conducted on the initial holding and the 8 positive holdings of the winter monitoring where BTV 11 was revealed. This inquiry included looking into all possible means of introduction of the virus: contacts with high-risk areas, artificial insemination, embryo transfer, use of cattle vaccines. Neither significant contacts nor common links between the holdings were shown.

None of the animals involved has shown clinical signs of disease. None of the holdings has had a particular history of diseased or other indications for the presence of BT.

Additional measures taken

In order to give additional guarantees for ruminants exchanged to other Member States, the FASFC decided on 16 February 2009 to delimit a temporary control zone around the farms that had come into contact with the BTV11. This zone includes at least a 20 km radius around the infected farms; its limits consist in the highways E40 and R4 and provincial borders (see figure 3).

Figure 3. Temporary control zone (shaded area) and BTV11 positive farms



The additional guarantees that apply since 17 February 2009 are consistent with the usual conditions set in Regulation 2007/1266/EC for BT. Ruminants for breeding and fattening kept within or having left this zone after 1 November 2008 are only allowed into intracommunity trade if they have been tested negative by PCR. The date of 1 November was chosen as a cut-off to add an additional period to the 60 day-period taken into account in the Regulation to refer to “safe” animals with regard to potential infectivity and virus spread.

The limited sampling performed so far in the framework of these additional guarantees has not revealed any additional BTV11 infected animals.

Discussion

The results of the serological and virological analysis of all BTV11 samples generally show high Ct values in PCR and high SNT titres, suggesting relatively old infections (see tables 1, 2 and 3). The repeated paired samples of the first detected animal indicate that indeed Ct values rise at a rate similar to what is known for BTV8 (see table 1). Furthermore, the absence of relevant epidemiological contacts between the farms and the geographical spread of the farms where BTV 11 was revealed (see figures 2 and 3) are consistent with the

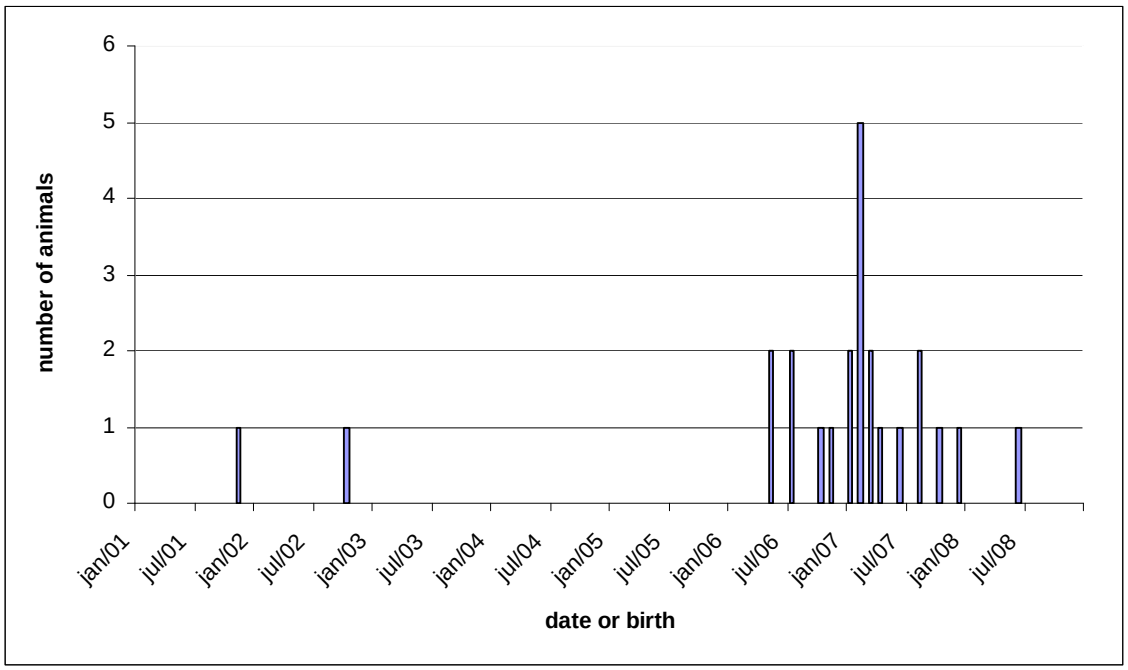
spread of virus by vector activity. The combined data suggest a spread in the second half of the 2008 BT season, probably in late summer and early autumn of 2008 when an ample vector population was active in Belgium.

However, the prevalence that BTV11 has been able to establish during the previous vector season is very low, both on herd (3,8 % of the farms sampled in the winter monitoring 2008-2009) and animal level (less than 0,2% for the animals sampled in the winter monitoring 2008-2009).

The absence of clinical signs, the very low prevalence, the absence of any indication of BTV11 in the nearly 2.000 dossiers of suspected animals during 2008 and the beginning of 2009, as well as the unnoticed spread of the virus during the 2008 vector season are not at all consistent with what has been observed with BTV8 in previous years, neither in Belgium nor in the neighbouring countries. Combined with the preliminary genetic analysis results, this suggests that not a BTV11 virulent field strain is involved, but rather a apathogenic vaccine virus.

The age class of the infected animals is rather particular. Despite a good representation of all age categories in the sampling, the vast majority of the animals been in contact with BTV11, are born from the end of 2006 till the end of 2007 (see figure 4). There is as yet no explanation for this finding.

Figure 4. Number of BTV11 positive animals per date of birth



Conclusions

A BTV11 has been introduced into the Belgian cattle herd during the 2008 vector season. Neither the source of the infection nor the way by which the virus was introduced are known.

The laboratory analysis of the samples performed both by the Belgian National Reference Laboratory VAR and the Community Reference Laboratory in Pirbright, the absence of relevant epidemiological links between the holdings, as well as the geographical spread of the holdings suggest that the subsequent spread of the virus into the cattle population occurred by culicoides, probably during late summer and early autumn of the 2008 vector season.

The absence of clinical signs, the very low prevalence both on herd level and on animal level, the unnoticed spread of the virus during the 2008 vector season as well as the genetic information that is available all suggest that the virus involved is most likely a vaccine strain and not a virulent field strain of BTV11.

The Belgian authorities, in order to give additional guarantees to other Member states, have delimited on 16 February 2009 a temporary control zone around the BTV11 positive holdings. Since, ruminants for breeding and fattening kept within or having left this zone after 1 November 2008 are only allowed into intracommunity trade if they have been tested negative in a virological test.

In view of the information currently available, it is appropriate to lift this control zone and the additional guarantees.

The Belgian authorities will therefore lift the control zone and the additional measures as of 5 March 2009.

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