Studies on verotoxigenic *Escherichia coli* O157 in Swedish cattle

From sampling to disease spread modelling

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Abstract

Verotoxigenic *Escherichia coli* O157:H7 (VTEC O157) is an important zoonotic pathogen capable of causing infections in humans, sometimes with severe symptoms such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). Cattle are considered to be the main reservoir of the bacterium. In this thesis, sampling strategies to detect VTEC O157 in a cattle herd, risk factors for the introduction and the spread of VTEC O157 in Swedish cattle herds, as well as options for control, are studied. A spatial data-driven stochastic model was developed to explore the spread of VTEC O157 by livestock movements and local transmission among proximal holdings in the complete Swedish cattle population. Overshoe sampling alone or in combination with dust and/or pooled pat sampling were established to be reliable for identifying cattle herds with animals shedding VTEC O157. Results from field studies and computer simulations show that animal movements and local spread are important for the transmission of VTEC O157 in the Swedish cattle population. However, simulated control measures based on reducing the between-herd VTEC O157 transmission by animal movements and local spread, had marginal effect in decreasing the prevalence. On the other hand, simulated control measures based on reducing the shedding and susceptibility, efficiently decreased the prevalence of VTEC O157 in the Swedish cattle population.

Keywords: Data-driven disease spread modelling, Computer simulations, Epidemiology, VTEC O157, Control, Zoonosis

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Widgren S**, Eriksson E, Aspán A, Emanuelson U, Alenius S, Lindberg A (2013). Environmental sampling for evaluating verotoxigenic *Escherichia coli* O157:H7 status in dairy cattle herds. *Journal of Veterinary Diagnostic Investigation* 25(2), 189–198. doi:10.1177/1040638712474814

II **Widgren S**, Söderlund R, Eriksson E, Fasth C, Aspán A, Emanuelson U, Alenius S, Lindberg A (2015). Longitudinal observational study over 38 months of verotoxigenic *Escherichia coli* O157:H7 status in 126 cattle herds. *Preventive Veterinary Medicine* 121(3–4), 343–352. doi:10.1016/j.prevetmed.2015.08.010

III **Widgren S**, Engblom S, Bauer P, Frössling J, Emanuelson U, Lindberg A (2016). Data-driven network modeling of disease transmission using complete population movement data: Spread of VTEC O157 in Swedish cattle. *Veterinary Research* 47:81. doi:10.1186/s13567-016-0366-5

IV **Widgren S**, Engblom S, Emanuelson U, Lindberg A (2016). Spatiotemporal modelling of verotoxigenic *Escherichia coli* O157 in cattle in Sweden: Exploring options for control. (manuscript)

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The contribution of Stefan Widgren (SW) to the papers included in this thesis was as follows:

I SW carried out the epidemiological analysis, and drafted the manuscript.

II SW participated in the design of the study, carried out the epidemiological analysis, and drafted the manuscript.

III SW participated in the design of the study, the design of the mathematical model, and the implementation of the `SimInf` R package. SW carried out the simulations and participated in the epidemiological analysis, and drafted the manuscript.

IV SW participated in the design of the study, the design of the mathematical model, carried out the simulations and participated in the epidemiological analysis as well as drafted the manuscript.

Abbreviations

CTMC	Continuous-time Markov chain
EHEC	Enterohemorrhagic *Escherichia coli*
GAM	Generalised additive model
GLMM	Generalised linear mixed model
HC	Hemorrhagic colitis
HUS	Haemolytic-uraemic syndrome
IMS	Immunomagnetic separation
LEE	Locus of enterocyte effacement
MLVA	Multi-locus variable number tandem repeat analysis
PCR	Polymerase chain reaction
STEC	Shiga toxin-producing *Escherichia coli*
VT	Verotoxin
VTEC	Verotoxigenic *Escherichia coli*

1 Background

1.1 Verotoxigenic *Escherichia coli* (VTEC)

Escherichia coli (*E. coli*) is a gram negative, rod-shaped (1.1–1.5 × 2.0–6.0 μm), aerobic and facultatively anaerobic, motile or non-motile bacterium of the family *Enterobacteriaceae* (Scheutz and Strockbine, 2015).

E. coli is an important part of the normal gut flora of humans and other mammals. However, there exists *E. coli* that are pathogenic (reviewed by Kaper et al., 2004), some of which produce verotoxin (Konowalchuk et al., 1977) (VT), an important virulence factor associated with disease in humans. Shiga toxin-producing *Escherichia coli* (STEC) is used synonymously with VTEC (O'Brien and LaVeck, 1983). VT can be divided into two main groups, verotoxin 1 (VT1) and verotoxin 2 (VT2), and then further divided into several subtypes e.g. VT1a (Scheutz et al., 2012). Other important virulence factors are intimin, an adhesin, with a central role in the type III secretion system that cause lesions to the host intestine, and enterohemolysin that damage the red blood-cells (reviewed by Mead and Griffin, 1998).

E. coli is classified by combinations of O (lipopolysaccharide), H (flagellar), and sometimes K (capsular) and F (fimbriae) antigens into serotypes (reviewed by Kaper et al., 2004). Verotoxigenic *Escherichia coli* O157:H7 (VTEC O157) is the most important serotype with respect to VTEC infection in humans (Karmali et al., 2003).

In veterinary medicine, the method used for qualitative analysis of VTEC O157 most often involves enrichment followed by immunomagnetic separaration (IMS) with beads coated with an antibody against VTEC O157 (Chapman et al., 1994). The beads are spread on sorbitol MacConkey agar plates supplemented with cefixime and potassium tellurite for culture of VTEC O157. Latex agglutination tests are performed on suspected colonies followed by polymerase chain reaction (PCR) assays to confirm the presence of the genes coding for e.g. VT1, VT2 and intmin (Gannon et al., 1997; Paton and Paton, 1998)

1.2 VTEC infection in humans

VTEC may cause gastrointestinal infection in humans, with clinical manifestations ranging from asymptomatic carriers, mild watery diarrhoea, to hemorrhagic colitis (HC), usually without fever (reviewed by Mead and Griffin, 1998). Most HC cases resolve spontaneously within a week. However, about 7–10% of the HC cases, although a higher incidence has been reported in some outbreaks, may develop haemolytic-uraemic syndrome (HUS), a severe and sometimes fatal complication, characterised by haemolytic anaemia with fragmented erythrocytes, thrombocytopenia and acute renal failure (Karmali et al., 1983)(reviewed by Mead and Griffin, 1998). HUS is more common among children under 5 years of age (reviewed by Pennington, 2010). Although several VTEC serotypes have been associated with HC and HUS, for example VTEC O26:H11, VTEC O103:H2 and VTEC 121:H19, the most commonly reported serotype is VTEC O157:H7 (Karmali et al., 2003).

VTEC O157 is a faecal-oral pathogen characterised by a very low infectious dose, suggested to be less than 50 bacteria (Tilden et al., 1996), causing person-to-person transmission to be important in many outbreaks (Garvey et al., 2016). However, ruminants, particularly cattle, are considered to be the main reservoir of VTEC O157 and infected animals excrete the bacteria in their faeces (Hancock et al., 2001). The bacteria can be transmitted to humans via various routes e.g. via contamination of the environment (Howie et al., 2003; Strachan et al., 2006), consumption of contaminated food (Cowden et al., 2001) or water (Swerdlow et al., 1992; O'Connor, 2002), or direct contact with infected ruminants (Crump et al., 2002).

Using phylogenetic analysis of single-nucleotide-polymorphisms (SNPs), VTEC O157 can be divided into nine distinct clades (Manning et al., 2008). Clade 8 is a hypervirulent variant of VTEC O157 which has caused several large outbreaks in North America (Manning et al., 2008). It has been associated with more severe disease compared to other types of VTEC O157 (Manning et al., 2008; Hartzell et al., 2011; Söderlund et al., 2012) and is often detected in HUS cases associated with cattle in Sweden (Eriksson et al., 2011; Söderlund et al., 2014).

The first VTEC O157 outbreak in Sweden occurred 1995, with a total of 110 reported cases distributed over several counties (Ziese et al., 1996). The source of the outbreak remained unknown, but was assumed to be food borne. The largest VTEC O157 outbreak in Sweden occurred 2005, when 135 persons became infected (11 HUS) after eating contaminated lettuce (Söderström et al., 2008). The lettuce was irrigated using water from a stream from which an identical VTEC O157 strain was found upstream

at a cattle farm. In Sweden, about one to five farms, particularly cattle farms, are investigated annually for an epidemiological link to VTEC infections in humans. When a link can be established the most common serotype is VTEC O157. Infections with VTEC O157 became notifiable in Sweden in January 1996. The notification was expanded 2004 to include all VTEC serotypes (Anonymous, 2016b). There were 320 domestic VTEC cases (3.3 cases per 100 000 inhabitants) notified in 2015 with an increasing trend. Most cases are notified during July to September and from southern Sweden. VTEC O157:H7 has historically been the most common serotype, however, other serotypes are increasingly reported, particularly VTEC O26:H11 and VTEC O103:H2. In a study to the estimate the burden of disease for VTEC infections in Sweden, the the total number of infections was estimated to eight times the reported cases, and direct and indirect costs associated with acute infection and the sequale HUS were estimated to 39 (CI: 17–83) million SEK annually (Sundström, 2010).

1.3 Epidemiology of VTEC O157 in cattle

VTEC O157 has been isolated from several ruminant species e.g. sheep and goats, as well as from non-ruminant mammals e.g. pigs but also from birds (reviewed by Caprioli et al., 2005). This thesis focuses on VTEC O157 in cattle. A better understanding of the VTEC O157 epidemiology in cattle could improve preventative measures aimed at reducing the prevalence of the VTEC O157 bacteria in the cattle population and thereby decrease the human exposure to this important zoonosis.

Cattle naturally infected with VTEC O157 shed the bacteria in faeces without any signs of illness (Garber et al., 1995). After the VTEC O157 infection ceases, animals become susceptible again (Cray and Moon, 1995; Wray et al., 2000). Age-related differences have been observed in the dose required to infect cattle, where calves become infected at a lower dose compared to adult cattle (Cray and Moon, 1995; Wray et al., 2000; Besser et al., 2001). Although VTEC O157 has been detected at various locations in the gastrointestinal tract in infected animals, the primary site for colonisation is the terminal rectum (Naylor et al., 2003).

A large within- and between-host variation in the, often intermittent, shedding pattern has been observed (Robinson et al., 2004; Smith et al., 2010). The duration of shedding varies widely, most animals shed for less than a week, but a month is not uncommon (Davis et al., 2006). Furthermore, calves are considered to shed for a longer period than adults (Cray and Moon, 1995). The majority of the infected animals shed in low numbers ($< 10^2$ CFU g^{-1} faeces), however, some excrete in much higher levels

($> 10^4$ CFU g^{-1} faeces) (Omisakin et al., 2003; Fegan et al., 2004; Ogden et al., 2004; Low et al., 2005). Cattle shedding at levels $\geq 10^4$ CFU g^{-1} faeces are referred to as super-shedders or high-level shedders (Chase-Topping et al., 2007, 2008). Although high-level shedders only constitute about 8-9% of infected cattle, they are estimated to account for over 96% of all VTEC O157 shed (Omisakin et al., 2003; Chase-Topping et al., 2007). Moreover, presence of high-level shedders on a farm has been observed to increase the proportion of low-level shedding animals (Chase-Topping et al., 2007).

Although the underlying mechanism is yet to be identified, the prevalence of infected cattle varies by season. A common finding in several regions is that the prevalence of infected cattle increase during the warmer months (Chapman et al., 1997; Mechie et al., 1997; van Donkersgoed et al., 1999; Conedera et al., 2001; Barkocy-Gallagher et al., 2003; Milnes et al., 2009). However, exceptions to the commonly observed seasonal pattern exist. A Swedish nationwide VTEC O157 monitoring study at the abattoirs found an insignificant seasonal variation in the number of positive faecal samples (Boqvist et al., 2009) and two studies from Scotland found that the prevalence was greater during the cooler months when the cattle were housed (Synge et al., 2003; Ogden et al., 2004). It has been hypothesised that endocrine effects, associated with the day length, may be responsible for the seasonal variation in the prevalence (Edrington et al., 2006, 2008). However, a recent experimental study suggests that the seasonal variation results from seasonal differences in the exposure dose, and not from intrinsic factors, such as the endocrine levels (Sheng et al., 2016).

Several management risk factors are associated with cattle shedding VTEC O157, for example purchase of new animals (Nielsen et al., 2002; Schouten et al., 2004), large group sizes (Vidovic and Korber, 2006; Ellis-Iversen et al., 2007), feeding and bedding material (Ellis-Iversen et al., 2007; Cernicchiaro et al., 2009). Furthermore, the prevalence at the herd level has been reported to be associated with production type (Cobbold et al., 2004; Cobbaut et al., 2009). The prevalence of VTEC O157 in cattle have been extensively studied in various regions, and was estimated to 5.68% (95% CI, 5.16–6.20) at the global level (reviewed by Islam et al., 2014).

The prevalence of VTEC O157 in Sweden has been monitored regularly at abattoirs since 1996 (Table 1). The increase observed 2005–2006 is most likely due to a change in the analytical technique, but a true increase in the prevalence cannot be excluded (Boqvist et al., 2009). The positive samples are mostly isolated from cattle in southern Sweden (Albihn et al., 2003; Boqvist et al., 2009; Anonymous, 2016b).

In a Swedish nationwide prevalence study conducted 1998-2000, VTEC

Table 1: Summary of monitoring verotoxigenic *E. coli* O157:H7 (VTEC O157) in faecal and ear samples collected from cattle at abattoirs in Sweden during 1996–2012. Positive faecal samples during 2011–2012 and 2011–2012 were further analysed to identify hypervirulent strains (clade 8).

	Faecal samples			Ear samples	
Year	Total	Positive (%)	Clade 8	Total	Positive (%)
1996–1997	3071	37 (1.2)			
1997–1998	2308	7 (0.3)			
1999	2057	14 (0.7)			
2000	2001	34 (1.7)			
2001	1998	36 (1.3)			
2002	2032	29 (1.4)			
2005–2006	1758	60 (3.4)		446	54 (12.1)
2008–2009	1993	65 (3.3)		500	41 (8.2)
2011–2012	2376	73 (3.1)	15		
2014–2015	1492	33 (2.2)	5		

O157 was isolated from 33 (8.9%) of the 371 randomly selected dairy herds. The prevalence was higher (23.3%) in the south-west of Sweden (Halland) compared to the rest of Sweden.

1.4 Control of VTEC O157 in cattle

It is important that slaughter of cattle is carried out properly to protect consumers. However, no matter how well slaughter is conducted the environmental transmission route remains. It is therefore necessary to find efficient interventions that could reduce the prevalence of VTEC O157 in the cattle population.

Principles for control involve: exposure reduction strategies; exclusion strategies; and direct antipathogen strategies (reviewed by LeJeune and Wetzel, 2007). Exposure reduction strategies consists of measures to control the pathogen in the cattle environment e.g. by securing feed (Crump et al., 2002) and water (Faith et al., 1996) quality, reducing animal density (Vidovic and Korber, 2006), and control house flies (Alam and Zurek, 2004; Ahmad et al., 2007). The main exclusion strategy involves the use of probiotics e.g. *Lactobacillus acidophilus* culture (Brashears et al., 2003; Elam et al., 2003; Younts-Dahl et al., 2005). Direct antipathogen strategies involve e.g. vaccination to prevent pathogen colonisation and faecal excretion (Thomson et al., 2009; Fox et al., 2009; McNeilly et al., 2010; Snedeker et al., 2012; Varela et al., 2013), and direct application of therapeutic agent to the terminal rectum (Naylor et al., 2007).

1.5 The Swedish livestock population

European Union legislation requires all bovine animals to be registered in national databases (Anonymous, 2000, 2004). The Swedish database, managed by the Swedish Board of Agriculture, contains geographical information on each holding, where and when a cattle was born, destination and date of movement, as well as date of death or slaughter (Nöremark et al., 2009).

In 2015, there were a total of about 1 475 000 cattle and approximatly 17 500 cattle farms, mainly located in the southern and central parts of Sweden. The number of holdings has decreased over recent decades, but the average herdsize has increased. In 2015, there were about 340 000 cows in 4200 dairy herds with an average of 81 cows per herd. The number of cows for calf production was approximatly 184 000 with an average herd of 18 cows (Anonymous, 2016b).

According to national legislation all cattle must be kept on pasture during the summer months (Jordbruksverket, 2016).

2 Aims of the thesis

A prerequisite for implementing interventions to reduce the prevalence of VTEC O157 in cattle is a good understanding of VTEC O157 epidemiology in cattle, and efficient sampling strategies for identifying cattle herds with animals shedding VTEC O157. The overall aim of the thesis was to gain further knowledge on the epidemiology, the spread and the control of VTEC O157 in cattle herds.

The specific aims of the thesis were to:

- Validate environmental sampling (consisting of dust, overshoe and pooled pat samples) of the farm environment as a strategy to determine the VTEC O157 herd status.
- Study the dynamics of VTEC O157 in the cattle farm environment over an extended period of time and to investigate potential risk factors for the presence of the bacteria.
- Develop a data-driven simulation framework capable of incorporating the within-herd population demographic and between-herd animal movements to study the spread of VTEC O157 in the complete Swedish cattle population.
- Explore options to reduce the prevalence of VTEC O157 in the cattle population using the developed simulation framework.

3 Material and Methods

This section gives a brief description of the material and methods used in the different studies. Details are presented in each of the papers (**I–IV**).

3.1 Livestock data (studies II, III and IV)

Data on the geographical location of all cattle holdings in Sweden as well as all reports to the national cattle database covering the period from 1 July 2005 to 31 December 2013 were obtained from Swedish Board of Agriculture. The data contained a total of 18 649 921 reports with detailed information for each individual about; *i)* the date and the holding for the birth, *ii)* the date and the source and destination holding for any movements, and *iii)* the date for slaughter or death (Nöremark et al., 2009). Each unique holding identifier (n = 37 221) in the data corresponds to a single geographical location where animals are kept, and could e.g. correspond to a farm building or pasture. Exact coordinates were found for 84% of the holdings. Coordinates for the other holdings were randomly sampled within the postal code area.

3.2 Study population (studies I and II)

The criterion for inclusion in study **I** was that VTEC O157 had recently been detected in the herd or that an animal from the herd had sampled positive at slaughter. The time period from the previous sampling occasion with a positive finding to the sampling in the current study ranged from 8 to 370 days with a median of 45 days. The farmers participating in the study voluntarily entered their herds, and 31 Swedish dairy herds were sampled.

The target populations in study **II** were dairy and suckler herds located in areas in Sweden where VTEC O157 had been detected in previous studies (Albihn et al., 2003; Eriksson et al., 2005; Boqvist et al., 2009). In four geographically separate areas in the southern part of Sweden, the regional

livestock association selected a convenience sample of herds within a limited geographical area served by the association. The VTEC O157 herd status was not known when farmers were asked to participate on a voluntary basis. A total of 126 herds were included from the following regions: *1)* Falköping (dairy = 20; suckler = 8), *2)* The Isle of Gotland (dairy = 19; suckler = 8), *3)* Halland (dairy = 18; suckler = 25), and *4)* Växjö (dairy = 19; suckler = 9).

3.3 Sampling (studies I and II)

In each herd in study **I** the animals were divided into three age categories: calves (6 weeks to 4 months), young stock (4–12 months), and adults (> 12 months). Within each age category, both individual faecal samples and environmental samples consisting of dust, overshoe, and pooled pat samples were collected. Overshoe sampling was performed by fitting gauze to the outside of each boot before walking around in all areas where the animals were kept. Pooled pat samples, consisting of fresh faeces were taken from the floor where the animals were kept. The dust samples were collected on paper cloths by wiping surfaces such as walls, gates, and water appliances where the animals were kept.

The selected herds in study **II** were visited by staff from the regional livestock association, approximately every sixth or eighth week from October 2009 to December 2013 (38 months). Overshoe and pooled pat samples (obtained as in study **I**) was used to determine the VTEC O157 herd status. At each visit, overshoe and pooled pat samples were collected from each of two age categories: calves (6 weeks to 4 months) and young stock (4–12 months). For animals kept indoors, the samples were collected as described in study **I**. For animals kept outdoors, areas with many recently voided faecal pats were selected. Each pooled pat sample consisted of approximately 50 g of recently voided faecal pats, collected from different places. Overshoe sampling was performed by walking on at least 20 of the recently voided faecal pats.

3.4 Bacteriological analysis (studies I and II)

In study **I** the individual faecal samples from each herd were pooled three and three with material from within the same age category. The pooled individual faecal samples, the overshoe samples, and the pooled pat samples, as well the dust samples were pre-enriched in modified tryptone soya broth (mTSB) for 18–24 hr followed by immunomagnetic separation (IMS). The immunomagnetic beads were spread on sorbitol MacConkey agar plates and

incubated for 18–24 hr. Suspected colonies were further analysed with biochemical methods and finally confirmed for VTEC O157 with polymerase chain reaction (PCR).

The overshoe samples and the pooled pat samples in study **II** were analysed as described in study **I**. The VTEC O157 herd status was classified as positive if VTEC O157 was detected in any of the environmental samples.

3.4.1 Molecular typing of isolates (study II)

On every occasion where a herd was positive, multi-locus variable number tandem repeat analysis (MLVA) typing was performed on isolates to provide data for molecular epidemiology, and a lineage-specific PCR assay was used to identify hypervirulent strains (clade 8) as previously described in Söderlund et al. (2014). Clustering of MLVA data was performed using the minimum spanning tree algorithm in Bionumerics 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium).

3.5 Statistical analysis (studies I and II)

3.5.1 Evaluation of environmental sampling (study I)

The ability of the environmental samples to detect VTEC O157 in a herd was evaluated in comparison to the prevalence of pooled individual faecal samples. Let π_h denote the within-herd pool prevalence in a herd, calculated as $\pi_h = 100 \times$(Number of positive pools)/(Number of sampled pools). Similarly, let π_g denote the within-group pool prevalence, calculated as $\pi_g = 100 \times$ (Number of positive pools)/(Number of sampled pools) in the group of animals in each age category.

The following seven combinations of environmental samples were evaluated: *1)* pooled pat only, *2)* dust only, *3)* overshoe only, *4)* dust and pooled pat, *5)* overshoe and pooled pat, *6)* dust and overshoe, and *7)* dust, overshoe, and pooled pat. To maximise sensitivity, each combination of the environmental samples was interpreted in parallel i.e. if any of the samples in the combination were positive, the result was considered positive (Gardner et al., 2000).

The probability to detect VTEC O157 in each of the seven combinations of environmental samples, given the pool prevalence of individual faecal samples, was estimated with a generalised linear mixed model (Breslow and Clayton, 1993) (GLMM). The candidate explanatory variables were the within-herd pool prevalence π_h, the within-group pool prevalence π_g, and the age category of the sampled group. To test if a contextual effect was present, π_h was added together with π_g, and to reduce collinearity, π_g was

Table 2: Longitudinal observational study of verotoxigenic *E. coli* O157:H7 (VTEC O157) in Sweden between October 2009 and January 2013. Description of the candidate explanatory variables in the statistical analysis of the samples from the cattle farm environment in study **II**.

Variable	Description
status_t	The VTEC O157 herd status with two levels (0 = negative, 1 = positive). The status at the sample point was classified as positive if any of the environmental samples were positive else negative
status_{t-1}	The previous herd status at $t-1$ with two levels (0 = negative, 1 = positive).
status_{t-2}	The previous herd status at $t-2$ with two levels (0 = negative, 1 = positive).
introduction	Animals were introduced to the herd within 90 days before sampling with two levels (0 = no, 1 = yes).
log(herd size)	The logarithm of average number of animals at the herd during the period 90 days before sampling
herd type	The type of herd containing the two levels: dairy or suckler
log(n_5000m)	The logarithm of average number of animals within a radius of 5000 m from the herd within 90 days before sampling.
inf_5000m	Herds within a radius of 5000 m from the herd with positive status during the period 90 days before sampling (0 = no, 1 = yes)
region	The geographical region of the herd containing the four levels: Falköping, Gotland, Halland and Växjö.
year	The year of the sampling containing the three levels: 2010, 2011 and 2012.
quarter	The quarter of the year of the sampling, containing the four levels: Q1 (January–March), Q2 (April–June), Q3 (July–September) and Q4 (October–December)

centred by subtracting π_h (Dohoo et al., 2009). Fischer's exact test (Agresti, 2002) was used to evaluate if the proportion of positive pools from calves was statistically different from those for young stock and adults. Comparison was also made between young stock and adults.

3.5.2 Risk factors for positive VTEC O157 status (study II)

A generalized estimating equations (GEE) (Liang and Zeger, 1986) model with binomial distribution and logit link function was used to study population averaged associations between herd status and explanatory variables (Table 2). The dependent variable was the binary environmental sampling result, status_t.

The herds were repeatedly sampled and thus data were clustered within herds with a serial correlation between sample occasions. A first-order autoregressive covariance structure was used to account for this dependence between observations. An initial model was created, with herd as cluster and status_{t-1}, status_{t-2}, introduction, log(herdsize), herdtype, log(n_5000m), inf_5000m, quarter of the year and region as fixed effects, where livestock data was used to determine introduction, herdsize and n_5000m. Moreover, to enhance the biological plausibility of the analysis, interaction terms were

added to the initial model between both status_{t-1} and status_{t-2} and each of introduction, log(herdsize), herdtype, log(n_5000m) and inf_5000m. The model was fitted with the `geepack` (Højsgaard et al., 2005) R package, using manual stepwise backward elimination of non-significant ($P > 0.05$) effects. The least-squares means was calculated for the interactions and the relevant contrasts using the `lsmeans` R package (Lenth, 2016). The first two observations from each herd were dropped, since they did not have information of status_{t-2}. The final dataset used in the statistical analysis contained 1959 complete observations from 124 herds.

3.6 Disease spread modelling (studies III and IV)

3.6.1 Disease spread model (studies III and IV)

The VTEC O157 infection dynamics was modelled with a stochastic within-holding model coupled to other holdings with animal movements. The within-holding spread model was a SIS_E compartment model with the two disease compartments: susceptible (S) and infected (I) and the environmental compartment (E) contaminated with VTEC O157 by infected animals. Susceptible animals were assumed to become infected indirectly through contact with VTEC O157 in the environment. Furthermore, infected animals were assumed to recover and return to the susceptible compartment. The susceptible and infected compartments were further divided into three age categories indexed by j: (1) calves - younger than 120 days, (2) young stock - between 120 and 365 days, and (3) adults - older than 365 days.

The transitions between the susceptible and infected compartments were modelled as a continuous-time discrete state Markov chain (CTMC) using the Gillespie's Direct Method (Gillespie, 1977). Demographic events and animal movements were incorporated from livestock data.

Let $\varphi_i(t)$ denote the concentration of the environmental infectious pressure in holding i. The transition from susceptible to infected depends on $\varphi_i(t)$ and the age dependent indirect transmission rate υ_j

$$S_{ij} \xrightarrow{\upsilon_j \varphi_i} I_{ij} \tag{1}$$

The transition from infected to susceptible depends on the age dependent recovery rate γ_j

$$I_{ij} \xrightarrow{\gamma_j} S_{ij} \tag{2}$$

The environmental compartment E_i was modelled as the time dependent environmental infectious pressure $\varphi_i(t)$ within each holding i. Let α denote the average shedding rate per day per infected individual that

contributed to $\varphi_i(t)$, and let $\beta(t)$ denote the rate per day of the bacterial decay and therefore reduction in $\varphi_i(t)$. Furthermore, let S_i and I_i denote the number of susceptible and infected in holding i, respectively, and $N_i = S_i + I_i$ the size.

In study **III**, a small background infectious pressure ε was included to allow for other indirect sources of environmental contamination (e.g. birds, rodents). The differential equation for the environmental infectious pressure in each holding was

$$\frac{d\varphi_i(t)}{dt} = \frac{\alpha I_i(t)}{N_i(t)} - \beta(t)\varphi_i(t) + \varepsilon \tag{3}$$

In study **IV**, the model was extended to include local spread of $\varphi_i(t)$ among proximal holdings within a radius $r = 5000$m. It was assumed that the magnitude of the local spread among proximal holdings decreased with the distance between them. Let D be the rate of the local spread and d_{ik} the distance between the two holdings i and k. The time dependent environmental infectious pressure $\varphi_i(t)$ was modelled as

$$\frac{d\varphi_i(t)}{dt} = \frac{\alpha I_i(t)}{N_i(t)} + \sum_k \frac{\varphi_k(t)N_k(t) - \varphi_i(t)N_i(t)}{N_i(t)} \cdot \frac{D}{d_{ik}} - \beta(t)\varphi_i(t) \tag{4}$$

To incorporate seasonality in the infection dynamics, $\beta(t)$ was allowed to vary over the year. In study **III**, the year was evenly divided by four quarters, with β_{q1}, β_{q2}, β_{q3}, and β_{q4} in each quarter, respectively. In study **IV**, the year was divided into the four seasons: spring, summer, fall and winter, with β_1, β_2, β_3, and β_4 reflecting the rate of decay in each season, respectively. The day of the year when each season started in each holding was determined from meteorological data.

3.6.2 Model calibration (studies III and IV)

The model calibration in the studies **III** and **IV** was addressed by replicating the observations in study **II** in simulations and measuring the difference between the observed and simulated data with an objective function $G(\theta)$, where θ was the vector of model parameters in the simulation. The objective functions differed between the studies **III** and **IV**. The parameter estimation was then approached as an optimisation problem to find the values of the parameters θ that minimised the objective function $G(\theta)$ with the Nelder-Mead algorithm (Nelder and Mead, 1965).

The observations in study **II** was replicated in simulations, as follows. Let Y^*_{in} denote the nth observed VTEC O157 status (1-positive; 0-negative)

in holding i at time t_n. Similarly, let $Y_{in}(\theta)$ denote the simulated status, corresponding to Y^*_{in}. To determine the status $Y_{in}(\theta)$ that could have been found if simulated holdings had been sampled, the environmental sampling was simulated. First, pools (pool size = 3) were randomly created within each age category from the number of susceptible and infected individuals in holding i at time t_n in the simulation. Then each pool was randomly classified as positive or negative, with P(positive) equal to the test sensitivity from Arnold et al. (2008), given the proportion of infected individuals in the pool. Similarly, using the estimated pool prevalence, the holding was randomly classified as positive or negative given the sensitivity of the environmental sampling protocol from study **I**.

In study **III**, a generalised additive model (Hastie and Tibshirani, 1986) (GAM) of the status against the day of the year was fitted to the observed, Y^*_{in}, and simulated, $Y_{in}(\theta)$, data. $G(\theta)$ was equal to the sum of the squared differences in the coefficients in the fitted GAM between the observed and simulated data.

In study **IV**, the objective function $G(\theta)$ was equal to the sum of two parts, $G(\theta) = G_1(\theta) + G_2(\theta)$, where $G_1(\theta)$ considered the number of infected holdings, and $G_2(\theta)$ the incidence cases of infected holdings i.e. the number of new holdings that was found with a positive status. $G_1(\theta)$ was equal to the sum of the squared differences in the number of positive statuses, aggregated quarterly, between the observed, Y^*_{in}, and simulated, $Y_{in}(\theta)$, data. $G_2(\theta)$ was equal to the sum of the squared differences in the number of incidence cases, aggregated quarterly, between the observed, Y^*_{in}, and simulated, $Y_{in}(\theta)$, data. Two models were fitted, one with local spread among proximal holdings, and one without local spread ($D = 0$).

3.6.3 Exploring spread on a national scale (studies III and IV)

In study **III** the following simulation experiment was conducted to explore the VTEC O157 spread model on a national scale. There are eight NUTS level 2 regions (Nomenclature of territorial units for statistics) (Anonymous, 2003) in Sweden, from south to north. In each of these regions, a set of 126 holdings were randomly selected. In each region, each selected holding was mapped to represent one herd in study **II**. These eight new sets of holdings represent what may have been found if study **II** had been conducted in each of the regions. Simulations were now conducted to explore the spread among these holdings. A multivariable linear regression model was used to assess the relationship between the proportion infected holdings and the NUTS 2 region and the quarter of the year.

Comparison of the spatial distribution of the simulated VTEC O157

holding status in the complete Swedish cattle population was performed between the two models in study **IV**.

3.6.4 Sensitivity analysis (study III)

Sensitivity analysis was performed to explore how variation in the model parameters α, β_{q1}, β_{q2}, β_{q3}, β_{q4}, γ_j, υ_j and ϵ would influence the outcome of the simulations on national scale.

3.6.5 Input data (studies III and IV)

The livestock data was used in the disease spread simulations in studies **III** and **IV** to model the demographic events (births, imports, ageing, slaughter, deaths) in each holding, as well as animal movements.

In study **IV**, data from the Swedish Meteorological and Hydrological Institute (SMHI) was used to determine when each season (winter, spring summer, fall) started in each holding (Anonymous, 2016a).

The data from study **II** with VTEC O157 herd-level statuses was used to calibrate the model parameters in studies **III** and **IV**.

3.6.6 Exploring options for control (study IV)

The effectiveness of control measures was investigated from numerical experiments comparing a baseline i.e. the outcome from simulations with the calibrated model parameters, with the outcome from simulations with parameters adjusted to reflect the control scenario in question, using the model with local spread.

The following control measurements were considered: *i)* 10% reduction of the average shedding rate α (e.g. by vaccination), *ii)* 10% reduction of the indirect transmission rates υ_1, υ_2 and υ_3 (e.g. by feeding probiotics), and *iii)* both *i)* and *ii)* together.

Effectiveness of control was also considered for pathogen transmission due to livestock movements. This was implemented in the model simply by changing the state, conditioned on network measures, of an infected individual to susceptible when it was moved, so that infection was not transferred to the destination holding. The reduction of the pathogen transmission due to livestock movements was based on the in-degree (ID) (Wasserman and Faust, 1994), out-degree (OD) (Wasserman and Faust, 1994), and ingoing contact chain (ICC) (Nöremark et al., 2011), as well as outgoing contact chain (OCC) (Dubé et al., 2008). The ID, OD, ICC and OCC over 90 days were calculated weekly for each holding. The following cut points were considered for ID and OD: > 1, > 2, > 3 and > 4, and for ICC and OCC: > 2, > 4, > 6, > 8 and > 10. In the simulations, if the ID or the ICC for a receiving holding exceeded the cut-point for a certain week all

incoming infected animals were shifted to become susceptible. Similarly, if the OD or the OCC for a sending holding exceeded the cut-point in any week all outgoing infected animals were shifted to become susceptible.

Finally, although unrealistic to consider for control, scenarios were also generated to compare the outcome when blocking all between-holding transmission routes via: *i)* movements, *ii)* local spread, and *iii)* both movements and local spread.

4 Results

4.1 Evaluation of environmental sampling (study I)

Positive overshoe samples, alone or in combination with dust and pooled pat samples were detected in 20 (0.83, 95% CI 0.63–0.95) out of the 24 herds where pooled individual faecal samples were positive.

The within-group pool prevalence π_g ranged from 0% to 100% and the within-herd pool prevalence π_h ranged from 0% to 57%. In the four herds with the lowest within-herd pool prevalence $\pi_h > 0$, there were no positive environmental samples. Moreover, a statistically significant contextual effect of the within-herd pool prevalence was found in the probability of detection for the 2 combinations "dust and overshoe" and "dust, overshoe, and pooled pat" within each age category.

Age was not found to be a significant predictor when modelling the outcome of the environmental sampling. However, the proportions of positive pooled individual faecal samples in calves (0.28) and young stock (0.27) were significantly different from those for adults (0.09). There was no statistically significant difference between the proportion of positive pools in calves and young stock.

4.2 Risk factors for positive VTEC O157 status (study II)

The herds were on average sampled at 17 occasions, on average 64 days apart, and VTEC O157 was detected at least once in 67 (53%) of the herds. Clustering of MLVA profiles yielded 35 clusters; most herds (n=46) had a single MLVA pattern, but 2 clusters were found in 18 herds, 3 clusters in two herds and 4 clusters in one herd. The clusters were distributed among the regions but nearby farms often belonged to the same MLVA cluster. Clade 8 isolates were found in twelve herds in Halland and six herds in Falköping.

The detection of VTEC O157 was significantly more likely if the herd was positive at status$_{t-2}$, increased with herd size, was more likely in 2010

than in the other years, and during the third and fourth quarters compared to the first quarter of the year. The other variables in the model were part of interactions. Thus:

- Having a positive neighbouring farm increased the odds of being positive for herds that were not already positive at the previous sampling.
- Introducing animals into a herd significantly increased the odds of finding VTEC O157 if the herd previously tested negative. In comparison, if the herd previously tested positive, introducing animals did not increase the odds.

Among herds that tested positive at least once, the percentage of positive test occasions ranged from 6 to 72% (mean = 19%). The odds for testing positive were higher in a herd with a previous positive sample, compared to a herd with a previous negative status. The odds increased more in a dairy herd compared to a suckler herd. If both a dairy and suckler herd were positive at the previous sampling, there was no statistically significant difference in the odds of testing positive again. In contrast, if they both were negative at the previous sampling, the odds were slightly less for a dairy herd compared to a suckler herd.

4.3 Demographic events and movements (studies III and IV)

The number of holdings decreased in the Swedish cattle population during the 8.5 year study period (1 July 2005 to 31 December 2013) with an evident seasonal pattern with more active holdings (having at least one animal) during the pasture season. The total cattle population in Sweden was about 1.6 million individuals with a slightly decreasing trend during the study period. There was a seasonal pattern in both the demographic events and animal movements. Births and imports peaked during spring each year. Slaughter and exports had a bimodal shape with a sharp decline at the end of each year. Animal movements and the proportion of connected holdings had one peak during spring and one peak during autumn.

4.4 Model calibration (studies III and IV)

4.4.1 Study III

The simulated outcome in study **III** showed no seasonal variation in the proportion positive holdings unless the decay of the environmental infectious pressure β was allowed to vary in each quarter of the year.

Table 3: Comparison between fitted parameters from two pathogen transmission models to study the infection dynamics of verotoxigenic *Escherichia coli* O157 in the complete Swedish cattle population. Both models included spread between holdings by livestock movements and one model also included local spread among proximal holdings.

		Local spread	
Parameter	Description (unit)	Yes	No
α	The rate of shedding from infected individuals (units per day)	$= 1.00 \times 10^{0}$	$= 1.00 \times 10^{0}$
β_1	The rate of the decay of the environmental infectious pressure during spring (per day)	1.09×10^{-1}	1.17×10^{-1}
β_2	The rate of the decay of the environmental infectious pressure during summer (per day)	1.03×10^{-1}	0.94×10^{-1}
β_3	The rate of the decay of the environmental infectious pressure during fall (per day)	1.14×10^{-1}	1.13×10^{-1}
β_4	The rate of the decay of the environmental infectious pressure during winter (per day)	1.25×10^{-1}	1.28×10^{-1}
υ_1	The indirect transmission rate of the environmental infectious pressure in animals younger than 120 days (per animal per day)	1.84×10^{-2}	1.56×10^{-2}
υ_2	The indirect transmission rate of the environmental infectious pressure in animals between 120 and 365 days of age (per animal per day)	1.84×10^{-2}	1.56×10^{-2}
υ_3	The indirect transmission rate of the environmental infectious pressure in animals older than 365 days (per animal per day)	0.98×10^{-2}	1.15×10^{-2}
γ_1	The recovery rate of infection in animals younger than 120 days of age (per animal per day)	$= 1.00 \times 10^{-1}$	$= 1.00 \times 10^{-1}$
γ_2	The recovery rate of infection in animals between 120 and 365 days of age (per animal per day)	$= 1.00 \times 10^{-1}$	$= 1.00 \times 10^{-1}$
γ_3	The recovery rate of infection in animals older than 365 days of age (per animal per day)	$= 1.00 \times 10^{-1}$	$= 1.00 \times 10^{-1}$
D	The rate of local spread among proximal holdings (per day per m)	0.10×10^{0}	$= 0.00 \times 10^{0}$

= Fixed value during model fit.

4.4.2 Study IV

The model with local spread reached a minimum at $G(\theta) = 1020$, where $G_1(\theta) = 641$ and $G_2(\theta) = 379$. The model without local spread reached a minimum at $G(\theta) = 1077$, where $G_1(\theta) = 528$ and $G_2(\theta) = 549$. Although, the overall fit $G(\theta)$ was similar between the two models, the model with local spread reached a lower value for $G_2(\theta)$, the fit against the number of new holdings that was found with a positive status each quarter of the year. In contrast, the model without local spread had a lower value for $G_1(\theta)$, the fit against number of positive statuses in each quarter.

When comparing the observed (**II**) and simulated data, quarterly, both the model with and without local spread consistently underestimated the

total number of infected holdings, as well as the incidence cases of infected holdings i.e. the number of new holdings that was found with a positive status each quarter, particularly in the third and fourth quarters of 2010. Furthermore, the numbers were consistently less for the model without local spread compared to the model that included local spread.

The overall pattern was similar between the two models, despite the differences in the fitted parameter values (Table 3). Both models had an indirect transmission rate that was higher for animals under one year of age compared to older animals. Furthermore, both models had the highest rate of the bacterial reduction per day during the winter and the least reduction during the summer.

Both models had a seasonal pattern in the holding-and individual-level prevalences that peaked at similar time points each year (Figure 1). The prevalences in each age category were similar in both models, where Adults < Calves < Young stock. However, the between-year variation in which date the prevalence peaked was greater in the model with local spread. The holding-level prevalence was higher and the between-year variability in the date for the peak prevalence was greater in the model that included local spread (Figure 1).

4.4.3 Exploring spread on a national scale (studies III and IV)

The multivariable linear regression model in study **III** showed that the proportion of infected holdings was significantly higher in the southern region SE22 (Sydsverige) compared to the other regions. Furthermore, it was significantly lower in quarter two and three and higher in quarter four compared to quarter one. The highest proportion of positive holdings were observed in SE22 (Sydsverige), on average between 8–10%. In contrast, the lowest proportion of positive holdings, on average between 2–3% was observed in SE32 (Mellersta Norrland). The levels in SE33 (Övre Norrland) were consistenly higher than in SE32 (Mellersta Norrland).

The sensitivity analysis in study **III** showed that the proportion of positive holdings decreased in all regions and all quarters of the year when the background infectious pressure, ε, and the indirect transmission rate, υ_j, was decreased and when the decay of environmental infectious pressure, β, was increased. In all regions except SE22 (Sydsverige) the average proportion of positive holdings was below 0.03 in all quarters of the year when the background infectious pressure, ε, was zero, regardless of the decay of environmental infectious pressure, β, in the investigated range of values. In contrast, in SE22 (Sydsverige) the average proportion of positive holdings was above 0.04. When varying the decay of environmental infectious pres-

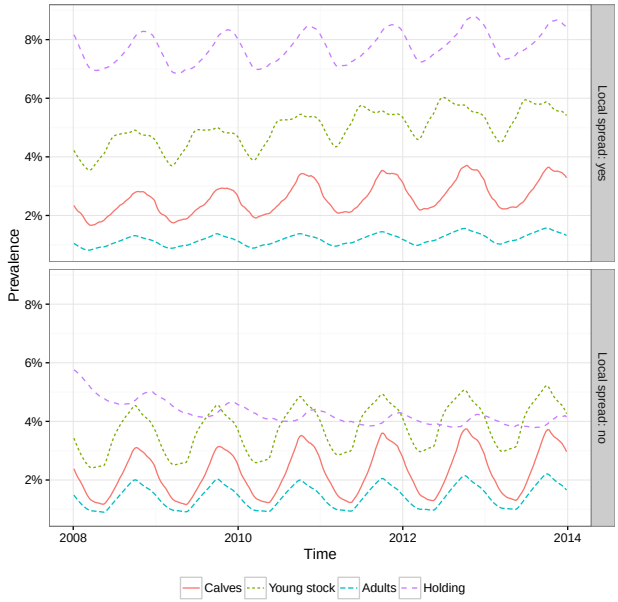

Figure 1: Comparison between two pathogen transmission models of the holding-level and the individual-level prevalence of verotoxigenic *Escherichia coli* O157 in the complete Swedish cattle population. Both models included spread between holdings by livestock movements and one model also included local spread among proximal holdings. Calves are younger than 120 days, Young stock are between 120 and 365 days, and Adults are older than 365 days. The holding prevalence was calculated among the the number of active holdings i.e. having at least one animal. The prevalences were averaged over 1000 trajectories.

sure, β, against the indirect transmission rate, υ_j, the average proportion positive holdings was above 0.01 in all regions and quarters of the year. The proportion of positive holdings decreased in all regions and all quarters of the year when the shedding rate, α, was decreased and when the recovery rate, γ_j, was increased. When varying the shedding rate, α, against the recovery rate, γ_j, the average proportion positive holdings was above 0.01 in all regions and quarters of the year.

The comparison between the two models in study **IV** showed that both had a similar global spatial distribution of the main clusters of infected holdings, which were located in the south (Skåne), south-west (Halland), the two south-east islands (Öland, Gotland), and at the western inland at the transition between the two southern quarters of Sweden (Falköping). However, the local pattern of infected holdings appeared more clustered in the model

Table 4: Comparison between various simulated intervention strategies to reduce the individual-level and the holding-level prevalences of verotoxigenic *Escherichia coli* O157 in the complete Swedish cattle population. The interventions started at 1 January 2009 and the reported prevalences in the table were estimated when simulated time reached 27 December 2013. The holding prevalence was calculated among the the number of active holdings i.e. having at least one animal. The prevalences were averaged over 100 trajectories.

Intervention	Prevalence [%]			
	Calves[a]	Young stock[b]	Adults[c]	Holding
None intervention	3.29	5.41	1.32	8.37
10% reduced shedding rate	1.21	1.82	0.26	2.16
10% reduced indirect transmission rate	1.20	1.82	0.26	2.17
10% reduced shedding rate and indirect transmission rate	0.50	0.59	0.04	0.58
Targeted control based on in-degree > 1	2.83	4.54	1.12	6.89
Targeted control based on out-degree > 1	3.05	4.89	1.18	7.19
Targeted control based on ingoing contact chain > 2	2.86	4.60	1.14	6.97
Targeted control based on outgoing contact chain > 2	2.99	4.91	1.16	7.23
Blocked transmission by animal movements	2.67	4.13	1.00	5.76
Blocked transmission by local spread	2.66	4.49	0.95	3.31
Blocked transmission by movements and local spread	2.07	3.27	0.67	1.87

[a] Calves: age 0–119 days. [b] Young stock: age 120–364 days. [c] Adults: age > 364 days.

that included local spread.

4.5 Explore options for control

Table 4 shows the prevalences at 27 December 2013 after that various control measures had been applied since 1 January 2009 (5 years) to reduce spread. Reducing the average shedding rate α, or the indirect transmission rate υ with 10%, alone or in combination decreased the prevalences at both the individual-level and the holding-level (Table 4). The decrease was more pronounced when reducing both α and υ in combination. Control based on the network measures ID (cut-point > 1), OD (cut-point > 1), ICC (cut-point > 2) and OCC (cut-point > 2) had a marginal effect on the reduction of the prevalences at both the individual-level and the holding-level (Table 4). The reduction for the other investigated cut-points of ID, OD, ICC and OCC was even less (data not shown). For the extreme scenarios, where animal movements and/or local spread was completely blocked, both the holding-level and the individual-level prevalencs were lower in comparison to not blocking the transmission routes (Table 4). The reduction of the holding-level prevalence was further pronounced when both the transmission routes were blocked in combination (Table 4).

5 Discussion

5.1 VTEC O157 in the farm environment

Individual faecal samples have commonly been used to determine the VTEC O157 herd status (Conedera et al., 2001; Eriksson et al., 2005; Arnold et al., 2008). However, due to the wide variability in the shedding pattern (Davis et al., 2006; Matthews et al., 2006a; Lim et al., 2007; Chase-Topping et al., 2008) and the within-herd prevalence (Matthews et al., 2006b), many animals must be sampled to determine the VTEC O157 herd status with a high level of confidence. Thus, it is costly to determine the herd status with individual faecal samples due to the time-consuming nature of the collection procedure and the large number of samples to analyse. A combination of environmental sampling methods consisting of pooled faecal material, overshoe and dust samples is considered to be a practical and cost-effective method to detect *Salmonella* in primary poultry production (Davies and Wray, 1996; Skov et al., 1999; Carrique-Mas and Davies, 2008).

5.1.1 Epidemiological aspects on evaluating environmental sampling

Study **I** was designed to evaluate environmental sampling consisting of dust, overshoe, and pooled pat samples in comparison to pooled individual faecal sampling for determining the VTEC O157 herd status under field conditions in naturally infected dairy herds. The epidemiological test performance to distinguish infected and non-infected is described by the test sensitivity (Se) and the test specificity (Sp). Se is the probability that an infected individual/herd is classified as infected, and Sp is the probability that a non-infected individual/herd is classified as non-infected.

An obstacle when evaluating tests, is to determine the true disease state to compare the test outcome against. The test that captures the true disease state is known as the gold standard. However, for VTEC O157 infection in cattle there is no such test. Due to this, the environmental sampling was evaluated against an imperfect test, namely pooled individual faecal samples

(Arnold et al., 2008; Sanderson et al., 2005). The samples were pooled to reduce costs of analysing individual faecal samples. To overcome the limitation of an imperfect test, many pooled individual samples were analysed from each farm.

Another complication is that the sensitivity of the environmental sampling might vary among populations or sub-populations of animals (Greiner and Gardner, 2000). For example, it is reasonable to assume that the sensitivity of environmental sampling increases with increased within-herd prevalence. The within-herd prevalence can be estimated using the results of bacterial culture of pooled, individual faecal samples, and several alternative methods exist (Cowling et al., 1999; Toribio and Sergeant, 2007). However, none of the methods can simultaneously account for both variation in pool size and in test sensitivity and specificity; therefore, the within-herd prevalence was not estimated, and the apparent prevalence of positive pools was used instead.

5.1.2 Evaluation of environmental sampling

The results in study **I** showed that environmental sampling is a reliable sampling strategy under field conditions to identify naturally infected cattle herds with animals shedding VTEC O157. The ability of overshoe samples to accurately classify the VTEC O157 herd status is in agreement with previous studies (Cobbaut et al., 2008, 2009). One obvious advantage of using overshoe sampling instead of individual faecal samples, is that overshoe sampling can be conducted without handling individual animals, and at the same time allow the sampler to collect either dust or pooled pat samples.

There was also a statistically significant effect on the probability of isolating VTEC O157 from environmental samples by increased within-group pool prevalence. The contextual effect implies that the probability of a positive outcome of the environmental sample depends both on the within-group pool prevalence and the average within-herd pool prevalence. Therefore, even if few or no animals are shedding in the sampled group, a high VTEC O157 load in the herd environment can spread by mechanical vectors, such as personnel and/or equipment and contaminate e.g. floors, walls, gates, and water appliances and thus give a positive environmental sample.

It was concluded from the results in study **I** that environmental sampling *per se* does not work better in younger animals. However, younger animals on average shed more VTEC O157, a finding which is consistent with previously published work (Wilson et al., 1998). Since environmental sampling is more likely to be positive in a group of animals younger than 12 months, sampling should be prioritised among cattle younger than one

year, if not all age categories could be included.

5.1.3 Presence of VTEC O157 in the farm environment

The results in study **II** indicated that local spread might be a relevant transmission route among nearby herds. This is consistent with a Scottish study that found evidence for local spread among cattle farms (Herbert et al., 2014). The underlying mechanism for the local spread was not investigated but previous studies have identified sharing of machines and staff between farms (Rosales-Castillo et al., 2011), wildlife (Synge et al., 2003; Cernicchiaro et al., 2012), direct contact between neighbouring cattle (Ellis-Iversen et al., 2008), as well as grazing animals contaminating water-courses shared among farms (Ellis-Iversen et al., 2007; Fremaux et al., 2010) to contribute to local spread.

It was further found that that introducing animals into a herd increased the risk of becoming infected, which is supported by several studies (Wilson et al., 1998; Nielsen et al., 2002; Schouten et al., 2004; Herbert et al., 2014). One obvious reason is that new, infected animals introduce the pathogen to the herd. On the other hand, introducing previously unexposed animals could also increase the total amount of VTEC O157 in the herd, if the new animals become infected from low levels in the environment (Lahti et al., 2003). Therefore, avoiding introduction of animals regardless of the underlying cause of the association, is likely to reduce the presence of VTEC O157 in the farm environment. Interestingly enough, simulated control measures in study **IV**, based on reducing the between-herd VTEC O157 transmission by animal movements, had marginal effect in decreasing the prevalence.

The results in study **II** showed that in the majority of infected herds, clearance of VTEC O157 occurred spontaneously within a limited time. A suckler herd was more likely to become infected compared to a dairy herd, but infection persisted longer in dairy herds. The transient appearance of VTEC O157 infection in a farm is commonly observed (Rahn et al., 1997; Robinson et al., 2004). Furthermore, the prevalence at the herd level has previously been reported to be associated with production type (Cobbold et al., 2004; Cobbaut et al., 2009). This might be related to differences in farm management practices e.g. feeding and bedding material (Ellis-Iversen et al., 2007; Cernicchiaro et al., 2009). The results suggest that intervention strategies might need to be different for dairy and suckler herds.

5.1.4 Presence of hyper-virulent strains in the farm environment

It is a public health concern that clade 8 strains was common in some regions in study **II** since that variant of VTEC O157 is often detected in HUS

cases associated with cattle in Sweden (Eriksson et al., 2011; Söderlund et al., 2014). The spatial distribution of hyper-virulent strains in Swedish cattle has changed over time, from Halland and later also other parts of Sweden (Aspán and Eriksson, 2010). Moreover, the subtype composition of VTEC O157 strains in Sweden has also changed over time (Söderlund et al., 2014). This calls for continuous monitoring of hyper-virulent strains to evaluate changes in risk.

5.2 Disease spread modelling of VTEC O157

Previous work has shown that it is important to consider the ambient temperature (season) (Gautam et al., 2011; Wang et al., 2013), the indirect transmission route via the environment (Ayscue et al., 2009), and the herd demographic (Turner et al., 2003, 2006, 2008) as well as between-farm transmission from cattle movements (Liu et al., 2007; Zhang et al., 2010) when modelling the VTEC O157 spread.

One interesting aspect that has not previously been explored is combining the various factors above in one model to further understand the spread on a regional and national scale. These questions were addressed in studies **III** and **IV**.

5.2.1 Data

Currently, an increasing amount of data is collected concerning epidemiologically relevant information, which could be incorporated into mathematical models to further understand pathogen transmission, as well as to study methods for surveillance and control (Brooks-Pollock et al., 2015).

European Union legislation requires all bovine animals to be registered in national databases (Anonymous, 2000, 2004). The Swedish database, managed by the Swedish Board of Agriculture, contains geographical information on each holding, where and when a cow was born, destination and date of movement, as well as date of death or slaughter (Nöremark et al., 2009). This enables realistic data-driven modelling of the population demographic and the time-varying contact network. Furthermore, geographical coordinates of the holdings enables modelling of local spread and integrating other spatial data sources e.g. meteorological data at each holding.

The livestock data was not structured in such a way that information relevant for the simulations in studies **III** and **IV** was easily retrieved. Furthermore, several issues with data completeness and correctness were identified during the data cleaning process, such as missing coordinates, erroneous movement records and cattle with ambiguous birth date. Although the livestock data is considered relatively complete and the quality improves over

time (Nöremark et al., 2011), similar data quality issues have been previously reported (Nöremark et al., 2009; Widgren and Frössling, 2010).

The data quality issue that is most concerning, is that exact coordinates were only found for 84% of the holdings. For the other holdings, coordinates were randomly sampled within the postal code area of the holding. This seems unlikely to affect the pattern on national scale, seemingly driven by livestock movements, but could have an impact on the local spread among proximal holdings. Although not assessed, one approach to evaluate the impact, could be to compare the simulation results with simulations where all holdings are randomly placed within their postal code area.

5.2.2 Computational simulation framework

It is essential to use a stochastic approach to model the VTEC O157 infection dynamics in a herd, since random events in small populations, like a herd, are important elements in the disease transmission, for instance after moving an infected individual to a herd. Although there are several approaches to incorporate stochasticity, the Gillespie Direct Method (Gillespie, 1977) correctly simulates a continuous-time Markov chain consisting in this case of rate laws between different epidemiological states of indviduals transitioning between the susceptible and infected compartments (Keeling and Rohani, 2007). For state transitions taking place in a noisy environment and in continuous time, a continuous-time Markov chain is the most natural mathematical model, based on clearly formulated first principles and assumptions. A limitation, though, of the Gillespie Direct Method is that the simulation time increases with population size, a challenge that must be addressed in order to model disease spread at national level.

Furthermore, to efficiently use data in computer simulations, it is paramount that a suitable algorithm for the specific task is used (Cormen et al., 2001), because the computational efficiency is determined not only by the speed of the processor, but also by how fast the data can be accessed from memory and delivered to the processor, and be used in the calculations (Grama et al., 2003).

Therefore, to address the computational challenges, the Unstructured Mesh Reaction-Diffusion Master Equation (URDME) (Drawert et al., 2012) framework for stochastic simulations in molecular systems biology was used as a starting point to develop the data-driven disease spread model. In that framework, reaction and diffusion processes in cells are studied in *in silico* experiments. The reason that this framework can be used as a starting point also for disease spread models, is that both chemical reactions in cells and the within-herd disease process are mathematically defined by a continous-time

Markov chain. URDME contained a flexible and efficient implementation in the compiled language C (Kernighan and Ritchie, 1988) suitable to build on.

In study **III**, the algorithm from URDME was adapted to make use of multi-core processors, and extended to include a data processing step, where scheduled events from livestock data were incorporated to model the population demographic and the time-varying contact network. The scheduled events modified the discrete state of individuals in a holding at a predefined time. Furthermore, an additional processing step was added to enable modelling of e.g. the environmental infecious pressure and local spread.

The following four types of scheduled events were defined to process the livestock data: enter; internal transfer; external transfer; and exit. The enter event handles births and imports from abroad. The internal transfer event happens the day an animal changes age category from calf to young stock or young stock to adult. The external transfer event occurs when an animal moves from one holding to another. The exit event implies slaughter, euthanasia or export of the animal to another country. From that day, the animal will no longer be included in the simulation. The scheduled events are executed when the simulation in continuous time reaches the time for any of the events. The individuals are randomly sampled from the compartments affected by the event.

This part of the thesis was made in collaboration with research focusing specifically on applied mathematics and computational challenges related to efficient use of multi-core processors, memory access as well as algorithms for these types of simulations, but will not be discussed further here (Bauer, 2015; Bauer et al., 2016). The simulation framework has been disseminated within the public domain as the `SimInf` R package (Widgren et al., 2016a,b). An added benefit of using the design of URDME as a starting point, is that the disease spread simulator is not specifically designed to model VTEC O157, but can easily be extended to other pathogens and research questions.

5.2.3 Considerations on model calibration

Before studying control measures the values of the parameters in the disease spread model had to be determined. This was addressed by replicating the observations in study **II** in simulations. In study **III** the agreement between observed and simulated data was measured with a GAM fitted to the two datasets. There are two limitations with the used approach. First, since the GAM was fitted to the data by day of the year, any year-to-year variation was not captured. The second limitation lies in the choice of using a GAM, since the smoothing of the data hides misfit. Thus, to overcome these limitations,

another approach was used in study **IV**.

In study **IV**, the agreement was measured by comparing the number of positive holdings aggregated quarterly over the complete study period in study **II**, thereby including year-to-year variation. Furthermore, the agreement also included newly infected holdings to capture that over time fewer new holdings became infected. The approach used in study **IV** improves evaluation of misfit compared to the approach in study **III**.

The model calibration in studies **III** and **IV** was based on the observations in the 126 holdings in study **II**, all located in the southern Sweden. Due to the limited size of that data set, careful judgements must be considered when generalising to a national scale. It is also possible that the small measurement errors of the observations in study **II**, due to misclassification bias from environmental sampling, could result in large errors in the estimate of the parameter values.

5.2.4 Model calibration

Several of the covariates in the livestock data used in studies **III** and **IV** had an evident seasonal pattern: more holdings were active (having at least one animal) during the pasture season; most births occurred during spring; and the number of animals moved and the proportion of connected holdings had one peak during spring and one peak during autumn. Interestingly enough, the data-driven disease spread model was unable to capture the seasonal herd-level prevalence in study **II**, unless a seasonal parameter was included, despite the inherently distinct seasonality in the livestock data that was used in the simulation.

The model in study **III** seemingly overestimated the prevalence of VTEC O157 in the two most northern regions in Sweden, SE32 (Mellersta Norrland) and SE33 (Övre Norrland). There are two plausible reasons for this. First, the length of the four seasons in Sweden differs by region, which could affect the decay of the environmental infectious pressure. Secondly, the density of holdings is higher in southern Sweden, suggesting that the environmental infectious pressure could influence neighbouring herds more.

The model fit improved in study **IV** after incorporating season (spring, summer, autumn, winter) according to the average temperature at the location of the holding. Furthermore, after adding distance-based local spread among proximal holdings in study **IV**, the holding-level prevalence were in better agreement with the reported prevalence in Sweden (Eriksson et al., 2005). The model seemed unable to capture the peaks in prevalence and incidence cases that were observed in the third and fourth quarters of 2010 in study **II**. One explanation for this might be that season was modelled

according to the average temperature for the reference period 1961–1990 (Anonymous, 2016a), and therefore not considering the year-to-year variation.

The results in study **IV** show that VTEC O157 spread occurs at a much lower rate in northern Sweden, and that in the event that infection is introduced, it gets extinct over time. This is consistent with previous observations, as the VTEC O157 bacteria has rarely been detected in cattle originating from northern Sweden in prevalence studies (Albihn 2003; Boqvist 2009). The rate of decay of the environmental infectious pressure had the least reduction during the summer, which leads to higher levels of the pathogen during that season. This finding is consistent with the hypothesis that the increased summertime VTEC O157 colonisation results from increased seasonal oral exposure to this pathogen (Sheng et al., 2016).

5.3 Options for control

It is reasonable to assume that targeted control based on network measures efficiently would reduce the disease spread between herds, considering that introduction of animals constitutes a risk for VTEC O157 infection (Wilson et al., 1998; Nielsen et al., 2002; Schouten et al., 2004; Herbert et al., 2014) and also observed in study **II**. However, the results in study **IV** showed, quite surprisingly, that the prevalence was in principle unaffected by targeted network interventions based on the measures in-degree, out-degree and in-going contact chain as well as outgoing contact chain. This is in agreement with Zhang and Woolhouse (2011) who reported that reducing movement-related transmission has, at best, a modest impact in reducing the steady-state prevalence of E. coli O157.

On the other hand, the combination of reducing the average shedding rate e.g. by vaccination (reviewed by Snedeker et al., 2012; Varela et al., 2013), and reducing the indirect transmission rate e.g. by feeding probiotics (reviewed by LeJeune and Wetzel, 2007), efficiently reduced the prevalence to low numbers. These findings are consistent with previous modelling studies because reducing the level at which infected cattle shed the pathogen (Matthews et al., 2006a; Turner et al., 2006; Zhang and Woolhouse, 2011), and decreasing the indirect transmission rate (Turner et al., 2003) has been shown to efficiently reduce the VTEC O157 prevalence in cattle.

6 Summary and concluding remarks

An increasing amount of epidemiologically relevant data is becoming available. In this thesis, several computational issues have been addressed to incorporate large amounts of data in disease spread simulations to explore surveillance and control of livestock infections.

Overshoe sampling alone or in combination with dust and/or pooled pat sampling were established to be reliable for identifying cattle herds with animals shedding VTEC O157. Environmental sampling should be prioritised among cattle younger than 12 months, if not all age categories could be included.

Animal movements and local spread are important for the transmission of VTEC O157 in the Swedish cattle population. However, simulated control measures based on reducing the between-herd VTEC O157 transmission by animal movements and local spread, had marginal effect in decreasing the prevalence. On the other hand, control measures based on reducing the shedding and susceptibility, efficiently decreased the herd- and individual-level prevalence of VTEC O157 in the Swedish cattle population.

Eradicating VTEC O157 seems unlikely, even implementing interventions in reducing the prevalence on larger scale have so far not been successful. However, successfully implementing zoonotic disease control programs on a regional and national scale is not only related to the epidemiological aspects of the disease transmission, but also to perceptions, motivators and economy among farmers, industry and government (Ellis-Iversen et al., 2010). These issues need further attention, since if control measures are to be applied, concerted action is a better strategy than to deal with herds on a case-by-case basis.

7 Future research and development

- What are the critical thresholds of herd density and animal movement intensity before clusters of infected herds occur in a region? If that was known, would it be possible to keep a low prevalence of VTEC O157 in the cattle population in those regions, given that the prevalence is first reduced?

- With an increased demand for monitoring emerging diseases in combination with limited resources, development of efficient and cost-effective surveillance systems are important (Stärk et al., 2006). Using readily available livestock and meteorological data in combination with environmental sampling and data-driven simulations could provide cost-efficient novel opportunities for disease monitoring.

- Further develop the computational model to also consider evolution of the bacteria to enable modelling of changes in e.g. VTEC viruelence factors or stochastic mutation processes relevant for antimicrobial resistance.

- Explore the relationship between human sporadic EHEC cases and the environmental load of VTEC generated by cattle to evaluate control strategies in the cattle population and the effect in incidence of human EHEC cases.

8 Populärvetenskaplig sammanfattning

Verotoxinproducerande *Escherichia coli* O157:H7 (VTEC O157) är en bakterie som kan orsaka enterohemorragisk *E. coli* (EHEC) infektion hos människa med blodig diarré, anemi och njursvikt. Nötkreatur smittade med VTEC O157 utsöndrar bakterien i avföringen utan att visa några tecken på sjukdom. Människor kan smittas av VTEC O157 genom att äta förorenad mat, dricka förorenat vatten eller vid direktkontakt med infekterade nötkreatur. Med en bättre kunskap om hur VTEC O157 sprids bland nötkreatur skulle bekämpningsåtgärder för att minska förekomsten av bakterien bland nötkreatur vara möjliga, vilket därmed skulle minska risken att människor blir sjuka av VTEC O157.

För att bestämma om det finns några VTEC O157 infekterade djur i en besättning behöver man undersöka träckprover från djur. Detta är kostsamt eftersom många djur behöver provtas och det därmed blir många träckprov att analysera. Eftersom VTEC O157 överlever en tid i stallmiljön går det istället att analysera prover från miljön, vilket också är enklare jämfört med att ta individuella träckprov från djur. Syftet med första studien var att utvärdera miljöprovtagning bestående av plock-, sock- och dammprov jämfört med individuella träckprov från djur. Resultaten från studien visade att miljöprovtagning är en tillförlitlig metod för att bestämma om en nötbesättning har djur infekterade med VTEC O157.

Det finns flera olika faktorer som påverkar risken att en besättning blir infekterad med VTEC O157. För att kunna prioritera bland övervaknings- och bekämpningsåtgärder är det viktigt att förstå hur bakterien beter sig på lokal och regional nivå över en längre tid. I den andra studien i avhandlingen följde vi totalt 126 besättningar från fyra regioner i södra Sverige under 38 månader. Ungefär var 6-8:e vecka togs ett miljöprov i varje besättning. I 67 av besättningarna hittades VTEC O157 vid minst ett tillfälle. Om VTEC O157 hittats vid föregående provtagning ökade risken att besättningen fortfarande var infekterad, men med tiden försvann infektionen. Dessutom ökade risken när en närliggande besättning var infekterad med

VTEC O157 samt om djur nyligen flyttats till gården.

Datorsimuleringar och matematisk modellering är ett effektivt sätt att studera hur sjukdomar sprids. Alla nötkreatur i Sverige är registrerade i en nationell databas med information om var och när djur föds, alla djurförflyttningar mellan besättningar och när djur går till slakt. Genom att använda den här informationen i simuleringar skulle det vara möjligt att bättre förstå hur VTEC O157 sprids mellan nötbesättningar. Dessutom skulle det vara möjligt att utvärdera strategier för att minska förekomsten av VTEC O157 bland nötkreatur. I tredje och fjärde studien i avhandlingen har programvara utvecklats för att genomföra detaljerade simuleringar som inkluderar alla besättningar, nötkreatur och hur de flyttas mellan besättningar. Resultaten från dessa studier visade att djurförflyttningar delvis förklarar varför det finns regionala skillnader i förekomsten av VTEC O157, men även att den viktigaste åtgärden för att minska förekomsten förmodligen är att minska utsöndringen hos enskilda djur genom t.ex. vaccination eller utfodring med probiotika.

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