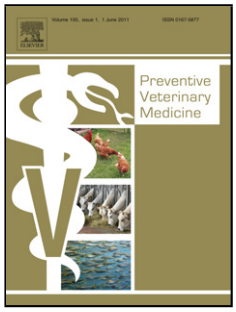

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Association of clinical signs after acute Schmallenberg virus infection with milk production and fertility in Swiss dairy cows

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Highlights:

- A significant drop in milk yield was observed during the Schmallenberg epidemic
- Return to previous milk production levels in animals with clinical signs was slow
- Number of inseminations per production cycle was increased in clinical animals
- Somatic cell counts in clinically affected cows were slightly elevated

Abstract

Since its first occurrence in August 2011 in Germany and the Netherlands, the Schmallenberg virus (SBV) spread rapidly across Europe, where it caused production losses and abortions in ruminants as well as congenital malformations in the offspring of affected animals. Several studies have investigated the impact of SBV on fertility and production parameters in dairy cows at herd level. However, the impact of clinical disease at the animal level remained undetermined. This study aimed at estimating the impact of clinical disease during and after an infection with SBV on production and fertility parameters in individual Swiss dairy cows. Sixty-seven case and twenty-four control herds were selected according to whether cows had been showing clinical signs indicative of SBV during the epidemic from July to December 2012 in Switzerland. Of these 91 farms, production and fertility data from 388 cows with clinical signs from case herds were collected over a time period

of four years, and compared to data from 932 cows without clinical signs originating from case or control herds. Milk yield, somatic cell count, number of inseminations and non-return at day 56 were analysed by means of hierarchical multivariable regression analysis.
A significant drop in milk yield was observed in all groups during the SBV epidemic compared to the time before the infection, which amounted to 1.9 kg per test day for clinical animals, 1.1 kg for non-clinical animals from case herds and 0.6 kg for non-clinical animals from control herds. A prolonged effect on milk yield was observed in clinical cows for about one year, suggesting that animals with clinical disease might not return to their previous milk production level in the current lactation after an acute infection with SBV. Clinical animals showed a significantly higher somatic cell count during the epidemic compared to the time before the infection. The number of inseminations per cow and production cycle was higher for clinical animals during the epidemic compared to the time periods before and after, but not significantly higher than for non-clinical animals from case and control herds. No difference regarding non-return at day 56 was found. Although the overall impact of the SBV epidemic in Switzerland was limited, the consequences could be substantial in farms with a high prevalence of clinical disease.

Keywords: Cattle, Symptom, Impact, Milk yield, Insemination, Non-return

1. Introduction In August 2011, the first cases of a new disease with acute clinical signs consisting of fever, drop in milk yield and diarrhoea were reported in cattle in Germany and the Netherlands. In November 2011, a novel causative virus was identified as an Orthobunyavirus of the Family Bunyaviridae and was named Schmallenberg virus (SBV) after the place of its first occurrence (Hoffmann et al., 2012).

Schmallenberg virus is transmitted by *Culicoides* midges (De Regge et al., 2012; Elbers et al., 2013) and affects domestic and wild ruminants. Besides acute clinical signs in adult ruminants (fever, reduction in milk production and diarrhoea) and depending on the time of infection, the virus can cause abortion, stillbirth and congenital malformations in offspring (Bayrou et al., 2014; van den Brom et al., 2012). The latter has been shown to be relatively rare in cattle as compared to small ruminants (Afonso et al., 2014; Veldhuis et al., 2014a; Wüthrich et al., 2016).

When it was recognized that the virus would spread rapidly across Europe, the Swiss Veterinary Services intensified their passive surveillance in February 2012 to ensure early detection of the virus in Switzerland (Schorer et al., 2012). The initial surveillance consisted of testing stillborn ruminants and new-born ruminants with malformations for SBV. In June 2012, these measures were extended to testing blood samples from animals with acute clinical signs suggestive of SBV infection. If several cows within a herd were showing acute clinical signs like fever, diarrhoea or a drop in milk yield, blood samples were tested with PCR and ELISA for virus and antibodies, respectively. The first cases in Switzerland were detected on 18 July 2012, when serum samples of four cows from two different herds in the canton of Berne tested positive for SBV (Schorer et al., 2012).

As expected, the virus spread rapidly throughout Switzerland thereafter. A representative nationwide survey of bulk tank milk samples testing for antibodies against SBV in Swiss dairy farms revealed that the herd level seroprevalence was already 19.7% in July 2012 and increased further to 99.5% until December 2012 (Balmer et al., 2014). These results are similar to findings in Belgium and the Netherlands, where the herd level seroprevalence was found to range between 95 and 100% in cattle after one vector season (Méroc et al., 2013; Veldhuis et al., 2013). The herd level impact of the SBV epidemic on milk production and fertility parameters in dairy cows has been studied in detail before (Toson et al., 2015; Veldhuis et al., 2014a, 2014b; Wüthrich et al., 2016). Veldhuis et al. (2014a) and Wüthrich et al. (2016) both performed a case-control study to assess the impact of clinical signs on production and fertility parameters by comparing herds with typical clinical disease manifestation and herds without clinical disease following an infection with SBV. Results showed a limited, yet statistically significant impact on milk production and fertility parameters. As both studies were performed at herd level, the impact of clinical disease on milk production and fertility parameters at individual cow level may have been diluted by the performance of cows without clinical signs. The objective of the present study was to investigate whether and to which extent Swiss dairy cows with and without clinical signs had altered milk yield, somatic cell counts and reproductive performance during and after an acute infection with SBV.

2. Materials and Methods

2.1. Study design and data collection

The present animal level investigation was based on a subsample of the study described by Wüthrich et al. (2016). In that study, health and productivity parameters of Swiss dairy cattle herds with animals having presented acute clinical signs during the SBV epidemic in the second half of 2012 (case herds) were compared to those of herds without animals with acute clinical signs (control herds). Case herds were recruited from a list of dairy farms that had reported acute clinical disease and had serum samples analysed at the Swiss reference laboratory (Institute of Virology and Immunology, IVI, Mittelhäusern, Bern). The local veterinarians were then asked to identify potential control farms in the vicinity of the case farm, and with similar average milk yield, breed and housing system. In close conformity with the definition of the European Food Safety Authority (EFSA, 2012), a herd was classified as a case herd if at least one animal was present with at least two of the following clinical signs during the SBV epidemic in Switzerland (July to December 2012): Fever, diarrhoea, milk loss or abortion. If abortion was the main symptom, at least two cows of the herd had to have aborted and shown at least one other clinical sign indicative of SBV infection (i.e. fever, diarrhoea or drop in milk production) to classify the herd as a case herd. Moreover, the herd had to have been confirmed infected with SBV in 2012 by the use of ELISA or PCR. Control herds should not have had any cows with suspicious clinical signs indicative of SBV (Wüthrich et al., 2016). At the end of 2012, over 99% of Swiss cattle herds tested positive for antibodies to SBV (Balmer et al., 2014). Therefore, a seronegative status of the herd was not applicable for control herds.

Recruitment of farms for the herd level analysis was performed by phone calls, carried out by two study veterinarians between October 2012 and April 2013 (Wüthrich et al., 2016). At that time, information on clinical signs observed in individual cows was collected in order to confirm whether the farm complied with the case definition. Between May and December 2013, all farms were visited once, and details on clinical signs were registered during face-to-face interviews with the farmers. Furthermore, treatment records and veterinary bills were collected. In combination with the farmers' reports and available results from the reference laboratory (SBV PCR and/or ELISA), the records were manually evaluated to assign individual cows to either the clinical or the non-clinical group, depending on the nature and number of clinical signs. Cows with at least two typical clinical signs (fever, diarrhoea, milk loss or abortion) were assigned to the group of clinical animals, whereas animals with no or only one of these clinical signs were assigned to the non-clinical group. By definition, animals in control herds had not shown clinical signs of SBV infection (Wüthrich et al., 2016). Yet to avoid any misclassification bias on the animal level, non-clinical animals were only included in the study if non-clinical status could be verified by farm records provided on the animal level. The inclusion criterion for the present investigation at animal level was thus good quality records on the presence or absence of clinical signs during the SBV epidemic for the individual animal. In addition, production and fertility data had to be available for the individual animal. It must be noted that both clinical and non-clinical animals were likely to be seropositive due to the extensive spread of SBV in the second half of 2012 (Balmer et al., 2014).

The study design, including the number of investigated herds and animals, is illustrated in Figure 1. From the 77 case and 84 control herds included in the herd level case-control study by Wüthrich et al. (2016), a total of 70 herds had to be excluded. Seven case and twelve control herds were not members of a Swiss breeding organisation, and reliable production and fertility records were therefore not available for individual cows. Another 3 case herds and 48 control herds had to be excluded because detailed health records at the individual animal level were lacking. Another 139 cows from case herds were excluded because their health records of clinical signs were incomplete.

Monthly milk yield recordings and somatic cell count (SCC) as well as calving dates and data on artificial inseminations (AI) for each individual cow were obtained from the Swiss breeding organisations. Data on milk yield, SCC and AI were retrieved over a period of 4 years, from January 2010 to December 2013. Milk yield and SCC were available on a monthly basis (data for one test day per cow per month).

The sample size was large enough to detect a difference in mean milk yield of 1.0 kg between clinical and non-clinical animals with 80% power and 95% confidence, or a difference in mean milk yield of 1.0 kg before and after an infection with SBV in the same animal (G*Power version 3.1.9.2; standard deviation = 5.0 kg)

2.2. Statistical analyses

All data were processed and analysed using Stata 13.1 software (StataCorp., 2013). Data were checked for completeness and plausibility before statistical analysis.

2.2.1 Primary exposure variable of interest and covariates

A total of four outcome variables were evaluated to investigate the effect of SBV infection on cow level: Milk yield, SCC, number of AI, and non-return at day 56 (NR56). Three conditions were of primary interest on how they affected production and fertility parameters: The clinical status of the cow (clinical or non-clinical), the herd status (case or control), and the time period in relation to the epidemic (before, during or after the epidemic). To avoid models with complex 3-way interaction terms, the clinical status of each animal and its herd status were combined with the time periods into one single variable 'Time&Status'. This led to three distinct categories within each time period (non-clinical animals from control herds / non-clinical animals from case herds / clinical animals from case herds). The time periods used for the different analyses are described in Table 1. For milk yield and SCC, the time periods corresponded to half-years (to account for seasonal effects, e.g., of feed quality), whereas the three periods before (P1), during (P2) and after the SBV epidemic (P3) were compared for fertility parameters.
In all models, clinically affected animals from case herds during the SBV period (July-December 2012) were set as baseline category of 'Time&Status'. The variable 'Time&Status' was forced into all models as it was the variable of main interest. For the post-hoc analyses comparing milk yield levels of the different animal groups over the time, the baseline category in the final model was changed in order to reveal the respective effect sizes and corresponding significances.
Covariates included in the respective models were defined as follows: Days in milk ('DIM'), 'Breed' (Simmental, Holstein Friesian (including Red Holstein), Swiss Fleckvieh, Braunvieh (including Brown Swiss) and other breeds (other)), 'Parity' (1, 2 , ≥3), 'Herd size' (<21, 21-30, 31-45, >45), 'Housing system' (tie-stall/free-stall), 'Alp' (alpine pasturing during summer 2012 (yes/no)), and the cumulative individual milk production level for each cow. The latter was calculated as the sum of the first five milk recordings in each lactation and was categorized based on the 33rd and 66th percentile of the cows included in the study (<124 kg, 124-154 kg, >154 kg).

2.2.2. General model approaches

In a first step, explanatory variables were tested in univariable regression models and considered for the multivariable model if they had a *p*-value below 0.2 (Wald test for the linear and logistic models and likelihood ratio chi-square test for the zero-truncated negative binomial regression model). Second, all models were fitted by a manual, stepwise backward elimination procedure where predictors were kept in the model if they tested significant at a *p*-level of 0.05 (log likelihood ratio test (LRT)). As matching was not ideal in the study at herd level (Wüthrich et al., 2016), matching of farms was not considered as a random effect for the present analysis on animal level.

Variables that caused a change in model coefficients of more than 20% were considered confounders and kept in the model. Multicollinearity among predictor variables was assessed by the use of correlation analysis (Pearson correlation coefficient for continuous data, Spearman's rank correlation coefficient for ordinal and phi-coefficient for categorical variables), the variance inflation factor (VIF) and tolerance values. Variables with a correlation coefficient ≥ 0.5, VIF ≥ 5 and tolerance values ≤ 0.7 were considered to be affected by multicollinearity; this was not the case for any of the variables. Two-way interactions were investigated according to their biological relevance in the respective final model, yet none of these interactions tested statistically significant. Normal distribution of residuals and equality of variance for the linear models were assessed by normal probability plots and scatterplots of standardised residuals versus predicted values. The Hosmer–Lemeshow test was used to confirm the fit of the logistic model.
Due to missing values for some predictor variables, the total number of animals in the final models slightly deviated from the original sample size as presented in Figure 1.

2.2.3. Milk yield and SCC

Somatic cell counts were natural log-transformed for linear regression analysis after removing implausible SCC values (SCC = 0) and observations in the colostral phase (<4 DIM, n=16). To model the effects of SBV infection on milk yield and SCC, two hierarchical multivariable linear regression models were built. To account for the seasonal pattern of milk yield and SCC, a sine harmonic was added to both models (Stolwijk et al., 1999). To model the peak in the lactation curve, the following peak production function was included in the milk yield model: $e^{DIM*-0.05}$ (Wilmink, 1987). For the SCC model, the parameter DIM was transformed into a categorical variable reflecting natural breaks (<61, 61-120, >120 days) and milk yield was included as a continuous variable (kg/day).

Random intercepts at the cow and herd levels were added to the models to correct for clustering of lactations within animals within herds. To adjust for autocorrelation between repeated test day recordings within lactations, an autoregressive correlation structure was selected for both models. This correlation structure resulted in the lowest Akaike Information Criterion (AIC) value compared to other competing correlation structures.

2.2.4 Fertility parameters

To investigate the effect of SBV infection on fertility, the number of AI and NR56 were analysed. For the number of AI, all inseminations of an animal per production cycle were considered, regardless of whether they lead to gestation or not. The NR56 was defined as the absence of a subsequent insemination up to 56 days after the first insemination. For the number of AI, an observation was assigned to the SBV period (P2) if at least one of the first three consecutive AI lay within P2. For NR56, at least one of the 56 consecutive days after the first AI had to lie within P2.

To model the number of AI, a zero truncated negative binomial distribution fitted the data substantially better than a negative binomial or a poisson distribution, assessed by the use of AIC, Bayesian information criteria (BIC) and the dispersion parameter alpha. This implied that, for this model, no corrections for the clustering of observations could be made. To model the effect of clinical signs on NR56, a multilevel logistic regression was applied. It included two random effects to account for the clustering of animals within herd and observations within animals.
For both models the same covariates were evaluated as described previously, except for DIM, which could not be evaluated since observations were at cow rather than test day level.

3. Results

3.1. Study population

A total of 1,320 animals from 91 herds were considered for the present investigation. Of these, 388 animals with clinical signs originated from 67 case herds, whereas 553 and 379 animals without clinical signs originated from 24 control herds and 67 case herds, respectively. The 91 herds were located in 10 different cantons across Switzerland, most of them in the cantons of Berne (27.8%, n=33), Luzern (28.6%, n=23) and Basel-Landschaft (18.9%, n=11). The majority of herds (56.0%; n=51) had free-stall housing systems. The median herd size was 29 cows, with an interquartile range (IQR) of 21-46 and a maximum herd size of 120 cows. The median number of cows per herd included in the statistical analyses was 11(IQR: 5-21). Forty-four percent (n=575) of the animals were first-lactating cows, 20% (n=264) were in their second and 36% (n=481) in their third or higher lactation at the time of the SBV epidemic.
Cows which later developed clinical signs of acute SBV infection differed from cows that remained non-clinical in some characteristics before the SBV period (Table 2). Clinical animals had a higher milk production and higher values of SCC than non-clinical animals in case and control herds before the epidemic, based on crude values.

3.2. *Clinical signs*

The within-herd prevalence of clinical signs in case herds ranged from 3 to 100%, with a median of 14.8%. Milk loss was the most frequently reported clinical sign and was observed in 90.2% of clinically affected animals (n=350), followed by diarrhoea (n=323, 83.2%), fever (n=226, 58.2%) and abortion (n=46, 11.9%).

3.3. Milk yield and SCC

3.3.1. Milk yield

Average milk yield was 25.4 ± 9.0 kg per animal per test day, assessing 25,849 test day records of 1,320 cows. The effect of SBV infection on milk yield resulting from the final multilevel linear regression model is presented in Table 3 and further illustrated in Figure 2. In clinical animals, milk yield decreased significantly by 1.9 kg per test day during the SBV epidemic compared to the half-year before (Table 3). A post-hoc analysis of the other animal groups also revealed an effect over time, resulting in a significant decrease of 1.1 kg in non-clinical animals from case herds ($p \leq 0.001$) and 0.6 kg in non-clinical animals from control herds ($p = 0.001$; results not shown).

In the time period one year after the epidemic (second half of 2013), milk yield in clinical animals was significantly lower by 1.0-1.9 kg per test day compared to all half-years before the epidemic (with one exception in the second half of 2010, where the difference was non-significant). Non-clinical animals from both case and control farms had similar milk yield levels in the second half of 2013 compared to the half-years before the epidemic, and were only significantly lower if compared to the two half-years just before the epidemic (0.6-1.1 kg).

Albeit non-significant in the multivariable model, clinical animals tended to have a higher milk production level compared to non-clinical animals before the SBV period. During the two half-years following the epidemic, the production level of clinical animals was similar to non-clinical animals from case herds (Figure 2).

A higher parity was associated with a significantly higher milk yield and Simmental cows had a significantly lower milk yield compared to Holstein Friesian and Swiss Fleckvieh (Table 3). The final statistical model for milk yield corrected for seasonal effects and peak production. 'Housing system' remained in the final model because it was confounding the parameter estimates of 'Herd size'.

3.3.2. Somatic cell count

Median SCC of cows included in the analysis (n=1,320) was 55,000 cells/ml (IQR: 26,000-117,000). The final multilevel linear regression model (Table 4; Figure 3) revealed that SCC of clinical animals was significantly higher during the SBV period, both if compared to non-clinical animals within the same time period and to clinical and non-clinical animals before the epidemic. In the first half-year 2013, just after the SBV period, clinical animals tended to have a higher SCC than during the SBV period, whereas non-clinical animals from both case and control herds remained on similar levels as during the SBV period. The SCC values of clinical animals as compared to the other groups remained also higher in the second half of 2013, although the difference was only significant if compared to non-clinical animals from case herds. Looking at the entire study period, SCC increased over time in all animal subgroups. Furthermore, SCC increased with an increasing parity as well as with increasing DIM, and was negatively associated with milk yield.

3.4. Fertility

3.4.1. Number of inseminations

The median number of inseminations per cow and reproduction cycle (n=1,242) was one (IQR: 1-2), with a maximum of nine. Incident rate ratios of the final zero-truncated negative binomial regression model are presented in Table 5. The effect of SBV on the number of AI is further illustrated in Figure 4. The number of AI in clinical animals was significantly higher during the SBV period compared to both clinical and non-clinical animals before and after the epidemic. No statistically significant difference was found between clinical and non-clinical animals during the epidemic period (P2). Simmental cows had the lowest, and Braunvieh and Holstein Friesian cows the highest number of AI. Small herds showed the lowest number of AI. Furthermore, a higher cumulative individual milk production level was associated with a significantly higher number of AI.

3.4.2. Non-return at day 56

Overall, NR56 was 65.9%, assessing 2,969 observations in 1,242 cows. Table 6 shows the results of the final hierarchical logistic regression model for NR56, and the odds ratios of 'Time&Status' are plotted in a graph over time in Figure 5. No significant differences were found, neither between different time periods nor between clinical and non-clinical animals. Animals from bigger herds and with higher parity showed significantly higher odds for NR56, whereas a high cumulative individual milk production level was associated with a decreased NR56.

4. Discussion

The aim of this study was to investigate the association between acute clinical signs and production and fertility parameters after an infection with SBV in individual Swiss dairy cows. Over the last few years, a number of studies have assessed the impact of a SBV infection on production and fertility parameters at herd level, either looking at the overall impact of the epidemic (Veldhuis et al., 2014b; Toson et al., 2015) or comparing herds with and without clinical signs indicative of SBV infection (Veldhuis et al., 2014a; Wüthrich et al., 2016). These studies identified limited effects on mortality and/or production and fertility parameters at herd level. Nonetheless, the economic impact of clinical disease in particular in farms with a high within-herd morbidity and a pronounced drop in milk production could be severe (Veldhuis et al., 2014b; Wüthrich et al., 2016).

To the extent of our knowledge, the present investigation is the first field study that assessed the impact of clinical disease at animal level. All animals were assumed to have been infected and to have seroconverted by the time of data collection. Hence, there were no seronegative control animals available. In addition, the assignment of animals to the clinical or non-clinical group was based in part on farmers' reports and health records, and thus relied on the ability of each farmer to detect and record respective clinical signs. Specifically, fever is expected to have been underreported, because it

cannot be detected by observation of the animal only. Nevertheless, the selection of both case and control animals was carried out very carefully, and misclassification bias was minimized by excluding all animals with incomplete or inconsistent information on their clinical status from the analyses. Even though infection had been confirmed by laboratory testing in all case herds, there is no proof for a causal association between the clinical signs observed and infection with SBV for each individual animal.

More than two thirds of the control herds had to be excluded, whereas only 10 of 77 case herds had to be excluded. A reason for missing or inconsistent health records in control herds could have been a lower motivation of the farmers who had not observed clinical disease in their animals during the course of the SBV outbreak. Recall bias might also be relevant, since nothing particular had happened on control farms during the SBV period. Thus, the motivation for recording complete data was likely higher in farmers of case herds, who had both clinical as well as non-clinical animals on their farm. Still, the number of non-clinical animals with reliable data was reasonably high, with 553 animals originating from control herds and 379 from case herds. In addition, the mixed models corrected for differences between herds by the inclusion of a random herd level intercept and potential herd level fixed effects such as herd size or housing system.

Milk loss was one of the first clinical signs observed in cows after acute infection with SBV (Hoffmann et al., 2012), and was part of the case definition for clinical animals. A stronger decrease in milk yield in clinical than non-clinical animals during the SBV period could therefore be expected, and thus no between-group comparisons were made for the time period during the epidemic. Nevertheless, an overall effect of the SBV epidemic on milk yield was visible. Our investigations revealed a remarkable drop in milk yield from 0.6 kg per test day in non-clinical animals from control farms up to 1.9 kg per test day in clinical animals during the SBV outbreak as compared to the time period before (January-June 2012). At herd level, Wüthrich et al. (2016) reported a median daily milk loss of 0.7 kg per cow in case farms during the same epidemic in Switzerland. Investigations on the herd level in the Netherlands by Veldhuis et al. (2014a) revealed a milk yield reduction of 1.7 kg per animal per day during the epidemic in herds including animals with clinical signs. Yet those findings were based on weekly milk yield records and the time interval only spanned four weeks during the peak of the epidemic, which might have led to less diluted and thus more accurate estimates at the herd level compared to Wüthrich et al.(2016).

The results of the present investigation also revealed a drop in milk yield for non-clinical animals, which is not unexpected as seroconversion had taken place in 99.5% of the herds by the end of the year (Balmer et al., 2014). This effect in cows classified as “non-clinical” might be due to the fact that either mildly affected animals which did not express a pronounced drop in milk yield, or animals with only one clinical sign, had been assigned to the non-clinical instead of the clinical group.

Results further revealed that clinical animals tended to have a higher milk yield before the SBV epidemic, which is in line with the findings reported on herd level by Wüthrich et al. (2016). Some farmers in that study also reported that cows in peak lactation as well as generally high yielding

animals were more often observed with clinical signs. It has therefore been hypothesized that animals under metabolic stress might be more likely to develop clinical signs after acute infection with SBV.

Despite higher milk production levels compared to non-clinical animals before the epidemic, the milk yield of clinical animals was similar to that of non-clinical animals from case herds in the two six-month periods following the SBV period. One year after the epidemic, the milk yield level of clinical animals was still significantly lower than before the epidemic. In contrast, milk yield of non-clinical animals from both case and control farms was similar to the level before the epidemic. Furthermore, the effect size of the difference in milk yield between the time periods before and after the epidemic was shown to be bigger in clinical animals, with a difference varying from 1.0-1.9 kg compared to 0.6-1.1 kg in non-clinical animals. This finding is remarkable, as previous studies did not reveal a prolonged effect on milk production at herd level. Hence, our findings suggest that animals with distinct clinical signs might not return to their previous production potential after infection with SBV, or at least not within their current production cycle. Especially in small herds with high within-herd incidence of clinical signs, the economic impact due to milk production losses can thus be severe.

Up to date, there had been no scientific evidence that an acute infection with SBV was associated with increased SCC in dairy cows. In the study of Wüthrich et al. (2016), no difference was found in bulk tank milk SCC between herds with and without animals showing acute clinical signs. Nevertheless, an increase in SCC and the occurrence of mastitis in relation with acute SBV infection in dairy cattle have been reported by veterinarians in Belgium and farmers in Switzerland (Martinelle et al., 2014; Wüthrich et al., 2016). Our results revealed a higher SCC in clinical animals compared to non-clinical animals both during and after the epidemic. However, whether or not clinical manifestation of SBV had a direct influence on SCC cannot be concluded from the present investigation. Clinical animals already had higher SCC before the SBV period compared to non-clinical animals. In addition, a trend towards an increasing SCC over the years was apparent in all groups.

During the SBV period, the number of AI was higher for all animal subgroups compared to the periods before and after, although the difference was only statistically significant for clinical animals. No effect was found on herd level, where the rate of cows with more than one insemination was compared between herds with and without cows with acute clinical signs of SBV infection (Wüthrich et al., 2016). Our results are comparable to the findings of Veldhuis et al. (2014b) in the Netherlands, where the number of inseminations was found to be slightly but significantly altered during the SBV epidemic in all herds, regardless of whether or not they had been reporting malformations indicative of an infection with SBV.
On the other hand, the NR56 of clinical animals was not significantly different during the SBV period compared to the periods before and after. This finding is in contrast to the results of Veldhuis et al. (2014b), where NR56 was found to be the most affected fertility parameter after an infection with SBV. The reason for the difference could be that the insemination rate increased particularly at the beginning of the epidemic, whereas some farmers stopped inseminating cows later in the SBV period because they felt that their cows would not become pregnant anyway (Wüthrich et al., 2016).

Consequently, NR56 may not have served as a proper indicator for a successful pregnancy anymore, i.e. NR56 as an indicator of pregnancy was likely overestimated during the epidemic period. Both fertility parameters showed no significant differences among subgroups during the SBV period. Although the comparison of clinical with non-clinical animals must be interpreted with caution because drop in milk yield and abortion were included in the definition of clinical cases, the impact of SBV on fertility and milk yield was constant, namely the effect during the SBV period was most pronounced in clinical animals, followed by non-clinical animals from case herds and finally animals from control herds.

5. Conclusions

The present study investigated the cow-level effects of SBV infection on production and fertility parameters in Swiss dairy cows during and after the epidemic in summer 2012, which allowed for the detection of effects at the individual level that had not been revealed by previous studies at herd level. Although a drop in milk yield was observed in all groups, the effect of an acute SBV infection was most pronounced in clinical animals. Moreover, clinical animals did not return to their previous milk production level after the epidemic, which suggests an extended effect on milk production after the expression of clinical signs. Even though the overall impact of the SBV epidemic might have been limited if considered on national level, milk production losses on specific farms and in individual animals were found to be substantial. Particularly, this applies to small farms with high within-herd prevalence of clinical disease. The present investigation highlights the strength of an assessment at the animal level, which can reveal the disease effect in the individual animal and circumvent dilution effects that may be present at herd level.

Conflict of interest

The authors declare no conflict of interest

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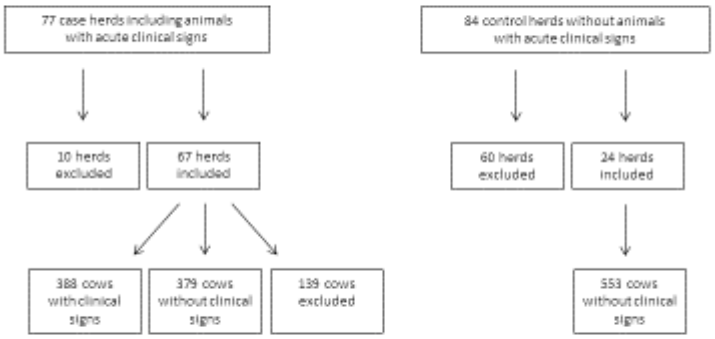

Figure 1

Selection of animals with and without clinical signs to assess the association between clinical signs and fertility and production parameters after acute Schmallenberg virus infection in Swiss dairy cows; herds and animals were excluded based on lack of reliable production data and/or uncertain clinical status.

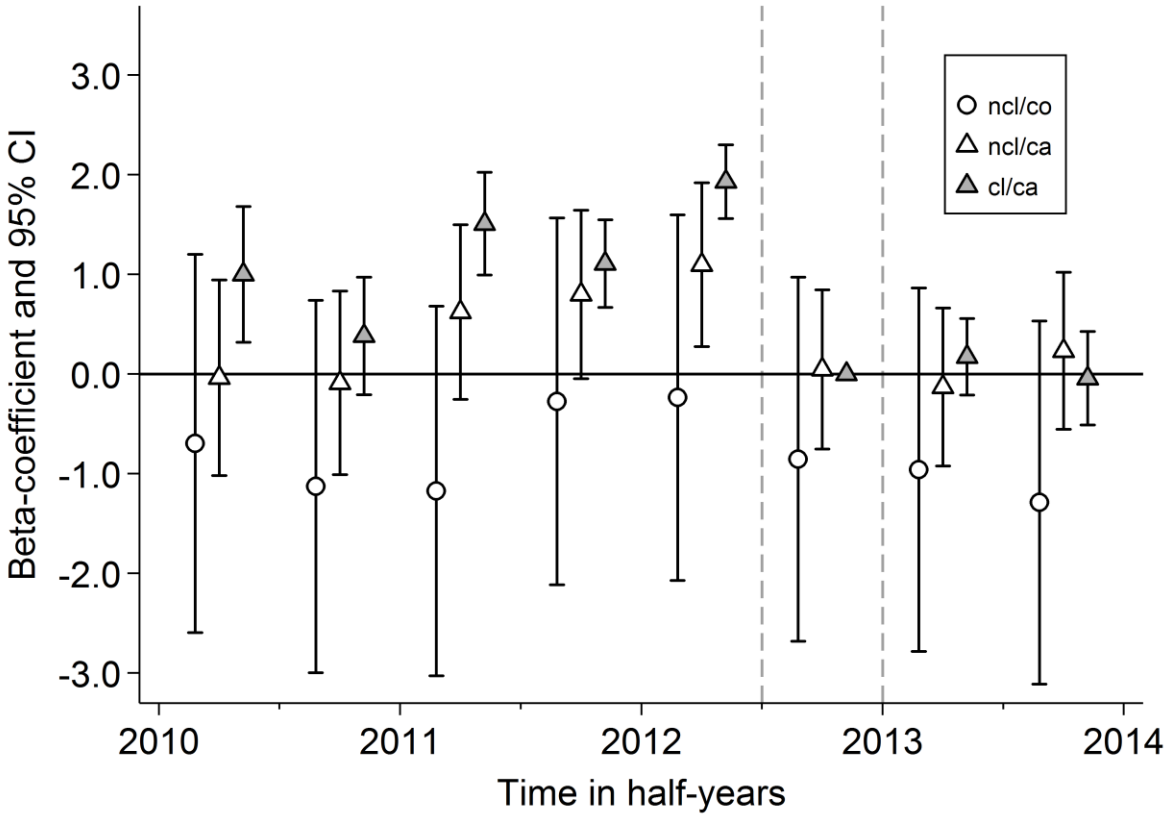

Figure 2

Effect estimates of the final model assessing the association between clinical disease and milk yield (kg/day) after acute infection with Schmallenberg virus in Swiss dairy cows. Beta-coefficients and 95% confidence intervals (CI) are presented for 3 categories: non-clinical animals from control farms (ncl/co), non-clinical animals from case farms (ncl/ca) and clinical animals from case farms (cl/ca). Results are plotted in relation to the baseline category, represented by clinical animals from case farms during the Schmallenberg virus epidemic (July - December 2012, dashed lines).

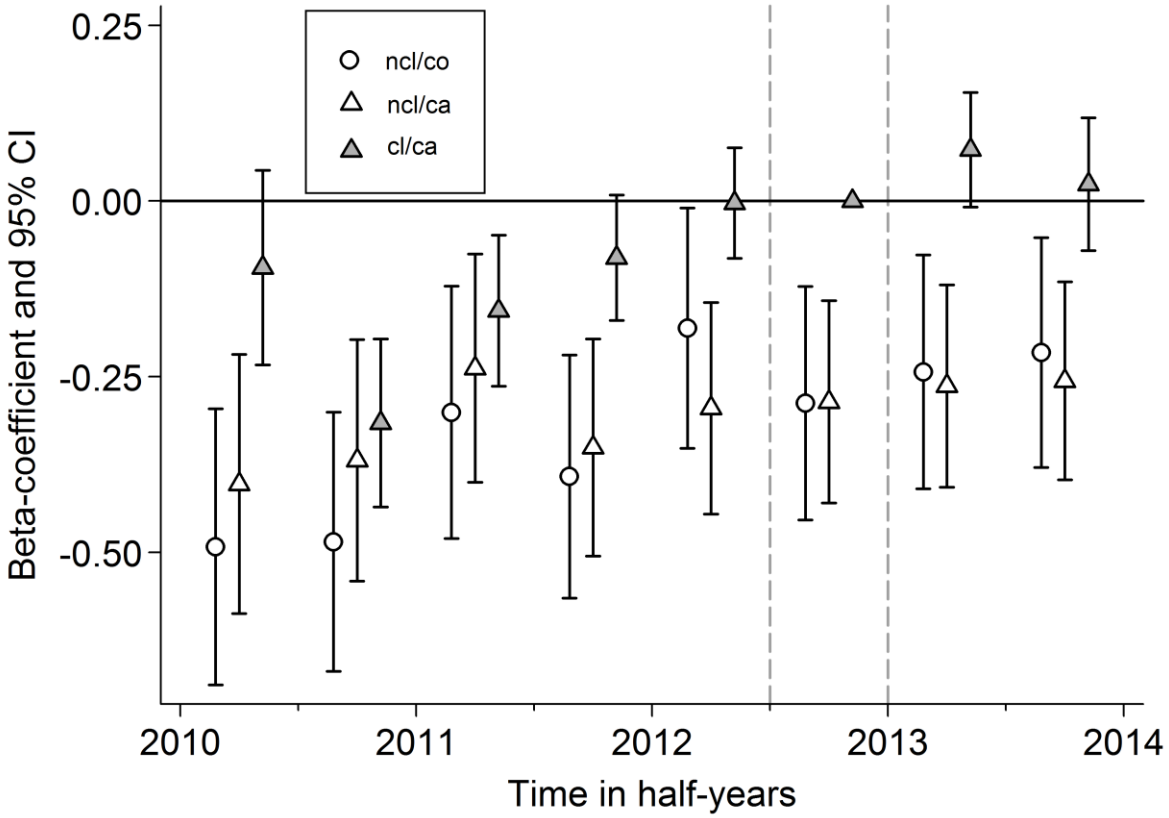

Figure 3

Effect estimates of the final model assessing the association between clinical disease and somatic cell count (10^3/ml) after acute infection with Schmallenberg virus in Swiss dairy cows. Beta-coefficients and 95% confidence intervals (CI) are presented for 3 categories: non-clinical animals from control farms (ncl/co), non-clinical animals from case farms (ncl/ca) and clinical animals from case farms (cl/ca). Results are plotted in relation to the baseline category, represented by clinical animals from case farms during the Schmallenberg virus (July - December 2012, dashed lines).

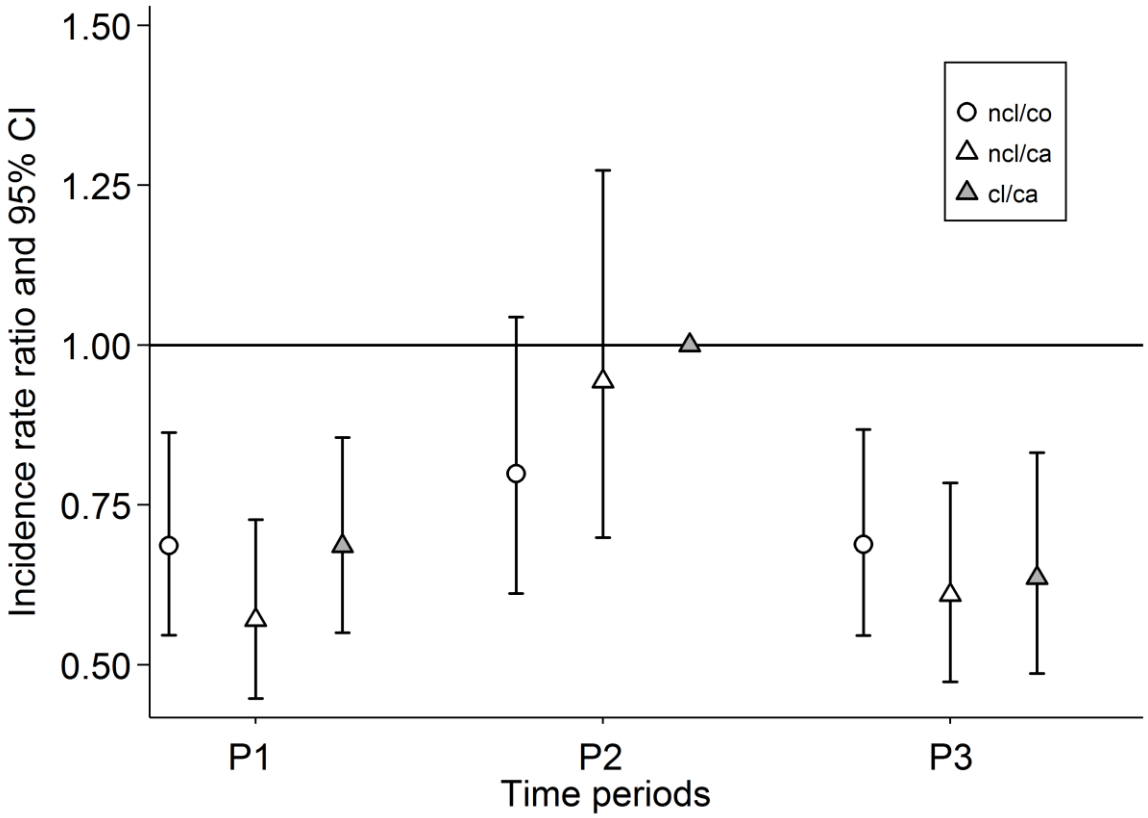

Figure 4

Effect estimates of the final model assessing the association between clinical disease and number of artificial inseminations after an infection with Schmallenberg virus in Swiss dairy cows. Incidence rate ratios (IRR) and 95% confidence intervals (CI) are presented for 3 categories: non-clinical animals from control farms (ncl/co), non-clinical animals from case farms (ncl/ca) and clinical animals from case farms (cl/ca). IRR's are plotted in relation to the baseline category, represented by clinical animals from case farms during the Schmallenberg virus epidemic (P2; July - December 2012). P1= January 2010 – June 2012; P3 = January – December 2013.

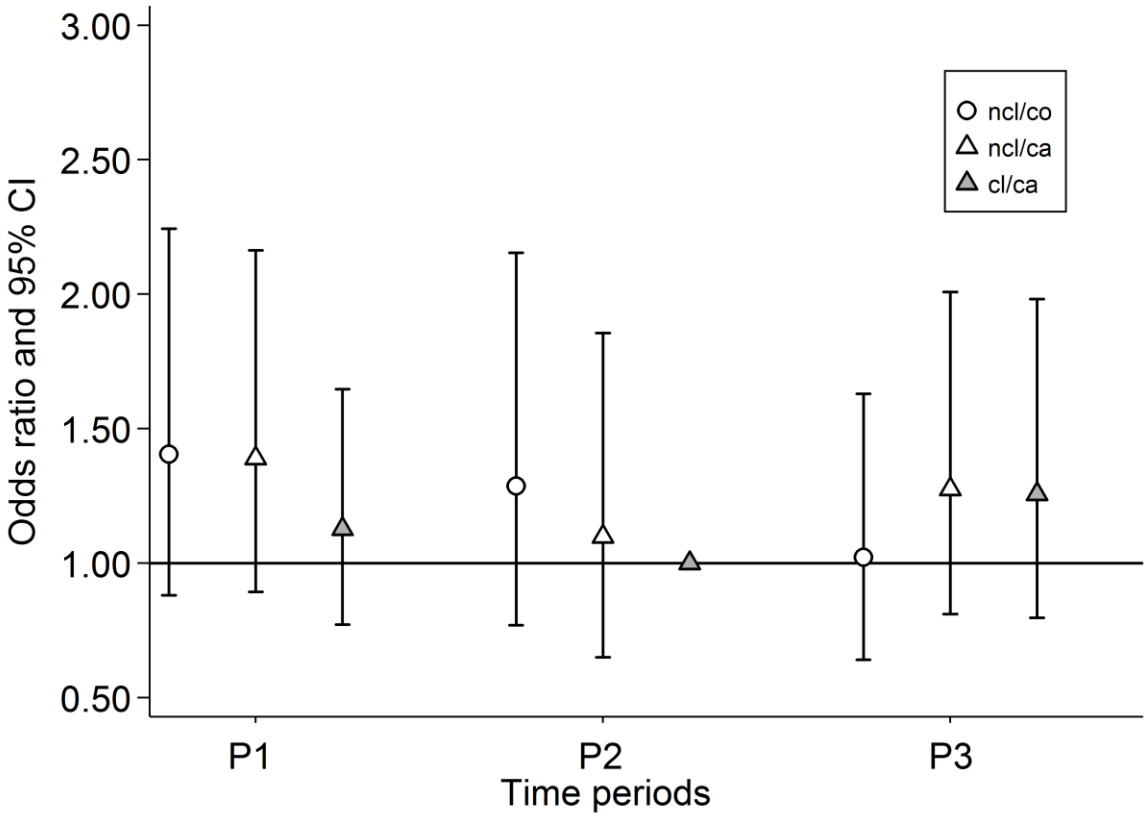

Figure 5

Effect estimates of the final model assessing the association between clinical disease and non-return at day 56 after acute infection with Schmallenberg virus in Swiss dairy cows. Odds ratios (OR) and 95% confidence intervals (CI) are presented for 3 categories: non-clinical animals from control farms (ncl/co), non-clinical animals from case farms (ncl/ca) and clinical animals from case farms (cl/ca). OR's are plotted in relation to the baseline category, represented by clinical animals from case farms during the Schmallenberg virus epidemic (P2; July - December 2012). P1= January 2010 – June 2012; P3 = January – December 2013.

Table 1 Time periods defined to investigate the effect of acute Schmallenberg virus infection on fertility and production parameters in Swiss dairy cows.

Time period assessed	Corresponding time span
Fertility parameters	
P1	January 2010 – June 2012
P2*	July – December 2012
P3	January 2013 – December 2013
Milk yield and somatic cell counts	
2010-1	January – June 2010
2010-2	July – December 2010
2011-1	January – June 2011
2011-2	July – December 2011
2012-1	January – June 2012
2012-2*	July – December 2012
2013-1	January – June 2013
2013-2	July – December 2013

* = Schmallenberg virus period in Switzerland

Table 2 Characteristics of cows included in the study by clinical and herd status to investigate the effect of acute Schmallenberg virus infection on production and fertility parameters. Results of the time period from January 2010 to June 2012 (prior to the Schmallenberg virus epidemic). IQR = Interquartile range.

	Non-clinical cows from control herds	Non-clinical cows from case herds	Clinical cows from case herds
	Median (IQR)	Median (IQR)	Median (IQR)
Herd size	24 (18-33)	34 (23-50)	33 (25-60)
Parity	2 (1-4)	2 (1-4)	2 (1-4)
Milk yield per animal (kg/day)	23.1 (18.3-28.7)	24.3 (19.3-30.2)	27.0 (21.0- 35.0)
Somatic cell count (10^3/ml)	55 (27-113)	50 (24-100)	59 (28-131)
Number of artificial inseminations per production cycle*	1 (1-2)	1 (1-2)	1 (1-2)
	Frequency (%)	Frequency (%)	Frequency (%)
Free-stall housing system	325 (58.8%)	235 (62.0%)	216 (55.7%)
Non-return at day 56	362 (67.9%)	275 (68.1%)	334 (66.8%)

* = Irrespective of a consecutive gestation

Table 3 Final multilevel linear regression model demonstrating the effect of acute Schmallenberg virus infection on milk yield (kg/day) in Swiss dairy cows (n=1,320). β = regression coefficient; CI = confidence interval; s.e. = standard error; ref. = reference category. Bold values are statistically significant (p< 0.05).

Explanatory variable		β	95% CI	*p*-value	Overall *p*-value
Time&Status					<0.001
2010-1	non-clinical / control	-0.70	-2.59 – 1.20	0.472	
	non-clinical / case	-0.04	-1.02 – 0.94	0.942	
	clinical / case	1.00	0.32 – 1.68	**0.004**	
2010-2	non-clinical / control	-1.13	-3.00 – 0.74	0.237	
	non-clinical / case	-0.09	-1.01 – 0.83	0.851	
	clinical / case	0.38	-0.21 – 0.97	0.202	
2011-1	non-clinical / control	-1.17	-3.03 – 0.68	0.216	
	non-clinical / case	0.62	-0.25 – 1.50	0.163	
	clinical / case	1.51	0.99 – 2.02	**<0.001**	
2011-2	non-clinical / control	-0.27	-2.12 – 1.57	0.771	
	non-clinical / case	0.80	-0.05 – 1.64	0.064	
	clinical / case	1.11	0.67 – 1.55	**<0.001**	
2012-1	non-clinical / control	-0.24	-2.07 – 1.60	0.801	
	non-clinical / case	1.10	0.27 – 1.92	**0.009**	
	clinical / case	1.93	1.56 – 2.30	**<0.001**	
2012-2	non-clinical / control	-0.85	-2.68 – 0.97	0.360	
	non-clinical / case	0.05	-0.75 – 0.84	0.909	
	clinical / case	ref.			
2013-1	non-clinical / control	-0.96	-2.78 – 0.87	0.303	
	non-clinical / case	-0.13	-0.92 – 0.66	0.750	
	clinical / case	0.17	-0.21 – 0.56	0.377	
2013-2	non-clinical / control	-1.29	-3.11 – 0.53	0.166	
	non-clinical / case	0.23	-0.55 – 1.02	0.561	
	clinical / case	-0.04	-0.51 – 0.42	0.859	
Days in milk		-0.07	-0.07 – -0.06	**<0.001**	<0.001
Peak production function		-7.34	-7.72 – -6.96	**<0.001**	<0.001
Breed					<0.001
Simmental		ref.			
Braunvieh		1.27	-0.70 – 3.24	0.207	
Holstein Friesian		3.42	1.67 – 5.17	**<0.001**	
Swiss Fleckvieh		2.41	0.69 – 4.12	**0.006**	

Other	0.61	-1.32 – 2.54	0.536	
Parity				<0.001
1	ref.			
2	3.20	2.90 – 3.50	**<0.001**	
≥3	5.52	5.19 – 5.84	**<0.001**	
Herd size				0.025
<21	ref.			
21-30	-0.82	-3.03 – 1.38	0.463	
31-45	-0.89	-3.60 – 1.81	0.519	
>45	2.23	-0.45 – 4.91	0.102	
Housing System				0.497†
Tie-stall	ref.			
Free-stall	0.68	-1.28 – 2.63	0.497	
Sine harmonic	0.22	0.09 – 0.35	**<0.001**	<0.001
Intercept	29.66	27.26 – 32.06	**<0.001**	
Random effect parameters	Variance	95% CI	s.e.	
Herd	11.85	8.50 – 16.53	2.01	
Animal	6.58	5.79 – 7.49	0.43	
Parity	<0.01	<0.01 – <0.01	<0.01	
Residuals (autoregressive)	19.93	19.32 - 20.56	0.32	

† = confounding variable

Table 4 Final multilevel linear regression model demonstrating the effect of acute Schmallenberg virus infection on somatic cell counts (10^3/ml) in Swiss dairy cows (n=1,320). β = regression coefficient; CI = confidence interval; s.e. = standard error; ref. = reference category. Bold values are statistically significant ($p< 0.05$).

Explanatory variable		β	95% CI	*p*-value	Overall *p*-value
Time&Status					<0.001
2010-1	non-clinical / control	-0.49	-0.69 – -0.30	**<0.001**	
	non-clinical / case	-0.40	-0.59 – -0.22	**<0.001**	
	clinical / case	-0.09	-0.23 – 0.04	0.179	
2010-2	non-clinical / control	-0.49	-0.67 – -0.30	**<0.001**	
	non-clinical / case	-0.37	-0.54 – -0.20	**<0.001**	
	clinical / case	-0.32	-0.44 – -0.20	**<0.001**	
2011-1	non-clinical / control	-0.30	-0.48 – -0.12	**0.001**	
	non-clinical / case	-0.24	-0.40 – -0.08	**0.004**	
	clinical / case	-0.16	-0.26 – -0.05	**0.004**	
2011-2	non-clinical / control	-0.39	-0.56 – -0.22	**<0.001**	
	non-clinical / case	-0.35	-0.50 – -0.20	**<0.001**	
	clinical / case	-0.08	-0.17 – 0.01	0.077	
2012-1	non-clinical / control	-0.18	-0.35 – -0.01	**0.038**	
	non-clinical / case	-0.29	-0.45 – -0.14	**<0.001**	
	clinical / case	-0.01	-0.08 – 0.08	0.944	
2012-2	non-clinical / control	-0.29	-0.45 – -0.12	**0.001**	
	non-clinical / case	-0.29	-0.43 – -0.14	**<0.001**	
	clinical / case	ref.			
2013-1	non-clinical / control	-0.24	-0.41 – -0.08	**0.004**	
	non-clinical / case	-0.26	-0.41 – -0.12	**<0.001**	
	clinical / case	0.07	-0.01 – 0.15	0.079	
2013-2	non-clinical / control	-0.21	-0.38 – -0.05	**0.010**	
	non-clinical / case	-0.26	-0.39 – -0.12	**<0.001**	
	clinical / case	0.02	-0.07 – 0.12	0.621	
Days in milk					<0.001
<61		ref.			
61-120		0.03	0.01 – 0.06	**0.043**	
>120		0.14	0.11 – 0.18	**<0.001**	
Breed					<0.001
Simmental		ref.			
Braunvieh		0.58	0.29 – 0.87	**<0.001**	

Holstein Friesian	0.60	0.33 – 0.87	**0.006**	
Swiss Fleckvieh	0.44	0.16 – 0.71	0.055	
Other	0.65	0.33 – 0.97	**<0.001**	
Parity				<0.001
1	ref.			
2	0.30	0.24 – 0.36	**<0.001**	
≥3	0.62	0.55 – 0.69	**<0.001**	
Milk (kg/day)	-0.05	-0.05 – 0.05	**<0.001**	
Sine harmonic	-0.10	-0.13 – -0.07	**<0.001**	<0.001
Intercept	4.57	4.28 – 4.85	**<0.001**	
Random effect parameters	Variance	95% CI	s.e.	
Herd	0.06	0.03 – 0.10	0.02	
Animal	0.24	0.21 – 0.27	0.02	
Parity	0.20	0.18 – 0.23	0.01	
Residuals (autoregressive)	0.62	0.61– 0.64	0.01	

Table 5 Final zero truncated negative binomial regression model demonstrating incidence rate ratios (IRR) for variables associated with the number of artificial inseminations in 3 groups of Swiss dairy cows (n=1,242) before (P1), during (P2) and after (P3) the Schmallenberg virus epidemic. CI = confidence interval; ref. = reference category. Bold values are statistically significant (p< 0.05).

Explanatory Variable		IRR	95% CI	*p*-value	Overall *p*-value
Time&Status					<0.001
P1	non-clinical / control	0.69	0.55 – 0.86	**0.001**	
	non-clinical / case	0.57	0.45 – 0.73	**<0.001**	
	clinical / case	0.69	0.55 – 0.86	**0.001**	
P2	non-clinical / control	0.80	0.61 – 1.04	0.100	
	non-clinical / case	0.94	0.70 – 1.27	0.703	
	clinical / case	ref.			
P3	non-clinical / control	0.69	0.55 – 0.87	**0.002**	
	non-clinical / case	0.61	0.47 – 0.78	**<0.001**	
	clinical / case	0.64	0.49 – 0.83	**0.001**	
Breed					<0.001
Simmental		ref.			
Braunvieh		2.46	1.68 – 3.62	**<0.001**	
Holstein Friesian		2.48	1.69 – 3.64	**<0.001**	
Swiss Fleckvieh		2.03	1.38 – 3.00	**<0.001**	
Other		1.96	1.19 – 3.21	**0.008**	
Herd size					<0.001
<21		ref.			
21-30		0.82	0.70 – 0.95	**0.011**	
31-45		0.70	0.60 – 0.81	**<0.001**	
>45		0.74	0.64 – 0.87	**<0.001**	
Individual production level (kg)					<0.001
<124		ref.			
124-154		1.29	1.11 – 1.47	**<0.001**	
>154		1.52	1.31 – 1.76	**<0.001**	
Intercept		0.54	0.35 – 0.83	**0.005**	<0.001

Table 6 Final multilevel logistic regression model demonstrating odds ratios (OR) for variables associated with non-return at day 56 in 3 groups of Swiss dairy cows (n=1,242) before (P1), during (P2) and after (P3) the Schmallenberg virus epidemic.
OR = odds Ratio; CI = confidence interval; ref. = reference category. Bold values are statistically significant (p< 0.05).

Explanatory Variable		OR	95% CI	*p*-value	Overall *p-value*
Time&Status					0.404
P1	non-clinical / control	1.41	0.88 – 2.24	0.154	
	non-clinical / case	1.39	0.89 – 2.16	0.145	
	clinical / case	1.13	0.77 – 1.65	0.535	
P2	non-clinical / control	1.29	0.77 – 2.15	0.337	
	non-clinical / case	1.10	0.65 – 1.86	0.727	
	clinical / case	ref.			
P3	non-clinical / control	1.02	0.64 – 1.63	0.929	
	non-clinical / case	1.28	0.81 – 2.01	0.293	
	clinical / case	1.26	0.80 – 1.98	0.325	
Parity					0.032
1		ref.			
2		1.37	1.08 – 1.75	**0.010**	
≥3		1.27	1.00 – 1.60	**0.048**	
Herd size					0.028
<21		ref.			
21-30		1.59	1.08 – 2.36	**0.020**	
31-45		1.50	0.99 – 2.27	0.056	
>45		1.83	1.20 – 2.78	**0.005**	
Individual production level (kg)					<0.001
<124.4		ref.			
124-154		0.70	0.56 – 0.87	**0.002**	
>154		0.53	0.41 – 0.70	**<0.001**	
Intercept		1.32	0.82 – 2.11	0.253	<0.001
Random effect parameters		Variance	95% CI	s.e.	
Herd		0.23	0.14 – 0.41	0.07	
Animal		0.16	0.04 – 0.61	0.11	