# Transmission and impact of bluetongue virus serotype 8 in dairy cattle

**Inge M.G.A. Santman-Berends**

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# Transmission and impact of bluetongue virus serotype 8 in dairy cattle

## Transmissie en impact van blauwtong virus serotype 8 in melkvee

(met een samenvatting in het Nederlands)

## Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr, J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op

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# Contents

Chapter 1

# General Introduction

Inge M.G.A. Santman-Berends

## 1. Vector borne diseases

Historically, vector borne diseases like malaria, dengue and plague were the most important cause for human disease and death. Because of prevention and control programs which focused on elimination of arthropod breeding sites in combination with insecticides, by the 1960s, vector-borne diseases were no longer a major public health threat (Gubler, 1998). However, after 1970, a number of vector-borne diseases began to re-emerge and advanced over time. Most important reasons for the increasing emergence of vector-borne diseases were demographic and societal changes in the human population, the climate change and the resistance against drugs and insecticides (Özer, 2005).

Besides being a human threat, vector borne diseases pose also an increasing threat for animal health. In the past years, important vector borne disease threats in Europe were Rift Valley fever, West Nile fever, African horse sickness, Crimean-Congo haemoragic fever and bluetongue virus (Moorman, 2008). Rift Valley fever, West Nile fever and Crimean-Congo haemoragic fever are of great importance because they are zoonotic diseases. Bluetongue emerged in the Mediterranean Basin, and African horse sickness emerged in Spain in the past. Both are transmitted by the same vector as bluetongue (Hoogstral, 1979; Laughlin et al., 1979; Marfins et al., 2001; Meiswinkel and Paweska, 2003). At this moment, north-western Europe is still free from all these emerging vector-borne diseases except for bluetongue.

Bluetongue (BT) is a dsRNA virus of the *Reoviridae* family within the genus *Orbivirus* (Parsonon, 1990; Mertens et al., 2004). Bluetongue virus causes an infectious non-contagious disease of ruminants and is a disease, of which outbreaks have to be notified to the world organization for animal health (OIE) (OIE, 2010). At the moment, 25 different bluetongue virus serotypes have been identified (Bonneau et al., 1999; Pritchard et al., 2004; Hofmann et al., 2008).

## 2. Vector of bluetongue: Culicoides

The vector that transmits bluetongue belongs to the order Diptera: Ceratopogonidae genus Culicoides. Culicoides are small biting midges, 1-3 mm in size and they are the only significant biological vectors of bluetongue virus. In total, approximately 1,340 species of Culicoides have been described (Borkent and Wirth, 1997; Tabachnik, 2004), but in 2004 only 32 species of Culicoides were considered to be

involved in the transmission of bluetongue virus (Meiswinkel et al., 2004). Female *Culicoides* require blood meals for egg production and can transmit the bluetongue virus when biting a host. *Culicoides* are usually most active during the afternoon, dusk and at night, preferring still, and warm conditions.

In Africa and the Mediterranean Basin, *C. imicola* is the principal vector of bluetongue virus. However, besides *C. imicola* also *C. pulicaris, C. scoticus, C. obsoletus and C. dewulfi* have been found to transmit BT in southern Europe (Savini et al., 2003; Caracappa et al., 2004). In northern Europe, *C. imicola* does not occur and *C. scoticus, C. obsoletus*, *C. chiopterus* and *C. dewulfi* are probably the most important vectors of BT (Meiswinkel et al., 2007; Meiswinkel et al., 2008).

**3. History of bluetongue**

BT was first described in South Africa in the late 19$^{th}$ and the early 20$^{th}$ century after the introduction of European breeds of sheep into South Africa (Hutcheon, 1881; Spreull, J., 1905; Howell and Verwoerd, 1971). The disease bluetongue was first described as 'malarial catarrhal fever' and 'epizootic catarrh of sheep'. The term 'bluetongue' was later used referring to the distinctive cyanotic tongue of some severely affected sheep (Spreull, 1905; Erasmus, 1975). Initially, bluetongue was only discovered in sheep. The first clinical cases of BT in cattle were reported in 1933 in a small percentage of cattle in South Africa (Bekker et al., 1934). Until the 1940s, bluetongue was thought to be confined to southern Africa. Gradually, however, thereafter, bluetongue became widespread on the African continent. The first well-documented outbreak amongst sheep was on Cyprus in 1943. Furthermore, in the second half of the 20$^{th}$ century, reports of bluetongue occurrence were described from countries in Asia, America and southern Europe (Bowne, 1967; Jubb, 1970; Mastroyanni et al., 1981; Walton, 2004).

It became internationally accepted that the global distribution of bluetongue occurred between latitudes of approximately 40-50$^{o}$N and 35$^{o}$S (Zhang et al., 1999; Mellor et al., 2008), which was related to warm climates and to the distribution of competent *Culicoides* vectors.

Between 1998 and 2005, BT spread further northwards and occurred in many countries around the Mediterranean Basin that had previously never recorded the virus (Calistri et al., 2004; Giovannini et al., 2004; Mellor et al., 2008). During these outbreaks, morbidity and mortality were observed in sheep (Calistri et al., 2004).

In endemic BT regions, clinical signs of bluetongue were often rare or non-existent in both cattle and sheep (MacLachlan, 2004). Furthermore, when clinical signs were observed in cattle, they were only mild (Nomikou et al., 2004; Shimshony, 2004; Purse et al., 2006). It seemed that outbreaks of bluetongue typically occurred after introduction of susceptible sheep into BT-endemic regions, or when the virus spread into seronegative sheep populations in non-endemic regions.
Between 1998 and 2005, five different bluetongue virus (BTV) serotypes (1, 2, 4, 9 and 12) were found in southern Europe (Mellor et al., 2008). BTV serotype 8 (BTV-8) was, prior to 2006, only identified in Pakistan and India, southern and western Africa and the Caribbean regions and never in Europe (Haresnape et al., 1988; Mo et al., 1994; Gerdes, 2004; Sreenivasulu et al., 2004). In most of these reports, cattle were only assumed to be exposed to BTV-8 because they showed antibodies against BTV-8. In African studies, indigenous cattle infected with BTV-8, in general did not show clinical signs (Haresnape et al., 1988).

**4. Life cycle and transmission of bluetongue**

The life cycle of BTV involves vectors i.e. *Culicoides* and hosts i.e. sheep, cattle, goats, dromedaries and wild ruminants (Stalnecht and Howerth, 2004; Maclachlan, 2004; Backx et al., 2007; Dercksen and Lewis; 2007; Vellema, 2008). In the host, the bluetongue virus replicates in the lymph nodes and then spreads to infect vascular endothelium and dendritic cells in many organs (DeMaula et al., 2002), which is followed by a transient cell associated viraemia (MacLachlan., 2004). In this period, the host is infectious and might infect feeding *Culicoides*. A *Culicoides* has a probability of less than 1% to become infectious after biting an infected host (Gerry et al., 2001: O'Connell, 2002). Whether a *Culicoides* becomes infectious depends on its vector competence and the animal's level of viraemia. The vector competence involves the ability of the *Culicoides* to support BTV replication and transmission, which is controlled by temperature. Increasing temperatures lead to a higher BTV replication rate within the vector and to earlier transmission of the virus, but will lead on the other hand, to a shorter life span of the vector (Paweska et al., 2002). Once a *Culicoides* is infected, it will become persistently infectious (Gibbs and Greiner, 1994).
A susceptible host can become infected with BTV-8 when bitten by an infectious *Culicoides*. BTV transmission from infectious *Culicoides* to susceptible hosts is

very efficient (100%), causing bluetongue infection in the host after one single bite (O'Connell, 2002). An infected host will recover from BTV-8 infection, but the time to recover depends on the lifespan of circulating red blood cells that carry the virus (Bonneau et al., 2002). Once a host recovers from a BTV-8 infection it will gain immunity against BTV-8 for the rest of its life. A simple graphical overview of the life cycle of bluetongue virus is presented in Figure 1.

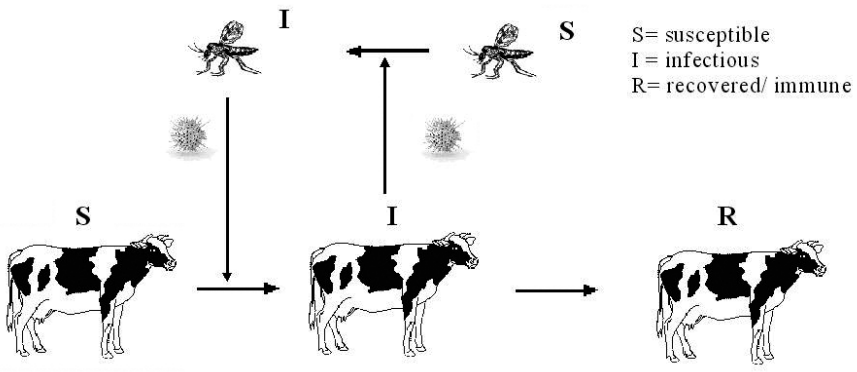


**Figure 1.** A simple graphical view of the life cycle of bluetongue virus

The numbers of susceptible hosts that can be infected by one infectious *Culicoides* depend on the longevity and biting rate of *Culicoides* after becoming infectious. A mature female *Culicoides* will feed once every three to five days and can live from a few weeks (usually) to several months (rarely) depending on the outside temperatures (Nevill 1971; Braverman et al., 1985).

Information about the transmission of BTV-8 is important to quantify the risk of BTV-8 in an epidemic situation. With this knowledge, decisions about monitoring, eradication or vaccination can be made in countries in which BTV-8 emerge. A parameter that describes the transmission of an infection in a susceptible population is $R_0$. This parameter is defined as the number of newly infected individuals that is generated by one infectious individual within a susceptible population.

To date, several studies have estimated the basic reproduction ratio ($R_0$) of BTV-8 (Gubbins et al., 2008; de Koeijer and Elbers, 2008; Hartemink et al., 2009). However, for these earlier studies field data on BTV-8 transmission in cattle were not available. They used assumptions based on density data and literature to quantify a range of

values for the $R_0$ of BTV-8. The majority of the values for $R_0$ ranged between 2 and 4, depending on the outside temperatures, vector and host densities (Gubbins et al., 2008; Hartemink et al., 2009).

**5. Incursion of bluetongue virus serotype 8 in north-western Europe**

Historically, it was thought that bluetongue was unlikely to spread to northern Europe, because there were no competent vectors and thus its occurrence was geographically limited to (sub) tropical regions. However, since the outbreaks of bluetongue in southern Europe in the late 20th and early 21th century, a risk assessment was made to determine the risk of the introduction of bluetongue in the Netherlands (Elbers et al., 2003). According to this study, it seemed possible that, because of the warmer climate, bluetongue associated vectors like *Culicoides imicola* would also settle in more northern regions and would be introduced in countries in North-West Europe.

In August 2006, two sheep farmers in the southern part of the Netherlands reported morbidity and mortality in their sheep to the Cattle Watch Monitoring system of Animal Health Service (GD). The ruminant watch is a reactive monitoring tool in which farmers and private practitioners are invited to report information of (un)known or changing symptoms and signs in small ruminants to a nationally operating group of ruminant specialists, for second line veterinary assistance (Van Wuijckhuise et al., 2007).
Because the cause of the reported problems was unknown, the flocks were visited on Monday the 14th of August (Van Wuijckhuise et al., 2006; Dercksen and Lewis, 2007). Because of the fact that clinical signs indicated a bluetongue infection, blood samples were taken, diagnostic tests were carried out, and animal movement restrictions were put in place. On Tuesday the 15th, the Central Veterinary Institute (CVI-Lelystad) reported positive PCR and serological test results, and two days later, on the 17th of August, IAH Pirbright, the EU reference laboratory, confirmed the results. At that moment, the Netherlands was declared bluetongue virus infected and measures were taken to prevent further spread (Elbers et al., 2007a). Shortly after the Netherlands found the first cases of bluetongue, Belgium (18th August) and Germany (21st August) also reported infected herds, and implemented restriction zones around infected herds. Retrospective epidemiological analyses

indicated that the area of the first introduction of BTV was probably in Belgium (Gerbier et al., 2008; Saegerman et al., 2010).

On the 28th of August 2006, the BTV in the Netherlands was identified as serotype 8. The introduction of this serotype came as a surprise, because BTV serotype 8 was not one of the BTV serotypes that occurred in southern Europe. However, it was believed that the BTV-8 infection could possibly be from sub-Saharan origin. Several hypotheses have been examined, which may explain the introduction of BTV-8 in north-western Europe (EFSA, 2007) namely, importation of BTV-8 infected ruminants, introduction of infected vectors with horses, introduction of infected vectors with exotic plants, and contaminated or unstable vaccines. Nevertheless, until this moment none of these possible introduction routes has been proven to have caused the BTV-8 epidemic, which started in August 2006.

Around BTV-8 infected herds a restriction zone with a radius of 20 km, a protection zone with a radius of 100 km and an additional surveillance zone of 50 km were implemented, leading to an area of in total 150 km around an infected herd. In the BTV-8 restriction zones of 20 km, animal transport was forbidden, farmers were obliged, if possible, to keep their cattle, sheep and goats indoors from one hour before sunset to one hour after sunrise, and cattle and sheep herds had to be treated with insecticides (LNV, 2006). After the first outbreak of BTV-8 was notified, the number of BTV-8 outbreaks increased and the protection zones increased up north. During the 2006 BTV-8 epidemic, 446 herds (268 sheep and 178 cattle) in the Netherlands, 682 herds (400 sheep and 282 cattle) in Belgium and 811 herds (297 sheep and 504 cattle) in Germany were reported to be infected with BTV-8 (SCoFCAH, 2007).

In north-western European winters, average daily temperatures decline to less than 10 ºC. Because of these low temperatures, *Culicoides* stop biting. This period was called 'the vector-free period'. In 2006, this *Culicoides* free period started mid-December and was subsequently followed by a bluetongue free period. With this phenomenon, the bluetongue epidemic in north-western Europe differed from bluetongue outbreaks in southern Europe and Africa, where no bluetongue free period occurs.

BTV-8 was known to spread by the dissemination of *Culicoides* by wind, but in the winter of 2007, it appeared that it could also spread by vertical transmission from a cow to her calf (Menzies et al., 2008). This vertical transmission did not occur in all infected cows and the relation between stage of gestation during infection and the BTV-8 status of the newborn calf was not known. It might be possible that these virus positive calves could infect *Culicoides* and thus potentially be the source from which BTV-8 started to spread in a former BTV-8 free area or even after a BTV-8 free period. Nevertheless, until this moment it has not been proven that calves that became BTV-8 virus positive by vertical transmission, can infect *Culicoides*.

The winter of 2006/2007 was fairly mild. Nevertheless, there was hope that BTV-8 would disappear. Unfortunately in July 2007, BTV-8 re-emerged in the Netherlands, Belgium and Germany and spread further, infecting neighbouring countries, i.e. Great-Britain, Luxembourg and France.

At the end of 2007, 3,182 cattle farmers in the Netherlands had notified clinical signs of BTV-8 in their herds and the economic consequences for the cattle industry were massive (Velthuis et al., 2009). The majority of these notifications were made by farmers in the southern and central region of the Netherlands. During this second phase of the BTV-8 epidemic, the virus just started to spread in the north and only a small number of cattle herds in that region became infected. In December 2007, the vector-free period began. At that moment BTV-8 had infected nine European countries involving the Netherlands, Belgium, France, Luxembourg, Germany, Switzerland, United Kingdom, Czech Republic and Bulgaria (OIE, 2010) and the European Union implemented regulations for BTV-8 infected countries to reduce further spread of the virus (European regulation 1266\2007). In the winter of 2007/2008, the European Commission decided to subsidize vaccination for countries that were infected with BTV-8. Each country was allowed to implement its own vaccination program, but the requirement for subsidy was that at least 80% of the sheep and cattle were antibody positive at the end of 2008. The Netherlands decided to implement a voluntary vaccination program and the first cattle were vaccinated in May 2008. In that year, most farmers decided to vaccinate their herd and at the end of 2008 seroprevalence of BTV-8 in sheep and cattle was over 80% in the Netherlands (Elbers et al., 2010).

## 6. Clinical signs of an infection with bluetongue virus serotype 8

The fact that farmers reported clinical signs in their cattle herds caused by bluetongue infection was remarkable because in literature, only occasionally, clinical signs in cattle were reported (Nomikou et al., 2004; Shimshony, 2004; Purse et al., 2006). Possibly, the BTV-8 strain that emerged in Europe was more virulent than other serotypes or maybe the clinical signs were more severe because BTV-8 emerged in a population that was fully susceptible. Nevertheless, clinical signs of bluetongue in affected animals were much more prominent in sheep flocks than in cattle herds (Elbers et al., 2008b; Elbers et al., 2008c, Van Schaik et al., 2008).
The clinical signs of bluetongue that were reported during the 2006 BTV-8 epidemic in cattle, started with an elevated rectal temperature up to 40$^{o}$C, subsequently congestion of the mucous membranes was followed by purulent nasal discharge (Dercksen and Lewis, 2007). Other clinical signs that were seen involved ulcerations and erosions of the oral mucosa, lesions or oedema of the nose and/or lips, stiffness of limbs, lameness and ulceration of the teats. In sporadic cases, infection with BTV-8 can lead to death (Toussaint et al., 2006; Dercksen and Lewis, 2007; Elbers et al., 2008b; Elbers et al., 2008c;Williamson et al., 2008). The purple coloration of the tongue from which bluetongue owned its name, occurred in 5% of the infected cattle herds in 2006 (Dercksen and Lewis, 2007). During the 2007 epidemic, symptoms of BTV-8 in cattle seemed more severe compared to 2006, and farmers also notified fertility and udder health problems in their herds (Elbers et al., 2008). However, quantitative data on the association between BTV-8 infection and both fertility and udder health parameters were until then not published. Furthermore, the rendering plant reported an increased number of rendered cattle in 2007, which they all assigned to the BTV-8 epidemic. However, in January 2007, regulations regarding the welfare of animals during transport were tightened (EG 1/2005, Regulation of the European Parliament, 2005), which also resulted in higher on-farm mortality. Therefore, the exact mortality associated with BTV-8 infections in the Netherlands remained unclear.

## 7. Knowledge gap on transmission and impact of BTV-8

After the emergence of BTV-8 in 2006, the Netherlands was officially declared 'bluetongue infected'. In the winter of 2006/2007, a sentinel program in cattle was implemented to monitor new BTV-8 infections, in the hope of regaining the BTV-8 free status. However, from July 2007 on, it became clear that BTV-8 re-emerged. Until that moment, transmission of BTV-8 was estimated based on models in which no field data on transmission of the virus in cattle was incorporated (Gubbins et al., 2008; de Koeijer and Elbers, 2008; Hartemink et al., 2009). However, including field data of the real transmission of BTV-8 in cattle gives the opportunity to calculate more accurate transmission parameters for the north-western European situation. A small number of the herds in the sentinel network that were located in heavily infected BTV-8 compartments had no, or only a small number of seroconversions in their cows. Some management factors in these herds may have worked protectively for BTV-8 transmission. However, no literature was available on the association between management factors and within-herd BTV-8 spread.

During the second phase of the BTV-8 epidemic in 2007, a larger number of cattle showed clinical signs associated with BTV-8 and the clinical signs reported seemed more severe than those reported in 2006 (Elbers et al., 2009). These BTV-8 related clinical signs reduced the productivity of cattle. However, objectively quantified information about the effect of a natural BTV-8 infection in a cow on production parameters such as fertility, udder health, mortality and vertical transmission in a field situation was not present.

In the beginning of 2008, the Animal Health Service (GD) in the Netherlands observed an increasing number of submissions of aborted and stillborn calves with severe developmental defects of the brain. This type of lesions has been described as hydranencephaly and appeared to be associated with BTV-8 infections in cattle during gestation (Wouda et al., 2008). Around the same time, evidence was found that BTV-8 was vertically transmittable from cow to her foetus, resulting in healthy looking calves that tested positive for bluetongue virus in a PCR (Menzies et al., 2008; Backx et al., 2009). Thus, there was need to investigate the impact of a natural BTV-8 infection in gestating cows and their subsequent calves.

## 8. Aim and objectives of this thesis

The objective of this thesis was to estimate the transmission and impact of BTV-8 in dairy cattle.

Transmission parameters on the BTV-8 spread within cattle herds were based on the spread of BTV-8 in cows in sentinel herds. Furthermore, a short questionnaire was conducted among the farmers of the sentinel herds to investigate possible associations between BTV-8 spread within cattle herds and management factors.
Cow-calf combinations were studied to quantify the percentage in which vertical transmission occurred, to evaluate what happened with virus positive calves after birth and to quantify at what stage of gestation cows were at the highest risk for giving birth to a virus positive calf.

Several field studies were conducted to objectively quantify the impact of BTV-8 infections on cattle health. In this thesis the results of BTV-8 infections on mortality, fertility, milk production and udder health are quantified. Quantification of the association between BTV-8 and these production parameters is important, because with this information, the economic consequences of BTV-8 infection in a dairy herd can be estimated. Based on the economic consequences, a farmer can evaluate the costs and benefits of control measures such as vaccination and farmers can decide whether or not to take preventive measures to reduce the risk of a BTV-8 outbreak.

## 9. Outline of this thesis

Based on the results of the sentinel network, which was implemented during the 2007 BTV-8 epidemic, within-herd transmission parameters were quantified (Chapter 2) and measures that can be taken by a farmer to reduce BTV-8 spread in the herd were identified (Chapter 3).
In Chapter 4, BTV-8 related mortality was estimated for herds that notified clinical signs of BTV-8 to the authorities, and for herds in BTV-8 infected regions.
Vertical transmission of BTV-8 was studied in 2007 (Chapter 5) and, in more detail, in 2008 (Chapter 6). Furthermore, in Chapter 6, the effect of BTV-8 infections on the fertility in dairy cows are presented and in Chapter 7, milk production and

somatic cell count around the time of BTV-8 seroconversion are investigated. Finally, a summarizing discussion of the results in this thesis, the implications for the Dutch dairy cattle industry including the economic consequences for a dairy herd in which BTV-8 emerges and the lessons that we have learned about BTV-8 infections in dairy cattle are described in Chapter 8.

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Chapter 2

# Quantitative transmission ($R_0$) of bluetongue virus serotype-8 within dairy herds based on serological field data

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**Abstract**

Bluetongue virus serotype 8 (BTV-8), emerged in North-West Europe in 2006. In 2007, one of the affected countries (the Netherlands) implemented a sentinel network of dairy cattle. Seronegative cattle within sentinel herds were selected and entered a monthly sampling program, from July until December 2007. The goal of this study was to quantify the transmission of BTV-8 ($R_0$) within a herd based on the 2007 sentinel data. $R_0$ was calculated using, a vector-borne transmission model. In the default model, transmission parameters like the proportion of infectious cows, infectious *Culicoides* and vector over host density were calculated from field data. Furthermore, literature and temperature based assumptions were made for recovery rate, *Culicoides* biting rate, extrinsic incubation period, mortality rate, the probability of BTV-8 transmission from vector to host and the probability of BTV-8 transmission from host to vector. $R_0$ could be estimated for 419 time intervals. The median $R_0$ was 2.9 ($5^{th}$ percentile=2.2; $95^{th}$ percentile=9.0). Median $R_0$ values differed between regions and months.

The median vector to host ratio was calculated from our field data at 159 ($5^{th}$ percentile 80; $95^{th}$ percentile=2132). The vector to host ratio differed between months, with the highest number of *Culicoides* per cow between October and November.

This study gives within-herd estimations for $R_0$ for the BTV-8 epidemic, based on serological field data. This $R_0$ seems to represent the spread of BTV-8 and these transmission rates may apply to countries in which BTV-8 emerge, given a similar climate, grazing patterns and barn type as North-West Europe.

*Keywords:* bluetongue; *Culicoides*; transmission; cattle

## 1. Introduction

Bluetongue virus (BTV) is an insect-borne virus, which can cause clinical disease and mortality in ruminants. BTV is transmitted by certain species of *Culicoides* midges and was historically only present between latitudes 35°S and 40°N (Lundervold et al., 2003). However, since 1998 BTV is present in Europe and the Mediterranean basin (Mellor et al., 2006). In 2006, bluetongue virus serotype 8 (BTV-8) emerged in northern Europe for the first time (Elbers et al., 2008; Van Wuijckhuise et al., 2006), and the Netherlands was one of the affected countries. In that year, BTV-8 infections remained restricted to the southern region of the Netherlands.
In the winter of 2006-2007, the Dutch government decided to start a sentinel network of 275 dairy cattle herds to see whether or not BTV-8 would re-emerge in 2007 (Santman-Berends et al., 2010).
The data from the sentinel study offered the opportunity for estimating transmission parameters for BTV-8. Information about the within-herd transmission of BTV-8 is important to quantify the risk of BTV-8 in an epidemic situation. Moreover, with this knowledge decisions concerning monitoring, eradication or vaccination programs can be made in countries in which BTV-8 emerges for the first time. The transmission of an infection is described by the basic reproduction ratio ($R_0$). This parameter is defined as the number of newly infected individuals that are generated by one infectious individual e.g. cows within a susceptible population in this study. Earlier modelling studies have estimated the $R_0$ for BTV-8, based on assumptions and field data on the spread of *Culicoides* (De Koeijer and Elbers, 2008; Hartemink et al., 2009). However, to our knowledge, $R_0$ estimates based on field data where transmission of BTV-8 in cattle was measured have hitherto not been published.
The goal of this study was to quantify the transmission of BTV-8 ($R_0$) within a herd based on the 2007 serological data from sentinel dairy herds.

## 2. Material and methods

### *2.1. Data collection*

For disease control purposes, the Netherlands was divided in 20 compartments based on geographic boundaries as proposed in Commission Decision 2005/393/EC. In the Netherlands, the geographic boundaries usually consisted of rivers or highways. Characteristics like temperatures and altitudes were comparable for all compartments.

Compartment 1 to 5 were located in the northern part, compartment 6 to 14 in the central part and compartment 15 to 20 in the southern part of the Netherlands (Figure 1).

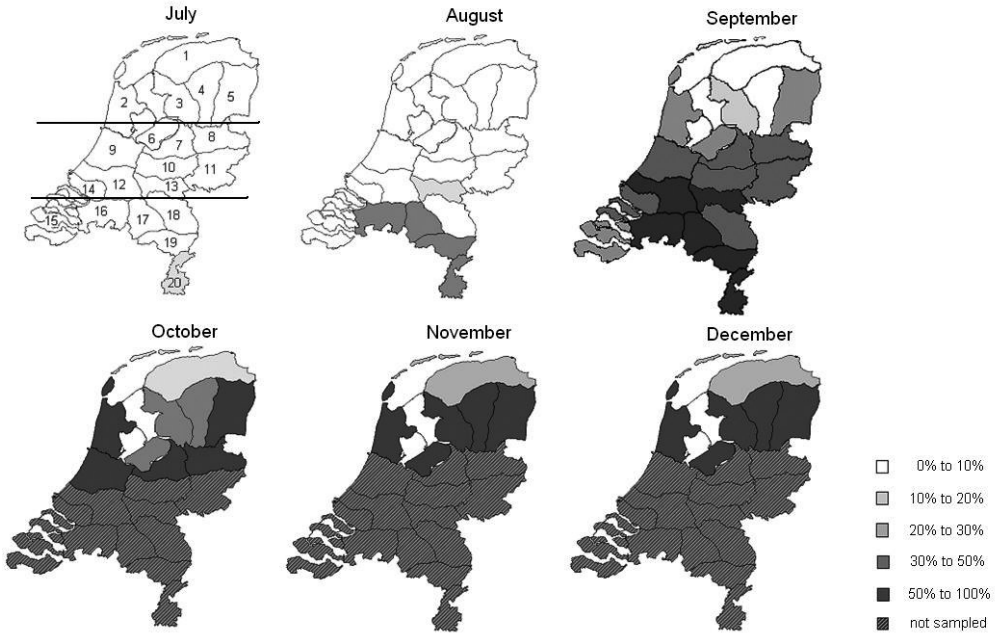


**Figure 1.** Average within-herd BTV-8 seroprevalence of 275 dairy herds in the Netherlands per compartment per month from July until December 2007.

In May 2007, on average 13 sentinel herds per compartment were selected for this sentinel network (for a detailed description of the selection process see Santman-Berends et al., 2010). The inclusion criteria for sentinel herds were that they had to have four-weekly test-day recording. In the sentinel herds, BTV-8 seronegative cows were selected at the start of the study and every four weeks, milk samples were collected from 16 randomly selected cows out of routinely collected milk samples. These samples were tested for antibodies against BTV-8 at the Dutch Animal Health Service (GD) with a commercially available ELISA test (sELISA; ID.VET, Montpellier, France; sensitivity = 98.1% and specificity = 99.0%). For a detailed description of the diagnostic test see Kramps et al. (2008).

Sampling for the sentinel network was stopped in September in compartments 10 to 20, and in October in compartment 7 to 9, because the prevalence in these compartments had increased up to almost 100% in most sentinel herds. Further sampling in these herds would not lead to more information.
Sampling was stopped in December 2007 in compartments 1 to 6, because outside temperatures were too low for BTV-8 to spread (Figure 1). In North-western Europe, BTV-8 only spreads in summer and autumn with highest transmission rates in the months July to October. In the winter and early spring, outside temperatures are too low for *Culicoides* to transmit BTV.

At the end of 2007, data of 275 sentinel herds was available. Each sentinel herd was sampled on average 3.8 times, starting in June 2007, after the first selection of cows. The complete dataset consisted of 1,323 observations and 1,048 between-measurement periods. Although in the sentinel network, herds had to be sampled once a month, in some herds the time between two consecutive measures was longer. To avoid large under- or overestimations in our results, we decided to remove an observation (one period) from the dataset when the number of days between two measurements exceeded 37 days. Eventually, 419 time periods in which BTV-8 transmission occurred within the herd remained for estimation of the $R_0$ values (Figure 2). The average number of days between two consecutive measurements in these herds was 29 days (minimum 16 days-maximum 37 days).

*2.2. Estimation of transmission parameters*

For the calculation of $R_0$, a vector-borne disease transmission model was used, which was derived from other vector-borne transmission models as described in literature (Anderson and May, 2002; De Koeijer and Elbers, 2008; Wei et al., 2008; Gubbins et al., 2008; Hartemink et al., 2009). In this model, the host population $h$ (cattle) at time $t$ was divided into three subclasses of proportions of cows that were susceptible ($S_h(t)$), infectious ($I_h(t)$) and recovered ($R_h(t)$). Susceptible hosts could become infected through bites from an infectious vector, which depended on the biting rate of a *Culicoides* (a), the proportion of infectious bites leading to virus transmission from vector to host (b), the vector over host density (m) and the proportion of infectious *Culicoides* ($I_v$).The recovery rate of the infectious hosts was given by $\gamma_h$. The recovered cows were assumed to have gained permanent immunity and there was no transmission from the $R_h$ class.

The following ordinary differential equations (ODE) were used to describe the dynamics of BTV-8 in the host population:

$$\frac{dS_h(t)}{dt} = -abmI_v(t) * (1 - (I_h(t) + R_h(t))) \quad [1]$$

$$\frac{dI_h(t)}{dt} = abmI_v(t) * (1 - (I_h(t) + R_h(t))) - \gamma_h * I_h(t) \quad [2]$$

$$\frac{dR_h(t)}{dt} = \gamma_h * I_h(t) \quad [3]$$

Where *dt* was time in days between two consecutive measurements. Variable $dR_h(t)$ denotes the increased proportion of seropositive cows for each dairy herd between two consecutive measurements, which was derived from our field data. Based on literature (Singer et al., 2001; Bonneau et al., 2002, Melville et al., 2004), the average time to recovery was assumed to be 25 days (default model) (Table 1). The recovery rate in cattle ($\gamma_h$) was assumed constant and was quantified at 0.04 per day (1/25=0.04) (default model).

Given the values for parameters $dR_h$, *dt* and $\gamma_h$, we estimated $I_h(t)$ and $S_h(t)$ ($S_h(t)$ = 1- $[R_h(t) + I_h(t)]$ and subsequently the difference in proportion of infectious cows $dI_h(t)$ per day was quantified from $I_h(t)$ and $I_h(t\text{-}1)$.

From the above, the infection rate per day ($abmI_v$), which reflected the probability of being bitten by an infectious vector (*Culicoides*) and transmission taking place, was quantified.

The *Culicoides* population was divided into a susceptible $S_v(t)$ and an infectious $I_v(t)$ population. Susceptible vectors $S_v(t)$ became infected by biting an infectious cow $I_h(t)$ at rate *ac*. Where parameter *a* reflects the *Culicoides* biting rate, which was calculated using the formula developed by Mullens et al. (2004) and including the average daily temperatures during the studied period in the Netherlands (Table 1). Parameter *c* represents the probability of BTV-8 transmission from the infectious cow to the *Culicoides* per bite, which was derived from literature (Sáenz et al., 1994; Venter et al., 1998; Gerry et al., 2001; Carpenter et al., 2008). Whether an infected *Culicoides* would become infectious depends on the chance of surviving the extrinsic incubation period (*EIP*). The duration of the *EIP* depends on temperature and the rate to become infectious is denoted by q (*1/EIP*) (Table 1). Given that the mortality rate is denoted by $\mu$, the probability to survive the *EIP* will be $q/q+\mu$.

When a *Culicoides* survives this incubation period, it is assumed that the midge will stay infectious throughout its live (Table 1). An infectious *Culicoides* can only leave the infectious state by dying at rate $\mu$ which is depending on temperature (Table 1).The system that describes the dynamics of the vectors is given by the following ODE's:

$$\frac{dS_v(t)}{dt} = -acI_h(t) * (1 - I_v(t)) * \left(\frac{q}{q+\mu}\right) - \mu * (1 - I_v(t)) \quad [4]$$

$$\frac{dI_v(t)}{dt} = acI_h(t) * (1 - I_v(t)) * \left(\frac{q}{q+\mu}\right) - \mu * I_v(t) \quad [5]$$

The proportion of $I_h$ cows were derived from our field data and from this information in combination with literature based assumptions for *a, c, q* and $\mu$, the proportion of infectious vectors ($I_v(t)$) could be derived.
Substituting this parameter in formula [2] in combination with literature based assumptions for *a, b* and $\gamma_h$, the vector over host ratio (*m*) was quantified per herd per month.

In the host transmission model, birth and mortality rates were excluded because the study population remained the same; no cows entered or left the study.
Based on the basic model, which captured the essentials of BTV-8 transmission in the host and the vector the basic reproduction ratio was calculated as:

$$R_0 = \sqrt{\frac{abm}{\gamma_h} * \frac{ac\left(\frac{q}{q+\mu}\right)}{\mu}} \quad [6]$$

**Table 1**. Description, estimates and sources on which assumptions were based for the calculation of $R_0$ parameters for cattle and *Culicoides* based BTV-8 serological field data

| Parameter | Description | Source | Estimation from the source | Point estimate used in the default model |
|---|---|---|---|---|
| $\gamma_h$ | Cattle recovery rate | Singer et al. (2001) | Mean 25 days<br>Range 14-63 days | 25 days[1] |
| | | Bonneau et al. (2002) | Maximum 21 days | |
| | | Melville et al. (2004) | Mean 20.6 days<br>Range 7-35 days | |
| *a* | *Culicoides* biting rate | Mullens et al. (2004) | Based on temperature: $\alpha$=0.0002 T (T-3.7) (41.9-T)$^{1/2.7}$ | Ranging from 0.005-0.13[2] |
| *b* | Effectiveness of BTV-8 transmission from vector to host | O'Connell (2002) | Mean 1.0<br>Range 0.8-1.0 | 1.0 |
| *c* | Effectiveness of BTV-8 transmission from host to vector | Carpenter et al. (2008) | Mean 2.8%<br>Range 0.4- 7.4% | 0.05 |
| | | Gerry et al. (2001) | Mean 0.4%<br>Range 0.0-2.2% | |
| | | Venter et al. (1998) | Range 1.9-9.8% | |
| *EIP* | Extrinsic incubation period (rate of becoming infectious) | Mullens et al. (2004) | Based on temperature: $EIP(T)$=0.0003 $T$ ($T$-10.4) | Ranging from 0.0-0.04[2] |
| $\mu$ | Vector mortality rate | Gerry and Mullens (2000) | Based on temperature: $\mu(T)$=0.009exp(0.16$T$) | Ranging from 0.01-0.15[3] |

[1] value is varied in sensitivity analyses

[2] depending on maximum monthly temperature between July and December 2007 in the Netherlands, in accordance with Hartemink et al. (2009).

[3] depending on the average monthly temperature between July and December 2007 in the Netherlands.

*2.3. Sensitivity analysis*

The value of the recovery rate ($\gamma_h$) of the infected cows was rather uncertain. Therefore we varied this parameter in a sensitivity analyses to see whether the change in value of this parameter have a small or large influence on the results of $R_0$. Rate $\gamma_h$ was included as a fixed value of 0.04 in the default model. In the sensitivity analyses this rate was varied to 0.017 which was equal to an infectious period of 60 days and to 0.1 which was equal to an infectious period of five days.

*2.4. General remarks concerning the model*

The transmission model was built in SAS version 9.1 (2006). Figures and frequency tables were used to describe the results of $R_0$ and univariate analyses methods (Kruskal-Wallis test, 1952) were used for comparison of $R_0$ values between regions.

**3. Results**

*3.1. BTV-8 transmission in cows within herds, per compartment and month*

The mean $R_0$ was 3.8 (95% CI: 3.5-4.1) and the median $R_0$ was 2.9 ($5^{th}$ percentile=2.2; $95^{th}$ percentile=9.0). $R_0$ values per herd-month varied from a minimum of 1 to a maximum of 38. $R_0$ values of 1 were mainly found in herds located in the north in which the BTV-8 infection spread slowly in the first months. Most $R_0$ values were between two and three (52%) (Figure 2).

$R_0$ values were estimated per compartment (Table 2). $R_0$ values for herds within compartments were not normally distributed and thus both mean and median $R_0$ values are provided. The number of time intervals in which $R_0$ could be estimated varied from eight in compartment 16 to 37 in compartment one (Table 2). These numbers of herds-months differed between compartments because the sampling period differed per compartment. Furthermore, $R_0$ was not estimated when no infectious cows were present.

Values of $R_0$ varied from a minimum of 1 in compartment one, two, three and five, to a maximum of 38 in compartment 10.

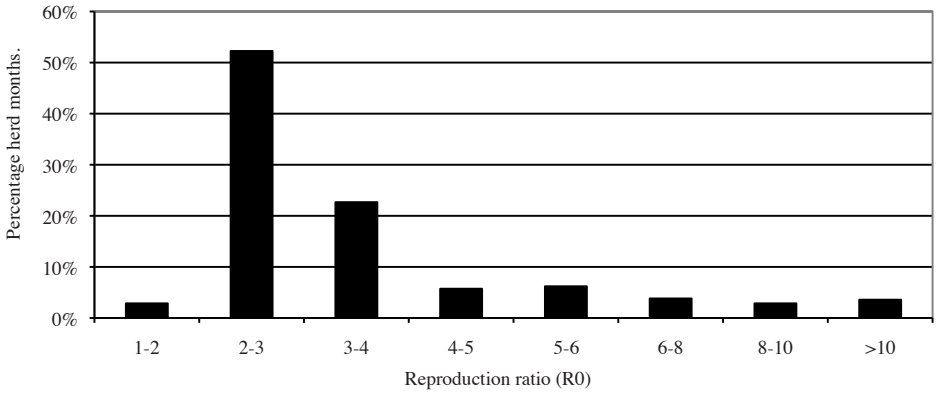


**Figure 2.** The distribution of the basic reproduction ratio ($R_0$) for BTV-8 within 275 Dutch dairy herds in 2007

**Table 2.** The number of herd-months, mean, minimum, 25th percentile, median, 75th percentile and maximum reproduction ratio ($R_0$) of BTV-8 per compartment in Dutch dairy herds in 2007.

| Compartment | N | Mean | Minimum | 25th percentile | Median | 75th percentile | Maximum |
|---|---|---|---|---|---|---|---|
| 1 | 37 | 2.9 | 1.4 | 2.5 | 2.8 | 3.2 | 5.1 |
| 2 | 27 | 2.9 | 1.4 | 2.4 | 2.7 | 3.1 | 7.1 |
| 3 | 22 | 3.8 | 1.0 | 2.5 | 3.0 | 4.0 | 12.0 |
| 4 | 29 | 3.0 | 1.7 | 2.4 | 2.8 | 3.3 | 5.6 |
| 5 | 36 | 4.0 | 1.0 | 2.6 | 3.0 | 4.7 | 12.3 |
| 6 | 17 | 3.0 | 2.2 | 2.5 | 2.8 | 3.4 | 4.1 |
| 7 | 19 | 4.2 | 2.2 | 2.4 | 2.8 | 4.2 | 15.2 |
| 8 | 20 | 3.2 | 2.3 | 2.5 | 2.8 | 3.4 | 6.6 |
| 9 | 12 | 3.4 | 2.2 | 2.4 | 2.7 | 3.4 | 9.0 |
| 10 | 17 | 5.5 | 2.4 | 2.5 | 3.1 | 3.2 | 38.2 |
| 11 | 20 | 3.5 | 2.4 | 2.6 | 3.0 | 3.6 | 7.2 |
| 12 | 23 | 4.2 | 2.3 | 2.5 | 2.6 | 5.7 | 11.1 |
| 13 | 23 | 4.8 | 2.4 | 2.5 | 3.2 | 5.6 | 15.1 |
| 14 | 19 | 3.9 | 2.3 | 2.5 | 2.9 | 5.1 | 9.0 |
| 15 | 14 | 3.1 | 2.4 | 2.6 | 3.0 | 3.6 | 4.2 |
| 16 | 8 | 4.7 | 2.3 | 2.5 | 3.2 | 6.3 | 11.2 |
| 17 | 23 | 4.4 | 2.4 | 2.6 | 3.8 | 5.7 | 11.6 |
| 18 | 13 | 3.6 | 2.3 | 2.5 | 3.0 | 3.6 | 7.3 |
| 19 | 25 | 4.4 | 2.4 | 2.6 | 2.9 | 3.8 | 19.7 |
| 20 | 15 | 4.2 | 2.3 | 2.6 | 3.3 | 4.5 | 10.2 |
| Total | 419 | 3.8 | 1.0 | 2.5 | 2.9 | 3.8 | 38.2 |

There was not a large variation in median values of within-herd $R_0$ between compartments (median $R_0$: 2.6-3.3) (Table 2). Mean values of $R_0$ were lowest in compartment one and two, which are located in the north of the Netherlands and highest in compartment 10 (Figure 1).

When dividing the compartments into three regions, North, Central and South, median values of $R_0$ were lowest in the northern region (mean 3.3; median 2.8 (5th

percentile=1.7; 95$^{th}$ percentile=7.1). In the central and southern region, mean and median $R_0$ values were 4.0; 2.9 (5$^{th}$ percentile=2.3; 95$^{th}$ percentile=9.0) and 4.1; 3.0 (5$^{th}$ percentile=2.4; 95$^{th}$ percentile=10.2) respectively. The $R_0$ values of BTV-8 in the northern region were significantly lower compared to the $R_0$ values in the southern region (Kruskal Wallis: *P*-value=0.01). No significant differences in $R_0$ values were found between the central and the southern region and between the central and northern region (Kruskal Wallis: *P*-value=0.14 and *P*-value=0.14).

Besides the differences in $R_0$ values between the regions, we also found an effect of month. Overall median $R_0$ values were highest between August and September and between September and October (3.5 in both months). However, per region the month in which the $R_0$ value was highest, differed. In the southern region the median $R_0$ value was highest between August and September (5.4), in the central region the median value was highest between August and September and between September and October (3.8 and 3.9, respectively) and in the northern region the median $R_0$ value was highest between September and October (3.3) (Figure 3).

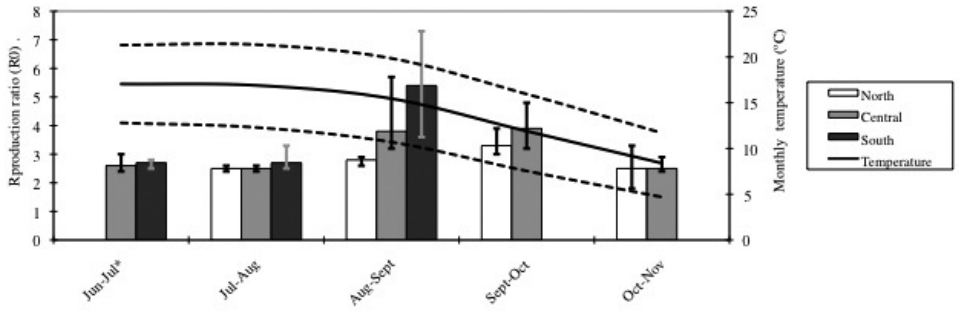


**Figure 3.** Median and inter quartile range of $R_0$ per month from June until December 2007 for region north, central and south within Dutch dairy herds and the average, minimum and maximum temperature.
* In region North, only in seven herds transmission occurred and therefore the interquartile-range was very large. Therefore the $R_0$ is not presented for region North in this figure.

*3.2 Vector to host ratio*

The median vector to host ratio (*m*) was calculated from our field data at 159 (5th percentile 80; 95th percentile=2132). Most values of m (27%) were between 50 and 100 (Figure 4).

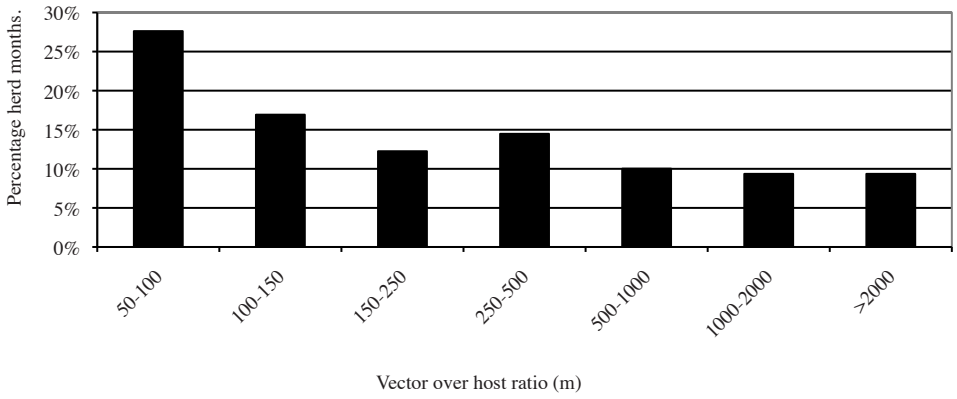


**Figure 4.** The distribution of the vector to host ratio (*m*) for BTV-8 within 275 Dutch dairy herds in 2007

Based on the field data the highest values of parameter m were found in the periods September-October and October-November, in which BTV-8 spread between cows within-herds, but in which conditions for *Culicoides* became less optimal (Figure 5).

In Figure 5, the vector to host ratio was presented for periods in which BTV-8 could spread. In November-December, the vector to host ratio (*m*) could not be estimated because, based on the assumptions made, the temperatures in that period were too low for *Culicoides* to be able to transmit the virus.

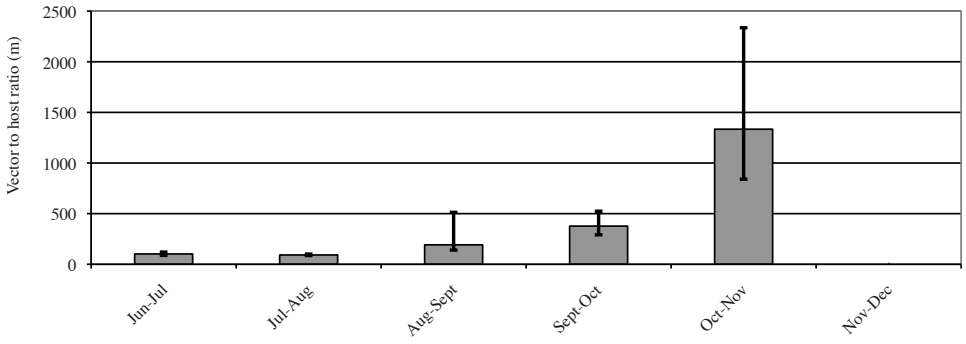


**Figure 5.** Median and inter quartile range of the vector to host ratio ($m$) per month from June until December 2007 within Dutch dairy herds.

*3.3. Sensitivity analyses*

When the infectious period ($\gamma_h$), which was assumed to be 25 days in the default model, was increased to 30 days or 40 days, the median $R_0$ value increased slightly from 2.9 in the default model to 3.1 or 3.3 respectively. Because cows stayed infectious for a longer period, there was an increased proportion of *Culicoides* becoming infectious after biting these cows and thus a higher $R_0$ (Table 3). Because the proportion of infectious *Culicoides* ($I_v$) is higher, less *Culicoides* per cow are needed to transmit the virus from *Culicoides* to the cow and thus parameter m also decreases when the infectious period for the cow increases.

When the infectious period was decreased to 20 days, the median $R_0$ decreased slightly to 2.7 and the median m increased to 195. However when the infectious period was assumed to be only 10 days the median $R_0$ was 2.3 and median m was 359.

**Table 3.** Results of the sensitivity analyses, the mean, median (the 5th percentile and the 95th percentile) of the reproduction ratio ($R_0$) and vector to host ratio of BTV-8 within 275 Dutch dairy herds in 2007.

| Assumptions | | | Vector over host ratio (25th and 75th percentile) | | Median $R_0$ (25th and 75th percentile) | |
|---|---|---|---|---|---|---|
| Default: | | | | | | |
| $\gamma_h$[1]: | 0.04 | (25 days) | 159 | (95-485) | 2.9 | (2.5-3.8) |
| $\gamma_h$ : | 0.1 | (10 days) | 359 | (106-539) | 1.3 | (1.7-3.1) |
| | 0.05 | (20 days) | 195 | (98-463) | 2.7 | (2.3-3.5) |
| | 0.03 | (30 days) | 129 | (92-345) | 3.1 | (2.8-3.7) |
| | 0.025 | (40 days) | 115 | (89-270) | 3.3 | (3.1-3.7) |

[1] Cattle recovery rate of BTV-8 per day

## 4. Discussion

In our study, a vector-borne transmission model was used to estimate $R_0$ for BTV-8 within Dutch dairy herds. The model calculated a median $R_0$ of 2.9. This $R_0$ exceeds one and will therefore lead to a major BTV-8 outbreak in a herd, when protective measures such as vaccination are not taken. Moreover, the proportion of infectious *Culicoides* and the vector over host ratio was estimated based on field data, which described the BTV-8 transmission in cows within herds.

This transmission model is a simplification of reality because it assumes that there is a linear increase in BTV-8 prevalence per herd-month. In reality, BTV-8 will start to spread slowly in a naïve herd; the rate of spread will increase when more cows become infectious and the rate of spread will decrease again when the proportion of susceptible cows becomes small. Thus, the estimates for $R_0$ overestimated the real transmission rate somewhat when the prevalence in the herds was very close to 0% or 100% and underestimated the real transmission rate when the within-herd prevalence was around 50%. Moreover, the estimated $R_0$ values are a simplification of reality, because the exact recovery rate, and the efficacy of host to vector transmission were unknown and fixed values based on literature, were assumed for these parameters. These assumptions were most likely values and were generalized for all herds in our study.

For the host part of the transmission model, field data of the within-herd

transmission was present from 275 dairy herds. For the vector part of the model no field data was gathered during our study and assumptions had to be made for the parameters in the model. Nevertheless, our field data gave the opportunity to estimate one of the parameters that were unknown in our model. It was decided to estimate the vector to host ratio based on our field data, because the number of *Culicoides* per cow will differ between different months, different cows, different housing systems and different herds. The number of *Culicoides* per cow varied greatly between cows and months. We calculated a median of 159 *Culicoides* per cow per day. Because exact estimations about the number of *Culicoides* per cow are not known for north-western Europe, earlier studies included a fixed number of *Culicoides* per cow based on light-trap or drop-trap catches (De Koeijer and Elbers, 2008; Hartemink et al., 2009). Carpenter et al. (2008) captured 2,184 *Culicoides* in total on 192 randomly selected sheep. Furthermore, Gerry et al. (2001) found that the amount of bites on cattle could range up to a maximum of 2,500 (a*m). In a study of Sáenz et al. (1994) on average 313 *Culicoides* were captured in one day using cattle as bait and a study in which *Culicoides* were captured in the Netherlands in 2006, found 0 to 8,722 *Culicoides* per light trap (Meiswinkel et al., 2008). Thus, numbers of captured *Culicoides* varies greatly within and between different studies, which is in accordance with the large variation we found for the number of *Culicoides* per cow.

We found that the number of *Culicoides* increased in time and was highest during the period in which conditions for *Culicoides* became less optimal because of the declining temperatures (October-November). In those months it was expected that BTV-8 transmission between cattle stopped. Nevertheless, our field data showed that, in rare cases, there still was transmission of the virus in the cattle population. It is known that the mortality rate of *Culicoides* decreases when temperatures decline. In addition, the extrinsic incubation period increases and the biting rate decrease (Gerry and Mullens, 2000). Thus, high numbers of *Culicoides* per cow must have been present for the BTV-8 virus to be able to spread.

In our field data, there were five herds in which BTV-8 transmission occurred between November and December. However, based on the assumptions for *Culicoides* relating parameters, BTV-8 transmission was not possible anymore because temperatures were too low. Therefore, our model could not estimate values for $R_0$ for these five observations.

The results of our model showed that the median $R_0$ value for BTV-8 in the northern

region was significantly lower compared to the southern region. The natural habitat of the *Culicoides* and the average daily temperatures do not differ between the regions in the Netherlands. Possibly, the lower $R_0$ values in the northern region are associated to the slower start of the epidemic in the north. From September-October on, BTV-8 transmission within-herds in the northern region increased. However, from that moment on, conditions for *Culicoides* became less favorable because of the decreasing temperatures and thus the $R_0$ remained low.

In the default model, the time to recovery was assumed to be 25 days. From literature it is known that BTV-8 PCR-positive cows can show the presence of viral dsRNA up to 200 days (Maclachlan, 1994) but for our transmission model we wanted to use the period in which a cow is infectious for the transmission of BTV-8 to the *Culicoides*. Because a lot of studies found different infectious periods (Singer et al., 2001; Bonneau et al., 2002; Melville et al., 2004; Monaco et al., 2006; Savini et al., 2009) we decided to vary this value in a sensitivity analysis. The results showed an increased $R_0$ value compared to the default model, when the infectious period was increased and a decreased $R_0$ value when the infectious period is decreased. This was as expected because when the infectious period of cows is prolonged they are able to infect susceptible *Culicoides* longer and BTV-8 transmission will increase.

In the model, also assumptions were made for the *Culicoides* biting rate (ɑ, the probability of BTV-8 transmission from vector to host (*b*), the probability of BTV-8 transmission from host to vector (*c*) the extrinsic incubation period (*EIP*), and the midge mortality rate μ. We did not vary the value of these parameters in our sensitivity analysis because both parameters are generally accepted and applied in other modeling studies (De Koeijer and Elbers, 2008; Gubbins et al., 2008; Hartemink et al., 2009).

Since the outbreak of BTV-8 in North-western Europe, several studies have tried to estimate the transmission of BTV-8 ((De Koeijer and Elbers, 2008; Gubbins et al., 2008; Hartemink et al., 2009; Szmaragd et al., 2009). Szmaragd et al. (2009) developed spatial transmission kernel which was able to predict the number of BTV-8 infected holdings. The model they used was based on the study of Gubbins et al. (2008), which estimated $R_0$ of BTV-8 in both cattle and sheep in Great-Britain. They found a median $R_0$ of 0.81 in the cattle population, which was lower than the $R_0$ found in our study. However, when the temperature was between 15 and 25°C in their model, the median values of $R_0$ seemed comparable to the values of $R_0$ we found in months in which the temperatures were comparable. Nevertheless, the

model of Gubbins et al. (2008) was different from ours, because their model also included sheep and did not include field data on cattle.
Furthermore, De Koeijer and Elbers (2008) and Hartemink et al. (2009), modelled the transmission of BTV-8 in the Netherlands. In their models, no data that described the transmission of BTV-8 in the host i.e. cattle or sheep was included. However, they did had data on the *Culicoides* catches in the Netherlands. De Koeijer and Elbers (2008) calculated values of $R_0$ between 0 and 4, depending on outside temperatures and Hartemink et al.(2009), found values of $R_0$ ranging between 0 and 31 depending on temperature and geographic location, with values of $R_0$ in the range 2 to 5 as most commonly seen. The values of $R_0$ found in both studies were in the same range as the $R_0$ values in our study.
In our model, only cattle were included as hosts for BTV-8, while in reality, also sheep are hosts for BTV-8 and can have an influence on transmission. *Culicoides* are more attracted to cows than to sheep (Braverman et al., 1971; Bartsch et al., 2009) and therefore it is possible that there is more BTV-8 transmission in cattle than in sheep. Thus estimating $R_0$ within cattle herds only, could give an overestimation of the total BTV-8 within-herd transmission. However, no data on monthly increases in within-herd BTV-8 prevalences in sheep were known. Therefore, it was decided not to include the sheep population in this model.

To conclude, this study presents the first estimates of the transmission rate ($R_0$) of BTV-8 within herds, based on serological field data from the 2007 epidemic in cows. The $R_0$ estimates obtained in this study are given the climate, grazing patterns and barn types used in North-West Europe. In an earlier study (Santman-Berends et al., 2010), we found that keeping the cattle indoors during the summer and autumn, reduces the BTV-8 transmission in the herd. When BTV-8 would emerge in a country where all cattle are kept inside or where the temperatures are lower compared to temperatures in the summer and autumn period in the Netherlands, $R_0$ can be somewhat smaller.
The $R_0$ seems to represent the within-herd spread of BTV-8 in the field and these transmission rates may apply to other countries in which BTV-8 emerges, given a similar moderate climate, grazing patterns and barn type as in North-West Europe.

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Chapter 3

# The increase in seroprevalence of bluetongue virus (BTV) serotype 8 infections and associated risk factors in Dutch dairy herds, in 2007

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**Abstract**

Bluetongue virus serotype 8 (BTV-8) emerged in the Netherlands 2006. In the winter of 2006/2007, the government decided to establish a sentinel network to monitor the re-emergence of BTV-8 in 2007.
Between June and December 2007, a sentinel network of 275 dairy herds with 8,901 seronegative cows at start, was in place for BTV-8 testing in milk samples. Besides estimates of the monthly BTV-8 within-herd prevalence, this sentinel was used to determine BTV-8 associated risk factors. Information on management and housing practices that were hypothesized to be related to the increase in BTV-8 prevalence were used. Complete information on BTV-8 testing and management was obtained for 234 herds. The increase in seroprevalence was defined as the total increase in seroprevalence among sentinel cows per herd during the sampling period divided by the number of sampled months. This parameter was used as dependent variable in the linear regression analysis. The final model revealed four risk factors in the final model. Herds in the central and southern region of the Netherlands had a higher monthly increase in seroprevalence 6.4% (95% CI: 3.1-9.9) and 10.1% (95% CI: 6.2-14.3) compared to herds in the northern region. Furthermore, there was a strong association with grazing. The monthly increase in seroprevalence in cattle pastured a few hours per day or throughout the day was 5.6% (95% CI: 1.4-10.2) to 11.4% (95% CI: 6.0-17.3) higher, relative to that for cattle kept indoors. For cattle that grazed outdoors throughout the day and the night, the monthly increase in seroprevalence was 13.6% (95% CI: 7.2-20.8). In addition, an association was found between the monthly increase in seroprevalence and some factors relating to stable design. Keeping the stable doors closed during the day was linked to a higher seroprevalence rate compared to that in stables with the door left open (3.6% (95 CI: 0.3-7.1). Furthermore, a horizontal ventilation opening (>30 cm) along the walls of the stable, and with a wind break curtain, appeared to offer some protection (-3.0% per month (95% CI: -6.0-0.2) as compared to stables that had no or, only a small, ventilation opening (<30 cm). Our study indicated that there were some management factors that may help limit exposure to BTV-8 and its consequences.

*Keywords:* bluetongue virus serotype 8; cattle; prevalence; risk factors; sentinel herds

## 1. Introduction

In August 2006, bluetongue virus serotype 8 (BTV-8) was diagnosed in the Netherlands for the first time (Van Wuijkhuise et al., 2006). A few days later, Belgium and Germany also reported cases of BTV-8. This discovery was remarkable because, prior to its unexpected appearance in northern Europe, BTV-8 was known only from Africa, Central America and parts of south-east Asia (Mo et al., 1994; Daniels et al., 2004; Gerdes et al., 2004).

In December 2006, the assumed BTV-8 transmission free period commenced. By this point, outbreaks of BTV-8 had been reported from five European countries: Belgium (695), France (7), Germany (952), Luxemburg (8) and the Netherlands with 460 outbreaks (260 in sheep flocks and 200 in cattle herds) (EFSA, 2007).

In the winter of 2006/2007, the European Union enforced a monitoring and surveillance program in all affected countries to detect new infections and to monitor disease status, in the hope of regaining its BTV-8 free status within the shorter term. The Dutch government decided to conduct a program using a network of sentinel dairy herds.

The monthly test results from the sentinel network made it possible to pinpoint the moment of introduction of BTV-8 and to predict the rate at which it would spread. Furthermore, by analyzing, the diagnostic results from the ELISA in milk in combination with the management and housing practices on the sentinel farms, risk factors for BTV-8 at herd level could be determined.

In this study, the spread of BTV-8 across the Netherlands, and its monthly increase in seroprevalence in dairy herds is quantified based on results obtained from the 2007 sentinel network program which has enabled us to identify risk factors down to herd level.

## 2. Material and Methods

### *2.1. Study population*

The Netherlands is divided into 20 compartments based on geographic boundaries as proposed in Commission Decision 2005/393/EC (Figure 1). Compartment 1 was divided in two sub-compartments (1a and 1b), because of its large size.

For the sentinel network, a sample size of 150 sentinel cows per compartment was needed to achieve 95% confidence and a precision of 2% (Dutch Ministry

of Agriculture, Nature and Food Quality, 2006). It was determined that in each compartment at least 10 randomly selected herds had to be sampled (with at least 15 cows per herd) to obtain the required sample size. Herds were not necessarily completely BTV-8 seronegative, but cows designated for the sentinel program had to be BTV-8 seronegative at moment of selection in May 2007. Dairy herds included in the program had to have at least 50 cows and had to participate in the dairy herd improvement program of the Cattle Improvement Cooperation (CRV) with test-day intervals monthly.

Thirteen or 14 herds per compartment, with at least 16 BTV-8 seronegative cattle per herd, were selected for sampling.

The 16 BTV-8 seronegative cattle were identified by collecting milk samples taken from 26 lactating cows per selected herd in compartments 1 to 13. In compartments14 to 20, milk samples were taken from all lactating cattle. All the cattle that tested negative were included in the sentinel network. In total, 8,901 initially seronegative cows housed in 275 Dutch dairy herds were selected for the BTV-8 sentinel program in 2007.

This method of selection was used because Van Schaik et al. (2008) found that seroprevalence rates in compartment 1 to 13 did not exceed 1% between January and June 2007 whereas prevalence rates in compartments 14 to 20 (in the south of the Netherlands) were higher. This selection method increased the likelihood of obtaining at least 16 seronegative cattle per herd for inclusion in the network.

All cattle that tested negative at first sampling in May 2007 were included in the study. Each month thereafter, and from this selected group, 16 randomly chosen cows were sampled in each herd.

*2.2. Study period*

The first round of monthly testing of sentinel cows was done in June 2007. The BTV-8 epidemic started in the south of the Netherlands and spread to the north. Sampling was halted in September in compartments 10 to 20 and in October in compartments 7 to 9. Because the prevalence of BTV-8 in these compartments was increasing rapidly, rendering further sampling was unnecessary. Initially, the sentinel study would end in October, but at that point a low number of cattle in the northern compartments of the Netherlands had seroconverted and it was decided to follow the herds in these compartments (1-6) up to and including December. Thus, cows located in the south were sampled for four months, those in the central

region for four, five or seven months and those in the north for seven months (Figure 1 and 2).
For the analyses, the test-results of the sentinel cows in the months in which BTV-8 did not spread on compartment level were excluded because risk-factors could not be determined in case there was no spread of BTV-8. Therefore, observations from sentinel cows located in central or northern compartments in June were excluded and observations from sentinel cows located in compartment 6 in June to August were also excluded.

*2.3. Data collection and management*

The monthly milk samples were tested at the Animal Health Service (GD) for antibodies to BTV-8 using a commercial ELISA (sELISA; ID.VET, Montpellier, France). The result of the ELISA is expressed in a S/P ratio. This ratio quantifies the amount of colouring (extinction) caused by the sample as compared to the amount of colouring (extinction) caused by the standard positive sample (extinction sample/extinction positive control x 100%). When the S/P ratio of the ELISA was >100 the sample was considered BTV-positive; those with an S/P ratio of <100 negative. The diagnostic test used is described in detail by Kramps et al. (2008).
Between March and May 2008, among the farmers of the sentinel herds, a short questionnaire was administered by telephone with questions concerning the type of housing and the grazing patterns of their cattle during 2007. All 275 sentinel herd owners were contacted of which 245 (88%) responded. The remaining 30 herds could not be reached by telephone or did not want to cooperate. Answers of the questionnaire were submitted in Net Q (Net Q, 2008), a program for preparation of questionnaire data. After validation, data from 241 herds remained for analysis. Of these, six herds were only tested once, and in one herd sampling errors were made. These seven herds were excluded from further analysis.
Additional data were obtained on net return of milk, herd size, and the purchase of cattle in the BTV-8 period (between May and December 2007).

*2.4. Statistical analysis*

Monthly within herd prevalences of the sentinel cows for each sentinel herd were calculated and aggregated at compartmental and regional level. These results were displayed geographically and depict infection levels to BTV-8 in 2007 (Figure 1). Univariate descriptive analyses were performed to obtain means and medians and

to compare herd characteristics (i.e. herd size, net return on milk) and the spread of BTV-8 between the regions south, central and north. Median tests (Kruskal-Wallis test: Kruskal and Wallis, 1952) were performed on the parameters that were not normally distributed. A Fisher's Exact test (Fisher, 1922) was used to compare proportions.

For the analysis of the risk factors, for each herd the diagnostic results were aggregated to one outcome variable which was calculated as:

$$MI_i = \frac{prev_{t_n} - prev_{t_0}}{N}$$

Where:

$MI_i$ = the mean monthly increase in seroprevalence amongst sentinel cows in each herd (i; i=1 to 234)

$Prev_{tn}$ = the within herd seroprevalence between the sentinel cows in the last month ($t_n$) in which the herd was sampled

$Prev_{t0}$ = the within herd seroprevalence between the sentinel cows in the first month ($t_0$) in which spread of BTV-8 occurred within the particular compartment in which the herd was located

$N$ = the number of months between $t_0$ and $t_n$

For the risk factor analyses, the outcome variables on herd level, which were based on the diagnostic results, were combined with the results of the questionnaire.
The outcome variable, the monthly increase in seroprevalence of BTV-8 was not normally distributed and a log transformation was used to normalize it. The log monthly increase in seroprevalence was used as a dependent variable and a general linear model in SAS 9.1 (SAS, 2006) was used to analyze risk factors related to the monthly increase in seroprevalence.

First, all variables derived from the questionnaire, and demographic data on the herds, were subjected to univariate analysis. When the *P*-value was below 0.20, the variable was used in the multivariate model. The multivariate analysis was done using a backward and a forward elimination procedure. After each run, the

variable with the highest *P*-value was excluded from the model until all variables had a *P*-value <0.05. Confounding was monitored by the change in the coefficient of a variable after removing another variable. If the change of the estimates exceeded 25% or 0.1 when the value of the estimate was between -0.4 and 0.4, the removed variable was considered a potential confounder and was re-entered in the model. In the final model, all possible two-way interactions were tested singly. The residuals of the final model were tested for normality and the $r^2$ was calculated to measure the proportion of variance explained by the model.

## 3. Results

### *3.1. Descriptive results*

Of the 275 sentinel herds that initially entered the Dutch sentinel program, 78 herds were located in the south, 114 in the central region and 83 in the north; respectively, 63, 100 and 71 of these herds, i.e. 85.1% overall, remained for final analysis.
Due to a lower dairy herd density, dairy herds in the southern region (2.0%) were significantly more often included in the sentinel study, than herds in the central (1.1%) or northern (0.9%) part of the Netherlands (Fisher's Exact test, P-value<0.001) (Table 1).

The monthly increase in whining herd seroprevalence and herd size differed significantly between regions (Kruskal-Wallis *P*-value=0.001 and *P*-value=0.02).
The percentage of herds in which all cattle tested negative in the last test round was not significantly different between regions (Kruskal- Wallis, *P*-value=0.59) (Table 1). However, we found that in 48% of the herds kept indoors all summer, all cattle tested negative in the last test round, as opposed to 14% for herds maintained outdoors (Kruskal-Wallis *P*-value<0.0001).
Nevertheless, the percentage of herds in which all cattle tested positive, and the average within-herd prevalence in the last test round, did not significantly differ between regions (Kruskal-Wallis, *P*-value=0.14 and *P*-value=0.64, respectively).

**Table 1.** Median and interquartile range (25th and 75th percentile) of the dairy herds included in the Dutch BTV-8 sentinel study in 2007 (n=234).

| | **South (71 herds) Median (25th-75th percentile)** | **Central (100 herds) Median (25th-75th percentile)** | **North (63 herds) Median (25th-75th percentile)** |
|---|---|---|---|
| Herd size: cows >2 years of age | 67[1] (57-78) | 65[1] (57-75) | 59 (54-67) |
| Net return on milk in euros/ cow/ lactation (in €) | 2,411 (2,254-2,583) | 2,316 (2,159-2,476) | 2,327 (2,081-2,539) |
| Monthly increase in BTV-8 seroprevalence (%) | 18.8[1] (3.1-31.3) | 13.3[1] (4.4-25) | 7.5 (1.4-16.3) |
| Percentage of herds with all cattle testing BTV-8 negative in the last test round[2] (%) | 19.7 | 15.0 | 20.6 |
| Average within-herd prevalence of BTV-8 in the last test round[2] (%) | 43.8 (6.3-93.8) | 50.0 (12.5-93.8) | 43.8 (7.1-81.3) |
| Herds with all cattle testing BTV-8 positive in the last test round[2] (%) | 22.5 | 23.0 | 11.1 |

[1] significantly different from region north

[2] the month in which the cows were sampled for the last time

In the southern (compartment 15 to 20) and central (compartment 6 to 14) region of the Netherlands, the seroprevalence in the sentinel groups started to increase rapidly from August or September on (Figure 1 and 2) while the rapid increase in the northern region (compartment 1 to 5) of the Netherlands did not commence until October.

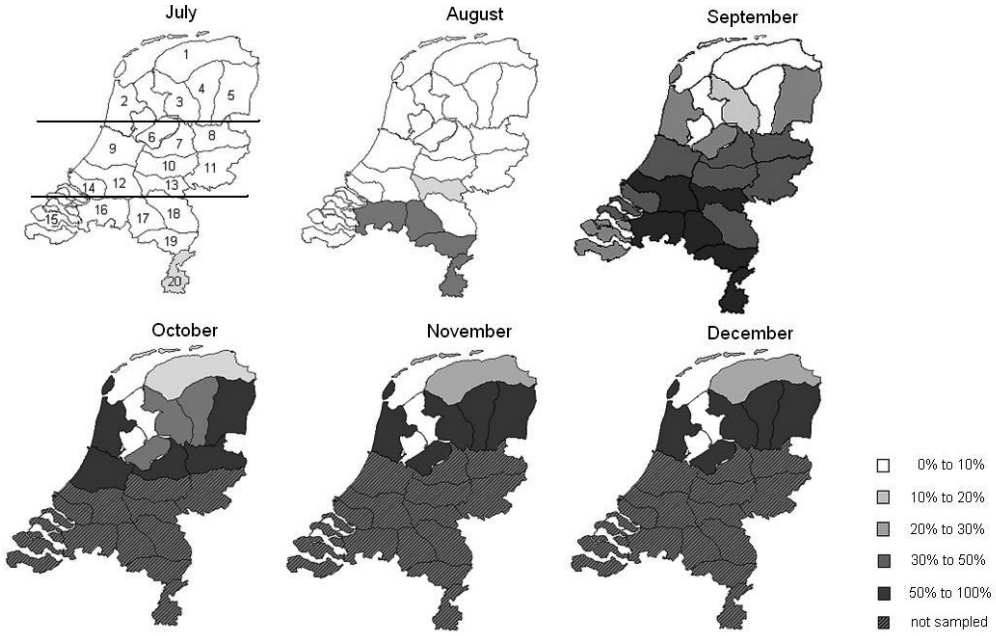


**Figure 1.** Mean within-herd BTV-8 prevalence of 234 dairy herds in the Netherlands per compartment per month from July until December 2007. With below the lines, the southern region of the Netherlands, between the lines, the central region of the Netherlands and above the lines, the northern region. In July the numbering of the compartments is included.

In compartment 6 (central region), the seroprevalence rate started to increase from September on. However, this increase was slower compared to that of other central compartments, where the seroprevalence rates remained at a low level for several months.

The increase in seroprevalence between months where highest in September for the central (mean 44.8%; 95% CI: 37.9-51.7) and southern region (mean 48.2%; 95% CI: 38.2-58.2) (Figure 2). This increase in seroprevalence in September was significantly higher than the increase in seroprevalence in the other months ($P$-value<0.0001 in the central region and $P$-value<0.0001 in the southern region). For region north the highest increase in seroprevalence was found in October (mean 20.9%; 95% CI: 15.0-26.9) and November (mean 19.5%; 95% CI: 14.5-24.4) (Figure 2). These increases in seroprevalence were significantly higher compared to the other months ($P$-value<0.0001 in October and $P$-value<0.0001 in November, respectively).

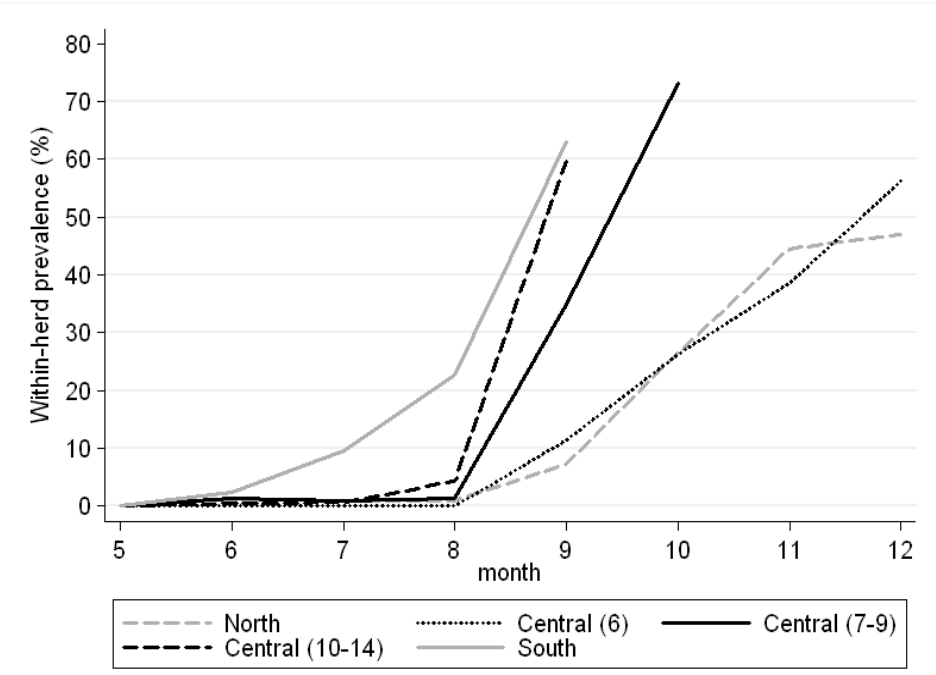


**Figure 2.** Mean within-herd prevalence per month for BTV-8 in the northern (compartment 1-5: sampled June-December), central (shown in three separate lines: compartment 6: sampled June-December, compartment 7-9: sampled June-October and compartment 10-14: sampled June-September) and southern region (compartments 15-20: sampled June-December) of the Netherlands in 2007.

*3.2. Multivariate analyses*

Nine of the 13 variables in the univariate analyses remained for multivariate analysis (*P*-value<0.20) (Table 2). The variables that were excluded based on the results of the univariate analyses, were purchase of cattle (*P*-value=0.37), stable doors open during the night (*P*-value=0.87), type of grazing management (rotation grazing, restricted grazing, siesta grazing, restricted grazing, grazing multiple weeks in the same field; *P*-value=0.99), and type of summer feed (maize, grass, both feeds; *P*-value=0.63).

**Table 2.** Univariate results of all farm management practices and housing variables with a *P*-value<0.20 in 234 Dutch dairy herds that participated in a testing program to estimate the monthly increase in seroprevalence of BTV-8 between June and September (compartments 16-20), June and October (compartments 7-15) or June and December (compartments 1-6).

| Variable | Category | Freq. | Mean monthly increase in seroprevalence (%) | P-value |
|---|---|---|---|---|
| Region | North (comp. 1-5) | 63 | 10.3 | 0.001 |
| | Central (comp. 6-14) | 100 | 16.7 | |
| | South (comp. 15-20) | 71 | 18.7 | |
| Herd size | 10% smallest herds (<50 dairy cows) | 23 | 19.0 | 0.08 |
| | 40% smaller herds (50-64 dairy cows) | 92 | 16.9 | |
| | 40% larger herds (64-90 dairy cows) | 96 | 14.9 | |
| | 10% largest herds (> 90 dairy cows) | 23 | 9.6 | |
| Cattle were grazed in 2007 | Yes | 207 | 16.6 | 0.003 |
| | No | 27 | 8.3 | |
| Grazing practices between the end of June and October | No grazing (0[1]) | 27 | 8.3 | 0.0005 |
| | A few hours per day between milking (1-1500[1]) | 126 | 14.1 | |
| | During the day (1501-2500[1]) | 55 | 19.7 | |
| | Day and night (> 2500[1]) | 26 | 21.7 | |
| Type of housing | Loose housed stable | 212 | 15.1 | 0.19 |
| | Different stable type | 22 | 20.4 | |

| | | | | |
|---|---|---|---|---|
| Horizontal ventilation openings at the side of the stable | < 30 cm | 72 | 18.3 | 0.02 |
| | > 30 cm without windbreak curtain | 103 | 16.4 | |
| | > 30 cm with windbreak curtain | 59 | 11.0 | |
| Stable doors open through the day in summer | Yes | 193 | 14.7 | 0.10 |
| | No | 41 | 20.0 | |
| Roof with opening of at least 20 cm | Yes | 182 | 14.7 | 0.13 |
| | No | 52 | 18.8 | |
| Net return on milk in euros/cow/ lactation | < € 1,838 (herds with 10% lowest net return) | 18 | 21.8 | 0.15 |
| | €1,838-€ 2,288 (herds with 40% lower net return) | 73 | 16.9 | |
| | € 2,289-€ 2,619 (herds with 40% higher net return) | 109 | 14.8 | |
| | > 2,620 (herds with 10% highest net return) | 34 | 12.0 | |

[1] The total number of hours was calculated as the number of hours per day times the number of days the cattle were in the field.

In the final multivariate model, the four factors 'region', 'grazing management between the end of June and October', 'keeping stable doors open during the day' and 'horizontal ventilation openings along the walls of the stable', were significantly associated with the monthly increase in seroprevalence of BTV-8 (Table 3).

**Table 3.** Variables in the final multivariate general linear model on the effect on the monthly increase in seroprevalence between June and December 2007 in the Netherlands and their categories, estimates (transformed from log transformation to real estimates), 95% confidence intervals and significance (P-value) (n=234).

| Variable | Category | Estimated monthly increase in sero-prevalence (%) | 95% Confidence interval (%) | | P-value |
|---|---|---|---|---|---|
| Intercept | | 0.73 | -4.1 | 6.1 | 0.77 |
| Region | North | Reference | | | |
| | Central | 6.4 | 3.1 | 9.9 | 0.0001 |
| | South | 10.1 | 6.2 | 14.3 | <0.0001 |
| Grazing management between the end of June and October | No grazing (0 [1]) | Reference | | | |
| | A few hours per day between milking (1-1500[1]) | 5.6 | 1.4 | 10.2 | 0.009 |
| | During the day (1501-2500[1]) | 11.4 | 6.0 | 17.3 | <0.0001 |
| | Day and night (> 2500[1]) | 13.6 | 7.2 | 20.8 | <0.0001 |
| Stable doors open throughout the day | Yes | Reference | | | |
| | No | 3.6 | 0.3 | 7.1 | 0.03 |
| Horizontal ventilation openings in the side walls the stable | None or smaller than 30 cm | Reference | | | |
| | Larger than 30 cm without windbreak curtain | -0.3 | -3.2 | 2.7 | 0.82 |
| | Larger than 30 cm with windbreak curtain | -3.0 | -6.0 | 0.2 | 0.07 |

[1]Number of hours in the field during the pasturing period were calculated as the number of hours per day times the number of days the cattle were in the field.

In herds in the central (6.4% (95% CI: 3.1-9.9)) and southern (10.1% (95% CI: 6.2-14.3)) parts of the Netherlands, BTV-8 spread more rapidly amongst cattle than in herds located in the north. Furthermore, it appeared that longer grazing hours were associated with a higher monthly increase in seroprevalence of BTV-8. Cattle that grazed outdoors for only a few hours a day (totaling between 1 to 1500 hours in summer and autumn), grazed outside all day (1501 to 2500 hours) or grazed outside day and night (>2500 hours), showed significantly higher monthly increases in seroprevalence of BTV-8 compared to cattle that remained indoors.
Keeping the stable door closed during the day was associated with a higher monthly increase in seroprevalence when compared to keeping the stable door open (3.6% (95% CI: 0.3-7.1).
In addition, herds with horizontal ventilation openings along the side walls of the stable (>30 cm) in combination with a windbreak curtain, tended to a lower monthly increase in seroprevalence, when compared to herds with no or only small horizontal ventilation openings (<30 cm) (-3.0% (95% CI: -6.0-0.2)). However, large horizontal openings along the side walls, and without windbreak curtain, did not reduce the monthly increase in seroprevalence, when compared to herds in stables with no or with only small horizontal openings.
The final model explained 21% of the variation in the monthly increase in seroprevalence. The variables in the final model were not significantly correlated amongst each other (Spearmann's correlation test). In the model one two-way interaction term was found to be significant: "grazing management" with "stable doors open at daytime". However, this interaction term did not improve the amount of variance explained by the model nor the fit of the model. In addition, the result did not seem biologically relevant and the interaction was not included in the final model. The residuals of the final multivariate model were normally distributed.

## 4. Discussion

The sentinel study was originally developed to establish whether the Netherlands could regain its BTV-8 free status in 2007. In July 2007, the first seronegative cattle in the sentinel herds seroconverted. At that point it became clear that BTV-8 had over wintered and re-emerged. Subsequent to this the sentinel network was used to determine the spread of BTV-8 across the Netherlands.
Herds in different regions were sampled for different periods. This could have

caused some bias in our data. However, within-herd prevalences in the south had increased to very high levels in September and further sampling would probably not provide additional information. At that point, BTV-8 within-herd prevalences were still very low in some central and northern compartments and for this reason it was decided to continue the testing in those compartments. To correct for the differences in sampling period for herds located in different compartments, the average monthly increase of seroprevalence within herds was determined. The median monthly increase in seroprevalence significantly differed between regions, with the highest increase in the south (18.8%) and the lowest in the north (7.5%). It is likely that the fraction of BTV-8 infected *Culicoides* in the southern and central region of the Netherlands was higher at the start of the *Culicoides* active period in 2007 compared to the fraction of infected *Culicoides* in the north, because BTV-8 had already spread in 2006 in the south and limited in the central region. Furthermore, these differences in the increase in the seroprevalence could be due to seasonal differences in the spread of BTV-8. In the south, BTV-8 spread in the summer months during which conditions were favourable and with temperatures high, ranging 15 and 25 degrees Celsius. In the north, BTV-8 started to spread from October to December. However, the conditions for the continued spread of BTV-8 deteriorated during these months due to declining temperatures.

Initially, we suspected that there would be a relationship between the purchase of cattle and the introduction of BTV-8 into a given herd. However, the results of the univariate analyses showed that there was no significant association between the purchase of cattle and increase in seroprevalence. To certify that the purchase (and movement) of cattle was not associated to the spread of BTV-8, we compared purchase of cattle (yes/ no) between June and December 2007 in herds that were BTV-8 positive with purchase of cattle between June and December 2007 in herds that had remained BTV-8 negative throughout the study period. Our data indicated no differences in purchase behavior between owners with herds that became BTV-8 positive and owners with herds that remained BTV-8 negative.

In compartment 6 (central region), the seroprevalence started to increase later than in the other central compartments and remained at a low level for a long time (Figure 1 and 2). The herd owners of only six of the 13 sentinel herds in compartment 6 responded to the questionnaire and so were included in our study. Of these six herds, four were kept stabled throughout the entire grazing season. Thus, the lower seroprevalence in this compartment may be linked by the specific management

practices or a lower of herd density.

In the sentinel program, 275 sentinel herds were included; of these, 234 were included in the final model. A fixed number of 13 to 14 herds per compartment were selected for the study. Because the south of the Netherlands has a lower dairy herd density than the north, the south was slightly overrepresented in our study. Moreover, in the north, fewer farmers responded to the questionnaire, perhaps due to lower BTV-8 morbidity and mortality rates and due to its belated arrival in October 2007 (Santman-Berends et al., 2010). Although the sentinel herds were not fully representative of the national situation, we believe nevertheless that the range of management practices, barn types, and risk factors involved, are applicable to all dairy herds in the Netherlands.

It was found that more hours of grazing were related to a higher monthly increase in BTV-8 seroprevalence. Herds maintained indoors stayed significantly more often BTV-8 free (48%) than herds maintained outdoors (14%) and therefore the maintenance of cattle indoors may reduce the spread of infection. Meiswinkel et al. (2000) found that the ratio outdoors vs. indoors catches of *Culicoides* differed per subspecies. However, almost all subspecies were captured outside more often than indoors. In addition, Baylis et al. (2009) trapped *Culicoides* indoors and outside the stable in the United Kingdom and they also found lower numbers of trapped *Culicoides* indoors compared to outdoors (6.5 times less). These findings support our results of grazing as a risk factor. However, Baldet et al. (2008) found that in France certain species of *Culicoides* were captured indoors as much as outdoors. Based on the fact that a relatively high proportion of the *Culicoides* trapped indoors were freshly blood fed, they assumed that these had been actively feeding. Meiswinkel et al. (2008) obtained similar results in the Netherlands that included captures in which up to 33% of the *Culicoides* were freshly blood fed, and which likely had fed inside the stable.

Their conclusions, in part, conflict with our results which, based on the significantly lower BTV-8 seroprevalence rates, indicate *Culicoides* attack rates to be higher outdoors than indoors. It is important to note that two of the potential BTV vectors in the Netherlands, i.e. *C. dewulfi* and *C. chiopterus,* breed exclusively in the fresh dung of cattle lying in the field outdoors; therefore, if all cattle at a dairy are being maintained indoors it will deprive *C. dewulfi* and *C. chiopterus* of their breeding habitat, leading to a decrease in their population levels, which, in turn, may impact on the dissemination of BTV locally.

We found an association between increase in seroprevalence and some factors relating to stable design. A horizontal air opening of more than 30 cm along the walls of the stable, combined with a windbreak curtain, seemed to be protective against BTV-8 infection; furthermore, keeping the stable doors open was associated with a significantly lower increase in seroprevalence compared to that found at stables where the doors were kept closed. Thus, it would appear that herds housed in stables with many air openings ("fresh stables") showed a lower increase in seroprevalence. It is possible that increased air circulation may inhibit *Culicoides*, resulting either in a reduced presence and/ or reduced biting frequency in fresh stables. However, stables with a large (>30 cm) horizontal opening but without windbreak curtain were not associated with a significantly lower increase in seroprevalence compared to that in herds maintained in stables with no or small horizontal openings in the walls. Windbreak curtains may in some way help to reduce entrance by *Culicoides* into stables, much as mosquito netting would do. Nevertheless, because of the fairly weak association, further research is required to prove causality.

In the second half of 2007, BTV-8 had spread over all regions in the Netherlands. Our study indicates that there are some management practices such as maintaining the herd indoors and characteristics relating to the stable design that may help limit exposure to BTV-8 and thus lead to a lower increase in BTV-8 prevalence.

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Chapter 4

# Mortality attributable to bluetongue virus serotype 8 infection in Dutch dairy cows

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Veterinary Microbiology, in press

**Abstract**

In 2007, bluetongue virus serotype 8 (BTV-8) re-emerged in the Netherlands and a large number of farmers notified morbidity and mortality associated with BTV-8 to the authorities. All dead cows in the Netherlands are registered in one of three age classes: newborn calves <3 days, calves 3 days-1 year, and cows >1 year. These registrations result in a complete data set of dead cattle per herd per day from 2003 until 2007. In this study, the mortality associated with BTV-8 for the Dutch dairy industry was estimated, based on this census data.

Default, mortality associated with BTV-8 was estimated for the confirmed notification herds. Moreover, an additional analysis was performed to determine if mortality associated with BTV-8 infection occurred in non-notification herds located in BTV-8 infected compartments. A multivariable population-averaged model with a log link function was used for analyses. Separate analyses were conducted for the three different age groups.

Confirmed notification herds had an increased cow mortality rate ratio (MRR) (1.4 (95% CI: 1.2-1.6)); calf MRR (1.3 (95% CI: 1.1-1.4)); and newborn calf MRR (1.2 (95% CI: 1.1-1.3)). Furthermore, in non-notification herds in BTV-8 infected compartments, mortality significantly increased 1.1 times (95% CI: 1.1-1.1) in cows, 1.2 times (95% CI: 1.2-1.2) in calves and 1.1 times (1.1-1.1) in newborn calves compared with BTV-8 non-infected months.

Using objective census data over a 5-year period, the MRRs indicated increased mortality associated with BTV-8 infection in herds of which the farmer notified clinical signs but also in non-notification herds in infected compartments.

*Keywords:* bluetongue; BTV-8; disease; mortality; cattle; epidemic; census data

## 1. Introduction

Bluetongue is a vector-borne viral disease of ruminants, caused by bluetongue virus (BTV), which is transmitted by BTV-infected biting midges of the genus Culicoides (Cêtre-Sosah et al., 2004).
In August 2006, bluetongue virus serotype 8 (BTV-8) emerged in northern Europe and was first detected in the Netherlands (Van Wuijckhuise et al., 2006; EFSA, 2007; Elbers et al., 2008a) and a few days later in Belgium and Germany (OIE animal Health Department, 2006; Toussaint et al., 2006). In 2006, the BTV-8 epidemic remained restricted to the southern part of the Netherlands and caused limited morbidity and mortality in cattle (Elbers et al., 2008a; Van Schaik et al., 2008).
At the end of July 2007, BTV-8 re-emerged in the Netherlands. Compared with the first BTV-episode, more farmers notified clinical signs of BTV-8 in their herds (3,182 vs 200 in 2006) and both farmers and veterinarians reported more severe clinical signs including increased mortality associated with BTV-8 infections (EFSA, 2007; Elbers et al., 2009).
At present, the overall effect of BTV-8 infection on mortality in dairy cattle is lacking. Sound estimates for mortality in the cattle population are important because increased mortality caused by BTV-8 results in economic losses for dairy herds. These economic losses will have impact on the level of control measures such as vaccination that will be implemented on herd or country level.
The aim of this study was to quantify cattle mortality associated with the BTV-8 outbreak in 2007 in the dairy industry in the Netherlands.

## 2. Material and Methods

### *2.1. Data*

Data of dairy herd owners that notified clinical signs of BTV-8 in 2007 (notification herds) were available from the Food and Consumer Product Safety Authority (VWA). Before the 10$^{th}$ of September 2007, notification herds were tested whether they were truly infected at the national reference laboratory (CVI) by an in-house developed serogroup-specific PCR-test based on S10 of BTV. After the 10$^{th}$ of September, only notifications of herds located in compartments in which BTV-8 prevalence was low had to be confirmed. Compartments, in total 20 are areas which are defined as proposed in Commission Decision 2005/393/EC.

In the Netherlands, all dead farm animals are collected at the farms, registered in one of three age categories: < 3 days, 3 days-1 year, >1 year, and rendered. These registrations, together with data on production and herd health per month were available for monitoring purposes for most Dutch dairy herds (97.5% gave permission) for a five year period from January 2003 until December 2007. These monitoring data were available from several sources i.e. the identification & registration system (I&R), the rendering plant (Rendac), the milk control station (Qlip), the Dutch Royal Cattle Syndicate (CRV) and the Animal Health Service (GD) and contained among others, a complete overview of all mortality for all Dutch dairy herds including both the cattle mortality before and during the BTV-8 period in the Netherlands.

All data were combined by unique herd identification (UHI) and aggregated to monthly level for each herd. Initially, all continuous explanatory variables, which we wanted to correct for, because they were relevant confounders, were divided in 10$^{th}$, 50$^{th}$, 90$^{th}$ percentiles because the variables were non-linear. Classifying the variables into different categories did not improve the model fit. Furthermore, we were not interested in their effect on mortality but our focus was to estimate the monthly mortality in the five year period as precisely as possible.

The final data set contained the following information:

- UHI
- Data on herd-level per month:
  - Notification of clinical signs of BTV-8: yes confirmed, yes not confirmed, not notified
  - Number of dead cattle collected by Rendac divided into three age groups: *1= cows >1 year, 2= calves between 3 days and 1 year, 3= newborn calves <3 days*
  - Herd size: *1 = <35 cows, 2= between 35 and 71 cows, 3 = between 72 and 27 cows, 4 = >127 cows*
  - Milk production level as net revenues per cow per lactation: *1 = < €1875, 2 = between € 1876 and 2308, 3 = between € 2309 and 2645, 4 >€ 2645, 5 = missing*
  - Percentage growth in herd size per year: *1= < -10%, 2 = between -10% and 0%, 3 = between 0% and 10%, 4 = >10%*
  - Geographic location divided into twelve provinces
  - Purchases of cattle *divided into three categories: 0 = not in previous 12 months, 1 = one in previous 12 month and 2 = more than one in previous 12 months*

- Season: *winter i.e. January-March, spring i.e. April-June, summer i.e. July-September, autumn i.e. October-December*
- Time in months.

In January 2006, regulations regarding emergency slaughter were tightened, laying down specific hygiene rules for food of animal origin (EG 853 and 854/2004, Regulation of the European Parliament, 2004). In January 2007, regulations regarding the welfare of animals during transport were tightened (EG 1/2005, Regulation of the European Parliament, 2005). Both changes resulted in higher on-farm mortality rates. An additional variable was included in the data set to adapt for these changes in regulations, i.e. new EU regulation 2006 and 2007; 0 = before regulations, 1 = regulations 2006 applied and 2 = regulations 2006 and 2007 applied.

*2.2. Assigning the period in which additional mortality associated with BTV-8 was assumed*

From the 3,182 cattle herds that notified clinical signs of BTV-8 to the authorities, 2,980 herds had complete data for the whole 5-year study period. The other 202 herds refused to cooperate with the national monitoring or had incomplete data. From the 2,980 herds that notified, which had complete data, 2,448 herds were dairy herds. The remaining 532 notifications were mainly done by suckling cow holders and small scale beef farmers. Only 598 of the 2,448 notifications from dairy farmers were diagnostically confirmed at the CVI. In case of the other 1,850 notifications, the farmers and their veterinarians reported clinical signs of BTV-8 in their herds but these suspicions were not confirmed with diagnostic tests. These herds were therefore excluded from the default analyses.

The mortality associated with BTV-8 was analyzed by comparing mortality in BTV-8 confirmed dairy herds in the month of notification to mortality in all cattle herds in BTV-8 non-infected i.e. free months. Although non-confirmed notification herds probably had a high probability to be truly infected based on a confirmation rate of 99%, we decided to estimate mortality associated with BTV-8 infection only on notification herds that were diagnostically confirmed to be truly infected, to avoid misclassification.

Besides the default analysis, the mortality associated with BTV-8 was also analyzed for all dairy herds located in BTV-8 infected compartments in the Netherlands. For this analysis, all dairy herds were divided into one of three classes, 1) all dairy herds

in BTV-8 non-infected/free months (defined the same as in the default model), 2) dairy herds located in BTV-8 infected compartments in 2007, and 3) confirmed notification herds in the month of notification.

In order to assign herds to the category 'dairy herds located in BTV-8 infected compartments', the results of a BTV-8 prevalence study in the Netherlands in 2007 described by Santman-Berends et al. (2010a) was used. In this study, on average 150 cows from 13 sentinel herds per compartment were tested each month to obtain monthly estimates of BTV-8 spread. Santman-Berends et al. (2010a) found that BTV-8 started to spread at the end of July 2007 in the south of the Netherlands and spread northwards. In infected compartments, most herds got infected within a month and once a herd was infected, the within-herd BTV-8 prevalence increased rapidly. Based on their results, it was assumed that compartment 7 and 10 to 20 (Figure 1) were infected from August until December 2007; that compartment 2 to 6, 8 and 9 were infected from September until December 2007; and that compartment 1 was infected from October to December 2007 (Figure 1). The BTV-8 period from July until December 2006 was excluded from all analyses because it was not possible to make a good differentiation between infected and non-infected compartments.

*2.3. Statistical analysis*

Descriptive analyses were performed with a Kruskal-Wallis test to describe the study population (Kruskal and Wallis., 1952). Mortality data followed a poisson distribution and there were repeated observations per herd. Therefore, a multivariable population-averaged model with a log-link function, using generalized estimated equations in Stata version 10 (Stata, 2007) was used. Separate analyses were conducted for three different age groups: newborn calves <3 days, calves between 3 days and 1 year, and cows >1 year. An independent correlation structure was included in the models and the number of cattle at risk was included as exposure variable. Potential confounding variables were included as fixed effects. The statistical model was formulated as:

$$Ln\left(\frac{Y_{it}}{n_{it}}\right) = \beta_{0i} + \beta_1 BTV8_{it} + \beta_2 X_{2it} + ..... + \beta_n X_{nit} + \varepsilon_{it}$$

In which:

| | | |
|---|---|---|
| $Y_{it}$ | = | number of dead cattle: number of dead cattle >1 year for estimation of cattle mortality, number of dead calves between 3 days and 1 year for estimation of calf mortality and number of dead cattle younger than 3 days for estimation of newborn calf mortality per herd (i) per month (t) |
| $n_{it}$ | = | number of cattle at risk: number of cattle >1 year for the estimation of cattle mortality, number of calves between 3 days and 1 year for the estimation of the calf mortality, and the number of cattle calving for the estimation of calf mortality younger than 3 days |
| $\beta_0$ | = | intercept |
| $\beta_1 BTV8_{it}$ | = | fixed effect of BTV-8 status |
| $\beta_n X_{nit}$ | = | fixed effects of potential confounders i.e. herd size, milk production, yearly growth in herd size, province, purchase of cattle, season, month, and new EU regulations 2006 and 2007. |
| $\varepsilon_{it}$ | = | standard error for each herd (i) per month (t) |

In the model, the estimate of $\beta_1 BTV8_{it}$ was the result of interest and represented the mortality rate ratio *(MRR)* in a BTV-8 infected herd relative to their mortality prior to BTV-8 infected i.e. non-infected months. BTV-8 non-infected months were defined as all months between 2003 and 2005, from January to June 2006 and from January to June 2007. The months July to December for both 2006 and 2007 were excluded from this category, because it was known that in that period also other than notification herds became infected with BTV-8 (Elbers et al., 2008a; Santman-Berends et al., 2010a).

When the *MRR* for BTV-8 was one, there was no association of BTV-8 status with mortality. When the *MRR* was lower than one, there was less mortality in BTV-8 infected herds compared to non-infected months, and in case the mortality rate exceeded one, there was more mortality in BTV-8 infected herds compared to non-infected months.

The goodness of fit of the model was determined by checking the correlation between the observed and predicted values of the model. Other correlation structures besides independent correlation were explored but did not improve the model fit. The mean and variance of the mortality were almost equal, which meant that overdispersion was not an issue in our model and the Pearsons overdispersion parameter was close to 1. We checked if the model was sensitive to the outliers by monitoring the difference in result between the model in which the outliers were included and the model in which outliers were excluded. The results between both models only differed slightly and did not alter the conclusions. Therefore it was decided not to exclude the outliers from the analysis of mortality.

## 3. Results

### *3.1. Descriptives*

The first case of BTV-8 in 2007 was notified on July 27$^{th}$ in a herd located in the south of the Netherlands and the last case notification was notified on December 29$^{th}$. The majority of the cases were notified in September (68.2%) and the number of notifications declined after that month (Table 1).

**Table 1.** Number (N) and percentage (%) of notifications of clinical signs of BTV-8 and the number and percentage of confirmations made in dairy herds between July and December 2007 in the Netherlands.

| Month | N notifications | % notifications | N confirmed notifications | % confirmed notifications |
|---|---|---|---|---|
| July | 7 | 0.3 | 7 | 100.0 |
| August | 247 | 10.1 | 243 | 98.4 |
| September | 1,669 | 68.2 | 243 | 14.6 |
| October | 421 | 17.2 | 43 | 10.2 |
| November | 75 | 3.1 | 36 | 48.0 |
| December | 29 | 1.2 | 26 | 89.7 |
| Total | 2,448 | 100.0 | 598 | 24.4 |

During the BTV-8 epidemic, most notifications were made by farmers in compartments 8 and 11, the eastern part of the Netherlands. The smallest proportion of farmers that notified were housed in compartment 1 and 3, which was the upper north of the Netherlands (Figure 1).

Herd characteristics such as herd size, net revenue per cow per lactation and purchase behavior did not differ between herds that did or did not notify clinical signs of BTV-8 in 2007 (Kruskal-Wallis, *P*-value>0.05). In 2007, the median herd size of a dairy herd was 69 cows >2 years, the median net revenue per lactation was € 2,336 and 52% of all dairy herds had not purchased any cattle in the previous year.

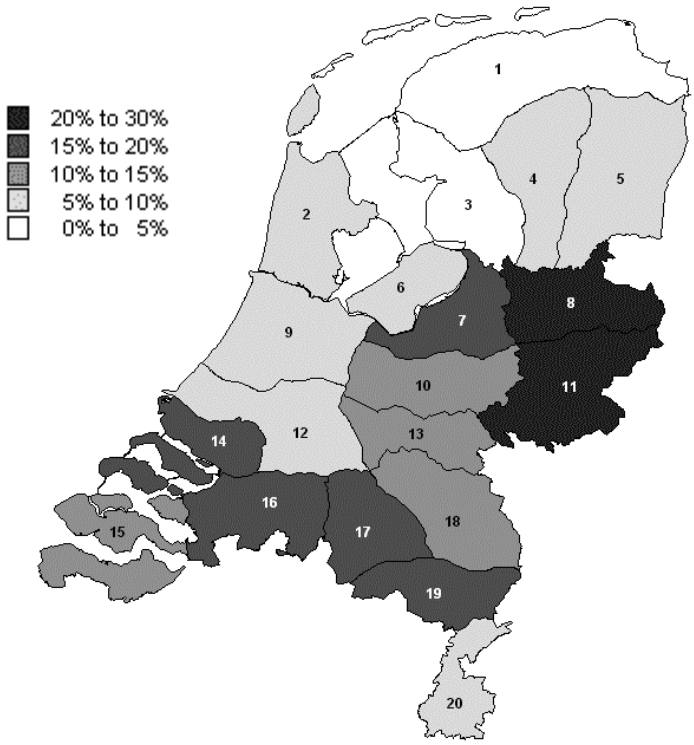


**Figure 1.** Percentage of farmers that notified bluetongue virus serotype 8 (BTV-8) related clinical signs in their herds to the Food and Consumer Product Safety Authority (VWA) between July and December 2007, per compartment (1 to 20) in the Netherlands.

*3.2. Multivariable analyses*

In the default model, confirmed notification herds had a 1.4 (95% CI: 1.2-1.6) increased cow *MRR* in the month of notification compared to BTV-8 non-infected months (Table 2). In addition, calf (3 days - 1 year) and newborn calf (< 3 days) mortality were significantly increased (*MRR*: 1.3 (95% CI: 1.1-1.4) and 1.2 (95% CI: 1.1-1.3), respectively) (Table 2).

In the model which estimated the mortality for the whole dairy industry in the Netherlands, mortality was significantly associated with BTV-8 in the non-notification herds that were located in BTV-8 infected compartments. In these herds, the *MRR* in cows (>1 year) increased 1.1 times (95% CI: 1.1-1.1), the *MRR* in calves increased 1.2 times (95% CI: 1.2-1.2) and the *MRR* in newborn calves increased 1.1 times (1.1-1.1) compared with the mortality in BTV-8 non-infected months (Table 2). The results of the notification herds were comparable to those of the default model.

The additional mortality associated with BTV-8 infection in non-notification herds in infected compartments in the BTV-8 period was smaller compared to the additional mortality in herds that notified clinical signs of BTV-8. Nevertheless, this difference in MRR between notification and non-notification herds became smaller when the month before or after notification was included in the category "herds that notified clinical signs" (results not presented). Thus it seemed that the clinical signs and the consequences of BTV-8 peaked in the month of notification.

**Table 2.** Results of the default multivariable log-linear regression analyses and sensitivity analysis on cow, calf and newborn calf mortality within Dutch dairy herds between 2003 and 2007 associated with bluetongue virus serotype 8 (BTV-8); with the mortality rate ratio (*MRR*), the 95% confidence interval (95% CI) and the *P* value.

| Model | Mortality | BTV-8 status | MRR (95% CI) [1] | P-value |
|---|---|---|---|---|
| Default | cattle (> 1year) | non-infected months (reference)[2] | | |
| | | confirmed notifications | 1.41 (1.22-1.63) | <0.01 |
| | registered calves (3 days - 1 year) | non-infected months (reference) | | |
| | | confirmed notifications | 1.29 (1.07-1.54) | <0.01 |
| | newborn calves (< 3 days) | non-infected months (reference) | | |
| | | confirmed notifications | 1.20 (1.08-1.33) | <0.01 |
| Sensitivity analysis | cattle (> 1year) | non-infected months (reference) | | |
| | | non-notified herds in affected period | 1.11 (1.08-1.13) | <0.01 |
| | | confirmed notifications | 1.36 (1.17-1.57) | <0.01 |
| | registered calves (3 days - 1 year) | non-infected months (reference) | | |
| | | non-notified herds in affected period | 1.20 (1.17-1.23) | <0.01 |
| | | confirmed notifications | 1.37 (1.14-1.63) | <0.01 |
| | newborn calves (< 3 days) | non-infected months (reference) | | |
| | | non-notified herds in affected period | 1.11 (1.09-1.12) | <0.01 |
| | | confirmed notifications | 1.23 (1.10-1.37) | <0.01 |

[1] The estimates for *MRR* are corrected for potential confounders i.e. herd size, milk production, yearly growth in herd size, province, purchase of cattle, season, month and EU regulations, which were implemented during the period that was studied.

[2] Non-infected months were defined as the months in which BTV-8 was not present or did not spread i.e. all monthly observations on herd level from 2003 until June 2006 and January to June 2007 and the monthly observations of herds located in BTV-8 free compartments in July-September 2007.

## 4. Discussion

In 2006, BTV-8 emerged in the Netherlands and the virus survived the subsequent winter period. At the end of July 2007, BTV-8 reservoirs seemed to be present from where the virus started spreading explosively over the Netherlands, causing morbidity and mortality in cattle.

Cow (> 1 year), calf (3 days- 1 year) and newborn calf (< 3 days) mortality associated with BTV-8 infection significantly increased in confirmed notification herds. We decided to estimate the mortality associated with BTV-8 infection in confirmed notification herds only and to exclude non-confirmed notification herds, to prevent misclassification. However, more than 99% of the herds that were confirmed appeared to be truly infected. Furthermore, results of the default model only differed slightly and conclusions did not change when we included all notification herds in the model and assumed them to be truly infected (results not presented).

When both confirmed notification herds and all non-notified herds in infected compartments were included in the model, we also found an increased mortality in the herds in infected compartments. However, the additional mortality associated with BTV-8 in these herds was lower compared to the BTV-8 mortality in the notification herds. The lower mortality can have several reasons. First, BTV-8 infected herds that did not notify clinical signs may have experienced a mild and sub-clinical BTV-8 infection without mortality. Second, not all non-notification herds in infected compartments would have suffered from BTV-8 infection, which would lead to an underestimation of the mortality in infected herds. However, given the rapid spread of BTV-8 in 2007 (Santman-Berends et al., 2010a; Santman-Berends et al., 2010b) it is not likely that many herds and cows remained free from BTV-8 infection in infected compartments.

In the model, we included a covariate to account for new regulations that were implemented in January 2006 and January 2007. These regulations caused additional mortality because, according to these regulations, cows that could have

gone to the emergency slaughter in the past had to be euthanized on the farm. The model estimates for these regulations were significant, thus partly explaining within-herd mortality. These regulations were implemented in January of each year. BTV-8 did not cause any mortality prior to August. The model had seven to eight months to estimate the additional mortality due to the changes in regulations before additional mortality due to BTV-8 occurred. Thus, the model was able to separate the mortality due to BTV-8 and the additional mortality due to the changes in regulations.

As we used monthly census data on mortality over a five-year period, we believe that our model gave a good indication of the mortality associated with BTV-8 in the Netherlands. However, a slight overestimation of the real mortality associated with BTV-8 might have occurred because in our model we attributed all additional mortality in the BTV-8 period to BTV-8, while there could have been other causes in the same period in the same compartments which contributed to increased mortality compared with previous years. However, it is unlikely that there were other reasons for an increased mortality in that period because, based on the cattle health monitoring and surveillance system in the Netherlands (Bartels et al., 2007), no other reasons for an increased mortality were found.

The MRR estimates for all non-notification herds in infected compartments give an indication of the total additional mortality associated with BTV-8, which can be expected in a country where BTV-8 emerges. Mortality rates due to BTV-8 that are only estimated on herds that notified clinical signs (Le Gal et al., 2008; Elbers et al., 2009) would be overestimated when extrapolated to national level. It is evident that notification is primarily done by farmers, who experience clear clinical signs of BTV-8 in their herds and less by farmers that see only few or no clinical signs.

Previous studies of bluetongue in relation to mortality have mainly focused on small ruminants (Breard et al., 2004; Calistri et al., 2004; Shimshony, 2004). There were only two studies which presented results on mortality in relation to infections with BTV-4, 9 and 16 in cattle (Mastroyanni et al., 1981; Nomikou, 2004). Both studies concluded that BTV outbreaks were not associated with increased mortality in cattle, and only in rare cases cattle seemed to die from a BTV-4, 9 or 16 infection. However, in our study a slight increase in mortality due to BTV-8 was found. Thus, it is possible that BTV-8 is more virulent in cattle than other BTV serotypes.

Previous studies estimated the losses associated with BTV-8 on national level with simulation models. The losses caused by morbidity and mortality in cattle were

based on losses for BTV-8 notification herds and were multiplied with the assumed percentage of herds that would undergo a BTV-8 infection i.e., which were assumed to suffer from these losses (Velthuis et al., 2010). Our mortality estimates can be used as input in the models to obtain a more precise indication of the losses of a BTV-8 outbreak on herd and national level and support decisions about control efforts.

## 5. Conclusion

The BTV-8 infection in 2007 in the Netherlands was associated with an increase in mortality in calves and cows. Infected herds that notified clinical signs and which were confirmed to be BTV-8 infected had a higher increase in mortality in the month of notification than herds in infected areas that did not notify clinical signs to the authorities.

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Chapter 5

# Vertical transmission of bluetongue virus serotype 8 virus in Dutch dairy herds in 2007

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**Abstract**

In February 2008, evidence was found for transplacental infection of bluetongue virus serotype 8 (BTV-8) in PCR negative, seropositive heifers in Northern Ireland originating from the Netherlands. The relevance of this route of transmission was studied in Dutch cow-calf combinations in the Netherlands of which the calves were born in the same time period of the year as the calves from the exported heifers, the first quarter of 2008. Blood samples were tested from 385 cows and their calves, housed in 43 dairy farms that became naturally infected with BTV-8 for the first time in 2007. All calves were at least ten days old at the moment of first testing. In total 229 cows tested seropositive for BTV-8. Eight of these cows were still PCR positive. Out of the 229 seropositive cows, 37 calves (16.2%; 95% CI: 11.4-21.0) were tested PCR positive in the first sample taken in April 2008. In the first week of June, 34 out of the 37 PCR positive calves were still available for resampling. Three calves were still PCR positive; one was five months old, the other two were three months old. One month later, in the first week of July, all initially PCR positive calves, including the three still tested positive one month earlier, were PCR negative. We showed that BTV-8 can be vertically transmitted from cow to calf and can result in healthy looking viraemic calves remaining PCR positive for up to five months. These PCR positive calves could play a role in the epidemiology, and in particular in overwintering of BT. However, further investigations are needed to evaluate the importance of this route of transmission.

*Keywords:* bluetongue, BTV-8; calves; vertical transmission

## 1. Introduction

In 2006, bluetongue virus serotype 8 (BTV-8) emerged in the Netherlands for the first time (BTV8/net2006 in Maan et al., 2008). The infection started in August and spread until November. In that year BTV-8 only spread in the southern part of the Netherlands and approximately 200 cattle herds notified clinical signs to the Food and Consumer Safety Authority (VWA) (Van Wuijckhuise et al., 2006; EFSA, 2007; Elbers et al., 2008). Overall, BTV-8 caused only limited morbidity and mortality during this first episode in 2006 (Elbers et al., 2008; Van Schaik et al., 2008).

In the spring of 2007, after the first BTV-8 episode in the Netherlands, a small scale study was performed to determine the possibility of overwintering of BTV-8 by vertical transmission. In this study 50 suckling cows and their newborn calves, originating from 14 BTV-8 infected herds, were tested for BTV-8. From the suckling cows 36 (72%; 95% CI: 59.6-84.4) tested seropositive and PCR negative in blood and colostrum. All 31 newborn calves were tested PCR negative, before and after colostrum intake (Van Wuijckhuise and Vellema, 2008). Based on these results, it was concluded that there was no or a small risk on vertical transmission to newborn calves.

However, in the third week of February 2008, one heifer and three calves from a group of 21 pregnant heifers imported from the Netherlands were tested BTV-8 PCR positive in Northern Ireland. Eight of these 21 heifers were BTV-8 seropositive, and all were PCR negative at the time of departure from the Netherlands and also at arrival in Northern Ireland. More than a month after arrival, one of the originally seronegative heifers tested PCR positive and three of four newborn calves from seropositive but PCR negative heifers were also tested PCR positive (Menzies et al., 2008). Based on these results and taken the *Culicoides*-free period into account, it was likely that BTV-8 was transmitted to the fetus after infection of the pregnant cow.

Recently, it was shown that experimental infection with BTV-8 of a cow in the final stage of pregnancy could result in the birth of a viraemic calf. In the same study, it was demonstrated that infection by BTV-8 could occur after uptake of BTV-8 contaminated colostrum (Backx et al., 2009). However, the relevance of transplacental and oral transmission for the epidemiology, the undesired movement of a possible BTV-8 reservoir, and in particularly the overwintering of BTV-8 was unknown.

Here we describe a study to determine the percentage of PCR positive calves born in the first quarter of 2008 from cows infected with BTV-8 in 2007. Subsequently, PCR positive newborn calves were resampled to determine the length of PCR- and ELISA-positivity.

## 2. Materials and Methods

### *2.1. Study population and data collection*

In the spring of 2008, dairy herds were selected from a region in the eastern part of the Netherlands in which massive spread of BTV-8 had occurred for the first time in September and October 2007 (Santman-Berends et al., 2009) (Figure 1). Based on an unknown expected prevalence of 50%, an accepted error of 5% and a confidence level of 95%, 400 cows and their newborn calves which were born in the first quarter of 2008 were blood sampled and BTV-8 PCR and serologically tested. The inclusion criteria for a cow-calf combination were that both the cow and her calf remained on the farm that was affected by BTV-8 in 2007. Furthermore, the calf had to be at least 10 days old and had to be born in the first quarter of 2008.

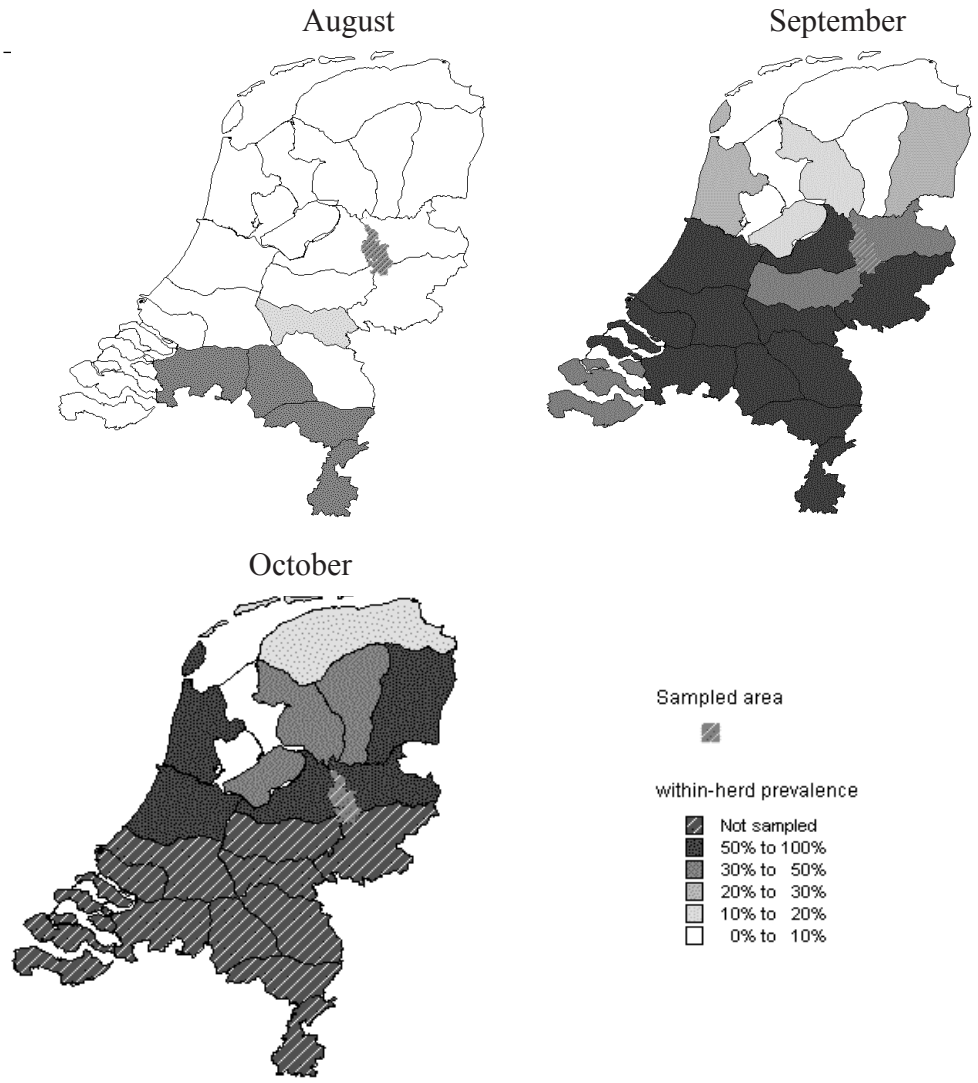


**Figure 1.** The Netherlands has been divided in 20 compartments. The within-herd prevalence per compartment in 2007 is indicated. The dairy herds selected for this study are located in the area indicated in blue.

Dairy herds should have at least eight calves that fulfilled the criteria to be finally selected (Figure 2). Fifty-five herds met these criteria and were contacted. Eventually 43 farmers were willing to cooperate in this study. From these 43 dairy herds, 388 cows and their calves were sampled for serum and EDTA-blood between March 27th and April 8th (Figure 2).

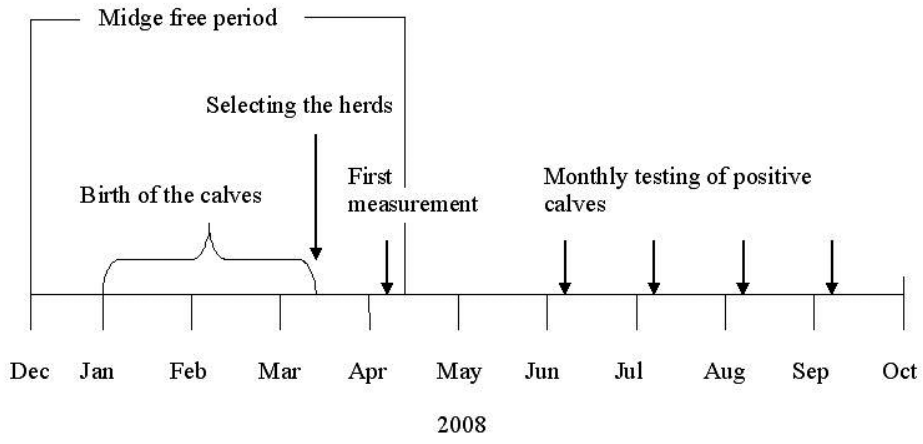


**Figure 2.** The timeline of the study in 43 Dutch dairy herds from January until September 2008. The period without proposed *Culicoides* activity (midge free period), the period of calving (birth of the calves) and the time points of sampling (arrows) are indicated.

Three combinations were excluded from the study because of improper samples. Eventually, 385 cow-calf combinations remained for analysis. Sera from cows and calves were tested for antibodies to BTV-8 with a competitive VP7 ELISA (Institut Pourquier, Montpellier, France). EDTA-blood samples were tested for BTV-8 with an in-house developed real time reverse transcriptase polymerase chain reaction assay (PCR test) (Van Rijn et al., 2009). If possible, PCR positive calves in the first sampling (Figure 2) were monthly resampled for serum and EDTA-blood, starting in June (Figure 2).

*2.2. Statistical analysis*

Proportion tests were used to determine the significance in difference between the number of cows and calves positive in ELISA or PCR test.

## 3. Results

### *3.1. Results of the first sampling*

From the 385 available combinations, 229 (8+221) cows (59.5%) and 234 (37+197) calves (60.8%) were seropositive (Table 1). Of these 229 seropositive cows, eight cows were PCR positive (3.5%), of which three had a relatively weak PCR-signal. Of the 234 seropositive calves, 37 calves were PCR positive (15.8%).

There was no significant difference between the number of seropositive calves and cows at first testing (proportion test; *P*-value: 0.77). However, the number of PCR positive calves was significantly higher compared to the number of PCR positive cows (proportion test; *P*-value:<0.001).

All 156 seronegative cows (40.5%; 95% CI: 35.6-45.4%) also were PCR negative. Fourteen calves from these seronegative cows (9.0%; 95% CI: 4.5-13.5%) were seropositive (Table 1).

**Table 1.** Results of the PCR test and ELISA on 385 cow-calf combinations tested between March 27th and April 8th , 2008.

| | PCR test | | Cows | | | | Total |
|---|---|---|---|---|---|---|---|
| | | ELISA | + + | + - | - + | - - | |
| Calves | + | + | 3 | 0 | 34 | 0 | 37 |
| | + | - | 0 | 0 | 0 | 0 | 0 |
| | - | + | 5 | 0 | 178 | 14 | 197 |
| | - | - | 0 | 0 | 9 | 142 | 151 |
| Total | | | 8 | 0 | 221 | 156 | 385 |

From 229 seropositive cows (59.5%; 95% CI: 54.6-64.4%), 37 (3+34) calves (16.2%; 95% CI: 11.4-21.0) were both PCR positive and seropositive. Eight of these seropositive cows were PCR positive. Their respective calves were all seropositive and three out of eight were also PCR positive (Table 1). The remaining 221 seropositive cows (57.4%; 95% CI: 52.5-62.3%) were PCR negative. From their 221 respective calves, 178 calves (80.5%; 95% CI: 75.3-85.8%) were seropositive and

PCR negative, however, 34 (15.4%; 95% CI: 10.6-20.1%) calves were positive in both tests and nine (4.1%; 95% CI: (1.5-6.8%) calves were negative in both tests (Table 1).

*3.2. Results of the follow-up period*

At first sampling, 37 out of 385 calves (9.6%; 95% CI: 6.6-12.6) were PCR positive (Table 1). These PCR positive calves were resampled monthly from June 2008 until the end of the study period in September 2008.

At second sampling in the first week of June, 34 calves were available for resampling; two had died for unknown reasons and one was no longer present on the farm. Three out of 34 initially PCR positive calves were still PCR positive (8.8%; 95% CI: 1.9-23.7) (Figure 3). From these three, one calf was born in January 2008 and the other two calves were born in March 2008.

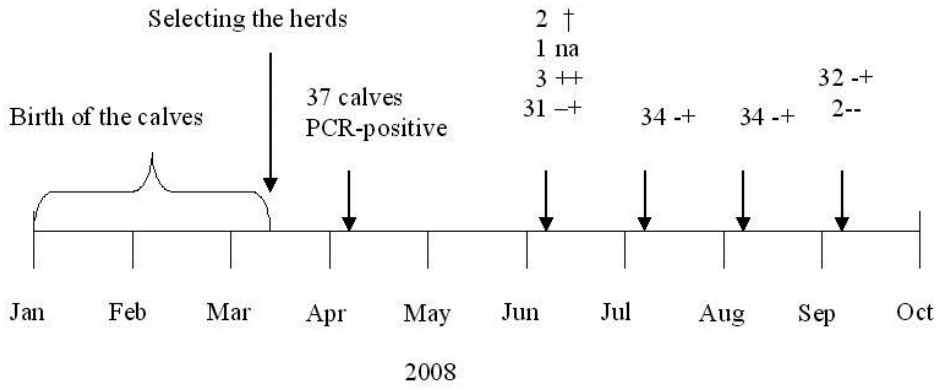


**Figure 3.** Results of the follow up period of PCR positive calves in Dutch dairy herds from April to September 2008.

† : dead

na : not available

++ : PCR positive and seropositive

-+ : PCR negative and seropositive

-- : PCR negative and seronegative

In the first week of July, all 34 initially PCR positive calves were PCR negative and remained PCR negative in following resamples (Figure 3).
All 34 initially PCR positive calves available for resampling were also seropositive in April. Of these 34 calves, 32 calves (94.1% 95% CI: 80.3-99.3) remained seropositive during the whole study period which was until September. Two initially PCR positive calves originating from different herds had no detectable levels of antibodies against BTV as determined by ELISA in September 2008 (Figure 3).

**4. Discussion**

In February 2008, BTV-8 seropositive but PCR negative heifers imported into Northern Ireland gave birth to PCR positive healthy looking calves during the *Culicoides*-free period, providing evidence of transplacental transmission of the virus (Menzies et al., 2008). Before this event, it was generally assumed that infection *in utero* by field strains of BTV could not result in healthy looking calves. *In utero* infection resulting in healthy looking calves seemed only possible for laboratory-adapted or vaccine strains (Osburn et al., 1971; Jochim et al., 1974; Waldvogel et al, 1992), whereas infection with field strains of BTV would result in abortion, malformed or weak calves, or dummy calves (De Clercq et al., 2008; MacLachlan and Osburn, 2008; Wouda et al., 2008; MacLachlan and Osburn, 1983). A small scale study performed in the Netherlands in the spring of 2007 (Van Wuijckhuise and Vellema, 2008), demonstrated that there was no or a small risk on transplacental transmission. Hence, it was unclear whether the findings of Menzies et al. (2008) were a common or rare observation and therefore a larger study was performed. From an area in the Netherlands which was heavily affected for the first time in 2007, 385 cow-calf combinations were selected in order to find cows which were likely to have been infected during gestation. In parallel to the observation in Northern Ireland, calves in this study were born in the first quarter 2008. From the 385 combinations selected for this study, 229 cows were seropositive at the end of March. This indicated that they were infected before calving, because the *Culicoides*-free period, which was based on outdoor temperatures and *Culicoides* activity, started the 13$^{th}$ of December 2007 and lasted until the 21$^{st}$ of April 2008. Furthermore, these cows probably were infected during gestation, as the area was first affected in 2007. Eight cows were still PCR positive at the end of March which is in agreement with previous observations that cattle can be PCR positive

for more than 200 days post infection. The calves from these eight PCR positive cows were all seropositive and three out of eight were also PCR positive. This latter finding indicated that BTV-8 was vertically transmitted from cow to calf, since birth and sampling occurred in the period without *Culicoides* activity. In other words, it is most unlikely that these newborn calves were infected by midges. From the remaining 221 calves, originating from BTV-8 infected cows, 178 were seropositive, but PCR negative, and 34 calves were PCR positive and seropositive. The first group of 178 seropositive calves can be explained by the uptake of colostral antibodies, or by seroconversion in combination with clearance of BTV-8.

In April 2008, the number of PCR positive calves (37) was significantly larger than the number of PCR positive dams (8). Apparently, the PCR-signal remained longer in the newborn calves than in the cows, the fetal infection was significantly delayed or the fetal infection occurred later with respect to that of the cow. Alternatively, these calves could be infected by uptake of colostrum contaminated with BTV-8 like this has been shown experimentally (Backx et al., 2009). There is no evidence that colostrum was contaminated by BTV-8, although colostrum can easily contain blood due to collapsed veins in the udder of freshly calved cows. Furthermore, nine calves from 229 seropositive cows were negative in both tests. Probably, these calves did not receive antibodies by insufficient uptake of colostrum with antibodies or by uptake of colostrum from a seronegative cow.

In total, 37 calves out of the 229 seropositive cows tested PCR positive in our study, indicating that the transplacental transmission rate in our study was 16.2% (95% CI:11.4-21.0). This rate is lower than the transplacental transmission rates found in the study of Galleau et al. (2009) and Batten et al. (2009). Galleau et al. (2009) tested 10 out of 24 calves (41.7%), born from seropositive dams, PCR positive within the first four days after birth. Batten et al. (2009) found 21 out of 61 calves (34.4%), born from seropositive dams, PCR positive within the first three days after birth. The difference between our study and theirs was that Batten et al. (2009) and Galleau et al. (2009) tested the calves within three or four days after birth, whereas the calves in our study could be up to three months old at the moment of sampling. Our study and the study of Batten et al. (2009) showed that the PCR positive calves became PCR negative during the time period of the study. Thus the 16.2% PCR positive calves we found in April 2008 is probably an underestimation of the real transplacental transmission rate, because calves born PCR positive, could have become PCR negative at the moment of first testing.

From the 156 PCR- and seronegative cows, 14 calves tested seropositive. The most logical explanation is that these calves were fed colostrum of a seropositive cow.
In our study, no acute infections were detected in the first sampling at the end of March, as there were neither cows nor calves PCR positive and seronegative. This is in agreement with the absence of *Culicoides* activity in the *Culicoides*-free period.

Fetal infection in the first three months of gestation has been assumed to result in abortion (De Clercq et al., 2008; MacLachlan and Osburn, 2008; Wouda et al., 2008; MacLachlan and Osburn, 1983). Fetal infection later in gestation could result in the birth of PCR positive calves up to six months after infection of the cow. At the time of birth, a new generation of *Culicoides* could be infected by feeding on these newborn viraemic calves and subsequently start spread of BTV-8 again after a *Culicoides*-free period. However, we were not able to cultivate virus from the samples of these calves with standard procedures, probably due to the presence of maternal neutralizing antibodies (data not shown). Nevertheless, the blood from the calves may be infectious to *Culicoides*. Mertens et al. (1996) showed that pretreatment of BTV-containing blood samples with serum or protease, increased the infectivity for *Culicoides* cells. We speculate that partial digestion in the gut of *Culicoides* could result in increased infectivity and thus in BTV-replication. In addition, in Dutch dairy herds about 10% to 25% of the newborn calves do not drink (sufficient) colostrum from their dams. In the absence of maternal antibodies, it was shown that BTV-8 could be easily cultivated from a viraemic newborn calf (Backx et al., 2009).

Our study also showed that calves could remain PCR positive up to five months of age. Although it is unknown whether PCR-positivity is related to infectivity of the midge and although the majority of the viraemic calves are PCR positive for only a short period of time, we emphasize that one or a few viraemic calves could induce the (re)start of BTV-8 circulation. Studies with species of *Culicoides* are needed to proof whether *Culicoides* can be infected by bites on these PCR positive calves. If so, BTV-8 could easily overcome a *Culicoides*-free period of 4-5 months by transmission to the fetus.
In seasons and/or in areas with a high level of *Culicoides* activity, vertical transmission will be of less or no importance for spread of BTV-8. However, in periods and/or areas without or with a very low level of *Culicoides* activity,

vertical transmission could become relatively important by lack of other routes of transmission. Further research will be needed to proof the significance of potential routes of transmission of BTV-8 in order to survive the winter in north-western Europe. Here we have shown that vertical transmission of BTV-8 in cattle is not rare and that, in the period without *Culicoides* activity, this mechanism could play an important role for the vector-borne disease bluetongue.

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Chapter 6

# Bluetongue virus serotype 8 (BTV-8) infection reduces fertility of Dutch dairy cattle and is vertically transmitted to offspring

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**Abstract**

In 2007, BTV-8 re-emerged for the second year in the Netherlands and caused morbidity and increased mortality in cattle herds. In addition, cattle farmers reported reduced fertility in their cows. For this study, fifteen herds that were not vaccinated were selected. These were matched to 10 vaccinated herds by geographic region. At the start of the study, in July 2008, all cattle in the non-vaccinated herds >1 year old were sampled. All seronegative cows entered the study program and blood samples from these cows were tested for antibodies against BTV-8 in an ELISA. Cows were sampled at intervals of three weeks and sampling was stopped once a cow tested seropositive. Sampling ceased in all remaining cows in December 2008. Newborn calves originating from infected dams or from vaccinated dams were tested by PCR for BTV-8. Fertility data were obtained from the Royal Dutch Cattle Syndicate (CRV). Multi-level generalized latent and linear models were used for analyses.

In 2008, 185 (17.2%) out of 1,074 initially seronegative non-vaccinated cattle seroconverted and were assumed to be infected with BTV-8. Infected cows were 5 (95% CI: 1.9-14.3) times more likely to return for insemination within 56 days after first insemination. In addition, these cows needed 1.7 (95% CI: 1.4-2.0) times more inseminations for an assumed pregnancy, and needed 2.5 (95% CI: 2.4-2.6) times more days between first and last insemination compared to the period prior to seroconversion and compared to cows not infected by BTV-8 in 2008. No association between BTV-8 infection and the chance to abort between 100 and 260 days after last insemination was found.

In total, 48 calves originating from infected cows were tested by PCR for the presence of BTV-8. Ten (20.8%) out of these 48 calves were born PCR-positive. None of 256 calves from vaccinated dams tested PCR-positive. Further, cows infected during the second half of gestation had a 15.5 times (95% CI: 1.3-190.4) higher chance of a PCR-positive newborn calf compared to cows infected in the first half of gestation. This study showed that BTV-8 has a negative effect on fertility of dairy cattle.

*Keywords*: bluetongue; vertical transmission; fertility; cattle

## 1. Introduction

Bluetongue virus serotype 8 (BTV-8) emerged for the first time in the Netherlands in 2006 (Van Wuijckhuise et al., 2006) In that year, 200 cattle farmers notified clinical signs of BTV-8 in their herds to the Food and Consumer Product Safety Authority (VWA). In 2006, symptoms of BTV-8 in cattle were mild and except for a small increase in abortions and stillbirths (Elbers et al., 2008), no effect of BTV-8 was found on reproductive parameters in cattle.
In July 2007, BTV-8 started to spread again in the Netherlands (Santman-Berends et al., 2010a). In that year, BTV-8 infected thousands of cattle herds. Symptoms of BTV-8 in cattle seemed more severe and farmers notified more fertility problems in their herds Elbers et al., 2006; Wouda et al., 2009) compared to 2006. However, the conclusion of increased fertility problems was mainly based on farmers' opinion. Except for an increased probability of abortions (Osburn, 1994; MacLachlan et al., 2000), to our knowledge, fertility problems in cows related to BTV based on objective data have not yet been published. From field data, it appeared that BTV-8 infection during gestation could lead to vertical transmission (De Clercq et al., 2008; Menzies et al., 2008; Batten et al., 2009; Galleau et al., 2009; Wouda et al., 2009, Santman-Berends et al., 2010b). There is no information available whether the moment of BTV-8 infection during gestation is associated with the probability of delivering a BTV-8 PCR-positive calf. In one experimental study, the birth of a viraemic calf due to vertical transmission in late gestation was shown, however, this single calf only lived for two days (Backx et al., 2009).
In this study, we describe the influence of BTV-8 infection on the fertility in cattle, and the effect of BTV-8 infection during gestation and vaccination on the BTV-8 status of the offspring.

## 2. Materials and Methods

### *2.1. Sample size considerations*

For this study, the aim was to obtain 200 cows that seroconverted, of which about 100 cows would be pregnant at seroconversion. This would ensure that, the proportion of PCR-positive calves could be estimated with 95% confidence and a precision of at least 10%. Assuming that 20% of the cows would seroconvert in 2008, a sample size of 1,000 seronegative cows in non-vaccinated was estimated. In

vaccinating herds, the aim was to obtain 300 samples from newborn calves to be able to show with 95% confidence that the proportion of PCR-positive calves was below 1%.

*2.2. Study population*

In 2008, the Dutch government implemented a voluntary vaccination program starting in May. Between January and May 2008, 15 dairy herds were selected of which the owners had decided not to vaccinate their cattle against BTV-8. In addition, 10 dairy herds in the same regions with an average distance to the closest case herd of 7.2 kilometers were selected of which the owners had planned to vaccinate their cattle against BTV-8 in 2008. Matching by geographic region was done to obtain information on the infection pressure of BTV-8 in vaccinated herds based on the transmission in the non-vaccinated herds in the same region.

At the start of the study in July 2008, all dairy cattle >1 year present in non-vaccinated herds were tested for BTV-8 antibodies in their blood and all 1,074 seronegative cows entered the study for being tested every three weeks between July and December 2008. When non-vaccinated pregnant cows seroconverted in the study period, BTV-8 infection was assumed, and their afterwards delivered calves were tested by PCR for BTV-8 shortly after birth. In addition, the reproductive performance of the vaccinated dairy cattle was followed during the BTV-8 period in 2008. Moreover, in the period of July-December 2008 samples of all newborn calves originating from vaccinated dams were PCR-tested for BTV-8. From January to June 2009, monthly samples of two or three newborn calves originating from vaccinated dams were tested by PCR for BTV-8.

*2.3. Study design*

After the first sampling in July 2008, blood samples from all 1,074 seronegative cows were collected every three weeks for antibody testing using a commercial competitive ELISA test (Pourquier, France). Sampling was ceased after a cow tested seropositive, assuming it to be infected, or at the end of the study period in December 2008, when the vector activity free period began. If a pregnant cow seroconverted, the expected calving date was obtained from the Dutch Royal Cattle Syndicate (CRV) and this date together with the unique identification number (ID) of the seroconverted cow, was sent to the veterinary practitioner involved. Shortly after birth, EDTA-blood samples were taken from these calves by the veterinary practitioner and were sent to the Central Veterinary Institute (CVI). At the CVI,

EDTA-blood samples were tested for BTV-8 by an in-house developed serogroup-specific PCR-test based on S10 of BTV.
Calves from seroconverted cows were born between September 2008 and June 2009. In the same period, every month, newborn calves from vaccinated cows were also sampled and tested in the PCR for BTV-8.

*2.4. Data description*
In July 2009, after all calves from seroconverted cows and a number of calves from vaccinated cows were born, fertility data of all cows in the vaccinated and non-vaccinated herds were obtained from CRV. This fertility data consisted of fertility indicators per cow per parity between 2005 and August 2009.
Eventually, three different data sets were available for this study:

1. data of cows in non-vaccinated herds containing unique herd identification (UHI), cow ID number, sampling date of seroconversion
2. newborn calves originating from both vaccinated and non-vaccinated herds containing UHI, calf ID number, dam ID number, sampling date, PCR-result
3. fertility data containing UHI, cow ID numbers, parities, insemination dates, calving dates

The three data sets were combined. All cows in the non-vaccinated herds included in the study program and all cows in vaccinated herds were assigned to one of three mutually exclusive BTV-8 categories: 1) cows in vaccinated herds were assigned to status 'V', vaccinated, 2) cows in non-vaccinated herds that remained seronegative throughout the study period (between July and December 2008) were assigned to status 'S', susceptible, and 3) cows in non-vaccinated herds that seroconverted during the study period were assigned to status 'I', infected.
The cattle in non-vaccinated herds that already tested seropositive at the first measurement were excluded from the analysis.
Four reproductive parameters were defined that covered different aspects of the reproductive performance and for which complete data were available: 1) non-return at 56 days after 1st insemination (i.e. a cow without return to service within 56 days after the 1st insemination was given the value 1. A cow re-inseminated within 56 days after the 1st insemination was given the value 0), 2) number of inseminations per assumed pregnancy, 3) days between first insemination and

insemination when the cow was assumed to be pregnant, and 4) abortion between 100 and 260 days after the last insemination.

*2.5. Statistical analyses*

Descriptive analyses were performed to describe the test results of cows in the non-vaccinated herds between July and December 2008. BTV-8 incidences in these herds were calculated per month and were compared using univariate analyses.

Regression analyses were used to determine the relation between BTV-8 seroconversion and reproductive parameters during the BTV-8 epidemic of 2008. Multi-level models were used to correct for repeated measurements within herds (multiple cows) and within cows (multiple parities). A multi-level Generalized linear Latent and Mixed Model (GLLAMM) in Stata version 10 (Stata, 2007) was used, which can be described as:

$$Y_{ij} = \mu_{ij} + \beta_1 PI_{ij} + \beta_2 yr_{ij} + \beta_3 S_{ij} + C_i + H_j + \varepsilon_{ij}$$

In which:

| | | |
|---|---|---|
| $Y_{ij}$ | = | Dependent variable for each cow (i) in each herd (j): non-return yes/no; number of inseminations; days between first and last insemination; abortion yes/no |
| $\mu_{ij}$ | = | Intercept for each cow (i) in each herd (j) |
| $\beta_1 PI_{ij}$ | = | Variable of interest for each cow (i) in each herd (j): the period in which non-vaccinated cows seroconverted (1) (1=Status P in 2008); cattle that did not seroconvert in 2008 (2) (2=Status V and S in 2008 and status: V, S, I in 2005, 2006, 2007, 2009) |
| $\beta_2 yr_{ij}$ | = | Year 2005 (for reproductive parameters: non-return, number of inseminations, and time between first and last insemination), 2006 until the first quarter of 2009 (all parameters) for each cow (i) in each herd (j). |
| $\beta_3 S_{ij}$ | = | BTV-8 status for each cow (i) in each herd (j): V, S or I |
| $C_i$ | = | Random cow effect |
| $H_j$ | = | Random herd effect |
| $\varepsilon_{ij}$ | = | Random error for each cow (i) in each herd (j) |

For the analyses of abortions and non-return a binomial distribution with a logit-link function was used and for the analyses of the number of inseminations and time between first and last insemination a poisson distribution with a log-link function was used. The variable of interest was $PI_{ij}$. This variable showed the level of fertility (equal, better or worse) in the period that cows were infected compared to uninfected cows, and compared to the period prior to seroconversion.
Reproductive parameters could have been influenced by BTV-8 if the cow was infected in the period in which it was inseminated (non-return, time between first and last insemination, number of inseminations) or if the cow was infected during gestation (abortion).
Frequency tables and univariate analyses methods (Fisher's Exact test, 1922; Kruskal-Wallis test, 1952) were used to describe the PCR results of the calves originating from infected dams and to compare these results to the PCR-results from calves originating from vaccinated dams. In addition, GLLAMM was used to determine if the PCR status of the newborn calf was correlated with the time of infection during gestation. A binomial distribution with a logit link-function was used for the analysis. The model can be described as:

$$PCR_i = \mu_i + per_i + H_i + \varepsilon_i$$

In which:

| | | |
|---|---|---|
| $PCR_i$ | = | PCR result of the calf in each herd (i): 1 (PCR-positive) or 0 (PCR-negative) |
| $\mu_i$ | = | Intercept for each herd (i) |
| $per_i$ | = | Period of gestation in which the cow seroconverted in each herd (i): 1) between last insemination and the fourth month of gestation or 2) between the fifth month of gestation and the time of birth |
| $H_i$ | = | Random herd effect |
| $\varepsilon_i$ | = | Random error for each herd |

## 3. Results

*3.1. Descriptive results*

For this study, 10 vaccinated herds and 15 non-vaccinated herds were selected. The average herd size was 109 cows >1 year (95% CI: 76-143) in vaccinated herds and 112 cows >1 year (95% CI: 88-137) in non-vaccinated herds.

In July 2008, 1,175 cows older than one year were sampled in non-vaccinated herds and 91% (1,074) were seronegative. From July to December 2008, 185 cows out of 1,074 cows (17.2%) seroconverted, which indicates that these non-vaccinated cows were infected by BTV-8. The highest percentage of these 185 cows (7.1%; N=72) seroconverted in September (Figure 1).

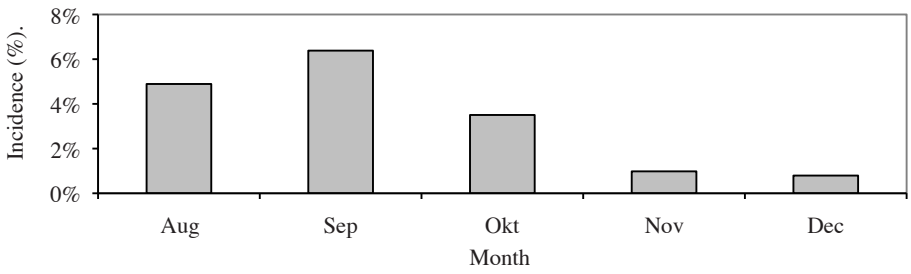


**Figure 1.** Percentage of seroconversion for bluetongue virus serotype 8 (BTV-8) per month in 185 non-vaccinated dairy cows in 2008.

The BTV-8 incidence in August, September and October was significantly higher compared to the BTV-8 incidence in November and December ($P$-value<0.01). In addition, the BTV-8 incidence in September 2008 was higher compared to the incidence in October ($P$<0.01).

In the month with seroconversion between the two samplings, 80 out of 185 cows were pregnant, 32 had just delivered a calf, and 31 cows were in the process of insemination. From the remaining 42 cows, fertility data were 1) incomplete because part of these cows were removed in 2009, or 2) were incomplete because of other reasons than reduced fertility.

*3.2. Results reproductive parameters*

Fertility results were graphically displayed on herd level, but no significant difference in fertility could be seen between vaccinated and non-vaccinated herds

during the period in which BTV-8 infections occurred. After dividing the cows in the non-vaccinated herds over the two different statuses S and I, there appeared to be an effect of BTV-8 seroconversion on three out of four reproductive parameters that were analyzed (non-return, number of days between first and last insemination and number of inseminations). Figure 2 shows the pattern for non-return for the different status groups. In Table 1, the results of the multi-level models for all four parameters are provided. BTV-8 seroconversion in 2008 did not seem to have an effect on abortions (Table 1).

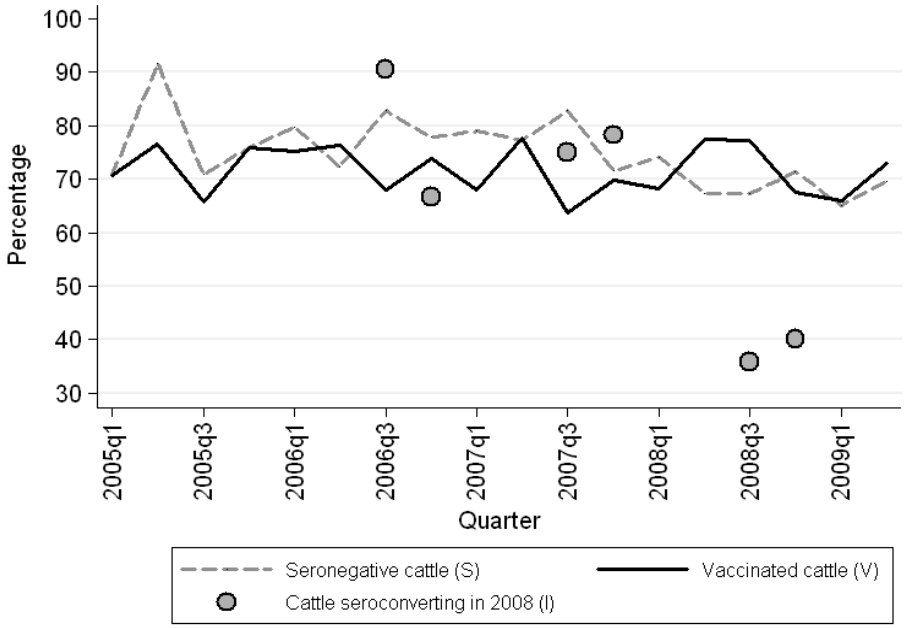


**Figure 2.** Non return rate (NR in %) for non-vaccinated cattle that remained seronegative (S), for non-vaccinated cattle that seroconverted and thus became infected during 2008 (I) in the quarters of seroconversion (3th or 4th quarter 2008) and in the previous years (3rd and 4th quarter of 2006 and 2007) and the NR for vaccinated cattle (V) per quarter from the first quarter in 2005 to the second quarter of 2009.

**Table 1.** Influence of BTV-8 infection on fertility, represented by the average percentage of non return, days between first and last insemination, number of inseminations, and percentage abortions in 2008.

| Reproductive parameter | Seroconv. cows prior to seroconversion in 2005-2007 | Status in 2008[1] **Seroconv.** | Seroneg. | Vacc. | *P*-value (Wald) |
|---|---|---|---|---|---|
| Non return (%) | 77.1 | **37.9** | 70.2 | 71.8 | <0.01 |
| Number of inseminations | 1.5 | **3.4** | 1.8 | 1.8 | <0.01 |
| Time between first and last insemination (days) | 26.4 | **128** | 39 | 35 | <0.01 |
| Abortions (%) | 11.1 | **9.5** | 11.0 | 9.6 | 0.75 |

[1] Vacc=vaccinated; Seroneg=not vaccinated and remained seronegative during the studied period; Seroconv=not vaccinated and seroconverted in 2008

The results of our multi-level model showed that cows that seroconverted (I) during the 2008 episode in herds that were not vaccinated had a 5.0 times (95% CI: 1.9-14.3) higher chance to return to service after the first insemination (NR=0) in 2008 compared to cows that did not seroconvert in that same year and compared to prior years (*P*-value<0.01). Furthermore, cows that seroconverted in 2008 (I) needed 1.7 times (95% CI: 1.4-2.0) more inseminations in the period in which they seroconverted (2008) compared to cows in status V or S and compared to the previous period (*P*-value<0.01). Finally, the results of our multi-level model showed that cows that seroconverted in 2008 (I) needed 2.5 times (95% CI: 2.4-2.6) more days before they appeared to be pregnant (not returned to service within 56 days after last insemination) compared to cows in status V and S and compared to the prior years (*P*-value< 0.01).

We found no difference in abortion percentage between the different statuses in the different periods of time (*P*-value=0.75).

*3.3. Results PCR-testing calves*

Out of 185 non-vaccinated, infected cows, 80 cows were pregnant at the time of BTV-8 infection. From these 80 cows, 67 gave birth to a calf in the study period, seven cows aborted and six calves were lost for the study. Samples from 48 calves were submitted for PCR testing since samples of 19 calves were missing due to several reasons. These missing samples were equally divided over the non-vaccinated herds and are assumed not to influence the interpretation of the observed results.

From the 48 PCR-results originating from calves that were delivered by a dam that became infected during the 2008 BTV-8 episode, 10 tested PCR-positive (20.8%; 95% CI: 9.3-32.3%) (Table 2).

Furthermore, samples from 21 calves that originated from dams that did not seroconvert during the study were submitted for PCR-testing. From the samples of these calves, eight originated from cows that remained uninfected (seronegative) and 13 calves originated from dams that had already been infected with BTV-8 before the start of the study (seropositive). One calf, out of the eight cows that remained uninfected during the 2008 BTV-8 episode, tested BTV-8 PCR-positive. None of the calves originating from cows already BTV-8 infected before the study tested PCR-positive (Table 2).

**Table 2.** BTV-8 PCR-results (number and percentage) of calves born between September 2008 and June 2009 relative to the BTV-8 status of their dam

| | | Dams[1] | | | Total |
|---|---|---|---|---|---|
| | | Seroconv. | Seroneg. | Seropos. | |
| Calves | BTV-8 PCR-positive | 10 (21%) | 1 (12%) | 0 (0%) | 11 (16%) |
| | BTV-8 PCR-negative | 38 (79%) | 7 (88%) | 13 (100%) | 58 (84%) |
| Total | | 48 | 8 | 13 | 69 |

[1] Seroconv=cows that became infected with BTV-8 during 2008; Seropos=cows that were infected with BTV-8 before the study in 2008; Seroneg=cows that remained uninfected from July-December 2008

The 10 PCR-positive calves were tested on average 5.1 days after they were born (95% CI: 1.9-8.3; minimum 0 days-maximum 14 days). The calves that tested PCR-negative were tested on average 7.6 days after birth (95% BI: 5.7-9.4; minimum 0 days-maximum 24 days). The difference in time from birth to PCR-testing was not significant between both groups (Kruskall-wallis: *P*-value=0.20). Five out of ten PCR-positive calves originating from seroconverted cows (status I) were followed in time. All five calves tested PCR-negative after approximately four months. From the cows that remained seronegative (status S), one calf tested PCR-positive for BTV-8. This calf was born on the 16th of August and was tested PCR-positive 46 days later on the 1st of October.

None of the 256 calves that originated from vaccinated dams tested PCR-positive (0%; 95% CI: 0-1.4).

The cows that delivered PCR-positive calves seroconverted mainly during the second half of their gestation (Figure 3).

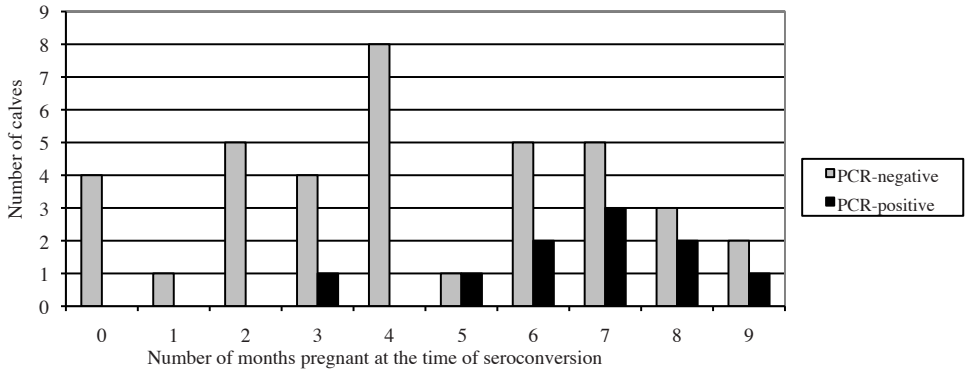


**Figure 3.** The month of gestation in which dams that delivered a BTV-8 PCR-positive or negative calf showed a seroconverted blood sample for BTV-8 in 15 non-vaccinated Dutch dairy herds in 2008.

Cattle that seroconverted in the second half of gestation (5th month of gestation until birth) had a significantly higher chance (15.5 times (95% CI: 1.3-190.4) of delivering a calf that tested PCR-positive at birth compared to cattle that seroconverted in the first half of gestation (last insemination until the 4th month of gestation).

**4. Discussion**

In 2008, 15 herds that were not vaccinated for BTV-8 were selected. At the start of the study, all cows >1 year old in these 15 herds were tested for bluetongue by ELISA and 91% of the cows still were seronegative and could be included in the study. The high percentage of seronegative cattle could be explained by the location of the herds. The non-vaccinated dairy herds in our study were mainly located in the north, which was just infested by BTV-8 at the end of 2007 (Santman-Berends et al., 2010a).

We analyzed four reproductive parameters, i.e. non-return, days between first and last insemination, number of inseminations and abortions, which are generally used to represent the reproductive performance and of which data were complete. Other parameters related to fertility of cattle, such as time between calvings, regular versus irregular return to service, age at first calving etc., were not analyzed in this study because they were superfluous or because the data of these parameters were incomplete.

With a total of 185 seroconverted cows in our study, the effect of BTV-8 on the reproductive parameters was based on a relatively small number of observations. However, the data provided evidence that a BTV-8 infection influenced three reproductive parameters, i.e. non-return, number of inseminations and days between first and last insemination. In agreement with our results, Elbers et al. (2009) found that farmers reported an increased chance of cows returning to service in 2007 in infected herds. In our study we could obtain more precise estimates by taking the precise moment on which individual cows seroconverted for BTV-8 into account.

We found no correlation between BTV-8 infection and abortion. In contrary, Elbers et al. (2008, 2009) did find an increased abortion rate in the Netherlands due to BTV-8 in 2007 (Elbers et al., 2006; Elbers et al., 2008). Furthermore, reports of studies in other countries affected by other BTV serotypes have also shown increased abortion rates (Osburn, 1994; MacLachlan et al., 2000). Possibly, the

relatively small number of dams that got infected during gestation (80 cows of which seven aborted) was too small to observe a relation between BTV-8 infection and abortion. Another explanation could be the standard definition of an aborted cow in the Netherlands, which was used in this study; re-insemination after a minimum of 100 days or delivery of a calf within 260 days after last insemination (CRV, 2009). Thus, if a cow returned to service between 46 and 100 days after the last insemination, we would have assigned an extra insemination instead of calling it an abortion. Elbers et al. (2008, 2009) defined abortion as return to service after >46 days after the last insemination (Elbers et al., 2006; Elbers et al., 2008).

The reduced fertility associated with BTV-8 may be slightly overestimated because in our model all reduced fertility in the BTV-8 period was attributed to BTV-8, while there could have been other causes in the same period that could have reduced fertility. However, in that period, no other reasons for a reduced fertility were known. Furthermore, vaccinated cows and cows that were housed in the same herds as the serocoverted cows and remained seronegative did not show reduced fertility during the BTV-8 period in 2008."

The results of the multi-level models showed that on average, more variation was explained on herd-level than on cow-level. This was to be expected because it is known that the management of the farmer plays an important role in the fertility performance of his cattle. The random-herd effect correct for the difference in management between herds.

The effect of BTV-8 on fertility was estimated in models with and without the random cow and herd effect. The model showed a better fit when random effects were included. The estimates from the models with and without random effects differed, but the conclusions from both models were the same.

The percentage PCR-positive calves that were found in this study was similar to that in our previous study in 2007 in which 16.2% (95% CI: 11.4-21.0%) of the calves tested PCR-positive (Santman-Berends et al., 2010b), but was smaller compared to others (Batten et al., 2009; Galleau et al., 2009). Batten et al. (2009) studied 61 calves, blood sampled within three days after birth, originating from dams BTV-8 infected during the 2007 epidemic. From these calves, 21 (33% (95% CI: 22.7-47.7%) tested PCR-positive. In the study of Galleau et al. (2009), 10 out of 24 calves (41.7%, 95% CI: 22.1-63.4) originating from BTV-8 infected dams tested PCR-positive. While 21 calves originating from vaccinated dams all tested PCR-negative. This last result is in agreement with our finding of no PCR-positive calves originating from 256 dams.

One calf from a seronegative dam was tested PCR-positive. This calf was sampled 46 days after birth, and could have been naturally infected. All PCR-positive calves (10) originating from BTV-8 infected dams were sampled within 14 days after birth. So, the chance of infection by bites of BTV-8 infected *Culicoides* midges in those calves was very small, and thus vertical transmission during gestation is the most likely cause of infection.

Infection by BTV-8 in the second half of gestation resulted in a higher chance of birth of a PCR-positive calf compared to infection in the first half of gestation. This finding was in agreement with the study of Batten et al. (2009) who also observed higher percentages of PCR-positive calves when BTV-8 was transplacentally transmitted later in gestation. It is possible that infections in the first months of gestation, before immunocompetence, can result in malformations, and death of the fetus (Osburn, 1994; MacLachlan et al., 2000). Consequently, it is likely that infection of dams early in gestation will result in abortion or return to service, but not in the delivery of a PCR-positive, healthy looking calf. Indeed, our study showed that BTV-8 infection is correlated with more inseminations needed for pregnancy.

BTV-8 infection in 2008 had a negative effect on fertility in dairy cows. The chance of a non-return was significantly lower, and BTV-8 infected cows needed significantly more inseminations to become pregnant. Furthermore, cows infected in the second half of gestation had a significantly higher chance of delivering a BTV-8 PCR-positive calf compared to cows infected in the first half of gestation.

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Chapter 7

# The Effect of Bluetongue Virus Serotype 8 on Milk Production and Somatic Cell Count in Dutch Dairy Cows in 2008

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**Abstract**

This study quantified the effect of BTV-8 infection on milk production and udder health. From July 2008 on, 1,074 seronegative cows in 15 herds that were not vaccinated against BTV-8 were tested every three weeks for BTV-8 antibodies. Sampling stopped when cows seroconverted and was ceased in all cows in December 2008. Test-day records were provided and three parameters were defined to evaluate the effect of BTV-8 on milk production and udder health; 1) the difference between observed and predicted fat and protein corrected milk production, 2) the natural logarithm of the SCC (lnSCC) and, 3) the occurrence of a new high SCC (HSCC). In the default model, the parameters were assumed to be potentially influenced by BTV-8 when the test-day record of the seroconverted cow was taken within 30 days prior to seroconversion, thus in the period in which the cow was infected. In sensitivity analyses, the time intervals in which BTV-8 was assumed to affect milk production and udder health were varied. During the study, 185 cows (17%) had a subclinical infection and seroconverted and 77 had a test-day result within 30 days prior to seroconversion. In this period, in cows that seroconverted, the fat and protein corrected milk production was 52 (95% CI: 26-77) kg less than in the period before and after seroconversion and was 51 (95% CI: 26-76) kg less than in cows that remained seronegative. When the time interval was increased to within 42 days prior to seroconversion, the milk production in BTV-8 seroconverted cows decreased with 61 (95% CI: 28-94) kg compared to the period before and after seroconversion and decreased with 59 (95% CI: 27-92) kg compared to cows that remained BTV-8 seronegative.
BTV-8 infection increased the lnSCC with 0.13 (95% CI: -0.08-0.34), compared to the period before and after seroconversion and with 0.20 (95% CI: -0.05-0.45) compared to cows that remained seronegative. In BTV-8 infected cattle the occurrence of a new HSCC increased with an odds ratio of 1.3 (95% CI: 0.6-2.6) compared to the period before and after seroconversion and with an odds ratio of 1.4 (95% CI: 0.7-2.8) compared to cows that remained seronegative.
BTV-8 infection in dairy cattle resulted in a significantly reduced milk production. In BTV-8 infected cows a non-significant increase of lnSCC and of new high SCC cows was found.

*Key words*: bluetongue, cows, milk production, SCC

## 1. Introduction

Historically, bluetongue did not occur in North-Western Europe, but in August 2006, bluetongue virus serotype 8 (BTV-8) emerged for the first time (Van Wuijckhuise et al., 2006). In that first year, clinical signs in cows seemed mild (Elbers et al., 2008) and dairy herds located in BTV-8 affected regions showed a slight decrease in 305-day milk production compared to the period before BTV-8 (Van Schaik et al., 2008). However, in 2007, BTV-8 reemerged, spread over a larger area and infected many herds (Santman-Berends et al., 2010a). In that year, clinical signs in cows appeared more severe and besides a higher morbidity, farmers reported reduced milk production, increased mastitis cases and increased somatic cell count (SCC) in BTV-8 infected cows (Elbers et al., 2009).

An association between BTV-8 and udder health is expected because BTV-8 replicates in endothelial cells, causing cell injury to small blood vessels. This may, among others, lead to vascular thrombosis, tissue infarction, erythema and necrosis of the udder skin and ulceration of the teats (Dercksen and Lewis, 2007; Williamson et al., 2008; Darpel et al., 2009). These lesions may lead to increased mastitis incidence and reduced milk production. Furthermore, BTV-8 infected cows can develop fever as one of the clinical signs, which can also lead to increased SCC and decreased production. Although, correct estimations of production losses are important to determine economic losses as a result of a BTV-8 infection, to our knowledge, no quantitative estimates about reduced milk production and mastitis as a result of BTV-8 infection on cow level are available in literature.

This paper presents the effect of BTV-8 infections on milk production and SCC in dairy cows.

## 2. Material and Methods

### *2.1. Study Population and Period*

For this study, the aim was to obtain data of 200 cows that seroconverted. Assuming that 20% of the seronegative cows would seroconvert in 2008, a sample size of 1,000 seronegative cows that were not vaccinated was required. In 2008, a voluntary vaccination program against BTV-8 was implemented in the Netherlands. At the start of the study in July 2008, 15 dairy herds of which the farmers decided not to vaccinate against BTV-8 were selected. All herds had Holstein Frisians in a loose

housing system with an average herd size of 109 cows older than one year.
All dairy cows >1 year present in the 15 herds (N=1,175) were tested for Bluetongue virus antibodies in blood using a commercial competitive ELISA test (Pourquier, Montpellier, France) with a sensitivity of 100% and a specificity of 99.8% (based on an unpublished internal validation at the animal health service in line with Kramps et al., 2008).
The 1,074 cows that tested seronegative for BTV-8 entered the study, and were subsequently tested every three weeks between July and December 2008. When cows seroconverted in this period, BTV-8 infection was assumed and further sampling was stopped. In the cows that remained seronegative throughout the study period, sampling was ceased in December 2008 when the vector activity free period began.

*2.2. Data Collection*

Milk production and SCC data from January 2006 until June 2009 of all cows in the 15 herds were provided by the Dutch Royal Cattle Syndicate (CRV). This data described four to six-weekly test-day results of every cow in lactation and contained the following information:

- Herd level: Unique herd identification (UHI)
- Cow level: ID number, date of the start of the lactation, parity
- Test-day level: date, number of days in lactation, delivered kg milk, delivered kg fat, delivered kg protein, SCC, predicted kg milk, predicted kg fat, predicted kg protein.

The complete dataset contained 18,689 test-day results of 1,074 cows with on average 21.5 (min. 1; max. 42) test-day results per cow. The data provided by CRV was combined with the serology results from the study, which contained results of 6,365 blood samples with on average 5 samples per cow (min. 1; max. 7).

*2.3. Milk Production and Somatic Cell Count*

For the analysis of the effect of BTV-8 on milk production and udder health, three parameters were analyzed; 1) the difference between observed and predicted fat and protein corrected milk production (*FPCM*) ($\Delta FPCM$), 2) the SCC and, 3) occurrence of a new high SCC (HSCC).
The predicted milk production is a standard parameter in the Netherlands and is calculated by CRV for each cow for every test-day. This parameter is corrected for

the observed 305 day milk production of the cow in the previous lactation (when parity >1), the predicted 305 day milk production in the previous lactation (when parity >1), the observed production on the prior test-day, the number of days in lactation, and parity (CRV, 2010a). For the analysis of milk production, the amount of milk delivered and predicted was corrected for the percentage fat and protein (*FPCM*) according to the following formula:

$$FPCM = 0.337 + (0.116 * \%fat) + 0.06 * \%protein * kg\_milk \quad [1]$$

Thereafter, Δ*FPCM* was calculated as the difference between the observed and predicted *FPCM*.

For the effect of BTV-8 on SCC, the natural log (lnSCC) was used. In addition, the probability of the occurrence of a new HSCC was analyzed in which a case of HSCC was defined as: 1) an increase in SCC from <150,000 cells/ml to >150,000 cells/ml in a heifer and 2) an increase in SCC from <250,000 cells/ml to >250,000 cells/ml in a multiparous cow. This definition was based on the currently used cut-off levels in the Netherlands (Sampimon et al., 2010).

*2.4. Statistical Analyses*

Descriptive analyses were performed on the data of the cows that remained seronegative and of those that seroconverted. In our study all cows>1 year were monitored for seroconversion. However, to study the effect of BTV-8 infections on milk production and SCC, only the data of lactating cows were included in the analyses.

The dependent parameters Δ*FPCM* and lnSCC were both normally distributed and new HSCC had a binomial distribution (yes=1 or no=0). For the analyses of Δ*FPCM* and lnSCC, multi-level linear mixed models with a Gaussian distribution and an identity-link function were used. For the analysis of the effect of a BTV-8 infection on a new HSCC a multi-level logistic regression with a logit-link function in Stata version 11 (Stata, 2009) was used.

For the analysis, all cows present in the studied herds were assigned to one of two mutually exclusive categories based on their status for BTV-8. Cows that remained seronegative throughout the study period between July and December 2008 were assigned to status 'S', susceptible, and cows that seroconverted during the study period were assigned to status 'I', infected.

The variable of interest was $AFP_{ij}$ (affected period), which gave an estimate of Δ*FPCM*, lnSCC and new HSCC for seroconverted cows around the moment of BTV-8 infection. This variable was estimated for three classes of cows, 1) cows that were infected with BTV-8 during the study in the period that was assumed to be influenced by BTV-8, 2) the same cows sampled either before or after the period they were assumed to be influenced by BTV-8, and 3) cows that remained seronegative during the whole study period. This division in categories was made to be able to compare the seroconverted cows in the affected period to themselves before and after BTV-8 infection and to cows that remained seronegative.

In the model, corrections were made for possible confounding factors such as year, BTV-8 status, parity, days in lactation and for clustering of observations within herds and cows.

Based on literature (Guyot et al., 2008; Dal Pozzo et al., 2009), it was assumed that BTV-8 infection occurred on average between 35 and 15 days prior to the date that seroconversion was detected. Therefore, a test-day measure was assumed to be influenced by BTV-8 when it was taken within 30 days prior to the date on which seroconversion was measured (Figure 1).

The models that were used can be described as:

$$Y_{ij} = \mu_{ij} + \beta_1 AFP_{ij} + \beta_2 yr_{ij} + \beta_3 mnd_{ij} + \beta_4 par_{ij} + \beta_5 dlac_{ij} + C_i + H_j + \varepsilon_{ij} \quad [2]$$

In which:

$Y_{ij}$ = Dependent variable for each cow (i) in each herd (j): Δ*FPCM*, lnSCC or HSCC

$\mu_{ij}$ = Intercept for each cow (i) in each herd (j)

$\beta_1 AFP_{ij}$ = Variable of interest for each cow (i) in each herd (j): test-day of seroconverted i.e. infected cows (I) that were affected by BTV-8 (1: seroconverted), test-day measures of seroconverted cows (I) prior to or post seroconversion (2: serocon. pre/post), thus between 2006 and June 2008 and after December 2008, and test-day measures that were not affected by BTV-8 because cows remained seronegative throughout the study (3: seroneg).

$\beta_2 yr_{ij}$ = Year 2006, 2007, 2008 or 2009.

$\beta_3 mnd_{ij}$ = Month 1, 2, 3…10, 11, 12.

$\beta_4 par_{ij}$ = Parity 1 (heifer), 2 or >2 for each cow (i) in each herd (j) for the

analyses of SCC and odds for a new HSCC. Parity was not included in the Δ*FPCM* model because the predicted FPCM was already corrected for parity.

$\beta_5 dlac_{ij}$ = Days in lactation (0-60, 60-120, >120) for each cow (i) in each herd (j) for the analyses of SCC and odds for a new HSCC. Days in milk was not included in the Δ*FPCM* model because the predicted FPCM was already corrected for days in milk.

$C_i$ = Random cow effect

$H_j$ = Random herd effect

$\varepsilon_{ij}$ = Random error for each cow (i) in each herd (j)

Normality of the residuals of the model was monitored with normal plots and skewness/kurtosis tests.

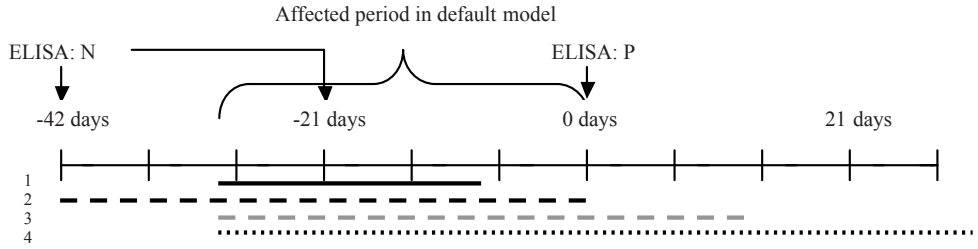


**Figure 1.** BTV-8 testing scheme with the default period in which the milk production and SCC of the cows were assumed to be BTV-8 affected, and with four alternative affected periods. N=seronegative and P=positive for the detection of antibodies.

1 BTV-8 affected period was 30 to 10 days prior to seroconversion.

2 BTV-8 affected period was 42 to 0 days prior to seroconversion.

3 BTV-8 affected period was 30 days prior to seroconversion until 15 days post seroconversion.

4 BTV-8 affected period was 30 days prior to seroconversion until 30 days post seroconversion.

It is possible that a cow was not recovered from the BTV-8 infection after seroconversion was detected. Then the Δ*FPCM*, lnSCC and the odds for a new HSCC could still be affected by BTV-8 for a short period after seroconversion was detected. Thus, the BTV-8 affected time interval was varied from 30 days

prior to seroconversion until 15 days post seroconversion and from 30 days prior to seroconversion until 30 days post seroconversion in the sensitivity analyses (Figure 1).

## 3. Results

### *3.1. Descriptives*

During the study, 185 (17%) out of the 1,074 initially seronegative cows seroconverted. The highest percentage of these seroconversion occurred in September (7.1%; N=72) (Santman-Berends et al., 2010b). None of the 185 seroconverted cows showed clinical signs of BTV-8 infection. In each of the 15 herds that were included in the study, seroconversion occurred. On average, 24% (95% CI: 9-39%; min. 2.5%, max. 100%) of the initially seronegative cows seroconverted in a herd.

From the 185 cows that seroconverted, 127 were lactating. For 77 of these cows, a test-day measurement was available within 30 days prior to the date that seroconversion was detected and could be included in the default analysis. Of the remaining 50 cows, 44 cows had the nearest test-day between 50 and 30 days prior to seroconversion and of 6 cows, the nearest test-day measure was more than 50 days prior to seroconversion.

The average difference between observed and predicted FPCM during the BTV-8 period in 2008 was -2.3 kg per day for cows that seroconverted within 30 days prior to seroconversion, while it was 0 kg in 2007, when no seroconversion occurred (Table 1). The average SCC and the percentage of cows that had a new HSCC also increased in the period in which the cows seroconverted compared to the same period in 2007 (Table 1).

**Table 1.** Descriptive results: mean (95% CI) difference between observed and predicted fat and protein corrected milk production, somatic cell count (SCC) and percentage cows with a new high SCC (HSCC) for cows that seroconverted, within a period of 30 days before seroconversion in 2008 and for the same period in 2007, and for cows that remained seronegative in the BTV-8 period in 2008 and for the same period in 2007.

| | Control period: month 8-11 in 2007 | | **BTV-8 period: month 8-11 in 2008** | |
|---|---|---|---|---|
| | Seroconv[1] (95% CI) | Seroneg[2] (95% CI) | **Within 30 days before seroconv[1] (95% CI)** | **Seroneg[2] (95% CI)** |
| Difference between observed and predicted milk production (kg/ day) | 0.0 (-0.4 - 0.4) | -0.1 (-0.3-0.1) | **-2.3 (-3.5 - -1.1)** | **-0.6 (-0.7 - 0.4)** |
| Mean SCC (x1000 cells/ml) | 100 (89 - 113) | 80 (76 - 85) | **111 (85 - 144)** | **80 (75 - 85)** |
| Percentage cows with a new HSCC based on test-day results (%) | 5.6 (3.0-8.2) | 7.4 (6.1-8.8) | **13.0 (5.3-20.7)** | **9.3 (7.9-10.6)** |

[1] Seroconv= seroconverted during the 2008 BTV-8 epidemic

[2] Seroneg=cows that remained seronegative during the whole study

Cows that remained seronegative had a slightly higher difference between observed and predicted FPCM and a higher percentage of cows with a new HSCC during the 2008 BTV-8 period compared to the same period in 2007 (Table 1). For cows that remained seronegative, the SCC did not differ between the BTV-8 period in 2008 and the same period in 2007.

*3.2. Effect of BTV-8 on Milk Production, SCC and Occurrence of New HSCC*

The multi-level model estimated that milk production (Δ*FPCM*) was significantly reduced in cows that seroconverted for BTV-8. A BTV-8 positive cow had a decreased Δ*FPCM* of 1.7 (95% CI: 0.9- 2.6, $P < 0.01$) kg per day compared to the period which was not affected by BTV-8. In addition, compared to cows that remained seronegative, Δ*FPCM* around seroconversion was decreased with 1.7 (95% CI: 0.9-2.5, $P < 0.01$) kg per day over a 30 day period. This meant that the milk production

in a BTV-8 infected cow was decreased with a total of 51 (95% CI: 26-76) to 52 (95% CI: 26-77) kg during the 30 day period (Table 2).

**Table 2.** Results of the multi-level models for the effect of BTV-8 infection within 30 days prior to seroconversion on milk production, somatic cell count (SCC) and odds for a high somatic cell count (HSCC) with the categories for BTV-8 status, estimates, 95% confidence intervals and significance (*P*-value) (n=77 valid observations from seroconverted cows).

| | BTV-8 cow status[1] | Estimate | 95% Confidence interval | *P*-value |
|---|---|---|---|---|
| Difference between observed and predicted milk production (kg) | Seroconverted | Reference | | |
| | Serocon pre/post | 51 | 26 - 76 | <0.01 |
| | Seronegative | 52 | 26 - 77 | <0.01 |
| lnSCC (x1000 cells/ml) | Seroconverted | Reference | | |
| | Serocon pre/post | -0.13 | -0.34 - 0.08 | 0.23 |
| | Seronegative | -0.20 | -0.45 - 0.05 | 0.11 |
| Odds for a HSCC (OR) | Seroconverted | Reference | | |
| | Serocon pre/post | 0.79 | 0.38 - 1.60 | 0.50 |
| | Seronegative | 0.72 | 0.36 - 1.45 | 0.37 |

[1] Seroconverted: seroconverted for BTV-8 during the 2008 BTV-8 epidemic in the period that could be affected by BTV-8; Serocon pre/post: cows that seroconverted during the 2008 BTV-8 epidemic prior to (before July 2008) or post (after December 2008) the period which could be affected by BTV-8; Seronegative: cows that remained seronegative during the 2008 BTV-8 epidemic.

Seroconverted cows had a statistically non-significant increase in lnSCC compared to the period before seroconversion and compared to cows that remained seronegative ($P = 0.23$ and 0.11 respectively). Cows that became BTV-8 seropositive had a 0.20 (95% CI: -0.05-0.45) higher lnSCC compared to cows that did not seroconvert (Table 2). Back transformation to the normal scale, revealed that for example, a cow 150 days in lactation and in her third parity that became BTV-8 positive had an average SCC of 159 $x10^3$ cells/ml while the same cow that remained seronegative had an average SCC of 130 $x10^3$ cells/ml.

Cows that seroconverted for BTV-8 had an odds ratio of 1.3 (95% CI: 0.6-2.6) for the occurrence of HSCC compared to the period prior to seroconversion and had an odds of 1.4 (95% CI: 0.7-2.8) for the occurrence of HSCC compared to cows that did not seroconvert. These odds ratios were not statistically significant ($P = 0.50$ and 0.37, respectively) (Table 2).

*3.3. Sensitivity analyses*

When the default interval during which BTV-8 could affect milk production and SCC, was changed into 30 to 10 days prior to seroconversion, the number of valid observations decreased to 56. The effect on $\Delta FPCM$ per day remained approximately the same at a reduction of 1.7 kg (95% CI: 0.7-2.7) per day. However, the by BTV-8 affected period was decreased to 20 days, which meant that the total reduced milk production per BTV-8 affected cow decreased to 34 (95% CI: 14-53; $P < 0.01$) kg compared to the period that was not affected by BTV-8 and decreased to 33 (95% CI: 13-53; $P < 0.01$) kg compared to cows that remained seronegative. The effect of BTV-8 on lnSCC and the occurrence of new HSCC however, increased (Table 3). Between 30 and 10 days prior to seroconversion, seroconverted cows had a higher lnSCC compared to cows that remained seronegative and a higher lnSCC compared to themselves prior to the period which could be affected by BTV-8 (Table 3). Additionally, also the odds for a new HSCC increased for seroconverted cows compared to the cows that remained seronegative (OR: 2.0; 95% CI: 1.0-4.1) and compared to themselves before and after the period which could be affected by BTV-8 (OR: 1.8; 95% CI: 0.9-3.8) (Table 3).

The results of lnSCC and for the odds of a new HSCC in which the BTV-8 affected interval was increased to 42 days prior to seroconversion (91 valid observations), were comparable to the results of the default model and to the model in which the affected interval was assumed to be between 30 and 10 days prior to seroconversion

(Table 3). In addition, the results of Δ*FPCM* per day decreased slightly to 1.5 (95% CI: 0.7-2.2; $P < 0.01$) per day compared to the period before or after the period that was affected by BTV-8 and to 1.4 (95% CI: 0.6-2.2; $P < 0.01$) kg per day compared to cows that remained seronegative. Nevertheless, when Δ*FPCM* was calculated for the whole affected period of 42 days, BTV-8 affected cattle had a reduced milk production of 61 (95% CI: 28-94) kg compared to themselves before and after the period that was affected by BTV-8 and a reduced milk production of 59 (95% CI: 27-92) kg compared to cows that remained seronegative.

When the BTV-8 affected time interval was prolonged to 15 days post seroconversion the associations between BTV-8 infection and the dependent variables decreased (Table 3). Furthermore, when the affected time interval was prolonged to as long as 30 days after seroconversion, the associations decreased even further (results not presented).

**Table 3.** Results of the sensitivity of the multi-level models for the effect of BTV-8 infection in different time-intervals around seroconversion on milk production, log transformed somatic cell count (lnSCC) and the odds for a new high somatic cell count (HSCC) with the categories for BTV-8 status, estimates, 95% confidence intervals and significance (*P*-value).

| | BTV-8 affected period | BTV-8 cow status[1] | Estimate | 95% Confidence interval | | *P*-value |
|---|---|---|---|---|---|---|
| Difference between observed and predicted milk production (kg) | 30 to 10 days prior to seroconversion (N=56) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | 34 | 14 | 53 | <0.01 |
| | | Seronegative | 33 | 13 | 53 | <0.01 |
| | 42 to 0 days prior to seroconversion (N=91) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | 61 | 28 | 94 | <0.01 |
| | | Seronegative | 59 | 27 | 92 | <0.01 |
| | 30 days prior to 15 days post seroconversion (N=138) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | 27 | -0.4 | 54 | 0.05 |
| | | Seronegative | 26 | -1.2 | 52 | 0.06 |

| | | | | | | |
|---|---|---|---|---|---|---|
| lnSCC (x1000 cells/ml) | 30 to 10 days prior to seroconversion (N=56) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | -0.21 | -0.46 | 0.03 | 0.09 |
| | | Seronegative | -0.29 | -0.57 | -0.01 | 0.04 |
| | 42 to 0 days prior to seroconversion (N=91) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | -0.15 | -0.35 | 0.04 | 0.13 |
| | | Seronegative | -0.23 | -0.47 | 0.01 | 0.06 |
| | 30 days prior to 15 days post seroconversion (N=138) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | -0.09 | -0.25 | 0.07 | 0.25 |
| | | Seronegative | -0.17 | -0.38 | 0.04 | 0.11 |
| Odds for new HSCC (OR) | 30 to 10 days prior to seroconversion (N=56) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | 0.55 | 0.26 | 1.14 | 0.11 |
| | | Seronegative | 0.51 | 0.24 | 1.04 | 0.07 |
| | 42 to 0 days prior to seroconversion (N=91) | Seroconverted | Reference | | | |
| | | Serocon. pri/po | 0.71 | 0.38 | 1.35 | 0.30 |
| | | Seronegative | 0.66 | 0.35 | 1.23 | 0.19 |
| | 30 days prior to 15 days post seroconversion (N=138) | Seroconverted | Reference | | | |
| | | Serocon. pre/post | 0.91 | 0.53 | 1.60 | 0.76 |
| | | Seronegative | 0.84 | 0.48 | 1.45 | 0.51 |

[1] Seroconverted: seroconverted for BTV-8 during the 2008 BTV-8 epidemic in the period that could be affected by BTV-8; Serocon pre/post: cows that seroconverted during the 2008 BTV-8 epidemic prior to (before July 2008) or post (after December 2008) the period which could be affected by BTV-8; Seronegative: cows that remained seronegative during the 2008 BTV-8 epidemic.

## 4. Discussion

In our study, 17% of the initially seronegative cows seroconverted for BTV-8, indicating that they became infected. All cows experienced a sub clinical infection. Besides a negative effect on fertility (Santman-Berends et al., 2010b), it appeared that a BTV-8 infection also reduced milk production and had a negative effect on

udder health. The total amount of reduced milk associated with a BTV-8 infection in our study was estimated at approximately 51 kg per cow, which is about 0.6% of the average yearly milk production per cow in the Netherlands (8218 kg (CRV, 2010b)), and relates to a economic loss of χ6.12 (95% CI: χ3.12 - χ9.12) per BTV-8 infected cow in a quotum situation (Huijps et al., 2008). A reduction in milk production associated with BTV-8 infections was also reported by Van Schaik et al. (2008) and Elbers et al. (2009). Van Schaik et al. (2008) found that the net return of 305-day milk production significantly decreased in dairy herds located in BTV-8 infected regions in the bluetongue period in 2006 compared to the period before the outbreak. Elbers et al. (2009) reported that, during the 2007 BTV-8 epidemic, 24% of the infected cows had a reduction in milk production. However, the exact amount of reduced milk production per infected cow was not described in these earlier studies. Possibly, the reduced milk production associated with BTV-8 infections were caused by the immunosuppressive effects of the virus. A similar result was found for dairy herds that suffered from a subclinical infection of BHV1 (Hage et al., 1998; Van Schaik et al., 1999).

Quantification of the exact amount of reduced milk production caused by BTV-8 infection is important for the calculation of the economic consequences of a BTV-8 outbreak. Velthuis et al. (2010) calculated the economic losses due to BTV-8 in the Netherlands and used expert assumptions for the effect of BTV-8 on milk production. In their study, BTV-8 was assumed to reduce milk production with 5.4 kg per day for a period of 10.5 days. This resulted in a total reduction in milk production of 56 kg per BTV-8 infected cow. The amount of reduced daily milk production in their study was higher compared to ours and the number of affected days was lower. Nevertheless, the total reduction of produced milk in their study was comparable to the reduction we found. However, in our study, all cows were subclinically infected with BTV-8, whereas in the study of Velthuis et al (2010) this amount of reduced milk was also extrapolated to clinically affected cows, which may have a larger reduction in milk production.

When cows show clinical signs of BTV-8, the effect on milk production might be higher. In the study of Kedmi et al. (2010), the effect of an epizootic hemorrhagic disease (EHDV) outbreak in Israel, a virus closely related to BTV, on milk production in cattle was studied. They found a total reduction in milk production of 207 (95% CI: 154 - 261) kg per cow in clinically affected herds. It might be that a part of the high milk losses in the study of Kedmi et al. (2010) were caused by the interaction of high

temperatures in Israel and clinical EHDV in cattle and may not be representative for EHDV outbreaks in other countries with colder climates. On the other hand, the effect of BTV-8 infections on milk production in our study possibly underestimates the reduction in milk production in clinically affected herds because all cattle in our study were subclinical infected.

The effect of BTV-8 infection on SCC was less clear. Although statistically not significant in the default model, the lnSCC and odds for the occurrence of new HSCC cases were higher in cows that seroconverted for BTV-8. To our knowledge there are no published papers on the relation between SCC, HSCC and BTV-8 infection. Elbers et al. (2009) however, did describe that in 2007, BTV-8 infected herds had more cows with red teats or lesions on teats, or both. Furthermore, BTV-8 infection is known to cause injury to small blood vessels caused by BTV replication in endothelial cells, which can lead to photosensitation, erythema, necrosis and ulceration (Dercksen and Lewis, 2007; Williamson et al., 2008; Darpel et al., 2009). Possibly, these injuries are associated with decreased milk production and increased SCC. The association between viral infections that induce teat lesions like BTV-8 does, and subclinical mastitis has been found before (Wellenberg et al., 2002). Nevertheless, the increased SCC and odds for a HSCC in our study was not significant, which might be related by the fact that the farmers in our study did not observe clinical signs associated with bluetongue infections such as damage of the udder skin.

The test used in our study was not serotype specific and theoretically other serotypes than BTV-8 could have been present in our study herds. During the study period, BTV serotype 6 was found in the eastern part of the Netherlands. Measures for prevention of spread of the virus were taken and herds surrounding the affected herds were sampled. It appeared that BTV-6 did not spread extensively and only 13 cows in 13 herds became infected (Promed, 2008; Promed 2009). The herds in our study were mainly located in the north of the Netherlands, far from the area in which BTV-6 emerged. Furthermore, in the monitoring and surveillance system in the Netherlands, which detected serotype 6, no other cases of BTV-6 than the 13 mentioned above, or other serotypes were found. Therefore, we believe that our results are only associated with BTV-8 and that other serotypes did not influence our results.

In the default model, the BTV-8 affected time interval was assumed to be 30 to 0 days prior to seroconversion. Based on literature, it is known that BTV-8 neutralizing

antibodies arise on average 14 days after infection (Dal Pozzo et al., 2009). In addition, in the prior seronegative sample 21 days before seroconversion was detected, the cows had a negative result for antibodies against BTV-8. Thus seroconversion occurred on average 10 days prior to the date on which seroconversion was detected. Based on these numbers we assumed that BTV-8 infection occurred between 35 and 15 days prior to the date on which seroconversion was detected. Because > 35 days prior to seroconversion, cows were assumed not yet to be infected by BTV-8 and clinical signs of BTV-8 are seen on average 7 to 11 days after BTV-8 infection (Guyot et al., 2008; Dal Pozzo et al., 2009), we determined that the test-day measure was influenced by BTV-8 when it was taken in this defined time interval of 30 to 0 days prior to seroconversion.

Because there was uncertainty about the exact time-interval in which BTV-8 infection would affect milk production and SCC, a sensitivity analysis was carried out with alternatives for the affected time-interval. The results of the two alternative intervals in which the BTV-8 affected time-interval was changed into 30 to 10 days prior to seroconversion, and to 42 days prior to seroconversion showed coherent results with the default model. All three intervals showed that BTV-8 had a significant negative effect on milk production. The estimates for the analyzed variables were not very sensitive for changing the BTV-8 affected time interval prior to seroconversion. Therefore, based on biological reasons and the results of the sensitivity analyses, the time interval from 30 to 0 days prior to seroconversion seemed to be the true period in which cattle suffer from a BTV-8 infection. When the BTV-8 affected time interval was prolonged to 15 or 30 days after seroconversion, effects of BTV-8 on production and udder health became smaller. This indicates that after seroconversion the milk production and SCC returned to normal levels.

## 5. Conclusions

BTV-8 infection has a negative influence on milk production of a cow. Cows that suffered from a subclinical BTV-8 infection had an average drop in milk production of about 1.7 kg per day for a period of 30 days. This meant that the total milk production of a BTV-8 infected cow decreased with about 52 kg. When the time interval in which it was assumed that cows were affected by BTV-8 was increased to within 42 days prior to seroconversion, the milk production in BTV-8 seroconverted cows decreased with 61 (95% CI: 28-94) kg compared to the period before and after

seroconversion and decreased with 59 (95% CI: 27-92) kg compared to cows that remained seronegative.
Subclinical BTV-8 infection was associated with a non-significant increase in lnSCC and in occurrence of new HSCC cows. Nevertheless, all cows in our study were subclinical infected with BTV-8 and a larger effect of BTV-8 on milk production and udder health might have been observed in cows suffering from clinical BTV-8 infections.

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Chapter 8

# General discussion

Inge M.G.A. Santman-Berends

## 1. Introduction

The general aim of this thesis was to investigate the transmission and impact of bluetongue virus serotype 8 (BTV-8) infections in dairy herds.
In this chapter, the main findings are summarized and discussed. Based on the impact of BTV-8 on the health of dairy cattle, a generalized calculation of the economic consequences of a BTV-8 outbreak is presented. In addition, strategies for monitoring and control of BTV-8 on herd, cattle industry and national level will be discussed, and the knowledge that was gained from the BTV-8 epidemic in north-western Europe will be presented. Finally, the main conclusions of this thesis are presented and suggestions for further research are provided.

## 2. Transmission of BTV-8

The transmission of BTV-8 differs from directly transmittable viral diseases by the fact that BTV-8 requires a vector, i.e. a competent species of *Culicoides*, to be transmitted from one animal to another. Because of this vector borne transmission, conditions that are important for the competent vector, e.g. temperature, wind, humidity, etc. play an important role in the spread of BTV-8. Furthermore, it is demonstrated that BTV-8 is vertically transmittable (Chapter 5 and 6).

### *2.1. Vector borne transmission of BTV-8*

During the 2007 BTV-8 epidemic in the Netherlands, it was found that one BTV-8 infectious cow infected on average 3.8 (median 2.9; $5^{th}$ percentile=2.2 and $95^{th}$ percentile=9.0) other susceptible cows within the same herd (Chapter 2). This study was the first that estimated the transmission ($R_0$) of BTV-8 based on serological field data. Earlier studies that quantified the transmission of BTV-8 often used data on vector and host densities but did not have the opportunity to include data of the infection status of the ruminant host (Gubbins et al., 2008; De Koeijer and Elbers, 2008; Hartemink et al., 2009).
The $R_0$ found in Chapter 2 was in the same range as those of De Koeijer and Elbers (2008) and Hartemink et al. (2009). However, the model described in this thesis did not include small ruminants. In theory, sheep could have influenced the within-herd transmission of BTV-8. However, there were no data available about sheep in the sentinel cattle herds. Nevertheless, the role of sheep on the transmission of

BTV-8 within cattle herds is probably minor given that cattle are the preferred host for competent *Culicoides* and that cattle are infectious for a longer period than sheep (Braverman et al., 1971; Bartsch et al., 2009).

The transmission model in chapter 2 showed that the values of $R_0$ within herds in the same months were comparable. However, there was variation between regions and months, which might be explained by the outside temperatures at the moment BTV-8 was introduced. In the northern region within-herd $R_0$ values were significantly lower than the within-herd $R_0$ in the south in which BTV-8 started to spread massively from June/July on. This could be explained by the later start of the BTV-8 epidemic in the northern region. In many northern herds BTV-8 did not spread until September/October. The difference in transmission speed between months was expected because it is known that BTV-8 transmission decreases when temperature declines. Furthermore, the assumption of the model that the infection in a herd starts with a single introduction of an infectious host or vector may have been violated in the south more often than in the north, resulting in an overestimation of $R_0$. From the serological field data that was used it could not be determined if the infection within a herd started with the introduction of one infection or with multiple infections. The dissemination of the virus in the warmer summer months was higher in the southern region than in the northern region. Thus, if infections within herds started with multiple introductions of the virus it was more likely that this would happen in the southern than in the northern region. In winter and early spring, north-western European temperatures are reported to be too low (<10ºC) for the vector to be able to replicate and spread the virus. However, in the field study, an increase in BTV-8 seroprevalence was observed between November and December in a few herds. It might be that the increase in seroprevalence was caused by the sampling method in which a group of 16-26 seronegative cows entered the study and every month a random sample of 16 cows were tested out of this group. Thus, in theory, cows that seroconverted in November that were not sampled in that month, could have been found in December. Another possibility is that in some herds, competent *Culicoides* entered the stables when temperatures declined. In the stable the temperature was higher than the temperature outside and thus *Culicoides* were able to spread BTV-8 for a prolonged time. However, from the results obtained in Chapter 3 it appeared that stabling cows worked protective against the introduction of BTV-8 infection and decreased the transmission ratio.

In the southern and central region, most herds became infected in the summer months and the BTV-8 within-herd seroprevalence increased rapidly to high levels because the conditions for *Culicoides* biting rate and survival were good. Nevertheless, there were some herds in those regions in which no cows became infected with BTV-8. It appeared that herd management and barn type can influence the BTV-8 transmission within a dairy herd (further discussed in paragraph 5).
The serological field data provided the opportunity to estimate one of the *Culicoides* related parameters, the vector to host ratio, that has been based on assumptions in other studies (Gubbins et al., 2008; De Koeijer and Elbers., 2008; Hartemink et al., 2009). It was found that the number of competent *Culicoides* per cow differed considerably between herds and between months. The high numbers of competent *Culicoides* estimated by the model in the months October and November are the result of the rate at which new infections were observed in these months and the knowledge of a reduced biting frequency, replication and transmission rate of *Culicoides* in colder periods (Gerry and Mullens, 2000).
In the transmission model, it was assumed that all infectious *Culicoides* became infected by biting cattle in the study herds while these *Culicoides* could also have been infected by cattle or sheep from other herds. Still, in general *Culicoides* disperse only short distances from their breeding sites and will thus in general stay with the same herd (Kettle and Lawson, 1951; Lillie 1985).
During 2008 seronegative cattle in herds of which the farmer had decided not to vaccinate were monitored and it was found that the transmission within these herds was lower compared to that within the herds monitored in 2007 (Chapter 6 and 7). In 2008 only 17% of the cattle seroconverted (Chapter 6 and 7).
The fact that less susceptible cows became infected in 2008 might be caused by the higher level of immunity in those herds and in the whole country in that year. In the spring of 2008, the majority of the cattle and sheep farmers decided to vaccinate their animals and the level of seropositive animals increased up to more than 80% either because prior BTV-8 infection or because of vaccination. This high level of immunity in the host population and partly immunity in the studied herds may have reduced the infection pressure within the herds.

*2.2. Vertical transmission of BTV-8*

It appeared that BTV-8 could be transmitted from cow to foetus resulting in either abortion, stillbirth (Wouda et al., 2008; Backx et al., 2009) or birth of BTV-8 positive

healthy-looking calves (Chapter 5, 6, Batten et al., 2009; Galleau et al., 2009).
It was found that 16.2% (95% CI: 11.4-21.0%) of the studied cows infected during the 2007 epidemic, gave birth to a BTV-8 positive calf (Chapter 5). Twenty-point-eight percent (95% CI: 9.3-32.3%) of the calves originating from cows that seroconverted during the 2008 BTV-8 epidemic tested BTV-8 positive (Chapter 6). It appeared that the majority of these positive calves originated from dams that became BTV-8 infected in the second half of gestation. These dams had a 15.5 (95% CI: 1.3-190.4) times higher probability on giving birth to a BTV-8 positive calf (Chapter 6) compared to cows that became infected in the first half of gestation. In contrary to BVD infections in which calves can become a persistent carrier of the virus after vertical transmission in the first trimester of gestation (Van Oirschot, 1983), BTV-8 positive calves in our study became negative again within a few months after birth. Higher rates of vertical transmission of BTV-8 were found in other studies (Batten et al., 2009; Galleau et al., 2009). Batten et al. (2009), found 33% (95% CI: 22.7-47.7%) of 63 calves born from an infected dam BTV-8 positive within three days after birth, and Galleau et al. (2009) even found 42% (95% CI: 22.1-63.4%) of 24 calves positive after birth.
Because of the vector season, the majority of the BTV-8 positive calves were born in winter and early spring. After birth they usually remained BTV-8 positive for a few months. It can therefore be hypothesized that these calves can start a new infection cycle when vectors become active again in the spring (Menzies et al., 2008). However, whether these BTV-8 positive calves can actually infect competent *Culicoides* has not been proven.
Vertical transmission can be prevented by vaccination; none of the calves originating from 256 vaccinated dams tested BTV-8 positive (Chapter 6). This result is consistent with the findings of Galleau et al. (2009), who did not find BTV-8 positive calves born from vaccinated dams either.

**3. The impact of BTV-8 infection in cattle**

Prior to the BTV-8 outbreaks in north-western Europe in 2006, infections with BTV-8 in cattle were only seen in Pakistan and India, South and West Africa and the Caribbean regions. However, in those countries, BTV-8 infections in cattle usually had a subclinical manifestation and only in some cases mild clinical signs were seen (Haresnape et al., 1988; Mo et al., 1994; Gerdes, 2004; Sreenivasulu et

al., 2004). Therefore, it was surprising that during the BTV-8 epidemic in north-western Europe, many BTV-8 infected cattle showed clinical signs of bluetongue (Toussaint et al., 2006; Dercksen and Lewis, 2007; Elbers et al., 2008a; Elbers et al., 2008b;Williamson et al., 2008; Elbers et al., 2009). In addition, during the large BTV-8 outbreak in 2007 in the Netherlands, the farmers reported reduced fertility, udder health problems and increased mortality (Elbers et al., 2009).

*3.1. Mortality*

A study was conducted to objectively quantify the association between BTV-8 infections and mortality during the 2007 BTV-8 epidemic, and it appeared that BTV-8 infections were associated with an increased mortality in dairy herds. In the month in which the farmer notified clinical signs of BTV-8 to the authorities, cattle (>1 year) mortality increased 1.4 (95% CI: 1.2-1.6) times, calf (3 days-1 year) mortality increased 1.3 (95% CI: 1.1-1.6) times, and newborn calf (<3 days) mortality increased 1.2 (1.1-1.4) times. Increased mortality in cattle had not been reported before the BTV-8 epidemic in north-western Europe, only in rare cases cattle seemed to die from BTV-4, 9 or 16 infections (Mastroyanni et al., 1981; Nomikou, 2004). Possibly, BTV-8 is more virulent in cattle than other BTV serotypes.

In Chapter 4, in herds in BTV-8 infected regions where clinical signs of bluetongue had not been notified, mortality rates were also increased. During the BTV-8 period, monthly cow (>1 year) and newborn calf (< 3 days) mortality in these herds were 1.1 (95% CI: 1.1-1.1) and 1.2 (95% CI: 1.2-1.2) times higher than in months that were not influenced by BTV-8 (Chapter 4).

When calculating the mortality associated with BTV-8 in herds with a confirmed BTV-8 outbreak, the additional mortality is very likely associated with BTV-8. However, extrapolating mortality rates derived from herds that notified clinical signs (Le Gal et al., 2008; Elbers et al., 2009) to the national level could easily overestimate the total mortality associated with BTV-8. Only herds with clear clinical signs of BTV-8 notified to the authorities and in these herds mortality associated with BTV-8 infections is likely to be higher than in herds with less clear clinical signs. The 5-year census data on mortality that was used for this study provided a good opportunity to estimate the additional mortality associated with BTV-8 in the Netherlands. Nevertheless, as whole compartments were classified as BTV-8 infected, it was not possible to extrapolate the mortality rates to individual herds. The additional mortality in herds located in infected regions based on the

census data underestimated the real mortality associated with BTV-8 on herd level, as probably not all herds in infected regions were infected. It was not known how many of the non-notification herds were BTV-8 infected and in how many of these herds clinical signs occurred. Nevertheless, in the sentinel network that was conducted in cattle herds during the 2007 BTV-8 epidemic in the Netherlands (Chapter 2 and 3), 82% of the herds in infected regions became infected. It is possible that the additional mortality only occurred in the proportion of non-notified herds that were clinically infected with BTV-8 and that the remaining herds did not show an increased mortality. Another possibility was that the BTV-8 outbreak caused clinical signs in only a few cattle in the majority of non-notification herds and that the increase in mortality was too small for the farmer to link it to a BTV-8 infection. Although an increase in cattle mortality associated with BTV-8 was observed during the BTV-8 epidemic in 2007 in the Netherlands, all additional mortality was initially assigned to the BTV-8 epidemic, while in the same period, new regulations were implemented. These implemented EC regulations 853 854/2004 and 1/2005 were responsible for the majority of the increase in mortality.

*3.2. Fertility*

An unfavourable effect of a BTV-8 infection was found on fertility of cattle (Chapter 6). Cows that became infected with BTV-8 had a 5.0 (95% CI: 1.9-14.3) times higher chance to return to service after first insemination, needed 1.7 (95% CI: 1.4-2.0) times more inseminations, and needed 2.5 (95% CI: 2.4-2.6) times more days between first and last insemination compared to cows that were not infected. The study into the effect of BTV infection on fertility parameters was carried out in cows with a subclinical infection. It is possible that the observed negative effect is even bigger in cows suffering from a clinical infection and thus that the estimates underestimated the effect for the whole population of BTV-8 infected cattle.

In the herds studied, no association between BTV-8 infections and the chance to abort was found. This was an unexpected result because a 2.6 (95% CI: 1.7-4.1) times increased abortion rate was reported by Elbers et al. (2009). In addition, during the BTV-8 epidemic from July until December 2007, on top of the average submission of blood samples from aborted cows in previous years, 220 blood samples from aborted cows per month were submitted to the Animal Health Service (GD) (quarterly reports, 2007). This increased number of submitted blood samples might be caused by a higher awareness of the farmers. In the period that

BTV-8 emerged, farmers were more alert to signs of BTV-8 in their cattle. Because an abortion could be a sign of BTV-8 infection, farmers were probably more likely to submit a blood sample from these cows to the GD.
It is possible that there was no significant association between BTV-8 infection and abortion because none of the studied cows that were infected with BTV-8 showed clinical signs. Increased abortion rates might be mainly associated with clinical signs and fever as a result of a BTV-8 infection. Another reason for the fact that an association between BTV-8 infection and abortion rate was not found could be the definition of an abortion, taking place between 100 and 260 days after last insemination. When a cow returned to service between 46 and 100 days after the last insemination, Elbers et al. (2009) defined the cow as having an abortion, while it was assigned an extra insemination in Chapter 6. Furthermore, the increased number of abortions that Elbers et al. (2009) reported was based on the reports of farmers of which the cattle showed clinical signs of BTV-8. These farmers probably assigned every aborting cow to BTV-8 while there could have been other causes for abortion in their herds.

*3.3. BTV-8 positive calves*

Although no relation was found between BTV-8 and abortions, it was found that a BTV-8 infection in the second half of gestation could lead to the delivery of a virus positive healthy- looking calf. However, after the large BTV-8 epidemic in 2007, 18 calves suffering from hydranencephaly were submitted to the GD. All 18 calves tested positive for bluetongue. From these calves, 11 were aborted and seven calves were born alive and lived for a maximum of 28 days (Wouda et al., 2008). Thus, vertical transmission of BTV-8 might lead to hydranencephaly and damaged the health of the calves. Furthermore, it might be possible that BTV-8 positive calves at birth that were not suffering from hydranencephaly were also weaker than virus negative calves.
However, during the study that investigated vertical transmission of BTV-8 (Chapter 5) in 2007, there were no indications that calves that were PCR positive at birth were not healthy. During the study that investigated vertical transmission in 2008 (Chapter 6) farmers were asked to report any health problems that occurred in the calves. In that study, ten virus positive calves were born and none of the farmers reported health problems. Thus, based on these results it appeared that all calves that tested virus positive at birth were clinically healthy. Furthermore, these calves became virus negative again within a few months after birth.

*3.4. Milk production and udder health*

A BTV-8 infection in a dairy cow reduced milk production for about 30 days with approximately 52 kg (95% CI: 26-77 kg) in total. Additionally, the somatic cell count (SCC) was increased in cows that became subclinically infected, but this increased SCC was not statistically significant (Chapter 7).

An association between BTV-8 infections and udder health was expected because BTV-8 is known to cause injury to small blood vessels as a consequence of virus replication in endothelial cells. These injuries can lead to photosensitation, erythema, necrosis and ulceration of the teats (Dercksen and Lewis, 2007; Williamson et al., 2008; Darpel et al., 2009). However, during the study, these clinical signs were not seen, which may explain the limited effect on udder health.

## 4. Economic consequences of BTV-8 outbreak in a dairy herd

The impact of BTV-8 infection on cattle could vary considerably. Infections could be subclinical, but might also lead to severe clinical signs, which sporadically even lead to death. Besides direct costs and losses due to clinical signs, BTV-8 also caused indirect losses, because farmers that were located within a radius of 150 km from an infected herd had to implement measures to prevent BTV-8 from further spreading according to EG regulation 393/2005/EG ((CEC, 2005); LNV, 2006a) and because export restrictions were put in place according to regulation 75/2000/EG (CEC, 2000).

Based on the direct and indirect costs and losses of the BTV-8 epidemic in the Netherlands, Velthuis et al. (2009) calculated the economic consequences of BTV-8 for the entire farm animal industry in the Netherlands at 32.4 million euros in 2006, and estimated that these consequences ranged between 164 and 175 million euros in 2007 (Velthuis et al., 2009). For an individual dairy farmer, information about the economic consequences of a BTV-8 outbreak on herd level is important. Based on this information, a farmer can decide how much he can invest to prevent his cattle from losses associated with BTV-8.

By combining the results of the impact of BTV-8 infection in dairy cattle, as quantified in Chapters 4-7 of this thesis, and the morbidity in cattle that was observed during the BTV-8 epidemic in the Netherlands (Elbers et al., 2008b; Elbers et al., 2009), the direct economic consequences of a BTV-8 outbreak on herd level were calculated. In this analysis, the losses caused by a BTV-8 outbreak

were calculated for an average Dutch dairy herd with 75 cows older than 2 years (Appendix 1). The economic consequences of BTV-8 outbreak were separately estimated for clinically and subclinically affected herds. The model used is:

$$EClin_{BTV-8} = Ctreat + Lmort + Lfert + Lmilk + Cmast$$

$$ECsubcl_{BTV-8} = Lfert + Lmilk$$

The population dynamics of the infection and thus the economic consequences ($EC_{BTV-8}$) at the moment of BTV-8 introduction in a dairy herd depended on the proportion of susceptible cattle. Economic consequences will differ greatly between a BTV-8 outbreak leading to clinical infections (*clin*) and a BTV-8 outbreak leading to only subclinical infections (*subcl*) in a dairy herd.

After introduction of BTV-8 in a herd, all susceptible cattle >1 years that were infected in subclinical affected herds, suffered from losses caused by reduced fertility (*Lfert*), and all susceptible cows >2 years suffered from reduced milk production (*Lmillk*) (Appendix 1).

In addition to losses caused by reduced fertility and reduced milk production, in clinically affected dairy herds also costs for sick cattle were made (*Ctreat*) and in clinical affected dairy herds also losses caused by additional mortality (*Lmort*) and udder health problems (*Cmast*) occurred (Appendix 1).

The economic consequence of a BTV-8 outbreak in a dairy herd was a combination of the number of cattle that become infected and the impact of infection on cattle health. It was assumed that a clinical BTV-8 infection would not occur when less than 40% of the cattle in a herd were susceptible to BTV-8 infection. This percentage was based on results that were obtained in this thesis. The 15 non-vaccinated dairy herds that were followed during 2008 were all subclinically infected and on average 17% (95% confidence interval: 3-39%) of the cattle seroconverted (Chapter 6 and 7).

By combining the estimates of the impact with the economic values of treatment, mortality, produced milk in a quota situation and inseminations (Appendix 1), the economic consequences of a BTV-8 outbreak in a dairy herd were calculated for clinically and subclinically affected herds with different proportions of susceptible cows. Instead of point estimates describing the impact of a BTV-8 infection, Pert distributions were used which included the lower confidence limit as the minimum value, the point estimate as most likely value and the upper confidence limit as

maximum value. A stochastic model that executed 1000 iterations in the program @Risk was used for the calculation of the economic consequences of BTV-8.

The results showed that the losses were €1962 (95% CI: €1284-€2806) when the introduction of BTV-8 would lead to a clinical outbreak in a completely susceptible dairy herd with 75 cows >2 years. From these losses, 32% was caused by diagnostic and treatment costs of diseased cattle, 21% by increased udder health problems, 18% by additional mortality, 16% by decreased fertility and 12% by reduced milk production (Appendix 1).
When the BTV-8 outbreak evolved subclinically, economic consequences for a completely susceptible dairy herd were €133 (95% CI: €56-€222) only (Figure 1).

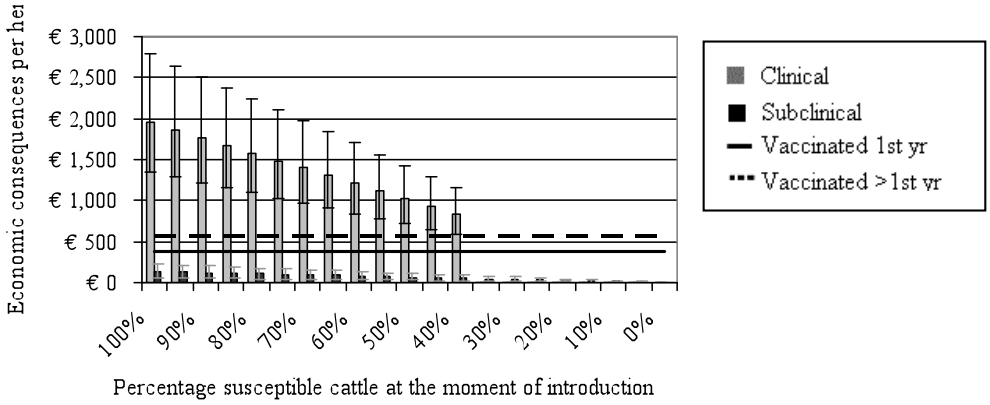


**Figure 1.** Economic consequences of a BTV-8 outbreak in a dairy herd. The average and 90% confidence interval of the economic consequences for (sub)clinically affected herds. The solid black line represents the yearly vaccination costs and the dashed black line represents the vaccination costs in the first year.

When less than 40% of the cattle in a herd are susceptible for a BTV-8 outbreak, an outbreak only seems to pass subclinically and the economic consequences are small, ranging from € 0 to € 78 (Figure 1).
Compared to other infections that are endemic in the Netherlands, the economic consequences of a clinical BTV-8 outbreak on herd-level are higher than for IBR

(infectious bovine rhinotracheitis) (€ 650) and neosporosis (€ 249) but lower than the losses due to paratuberculosis (€ 2064), salmonellosis (€ 2230) or BVD (bovine viral diarrhoea) (€ 3606) (Hogeveen et al., 2003).

The economic losses due to costs of treatment, mortality, reduced milk production, decreased udder health, and reduced fertility gave a good indication of the direct production losses when BTV-8 emerges in a dairy herd. Vaccination of the herd will prevent BTV-8 related symptoms and economic losses (Wäckerlin et al., 2010). Vaccination of an average dairy herd costs € 478 in the first year and € 312 per year in the following years (Figure 1). Vaccination of the herd is only cost-effective when at least 40% of the cattle are susceptible for BTV-8, and BTV-8 infection manifests itself clinically. Although the costs of vaccination might be higher than the production losses caused by a subclinical BTV-8 outbreak, vaccination also prevents indirect losses from BTV-8 infections such as secondary infections as a result of reduced resistance that were not included in this study. In addition, with vaccination it is possible that the BTV-8 free status can be regained sooner and in that case losses due to movement and export restrictions will be lower (Velthuis et al., 2010).

Whether a farmer should decide to vaccinate his cattle or not, depends on the proportion of susceptible cattle in the herd and the risk of a BTV-8 outbreak. An approximate indication of the proportion of susceptible cattle in the herd can be obtained by performing a bulk milk test on BTV-8 antibodies (Kramps et al., 2008; Mars et al., 2010). When the test result is negative or low positive, the proportion of susceptible cows will be high enough for a new clinical outbreak of BTV-8. When the test is highly positive, the proportion of susceptible cattle in the herd is probably too small for a new clinical outbreak.

When both the proportion of susceptible cattle and the risk on a BTV-8 outbreak are high, it is recommendable to vaccinate. Moreover, because of the average replacement rate in Dutch dairy herds of 27% per year, the proportion of susceptible cattle will increase to sufficient levels for a new clinical outbreak of BTV-8 within two years after a major outbreak of BTV-8.

## 5. Control and monitoring of BTV-8

From the studies in this thesis it appeared that, once BTV-8 has been introduced into a dairy herd, it can infect a large proportion of cattle in a short period of time (Chapter 2).
Measures to control BTV-8 can be taken on herd level to prevent or reduce the risk of cattle becoming infected. Measures for monitoring and control of BTV-8 can be implemented on a national level. However, to eradicate BTV-8, measures have to be taken on an (inter)national level.

### *5.1. Control of BTV-8 on herd level*

Measures aiming at controlling BTV-8 in cattle should focus on adapting herd management in order to reduce the risk of BTV-8 transmission (Chapter 3), on protecting the cattle from BTV-8 by vaccination, or on reducing vectoral capacity like biting rate and *Culicoides* survival with insecticides. When an effective vaccine is on the market, vaccination is probably the best method of defence. However, in 2006 and 2007, no BTV-8 vaccine was available and thus, farmers could only reduce the transmission and impact of BTV-8 by adapting management or by trying to reduce vectoral capacity.

#### 5.1.1. Adapting herd and grazing management

Prior to the study described in Chapter 3, no literature was available on the relation between herd management and bluetongue. Earlier studies only looked at more regional risk factors such as temperature, humidity, soil type and altitude (Ward and Thurmond, 1995; Green et al., 2005; Racloz et al., 2008).
Keeping cattle indoors was a protective measure against BTV-8 transmission. The longer cattle were grazing, the higher the increase in within-herd BTV-8 seroprevalence was. The monthly increase in seroprevalence in cattle during the 2007 BTV-8 epidemic was 5.6% (95% CI: 1.4-10.2) higher for cattle that were outside for a few hours per day, and was 13.6% (95% CI: 7.2-20.8) higher for cattle that were outside day and night compared to cattle kept indoors only. In addition, cattle kept indoors stayed BTV-8 free (48%) significantly more often compared to cattle kept outside (14%).
These findings might be explained by results of Meiswinkel et al. (2000) and Baylis et al. (2009) who captured significantly lower numbers of *Culicoides* indoors

compared to outdoors. However, other studies demonstrated that *Culicoides* counts in indoor traps can be as high as those in outdoor traps and that part of the indoor captured *Culicoides* had also been actively feeding inside the stable (Baldet et al., 2008; Meiswinkel et al., 2008a). Nevertheless, according to the results described in this thesis, competent *Culicoides* were more likely to feed on cattle kept outside than on cattle kept inside. Although stabling of cattle works protectively against BTV-8 infection, it may not be applicable for the whole grazing season for all herds. Furthermore, keeping cattle indoors in summer may reduce the general cattle health and welfare.

Other protective factors BTV-8 transmission were open stable doors versus closed doors and large horizontal openings with wind break curtain on the side of the barn versus no or small openings at the horizontal side of the barn, which decreased the monthly increase in BTV-8 seroprevalence with 3.6% (95 CI: 0.3- 7.1) and 3.0% (95% CI: -0.2-6.0), respectively (Chapter 3). It might be that *Culicoides* do not like light and airy stables and prefer dark stables without a lot of air movement.

This effect of stable type might explain the difference in results between the study described in Chapter 3 and the studies of Baldet et al. (2008) and Meiswinkel et al. (2008a), who found that competent *Culicoides* were as likely to feed inside as outside the barn in north-western Europe. Maybe, they studied *Culicoides* in stables with no or only a few air openings, which appear to be a more favourable habitat for *Culicoides*.

Although large openings with windbreak curtain reduced the monthly increase in BTV-8 prevalence, stables with a large horizontal opening but without windbreak curtain were not associated with a significantly lower increase in seroprevalence compared to stables with no or small horizontal openings in the walls. Windbreak curtains may in some way help to reduce entrance of *Culicoides* into stables, much as mosquito netting would do.

### 5.1.2. Reducing vectoral capacity

The scope of this thesis did not include reduction of the vectoral capacity. However, in the 15 non-vaccinated herds that were studied during the 2008 BTV-8 epidemic (Chapter 6 and 7), a short questionnaire was conducted including a number of questions concerning the use of insecticides on the cattle and in the stable. From the 14 herds that cooperated with the questionnaire, three herds used insecticides on their cattle. In these herds on average 22.4% of the cattle seroconverted during

the 2008 BTV-8 epidemic vs. 3.11% in the remaining 11 herds. In addition, two out of 14 herds used insecticides in their stables and the percentage of seroconversions between the herds that did and did not use insecticides in their stables was comparable (7.4% versus 7.1%). Although the number of herds was very small, there was no indication that using insecticides reduced the risk of BTV-8 infection in cattle. These results were in agreement with the studies of Mullens et al. (2000 and 2001) and Satta et al. (2004) who also found limited to no effect of the use of insecticides. Thus, protecting cattle against BTV-8 infection by trying to reduce vectoral capacity using insecticides seems at this moment not very effective.

### *5.2. Monitoring, control and eradication of BTV-8 on cattle industry and national level*

#### 5.2.1. Monitoring, early detection and surveillance of BTV-8

Monitoring and surveillance systems are important to identify the introduction of a new disease. In the Netherlands, a voluntary passive monitoring and surveillance system, which is based on the reporting of unusual clinical signs in animals to the GD, is in place. For the ruminant industry in the Netherlands, a helpdesk phone service is available where farmers and private veterinary practitioners can get assistance for animal problems they encounter. The “ruminant-watch” is a nationally operating group of ruminant health specialists, located at the Animal Health Service (GD) (Bartels et al., 2007). This system, in which farmers can call the GD in the Netherlands when they notice unusual or unknown clinical signs in their animals, was successful in identifying the BTV-8 outbreak in 2006 (Van Wuijckhuise et al., 2006; Dercksen and Lewis, 2007). Bluetongue was first discovered in the Netherlands, while retrospectively, Belgium and Germany probably had already been infected before the Netherlands. However, the neighbouring countries initially did not link the problems to the bluetongue virus (Saegerman et al., 2010), whereas the Netherlands did.

According to the Terrestrial Animal Health Code for bluetongue (OIE, 2010a), there are several methods for the surveillance of bluetongue. Clinical surveillance is preferably done in sheep because this species is most likely to exhibit clinical signs. Other methods of surveillance can be serological surveillance, virological surveillance, sentinel animals and vector surveillance.

For the Netherlands, three surveillance methods were applied during the BTV-8

outbreak. After the 2006 BTV-8 epidemic, the seroprevalence of BTV-8 was studied by means of a cross sectional study (Van Schaik et al., 2008). An entomological investigation was done to investigate the geographical distribution and abundance of *Culicoides* species in the Netherlands (Meiswinkel et al., 2008b) and a sentinel network of dairy cattle was implemented to monitor new BTV-8 infections in 2007 (Chapter 2 and 3) (LNV, 2006b). For this network, cattle were chosen as being the most appropriate sentinels because they were the preferred host for competent *Culicoides* and because BTV-8 is known to spread more likely in cattle than in sheep (Braverman et al., 1971; Bartsch et al., 2009).
The cattle sentinel program was effective, because in the sentinel cows new BTV-8 infections were detected prior to the first notifications of clinical signs of BTV-8 (unpublished data). Furthermore, by monthly sampling of the sentinel cows valuable information about the BTV-8 transmission in space and time was gathered, which resulted in a quantification of the BTV-8 transmission (Chapter 2) and in identification of management factors that can be taken to reduce the spread of BTV-8 (Chapter 3).

### 5.2.2. Control of BTV-8 in the Netherlands

During the BTV-8 epidemic in north-western Europe, vaccination appeared the only effective control measure. In the spring of 2008, an emergency vaccine became available. In that year, the Dutch government decided to implement a voluntary vaccination program and the majority of the cattle, sheep and goat farmers decided to have their animals vaccinated (Elbers et al., 2010).
Vaccination was proven to prevent cattle from viral replication and clinical disease after experimental challenges with BTV-8 (Wäckerlin et al., 2010). In the study where a group of vaccinated and non-vaccinated cattle were monitored (Chapter 6), the fertility of vaccinated cows did not differ before and after vaccination. In addition, vaccination of cattle appeared to protect their unborn calves against vertical transmission of BTV-8 as none of the calves from 256 vaccinated dams were BTV-8 positive (Chapter 6). Furthermore, during that study, the farmers and veterinarians were also asked to notify possible BTV related health problems in the calves born from vaccinated dams and no problems were notified.

### 5.2.3. Eradication of BTV-8 in the Netherlands

After the introduction of BTV-8 in north-western Europe, the aim was to try to

eradicate the virus again. With the availability of a vaccine against BTV-8, from 2008 on, an attempt to eradicate the virus was made. From prior experiences in the Mediterranean area, it seemed possible to eradicate the virus when the antibody level in the host population was over 80%. Therefore, the European Commission decided to subsidize part of the vaccination costs in countries that were affected by BTV-8 and that could prove that the antibody level in the susceptible host population was over 80% at the end of 2008.

In the Netherlands, the antibody level was proven to be over 80% at the end of 2008 and subsequently no new BTV-8 infections were observed until November 2010.

During 2008, BTV-8 infections were monitored in a group of unvaccinated seronegative cattle; only 17% of them seroconverted (Chapter 6 and 7). In addition, in 2009, 75 farmers notified suspicion of BTV-8 infection to the authorities but not a single one was confirmed in the PCR test at the National Reference Laboratory, Central Veterinary Institute of Wageningen UR (CVI-Lelystad). Thus increasing the proportion of immune hosts by vaccination ar field infection, seemed very effective in reducing BTV-8 transmission. Nevertheless, after 2008 the willingness to vaccinate slowly declined.

In order to regain the BTV-8 free status again, countries have to prove that they have an effective surveillance program that is able to detect new infections. Subsequently, countries have to prove that there is no transmission of the bluetongue virus for 24 months in susceptible domestic ruminant populations (OIE, 2010a).

BTV-8 was still circulating in the Netherlands in 2008. Farmers are obliged to notify a bluetongue suspicion and in 2009, none of the 75 herds in which suspicion of BTV-8 infection was notified was confirmed to be truly infected. After the vector-active period of 2009, a virological survey was carried out to study if there had been virus transmission in 2009. For this survey, 346 random blood samples stratified at compartment level were tested for BTV in a serogroup-specific PCR-test between 20 December 2009 and 12 February 2010. All samples tested PCR-negative (Muskens et al., 2010). However, it appeared that in 60% of the randomly-selected herds, at least a part of the cattle were vaccinated, and were therefore not susceptible to BTV-infection. This reduced the confidence to find BTV-circulation. Therefore, a third survey was carried out to increase the certainty about the absence of circulation of BTV-8 in 2009.

Between the 26$^{th}$ of July and the 7$^{th}$ of September 2010, 1,989 blood samples from unvaccinated heifers, equally distributed over the country, born out of vaccinated

dams and at least eight months old, were sampled. If there had been no virus transmission in 2009, these heifers had to be BTV seronegative. Therefore the samples were tested for antibodies against BTV. All samples tested negative. On a national level, the prevalence of BTV-seropositive heifers in 2009 was <0.15% with 95% confidence. When the Netherlands are subdivided into three regions, south, central and north, the seroprevalence in each of these regions was <0.65% with 95% confidence. The design prevalence was set at 1%, which seems sufficient to detect virus circulation because with an average within-herd $R_0$ of 3.8 in a susceptible population (Chapter 2) the probability of a large outbreak is fairly high. Thus, assuming a design prevalence of 1%, the results were adequate to conclude with 95% confidence that the population was free from BTV circulation in 2009 on national and regional level. Although focused on BTV serotype 8, the same conclusion can be drawn for introduction and circulation of other BTV-serotypes (e.g. serotype 1), since the used diagnostic tests detect all BTV-serotypes (Van Wuyckhuise et al., 2010).
In order to regain the BTV free status again, the authorities have to prove that the Netherlands were also free from BTV circulation in 2010. This will be done by testing a large random sample of non-vaccinated young stock born in 2009 or 2010, after the start of the *Culicoides*-free period at the end of 2010.
In the current situation, some of the countries that surround the Netherlands still seem to be BTV-8 infected and thus virus appears to be present in *Culicoides* located in north-western Europe (OIE, 2010b). Therefore, BTV-8 can only be eradicated when the proportion of immune hosts, i.e. cattle, sheep, goats and wild ruminants, is sufficiently high. With the high replacement rates in cattle herds and sheep flocks in the Netherlands, the immunity level in the host population will decrease rapidly if measures as vaccination are not taken. In order to regain the BTV free status it is very important that farmers keep vaccinating their animals for as long as BTV-8 reservoirs are present in north-western Europe.

When, eventually, the Netherlands regain the BTV-8 free status, re-emergence of the bluetongue virus can be monitored with the surveillance system in ruminants that is in place in the Netherlands (Bartels et al., 2007). The reactive part of the surveillance, the GD-Veekijker or ruminant-watch, was successful in detecting the introduction of BTV-8 in 2006 and will possibly do so again in the future. Additionally, diagnostics on submitted blood samples from aborted ruminants,

bulk milk and/or necropsy on relevant cases may increase the sensitivity of the surveillance for early detection.

**6. Knowledge gained from the bluetongue epidemic: measures that can be taken in case a new vector borne disease emerges**

The BTV-8 epidemic in north-western Europe provided us with new insights on three important points.

First, the trend towards the increasing amount of (animal) movements between countries may cause an increased risk for the introduction of emerging diseases. Before the BTV-8 outbreak in north-western Europe in 2006, bluetongue only occurred in warmer climates and the closest area in which bluetongue occurred was southern Europe. It was thought that a possible introduction of bluetongue in northern Europe would be associated with an upwards migration of its most competent vector *Culicoides imicola* (Elbers et al., 2003). Nevertheless, during the 2006 BTV-8 outbreak, vector species *C. dewulfi, C. chiropterus*, *C. scoticus* and *C. obsoletus*, which are widely dispersed within north-western Europe, also were supposed to be competent to transmit the BTV-8 virus (Meiswinkel et al., 2007, 2008b, 2008c). Furthermore, bluetongue virus serotype 8 was not one of the serotypes that already occurred in southern Europe. There are some hypotheses about the possible introduction route of BTV-8 (EFSA, 2007; Saegerman et al., 2010), but the exact source has never been discovered.

Secondly, the BTV-8 outbreak was not comparable to other BTV outbreaks and showed, that it is uncertain that a new emerging disease will behave itself in the same way as it did in other countries in the past. Before BTV-8 emerged in the Netherlands, the same serotype was already identified in Pakistan, India, South and Western Africa, and the Caribbean regions (Haresnape et al., 1988; Mo et al., 1994; Gerdes, 2004; Sreenivasulu et al., 2004). In those countries, clinical signs of BTV-8 were rarely seen (Haresnape et al., 1988), while clinical signs of BTV-8 in north-western Europe occurred in many infected animals (Van Wuijckhuise et al., 2006; Dercksen and Lewis 2007; Elbers et al., 2008a). Furthermore, the fact that BTV-8 appeared to be vertically transmittable (Chapter 5 and 6) was never documented before in any of the known bluetongue serotypes, except for modified live vaccines of BTV which have previously been described to be able to cross the placenta and have teratogenic potential (MacLachlan and Osburn 1983; MacLachlan et al., 2000)

However, vertical transmission of BTV may not have been studied extensively before because this route of transmission is of minor importance in countries in which temperatures are always sufficiently high for *Culcicoides* to transmit the virus. Thus, one should be careful to rely on historical research information, because an emerging vector-borne disease may behave differently in countries with different natural habitats, climates and different animal populations.

And thirdly, it appeared that bluetongue only stopped from spreading in the Netherlands when the protection level was high enough. This was accomplished by the massive number of cattle and small ruminants that became infected in 2007 and the high proportion of farmers that decided to vaccinate their cattle in 2008. In theory, the cold winter of 2007/2008 might also have resulted in a reduction of total BTV-8 transmission. Nevertheless, from repeated BTV epidemics in countries with colder winters than in the Netherlands it appeared that the virus can somehow survive cold and long winters.

During the first outbreak of BTV-8 in 2006, there was no appropriate vaccine available. At that moment the use of insecticides was obliged and movement restrictions were put in place according to the prescriptions of Council Directive 2000/75/EC (CEC, 2000) and Commission Decision 2005/393/EC (CEC, 2005). These restrictions were not able to stop the transmission of BTV-8 between herds because the vector movements could not be limited and the use of insecticides appeared not to be effective in reducing the vector capacity (Mullens et al., 2000, 2001; Satta et al., 2004). However, there are indications that the movement restrictions slowed down the transmission of BTV-8. In 2006, BTV-8 migrated from its initial introduction site about 50-100 km up north, but at the end of this first episode, the northern half of the country still was BTV-8 free. In contrast, in that same year, Belgium decided to declare the whole country BTV-8 infected and loosened the restrictions for animal transport. This resulted in a faster and wider spread of BTV-8 across Belgium (Mintiens et al., 2008).

## 7. Suggestions for future research

During the BTV-8 outbreak in 2007, transmission of the virus was monitored in sentinel cows. These data were used to quantify the overall transmission ratio of BTV-8 from cattle to *Culicoides* and vice versa. The sentinel data gave the opportunity to estimate one of the *Culicoides*-related parameters. Because of lack of good data

on *Culicoides* from north-western Europe, estimates for the remaining *Culicoides*-related parameters were obtained from different studies in different countries and from different *Culicoides* species. Therefore, it would be recommended to perform studies on competent *Culicoides* specoes in north-western Europe. These studies should focus on gaining better estimates for Culicoides-related parameters such as *Culicoides*-biting rates, and the probability to transmit the bluetongue virus from different hosts to different *Culicoides* species and vice versa in the north-western European situation.

After the second episode of the BTV-8 epidemic in 2007, BTV-8 appeared to be vertically transmittable (Chapter 5 and 6). However, until this moment, there is uncertainty which role these BTV-8 positive, healthy-looking calves play in the epidemiology, and in particular, in overwintering of BTV-8. Therefore it is recommended to perform further investigations to evaluate the importance of this route of transmission by means of experimental trials. In these trials, cows should be infected with BTV-8 during the second half of gestation. When BTV-8 positive calves are born, it is important to study whether the blood of these calves can infect other cattle. This can be studied by either injecting blood from infectious calves into susceptible cattle to see whether the susceptible cattle become infected with the virus or by placing these calves under circumstances where *Culicoides* species are able to feed on those calves and, subsequently, are kept together with susceptible cattle.

In 2008, the majority of the Dutch sheep and cattle were vaccinated or already had antibodies against BTV-8 as a result of infection with the field virus. Therefore, in that year also less competent *Culicoides* became infectious and thus the infection pressure decreased. During the study that was conducted during 2008, BTV-8 was transmitted among seronegative cattle and almost 200 of the study cows became infected. Nonetheless, in none of the cows clinical signs were observed, while in 2007, when the infection pressure was higher, numerous cattle showed clinical signs. It might be possible that there is a relation between viral load that is transmitted to a cow, the severity of the clinical signs and the duration of the infectious period in the cow. Therefore, it is interesting to study whether there is a relation between the dose of BTV-8 and the clinical response from cattle.

Although none of the cattle that became infected with BTV-8 during 2008 showed clinical signs (Chapter 6 and 7), a clear association between BTV-8 infection and three out of four studied fertility parameters was found and subclinical BTV-8 infections also resulted in a temporarily reduction in milk production. Nonetheless, there appeared to be no significant association from a BTV-8 infection on udder health and abortion rate, while, in earlier studies, there were indications that BTV-8 infections were associated with higher abortion rates and, hypothetically, an association between BTV-8 infections and udder health was expected. Therefore, it would be interesting to study the association of a BTV-8 infection with abortion rate and udder health in cows that suffer from a clinical BTV-8 infection in a field situation or in an experimental trial.

## 8. Concluding remarks

Based on the studies that were conducted as part of this thesis, the following conclusions can be drawn:

### *8.1. Transmission of BTV-8 within cattle herds*

In a fully susceptible herd, one BTV-8 infectious cow can infect on average 3.8 (median 2.9; 5$^{th}$ percentile=2.2 and 95$^{th}$ percentile=9.0) other cattle. However, the transmission ratio can vary between herds and months, depending on outside temperatures and herd management. Stable design and grazing management can reduce BTV-8 transmission. Keeping cattle indoors, and stables with large horizontal openings in combination with windbreak curtain and open stable doors decrease BTV-8 transmission.

In addition, BTV-8 infections during gestation can result in the birth of healthy-looking, BTV-8 positive calves. These virus-positive calves mainly originated from cows that became infected during the second half of gestation and became virus negative again within five months after birth. These virus positive calves might play a role in the overwintering of BTV-8. However, whether virus positive calves can actually start a new infection remains unclear. Vaccination seems to work fully protective against vertical transmission of BTV-8.

*8.2. Impact of BTV-8 infections*

BTV-8 infections were associated with increased mortality, reduced fertility and reduced milk production. No statistically-significant association was found between BTV-8 infections increased abortion rates and increased somatic cell count.

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## Appendix 1. Input parameters for the calculation of the economic consequences of a BTV-8 infection in a dairy herd

**Table 1.** General characteristics of Dutch dairy herds in 2010

| Parameter | mean | Reference |
|---|---|---|
| **Herd size** | | |
| Cows >2 year | 75 | Based on dairy herd census data in 2010, from the cattle health monitor. Bartels et al., 2007. |
| Young stock 1-2 years | 27 | |
| Calves 3 days- 1 year | 26 | |
| Newborn calves <3 days | 6 per month | |
| Replacement percentage | 27% | |
| **Reproductive performance of the herd** | | |
| % cattle >1 year in gestation | 66.2% | Based on:<br>• a between calving interval of 423 days<br>• a gestation period of 280 days<br>• a insemination period of 35 days<br>• a 108 day period after giving birth before the start of insemination.<br>CRV, 2010 |
| % cattle >1 year not in gestation and not in insemination process | 25.5% | |
| % cattle >1 year in insemination process | 8.3% | |
| **Milk production of the herd** | | |
| % dry cows | 12.1% | Based on a lactation period of 356 days, CRV, 2010 |

**Table 2.** Epidemiological input parameters with the value in the normal situation (baseline), the alteration for subclinical and clinical affected herds and the reference.

| Parameter | Baseline | Alteration associated with BTV-8 | | Reference |
|---|---|---|---|---|
| | | Subclinical affected herds | Clinical affected herds | |
| **% infected cattle** | | 17% (3-39%) | 60% (40-100%) | Subclinical affected herds: chapter 6 and 7. Clinical affected herds assumed based on data from chapter 2 and 3. |
| **Morbidity** | | | 4.1% (0-32.4%) | Elbers et al., 2009 |
| **Mortality** | | | | |
| Cow >1 year per month | 0.24% | | 1.36[1] (1.17-1.57) | Chapter 4 |
| Calves 3 days-1 year per month | 0.91% | | 1.37[1] (1.14-1.63) | Chapter 4 |
| Newborn calves <3 days per month | 0.76% | | 1.23[1] (1.10-1.37) | Chapter 4 |
| **Fertility** | | | | |
| Inseminations per pregnancy per cow | 1.8 times | 1.7[2] (1.4-2.0) | 1.7[2] (1.4-2.0) | Chapter 6 |
| Time between first and last insemination | 35 days | 2.5[2] (2.4-2.6) | 2.5[2] (2.4-2.6) | Chapter 6 |
| Abortion | 2% | | 2.6[2] (1.7-4.1) | Elbers et al., 2009 |
| Early calving | 3% | | 2.9[2] (1.6-5.4) | Elbers et al., 2009 |

| | | | | |
|---|---|---|---|---|
| **Reduced milk production** | | | | |
| Milk production in kg per day | 27 kg | 1.7 kg (0.9-2.6) | 1.7 kg (0.9-2.6) | Chapter 7 |
| Total reduced milk production | | 52 kg (26-77) | 52 kg (26-77) | Chapter 7 |
| **Udder health** | | | | |
| New high somatic cell count | 10% | | 2.0[2] (1.0-4.2) | Elbers et al., 2009 |

[1] MRR: mortality rate ratio

[2] OR: odds ratio

**Table 3.** Economical input parameters with the economic values and the references

| Parameter | Economic value | Reference |
|---|---|---|
| **Morbidity** | | |
| Diagnose of BTV-8 | € 80.00 | Half hour consultation by a local veterinarian |
| Treatment corticico steroids (cattle that are not in gestation) | € 50.00/ case | Dercksen et al., 2007, costs veterinarian |
| NSAIDS (Non-Steroidal Anti-Inflammatory Drugs) | € 15.00/ case | Dercksen et al., 2007, costs veterinarian |
| Antibiotics | € 50.00/ case | Dercksen et al., 2007, costs veterinarian |
| Material | € 0.75/ case | Dercksen et al., 2007, costs veterinarian |
| **Mortality** | | |
| Cow >2 years | € 1050.00/ case | Lei, 2009 |
| Young stock 1-2 years | € 1000.00/ case | Lei, 2009 |
| Calf 3 days-1 year | € 550.00/ case | Lei, 2009 |
| Newborn calf <3 days | € 85.00/ case | Lei, 2009 |
| **Fertility** | | |
| Extra inseminations | € 14.00/ insem. | Velthuis, 2010; Lei, 2009 |
| Extra time until gestation | € 9.00/ cycle | Jalvingh and Dijkhuizen, 1997; Velthuis, 2010 |
| Abortion | € 85.00/ case | Lei, 2009 |
| **Production** | | |
| Losses caused by decreased milk production in a quotum situation | € 0.12/ kg | Huijps et al., 2008 |
| **Udder health** | | |
| Subclinical mastitis | € 94.00/ cow | Huijps et al., 2008 |
| | € 72.00/ heifer | Huijps et al., 2008 |

| **Vaccination** | | |
|---|---|---|
| Implementing vaccination by a local veterinarian | € 273.33 first year in which all cattle have to be vaccinated twice | costs veterinarian |
| | € 188.33 following years in which only newborn calves have to be vaccinated twice | costs veterinarian |
| Costs vaccination | € 0.80/ vaccination/ cow | Pfizer, personal communication |

Chapter 9

# Summary

# Transmission and impact of Bluetongue virus serotype 8 in dairy cattle

Inge M.G.A. Santman-Berends

Bluetongue virus (BTV) is a double-stranded RNA virus of the Reoviridae family within the genus Orbivirus. At this moment, the serogroup of BTVs contains 24 different serotypes and, recently, a 25th BTV serotype has been proposed. Bluetongue virus causes an infectious non-contagious disease in ruminants, which is transmitted by a vector, specific species of Culicoides. Before the BTV serotype 8 (BTV-8) outbreak in north-western Europe, BTV-8 was known to cause clinical signs in sheep whereas in cattle, clinical signs of BTV-8 were only observed in sporadic cases.

The transmission of BTV-8 differs from directly transmittable viral diseases because BTV-8 requires a vector, i.e. a competent species of Culicoides, to be transmitted from one animal to another. Because of this vector borne transmission, conditions that are important for the competent vector like temperature, wind, humidity, etc., play a significant role in the spread of BTV-8.

Historically, it was believed that spread of bluetongue to northern Europe was unlikely, because the occurrence of the known competent vector, Culicoides imicola, was geographically limited to (sub)tropical regions. However, in August 2006, BTV-8 was discovered in the Netherlands for the first time. Shortly after the first detected case of bluetongue in the Netherlands, Belgium and Germany reported infected herds as well.

During the 2006 BTV-8 epidemic, 446 herds (268 sheep and 178 cattle) in the Netherlands, 682 herds (400 sheep and 282 cattle) in Belgium and 811 herds (297 sheep and 504 cattle) in Germany reported clinical signs of BTV-8 to the authorities. In 2007, BTV-8 re-emerged and, during this second epidemic, clinical signs of bluetongue in sheep and cattle seemed more severe and more herds became infected. At the end of 2007, 3,182 cattle farmers in the Netherlands had notified clinical signs of BTV-8 in their herds and the economic consequences for the cattle industry seemed considerable.

During the first and second episode of the BTV-8 epidemic, BTV-8 seemed to spread fast, and infected a large number of cattle herds. However, objectively quantified field information about the effect of BTV-8 infection on fertility, udder health, mortality and vertical transmission were not available. With such information, the economic consequences of BTV-8 on herd and national level can be quantified more accurately, and weighted against costs of control measures. Therefore, the aim of this thesis was to quantify the transmission and impact of BTV-8 in dairy cattle. Eventually, a general economic model was used to calculate the economic consequences of a BTV-8 infection in a dairy herd.

**Within-herd transmission of BTV-8**

The quantification of the transmission of BTV-8 and measures that can be taken to reduce the speed of transmission were studied in chapter 2 and 3. After the first episode of BTV-8, the Dutch government decided to start a sentinel network of 275 dairy cattle herds in which cattle were sampled every four weeks. With this sentinel network, the within-herd spread of BTV-8 was monitored during 2007. Transmission parameters were calculated and described the number of secondary infected cows in the next generation that became infected through bites of infectious Culicoides infected by one initially infectious cow.

During the 2007 BTV-8 episode, one infectious cow infected on average 3.8 (median 2.9; 5th percentile 2.2; 95th percentile 9.0) other cows through bites of infectious Culicoides. The transmission rates were highest in summer and early autumn months and declined in the months in which temperatures became lower. Furthermore, values of R0 were lower in the northern region (3.3) than in the central and southern region (4.0 and 4.1). This difference between regions could be explained by the later start of the BTV-8 epidemic in the northern region. In many northern herds, BTV-8 did not spread until September/October. In these months, conditions for Culicoides became less favourable because of declining temperatures and the transmission ratio was relatively low.

The serological data from the field that was used to quantify transmission enabled estimation of the vector to host ratio, which is assumed to vary between cows, herds and months. The median vector to host ratio was calculated at 159 (5th percentile 80; 95th percentile 2132). Based on these field data, the highest values for the vector to host ratio were found between September and November, in which BTV-8 spread between cows within-herds, but in which conditions for Culicoides became less optimal. So, more Culicoides were needed to spread the infection between cattle.

There were herds in heavily infected regions in which no or only a few cows became infected with BTV-8. Therefore it was decided to conduct a questionnaire in all sentinel herds to investigate if there were management factors and housing practices that were related to the within-herd BTV-8 transmission (chapter 3).

There appeared to be a strong association between BTV-8 transmission and grazing. Farmers that kept their cattle in the barn during the summer and autumn months stayed seronegative significantly more often, 48% vs. 14% in herds that were grazed. In addition, the longer the cattle were outside in the field, the higher

the monthly increase in BTV-8 within-herd prevalence. The monthly increase in prevalence for cattle that were outside in the field a few hours per day, throughout the day, or day and night was 5.6% (95% CI: 1.4-10.2), 11.4% (95% CI: 6.0-17.3) and 13.6% (95% CI: 7.2-20.8), respectively, relative to cattle kept indoors. In addition, an association was found between the monthly increase in seroprevalence and some factors relating to stable design. Keeping the stable doors closed during the day was linked to a higher seroprevalence rate compared to that in stables with the doors left open (3.6% (95 CI: 0.3-7.1). Furthermore, a horizontal ventilation opening (>30 cm) along the walls of the stable, and with a wind break curtain, appeared to offer some protection (-3.0% per month (95% CI: -6.0-0.2) as compared to stables that had no or, only a small, ventilation opening (<30 cm). However, stables with a large (>30 cm) horizontal opening but without windbreak curtain were not associated with a significantly lower increase in seroprevalence.

**Vertical transmission of BTV-8**

During the BTV-8 epidemic in north-western Europe, BTV-8 infections during pregnancy appeared to lead occasionally to the birth of healthy looking PCR-positive calves. The relevance of this route of transmission was studied in the Netherlands in cow-calf combinations of which the cow was infected during the 2007 BTV-8 episode (chapter 5). From the 229 cows that seroconverted for BTV-8, 37 calves (16.2%; 95% CI: 11.4-21.0) tested PCR-positive for BTV-8 within three months after birth. These PCR-positive calves were subsequently resampled every month to study their BTV-8 status. All initially PCR-positive calves became negative again within five months after birth.

After the 2008 BTV-8 episode, 48 newborn calves originating from cows that became infected during the 2008 episode were tested by PCR for BTV-8 (chapter 6). In addition 256 newborn calves from vaccinated dams were also tested for BTV-8 virus. Out of the cows that became infected during the 2008 BTV-8 epidemic, ten calves (20.8%; 95% CI: 9.3-32.3%) were born PCR positive. The majority of these positive calves were born out of dams that became infected in the second half of gestation. The odds in the second half of gestation were 15.5 times (95% CI: 1.3-190.4) higher than in the first half of gestation. Furthermore, from vaccinated dams none of the 256 calves tested PCR-positive.

**Impact of BTV-8**

Studies into the impact of a BTV-8 infection on cattle are described in chapter 4 to 7. In chapter 4, additional mortality associated with BTV-8 infections was quantified for herds that notified clinical signs to the authorities, which were confirmed to be caused by BTV-8 during the 2007 episode. Moreover, an additional analysis was performed to determine if mortality associated with BTV-8 infection occurred in non-notification herds located in BTV-8 infected compartments. Because the rendering plant distinguishes three separate age groups when collecting dead cows (newborn calves <3 days; calves from 3 days until 1 year; cows >1 year), separate analyses were conducted for the three different age groups.

In non-notification herds in BTV-8 infected compartments, mortality significantly increased 1.11 times (95% CI: 1.08-1.13) in cows, 1.20 times (95% CI: 1.17-1.23) in calves and 1.11 times (1.09-1.12) in newborn calves compared with BTV-8 non-infected months. Furthermore, confirmed notification herds had an additionally increased cow mortality rate ratio (MRR) of 1.41 (95% CI: 1.22-1.63), calf MRR of 1.29 (95% CI: 1.07-1.54), and newborn calf MRR of 1.20 (95% CI: 1.08-1.33) in the month of notification. From these results it was concluded that the BTV-8 epidemic in 2007 was associated with a slightly higher mortality. Herds that notified clinical signs that were confirmed to be caused by BTV-8 had the highest additional mortality.

To study the effect of a BTV-8 infection on fertility, milk production and udder health, 1,074 cows that were seronegative for BTV-8 in the spring of 2008 were sampled every three weeks to monitor BTV-8 seroconversion and 185 (17.2%) of the initially seronegative non-vaccinated cattle seroconverted.

These cows were 5 (95% CI: 1.9-14.3) times more likely to return to service within 56 days after first insemination, needed 1.7 (95% CI: 1.4-2.0) times more inseminations for an assumed pregnancy, and needed 2.5 (95% CI: 2.4-2.6) times more days between first and last insemination compared to the period prior to seroconversion and compared to cows not infected by BTV-8 in 2008. No association between BTV-8 infection and the chance to abort between 100 and 260 days after last insemination was found.

To evaluate the effect of BTV-8 on milk production and udder health in these cows, three parameters were defined: 1) the difference between observed and predicted fat and protein corrected milk production, 2) the natural logarithm of the somatic

cell count and, 3) the occurrence of a new high somatic cell count (>150 x103 cells/ml for heifers; > 250 x103 cells/ml for cows).
From the 185 cows that seroconverted, 77 cows had a test-day result within 30 days prior to seroconversion. In this period, the fat and protein corrected milk production was reduced with 51 kg (95% CI: 26-77 kg). BTV-8 infection seemed to increase the somatic cell count and the occurrence of new high somatic cell count but these results were statistically not significant.
By combining the estimates of the impact for mortality, morbidity, fertility, milk production and udder health with their economic values, the economic consequences of BTV-8 in a dairy herd were calculated at χ1962 (95% CI: 1284-2806) when the BTV-8 infection would lead to a clinical outbreak in a herd, and χ 133 (95% CI: 56-222) when the BTV-8 infection evolved subclinically. Vaccination of the herd will prevent these consequences of a BTV-8 infection. In herds with a subclinical outbreak, the costs of vaccination may be higher than the production losses. In herds with a clinical outbreak of BTV-8, the losses of the outbreak are probably higher than the costs of vaccination.

**Conclusions from this thesis**

In a fully susceptible herd, one BTV-8 infectious cow can infect on average 3.8 (median 2.9; 5th percentile=2.2 and 95th percentile=9.0) other cattle through bites of Culicoides. However, the transmission ratio can vary between herds and months, depending on outside temperatures and herd management. Stable design and grazing management can reduce BTV-8 transmission. Keeping cattle indoors and stables with large horizontal openings in combination with windbreak curtain and open stable doors decrease BTV-8 transmission. It seems that Culicoides are more likely to feed on grazing cows than on stabled cows and that well-ventilated stables further prevent Culicoides from entering and/or biting.
BTV-8 infections during gestation can result in the birth of healthy looking, BTV-8 PCR-positive calves. These virus-positive calves mainly originate from cows that became infected during the second half of gestation, and become virus-negative again within five months after birth. These virus-positive calves might play a role in the overwintering of BTV-8. However, whether virus-positive calves can actually start a new infection remains unclear. Vaccination seems to work fully protective against vertical transmission of BTV-8.

The BTV-8 outbreak in north-western Europe was not comparable to other BTV-outbreaks and showed that it is uncertain that a new emerging disease will behave itself in the same way as it did in other countries in the past. BTV-8 infections were associated with increased mortality, reduced fertility and reduced milk production. The economic consequences of a BTV-8 outbreak depended on the course of infection, clinical or subclinical. Whether or not it is economically beneficial for a farmer to vaccinate depends mainly on the risk of a clinical outbreak.

Chapter 10

# Samenvatting

# Transmissie en impact van Blauwtong virus serotype 8 in melkvee

Inge M.G.A. Santman-Berends

Blauwtongvirus (BTV) is een dubbelstrengsRNA virus dat behoort tot het genus *Orbivirus* van de *Reoviridae* familie. Op dit moment bestaat de groep van blauwtongvirus uit 24 serotypen en recentelijk is er een 25ste serotype voorgesteld. Het blauwtongvirus veroorzaakt een infectieuze niet-besmettelijke ziekte in herkauwers die verspreid wordt door de vector, specifieke *Culicoides*-soorten. Voor de uitbraak van blauwtongvirus serotype 8 (BTV-8) in Noordwest-Europa, stond blauwtong vooral bekend als veroorzaker van ziekteverschijnselen in schapen. In runderen werden ziekteverschijnselen niet of nauwelijks waargenomen.

Historisch gezien was het onwaarschijnlijk dat blauwtong zou kunnen spreiden in Noordwest-Europa omdat de temperaturen te laag waren voor de *Culicoides* soort die bekend stond als verspreider van blauwtong. Echter, in augustus 2006 werd BTV-8 ontdekt bij een aantal schapen in Zuid-Nederland. Vlak na deze ontdekking waren er ook meldingen van blauwtong in België en Duitsland. Gedurende de blauwtongepidemie in 2006 werden 268 schapen- en 178 rundveebedrijven in Nederland, 400 schapen- en 282 rundveebedrijven in België, en 297 schapen- en 504 rundveebedrijven in Duitsland geïnfecteerd met BTV-8.

In de zomer van 2007 begon BTV-8 opnieuw te spreiden. In dit jaar meldden meer veehouders ziekteverschijnselen van blauwtong in hun vee en de ziekteverschijnselen leken ook erger dan in 2006. In totaal zijn er in 2007 door 3.182 veehouders ziekteverschijnselen van blauwtong gemeld.

In de eerste twee jaar van de blauwtong epidemie leek BTV-8 zich snel te verspreiden en infecteerde een groot aantal bedrijven met runderen, schapen of geiten. Echter, objectief verzamelde gegevens waarmee een precieze schatting van het effect van blauwtong op de gezondheid van rundvee kon worden bepaald waren niet aanwezig. Deze gegevens zijn nodig om een schatting te kunnen maken van de economische gevolgen van blauwtong en vast te stellen of deze economische gevolgen opwegen tegen de kosten van het controleren van de epidemie door bijvoorbeeld vaccinatie.

Het doel van dit proefschrift was om de verspreiding van blauwtong onder melkvee te kwantificeren en om te onderzoeken wat het precieze effect van blauwtong was op de productiviteit van melkvee. Daarnaast is een algemeen economisch model gemaakt waarmee de economische gevolgen van een blauwtong uitbraak op een individueel bedrijf kunnen worden berekend.

**Binnenbedrijfsverspreiding van BTV-8**

De verspreiding van blauwtong verschilt van direct overdraagbare aandoeningen doordat blauwtong een vector nodig heeft om zich te kunnen verspreiden. Blauwtong wordt namelijk verspreid doordat een *Culicoides* een besmettelijke koe bijt waardoor de *Culicoides* ook besmet raakt en het virus, na vermeerdering, weer kan overdragen aan een gevoelige koe. Doordat *Culicoides* betrokken zijn bij de verspreiding van blauwtong zijn temperatuur, luchtvochtigheid en windsnelheid belangrijke factoren bij de verspreiding van blauwtong.
De verspreiding van blauwtong en maatregelen die genomen kunnen worden om de verspreiding terug te dringen zijn beschreven in hoofdstuk 2 en 3. Na het eerste jaar waarin blauwtong zich verspreidde in Nederland, besloot de Nederlandse overheid om een sentinel netwerk van melkveebedrijven op te zetten. Hierin werden runderen die nog negatief waren voor blauwtong elke maand te bemonsterd. Met de data van dit netwerk kon de binnenbedrijfsverspreiding van blauwtong worden berekend. De binnenbedrijfsverspreiding wordt weergegeven door middel van een transmissie coëfficiënt, $R_0$, en betreft het aantal runderen dat geïnfecteerd raakt door één met blauwtong besmet rund.
In 2007 besmette één geïnfecteerde koe gemiddeld 3,8 (mediaan=2,9; 5% percentiel=2,2; 95% percentiel=9,0) andere koeien door beten van besmette *Culicoides*. Blauwtong verspreidde zich het snelst in de zomer en vroege herfst. Daarna vertraagde de verspreiding doordat de temperatuur mogelijk minder gunstig werd voor *Culicoides* om het virus te vermenigvuldigen en te verspreiden. Bovendien besmette één geïnfecteerde koe in het noorden minder koeien (3,3) dan een geïnfecteerde koe in het midden of zuiden van Nederland (4,0 en 4,1). Dit verschil tussen regio's kan worden verklaard doordat BTV-8 pas later in het jaar ging spreiden in het noorden. In deze herfstmaanden waren de temperaturen lager.
De data uit de veldstudie gaven ons de mogelijkheid om één parameter te schatten die normaal gebaseerd werd op literatuurgegevens. Deze parameter betrof het aantal *Culicoides* per koe. Deze parameter zal namelijk variëren per koe, bedrijf en meetmoment. De mediaan van het aantal *Culicoides* per koe werd geschat op 159 (5% percentiel=80; 95% percentiel=2132). Het hoogste aantal *Culicoides* per koe werd gevonden in de koudere periode van september tot november waarin blauwtong wel verspreidde maar condities voor de verspreiding, zoals temperatuur, minder gunstig werden.

Ondanks de verspreiding waren er een aantal bedrijven in ernstig besmette gebieden waar geen of maar enkele koeien geïnfecteerd raakten. Er werd een enquête gehouden om uit te zoeken of er misschien management factoren waren die de binnenbedrijfsverspreiding van blauwtong beïnvloedden (hoofdstuk 3).
Uit deze enquête bleek dat er een associatie was tussen de verspreiding van BTV-8 en het weiden van rundvee. Runderen die op stal werden gehouden raakten minder vaak besmet dan runderen die werden geweid (48% vs. 14%). Daarnaast bleek dat de maandelijkse toename in percentage besmette runderen hoger was naarmate runderen langer buiten gehouden werden. Indien runderen enkele uren per dag, de hele dag, of dag en nacht buiten werden gehouden, was de maandelijkse toename 5,6% (95% BI: 1,4-10,2%), 11,4% (95% BI: 6,0-17,3%) en 13,6% (95% BI: 7,2-20,8%) hoger ten opzichte van runderen die binnen bleven. Daarnaast bleek dat blauwtong zich minder snel verspreidde in luchtige stallen. Staldeuren open houden (-3,6%; 95% BI: -7,1-0,3%) en een grote luchtinlaat aan de zijkant van de stal met windbreekgaas (-3,0%; 95% BI: -6,0-0,2) waren geassocieerd met een minder snelle stijging van het aantal met blauwtong besmette runderen.

**Transmissie van blauwtong van moeder naar het ongeboren kalf**

Gedurende de BTV-8 epidemie in Noordwest-Europa werd ontdekt dat blauwtong infecties gedurende de dracht kunnen worden overgedragen op het ongeboren kalf. Deze kalveren zijn positief voor genetisch materiaal van het blauwtongvirus (PCR-positief ) op het moment dat ze geboren worden. In hoofdstuk 5 en 6 werd deze mogelijkheid van virusverspreiding onderzocht. In hoofdstuk 5 werden 229 koeien die in 2007 besmet waren geraakt met het blauwtongvirus samen met hun kalveren onderzocht. Uit deze 229 koeien bleken 37 PCR-positieve kalveren te zijn geboren (16,2%; 95% BI: 11,4-21,0). Al deze kalveren werden uiteindelijk binnen vijf maanden na de geboorte weer PCR-negatief.
Tijdens de BTV-8 epidemie van 2008 zijn 48 kalveren uit koeien die besmet waren geraakt onderzocht met de PCR-test voor blauwtongvirus. Daarnaast werden ook 256 kalveren uit runderen die in 2008 gevaccineerd waren onderzocht op blauwtongvirus (hoofdstuk 6). Van de 48 kalveren uit besmet geraakte runderen testten er 10 PCR-positief voor het blauwtongvirus (20,8%; 95% BI: 9,3-32,3%). De meerderheid van deze kalveren werd geboren uit moeders die in de tweede helft

van de dracht besmet raakten met het blauwtongvirus. Van de kalveren uit de gevaccineerde moeders testte er geen enkele positief voor het blauwtongvirus.

**Effect van blauwtong op de productiviteit van melkvee**

Het effect van BTV-8 op de productiviteit van melkvee is beschreven in hoofdstuk 4 tot en met 7. In Hoofdstuk 4 werd onderzocht of een BTV-8 infectie geassocieerd was met verhoogde sterfte. Hiervoor werd gekeken naar de sterfte op melkveebedrijven waarvan de veehouder ziekteverschijnselen van BTV-8 meldde bij de Nederlandse autoriteiten en is er gekeken naar de sterfte op melkveebedrijven die wel gelegen waren in BTV-8 besmette gebieden, maar die geen ziekteverschijnselen van blauwtong hebben gemeld. De sterfte werd onderzocht voor drie leeftijdsgroepen namelijk pasgeboren kalveren jonger dan 3 dagen, kalveren tussen 3 dagen en 1 jaar, en runderen ouder dan één jaar. Op bedrijven in besmette gebieden die geen ziekteverschijnselen meldden was de sterfte 1,11 keer (95% BI: 1,08-1,13) verhoogd in runderen, 1,20 keer (95% BI: 1,17-1,23) in kalveren en 1,11 keer (95% BI: 1,09-1,12) in pasgeboren kalveren, ten opzichte van bedrijven in BTV-8 vrije gebieden. Op bedrijven waarvan de veehouder wel ziekteverschijnselen van blauwtong in zijn runderen meldde was de rundersterfte 1,41 keer (95% BI: 1,22-1,63) hoger, was de kalversterfte 1,29 keer (95% BI: 1,07-1,54) hoger en was de sterfte bij pasgeboren kalveren 1,20 keer (95% BI: 1,08-1,33) hoger in de maand van melding, ten opzichte van bedrijven in BTV-8 vrije gebieden. Op basis van deze resultaten werd geconcludeerd dat blauwtong een lichte stijging in sterfte veroorzaakt. Bedrijven waarvan de dieren ziekteverschijnselen van blauwtong hebben, hebben ook meer sterfte.

Gedurende 2008 werden 1.074 runderen die in de lente van 2008 nog geen afweerstoffen tegen BTV-8 hadden, regelmatig getest om te onderzoeken of er een associatie is tussen blauwtong en vruchtbaarheid en melkproductie. Gedurende 2008 raakten 185 (17,2%) van deze runderen besmet.

Besmet geraakte runderen kwamen 5 keer (95% BI: 1,9-14,3) vaker terug voor herinseminatie binnen 56 dagen na de eerste inseminatie dan koeien die niet besmet raakten, hadden 1,7 keer (95% BI: 1,4-2,0) meer inseminaties nodig om drachtig te worden en hadden 2,5 keer (95% BI: 2,4-2,6) meer dagen nodig om drachtig te worden. Er werd geen associatie gevonden tussen blauwtong en de kans op abortus.

Om het effect van blauwtong op de uiergezondheid te bepalen werden drie parameters gedefinieerd namelijk: 1) het verschil tussen de werkelijke en voorspelde melkproductie, 2) de log van het celgetal en 3) de kans op subklinische mastitis berekend op basis van een verhoogd celgetal (>150 x$10^3$ cellen/ml voor vaarzen en >250 x$10^3$ cellen/ml voor koeien).
Van de 185 koeien die besmet raakten waren er van 77 koeien gegevens over melkproductie en celgetal op basis van melkproductiegegevens, in de 30 dagen waarin de besmetting waarschijnlijk plaatsvond. In deze periode was de melkgift verlaagd met gemiddeld 51 kg (95% BI: 26-77 kg). Blauwtong leek ook het celgetal en de kans op subklinische mastitis te vergroten, maar deze resultaten waren niet statistisch significant.
Het effect van blauwtong op sterfte, melkproductie en vruchtbaarheid en de economische waarden van deze factoren werden gecombineerd om de economische consequenties van een blauwtong uitbraak op een melkveebedrijf te kunnen berekenen. Op het moment dat een BTV-8 uitbraak leidt tot ziekteverschijnselen, kunnen de economische gevolgen oplopen tot € 1.962 (95% BI: € 1.284-2.806). Wanneer een uitbraak niet leidt tot klinische verschijnselen, bedragen de economische gevolgen € 133 (95% BI: € 56-222). Vaccinatie van runderen voorkomt schade door blauwtong. Echter, wanneer BTV-8 alleen maar leidt tot een subklinische infectie zullen de kosten van vaccinatie hoger zijn dan de schade door BTV-8. Wanneer BTV-8 leidt tot ziekteverschijnselen is het goedkoper om te vaccineren.

**Conclusies**

Op een bedrijf waar alle koeien gevoelig zijn voor BTV-8 kan een besmette koe gemiddeld 3,8 (5% percentiel=2,2; 95% percentiel=9,0) andere koeien besmetten door beten van besmette *Culicoides*. Echter, de verspreiding kan variëren tussen verschillende bedrijven en verschillende maanden, afhankelijk van de temperatuur en het management. Het staltype (luchtig) en het binnen houden van rundvee werken beschermend tegen verspreiding van blauwtong.
Blauwtong besmettingen gedurende de dracht kunnen resulteren in de geboorte van blauwtong PCR-positieve kalveren. Deze kalveren worden voornamelijk geboren uit runderen die in de tweede helft van de dracht besmet zijn geraakt en worden over het algemeen weer PCR-negatief binnen vijf maanden na de geboorte.

Het is mogelijk dat deze kalveren een rol spelen in de overwintering van BTV-8 maar het is op dit moment niet duidelijk of deze PCR-positieve kalveren werkelijk een nieuwe infectie kunnen starten. Verticale transmissie kan worden voorkomen door vaccinatie.

De blauwtongvirus serotype 8 uitbraak in Noordwest-Europa was niet vergelijkbaar met eerdere blauwtong uitbraken elders. Op basis van deze ervaring moeten we er in de toekomst rekening mee houden dat een nieuwe ziekte zich in onze regio anders kan gedragen als eerder het geval was in andere regio's.

BTV-8 besmettingen waren geassocieerd met verhoogde sterfte, verminderde vruchtbaarheid en een verlaging van de melkproductie. De economische consequenties van een BTV-8 uitbraak zijn afhankelijk van de kans op insleep en de kans dat BTV-8 ziekteverschijnselen in de runderen veroorzaakt. Of het economisch gezien verstandig is om te vaccineren is afhankelijk van de kans op insleep en de kans op klinische verschijnselen door de BTV-8 uitbraak.

Chapter 11

# Dankwoord

# Curriculum vitea

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Inge M.G.A. Santman-Berends

**Dankwoord**

Het maken van elk willekeurig proefschrift bestaat grofweg uit drie delen. Het eerste deel is het onderzoeksplan en het opstarten van het traject. Daarna volgt er een periode waarin het eigenlijke onderzoek gebeurt en als laatste worden alle resultaten van het gedane onderzoek samengevoegd tot een boekje 'het proefschrift'.
In mijn geval begon de officiële aftrap van mijn promotietraject op dinsdag 26 augustus 2008. Op deze dag was er een bespreking met alle mensen die binnen de Gezondheidsdienst voor Dieren betrokken waren bij het onderzoek naar Bluetongue. In deze bespreking werd er gevraagd of er bezwaren waren indien ik zou gaan proberen om op het onderwerp Bluetongue te gaan promoveren. De mensen binnen deze groep reageerden allen erg enthousiast en daarmee was de start van mijn promotietraject een feit.

Er zijn vele mensen die in meer of mindere mate bijgedragen hebben bij de ontwikkeling van dit proefschrift, maar er is toch één persoon die hierin van onschatbare waarde is geweest en dat is Gerdien van Schaik. Gerdien, als er één iemand is die dit hele proefschrift van de eerste tot en met de laatste letter wel minimaal drie keer gelezen heeft, dan ben jij het wel. Jij was degene met wie ik alles kon bespreken van inhoudelijke discussies tot en met de kaft van het boekje. De enkele regels in het dankwoord doen eigenlijk te kort aan jouw bijdrage aan dit proefschrift, maar het mag duidelijk zijn dat dit proefschrift zonder jou niet geworden was wat het nu is.

Arjan, bedankt dat jij mijn promotor hebt willen zijn. Je inhoudelijke input, frisse blik en betrokkenheid bij de verschillende studies was erg waardevol. Bedankt voor alle tijd die je vrij gemaakt hebt voor mij, ondanks je drukke agenda. Piet Vellema, jij was eigenlijk de aanstichter van mijn promotietraject en was als co-promotor en lid van mijn begeleidingscommissie erg betrokken bij mijn onderzoek. Jij was als inhoudsdeskundige op het gebied van Bluetongue altijd als geen ander in staat om van een afstand naar mijn artikelen te kijken en van goede inhoudelijke input te voorzien. Daarnaast was ook Piet van Rijn een van de leden van mijn begeleidingscommissie. Piet bedankt voor je enthousiasme en inzet tijdens mijn promotietraject. Jij was door je positieve en enthousiaste manier van meedenken een waardevolle aanvulling van mijn begeleidingscommissie en wist altijd hoe je

mij kon motiveren. Als het even tegenzat, kon jij dit goed relativeren ('ach je zal wel een Italiaanse reviewer hebben gehad, die zitten altijd te zeuren') en als het meezat was jij altijd de eerste die mij feliciteerde.

Naast de leden van mijn begeleidingscommissie waren ook de mensen van onze Epidemiologie afdeling nauw betrokken bij mijn promotieonderzoek. Zo was Bluetongue een vast agendapunt tijdens de wekelijkse werkoverleggen en was mijn promotie toch regelmatig een van de gespreksonderwerpen op onze 'epi-kamer'. Chris, bedankt voor de input die je hebt geleverd, niet alleen bij de verschillende artikelen, maar zeker ook bij de algemene introductie en discussie. Het was echt superfijn om iemand op de kamer te hebben waaraan ik tussen het werk door even inhoudelijke dilemma's voor kon leggen.
Henriëtte, jij was mijn steun en toeverlaat tijdens het schrijven van mijn proefschrift en wist als geen ander wat ik doormaakte aangezien je in hetzelfde schuitje zit. Ik ben er trots op dat jij straks naast mij staat als mijn paranimf bij het verdedigen van mijn proefschrift. Ik ben nu klaar, jij moet nog even maar ik weet zeker dat het slechts een kwestie van tijd is voordat jij op mijn plaats staat om je proefschrift te verdedigen.
Wim, jij was niet zozeer inhoudelijk bij mijn proefschrift betrokken maar ik zal je luisterende oor, je talrijke psychologische analyses van mijn karakter en je nuchtere kijk op zaken niet snel vergeten. Bedankt daarvoor.
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Frens, als overkoepelend hoofd van de afdeling Diagnostics, Research and Epidemiology (DRE) waar onze afdeling onder valt, was jij altijd erg geïnteresseerd en enthousiast over mijn artikelen. Dit heb ik altijd erg gewaardeerd.

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je gedaan wou krijgen. Onze samenwerking heeft op een erg ontspannen en leuke manier tot een mooi resultaat geleid.
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Voor het stuk waarbij gekeken is naar verspreiding van Bluetongue hebben Aline de Koeijer en Nienke Hartemink een belangrijke bijdrage geleverd. Aline, bedankt voor het bijbrengen van de beginselen van het berekenen van transmissiecoëfficiënten. De vrijdagen dat ik bij jou heb gezeten waren erg gezellig en leerzaam. Nienke, bedankt voor het meedenken. Jouw kennis bracht ons op nieuwe ideeën en je maakte de cryptische omschrijvingen van transmissiemodellen begrijpbaar.

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Een aantal van mijn artikelen, de algemene introductie en de discussie zijn doorgelezen op juist grammaticaal gebruik van de engelse taal door Hanneke Resius. Hanneke, bedankt dat je altijd bereidt was om stukken van mij door te lezen. De uitleg die jij mij gaf over de fouten die ik maakte hebben mij tot een betere schrijfster gemaakt en heeft de kwaliteit van mijn artikelen verhoogd.

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Naast de al eerder genoemde personen zijn er natuurlijk nog veel meer mensen die op enige wijze hebben bijgedragen bij het tot stand komen van dit proefschrift. Bij dezen wil ik daarom ook iedereen die ik nog niet genoemd heb bedanken voor het luisterende oor, het geven van advies en de mentale steun. Bedankt!!!

## Curriculum vitea

Inge Santman-Berends werd geboren op 29 november 1982 te Huissen. In 2000 studeerde zij af op de HAVO van het Overbetuwe College te Bemmel en begon zij haar studie dier- en veehouderij aan de Hogere Agrarische School te Den Bosch. In 2004 studeerde zij af met als afstudeerproject 'het ontwikkelen van een bedrijfseconomische scan voor de paardenhouderij'. Vervolgens is zij dierwetenschappen gaan studeren aan de Wageningen Universiteit te Wageningen. In 2005 begon zij haar afstudeerproject bij de leerstoelgroep Kwantitatieve Veterinaire Epidemiologie welke uitgevoerd werd bij de Gezondheidsdienst voor Dieren (GD) te Deventer. In dit project werd er gekeken naar de effecten van BVD-vrij worden op vruchtbaarheid. In juni 2006 studeerde Inge af en sinds 21 augustus 2006 is zij werkzaam als epidemiologisch onderzoeksmedewerker in de Epidemilogie groep (DRE) van de GD.

## Biography

Inge Santman-Berends was born on the 29th of November 1982 in Huissen, the Netherlands. In 2000, she graduated from the Overbetuwe high school in Bemmel. In the same year she started with her bachelor study, 'companion animal and livestock' at the higher Agricultural College in Den Bosch (HAS). She graduated in 2004 on the topic, 'developing an economic scan for the horse industry'. Thereafter, she started her master study, 'Animal Ssciences' at Wageningen University. In 2005, a major thesis was performed in the Quantitative Veterinary Epidemiology group, which was carried out at the Animal Health Service in Deventer. The topic of this project was to estimate the effect of becoming BVDV free on fertility.
In June 2006, Inge obtained her MSc at Wageningen University and since then she is working as a junior epidemiologic researcher in the epidemiology group of the Animal Health Service.

## List of publications

*Scientific publications about BTV-8*

Santman-Berends, I.M.G.A., Hage, H., Lam, T.J.G.M., Sampimon, O.C., Schaik van, G., 2010. The effect of bluetongue virus serotype 8 on milk production and somatic cell count in Dutch dairy cows in 2008. J. Dairy Sci. In press.

Santman-Berends, I.M.G.A., Schaik van, G., Bartels, C.J.M., Stegeman, J.A., Vellema, P., 2010. Mortality attributable to bluetongue virus serotype 8 infection in Dutch dairy cows. Vet. Mic. In press.

Santman-Berends, I.M.G.A., Hage, J.J., Rijn van, P.A., Stegeman, J.A., Schaik van, G., 2010. BTV-8 in Dutch cows in 2008; fertility performance and the infection status of the offspring. Theriogenology. In press.

Santman-Berends, I.M.G.A., Bartels, C.J.M., van Schaik, G., Stegeman, J.A., Vellema. P., 2010. The increase in seroprevalence of bluetongue virus (BTV) serotype 8 infections and associated risk factors in Dutch dairy herds, in 2007. Vet. Mic. 142:268-275.

Santman-Berends, I.M.G.A., van Wuijckhuise, L., Vellema, P., van Rijn, P. A., 2009b. Vertical transmission of bluetongue virus serotype 8 virus in Dutch dairy herds in 2007. Vet. Mic. 141:31-35.

Van Schaik, G., Berends, I.M.G.A., Langen van, H., Elbers, A.R.W., Vellema, P., 2008. Seroprevalence of bluetongue serotype 8 in cows in the Netherlands in spring 2007, and its consequences. Vet. Rec. 163:441-444.

*Other scientific publications*

Berends, I.M.G.A., Holzhauer, M., Giessen van der, J.W., Schaik van, G., 2009. Risk of Echinococcus granulosus becoming endemic in Dutch cattle. In Dutch. Tijdschr. Diergeneeskd. 134:104-109.

Berends, I.M.G.A., Graat, E.A., Swart, W.A.J.M., Weber, M.F., Giessen van de, A.W., Lam, T.J.G.M., Heuvelink, A.E., Weering van, H.J., 2008. Prevalence of VTEC O157 in dairy and veal herds and risk factors for veal herds. Prev. Vet. Med. 87, 301-310.

Berends, I.M.G.A., Swart, W.A.J.M., Frankena, K., Muskens, J., Lam, T.J.G.M., Schaik van, G., 2008. The effect of becoming BVDV-free on fertility and udder health in Dutch dairy herds. Prev Vet Med. 84, 48-60.

Sampimon, O., van den Borne, B.H., Santman-Berends, I., Barkema, H.W., Lam, T.J.G.M., 2010. Effect of coagulase-negative staphylococci on somatic cell count in Dutch dairy herds. J. Dairy Res. 77:318-324.

Sampimon, O., Barkema, H.W., Berends, I.M.G.A., Sol, J., Lam, T.J.G.M., 2009. Prevalence of intramammary infection in Dutch dairy herds. J Dairy Res. 76, 129-136.

Sampimon, O.C., Barkema, H.W., Berends, I.M.G.A., Sol, J., Lam, T.J.G.M., 2009. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Vet Mic. 134, 37-44.

Muskens, J., Swart, W.A.J.M., Berends, I.M.G.A, Schaik van G., Lam T.J.G.M.,2008. Udder health: the influence of BVD-free status. Article in Dutch. Tijdschr. Diergeneeskd. 133, 562-565.

*Contributions to conferences and symposia*

Santman-Berends, I.M.G.A., Hage, J.J., Rijn van, P.A., Schaik, G., The reproductive performance and the PCR status of calves of cattle that seroconverted for BTV-8 in the Netherlands in 2008. EPIZONE IV Saint Maló, France, 7-10 June 1010. (oral presentation)

Santman-Berends, I.M.G.A., Stegeman, J.A., Koeijer, A.A., Vellema, P., Schaik van, G., 2010. Quantification of transmission ($R_0$) of bluetongue virus serotype-8 within dairy herds based on serological field data. EPIZONE IV Saint Maló, France, 7-10 June 1010. (poster/ abstract)

Santman-Berends, I.M.G.A., Bartels, C.J.M., Roo de, B., Vellema, P., 2009. Monthly within herd prevalence of BTV-8 in 2007 in the Netherlands and risk factors of BTV-8 on Dutch dairy herds, based on a cattle sentinel study. ISVEE XII Durban, South Africa, 10-14 August 2009. (oral presentation).

Berends, I.M.G.A., Holzhauer, J., Giessen van der, J.W.B., Schaik van, G., 2007. The risk for the re-emergence of E.granulosus in cattle in the Netherlands (in Dutch), VEEC, the Netherlands, 28 November 2008. (oral presentation).

Berends, I.M.G.A., Swart, W.A.J.M., Frankena, K., Muskens, J., Lam, T.J.G.M., Schaik van, G., 2007. The effect of becoming BVDV-free on fertility and udder health in dairy herds. SVEPM Helsinki, Finland, 27-30 March 2007. (poster)

Van Schaik, G., Berends, I.M.G.A., Vellema, P., Brouwer-Middelesch, H., Muskens, J., Bartels, C.J.M., Langen van, H.H., 2008. Mortality and abortions in Dutch cattle attributed to bluetongue. EPIZONE Bluetongue satellite symposium Brescia, Italy, 7 June 2008. (oral presentation by G. van Schaik).

Sampimon, O.C., van den Borne, B.H.P., Santman-Berends, I.M.G.A., Barkema, H.W., Lam, T.J.G.M., 2010. Coagulase-negative Staphylococci and somatic cell count in Dutch dairy herds. International Seminar on coagulase-negative staphylococci in the bovine, Ghent, Belgium, 15-16 September 2010. (Oral presentation by O. Sampimon).

Sampimon, O.C., Barkema, H.W., Berends, I.M.G.A., Sol, J., and Lam, T.J.G.M., 2007. Prevalence of coagulase- negative staphylococci on dairy farms in The Netherlands. International Heifer Mastitis Conference, Ghent, Belgium, 24-26 June 2007. (Oral presentation by O. Sampimon).

Sampimon, O.C., Barkema, H.W., Berends, I.M.G.A, Van den Borne, B.H.P., and Lam, T.J.G.M., 2010. Coagulase-negative staphylococci and somatic cell count in Dutch dairy herds. The 5th international IDF mastitis conference. Christchurch, New Zealand, 21-24 March 2010. (Poster).