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### Summary

### Zusammenfassung

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## The effect of bovine viral diarrhoea virus on fertility in dairy cows: two case-control studies in the province of Styria, Austria

### *Der Einfluss des Bovinen Virusdiarrhoe-Virus auf die Fruchtbarkeit von Milchkühen: zwei Fall-Kontrollstudien im Bundesland Steiermark, Österreich*

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Bovine viral diarrhoea (BVD) leads to substantial economic losses in beef and dairy herds worldwide. Two case-control studies were carried out using production data from 1996 to 2012 to analyse the impact of BVD virus (BVDV) on fertility in dairy herds in the province of Styria during an eradication programme. In study 1, herds in which at least one persistently BVDV-infected (PI) animal was detected (case herds) were compared to a group of control herds proven free from BVDV infection (control herds). In study 2, within BVD infected herds the period during which PI animals were present (exposed period) was compared to the period after successful BVD eradication (unexposed period). Calving interval (CAI) and the probability of a first service conception (FSC) were used as indicators in a mixed regression model to investigate the impact of BVD on reproductive performance. The model results indicated that BVD had a significant influence on CAI and FSC. Cows from control herds were 1.1 times more likely to conceive at first service compared to cows from case herds and cows served during the BVDV unexposed period were 1.3 times more likely to conceive at first service than those inseminated during the exposed period. In BVD-infected herds the CAI averaged seven days shorter in unexposed periods than in exposed periods. Besides BVD the animal breed and the parity substantially impact the analysed fertility indicators.

**Keywords:** BVD-eradication programme, mixed models, first service conception, calving interval, BVDV, Austria

Die Bovine Virusdiarrhoe (BVD) führt weltweit zu substanziellen wirtschaftlichen Verlusten in Milch- und Fleischrinderherden. Zwei Fall-Kontrollstudien wurden unter Verwendung von Leistungsdaten aus den Jahren 1996 bis 2012 durchgeführt, um den Einfluss der BVD auf die Fruchtbarkeit in Milchviehherden im Bundesland Steiermark in Österreich während eines laufenden BVD Eradikationsprogrammes zu untersuchen. In Studie 1 wurden Betriebe, in denen zumindest ein persistent BVD-Virus (BVDV) infiziertes Tier nachgewiesen wurde (Fallbetriebe), mit BVD virusfreien Kontrollbetrieben verglichen. In Studie 2 wurde in Fallbetrieben der Zeitraum, in dem persistent BVD-Virus infizierte Tiere nachgewiesen wurden, mit dem Zeitraum nach der erfolgreichen BVD-Sanierung verglichen. Als Indikatoren für die Fruchtbarkeit wurden die Zwischenkalbezeit sowie der Erstbesamungserfolg verwendet. Die Ergebnisse der gemischten Regressionsmodelle zeigen, dass die Chance, dass bereits die erste Besamung nach der Abkalbung zu einer neuerlichen Trächtigkeit führt, bei Abwesenheit von BVDV am Betrieb signifikant steigt. Die Chance einer erfolgreichen Erstbesamung war in BVDV freien Kontrollbetrieben um einen Faktor von 1.1 höher als in den Fallbetrieben. In BVD infizierten Betrieben stieg die Chance für eine erfolgreiche Erstbesamung nach der BVD-Sanierung um einen Faktor von 1.3. Die Zwischenkalbezeit verkürzte sich in BVD infizierten Betrieben nach der BVD-Sanierung um sieben Tage. Neben der BVD beeinflussten vor allem die Rasse und die Laktationszahl die analysierten Fruchtbarkeitsparameter.

**Schlüsselwörter:** BVD-Eradikationsprogramm, gemischte Modelle, Erstbesamungserfolg, Zwischenkalbezeit, BVDV, Österreich

## **Introduction**

Bovine viral diarrhoea (BVD) is economically one of the most important infectious diseases in cattle worldwide (Baker, 1995; Reichel et al., 2008; Nickell et al., 2011; Häslér et al. 2012; Rodning et al., 2012; Smith et al., 2014). The production losses caused by infection with bovine viral diarrhoea virus (BVDV) result from lower milk yield, extended calving intervals, reduced first service conception and increased abortion rates (Grein, 1995; Niskanen et al., 1995; Taylor et al., 1997; Rufenacht et al., 2001; Houe, 2003). In a review by Houe in 2003, the calculated economic losses to Danish herds were estimated to be between \$25 and 160 per cow and at the national level between \$10 and 40 million per million calvings. Calculations of economic consequences vary (Carman et al., 1998; Bennett et al., 1999; Chi et al., 2002; Gunn et al., 2005), depending on the prevalence and variability of BVDV infections (Fourichon et al., 2005). It remains difficult to quantify the damage caused by BVD and to transform this into economic terms.

In BVDV persistently infected cattle (PI), growth retardation and a variety of diseases due to BVDV-related immunosuppression predominate. In PI cattle developing mucosal disease, severe diarrhoea causes the death of the animals within a few days. PI animals shed large amounts of virus but do not develop specific antibodies to BVDV (Moennig and Liess, 1995). In immunocompetent cattle, BVDV predominantly affects all phases of gestation (Grooms, 2004; Robert et al., 2004). These animals generally demonstrate mild respiratory and enteric symptoms and excrete small amounts of viral particles (Brownlie et al., 1987). BVDV infections during gestation depending on stage, however, can result in cows returning to oestrus following early embryonic death and abortions, the birth of weak or malformed calves and – crucial for the spread of the infection – the birth of PI calves.

Farmers and livestock businesses have several options either to control or totally eradicate BVDV to reduce or avoid BVD-associated losses in both dairy and beef herds (Brock, 2004). Eliminating the PI animals is the key issue. In recent years, several European countries have implemented BVDV eradication campaigns (Houe et al., 2006). On a regional level, a BVDV eradication campaign was initiated in the province of Styria, Austria, in 2001 in cattle herds participating in performance recording. From mid-2004, participation in the programme became compulsory for every cattle herd in Austria, with the exception of specialized fattening units. The eradication programme followed the example of the Scandinavian eradication scheme, which is based on assessing the BVD status of herds through serological testing, on clearance of the virus from infected herds, and on livestock movement controls (Lindberg and Alenius, 1999). In 2013, the Austrian cattle sector consisted of 1.95 million animals and approximately 66 thousand livestock holdings, with 39% of the cattle being cows. Totally, 16% (322 467) of the cattle were located in Styria (Statistik Austria, 2014); the cattle were housed in approximately 12 000 farms. During the eradication programme from 2001 onwards, 2595 PI animals were detected in the province of Styria; with the last persistently infected calf being reported in 2011.

Besides BVD however, fertility strongly depends on management factors (Farin and Slenning, 2001) and is

influenced by production environments (Horn et al., 2013; Butler, 2014; Piccardi et al., 2014). Additional information, that was available for study herds (animal breed, production region, parity), was analysed with regard to fertility indicators. Production regions were considered because farming conditions differ widely across the province of Styria, ranging from mountainous areas to lowlands.

The main aim of this study was to analyse the impact of BVDV on fertility in Styrian dairy herds. We hypothesised that, eliminating BVDV from infected herds during a period of on-going BVD eradication measures, results in improved reproductive performance. This scientific issue was tested using two case-control studies, which were evaluated by means of mixed model regression.

## **Material and Methods**

### **Databases**

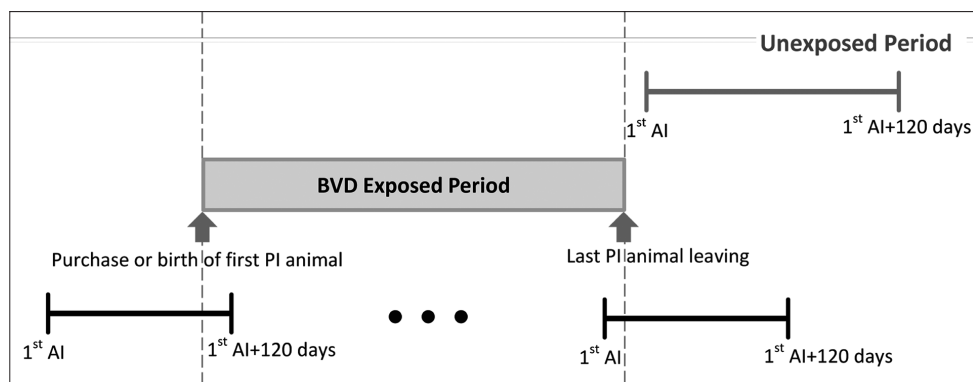
In order to analyse whether BVD has an impact on fertility in dairy herds, BVDV relevant data such as BVD status of the herds, the period between the arrival (birth or purchase) of the first verified PI animal and the exit of the last PI animal (exposed period), as well as sex, date of birth and movement data of PI animals were extracted from the official BVD database of the provincial government of Styria, Austria. Information on breed, parity, calving date, date of purchase or culling and data on artificial insemination of all single cows, as well as herd information (production regions, average milk production per herd per production year) were provided by the official Austrian milk performance recording database in order to investigate the impact of generic factors.

### **Study herds**

To analyse the impact of BVD on reproductive performance based on data from 1996 to 2012, two studies were carried out on dairy herds participating in milk performance recording in Styria. At least one PI animal was identified on 429 of these farms from 1996 until 2009. The number of PI animals per herd ranged between one and 13 (median 2). The age of the PI animals when leaving the herds ranged between six and 2357 days (median 201). The total number of PI animals identified in study herds was 967, infected herds were referred to as case herds.

Herds, which proved BVDV free during the whole period covered by the eradication programme from 2001 to 2012, in accordance with the predefined criteria of the eradication scheme, were preselected. Each case herd was matched to one BVDV free herd, referred to as control herd, with respect to the calendar year before infection, the production region, the predominant cattle breed, the number of lactating cows in the herd, the number of calvings within the year of observation and the herd's annual milk yield within the year of observation in order to obtain a comparable sample where a variation of less than 20% was considered acceptable. Herds were classified as one breed if at least 80% of the cows belonged to that breed.

In the first study, the reproductive performance of cows of case herds was compared to the reproductive performance of cows of control herds. Overall the number of animals per herd (case and control herds) ranged from one to 150 (median 18 cows per herd). With respect to



**FIGURE 1:** Definition of exposed and unexposed periods for case herds. No time distinction was necessary for control herds (AI = artificial insemination, PI = persistently BVDV infected).

cattle breeds, 72% of the herds were Simmental cattle, 22% Brown Swiss and 6% Holstein Friesian. The median calving interval was 375 days, ranging from 301 to 666 days and the first service conception rate was 62%.

In the second study, only BVDV-infected herds were considered in which the eradication of the infection could be proven. Within these herds periods of a current BVDV infection (exposed periods) were compared to periods after the eradication of BVDV from these herds (unexposed periods, Fig. 1). The size of herds used in the second study ranged from three to 100 (median 24 cows per herd) animals, the distribution of breeds was 63% Simmental, 28% Brown Swiss and 8% Holstein Friesian. The median calving interval was 377 days, ranging from 295 to 661 days and the overall first service conception rate was 60% for cows in the second study.

**Data selection**

The period between the arrival of a PI animal on the farm and the exit of the last PI animal from that farm was defined as the BVD exposed period (Fig. 1). Conception failure and early embryonic death are frequent consequences of a BVDV infection during the initial 120 days after service inducing a subsequent return to

service. If the arrival of the first PI animal occurred up to 120 days after the first insemination, then those first services were considered part of the exposed period and were used for analysis (Fig. 1). Records of case herds were allocated to the unexposed period if the first insemination took place after the last PI animal was removed from the herd. Inseminations of maiden heifers were excluded as the first service conception rates of young stock are known to be higher than those of lactating cows and no calving interval can be calculated for heifers. To analyse calving intervals (CAI) gestation periods reported to be shorter than 63 days or longer than 305 days were also excluded from analysis, due to implausible mating or implausible gestation length, respectively. Gestation periods between 63 and 264 days were considered to have been abortions and also were not included in the calculation of the CAI.

Apart from the effect of BVDV on fertility, breed, production regions, parity and year of calving were considered as confounders. To create a balanced dataset, only data on Simmental, Brown Swiss, Holstein Friesian and Montebeliarde breeds were used. Records from other breeds were excluded due to the low number of observations. Due to the same reason records of cows with parities 10 and higher were summarised (10+), also production region C1–3 were summarised to C.

A total of 22 herds were excluded from the second study as they did not have records from both exposed and unexposed periods. Records of cows which moved from one farm to another were also excluded from both study designs.

Consequently, in the first study, data on 321 BVDV-infected herds were included as case herds. 5865 calving events were selected for modelling, as only for these calving events BVDV infections can be deemed relevant. Records of the control herds consisted of 20 670 calving events from 328 herds. In the second study, data on 375 BVDV infected herds were used: 7407 records from the period of a current BVDV infection (exposed period) were compared to 10 699 records from the period after the eradication of BVD on the affected farm (unexposed period) (Tab. 1).

**Parameters examined**  
Reproductive performance was assessed based on calving interval (CAI) and first service conception (FSC) rate. CAI is a useful retrospective measure of reproductive performance for an individual cow, being a function of oestrus detection adequacy, conception rate and calving to first service interval (Wapenaar et al., 2008). CAIs were calculated of all cows and for all parities in both BVDV infected and control herds if at least two consecutive calvings were recorded during the period from 1996 to 2012.

FSC was used because BVD infections severely affect early pregnancy stages, leading to embryo mortality (Whitmore et al., 1981; Yavru et al., 2013). First service

**TABLE 1:** Figures on data used in study 1 and 2

Total number of herds participating in milk performance recording with at least 1 PI	588	
Declaration of consent to the forwarding of information available	447	
Total number of case herds used in study 1 and 2	429	
	<b>study 1</b>	<b>study 2</b>
Number of herds (case/control)	321/328	375/-
Number of PI animals (min-median-max)	1-2-13	1-2-13
Number of days on which confirmed PI animals were present in the herd (min-median-max)	1-174-2273 days	1-201-2273 days
Number of calving events: case (without abortions)/control (without abortions)	5865 (5764)/20 670 (20 307)	-
Number of calving events: exposed (without abortions)/unexposed (without abortions)	-	7407 (7291)/10 699 (10 498)
Median calving interval for all considered herds	375 days	377 days
First Service Conception	62%	60%

was defined as successful if the first service within a lactation led to a calving event. If the first service to conception interval did not exceed ten days, the service was considered successful. A timeframe of ten days was chosen to exclude records representing incorrect heat detection.

### Statistical modelling

For each of the both study designs, two models were applied to investigate the impact of BVD, breed, number of lactations, production region and year of calving on CAI and FSC, where the cow serves as experimental unit. The CAI was logarithmically transformed to comply with a normal distribution and modelled using a linear mixed model. As the FSC was defined as a binary variable (first service was either successful/not successful), this parameter was modelled using a logistic mixed model.

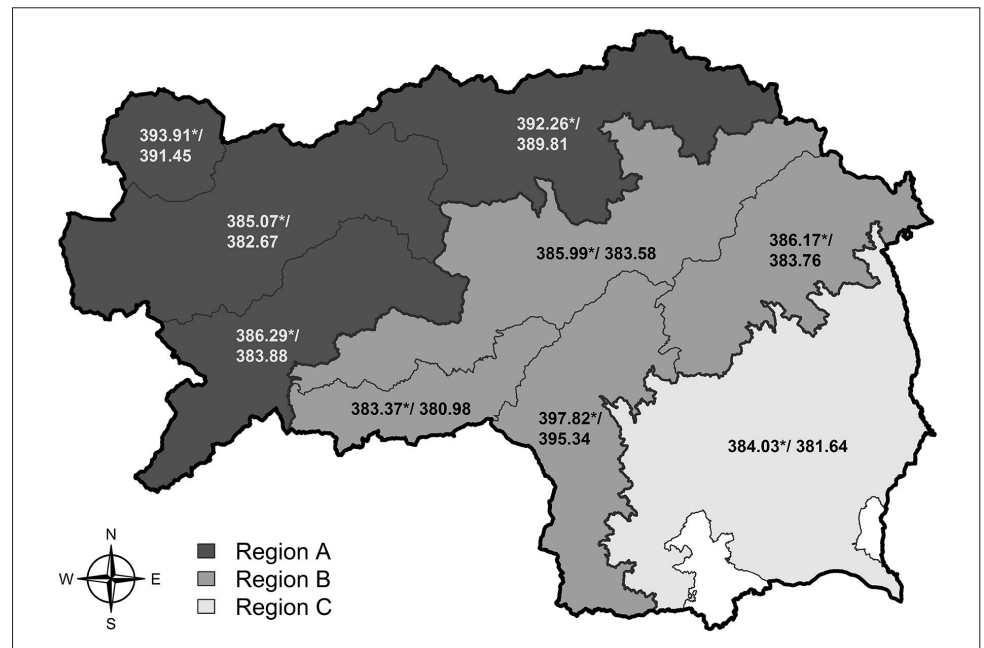
The year of calving was considered as a continuous variable, with 1996 coded as year 0, and the last year, 2012, as year 16. Geographical variations were considered using production regions, which were included in the models as categorical variables. The unique identification number of each animal and the herd identification number were taken into account as random effects in order to allow for herd and animal specific variations.

To display the results, a 'reference' cow was set as Simmental breed, production region A1 (mountainous), first lactation, year 1996, control herd (in the first study) and unexposed period (in the second study). The significance level was utilised to determine significant factors. The models were implemented using the R statistical computing environment (R Development Core Team, 2014).

## Results

### First study (case vs. control herds)

The model results of the first study show that breed, year of calving, production region and number of lactations exert a significant effect ( $p \leq 0.05$ ) on CAI. The difference of the CAI between cows of case and control herds was not significant, but increased by a factor of 1.006 (this corresponds to two days for a reference animal) (Tab. 2). The intercept in Table two represents the estimated expected CAI (in days) for a 'reference' cow. Hence, the expected CAI of a Simmental cow during her first lactation in a control herd situated in the production region A1 in 1996 is 384 days. As an example Figure two illustrates the differences in the expected CAI for a primiparous Simmental cow in 2006 depending on production region and BVD status (case/control). The expected CAI is two days longer for animals from case herds compared to control herds (Fig. 2). Despite this, the expected CAI



**FIGURE 2:** Calving Intervals (CAI) in case (\*) and control herds for a Simmental cow during her first lactation in 2006 in Styria, Austria. Production regions A1–A4 describe mountainous farming conditions, B1–B4 are regions at the eastern edge of the Alps with hilly grassland and C1–C3 regions are flat with arable land.

for animals from production region B1 (hilly grassland) is around nine days shorter than the expected CAI in production region A3 (mountainous).

For the FSC, breed, lactation number and year of calving were identified as statistically significant variables at the 0.05 significance level (Tab. 3). Furthermore, the effect of BVD (case herd) was significant at the 0.05 significance level. The chance of conception after the first service was 1.1 times higher for insemination in control herds compared to those in case herds.

### Second study (exposed vs. unexposed periods)

In the second study, exposure to BVDV, breed, lactation number and year of calving had a statistically significant influence on CAI at the 0.05 significance level (Tab. 2). CAI increased by a factor of 1.018 in cows served during the BVDV exposed period. This corresponds to a seven day longer CAI in a primiparous Simmental cow, which calved in 1996 and was served during a BVDV exposed period. Furthermore, CAI increased by a factor 1.003 (equating to approximately 1 day) each year in the period from 1996 to 2012. The CAI extended as the lactation number increased.

Parity, breed, year of calving as well as exposure to BVDV had a statistically significant influence on FSC (Tab. 3). The odds of a successful first service during the exposed period are 0.68 times the odds during the unexposed period. With increasing numbers of lactations and hence the age of the cow, the chance of a successful first service decreased.

## Discussion

The influence of BVDV on the reproductive performance of dairy herds was assessed by examining two fertility parameters (CAI and FSC). These parameters were used



to analyse and underpin results presented by other authors (Niskanen et al., 1995; Frederiksen et al., 1998; Valle et al., 2001; Robert et al., 2004). Our data is showing that BVDV deteriorates dairy herds' reproductive performance. Consequently reproductive performance is positively influenced by eliminating BVDV following eradication programmes. CAI is a useful parameter to compare the reproductive performance of individual cows, but it is strongly influenced by management and environmental production factors. CAI cannot be calculated for cows, which fail to conceive (Wapenaar et al., 2008). FSC was taken into analysis to show the impact of BVD on especially early stages of gestation.

In addition possible confounders like breed, parity and production region were considered as fixed effects in the model in order to verify their influence on CAI and FSC. Further herd or animal specific attributes were moreover considered through farm and animal individual random effects in the models to correct for the effect of these attributes.

Our results verify a significant influence of BVD on CAI and FSC for cows in BVD infected Styrian dairy herds comparing exposed to unexposed periods. CAI increased by approximately seven days and the odds of FSC decreased by a factor of 0.68 during BVDV exposed periods. Comparing case and control herds only

FSC was significantly improved in herds proven not BVD infected. No significant effect could be shown for CAI between case and control herds. This conflicting result might be explained by different designs in study 1 and 2. Different herds were compared in study one, different periods in the same herd were compared in study 2. Another explanation for the inconsistent observations in the two models for the CAI in the present study might be found in the multitude of factors related to management, farm set-up, training of the farmer, husbandry and nutrition. All these factors could have influenced the calving to first service interval, the conception rate, the calving to conception interval (hence influencing CAI and FSC) and the abortion rate.

As well study results of the effect of BVD on reproductive performance as represented in the literature are inconsistent. The results for CAI of the present study correlate well with a previous study carried out by Niskanen et al. (1995). Valle et al. (2001) reported no differences between BVDV affected and non-affected herds with respect to average CAI. Other studies however have reported the opposite to be the case. For instance, in a case-control study in Norwegian dairy herds, the CAI tended to improve over a three year period in BVDV infected groups (Fredriksen et al., 1998).

In addition, the model results in this study showed that the odds for FSC for cows from BVDV case herds are 0.90 times lower as for

**TABLE 2:** Results for the response calving interval (CAI). Estimation of fixed effects for study 1 and 2, where all studied parameters, except case herd in study 1 and production region in study 2 are significant ( $p \leq 0.05$ ) Exponent of estimated coefficients represents a multiplicative change in number of days

	Study 1			Study 2			
	Case/Control Herds			Exposed/Unexposed Period			
	Estimated coefficients	Standard error	Exp. of estimated coefficients		Estimated coefficients	Standard error	Exp. of estimated coefficients
(Intercept)	5.9504	0.0120	383.8901		5.9140	0.0072	370.1860
Control herd (reference)				Exposed period (reference)			
Case herd	0.0063*	0.0034*	1.0063*	Unexposed period	0.0178	0.0037	1.0179
Year	0.0019	0.0003	1.0020		0.0025	0.0006	1.0025
Lactation number 1 (reference)							
Lactation number 2	-0.0026	0.0021	0.9974		0.0002	0.0026	1.0002
Lactation number 3	0.0018	0.0023	1.0018		0.0051	0.0029	1.0051
Lactation number 4	0.0064	0.0025	1.0064		0.0133	0.0032	1.0134
Lactation number 5	0.0050	0.0029	1.0050		0.0129	0.0037	1.0130
Lactation number 6	0.0116	0.0037	1.0117		0.0153	0.0046	1.0154
Lactation number 7	0.0114	0.0046	1.0115		0.0163	0.0058	1.0164
Lactation number 8	0.0175	0.0064	1.0176		0.0231	0.0077	1.0234
Lactation number 9	0.0146	0.0093	1.0147		0.0103	0.0120	1.0103
Lactation number 10+	0.0094	0.0112	1.0094		0.0343	0.0140	1.0349
Simmental (reference)							
Brown Swiss	0.0518	0.0040	1.0531		0.0524	0.0044	1.0537
Holstein Friesian	0.0433	0.0052	1.0443		0.0552	0.0063	1.0567
Montbeliarde	0.0137	0.0165	1.0138		n. c.		
Prod. region A1 (reference)					n. s.		
Prod. region A2	-0.0227	0.0123	0.9776				
Prod. region A3	-0.0042	0.0152	0.9958				
Prod. region A4	-0.0195	0.0128	0.9807				
Prod. region B1	-0.0271	0.0127	0.9733				
Prod. region B2	-0.0203	0.0119	0.9799				
Prod. region B3	0.0099	0.0135	1.0099				
Prod. region B4	-0.0198	0.0120	0.9804				
Prod. reg C (C1, 2, 3)	-0.0254	0.0155	0.9749				
Variance component of random effect							
Animal Unique identification number		0.0014			0.0015		
Farm		0.0010			0.0012		

n. s. = not considered, because the effect was not significant at the significance level  $\alpha = 0.05$

n. c. = not considered because of too few observations

\* significant at the significance level  $\alpha = 0.10$ . Despite the lower significance this variable was considered because it is in the main focus of the study.

Production regions C1–C3 were summarized as well as records of lactations with lactation numbers of 10 or higher into the category '10 plus' due to the low number of observations.

cows from control herds. Results for study two showed that the odds for FSC during exposed periods are 0.68 times lower as during unexposed periods. Houe et al. (1993) reported a 16% to 64% higher conception rate during post-risk periods of five BVDV-infected dairy herds in Denmark. Another study by Larson et al. (1994) concluded that the mean number of inseminations per pregnancy in a herd comprising approximately 60 cows was higher during the four-month BVDV risk period (1.92 inseminations per pregnancy) compared to the time before and after this period (1.36–1.51 respectively). Further studies which underpin our results can be found in the literature by Whitmore et al. (1981), Virakul et al. (1988), McGowan et al. (1993), Yavru et al. (2013) for dairy cows and Munoz-Zanzi et al. (2004) for heifers. However, some literature also demonstrates no or even a positive effect of BVDV on conception rates (Rüfenacht et al., 2001; Valle et al., 2001). Berends et al., 2008 performed a study on the effect of BVDV on fertility calculating ten parameters, concluding that there is no difference between case and control herds, except a higher abortion rate in case herds. However, even between similarly structured studies it is difficult to draw comparisons as they frequently utilize different methods and/or different definitions for the duration of exposure to BVDV infected animals.

Cattle breed was determined to have a significant influence on fertility, with Simmental cattle attaining better figures for CAI and FSC in all model results. Compared to the results for Simmental cows, the average CAI in Brown Swiss cows was 20 days longer in both studies (factor 1.053 and 1.054) and in Holstein Friesian cows 17 to 21 (factor 1.044 and 1.057) days longer. The odds for FSC decreased by a factor of 0.79 in Brown Swiss cows and by 0.56 respectively 0.66 in Holstein Friesian cows compared to Simmental cows. The impact of breed on fertility is determined by genetics and related to the quantity of milk produced by cows of different breeds. In our study population, the majority of cows were dual-purpose Simmental cattle. Compared to the pure dairy Holstein Friesian cows, the Simmental cows had a significantly lower milk production of 7039 compared to 8473 kg in Austria in 2012 (ZAR, 2013). An increase in milk production is commonly linked to reduced fertility (de Kruif et al., 2008; Albarrán-Portillo and Pollott, 2013).

Higher lactation numbers (and therefore the age) of the cows induced higher CAIs and decreased the probability of a FSC. These results are in line with literature documenting the decrease of fertility in female cattle due to ageing (Hillers et al., 1984).

With respect to production regions, a significant influence of the regions on reproductive performance could only be shown for the CAI in the first study. The impact of the production regions on fertility is caused by farming conditions depending on different agricultural landscapes. The conditions for milk production diverge widely across the province of Styria. Arable land allows the farmer to grow high energy and digestible feedstuffs for dairy

cows, whereas alpine surroundings do not, due to climatic and practical reasons. Husbandry conditions vary too: tethered housing in sheds in winter, with access to outdoor grazing in summer is more common in alpine surroundings whereas permanent cubicle housing and no access to outdoor grazing are more likely to be used in arable lowland regions. Herd size is linked to the production region. In study 1 and 2 dairy herds located in mountainous areas (region A) tend to be smaller (median 15, respective 18 cows per herd) compared to herds in hilly grassland (20 respective 25 cows, region B). The influence of the production region on CAI might possibly be linked to seasonal calving, which is more common in alpine regions, and low energy density in feedstuffs. Dairy cows are more likely to become pregnant when their energy balance becomes positive again after calving. This occurs earlier in cows fed a high energy ration. Assuming that farm management is perfectly adapted to the regional conditions, the success rate for the first insemination is the same in every region, even though the calving to first service interval might be longer in regions with poor agricultural conditions (Butler, 2014).

It should, however, be noted that the results of the analysis presented here are subject to the following limitations: The study population represents Austrian conditions for one federal state (Styria), wherefore these differ not only between but also within countries. Other diseases of animals and on-farm factors, such as different management practices of the case and control herds, which may also have affected reproductive per-

**TABLE 3:** Results for the response success of first service (FSC). Parameter estimates of fixed effects are stated as estimated odds ratios regarding the reference categories for study 1 and 2

	Study 1		Study 2		
	Case/Control Herd		Exposed/Unexposed Period		
	Exp. of estimated coefficients (Odds ratio)	p-value		Exp. of estimated coefficients (Odds ratio)	p-value
(Intercept)	2.89	< 0.001		5.370	<0.001
Control herd (reference)			Unexposed Period (reference)		
Case herd	0.90	0.031	Exposed Period	0.68	<0.001
Year	0.96	< 0.001		0.91	<0.001
Lactation number 1 (reference)					
Lactation number 2	0.99	0.727		0.97	0.539
Lactation number 3	0.92	0.054		0.85	< 0.001
Lactation number 4	0.90	0.013		0.82	< 0.001
Lactation number 5	0.87	0.006		0.74	< 0.001
Lactation number 6	0.77	< 0.001		0.79	0.003
Lactation number 7	0.76	< 0.001		0.76	0.004
Lactation number 8	0.65	< 0.001		0.71	0.007
Lactation number 9	0.61	0.002		0.75	0.144
Lactation number 10+	0.78	0.199		0.71	0.139
Simmental (reference)					
Brown Swiss	0.79	< 0.001		0.79	< 0.001
Holstein Friesian	0.56	< 0.001		0.66	< 0.001
Montbeliarde	0.99	0.961		n. c.	
Variance component of random effect					
Animal Unique identification number	0.122			0.126	
Farm	0.156			0.126	

n. c. = not considered because of too few observations

formance, were not considered. Some bias might have been produced by taking data from the period in which only herds participating in performance recording were involved in the BVD eradication program, speculating that especially good managed farms have taken part in that time. There might have been some new infections originating from herds not involved in the eradication programme before 2004. It shall be pointed out that only data from herds participating in milk performance recording were used in this study and only farms proven free from BVDV since the beginning of the eradication measures in 2001 were used as control herds. An underestimation of the effect of BVD on fertility in our data might have been caused by the fact that the entry of the first verified PI animal defined the beginning of the exposed period. This animal might not have been the real first PI on a specific farm in particular when the first PI entered the farm before the beginning of the eradication campaign in 2001.

Before the beginning of the eradication campaign in 2001 in fact most herds were protected by high BVD antibody titres (Rossmann et al., 2010). The impact of BVD on reproductive performance would likely have been much bigger, if BVD naive herds had been affected (Houe et al., 1993). BVDV seroprevalence is positively related to the ELISA absorbance value of antibodies to the virus in bulk tank milk (Niskanen, 1993). In 2002 bulk milk of 56% of all dairy herds in Styria tested for BVD antibodies had ELISA absorbance values, which gave evidence that more than 30% of the cows in the herds were BVD antibody positive. Due to the successful eradication of BVD in Styria, only 5% of the herds tested for BVD antibodies in the bulk milk were classified above this absorbance value in 2011. As Austria is carrying out an eradication program without vaccination, no interference with vaccine caused antibodies is possible.

Reproductive efficiency is mandatory for the profitability of dairy herds. As shown in this study, BVD affects reproductive performance. Compulsory eradication programmes, when performed in a targeted manner, decrease the relative risk of new infections (Obritzhauser et al., 2005), but BVD naive herds remain highly susceptible. It could be shown that BVDV has a negative effect on both CAI and FSC, which consequently results in economic losses for the farmer. Stakeholders in the dairy industry and veterinary administration can use the information in the presented paper as strong arguments to implement further BVD monitoring programmes after termination of the eradication programme to safeguard the BVD-free status of the Styrian cattle population.

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## Conflict of interest

All authors declare that they have no conflict of interest regarding the present study and manuscript.

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