The Epidemiology and Evolution of Marek's Disease Virus

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I declare that this thesis has been composed by myself and that the research reported therein has been conducted by myself unless otherwise indicated.

Katherine Atkins, Edinburgh.

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Abstract

Marek's disease (MD) is an oncogenic disease affecting chickens and is estimated to cost the worldwide poultry industry $1-2 billion annually. The causative agent of MD, Marek's disease virus (MDV), provides a well-documented example of virus virulence evolution occurring over a period of sixty years. The reason behind this evolution is unknown, although certain untested hypotheses have been suggested. These include vaccination (with increasingly potent vaccines) and other aspects of industrialisation, such as the decreased cohort duration of successive generations and an increased stocking density of the broiler flocks.

In this thesis, four sections of work are undertaken. First, estimation of epidemiological parameters is tackled: virulence of MDV is quantified by looking at host mortality and virus shedding rates in vaccinated and unvaccinated birds. This is achieved via maximum likelihood estimation and Bayesian McMC techniques. Second, viral fitness is quantified by defining multiple lifetime fitness functions using the parameters previously estimated to understand the direction and force of virulence selection for different farm environments. Third, the impact of an outbreak of MDV on a broiler flock is examined by simulating a whole flock of birds. This provides an epidemiological understanding of the virus at the flock level and can help elucidate methods for disease control and surveillance and can also give a fitness measure to understand on-farm evolution of the virus. Fourth, a between-farm model is analysed to evaluate which MDV strains are able to persist in a network structure of farms and how this might be affected by biosecurity measures, different farm networks, farm size, bird lifespan and vaccination. This provides insights into how quickly a different strain can invade a farm network and the plausibility of it becoming endemic.

Parameter estimation results show that the time to death for an infected bird decreases and its virus shedding rate increases with previous definitions of virulence in the literature. Model results suggest that the choice of fitness measure alters the conclusions reached. Increasing the amount of demographic structure introduced into the fitness measure shows that neither vaccine introduction nor decreasing a bird's lifespan changes the ability of more virulent virus strains to outcompete less virulent strains. In any environment, more virulent strains are always selected for. Epidemiological results suggest

that vaccination allows a low prevalence of virus on a farm although there are no deaths from the disease itself. Analogous results for between-farm spread suggest that if on-farm cleaning efficiency is low enough, a high prevalence of disease throughout a network of vaccinated flocks can exist but the farms themselves show no signs of increased mortality from the disease.

The hypotheses for explaining the increase in virulence of MDV may not be consistent with the results of this work. Despite previous arguments that vaccines are driving the evolution of virulence of MDV, this first quantitative work on the subject demonstrates that this might not be the case. This work also formulates new hypotheses to explain why MDV virulence has increased over the past sixty years which will pave the way for ongoing research in the area of virulence evolution in farm environments.

Contents

1 Introduction

1.1 Motivation

This thesis is concerned with human influence on the lethality of pathogens, and examines the extent to which observable increases in this deadliness can be attributed to our actions. Pathogens cohabit an environment that includes humans, livestock and wildlife. Humans have been responsible for numerous, abrupt changes to this environment, such as the introduction of vaccines and alterations to livestock farming practices. The survival of pathogens has relied upon their ability to adapt to these changes. These adaptations can be observed in phenotypic changes of the pathogen, for example the malignancy of an infection.

I will study whether the changes in a farm system are able to govern the virulence of a pathogen which infects farm animals. The pathogen of interest is Marek's disease virus (MDV), a pathogen infecting poultry farms, which is known to have been progressively increasing in virulence since World War II.

There are few examples of observed virulence evolution and the explanations of why these changes have occurred are usually qualitative. Relying on these descriptive reasons to explain systems often involving complicated host-pathogen life-history interactions does not seem adequate. The evolutionary dynamics of a system must be studied quantitatively if reliable predictions of the direction and force of selection are to be made.

There is no shortage of mathematical analyses devoted to the theoretical exam-

ination of virulence (Bull, 1994; Frank, 1996; Boots and Sasaki, 1999; Dieckmann, 2002) or the effect of vaccine treatment on its evolution (Gandon et al., 2001, 2003). However, the flurry of activity in this field in recent years relies on many assumptions, and indeed there is a growing body of literature concerned with documenting just how sensitive the results are on the form these assumptions take (Ganusov and Antia, 2003; Bowers et al., 2005). It is important to take a step back to better understand how we should formulate our models of disease transmission and exactly what we need to include about the life history of the disease. Justification of model choice and parameter values can only feasibly be done with good data. There is much scope for testing predictions generated by theory, and it is imperative that not only these models use data but the models themselves are driven by data and the real phenomena of disease processes.

One of these assumptions in the field of virulence evolution modelling is the trade-off between virulence and transmission of a pathogen. In this introduction it will be demonstrated that there is scant evidence that this occurs. If it does, the functional form is sometimes unclear. However, most mathematical analysis concerning virulence evolution will include this trade-off in some form. Therefore before continuing to develop a model of a Marek's disease system, I develop an intuitive notion of virulence which is extended from the experimentalists' interpretation. This quantitative notion of virulence is then used throughout the work to formulate measures of virulence in order to answer the question, *why did Marek's disease virus evolve to be more virulent, and did humans play a part?*

In particular, the following questions are addressed:

- Can MDV strains be pathotyped according to their mortality effects?
- Can key MDV epidemiological parameters be estimated?
- What is the impact of vaccination on MDV and host mortality?
- How can fitness be defined for MDV?
- In what environment do more virulent MDV strains do better? Specifically, do vaccination, host lifespan or density of hosts affect the optimum virulence of MDV?

- What is the probability of an MDV outbreak on a farm and is it difficult to implement biosecurity against MDV?
- Do MDV mutants persist and spread through a population of farms or are they independently arising on multiple farms?

1.2 Introduction

1.2.1 Marek's Disease

1.2.1.1 Prevalence and Economic Impact

Almost all of the industrialised countries have experienced MD losses in their poultry industry and a crude estimate of the cost of Marek's disease is said to be in the range of $1-2 billion annually (Morrow and Fehler, 2004). The Food and Agriculture Organisation (FAO) valued the 2002 worldwide poultry industry (consisting of about 45 billion broilers (birds bred for meat) and 57 million tonnes of eggs) between $100-200 billion, giving the MD-associated damage as 1% of the total value. Comprehensive reports of the situation worldwide of MD are difficult to obtain for four reasons elucidated by Morrow and Fehler (2004):

1. MD is not a notifiable disease.
2. Vaccination failure is accepted and small losses are viewed as normal.
3. Records of cases of MD are usually related to financial claims between farms and hatcheries or vaccine manufacturers and are not made public.
4. Companies are not willing to reveal cases of MD as outbreaks in case of bad publicity.

There is variation in prevalence between countries, with some suffering from increased mortality in layers (e.g. France and Germany), while others have problems with broilers (e.g. Italy) (Morrow and Fehler, 2004). There is also an increased chance of losses in developing countries where lack of temperature-controlled climates, improper vaccine storage and use of multi-age farm structures lead to a heightened risk of outbreaks (Morrow and Fehler, 2004).

1.2.1.2 Control and Prevention

Control of Marek's disease is predominantly via vaccination of chickens. Indeed the MDV vaccine was the first vaccine to be developed against oncogenic (tumour-inducing) viruses (Davison and Kaiser, 2004). The use of mass vaccination strategies is common across both the developed and developing worlds, although there is some degree of heterogeneity in the use and type of vaccine between countries (Morrow and Fehler, 2004).

There have been four types of vaccines which have been developed for control of MD: a HVT (Herpesvirus of turkeys) based formula, a bivalent HVT/GaHV-3 (Gallid Herpesvirus Type 3) vaccine and two attenuated forms of MDV (the first superceded quickly and no longer in use, the second known as CVI988 or Rispens) (Bublot and Sharma, 2004). The three vaccines in current commercial use are listed in increasing potency and thus increasing protection. HVT has been used worldwide since the early 1970s, bivalent vaccines have been in widespread use since the early 1980s and Rispens has been used outside the US since the early 1970s, although US use only commenced at the start of the 1990s (Witter, 2001). Poultry companies have a vaccination policy for either just layers or both broilers and layers (Morrow and Fehler, 2004). Broilers are sometimes not vaccinated since they live for a much shorter time than the layers and are therefore less likely to develop the disease over their lifespan.

Vaccinated hosts are still able to become infected with, and transmit, MDV. These vaccines are therefore examples of 'leaky vaccines'. In successful MDV vaccination, hosts maintain the "latent" stage of infection, albeit with a reduced viral load, with a fully productive infection in their feather follicle epithelium (FFE), causing them to be infectious (Baigent and Davison, 2004). Vaccination is thought to target viral replication in the cytolytic and transformative phase of infection and prevents lymphoma. It achieves this by stimulating innate, antibody and cell-mediated immunity (Davison and Kaiser, 2004).

There is a delay between vaccination and full efficacy, of between 5 and 8 days (Okazaki and Burmester, 1971; Witter and Lee, 1984; Islam et al., 2007). In commercial situations, it is difficult to completely reduce the risk of exposure immediately after introduction to a layer or broiler farm. To combat this, vaccination *in ovo* is becoming commonplace in the US and is recognised as an efficient way to reduce the chance of symptomatic MD (Witter, 2001). Vacci-

nation does however deplete any protection given by maternal antibodies in the vaccinated bird, although vaccine-derived antibodies will be passed down to the offspring of vaccinated individuals.

1.2.1.3 Discovery and the Causative Agent

Marek's disease (MD) was described a century ago by Hungarian veterinarian Joszef Marek (Marek, 1907). It was first described as a fowl paralysis and was not differentiated from Avian Lymphoid Leukosis until the causative agent was found in around 1950 (Pastoret, 2004). It is now viewed as "… one of the most potent oncogenic herpesvirus known" (Nair and Kung, 2004).

The causative virus, MDV, is a DNA virus and a member of the *mardivirus* genus, which belongs to the subfamily *Alphaherpesvirinae* (other example members are the human infections Herpes simplex virus 1 and 2). MDV was originally described as a *Gammaherpesvirus* (Roizman, 1990) because its infection pathway is similar to that of other oncogenic members of the subfamily (for example the Epstein-Barr Virus (EBV) or Kaposi's sarcoma herpesvirus (KSHV)) in that it maintains latency in lymphocytes, which may undergo subsequent transformation (Nair and Kung, 2004). The original classification, based on similarities between biological properties, was superceded by a more complex system which also incorporates tissue tropism, genomic organisation and protein comparison (Silva et al., 2001).

There are two other members of the *mardivirus* genus: *Gallid Herpesvirus* 3 (GaHV-3, also known as MDV-2) and Herpesvirus of Turkeys (HVT). These three sole members, which all have at least one sequenced representative, are often referred to as *serotypes*, although the large variation in base compostition and genome size suggests independent evolution and thus different species (Osterrieder and Vautherot, 2004). However, the three viruses do share many antigenic properties. MDV has been termed MDV-1 in the past, but in line with observed current nomenclature (Osterrieder and Vautherot, 2004), MDV, GaHV-3 and HVT shall be employed throughout this work. MDV is the only member of the genus to be pathogenic in chickens.

The origin of MDV is unknown although both highly virulent MDV and non-pathogenic serotypes have been found to occur in wild geese (Murata et al., 2007).

1.2.1.4 Natural History of Marek's Disease

MDV is an airborne virus and infection occurs via inhalation (Osterrieder et al., 2006). Other possible transmission routes have been postulated as faecal/oral (Witter et al., 1968) although there has been little evidence of this in any other experimental systems. Virus shedding occurs via infected FFE (feather follicle epithelium) by dead stratified cells and moulted feathers (Carrozza et al., 1973). The resulting dust and dander can then remain in the environment and act as a reservoir for chicken infection. Early studies on the lifespan of the excreted virus have suggested that chickens inoculated with 200 day old infective dust can cause MD-associated lesions (Carrozza et al., 1973). The long duration of infectivity of the virus has been attributed to the highly cell-associated nature of the virus, with shedding occurring via keratinized desquamated cells, which provide protection for the virion, although rendering it less infectious (Baigent and Davison, 2004). Cell-free particles shed offer a labile yet highly infectious source of transmission (Baigent and Davison, 2004).

Chickens are the most affected of bird species, and receive the largest focus of attention (see Sections 1.2.1.1 and 1.2.1.2). However quail can also be naturally infected and there have been recent reports of outbreaks in turkey flocks in France, Israel and Germany (Merck et al., 2005), although turkeys are normally naturally infected with HVT.

According to the 'Cornell Model', the infection pathway of MD, once it has infected a chicken, is described by four stages (Biggs, 1997; Calnek and Witter, 1986; Baigent and Davison, 2004; Nair, 2005): cytolytic, latent, late cytolytic and transformative. Once the virus has entered the lungs, it moves to the lymphoid tissue of the spleen, thymus and bursa via macrophages. The virus undergoes a cytolytic stage. Current literature estimates this occurs between 2-7 days post infection (dpi) when B cells are infected and inflammation occurs. This is followed by a so called "latent" infection of many tissues as the immune response is triggered and T cell production is activated. This is thought to occur between 6-7 dpi onwards. There is a resulting immunosuppression and a period of viraemia as the T cells are attacked. This is followed by a secondary cytolytic infection around 14-21 dpi, which affects the thymus, bursa, some epithelial tissues (including FFE), kidney, adrenal gland and proventriculus (a digestive division of avian stomachs). There is then a transformation of

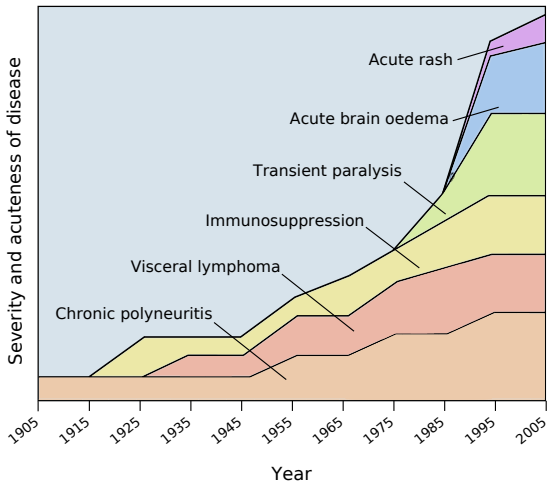

Figure 1.1: The rise in severity and variability of clinical signs after infection with Marek's disease virus. Diagram taken from Nair (2005) and redrawn.

CD4+ T cells, holding latent virus (although the source of these CD4+ T cells is contested in different models (Baigent and Davison, 2004)), which eventually results in malignant tumours. This final stage may occur weeks or months post infection.

Both the "latent" and transformative stages are non-productive since no replication nor progeny release occur and gene expression is limited. Both cytolytic phases are semi-productive since non-enveloped intranuclear particles are produced. Fully productive infection occurs solely in the FFE, which has been estimated to take place around 13 dpi once the virus has migrated during latency via the peripheral blood lymphocytes Baigent and Davison (2004). However more recent quantitative estimates have found significant quantities of MDV in the feather tips at 7 dpi (Baigent et al., 2005).

Note that the "latent" stage in the infection pathway is defined via gene expression and immunological response. However, this differs from the epidemiogists' definition of 'latent period', which is defined as the delay between infection and infectivity (Anderson and May, 1992; Murray, 2002; Britton, 2003). Using this definition current estimates of the 'latent period' for MDV therefore stand around 6-7 days (Baigent et al., 2005). The latter definition will be used throughout this thesis from Chapter 2 onwards when the epidemiology of MDV will be focused on.

Clinical signs are varied and result in morbidity and mortality (Baigent et al.,

2006). Onset of lymphoma-like signs have usually occur within a few weeks of infection during the transformative stage of the infection and death in this case can happen weeks or months after infection (Witter, 1997). However there are a wide range of symptoms which cause death anywhere between a few days and a month (Witter, 1997, 1999). Symptoms of MDV can include polyneuritis (an enlargement of multiple peripheral nerves), visceral lymphoma (tumours affecting organs such as the heart, liver, spleen etc.), acute transient paralysis (synonomous with one leg held back, the other forward and 'floppy-chick' syndrome), immunosuppression, brain oedema and acute rash. There has been a change in the types of clinical signs since the disease was first noted (Morrow and Fehler, 2004) (see Figure 1.1), when chronic polyneuritis was the only sign. Since then, the previous list has been gradually populated over the decades (Osterrieder et al., 2006). Current common MD symptoms for many pathotypes are chronic transient paralysis (Witter, 1999) and lesions (inflammatory, occurring in early pathogenesis in direct response to the cytolytic infection of B cells; proliferative, occurring as lymphoma; and, degenerative occurring as arterial atherosclerosis) (Nair and Kung, 2004).

The disease outcome is varied and is known to be affected by five factors Baigent and Davison (2004):

1. *Virus Serotype and Pathotype*. All mardivirus serotypes are infectious for, and productive in, chickens, although only MDV is pathogenic. There are various levels of pathogenicity of MDV, which are discussed in Section 1.2.2.2.

2. *Host Genotype*. The existence and severity of the disease depending on the genotype of the host was investigated by Emara et al. (2000), who noticed a significant correlation between host growth rate and the development of MD paralysis in commercial birds. Genetic resistance has been found to be both associated and non-associated with the major histo-compatibility complex (MHC). However, all chickens are in some way susceptible to MDV (Davison and Kaiser, 2004).

3. *Maternal Antibodies*. The effects of early challenge can be lessened by inherited immunity (see also Eidson et al. (1972)).

4. *Age of Infection*. Development of a functional immune system reduces the pathogenicity (see also Witter (1999))

5. *Stress and Immunosuppression*. Concurrent infections and transportation stress may impact on disease outcome. Indeed, Witter and Gimeno (2006) suggests this may be a explanation for late outbreaks in flocks, which have suffered some immunosuppression, allowing a delayed increase in MD symptoms. Immunosuppression has been associated with a reduction in the weight of the thymus and bursa of Fabricius, the numbers of circulating T lymphocytes and B lymphocytes, and titres of antibodies specific to other viruses.

1.2.1.5 Genetics of MDV

MDV has a *Herpesviridae* genome structure, with the 180kb double stranded DNA arranged into two unique regions (long, U_L and short, U_S), which are both flanked by inverted repeat regions TR_L, IR_L, TR_S and IR_S (Nair, 2005).

The replication of alphaherpesviruses is known to be efficient, leading to a low nucleotide substitution rate of $3x10^{-8}$ per site per year (Sasaoka et al., 1994). The latter is still an order of magnitude greater than the mammalian/avian host species these viruses can infect (Shackelton and Holmes, 2004), leading to the possibility that these viruses can evolve faster than their host species (Thiry et al., 2005). Possible high levels of recombination within an alphaherpesvirus genome is suggested as the major cause of variation on which selection can act (Thiry et al., 2005).

The vast majority of the genes found in the *mardivirus* genus consist of open reading frames (ORFs) that are homologous to other members of *Alphaherpesvirinae* (Osterrieder and Vautherot, 2004), but there are a number of MDV-specific genes whose putative function has been associated with the transition to latency and transformation, most notably Marek EcoRI-Q-encoded protein (Meq) and the phosphorylated protein gene pp38. Meq is sometimes described as the major oncogene (Nair and Kung, 2004) with validation from knockout experiments performed by Anderson et al. (1998), which showed the Meq-null mutant virus replicated well *in vitro* but was not oncogenic *in vivo*. However, there is still very little evidence of any correlation between genotype and

Virulence Rank	Year first observed	Vaccine introduced
mild	pre 1940	
virulent	1950	HVT 1970
very virulent	1980	Bivalent 1985
very virulent+	1990	Rispens 1995

Table 1.1: Emergence of more virulent strains of MDV and associated vaccine introductions.

pathotype despite efforts to that effect (Osterrieder and Vautherot, 2004).

1.2.2 Marek's Disease: the Evolution of Virulence

1.2.2.1 Trend of Increased Virulence

Marek's disease provides an example of a trend of increased virulence in virus strains (Witter, 1997) (for a detailed discussion of the definitions of this concept, please refer to Section 1.2.2.2). This evolution has been documented since the 1950s (Osterrieder et al., 2006) (see Table 1.1) and is of interest and concern to the poultry industry, poultry experts and evolutionary biologists alike. The evidence for this shift in virulence has been recognised due to increased chicken losses and the ensuing need for better vaccines to quell this rise (Nair, 2005). However, actual data from sampled strains and pathotyping have proved a less than robust method of assessing the rise in virulence, due to small sample sets from pre-1990s strains and continued bias in isolate samples (Witter, 1997).

The most-cited theory to explain this trend has been the 'vaccination hypothesis', whereby pathogen evolutionary pressures are directly dictated by the presence of a vaccine in the environment. The sudden, pronounced rises in poultry losses are synonomous with 'vaccine breaks', whereby one or more parasite genotypes become resistant to the current vaccine and the greater fitness of these genotypes allows subsequent invasion of an area (Witter, 2001).

However, the first vaccine was developed as a consequence of increased mortality in the poultry industry, and so this first wave of evolution requires an alternative explanation. The initial rise in observed virulence (empirically judged by the case mortality due to the MD per farm or area) has since been

attributed to the emerging industrialisation of commercial poultry holdings (Nair, 2005), with the availability of comparatively huge farms being an ideal environment for the invasion of more virulent strains of a virus.

Other hypotheses exist for the continued virulence evolution which include: 'resistant chicken' (better housing and nutrition have led to increased resistance to MD, thereby producing a selective pressure for more virulent strains which can thrive in a resistant host, although there is little evidence for direct selection of MDV-resistant chickens), 'homogeneous chicken' (reduction in genetic diversity has left the population more susceptible to disease), 'broiler lifespan' (a halved lifespan of broilers from around 70 days fifty years ago has allowed more virulent strains to proliferate since there is no incentive for a virus to keep its host alive if the host's lifespan is very short), 'chicken density' (larger farms have lead to greater virulence since there is less of a disadvantage in killing a host if there are other hosts to infect). They are all consistent with the established evolutionary biology theory, which is elaborated in Section 1.2.3.1. This thesis will only be examining the hypotheses concerned with broiler lifespan, host density and vaccination, due to the limited data available on the host genotype over the past half century.

1.2.2.2 Definition of Virulence

Definitions of virulence are numerous and the precise view depends on the perspective of the researcher. Microbiologists equate virulence with the notions of both infectivity and severity of disease; whereas, evolutionary biologists add a third component, that being evolutionary fitness (either the pathogen's or the host's) (Read, 1994). Zoologists tend to focus on host fitness (e.g. Levin and Pimentel, 1981; Anderson and May, 1982) with most mathematical models describing this specifically as host mortality (e.g. Anderson and May, 1992; Tompkins et al., 2002).

The effect of MD on chickens has been described above and an infecting MDV strain determines incubation period (time from infection to clinical signs) (Osterrieder et al., 2006), time to death (Witter, 1999) and severity of clinical signs (Witter, 1997) within a host.

There have been various attempts at defining MDV virulence and subsequent pathotyping of strains (e.g Witter, 1997; Witter et al., 2005). It is normally

measured by a strain's propensity to induce lymphoproliferative lesions in vaccinated chickens (Witter et al., 2005). In the pathotyping study done by Witter et al. (2005), Virulence Rank measured the average percentage of individuals displaying gross lesions or dying over an eight week period in two groups of chickens, one vaccinated with HVT, the other with Bivalent vaccine.

Currently there are four recognised groups of virulence, each containing part of the 'continuum of virulence' (lying between Virulence Rank 0-100%) (Witter, 1997), known as: mild, m; virulent, v; very virulent, vv; very virulent plus, vv+. The definition of virulence of MDV is in vaccinated hosts, which is in contrast to evolutionary biologists' accepted definition involving naïve hosts. With the former approach, vaccine-induced virulence evolution becomes self defining and is more akin to the notion of drug resistance.

There have been studies to associate Virulence Rank with various host infection outcomes. There is a significant association between density of gross visceral lesions with different vaccines and Virulence Rank (Witter, 1999). The time-to-lesion production is not significantly correlated with Virulence Rank (Witter, 1999). There have been efforts to find other correlates with virulence of MDV isolates, most notably with viral load. This has been achieved by cell culture techniques (e.g. Calnek and Witter, 1986) and by quantitative PCR methods (Bumstead et al., 1997; Burgess and Davison, 1999; Reddy et al., 2000; Baigent et al., 2005; Yunis et al., 2004; Islam et al., 2006). However, the relationship between virus load (or replication) and Virulence Rank has not been convincingly tested (Witter et al., 2005).

1.2.3 Evolution of Virulence

1.2.3.1 Theory, Models and the 'Trade-Off' Hypothesis

The notion of virulence evolution has been established as an important factor in the understanding of infectious diseases. It was not, however, until the end of the C20th when a paradigm shift exposed a new way with which biologists could understand the concept (Ewald and de Leo, 2002). Previous appreciation of the idea of virulence centred around recognising the necessary evolutionary end-point of a pathogen as ultimately benign. This was termed the classical Panglossian view (Sigmund et al., 2002) and has now been su-

perceded by a thesis allowing virulence to evolve to any point on a pathogen's virulence spectrum (Ebert, 1999).

Central to this current theory is both empirical (e.g. Ewald, 1983, 1991, 1994) and theoretical (e.g. Anderson and May, 1981, 1982; Frank, 1996) results. Mathematical models, often coupled with an epidemiological framework (such as the SIR model), have been employed to describe the often complicated behaviour afforded by particular host/pathogen systems (e.g Levin and Pimentel, 1981). However Ganusov and Antia (2003) show that the precise details of the epidemiology of the pathogen, such as host pathogenesis and mechanisms of transmission, have a crucial role to play in predicting the evolutionary outcome of virulence of a particular pathogen. The authors conclude that simple models are not robust in their assertions and more complicated models incorporating the biology of host-pathogen interaction are required.

The current understanding of virulence evolution is based on theoretical frameworks, often themselves not mutually exclusive. These have been reviewed by many authors (e.g. Bull, 1994; Read, 1994; Frank, 1996; Ebert, 1999; Galvani, 2003) and include:

1. *Maximisation of Parasite Fitness*
   a) Transmission is directly increased by a particular feature of virulence (e.g. the induction of cold symptoms in the host by *rhinoviruses* causing increased likelihood of transmission between contacts).
   b) Virulence maximises transmission (the 'Trade-Off Hypothesis') whereby virulence will always decrease parasite fitness in the long term since it harms its host, but is necessary to increase transmission. The result is a trade-off for optimum lifetime fitness. This is often seen as a central axiom of virulence evolution and has been formulated by mathematical models, probably due to both the relative ease of inclusion computationally and explanation biologically. A pathogen's transmission and virulence capabilities are commonly modelled by a trade-off relationship (Day, 2002; Fenner et al., 1956; Frank, 1996; Gandon et al., 2001; Ganusov and Antia, 2003). There have been an number of empirical results suggesting that this functional form may be a reasonable assumption (Anderson and May, 1982; Lipsitch, 1997; Mackinnon and Read, 1999; de Roode et al., 2008) for such pathogen infections as Myxoma in rabbits (Dwyer et al., 1990), Malaria in mice (Mackinnon and

Read, 1999), Malaria in chickens (Paul et al., 2004), *Pasteuria ramosa* in *Daphnia magna* (Jensen et al., 2006), HIV in humans (Fraser et al., 2007) and *Ophryocystis elecktroscirrha* in monarch butterflies (de Roode et al., 2008) however there is also evidence that a trade-off may not exist for other systems such as ranavirus infection in salamanders (Brunner and Collins, 2009). This is a key assumption for evolutionary biology theory but there is scant evidence for it in practice.

2. *Maximisation of Host Fitness*
   Hosts' response (or failure to respond) causes the observed virulence (e.g. it may be better to tolerate a small worm burden, say in the case of a nematode infection of a host within an endemic area, than to expel it by costly physiological means suggested by Behnke et al. (1992)).

3. *Coincidental Selection*
   Movement of parasites to a new host species or tissues produces a more virulent response. Any virulence is therefore a dispersive phenomenon and not adaptive. An example of this has been postulated by Levin and Eden (1990), who suggest that *E. coli* genes that code for bacterial organelles that bind to host cells are selected for in other regions of the body (e.g. in the gastrointestinal tract, where they do not cause disease) and selected against in some regions of the body (e.g. in the urinary tract, where the inflammatory response causes excretion of the parasite) (Read, 1994). Any virulence observed would therefore arise due to relocation from the gastrointestinal to the urinary tract.

4. *Short Term Selection*
   Local within-host growth allows persistence of mutants, even though transmission is depleted partially or completely by the virulence. An example of this may be infection of the central nervous system by a parasite strain. While serving the pathogen well in the short term, subsequent transmission may be compromised due to difficulty exiting the host (HIV, polio and bacterial meningitis have been postulated as examples of this phenomenon by Bull (1994) and Levin and Bull (1994)).

5. *Coevolution*
   The continued evolution of both parties and the genetic diversity of

the hosts both act as determinants of the virulence expressed by the parasites and experienced by the hosts. The best example of this has been the introduction of the myxoma virus into Australia, which is discussed in Section 1.2.3.2.

In the context of Marek's disease, all but the 'homogeneous chicken' hypothesis are examples of the 'Trade-Off Hypothesis'. The 'homogeneous chicken' hypothesis is an example of virulence change through a shift in the host genotype.

1.2.3.2 Documented Examples of Virulence Evolution

There are relatively few naturally occurring examples of empirical evidence of virulence evolution to support the vast theoretical literature in any rigorous way (Bull, 1994). Nevertheless there are a few important examples outlined below:

Myxoma

The story of the introduction of the myxoma virus into Australia in the 1950s to control the rabbit population, centres around the coevolution of the host and pathogen population. Mortality in the rabbits reached 99.8% during the early stages of the epidemic. Less virulent strains were then isolated in the population, which might have been attributable to the scarcity of mosquitoes, which act as vectors, during the winter months, whereby transmission rates are increased if host mortality is reduced (Ewald and de Leo, 2002). Strains have been isolated over the last 50 years and given a virulence rank (I-VI) based on the clinical survival time (short to long respectively) of inoculated rabbits. Grade III predominated after the initial reduction of mortality rates to around 90% of infected rabbits (de Leo et al., 2002). Data have suggested that selection for resistant hosts has occurred, which has been followed by an increase in the virulence of the pathogen, leading to an observable stable state (Fenner and Fantini, 1999). The continuous adaptation of a system like this without observable change has been termed the 'Red Queen Hypothesis' (van Valen, 1993).

Infectious Bursal Disease

Failure of vaccines protecting against IBD have been noted throughout industrialised farming countries since the mid 1980s. These new strains can cause up to 60% mortality in unvaccinated birds (Read and Mackinnon, 2008). Interestingly the more virulent strains in Europe differ from those across the Atlantic, with the former harbouring more virulent ancestral forms of IBDV compared with the US, where novel antigenic strains have emerged (van den Berg, 2000). This is a case where evolution of increased virulence has emerged via two independent causes. The resulting increased mortality is, however, the same.

1.2.3.3 Vaccination and Evolutionary Pressure

There are various host-pathogen systems in which a vaccine program has been put in place to reduce the effects of disease and a resulting increase in virulence has been detected (Read and Mackinnon, 2008). These include bacteria (Pertussis, Pneumococcal disease, Diphtheria); RNA viruses (avian Influenza), and DNA viruses (Hepatitis B, Infectious Bursal disease, Marek's disease). The question of why we have not seen more vaccine escape mutants has been addressed by Read and Mackinnon (2008) who suggest two possible reasons: (1) the cases where vaccines have been successful have been predominantly acute childhood infections which might be easier to eradicate than both the chronic diseases, such as sleeping sickness, HIV, malaria etc. or the diseases where reinfection of hosts by a multitude of strains is common, such as influenza, pneumococcal disease, malaria etc., (2) it might be too soon in the evolutionary timescale to understand the true ramifications of vaccination thus far, indeed it might take decades for a very rare mutant to become established in a population.

1.2.3.4 Quantifying Virulence Evolution

The theoretical approach to analyse the evolution of virulence centres around the concept of an Evolutionary Stable Strategy (ESS), first formalised by Smith and Price (1973). This has been calculated via maximisation techniques (in

particular R_0 (the number of infected individuals arising from a single infected individual in an otherwise fully susceptible population) maximisation, known as the 'Game Theoretic' approach), Adaptive Dynamics and methods similar to those used in quantitative genetics and population genetics theory. The most useful technique will be governed by the evolutionary and epidemiological properties of the system. By assessing a general epidemiological model, Dieckmann (2002) explain how the the Game Theoretic and Adaptive Dynamics approaches are equivalent whenever one of the following is true: epidemiogical rates don't depend on host density, the host population size either stays constant or changes only due to infection.

In the theory, mathematical models have often focussed on the factors governing the evolution of virulence which may provide scope for contributions to human and animal welfare (Bull, 1994). Ewald and de Leo (2002) discuss the implications of mobility of the parasite and host as potential determinants of virulence and Gandon et al. (2001, 2003) study the evolutionary implications of *imperfect* vaccines (in the sense they do not provide a complete block for initial infection and/or subsequent transmission of the parasite) in the context of an epidemiological framework. In both these latter papers, the authors equate virulence with parasite-induced mortality rate and increased host exploitation, which is also associated with increased transmission of the parasite (a relationship which has been suggested by some empirical systems (e.g. Lipsitch, 1997; Mackinnon and Read, 1999)). The studies found that targeting vaccination on different parasite life-cycle stages led to qualitatively distinct evolutionary consequences. Using R_0 maximisation methods, vaccinations working to decrease infection or transmission, in general, selected for less virulent parasites, whilst those decreasing within-host exploitation or levels of toxicity selected for more virulent parasites. Epidemiological feedback enabled systems to be bistable (one high, one low virulence state), with the evolutionary stable outcome determined by initial conditions.

Another important aspect of modelling virulence is understanding different definitions of virulence. Although virulence can correspond to any reduction in fitness of the host, such as loss of fecundity or movement, it is usually associated with death rate of the host. Day (2002) highlights the difference between four different definitions of virulence as host mortality:

- *Parasite-induced instantaneous mortality rate*
  The standard form of denoting virulence in mathematical models, e.g. infected host death rate.

- *Case mortality*
  The most popular non-mathematical notion of virulence, seen as the likelihood of death given infection.

- *Expected lifespan*
  Corresponding to the lifespan of an infected individual, given that it dies from the disease.

- *Lethal dose*
  Used as a measure in empirical studies and defines the size of the parasite inoculum required to produce a specific % case mortality.

Using a simple deterministic SI model with both disease-mortality and parasite clearance (although the results are true for a model where there is no recovery), the oft-tested result that virulence should evolve to be higher with increasing background mortality rate, is shown to be dependent on the definition of virulence: it is true for an instantaneous mortality rate (since this is the standard definition for theoretical models) but false for a case-mortality definition (with a *decrease* in the virulence level). Day (2002) recognises the fact that host death rate does not capture the full extent to which the parasite induces host mortality, since a high case mortality might be achieved by a relatively low host death rate in the case of a small clearance rate or background mortality rate.

1.2.4 Modelling Infectious Diseases: Relevant Sources

Predominantly, the published mathematical models of infectious diseases in chickens concern Highly Pathogenic Avian Influenza (HPAI). Currently these divide between 'on farm' epidemiological analyses (e.g. the effect of vaccination strategies on H5N1 (Savill et al., 2006)) and 'area wide' network models (e.g. parameterised for the H7N7 2003 Dutch outbreak (Le Menach et al., 2006)). These two models exemplify two approaches of models: 'what if' and data-fitting respectively. Savill et al. (2006) use past studies to estimate the required parameters (and then conduct sensitivity analyses around these values)

to predict the dynamics arising from different culling and vaccination strategies. Le Menach et al. (2006) fit their model to actual epidemic data via maximum likelihood methods to achieve an accurate representation of the spread during an epidemic.

There are also publications concerned with the estimation of R_0 via statistical methods for different pathotypes of AI (e.g. van der Goot et al., 2003a,b) and host vaccination status (e.g. van der Goot et al., 2005).

Klinkenberg and Heesterbeek (2005) have produced a discrete time epidemic model of within-host dynamics of *Eimeria* spp. in chickens.

Modelling of the combined evolution and epidemiology of a system has been explored previously by Gandon et al. (2001, 2003) as described above, which gives a parameterised framework for malaria transmission. Grenfell et al. (2004) also couple epidemiological dynamics, immunodynamics and evolutionary biology to build a framework for understanding RNA viruses of vertebrates.

Current mathematical models for evolution and epidemiology usually employ continuous dynamics (Tompkins et al., 2002; Swinton et al., 2002), while those for livestock do not tend to impose a strong cohort structure for on-farm dynamics (modelling instead the spread through one farm over the course of one cohort (Savill et al., 2006) or the spread through a network over time with a continuous transmission kernel (Truscott et al., 2007)). However, Klinkenberg and Heesterbeek (2007) extended their previous model (Klinkenberg and Heesterbeek, 2005) to include a cohort structure within the model and allowing multiple cohorts through time. The results explore the epidemiology of coccidiosis with varying levels of hygiene between the cohorts. This seems to be the only published example of an explicitly modelled multiple cohort system.

1.3 Thesis Plan

The overarching theme of this work concerns understanding the factors which influence pathogen virulence evolution; and how man-made changes have influenced the scale and rate of this evolution. In particular, the work is

concerned with combining the aspects of the epidemiology and ecology of a pathogen, its host demography and its external environment with the theoretical framework ubiquitous to virulence evolution literature. Specifically, the work here focuses on explaining past trends of virus evolution towards greater virulence, seen in the case of Marek's disease virus, a pathogen naturally infecting chickens.

Chapter 2 investigates the epidemiologically important parameters for describing Marek's disease virus as it spreads throughout a population. The parameters of interest are formally estimated via statistical methods from experimental data. From this analysis I propose a new definition of virulence for Marek's disease requiring only mortality effects in unvaccinated birds, which suggests a possible trade-off between virulence and transmissibility of the virus.

Chapter 3 examines the evolutionary fitness of strains within the specific demography of a farm of broiler birds. Key measures used in evolutionary life-history and epidemiological theory, which are used to imply selection direction, are calculated for this system. I will argue that the host population structure might render these traditional methods suboptimal at explaining the selection encountered by the pathogen.

Chapter 4 explores the effect of MDV exposure on a broiler flock and assesses the extent to which the quantity of virus existing after an outbreak can determine the selection force and direction on the virulence of virus strains.

Chapter 5 considers a group of interconnected farms to understand how Marek's disease virus can persist in a network of host farms. This work can shed light on whether new occurrences of more virulent strains are very rare, but spread quickly, or whether many new strains are constantly introduced into the farm population.

Chapter 6 discusses the main conclusions of the previous four chapters, and merges their evidence to determine the most plausible answers to the questions posed in this introduction. It contains an interpretation of where this thesis sits in relation to other studies done and its potential for directing future work on the topic.

Together these chapters will provide the first quantitative assessment of how

this economically important worldwide disease has persisted and thrived in the face of sustained and adaptive interventions. The work uses and extends epidemiological and evolutionary frameworks to both explain the causes of historical change in Marek's disease virus virulence and to highlight potential future problems. This approach, I hope, will light the way for future work, both experimentally, theoretically and in the field, so we might test the predictions made herein and further understand the implications of our farming system on its virus denizens.

1.3.1 Notation

In each chapter, where appropriate, the abbreviations, mathematical notation and formulae are given in a glossary at the end of each Methods section. For ease of notation, log is written to represent $\log_{10}$ throughout the paper.

2 Parameter Estimation

2.1 Introduction

In this chapter, fundamental parameters relating to the epidemiology of MDV are estimated using mathematical methods. These parameters will be used in later chapters as the evolution and epidemiology of MDV is explored further. However, many of these parameters have not been formally estimated, to the best of my knowledge, and their values are important in their own right in explaining the character of an important poultry disease.

Outbreaks of MDV are very damaging to the farming industry and effective control methods are required to limit the impact of the disease. Successful control strategies for MDV require an understanding of the epidemiology of the disease, which this chapter explores.

There will be four groups of parameters to estimate; those concerned with

- dust production by a bird;
- viral shedding capabilities;
- mortality of infected individuals;
- transmission of the virus to susceptible individuals.

In each section that follows, all the groups of parameters will be covered in turn.

2.1.1 Dust Shedding

The aim of this analysis is to characterise the dust shedding profiles for layer birds. The results will be used later in this chapter.

2.1.2 Viral Shedding

Viral shedding rate is defined in this chapter as the viral copy number (VCN) per *mg* of dust shed by a single infected bird over the infectious period. This section estimates the quantity of virus material shed by an infected bird and also when this viral shedding begins (i.e. the latent period of the infection). Both these parameters are estimated for birds infected with different strains of MDV and vaccinated with different vaccines.

Estimation of viral production in the dust is important epidemiologically since MDV transmits via the inhalation of infected dust and quantifying the virus in the dust enables the estimation of the transmission potential.

2.1.3 Host Mortality

Trying to quantify the impact of a disease on a host is a difficult matter. The concept of 'virulence' can evoke different meanings depending on your organism viewpoint (host or parasite). In this work it must be clear what virulence means. Therefore a short discussion is presented in the context of host mortality in Box 2.1.

Box 2.1: Virulence Definitions

Definitions of virulence are numerous and varied (Dieckmann, 2002). Microbiologists equate virulence with the notions of both infectivity and severity of disease, whereas evolutionary biologists focus on evolutionary fitness (either the pathogen's or the host's) (Read, 1994). Zoologists tend to focus on host fitness (e.g. Levin and Pimentel, 1981) with most mathematical models describing this specifically as host mortality (e.g. Anderson and May, 1982; Tompkins et al., 2002). There have been various attempts at virulence definitions in the context of MDV and pathotyping of strains (e.g Witter, 1997; Witter et al., 2005). It is normally measured by a strain's propensity to induce lymphoproliferative lesions in vaccinated chickens (Witter et al., 2005). In the pathotyping study done by Witter et al. (2005), a higher Virulence Rank was attributed to strains inducing a more severe clinical response (measured as the proportion of individuals displaying gross lesions in a group of chickens) in a combination of HVT and Bivalent vaccinated chickens. Currently there are four recognised MDV pathotypes, each occupying part of the 'continuum of virulence' (Witter, 1997), known as: mild, m; virulent, v; very virulent, vv; very virulent plus, vv+. Thus, the definitions of virulence associated with MDV have mostly been measured in vaccinated hosts. I am unaware of any attempt to directly test for an association between Virulence Rank and host mortality rate in unvaccinated chickens. The data collected by Witter (1997) and Witter et al. (2005) only report the total clinical signs and death within a given period, making it difficult to assess the disease-induced mortality by itself.

From an evolutionary standpoint, usually the most important aspect of virulence is its effect on host mortality. There are of course examples where this is not true (for example, there is pathogenicity by non-lethal morbidity in the case of the common cold; sterlising effects, both physical in the case of Chlamydia and through changes in host behaviour, such as certain HPV infections). Yet for the industrial chicken system, where dead chickens are removed from the population at regular intervals and movement of broiler chickens is restricted, focusing on a measure of mortality as a measure of virulence seems sensible, especially since dead chickens do not continue to transmit the virus. Therefore, the parameter estimation work is based on a definition of virulence of MDV in terms of host mortality alone.

2.1.4 Transmission

In most MDV-related experimental work, transmission usually occurs via insufflation (Baaten et al., 2004) or subcutaneous injection (Sharma, 1984). However, the natural route for infection is by inhalation of virus-contaminated dust (Carrozza et al., 1973). To understand the consequences of viral shedding in a flock, it is vital to understand the role of natural transmission and susceptibility to infection. The aim is to quantify the daily transmission rates to susceptible birds, who are either unvaccinated or HVT-vaccinated. Vaccinated hosts are still able to become infected with MDV. However, an assumption in the literature is that vaccination protects against clinical signs of the disease, not infection (Nair, 2005), but this is largely an untested assumption and the methods employed aim to elucidate the relationship between vaccination and susceptibility to infection.

2.2 Data

2.2.1 Dust Shedding

In an experiment conducted and described by Renz (2008), groups of layer chickens were raised from an age of one day in isolators (a raised glass box housing a group of birds, which allows air flow to be regulated via an exhaust and air filter). All the dust from each isolator and its exhaust was retrieved every 24 or 48 hours. With knowledge of the number of chickens per isolator each day and the total dust, the total mass of dust shed per day per bird was found for weeks 1-8, giving a total of 8 data points per isolator with confidence intervals, from repeated sampling.

2.2.2 Virus Shedding and Host Mortality

The analysis looks at a single experiment conducted by Renz (2008) with two response variables measured. Groups of maternal antibody positive Rhode Island Red layer chickens were inoculated at one day old with one of three vaccine treatments: sham (saline solution), HVT (first generation industry vac-

cine) or Bivalent (second generation industry vaccine). At 5 days post vaccination (dpv), they were challenged (via injection) with one of three MDV strains: 04CRE, MPF57 and 02LAR. The experiment was therefore a 3x3 factorial design study, with vaccine type fully cross-factored against virus strain (i.e. for each vaccine strain used there was a different virus isolate). There were either 26 or 27 birds in each group treated with the same virus-vaccine combination and each group was housed in a separate isolator. The air in each isolator was changed 8-20 times per hour. The whole experiment was replicated once to give a total of 484 birds.

Due to regulatory requirements, birds who were thought to be nearing death were removed from the isolator. The experiment lasted 56 days post infection (dpi) and many of the birds were recorded as dying on the last day. Therefore for the purposes of this analysis, the data were censored after day 55. All birds were given a large dose of MDV isolate (500 pfu) via injection and it was therefore assumed that all the birds were infected and would shed the virus.

The two variables recorded were the time to death of a bird (measured in days) and the density of virus within each isolator every week (measured in VCN per *mg* of dust in the environment). The virus titres in the dust were determined via PCR of dust in the isolator air filters.

The independently sampled isolates were Australian in origin and had been pathotyped at Virulence Ranks of 16.5, 36 and 46 respectively (Walkden-Brown et al., 2008) as shown in Table 2.1 according to the scheme developed by Witter (1997) and Witter et al. (2005). In the last chapter, the metric 'Virulence Rank' for a strain of MDV was defined and discussed. To recapitulate, Virulence Rank was defined by Witter (1997) to be the average percentage of birds who either died or developed gross clinical signs during a time period of 56 days. The average was taken with respect to two groups of birds - one vaccinated with HVT, the other with the Bivalent vaccine. In the terminology used by Witter, this leads to classifying 04CRE and MPF57 as v and 02LAR as vv.

	Vaccine Treatment			Virulence Rank
	Sham	HVT	Bivalent	*(mean HVT/Bivalent)*
04CRE	59	20	13	16.5
MPF57	69	43	29	36
02LAR	84	61	31	46

Table 2.1: Pathotyping of Australian Isolates: The percentage of infected birds who either died or showed gross clinical signs within 56 days of inoculation with one of the strain-vaccine combinations. The number of birds in each of the nine groups was between 22 and 27. The mean value of the % clinical signs and death of the two vaccinated groups was used to find the Virulence Rank, as defined by Witter (1997).

2.2.3 Transmission

The data on infection of susceptible birds were collected by Islam et al. (2008). There were 10 identical floor pens with woodshavings, housing 60-72 infected chicks in each. In each pen, at 21 dpi, 40 day old sentinel chicks (20 HVT-vaccinated, 20 unvaccinated) were placed in a netted corner of the pen. 21 dpi corresponds to 0 days post exposure (dpe). 5 unvaccinated and 5 vaccinated sentinel chicks were removed at 5, 10, 15 and 20 dpe unless otherwise stated. Their spleens were assayed for detection of MDV via PCR techniques. The dust content of each of the pens was measured at regular intervals and calculated in mg/m^3) along with a quantification of MDV via quantitative PCR (qPCR), so that the VCN per m^3 was calculated for each pen at regular intervals.

2.3 Methods

For all the groups of data given in Section 2.2, parameters were estimated which provided:

- daily dust shedding for layer birds;
- latent period (the time until an infected bird becomes infectious);
- viral shedding rate (VCN/mg dust);
- host lifespan for an MDV infected host (without any other mortality);
- daily probability of transmission to a susceptible individual.

All statistical tests were completed in R-2.6.2 (R Development Core Team, 2005) unless otherwise stated and all graphs were plotted in Matlab® (2007)

2.3.1 Dust Shedding

A cubic spline method is used, fitting three splines (one to the central estimate, one to both the lower and upper confidence estimates). Matlab® (2007) was used for this computation.

2.3.2 Viral Shedding

Latent period and rate of viral shedding cannot be estimated directly from the data since the response variable (measured weekly in VCN per *mg* dust) could vary with the number of birds housed together, which varies as birds die. Therefore a discrete time dynamic model capturing this variability was used to estimate the two infectiousness parameters. These parameters were estimated for different virus strains infecting birds vaccinated with one of three vaccine treatments.

To model the viral shedding of MDV from a dataset, there are several assumptions:

1. After MDV infection, there is a delay before virus is first shed (Baigent and Davison, 2004). This latent period is assumed to be constant across all the birds in a single isolator, but could vary between isolators.

2. Once shedding has begun, the rate of shedding (measured in VCN per *mg* of dust) is constant over the duration of the experiment and is the same for all birds within an isolator, but could vary between isolators. Not much is known about the viral shedding rates of infected birds, but this is the most parsimonious model to start with and there is evidence from the data that the density of MDV in the dust plateaus (indeed if this is true, the true asymptote should be the constant rate of viral shedding).

3. The density of virus (VCN per *mg* dust) is calculated at the end of each day and any removal of birds is assumed to occur at the start of the day.

There are therefore two parameters estimated per isolator: latent period (in days) (i.e. the time elasped before viral shedding occurs after infection) and rate of shedding (VCN/mg dust). A discrete time dynamic model was set up accounting for the removal of birds at the start of days given in the mortality data.

The latent period is denoted by T_s. Birds will then shed virus from T_s+1 at a constant rate, a (VCN per mg dust), for the remaining time of the experiment. The density of MDV in the dynamic model can be compared to the amount of virus recorded in the data (sampled every 7 days). Modelling all experiments, both $a(v, j)$ and $T_s(v, j)$ were estimated for each Virulence Rank $v \in \{16.5, 36, 46\}$ and each vaccine status $j \in$\{sham, hvt, bivalent\}.

In order to fit the model to the available data, a metric must be defined for disparity between the model and data. MDV density in dust was only sampled once at each time point for each isolator. This means that there is no direct way of calculating the measurement error in a sample of dust since the replicate isolators are themselves subject to different bird deaths at varying times in the sampling period. The difference in MDV density between these replicates at each time point can therefore *not* be taken as samples from the true error distribution. Another method is sought.

Assuming that a final MDV density has been reached at some time before the end of the sampling period, the sample points after that are assumed to be replicates. Thus, assuming measurements errors are normally distributed, an approximate estimate of error variance can be found. Let Y_1 and Y_2 be independent measurements such that $Y_1, Y_2 \sim N(\mu, \sigma^2)$ then $Y_1 - Y_2 \sim N(0, 2\sigma^2)$.

To find the time point after which the data is assumed to have plateaued, sets of consecutive points were examined: weeks 7-8, 6-8 and 5-8 (Table 2.2). The first sample (Weeks 7-8) provides the highest probability that the Anderson-Darling statistic is obtained from a distribution drawn from a normal distribution (p=0.79) given that the underlying distribution has a true mean of zero (p=0.40). The resulting estimated standard deviation is 0.33. The distribution is displayed in Figure 2.1. The Anderson-Darling algorithm is used to test for normality for all cases due to the small sample sizes.

This analysis gives an error distribution of the random variable, X, a random sample of MDV density (VCN/mg dust). Now, let $\log X = Y$, then $\log X \sim$

k	Weeks (comparison)	Sample Size	Norm. Test p value	t test 95% CI
1	7-8 (7)	18	0.79	(-0.096, 0.23)
2	6-8 (6)	34	0.44	(-0.22, -0.034)
3	6-8 (7)	35	0.95	(0.013, 0.21)
4	6-8 (8)	35	0.36	(-0.098, 0.12)
5	5-8 (5)	53	0.69	(-0.19, -0.044)
6	5-8 (6)	51	0.90	(-0.14, -0.0019)
7	5-8 (7)	53	0.97	(0.064, 0.21)
8	5-8 (8)	53	0.50	(-0.042, 0.14)

Table 2.2: Anderson-Darling normality test results (with the null hypothesis $Y_k \sim N(\hat{\mu}_k, \hat{\sigma}_k)$) and t test results (with the null hypothesis $Y_k \sim N(0, \hat{\sigma}_k)$ where σ_k is estimated). The comparison week in brackets denotes the value to which the other weeks (in the range) were compared.

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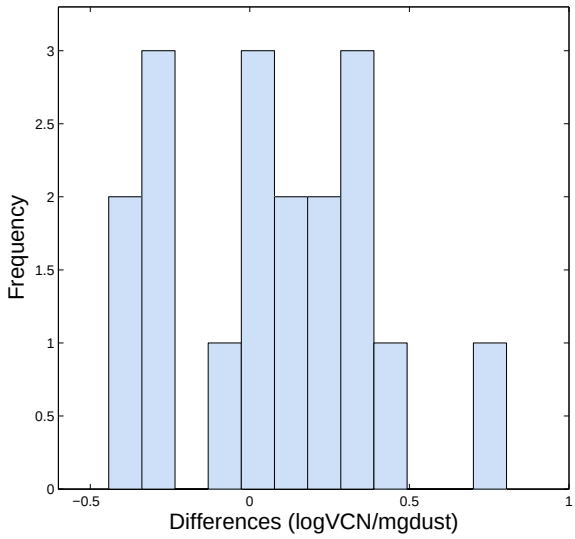


Figure 2.1: Viral Shedding: Differences between virus density (logVCN per mg dust) data points within each isolator between weeks 7 and 8.

N(μ,σ^2), where μ is the underlying mean given time and environment and σ^2=$0.33^2/2$.

Thus a distance function for mapping the data set, Y, to the model set, Θ, is defined as the probability of the logged data point being sampled from a normal distribution with mean defined as the logged model value at t and constant variance, σ^2 as defined above. Multiplying over all t, such that $|Y|$=n, the likelihood is calculated. More formally:

$$\begin{aligned}\mathcal{L}(a, T_s) &= P(Y|\Theta) \\ &= \prod_{t\in T} P(y_t|\theta_t) \\ &= \frac{1}{\left(\sigma\sqrt{2\pi}\right)^n} \prod_{t\in T_s} \exp\left(\frac{y_t - \theta_t}{2\sigma}\right)^2\end{aligned}$$

This can be calculated for combinations of the two parameters to create a discretised likelihood profile, which is maximised to find the maximum likelihood. Equivalently, the negative log-likelihood is found, which is usually quicker and easier to calculate. For the normal distribution:

$$-\ln \mathcal{L}(a, T_s) = n\ln(\sigma\sqrt{2\pi}) + \frac{1}{2\sigma^2}\sum_{t\in T}(y_t - \theta_t)^2$$

Moreover, since this is an order isomorphism of the likelihood, it is minimised to find the maximum likelihood, by finding the maximum likelihood estimates of a and T_s for each experiment. This is done by calculating a likelihood surface for a complete parameter state subspace (to a certain specified level of accuracy, in this case 3 significant figures) since the parameters can be bounded easily by observing the data.

Once the maximum likelihood estimates for each parameter have been found the full realisation of the model can be plotted against the data. Credible intervals are calculated by means of Markov chain Monte Carlo (McMC) realisations. For this, the McMC algorithm samples values from the respective updated posterior distributions of the 2 parameters. After a burn in period, these parameters can either be accepted or rejected according to the algorithm rules. If accepted they enter the set of possible (repeated) values for the estimated parameters (Carlin and Lewis, 2008). The model output for each of these sets of parameter values is calculated. At every time point for which we have data, a 95% credible interval is found for the distribution of this output.

The prior distribution of each parameter was assumed to be uninformative and were thus taken as uniform, to ensure equivalence to maximum likelihood estimation. The burn in period was set to 20,000 iterations. Details on the method with diagnostics and posterior distributions are given in Appendix

A.1.

The code for this analysis was written from scratch in *C++* and compiled using DevC++ (Busbee, 2009) .

2.3.3 Host Mortality

A survival analysis was undertaken for the mortality data because multiple covariates need to be included to find the host lifespan. These covariates are vaccine treatment and virus Virulence Rank.

The biology of Marek's disease suggests that the probability of death by MDV infection changes through time, since the virus enters a "latent" phase after infection (note that this is not the same as the latent period of the infection, but denotes a specific time in the infection pathway of the virus described in Chapter 1). The lytic and transformation stages that follow are pathologically distinct and may lead to a different mortality rate. Therefore, a Weibull distribution was chosen as a candidate distribution for modelling survivorship curves, which is often used for time to death data since it is flexible and can mimic other distributions but only has two parameters in its non-location form. We therefore assume that time to death can be modelled as a random variable, T, such that $T \sim W(r, \lambda)$ where $r, \lambda > 0$ are the shape and scale parameters respectively (Hosmer and Lemeshow, 1999). The associated probability density function is

$$f(t) = \begin{cases} \frac{r}{\lambda}\left(\frac{t}{\lambda}\right)^{r-1} e^{-\left(\frac{t}{\lambda}\right)^r} & \text{if } t \geq 0 \\ 0 & \text{else} \end{cases}$$

A Weibull model was fitted to all the data in the study. In the case when r=1, the distribution collapses to an exponential distribution. When $r > 1$ or $r < 1$ there is an increasing or decreasing chance of death respectively (see Figure 2.2). Coefficients, $\boldsymbol{\beta}$, are such that $\lambda = \exp(\boldsymbol{\beta}.\mathbf{x})$, where $\boldsymbol{\beta} = [\beta_0, \beta_1, \beta_2, \beta_3]$ are the covariate coefficients and $\mathbf{x} = [1, x_1, x_2, x_3]^T$ are the covariates. In this analysis, there are three covariates (one continuous: the arcsine square-root transformation of the Virulence Rank of an isolate (x_1) and two binary: the presence or absence of HVT (x_2) and Bivalent (x_3) vaccine). Therefore there are 9 combinations on the set of possible covariates, thus $\lambda_j = \boldsymbol{\beta}.\boldsymbol{x}_j$ where

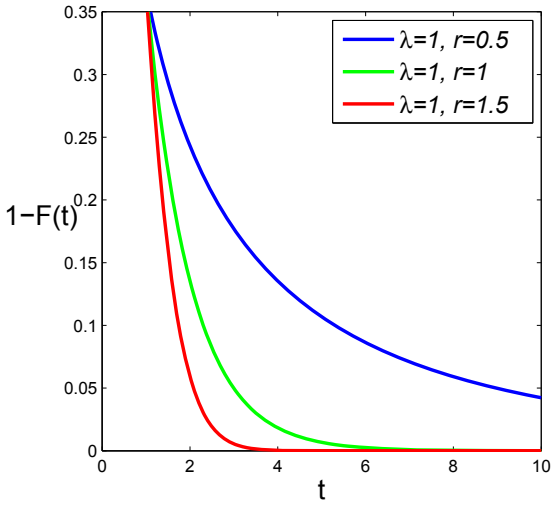


Figure 2.2: Host Mortality: Effect of changing the shape parameter, r, on the Weibull survival function.

$j \in [1, 9]$. The likelihood function can therefore be written

$$\mathcal{L}(\lambda_j, r) = \prod_{j=1}^{9} \prod_{i=1}^{n} \left\{ \frac{r}{\lambda_j} \left(\frac{t_i}{\lambda_j} \right)^{r-1} \right\}^{\delta_i} \exp\left(-\frac{t_i}{\lambda_j} \right)^{r}$$

where δ_i is zero when the ith observation is censored and unity elsewhere. This function was maximised via the Newton-Raphson algorithm such that $\mathcal{L}(\hat{\lambda}_j, \hat{r}) = \max \mathcal{L}(\lambda_j, r)$ where $\hat{\lambda}_j = \exp(\hat{\boldsymbol{\beta}}.\mathbf{x}_j)$ and $\hat{r}_j = \hat{r}$. Note that the Virulence Rank, v, of an isolate is a percentage measure and to be used as an explanatory variable in a regression analysis, it should be transformed such that $v_T = \arcsin \sqrt{0.01 v}$. The regression fits the maximum likelihood estimates for $\boldsymbol{\beta}$, however further Bayesian analysis is required to estimate the associated credible intervals when the covariate covariance matrix does not approximate the identity matrix.

To test whether vaccine status is a possible covariate in the survival analysis, the shape parameter, r, is fitted to the Sham vaccinated data with the covariate being transformed Virulence Rank only. This is denoted as r_0. Next, two models are fitted to both the HVT and Bivalent vaccinated data: the restricted model where r is set to be r_0 and secondly, an unrestricted model where r is chosen to as the Maximum Likelihood Estimate (MLE), denoted by r_1. The log-likelihoods for each of these two models are denoted by LL_0 and LL_1 respectively. Twice the likelihood difference follows a χ^2 distribution, which can

be the basis for a statistical test where the null hypothesis states that the shape parameter is the same for both models.

In the HVT case, $r_1 = 3.0864$ (H_0: r_0=r_1), so $X^2 = 2(LL_1\text{-}LL_0) = 2(\text{-}69.1+70) = 1.8 < \chi^2(1)_{0.05}$ (p=0.1797). Therefore the null hypothesis is accepted. In the Bivalent case, the restricted model $r_1 = 6.4516$. $X^2 = 2(LL_1\text{-}LL_0) = 2(\text{-}59.2+59.7) = 1.0 < \chi^2(1)_{0.05}$ (p=0.3173). Therefore the null hypothesis is accepted.

A likelihood test on the possible covariates suggests that the vaccine treatment of a bird can be considered as a covariate and all the vaccine treated birds (Sham, HVT and Bivalent) can be modelled together. The three vaccine groups of data will therefore be analysed together with only one hazard function.

The prior distribution of each parameter was assumed to be uninformative and were thus taken as uniform, to ensure equivalence to maximum likelihood estimation. The burn in period was set to 22,000 iterations. Details on the method with diagnostics and posterior distributions are given in Appendix A.2.

R-2.4.2 (R Development Core Team, 2005) was used initially to find the maximum likelihood estimate and to fit the model using the function `survreg` within the package *Survival*. WinBUGS 14 (Lunn et al., 2000) was used for the McMC analysis.

2.3.4 Transmission

Daily transmission probabilities for unvaccinated and HVT vaccinated birds were calculated independently and directly from the data via a statistical model. Each pen was analysed separately for unvaccinated and HVT vaccinated birds and a maximum likelihood approach was used.

Let X be defined as the random variable, the number of sampled individuals who are infected. At each sample time, $i = 1, 2, 3, 4$ (corresponding to 5, 10, 15, 20 dpe respectively) birds are sampled without replacement. Therefore the number of sampled infected individuals follows a hypergeometric distribution (Kalbfleisch, 1985).

$$P(X = k_i) = \frac{\binom{M_i}{k_i}\binom{N_i - M_i}{n_i - k_i}}{\binom{N_i}{n_i}}$$

where,

M_i	total number of infecteds in the population at time i before sampling
N_i	total population at time i before sampling
n_i	sample size at time i

Now the likelihood can be defined such that

$$\mathcal{L}(k_1, k_2, k_3, k_4 | M_1, M_2, M_3, M_4) = \prod_{i=1}^{4} P(X = k_i | M_i)$$

where k_i is the observed number of infected individuals in each sample at time i and M_i are the parameters to estimate.

$$\max\{\mathcal{L}\} = \max_{M_j \leq N_j} \prod_{i=1}^{4} P(X = k_i | M_i)$$

Since k_i, n_i and N_i are known, $M_i = \hat{M}_i$ can be calculated directly for the maximum likelihood estimate. There will therefore be a set of $\hat{M}_i$ for each pen, for each vaccination group. The newly infected individuals between each time point, $m_i = \hat{m}_i$ can be calculated trivially, giving us the total number of infected individuals in each group in each pen between each sample time.

Assuming the number of newly infected individuals between each time point, L_i, follows a binomial distribution, with $\mathbb{E}(L_i) = m_i$, $L_i \sim \text{Bin}(N_i - \sum_{j<i} m_j + \sum_{j<i} k_j, q_i)$. The first parameter is the effective population size available to be infected at timestep i, which is the number of unsampled individuals at time i before sampling ($N(i)$), minus the number of infected individuals who have not yet been sampled ($\sum_{j<i} m_j - \sum_{j<i} k_j$). The second parameter is the probability of infection between sample time $i-1$ and i. The maximum likelihood estimate of the expected probability of transmission within time period i is therefore

$$\hat{q}_i = \frac{\hat{m}_i}{N_i - \sum_{j<i} m_j + \sum_{j<i} k_j}$$

Since q_i is the probability of infection over 5 days and assuming there is an equal chance of infection on any of the 5 days between sampling and the daily infection per bird is p_i,

$$\begin{aligned} \hat{q}_i &= 1 - (1 - \hat{p}_i)^5 \\ \Rightarrow \hat{p}_i &= 1 - (1 - \hat{q}_i)^{1/5} \end{aligned}$$

The likelihood surfaces were characterised and solved in Matlab® (2007).

2.4 Results

2.4.1 Dust Shedding

An additional datapoint of $d(0) = 0$ is used to interpolate before the first value to fit the cubic spline. The results are shown in Figure 2.3.

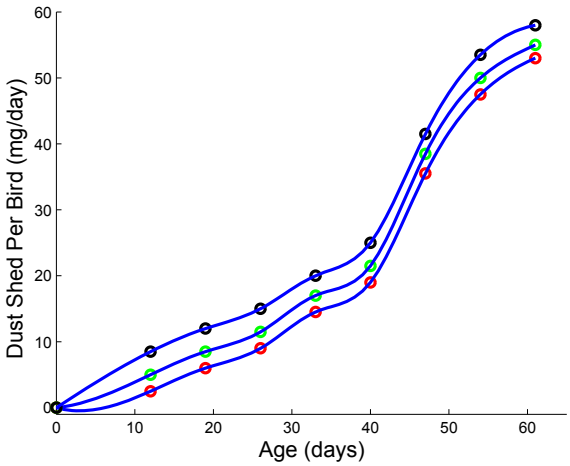
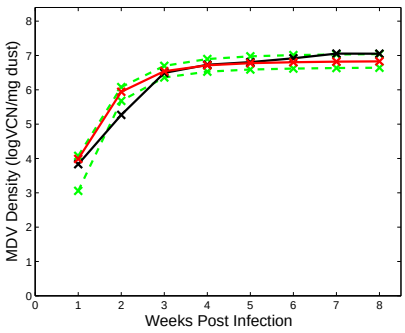


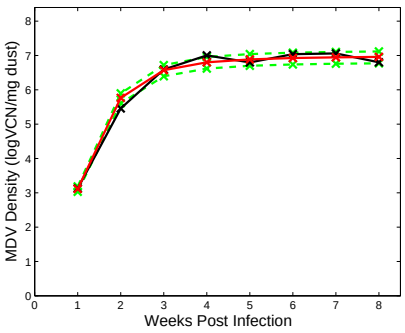
Figure 2.3: Dust Shedding: The amount of dust shed over time by a layer chicken (green, red and black circles are the mean, lower confidence interval and higher confidence interval). Lines in blue are the cubic spline fits.

2.4.2 Viral Shedding

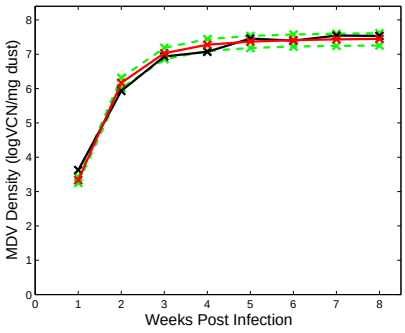
The dynamic model output records the density of virus (measured in log VCN/*mg* dust). The dynamic model is fitted to the data from each isolator independently and the model fits can be compared directly in Figures 2.4-2.6.



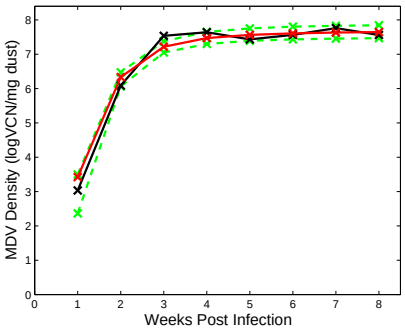
(a) $v = 16.5$, Replicate 1



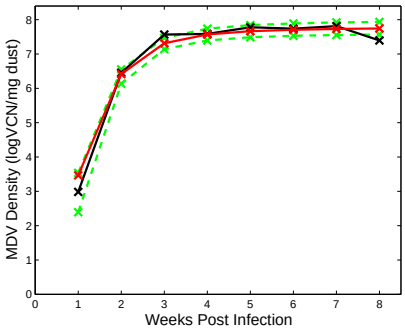
(b) $v = 16.5$, Replicate 2



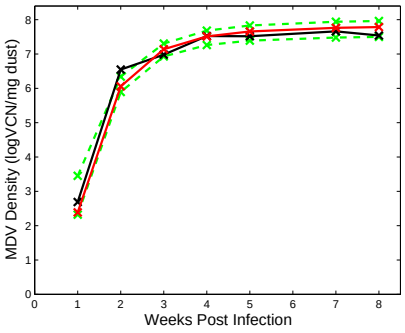
(c) $v = 36$, Replicate 1



(d) $v = 36$, Replicate 2



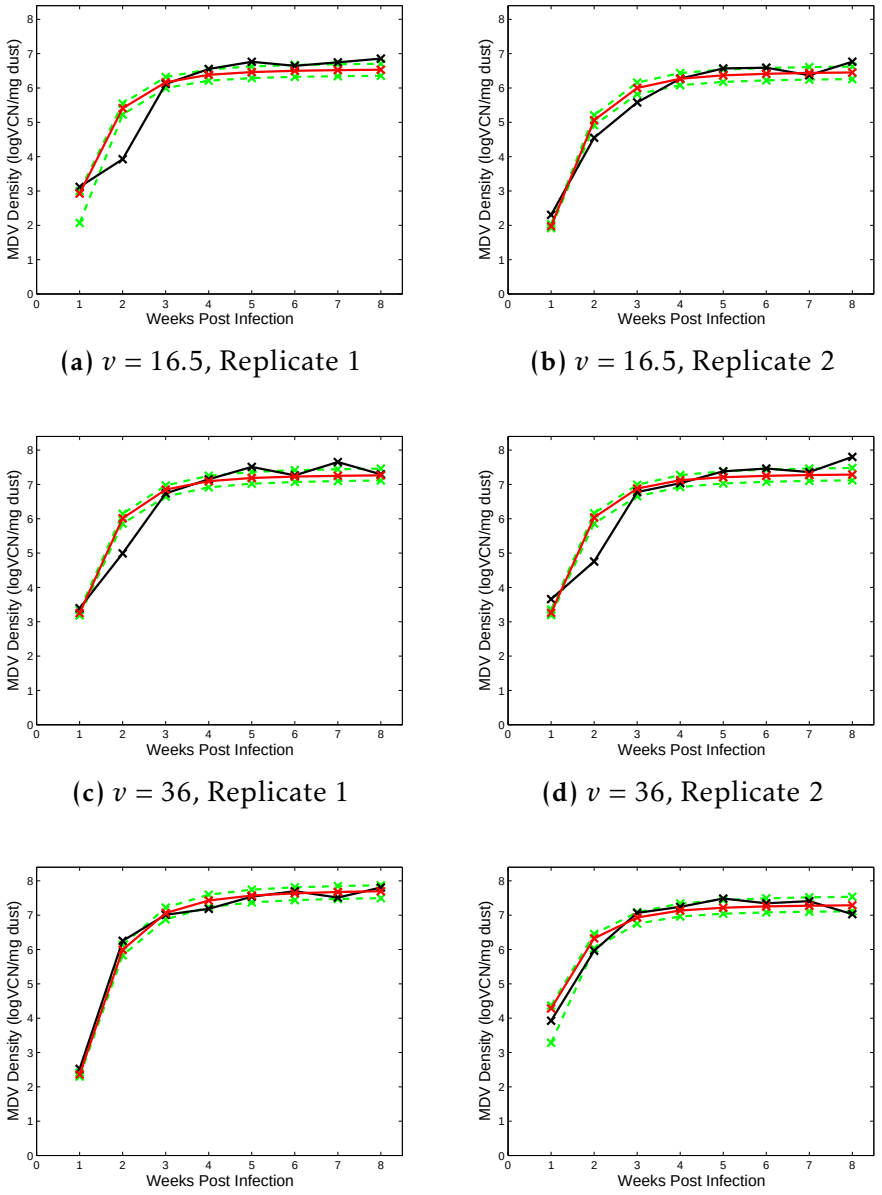
(e) $v = 46$, Replicate 1



(f) $v = 46$, Replicate 2

Figure 2.4: Viral Shedding: Sham vaccinated experiments. Graphs include the data (black), the maximum likelihood realisation (red) and the 95% credible interval (green)

For each isolator, the maximum likelihood estimate for the two parameters in



(a) $v = 16.5$, Replicate 1

(b) $v = 16.5$, Replicate 2

(c) $v = 36$, Replicate 1

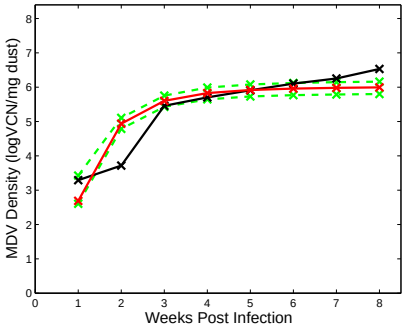
(d) $v = 36$, Replicate 2

(e) $v = 46$, Replicate 1

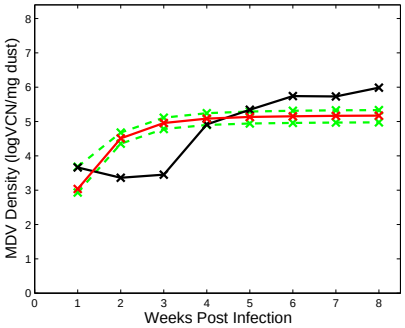
(f) $v = 46$, Replicate 2

Figure 2.5: Viral Shedding: HVT vaccinated experiments. Graphs include the data (black), the maximum likelihood realisation (red) and the 95% credible interval (green).

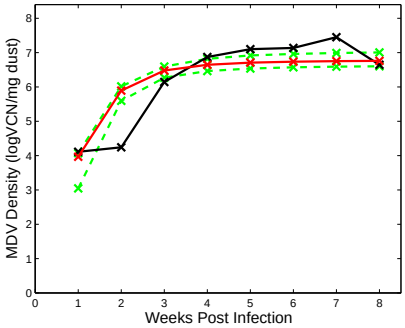
the model (latent period and rate of viral shedding) are given in Tables 2.3-2.4, where the dust shed function is the cubic spline fit to the means given in Section 2.4.1. The credible intervals in all cases are calculated by the Bayesian method as described in the Methods section. The diagnostics and posterior distributions for the Markov chains are shown in Appendix A.1.



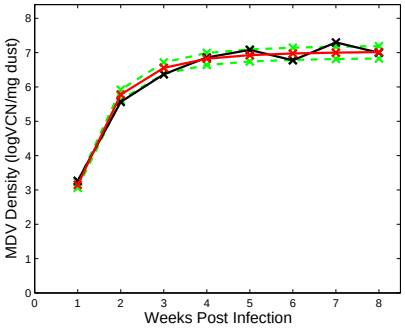
(a) $v = 16.5$, Replicate 1



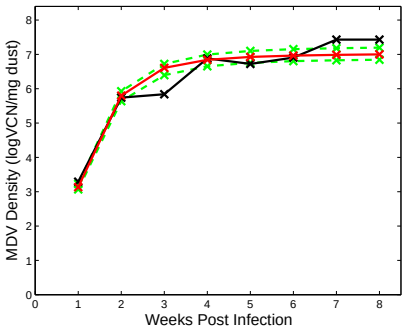
(b) $v = 16.5$, Replicate 2



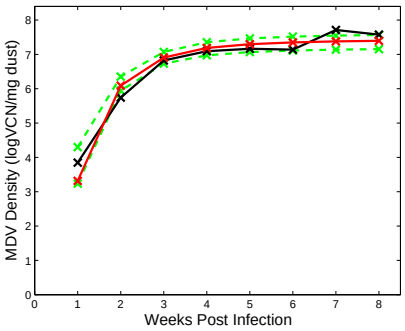
(c) $v = 36$, Replicate 1



(d) $v = 36$, Replicate 2



(e) $v = 46$, Replicate 1



(f) $v = 46$, Replicate 2

Figure 2.6: Viral Shedding: bivalent vaccinated experiments. Graphs include the data (black), the maximum likelihood realisation (red) and the 95% credible interval (green).

	Replicate 1	Replicate 2
$v = 16.5$	6.94 (6.79,7.08)	7.14 (6.99,7.29)
$v = 36$	7.64 (7.40,7.79)	7.85 (7.70,8.01)
$v = 46$	7.95 (7.80,8.11)	7.81 (7.64,7.98)

(a) log Shedding Rate, Sham

	Replicate 1	Replicate 2
$v = 16.5$	6.71 (6.56,6.87)	6.51 (6.35,6.73)
$v = 36$	7.51 (7.36,7.67)	7.53 (7.37,7.69)
$v = 46$	7.74 (7.58,7.89)	7.40 (7.25,7.54)

(b) log Shedding Rate, HVT

	Replicate 1	Replicate 2
$v = 16.5$	6.18 (5.93,6.32)	5.26 (5.09,5.41)
$v = 36$	6.91 (6.77,7.07)	7.25 (7.07,7.39)
$v = 46$	7.25 (7.09,7.40)	7.39 (7.25,7.65)

(c) log Shedding Rate, Bivalent

Table 2.3: Viral Shedding: maximum likelihood estimations of the logged viral shedding rate (logVCN/mg dust) for both replicates of the factorial study using mean value of dust profile function. The 95% credible interval is calculated via McMC, with the full posterior distributions given in Appendix A.1.

	Replicate 1	Replicate 2
$v = 16.5$	4 (4,4)	5 (5,5)
$v = 36$	5 (5,5)	5 (5,5)
$v = 46$	5 (5,5)	5 (5,5)

(a) Latent Period, Sham

	Replicate 1	Replicate 2
$v = 16.5$	5 (5,5)	5 (5,6)
$v = 36$	5 (5,5)	5 (5,5)
$v = 46$	5 (5,5)	4 (4,4)

(b) Latent Period, HVT

	Replicate 1	Replicate 2
$v = 16.5$	5 (4,5)	4 (4,4)
$v = 36$	4 (4,4)	5 (5,5)
$v = 46$	5 (5,5)	4 (4,5)

(c) Latent Period, Bivalent

Table 2.4: Viral Shedding: maximum likelihood estimations of the latent period (days) for both replicates of the factorial study using mean value of dust profile function. The 95% credible interval is calculated via McMC, with the full posterior distributions given in Appendix A.1.

Using the mean dust profile fit (shown in Figure 2.3), the estimates of the two parameters for all the experiments are shown in Table 2.3 (viral shedding rate) and Table 2.4 (latent period). The credible intervals were calculated via McMC and the diagnostics and posterior distributions are shown in Appendix A.1. The variation between replicates and between treatments is very low for the estimated latent period parameter. A linear model was fitted to try to account for this variation, with the latent period as the response variable (Table 2.5). None of the results are significant, showing that the latent period does not differ among virus strains and nor is it altered by vaccination.

	Df	Sum Sq	Mean Sq	F value	P(>F)
Treatment	1	3.3e-1	1.7e-1	4.9e-1	6.3e-1
Transformed Virulence Rank	2	3.2e-1	9.3e-1	3.2e-1	3.5e-1
Interaction	2	1.2	6.2e-1	1.8	2.1e-1
Residuals	12	4.1	3.4e-1		

Table 2.5: Viral Shedding: linear regression and resulting Anova model output with interactions for latent period and effect of Virulence Rank and vaccine treatment. Adjusted R^2=0.039, p=0.39.

An association is wanted between the Virulence Rank pathotype and the estimated viral shedding rate. As a first approach, a linear regression was applied between the arcsine square-root transformed Virulence Rank and the estimated rate of viral shedding. Results for this regression are in Tables 2.6a-2.6c with the graph displayed in Figure 2.7. All the regression lines (testing the fit between viral shedding rate as a function of Virulence Rank) for the three vaccine treatments are significantly different from zero (p=0.0068, 0.025, 0.0093 respectively).

The regression lines themselves can now be tested to find whether they are indeed significantly different from each other. Results are displayed in Table 2.7 and show that the only gradients significantly different to each other are Sham and Bivalent (p=0.0066).

In summary, increasing the Virulence Rank of the MDV isolate increases the viral shedding rate of the infected bird (measured in VCN per *mg* dust) whereas vaccination of the bird with Bivalent vaccine reduces the viral shedding. The latent period for all infected birds is 4-6 days.

	Estimate	Std. Error	t value	$P(>\|t\|)$
Intercept	-7.3e+7	2.4e+7	-3.0	3.9e-2
Transformed Virulence Rank	2.0e+8	3.9e+7	5.1	6.8e-3

(a) Unvaccinated (adjusted R^2=0.84)

	Estimate	Std. Error	t value	$P(>\|t\|)$
Intercept	-4.2e+7	2.0e+7	-2.1	1.0e-1
Transformed Virulence Rank	1.1e+8	3.2e+7	3.5	2.5e-2

(b) HVT (adjusted R^2=0.69)

	Estimate	Std. Error	t value	$P(>\|t\|)$
Intercept	-2.5e+7	8.0e+6	-3.1	3.5e-2
Transformed Virulence Rank	6.1e+7	1.3e+7	4.7	9.3e-3

(c) Bivalent (adjusted R^2=0.81)

Table 2.6: Viral Shedding: Statistics corresponding to a linear regression between the arcsine square-root transformed Virulence Rank of an isolate and the fitted shedding rate, $a(v, j)$, for each replicate. The three graphs correspond to the three vaccine treatments labelled.

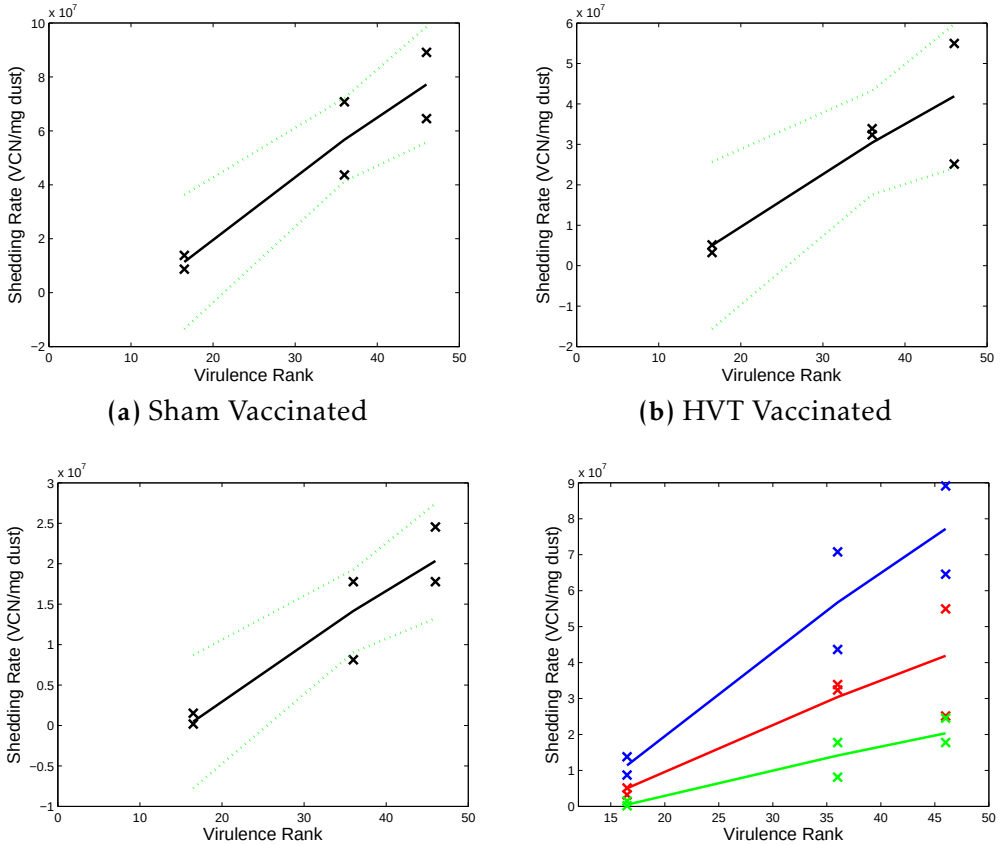
	Estimate	Std. Error	t value	$P(>\|t\|)$
Intercept	-7.3e+7	1.9e+7	-3.9	2.1e-03
Transformed Virulence Rank	2.0e+8	3.0e+7	6.7	2.4e-05
HVT	3.1e+7	2.0e+7	1.2	2.7e-1
Bivalent	4.8e+7	2.6e+7	1.8	9.6e-2
TVR:HVT	-8.9e+7	4.3e+7	-2.1	6.1e-2
TVR:Bivalent	-1.4e+8	4.3e+7	-3.3	6.6e-3

(a) Comparison with Sham regression line

	Estimate	Std. Error	t value	$P(>\|t\|)$
Intercept	-4.2e+7	1.5e+7	-2.8	2.5e-2
Transformed Virulence Rank	1.1e+8	2.5e+7	4.6	1.8e-3
Bivalent	1.7e+7	2.2e+7	8.0e-1	4.0e-1
TVR:Bivalent	-5.2e+7	3.5e+7	-1.5	1.7e-1

(b) Comparison with HVT regression line

Table 2.7: Viral Shedding: Comparison of regression lines associating the arcsine square-root transformed Virulence Rank of the strain to its shedding rate, $a(v, j)$.



(a) Sham Vaccinated (b) HVT Vaccinated

(c) Bivalent Vaccinated (d) All Treatments

Figure 2.7: Viral Shedding: Linear regression analysis of the arcsine square-root transformed Virulence Rank and the fitted shedding rate for all experiments. The 95% confidence intervals for the mean response are given by the dotted lines. All three regression lines give significant associations (see Table 2.6). The blue, red and green lines in (d) corresponds to unvaccinated birds, HVT vaccinated birds and Bivalent vaccinated birds respectively.

2.4.3 Host Mortality

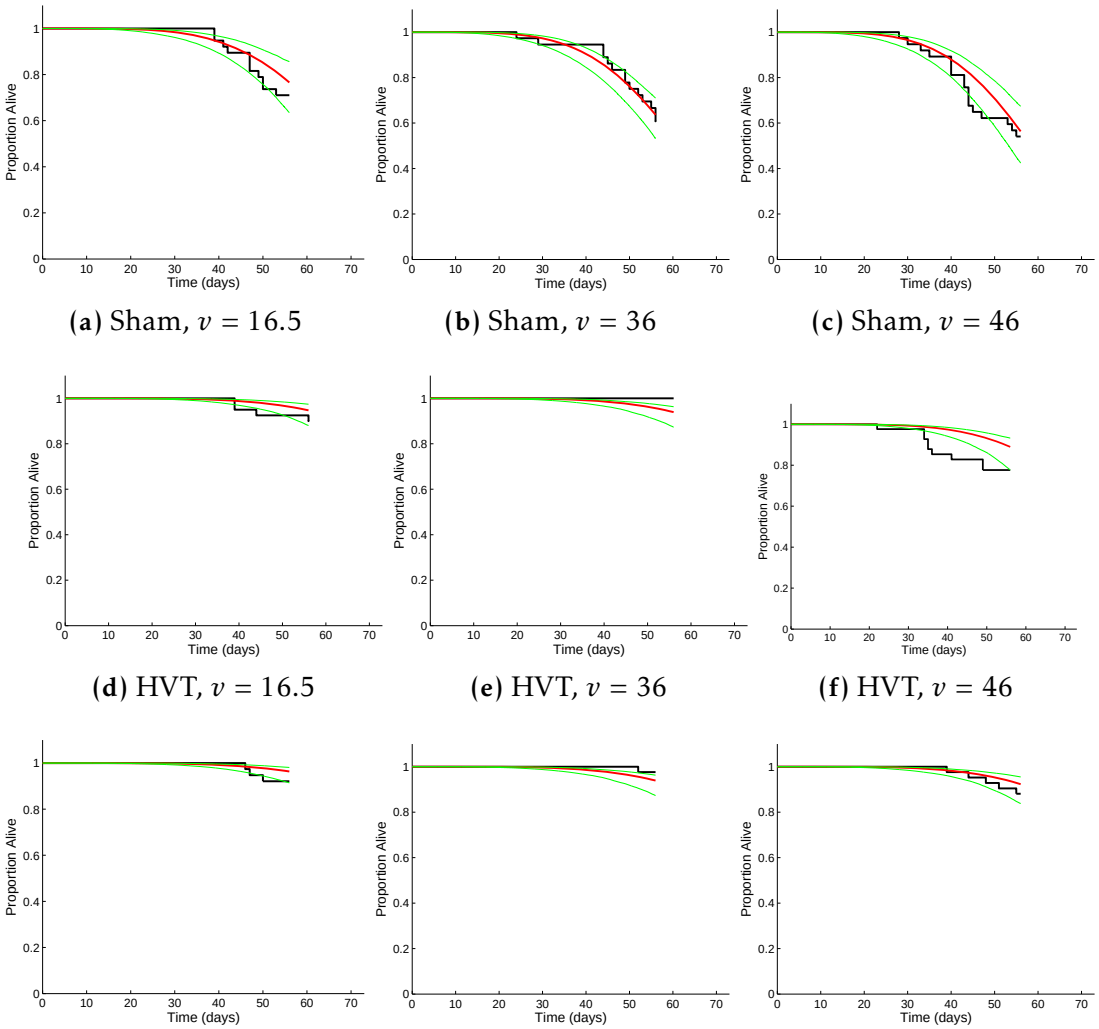
A multiplicative covariate Weibull regression model was fitted to the bird survival data, but since the interaction terms were not significant, a more parsimonious additive model was then fitted. The model estimates are given in Table 2.8. The p value for this Weibull model (with fixed shape parameter, r) is 2.8e-09 with three degrees of freedom. The resulting graphs showing the data and Weibull model fit are displayed in Figure 2.8. The model shows that host lifespan decreases with Virulence Rank and increases with vaccination (Figure 2.9 and Table 2.8), although there is no significant difference between the effect of either vaccine on lifespan (Table 2.8). The effects of vaccine and Virulence Rank on expected lifespan are seen in Figure 2.9.

Symbol	Coefficient	Value (95% CI)	z	p
r	Shape Parameter	4.1 (3.3, 5.1)	-12	4.2e-31
β_0	Intercept	4.5 (4.2, 5.0)	26	7.0e-150
β_1	Transformed Vir. Rank	-0.53 (-1.1, -0.061)	-2.0	4.1e-02
β_2	HVT vaccine	0.36 (0.21, 0.55)	4.0	7.8e-05
β_3	Bivalent vaccine	0.44 (0.27, 7.0)	4.3	1.8e-05

Table 2.8: Mortality: Regression statistics between mortality and arcsine square-root transformed Virulence Rank as a continuous covariate and vaccine treatment as a categorical covariate with no interactions. The credible intervals were calculated via McMC.

For each maximum likelihood estimate of a parameter, there is an associated standard error on that estimate. A confidence interval of the full distribution can be drawn directly from this estimate only if the covariance of each pairwise set of parameters is equal to zero. Since this is not the case, the joint distributions of the parameters were calculated via McMC. The diagnostics and posterior distributions are given in Appendix A.2.

In summary, the lifespan of infected birds was modelled with a Weibull distribution. The conclusion is twofold: that a bird infected with an isolate of higher Virulence Rank has a shorter lifespan whereas vaccination of that bird increases its lifespan significantly.



(a) Sham, $v = 16.5$ **(b)** Sham, $v = 36$ **(c)** Sham, $v = 46$

(d) HVT, $v = 16.5$ **(e)** HVT, $v = 36$ **(f)** HVT, $v = 46$

(g) Bivalent, $v = 16.5$ **(h)** Bivalent, $v = 36$ **(i)** Bivalent, $v = 46$

Figure 2.8: Host Mortality: Three different treatments with three different Virulence Ranks as stated. Graphs show data (black), maximum likelihood estimate of Weibull distribution (red) and 95% credible interval (green). Arcsine square-root ransformed Virulence Rank (continuous) and vaccine treatment (categorical) are the (additive) covariates.

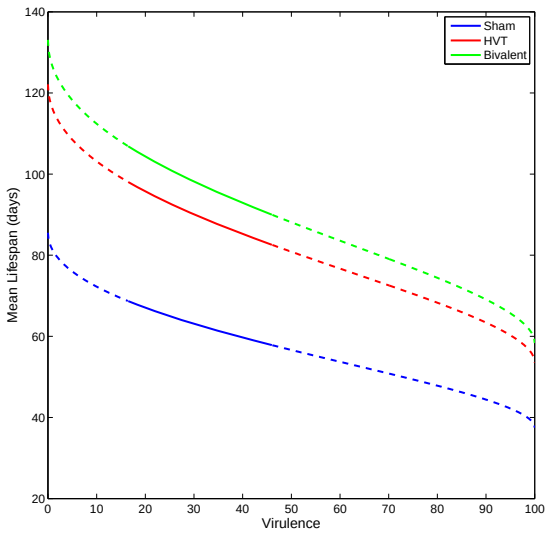


Figure 2.9: Host Mortality: Maximum likelihood estimates of λ and r give the mean of the lifespan as $\lambda\Gamma(1+\frac{1}{r})$, where $\Gamma(z) = \int_0^\infty t^{z-1}e^{-t}\,\mathrm{d}t$. The dashed lines represent extrapolation outside Virulence Ranks given in the data.

	mean	sd	MC error	Lo 95% CI	median	Hi 95% CI
r	4.159	0.46	0.062	3.34	4.1	5.1
β_0	4.5	0.18	0.0095	4.2	4.5	5.0
β_1	-0.53	0.26	0.014	-1.1	-0.51	-0.061
β_2	0.36	0.088	0.0061	0.21	0.36	0.55
β_3	0.45	0.11	0.0077	0.27	0.45	0.70

Table 2.9: Host Mortality: Results from an additive regression using McMC between the two covariates (arcsine square-root transformed Virulence Rank as a continuous covariate and vaccine treatment as a categorical covariate) and the lifespan of an infected individual. McMC diagnostics and posterior distributions are given in Appendix A.2.

	Pen Number									
	1	2	3	4	5	6	7	8	9	10
k_1	0	0	0	1	1	0	0	0	1	1
k_2	3	3	2	3	2	2	1	3	3	5
k_3	4	5	4	5	4	5	5	5	4	5
k_4	5	5	5	5	5	5	5	5	5	5
m_1	0	0	0	4	4	0	0	0	4	4
m_2	9	9	6	6	3	6	3	9	6	12
m_3	2	4	4	4	4	6	8	4	2	0
m_4	1	0	1	0	1	0	0	0	1	0
M_1	0	0	0	4	4	0	0	0	4	4
M_2	9	9	6	9	6	6	3	9	9	15
M_3	8	10	8	10	8	10	10	10	8	10
M_4	5	5	5	5	5	5	5	5	5	5
q_1	0	0	0	0.2	0.2	0	0	0	0.2	0.2
q_2	0.6	0.6	0.4	0.5	0.25	0.4	0.2	0.6	0.5	1
q_3	0.5	1	0.67	1	0.67	1	1	1	0.5	-
q_4	1	-	1	-	1	-	-	-	1	-
p_1	0	0	0	0.044	0.044	0	0	0	0.044	0.044
p_2	0.17	0.17	0.10	0.13	0.056	0.10	0.044	0.17	0.13	1
p_3	0.13	1	0.20	1	0.20	1	1	1	0.13	-
p_4	1	-	1	-	1	-	-	1	-	-

Table 2.10: Transmission: Maximum likelihood estimates for quantities from the hypergeometric distribution. For timestep i: k_i is the observed number of infected individuals; m_i is the number of newly infected individuals; M_i is the cumulative number of infected individuals; q_i is the probability per timestep of infection per bird, and p_i is the daily probability of infection per bird. The sampling was conducted in an unvaccinated population of birds where the number of newly infected individuals within a timestep is assumed to be binomially distributed.

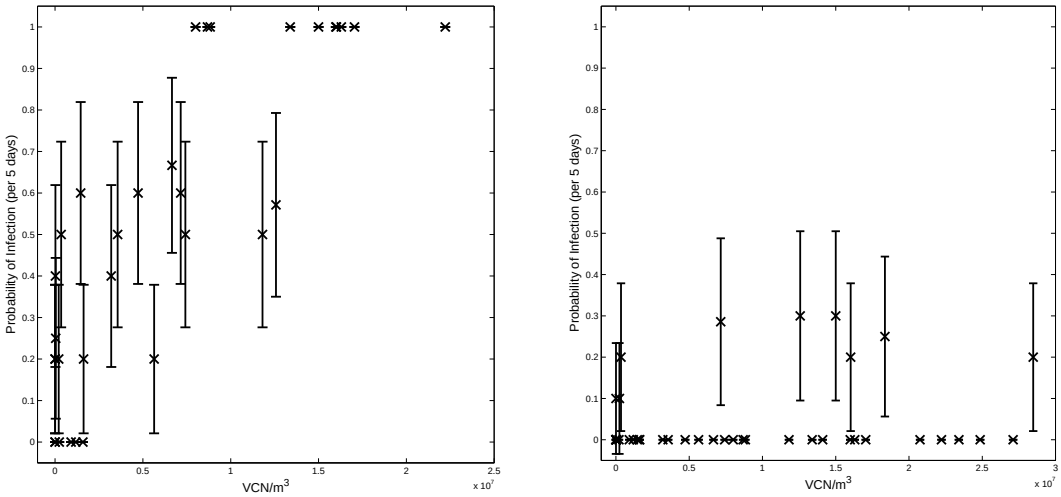
2.4.4 Transmission

The maximum likelihood estimates for the number of newly infected individuals, $\hat{m}_i$, and the daily probabilities of becoming infected, $\hat{p}_i$, can be calculated for each replicate (pen) and are given in Table 2.10 and Table 2.11 for unvaccinated and HVT vaccinated birds respectively.

The amount of virus in each pen is known at certain days, and linear interpolation estimates the average amount of virus (measured in VCN/m^3) between days 0-5, 5-10, 10-15 and 15-20. The average amount of virus in each pen and the probability of infection within that period is shown in Figures 2.10a and 2.10b. The associated probabilities per day are shown in Figure 2.11.

	Pen Number									
	1	2	3	4	5	6	7	8	9	10
k_1	1	0	0	0	0	0	0	2	0	0
k_2	1	2	0	0	0	0	0	0	1	0
k_3	0	1	0	1	2	0	2	0	1	0
k_4	0	1	0	2	1	0	1	0	1	1
m_1	2	0	0	0	0	0	0	2	0	0
m_2	0	4	0	0	0	0	0	0	3	0
m_3	0	0	0	2	3	0	3	0	0	0
m_4	0	0	0	1	0	0	0	0	0	1
M_1	2	0	0	0	0	0	0	2	0	0
M_2	1	4	0	0	0	0	0	0	3	0
M_3	0	2	0	2	3	0	3	0	2	0
M_4	0	1	0	2	1	0	1	0	1	1
q_1	0.1	0	0	0	0	0	0	0.1	0	0
q_2	0	0.29	0	0	0	0	0	0	0.2	0
q_3	0	0	0	0.2	0.3	0	0.3	0	0	0
q_4	0	0	0	0.25	0	0	0	0	0	0.2
p_1	0.021	0	0	0	0	0	0	0.021	0	0
p_2	0	0.065	0	0	0	0	0	0	0.044	0
p_3	0	0	0	0.044	0.069	0	0.069	0	0	0
p_4	0	0	0	0.056	0	0	0	0	0	0.044

Table 2.11: Transmission: Maximum likelihood estimates for quantities from the hypergeometric distribution. For timestep i: k_i is the observed number of infected individuals; m_i is the number of newly infected individuals; M_i is the cumulative number of infected individuals; q_i is the probability per timestep of infection per bird, and p_i is the daily probability of infection per bird. The sampling was conducted in a HVT vaccinated population of birds where the number of newly infected individuals within a timestep is assumed to be binomially distributed.



(a) Sham Vaccinated **(b)** HVT Vaccinated

Figure 2.10: Transmission: 5 day probabilities for infection for different atmospheric virus concentrations (measured in VCN per m^3). Error bars are twice the standard error of the estimate.

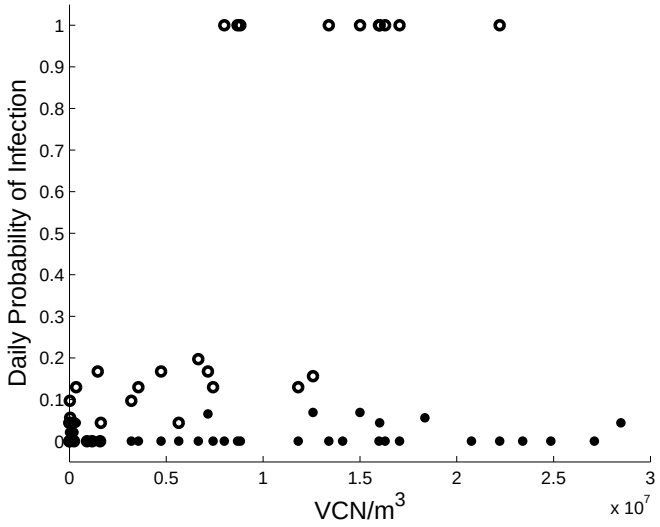


Figure 2.11: Tranmission: Daily probability of infection per bird with different vaccination treatments. The circles are the maximum likelihood point estimates for p given for the different timesteps and pens (open for unvaccinated, filled for HVT vaccinated).

2.5 Discussion

This chapter uses a mathematical approach to estimate key epidemiological parameters for MDV. All the parameters were estimated from experimental data using a range of statistical and mathematical techniques. Daily dust shedding rates were estimated for layer birds; viral shedding rates and latency times were found for a range of virus strains under different host vaccination conditions; times to death for infected hosts were calculated for different infecting strains and vaccination host status; and daily transmission probabilities to fully susceptible and vaccinated individuals were estimated.

Data for the daily amount of dust shed in *mg* per layer bird were used to fit a cubic spline to estimate the daily rate of dust shedding per bird.

Data from a single experiment were modelled to estimate the latent period and viral shedding rate of birds. The birds were infected with one of three MDV strains and inoculated with one of three treatments: sham (saline), HVT (first generation vaccine) or Bivalent (second generation vaccine). I developed a dynamic model to simulate the shedding of virus by a group of birds and used maximum likelihood techniques to estimate the two key parameters: latent period and viral shedding rate. The credible intervals for these two parameters were calculated via Bayesian techniques. The dynamic model was fitted to the virus shedding profiles assuming a fixed delay to viral shedding and rate of viral shedding for each group of chickens sharing an isolator (and infected with the same vaccine strain and MDV isolate). The delay from infection to viral shedding (latent period) does not change significantly with vaccine nor infecting isolate (Table 2.5). An increase in Virulence Rank (again, as defined by Witter (1997) and Witter et al. (2005)) correlates significantly with an increase in viral shedding (Figure 2.7).

A sensitivity analysis varied the amount of dust shed by each bird based on the spline fitting done in Section 2.4.1. In the main analysis, the mean dust shed by each bird was used. The parameters were re-estimated using the low and high confidence interval values of dust but this does not affect the results in any significant way.

Overall, the model tends to overestimate the density of MDV in dust in week two. This may be because the viral shedding rate increases as infection progresses, contrary to our assumption that the shedding rate is constant. Fitting a model with a further two parameters characterised by a delay to a higher viral shedding rate would improve the fit of the model. However, this would drastically increase the complexity of the problem, with a more intractable likelihood surface and more difficult interpretation of the fitted parameter set. Given the low sensitivity of the results to the amount of dust produced by each bird, the simpler model would seem the best for understanding the biological interpretation the effect of Virulence Rank and vaccination on the shedding of virus strains.

Vaccination is thought to reduce the MDV load in an infected host (Baigent and Davison, 2004), which is backed up by other empirical studies (e.g. Islam et al., 2007, 2008) and the results presented here do not contradict this. My estimate of the latent period is much shorter than earlier estimates of around 13 dpi (Baigent and Davison, 2004) but only slightly shorter than more recent estimates based on new PCR techniques which have detected significant quantities of virus in feather tips on 7 dpi (Baigent et al., 2005) (viral shedding is estimated in this work to begin between 5 and 7 dpi (the day after the latent period ends)).

There have been efforts to find other correlates with virulence of MDV isolates, most notably with viral load (the density of virus within bird tissue). This has been achieved by cell culture techniques (e.g. Calnek and Witter, 1986) and intra-cellular detection during the first 10 days of infection (Yunis et al., 2004). However, the relationship between viral load (or replication) and Virulence Rank had not been convincingly tested (Witter et al., 2005). Current PCR methods enable viral loads in shed dust to be directly measured (Baigent et al., 2005; Bumstead et al., 1997; Burgess and Davison, 1999; Islam et al., 2006; Reddy et al., 2000; Yunis et al., 2004) and this enabled a more quantitative analysis as used in this chapter.

Data from the same experiment (as discussed previously in the context of viral shedding) was modelled to estimate the host lifespan. The birds were infected with one of three MDV strains and inoculated with one of three treatments: sham (saline), HVT (first generation vaccine) or Bivalent (second generation

vaccine). Survival analysis, with a Weibull mortality function as a statistical model, was used to describe the lifespan of birds infected with MDV. The relative impact of Virulence Rank and vaccine covariates were compared by calculating credible intervals again using Bayesian methods.

Applying these statistical methods to the data for three strains, it was found that increasing Virulence Rank of a virus strain decreases the lifespan of the host (Figure 2.9). Vaccinating the host with either the first or second generation vaccine increases its lifespan, but neither one to any significantly greater degree (Table 2.8). For example, a Virulence Rank of 20 produced a host lifespan of 67 days for unvaccinated birds compared with 96 and 104 days for HVT and Bivalent vaccinated birds respectively.

The value of $r = 4.09$, for the shape parameter implies there is an increasing death rate with age.

Analysis of transmission experiments allowed a formal estimation of daily transmission rate of MDV from infectious individuals to susceptible, unvaccinated or HVT vaccinated birds. Maximum likelihood methods were used to estimate the number of newly infected individuals within each time frame, and binomial theory allowed conversion of these estimates to daily probability values. The daily probability of transmission had large variability, especially for small quantities of dust in the unvaccinated case and a precise model fit was not analysed here. It will be revisited in the context of later chapters.

However, it was clear that vaccination can protect a bird from initial infection. This result is not recognised in the literature on Marek's disease, which usually focuses on a vaccine's ability to prevent clinical signs or death in an infected individual (Baigent and Davison, 2004). However, the way the vaccine protects against a disease dictates the direction and force of selection applied on the pathogen, as demonstrated by Gandon et al. (2001). Providing no protection from initial infection but serving only to reduce the clinical signs of a disease (albeit with the ongoing transmission to other individuals reduced), can drive virulence to higher levels very rapidly. On the other hand, vaccines which provide protection from initial infection from disease can drive virulence to lower levels. This result perhaps suggests that there may be other factors at play and that vaccination may not be single-handedly governing the drive to more virulent viruses, since initial infection by MDV is

reduced by vaccination.

The results described here are qualitatively in line with current thinking on MDV pathogenesis, but the new methods presented allow a formal quantitative comparison of strains in an epidemiological meaningful way. It is anticipated that this analysis will allow a more quantitative understanding of the aetiology of MDV and the mechanisms governing a functional rise in the virulence of a strain. With this analysis is it hoped that a more epidemiologically relevant characterisation of MDV strains will emerge which will eventually provide not only insight into the reasons for evolution to highly virulent strains but also a metric to evaluate future risks and the efficacy of possible control strategies.

The pathotyping of isolates pioneered by Witter (1997) and Witter et al. (2005) is a useful tool for comparing the pathogenicity of strains with respect to others in vaccinated birds. However several of the more recently isolated, highly virulent strains have ranks above 80, in some cases 90. Should more virulent strains emerge, it will be difficult to categorise them via this method, as the scale is truncated at 100. The method of fitting a Weibull distribution proposed in this chapter allows for a greater flexibility of pathotyping, in that an isolate may be categorised according to its mean time to kill the host. The subsequent effect of vaccination of the host on this metric can then be established. Biologically, it may seem more logical to pathotype according to the lifespan of an unvaccinated bird, but experimentally it may also be a solution to the costly procedure of having two groups of birds, each vaccinated with a different treatment. Even a simple 'time to death' metric can be estimated using relatively few birds, as was the case for the myxoma virus in rabbits in Australia. Myxoma strains were pathotyped effectively when the case fatality rate proved experimentally intensive and ineffective in discriminating between similarly virulent strains (Fenner and Fantini, 1999).

Furthermore, the approach adopted by Witter implies that the change in pathotype is a form of 'vaccine break' where the resistant strains are less pathological in vaccinated hosts than the wild-type (non vaccine-resistant) strains. This is in contrast to evolutionary biologists' accepted definition involving naïve hosts, such that a more virulent infecting strain causes more morbidity or mortality in an unvaccinated host. To understand MDV viru-

lence evolution it may be necessary to adopt an evolutionary approach to the notion of MDV virulence.

Many of the evolutionary arguments for virulence evolution are based on the trade-off hypothesis, which has been mentioned in Section 1.2.3.1 and will be returned to in Chapter 3. Before a more confident assertion can be made, it may be necessary to expand the analysis to include more strains of higher Virulence Rank. Nonetheless, there are sufficient clues to suggest that MDV may well show a trade-off between virulence and transmissibility and that an optimum virulence could be selected for which maximises the potential for survival based on its viral shedding rates and host lifespan.

3

Evolution of MDV

3.1 Introduction

There is evidence that Marek's Disease Virus (MDV) has been evolving to higher virulence over the past sixty years (Witter, 1997, 1998; Nair, 2005). The aim of this chapter is to test hypotheses for the causes of virulence evolution of MDV in broiler chickens. These hypotheses have been discussed by authors including Morrow and Fehler (2004), Davison and Nair (2005), Nair (2005) and Gimeno (2008) and include:

- introduction of vaccination;
- broiler lifespan reduction;
- increase in size of chicken flock population and stocking density of birds.

These hypotheses are examined in this chapter for their feasibility of driving evolution of MDV virulence. The hypotheses above for the continued evolution of MDV virulence can all be explained within the context of the Trade-Off Hypothesis (discussed in Section 1.2.3.1). The Trade-Off hypothesis states that virulence (here defined as the ability for the pathogen to cause mortality in its host) is positively associated with its ability to transmit infection to a new host. This hypothesis has been shown to be consistent with only a few systems where a functional form has been found (Mxyomatosis in European rabbits (Dwyer et al., 1990), *Plasmodium chabaudi* in mice (Mackinnon and Read, 1999), *Plasmodium gallinaceum* in chickens (Paul et al., 2004), *Pasteuria ramosa* in *Daphnia*

magna (Jensen et al., 2006), HIV in humans (Fraser et al., 2007) and *Ophryocystis elektroscirrha* in monarch butterflies (de Roode et al., 2008)). However, as Chapter 2 showed, there is reasonable evidence that MDV does indeed exhibit a virulence-transmission trade-off which might suggest that the hypotheses for virulence evolution of MDV are consistent with the epidemiology and life history of the virus.

It may be helpful to reiterate the arguments for why these changes to broiler farming may have promoted higher virulence in MDV strains (as discussed in Section 1.2.2.1), and this is done in Box 3.1.

Box 3.1: Arguments for MDV Virulence Selection

Using the assumption that there is a trade-off between transmission and host death the possible reasons for MDV virulence evolution are as follows:

Vaccination: This can select for increased virulence since infection will still occur, but the fitness costs (i.e. host death) of the infection will be greatly reduced. The parasite can then become more virulent (and reap the increased transmission) without incurring such a great disadvantage by killing its host.

Reduced Cohort Duration: A more virulent pathogen will have a greater transmission rate for the lifespan of the host than a less virulent pathogen will. However the more virulent pathogen does not have a decreased fitness cost of host mortality since the host lifespan will be shorter for both pathogens (assuming the host lifespan due to both pathogens is greater than the length of the cohort). This argument implies that virulence will be greater in shorter-lived hosts.

Increased Size of Cohort: If a host population is much larger and kept in higher densities, this may give rise to evolution towards a higher virulence, since there are more susceptible birds to infect in the population and there is less chance that a parasite could run out of hosts and consequently be driven to extinction by being too deadly.

However, the evolutionary constraints and pressures on such a managed cohort system may be vastly different to a wild population, for which these arguments are generally based. This analysis incorporates the life history of the virus (examined in Chapter 2) with the demographic-specific parameters for a

broiler system in order to directly test the ability of industry changes to have driven virulence evolution over the past sixty years.

To examine drivers for evolution it is necessary to define functions to compare the relative success of virus strains in a given environment. The success of an individual genotype in its environment is known as its 'fitness' (Harvey, 1994). Defining the fitness of an individual organism can be a difficult task and many authors use different and sometimes conflicting definitions (Dawkins, 1982; Henle, 1991). It is therefore imperative to be explicit about what one means when the term 'fitness' is used and to explain why it is a good metric for evolutionary study (Mylius and Diekmann, 1995; Dieckmann, 2002). This chapter employs three different measures of fitness:

- The maximum amount of virus produced by a single infected bird over its lifespan (which is analagous to LTS (Lifetime Transmission Success), described in Jensen et al. (2006), which measures the transmission potential of the parasite over the lifetime of its host);
- The basic reproductive number, R_0, of the virus strain (the multiplicative population growth rate in discrete population generations (Mylius and Diekmann, 1995));
- The rate of infection of susceptible birds by one infected bird (which is similar to the Malthusian parameter for exponential growth in continuous time (Mylius and Diekmann, 1995)).

The first measure has been used by experimentalists as stated above. The last two have been used to define fitness in previous studies in relation to parasite fitness and are described in numerous studies (e.g. Roughgarden, 1979; Yodzis, 1989).

It is also worth pointing out that the fitness of an individual strain of MDV refers solely to the fitness given the particular *virulence* of that strain (defined in Chapter 2 as the mean time to cause the death of its host). The virulence of a strain of MDV is assumed to be a heritable trait. Implicit in this assumption is that viral phenotype has a one-to-one mapping with its viral genotype. Therefore the 'fitness of the virulence genes' is interchangable with the 'fitness of the individual MDV strain' in this chapter. Also the 'virulence' of a virus strain is used interchangably with 'Virulence Rank' as defined in Box 2.1.

Floor-reared broiler birds are raised from just after hatch to slaughter with birds of the same age. Once the whole cohort are at a finishing weight (after what is defined as the 'cohort duration') they are all removed, whereupon the barn is cleaned pending a new cohort arrival. This artificially age-structured population changes the quantitative fitness definitions from those used for natural populations. The aim of the chapter is to identify fitness definitions which have been used in other studies and, having adapted them for their use for MDV, elucidate the reasons for MDV virulence evolution with these measures.

3.2 Methods

All the notation for this chapter is found in Box 3.2 while the mathematical equations and parameter values are in Box 3.3 at the end of the Methods section.

3.2.1 Infectiousness Potential, W

The maximum amount of an MDV strain that can be shed by a single bird over its lifetime is defined as its infectiousness potential, W. During the cohort duration, a bird can be removed from the population by natural mortality (any cause of death other than MDV-related), by MDV-related death and by final removal from the cohort (along with the other birds at the end of day number T_c). The lifespan and viral shedding of an individual depend on the infecting virus strain virulence, v, and the vaccine status, j, of an individual (see Sections 2.4.2 and 2.4.3). A bird starts shedding virus after infection at time $T_s + 1$ (after the latent period) and does so until the end of the cohort duration at T_c, unless it dies and is removed from the population. The amount of virus shed on day t by a single bird is defined as $m(t, T_c, v, j)$ and the probability that the infected bird is alive at time t is $L(t|v, j)$, given the bird is infected with a strain of virulence v and has vaccination status j.

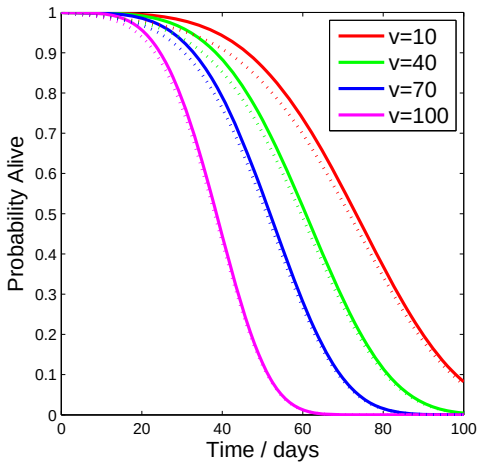


Figure 3.1: The probability of survival until at least day t for an individual infected with a strain of virulence v. The solid and dotted lines correspond to daily background mortality probabilities of 0.0001 and 0.001 respectively. The function is a compound of Weibull (MDV-induced death) and Binomial (non-MDV-induced death) survival curves. The Weibull distribution is given by $f(t|v,j) = (r/\lambda)(t/\lambda)^{r-1}e^{-(t/\lambda)^r}$ where both r and λ have been fitted to the survivorship curves analysed in Chapter 2.

3.2.1.1 Lifespan of an Individual

The probability that a bird survives until t days in the cohort is the probability of not dying from either MDV or background mortality until that time. The daily background mortality probability per bird is denoted μ. The probability of a bird lifespan being t days (with death due to MDV) is $f(t|v,j)$, given infecting virus virulence, v and vaccination status j. Therefore the probability of dying from MDV on or before time t is $P(T \leq t) = F(t|v,j) = \sum_{T=1}^{t} f(T,v,j)$. The probability of survival until t days within the cohort is therefore

$$L(t|v,j) = (1-\mu)^{t-1}(1-F(t-1|v,j))$$

A graph showing the dependency of L on v is shown in Figure 3.1.

3.2.1.2 Dust Shedding

The amount of dust shed daily by a broiler bird of age t days in mg is calculated in Appendix B, and is found to be

$$d(t, T_c) = 368 \exp(-P(T_c)/t^{1.64}) + 10.8$$

where P is a function of the duration of the cohort, T_c. A value for $P(45)$ is found in the calculation (see Appendix B) since the slaughter time for the birds in the experiment would usually be 45 days (S.W. Walkden-Brown, Personal Communication, May 2008). If the cohort duration changes, the amounts of dust shed by birds will also change, since the birds grow at a different rate to reach the desired finishing weight at the end of a cohort duration. Therefore when a different cohort duration is used, $P(T_c)$ is reestimated. For example $P(45)$=326 gives $d(45, 45)$=206, so $P(70)$, for example, can be estimated by solving $206 = 368 \exp(-P(70)/70^{1.64}) + 10.8$. This then gives the new $d(t, T_c)$ which can be used for estimating the quantity of dust produced by a broiler on day t when in a cohort of duration 70 days. A graph depicting $d(t, T_c)$ is displayed in Figure 3.2 for birds bred to live through different cohort durations.

3.2.1.3 Total Viral Shedding

Since the amount of virus is related to the mass of the dust shed, the virus shed will also be a function of time. The virus shed (VCN) per mg of dust is given by $a(v, j)$ and therefore the daily amount of virus (measured in VCN) is given by

$$m(t, T_c, v, j) = d(t, T_c)a(v, j)$$

3.2.1.4 Formulating W

Combining the two results above, the expectation of the mean quantity of virus produced in a bird's lifetime (given infection at $t = 0$, infecting strain virulence v and vaccine status j) is therefore

$$W(T_c, v, j) = \sum_{t=0}^{T_c} L(t|, v, j)m(t, T_c, v, j)$$

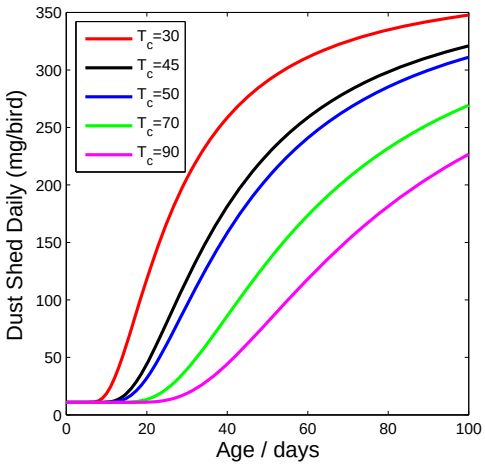


Figure 3.2: The amount of dust shed by a bird at different ages given that it resides in a cohort of duration T_c. The black line corresponds to the initial fitted line for a cohort duration of 45 days.

Since $m(t, T_c, v, j)$ is zero when the bird is not shedding virus in its latent period (i.e. when $t \leq T_s$, where T_s is the latent period), this leads to the following equation:

$$W(T_c, v, j) = a(v, j) \sum_{t=T_s+1}^{T_c} (1-\mu)^{t-1}(1 - F(t-1|v, j))d(t, T_c)$$

3.2.2 Basic Reproductive Number, R_0

The calculation of W, the maximal expected amount of virus produced by a single bird over the course of its life, did not take into account the transmission potential of that virus, and hence the expected number of chickens infected by this virus material. By using the transmission parameters gleaned from the experimental data, the key epidemiological parameter R_0 can be calculated for each strain under a different set of environmental conditions. R_0 is the number of individuals that are directly infected by a single infected bird in an otherwise susceptible population. Transmission of virus between birds is indirect through the dust.

3.2.2.1 Reduction in Transmissible Virus

In floor-reared broiler barns, there is thought to be an equilibrium of aerial contaminants brought about by the continued production and removal of dust and airborne material (Wathes, 1994). There is a growing realisation that constant exposure to high levels of contaminants in the dust (such as ammonia) harms not only the livestock, but also the workers on the establishments (Wathes, 1998) and there have been implementations of Optional Exposure Limits (OELs) in North America and Europe to reduce dust pollutants to a reasonable level within broiler barns (Donham et al., 2000). Studies around Northern Europe have revealed the density of inhalable dust in broiler buildings (sampled at around twenty eight days into the cohort duration) (Takai et al., 1998). The mean inhalable dust ranged from 3.8-10.4mg/m^3. Therefore in this model of a broiler farm, the density of dust is assumed to stay constant once this limit has been reached. For the purposes of calculation, the new dust (and virus) is assumed to be produced at the start of each day, is thoroughly mixed with the old dust (and virus) and a proportion removed to regain the equilibrium level of dust, before any new infection takes place that day.

If the limit for the density of dust in the barn is set to E (mg/m^3), the fraction of remaining dust at each time point t is therefore

$$\gamma(t, T_c, s_d) = \min\left[\frac{EV(S_0, s_d)}{\min[\sum_{s=1}^{t-1} S_0 d(s, T_c), EV(S_0, s_d)] + S_0 d(t, T_c)}, 1\right]$$

where the volume, V, of the barn can be calculated by the initial number of birds, S_0, and the stocking density, s_d (kg/m^2) given the finishing weight, w (kg), is fixed for all calculations. The equilibrium value of dust can vary between farm to farm, however in the study done by Takai et al. (1998) values for Denmark, England, Germany and the Netherlands found averages of 3.8, 9.9, 4.5 and 10.4 mg/m^3, giving an mean value of 7.15mg/m^3. This value was used for the concentration limit of dust in the atmosphere, E.

Therefore the *effective* amount of virus produced by a single bird, $M_e(t, T_c, v, j)$(VCN) in the atmosphere at a given time t when $m(t, T_c, v, j)$ virus is released into the atmosphere by a single bird at time t is therefore

$$M_e(t, T_c, v, j) = \gamma(t, T_c, s_d)[M_e(t-1, T_c, v, j) + m(t, T_c, v, j)].$$

3.2.2.2 Calculation of Basic Reproductive Number

Most calculations of R_0 are derived from assumed infinite (and continuous) populations occurring over continuous time and described using differential equations. However this system requires an individual-based approach with R_0 being calculated in a more heuristic way. Indeed Keeling and Grenfell (2000) describe the differences in the formulation of R_0 under these two different systems and give a method for the calculation under the individual-based setting. Keeling and Grenfell (2000) is therefore the motivation behind the following formulation:

$$R_0(T_c, v, j, s_d) = \sum_{t=T_s+1}^{T_c} S(t) p\left(\frac{M_e(t, T_c, v, j)}{V(S_0, s_d)}, j\right) L(t|v, j)$$

where p is the daily probability of transmission to a single uninfected bird. Assuming that the number of susceptibles stays approximately constant throughout the cohort duration, $S(t) = S_0$ (i.e. that the number of infecteds remains small compared to the cohort size)

$$R_0(T_c, v, j, s_d) = S_0 \sum_{t=T_s+1}^{T_c} p(\frac{M_e(t, T_c, v, j))}{V(S_0, s_d)}, j) L(t|v, j)$$

where R_0 is the summation over all infectious days, of the probability that a bird is alive on that day times the expected number of susceptibles that will get infected (Keeling and Grenfell, 2000).

R_0 is dependent upon the vaccination status of a bird in the above formulation and although vaccination is technically a control measure, this chapter sticks to denoting the reproductive number, R_0, under vaccination (compared with R_u which is sometimes used (Porco et al., 2005)).

3.2.3 Rate of Infection of Individuals

The number of infected individuals caused by a single bird at time t follows simply from the definition of R_0 and may be written

$$R(t, T_c, v, j, s_d) = S_0 \sum_{s=T_s+1}^{t} p(\frac{M_e(s, T_c, v, j))}{V(S_0, s_d)}, j) L(s|v, j)$$

Box 3.2: Glossary of Definitions

$a(v,j)$	Amount of virus present (VCN per mg dust) given strain virulence v and vaccine status j
E	Maximum Dust Concentration (mg/m^3)
$d(t,T_c)$	Dust shed by one bird on day t in a T_c day cohort
$f(t\|v,j)$	Lifespan (days) probability distribution due to MDV infection given strain virulence v and vaccine status j
$F(t\|v,j)$	Lifespan (days) cumulative distribution due to MDV infection given strain virulence v and vaccine status j
D_{total}	Total death % in cohort
h	Height of barn (m)
j	Vaccination status (Sham, HVT or Bivalent)
$L(t\|v,j)$	Survival duration (days) cumulative distribution function given strain virulence v and vaccine status j
$m(t,T_c,v,j)$	Virus produced by a bird infection on day t(VCN) given strain virulence v and vaccine status j
$M_e(t,T_c,v,j)$	Cumulative virus produced by a bird remaining in the barn by day t (VCN) given strain virulence v and vaccine status j
$p\left(\frac{M_e(t,T_c,v,j)}{V(S_0,s_d)},j\right)$	Probability of transmission to a single individual on day t
r	Shape parameter for Weibull distribution of host lifespan
$R_0(T_c,v,j,s_d)$	Basic reproductive number
s_d	Stocking density of birds (kg/m^2)
S_0	Initial number of susceptible individuals in cohort
$S(t)$	Number of susceptible individuals on day t
T_c	Cohort duration (days)
T_s	Latent period of virus (days)
v	Virulence Rank of MDV strain
v_T	Transformed Virulence Rank of MDV strain
$V(S_0,s_d)$	Volume of the barn (m^3)
w	Final weight of broiler bird (kg)
$W(T_c,v,j)$	Expected maximum virus production in one bird's lifetime (VCN) given strain virulence v and vaccine status j
$\alpha(j)$	Gradient of linear regression in transmission rate estimation
$\gamma(t,T_c,s_d)$	Fraction of dust remaining in barn after extraction on day t
$\lambda(v,j)$	Scale parameter for Weibull distribution of host lifespan
μ	Daily background mortality probability per bird

Box 3.3: Glossary of Parameter Values

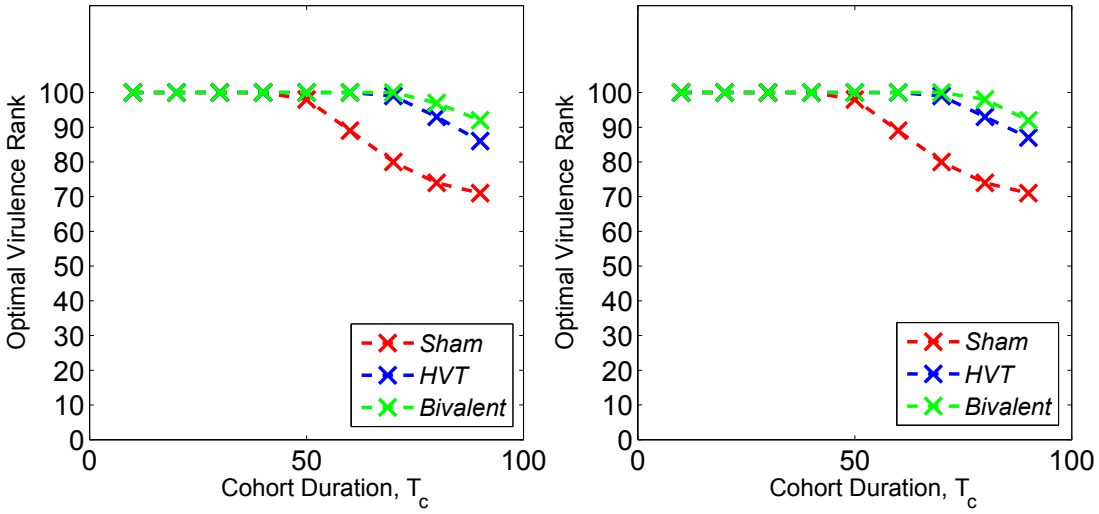
$$
\begin{aligned}
a(v,\text{sham}) &= -7.27\text{x}10^7 + 2.01\text{x}10^8 v_T \text{ (Chapter 2)} \\
a(v,\text{hvt}) &= -4.21\text{x}10^7 + 1.13\text{x}10^7 v_T \text{ (Chapter 2)} \\
a(v,\text{biv}) &= -2.49\text{x}10^7 + 6.07\text{x}10^7_T \text{ (Chapter 2)} \\
E &= 7.15 mg/m^3 \text{ (Takai et al., 1998)} \\
d(t,T_c) &= 368\exp(-P(T_c)/t^{1.64}) + 10.8 \text{ (Appendix B)} \\
D_{total} &= 3.6 - 6.8\% \text{ (Sheppard, 2004)} \\
F(v,t,j) &= 1 - exp(-(t/\lambda(v,j))^r) \\
h &= 2.5m \text{ (S.W. Walkden-Brown, } \textit{pers. comm.}\text{, 2008)} \\
L(t|v,j) &= (1-\mu)^{t-1}(1-F(v,t-1,j)) \\
m(t,T_c,v,j) &= d(t,T_c)a(v,j) \\
p\left(\frac{M_e(t,T_c,v,j)}{V(S_0,s_d)},j\right) &= \alpha(j)\frac{M_e(t,T_c,v,j)}{V(S_0,s_d)} \\
P(T_c) &= -T_c^{1.64}\ln\left(\frac{d(45,45)-10.8}{368}\right) \\
r &= 4.09 \text{ (Chapter 2)} \\
s_d &= 5,\ 20,\ 35 kg/m^2 \text{ (N. Sparks, } \textit{pers. comm.}\text{, 2008)} \\
S_0 &= 500,\ 5{,}000,\ 30{,}000 \text{ Birds (N. Sparks, } \textit{pers. comm.}\text{, 2008)} \\
T_c &= 30,\ 50,\ 70,\ 90 \text{ days (Sheppard, 2004)} \\
T_s &= 4 \text{ days (Chapter 2)} \\
v_T &= \arcsin\sqrt{0.01v} \\
V(S_0,s_d) &= S_0 wh/s_d \\
\alpha(\text{sham}) &= \text{8.97e-9 (probability of infection per bird per day per VCN}/m^3) \\
\alpha(\text{hvt}) &= \text{1.47e-9 (probability of infection per bird per day per VCN}/m^3)) \\
\lambda(v,\text{sham}) &= 4.541 - 0.525 v_T \text{ (Chapter 2)} \\
\lambda(v,\text{hvt}) &= 4.541 - 0.525 v_T + 0.356 \text{ (Chapter 2)} \\
\lambda(v,\text{biv}) &= 4.541 - 0.525 v_T + 0.442 \text{ (Chapter 2)} \\
\mu &= 1 - \sqrt[T_c]{1 - D_{total}}
\end{aligned}
$$

3.3 Results

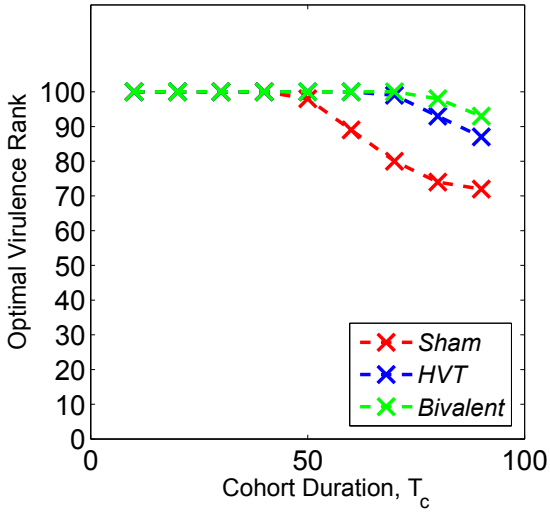
3.3.1 Infectiousness Potential, W

Figure 3.3 shows the Virulence Rank at which the maximum W is reached, for different values of T_c, μ and j. For unvaccinated populations, decreasing the cohort duration, T_c, serves to increase the Virulence Rank at which there exists a fitness peak. In HVT vaccinated populations, the same trend is apparent, although only for longer cohort durations (since at shorter cohort durations, the maximum Virulence Rank is reached). For Bivalent vaccinated populations, there is no change in the location of the fitness peak, since the maximum Virulence Rank is selected for in all cases. Thus, introducing a first generation vaccine and a second generation vaccine both serve to increase the Virulence Rank at which there exists a fitness peak, unless the cohort duration is small enough such that the maximum fitness has already been reached. The background mortality has little effect on the fittest Virulence Rank. The fittest Virulence Rank is made ever so slightly higher in the presence of a greater background mortality (when the maximum virulence has not been reached).

In summary, this fitness measure is defined as the total infectious material produced over the course of the cohort duration by a single infected bird. Optimising this fitness measure locates the fittest virulence. A reduction in cohort duration and vaccination (or vaccination with a more recent vaccine) both serve to select for higher virulence, unless the maximum virulence has already been reached.



(a) Background Mortality, $\mu = 0.0001$ **(b)** Background Mortality, $\mu = 0.0005$



(c) Background Mortality, $\mu = 0.001$

Figure 3.3: W: Optimal virulence (the virulence at which W is largest) for different cohort durations (measured in days) with different vaccines and background mortalities.

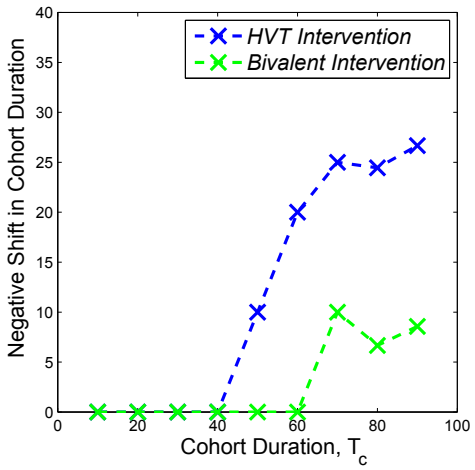


Figure 3.4: *W*: Impact of moving vaccine status from unvaccinated to HVT (blue line) and from HVT to Bivalent (green line), measured in terms of the reduction in cohort duration (days) to give the same virulence shift.

3.3.1.1 Relative Importance of Industry Changes

The optimal virulence in an environment is influenced by both cohort duration and vaccine status of the hosts. To distinguish between these two effects, the relative impact of vaccination can be viewed in terms of how much the cohort duration would have to be decreased for the shift in optimal Virulence Rank to be the same. A plot of the impact of vaccination *relative* to the alternative reduction in cohort duration is given in Figure 3.4. The blue and green lines correspond to the impact of moving from an unvaccinated population to an HVT vaccinated population and from an HVT vaccinated population to a Bivalent one respectively. Since cohort durations have reduced from around 70 days to 40 days in the past sixty years (Morrow and Fehler, 2004; Sheppard, 2004), this would suggest that the effect of vaccine introduction and cohort duration reduction are of the same order.

In summary, vaccine introduction and cohort duration reduction affect the infectiousness potential *W* by the same order of magnitude.

3.3.2 Basic Reproductive Number, R_0

If the equations used to calculate R_0 are studied more closely, it is found that R_0 itself is independent of the number of individuals within the cohort.

$$\begin{aligned}
R_0(T_c, v, j, s_d) &= S_0 \sum_{t=T_s+1}^{T_c} p\left(\tfrac{M_e(t,T_c,v,j)}{V(S_0,s_d)}\right) L(t,v,j) \\
&= S_0 \sum_{t=T_s+1}^{T_c} \alpha(j) \frac{M_e(t,T_c,v,j)}{V(S_0,s_d)} L(t,v,j) \qquad \text{(see Figure 3.5)} \\
&= \frac{S_0\alpha(j)}{V(S_0,s_d)} \sum_{t=T_s+1}^{T_c} M_e(t,T_c,v,j) L(t,v,j) \\
&= \frac{S_0\alpha(j)}{hwS_0/s_d} \sum_{t=T_s+1}^{T_c} M_e(t,T_c,v,j) L(t,v,j) \\
&= \frac{\alpha(j)}{hw/s_d} \sum_{t=T_s+1}^{T_c} \gamma(t,T_c,s_d)[M_e(t-1,T_c,v,j) + m(t,T_c,v,j)] L(t,v,j)
\end{aligned}$$

Now since the reduction, $\gamma(t, T_c, s_d)$, can be further be broken down:

$$\begin{aligned}
\gamma(t,T_c,s_d) &= \min\left[\frac{EhS_0/(s_d/w)}{\min[\sum_{s=1}^{t-1} S_0 d(s,T_c), EhS_0/(s_d/w)] + S_0 d(t,T_c)}, 1\right] \\
&= \min\left[\frac{EwhS_0/s_d}{\min[\sum_{s=1}^{t-1} S_0 d(s,T_c), EwhS_0/s_d] + S_0 d(t,T_c)}, 1\right] \\
&= \min\left[\frac{Ewh/s_d}{\min[\sum_{s=1}^{t-1} d(s,T_c), Ehw/s_d] + d(t,T_c)}, 1\right]
\end{aligned}$$

it is clear that R_0 is independent of S_0, but not s_d, the stocking density. Supposing the equilibrium value of dust has been reached, then since γ is a function of s_d, increasing the stocking density will reduce the fraction of dust remaining. Mathematically, this is true since the numerator is reduced by increasing

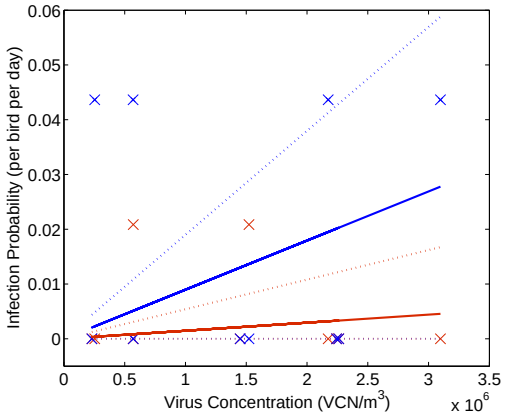
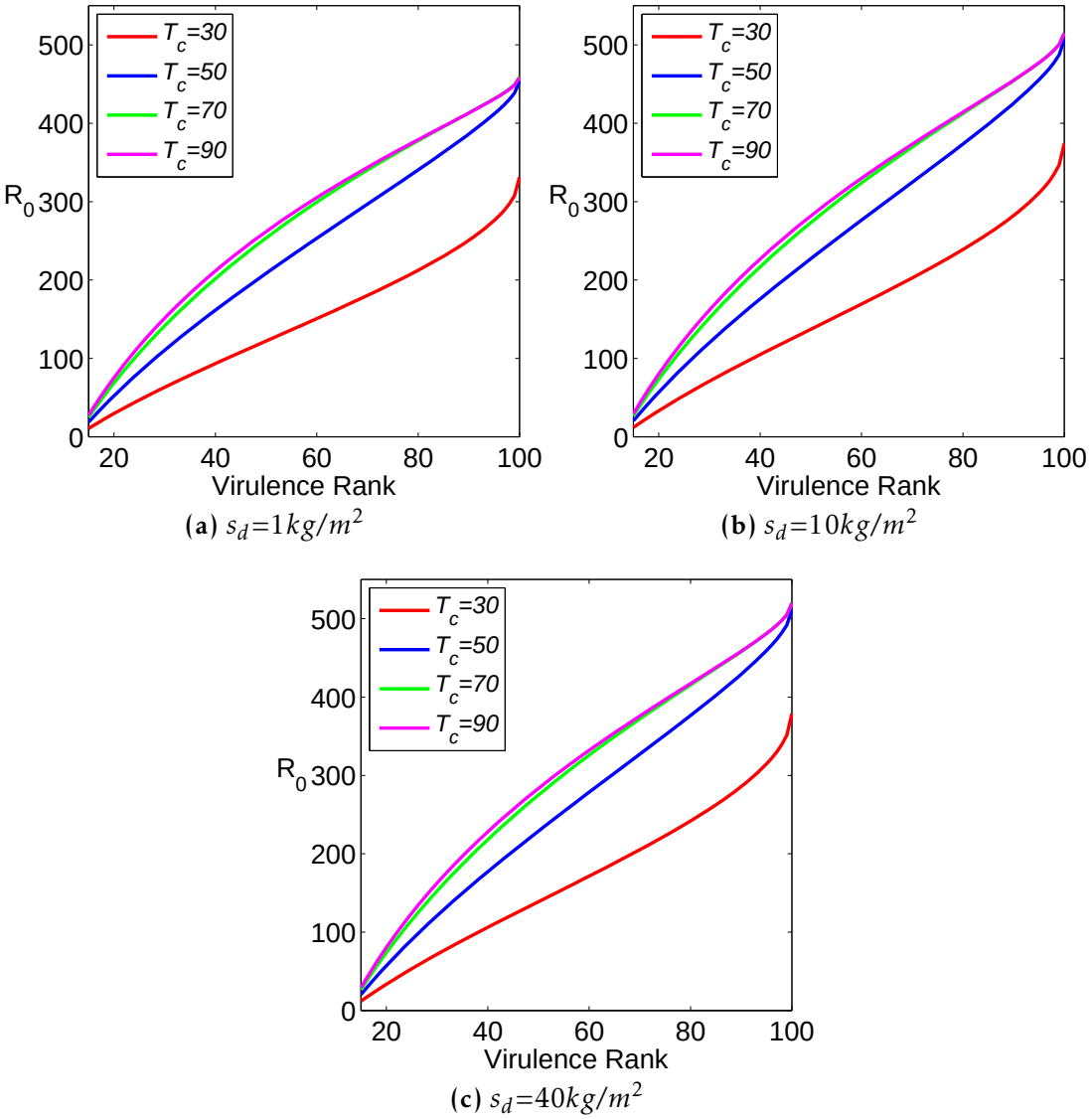


Figure 3.5: Transmission for Small Virus Concentrations: the probability of infection per day per bird as calculated in Chapter 2, with the average estimated quantities of virus concentration in the atmosphere. The blue and red crosses are the unvaccinated and HVT vaccinated birds respectively. The blue and red lines give the least squares estimate of the line of best-fit to the unvaccinated and HVT vaccinated birds respectively. The dotted lines give the 95% confidence intervals on the regression line. Note that the dotted line at $y = 0$ is the limit for the lower confidence interval for both lines.

s_d more than the denominator in the above formulation. This makes intuitive sense since the higher the stocking density, the more birds per unit volume and the more dust per unit volume which implies that more dust must be taken out if the equilibrium is to be maintained.

The transmission function, relating the concentration of virus in the atmosphere (VCN/m^3), is calculated by using the results from Section 2.4.4. Since the virus shed for one bird is much smaller than the quantities of virus examined in the experiment from Section 2.2, only the first datapoint (dpe 5) from each replicate is used to fit a linear regression between virus concentration and probability of infection per bird per day. Since the value of the intercept was not significantly different to zero and it makes biological sense to fit the line through the origin, the gradient was calculated as $\alpha(sham)$=8.97e-09 (p=0.07) for the unvaccinated birds and $\alpha(hvt)$=1.47e-09 (p=0.42) for the vaccinated birds. This relationship is shown in Figure 3.5.

Using $E = 7.15mg/m^3$, and the lines of best-fit for the transmission probabilities, results are displayed for unvaccinated (Figure 3.6) and HVT vaccinated birds (Figure 3.7). In all cases, a more virulent strain always has a higher fitness.

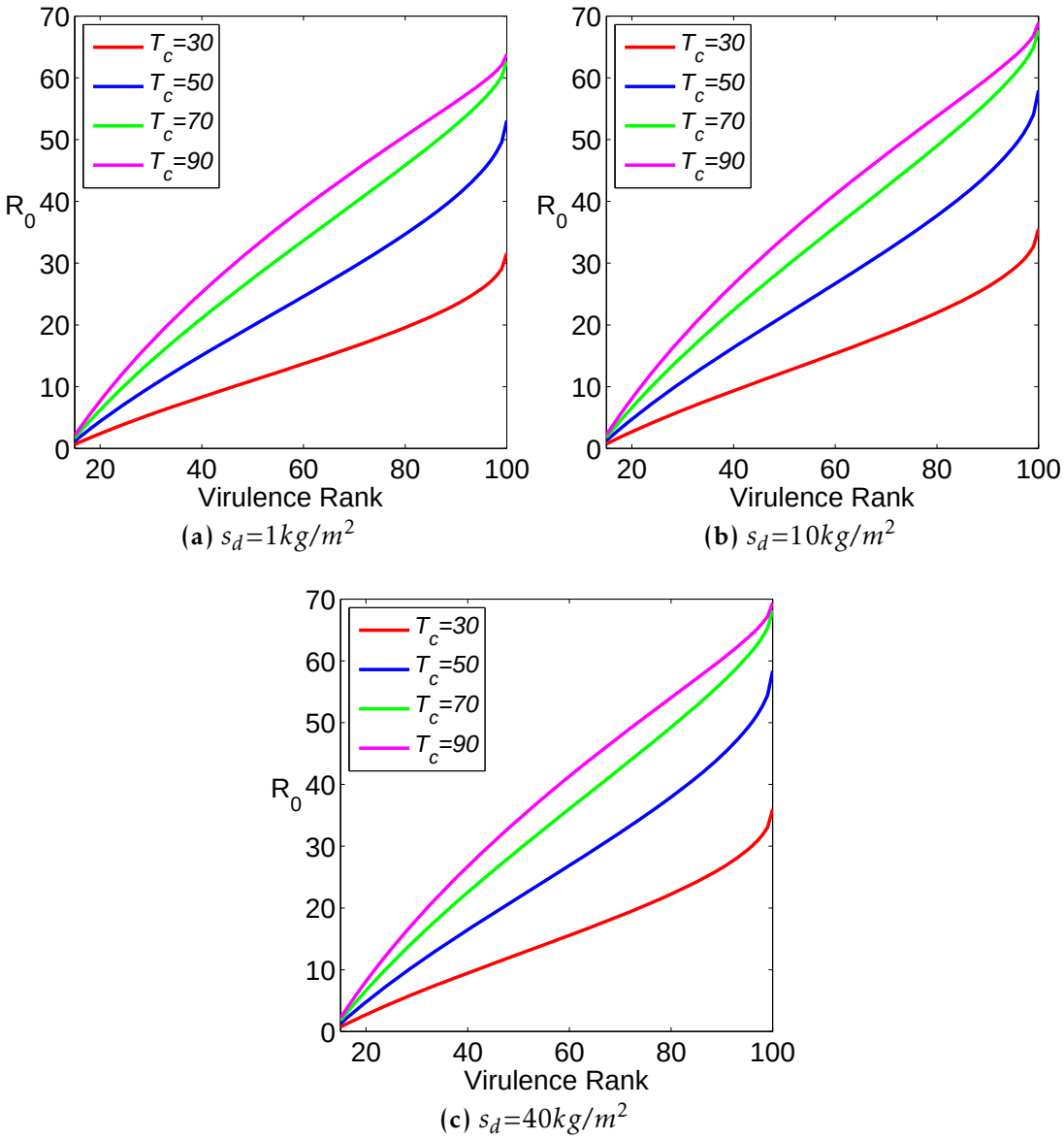


(a) s_d=1kg/m^2 **(b)** s_d=10kg/m^2

(c) s_d=40kg/m^2

Figure 3.6: R_0: Finding the value of R_0, and hence the optimal virulence, for different stocking densities, s_d, and cohort durations, T_c. Background mortality μ=0.0005, equilbrium E=7.15mg/m^3, unvaccinated population and population size, S_0=40,000.

The time step of the summations in calculating R_0 is set to one day, which approximates the continuous system well if there is an ever increasing amount of dust. Since there is another continuous process occurring i.e. not just the production of new dust, but also the removal of dust, this time step was changed.



(a) s_d=1kg/m^2 (b) s_d=10kg/m^2

(c) s_d=40kg/m^2

Figure 3.7: R_0: Finding the value of R_0, and hence the optimal virulence, for different stocking densities, s_d, and cohort durations, T_c. Background mortality μ=0.0005, equilbrium E=7.15mg/m^3, HVT vaccinated population and population size, S_0=40,000.

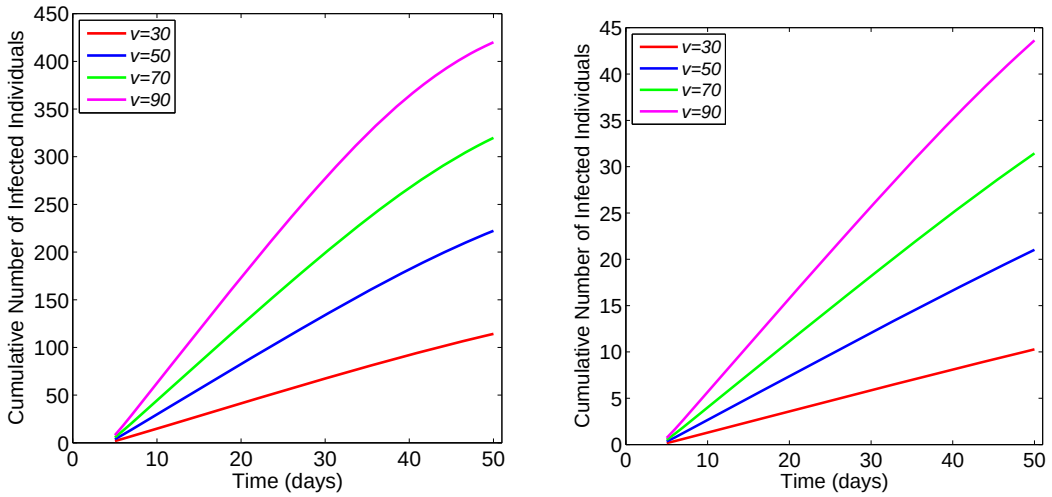
However the results do not vary a huge amount and the results are qualitatively the same.

The transmission function chosen will change the results of R_0 quantitatively. However, varying both gradients within their respective confidence intervals does not alter the qualitative results, such that more virulent strains will always have a greater fitness.

In summary, using a realistic dust environment, the R_0 of a strain increases with strain virulence. This result shows that there is always a fitness advantage for the more virulent strains, regardless of environmental pressures.

3.3.3 The Rate of Infection of Individuals

The rate at which susceptible individuals become infected is an alternative measure of the fitness of a strain. Figure 3.8 shows this for four different strains. In each case a greater Virulence Rank shows a larger number of infected individuals at each time step. This implies that in all cases a more virulent strain always has a higher fitness.



(a) Sham vaccinated Population **(b)** HVT vaccinated Population

Figure 3.8: Rate of Infection of Individuals: The total infected individuals over time where stocking density s_d=10kg/m^2, background mortality μ=0.0005, equilbrium E=7.15mg/m^3 and cohort duration T_c=50 days and population size S_0=40,000.

3.4 Discussion

The work presented here proposes fitness functions for Marek's disease virus strains entering a cohort of broiler birds with one infected bird at the start of the cohort duration and examines the evolutionary fitness for a spectrum of strain virulences under different environmental conditions. Three fitness functions were defined: the maximum expected amount of virus produced by a single infected bird in a cohort, W; the basic reproductive ratio, R_0; and, the rate of increase in the number of infected individuals.

For W, results suggest that decreasing the cohort duration drives virulence to higher levels, until they could increase no more on the virulence scale. Once a selected virulence was at the maximum on the scale, selection forces it to remain there. This result is in accordance with evolutionary theory (Anderson and May, 1982; Sasaki, 1991; Day, 2002).

Results also indicate that introducing the HVT vaccine (the first generation vaccine) serves to drive virulence to higher levels and again with Bivalent vaccine (the second generation vaccine). Again this result is in line with evolutionary theory which suggests that the introducing imperfect vaccination into a population selects for higher virulence (Gandon et al., 2001, 2003).

Increasing the background mortality decreases the fitness of a viral strain, all else being equal, however it only very slightly changes the fittest virulence, almost imperceptibly on the graphs. Increasing the mortality slightly increases the Virulence Rank selected. This result is in line with the theory stating that increasing background mortality selects for higher virulence (Kakehashi, 1992; Lenski, 1994; Ebert and Weisser, 1997).

The relative effects of vaccination and cohort duration were studied in Figure 3.4. Introducing a vaccine at longer cohort durations increases the selection for more virulent strains. There is no effect on virulence if a vaccine was introduced at very short cohort durations (under about 40 days if HVT was introduced in an unvaccinated population and under about 60 days if Bivalent was introduced in a HVT population). Since both cohort duration reduction and vaccination select for higher virulence in most cases, for the instances where vaccine introduction caused a change in virulence selection, the relative effect of vaccine in terms of cohort reduction was determined. Vaccine introduction

(from unvaccinated to HVT and HVT to Bivalent) had a similar effect as a reduction in the cohort duration of up to 10 and 27 days respectively depending on the length of the cohort duration. Over the course of 30 years (1960-1990), for example in the USA, 2 vaccines were introduced (HVT then Bivalent) and cohort duration was reduced by about 30 days (75 days to about 45 days). This suggests that the strength of selection by both vaccination and cohort duration reduction are of the same magnitude.

The results from the optimisation of W agree with evolutionary theory in that reducing cohort duration, increasing background mortality and introducing an imperfect vaccination all serve to select for higher virulence. If this was the only information available, a reasonable conclusion would be that both cohort duration reduction *and* vaccination are likely candidates for driving the virulence of MDV higher. Since vaccination was initially brought in to stem the first losses from more virulent strains emerging in the 1950/1960s, the reduction of cohort duration from 75-80 days to 60 days is a reasonable explanation for the first move from benignity to malignity.

When the role of transmission was included in the W measure, a value for the basic reproductive number, R_0, could be calculated. However, since ventilation is known to constantly remove dust to keep airborne dust contaminants to a safe level, a more realistic broiler environment was modelled by introducing a threshold above which the dust density did not increase. In this situation, more virulent strains are always selected for regardless of other environmental conditions, because the relative advantage of more virulent strains is increased when there is reduced transmission (due to the constant removal of the virus).

If a realistic quantity of dust is kept in the environment, increasing the cohort duration increases a strain's fitness, as one would expect and vaccination always results in lower R_0 for a strain, everything else being equal. This can be explained by R_0 explicitly including transmission, which is reduced for HVT vaccinated birds. Since no data was available for transmission to Bivalent vaccinated individuals, R_0 was not calculated.

R_0 does not depend on the initial number of susceptible individuals in a population. This is apparent, since the model setup changes the volume of the barn based on parameters including the stocking density and number of individuals. If the number of individuals increases, then the volume of the barn increases linearly, but the stocking density remains the same. Therefore for

each area of the barn, there is the same density of birds.

It can also be seen that stocking density does change the value of R_0, but not greatly, especially towards higher stocking densities. The overall trend that an increasing stocking density increases the R_0 of a particular strain might be expected. However the reason why this is is perhaps not so intuitive. There are two opposing factors at play: firstly, with a higher stocking density, there is a larger number of hosts per area, so the possible number of susceptibles to infect increases, which increases R_0; however secondly, as the stocking density increases, the dust remaining fraction, γ, decreases since more dust is being produced per volume. Therefore if R_0 is seen as a function of s_d, it is sublinearly increasing, due to the linear increase for the first factor and the negative weighting effect of γ, the second factor.

The conclusions for the causes of evolution are different if R_0 or the rate of infection of individuals is used for fitness. For the range of Virulence Ranks considered, optimising either of these two fitness functions always results in the maximum virulence chosen for any environment. Since the R_0/Rate of infection calculations are always maximised for more virulent strains, the trade-off between virulence and transmission was not realised. The more virulent strains were not reducing the host lifespan enough to be reducing their fitness to levels lower than other less virulent strains. This is in contrast to Anderson and May (1982) suggesting that a medium virulence could exist if the virulence-transmission trade-off exists. The conclusion from this analysis is that the three hypotheses for the reasons for virulence evolution are not factors in promoting MDV virulence to higher levels. Indeed they are all implicated in slowing the evolution by reducing the evolutionary pressure for more virulent strains.

In this analysis data relating to maternal antibody positive birds were used, however a discussion on the possible effects on virulence evolution within a population of maternal antibody negative birds is in Appendix C. A changing epidemiological background could not only have been associated with a move from maternal antibody negative to positive birds but also with host genetics. Genetic resistance to MDV (where chickens were less likely to harbour the infection) or indeed tolerance of the virus (where MDV infection would occur but without severe morbidity) could have played a role similar to that of the introduction of maternal antibodies into the host population. However the se-

lection for this would be in the breeder farms and since there is little evidence for direct selection in response to MDV infection, it is unclear the extent to which inadvertent selection of birds has occurred.

Since R_0 takes into account more information about the environmental transmission route of MDV infection, it is suggested that this may be the best fitness measure studied in this chapter. An extension to this measure will be discussed in Chapter 4.

4 MDV Epidemiology

4.1 Introduction

In Chapter 3 the notion of 'fitness' was introduced to differentiate the environments in which more virulent strains did better than less virulent ones. This chapter looks more closely at the epidemiology of Marek's disease virus within its natural setting of a flock of chickens in order to determine the epidemiological dynamics of a strain of MDV. The aim of this approach is twofold: to understand on-farm MDV dynamics to aid disease control in the future; and to understand why MDV has become more virulent over the past half century. This chapter studies the outcome in both the virus and chicken populations when dust infected with a single strain of MDV enters a single barn of chickens.

I know of only one published mathematical model looking explicitly at the epidemiology of MDV; Gao et al. (2005) quantified the dynamics of infected broiler birds within one cohort by a system of differential equations. The aim was for the model to be used as a tool for farms to understand the effect of different MDV strains under different management regimes. However, this differs from the method presented here since the parameter values used in this work are taken directly from novel parameter estimation work (described in Chapter 2) and the model approach is a stochastic individual-based model, to more easily incorporate the parameter estimates. Gao et al. (2005) did not formally estimate the parameters used in their model (instead relying on conjectured values, based on data observation) and a complete exploration of the model was not published.

Dynamics within a cohort structure have not been well-studied, with many more models concerned with the spread of disease in either well-mixed populations (Anderson and May, 1992; Keeling and Rohani, 2008), or completely spatially-explicit systems (Boots and Sasaki, 2000; Cook et al., 2007). However, few have developed the theory for temporally-explicit dynamics for situations such as these, where pathogens infect cohort-structured populations. The only similar work I know of concerns *Coccidiosis* in broiler chickens (Klinkenberg and Heesterbeek, 2007). Those authors model multiple cohorts by a series of mean-field difference equations. They studied the free-living and within-host stages of the parasite to describe optimal strategies for damage limitation for flock infection.

This chapter simulates one cohort of broiler birds when exposed to MDV and quantifies the behaviour of the virus strain and the effect on its hosts.

4.2 Methods

The model simulates a single cohort of susceptible broiler birds in a barn from start to finishing time during a potential outbreak of MD initiated by MDV infected dust entering the cohort barn at the same time as the birds.

Through the work in Chapter 2, the distributions of basic epidemiological parameters were found. These were estimates for different virus strains infecting hosts of different vaccine status. The parameters estimated were the latent period of MDV, the viral shedding rate, the time to death due to MDV infection and the transmission rate. The uncertainty in the value of these parameters included the variability between different groups of hosts in their response to a MDV infection. Allowing these parameters to vary enables a broiler farm to be modelled in a fully stochastic manner with every individual bird having a different combination of 'Infection Attributes', given by sampling values from the parameter distributions.

A full description of the stochastic model is laid out in this section, with all the parameters of interest and mathematical formulae laid out in Boxes 4.1 and 4.2 respectively.

4.2.1 Host Population

The initial host population is a single cohort of S_0 floor-reared broiler chickens. The population is subject to no immigration, although there is a possibility of host mortality, either non-MDV or MDV related. The removal of sick or dead birds is done daily. Each bird sheds an amount of dust per day, $d(t, T_c)$, which is set at $326\exp(-P(T_c)/t^{1.64}) + 10.8$ where P is a function of the duration of the cohort. This function is derived in Appendix B and explained in detail in Section 3.2.1.2. At the end of each cohort duration, at the end of day T_c, the remaining chicken population is removed for slaughter.

4.2.2 Cohort System

At the start of a cohort, the barn is seeded with an amount of infected dust, D_0, (which is unknown, but varied) which determines the total amount of infected virus, Z_0, measured in viral copy number (VCN). Since two values for the concentration of virus per mg of dust were calculated in Section 2.4.2 for each of the three virus isolates (04CRE, MPF57 and 02LAR, one value for each replicate), an approximation for Z_0 is the virus concentration per mg of dust as the average of the two values from each replicate, $\bar{a}(v, j)$, multiplied by D_0. It is assumed the total amount of uninfected dust is set to zero.

If at any time the virus concentration in the air drops below a critical point, z_e (VCN/m^3), the virus is assumed extinct as it becomes untransmissable. There is no explicit virus decay rate in the model, since Jurajda and Klimes (1970) showed that 44 day old virus had a similar infection potential as recently shed virus and Carrozza et al. (1973) demonstrated that even infected dust 205 days old was still able to infect birds to a high degree.

Floor-reared broiler barns can produce vast quantities of dust throughout the lifespan of the cohort and ventilation mechanisms exist for the removal of dust and its constituent air-borne contaminants, for the health of both the birds and the farm workers (Wathes, 1998). Studies sampling the quantity of dust at approximately twenty eight days at various farms over Northen Europe gave a range of 2-10mg/m^3 in the barn atmosphere (Takai et al., 1998). It is therefore assumed that the amount of dust never rises beyond a limit and a baseline for this value is taken as the average of the samples, 7.15mg/m^3. The production

of (and subsequent removal of any excess) dust is assumed to happen at the start of each day and any infection of new birds occurs after.

4.2.3 Host Infection

Infection is known to be via inhalation of virus in the atmosphere, which is shed by infectious birds (Calnek et al., 1970). Infection probability is therefore modelled as a scaled concentration of virus in the air (VCN/m^3). The amount of virus in the air can be calculated since there is a density of virus in the dust (VCN/mg), a known quantity of dust in the barn (mg) and a fixed barn volume (m^3). The probability of infection depends only on the concentration of airborne virus and the host's vaccination status. The probability of infection was estimated as a function of virus concentration via linear regression with no intercept (from the data and estimates in Section 2.4.4). The gradients are defined as 4.93e-8 (s.e.=4.50e-9, $P < 0.005$) and 8.26e-10 (s.e.=2.57e-10, $P < 0.005$) per bird per VCN/m^3 for unvaccinated and HVT vaccinated birds respectively. A graph of these functions is shown in Figure 4.1. The standard errors on the estimates define a normal distribution of the possible gradients which are used in the model (see Section 4.2.4).

4.2.4 Host Pathogenesis

Each bird has certain 'Infection Attributes' which have been estimated previously: latent period, viral shedding rate, Weibull parameters determining time to death and gradient of infection rate determined by virus concentration. Each bird has a value of each parameter which will determine its infection course and whether it becomes infected. A value for each of these parameters is sampled from the relevant distribution obtained in Sections 2.4.2, 2.4.3 and 4.2.3.

Once infection occurs there is a delay until the individual becomes infectious (Baigent and Davison, 2004) as the virus infects the feather follicle epithelium, the only source of a fully productive infection. This delay has been estimated as between four and six days (Chapter 2). Once infectious, the bird has a constant daily viral shedding rate, $a(v, j)$ (VCN/mg dust) until it is removed from the population. Once infected, each individual has an infection-specific

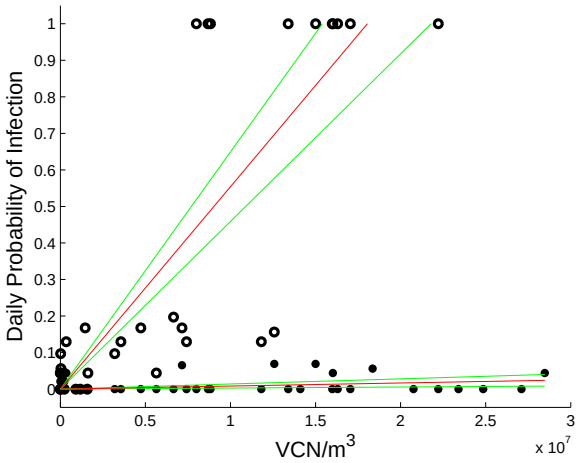


Figure 4.1: Tranmission: Daily probability of infection per bird with different vaccination treatments. The circles are the maximum likelihood point estimates for p given for the different timesteps and pens (open for unvaccinated, filled for HVT vaccinated). The upper and lower red lines are the lines of best-fit for unvaccinated and HVT vaccinated hosts respectively and the green lines are the associated 95% confidence intervals.

lifespan dependent on the virulence of the infecting virus and its own vaccination status. There are thus four states of the individual in the model - *Susceptible*, *Exposed*, *Infectious* and *Removed*. Note that there is an underlying mortality probability, μ (per bird per day), which is constant across chicken states which acts to remove chickens from the population for reasons other than MDV-related. The bird may then die as a result of MDV infection only if it has not yet died of other causes or been slaughtered at the end of the cohort time. A schematic of the chicken states and environmental conditions is shown in Figure 4.2.

4.2.5 Virus Strain

MDV is characterised by the Virulence Rank, which is the gold standard pathotype as devised by Witter (1997) and Witter et al. (2005). It is the percentage of cases of gross lesions produced by a strain when infecting HVT and Bivalent vaccinated birds. The strain rank, v, of the virus determines the viral shedding rate of an infected bird and its lifespan (if there are no other mortality causes). The cumulative amount of virus strain in the atmosphere at time t is defined as $Z(t)$ (VCN).

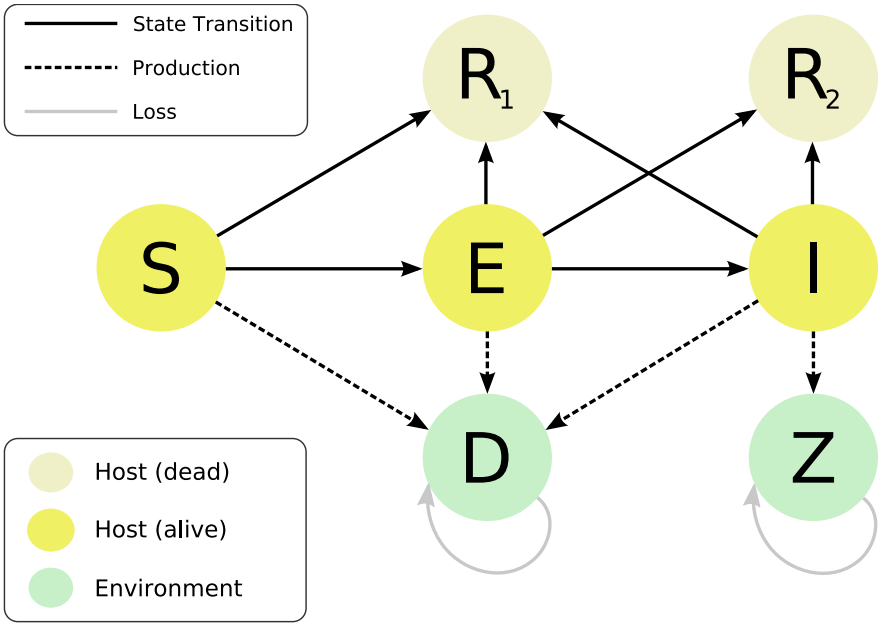


Figure 4.2: Schematic of the state transitions of the host individuals in one cohort. Susceptible (S), Infected-Uninfectious (E), Infected-Infectious (I), Removed-other cause (R_1), Removed-MDV related (R_2) and production/loss of dust (D) and virus (Z).

4.2.6 Between Cohort Dynamics

At the end of each cohort all the birds are removed and a thorough cleaning of the barn takes place (van de Giessen et al., 1996). The dust removed is therefore a fraction, ϵ (a contamination parameter), of the dust at the end of the cohort. The model assumes the virus is well-mixed in the dust and therefore the same fraction of virus will be left.

4.2.7 Model Outcome

The probability of an outbreak was calculated. Also found were the amounts of virus at the end of the cohort duration and the number of infected individuals and the total removed individuals throughout the cohort duration. An increasing amount of infected dust was used to seed a single cohort. Realisations of the model were performed 100 times.

Box 4.1: Glossary of Definitions (1 of 2)

Variables

(denoted at time t for infecting strain virulence v and host vaccine status j)

$S(t,v,j)$	Number of susceptible birds
$E(t,v,j)$	Number of exposed birds
$I(t,v,j)$	Number of infectious Birds
$R_1(t,v,j)$	Number of dead (non-MDV-induced) birds
$R_2(t,v,j)$	Number of dead (MDV-induced)
$Z(t,v,j)$	Amount of virus within barn (viral copy number (VCN))

Fixed Parameters

$\bar{a}(v,j)$	Estimated viral shedding (VCN/mg dust) for bird of vaccine status j infected with strain virulence v
$d(t,T_c)$	Dust shed per bird age t per day in cohort duration of T_c days (mg)
D_{total}	Total background mortality (% in cohort)
e	Age at introduction (days)
h	Height of barn (m)
T_{mat}	Maturation time for Rhode Island Red birds (days)
w	Final bird weight (kg)

Derived Parameters

A	Area of barn floor (m^2)
$P(T_c)$	Shape parameter for the per bird dust function
$V(S_0,s_d)$	Volume of barn
Z_0	Initial amount of virus (VCN)
μ	Non-MDV mortality probability per bird per day

Box 4.1: *cont.* Glossary of Definitions (2 of 2)

Sampled Parameters - estimates/distributions estimated in Chapter 2

T_s	Latent period (days)
$a(v, j)$	Virus present in dust (VCN/mg dust) for bird of vaccine status j infected with strain virulence v
r	Weibull shape parameter for host lifespan distribution
$\lambda(v, j)$	Weibull scale parameter for host lifespan distribution
$p(\frac{Z(t)}{V(S_0, s_d)}, j)$	Transmission probability per bird per day for bird of vaccine status j infected with strain virulence v

Farm Controlled Parameters

E	Maximum dust quantity in barn (mg/m^3)
D_0	Initial dust (mg)
j	Vaccine treatment (Sham or HVT)
s_d	Stocking density (kg/m^2)
S_0	Initial number of birds
T_c	Cohort duration (days)
ϵ	Dust reduction at end of cohort duration

Uncontrollable Parameters

v	Virulence Rank of virus (%)
v_T	Arcsine square-root trasnformed Virulence Rank
z_e	Virus extinction parameter (VCN/m^3)

Box 4.2: Glossary of Values

Fixed Parameters

$d(t, T_c)$	$368\exp(-P(T_c)/t^{1.64}) + 10.8$ (Appendix B)
D_{total}	3.6-6.8% (Sheppard, 2004)
e	2 days (Sheppard, 2004)
h	2.5 m (S.W Walkden-Brown, *pers. comm.* 2008)
T_{mat}	45 days (S.W. Walkden-Brown, *pers. comm.* 2008)
w	2.5 kg (S.W. Walkden-Brown, *pers. comm.* 2008)

Derived Parameters

A	$S_0 w/s_d$
$P(T_c)$	$-T_c^{1.64}\ln\left[\frac{d(45,45)-10.8}{368}\right]$
Z_0	$\bar{a}(v,j)D_0$
μ	$1 - \sqrt[T_c]{(1-(D_{total}))}$

Farm Controlled Parameters

E	7.15 mg/m^3 (Takai et al., 1998)
D_0	0-560 mg (unknown)
j	Sham, HVT (Bublot and Sharma, 2004)
s_d	5, 20, 35 kg/m^2 (N. Sparks, *pers. comm* 2008)
S_0	500, 5,000, 30,000 Birds (N. Sparks, *pers. comm* 2008)
T_c	30, 60 days (Sheppard, 2004)
ϵ	0-1 (unknown)

Uncontrollable Parameters

v	16.5, 36, 46% (Walkden-Brown et al., 2008)
z_e	10^{-9},10^{-5} VCN/m^3 (unknown)

4.3 Results

4.3.1 The Probability of an Outbreak, P

An outbreak is defined such that there is at least one individual infected in the cohort. The probability of an outbreak occurring is calculated as a function of the initial inoculum of dust in a cohort. The results are shown in Figure 4.3.

The coloured lines show the probability of an outbreak occurring for three isolates of different virulences, while each plot displays results from different environmental conditions.

A higher Virulence Rank increases the probability of an outbreak for a given dust inoculum. Vaccination greatly decreases the probability of an outbreak. Increasing the stocking density of individuals increases the probability of an outbreak, whereas changing the population size has no effect. Increasing the extinction parameter, z_e, increases the time available for the virus to seed an outbreak in theory; however, the virus is removed relatively quickly and the probability of infection at very low virus concentrations is very small, so varying z_e between 10^{-9} and 10^{-5} does not change the probability of an outbreak.

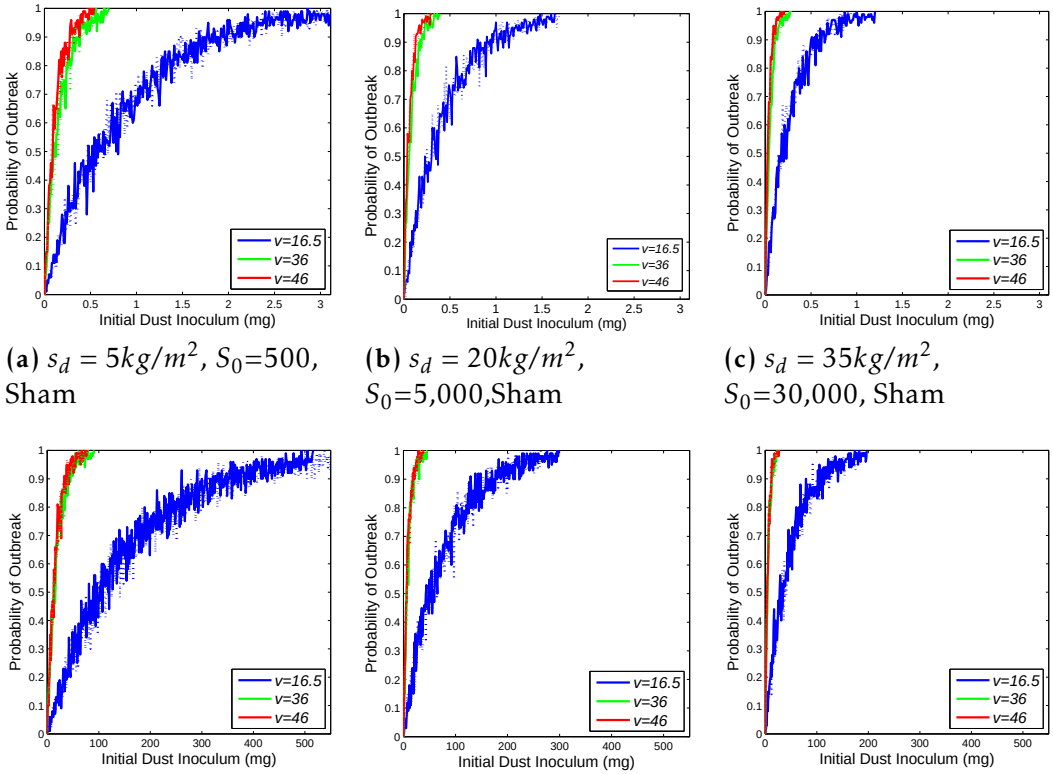
The simulations can be checked against calculating the expected probability of an outbreak. The definition that a viral inoculum will create an outbreak if and only if it infects at least one individual is defined by the following equation:

$$\begin{aligned} P(\text{no infection from inoculum}) &= \prod_{s=1}^{T_c} P(\text{no transmission to bird on day } s)^{S(s)} \\ &= \prod_{s=1}^{T_c} [1 - p(j, Z(s,v,j)/V(S_0, s_d))]^{S(s)} \end{aligned}$$

where p is the probability of a single bird being infected on day t given vaccination status, j and atmospheric density of virus, $Z(t,v,j)/V(S_0,s_d)$. This function is plotted is Figure 4.4, which shows that the expected probability of an outbreak is the same as the complement of the probability that no birds are infected with the initial inoculum.

4.3.2 The Outcome of an Outbreak

The following measures are not affected by the extinction parameter z_e.



(a) $s_d = 5kg/m^2$, S_0=500, Sham

(b) $s_d = 20kg/m^2$, S_0=5,000,Sham

(c) $s_d = 35kg/m^2$, S_0=30,000, Sham

(d) $s_d = 5kg/m^2$, S_0=500, HVT

(e) $s_d = 20kg/m^2$, S_0=5,000, HVT

(f) $s_d = 35kg/m^2$, S_0=30,000, HVT

Figure 4.3: Probability of Outbreak: The probability that there is at least one individual infected during the cohort duration given a certain quantity of infected dust inoculum, for given stocking densities (s_d), number of individuals (S_0) and vaccination status. The solid line corresponds to a cohort duration , T_c, of 30 days, while the dotted line for one of 60 days. The probability is calculated as the proportion of 100 cohort simulations where there has been at least one infected individual. Note the scale on the x-axis changes for different vaccine treatments.

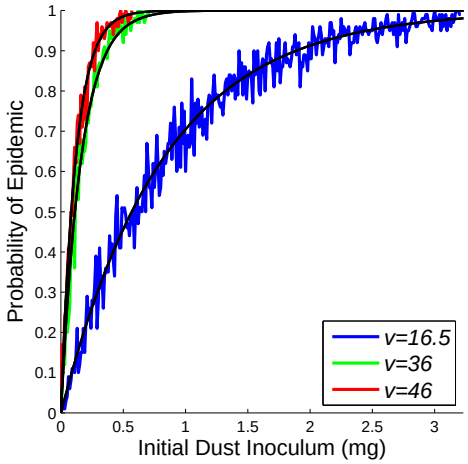


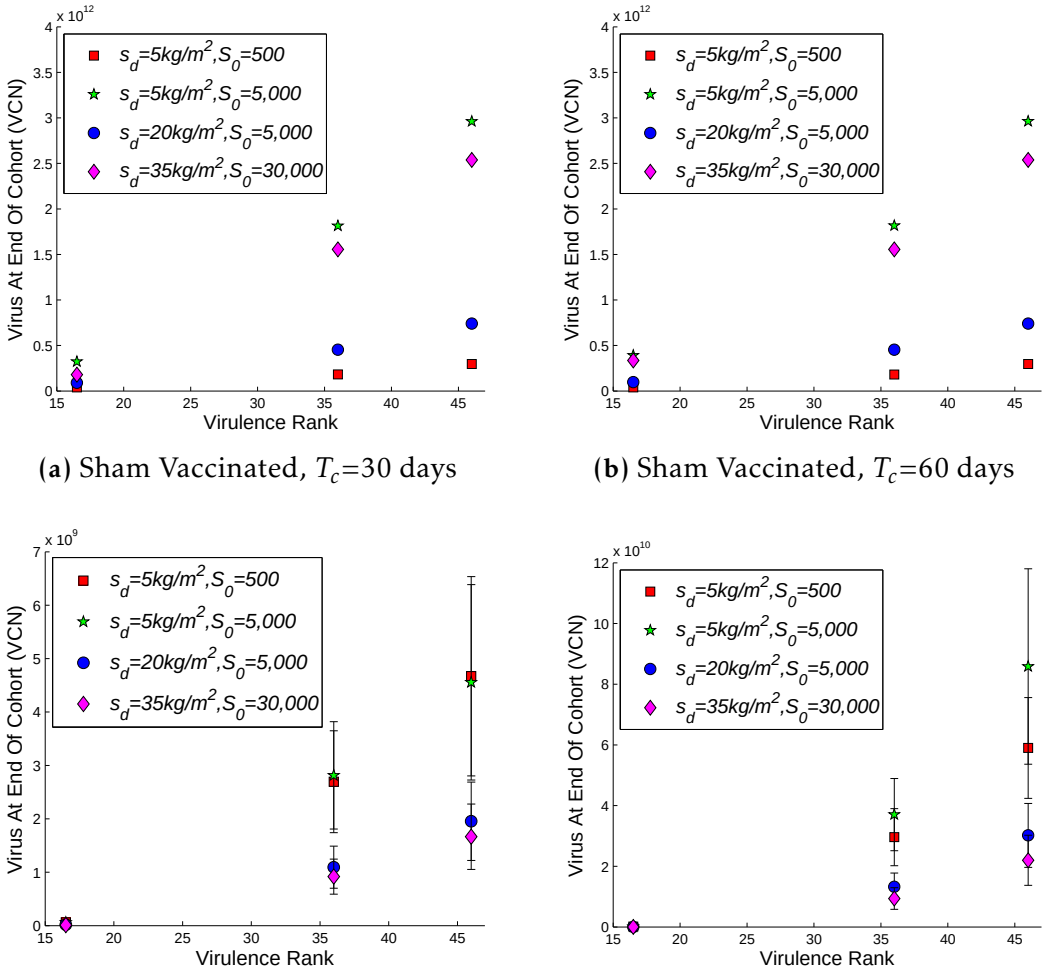
Figure 4.4: Probability of Outbreak: The probability that there is at least one individual infected during the cohort duration given a certain quantity of infected dust inoculum. The probability is calculated as the proportion of 100 cohort simulations where there has been at least one infected individual. The black line is the expected probability that there is at least one infection from the initial inoculum before the inoculum is removed from the barn. Stocking density, s_d=5kg/m^2, background mortality, μ=0.0005, number of individuals, S_0=5000, sham vaccinated, cohort duration, T_c=30 days.

4.3.2.1 Virus at the End of the Cohort Duration, Z_c

The amount of virus in the atmosphere at the end of a cohort (given an outbreak occurs) is plotted against Virulence Rank for different environments in Figure 4.5.

In the sham vaccinated case, increasing the Virulence Rank and population size increases the quantity of virus left at the end of a cohort in all cases. However, decreasing the stocking density increases the total amount of virus left since the volume of the barn is increased, leading to a greater amount of virus in total (due to the upper limit of dust being enforced). There is little or no effect of lengthening the cohort duration.

For the HVT vaccinated case, increasing the population size has no significant effect on the amount of virus left. There is a rise in amount of virus due to both increased Virulence Rank and decreased stocking density. A longer cohort duration increases the quantity of virus at the end of the duration.



(a) Sham Vaccinated, T_c=30 days

(b) Sham Vaccinated, T_c=60 days

(c) HVT Vaccinated, T_c=30 days

(d) HVT Vaccinated, T_c=60 days

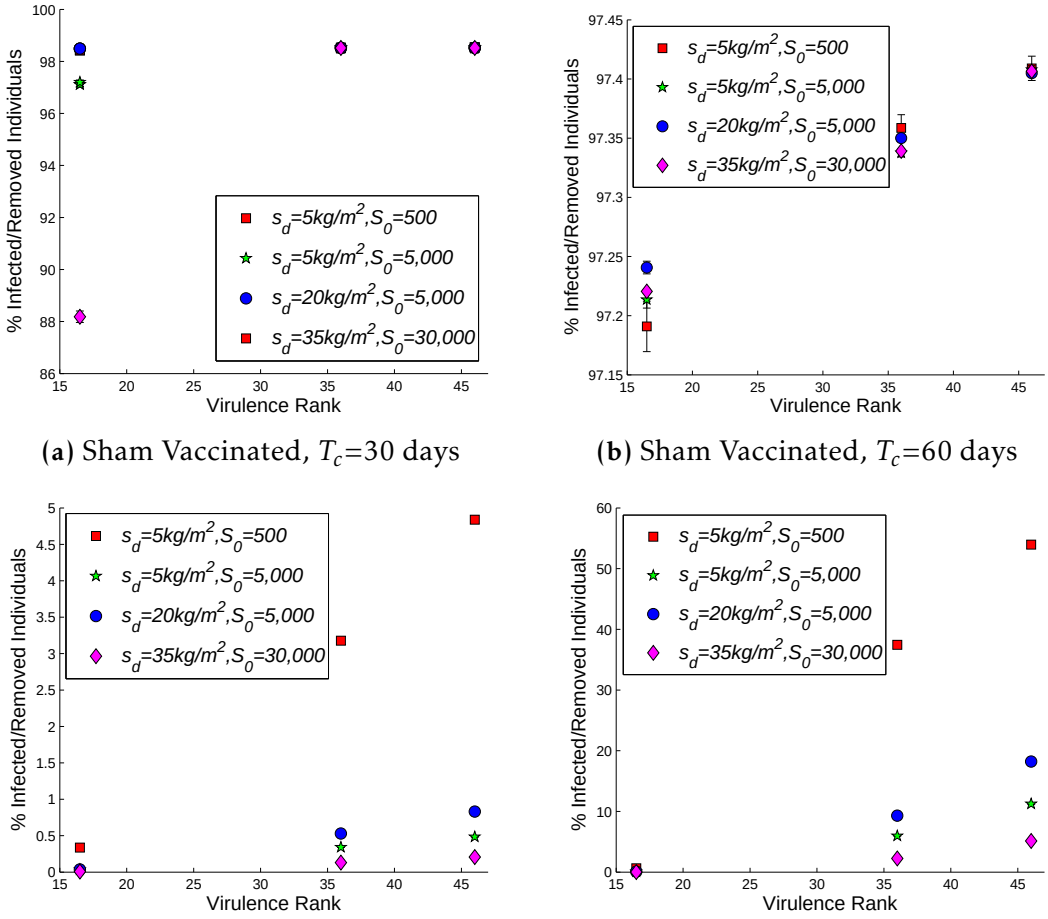
Figure 4.5: Mean virus (VCN) at end of cohort duration given outbreak: Background mortality, μ=0.0005. The mean is calculated from all the 100 simulations of the different inocula and 95% confidence intervals are given in all cases (although too small to see in the unvaccinated cases). Note the different scales on y-axes.

4.3.2.2 Number of Infected Cases

The percentage of infected and removed individuals at the end of the cohort is plotted for various environments in Figure 4.6. This measure does not include any birds dying of other reasons.

For unvaccinated birds with a short cohort duration, the two most virulent strains cause the same amount of infection, the least virulent strain, slightly less. The two most virulent strains infect all the cohort, the rest of the population accounted for by being removed for some other non-MDV related reason. For a longer cohort duration, all the birds are infected, with the rest being removed for other reasons. The increase in the number infected or removed due to Virulence Rank can be accounted for by the increased propensity for more virulent strains to kill their host faster, which can only be seen when the cohort duration is long enough. The slight decrease in the numbers infected or removed for the higher virulent strains when a longer cohort duration is used can be explained by the death of infected individuals from other causes during this time.

For vaccinated birds, there is a vastly reduced chance of infection or removal due to MDV. The longer the cohort duration is, the more infection and removal of birds there will be. The more virulent a strain is, the more infected and removed birds will result in a cohort. If the size of the population increases, the volume of the barn increases, so the initial inoculum is reduced in concentration and therefore the rate of infection is reduced. Similarly, if the stocking density increases, there is more infection, since there is a higher concentration of virus in the smaller volume.



(a) Sham Vaccinated, T_c=30 days

(b) Sham Vaccinated, T_c=60 days

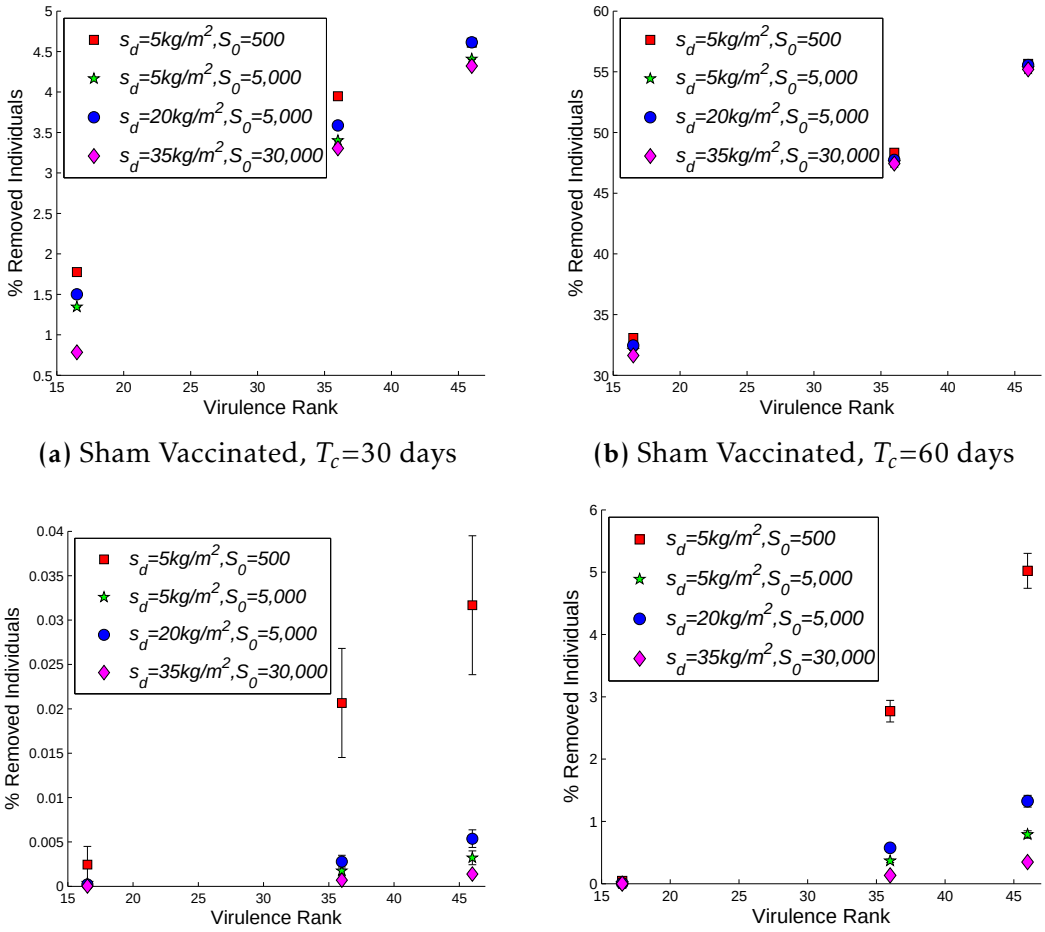
(c) HVT Vaccinated, T_c=30 days

(d) HVT Vaccinated, T_c=60 days

Figure 4.6: Mean % of infected individuals at the end of a cohort duration given outbreak: Background mortality, μ=0.0005. The mean is calculated from all the 100 simulations of the different inocula and 95% confidence intervals are given in all cases (although too small to see in the vaccinated cases). Note the different scales on y-axes.

4.3.2.3 Removed Cases

The percentage of removed individuals is the farm's yardstick for MDV prevalence and severity. The percentage of removed individuals who have died through MDV-induced disease is shown in Figure 4.7 and results concur with those found relating to the total infection and removed individuals. Namely, increasing Virulence Rank and stocking density, in general, increases the removed individuals, whereas introducing vaccination and increasing population size reduces the number of removed individuals. Increasing cohort duration in a vaccinated population will increase the total removed individuals, which is not true in the unvaccinated population since the population is saturated with infection in both cases.



(a) Sham Vaccinated, T_c=30 days

(b) Sham Vaccinated, T_c=60 days

(c) HVT Vaccinated, T_c=30 days

(d) HVT Vaccinated, T_c=60 days

Figure 4.7: Mean % of removed individuals due to MDV as percentage of initial population size at the end of a cohort duration given an outbreak: Background mortality, μ=0.0005. The mean is calculated from all the 100 simulations of the different inocula and 95% confidence intervals are given in all cases (although too small to see in the unvaccinated cases). Note the different scales on y-axes.

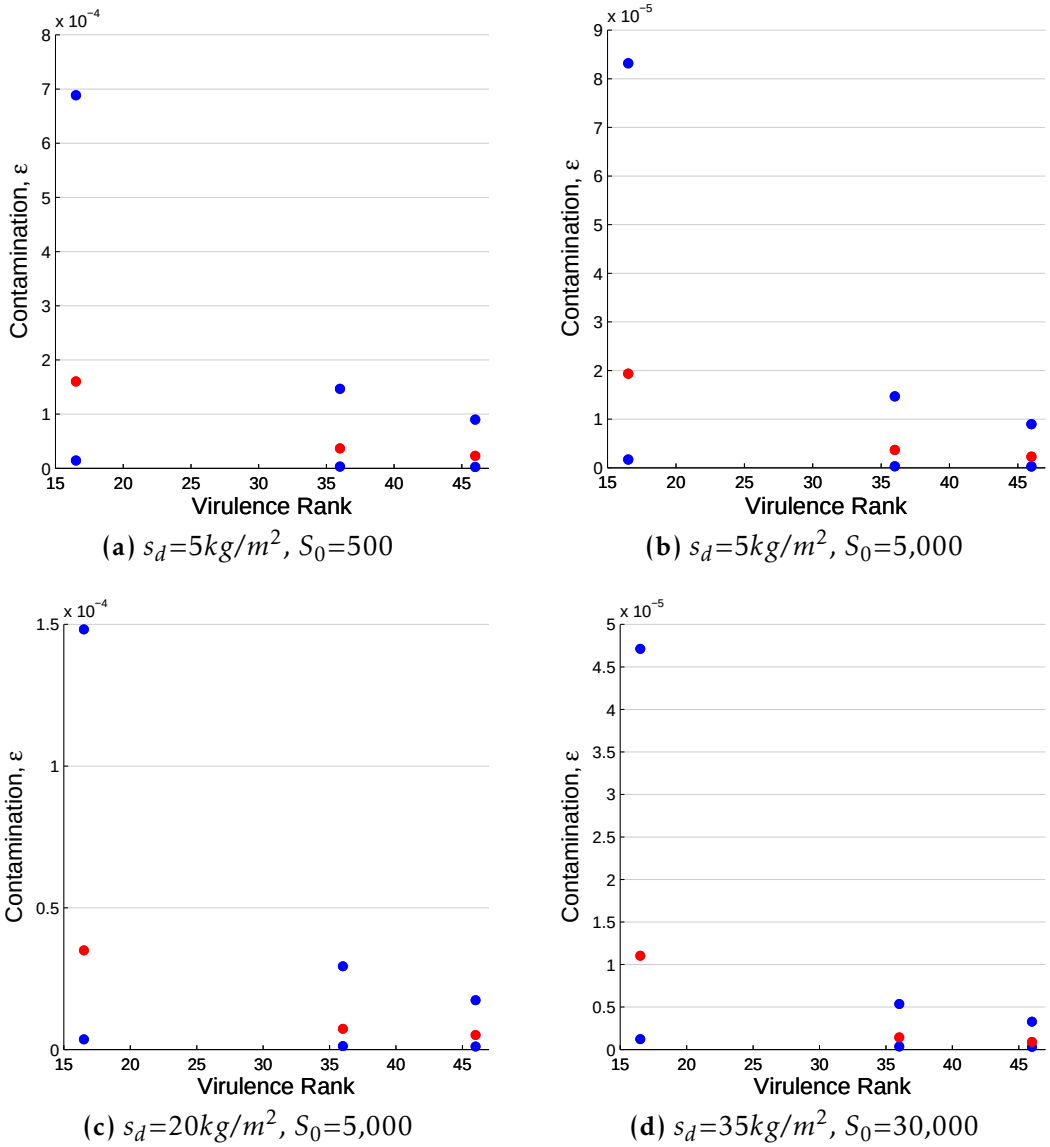
4.3.3 Impact of Contamination Parameter

Given that there has been an MDV outbreak on a farm, the virus in the barn must be removed to a certain level to reduce the risk of an outbreak occurring in the following cohort. The barn is assumed to be cleaned with an efficiency of $1 - \epsilon$ before the next generation cohort of chickens is added to the barn.

The reduction needed to maintain a δ probability of an outbreak is plotted in Figure 4.8 for sham vaccinated birds and in Figures 4.9 and 4.10 for HVT vaccinated birds.

For Sham vaccinated environments, the reduction factor reduces with the virulence of a strain, implying that if a farm is infected with a more virulent strain of MDV, more cleaning is required to reduce the probability of a new outbreak occurring to the same level. However in more populated and denser cohorts, it becomes increasingly difficult to reduce the amount of virus to reduce the chance of an outbreak in the next cohort to below 5%. Since the amount of virus does not change with cohort duration, the effect of the contamination parameter does not change with the cohort duration.

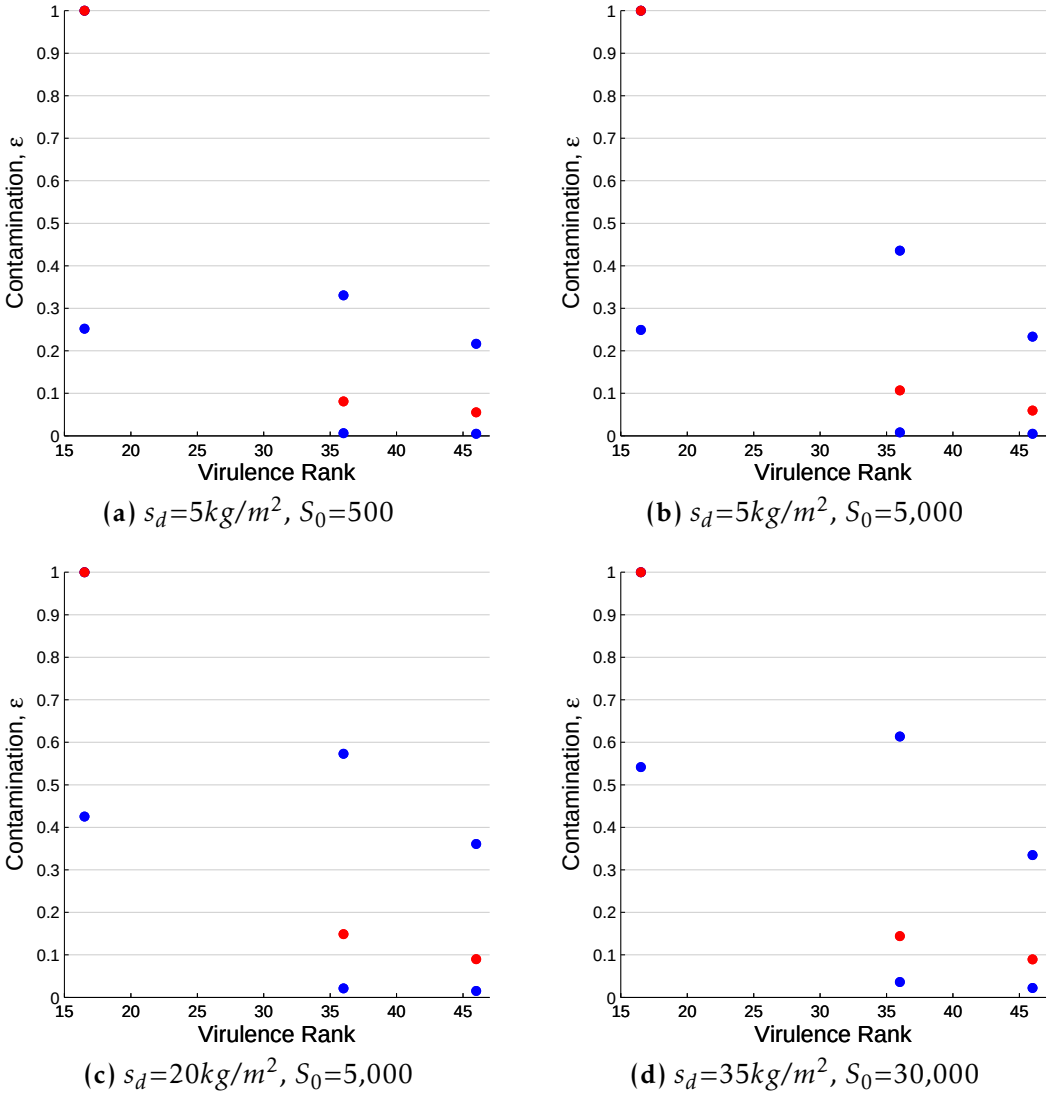
For HVT vaccinated it can require less cleaning to reduce the probability of an outbreak to 5%. Since the length of the cohort increases the quantity of virus left at the end of a cohort duration, increasing the cohort duration for a vaccinated farm serves to increase the probability of an outbreak in the next cohort (given the same level of contamination).



(a) s_d=5kg/m^2, S_0=500 **(b)** s_d=5kg/m^2, S_0=5,000

(c) s_d=20kg/m^2, S_0=5,000 **(d)** s_d=35kg/m^2, S_0=30,000

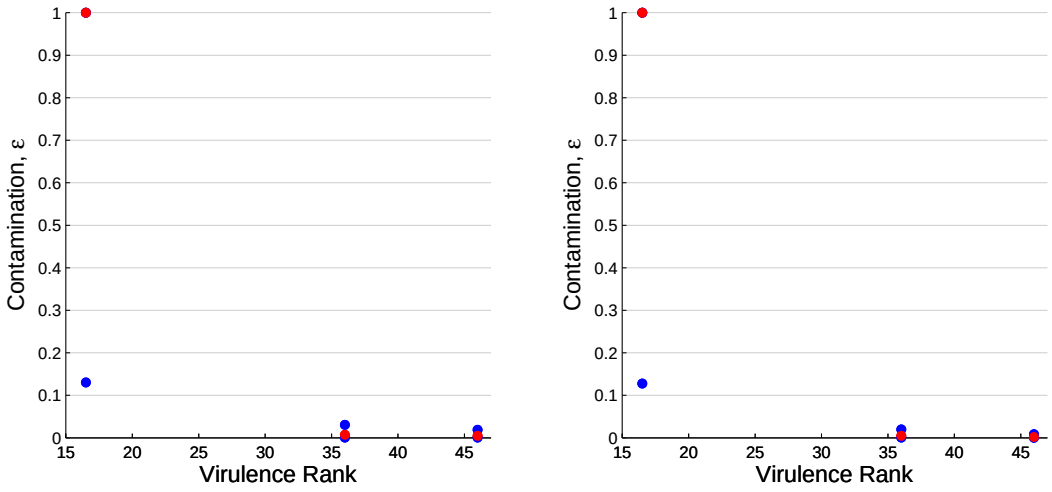
Figure 4.8: Contamination Parameter: Sham vaccinated, background mortality, μ=0.0005, cohort duration, T_c=30 days. The red circles give the fraction to reduce the virus by at the end of the cohort to give a δ=0.5 probability of an outbreak the next cohort, the upper and lower blue circles give the reduction to obtain a δ=0.05 to δ=0.95 probability of an outbreak respectively.



(a) s_d=5kg/m^2, S_0=500 **(b)** s_d=5kg/m^2, S_0=5,000

(c) s_d=20kg/m^2, S_0=5,000 **(d)** s_d=35kg/m^2, S_0=30,000

Figure 4.9: Contamination Parameter: HVT vaccinated, background mortality, μ=0.0005, cohort duration, T_c=30 days. The red circles give the fraction to reduce the virus by at the end of the cohort to give a δ=0.5 probability of an outbreak the next cohort, the upper and lower blue circles give the reduction to obtain a δ=0.05 to δ=0.95 probability of an outbreak respectively.



(a) s_d=5kg/m^2, S_0=500 **(b)** s_d=5kg/m^2, S_0=5,000

(c) s_d=20kg/m^2, S_0=5,000 **(d)** s_d=35kg/m^2, S_0=30,000

Figure 4.10: Contamination Parameter: HVT vaccinated, background mortality, μ=0.0005, cohort duration, T_c=60 days. The red circles give the fraction to reduce the virus by at the end of the cohort to give a δ=0.5 probability of an outbreak the next cohort, the upper and lower blue circles give the reduction to obtain a δ=0.05 to δ=0.95 probability of an outbreak respectively.

4.4 Discussion

A cohort of chickens exposed to MDV infected dust was simulated and the probability of an outbreak, the total virus at the end of an outbreak, the total number of infected individuals and the total removed individuals throughout the cohort were tracked. The results obtained in this chapter provide a novel way of assessing the impact of an MDV strain on a cohort of broiler chickens.

The probability of an outbreak given a certain quantity of infected dust was dependent on the Virulence Rank of the strain, unsurprisingly because the probability of infection depends only on the quantity of virus material and the concentration of virus material in dust increases with Virulence Rank (detailed in Section 2.4.2). The extinction of virus within a cohort occurs if only if there is no infection by the viral inoculum, which is a tractable way of defining whether an outbreak occurs or not given an initial inoculum.

The probability of an outbreak increases with the stocking density of individuals, since the number of opportunities to infect remains the same (since the number of birds stays constant), but the probability of infection per opportunity increases since the virus concentration increases. Conversely, if the number of individuals doubles, the chances for new infections doubles, however, the volume of the barn also doubles, which means the virus concentration halves and thus the probability of an outbreak remains the same. Therefore an increase in both the stocking density and the number of individuals to current industrial conditions will have increased the chance of an outbreak if the farm was exposed to any MDV.

Whatever the other cohort conditions are, if the flock is unvaccinated, most, if not all, of the birds will become infected/removed, if they do not die of other causes prematurely. For vaccinated cases the situation changes drastically. For longer cohort times (perhaps akin to a free range farm) with a small flock size and low stocking densities (e.g. 500 birds at $5kg/m^2$) a less virulent strain gives only 1% infection/removed, compared with 54% for more virulent strains. For shorter cohort durations in a more industrial farm (e.g. 30,000 birds at $35kg/m^2$), for all Virulence Ranks studied here, there is less than 0.2% infection/removal during the cohort lifetime. In the unvaccinated case, the population is saturated by infection and therefore a bigger population will result in more infections.

The total number of birds removed due to MDV follows the same trend. For shorter cohort durations in a more industrial farm (e.g. 30,000 birds at $35kg/m^2$), for all Virulence Ranks studied here, a less virulent strain gives 2 birds removed due to MDV (0 for a vaccinated flock) compared with 1320 (0-1 for a vaccinated flock) for more virulent strains. For longer cohort times (perhaps akin to a free range farm) with a small flock size and low stocking densities (e.g. 500 birds at $5kg/m^2$) a less virulent strain gives about 165 birds removed due to MDV (0-1 for a vaccinated flock), compared with 280 (27 for a vaccinated flock) for more virulent strains.

For currently realistic environments (e.g. 30,000 birds at $35kg/m^2$), where the population is vaccinated, the cleaning efficiency would only have have to be 98% to remove enough virus to reduce the probability of an outbreak in the next cohort to 5%. However if the population is unvaccinated, for there to be a 5% chance of an outbreak in the next cohort the efficiency of cleaning needs to be greater than $1 - 10^{-9}$.

If an outbreak does occur the quantity of virus left at the end of the cohort duration is a good measure of viral fitness since it is a fraction of this amount which will enter the next generation through contamination. In both unvaccinated and vaccinated cases, the amount of virus left increases as the Virulence Rank increases, implying that in all environments, selection always favours more virulent strains. Using the amount of virus as a measure of fitness of a virus strain, the results imply that any increase in virulence over the past sixty years has been due to the continued shift of strains towards a higher virulence. Regardless of the extent of industrialisation of the farming industry, more virulent strains are always selected for. In fact, increasing the stocking density of the birds and introducing vaccination both serve to decrease the selection pressure for more virulent strains.

There is very little information on MDV prevalence and severity around the world, and even less data tracking MDV incidence within one farm. However, Heier et al. (1999) present results from a longitudinal study with data collected in Norway on flock-level cumulative incidence and mortality for Norwegian and imported layer birds in caged housing. The mortality in this case refers to the percentage of birds within the flock, when examined portmortem, had evidence of MD. For layer flocks living together between the ages of 16 and 68 weeks, in groups of around 7,000, there was a range of 5-8.2% mortality. These

birds in all the farms were vaccinated at hatch. It is difficult to compare these data directly with the results shown in this chapter since the method of housing and chicken population are very different. However, for cohort durations of 2 months, there is about a 1% total mortality when 5,000 vaccinated birds are kept together. This is at least in the right order of magnitude (allowing for the cohort duration) and the results do not falsify the validity of the results presented here.

Since there is very little known about the prevalence of MDV on farms around the world, this work sheds lights on the extent to which farms may be affected by MDV. For real industrial broiler farms, where there are currently about 30,000 birds living together at a stocking density of $35kg/m^2$ for around 30 days, usually there is no vaccination and, although the total mortality will rise by only 0.7% (210 birds), there will be an MDV prevalence before slaughter of 88% for a relatively low virulence strain (04CRE). Therefore choosing shorter cohort durations masks the huge infection rate on the farm. Suppose that the same farm was infected with a more virulent strain (02LAR) and the owners felt that the losses due to MDV were too high so they decided to vaccinate the flock, the removed cases would drop to a negligible quantity. However, despite there being no visible signs of Marek's disease, it would still require a 98% reduction in the MDV persisting at the end of the cohort to reduce the chance of MDV persisting the next generation to 5%. A combination of short cohort durations and vaccination can therefore allow MDV to persist undetected in farm environments. This would suggest to industry that the death toll from a cohort may not be the best indicator for disease prevalence in the flock. Furthermore, undetected virus within environments will not only allow undetected spread from farm to farm, but will increase the risk of evolution of more virulent strains as predicted by the model.

This is the first exposition of the effect of exposure to MDV on a flock of broiler birds where all parameters have been formally fitted to data. This approach enables understanding of the extent to which a flock of birds may be affected by disease and how difficult it is for the farm to return to a disease-free state. Quantification of the epidemiology of MDV can not only help to elucidate hypotheses for evolution and persistence of more virulent strains of the virus but can provide evidence for implementing control methods to reduce the impact of the virus and its evolution in the future.

5 Farm Networks & MDV

5.1 Introduction

The last two chapters were concerned with the invasion and fitness of Marek's disease virus strains on a single farm in one cohort of birds. This approach is extended to look at a network of poultry farms, using parameters estimated from the past three chapters.

This work is the first attempt to track the persistence of MDV strains in a network of farms. It will concern the extent to which cleaning, vaccination, farm size, length of cohort duration, transmission network size and network connectivity affect the persistence of the virus within that network.

There has been much modelling of pathogen spread through host networks which have shown that there is much scope for complicated parameterisations of the demographic structure, disease transmission processes and biosecurity measures. For example, Watts and Strogatz (1998) quantified the effect of 'small-world networks' on diverse modelling applications; May and Lloyd (2001) assessed the impact on scale-free networks on qualitative disease dynamics; van Baalen (2002) introduced simple epidemiological models on network structures and assess the implications for the evolution of virulence; Read and Keeling (2003) studied the evolution of parasite traits within different types of network; Keeling and Eames (2005) reviewed the implications of network structures on disease outcome and control; Woolhouse et al. (2005) analysed specific cattle networks and assessed the impact the network strucutre on the size of the basic reproductive number of an infection.

However, choosing a good network model and estimating parameters comes at the price of good data, which is unfortunately lacking for MDV. The reasons behind this were noted in Chapter 1, but mainly hinge on the fact that MD is not a notifiable disease. Thus, a clear picture of MDV prevalence within farm networks is patchy at best, although there is evidence that in addition to strains becoming more virulent, these strains have increased in prevalence among farms (Witter, 2001). With this in mind, this chapter limits itself to disease transmission on a simple network of farms, which can be easily parameterised with information gleaned from earlier studies. It is hoped that this work may generate hypotheses concerning the prevalence of MDV strains which can be tested in the field.

5.2 Methods

5.2.1 Network Epidemiological Model

A network of broiler farms is modelled such that each farm is in either one of two states: 'susceptible' or 'infected'. An infected farm is defined as having at least one MDV infected bird during the life of the cohort. A susceptible farm is one in which there is has never been an MDV infected bird during the life of the cohort. For simplicity, I make a number of assumptions: that each farm is assumed to have only one barn; that all farms have equal numbers of birds; and, all other important parameters are the same.

An infected farm is assumed to have the potential to transmit to other farms when the cohort of chickens reaches the end of its life in the barn. Therefore transmission between farms is naturally modelled by using a discrete time model, where each time step corresponds to the end of a cohort generation. At the end of every cohort generation two processes are modelled in turn:

1. the removal of birds, by a catcher company, to slaughter. Infectious farms infect the fomites (e.g. bird crates of the catcher company) and the virus is transmitted to other farms in the network (note that the order in which farms are visited is not considered), then;

2. the cleaning of farms before new cohorts arrive.

The contamination of farms can occur via human movement, especially catcher companies whose responsibility it is to remove a cohort of birds and take them for slaughter. These catcher companies will service multiple broiler farms (surveys suggest that each UK catcher company services between 1-200 farms (Dent et al., 2008)). The effective transmission network for a single farm will be defined as either 1) all of the farms in the network (well-mixed network) or 2) the closest four neighbours in a farm lattice model (von Neumann neighbourhood). Any transmission between farms is within this effective transmission network.

At the end of a cohort generation before cleaning, a constant quantity of virus is left in a barn at the end of a cohort, Z_c (Section 4.3.2.1), which is assumed to be the same for all infected farms. Let the number of infected farms able to transmit to farm j at time n, be $I_j^{(n)}$. The total virus in all the farms able to transmit to farm j is therefore $Z_c I_j^{(n)}$. The virus that contaminates farm j is a proportion, θ, of this amount, $\theta Z_c I_j^{(n)}$, where θ is a contamination parameter. All farms are then cleaned and a proportion, ϵ, of this virus is left.

Therefore the amount of virus on a susceptible farm j at the start of the next cohort is $\epsilon\theta Z_c I_j^{(n)}$, where 1-$\epsilon$ is the on-farm cleaning efficiency; and the amount of virus on an infected farm at the start of the next cohort is $\epsilon Z_c[1 + \theta I_j^{(n)}]$ because Z_c is left before cleaning from the previous cohort.

Therefore, the probability of a farm j moving from a susceptible state to an infected state at time n is

$$p_j^{(n)} = P\left(\epsilon\theta Z_c I_j^{(n)}\right)$$

Whilst the probability of a farm j moving from an infected state to a susceptible state at time n is

$$q_j^{(n)} = 1 - P\left(\epsilon Z_c[1 + \theta I_j^{(n)}]\right)$$

where the probability of remaining in a susceptible or infected state is $1 - p_j^{(n)}$ or $1 - q_j^{(n)}$ respectively and P is the probability of an outbreak given an initial inoculum of virus (see Section 5.2.4).

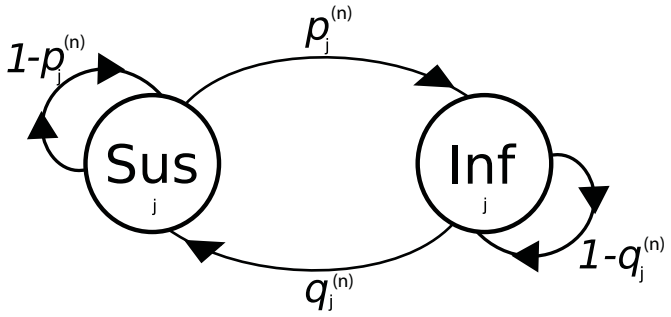


Figure 5.1: Transition diagram showing the possible transitions to and from each state (Susceptible or Infected) at time n. The possible movements are associated with probabilities.

The proportion of farms at time n in an uninfected state is denoted by s_n and the proportion of infected farms by i_n. The total number of farms in the population, f, is assumed to be constant and therefore $s_n + i_n = 1$ for any $n \in \mathbb{N}$. For a given farm the probabilities can be represented in a transition diagram depicted in Figure 5.1. This system is a Markov chain since the state of the system at time n only depends on the state of the system at $n-1$ (Norris, 1997).

In the well-mixed transmission case, $p_j^{(n)} = p^{(n)}$ and $q_j^{(n)} = q^{(n)}$. At any time, n, this system can be represented by the following equation,

$$\begin{pmatrix} s_n \\ i_n \end{pmatrix} = \mathbf{Q} \begin{pmatrix} s_{n-1} \\ i_{n-1} \end{pmatrix} = \begin{pmatrix} 1-p^{(n)} & q^{(n)} \\ p^{(n)} & 1-q^{(n)} \end{pmatrix} \begin{pmatrix} s_{n-1} \\ i_{n-1} \end{pmatrix}$$

Since the transition probabilities depend on the state of the chain at time n, the Markov chain is classified as time-inhomogeneous. Most theory for Markov chain systems is concerned with time-homogeneous systems (e.g. Durrett, 1999) and therefore this system is solved numerically.

5.2.2 Parameter Values

Two sizes of networks will be considered: small (f=20 farms) and large (f=200 farms). There are two scenarios: every farm is assumed to be equivalent to a barn of either a small number of chickens (S_0=500 birds at a low stocking density, $5kg/m^2$) or a large number (S_0=30,000 birds at $35kg/m^2$) where all the farms in the network are all unvaccinated or HVT vaccinated. For every

farm in the network, the cohort duration will either have a short cohort duration (T_c = 30 days) or a long cohort duration (T_c = 60 days). Contamination parameters (θ, ϵ) will be varied over the range 1x10^{-5} to 1.5x10^{-1}.

The number of simulations for each parameter combination is set to 300, since preliminary analysis showed an acceptable level of convergence on the mean prevalence was reached with this number.

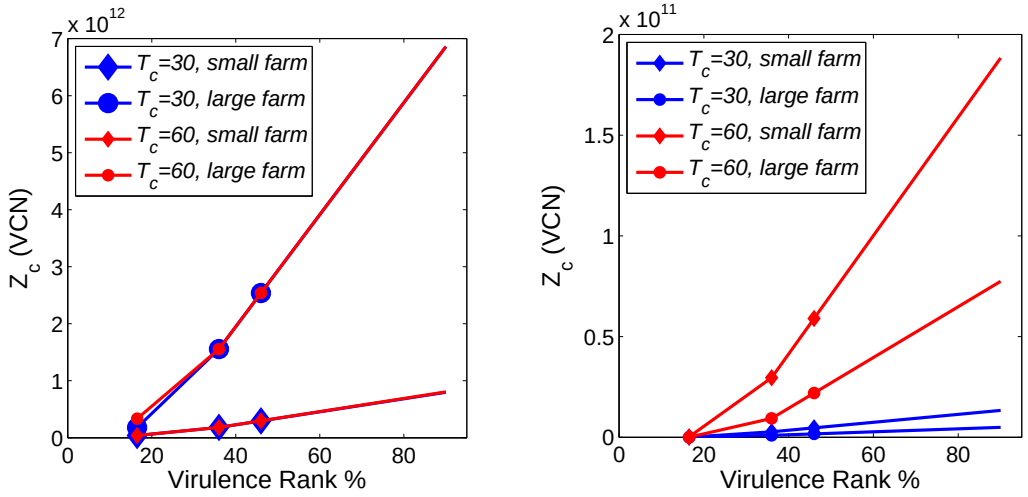
Note that the transmission parameters are not scaled with network size and thus larger networks potentially have larger basic reproductive numbers.

5.2.3 Virus at the End of the Cohort Duration, Z_c

The mean amount of virus left at the end of a cohort duration was calculated in Chapter 4. Graphs of this are shown in Figure 5.2a (Unvaccinated birds) and Figure 5.2b (HVT Vaccinated birds). In the unvaccinated case the farm is saturated with infection and a larger barn, with more infected individuals, harbours more virus. In the vaccinated case the amount of virus does not change with population size because the farm is not saturated with infection. Although, when the individuals are more densely stocked, the volume of the barn is reduced and therefore both the amounts of dust and virus in the atmosphere are reduced too.

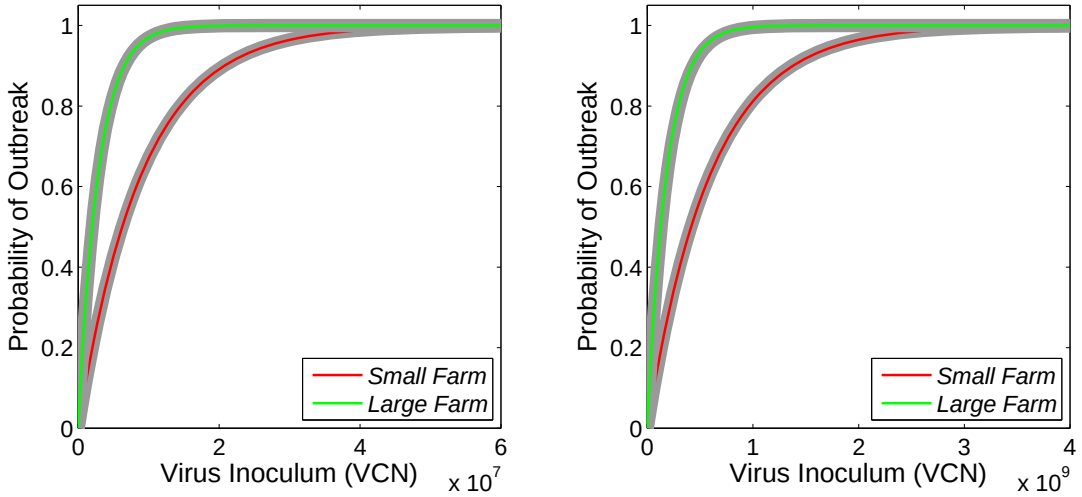
5.2.4 Probability of an Outbreak, P

The probability of an MDV outbreak was calculated in Chapter 4. A function relating this probability, P, to the virus inoculum, V_0, was fitted to this expectation such that $P = 1 - \exp(-u V_0)$ where the constant, u, was estimated via least-squares (Figure 5.3). For an unvaccinated population, $u_{unvacc/small}$ = 1.11x10^{-7}, $u_{unvacc/large}$ = 3.51x10^{-7} for a small and large farm respectively. For a HVT vaccinated population, $u_{vacc/small}$ = 1.67x10^{-9}, $u_{vacc/large}$ = 5.46x10^{-9} for a small and large farm respectively. Curve fitting was carried out in Matlab® (2007).



(a) Unvaccinated **(b)** HVT Vaccinated

Figure 5.2: Total Virus at Cohort Duration End: the estimates of virus left at the end of a cohort duration from Chapter 4. This can vary with Cohort Duration, T_c, measured in days, depending on whether the farm is small (S_0=500 birds at stocking density of $5kg/m^2$) or large (S_0=30,000 birds at stocking density $35kg/m^2$). Note the different y-axis scales.



(a) Unvaccinated **(b)** HVT Vaccinated

Figure 5.3: Probability of Outbreak: An increasing probability of an outbreak on a small farm (S_0=500 birds at $5kg/m^2$) and large farm (S_0=30,000 birds at $35kg/m^2$), where the farm is either unvaccinated or HVT vaccinated. The grey lines correspond to the expected value based on calculations discussed in Chapter 4, the red/green lines are the best fit of $P = 1 - \exp(-uV_0)$ to this expected value (V_0 is the virus inoculum variable and u is $u_{unvacc/small}$, $u_{unvacc/large}$, $u_{vacc/small}$ or $u_{vacc/large}$). Note the different x-axis scales.

5.3 Results

For all the results presented here, the time-evolution of the Markov chain showing the prevalence of the infected farms was run for 100 years. This corresponds to 1216 and 608 cohort generations of durations 30 days and 60 days respectively.

The results show three qualitatively different types of Markov chain time-evolutions: extinction (characterised by an immediate decline in the number of infected farms), epidemic (characterised by an initial rise in the number of infected farms, but an eventual decline to zero prevalence) and endemic (characterised by an initial rise in the prevalence of infected farms such that the prevalence rises to nearly, or equal to, 100%). Therefore there are two outcomes to consider: 1) the maximum mean prevalence of a strain (i.e. the maximum prevalence an epidemic reaches, when the average of the simulations is calculated) 2) the rate at which this maximum mean prevalence is reached (or the rate at which an stable endemic prevalence is reached).

Figures 5.4-5.13 show the effect on outcomes 1) and 2) when parameters of interest are changed which dictate the following variables: Virulence Rank, contamination of farms, transmission network size, farm size, network size, vaccination and cohort duration.

5.3.1 Effect of Virulence Rank, v

In all the figures presented in Section 5.3, the effects of different Virulence Ranks on the prevalence of infected farms through time are shown. A strain with a greater Virulence Rank has a greater maximum mean prevalence than less virulent strains (clearly seen in Figures 5.4-5.13). A higher Virulence Rank will also increase the rate at which that maximum prevalence is reached.

5.3.2 Effect of Contamination Parameters (θ,ϕ)

Increasing either parameter θ or ϵ increases the maximum mean prevalence for all the strains and the rate at which that prevalence is reached, although the effect of ϵ is much greater (compare Figure 5.4c to 5.5a and 5.5c, Figure

5.4d to 5.5b and 5.5d) because θ does not affect the amount of virus remaining on an infected farm after cleaning.

Therefore in all cases, increasing the contamination parameters θ and ϵ together increases the maximum prevalence for all the strains and increases the rate at which the endemic prevalence is reached (seen in all figure columns e.g. Figures 5.6a, 5.6c, 5.6d through to 5.13a, 5.13c, 5.13d).

5.3.3 Effect of Effective Transmission Network Size

Changing from a well-mixed network to nearest neighbour transmission slows the rate at which that prevalence is reached (e.g. compare Figures 5.4 to 5.7 and Figures 5.6 to 5.8).

5.3.4 Effect of Farm Size (s_d,S_0)

For either unvaccinated or vaccinated networks, changing the farm structure to a more industrial cohort size and density (from 500 birds at $5kg/m^2$ to 30,000 birds at $35kg/m^2$) increases the maximum mean prevalence of a strain (compare Figure 5.4 to Figure 5.6 and Figure 5.9 to Figure 5.10).

5.3.5 Effect of Network Size

Increasing the number of farms in the transmission network decreases the variability of the prevalence, giving a narrower confidence interval on the mean prevalence for each strain over time (compare left hand columns of Figures 5.4-5.13 with right hand columns in the same figures). The consequence of this stochasticity is that, in some cases, a small network of farms leads to a extinction of the strain which is not the case for larger networks (e.g. compare Figure 5.9a to 5.9b). This effect decreases when other parameters are such that the probability of strain extinction is very low. However, when all strains in each case can persist (with a combination of intermediate values for θ and ϵ and high virulences or simply large θ and ϵ values (e.g. compare Figure 5.8e to 5.8f), the size of the network only has a big positive effect on the *rate* at which the maximum strain prevalence is reached. In nearest neighbour transmission,

a larger network slows the rate of the spread, whereas when the transmission is well-mixed, a larger network increases the rate at which the maximum mean prevalance is reached as there are more contacts and the dynamics are accelerated.

5.3.6 Effect of Vaccination (sham or HVT)

The prevalence of infected farms housing vaccinated birds was calculated for well-mixed transmission (Figure 5.9 (small farms) and Figure 5.10 (large farms)) and nearest neighbour transmission (Figures 5.11 (small farms)). Using a network of farms in which all the birds are vaccinated drastically decreases the maximum mean prevalence of all strains. Note the large increase in the size of the contamination parameters θ and ϵ to achieve similar prevalences for some strains (e.g. compare Figure 5.4e to Figure 5.9e, Figure 5.6e to Figure 5.10e and Figure 5.7e to Figure 5.11e).

5.3.7 Effect of Cohort Duration (T_c)

There is no effect of changing the cohort duration for unvaccinated networks on the maximum mean prevalence and the number of cohort generations is takes to reach that maximum mean prevalence. Although using a cohort duration of 30 days instead of 60 days will half the time it takes to reach the maximum mean prevalence. For vaccinated networks, there is a substantial rise in the maximum mean prevalence of a strain for longer cohort durations because there is more virus is produced at the end of each cohort duration. Figures 5.9 and 5.10 show well-mixed transmission results for vaccinated populations for T_c=30 days which should be compared to Figures 5.12 and 5.13 showing the same results for T_c=60 days.

5.3.8 Rate at Which The Maximum Prevalence Is Reached

The time taken to reach a maximum prevalence (in the case of an epidemic or endemic situation) varies widely. In most cases only a couple of generations are needed (e.g. Figure 5.10f), for others a number of years is required (e.g. Figure 5.4e). In the case where an epidemic occurs and the strain subsequently

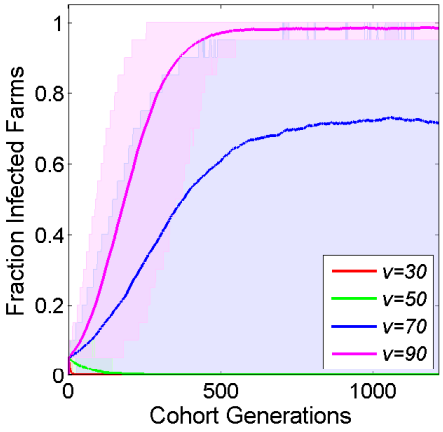
Increasing Variable	Rate to Maximum Prevalence	Maximum Mean Prevalence
Virulence Rank	+	+
Contamination, θ	+	+
Contamination, ϵ	+	+
Farm Size (S_0,s_d)	+	+
Network Size, f	+/-*	+
Transmission Network Size	+	+
Vaccination (introduction)	na	-
Cohort Duration, T_c	+	+

Table 5.1: Effect of Increasing Variables on both the rate at which an epidemic occurs or the rate at which an endemic prevalence is reached and the maximum mean prevalence of the strain. *There is an increase in the rate to maximum prevalence for well-mixed networks, but a decrease in the nearest neighbour transmission networks.

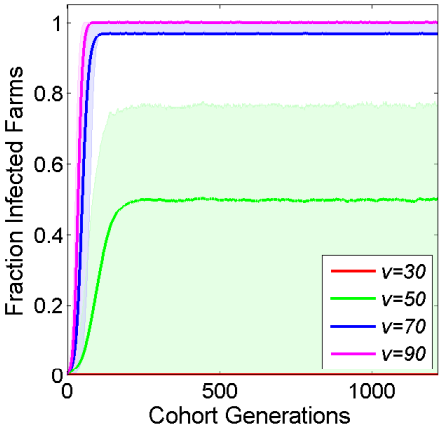
becomes extinct in the population, the rate of decline may be so slow that it appears to be endemic (e.g. Figure 5.11e for a strain where v=50).

5.3.9 Summary

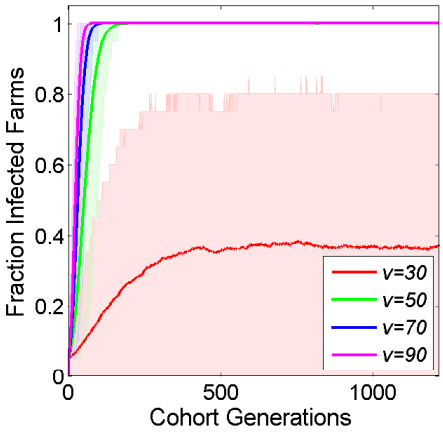
A table of the main results of this chapter are given in Table 5.1. Included are the outcomes: rate to maximum prevalence and maximum mean prevalence. The rate at which an epidemic occurs correlates with how severe it is (i.e. the rate to the maximum prevalence changes positively with the maximum mean prevalence). This can be seen by noting that if one outcome is changed by a variable in one way, the other outcome changes in the same way.



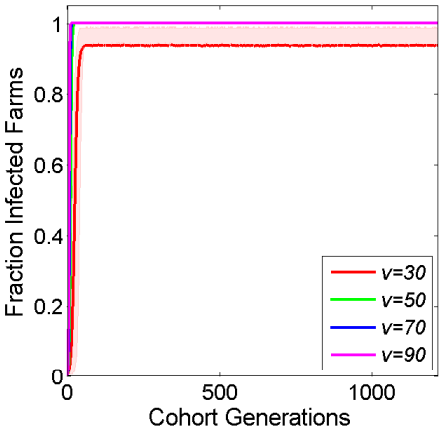
(a) $f = 20, \theta, \epsilon$=0.0001



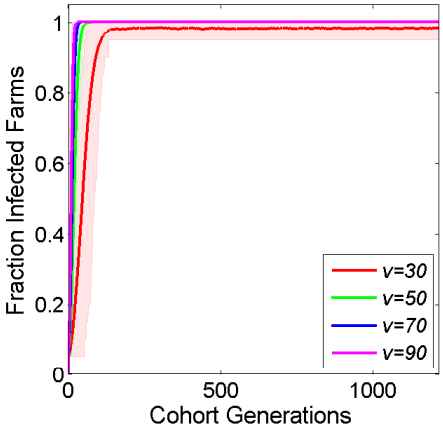
(b) $f = 200, \theta, \epsilon$=0.0001



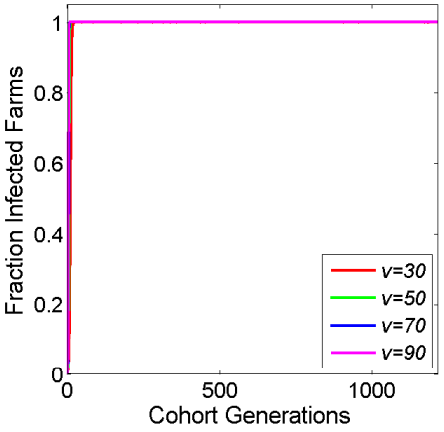
(c) $f = 20, \theta, \epsilon$=0.0003



(d) $f = 200, \theta, \epsilon$=0.0003

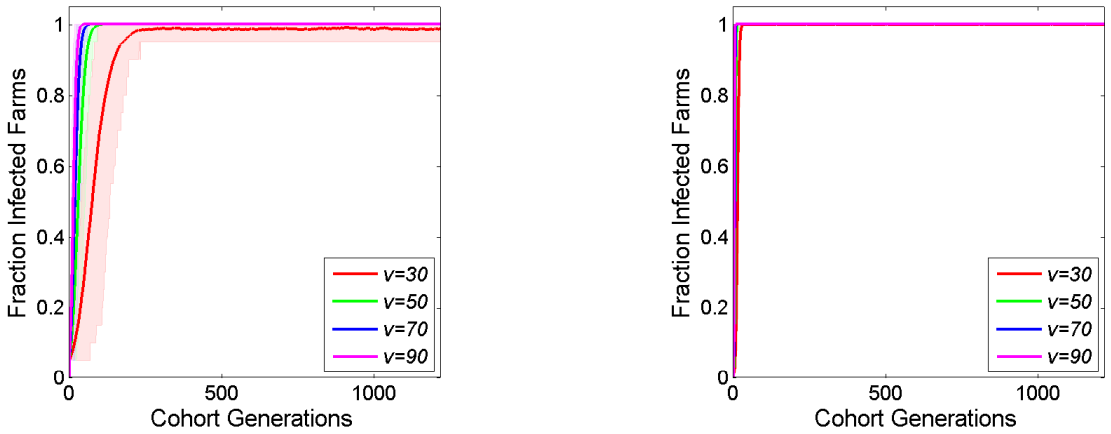


(e) $f = 20, \theta, \epsilon$=0.0005



(f) $f = 200, \theta, \epsilon$=0.0005

Figure 5.4: Convergence of Markov chain with Well-Mixed Transmission (Unvaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 500$ and $s_d = 5kg/m^2$ and T_c=30 days.



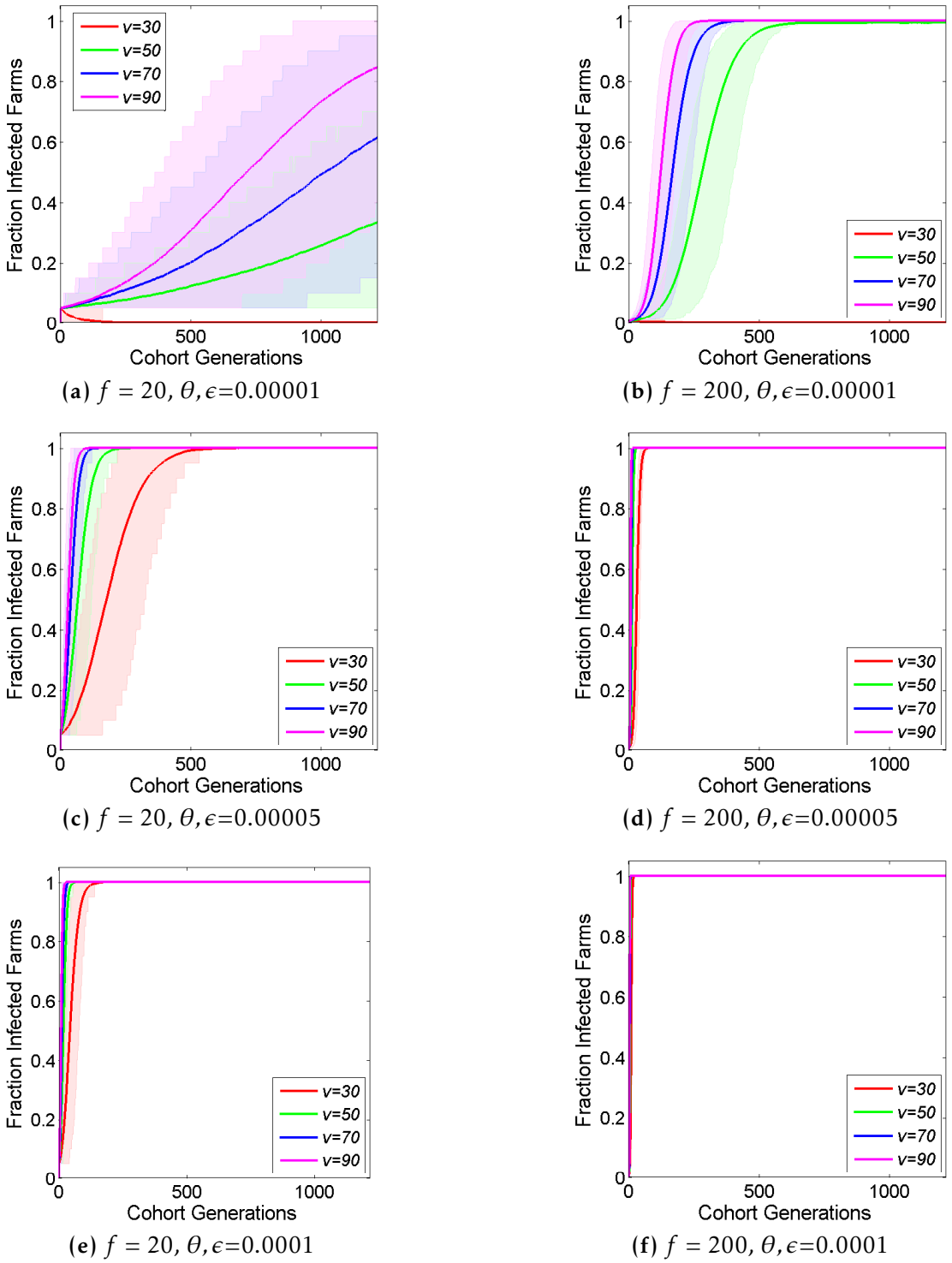
(a) $f = 20$, θ=0.0003,ϵ=0.0005

(b) $f = 200$,θ=0.0003,ϵ=0.0005

(c) $f = 20$, θ=0.0005,ϵ=0.0003

(d) $f = 200$,θ=0.0005,ϵ=0.0003

Figure 5.5: Convergence of Markov chain with Well-Mixed Transmission (Unvaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 500$ and $s_d = 5kg/m^2$ and T_c=30 days.

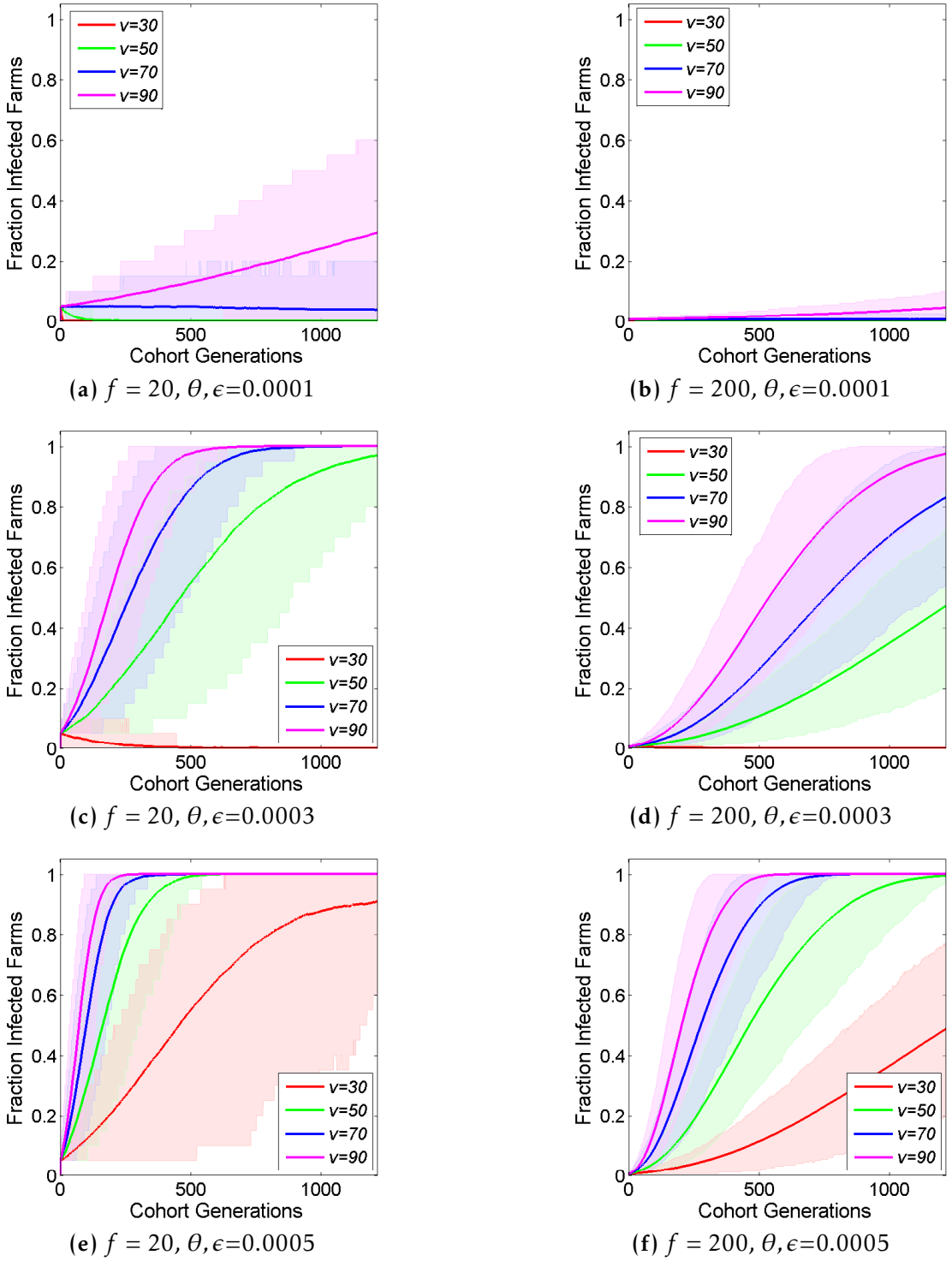


(a) $f = 20$, θ, ϵ=0.00001 (b) $f = 200$, θ, ϵ=0.00001

(c) $f = 20$, θ, ϵ=0.00005 (d) $f = 200$, θ, ϵ=0.00005

(e) $f = 20$, θ, ϵ=0.0001 (f) $f = 200$, θ, ϵ=0.0001

Figure 5.6: Convergence of Markov chain with Well-Mixed Transmission (Unvaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 30,000$ and $s_d = 35kg/m^2$ and T_c=30 days.

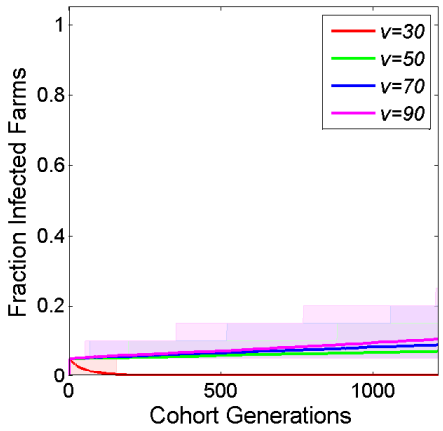


(a) $f = 20$, θ, ϵ=0.0001 (b) $f = 200$, θ, ϵ=0.0001

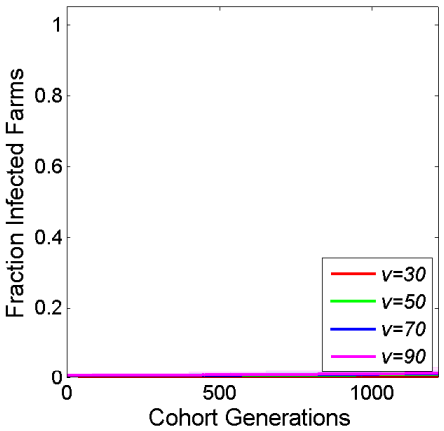
(c) $f = 20$, θ, ϵ=0.0003 (d) $f = 200$, θ, ϵ=0.0003

(e) $f = 20$, θ, ϵ=0.0005 (f) $f = 200$, θ, ϵ=0.0005

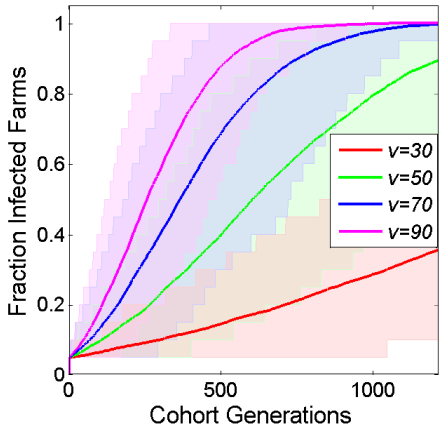
Figure 5.7: Convergence of Markov chain with Nearest-Neighbour Transmission (Unvaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 500$ and $s_d = 5kg/m^2$ and T_c=30 days.



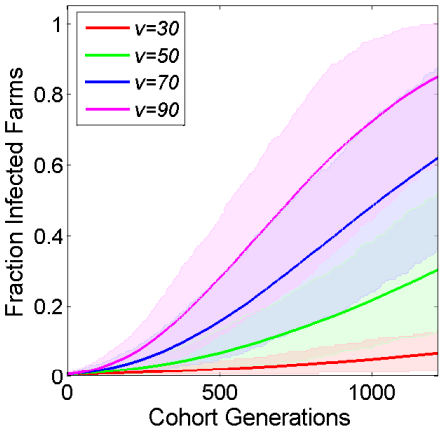
(a) $f = 20$, θ, ϵ=0.00001



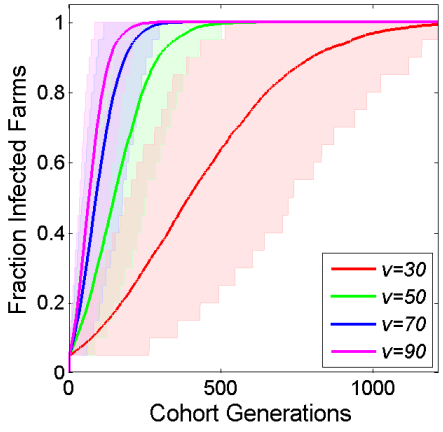
(b) $f = 200$, θ, ϵ=0.00001



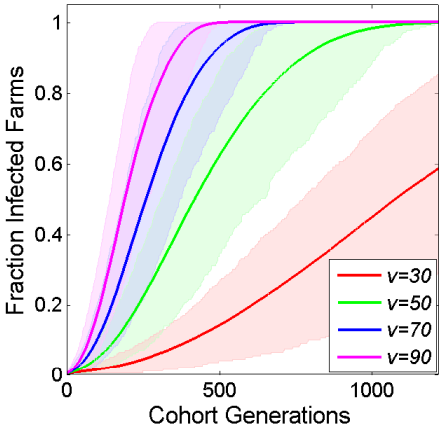
(c) $f = 20$, θ, ϵ=0.00005



(d) $f = 200$, θ, ϵ=0.00005

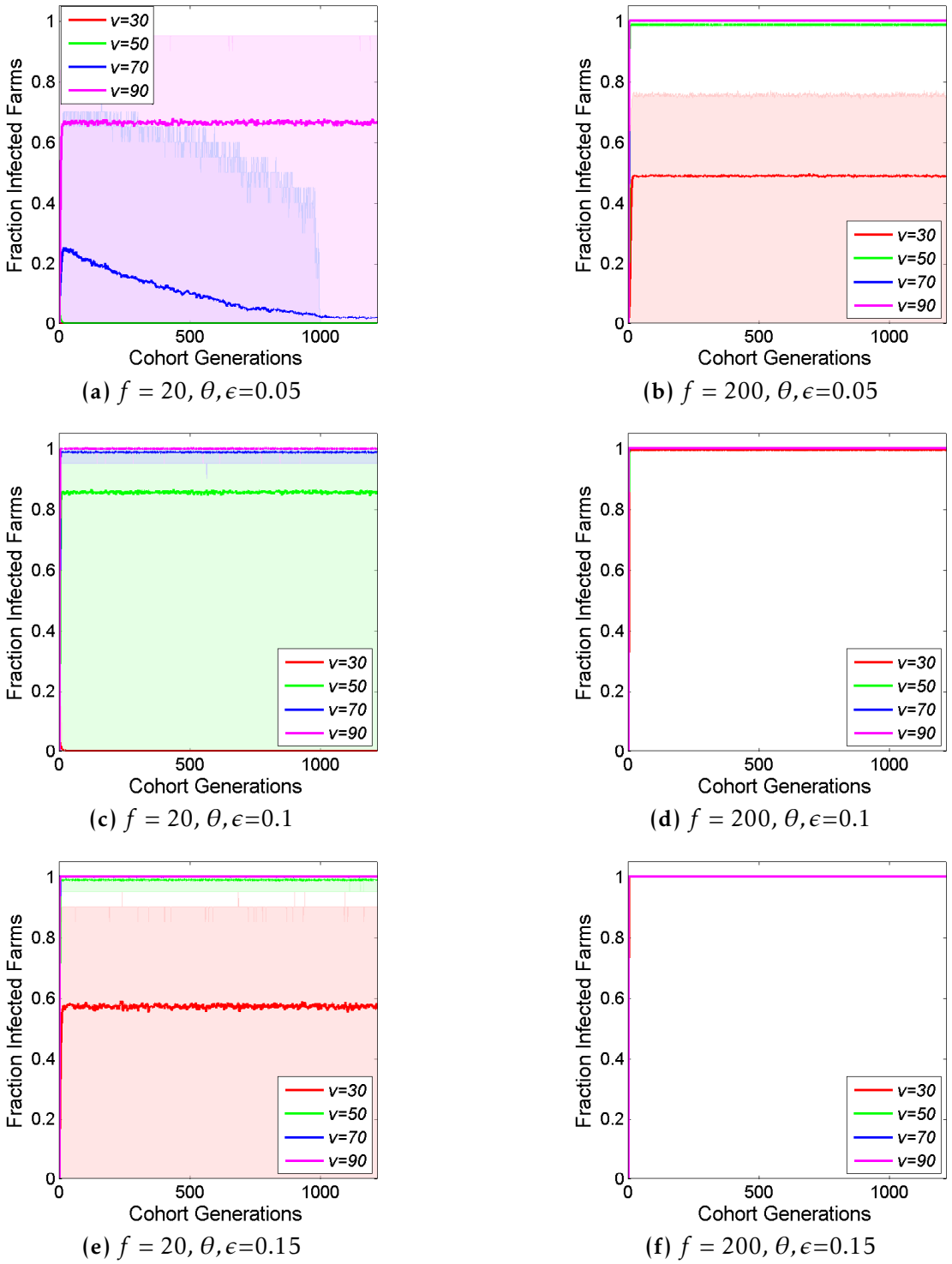


(e) $f = 20$, θ, ϵ=0.0001



(f) $f = 200$, θ, ϵ=0.0001

Figure 5.8: Convergence of Markov chain with Nearest-Neighbour Transmission (Unvaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 30,00$ and $s_d = 35kg/m^2$ and T_c=30 days.

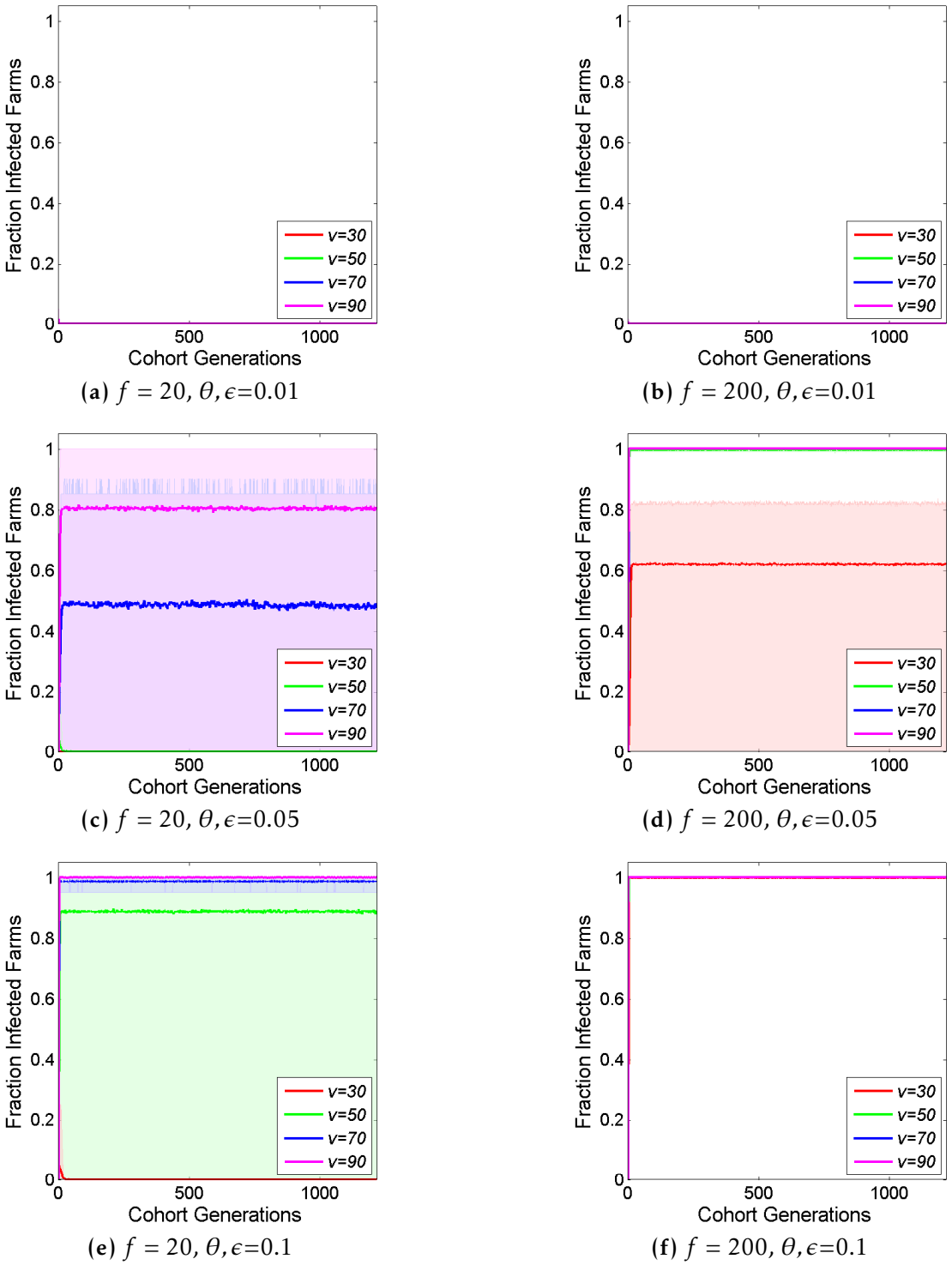


(a) $f = 20, \theta, \epsilon$=0.05 **(b)** $f = 200, \theta, \epsilon$=0.05

(c) $f = 20, \theta, \epsilon$=0.1 **(d)** $f = 200, \theta, \epsilon$=0.1

(e) $f = 20, \theta, \epsilon$=0.15 **(f)** $f = 200, \theta, \epsilon$=0.15

Figure 5.9: Convergence of Markov chain with Well-Mixed Transmission (HVT vaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 500$ and $s_d = 5kg/m^2$ and T_c=30 days.



(a) $f = 20$, θ, ϵ=0.01 **(b)** $f = 200$, θ, ϵ=0.01

(c) $f = 20$, θ, ϵ=0.05 **(d)** $f = 200$, θ, ϵ=0.05

(e) $f = 20$, θ, ϵ=0.1 **(f)** $f = 200$, θ, ϵ=0.1

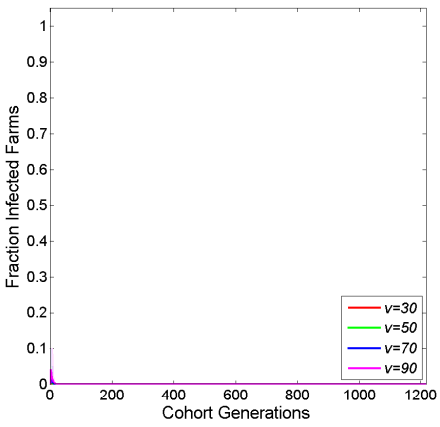
Figure 5.10: Convergence of Markov chain with Well-Mixed Transmission (HVT vaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 30,000$ and $s_d = 35kg/m^2$ and T_c=30 days.

5.4 Discussion

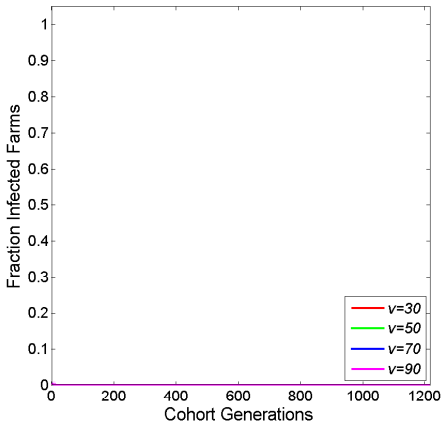
Three types of phenomena can be observed in these results: extinction, epidemic, or persistence at endemic prevalences near, or equal to, 100%. It is anticipated that the limit distributions of the system are $i_n = 0$ and $i_n = 1$, since the epidemic strains always include a zero prevalence in their confidence intervals and the endemic strains never include a zero in theirs. The limit distribution would then depend on the initial values of the variables which have been highlighted in Section 5.3.

The more virulent a strain is the better able it is to persist in a network of farms, the better able it is to persist at a higher maximum mean prevalence (in an epidemic situation) and the better able it is to reach that positive prevalence at a faster rate. This is expected because Virulence Rank is positively correlated with a greater amount of virus per *mg* of dust.

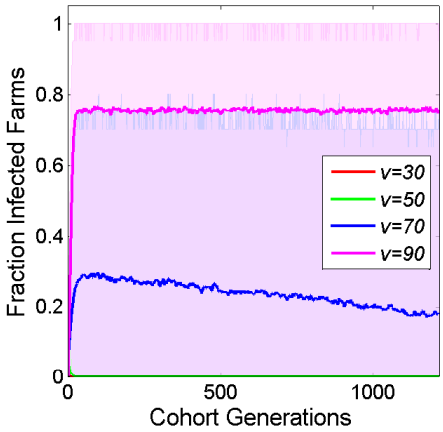
Improved cleaning on a farm and reduced contamination between farms can provide a better chance of the virus becoming extinct in the farm population, or at least can reduce the mean maximum prevalence of the strains within the network in the epidemic situation. This result is intuitively correct and should be expected in this simple network model. The effect of on-farm cleaning has a greater impact on reducing the maximum mean prevalence of a strain and its rate of increase within the network than between-farm cleaning. This can be expected due to the fact that virus extinction on one farm reduces the network prevalence by both that one farm plus any reduction in transmission to other



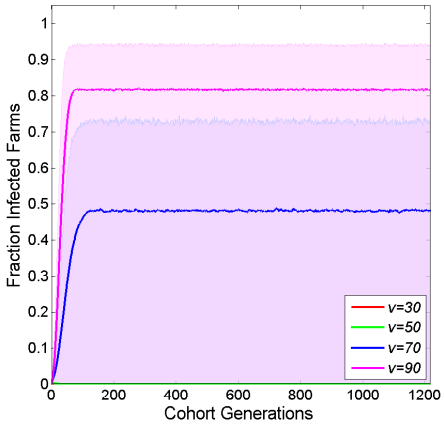
(a) $f = 20, \theta, \epsilon$=0.05



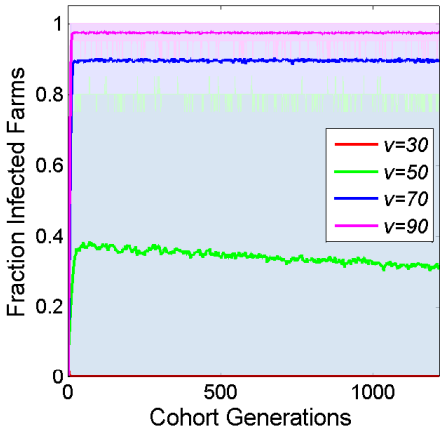
(b) $f = 200, \theta, \epsilon$=0.05



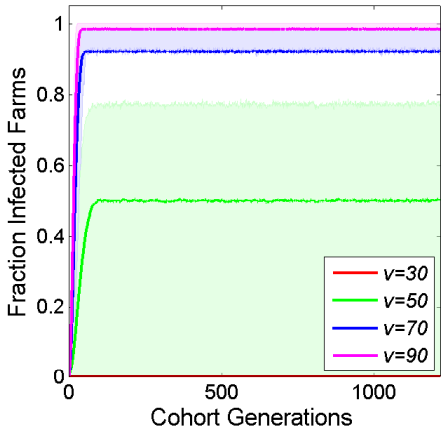
(c) $f = 20, \theta, \epsilon$=0.1



(d) $f = 200, \theta, \epsilon$=0.1

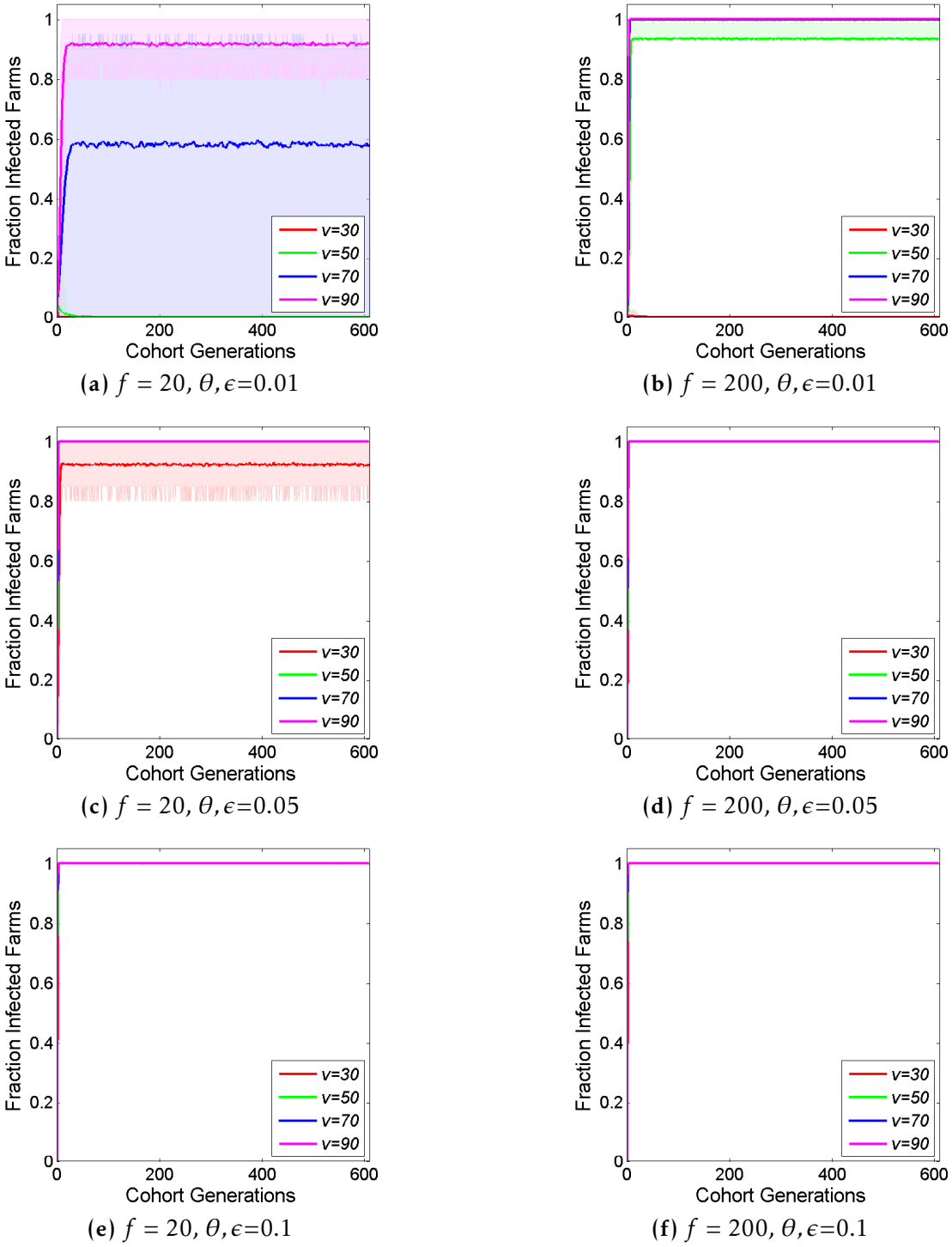


(e) $f = 20, \theta, \epsilon$=0.15



(f) $f = 200, \theta, \epsilon$=0.15

Figure 5.11: Convergence of Markov chain with Nearest-Neighbour Transmission (HVT vaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 500$ and $s_d = 5kg/m^2$ and T_c=30 days.

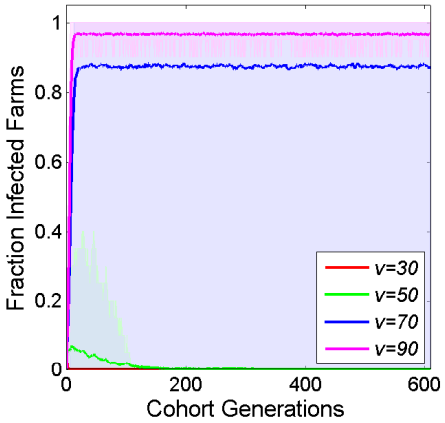


(a) $f = 20, \theta, \epsilon$=0.01 **(b)** $f = 200, \theta, \epsilon$=0.01

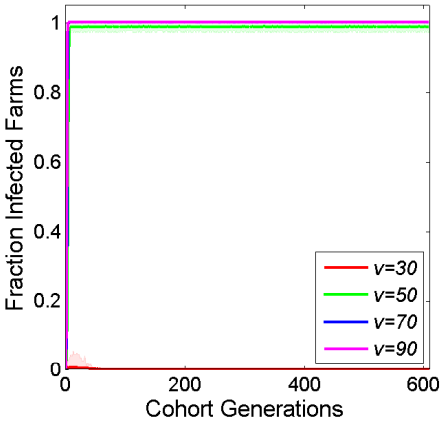
(c) $f = 20, \theta, \epsilon$=0.05 **(d)** $f = 200, \theta, \epsilon$=0.05

(e) $f = 20, \theta, \epsilon$=0.1 **(f)** $f = 200, \theta, \epsilon$=0.1

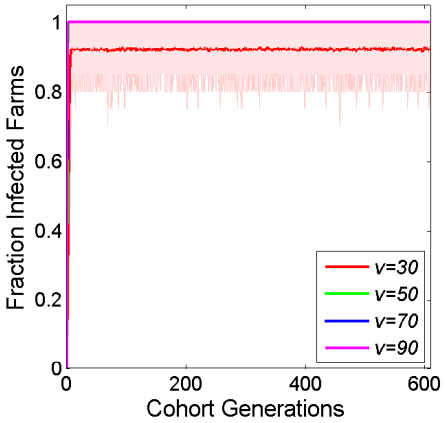
Figure 5.12: Convergence of Markov chain with Well-Mixed Transmission (HVT vaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 500$ and $s_d = 5kg/m^2$ and T_c=60 days.



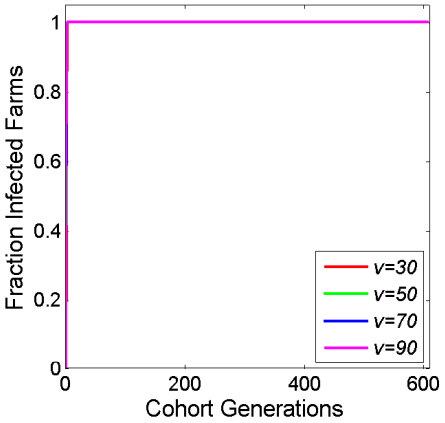
(a) $f = 20, \theta, \epsilon$=0.01



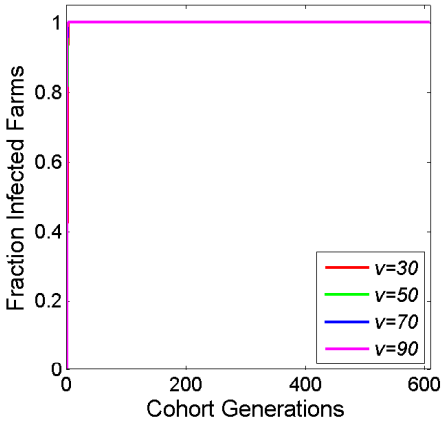
(b) $f = 200, \theta, \epsilon$=0.01



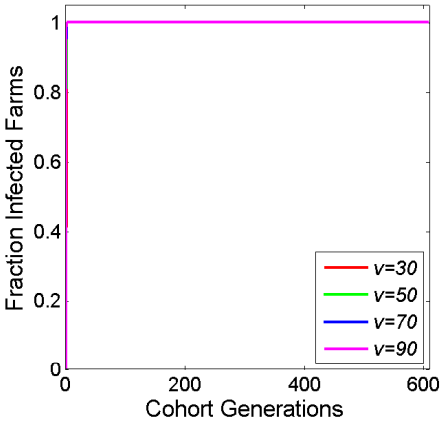
(c) $f = 20, \theta, \epsilon$=0.05



(d) $f = 200, \theta, \epsilon$=0.05



(e) $f = 20, \theta, \epsilon$=0.1



(f) $f = 200, \theta, \epsilon$=0.1

Figure 5.13: Convergence of Markov chain with Well-Mixed Transmission (HVT vaccinated Population): The trajectory of the proportion of infected farms, when initially one farm is infected with a strain of virulence, v. The coloured regions show the 95% confidence interval for their respectively mean values. In all cases, $S_0 = 30,000$ and $s_d = 35kg/m^2$ and T_c=60 days.

farms. Reduction of contamination between-farms serves to limit the rate of spread between farms, but does nothing to stop the reinfection of a farm infected in its last cohort.

The bigger the farms are in a network, the greater the maximum mean prevalence at which a virus can persist in that network. Farm size positively affects the total virus produced by a cohort for unvaccinated farms (Figure 5.2a), but negatively affects it on vaccinated farms (Figure 5.2b). However, the probability that an inoculum creates an outbreak is always less on smaller farms. The net result in both cases is that there is always a larger maximum mean prevalence when the farm is large.

Small farm networks lead to more variability in the prevalence of a virus strain, and hence the strains are more likely to go extinct in both well-mixed and nearest-neighbour transmission models. Larger networks of farms reduce this variability and so have higher persistence probabilities when there is a chance of extinction for the smaller networks. The stochastic effects of small networks are well-documented, and these results are in line with the theory (Murray et al., 1986; Mollison, 1995; Shigesada and Kawasaki, 1997).

Nearest-neighbour transmission increases the probability of virus extinction for small contamination probabilities. The increased chance of extinction can be accounted for by the fact that, at the start of an outbreak, there are only a few farms to which transmission can occur and stochastic effects can force the initial outbreak to zero more easily due to the reduced transmission. This limited transmission also slows the rate at which the strain mean maximum prevalence is reached. This is because the spatially explicit nature of the network allows an epidemic wave front to limit the size of the epidemic at each generation.

Cohort duration only changes the rate at which the maximum mean prevalence is reached when the network is comprised of unvaccinated farms (because the duration of each generation changes). This is because the probability of an outbreak does not depend on the duration of a cohort, and the quantity of virus is not affected by cohort duration in any significant way (Figure 5.2a). However, for vaccinated networks, since a longer cohort duration is associated with a large quantity of virus within a barn (Figure 5.2b), a longer cohort duration is associated with an increased maximum mean prevalence for a virus strain in the network.

Vaccinated networks require a much greater level of contamination to allow any of the strains to reach the same maximum mean prevalences. For any of the more virulent strains to reach the same probabilities of persistence as the unvaccinated network, both contamination parameters θ and ϵ have to increase by over 2000 times (e.g. compare Figure 5.4c (unvaccinated) to Figure 5.4e (vaccinated)). If the contamination parameters are this high (i.e. for strains to reach even low prevalences in vaccinated networks) in unvaccinated networks, all strains examined here will become endemic in the network and persist at 100% prevalence.

If a strain is able to persist in the farm network, and the network is well-mixed, it can usually reach maximum prevalence in less than 30 generations. Only in the cases where either transmission is by nearest neighbour or when the contamination parameters are very small is the maximum mean prevalence reached in over 10 years (indeed the extreme cases suggest that the strains cannot reach their maximum prevalence even after 100 years (e.g. Figure 5.7a)). Since there has been approximately one vaccine introductions every 10-15 years in response to the waves of more virulent strains, each strain would have to spread significantly through the farm network in less than 10-15 years to cause concern. It is possible that a more virulent strain, having entered a system of farms, could spread quickly (if the transmission occurred frequently between farms), and an introduction of a single virulent strain would be sufficient to generate a country-size epidemic. In a well-mixed farm network, increasing the number of farms will only increase the rate at which the maximum mean prevalence is reached and therefore a more virulent strain could spread through a network of broiler farms the size of the UK in less than 10 years. However, in the more realistic case where transmission is more restricted (i.e. there is either limited contact between the different farms in a country or the contamination levels on farms is so low), an invading strain would not be able to create an epidemic on the time scale that MDV has been evolving. In the latter case for a country the size of the UK with 3000 broiler farms (Sheppard, 2004), it would require multiple introductions of more virulent strains to create an country-wide epidemic of MDV over a time scale of decades.

Despite some strains eventually becoming extinct, the length of time they may take to do this can be in the order of decades, if not longer. For example, Figure 5.11f shows a strain of virulence 70 reaching a mean prevalence of 30% before slowly decreasing its prevalence in 95 years to 20%. This phenomenon of an

epidemic appearing as an endemic has been postulated previously; Woolhouse et al. (2001) looked at sheep scrapie in the UK and explained how its epidemic behaviour could have been masked by the long generation time of the disease, making it seem like an endemic problem.

This model only looks at the situation where there is one circulating strain in the network. Past work in Chapter 4 has shown that the cohort structure of broilers always favours more virulent strains, which suggests that if two strains enter a cohort of birds, the more virulent strain will always outcompete the less virulent strain. This study is therefore collapsing this competition and assuming that the circulating strain is the most virulent at the time. Implicit in this model is the assumption that the presence of a less virulent strain has no effect on a more virulent strain. There is limited information about the outcome of superinfection with two MDV strains. Most work is concerned with the dynamics of a vaccinal (non-pathogenic) strain in response to a superinfecting MDV (pathogenic) strain (e.g. Islam et al., 2007) and there is no reason to assume the same dynamics occur with two pathogenic strains. However, this chapter assumes that superinfection with a more virulent strain can disadvantage a less virulent strain either by simply killing off its host quicker or by both killing off its host quicker and suppressing its replication potential (and therefore its shedding rate). If there is some competition between strains, then the invasion of a more virulent strain would be slower.

In the case where short cohort durations are used instead of vaccination as a control to curb the losses due to MDV, it is apparent that the disease is more likely to be endemic than in the case where longer cohort times are used but where broilers are vaccinated.

The prevalence in a farm network may not correlate with the prevalence of disease-induced death on a farm. For example, when a vaccinated large farm has an outbreak of MDV of Virulence Rank 46, there is little or no MDV seen in terms of clinical signs (see Chapter 4 where a Virulence Rank of 46 causes 0.002% extra mortality), however the disease may persist at high prevalence if the biosecurity both on- and between-farm is lax (see Figures 5.10c-f). Indeed it may well be the case that a decreased perception of risk may lead to risky behaviour with a farm's disease-protection measures. A high prevalence of virus circulating in the farm network could then be acting as a reservoir for the emergence and proliferation of new more virulent strains.

The results of the model are consistent with reports that smaller back-yard poultry holdings suffer from MDV less than large commercial holdings (Nair and Kung, 2004) and that more virulent strains exist at higher prevalences than less virulent strains (Witter, 2001). The latter case also highlights the problem that future, more virulent strains may be harder to eliminate from the farm networks than those which have previously been (relatively) successfully controlled.

To reduce the ability of MDV strains to persist in the network, a farm must first and foremost:

- vaccinate their stock;
- reduce the dust contamination within the barn during the cohort duration.

Other effective control measures are then to:

- reduce the contamination between each cohort and have better hygiene facilities for on-farm visitors/catchers;
- maintain shorter cohort durations if the flock is vaccinated;
- use smaller catcher companies and avoid contact with other farms.

This model highlights the situation in which there are strains circulating in the farm networks but are not currently observable. This fact reinforces the need for a clear understanding of the actual prevalence of MDV strains and to investigate the spectrum of virulences currently infecting our poultry flocks. This will provide us forewarning of the potential risk from new, more virulent, strains, which cannot be so easily controlled by our current vaccines and which are harder to control than previous less virulent strains.

6 Discussion

This thesis is the first quantitative assessment of Marek's disease virus (MDV) to answer the question of why the virus has evolved to higher virulence over the past sixty years. The work examines the effect of the virus on its chicken host and the ability of the virus to persist in a host population. The work draws on epidemiological and evolutionary theory to formalise and test hypotheses related to the increase in MDV virulence.

MDV is an ongoing concern in the poultry industry and vaccines are routinely used to control the disease; indeed new vaccines are brought into circulation every decade or so to quell the tide of problems which emerge as the virus strains increase in virulence and the previous vaccine efficacy wanes. However, since there has been no testing of the causes of this virulence evolution it is hoped that this work will provide crucial first steps in both explaining the problem caused by MDV virulence increases and shed light on the best course of action to limit the damage caused by future evolution.

Little was known about many important parameters needed for an evolutionary analysis of MDV strains. Chapter 2 was concerned with formally estimating parameters for viral shedding, host mortality and the transmission process. There was good evidence that viral shedding and host mortality positively correlate with the notion of virulence defined by previous authors (see Witter (1997) and Witter et al. (2005)). It was demonstrated that the probability of infection increases with the virus density in the atmosphere. There was good support for the hypothesis that the first and second generation vaccines reduce host mortality due to MDV. It was shown that the first generation vaccine did

not effect the viral shedding rates, although it did significantly lower the probability of infection for a susceptible bird. The second generation vaccine did lower the viral shedding rates of the birds significantly but was not examined in the infection experiment. The work also supports the claim that there exists a trade-off between virulence and transmission for MDV. Host mortality rate in unvaccinated individuals is proposed as a better definition of virulence for future pathotyping studies.

The fact that this work estimated the latent period of the virus as shorter than previously published (4-6 days compared with 6-7 days (Baigent et al., 2005)), may highlight the different methods and virus strains used to obtain the estimates. Baigent et al. (2005) worked with a vaccine strain (CVI988), whereas the estimates used in this work are based on pathogenic forms of MDV.

It is not certain that all virus produced is viable. However, the transmission experiments measured the total viral copy number (VCN) in the atmosphere and the estimated probability of infection could be correlated with this figure. Therefore the issue of how one relates VCN to viable virus can be side-stepped. However, the estimates of transmission probability in either low or high viral concentrations showed a wide variation. Unfortunately, this may be due to the limitations of the data, which have small samples sizes and from which only indirect estimates of transmission probability are obtained.

Only three different strains of MDV are examined in this study and extrapolation beyond the virulence ranges were undertaken in some cases for explorative purposes. To improve the validity of the conclusions, more strains, constituting a broader range of virulences could be used in further analyses.

Using current evolutionary biology methods, the fitness of MDV strains was defined in Chapter 3. The fitness of a virus measures how well a strain persists in a certain environment. By changing the environment in which the strains reside, one may elucidate which environmental factors lead to the persistence of more virulent strains. Three definitions of fitness were formalised in this work. Without full inclusion of the transmission processes and specific demography of the host system different conclusions were reached. Without these inclusions, the model concluded that both reducing cohort duration and introducing vaccination both select for more virulent strains. In the more complex model, more virulent virus strains are always more able to persist

compared to their less virulent counterparts whatever the environment. This meant that changing neither the duration of time the birds live, nor the density and size of the population in which the birds live, nor the vaccine status of the birds alters the selection for more virulent strains of MDV. This was the first study to test these hypotheses. It was concluded that under present conditions (most notably when broiler chickens are vaccinated or have maternal antibodies for MDV) changing farming conditions does not change the direction of selection for more virulent strains, but can change the strength of selection. In particular, vaccination dramatically reduces the strength of selection for more virulent strains. These findings therefore imply that the rate of evolution of more virulent strains and their subsequent persistence in farm networks might be limited by virus genetics.

The role of host mortality and its association with virus shedding was found to be extremely important in the conclusions. MDV would have experienced maternal antibody negative hosts when it first emerged in chicken flocks at the turn of the last century. Therefore the selection on MDV could have occurred first in maternal antibody negative birds. Consequently, it is necessary to understand the precise role that maternal antibodies have on the life-history of the virus before one can draw definite conclusions about the most plausible route of MDV becoming more virulent.

The role of broilers are studied since all the hypotheses for virulence evolution can be tested: vaccination introduction, cohort duration reduction and bird density and population size increases. However, it is unclear at this current time in which sector of the poultry industry the evolution of MDV is occurring (i.e. broilers, layers or breeders).

In Chapter 4 a farm of broiler chickens was simulated to measure the effect of a virus entering a cohort of hosts. The total amount of virus at the end of each cohort was used as a fitness measure and the results concurred with Chapter 3, such that host vaccination, cohort duration and host population structure do not change the selection direction for more virulent strains, although again vaccination in particular reduced the strength of this selection. The epidemiological impact of an MDV outbreak in a chicken population was examined by tracing the number of infected individuals and the number of dead or removed individuals over the life of the cohort. Vaccination accounts

for a drastic reduction in the perceived impact of MDV infection (such that there are no removed or dead individuals in many cases) but a pool of virus could still be maintained undetected on the farm.

It is clear from this work how introducing vaccination into a farm suffering from substantial MDV losses can radically improve the situation. This result is corroborated by the substantial use of vaccines within the field and their success to alleviate MDV losses (Bublot and Sharma, 2004).

The important epidemiological variables, such as quantity of virus emerging from a cohort and outbreak probability, found in Chapter 4, were used to create a stochastic model of between-farm spread of MDV in Chapter 5. As expected, the probability of an epidemic of MDV in a network of farms increases with the virulence of the strain causing an outbreak. For certain parameter ranges vaccination and good hygiene methods are both highly effective in limiting the probability of an endemic situation occurring.

This result is in line with anecdotal evidence that not only have strains increased in virulence in the post-war period, they have also increased in prevalence (Witter, 2001).

There is no evidence that vaccination of broilers or increases in farm size have contributed to the selection for more virulent strains of MDV. With the current situation, it is concluded from this work that MDV strains are in evolutionary transition and the rise in virulence is the observation of a virus not having reached its evolutionary stable strategy. It is not clear in which part of the poultry sector the selection of more virulent strains emerged. However the conclusions that vaccination, nor increases in farm size, have selected for greater virulence should hold when the MDV hosts are not just restricted to broilers, but include layers and breeders also. Broiler cohorts may only be the source of the rise in virulence if the cohort duration of broiler birds, which were maternal antibody negative (the case for rare non-endemic diseases), was reduced. Decreasing cohort duration may have given rise to more virulent strains if an absence of maternal antibodies can confer a strong selection for less virulent strains (if maternal antibodies have a very large effect on the survival of a bird, but little effect on viral shedding). To test this result properly, a quantitative assessment of the effect of maternal antibody negative birds must

first be established. Alternatively, if the initial rise in virulence occurred in other birds (layers or breeders), it is plausible that if maternal antibodies produce a great enough protection against disease mortality, the spreading of the virus itself was enough to allow MDV evolution to more virulent strains. If the maternal antibody hypothesis is incorrect, the alternative plausible hypothesis is that the rise in virulence was inevitable and could occur in every poultry farm. There are two competing hypotheses for the emergence, spread and persistence of more virulent strains; the first is that is it caused by the emergence of a single strain which can spread through the entire population of farms, the second that it is caused by multiple lineages of strains, introduced at different points in the population. This work shows that the rate of spread through a network of poultry farms is largely determined by the transmission mechanisms involved and without further information on between-farm transmission parameters neither hypothesis can be ruled out.

6.1 Future Work

Since relatively mild strains were used in this analysis it would be sensible to look at the effect of some more virulent strains on a farm. It would then be possible to calculate whether there was a point after which our current vaccines would not be sufficient to prevent larger losses from MDV. Furthermore, it would then be possible to work out how efficacious a future vaccine would have be to prevent huge losses in the face of much more virulent MDV strains.

It is difficult to estimate the prevalence of MDV within farm networks. If data were available, the model in Chapter 5 could be formally fitted to prevalence samples in the field. It still remains a open question as to how much virus there is circulating in the farm networks. If the virus is not persisting in the farm networks then there must be constant introduction from another source, such as wild birds, which could also be acting as a means of spread. It would therefore be sensible to test for MDV in wild birds (in the way that Murata et al. (2007) have done) specifically around a variety of poultry houses. Müller et al. (1999) attempt a similar analysis for the risk of Newcastle disease in Europe due to the prevalence in migratory birds. This, together with understanding of the MDV within a network of farms, could provide better instruction for a more complex model of between-farm MDV spread to ascertain the

most plausible contamination process. Furthermore, having a large sample of recent and historic isolates would allow a formal phylogenetic tree to be constructed which can provide crucial facts about the introduction, persistence and evolution of a population's MDV strains and the host size in which they have remained.

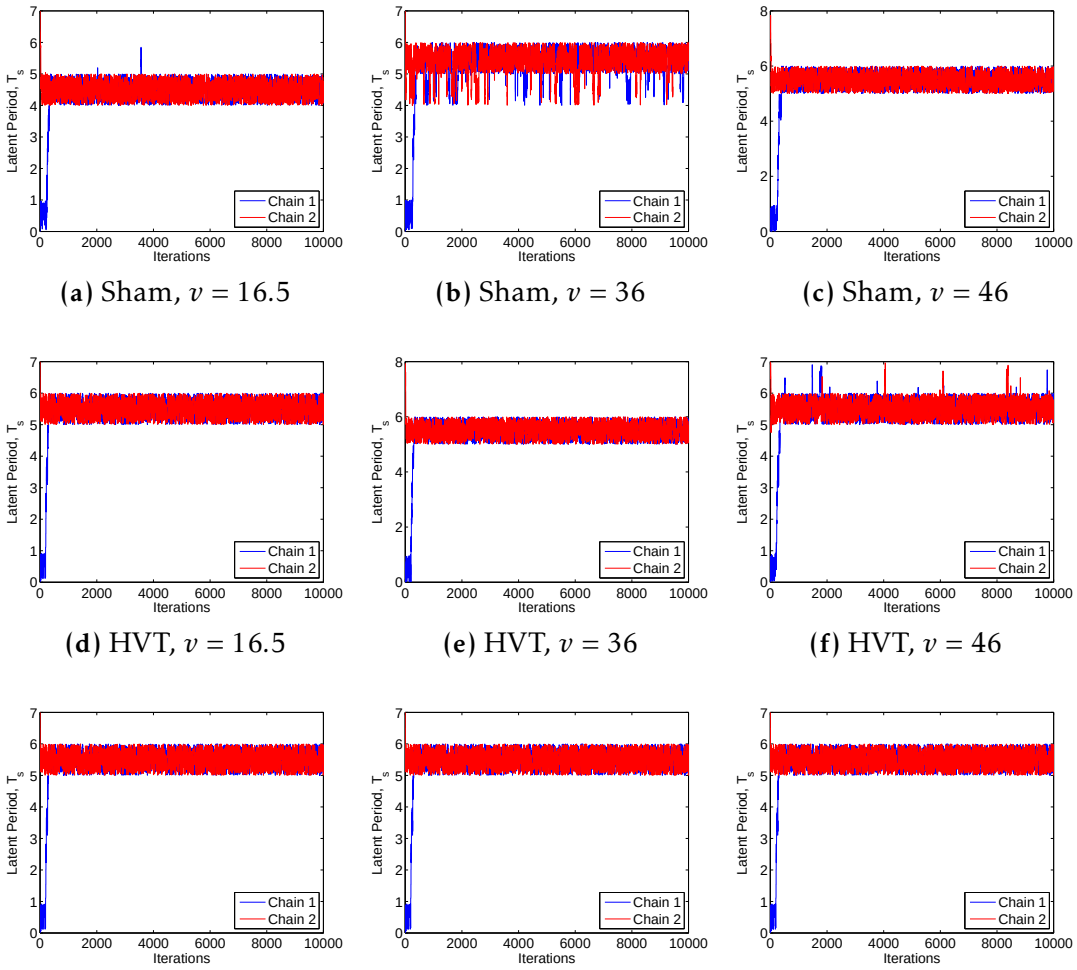
If strains are being sampled from the field to test for prevalence, the same strains can also be used for pathotyping experiments. Not only is the prevalence of MDV unknown, but so is the current virulence of strains. It is not clear for how long circulating strains can be controlled by current vaccines. To understand the current situation is to understand whether more virulent strains are possible and if so, how quickly they could render out current control methods inadequate.

A

McMC Results

A.1 Viral Shedding

Two chains are used to diagnose convergence in the estimation of both the latent period, $T_s(v, j)$, and the shedding rate of the virus, $a(v, j)$. These chains are shown in Figures A.1 and A.2, with the Brooks-Gelman-Rubin statistic for each parameter given in Figures A.3 and A.4. The Brooks-Gelman-Rubin statistic is described in more detail in Box A.1. The final posterior distributions are shown in Figures A.5 and A.6. The burn-in time was set to 20,000 and the posterior distribution was calculated from every 10th accepted value from the following 25,000 iterations. Raftery and Lewis (1992) suggest taking every kth sample to ensure independence of consecutive samples, where $k \geq 10$. Results correspond to credible intervals used in Section 2.4.2.

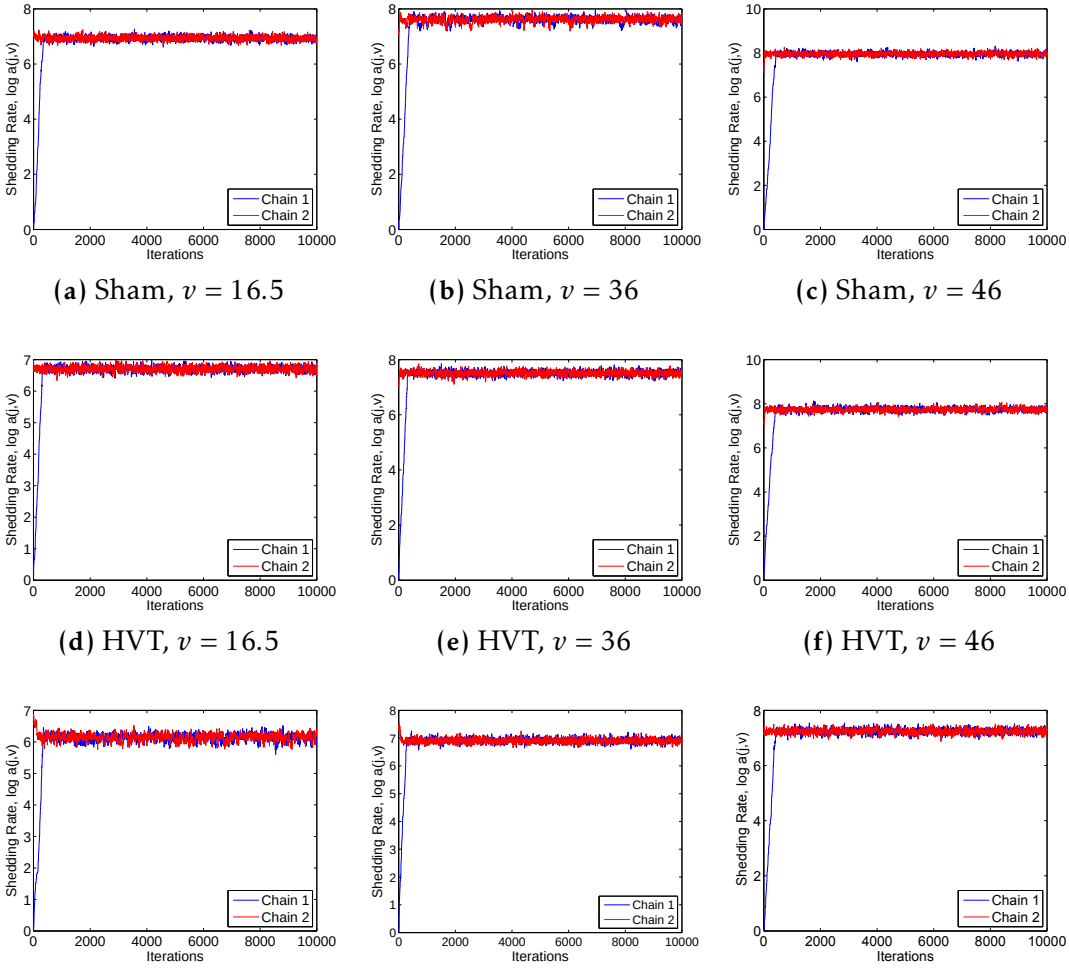


(a) Sham, $v = 16.5$ **(b)** Sham, $v = 36$ **(c)** Sham, $v = 46$

(d) HVT, $v = 16.5$ **(e)** HVT, $v = 36$ **(f)** HVT, $v = 46$

(g) Bivalent,$v = 16.5$ **(h)** Bivalent,$v = 36$ **(i)** Bivalent,$v = 46$

Figure A.1: Viral Shedding: Two parallel Markov chains estimating the latent period, T_s, for each combination of vaccine and Virulence Rank as stated (first replicate).

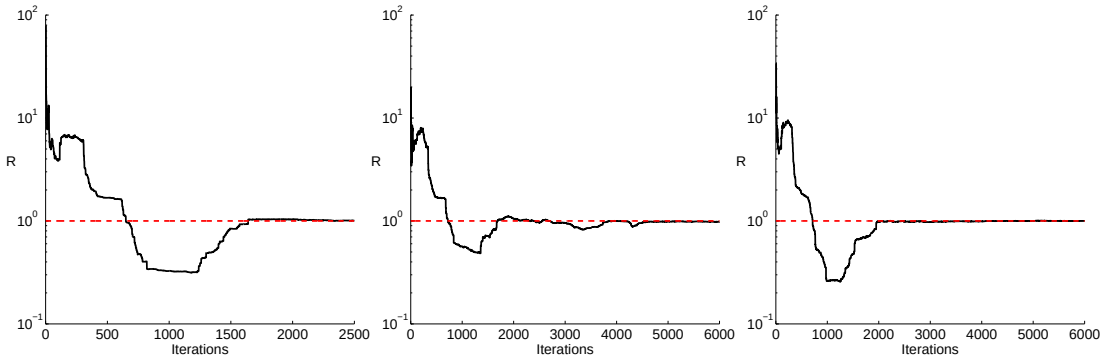


(a) Sham, $v = 16.5$ **(b)** Sham, $v = 36$ **(c)** Sham, $v = 46$

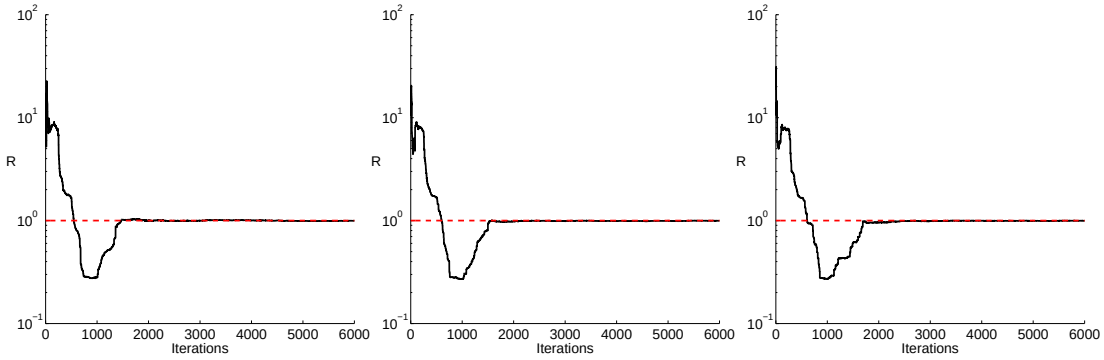
(d) HVT, $v = 16.5$ **(e)** HVT, $v = 36$ **(f)** HVT, $v = 46$

(g) Bivalent, $v = 16.5$ **(h)** Bivalent, $v = 36$ **(i)** Bivalent, $v = 46$

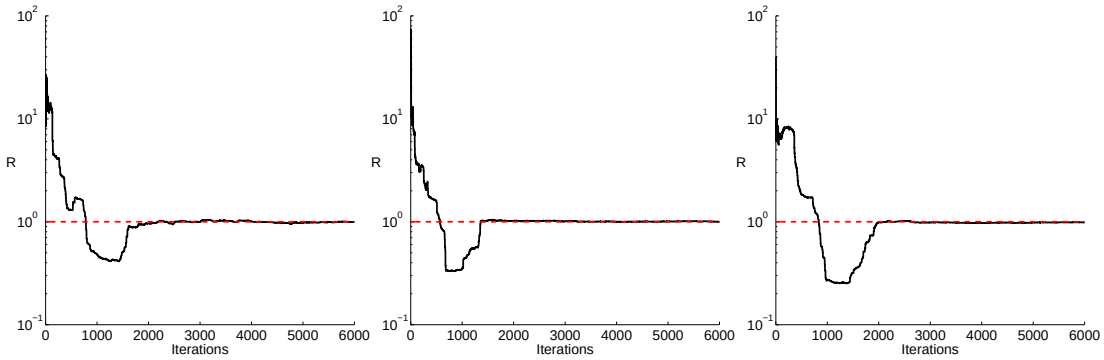
Figure A.2: Viral Shedding: Two parallel Markov chains estimating the logged shedding rate, $\log a(v, j)$, for each combination of vaccine and Virulence Rank as stated (first replicate).



(a) Sham, $v = 16.5$ **(b)** Sham, $v = 36$ **(c)** Sham, $v = 46$

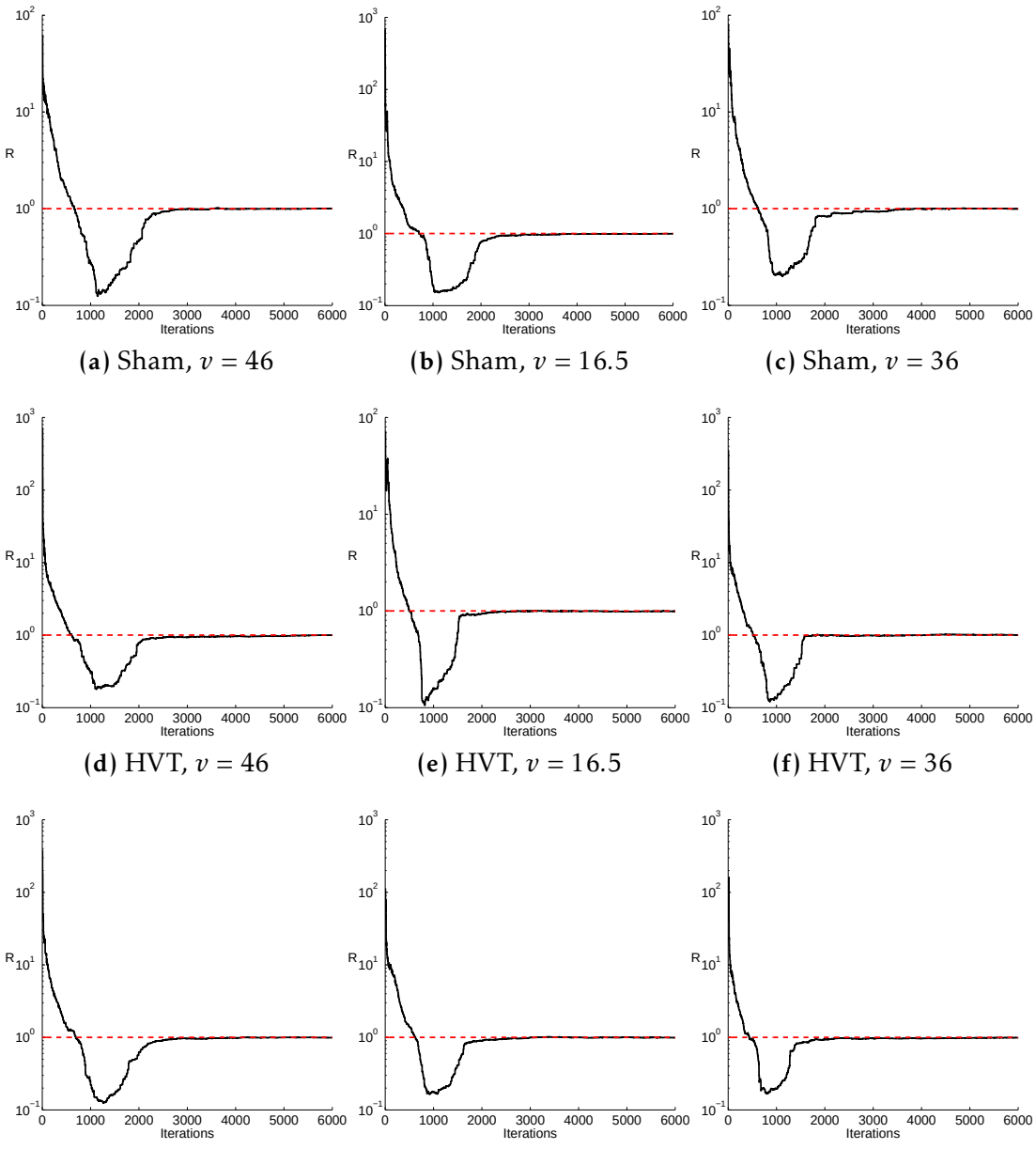


(d) HVT, $v = 16.5$ **(e)** HVT, $v = 36$ **(f)** HVT, $v = 46$



(g) Bivalent, $v = 16.5$ **(h)** Bivalent, $v = 36$ **(i)** Bivalent, $v = 46$

Figure A.3: Viral Shedding: Brooks-Gelman-Rubin statistics for the latent period, T_s, for each combination of vaccine and Virulence Rank as stated (first replicate).

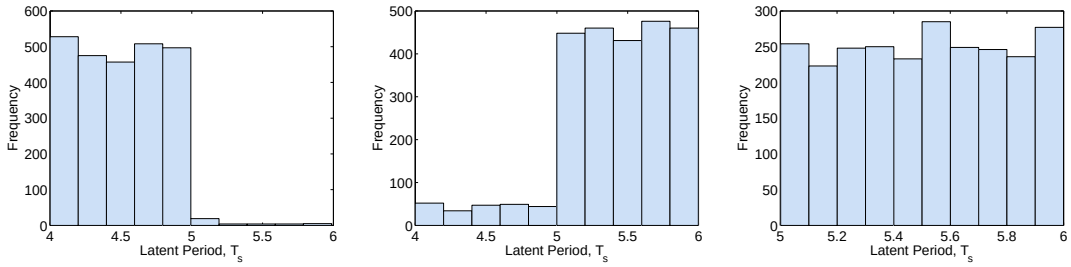


(a) Sham, $v = 46$ **(b)** Sham, $v = 16.5$ **(c)** Sham, $v = 36$

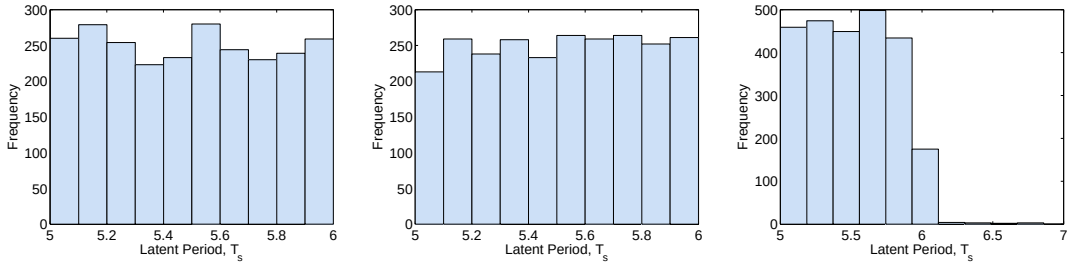
(d) HVT, $v = 46$ **(e)** HVT, $v = 16.5$ **(f)** HVT, $v = 36$

(g) Bivalent, $v = 46$ **(h)** Bivalent, $v = 16.5$ **(i)** Bivalent, $v = 36$

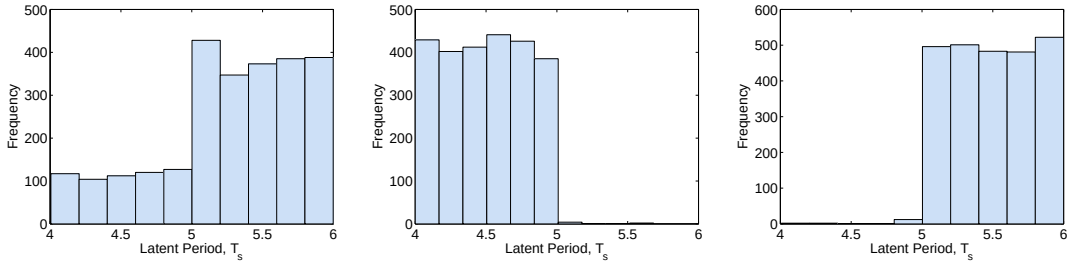
Figure A.4: Viral Shedding: Brooks-Gelman-Rubin statistics for the logged shedding rate, $\log a(v, j)$, for each combination of vaccine and Virulence Rank as stated (first replicate).



(**a**) Sham, $v = 16.5$ (**b**) Sham, $v = 36$ (**c**) Sham, $v = 46$

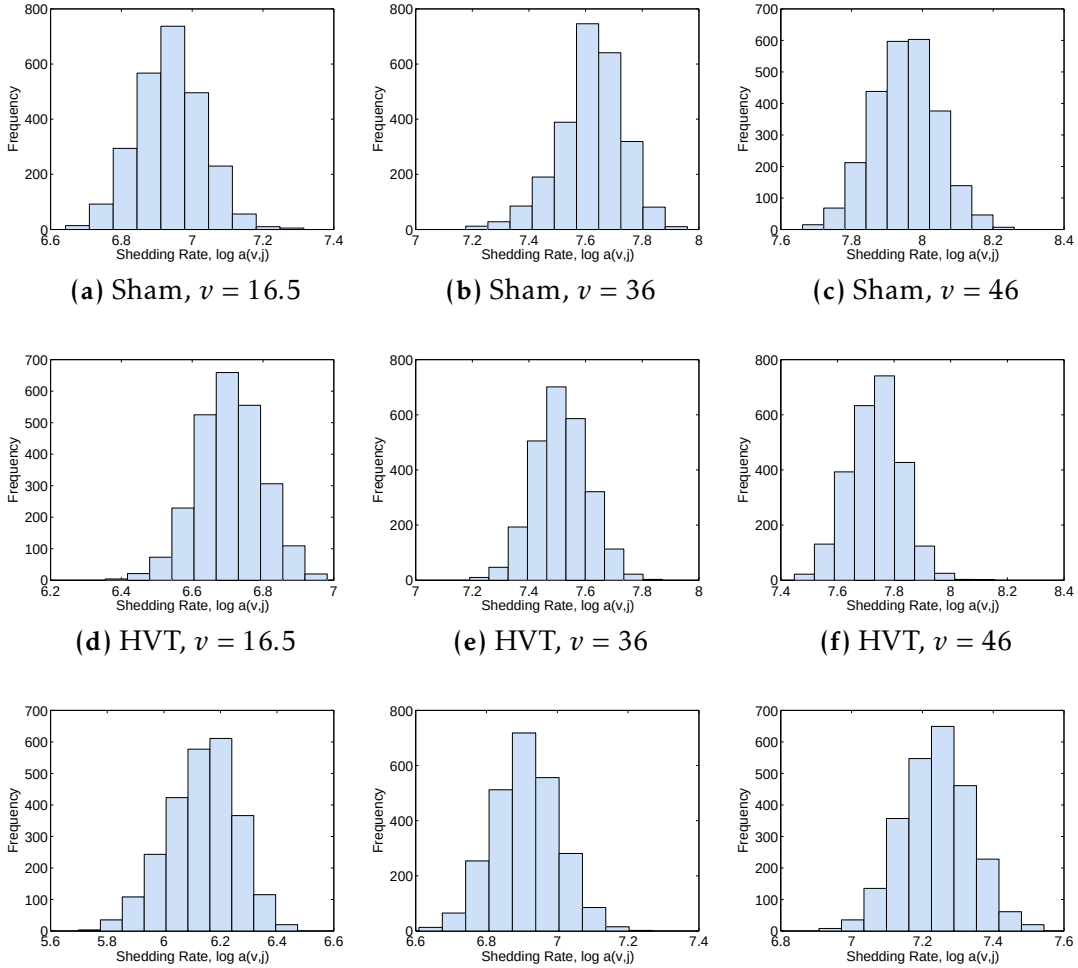


(**d**) HVT, $v = 16.5$ (**e**) HVT, $v = 36$ (**f**) HVT, $v = 46$



(**g**) Bivalent, $v = 16.5$ (**h**) Bivalent, $v = 36$ (**i**) Bivalent, $v = 46$

Figure A.5: Viral Shedding: Posterior distribution for the latent period, T_s, for each combination of vaccine and Virulence Rank as stated (first replicate).



(a) Sham, $v = 16.5$ **(b)** Sham, $v = 36$ **(c)** Sham, $v = 46$

(d) HVT, $v = 16.5$ **(e)** HVT, $v = 36$ **(f)** HVT, $v = 46$

(g) Bivalent, $v = 16.5$ **(h)** Bivalent, $v = 36$ **(i)** Bivalent, $v = 46$

Figure A.6: Viral Shedding: Posterior distribution for the logged shedding rate, $\log a(v, j)$, for each combination of vaccine and Virulence Rank as stated (first replicate).

Box A.1: Brooks-Gelman-Rubin

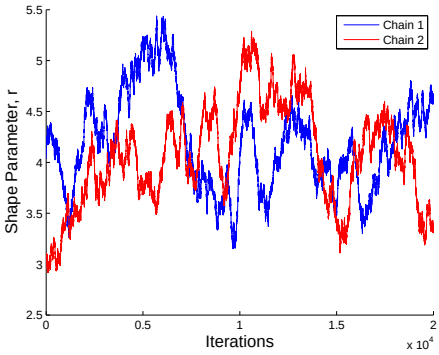
It is often stated that it is difficult to prove whether a Markov chain has converged, only that it has not (Carlin and Lewis, 2008). Brooks and Gelman (1998) build on previous work done by Gelman and Rubin (1992) and provide a method for testing the convergence of a Markov chain without using the variance, thereby bypassing the need for the assumption of normality. The statistic is R and is defined thus:

$$\begin{aligned} A &= \text{width of central 80\% interval of pooled runs} \\ B &= \text{average width of the 80\% intervals within individual runs} \\ R &= A/B \end{aligned}$$

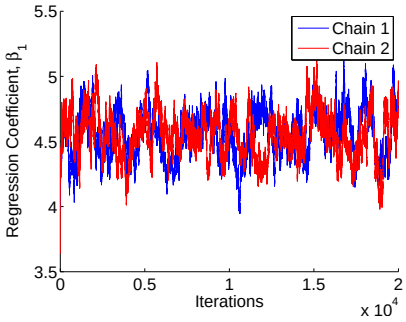
$R \to 1$ as more iterations are included in the calculation. In an analysis, the chain is thought to have converged when $R \approx 1$. Initially R should be greater than 1 to ensure over-dispersal of the initial values (Carlin and Lewis, 2008).

A.2 Host Mortality

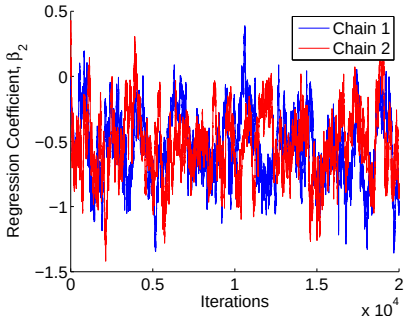
The burn in for two Markov chains corresponding to the estimation of the Weibull shape and scale parameters, r and λ are shown in Figure A.7. Convergence diagnostics were calculated by the Brooks-Gelman-Rubin statistic (Brooks and Gelman, 1998) described in Box A.1. The convergence diagnostics are shown in Figure A.8. After a burn in period of 22,000 iterations, the chain was run for a total of 100,000 iterations, every 10th accepted figure was recorded to calculate the posterior distributions of the five parameters, shown in Figure A.9. Results correspond to credible intervals used in Section 2.4.3.



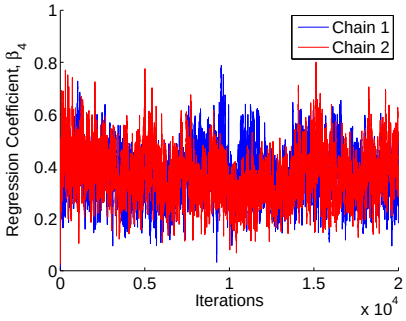
(a) r



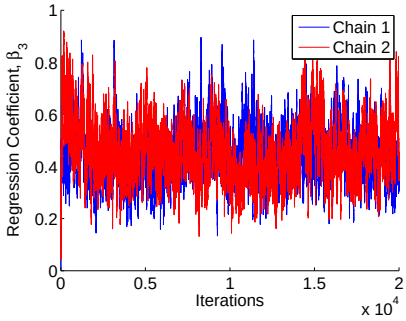
(b) β_0



(c) β_1

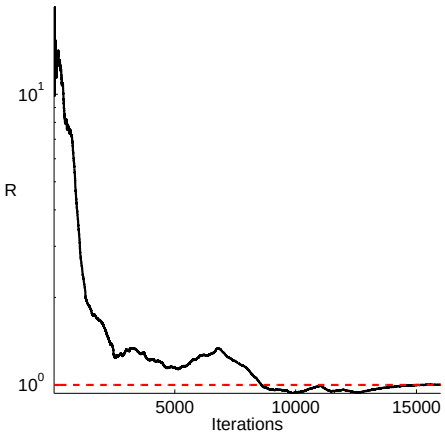


(d) β_2

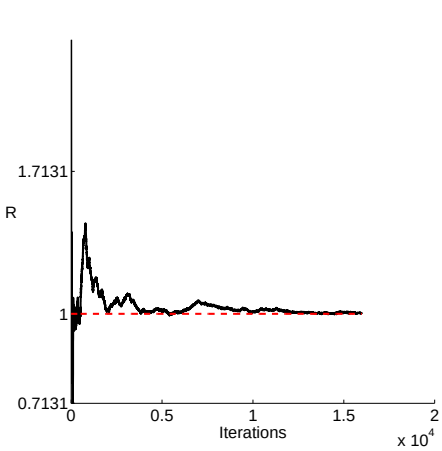


(e) β_3

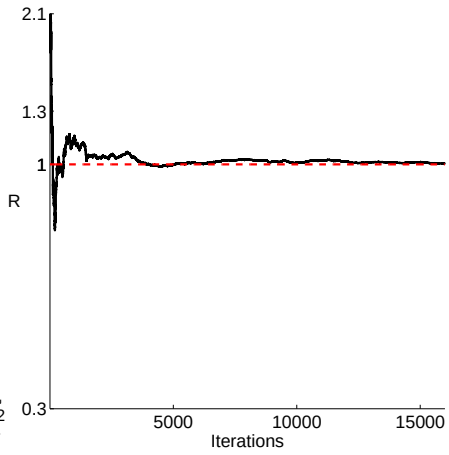
Figure A.7: Host Mortality: Mixing of 2 independent chains for each estimated parameter. The chains have different (overdispersed) initial values.



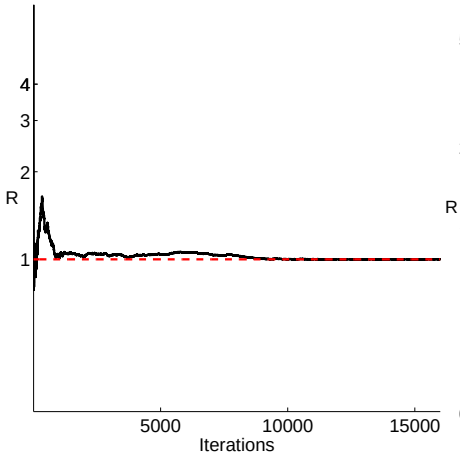
(a) r



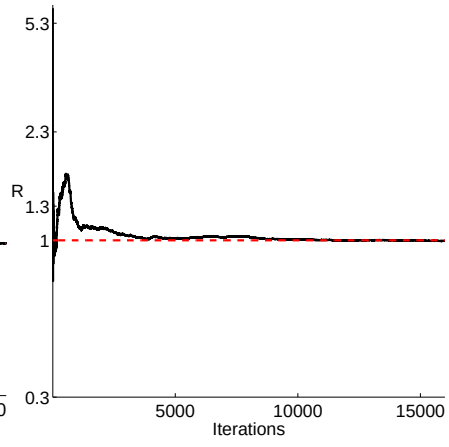
(b) β_0



(c) β_1

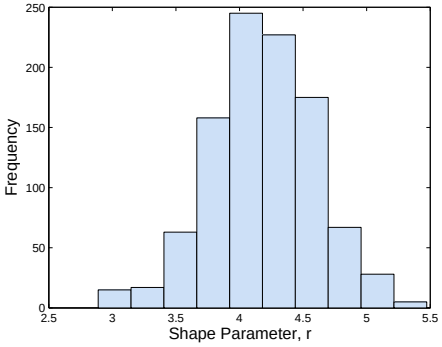


(d) β_2

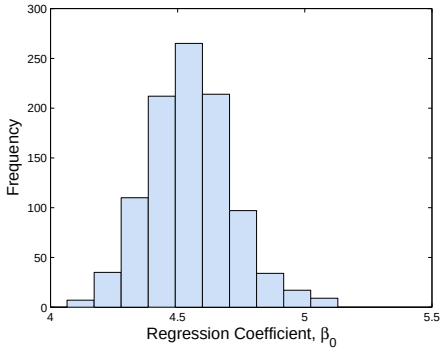


(e) β_3

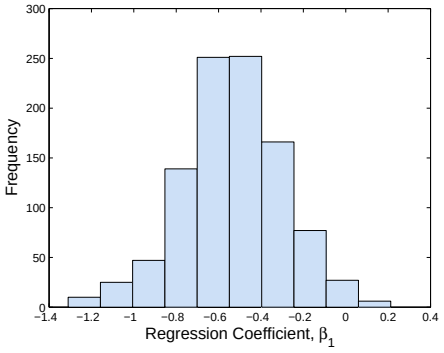
Figure A.8: Host Mortality: Brooks-Gelman-Rubin statistic for diagnosing chain mixing for each estimated parameter. Mixing is considered to have occurred when the statistic, R equilibriates at 1.



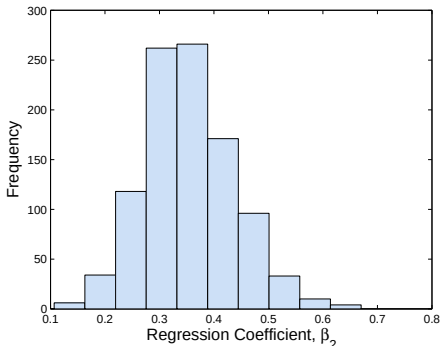
(a) r



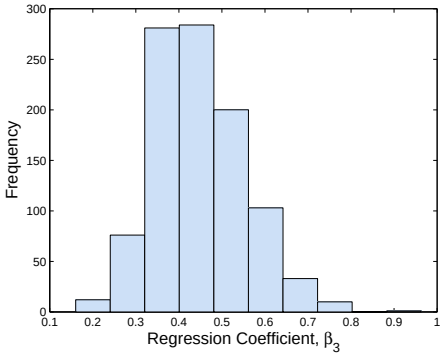
(b) β_0



(c) β_1



(d) β_2



(e) β_3

Figure A.9: Host Mortality: Posterior distributions of the five fitted parameters included in the Weibull scale parameter, λ.

B

Broiler Dust

Broilers and layer birds are known to have different growth patterns (Sewalem et al., 2002), with broilers having a faster growth rate and also growing to a larger size.

B.1 Data

In an experiment conducted and described by Islam et al. (2007), groups of broiler chickens were raised from an age of one day in isolators. All the dust from each isolator and its exhaust was retrieved every 24 or 48 hours. With knowledge of the number of chickens per isolator each day and the total dust, the total mass of dust shed per day per bird was found for weeks 1-8, giving a total of 8 data points per isolator.

B.2 Methods

The aim is to fit a function of the form $y = d(t)$ where y is the mass of the dust shed per day (mg) and t is the age of the bird. Biologically, the dust produced by a recently hatched bird will not be zero. Other assumptions are the dust shed is a non decreasing function of age and that the bird will shed an amount of dust which will tend to a fixed amount as the growing process ends. A possible candidate model for d is therefore:

$$d(t) = \eta_1 exp(-\eta_2/t^{\eta_3}) + \eta_4$$

where η_i, $\forall i \in [1,4]$ are parameters to be estimated. Since the aim is to identify a good fit of the model to the data, point estimates for the parameters are sought. The least squares method is used, together with the Levenberg-Marquardt algorithm to minimise the resulting sum function. Matlab® (2007) was used for this analysis with the `lsqcurvefit` function.

Since we have multiple dust profile curves and need to estimate a dust shedding function, it can be proved that in this case minimising the sum (over all observations) of the distances from each data point to the model point is equivalent to minimising the distance from the arithmetic mean of multiple data points to the model point.

To show this, consider one time point at which the sum of the distance, g, from all data points, x_i, to the model value, y, is to be minimised by y^*:

$$\begin{aligned} g(x) &= \sum_{i=i}^{n} (x_i - y)^2 \\ \frac{dg}{dy} &= -\sum_{i=1}^{n} 2(x_i - y) \\ 0 &= \sum_{i=1}^{n} (x_i - y) \\ \Rightarrow \qquad & y^* = \frac{1}{n} \sum_{i=1}^{n} x_i \end{aligned}$$

Similarly, minimising the distance from the mean of the data points, $\bar{x}_j$, to a single model value, y^*, is given thus:

$$
\begin{aligned}
h(x) &= \left(\frac{1}{n}\sum_{i=i}^{n} x_i - y\right)^2 \\
\frac{dh}{dy} &= -2\left(\frac{1}{n}\sum_{i=1}^{n} x_i - y\right) \\
0 &= \frac{1}{n}\sum_{i=1}^{n} x_i - y^* \\
\Rightarrow \quad & y^* = \frac{1}{n}\sum_{i=1}^{n} x_i
\end{aligned}
$$

Since it is true for one point, without loss of generality, it is true for fitting multiple y_j to each $\bar{x}_j$.

B.3 Results

A least squares fitting gives the quantitity of dust (in *mg* per day) as

$$d(t) = 368\exp(-326/t^{1.64}) + 10.8$$

with the graph displayed in Figure B.1.

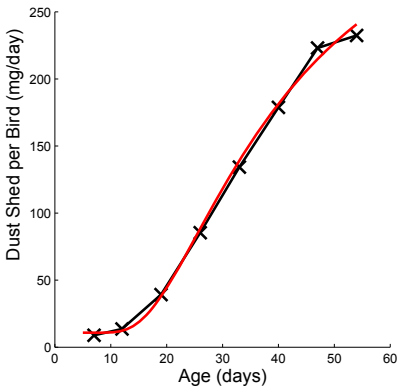


Figure B.1: Dust Shedding: The amount of dust shed over time by a broiler chicken (black line) and the fitted function, $d(t)$ (red line).

C

Maternal Antibodies

All the data being used in the analysis in Chapter 3 are from experiments using maternal antibody positive birds. However, in a situation where the disease is not endemic, maternal antibody positive birds would not be widespread in the population. There is evidence that maternal antibodies have an important role to play in protecting unvaccinated birds against MDV-induced mortality (Calnek, 1972; Eidson et al., 1972). Maternal antibody negative chickens show increased mortality effects. Using maternal antibody negative birds, infection with a very virulent strain (for example Virulence Rank 85) can kill a group of birds within 9 days of infection, compared to 45 days for those infected with a less virulent strain (Virulence Rank 20) (A.F. Read, Personal Communication, January 2008.). The following extension to the results will take this phenomenon into account by looking at the fitness of strains (R_0) in a maternal antibody negative environment, assuming there is no effect of maternal antibodies on the transmissibility of the parasite.

The increase in MDV-induced mortality due to the absence of maternal antibodies is simulated by reducing the expected lifespan of an infected bird if it is maternal antibody negative. This can be naturally achieved by reducing the regression parameter which is responsible for taking into account the effects of Virulence Rank on the survival of the infected bird, β_1. By choosing β_1=-2.0, the expected lifespan of an maternal antibody negative bird infected with a strain of Virulence Rank 20 or Virulence Rank 85 is 32 and 7 days respectively. This is the correct order of magnitude for the data provided (A.F. Read, Personal Communication, January 2008). Figure C.1 shows the expected lifespan of an infected individual with reduced β_1. Figure C.2 shows R_0 when β_1 is reduced. Changing the regression parameter β_1, to reflect a greater effect

of a virus on the lifespan of an individual, reduces the R_0 of a strain. Also, R_0 is optimized at an intermediate virulence when β_1 is reduced. In this case, decreasing the cohort duration may serve to select for higher virulence.

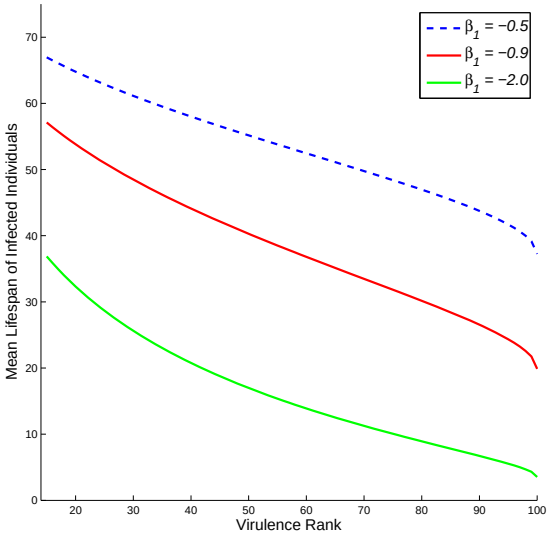


Figure C.1: Lifespan: Background mortality, μ=0.0005, equilbrium, A=7.15mg/m^3, unvaccinated population. Lifespan for maternal antibody positive bird (blue dash), increased susceptibility to death due to MDV (red solid) and simulated maternal antibody negative (green solid).

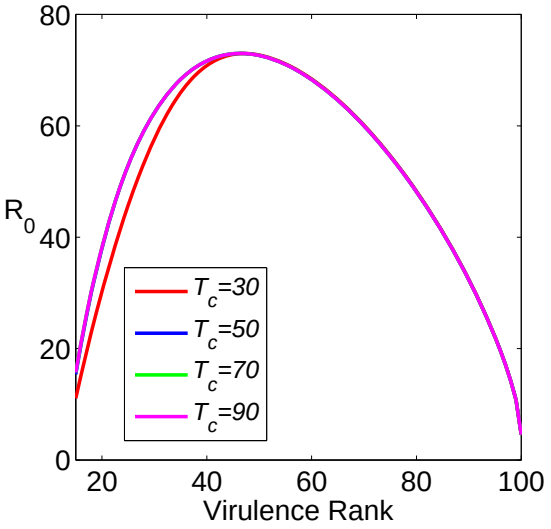


Figure C.2: R_0: Simulating maternal antibody negative birds where $\beta_1 = -2.0$. Background mortality μ=0.0005, equilbrium A=7.15mg/m^3, unvaccinated population, population size, S_0=40,000.

These findings suggest that if mortality effects were originally higher than they are for current birds, the trade-off between virulence and transmission would indeed lead to a medium virulence being selected. Since there is no evidence that maternal antibodies would act in a way much different to vaccine-derived antibodies, maternal antibody negative birds, although showing a higher mortality rate, might also have a higher viral shedding rate (as the vaccinated birds do, compared with the unvaccinated). However if maternal antibodies do not have enough of an effect on viral shedding rate to negate the effects of the reduced lifespan, then the shift from the farming of maternal antibody negative birds (when MDV was not endemic in the bird population) to the farming of mainly maternal antibody positive birds (when MDV became endemic in the bird population) may have been the reason for the initial rise in the virulence of MDV.

There are numerous theories consistent with the results shown in this section, beginning with the assumption that in an almost disease-free state, a cohort of birds will not possess maternal antibodies for MDV, leading to selection for less virulent strains. Then,

1. If the industry introduced shorter cohort durations for broilers this might lead to selection for more virulent strains. The response would be the introduction of the vaccines to stem the wave of these more virulent isolates and selection would favour more and more virulent strains.

2. Again, if changes were made to reduce the cohort duration (which led to more virulent strains) these more virulent strains may be able to survive at higher prevalences in the environment (since they have a higher fitness in this environment) and lead to an endemic state in which maternal antibody birds would become the majority. In this situation, more virulent strains would therefore always be selected for.

3. Less virulent strains may be able survive at higher prevalences in the environment (since they have a higher fitness in this environment) and lead to an endemic state where the emergence of maternal antibody positive birds would lead to a selection for more virulent strains whatever the environment.

These three suggestions use different factors as their evolution drivers. They in turn use 1) cohort duration reduction then vaccine introduction, 2) cohort

duration reduction and the emergence of an endemic disease state in the bird population 3) only the emergence of an endemic disease state in the bird population.

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