

Bluetongue serotype 8 outbreak in Norway

Surveillance and monitoring of ruminants and
vectors in the years 2007 to 2010

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Bluetongue serotype 8 outbreak in Norway

Surveillance and monitoring of ruminants and vectors in the years 2007 to 2010

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Summary

When the outbreak of bluetongue, serotype 8 (BT 8), started in the Netherlands in 2006, the Norwegian authorities and the Norwegian Veterinary Institute carefully followed the development of the disease. Diagnostic methods for detection of BTV had already been implemented, and the staff was skilled to handle an eventual outbreak in Norway. In 2007 information about bluetongue was distributed to veterinarians, farmers, stakeholders and other people dealing with farm animals. A surveillance program for vectors was established during the summer 2007, and a surveillance program for cattle started in 2008. Two dairy herds infected with BTV 8 were detected in the southern part of Norway, in Vest-Agder county, in February 2009. A following comprehensive investigation of the outbreak was carried out before the vector season began late April 2009, and two more infected herds were detected. One of these herds, a beef herd, was situated close to one of the index herds, and the other one, a dairy herd, was located in the neighbouring county, Aust-Agder. Based on the low virus level in the infected animals the Institute of Animal Health in Pirbright estimated the most probable time for infection of the herds to be between August and October 2008.

On the basis of the results from the investigation, monitoring and surveillance, and the fact that the BT-outbreak in Norway was very limited, regarding the number of infected herds and the geographical distribution of the outbreak, it was decided not to vaccinate the ruminant population against BTV 8.

In the vector seasons 2009 and 2010 comprehensive monitoring and surveillance programmes for bluetongue were carried out in 10 counties in the southern part of Norway, in the restricted zone and in the free zone. The monitoring and surveillance fulfil the criteria laid down in Regulation 1266/2007/EC. No more BTV positive ruminants or herds have been detected during these two years. The Norwegian authorities therefore conclude with that Norway can be considered free of BTV from 28th of March 2011.

Restricted zones

The restricted zones were established initially around the two first identified farms 20th of February 2009 (zones established according to Directive 2000/75/EC). The zones were extended with the third confirmation. Due to the control policy and the need for movement of sheep to mountain pastures during the vector active season, the restricted zone after some weeks, was further extended to cover these areas.

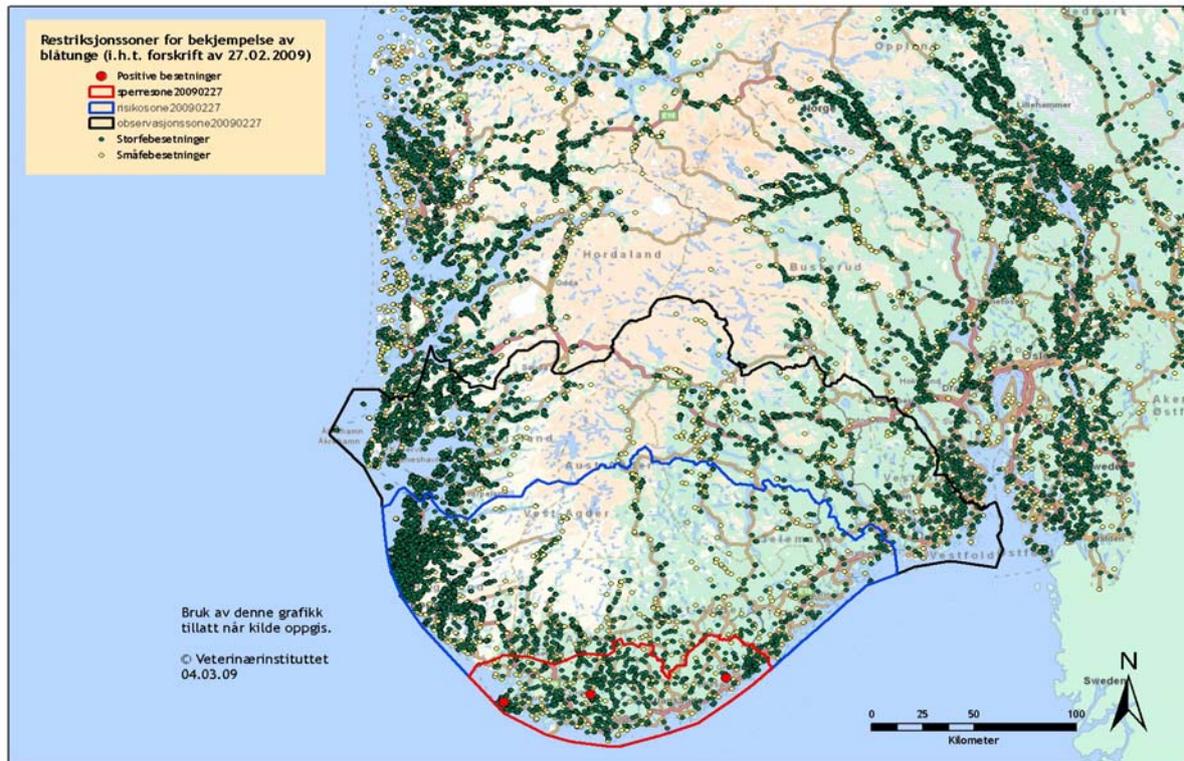


Figure 1. Map of the southern part of Norway with the control zone (red line) and the protection zone (blue line) and surveillance zone (black line). Cattle herds (green dots), small ruminant herds (yellow dots) and bluetongue positive herds (red dots) are indicated in the map.



Figure 2. Map of the southern part of Norway with the extended restriction zone (blue line) and the control zone (red line).

The aim of the bluetongue monitoring and surveillance programme

Before the detection of the first positive animals in 2009, the aim of the National bluetongue surveillance programme was to detect potential BTV that could be introduced into the country by animal import or by infected midges that could cross the border.

In 2009, after detection of the first positive animals and the establishment of a restricted zone, the aim of the monitoring and surveillance programme has been firstly to define the geographical distribution of the BTV-infection and to identify risk animals, and secondly to detect virus circulation during the following seasons.

Legislation

According to the EEA Agreement Norway follows the EU's legislation regarding animal health. Thus Norway established restricted zones and a ban of animal movement according to the directive 2000/75/EC, when the BTV 8 infection was detected in February 2009.

The monitoring programme was carried out according to the Bluetongue Regulation (EC) No. 1266/2007, Article 4 and Annex I, point 1.1.1 and 1.1.2.

The surveillance programme was carried out according to Regulation (EC) No. 1266/2007, Article 4 and Annex I, point 2.1 and 2.2. The surveillance setup was designed to detect a 2 % prevalence of BTV 8 in the ruminant population with 95% confidence.

Vector surveillance was set up according to Regulation (EC) No.1266/2007, Annex 1.1.2. The definition of vector free season was according to Annex V in the regulation.

The ruminant population in monitored parts of Norway

Because the sheep population in Norway is dependent of pasturing at outlying fields and mountain areas, it was opened up for the sheep from the control zone to go to pasture in the protection zone and surveillance zone (see figure 2). These two zones were joined and extended to a restricted zone. Outside the restricted zone there was a buffer zone that was thoroughly monitored.

This area comprised of twelve counties. For some counties only a part was included. Within the monitored area there were more than 7300 cattle herds with altogether approximately 360 000 cattle. Of these herds, 4000 were dairy herds with more than 81 000 dairy cows, whereas the number of suckling cows was 30 000 in 2500 herds. The small ruminant population comprised of 7900 sheep flocks and more than 100 dairy goat herds with 530 000 sheep and 9000 dairy goats, respectively.

The county of Rogaland in the southwestern part of Norway has the most dense cattle and sheep population in Norway.



Figure 3. Map of the southern part of Norway with the monitored area in the free zone (black line) and the extended restriction zone (blue line).

Selected animal populations for bluetongue monitoring and surveillance

The Norwegian Veterinary Institute (NVI) preferred to use 'the region' as the geographical unit of reference, cf. 1266/2007 (EC) Annex 1, 1.

The most probable introduction of BTV into Norway from affected countries in the EU was with infected midges from overseas. The shortest overseas distance from the Northern Jutland to the southern part of Norway is 120 km. However, when Sweden experienced their BTV outbreak in

September 2008 at the west coast, the risk picture changed and it was more likely that the infection could spread north in Sweden towards the Norwegian border, than coming from Denmark overseas. Thus the surveillance was intensified in the southeastern part and continued in the southern part of Norway.

Surveillance of dairy cattle by antibody testing of bulk milk samples was considered as the best and most efficient way to monitor the situation. In Norway it is regulated by law that all the cattle in tie stalls have to be outside the barn for at least 8 weeks during the summer. Additionally blood samples from beef cattle were collected in areas with less dairy herds.

In the surveillance programme for 2008 selected dairy and beef herds in certain geographical regions at risk were tested. After the two first BTV-positive herds were detected in 2009, bulk milk samples from dairy herds were still considered as the most important testing parameter in the monitoring and surveillance of BT. In the investigation of the outbreak and later in the monitoring and surveillance programme almost all the dairy herds in the restricted zones were repeatedly tested. In herds with seropositive bulk milk, individual blood samples from all the lactating cows in the herd were analyzed for antibodies against BTV. Approximately 0.6% of the bulk milk samples became false seropositive for BTV 8.

Additionally blood samples from beef cattle and sheep were collected and analyzed in the investigation of the outbreak and also in the monitoring and surveillance programme. Spleens and blood from wild cervids shot during the hunting season in 2009 in the counties of Østfold, Aust-Agder and Vest-Agder, were analyzed for BTV.

Methods used for monitoring and surveillance of bluetongue

Immediately after the confirmation of the diagnose the Norwegian Food Safety Authority extended the measures provided for in article 4 to holdings located within a radius of 20 km around the infected holdings, according to the Council Directive 2000/75/EC, article 6,1c. A 100 km protection zone and a 150 km surveillance zone were established around the infected holdings, according to Council Directive 2000/75/EC, article 8, to prevent movements of animals. During the investigation of the outbreak all the herds of cattle and small ruminants within the 20 km area were investigated.

Bulk milk samples from dairy herds were tested with the ID Screen® Blue Tongue Milk Indirect ELISA for detection antibodies against BTV. By positive bulk milk test, blood samples from all dairy cows in the herd were examined for antibodies with the ID Screen® Bluetongue Competition ELISA.

In beef cattle herds blood samples from all heifers and cows, and in small ruminant flocks, blood samples from up to 50 animals per flock, were examined for antibodies.

All animals in the positive herds were examined for BTV. A real time RT-PCR (Shaw 2007) was used for detection of BTV.

Criteria for choosing a “no vaccination strategy”

Norway is situated north of the 57th latitude north with a relatively cold climate. The vector free season is from the beginning of May until the end of October / beginning of November. The country is surrounded by sea in the southern, western and northern directions that constitutes a natural barrier against contagious diseases from abroad. Eastwards there is a long common border towards Sweden. The topography in Norway with hills and valleys makes it difficult for long distance transfer of *Culicoides* from one local area to another. Generally there are few ruminants per area and the herds are small compared to the rest of Europe. The ruminant population with the highest density is located in the south-western part of the country. The imports of live animals or animal products are very limited.

The bluetongue infection was detected in the middle of the vector free season which provided time to consider which strategy too choose to eradicate the disease in the most efficient way. There

were only four infected herds whereby three of them were rather small. No infected animals had shown clinical signs and no animals were viremic at the time of detection.

The fact that Sweden and Denmark, and other EU-countries infected with BTV8, had decided to vaccinate their ruminant populations at risk against BTV 8, prevented airborne infection from abroad to come into Norway. This was an important measure and a condition for the choice of strategy. With the knowledge about the limited distribution of the disease at that time, and that the likelihood of reinfection from abroad was considered low, the Food Safety Authority decided not to initiate vaccination as the first measure of choice. Instead a comprehensive surveillance program in twelve counties in the southern part of Norway should be launched and a continuously consideration of the strategy according to the actual situation should be done. A decision three was set up to help deciding in which situations a vaccination campaign had to be launched. A vaccination plan for the southern part of Norway was prepared and 300 000 doses of BTV 8 vaccine were obtained from one of the companies.

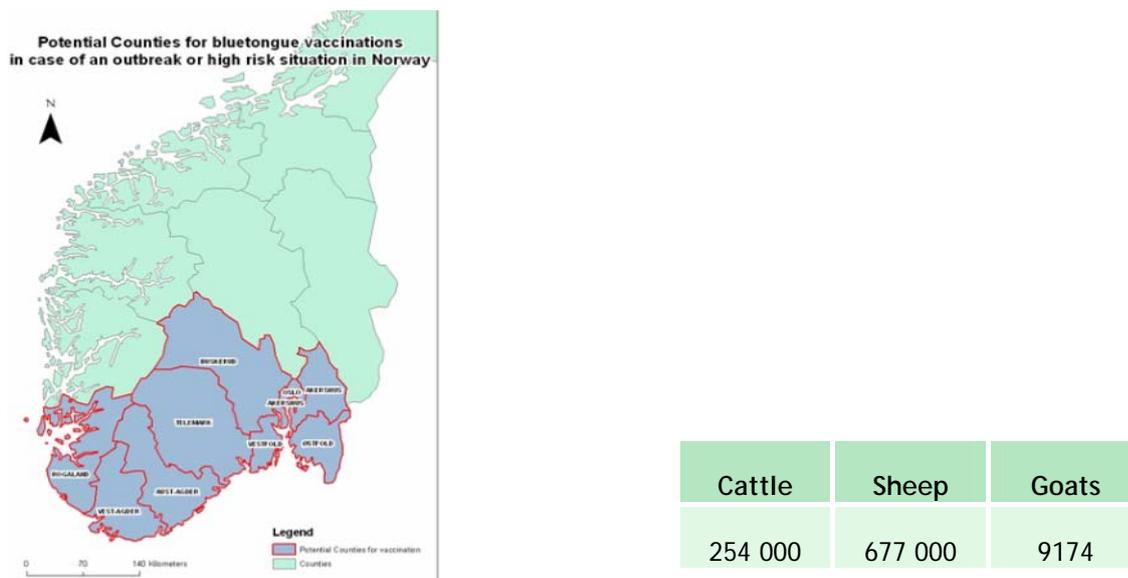


Figure 4. Map of the southern part of Norway where the potential area for bluetongue vaccination is indicated. Figures relevant for ruminant population to be vaccinated are given in the table.

The surveillance programme for bluetongue in 2008

The surveillance programme for bluetongue was devised in the fall of 2007. At the time, there was only one confirmed case of bluetongue in Denmark. A large area of Southern Sweden was in a restricted zone as a result of the Danish outbreak, but there were no diagnosed incidences of bluetongue in Sweden.

The plan was to carry out serological testing of 600 tank milk samples from the southern part of Norway, including Aust- and Vest-Agder counties. An additional 400 blood samples from 40 beef cattle holdings in the same geographical regions, preferably from areas with few dairy cattle, were also to be analysed.

When Sweden found their first cases of bluetongue in Halland in September 2008, the surveillance programme was intensified in the areas of southeastern Norway bordering Sweden.

A number of the bulk milk samples collected from the Agder in December 2008 were analysed in February 2009. Of these two herds tested positive for antibodies against BTV. Later investigations revealed that they were also PCR-positive for BTV-8.

Table 1. Number of samples and number of cattle herds sampled in the surveillance program for bluetongue in Norway in 2008

| Region | Dairy cows | | | Beef cows | | |
|-----------|---------------|-------|----------|---------------|-------|----------|
| | Number tested | | Positive | Number tested | | Positive |
| | Samples | Herds | Herds | Samples | Herds | Herds |
| Southeast | 561 | 547 | | 85 | 10 | 0 |
| Southwest | 229 | 229 | 2 | 174 | 22 | 0 |
| Total | 790 | 776 | 2 | 259 | 30 | 0 |

Investigation of the outbreak

An extensive investigation designed to reveal as many BTV 8-infected animals and premises as possible were implemented.

All dairy herds within the control zone (20 km) were examined serologically by using bulk milk samples. By seropositive bulk milk sample individual blood samples from all cows in the dairy herd were analyzed to verify the bulk milk diagnosis. In beef herds with less than 20 animal all animals were tested and in flock with small ruminants with less than 50 animals all animals were analyzed, whereas in bigger herds or flock blood from only a proportion of the animals were bled.

In the protection zone bulk milk samples from all dairy herds were tested. Additionally, blood samples were taken from roughly 100 suckling cow herds that were located in two areas with few dairy herds close to the control zone.

Within the surveillance zone and the free zone, bulk milk samples were taken from all dairy in Rogaland county north to the islands of Boknafjord, southern portions of Buskerud and Oppland county, Vestfold county, Akershus county, southern Hedmark county and Østfold county.

The investigation of the outbreak detected infection in two more cattle herds; a dairy herd in Aust-Agder and a beef herd in Vest-Agder.

Table 2. Number of herds and number of samples in different zones and categories analyzed for antibodies against BTV in the investigation of the bluetongue outbreak in 2009.

| Zone | Category of herds | Analyzed herds | Analyzed samples |
|--------------|-------------------|----------------|------------------|
| Control | Dairy cattle | 252 | 258 |
| | Beef cattle | 247 | 4289 |
| | Sheep | 420 | 8088 |
| Protection | Dairy cattle | 1238 | 1243 |
| | Beef cattle | 147 | 1594 |
| | Sheep | 20 | 841 |
| Surveillance | Dairy cattle | 415 | 415 |
| | Beef cattle | 8 | 51 |
| | sheep | 3 | 77 |
| Free | Dairy cattle | 1527 | 1549 |
| | Beef cattle | 11 | 41 |
| | Sheep | 57 | 310 |
| | Total | 4345 | 18756 |

Table 3. Number of animals in different age categories and number of animals infected with BTV in 4 BTV 8 infected herds in Norway in 2009.

| Herd | Calves and young cattle | | Cows and bulls | |
|--------------|-------------------------|----------|----------------|-----------|
| | Individuals | Positive | Individuals | Positive |
| L | 40 | 2 | 28 | 25 |
| K | 31 | 1 | 20 | 2 |
| U | 59 | 0 | 15 | 1 |
| N | 216 | 1 | 116 | 1 |
| Total | 346 | 4 | 179 | 29 |

The positive herds and measures implemented

Four cattle herds were infected with BTV 8 virus. In three of the herds from one to three animals were seropositive and virus positive (PCR) in each herd, whereas in the fourth herd 27 out of 67 animals were seropositive. Most of them were also virus positive (PCR). Only two of the 27 seropositive animals in this herd were young stock, the other animals were dairy cows. The cows had been on a pasture with a lot of bushes and trees while the majority of the young stock had been on another pasture some kilometres from the cow's pasture. The interpretation of this was that there had been a replication of BTV 8 in local midges at the cow's pasture which had infected more cows in the herd. In the same herd, a transplacentally infected calf was born in the end of December 2008. In March the virus concentration in the blood from the calf corresponded to viremia (PCR-ct-value like 24.08). The calf had no traceable antibodies against BTV 8 in the blood. From the same herd post mortems were performed on two fetuses from two seropositive cows that were slaughtered. One of the fetuses had malformation on the brain and BTV 8 was detected in the foetal fluid and in the liver from the foetus.

The four positive herds were followed up with blood samples from the majority of the animals in the herd every month from May to October 2009. To eliminate potential transplacentally infected calves before the vector season started in the end of April, it was decided to slaughter all pregnant cows and heifers that were expected to calve before the first of June. Virus positive calves (PCR) were also slaughtered. In all, four pregnant cows and two calves were culled.

The monitoring and surveillance programme in 2009

The surveillance program started in the beginning of the vector season which began 20th of April.

Bulk milk samples from every dairy herds in the counties of Rogaland, Vest-Agder, Aust-Agder, Telemark, Vestfold, Akershus, Oslo and Østfold, as well as the southern areas of the counties Hordaland, Buskerud, Oppland and Hedmark, were tested monthly from May to November. This comprised approximately 3200 samples per month. Additionally, all dairy herds in Hordaland and holdings in Sogn og Fjordane south of the Sognefjord were tested in November. In total, 18 300 bulk milk samples were analysed and 124 of these samples tested positive. The holdings that tested positive were followed-up with blood testing from the lactating individuals; all were seronegative. This meant that 0.7% of the bulk milk samples had given false positive results.

Individual blood samples from animals in the BTV positive herds were tested regularly during the vector active season. In addition, all ruminant holdings located within five km of the (most infected) operation in Vest Agder were tested once before being sent to pasture. Blood samples from about 4500 beef cattle and almost 200 sheep were collected at the abattoir and examined serologically.

Additionally 152 suspicious cases (97 cattle) were analyzed serologically and by PCR.

Spleen and blood samples from 287 wild ruminants, mostly moose, in Vest-Agder, Aust-Agder and Østfold were submitted by hunters during the hunting season. The spleen was examined for the presence of virus with the use of PCR, and the blood was examined for the presence of antibodies.

No more animals were found to be positive for BTV 8 under the surveillance programme of 2009.

Table 4. Number of herds and number of samples in different zones and categories analyzed for antibodies and virus (cervids, suspicious cases) against BTV in the surveillance programme for bluetongue in 2009.

| Category | Number of examined | | Total number of Herds in the Category |
|------------------------|--------------------|-------|---------------------------------------|
| | Samples/ Animals | Herds | |
| Dairy: Restricted zone | 13181 | 2545 | 2572 |
| Dairy: Free zone | 5056 | 1578 | 1599 |
| Beef: Restricted zone | 2803 | 288 | 1816 |
| Beef: Free zone | 1318 | 222 | 973 |
| Sheep: Restricted zone | 190 | 16 | 3466 |
| Suspicious cases | 152 | 38 | |
| Wild cervids (Moose) | 287 (252) | | |

The monitoring and surveillance programme in 2010

The surveillance of bulk milk of 2009 was continued in 2010, but the period of surveillance was reduced: Bulk milk samples were collected in the months May, August, September, October and November. Blood samples from 3200 beef cattle and 800 sheep have been analysed.

Every animal of the most infected holding in Vest Agder was tested once before being sent to pasture. One calf that was born in November 2009, from a serologically positive cow, tested positive for antibodies against BTV 8 despite being over 5 months of age at the time of sampling. The calf was retested in November, but was found to be negative. The positive result of the first test was due to maternal antibodies.

Bulk milk samples from eight dairy goat herds in the Sogn og Fjordane county (in the free zone) were tested for antibodies against BTV 8. Six bulk milk samples became positive. Blood samples from individual goats showed that 5 herds had false positive bulk milk samples. However, in one herd 43 out of 66 goats had antibodies against BTV. None of the goats were positive for virus detection by PCR (pan-BTV real time RT-PCR assay "Shaw"). Blood samples from 15 kids were sent to reference laboratory, Institute of Animal Health in Pirbright, UK. All 15 blood samples tested positive for a special PCR assay called "Orru". The attempt to sequence the PCR-product failed and they did not succeed to grow the virus.

In the serum neutralization test, the antibodies were negative for all the 24 known serotypes of BTV. This is probably a new orbivirus that is similar to the "Toggenburg" virus firstly detected in Switzerland.

Table 5. Number of herds and number of samples in different zones and categories analyzed for antibodies against BTV in the surveillance programme for bluetongue in 2010.

| Category | Number of examined | | Total number of Herds in the Category |
|------------------------|--------------------|-------|---------------------------------------|
| | Samples/ Animals | Herds | |
| Dairy: Restricted zone | 9558 | 2348 | 2530 |
| Dairy: Free zone | 4271 | 1471 | 1606 |
| Beef: Restricted zone | 2496 | 455 | 1768 |
| Beef: Free zone | 710 | 106 | 973 |
| Sheep: Restricted zone | 804 | 48 | 3466 |

The surveillance of the *Culicoides*

The surveillance of *Culicoides* started in July 2007 and it has been going on every year until 2010. Onderstepoort blacklight suction traps have been used for collection of the midges. The traps have been located at 15 - 20 farms along the Norwegian south coast (figure 5) and the farmers have been responsible for capturing and submission of the samples to the laboratory.

In 2008 and 2009, the trap on each location has been in use one day a week from the mid April to the end of the season in October or November. The five species of *Culicoides* that are known to transmit BTV 8 in the Northern Europe are found in Norway, but the dominating species was the *C. obsoletus* complex which constituted more than 80% of the captures.

It was made attempts to detect BTV by PCR-assay from a proportion of the midge captures that were collected in 2008 and 2009, but we did not detect any virus in the midges.

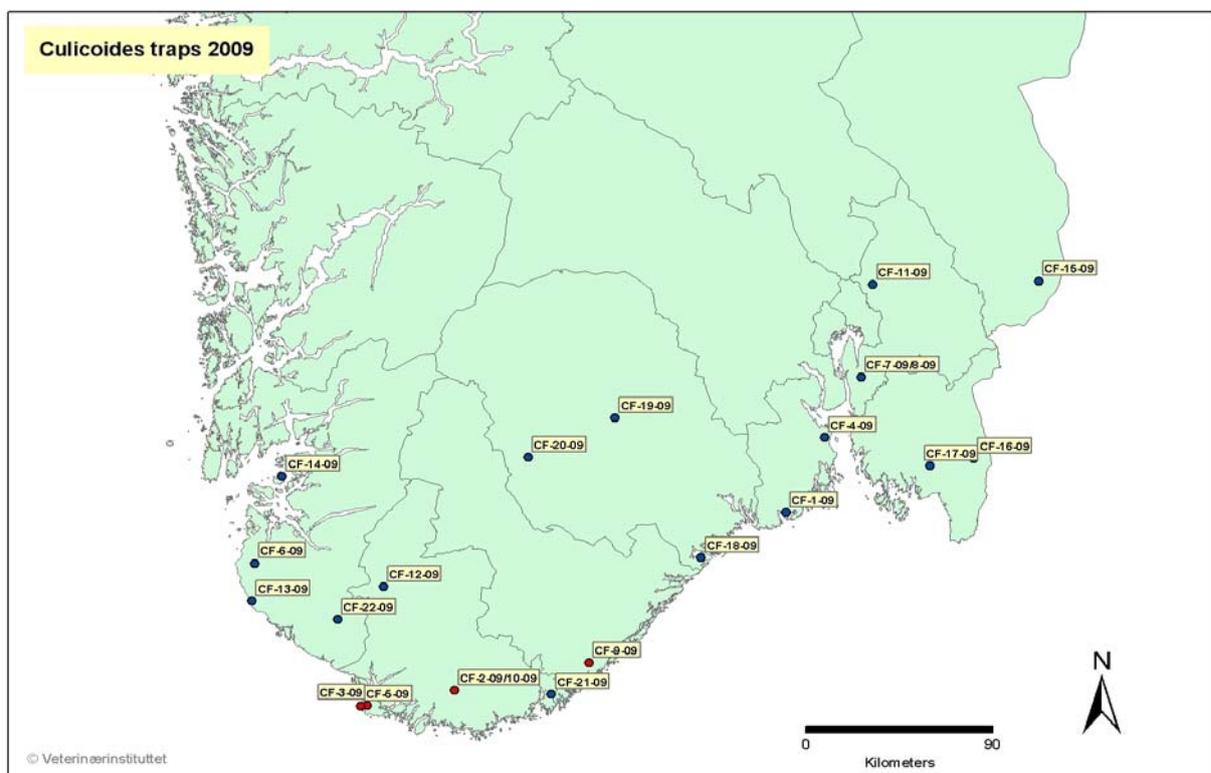


Figure 5. Map of the southern part of Norway where the locations for the midge traps in 2009 are indicated.

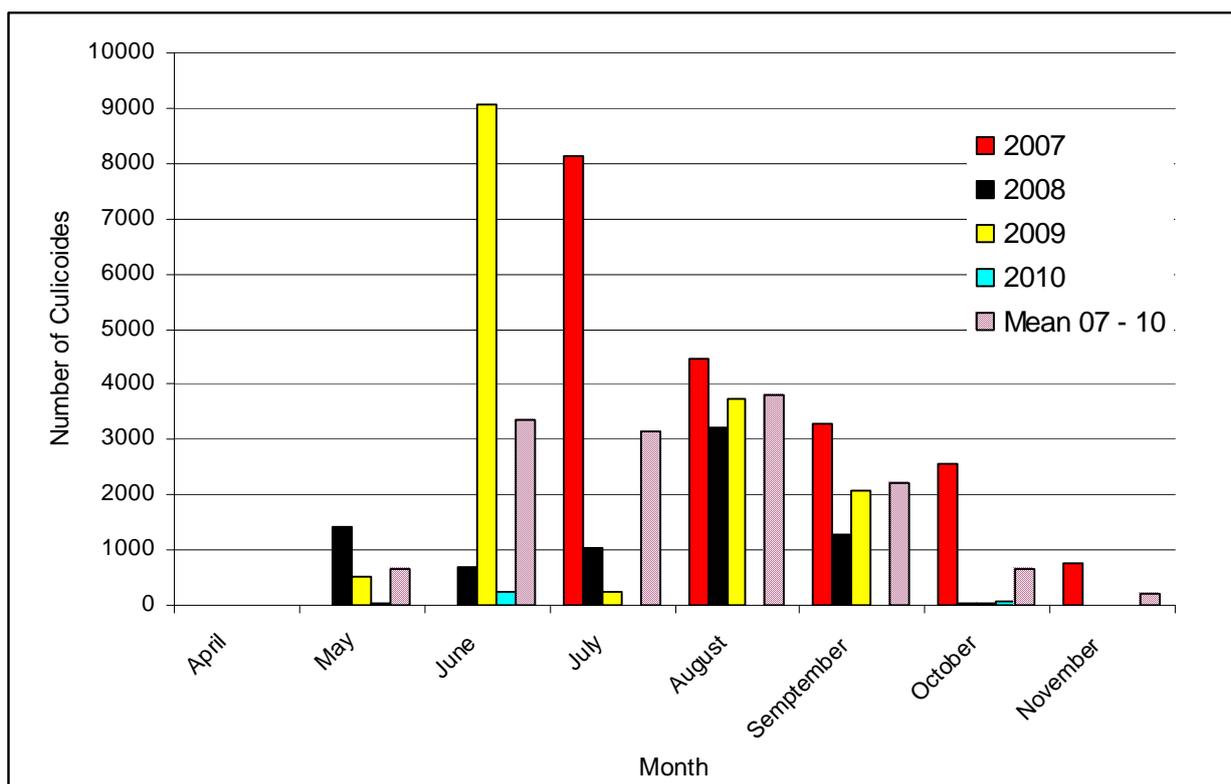


Figure 6. Average number of Culicoides captured per trap and month in the vector season for the years 2007 - 2010.

Key points of the surveillance

- The bluetongue outbreak in Norway was small and located to a limited area. No clinical signs could be observed in the infected herds
- Transplacental infection was considered as the most important mechanism for the BTV to survive the winter in Norway
- It was decided not to vaccinate the ruminant population. All the ruminant animals were in fact sentinel animals
- Bulk milk samples from dairy cattle herds constituted the main parameter in the surveillance program
- All dairy herds from a defined part of Norway were regularly tested during the vector season for two years
- All testing was carried out according to regulation (EC) No. 1266/2007
- No animal positive for bluetongue serotype 1 - 24 was diagnosed after 10.03.2009.



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