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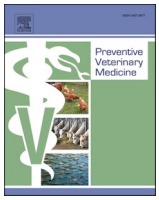

# Seroprevalence of Bluetongue serotype 3 and associated risk factors in Dutch sheep: An analysis of the variation of between-and within-farm prevalences following the first epidemic year

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ABSTRACT

In September 2023, bluetongue virus serotype 3 (BTV-3) emerged in the Netherlands. Thousands of sheep farms became infected and over 55 thousand sheep died between September and December 2023. This study aimed to determine the final size of the epidemic after the vector-active season of 2023. To help farmers prepare for possible resurgence in 2024, risk factors were identified that were associated with presence of BTV antibodies at farm level, and if so, the proportion of affected sheep in positive farms. Archived serum samples from adult sheep (N = 387 farms; 13 samples per farm) submitted to Royal GD for monitoring purposes between January and April 2024 (prior to the vector active season of 2024) were selected and tested for presence of BTV-specific antibodies. Farm prevalence, within-farm prevalence in seropositive farms and overall (national) animal level BTV prevalence were calculated from the test results. Additionally, farmers that agreed to participate in the study were surveyed in spring 2024 regarding their farm management in 2023. Multivariable regression analyses with appropriate distributions were conducted to quantify associations between potential risk factors and i) the probability of sheep farms being BTV-seropositive, and if so, ii) the proportion of affected sheep within the farm. For the latter, the apparent within-farm seroprevalence was combined with the mortality recorded at farm level between Sept-Dec 2023, to correct for infected sheep that died in affected farms. Farm-level seroprevalence in sheep was estimated at 47 % (95 % CI: 41–52) and the national animal-level seroprevalence was estimated to be 10 % (95 % CI: 8–13). Within BTV seropositive farms, a median percentage of 15 % (mean 23 %) of sheep tested seropositive. Flocks kept indoors to prevent infection were less frequently seropositive (OR: 0.25, 95 % CI: 0.09–0.67). Within seropositive farms, shearing in summer, between July and September, was associated with a higher proportion of affected sheep compared to shearing in winter months (OR: 2.22, 95 % CI: 1.27–3.86). Although several protective factors were found, they might not fully prevent sheep from becoming infected resulting in seropositivity. Nevertheless, these measures likely offer farmers possible measures to reduce the spread and subsequent impact of BTV-3 in their sheep farms, beyond vaccination.

## 1. Introduction

Bluetongue (BT) is an infectious, non-contagious, arthropod-borne disease primarily affecting ruminants. It is caused by the bluetongue virus (BTV), a segmented double-stranded RNA virus belonging to the genus Orbivirus within the Reoviridae family. BTV is almost exclusively transmitted by midges of the genus *Culicoides* (Maclachlan et al., 2015, 2009). The virus induces haemorrhagic disease in ruminants, characterized by high fever, depression, mucosal lesions in the mouth and nose, congestion of the coronary band, hypersalivation, nasal discharge, lameness and mortality (Saminathan et al., 2020; Elbers et al., 2008). Although BTV affects most species of wild and domestics ruminants,

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clinical signs and mortality are generally more severe in (domestic) sheep compared to cattle and goats. The first outbreak of BTV in the Netherlands was identified in 2006 and was attributed to BTV serotype 8. During the 2006 outbreak, 7 % of sheep farms were affected (Elbers et al., 2008). After successful overwintering in the region, BTV subsequently re-emerged in 2007, in a far more extensive way than the first wave of the epidemic in 2006, leading to an estimated farm prevalence of 70 % in sheep farms in the Netherlands (Elbers et al., 2008). Factors associated with the BTV-8 seroprevalence after the 2007 epidemic included the region the farm was located, grazing and ventilation during housing (Santman-Berends et al., 2010). Following the acquisition of natural immunity and a successful vaccination campaign, the virus ceased to spread in 2008, and the Netherlands regained its official free status in February 2012 (Santman-Berends, 2011).

On 3 September 2023, two veterinary practises notified BT-like symptoms in five sheep farms located in close proximity in the centre of the Netherlands, which was later that week identified by the national reference laboratory (WBVR, Lelystad, The Netherlands) as bluetongue virus serotype 3 (BTV-3) (Holwerda et al., 2024). In the following weeks, BTV-3 spread rapidly over a large part of the country, resulting in thousands infected sheep and cattle farms (van den Brink et al., 2024). The impact was substantial, with severe clinical signs and significant high mortality in both cattle and sheep farms (van den Brink et al., 2025a & 2024; Santman-Berends et al., 2024).

Having experienced successful overwintering of BTV in the Netherlands in 2006/2007, there was a need to gain insight into the proportion of the population that would still be susceptible for infection, after the epidemic wave in 2023. For BTV it is known that neutralizing antibodies following natural infection protect against homologous virus infections (Schwartz-Cornill et al., 2008; Maclachlan et al., 2014). Therefore evaluation of the final size of the epidemic of 2023 in terms of seroprevalence will give insight in the proportion of protected sheep in the population, and which proportion is still susceptible for infection in 2024. Additionally, there was a need to know how effective preventive measures had been (if any), to aid farmers in protecting susceptible sheep from the virus if it would overwinter. Knowledge on effective preventive measures was particularly needed as up to April 2024 it was uncertain whether vaccination options would become available in time. Consequently, it was decided to combine the prevalence study with a risk factor evaluation.

The primary aim of this study was to estimate the final size of the BTV-3 epidemic in sheep after the vector-active season of 2023 in the Netherlands in terms of seroprevalence at farm-level, within-farm level (i.e. seroprevalence within seropositive farms) and national animal-level. Secondly, this study aimed to identify factors associated with presence of BTV antibodies at farm level and subsequent percentage of affected sheep within positive farms after the 2023 vector-active season.

## 2. Material and methods

### 2.1. Study population

In the spring of 2024, 876,616 sheep were registered in the official national identification and registration database (I&R) (RVO, Assen the Netherlands), distributed over 28,959 different farms in the Netherlands. Of these sheep, 91 % were located in 10,327 farms that housed more than ten sheep. To estimate BTV seroprevalence in sheep farms after the vector-active season of 2023, a convenience sample of archived serum samples that were collected from adult sheep for monitoring purposes in the first quarter of 2024 was used. The origin of the samples was as follows. Each year, a random sample of 1475 farms with more than ten animals are selected to submit serum samples from 13 animals older than one year of age to Royal GD for the official national *Brucella melitensis* monitoring programme (van den Brom et al., 2012a; 2012b; Veldhuis et al., 2013). From farms with up to 13 sheep, all animals are sampled. From the 1475 farms that were sampled in the first months of 2024, 500 sheep farms were selected for inclusion in this study. These farms were stratified across twenty geographical compartments, with 25 randomly selected farms per compartment, based on geographic boundaries as proposed in Commission Decision 2005/393/EC (Figure 2). This sampling strategy aligns with the European Commission's recommendations for monitoring BTV in a free situation without vaccination (European commission, 2020). With 13 samples per farm, it is possible to detect the presence of BTV in sheep farms with 95 % confidence assuming a minimum detectable within-farm seroprevalence of 21 % (Stata, version 17).

In April 2024, the 500 selected farmers were invited to participate in this study. A total of 466 farmers agreed to participate. They were then asked to complete a short survey and consented to the use of their archived sheep samples for BTV antibody evaluation. Since the antibody test used in this study are sensitive to all BT serotypes, antibodies against other BTV-serotypes induced by vaccination could potentially interfere with the study results leading to false-positives. Consequently, data from farms where the farmer indicated having vaccinated against BTV in the previous five years (as noted in survey responses) were excluded from the study (n = 12), leaving 454 farms for inclusion in the study. From those, sufficient material could be retrieved from 387 farms. Based on a total of 387 of participating farms the farm-level seroprevalence could be determined with 5 % accuracy and 95 % confidence (assuming a prior estimate of 50 %). In addition, this sample size enabled identification of risk factor associations with an odds ratio (OR) of 1.9 and higher with 95 % confidence (Stata® version 17; Stata, 2022).

### 2.2. Diagnostic evaluation

From each participating farm, generally 13 serum samples submitted between January and April 2024, were included. The Idexx® Bluetongue competition antibody test, accredited under ISO17025 at Royal GD (Royal GD, Deventer, the Netherlands), was used (Niedbalski, 2011). This ELISA detects antibodies against all BT serotypes, with a sensitivity of 100 % (95 % CI: 97.5–100) relative to the test of the Dutch national reference laboratory (WBVR, Lelystad, the Netherlands). The specificity of the test is 99.91 % (95 % CI: 99.85–99.97). Given the number of sampled sheep per farm this will result in an estimated farm-level sensitivity of 100 % and a farm-level specificity of approximately 98.8 % (99.91 %^13).

### 2.3. Available data

A short survey was developed to collect information on various aspects potentially related to the BTV-3 status of the farm. The survey included questions about observed clinical signs of BT in 2023, confirmation of infection by PCR, administration of BTV vaccination against other serotypes in the previous five years and farm management practises in 2023. The management questions addressed factors potentially associated with vector borne diseases such as BT and Schmallenberg virus, as identified in previous studies (Veldhuis et al., 2014; Santman-Berends et al., 2010). These included details about grazing (which flocks of sheep and when), housing, the size of ventilation openings in the barn, the use of mechanic ventilation, use of insecticides and/or repellents, the moment sheep were shorn (in the Netherlands it is common practice to shear all sheep in a flock on the same day), presence of other BTV-susceptible species on the farm and other possible precautionary measures that were applied. The factor "whether or not all sheep were kept indoors" combined with the moment at which it was decided to put the sheep indoors (i.e. before clinical signs were observed or during the period in which clinical signs were observed), was retained for further analysis. The survey can be found in Supplementary Material 1 (S1).

All selected farmers were asked by email or post on 16 April 2024 to complete the survey. On 23 April a reminder was sent and on 10 May 2024, the survey was closed to ensure that responses were not

influenced by their knowledge on their BTV antibody results, which were send to the farmers on 19 May. Participating farmers were asked to grant access to their data from the national I&R-database, where all individual sheep movements are mandatory recorded within seven days (Dijkstra et al., 2022; Dijkstra et al., 2023). These data were used to determine farm size, mortality during the BTV-3 epidemic, the introduction of sheep into a farm during the BTV-3 epidemic (between September and December 2023), and the origin of the introduced sheep. Eventually, data from three sources were available for analysis: diagnostic results, survey responses, and relevant parameters derived from the I&R-database.

### 2.4. Analyses

Stata® 17 (Stata, 2022) was used for data validation, descriptive statistics and multivariable analyses.

#### 2.4.1. Prevalence estimation

farm-level seroprevalence was defined as the percentage of farms in which at least one out of the sampled sheep tested BTV seropositive. An overview of the results per compartment is provided in Supplementary Material 2 (S2). Within-farm seroprevalence was defined as the percentage of seropositive sheep out of the sampled sheep from farms with at least one BTV seropositive sheep. The results were subsequently combined across all sheep farms to get a national estimate of within-farm seroprevalence in BTV seropositive farms. Both mean and median within-farm prevalence is presented, due to non-normality of the data. Seroprevalence at animal level was defined as the (national) proportion of seropositive sheep amongst all farms and was estimated using a generalized estimating equations model (‘*xtgee*, family (binomial)’), including a random farm effect. This method takes into account the clustering of animals (or test results) within farms. Additionally, sampling weights were considered during this estimation procedure to reflect the fact that a fixed sample of sheep from larger farms represents a larger proportion of the target population than sheep sampled from smaller farms. Sampling weights were determined as the inverse of the animal’s sampling probability. This probability was calculated based on the proportion of sampled sheep per farm relative to the total number of sheep (>1 year old) present on the farm in fourth quarter of 2023.

#### 2.4.2. Risk factor evaluation

diagnostic results were combined with I&R-data as well as survey responses. For unknown reasons, 3 farmers did not complete the questionnaire and could therefore not be included in the risk factor evaluation. Eventually, complete questionnaire data were available for 384 out of 387 farms, which were included in the multivariable analysis.

As BT-related case-fatality rates were very high in sheep in 2023 (74,8 %, van den Brink et al., 2024), it was possible that some farms tested seronegative even though they stated that presence of BTV-3 in their farms was diagnostically confirmed by the national reference lab in 2023. In other words, if a large proportion of a farm’s sheep flock did not survive the BTV-3 infection in 2023, this may have resulted in a low ‘remaining’ seroprevalence in such farms at the end of the epidemic. For the risk factor evaluation, the aim was to identify farm management factors that are associated with BTV infection in 2023 (and its magnitude within affected farms), which was hampered by the above described phenomenon. Consequently it was decided to consider farms for which the farmer responded in the survey that presence of BTV-3 had been confirmed in their farm in 2023 but tested seronegative in our serosurvey (n = 16) as infected in the risk factor evaluations. Two types of models were developed: (1) a model to identify risk factors for the presence of BTV antibodies at the farm level, and (2) a model to identify risk factors associated with the proportion of affected sheep in positive farms. For each model, regression analyses were conducted using appropriate distribution functions based on the nature of the dependent variable. An overview of the study design is provided in Figure 1.

In Model 1, to identify risk factors for BTV infection at farm level, a logistic regression model was applied with a logit link function (‘*logit*’), using the farm-level BTV infection status as the dependent variable. Farms were classified as either BTV positive (at least one out of the

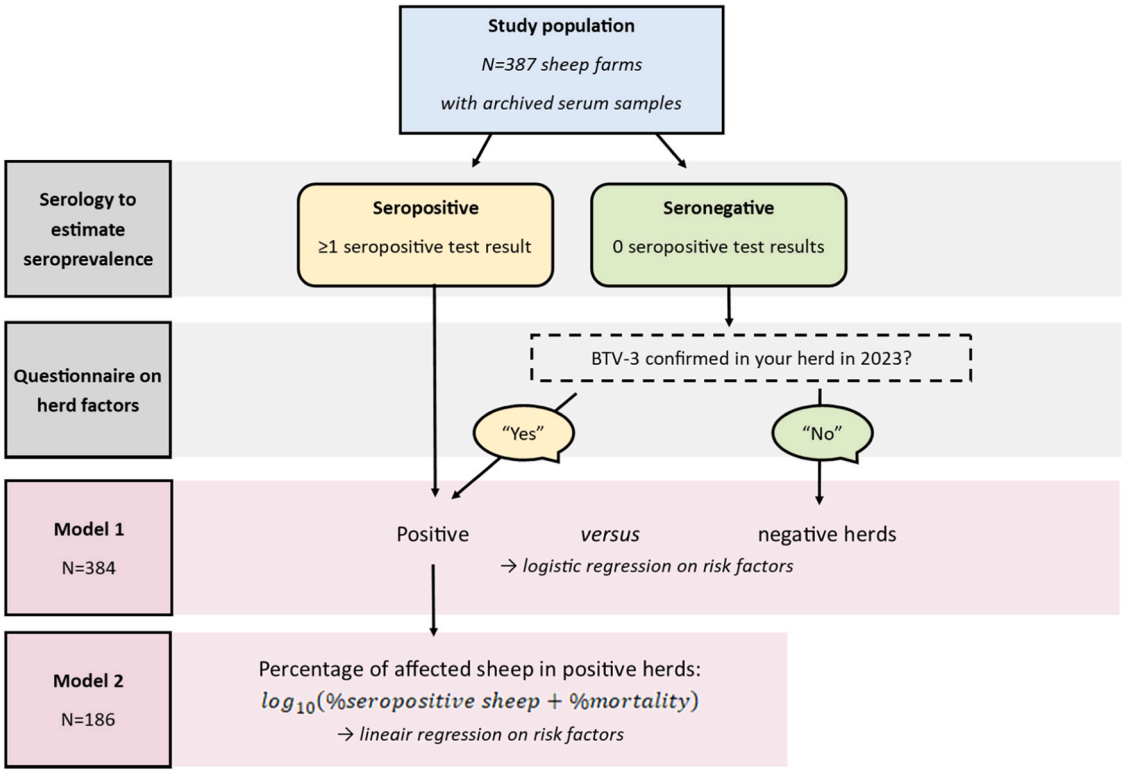


**Fig. 1.** Study overview.

sampled sheep tested BTV seropositive or BTV-3 was diagnostically confirmed during the epidemic in 2023, according to the farmer) or BTV seronegative. The following explanatory factors were evaluated: preventive indoor housing of sheep, use of mechanical ventilation when sheep are kept indoors, use of insecticides and/or repellents in autumn 2023, most recent month in which the sheep were shorn, farm size and whether farmers indicated that other precautionary measures were taken. An additional explanatory variable was included to control for the geographical location of the farm relative to the initial outbreak area (low, medium or high risk) as confounder. The risk qualification was based on the number of official notifications of BT suspicions to the authorities for each individual area, as shown in Figure 2. Furthermore, an interaction term was incorporated to account for the on-farm movement of sheep between September and December 2023, considering the risk profile of the location of origin (i.e., no introduction of sheep, or introduction of sheep originating from low, medium or high BTV-3 risk areas as shown in Figure 2). This approach was taken to ensure that regional variations in farm management were not incorrectly attributed to the farm's BTV serostatus. Risk factors are expressed as odds ratios (OR).

For Model 2, the within-farm seroprevalence from the serosurvey was added to each farm's mortality rate between September and December 2023, into a 'mortality-adjusted percentage of BTV affected sheep in 2023'. This was done for all BTV seropositive farms as well as for seronegative farms for which the farmer responded in the survey that presence of BTV-3 had been diagnostically confirmed by PCR in their farm in 2023 (n = 16). For the within-farm risk evaluation, these mortality-adjusted percentages of affected sheep per farm were log-transformed (log10 of the within-farm seroprevalence measured in the serosurvey + the percentage of sheep that died between September and December 2023) and subsequently analysed via linear regression (*'regress'*) (Figure 1). As the linear regression model was ran on log-transformed data, the coefficients from the model were exponentiated for interpretation purposes, i.e. exponentiating would result in form where a 1 unit change in x would result in a percent change in y (like IRR). The percentage of sheep affected by BTV-3 in 2023 (either survived and seropositive in early 2024, or dead) was included as the dependent variable. The same potential risk factors as in Model 1 were included as explanatory variables for the within-farm exposure to BTV.

As the number of variables in the models were limited, it was deemed unnecessary to conduct a univariable pre-screening; all variables were directly included in the full multivariable models. For final model selection, a backward selection and elimination method was used. In each round, the variable with the highest *p*-value was excluded until all remaining variables were significantly (*p*-value <0.05) associated with the BTV serostatus. During this process, confounding was monitored by examining changes in the coefficient of a variable after removing another variable. If the change in estimates exceeded 25 %, the removed variable was considered a potential confounder and was re-entered into the model. In the final model, all biological plausible logical two-way interactions were evaluated and included if significant. Normality of linear model residuals was checked using normal probability plots and the normality assumption was met for the within-farm risk evaluation after log transformation. The goodness-of-fit of the logistic model was assessed using Pearson's goodness of fit test (*'estat gof'* postestimation) and visual inspection of diagnostic plots of deviance residuals. Statistical dependence between independent variables, potentially leading to multicollinearity, was monitored by examining pairwise correlation coefficients prior to multivariable analysis. A maximum of 0.50 between two variables was set.

## 3. Results

### 3.1. Prevalence estimation

A total of 4947 antibody test results from 387 sheep farms were available, of which 367 farms (95 %) had 13 samples. The sampled farms kept on average 49 adult sheep and 85 sheep in total (median 23 and 37, respectively), which was comparable to the number of adult sheep in the target population of all sheep farms with more than 10 sheep in the Netherlands in 2023 (Santman-Berends et al., 2024). Of the 4947 samples sheep, 527 sheep tested BTV seropositive. In 47 % (n = 180) of tested sheep farms (95 % CI: 41–52), at least one sheep tested BTV seropositive. In 83 of the 180 seropositive sheep farms, only one out of the tested sheep tested BTV seropositive. The highest farm-level seroprevalences were found in the compartments where BTV-3 was first detected in 2023: compartment 6 (83 % of tested farms), compartment 9 (93 % of tested farms), and compartment 10 (72 % of tested farms). This seroprevalence declined with increasing distance from the initial outbreak area (Figs. 2 and 3). Within BTV seropositive farm, a median percentage of 15 % of sheep tested seropositive (mean 23 %). Similarly to the farm-level seroprevalence, the within-farm seroprevalence decreased with increasing distance from the initial outbreak area, varying between 53 % in compartment 6 and 8 % in compartment 17 (Fig. 3). Overall, at animal-level 10 % (95 % CI: 8–13) of the examined sheep tested antibody positive (Fig. 3). The highest animal-level seroprevalence was found in compartment 9 (37 %, 95 % CI: 32–42). The lowest animal-level seroprevalences were found in the northern compartment (compartment 1: 1 %, 95 % CI: 0–3) and southern compartments (compartment 15 and 20: both 1 %, 95 % CI: 0–5).

### 3.2. Risk factors associated with Bluetongue seropositivity (yes/no)

To evaluate the risk factors associated with BTV farm status, sheep farms were classified as either BTV seropositive (at least one out of thirteen sheep tested BTV seropositive; n = 180 or the farmer stated that BTV-3 was diagnostically confirmed in 2023 by the national reference lab; n = 16), or BTV seronegative (all sheep tested negative and no confirmed BTV-3 infection; n = 191). Of the assessed risk factors (location, shearing, use of insecticides, farm size, putting sheep indoors as a preventive measure for BTV, ventilation, introduction of sheep), three were significantly associated with farm-level BTV serostatus (Table 1; Model 1). Flocks of farmers who decided to put or keep their sheep indoors as a preventive measure were four times less likely to be BTV seropositive (OR: 0.25 (95 % CI: 0.09–0.67)), compared to flocks

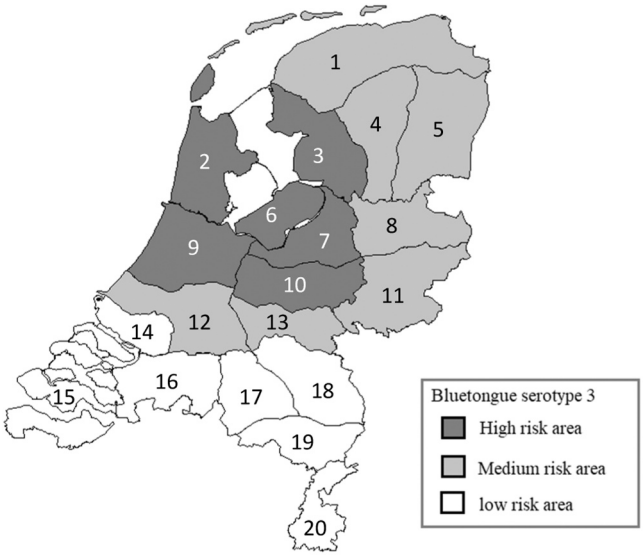


**Fig. 2.** The Netherlands subdivided in twenty compartments and the BTV-3 risk areas in Sept-Dec 2023 based on the number of BTV notifications per compartment are indicated as white (<2 % of sheep farmers notified), grey (≥2-<10 % sheep farmers notified) or dark grey (≥10 % of sheep farmers notified). Source: NVWA, Utrecht, the Netherlands.

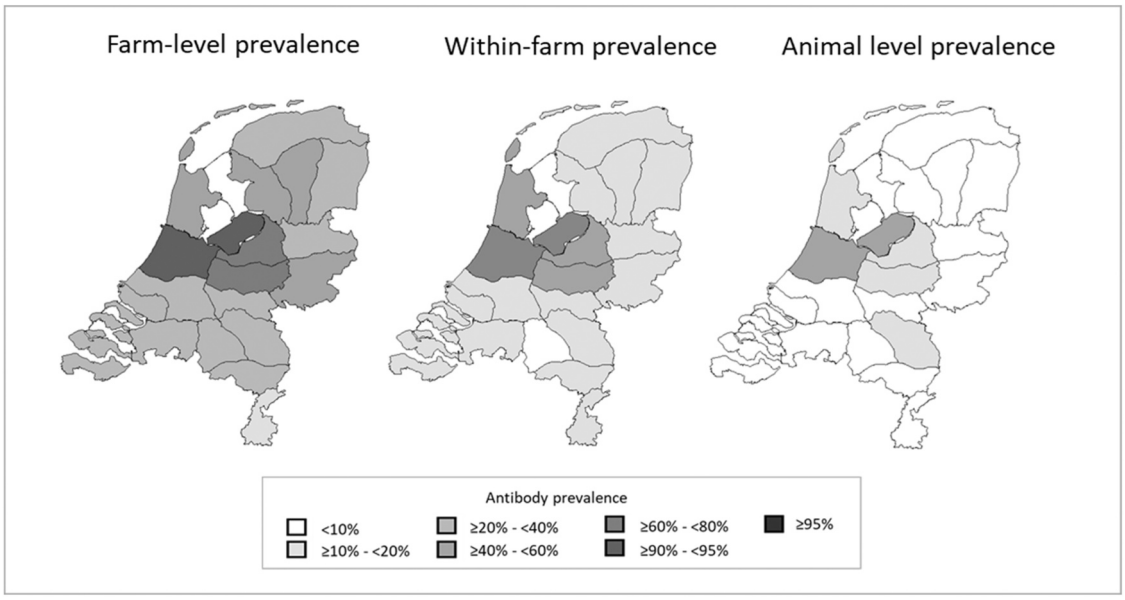


**Fig. 3.** Farm-level, within-farm (seroprevalence within seropositive farms) and (national) animal-level bluetongue seroprevalence per compartment based on 4947 sheep in 387 sheep farms following the BTV serotype 3 epidemic in the Netherlands in 2023.

**Table 1**
Risk factors associated with presence of BTV (Model 1) or increasing within-farm proportion of affected sheep (Model 2) in Dutch sheep farms after the BTV-3 epidemic in 2023 based on multivariable regression models, with odds ratio (OR) or incidence rate ratio (IRR), 95 % confidence interval (CI) and *p*-value. Associations with a *p*-value < 0.05 are displayed in bold.

| | **Model 1** | | | | **Model 2** | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Farm level BTV status (positive/ negative) N = 384 | | | | Prevalence of BTV affected sheep within-farm N = 186 | | | |
| | OR | 95 % CI | | *p*-value | IRR | 95 % CI | | *p*-value |
| **Did you put your sheep indoors to prevent them from acquiring BTV−3** | | | | | | | | |
| No | Ref. | | | | Ref. | | | |
| Yes | **0.25** | **0.09 -** | **0.67** | **< 0.01** | 0.61 | 0.34 - | 1.10 | 0.09 |
| **When was the last time you sheared your sheep in 2023** | | | | | | | | |
| January-March | Ref. | | | | Ref. | | | |
| April-June | 1.59 | 0.95 - | 2.66 | 0.08 | 1.05 | 0.81 - | 1.37 | 0.69 |
| July-September | 0.76 | 0.31 - | 1.85 | 0.54 | **2.22** | **1.27 -** | **3.86** | **< 0.01** |
| October-December | 3.03 | 0.77 - | 11.9 | 0.11 | 1.56 | 0.85 - | 2.85 | 0.15 |
| **Location** | | | | | | | | |
| Low BTV−3 risk area | Ref. | | | | Ref. | | | |
| Medium BTV−3 risk area | **2.03** | **1.10 -** | **3.75** | **0.02** | 1.32 | 0.88 - | 1.98 | 0.18 |
| High BTV−3 risk area | **8.39** | **4.24 -** | **16.6** | **< 0.01** | **3.30** | **2.23 -** | **4.88** | **< 0.01** |
| **Farm size (adult sheep)** | | | | | | | | |
| Smallest farms (0–9 sheep) | | | | ns | Ref. | | | |
| Smaller farms (10–23 sheep) | | | | | 1.10 | 0.73 - | 1.66 | 0.63 |
| Larger farms (24–99 sheep) | | | | | 1.10 | 0.70 - | 1.59 | 0.80 |
| Largest farms (>99 sheep) | | | | | **0.51** | **0.30 -** | **0.86** | **0.01** |

that were kept outside. Additionally, shearing sheep in spring tended to be associated with a higher probability of testing BTV seropositive compared to shearing in winter (OR: 1.6 (95 % CI: 0.95–2.66)). Moreover, farms located in medium or high BTV-3 risk areas were associated with a higher probability of testing BTV seropositive (Table 1; Model 1).

### *3.3. Risk factors associated with magnitude of Bluetongue infection in affected farms*

From the 180 seropositive farms, ten were excluded from the within-farm risk factor evaluation because of a large number of missing values in the survey (n = 2) or because no consent to use their I&R-data was provided (n = 8). From the seronegative farms with complete data, 16 farmers indicated that BTV-3 was diagnostically confirmed in their farm and, for these farms, the percentage of BTV affected sheep was calculated. Thus, data from 186 farms were included in this analysis with a percentage of BTV affected sheep varying between 0.5 and 100 percent (mean: 33 %, median: 22 %). Within positive farms (N = 186), shearing between July and September was associated with a higher BTV affected sheep prevalence (IRR: 2.22 (95 %CI: 1.27–3.86)) compared to shearing between January and March (Table 1; Model 2). Additionally, the largest farm size (>99 sheep) was associated with a lower within-farm proportion of affected sheep (IRR: 0.51 (95 %CI: 0.30–0.86)). Farms located in medium or high BTV-3 risk areas were associated with a higher proportion of affected sheep (IRR 3.3 (95 %CI: 2.23–4.88)). Ventilation openings in the barns, keeping flocks indoors as a preventive measure, purchase of sheep (regardless of the risk area of origin), and the use of insecticides were not significantly associated with either the farm-level BTV status or within-farm proportion of BTV affected sheep.

## 4. Discussion

In this study, we evaluated the BTV seroprevalence in sheep following the BTV-3 epidemic in the Netherlands in 2023 and identified associated risk factors. During the first year of the BTV-3 epidemic, an increased mortality of 55,000 sheep compared to 2020–2022 was observed, and at farm-level was estimated to be five to fifteen times higher than mortality in BTV-free years (Santman-Berends et al., 2024). The primary research question of this study was to determine the percentage of sheep that would be protected against a new BTV-3 epidemic in 2024, and the study design successfully addressed this question. For this study, a convenient sample of serum of 13 sheep older than one year in Dutch sheep farms which were part of the *Brucella melitensis* monitoring programme, was investigated for presence of BTV antibodies. In farms where less than 13 animals were tested, the sample size existed of all sheep present. Although this sampling scheme was chosen for convenience, the farms selected for *Brucella melitensis* monitoring were randomly chosen, making them a representative study population for all Dutch sheep farms. Therefore, we believe that our results can be extrapolated to the national level. A sample of 13 sheep per farm sheep allows for the detection of BTV if at least 21 % of the farm had been seropositive. A sheep farm was classified as BTV seropositive when at least one sheep tested seropositive or BTV-3 was diagnostically confirmed in 2023 (according to the farmer). The prevalences we found in this study are relatively low; 46 % of farms and 10 % of sheep were seropositive. In cattle a farm-level seroprevalence of 64 % (95 % CI: 63–65) and an animal-level seroprevalence of 23 % (95 % CI: 22–24) was found in the Netherlands after the 2023 outbreak (van den Brink et al., in preparation). These differences are likely the result of the high case-fatality observed among sheep during the outbreak in 2023 (van den Brink et al., 2024), which likely decreased the proportion of seropositive animals (that survived) and farms in this study. Moreover, some farms that tested seronegative in our final size estimation may have been misclassified as such, and the magnitude of infection in affected farms may be underestimated when looking at seroprevalence alone. Evaluating the risk factors therefore posed a challenge due to the high mortality rates observed in 2023. In the risk factor analysis we aimed to assess factors associated with exposure to BTV-3 in 2023, rather than the percentage of infected animals that survived until spring 2024. To address this, we adjusted our approach for the risk factor evaluation. For the model to identify risk factors for infection with BTV-3, sixteen seronegative farms that stated BTV-3 had been confirmed in their farm in 2023 were included as positives. Subsequently, for the within-farm risk factor evaluation, we combined the estimated within-farm seroprevalence in farms classified as BTV seropositive with recorded sheep mortality from September to December 2023 at farm level, to calculate the percentage of BTV affected sheep per farm. The resulting percentage of BTV affected sheep may be slightly overestimated as not all deceased sheep may have died due to BTV-3 infections, although case-fatality rates up to 75 % were observed in 2023 (van den Brink et al., 2024). However, it was assumed that this potential noise was present in the data for all farms that were classified as positive, and that the approach did not significantly bias the identification of risk factors. In addition, for both models, sensitivity analyses showed that including the 16 seronegative farms as positive did not significantly impact the results and conclusion of the risk factor analysis (*results not shown*).

A large review evaluating seroprevalence and risk factors for bluetongue in Africa, where the virus spreads endemically, also found lower seroprevalences in sheep compared to cattle (36.3 % and 49.7 %, respectively; Medrouh et al., 2024). In their study, it was suggested that this difference might be associated with host preferences by the vector, differences in longevity between cattle and sheep and variations in environmental habitat factors for different animal species. Although host preference may have contributed to the differences in farm and animal-level seroprevalences in our study, we believe that the differences in longevity and environmental factors are unlikely to have influenced the disparities between cattle and sheep observed after one year of BTV-3 spread in the Netherlands.

Comparing the risk factors identified in our study to those in previous research presents a challenge. Most existing research is conducted in areas where BTV-3 is endemic and primarily focused on identifying animal-level risk factors, such as age, gender and breed or climate factors (Cappai et al., 2018; Liu et al., 2021; Ishaq et al., 2023). In contrast, our study aimed to identify farm-level factors that could help farmers reduce the risk of BTV introduction and spread within their farms. Our risk factor evaluation indicated that keeping sheep indoors as a preventive measure (i.e. before BTV-3 clinical signs were observed), was associated with lower odds of testing BTV seropositive. This result aligns with previous findings on risk factors associated with BTV-8 infections in cattle in the Netherlands (Santman-Berends et al., 2010) and has also been observed in other parts of the world (Sbizera et al. 2024; Gong et al., 2021). The protective effect of keeping animals indoors also agrees with previous findings related to another vector-borne diseases in the Netherlands, namely Schmallenberg virus (Veldhuis et al., 2014). This protective effect is suggested to be associated with a reduced probability of being bitten by midges of the *Culicoides* species, as they may be less active indoors compared to outdoors (Baylis et al., 2010). Although keeping animals indoors will not prevent all infections with BTV, it could be a worthful measure when no other effective preventive measures, such as vaccination, are available. Another factor found to be associated with both farm-level and within-farm level BT prevalence was shearing. Within positive farms, the percentage of affected sheep was significantly lower in farms with sheep shorn between January and March compared to farms with sheep shorn in summer months (i.e. between July and September). To our knowledge there are no previous studies investigating this factor. A possible hypothesis for this finding could be that fleece regrowth in sheep sheared early in the year partly prevented them from being bitten by *Culicoides* spp. during the outbreak in late summer. Nonetheless, we believe that this finding requires further investigation.

In our study, the use of insecticides was found not to be significantly associated with either the farm-level BTV infection status or the percentage of BTV affected sheep within farms. Previous studies showed varying results from no effect (Cappai et al., 2018) to a protective effect in decreasing BTV transmission (Şevik, 2023). Nevertheless, a recent scientific opinion of EFSA also concluded that there is no evidence that the use of insecticides and repellents reduces BTV transmission in the field (EFSA, 2017; Versteirt et al., 2017).

## 5. Conclusion

The results indicated that nearly half of the Dutch sheep farms encountered BTV-3 after the first epidemic year in 2023. The findings provided an estimate of the proportion of the population that would be protected against BTV-3 in 2024. The within-farm and animal-level seroprevalences showed that the majority of the sheep population remained susceptible to BTV-3 in 2024. Although a number of management factors were found to be protective against BTV-3 infection or the magnitude of the infection in 2023, they could not fully prevent sheep from becoming infected resulting in seropositivity. Nevertheless, this study offers farmers insight in measures to reduce the impact of BTV-3 in their farms, beyond just vaccination.

## CRediT authorship contribution statement

**Veldhuis A. M. B.:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation. **Mars M. H.:** Writing – review & editing, Supervision, Methodology, Investigation. **Bogt-Kappert C. ter:** Writing – review & editing, Resources, Project administration. **Fabri N.:** Writing – review & editing, Validation, Formal analysis. **van den Brom R.:** Writing – review & editing, Supervision, Conceptualization. **van den Brink K.M.J.A.:** Writing – review &

editing, Visualization, Investigation, Conceptualization. **Dijkstra E.:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **I.M.G.A. Santman-Berends:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106699.

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