AHEAD IN
ANIMAL HEALTH

Epidemiological situation of bluetongue in cattle and small ruminants in August 2023.

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We own one of the largest veterinary laboratories in the world, in which we perform over almost 5 million laboratory analyses annually. Furthermore, GD employs a team of veterinarians, specialists and scientists who execute animal health monitoring, conduct practical research and develop programmes for animal disease prevention and control.

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Epidemiological situation of bluetongue in cattle and small ruminants in August 2023.

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Summary

On 3 and 4 September 2023, two veterinary practices made notification to the Netherlands Food and consumer product safety authority (NVWA) of clinical signs indicative of bluetongue (BTV) at five sheep farms in the central part of the Netherlands. On 5 September, a BTV infection was confirmed by Wageningen Bioveterinary Research (WBVR) and on 8 September serotype 3 was determined as most likely serotype. This serotype was until that moment in the European Union only found at Sicily and Sardinia. Therefore, there was a need to know whether BTV was already widespread in the Netherlands or whether BTV was present only locally in the region were notifications of clinical signs came from.

Routinely collected serum samples from small ruminants and bulk milk samples from dairy cattle farms from August 2023 that were available at Royal GD were tested for antibodies against bluetongue to obtain insight in what happened in the month before the first clinical signs were notified. For this study, 1,003 serum samples from small ruminants from 89 different farms and thousand bulk milk samples (fifty from each of the twenty compartments in the Netherlands) were selected.

Antibody positive results were found in 3.4% (95% CI: 0.9-7.5%) of sampled sheep flocks and in 2.8% (95% CI: 1.9-4.1%) of the bulk tank milk samples. Out of 991 tested bulk tank milk samples, 955 tested negative, eight tested doubtful and 28 tested antibody positive. However, after combining the bulk tank milk antibody results with vaccination data, 24 out of the 36 bulk tank milk samples with doubtful or positive results appeared to be from farms with a history of vaccination against BTV-8. In comparison, only 7 of the 955 bulk milk samples that tested negative were linked to vaccination. For small ruminant herds, no historical vaccination data were available.

In conclusion, based on the results of this screening and considering that a large part of the seropositive BTM samples could be explained by a history of vaccination, it seems likely that BTV was not widespread in the Netherlands in August 2023. The notifications of the disease were mainly in an area in the middle of the Netherlands and it seems that the monitoring system picked up the outbreak in an early phase.

1 Introduction

Bluetongue virus (BTV) is an arthropod-borne virus which can cause clinical disease and mortality in ruminants. Exclusively, all types of ruminants are susceptible for infection with BTV. The virus is not transmittable to other species nor are human susceptible for infection i.e. BT is not a zoonosis. BTV is transmitted by certain species of *Culicoides* midges and was historically only present between latitudes 35ºS and 50ºN (Zhang et al., 1999; Mellor et al., 2008). The BTV serogroup consists of at least 27 serotypes. There is no or limited cross-protection between serotypes. Since 1998, several BTV serotypes have been present in Europe and the Mediterranean basin (Lundervold et al., 2003) and in 2006, bluetongue virus serotype 8 (BTV-8) emerged in north-western Europe for the first time, and the Netherlands were the first country where the infection was detected (Van Wuijckhuise et al., 2006; Elbers et al., 2008). After a major BTV-8 outbreak in 2006 and 2007, in 2008 an emergency BTV-8 vaccine became available (Wäckerlin et al., 2010) and many cattle herds and small ruminant flocks participated in the voluntary vaccination programme that was implemented by the Dutch government (Elbers et al., 2010). This resulted in a dramatic decline in the number of clinical notifications at the Netherlands Food and consumer product safety authority (NVWA). At the end of 2008, over 80% of the susceptible host population tested positive for antibodies due to natural infection or vaccination. From 2009 on, no new infections were seen and after three years of screening of BTV circulation, the Netherlands regained its official BTV free status in February 2012. This disease-free status was monitored annually according to EU regulation 1108/2008/EC and confirmed without interruption up to December 2022. However, based on the risk of introduction of BTV from other countries, vaccination was allowed and therefore some farmers vaccinated their animals.

On 3 and 4 September 2023, NVWA was notified of clinical signs that were indicative for BT at five sheep farms in the central part of the Netherlands. Sheep showed signs of fever, lethargy, hypersalivation, ulcerations and erosions of the oral and nasal mucosal membranes, facial oedema, and lesions of the coronary band, lameness and mortality. Flocks were visited by a team of veterinary specialists and sheep were sampled. On 5 September, a BTV infection was confirmed by Wageningen Bioveterinary Research (WBVR), the national veterinary reference laboratory in the Netherlands. On 8 September, WBVR announced that genotyping revealed that the BTV outbreak was caused by BTV serotype 3. Hitherto, within the European Union, this serotype was only found at Sicily and Sardinia. In the days subsequent to the first confirmation of BTV infection, additional notifications of clinical signs were made, both in sheep flocks and cattle herds.

In the first days after detection of BTV-3, many questions arose. The route of introduction is unknown although possible routes might be considered such as introduction of infectious animals, incursion of infected *Culicoides* by wind or through the largest national airport, Schiphol, or use of contaminated live vaccines. Also, it is unknown how long the infection is present in the Netherlands and whether transmission is limited to a small number of flocks/herds in a specific region or is already widespread in the Netherlands. Given the fact that BTV can cause clinical signs, but may also evolve subclinically (Santman-Berends et al., 2011), clinical notifications of BT might only present the tip of the iceberg.

Royal GD coordinates the national monitoring and a number of surveillance programmes in both cattle and small ruminants (Santman-Berends et al., 2016; Dijkstra et al., 2022). Additionally, GD coordinates many mandatory and voluntary disease control programmes in the Netherlands (Santman-Berends et al., 2021) and farmers can submit samples for laboratory investigations. All these tasks result in a continuous flow of serum and bulk milk samples from

all over the country. This offered the possibility to screen samples originating from cattle and sheep that were submitted in August 2023 for the presence of BTV antibodies.

Therefore, the aim of this study was to get an impression of possible spread of BTV in August 2023 in the cattle and small ruminant population in the month before the outbreak was detected in the Netherlands.

2 Materials and Methods

2.1 Study population

For this study, the target population was defined as either cattle or small ruminants that had the highest risk to have come into contact with *Culicoides*, i.e. that were grazed during the summer of 2023. Given that we were interested to detect whether BTV was present before the moment of detection, milk and blood samples that were submitted to Royal GD for routine laboratory testing in August 2023 and that were still available were initially selected. For small ruminants, 1,008 serum samples from 89 herds that were submitted for screening for *Brucella melitensis* in August were available (van den Brom et al., 2013). All these samples entered the study. On herd level, when testing 89 herds, BTV circulation can be detected if at least 3.5% of the small ruminants test antibody positive.

For cattle, it was possible to conduct a nation-wide herd level screening based on bulk milk samples. It was decided to select bulk milk samples that were submitted for i) one of the voluntary or mandatory disease control programmes that are coordinated by GD (i.e. BVD, IBR, Leptospirosis, Salmonellosis or Neosporosis (Santman-Berends et al., 2021)) or ii) one of the voluntary bulk milk screening programmes (claw health). From all Dutch dairy herds, the most recent bulk milk sample that was submitted to GD in the month of August was selected for inclusion. Bulk milk samples were available from approximately 90% of the Dutch dairy herd population (more than 12,000 dairy herds). Given that the bulk milk screening is an antibody test that detects BTV antibodies regardless of the subtype, and is very sensitive, it was decided to only select dairy herds that did not purchase any cattle during the vector active season in 2023 (from April on) and that only housed Dutch bred animals. To enable this selection, animal movement data was retrieved from the identification and registration database (RVO, Assen, the Netherlands). The risk based selection was made to minimize the risk of finding antibody positive results related to cattle movements and to import of cattle from non-BTV free regions in Europe. After this selection, bulk milk samples from approximately 7,900 dairy herds remained for inclusion in this study.

The Netherlands is divided into twenty compartments based on geographic boundaries as proposed in Commission Decision 2005/393/EC (Fig. 1). A random sample of thousand bulk milk samples was selected stratified to the twenty compartments. This resulted in approximately fifty herds per compartment. This number is sufficient to detect BTV assuming a prevalence of BTV antibodies in at least 6% of the Dutch dairy herds, with 95% confidence (Stata 17). In case transmission would be detected, a sample of fifty herds per compartment would provide a prevalence estimate with a precision of 14% and 95% confidence. With thousand samples, a prevalence estimate could be given with a precision of 3% with 95% confidence.

Figure 1. The Netherlands is divided in twenty compartments, including its numbering, according to EU regulation 202/393/EC.

2.2 Laboratory testing

For serum, a competition ELISA based on a recombinant VP7 protein (Idexx Bluetongue competition Ab test) has been used. This ELISA has a reported sensitivity of 100% (as compared to VMRD ELISA and IDvet ELISA). Specificity was 99.8% (95% Confidence interval 99.7-99.9%). Competition ELISAs are suitable according to the WOAH for detection of immune responses for prevalence and surveillance studies.

For bulk milk, an indirect ELISA based on the recombinant VP7 protein (ID Screen ® Bluetongue Milk Indirect, IDvet, Montpellier, France) was used. Antibodies present in the sample will bind to the VP7 antigen and will be detected by adding anti-ruminant-IgG-conjugate and substrate. The intensity of staining is correlated with the amount of antibodies in the sample. VP7 is conservative in all BT serotypes. This ELISA has been validated in 2007 in the Netherlands, comparing ELISA results in bulk milk samples and individual milk samples from 92 herds in BTV-8 infected areas and 88 historically negative milk samples (Mars M.H. et al., 2010). In this study, sensitivity and specificity in bulk milk were high. Using a S/P cut off of 35% results in a sensitivity of 90% and a specificity of 97.4% to detect a within-herd-prevalence of 3%. Herds without positive animals and historically negative bulk milk samples were all clearly negative. All bulk milk samples from herds with more than 10% milk-antibody positive cows had S/P ratios >100%. To study the herd prevalence of BT infections in 2023, the cut off was used as prescribed in the

manual: S/P≤30% is considered negative, 30>S/P%≤40% is considered doubtful and S/P>40% is considered positive.

2.3 Statistical Analyses

Besides the data from the identification and registration system and the laboratory results, for the dairy cattle herds also data on BTV-8 vaccination registrations after the BTV-8 epidemic between 2008 and 2012 (Royal GD) and purchase figures of vaccination between September 2017 and July 2023 were available (MediRund). These data were combined with the results of the bulk tank milk screening to enable to correct for the effect of vaccination. All selected samples were anonymized, so that they could not be traced back to individual animals or farms. Only the two-digit postal code and compartment were retained to enable evaluation of regional BTV antibody status.

2.3.1 Serological evaluation individual small ruminant samples

The results of the serological evaluation are presented descriptively on individual and at farm level. The number of sampled small ruminants and farms is presented in a thematic map. The results of the BTV screening are presented on both compartment and country level on individual level and at herd level. The results of the individual screening will be corrected for within-herd clustering of sampled small ruminants. Given the low number of sampled herds per compartment, the number of farms where antibodies are detected are presented instead of a percentage. When positive results are found, additional information will be presented on the exact date the original samples were submitted and the animal species involved i.e. sheep or goats. All analyses are conducted in Stata version 17® (Statacorp, 2021).

2.3.2 Bulk milk screening in dairy cattle herds

The results of the bulk milk screening are presented in a descriptive manner on country and compartment level. The number and percentage of samples with either a doubtful or positive outcome are provided in tables and are graphically displayed in thematic maps of the Netherlands. In compartments in which all samples tested negative for BTV antibodies, the maximum possible prevalence is calculated with its 95% confidence interval. When part of the samples in a compartment test positive, the estimation of the prevalence with its 95% confidence interval is provided. The results are presented with and without correction of the possible interference of vaccination. All analyses are conducted in Stata version 17® (Statacorp, 2021).

3 Results

The results of this study are both presented on 2-digit postal code and on compartment level. In Figure 1 on the left, the compartments with their respected numbers are presented. In Figure 1 on the right, the reason is presented why we are conducting this study. The map presents the number of confirmed cases of BTV infection up to 12 September 2023 (source: NVWA).

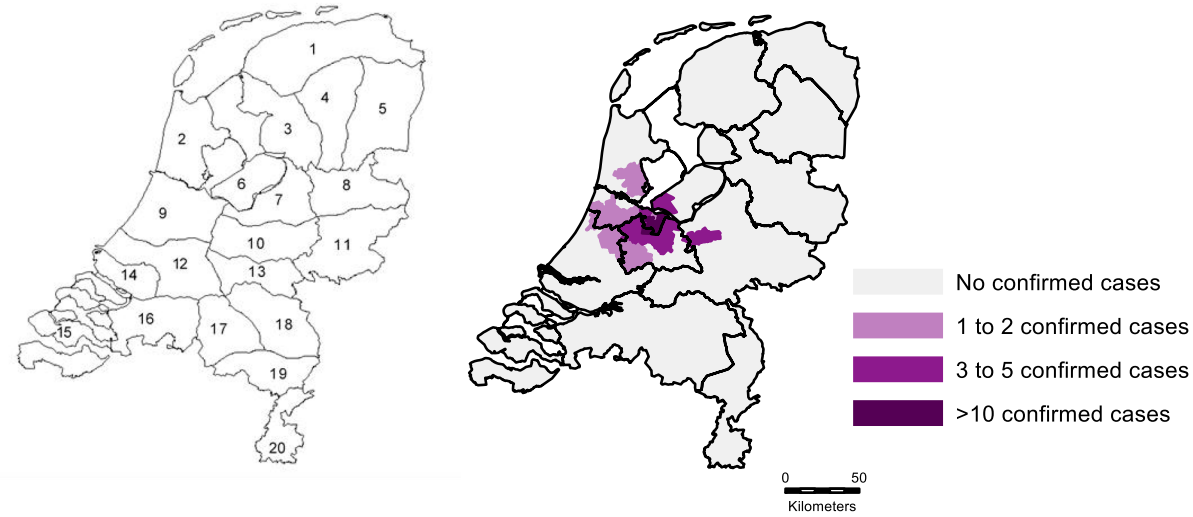

Figure 1. On the left: The Netherlands is divided in twenty compartments, including its numbering, according to EU regulation 202/393/EC. The graph on the right depicts the number of confirmed cases of BTV in a 2-digit postal code area. Source: NVWA at 12 September 2023.

3.1 Serological survey of serum samples of small ruminants

Out of 1,008 serum samples from small ruminants, samples of five sheep appeared in duplicate and from these animals only one result was retained. Eventually, 1,003 samples remained from 85 goats and 918 sheep originating from 89 farms with small ruminants (82 sheep farms, six goat farms and one farm with both sheep and goats). The samples originated from all twenty compartments. The number of farms per compartment that were included in the survey remained limited, and varied between one (compartment 10 and 16) and eleven (compartment 1) (Figure 1 and 2b).

The number of samples included per compartment varied between five samples (this concerned 1 flock that submitted five samples) (compartment 10) and 127 (compartment 1) (Figure 2a). The number of samples per flock or herd was on average twelve (median 13), which was according to the regulations of the *Brucella melitensis* control programme. The moments of submission of samples were fairly equally distributed over the whole month of August (Figure 3).

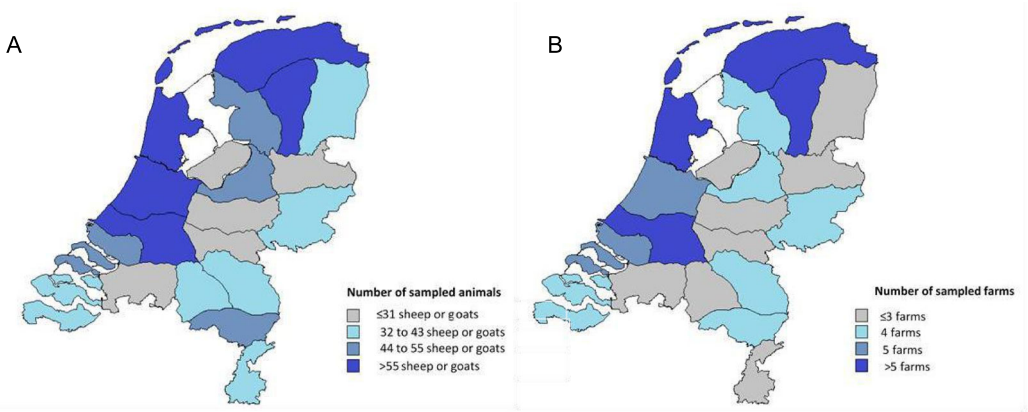

Figure 2. The number of serum samples from sheep and goats (A) and the number of small ruminant farms (B) that were included in the serological survey to determine the presence of bluetongue, in August 2023, at the onset of the outbreak in the Netherlands.

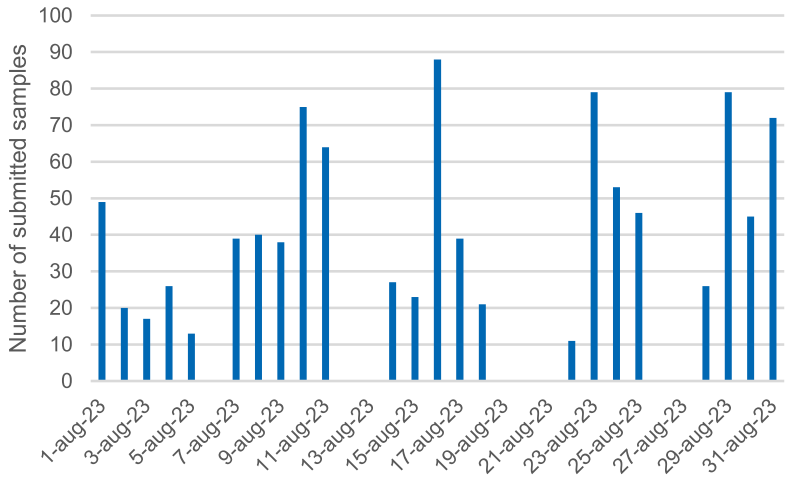

Figure 3. Distribution of dates of routinely submission of serum samples from small ruminants in August 2023 in the Netherlands.

In total 983 of the submitted samples tested negative for antibodies against BTV, fourteen samples had a doubtful result and six samples tested positive. Samples with a doubtful or positive result were considered positive for further analysis. Based on these samples and corrected for clustering within herds (i.e. that samples from sheep or goats within herds are correlated), the estimated antibody prevalence in August in the small ruminant population was estimated at 1.5% (95% confidence interval 0.8-2.5%) on animal level and at 6.7% (95% CI: 2.5-14.1%) on herd level. When only antibody positive samples were considered the herd level prevalence was estimated at 3.4% (95% CI: 0.9-7.5%).

All samples that tested positive or doubtful were sheep serum samples. The twenty samples that did not test negative, originated from fourteen different sheep flocks. From these, in eleven farms, only one sample reacted (either positive or doubtful), in two farms two samples reacted (both samples doubtful in both farms) and in one farm, five samples reacted (4 positive and 1 doubtful). The samples that reacted in the antibody test were submitted between the first and 29th of August. The flock in which five samples reacted, submitted the samples on 9 August 2023.

The farms from which samples were found to be doubtful or positive in the antibody test originated from all parts of the Netherlands (Figure 4). The farm from which four samples were found to test antibody positive was located in the central part of the Netherlands (in compartment 17) (Figure 4 and Figure ??).

The results of both animal and farm level per compartment are presented in Table 1. In 8/20 of the compartments none of the samples were found to test antibody positive, in 9/20 compartments animals were found that tested doubtful for antibodies and in 3/20 compartments antibody positive results were detected (Table 1).

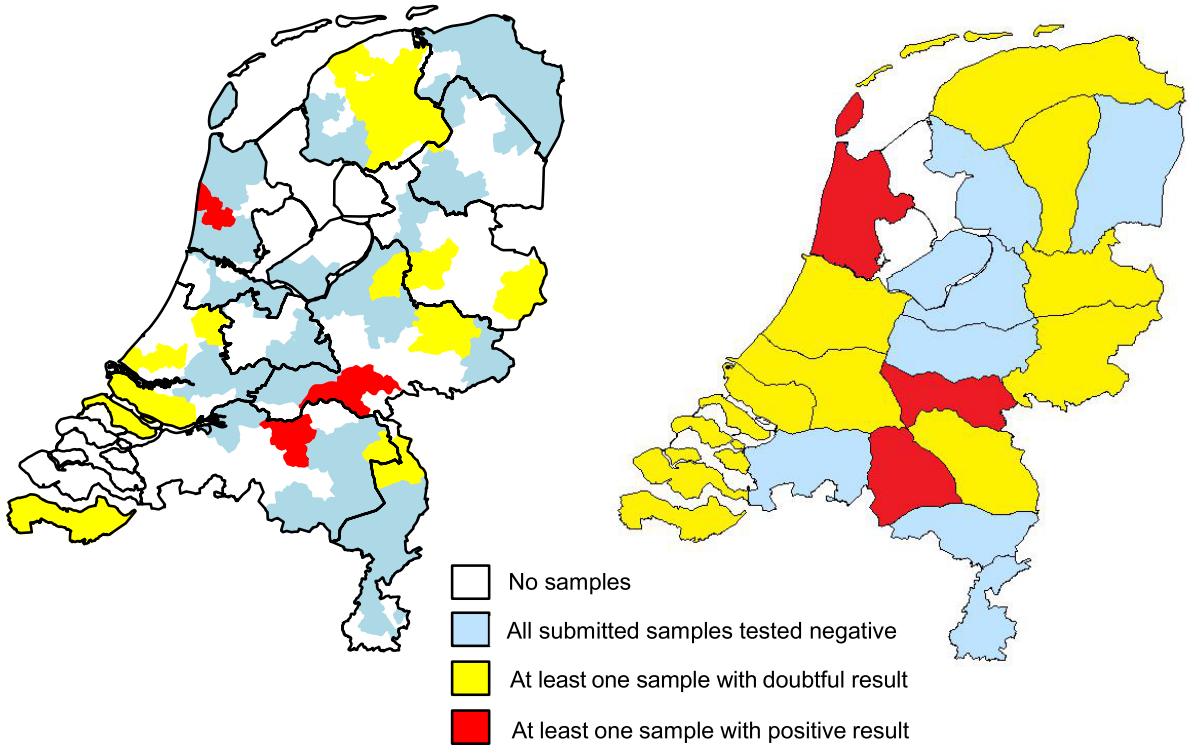

Figure 4. Thematic map of the Netherlands in August 2023, showing the results of an antibody screening of bluetongue in serum samples of 1,003 small ruminants housed in 89 farms on 2-digit (left) and compartment level (right).

Table 1. Results of the antibody screening of bluetongue in serum samples of 1003 small ruminants in 89 different farms at compartment level, in August 2023 in the Netherlands.

Compartment	Number of Sheep or goats			Number of farms		
	Tested	Positive result	Doubtful result	Tested	Positive result	Doubtful result
1	126	0	3	11	0	2
2	121	1	0	10	1	0
3	52	0	0	4	0	0
4	69	0	1	6	0	1
5	36	0	0	3	0	0
6	13	0	0	1	0	0
7	45	0	0	4	0	0
8	20	0	1	2	0	1
9	72	0	1	6	0	1
10	5	0	0	1	0	0
11	43	0	2	4	0	2
12	101	0	1	8	0	1
13	27	1	0	3	1	0
14	46	0	1	5	0	1
15	51	0	2	5	0	1
16	7	0	0	1	0	0
17	39	4	1	4	1	1
18	43	0	1	4	0	1
19	48	0	0	4	0	0
20	37	0	0	3	0	0

3.2 Bulk milk screening in cattle herds

Bulk tank milk results were produced for 991 dairy cattle herds (from the remaining nine herds an insufficient amount of milk was available for testing). The samples were taken between 4 and 24 August, but the fast majority of the samples (99.5%) was taken between 4 and 16 August. From the 991 bulk milk samples, 955 (96.4%) tested antibody negative, eight tested doubtful for antibodies (0.8%) and 28 tested antibody positive (2.8%). Including both doubtful and positive results, the antibody prevalence in Dutch dairy herds at herd level was 3.6% (95% CI: 2.6-5.0%) in August 2023. When only positive samples were classified as positive, the herd level prevalence would be 2.8% (95% CI: 1.9-4.1%). When only high positive samples with an SP percentage >100 were classified as positive, the herd level prevalence would be 1.2% (95% CI: 0.6-2.1%). An additional 35 bulk milk samples from the months prior to August were also tested and from these, 34 samples tested negative and 1 tested doubtful for antibodies. This sample with the doubtful result was submitted in July and had a value just above the cut-off value of the test (30.16: cut-off value=30).

The dairy herds that tested positive for BTV antibodies were mainly located in the southeast of the Netherlands (twenty out of the 28 dairy herds that tested BTM positive) (Figure 5).

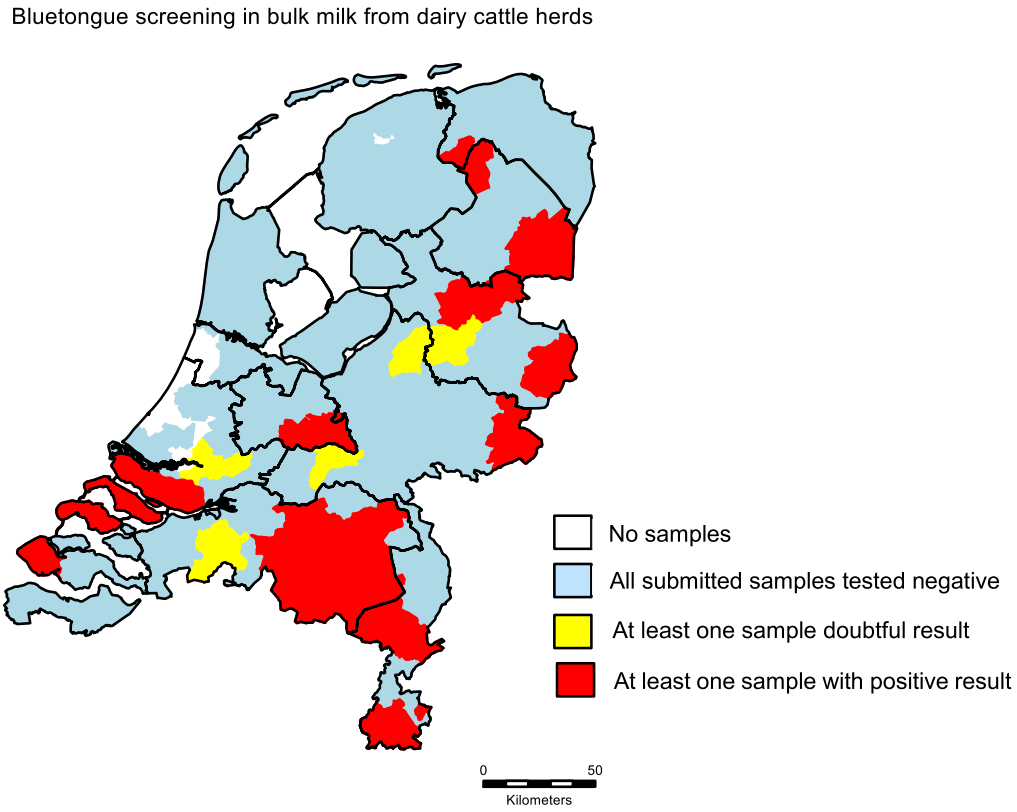

Figure 5. Map of the Netherlands in August 2023, showing the results of an antibody screening of bluetongue in bulk milk samples of 991 dairy herds.

The number of samples in which antibodies were found, varied between 0 and nine out of the approximately fifty samples that were tested per compartment (Table 2). The bulk tank milk samples that tested high positive, mainly originated from compartments 17 to 20 that are located in the southeastern part of the Netherlands (Figure 7).

The raw data of the ELISA results showed that the S/P percentages varied between a minimum of 6 to a maximum of 268% (Figure 7). Again the highest values were found in compartment 17 to 20.

Table 2. Results of the antibody screening of bluetongue in 991 bulk milk samples originating from dairy herds at compartment level, in August 2023 in the Netherlands.

Compartment	Number of herds			
	Tested	Highly positive result (HP)*	Positive result (P)	Doubtful result (D)
1	49	0	0	0
2	49	0	0	0
3	51	0	0	0
4	49	0	1	0
5	47	1	2	0
6	49	0	0	0
7	52	0	0	0
8	50	0	0	1
9	51	0	0	0
10	48	0	1	0
11	48	0	2	0
12	50	0	0	1
13	53	0	0	1
14	48	0	2	2
15	50	0	0	0
16	47	0	0	1
17	52	6	8	1
18	52	2	5	0
19	48	2	4	1
20	48	1	3	0

*The samples that tested highly positive for antibodies are also included in the column “positive result”.

The percentage of dairy herds in which antibodies were detected varied from none in seven compartments, to 17.3% in compartment 17 (Table 2), when both doubtful and positive results were considered as positive. When only positive samples were considered, the antibody prevalence on herd level varied from zero (in eleven compartments) to 15.4%. When only high positive samples were considered, antibodies were detected in five out of twenty compartments and varied between 0 and 11.5% (Table 3).

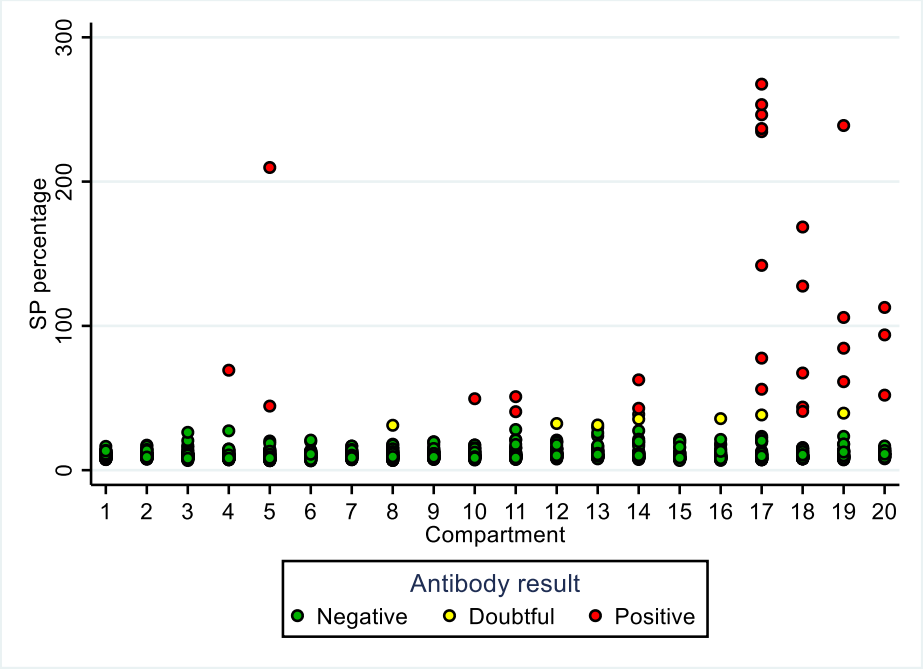

Figure 6. S/P values of ELISA of 991 bulk milk samples collected in August 2023, originating from Dutch dairy herds.

Table 3. Herd level antibody prevalence with 95% confidence interval in 991 cattle bulk milk samples at compartment level, in August 2023 in the Netherlands.

Compartment	Tested	Antibody doubtful or positive (SP percentage>30)		Antibody positive (SP percentage>40)		Antibody highly positive (SP percentage>100)	
		Percentage	95% Confidence interval	Percentage	95% Confidence interval	Percentage	95% Confidence interval
1	49	0	0-5.9%	0	0-5.9%	0	0-5.9%
2	49	0	0-5.9%	0	0-5.9%	0	0-5.9%
3	51	0	0-5.7%	0	0-5.7%	0	0-5.7%
4	49	2.0	0.1-10.9%	2.0	0.1-10.9%	0	0-5.9%
5	47	4.3	0.5-14.5%	4.3	0.1-14.5%	2.1	0.1-11.3%
6	49	0	0-5.9%	0	0-5.9%	0	0-5.9%
7	52	0	0-5.6%	0	0-5.6%	0	0-5.6%
8	50	2.0	0.1-10.6%	0	0-5.8%	0	0-5.8%
9	51	0	0-5.7%	0	0-5.7%	0	0-5.7%
10	48	2.1	0.1-11.1%	2.1	0.1-11.1%	0	0-6.1%
11	48	4.2	0.5-14.3%	4.2	0.1-14.3%	0	0-6.1%
12	50	2.0	0.1-10.6%	0	0-5.8%	0	0-5.8%
13	53	1.9	0.0-10.7%	0	0-5.5%	0	0-5.5%
14	48	8.3	0.0-10.1%	4.2	0.1-14.3%	0	0-6.1%
15	50	0	0-5.8%	0	0-5.8%	0	0-5.8%
16	47	2.1	0.1-11.3%	0	0-6.2%	0	0-6.2%
17	52	17.3	8.2-30.3%	15.4	6.9-28.1%	11.5	4.4-23.4%
18	52	9.6	3.2-21.0%	9.6	3.2-21.0%	3.8	0.5-13.2%
19	48	10.4	3.5-22.7%	8.3	3.5-22.7%	4.2	0.1-14.3%
20	48	6.3	1.3-17.2%	6.3	1.3-17.2%	2.1	0.1-11.1%

The majority of compartments in which no antibodies were found in August 2023, were located in the western and northern part of the country (Figure 7). When both doubtful and positive bulk tank milk were considered positive, antibodies were found in the whole eastern and southern part of the Netherlands (Figure 7). When only highly positive bulk tank milk samples were considered positive, the compartments in which antibodies were found were mainly limited to the southeastern part of the country.

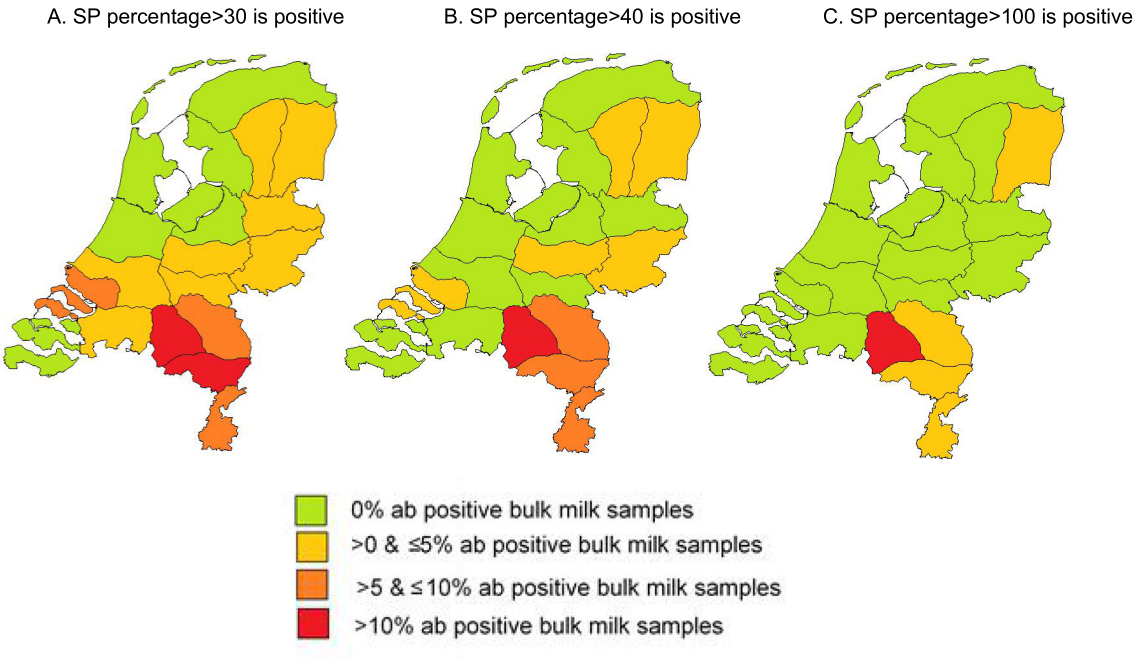

Figure 7. Map of the Netherlands in August 2023, showing the prevalence results on compartment level of an antibody screening of bluetongue in bulk milk samples of 991 dairy herds when different cut-off values are used to classify bulk milk samples as being antibody positive.

3.3 Influence of vaccination on the results of the bulk tank milk screening

Data of vaccination became available after initial analysis of the data. Between September 2017 and July 2023, 1,390 vaccination records against BTV-8 were reported. The vaccines were used in 342 different cattle herds. Most herds vaccinated multiple years in a row and if vaccination was administered, farmers vaccinated twice per year. Most vaccinations were administered in 2019, the year in which BTV-8 emerged in Belgium (Figure 8).

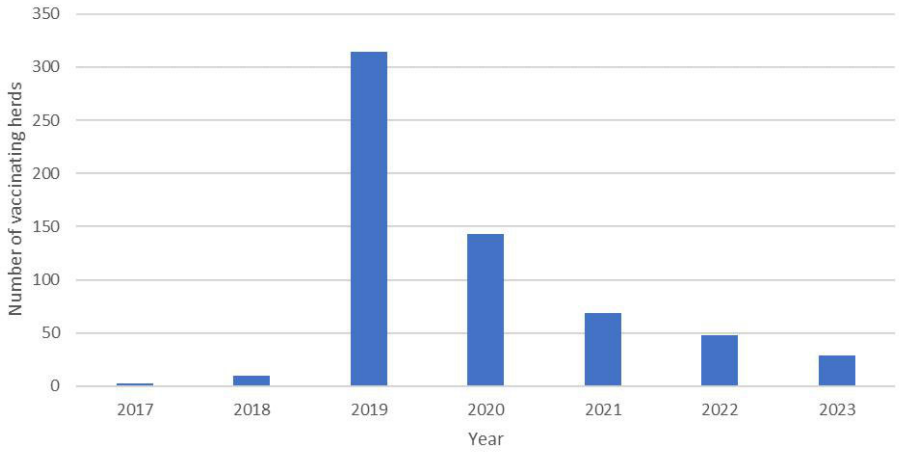

Figure 8. The number of dairy herds of which vaccinations against BTV-8 were recorded per year.

Out of 342 cattle herds with vaccination, bulk milk samples of 28 herds appeared to be included in the screening. Out of these, seven herds tested negative for antibodies (25%) and 21 tested either doubtful or positive (75%). Vaccinated herds that were included in the bulk tank milk screening had a significantly higher probability to have an antibody doubtful or positive result (*P-value Z-test<0.001*).

In the herds that tested negative in the bulk tank milk screening, only 0.7% (7/955) appeared to be vaccinated in the past five years. Of the herds with a doubtful or positive antibody bulk tank milk sample, 58% (21/36) used vaccination in the past five years. Cattle herds that tested bulk tank milk antibody positive, used vaccination significantly more often than herds that tested negative for antibodies (*P-value Z-test<0.001*). From the remaining 15 herds that tested either doubtful or positive for which no evidence of vaccination was found, in three herds, old cows were present of which could be traced that they received vaccination in the past (between 2008 and 2012). The detailed results can be found in Appendix I of this report.

Table 4. Bulk tank milk antibody results relative to the vaccination status between September 2017 and July 2023.

Bulk tank milk result	Vaccinated in the period of interest (Sept '17 – Jul '23)		Total
	No	Yes	
Negative	948 (99.3%)	7 (0.7%)	955
Doubtful	3 (37.5%)	5 (62.5%)	8
Positive	12 (42.9%)	16 (57.1%)	28
Total	963	28	991

BTV antibodies that could not be linked to a recorded history of vaccination against BT were found in twelve out of the 991 bulk tank milk samples. In case that these results would reflect an indication of a BTV infection, this would result in an antibody prevalence of 1.2% (95% CI:0.6-2.1%) in August 2023 in the Netherlands. However, the twelve positive samples were not clustered and showed a somewhat similar distribution as the positive samples that originated from vaccinated herds (Figure 9). Unfortunately, there was no data available of vaccinating herds between 2012 and 2017.

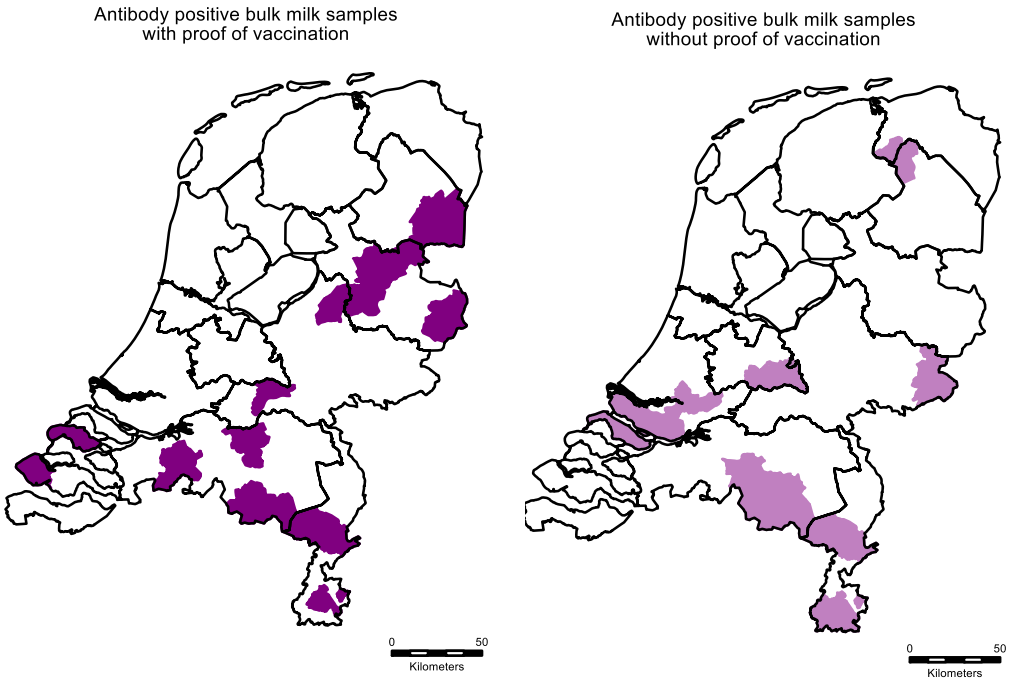

Figure 9. Distribution of location from which the bulk milk samples originated that tested doubtful or positive for antibodies against BTV, for herds where evidence of vaccination in the last five years was found (left) or no evidence of vaccination in the most recent five years was found (right)

4 Discussion and Conclusion

The detection of antibody positive sheep sera and bulk milk samples can have several possible explanations. Specificity of tests might influence test results. With a specificity of 99.8% for the serum test, we might expect two false positive samples in the small ruminant sera. We detected six positive sera. Four of these positive results were found on one sheep farm in compartment 17. This finding may reflect an unnoticed BTV infection, or vaccination. Because the study results were anonymised, we could not check this. More sheep sera tested doubtful, and these findings were scattered over the country, which is more indicative for non-specific results. For bulk milk, specificity was estimated to be 100%. Nevertheless, false positive results can never be ruled out totally. In our study however, the number of positive results is higher than expected based on test specifications.

In August, the antibody prevalence on herd level was 6.7% (95% CI: 2.5-14.1%) in small ruminant farms and 3.6% (95% CI: 2.6-5.0%) in dairy cattle herds when both doubtful and antibody positive samples were considered When only positive were considered, the antibody prevalence at herd level was estimated at 3.4% (95% CI: 0.9-7.5%) in small ruminants and at 2.8% (95% CI: 1.9-4.1%) in cattle.

Both ELISAs that were used, detect antibodies to all BTV serotypes and vaccines. Therefore, not only recent BTV infected animals but also vaccinated animals, or animals that had been infected with BTV8 might have caused antibody positive results in this study. BTV-8 has circulated in previous years in Western Europe. In Germany as well as in Belgium, ruminants were vaccinated in recent years. We tried to rule out the risk for test positives due to imported vaccinated or infected animals, by selecting cattle herds that exclusively housed Dutch cattle and that did not introduce any cattle during the vector active season in 2023 (from April on). However, for sheep we could not make this selection.

Infections with other BTV serotypes cannot be excluded at this moment, and neither can the presence of vaccinated Dutch cattle, since there might be missing records in the vaccination database. In the past, in Germany and Belgium BTV-8 vaccination was subsidised or even obligatory. It is possible that animals that had been raised in Germany or Belgium more than three years ago, and were imported afterwards, have been vaccinated in Germany or Belgium. No data were available about vaccinations in sheep in the Netherlands.

Using data on BTV vaccination in the Netherlands from 2017 on, we could show a clear association between vaccination and antibody detection in bulk milk ELISAs. Out of the 36 bulk milk samples that tested doubtful or positive, 21 had vaccinated in the past. These vaccinating herds were mainly detected in the southern part of the Netherlands. Bulk milk samples with positive ELISA results of herds of which no vaccination was recorded, might be associated with recent or older BTV infections or vaccinations. Based on the location of the herds and the relatively weak positive results of these samples, spread of BTV3 is not the most likely explanation. It was not possible to rule out vaccinating herds beforehand as the vaccination data (MediRund) only came available after the results of the testing were known.

It might have been expected that antibodies would first be detected in the region in which the first clinical signs have been reported. However, in that region most of the samples tested negative. Probably the antibody prevalence in that specific region was still zero or very low in August, however it might be possible we did not test enough samples from that specific area to detect infections.

In conclusion, it is most probable that BTV has not led to many seroconversions in August and the clinical cases we have detected in September were the first BTV infections in the Netherlands.

5 Suggestions for further research

In order to shed a light on the moment of introduction and speed of transmission in the region in the central part of the country where the first and most clinical notifications have been made, more samples from sheep and cattle housed in the specific region that were sampled in August and September could be tested.

To monitor the further spread of BTV infections, it is recommended to test serum samples from small ruminants and bulk milk that will be collected in September and October. For dairy herds, bulk milk samples of herds that tested negative in the August evaluation could be included (when samples from these herds are available, which will probably the case for part of the bulk milk samples).

6 Literature

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7 Appendix 1. Raw ELISA data of the Bulk milk samples that tested doubtful or positive for antibodies with the vaccination status of these herds between Sep ’17 and Jul ‘23

	Old vaccinated cows	Times that vaccination was purchased								
ubn	present*	2019	2020	2021	2022	Jan-jul ‘23	PP%	Result	pc2	Compartment
1		2	1	0	0	0	210	P	77	5
2		2	0	0	0	0	31	D	40	13
3		2	0	0	0	0	39	D	43	14
4	yes	2	0	0	0	0	40	D	60	19
5		3	2	2	2	2	235	P	50	17
6		2	0	0	0	0	41	P	54	18
7		2	0	0	0	0	169	P	54	18
8		2	0	0	0	0	44	P	54	18
9	yes	2	4	0	0	0	67	P	57	18
10		2	1	0	0	0	128	P	57	18
11		2	2	2	2	2	237	P	50	17
12		2	2	2	2	2	246	P	50	17
13		2	4	0	0	0	78	P	52	17
14		3	2	2	3	3	253	P	50	17
15		2	2	2	0	0	142	P	50	17
16		2	0	0	0	0	98	P	63	20
17		2	0	0	0	0	31	D	81	8
18		2	2	0	0	0	85	P	60	19
19		2	2	2	2	2	239	P	55	19
20		2	2	2	2	2	268	P	50	17
21		3	2	0	0	0	36	D	48	16
22							69	P	93	4
23	yes						51	P	75	11
24							41	P	71	11
25							32	D	29	12
26							35	D	32	14
27							63	P	32	14
28	yes						43	P	43	14
29							56	P	56	17
30							61	P	55	19

		Times that vaccination was purchased								
ubn	Old vaccinated cows present*	2019	2020	2021	2022	2023	PP%	Result	pc2	Compartment
31							106	P	60	19
32							52	P	63	20
33							50	P	39	10
34							113	P	62	20
35	yes						44	P	78	5
36							38	D	50	17

* Old cows that were vaccinated between 2008-2012 are present in the herd