

## ORIGINAL RESEARCH

# Bluetongue virus serotype 3 in ruminants in the Netherlands: Clinical signs, seroprevalence and pathological findings

Katrien M. J. A. van den Brink<sup>1</sup>  | Inge M. G. A. Santman-Berends<sup>1</sup> |  
Liesbeth Harkema<sup>1</sup> | Christian G. M. Scherpenzeel<sup>1</sup> | Eveline Dijkstra<sup>1</sup>  |  
Petra I. H. Bisschop<sup>1</sup> | Karianne Peterson<sup>1</sup> | Nienke Snijders van de Burgwal<sup>1</sup> |  
Hubert W. F. Waldeck<sup>1</sup> | Thomas Dijkstra<sup>1</sup> | Melle Holwerda<sup>2</sup> |  
Marcel A. H. Spierenburg<sup>3</sup> | René van den Brom<sup>1</sup>

<sup>1</sup>Royal GD, Deventer, The Netherlands

<sup>2</sup>Wageningen Bioveterinary Research, Lelystad, The Netherlands

<sup>3</sup>Dutch Food and Consumer Product Safety Authority (NVWA), Utrecht, The Netherlands

## Correspondence

Katrien M.J.A. van den Brink, Royal GD, Deventer, The Netherlands.  
Email: [k.vd.brink@gdanimalhealth.com](mailto:k.vd.brink@gdanimalhealth.com)

## Funding information

Ministry of Agriculture, Nature and Food Quality (LNV)

## Abstract

**Background:** The bluetongue virus serotype 3 (BTV-3) outbreak in the Netherlands in 2023 caused severe clinical signs in ruminants. The clinical and pathological signs in ruminants and their spread during the outbreak in 2023 are described.

**Methods:** Data from the Dutch monitoring and surveillance system were available to describe clinical signs and pathological findings related to BTV-3 in sheep, cattle and goats. During the outbreak, 13 farms (five sheep, five cattle and three dairy goats) were closely monitored.

**Results:** In 2023, BTV-3 infections were confirmed by real-time polymerase chain reaction in sheep flocks ( $n = 1807$ ), cattle herds ( $n = 1864$ ), goat herds ( $n = 62$ ), alpaca and/or llama herds ( $n = 15$ ) and one dog. Sheep exhibited the most severe clinical signs and had the highest mortality. In other animal species, a large variation in both occurrence and severity of clinical signs was observed.

**Limitation:** Only 13 farms were closely monitored.

**Conclusions:** The clinical signs observed in affected animals during the 2023 BTV-3 outbreak seem to be more severe than those observed during the BTV-8 outbreak between 2006 and 2008. It seems likely that BTV-3 will overwinter, similar to BTV-8. Therefore, the availability of an effective and safe vaccine is crucial to limit the future impact of BTV-3.

## KEYWORDS

bluetongue virus serotype 3, cattle, clinical signs, outbreak, sheep, transmission

## INTRODUCTION

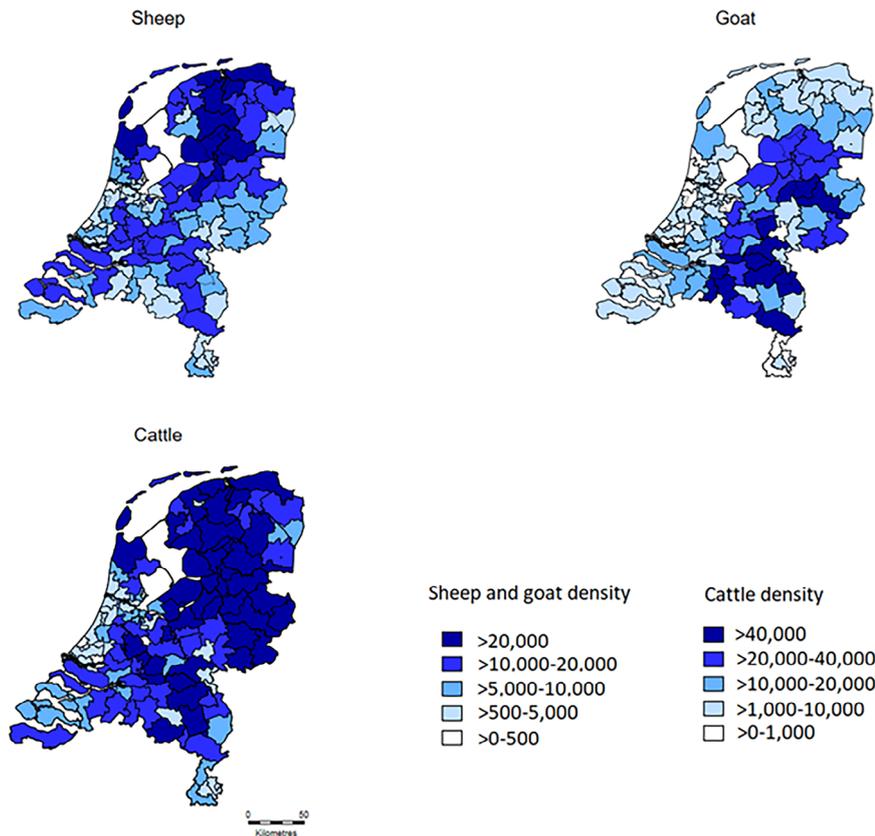
Bluetongue (BT) is an infectious, non-contagious vector-borne viral disease of ruminants caused by bluetongue virus (BTV), which is an Orbivirus from the Reoviridae family and is mainly transmitted by midges of the genus *Culicoides*.<sup>1,2</sup> Transmission via needles, semen and embryos has also been described.<sup>3,4</sup> Additionally, some BTV serotypes can be transmitted verti-

cally from dam to offspring.<sup>5</sup> BT is listed by the World Organisation for Animal Health as a multispecies disease with a large economic impact<sup>6</sup> and is categorised as a category C + D + E disease by the European Animal Health Law.<sup>7,8</sup> To date, more than 30 different BTV serotypes have been described, although only the first 24 are notifiable.<sup>9,10</sup>

BT causes morbidity and mortality in domestic and wild ruminants and has also been described

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *Veterinary Record* published by John Wiley & Sons Ltd on behalf of British Veterinary Association.



**FIGURE 1** Density of sheep, cattle and goats per two-digit postal code area in the Netherlands in 2023, generated with identification and registration (I&R) data

in camelids and carnivores.<sup>10–12</sup> Sheep develop the most severe clinical signs from BT; however, the BTV serotype 8 (BTV-8) outbreak in the Netherlands between 2006 and 2008 also resulted in clinical illness in cattle and goats.<sup>13,14</sup> Clinical signs are mainly a result of damage to the endothelial cells of blood vessels, leading to extensive systemic vascular damage with fluid leakage, bleeding and ischaemic degeneration and necrosis of the affected tissues.<sup>1</sup>

On 3 September 2023, suspicions of BTV infections were reported to the Dutch Food and Consumer Product Safety Authority (NVWA) by two veterinary practices in the centre of the Netherlands. BTV was confirmed on 5 September and was identified as BTV serotype 3 (BTV-3) by the Dutch National Reference Laboratory Wageningen Bioveterinary Research (WBVR) and the European Reference Laboratory in Spain.<sup>15,16</sup> A retrospective study based on stored bulk milk samples did not indicate extensive circulation of BTV before early September 2023, and the source and route of virus introduction remain unclear.<sup>15</sup> Following the detection of BTV-3 in the Netherlands, spread to Belgium, Germany and the UK was confirmed in 2023.<sup>16</sup>

This manuscript aims to: (1) describe the clinical signs and pathological findings associated with the BTV-3 outbreak in multiple animal species; (2) give insight into within-herd and between-herd spread on five sheep, five cattle and three goat farms; and (3) discuss challenges and insights related to BTV-3 during the outbreak in the Netherlands in 2023.

## MATERIALS AND METHODS

### Study population and study period

This manuscript focuses on the main BTV-susceptible domestic ruminants in the Netherlands: sheep, cattle and goats. The study period started at the beginning of the outbreak on 3 September 2023 and continued until 31 December 2023. During this period, about three million cattle (excluding veal calves), one million sheep and 650 thousand goats were present in the Netherlands. Figure 1 shows the density of cattle, sheep and goats in the Netherlands in 2023. All maps were generated using STATA (version 17).

### Notification data

Since BT is a notifiable disease, data from notified and confirmed BT cases from the NVWA were analysed to obtain an impression of the spread of BTV-3. Blood samples from animals with clinical signs of BT were sent to the WBVR and analysed with real-time polymerase chain reaction (PCR).<sup>15</sup> NVWA listed PCR-confirmed cases as well as clinical cases reported to NVWA by veterinary practitioners and/or farmers for which no blood samples were submitted for confirmation.

### Dutch monitoring and surveillance system

Monitoring and surveillance systems in both cattle (Cattle Health Surveillance System [CHSS]) and small

ruminants (Small Ruminant Health Surveillance System [SRHSS]) in the Netherlands were created with the aim to: (1) identify outbreaks of specified animal diseases not endemic in the country, (2) identify previously unknown clinical and pathological findings, and (3) follow trends and developments in endemic diseases in (small) ruminants.<sup>17,18</sup> The CHSS and SRHSS were used to map the clinical signs and pathological findings of BTV-3 infection in sheep, cattle and goats.

## Veterinary helpdesk and pathological examination

Clinical signs displayed by sheep, cattle and goats were reported by telephone to the veterinary helpdesk (Royal GD Veekijker) as part of the CHSS and SRHSS. In addition to clinical reports from farmers and veterinarians, clinical aspects were complemented by findings acquired during visits to BTV-3-infected farms conducted by Royal GD veterinarians. Post-mortem examinations of sheep, cattle and goats were conducted to characterise the lesions associated with BTV-3 infections. Several infected animals that either died or were euthanased were submitted for postmortem examination at Royal GD. An overall macroscopic examination was conducted as well as tissue collection for histopathological review based on macroscopic lesions and the reported clinical signs. A sample of splenic tissue was also submitted to WBVR for BTV PCR testing. Taken together, these findings were used to gain a better understanding of the presence of BTV-3 in the affected species, species differences, variations in disease severity, prognosis and possible treatment options.

## Prospective follow-up in 13 case farms

A total of five sheep farms, five dairy cattle farms and three dairy goat farms were monitored over 13 weeks by cattle and small ruminant veterinarians from Royal GD. The inclusion criteria consisted of herd or flock sizes with at least 100 animals, having a BTV-3 infection confirmed by PCR in October 2023 and not having vaccinated against BTV (any serotype) in the last 5 years. For cattle herds and sheep flocks, the study period started when a first farm visit was performed, clinical signs were scored and 50 randomly selected animals were blood sampled (serum and EDTA). Based on 50 samples, and assuming an unknown prevalence (i.e., 50%), a point estimate with 14% precision and 95% confidence could be determined (Stata). The samples collected at the start of the study period were submitted to WBVR for PCR testing and to Royal GD for an IDEXX Bluetongue Competition Antibody Test (Idexx), which detects antibodies against the highly conserved protein VP7.

At the sheep and cattle farms, clinical signs were followed up by a veterinarian during a second farm visit that was scheduled 2 weeks after the initial visit. Dur-

ing the second visit, the animals showing clinical signs were examined and recorded. In every other week during the study period, animals showing clinical signs were recorded by the farmer, and these cases were discussed during weekly telephone contact between the farmers and the veterinarian. The number of ill, recovered and deceased animals, as well as the clinical signs observed in affected animals, were recorded by farmers and veterinarians using predefined protocols. The three dairy goat farms were monitored by weekly telephone contact to record and monitor clinical signs. In January 2024, the study period of 12 of the participating cattle, sheep and dairy goat farms was finished with a farm visit to determine the BTV-3 seroprevalence based on serum samples of 50 randomly selected animals. On one sheep farm, the final visit took place in February 2024.

In addition to the on-farm data collected, mortality data were obtained from the national identification and registration (I&R) database (Netherlands Enterprise Agency [RVO]).

## RESULTS

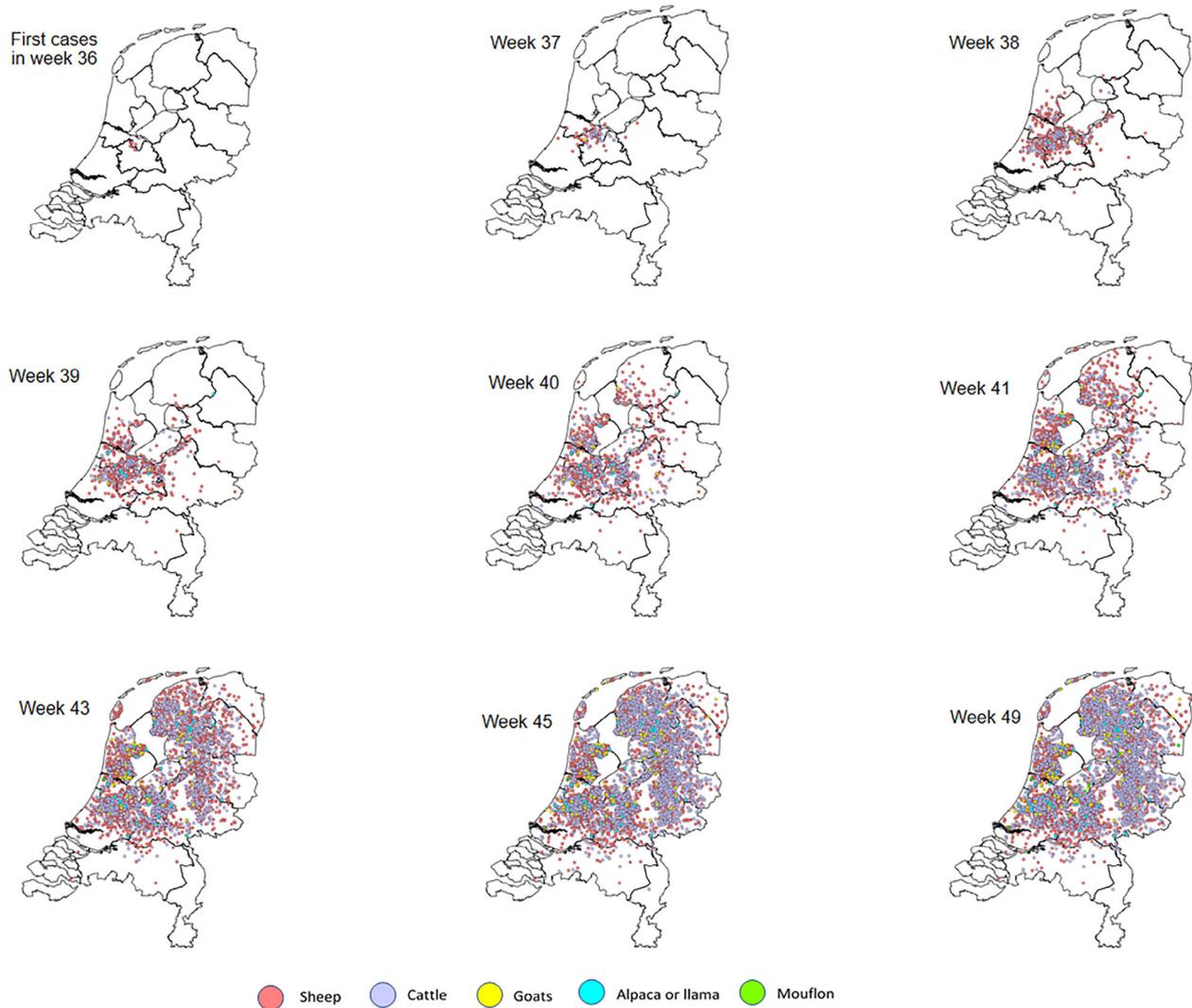
### Spread of BTV-3 in the Netherlands

After the onset of the outbreak in September 2023, BTV-3 spread rapidly through the country, affecting many flocks and herds across species (Figure 2). The NVWA data showed that, up to 31 December 2023, infections with BTV-3 were confirmed by PCR in 1807 sheep farms and 1864 cattle farms. In addition, infections were detected and confirmed by PCR in (dairy) goats (62 farms), alpacas (14 farms), llamas (one farm), vicuña (one zoo), two mouflons and one dog. Furthermore, signs consistent with BT had been reported by 1456 farmers (75% sheep farmers, 23% cattle and 2% other) on farms where BTV-3 had not yet been confirmed with PCR.

### BTV-3 in sheep

#### Clinical signs

During the BTV-3 outbreak, affected sheep exhibited apathy, isolation from others, lameness/stiffness and/or excessive salivation and nasal discharge. Inspection of the head revealed oedematous swellings (Figure 3), sometimes limited to the lips, and swelling, redness, erosion and/or ulceration of the mouth (Figure 4), conjunctiva and nasal mucous membranes. Some of the surviving sheep showed (peri)arthritis and shedding of claw horn in the weeks after the first clinical signs were seen. In this period, stiffness as a consequence of muscle degeneration was also reported. High mortality was reported on sheep farms. A table of the clinical signs observed during the prospective case study can be found in [Supporting Information](#).



**FIGURE 2** Spread of bluetongue virus serotype 3 (BTV-3) through the Netherlands from the start of the outbreak until December 2023



**FIGURE 3** Sheep with oedematous swelling of the head and jaws

## Pathological findings

Pathological examination of 23 BTV-3-infected sheep revealed severe pulmonary oedema, with or without hydrothorax and hydropericardium. Severe ulcerations and haemorrhages in the ruminal pillars (Figure 5) and/or smaller haemorrhages in the myocardium were also observed. Histological examination confirmed the severity of organ damage. Lung tissue exhibited marked alveolar oedema, with microangiopathy, vasculitis, microhaemorrhages, occasional microvascular thrombosis and multifocal damage to alveolar septa (Figure 6). Oral mucous membranes and ruminal pillars showed subepithelial microangiopathy, vasculitis and haemorrhages associated with interstitial oedema and ischaemic necrosis and ulceration of the overlying epithelium. Acute muscle degeneration was occasionally observed in the oesophagus, ruminal pillars, myocardium and skeletal muscle tissue. In severely affected cases, animals showed extensive acute tubular degeneration, microangiopathy and vasculitis in the kidneys. Small blood vessels in the dermal lamina of the claw were



**FIGURE 4** Mouth of a sheep with lesions on the dental plate



**FIGURE 5** Ulceration and haemorrhages in the ruminal pillars of a sheep

often affected, with oedema, haemorrhages, neutrophilic laminitis and detachment of hoof horn. In more chronic cases, secondary purulent inflammation was seen in the hooves. In one aborted lamb from an ewe suffering from BT, BTV-3 was detected in the fetal spleen without additional malformations of the fetus.

### Morbidity, mortality and within-herd prevalence

The morbidity rate across the five more intensely monitored sheep farms was 7.5%. During the study period, 202 sheep on these farms exhibited clinical signs indicative of BTV-3 infection. The percentage of animals showing clinical signs varied between 0.9% and 14.2%. The case fatality rate was 74.8%. All five

sheep farms experienced significantly higher mortality during the study period than during the same period in 2022 (Figure 7). On average, 45 (10%, min 3%, max 17%) sheep per farm died in the study period compared to an average of 13 sheep per farm in the previous year. Mortality increased in both lambs (<1 year old) and sheep (>1 year old) (Figure 7).

The diagnostic evaluation at the initial visit showed that the average percentage of animals that tested PCR positive (13%) was similar to the percentage of antibody-positive animals (12%). Unexpectedly, on four of the five sheep farms, the percentage of animals with antibodies did not increase between the first and final measurements (Figure 8). The diagnostic results from the initial farm visit in October and the I&R data for individual animals revealed that, out of 18 antibody-positive sheep for which I&R data were available, seven sheep died by the end of the study period (38%, 95% CI: 17%–64%). For the sheep that tested antibody negative during the initial visit and for which I&R data were available, the mortality was significantly lower (4%, 95% CI: 2%–8%). Therefore, the final antibody prevalence measurement (7%) in January 2024 primarily indicates which portion of sheep is likely to be protected against BTV-3 in 2024. It does not reflect the extent of virus spread in 2023.

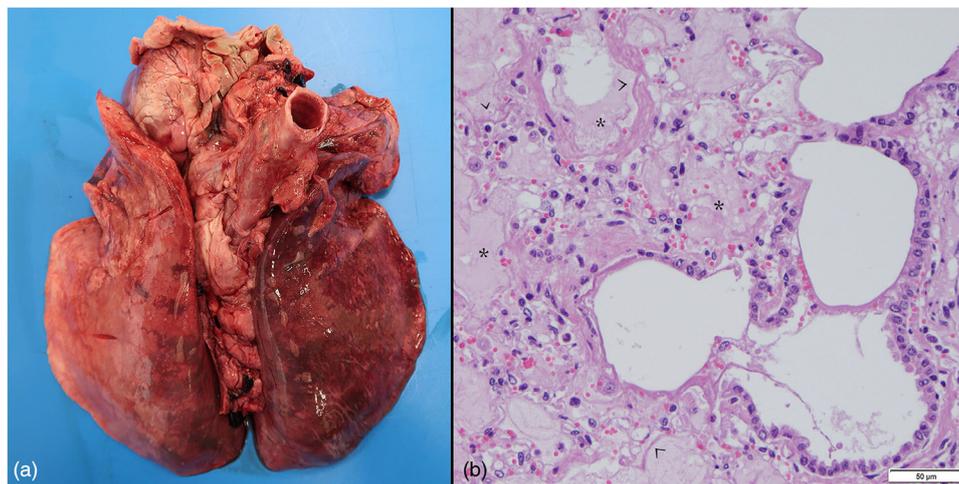
## BTV-3 in cattle

### Clinical signs

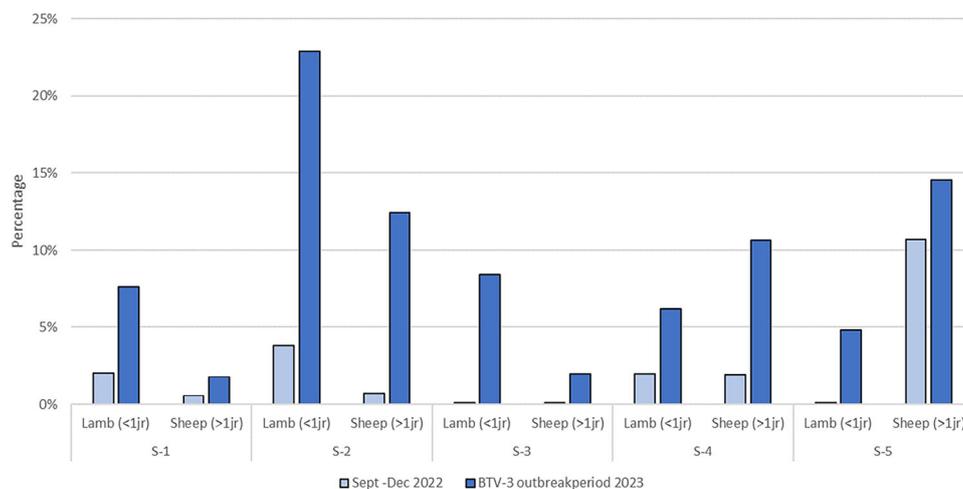
Clinical signs observed in cattle during the BTV-3 outbreak included fever, lesions, such as erosions, ulcerations and bleedings on the nasal planum (Figure 9), in the mouth (Figure 10) and on the teats with hyperaemia (Figure 11), as well as nasal discharge, conjunctivitis and salivation. Lameness and stiffness were prominent signs, with red and swollen coronary bands (coronitis) and swelling of the legs (Figure 12). Additionally, regurgitation was reported. The course and duration of the disease, the number of sick cattle per farm and the severity of clinical signs in cattle varied, ranging from subclinical and mild disease to severe cases with serious illness and claw horn detachment. In particular, cows around the time of calving were found to be severely ill. Animals with severe clinical signs, such as severe lameness or severe pulmonary oedema, died, or in cases of unacceptable suffering, they had to be euthanased. Additionally, a clear and significant drop in milk production was observed, which was most prominent in areas that were infected in September 2023. A table of all clinical signs observed during the prospective case study can be found in the [Supporting Information](#).

### Pathological findings

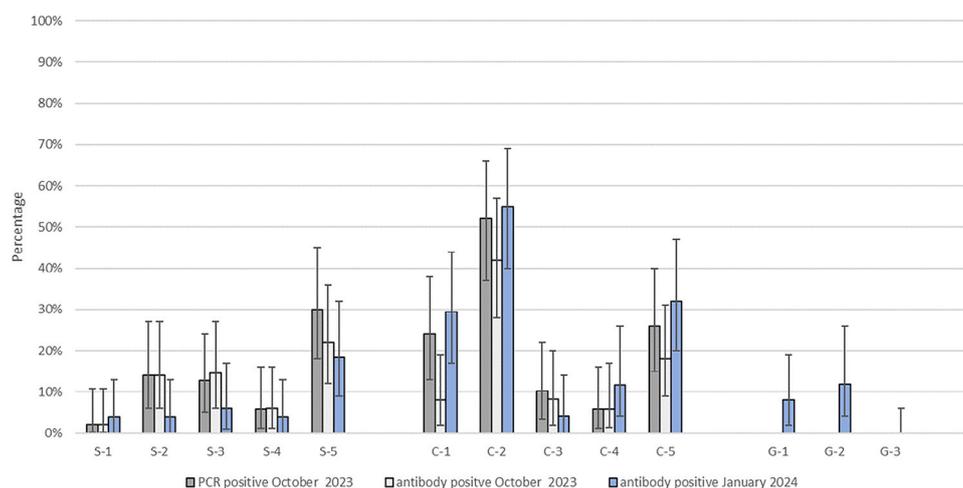
Pathological examination of 42 cattle with BT primarily showed erosions and ulcerations on the mucocutaneous junctions of the lip and nasolabial planum, ulcerations and petechial haemorrhages in



**FIGURE 6** (a) oedematous lungs of a sheep. (b) microscopic image of lung tissue with oedema (\*) and damage to alveolar septa (>)



**FIGURE 7** Mortality of sheep (>1 year) and lambs (<1 year) during the bluetongue virus serotype 3 (BTV-3) outbreak period in 2023 (dark blue) compared to the same period in 2022 (light blue) on the sheep farms (S1–S5)



**FIGURE 8** Proportion of animals on individual sheep (S1–S5), cattle (C1–C5) and goat (G1–G3) farms testing positive for bluetongue virus by polymerase chain reaction (PCR) and antibody testing during the study period

the oral cavity and pharynx, and petechial haemorrhages on the heart, pulmonary artery and aorta. In three aborted and/or stillborn calves, BTV-3 was detected in the spleen by PCR testing without further

morphological abnormalities being found in these calves.

Histopathological examination of affected mucous membranes revealed histopathological lesions similar



**FIGURE 9** Nasal planum of a cow with lesions



**FIGURE 11** Udder of a cow with redness of the skin and lesions on the teats



**FIGURE 10** Mouth of a cow with lesions



**FIGURE 12** Leg of a cow with red and swollen coronary bands (coronitis)

to those in sheep: ulceration, subepithelial microhaemorrhages, oedema, vasculitis, microangiopathy and microvascular thrombosis. Acute muscle degeneration was occasionally observed in the tongue, oesophagus, rumen pillars, skeletal muscles and tunica media of the aorta. The muscle degeneration observed in the oesophagus in some animals may explain the regurgitation reported in the field.

### Morbidity, mortality and within-herd prevalence

Morbidity across the five case farms was 24.5%, with a total of 214 cows in the five study herds exhibiting clinical signs indicative of a BTV-3 infection during the 13-week study period. The percentage of animals showing clinical signs varied between 8.1% and 50.4% per farm. At the end of the study, 196 of the cows that were reported as being ill at some

point during the study were documented as recovered (91.6%). On average, cows were ill for 22 days. During the BTV-3 outbreak period, four of the monitored dairy farms registered a deceased cow, compared to no mortality in the same period of the previous year.

There was no remarkable difference in calf mortality between the period of the BTV-3 outbreak and the same period in 2022. At the start of the study, the prevalence of BTV-3 PCR-positive cows was higher than the prevalence of antibody-positive animals. At the final measurement, the percentage of animals testing positive for antibodies against BTV-3 had increased on four of the five farms, with an average antibody prevalence of 26% (min 4%, max 55%) (Figure 8).

## BTV-3 in (dairy) goats

### Clinical signs

In general, BTV-3-related clinical signs in goats appeared milder than those in sheep and cattle, although deaths were reported. The clinical signs that were observed included fever, swollen lips, ocular and nasal discharge, decreased milk production, lameness, abnormal signs of the central nervous system, hyperaemia and haemorrhages of the udder skin. A table of all the clinical signs observed during the prospective case study can be found in the [Supporting Information](#).

### Pathological findings

Pathological examination of seven BTV-3-infected dairy goats showed subcutaneous oedema in the lips and/or head, mild erosions and ulcerations in the mouth, occasional mild ulceration of the ruminal pillars and hyperaemia of the udder skin. Histopathological examination revealed marked acute skeletal muscle degeneration and muscle degeneration in the tongue and oesophagus. Microangiopathy and perivascular haemorrhages were observed throughout the brain and in the claw lamina.

### Morbidity, mortality and within-herd prevalence

The morbidity across the three goat farms was 1.1%, with a total of 39 animals exhibiting clinical signs indicative of a BTV-3 infection during the 13-week study period. The percentage of animals showing clinical signs varied between 0.3% and 3.6% per farm. The case fatality rate was 44%. Of the 39 goats reported as sick, 17 died by the end of the study, 15 were reported as fully recovered by the farmers (28%), two were sent for slaughter and the remaining five has still not fully recovered at the end of December 2023. On these three goat farms, mortality during the study period in 2023 was not significantly different from that in 2022. However, it should be noted that the infection rate on these three farms also appeared to be limited, with the prevalence of antibodies ranging from 0% to a maximum of 12% (Figure 8).

## DISCUSSION

The findings of this study provide insight into the impact of BTV-3 on the health of multiple animal species. The clinical signs observed during the BTV-3 outbreak appeared similar to those observed during the previous BTV-8 outbreak, although more severe. Overall, the case fatality rate was high, especially in sheep.

Although it is too early to quantify the full impact of this BTV-3 outbreak, it seems that the spread of BTV-3

occurred faster and had a more severe impact<sup>19</sup> than did the BTV-8 outbreak in the period 2006–2008.<sup>20</sup> In August 2006, the BTV outbreak was detected in the southern Netherlands, and movement restrictions for animals were put in place immediately. In that year, spread remained limited to the southern region, with approximately 400 notified cases. In 2023, the situation was completely different. The first cases were detected in the centre of the Netherlands at the beginning of September, and no movement restrictions were implemented. During the following months, BTV-3 spread rapidly throughout large parts of the country, infecting over 5000 farms. During the BTV-8 outbreak, the virus started to spread again from July 2007 onwards and infected the majority of ruminant herds and flocks in the Netherlands.<sup>21</sup> Most BTV-8 infections and the highest impact on animal health were observed in the second year of the outbreak (2007).<sup>20</sup>

The severity of clinical signs associated with BTV-3 varied greatly within and among animal species (sheep, cattle and dairy goats) on the 13 monitored farms that were followed up, as did morbidity and mortality. However, the seroprevalence on all monitored farms at the end of the study was relatively low. We were able to use the antibody results at the end of the study period to measure the prevalence because, without spread of BTV-3, the studied cattle should have been antibody negative. Before the BTV-3 outbreak, no BTV infections had been detected in the Netherlands since 2009, with freedom from infection proven every year between 2009 and 2023 according to EU legislation. This resulted in the Netherlands obtaining official BTV-free status since February 2012. This fact, combined with the fact that we ensured that only non-BTV-vaccinated herds were included in our study, allowed us to conclude that the antibodies found during the study were acquired through a BTV-3 infection. During the study period, mortality was significantly higher in sheep that tested antibody positive in October than in antibody-negative sheep. The seroprevalences of sheep farms unexpectedly decreased during the study period, most likely due to the high case fatality rate in sheep. There was a clear increase in seroprevalence in cattle, possibly due to the fact that most cattle survived the infection and antibodies were subsequently detected in these animals at the final measurement. Based on the overall low seroprevalences found on BTV-3-infected farms in our study (Figure 8), it may be that the majority of the Dutch livestock population is still susceptible to BTV-3 infections, even in infected herds. Based on that and the experiences during the previous outbreak, it is likely that BTV-3 will overwinter, similar to BTV-8 in 2006 and 2007, when the highest impact was seen in the second year. There is a major concern about what to expect in 2024 if no licensed vaccine is available in time. Additionally, BTV-3 could overwinter in more locations in 2023/2024 than in 2006/2007.

Similar to the outbreak of BTV-8, we found that BTV-3 was vertically transmitted from cows and ewes to their unborn offspring.<sup>5,16,22</sup> It is not yet clear

whether vertical transmission can lead to the birth of clinically healthy calves and lambs positive for BTV-3 or to the birth of animals with congenital abnormalities. All these outcomes were observed during the previous BTV-8 outbreak.<sup>23</sup>

The remaining questions are whether the virus will overwinter and which preventive measures could be taken by farmers in order to prevent new infections in 2024. Based on previous experience with BTV-8 and the relatively mild winter of 2023/2024, overwintering of BTV-3 is a realistic scenario. In the case of successful overwintering of BTV-3, applying an effective and safe inactivated BTV-3 vaccine is likely to be the best measure to prevent new infections. In spring 2023, it was reported that a German autogenous vaccine was found to cause clinical signs of BT after vaccination, resulting in BTV PCR-positive animals. At the time of writing, several BTV-3 vaccines are available for the Dutch, Belgian and German markets. Besides vaccination, management measurements could be taken by farmers to protect susceptible animals from being bitten by *Culicoides* midges. Based on an EFSA report,<sup>24</sup> it may be concluded that the use of insecticides and repellents reduces host/vector contact, but no evidence for a positive effect on the reduction of transmission of BTV in the field was found. Based on preliminary experience from this outbreak and scientific proof during the previous outbreak,<sup>25</sup> housing of animals, especially during midge activity periods, combined with proper ventilation seems to be the best known protective measure.

The Dutch CHSS and SRHSS, in which farmers and veterinary practitioners play an important role in the early detection and monitoring of animal diseases, in close collaboration with the Royal GD, WBVR, NVWA and the Ministry of Agriculture, Nature and Food Quality, has proved to be essential in the early detection of the BTV-3 outbreak. This study revealed that seroprevalence across various animal species remained low after 2023. However, there is a significant risk of BTV overwintering. Therefore, it remains crucial for veterinarians and livestock owners to continue reporting new clinical suspicions to the NVWA and the Royal GD telephone helpdesk. The Dutch monitoring and surveillance system will remain crucial in identifying future infections.

## CONCLUSIONS

BTV-3 rapidly spread in a large part of the Netherlands in 2023, infecting several ruminant species. Sheep exhibited the most severe clinical signs, with high mortality and case fatality rates. A wide variety of clinical signs and disease severities were observed in other species. Overall, the clinical signs related to this BTV-3 outbreak seem more severe than those related to the BTV-8 outbreak in the Netherlands in the period 2006–2008. The availability of a licensed inactivated vaccine is crucial to combat BTV-3.

## AUTHOR CONTRIBUTIONS

Katrien van den Brink, Inge Santman-Berends, Christian Scherpenzeel and René van den Brom contributed to the study design, methodology and writing and reviewing of the manuscript. Liesbeth Harkema was responsible for the pathology data, including the text related to that topic. Data analysis was performed by Irene Bisschop and Inge Santman-Berends. Data collection was done by Christian Scherpenzeel, Frederik Waldeck, Katrien van den Brink, Karianne Peterson, Eveline Dijkstra, Thomas Dijkstra, Nienke Snijders van de Burgwal, Melle Holwerda and Marcel Spierenburg. All authors reviewed and edited the manuscript.

## ACKNOWLEDGEMENTS

The authors would like to thank all the farmers and veterinarians in the field who reported the clinical signs and submitted animals for pathological examination, as well as the farmers who participated in the prospective follow-up study. We also thank our colleagues at Royal GD, NVWA and WBVR for their contributions in managing this outbreak. This research was partly sponsored by the Ministry of Agriculture, Nature and Food Quality (LNV) contract number IUCEZK202310168 and project number 1600002757 'WOT VZVD', which was granted to WBVR.

## CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are not publicly available due to privacy or ethical restrictions. They can be made available by the corresponding author upon reasonable request.

## ETHICS STATEMENT

The Animal Welfare Body of Royal GD gave permission to perform the study on the 13 case farms. This was approved by TaskNumber IvD 2023-063. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and that appropriate ethical review committee approval has been received. Directive 2010/63/EU was followed.

## ORCID

Katrien M. J. A. van den Brink  <https://orcid.org/0000-0001-8241-3658>

Eveline Dijkstra  <https://orcid.org/0000-0002-2367-3393>

## REFERENCES

1. MacLachlan NJ, Drew CP, Darpel KE, Worwa G. The pathology and pathogenesis of bluetongue. *J Comp Pathol.* 2009;141(1):1–16.
2. MacLachlan NJ, Mayo CE, Daniels PW, Savini G, Zientara S, Gibbs EP. Bluetongue. *Rev Sci Tech.* 2015;34(2):329–40.
3. Suttmoller P, Wrathall AE. The risks of disease transmission by embryo transfer in cattle. *Rev Sci Tech.* 1997;16(1):226–39.

4. Suttmoller P, Wrathall AE. A quantitative assessment of the risk of transmission of foot-and-mouth disease, bluetongue and vesicular stomatitis by embryo transfer in cattle. *Prev Vet Med.* 1997;32(1–2):111–32.
5. Santman-Berends IMGA, van Wuijckhuise L, Vellema P, van Rijn PA. Vertical transmission of bluetongue virus serotype 8 virus in Dutch dairy herds in 2007. *Vet Microbiol.* 2010;141(1–2):31–35.
6. World Organisation for Animal Health. Terrestrial animal health code. Section chapter 8.3: Infection with bluetongue virus. Accessed 2 Apr 2024. [https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmlfile=chapitre\\_bluetongue.htm](https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmlfile=chapitre_bluetongue.htm)
7. European Union. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law'). 2016. Accessed 2 Apr, 2024. <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32016R0429&qid=1721909751854>
8. Hodnik JJ, Acinger-Rogic Z, Alishani M, Autio T, Balseiro A, Berezowski J, et al. Overview of cattle diseases listed under category C, D or E in the animal health law for which control programmes are in place within Europe. *Front Vet Sci.* 2021;8:688078.
9. van Rijn PA. Prospects of next-generation vaccines for bluetongue. *Front Vet Sci.* 2019;6:407.
10. Saminathan M, Singh KP, Khorajiya JH, Dinesh M, Vineetha S, Maity M, et al. An updated review on bluetongue virus: epidemiology, pathobiology, and advances in diagnosis and control with special reference to India. *Vet Q.* 2020;40(1):258–321.
11. Alexander KA, MacLachlan NJ, Kat PW, House C, O'Brien SJ, Lerche NW, et al. Evidence of natural bluetongue virus infection among African carnivores. *Am J Trop Med Hyg.* 1994;51(5):568–76.
12. Dubovi EJ, Hawkins M, Griffin RA Jr., Johnson DJ, Ostlund EN. Isolation of bluetongue virus from canine abortions. *J Vet Diagn Invest.* 2013;25(4):490–92.
13. Elbers AR, Backx A, Meroc E, Gerbier G, Staubach C, Hendrickx G, et al. Field observations during the bluetongue serotype 8 epidemic in 2006. I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium, France and the Netherlands. *Prev Vet Med.* 2008;87(1–2):21–30.
14. Backx A, Heutink CG, van Rooij EM, van Rijn PA. Clinical signs of bluetongue virus serotype 8 infection in sheep and goats. *Vet Rec.* 2007;161(17):591–92.
15. Holwerda M, Santman-Berends IMGA, Harders F, Engelsma M, Vloet RPM, Dijkstra E, et al. Emergence of bluetongue virus serotype 3 in the Netherlands in September 2023. *bioRxiv.* 2023. 20230929560138. <https://www.biorxiv.org/content/10.1101/2023.09.29.560138v1>
16. van den Brink KMJA, Santman-Berends IMGA, Harkema L, Scherpenzeel CGM, Dijkstra E, Mars MH, et al. Uitbraak van blauwtong serotype 3 in Nederland. *Tijdschrift voor Diergeneeskunde.* 2023;148(12):45–49.
17. Dijkstra E, Vellema P, Peterson K, Bogt-Kappert CT, Dijkman R, Harkema L, et al. Monitoring and surveillance of small ruminant health in The Netherlands. *Pathogens.* 2022; 11(6):635.
18. Santman-Berends IMGA, Brouwer-Middelesch H, Van Wuijckhuise L, de Bont-Smolenaars AJG, Van Schaik G. Surveillance of cattle health in the Netherlands: monitoring trends and developments using routinely collected cattle census data. *Prev Vet Med.* 2016;134:103–12.
19. Boender G-J, Hagenaars TJ, Holwerda M, Spierenburg MAH, van Rijn PA, van der Spek AN, et al. Spatial transmission characteristics of the Bluetongue virus serotype 3 epidemic in the Netherlands, 2023. *Viruses.* 2024;16:625.
20. Santman-Berends IMGA. Transmission and impact of bluetongue virus serotype 8 in dairy cattle. Deventer, The Netherlands: Utrecht University; 2011.
21. van Schaik G, Berends IMGA, van Langen H, Elbers ARM, Vellema P. Seroprevalence of bluetongue serotype 8 in cattle in the Netherlands in spring 2007 and its consequences. *Vet Rec.* 2008;163(15):441–44.
22. Santman-Berends IMGA, Hage JJ, van Rijn PA, Stegeman JA, van Schaik G. Bluetongue virus serotype 8 (BTV-8) infection reduces fertility of Dutch dairy cattle and is vertically transmitted to offspring. *Theriogenology.* 2010;74(8): 1377–84.
23. Wouda W, Peperkamp NH, Roumen MP, Muskens J, van Rijn A, Vellema P. Epizootic congenital hydranencephaly and abortion in cattle due to bluetongue virus serotype 8 in the Netherlands. *Tijdschr Diergeneeskd.* 2009;134(10):422–27.
24. EFSA Panel on Animal Health and Welfare. Bluetongue: control, surveillance and safe movement of animals. *EFSA J.* 2017;15(3):e04698.
25. Santman-Berends IMGA, Bartels CJM, van Schaik G, Stegeman JA, Vellema P. The increase in seroprevalence of bluetongue virus (BTV) serotype 8 infections and associated risk factors in Dutch dairy herds in 2007. *Vet Microbiol.* 2010;142(3–4): 268–75.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** van den Brink KMJA, Santman-Berends IMGA, Harkema L, Scherpenzeel CGM, Dijkstra E, Bisschop PIH, et al. Bluetongue virus serotype 3 in ruminants in the Netherlands: Clinical signs, seroprevalence and pathological findings. *Vet Rec.* 2024;e4533. <https://doi.org/10.1002/vetr.4533>