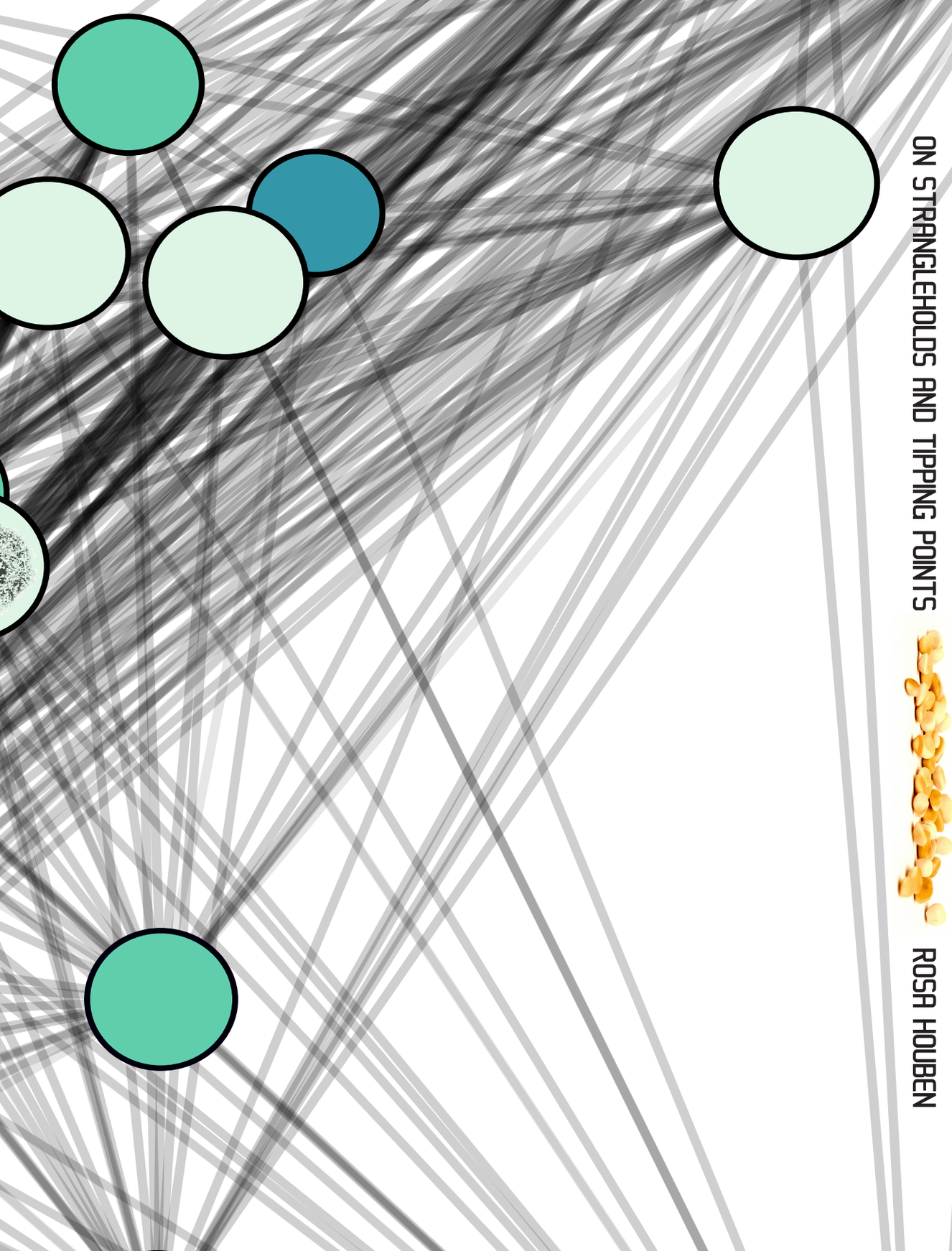

ON STRANGLEHOLDS AND TIPPING POINTS

ROSA HOUBEN

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On strangleholds and tipping points

Transmission dynamics of *Streptococcus equi* subspecies *equi* and equine herpesvirus 1

Wurggrepen en kantelpunten
Dynamiek van transmissie van *Streptococcus equi* subspecies *equi* en equine herpesvirus 1

(met een samenvatting in het Nederlands)

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You've got questions,
we've got assumptions.
Randall Munroe/XKCD

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Part I

THE PATHOGENS AND THE PROBLEMS

Chapter 1

GENERAL INTRODUCTION AND THESIS OUTLINE

INTRODUCTION

The focus of this thesis is on the equine infectious agents *Streptococcus equi* subspecies *equi* (*S. equi*) and equine herpesvirus type 1 (EHV-1), which both weigh heavily on the global equine population.

Infection with *S. equi* can cause retropharyngeal lymphadenopathy (Figure 1.1) which is the reason why the disease is colloquially known as strangles.

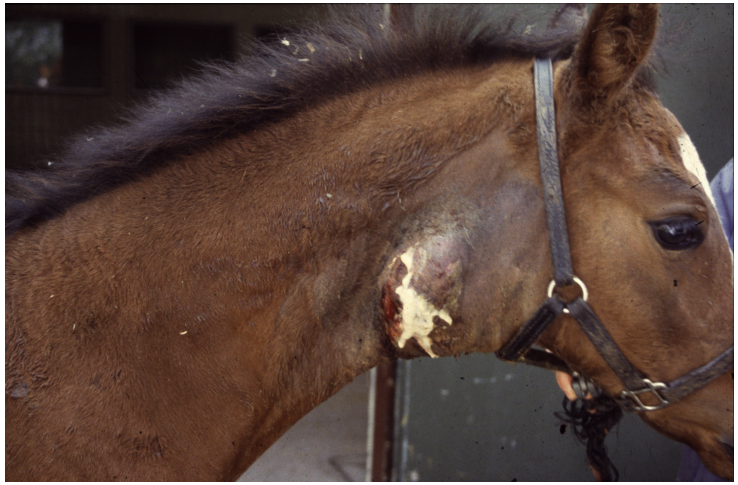


Figure 1.1: *A foal with typical strangles (M. Sloet, faculty of Veterinary Medicine, Utrecht University).*

Initial infection with EHV-1 typically causes mild respiratory disease known as "rhinopneumonitis". EHV-1 can also cause other disease syndromes in adult horses, of which neurological disease, presenting as ataxia or paralysis, is arguably the most feared, as it can transform even the fittest equine athlete into a euthanasia candidate (and news headline - Figure 1.2) in a matter of days, with no definitively proven preventive measure or cure currently available.

Both infectious agents occur worldwide and are currently endemic in the Netherlands. Both are also infections that most horses worldwide will not manage to escape throughout their lifetime, with the exception of those residing in Iceland, a remote island nation which has not allowed import of horses for 1000 years and whose resident horse population up to today remains free of both infectious agents. Year upon year, questions concerning *S. equi* and EHV-1, when combined, constitute around 3/4 of all inquiries from Dutch veterinarians to a national equine infectious diseases helpline (unpublished data, Royal GD). Wondering how

to understand and mitigate this stranglehold on the equine community was the starting point and motivation for this thesis.

Figure 1.2: *A news report on the 2021 Valencia EHV-1 outbreak. Science.org, dated 24 March 2021 (Lesté-Lasserre 2021).*

THE PATHOGENS

An American College of Veterinary Internal Medicine (ACVIM) Consensus Statement is available for both EHV-1 and *S. equi* (Lunn et al. 2024; Boyle et al. 2018). Both agents also feature in the American Association of Equine Practitioners Guidelines and in the UK's Horserace Betting Levy Board Codes of Practice. In the Netherlands, the KNMvD has issued a Richtlijn Rhinopneumonie; Royal GD and the Utrecht University, Department of Clinical Sciences have jointly issued a Leidraad Droes. The existence of these documents underscores the importance of EHV-1 and *S. equi* to the global equine (veterinary) community.

Natural history of disease

S. equi

Infection with *S. equi* via the respiratory or oral mucosa leads to abscessation of the lymph nodes of, most commonly, the head and neck region. The retrophayngeal and mandibular lymph nodes are most often affected. Strangles is typically accompanied by fevers and purulent nasal discharge. Inoculation

Figure 1.3: *A mare and her foal recovering from strangles (R. Houben, personal collection).*

with a sufficiently large infectious dose will reliably result in disease (Knight et al. 1975). Pharyngitis and inappetence are also frequently observed clinical signs. Fever commonly appears as the first sign 3–14 days after inoculation, and reliably appears 1-2 days before nasal discharge and infectiousness commence. Although most horses recover uneventfully from clinical disease within 2–3 weeks, complications are not uncommon, occurring in around 20% of clinical cases. Complications include dysphagia leading to aspiration pneumonia, upper respiratory tract obstruction, abscess metastasis (also called "bastard strangles") and immune-mediated complications such as vasculitis and myositis (Sweeney et al. 1987; Ford and Lokai 1980). Mortality is 1% to 10% of strangles cases (Todd 1910; Duffee et al. 2015; Piché 1984; Christmann and Pink 2017).

After recovery, animals are resistant to re-challenge for some period of time: at least 28 weeks (Galan and Timoney 1985; Hamlen et al. 1994) and presumably several years (Boyle et al. 2018). It is generally believed that a proportion of horses (around 25%) do not mount lasting convalescent immunity after clinical recovery from strangles, and become susceptible to re-infection within months. The origins and validity of this assumption are explored in **Chapter 3**.

It is thought that protective immunity in convalescent horses may be prolonged by repeat challenges (possibly by carrier animals) causing repeated immune stimulation without establishing infection or causing clinical signs in partially immune hosts (Boyle et al. 2018). However, this theory is currently unproven. Most foals have protective maternal immunity for at least the first two months of life (Galan et al. 1986) with progressively declining antibody levels over time since colostrum consumption. Foals are assumed to have lost all maternally derived immunity by six months of age. Some partially immune hosts, when challenged, may display clinical signs such as mild fever and mild nasal discharge. Despite not presenting clinically as "typical strangles", these cases are infectious to other horses (Newton et al. 1997). More severe disease and shorter incubation periods likely result from higher inoculation dose challenges and *vice versa* (Boyle

et al. 2018).

Asymptomatic infections are not considered a main feature of strangles, although outbreak reports exist in which horses seroconverted without having displayed clinical signs (Christmann and Pink 2017; Tscheschlok et al. 2018); in addition, outbreaks have been described in which the pathogen produced notably milder clinical symptoms, in fewer than expected susceptible horses, and a number of horses in the affected herds seroconverted or tested positive on nasal swab PCR without having displayed clinical signs. In the case of Christmann and Pink (2017), the immune status of the horses in the herd at the time of the outbreak was unknown. The decreased morbidity described for some outbreaks may be attributable to loss of virulence of the bacteria during guttural pouch persistence (Tscheschlok et al. 2018; Prescott et al. 1982; Chanter et al. 2000).

After recovery, around 10-20% of horses of horses fail to clear their guttural pouches of purulent debris containing *S. equi* (Newton et al. 1997; Boyle et al. 2018; Riihimäki et al. 2018; Pringle et al. 2019; Duffee et al. 2015). Although apparently healthy, these horses continue to harbour, and periodically shed, virulent bacteria and remain infectious for months to years, potentially for life (Newton et al. 1997; Newton et al. 2000; Boyle et al. 2018; Riihimäki et al. 2018; Pringle et al. 2019). Around half of these carriers may intermittently display mild, nonspecific clinical signs of (often unilateral) nasal discharge (Judy et al. 1999). *S. equi* carriers are discussed more in-depth in **Chapter 4**.

There is limited prospective data on the dynamics of seropositivity after natural infection with *S. equi*; in a group of Icelandic horses in Sweden followed-up after an outbreak with 100% morbidity, 88% of untreated, non-carrier convalescent horses animals had detectable antibody 10 months after the outbreak (Pringle et al. 2020a; Pringle et al. 2020b).

EHV-1

EHV-1, an alpha-herpesvirus, is widely endemic in the EU (Nielsen et al. 2022) and in most of the world beyond the EU (Lunn et al. 2024).

Most horses are first infected early in life as foals, and the source of infection are thought to be their dams (Gilkerson et al. 1998; Gilkerson et al. 1999; Gilkerson et al. 2000; Brown et al. 2007). On endemic farms, foals have detectable neutralising antibody, which decreases over the span of several months (Brown et al. 2007). The initial infection in young animals typically produces only mild upper respiratory disease and fever. This disease syndrome of respiratory signs can also becaused by the closely related virus EHV-4 and respiratory disease caused by either virus is called rhinopneumonitis.
Older horses may either undergo silent infection, present mild respiratory disease, or develop one of the more severe disease presentations. Infection with EHV-1 in adult horses can result in late term abortion or birth of a live infected foal

that quickly deteriorates due to respiratory failure (van Maanen et al. 2000; Barrandeguy et al. 2002; Schulman et al. 2015; Slater 2014), or in neurological disease characterised by ataxia, hindlimb paralysis, and bladder paralysis (WOAH 2022). Neurological disease caused by EHV-1 is called equine herpesvirus myeloencephalopathy (EHM). In outbreaks of EHM, 1% to 50% of infections may result in neurological disease (van Maanen et al. 2001; Goehring et al. 2006; Henninger et al. 2007; Barbic et al. 2012; USDA 2013; Weese 2017). Prognosis for recovery after EHM is favourable as long as the horse does not become recumbent; recumbency greatly increases the risk of fatal complications (van Maanen et al. 2001; Slater 2014).

Viremia is thought to be a prerequisite for abortion and for EHM (Pronost et al. 2012; Lunn et al. 2024). Studies on host risk factors for the development of neurological disease have consistently concluded that increasing age is a risk factor; EHM in non-adults (horses less than three years of age) is rare (Goehring et al. 2006), and horses over five years old (Henninger et al. 2007), or over nine years old (Couroucé et al. 2023) have been reported to be at increased risk of EHM, however in one outbreak, most horses with EHM were 5-9 years old (Traub-Dargatz et al. 2013). One report collating data of multiple European outbreaks reported an odds ratio of developing EHM after exposure to EHV-1 of 1.06 for each increasing year of age (Klouth et al. 2022) while another found a significant effect of age only for "severe" neurological disease and not for all EHM (Goehring et al. 2006).

A single nucleotide polymorphism resulting in an amino acid shift (N_{752} to D_{752}) was for a while thought to be a determinant for neurological disease, as the D_{752} mutant genotype was observed to cause higher viremia than the N_{752} genotype (Nugent et al. 2006; Allen and Breathnach 2006; Goodman et al. 2007; Van de Walle et al. 2009). The distinction between neuropathogenic (D_{752}) and non-neuropathogenic (N_{752}) genotype, which was made upon that discovery, has not fully stood the test of time. Outbreaks of EHM continue to be caused by N_{752}, as was the case in Valencia in 2021. The N_{752} variant remains the predominant variant found in horses in the USA, and nasal swab viral loads did not differ between the variants (Goodman et al. 2007; Van de Walle et al. 2009; Pusterla et al. 2023a), consistent with earlier experimental studies where nasal shedding was measured for both variants. In a 2020 study of 65 EHM cases from the USA (Pusterla et al. 2020b), the N_{752} to D_{752} genotype were detected with similar frequency, however, that study did not have information on the total number of infections that each of the genotypes had caused in total. It is clear however, that outbreaks of EHM caused by both variants continue to occur. More recently, a new mutant genotype H_{752} was described as a cause of two apparently unrelated outbreaks of EHM in the USA and in Europe (Sutton et al. 2020; Pusterla et al. 2021), which further draws into question the appropriateness of the neuropathogenic *vs* non-neuropathogenic distinction of D_{752} *vs* N_{752} genotypes.

After clinical recovery, most horses remain latently infected, with the virus present in the lymphoreticular system and neurons within the trigeminal ganglia (Slater et al. 1994; Slater 2014). Horses with latent infection are thought to be the principal reservoir for EHV-1, and prevention of latency is not currently possible (Paillot et al. 2008; Lunn et al. 2024). The presence or absence of latent EHV-1 was not influenced by the horses' vaccination histories in one study in horses presented for post-mortem examination in France (Taouji et al. 2002).

Latent EHV-1 virus can undergo re-activation within the host and cause all forms that are caused by new infections: rhinopneumonitis, abortion, neurological disease, viremia, and also silent shedding and seroconversion (Paillot et al. 2008; Slater 2014). Re-activation of herpesvirus from latency has been investigated after various forms of stress. After pharmacological immunosuppression with high doses of dexamethasone, EHV-1 nasal shedding was detected in all four dexamethasone-treated horses, however, no co-mingling sentinel horses became infected during that trial (Pusterla et al. 2010a). In a survey of predominantly young horses and weanlings attending sales and other events in the USA, nasal shedding of EHV-1 was detected in 3.3% of horses tested upon arrival at the event (Carlson et al. 2013). Horses in mandatory quarantine in California, USA (median age six years) after international transport were tested by nasopharyngeal swabs or in peripheral blood; 1% was PCR-positive on arrival, and another 1.3% of horses seroconverted during quarantine, suggesting they had undergone reactivation or recent subclinical infection (possibly acquired en route; Pusterla et al. 2009). In a small study of eight pregnant mares consigned for sales, nasal shedding of EHV-1 was detected in one mare (Schulman et al. 2014).

Sometimes, no nasal shedding is detected when horses are tested after stressful events. Examples are a study in two-year old racehorses horses being gathered for sales (Badenhorst et al. 2015), and a study of 12 horses undergoing an 8-hour transport (Muscat et al. 2018). Two studies in hospitalised horses, one in horses in acute abdominal crisis (Carr et al. 2011), and one in horses that were febrile during hospitalisation (Sonis and Goehring 2013), detected no viral shedding of EHV-1 in any of the horses. All in all, predicting whether stressors will cause re-activation and shedding appears to be difficult.

The duration of protective convalescent immunity to EHV-1 is not known, but has been estimated to be three to eight months (Paillot et al. 2008). Anecdotally, mares are rarely observed to abort due to EHV-1 twice in consecutive years (Slater 2014). After three rounds of experimental infection in six weanlings, serum antibody was detected until the end of the study period at 13 weeks after the last infection (Breathnach et al. 2001).

Transmission

S. equi

S. equi is transmitted by infectious discharge from horses with strangles, transmitting infectious material by direct (nose-to-nose) or indirect contact via fomites such as halters, housing surfaces, drinking troughs, personnels clothing, and potentially flies (Boyle et al. 2018; Durham et al. 2018; Pusterla et al. 2020a). Intranasal exposure of a dose of $10^6 - 10^8$ CFU consistently induces typical disease in susceptible horses (Galan and Timoney 1985; Robinson et al. 2020); lower doses do not reliably induce disease even in naive horses (Boyle et al. 2018). Survival of *S. equi* in the environment outside of its host on a variety of fomites, climatic conditions and during cleaning protocols has been investigated; *S. equi* survival is prolonged in wet conditions and cool temperatures; contaminated water outlets or buckets may therefore be important routes of transmission (Todd 1910; Durham et al. 2018; Frosth et al. 2018; Pusterla et al. 2020a; Ryden et al. 2023).

EHV-1

EHV-1 is principally transmitted via inhalation of aerosolised respiratory droplets; other routes of transmission are direct contact or via fomites, or the ingestion of droplets from surfaces. After an abortion caused by EHV-1, fetal and placental tissues are highly infectious (Slater 2014). EHV-1 can survive and remain infectious in water for 2-3 weeks (Dayaram et al. 2017) and in outdoor or indoor environments for up to 48 hours (Saklou et al. 2021).

Diagnosis

S. equi

Once typical clinical signs of fever and abscess-forming lymphadenopathy of the head and neck are apparent, alternate diagnoses are scarce and the diagnosis of strangles is often made clinically (Boyle et al. 2018). However, highly pathogenic *S. zooepidemicus* strains can, in rare cases, cause a similar disease presentation (Lindahl et al. 2013). For confirmation, and for early stage disease or mild and atypical typical disease, ancillary diagnostics are necessary.

Presence of *S. equi* can be confirmed by culture or molecular methods (PCR) in nasal or lymph node secretions, with PCR being the more sensitive option (Båverud et al. 2007; Webb et al. 2013; Pringle et al. 2019). During the initial 24-48 hours of clinical signs, when fever and pharyngitis dominate, nasopharyngeal samples can return a false negative result as during this time, it is possible for the pathogen to only be present submucosally and not appear in samples of mucosal surfaces (Boyle et al. 2018).

To test past exposure and infection, two ELISA serologic tests are available; for the *S. equi* SeM protein (which has partial cross-reactivity with *S. zooepidemicus*, Robinson et al. 2013) or for combined antigen A/C antibodies. Both can be used to reliably demonstrate recent infection or non-DIVA vaccination. Serology can not be used to estimate the level of protective immunity of individual horses, as resistance to (re)infection seems to have an important local mucosal and cell-mediated component and serum antibody levels do not appear to be closely correlated with the level of protective immunity (Galan and Timoney 1985; Timoney and Eggers 1985; Davidson et al. 2008; Robinson et al. 2020).

S. equi serology cannot reliably predict carriership or freedom from carriership (Durham and Kemp-Symonds 2020). The challenges in diagnosis of carriers are described in detail in **Chapter 4**.

EHV-1

EHV-1 cannot be diagnosed based on clinical signs alone, especially if only respiratory signs are present. When accompanied by abortion and/or typical neurological signs, a tentative clinical diagnosis of infection with EHV-1 can be made, but additional confirmatory testing is nonetheless required. Since EHV-1 is a World Organization for Animal Health (WOAH) listed disease, recommendations for diagnostic testing are provided in their manual (WOAH 2022). PCR or virus isolation of nasal secretions, peripheral blood mononucleated cells, placental or fetal tissues, or paired serology 10-14 days apart can confirm EHV-1 as the etiological agent (WOAH 2022).

Ante-mortem diagnosis of latent EHV-1 infection can be difficult. Detection of EHV-1 in mandibular lymph nodes can confirm latent infection (Allen 2006), but absence of EHV-1 in these (accessible) lymph nodes does not prove absence of latent infection, as the virus can be present in other, less accessible sites (Pusterla et al. 2010b). Serology is also a poor predictor of latent infection (Dunowska et al. 2015).

Treatment

S. equi

Treatment of acute strangles cases is usually symptomatic supportive with anti-inflammatory drug treatment as required and nursing care.
Only in the most severe cases, if potentially life-threatening or if complications occur, may anti-microbial treatment be considered. *S. equi* is reliably sensitive to penicillin and no reports of penicillin-resistant *S. equi* cultures were found in a 2021 EFSA review (Nielsen et al. 2021), although this situation may not last forever, or even much longer. The sustained susceptibility to β-lactam antimicrobials is somewhat out of character for *Streptococcus* species, but is also observed for *S. pyogenes*, a streptococcal infection of great human health impact. Emergence of penicillin resistance in *S. pyogenes* is thought to be

a case of "if not when" (Beres et al. 2022), and the same could be true for *S. equi*.

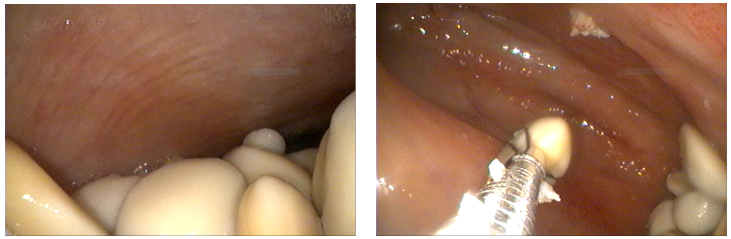


Figure 1.4: *Left: Chondroids (inspissated purulent material) in situ in a S. equi carrier's guttural pouch. Right: The same chondroids during the author's ultimately abandoned attempt at endoscopically-aided clearing of the infectious debris. Due to the large number of chondroids present, a decision was eventually made to access and empty the guttural pouch surgically under standing sedation, which was performed successfully a few days later. The pony made a full recovery and could be re-introduced to his herd within weeks. Source: R. Houben, Utrecht University.*
NB These chondroids also feature on the cover of this thesis.

S. equi carriers can be treated by removal of infectious debris from their guttural pouches, either endoscopically or surgically (Figure 1.4).

EHV-1

Treatment of disease caused by EHV-1 is mostly symptomatic. Treatment is not usually required for the mild respiratory disease. Mares which have aborted require routine post-partum care. Horses with neurological disease are treated as needed with supportive therapy such as anti-inflammatory drugs, anti-coagulants, fluids and nutritional support if needed, bladder catheterisation, and hoists to prevent injury from falls caused by profound ataxia and weakness (Figure 1.2). There is no treatment that will prevent or resolve latent infection with EHV-1 (Paillot et al. 2008).
Antiviral medications to prevent the viremia that is a prerequisite for EHM and abortion have been investigated, with acyclovir and its various prodrugs generating the most interest. So far, inconsistent results have been reported as to their efficacy for outcomes such as viremia, nasal shedding, and neurological disease. Valacyclovir currently appears to have the most favourable combination of affordability and apparent clinical efficacy (Vissani et al. 2016; Maxwell 2017; Thieulent et al. 2022; Goehring et al. 2024).

Prevention

S. equi

For strangles, biosecurity remains the most effective measure to prevent an outbreak. Introduction of *S. equi* into a herd can be prevented entirely by imposing a 14-day quarantine, and infection screening for all new arrivals or residents that have been away and have contacted other horses off the premises. Carrier-free status for new arrivals can be established before introduction into the herd by guttural pouch endoscopy and lavage together with a bilateral nasopharyngeal wash, and only allowing contact with the resident herd if all samples have returned a negative result. For returning horses which have been in contact with other horses but have not been ill, a 2-week quarantine should suffice, without the need to re-check for carriership. As a further preventative measure to prevent spread among the resident herd, e.g. if a carrier is already present in the herd or if a quarantine breach occurs, daily temperature checks will allow for quarantining of most if not all acute cases before they become infectious. Incidentally, the efficacy of daily clinical checks as a preventive measure for strangles outbreaks was already reported more than a century ago (Todd 1910).

Currently used vaccines against *S. equi* include an attenuated live intranasal vaccine (Borst et al. 2011), which needs to be boostered annually, and a submucosal live deletion-mutant strain vaccine which is injected into the lip and boostered every three months (Jacobs et al. 2000). More recently, a recombinant intramuscular vaccine with recommended annual boostering was introduced (Robinson et al. 2020), which has the added benefit of being a DIVA (Differentiating Infected from Vaccinated Animals) vaccine. Two strangles vaccines are currently available in the Netherlands: the submucosal live deletion-mutant and the recombinant intramuscular vaccine.

So far, strangles vaccines have seen limited uptake and some are not, or no longer, widely available. In a 1990 report from stud farms in Australia (Jorm 1990), vaccination with products available at the time had no significant effect on the likelihood of an outbreak occurring. A 2015 USDA report notes that out of equine operations which used any vaccines, 27% had applied a strangles vaccination in the previous 12 months. The main reason *not* to vaccinate against strangles was the perceived low risk of exposure to the disease (USDA 2016).

EHV-1

Prevention of transmission of EHV-1 is more difficult than for *S. equi*. If horses share an airspace or building, even extensive barrier precautions cannot fully prevent transmission (Lunn et al. 2024). In addition, unlike *S. equi* carriers, latent infection of EHV-1 cannot be tested reliably ante-mortem, so prevention of introduction of horses with latent infection onto a premises is not achievable.

Vaccines for EHV-1 are available and are effective at preventing outbreaks of respiratory disease (Bannai et al. 2014) and are partially effective at preventing abortions (Heldens et al. 2001), although one field study found no significant difference in the incidence of abortions with vaccination (Bresgen et al. 2012). Vaccination does not appear to be able to prevent neurological disease (Carvelli et al. 2022; Lunn et al. 2024).
Two meta-analyses have recently been published examining the effect of vaccination on clinical parameters, including nasal shedding, the principal route of EHV-1 transmission. Marenzoni et al. (2022) included only studies with experimental infection in randomised controlled trials, and found no significant effect of vaccination on the incidence of nasal shedding. Osterrieder et al. (2023) had a similar study design as Marenzoni et al. (2022), but included a broader range of experimental designs, and also excluded studies where nasal shedding was observed in all horses in both arms of the study. No significant effect of vaccination on the incidence of shedding was found in their main analysis, but a small but significant reduction in the risk rate for developing nasal shedding was found in the subgroup of commercially available modified live virus vaccines (i.e. excluding inactivated vaccines from analysis).

Prior EHV-1 vaccination was for a while thought to be a risk factor for development of EHM, with recent vaccination a risk factor (Traub-Dargatz et al. 2013) or higher total lifetime doses of vaccine a risk factor (Henninger et al. 2007) for EHM, but those results were not consistently confirmed (Klouth et al. 2022). Age (Henninger et al. 2007) or horses' location in the tent (Couroucé et al. 2023) may have been confounders in theses reports.
A European Food Safety Authority assessment panel in 2022 recommended the promotion of vaccination (Carvelli et al. 2022), as did the most recent EHV-1 Consensus Statement (Lunn et al. 2024), both while acknowledging the limitations of the effectiveness of the currently available vaccines. Vaccination for EHV-1 is discussed more in-depth in **Chapter 5**.

There is currently no information on uptake of vaccination against strangles or EHV-1 in the Netherlands.

Zoonotic potential

S. equi is host-specific for equids, but has sporadically infected humans, usually immuno-compromised individuals (Sleutjens et al. 2019; Torpiano et al. 2020).

EHV-1 is an equid-specific infectious agent, but has been recorded as a cause of mortality in zoological species (Wohlsein et al. 2011; Flanders et al. 2018). There have been no reports of EHV-1 infection in humans.

Global disease burden

Outbreaks of infectious disease affect horse welfare due to illness, but also due to movement restrictions during outbreaks, which leads to less opportunities for exercise and social contact, and increased time spent stabled in individual boxstalls. Outbreaks cause economic losses due to yard closures, limiting trading and breeding opportunities, prohibiting attendance or hosting of events, increased labour and feed cost as communal grazing becomes impossible, loss of livery clientele, and veterinary fees.

An overview of the annual incidence in selected countries and regions of *S. equi* and EHV-1 outbreaks, based on data from the International Collating Centre (ICC) and Surveillance of Equine Strangles (SES) websites, is presented in Figure 1.5. It is important to note that in most countries neither disease is notifiable by law, and under-reporting is likely, meaning that these data cannot be relied on for an accurate estimate of the global or even national burden of disease, nor should trends in reported incidence be directly interpreted as trends in true incidence.

S. equi

S. equi is a notifiable diagnosis in Sweden, which makes Swedish incidence reports more reliable than those from elsewhere. Swedish outbreak data are not reported by the ICC, but a report by the Swedish National Veterinary Institute indicated that in 2022, 66 outbreaks of *S. equi* were confirmed (SVA 2022). Strangles is not a notifiable disease in most other countries. Voluntary reporting schemes are in place in numerous other countries: Equine Infectious Disease Surveillance (EIDS) and SES in the UK, Surveillance Equine Infectieziekten Nederland (SEIN) in the Netherlands, and many more.

In countries where infection with *S. equi* is not a notifiable disease, a substantial proportion of outbreaks may remain unreported, due on the one hand to the stigma associated with strangles outbreaks, and on the other hand due to diagnosis not always being pursued. Particularly in large groups of young horses, owners may choose to let the outbreak run its course and have the affected animals develop protective immunity. Under-reporting of *S. equi* outbreaks in countries where the disease is not notifiable is therefore likely.

Although strangles is endemic in nearly all parts of the world, local incidence likely varies. A 1990 survey (Jorm 1990) among stud farms in New South Wales found an incidence of 2.1 cases of strangles per 100 horses per year. A 2010 UK study (Parkinson et al. 2011) estimated that around 600 outbreaks occur in the UK annually; a 2011 paper suggested an incidence of 700 outbreaks per year (Waller et al. 2011). A 2005 USA Department of Agriculture report (USDA 2005) listed strangles as a cause in 0.8% of all deaths recorded in their survey of equine premises.

The prevalence of apparently healthy carriers has been reported as 2.3% in Brazil, 3.1% in the UK, and 13.5% in Colombia (Libardoni et al. 2016; Durham and Kemp-Symonds 2020; Jaramillo-Morales et al. 2022). The prevalence of *S. equi* carriers in the Netherlands is the subject of **Chapter 4**.

Reported seroprevalence for *S. equi* antibodies ranges from 5.6-74% (Ling et al. 2011; Al-Ghamdi 2012; Tirosh-Levy et al. 2016; Minai and Araghi-Sooreh 2020; van Maanen et al. 2021; Stritof et al. 2021). Possible explanations for the large range of estimates include national or regional differences in seroprevalence (such as suggested by Stritof et al. 2021) and the effect of husbandry and housing practices, with highest prevalence reported in unregulated horses (van Maanen et al. 2021).

Surveillance initiatives provide some insight into the age distribution of the burden of disease for *S. equi*. Strangles is historically considered a disease of mostly young horses; older horses reportedly show milder symptoms, and therefore younger horses were traditionally considered more susceptible. Reports of field observations do not always support the historical assumption of strangles being a typical "childhood disease". Todd (1910) recounts an outbreak that occurred in a group of adult horses who were all five years or older. According to a 2011 survey (Pusterla et al. 2011), in horses aged 4-10 the most frequently detected infectious agent is *S. equi*, however no comment is made in that paper with regards to the incidence in the 4-10 year group *vs* other age groups. In a large outbreak in the UK, the average age of clinical cases of strangles was seven years (not including the seven foals out of 62 cases in total), but the age distribution of the animals at risk during that outbreak was not reported (Christmann and Pink 2017). In a report on samples submitted for diagnostic testing from horses with fever and respiratory signs in the USA (Jaramillo-Morales et al. 2023), 26% of positive samples were from horses aged 1-5 years, and 42% were from horses aged 6-15 years; only 9.5% of positive samples were from horses less than one year of age, despite samples from horses of less than one year old constituting 16% of all samples submitted. For *S. equi* diagnoses reported through the SES from 2019 to 2023, the median age of the sampled horse was 8 years, with a range of 2 weeks to 33 years.

EHV-1

Reliable global data on the incidence of disease caused by EHV-1 is not available; Figure 1.5 is likely based on incomplete reporting.

In Kentucky, USA, a region with a high density of breeding mares, the incidence of abortion due to EHV-1 dropped from 17/1000 pregnant mares in the 1960s to 2/1000 pregnant mares in 2002. This drop was partly attributed to the widespread uptake of vaccination on breeding farms in the area (Paillot et al. 2008). More recently, an incidence of 0.3 EHV-1 abortions per 100 pregnancies was reported (Roach et al. 2020).

The prevalence of nasal shedding in apparently healthy horses has been reported for a variety of circumstances. A survey of weanlings sampled on their home premises in Australia demonstrated presence of EHV-1 in peripheral blood mononuclear cells in 3.6% of foals, but none in nasal swabs of the same foals (Wang et al. 2007). In a longitudinal study of French Standardbred racehorses in training, nasal shedding was detected 1.9 times per 100 horses per month (Doubli-Bounoua et al. 2016). In apparently healthy horses in South Korea, sampled at their premises of residence, 12% of nasal swabs was PCR positive for EHV-1 (Seo et al. 2020). Nasal shedding was detected in 0.5% of horses presenting for routine dental care in the USA (Pusterla et al. 2020c).
Nasal shedding was not found in any of 111 apparently healthy Ethiopian equids, but was detected frequently in animals with respiratory disease (Negussie et al. 2017). A survey of Polish national studs also did not detect any nasal shedding in horses that were sampled at their home premises (Stasiak et al. 2018).
Several reports on nasal shedding at sports events have been published, all in California. When stalls, rather than horses, were sampled at one event site during the course of the winter competitive season, EHV-1 was detected by PCR in 0% to 1.6% of stalls (Lawton et al. 2023) while a similar testing protocol during the summer months did not detect any EHV-1 in nasal swabs nor in stall samples (Pusterla et al. 2023b). In the spring, no EHV-1 was detected in nasal swabs of 162 horses, but 3% of stall samples were positive for EHV-1. In the latter study, the authors could not be sure of EHV-1 PCR-negative status of the stalls prior to entry of the horses (Pusterla et al. 2022b).

The reported prevalence of latent EHV-1 infection on post-mortem investigations from various geographic regions has varied from 10% to 88% (Carvalho et al. 2000; Taouji et al. 2002; Allen et al. 2008; Pusterla et al. 2010b; Pusterla et al. 2012; Dunowska et al. 2015; Radalj et al. 2018; Bueno et al. 2020). Differences in reported latency prevalence may reflect true differences in prevalence, or differences in detection methods, as the presence of EHV-1 differs per anatomical site, with latency detected in the trigeminal ganglia more often than in the mandibular lymph nodes (Pusterla et al. 2010b).

There is no international standard for serologic tests for EHV-1, and many serologic tests have cross-reactivity with EHV-4 (WOAH 2022), therefore, seroprevalence reports are not discussed here.

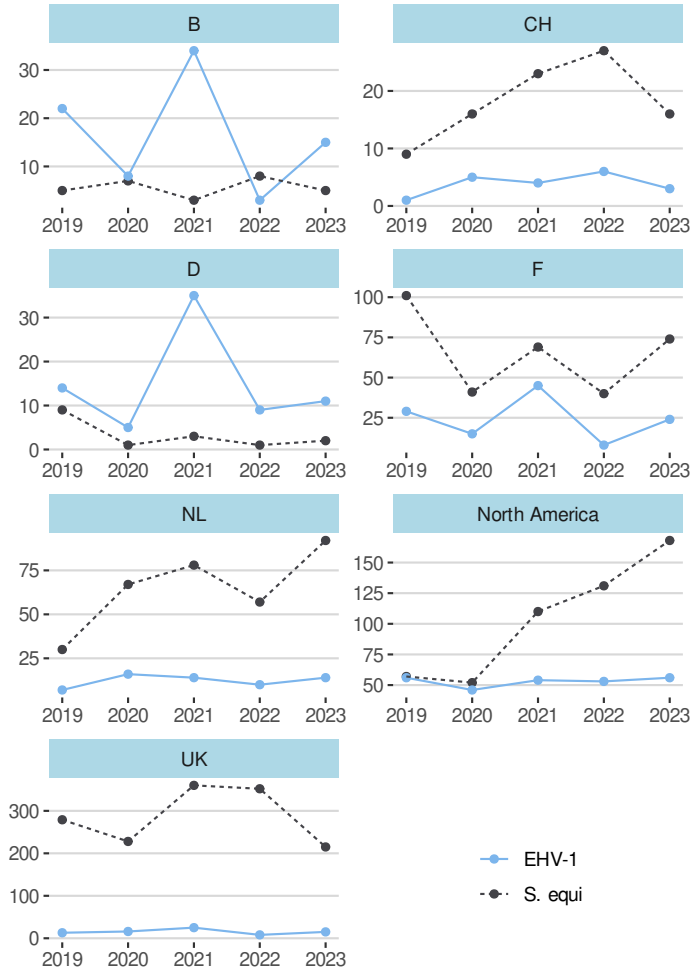


Figure 1.5: *Collated annual incidence of S. equi and EHV-1 outbreaks (2019-2023), according to the International Collating Centre and Surveillance of Equine Strangles websites. B–Belgium; CH–Switzerland; D–Germany; F–France; NL–the Netherlands. North America refers to Canada & USA. Under-reporting is likely in all depicted regions. The level of under-reporting may vary by country and by disease.*

Economic impact

S. equi

In a description of outbreaks on two Dutch premises, the cost per premises were estimated to be €40,000 to €90,000 with premises closed up to two months (C. van Maanen, GD Academy, 2018). A per-premises cost of an *S. equi* outbreak of up to $425,000 has also been reported (Waller 2014). In an impact report of 37 outbreaks in Sweden, the median cost incurred per premises was €4144 per outbreak (range €0 – €55,061) with closing of the premises necessary for a median of 6 weeks (Gröndahl and Ekmann 2019).

EHV-1

The threat of EHV-1 often complicates the work of equine veterinarians, in particular of those working in equine hospitals, and hospital outbreaks have occurred (Goehring et al. 2010a; Vandenberghe et al. 2021). Given EHV-1's various possible clinical presentations, from mild fever, to neurological disease without fever, to a dyspneic neonate, many patients that present to a veterinary hospital qualify as a potential EHV-1 case. This complicates the delivery of adequate care to these patients without jeopardising the other patients in the hospital.

In an outbreak in a Dutch equine referral clinic in 2018 (Vandenberghe et al. 2021), it was estimated that the total cost of that single outbreak amounted to €151,000. In a 2008 USA survey among stakeholders about the costs of EHV-1 outbreaks, lab costs were estimated at $12,000 - $20,000 per outbreak, board costs for horses held due to movement restrictions at $20,000, and the total cost of a hospital outbreak at $755,000 (USDA 2008). A European commission report on EHV-1 (Nielsen et al. 2022) reported cost estimations for equine premises in France involved in a nationwide outbreak in 2018. Per-premises cost estimates of €15,300 to €47,400 (depending on the premises type) were cited, with the highest costs incurred by "Leisure Centres". Besides the direct costs to the affected premises, the estimated cost to the French equine industry of that outbreak, incurred by the need for additional biosecurity measures, testing, and health certificates, was €1.1 million.

THE CURRENT CONTROL EFFORT

Few equine infectious disease of importance are amenable to eradication simply by lacking the properties that make eradication a realistic endeavor (Weese 2014). Table 1.1 lists these factors and assesses how well EHV-1 and *S. equi* fulfill theses categories.

Herds of feral or semi-feral horses and other equids exist worldwide, with population sizes up to an estimated 400,000 (Australian Brumby) and 73,000

Properties	*S. equi*	EHV-1
Readily identiable clinical disease	Yes	Silent infection cycles common
No chronic disease state with active infection	Yes	Yes
No long-term carrier state Carriers state is detectable Carrier state is treatable	No Yes Yes (but resource-intensive)	No Not reliably No
Only infects horses, with no wildlife reservoir[1]	Only equids susceptible	All equids and some non-equids susceptible[2]
Highly sensitive diagnostic tests available	Yes	Yes (but not for latent infection)
Highly effective vaccines available	Clinically yes, effect on transmission not yet quantified	No

Table 1.1: *Properties that facilitate eradication of an equine pathogen. Adapted from Weese (2014).* [1]*Pockets of feral and semi-feral equine populations exist worldwide, which could be considered wildlife reservoirs.* [2]*Wohlsein et al. (2011)*

(North American Mustangs and Burros). Other populations of free roaming feral horses exist: the Przewalski's horses of central Asia, the Lavradeiro horses of Brazil, and many more. The feral Konik horse population in the Netherlands is fenced in and does not come into direct contact with domesticated horses, but indirect contact is possible. Many other (semi-)feral horse populations do have opportunities for direct contact with domesticated horses. Some of these feral horse populations are protected by law (e.g. The Wild Free-Roaming Horses and Burros Act of 1971, USA) which means that removing infectious individuals from herds, even if required in an eradication effort, may not be possible (Bender 2007).

Clear written consensus guidelines on how to manage outbreaks and how to apply quarantine measures to avoid introduction of *S. equi* into herds were first published 15 years ago (Sweeney et al. 2005). Historical longitudinal data on the incidence of strangles in the Netherlands is not available, but empirically, the incidence of *S. equi* outbreaks does not appear to have diminished in the past 15 years. A similar observation is noted in the most recent consensus statement (Boyle et al. 2018).

The feasibility of eradication of strangles was coined in 2007; Prescott and Timoney (2007) suggested that eradication of strangles should theoretically be possible given the characteristics of the disease and available options for detection of diseased or carrier animals, treatment and prevention. The authors went as far as to propose an eradication plan (Figure 1.6). Practical objections which were then raised included the economic cost of eradication efforts, and the difficulty in addressing the disease in herds of free-ranging and feral horses which could continue to serve as reservoirs for the disease (Bender 2007). In a 2013 paper, the topic was once again addressed, with a focus on how, since

infectious horses can be traced and treated and outbreaks can be effectively managed, eradication should be technically feasible (Waller 2013). So far, no attempt seems to have been made to quantify the challenges of eradication through mathematical modelling.

Control step	Comment
Strangles becomes a reportable disease within discrete segments of the horse industry (eg, Thoroughbreds, Standardbreds, Quarter Horses, Saddlebreds, etc)	Program would be mandatory, with published findings and agreed outbreak investigation procedures. Funding would be from a levy on horse sales, betting, or an industry insurance scheme.
Each horse will be issued a health passport that records, among other things, exposure to strangles. Investigation of outbreaks would be done by certified personnel under veterinary supervision.	If not exposed, horses could proceed with racing, breeding, dressage competition, etc. For horses not in the passport scheme, quarantine and screening procedures would be included before they entered susceptible populations (eg, child's pony on a Thoroughbred farm). If exposed, institute movement controls. Horse would be monitored for signs of disease during subsequent 2 weeks. Bacterial culture or PCR assay would be performed if signs developed.
Import-export	Require health passport and recent culture or PCR assay.

Figure 1.6: *The eradication scheme proposed by Prescott and Timoney (2007).*

EHV-1 is listed by the WOAH as a disease of importance for equids (WOAH 2023), but infection with EHV-1 is not notifiable. The WOAH publishes recommendations regarding the import of horses: namely that a shipment includes an international veterinary certificate attesting that the animals: 1) showed no clinical signs of EHV-1 infection on the day of shipment; and 2) were kept for the 21 days prior to shipment in an establishment where no case of EHV-1 infection was reported during that period (Article 12.8.2). As silent infection cycles are common for EHV-1, this does not prevent horses from embarking on transport while infectious. In addition, horses can become infectious during their trip, either through re-activation from stress-related immunosuppression or by becoming infected *en route* by a travel companion.

Extensive recommendations for prevention and control of EHV-1 outbreaks on premises were published in 2002 (Allen 2002), and these continue to be the basis for current recommendations. The original EHV-1 Consensus statement (Lunn et al. 2009) described measures for control of EHV-1 at the premises level, and no changes on advice on control were made for the 2024 update.

At the time of publication of the original EHV-1 Consensus Statement (Lunn et al. 2009), EHV-1, and in particular EHM, were thought to be increasing in incidence. Whether that trend has continued or reversed is not clear; global incidence data have not been systematically collected in the period between.

Neither *S. equi* nor EHV-1 are among the infectious diseases covered by the WOAH's "High Health, High Performance" framework which aims to control the risk of infectious disease spread by the international transport of elite sport horses.

THE USE OF MODELLING TO STUDY THE DYNAMICS OF EQUINE INFECTIOUS DISEASES

The use of (mathematical) models to predict the effect of interventions (or different combinations thereof) is an integral, informative, and accepted part of considerations of control scenarios for many infectious diseases in humans and in farm animal species (Heesterbeek et al. 2015), yet for equine diseases, this approach appears underused. Equine infectious disease modelling studies so far have mostly focused on equine influenza (summarised by Daly et al. (2013)); glanders (Cárdenas et al. 2019), and the vector-borne infections African Horse Sickness (Klerk et al. 2023), West Nile Virus (Gonzálvez et al. 2023) and Equine Infectious Anemia (Machado et al. 2021).

No studies modelling the dynamics of infection by EHV-1 or *S. equi* are available, and in none of the disease control guidelines that were listed at the beginning of this chapter are the recommendations for control of the disease model-based, which seemed like an omission in need of addressing.

AIMS OF THIS THESIS

This PhD will aim to estimate transmission and other epidemiologically relevant parameters for *S. equi* and EHV-1, which will enable parameterisation of future predictive models of interventions for these globally endemic, and so far insufficiently understood, equine infectious diseases.

OUTLINE OF THIS THESIS

In **Chapter 2**, $\hat{R}_0$ is estimated for *S. equi* by analysing records from naturally occurring outbreaks. In **Chapter 3** this estimate is applied to parameterise mathematical models for *S. equi* transmission dynamics, and used to find estimates for thus far unknown or uncertain key parameters in *S. equi* natural history and epidemiology. To help asses the risk of introduction of *S. equi* via post-symptomatic carriers, a cross-sectional survey of outwardly healthy horses and ponies in the Netherlands was carried out to determine the prevalence of *S. equi* carriers, and is described in **Chapter 4**. Using preliminary contact network analysis, the opportunities of *S. equi* transmission from carriers to susceptible horses at competitive equestrian events in the Netherlands is also evaluated.

In **Chapter 5** the focus is turned to EHV-1 and here, outbreak data is analysed to find $\hat{R}_0$ for EHV-1 and also to evaluate the effect of vaccination of the herd on $\hat{R}$.

Chapters 2, **3** and **5** describe transmission dynamics within groups of horses with (more or less) random mixing. This assumption is defensible within a premises, but not for a national or global population of horses. To really understand disease transmission on a national scale, and predict outcomes on populations at a national or global level, the contact network of horses should be taken into account. The equine contact network is likely unique to the species: horses are housed in herds like livestock, but socialise like their human owners. Yet humans don't typically live in households of several dozens to several hundred individuals. Interventions to infectious disease transmission that are effective as well as practical therefore probably cannot be copy-pasted from strategies that are applied in humans or livestock. A short assessment of current biosecurity practices around horse movements onto premises is described in **Chapter 6**, and in **Chapter 7** the contact network of horses participating in competitive events in the Netherlands is described.

In **Chapter 8**, the general discussion, the new information gained through the work of this thesis is placed into context and directions for future work are suggested.

Part II

S. EQUI

Chapter 2

BASIC REPRODUCTION NUMBER FOR *S. EQUI* OUTBREAKS

Houben RMAC, van Maanen K, Kemp-Symonds JG, Waller AS, Sloet van Oldruitenborgh-Oosterbaan MM, Heesterbeek H (2023): Estimation of the basic reproduction number for *Streptococcus equi* spp. *equi* outbreaks by meta-analysis of strangles outbreak reports. *Equine Veterinary Journal* 55(3):506-514. doi:10.1111/evj.13865

Abstract

Background *Streptococcus equi* subspecies *equi* (*S. equi*), the cause of strangles in horses, is considered a highly contagious pathogen affecting equines and the equine industry worldwide. Fundamental epidemiological characteristics of outbreaks, such as the basic reproduction number (R_0), are not well described. **Objectives** Estimate R_0 for *S. equi* in equine populations from outbreak data. **Study design** Systematic review and meta-analysis of published and unpublished outbreak data. **Methods** A literature search for outbreak reports was carried out. Depending on data available in the reports, the early epidemic growth rate or final attack rate (AR) approach was used to estimate the basic reproduction number for that outbreak. Other recorded outbreak characteristics were the type of housing (group *vs* individual). An overall estimate for R_0 was computed by meta-analysis. **Results** Data from eight outbreaks were extracted from peer-reviewed publications. Data from two additional, non-published outbreaks was also included in the meta-analysis. A conservative estimate for R_0 was 2.2 (95% CI 1.9- 2.5). A less conservative estimate, including outbreaks with a 100% AR for which a lower limit R_0 was estimated, was 2.7 (95% CI 2.1- 3.3). **Main limitations** Few papers describing longitudinal incidence data were found so most estimates were based on the outbreaks' final size. Several outbreaks had a 100% attack rate and could therefore only be included as a lower limit estimate in the meta-analysis. The reported result therefore may be an underestimation. **Conclusions** This estimate for R_0 for *S. equi* informs parameters for future mathematical modelling, quantifies desired preventive vaccine coverage and helps evaluate the effect of prevention strategies through future modelling studies.

INTRODUCTION

Strangles is a disease in equids caused by infection with *Streptococcus equi* spp *equi* (*S. equi*) which is endemic nearly worldwide(Mitchell et al. 2021). *S. equi*'s impact on the global equine industry is severe enough to warrant a Consensus Statement by the American College for Veterinary Internal Medicine (Boyle et al. 2018) and incorporation in the International Codes of Practice on infectious diseases of the Horserace Betting Levy Board in the United Kingdom (HBLB Code of Practice: Strangles n.d.). It is a notifiable disease in several countries, including the USA.
Despite recent advances in understanding the epidemiology of *S. equi* and widespread knowledge on possible measures for the prevention of introduction and transmission, no trend towards decreasing incidence of illness due to *S. equi* has been reported over the past decade (Boyle et al. 2018).

Mathematical models of *S. equi* epidemiology may help identify key interventions which are practical and effective for reducing the impact of *S. equi*. Control of epidemics caused by *S. equi* transmission is complicated by the presence of silent, post-clinical "carriers" which can shed infectious material from their guttural pouches or paranasal sinuses without showing clinical signs (Newton et al. 1997; Boyle et al. 2018).
S. equi is usually considered to be a highly contagious pathogen (Boyle et al. 2018), however no estimate of the basic reproduction number (R_0) for acute strangles was found after review of the available literature. The basic reproduction number is defined as the average number of new infections of a pathogen caused by the introduction of one infectious individual into a completely susceptible population (Diekmann et al. 2013).
Estimates of the value of R_0 are useful as they can provide information on key parameters of mathematical models (Diekmann et al. 2013) that are difficult to measure in the field, such as transmission rates. Such models can be used to study how interventions influence transmission dynamics, such as a change in husbandry practices or vaccination strategy. Estimates of R_0 can be used to provide a rough estimate of minimal vaccine coverage $(1 - 1/R_0)$(Diekmann et al. 2013) required to reach the herd immunity threshold for the prevention of outbreaks. One could also interpret the quantity in terms of the probability that an introduction of an infectious individual into a well-mixed naive population will result in a major outbreak. This probability is $1 - 1/R_0$ (for $R_0 > 1$ and when the length of the infectious period follows an exponential distribution) (Diekmann et al. 2013).

The aim of this study was to estimate the basic reproduction number of strangles by analysing data from naturally occurring outbreaks.

METHODS

Search Strategy

The PUBMED/MEDLINE and CAB Abstracts databases were searched with the query:

(equine OR horse AND "*Streptococcus equi*") OR (equine OR horse AND strangles)

The resulting hits titles and abstracts were screened for mentions of *S.equi* horse-to-horse transmission or an outbreak, and full-text manuscripts were retrieved where possible. As clinical signs of strangles are highly specific, no limitation of the year of publication was applied as the authors considered that descriptions of outbreaks occurring at times preceding molecular diagnostics might still carry relevant information.

Studies were considered for inclusion if they contained, in the English language and within the manuscript or abstract, a description of naturally occurring horse-to-horse transmission of *S. equi* in a herd of likely naive horses and/or ponies. Information on the final size of the epidemic (attack rate) and/or early outbreak longitudinal incidence data of unmitigated strangles outbreaks were collected. Reports were excluded if from the outbreak description it seemed likely that multiple infectious individuals were introduced to the susceptible group; reports were only considered eligible if one point source of the outbreak was detected or if the source of the outbreak was not determined but assumed to be from a single source. Reports describing experimental infection were not included. Information was recorded, where available, on the herd composition and husbandry; in particular, whether horses were housed in groups for at least a significant part of the day, or were kept in individual boxstalls. For horses kept in individual boxstalls, the assumption of random mixing within the population is likely violated, however the information obtained by evaluating R_0 for this husbandry practice can be potentially useful in future studies. The type of premises and main use of the herd and this information was used to assess whether the herd was likely naive to *S. equi* at the time of the introduction of *S. equi*, and whether horses were group or individually housed, when this was not mentioned explicitly.

In addition to published outbreak reports obtained through the systematic review, unpublished data from the Animal Health Trust (HT) records on numerous outbreaks in the UK, collated prior to its closure in 2020, were checked for adherence to the inclusion criteria and outbreaks were added to the meta-analysis if the criteria were met.

Data extraction and calculation of the basic reproduction number

Data extracted from the reports, when available, were: type of housing (housed in groups with unhindered mingling, such as grazing or paddock turnout, with horses in boxstalls but with 'daily turnout' classed as group housed) or housing in individual boxstalls; herd immune status prior to the outbreak (whether or not some animals in the herd were likely to have at least partial protective immunity, this information was often assumed based on descriptions of the herd and its history); number of animals at risk, the number of animals infected by the end of the outbreak; and method for diagnosing infection with *S. equi*.
An estimate of the basic reproduction number was computed using two common estimators from early epidemic (exponential) growth rate data or based on the final attack rate (AR). The latter is the total fraction of the initial population that eventually becomes infected in the outbreak (1 AR). This is also referred to as the final size of the outbreak, $1 - s(\infty)$, the fraction of the original population that has escaped infection when the outbreak has run its course. For these estimates there are a number of assumptions (Diekmann et al. 2013). We assume that the herds are closed for the duration of the outbreak in the sense that there are no births or deaths or migration into or out of the herd in that time period. In addition, we assume that mixing inside the herd is homogeneous in the sense that a contact of the type that can potentially lead to transmission is equally likely for any pair of individuals in the herd (the herd is well-mixed). We assume that immunity that arises from infection lasts at least for the duration of a typical outbreak. We assume that all individuals in the herd are equal in their susceptibility, infectivity and contact pattern. Finally, it is assumed that the outbreaks run their course without mitigation of control measures of any kind.

The estimator based on the final size/attack rate is given by

$$\hat{R}_0 = \frac{\ln(s(0)) - \ln(s(\infty))}{s(0) - s(\infty)} \tag{2.1}$$

where $\ln$ is the natural logarithm, $s(0)$ is the fraction of the herd that is susceptible at the start of the outbreak (so $s(0) = 1$ in a fully susceptible herd) and $1 - s(\infty)$ is the final size (Dietz 1993).

The simplest estimator based on early outbreak exponential growth rate, denoted by r, is given by

$$\hat{R}_0 = e^{rT} \tag{2.2}$$

where T is the generation interval of the epidemic (see (Roberts and Heesterbeek 2007; Wallinga and Lipsitch 2007) for this and related estimators). We assumed the mean generation interval, or the time interval between successive cases in a chain of transmission, to be 13 days (standard deviation of 5 days) as suggested by (Sweeney et al. 1989). For Estimator 2, a compartmental

model of susceptible - infectious - recovered (and resistant) or SIR model is assumed (Dietz 1993), which we believed was an appropriate assumption for the timespan of the early exponential growth period of a typical *S. equi* outbreak.

Both estimators, including 95% confidence intervals (CIs) were calculated using the R_0-package (Obadia et al. 2012) in R (R Core Team 2021) which incorporates both, following the methods as described by Dietz *et al.*(Dietz 1993) and Wallinga & Lipsitch(Wallinga and Lipsitch 2007). The meta-analysis was then performed using the meta-package (Balduzzi et al. 2019). A generic inverse variance meta-analysis was applied, and a random effects model was selected for the meta-analysis to address heterogeneity in herd composition between the included outbreaks (Higgins et al. 2019). As the CIs produced by the R_0 – package were derived via a log transformed variable they were not always symmetrical around the mean (but usually very close). However, the meta-package assumes a symmetrical estimate and calculates the standard error as $(CImax - CImin)/3.92$. As the deviations from this assumption were minimal, we did not consider this issue problematic.

Estimator 1 cannot not be applied to reports in which the AR is 100%, as the resulting $R_0 = \infty$. *S. equi* outbreaks with a 100% AR can occur, and longitudinal data is not always available for such outbreaks, meaning that a point estimate and confidence interval for these outbreaks cannot be obtained and they cannot be included in the meta-analysis. In order to circumvent under-estimation of the overall R_0 estimate due to the exclusion of these 100% AR outbreaks, a lower limit R_0 estimate was calculated by assuming one horse in the herd escaped infection. Applying this method, only a lower bound for the 95% CI could be calculated via the method described by Dietz 1993 (Dietz 1993), as a result of the upper bound on the 95% CI AR being >1. For a weighted inclusion in the meta-analysis an upper bound is required, therefore a symmetrical CI was assumed $CI_{upper} = R_0 + (R_0 - CI_{lower})$ for these outbreaks.

Sensitivity analyses

To evaluate the effect of an alternative assumption on the generation interval for *S. equi* infection, a 'worst-case scenario' short interval of 4±1 days was assumed for R_0 estimations of outbreaks for which longitudinal data was available. The interval of four days was chosen as the shortest possible interval between one infection to the next, assuming horses become febrile three days after a large challenge dose (Guss et al. 2009) and becoming infectious one day later. This has been observed following experimental challenge of naive ponies with a dose of 10^8 CFU of *S. equi*. The meta-analysis was then repeated with these alternative R_0 estimates.

A second sensitivity analysis was performed where all outbreaks that had a 100% AR were included by applying Estimator 1 when assuming not n, but $n - 1$ animals were involved in the outbreak, i.e. assuming that one animal escaped infection.

The meta-analysis was also repeated after excluding outbreak data from non-published sources, i.e. the outbreaks marked HT.

Individual housing

A meta-analysis was also performed combining reports on outbreaks which did not meet the criterium of group housing, but which met the other inclusion criteria.

RESULTS

Outbreak reports retrieved

PUBMED/MEDLINE and CAB Abstracts (1910-present) databases were accessed between 26-12-2020 and 30-12-2020. The queries resulted in 562 and 1574 hits respectively. Eight of these titles described horse-to-horse transmission or an outbreak meeting the inclusion criteria and contained sufficient information for an R_0 estimate in their full-text (where available) or abstract (Piché 1984; Dalgleish et al. 1993; Zadeh et al. 1992; Newton et al. 2000; Katayama et al. 2003; Wilsher and Allen 2006; Davidson et al. 2008; Bhardwaj and Taku 2010; Riihimäki et al. 2018; Tscheschlok et al. 2018), of which(Newton et al. 2000) contained information on two outbreaks and only one of the outbreaks met the inclusion criteria. Two additional outbreak reports (Zadeh et al. 1992; Katayama et al. 2003) were retrieved which met the inclusion criteria except for the assumption of random mixing. The CAB Abstracts search returned 1 suitable report (Zadeh et al. 1992) which was not found by the PUBMED/MEDLINE search. The reverse did not occur. Attack rates were available for four additional outbreaks from HT records, two of which did not meet the criterium of random mixing (HT27 and HT52) ; three of the HT outbreaks (HT22, HT27, and HT52) were featured in Mitchell et al. 2021 as UK outbreak 44, 15 and 43. Two of the included outbreak reports contained suitable longitudinal incidence data for an R_0 estimate based on the outbreak exponential growth rate (Newton et al. 2000; Tscheschlok et al. 2018). All other outbreak R_0 estimates were based on the reported AR (Dietz 1993). A summary of herd, husbandry, and numerical data for each included outbreak is given in Table 2.1.

Table 2.1: *Overview of outbreaks included in the meta-analysis. *Note: Outbreaks in individually housed herds were analysed separately.*

Reference	Text	Group size	Attack rate	R0 Estimate	Herd description	Housing	Premises type	Country	Diagnosis of cases	Interventions
Tscheschlok et al. 2018	Fulltext	112	0.83	2.2	Yearlings with no history of strangles	Group	Group housing with direct contact between groups.	Germany	Clinical, culture, PCR, serology	None
Riihimaki et al. 2018	Fulltext	41	1	3.81 (n-1)	Adult icelandic horses	Group	Boxstalls with direct contact and turnout in small groups with indirect contact between groups until 10 months after the index case.	Sweden	All animals clinical signs and culture positive	None untill 10 months after index case
Bhardwaj et al. 2010	Fulltext	43	0.88	2.43	Working horses	Assumed group	Brick kiln	India	Clinical and culture	Penicillin for severe cases
Davidson et al. 2008	Fulltext	30	0.93	2.9	Naive pony weanlings	Group	Research herd	UK	Clinical and serology	None
Wilsher et al. 2006	Fulltext	20	1	3.15 (n-1)	Out of training TB fillies 2-3 research herd	Group	Paddocks 24/7	UK	Not mentioned, assumed clinical	None
Katayama et al. 2003*	Abstract only	58	0.43	1.31	Riding school	Assumed individual	Riding school	Japan	Culture of index case, clinical	
Newton et al. 2000-1	Fulltext	85	0.66	2.07	Closed herd	Assumed group	Unknown	UK	Culture of index case, clinical	Splitting into risk groups, but final AR not used for this outbreak.
Dalgleish et al. 1993	Abstract only	19	0.84	2.19	Young ponies	Assumed group	Research herd	UK	NP swab culture of all horses; S. equi not identified in all, possible overgrowth of S. zooepidemicus?	Some animals euthanised while still showing clinical signs
Zadeh et al. 1992-1	Fulltext	16	1	2.96 (n-1)	Adults 8-16 year old	Individual (direct contact possible)	Not mentioned	Iran	Nasal swab culture of some horses	All horses with clinical signs treated with penicillin
Zadeh et al. 1992-2	Fulltext	9	0.89	2.47	Adult horses 6-18 years old.	Assumed individual	Not mentioned	Iran	Nasal swab culture of some horses	All horses with clinical signs treated with penicillin
Piché et al. 1984 (youngsters group)	Fulltext	131	1	4.91 (n-1)	No strangles for 11 years; transient population, resident youngstock (<4yo) housed in separate (crowded) group; outbreak originated from mares	Group	Stud farm	Canada	Clinical	None except treatment of severely affected animals
HT22 2007	n/a	23	0.87	2.34	Mostly adult horses. Yard had seen an outbreak ±15 years prior.	Mostly group	Charity yard	UK	Clinical, serology, NP swab culture and/or PCR on all cases	
HT27 2006	n/a	21	0.67	1.65	Mostly adult horses. Yard had seen an outbreak ∼15 years prior.	Individual	Livery yard	UK	Clinical, serology, NP swab culture and/or PCR on all cases	
HT38 2006	n/a	26	0.62	1.55	TB mares and youngstock	Mostly group	Stud farm	UK	Clinical, serology, NP swab culture and/or PCR on all cases	
HT52 2007	n/a	8	0.63	1.57	Adult mixed	Individual	Livery yard	UK	Clinical, serology, NP swab culture and/or PCR on all cases	

Basic reproduction number

A forest plot of per-outbreak R_0 estimates and 95% CI and overall R_0 is presented in Figure 2.1. The overall estimate was $R_0 = 2.1$ (95% CI 1.8- 2.4).

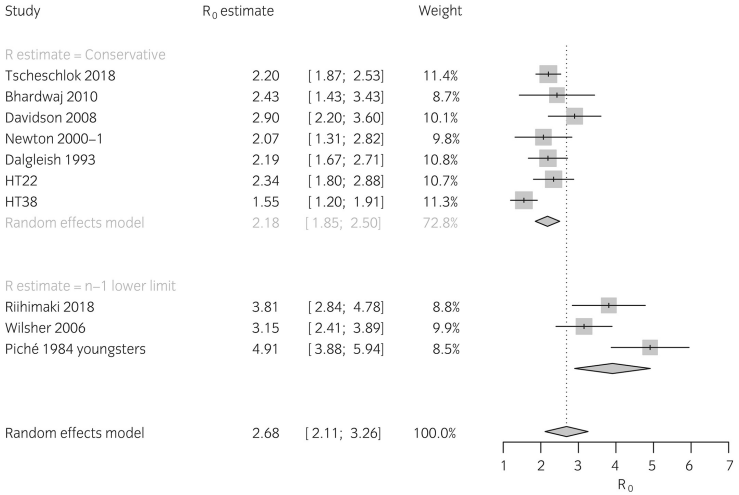


Figure 2.1: *Overall estimate by meta-analysis of the basic reproduction number (R_0). An overall estimate, when outbreaks with 100% attack rate (AR) are included by assuming $n - 1$ animals became infected (as described in the text), is provided (lower diamond) as well as a more conservative estimate, where the outbreaks with a 100% AR (which required a workaround to produce a lower limit estimate), were not included (upper diamond).*

Sensitivity analyses

Adding the 100% AR outbreak reports, as described in the methods section, resulted in an overall estimate of $R_0 = 2.7$ (95% CI 2.1 - 3.3).

Removing the data from the two outbreaks that were obtained from non-published sources, but including the 100% AR outbreaks, resulted in an overall estimate of $R_0 = 2.9$ (95% CI 2.3 - 3.5).

An overview of the R_0 estimates produced by the different generation interval assumptions is given in Table 2. The resulting overall R_0 estimate in the meta-analysis, when including the lower per-outbreak estimates which resulted from applying the shorter generation interval to the two outbreaks for which an R_0 was obtained by estimator 2, and including the 100% AR outbreaks was $R_0 = 2.5$ (95% CI 1.8 - 3.2).

	Generation interval	13±5 days	4±1 days
		R_0 estimate	
Reference	Tscheschlok et al. 2018	2.20 [1.90 - 2.56]	1.32 [1.25 - 1.39]
	Newton et al. 2000	2.07 [1.47 - 2.98]	1.29 [1.14 - 1.47]

Table 2.2: *A comparison of per-outbreak R_0 estimates based on epidemic growth rate, when assuming a realistic or a very short generation interval (see text) between successive cases.*

Individual housing

The overall estimate for the five outbreaks which occurred in herds which were housed in individual boxstalls was $R_0 = 2.0$ (95% CI 1.3 - 2.6). The corresponding forest plot is available as Supplementary Item B.1.

DISCUSSION

In this study we provide the first estimate of the value of the basic reproduction number R_0 for *S. equi*, the causal agent of strangles, based on a range of published descriptions of outbreaks. The overall estimate for R_0 of 2.1 (or, less conservatively, 2.7) found in this study does not support the assumption that *S. equi* is a highly contagious pathogen. Notorious, highly contagious human diseases (such as rubella: $R_0 = 6 - 7$, measles: $R_0 = 12 - 18$, or pertussis: $R_0 = 12 - 17$) (Vynnycky and White 2010) have substantially higher basic reproduction numbers. Given that the infectious period for strangles is not particularly short, usually assumed to be 14-21 days (Boyle et al. 2018) the number of daily contacts sufficient for disease transmission of disease per day per infectious horse likely is small.

Few basic reproduction numbers for equine infectious diseases are available for comparison. Estimates for equine influenza have been computed by outbreak analysis and/or modelling and have resulted in estimates of 2-5 to 10 (Satou and Nishiura 2006; Daly et al. 2013). The higher R_0 estimates for equine influenza, compared to *S. equi*, can probably to some extent be explained by the fact that equine influenza, unlike *S. equi*, can be transmitted via aerosols (Landolt et al. 2014), and is known to travel further distances and more easily spreads between premises without horse or fomite movement.

Some variation of estimates of R_0 for each of the individual outbreaks was found in the present study. Contributing to this variation, besides factors such as herd composition and husbandry, might be the previously observed reduction of virulence of the organism after prolonged persistence in a guttural pouch (Chanter

et al. 2000; Harris et al. 2015; Pringle et al. 2019). This was the proposed cause for the low morbidity in the outbreak described by (Tscheschlok et al. 2018), but may also have been a factor in other outbreaks which reported AR at the lower end of the spectrum. It is also important to note that in smaller herds, the effects of chance play a more important role in determining the final size of the outbreak. The size of the major outbreak from simple stochastic models has been shown to have a Normal distribution around the mean $N(1 - 1/R_0)$ (Diekmann et al. 2013), which can explain the chance occurrence of a relatively small (or indeed a relatively large) AR, given the value of R_0.

The overall estimate for R_0 found in this study was relatively low compared to other notorious veterinary and human diseases. This should be considered a favourable finding as a comparatively low R_0 suggests that measures such as enhanced biosecurity, pre-entry diagnostic screening and other prevention strategies could effectively minimise the probability of an outbreak occurring. For example, if vaccination of a herd is considered, the herd immunity threshold $(1 - 1/R_0)$ based on an R_0 of 2.7 is 62% which, considering the efficacy reports of some strangles vaccines (Jacobs et al. 2000; Robinson et al. 2020) could be an achievable goal. Vaccines would only need only be >68% effective to be able to attain the herd immunity threshold, and for vaccines with substantially higher efficacies, vaccination coverage may not need to be 100%. Care should be taken however, to consider other, unexpected and/or unwanted effects of various levels of vaccine coverage, and cost/benefit ratios should be established to determine the optimum vaccination strategy, which may vary depending on age distribution and husbandry practices within the herd. An important example of a potential adverse effect is a shift in the age groups which carry the majority of the burden of the disease (Anderson and May 1983; Panagiotopoulos et al. 1999), which might lead to strangles cases increasingly occurring at ages where the horse's economic potential is more seriously affected than it would have been had they been infected as a youngster. Whether or not, and under which circumstances, this or other unwanted effects are likely to occur should be the subject of future modelling studies.

It is worth pointing out that the estimates for R_0 reported in this paper are dependent on the assumptions underlying the estimators being met to a reasonable degree (Delamater et al. 2019), and deviations from these assumptions affect the accuracy of the R_0 estimate. In addition to this, the accuracy of the R_0 estimates are dependent on the accuracy of the data extracted from each of the reports; reports where diagnosis was based on clinical signs only may underestimate the R_0 for that particular outbreak as silent infections may potentially have been missed. Silent infections with *S. equi* in naive horses are not common, but have been described (Tscheschlok et al. 2018). The overall estimate for R_0 provided in this current work is intended to serve as a rough initial estimate, with the understanding that it may be an underestimation to some extent, but an estimate nevertheless which we hope will be of use in future studies evaluating possible interventions against transmission of *S. equi*. Alternative methods to calculate R_0

exist, in particular through mathematical models where all relevant parameters of disease transmission are assigned a certain value, transmission is described by equations and R_0 is calculated by solving these equations (Diekmann et al. 2013; Delamater et al. 2019). However, although much is currently known with some certainty about the natural history of *S. equi* infection, such as duration of the incubation period and average duration of infectiousness, key parameters such as most importantly the effective contact rate, were still absent. One way to find values for these missing parameters would be through infection experiments, where transmission can be followed in real-time and which would give the most accurate information. (Hamlen et al. 1994) conducted an infection experiment in foals, with the aim of evaluating the effect of prior exposure to strangles illness upon re-exposure; however, in their setup, foals with prior exposure and naive foals co-mingled; also, no longitudinal data was available from this report. Setting up a suitable infection experiment, although a very reliable way to find R_0, will come at a significant financial and animal welfare cost. The current project was devised to find an estimate for R_0 from already available data.

Sparse data was available to evaluate the effect of housing on R_0 so it is not possible to draw a meaningful conclusion on the effect of this husbandry practice on R_0, other than that R_0 is likely lower in purely individually housed animals, which would be a plausible effect of a reduction of the opportunities for effective contact for transmission both direct (social behaviours e.g. nosing) and indirect (e.g. shared water sources) contact.

Exclusion of non-published outbreaks had only a minor effect (increase from 2.1 to 2.3) on the overall estimate for R_0. Inclusion of the lower limit R_0 estimate for the 100% AR outbreaks did have a notable effect (increase from the conservative estimate of 2.1 to 2.7). It is not immediately evident why these particular outbreaks had such a high AR; they did all occur in group-housed, juvenile or naive (Riihimäki et al. 2018) horses, but so did other outbreaks with lower attack rates (Davidson et al. 2008; Tscheschlok et al. 2018). Herd sizes of the 100% AR outbreaks were mostly smaller (20-41 animals) (Davidson et al. 2008; Riihimäki et al. 2018) and as such were more susceptible to outcomes occurring by the effects of chance, as described earlier.

Using an alternative, very short estimate for the generation interval had a substantial impact on the R_0 estimates for those particular outbreaks (as summarised in Table 2) which, as could be expected, were lower than when a longer generation interval was assumed (Wallinga and Lipsitch 2007). Due to the relatively few outbreaks for which the R_0 estimation was based on the growth rate, the effect on the overall estimate for R_0 was limited (decrease from 2.7 to 2.5). In the authors clinical experience, the estimate of 13ś5 days (Sweeney et al. 1989) is probably closer to the real-life transmission dynamics than the assumption of a worst-case scenario generation interval, where animals are assumed to become infectious in the shortest possible time and to always infect another horse within 1-2 days of becoming infectious. Going forward, the

authors do not recommend using such a short generation interval for modelling the dynamics of *S. equi* transmission.

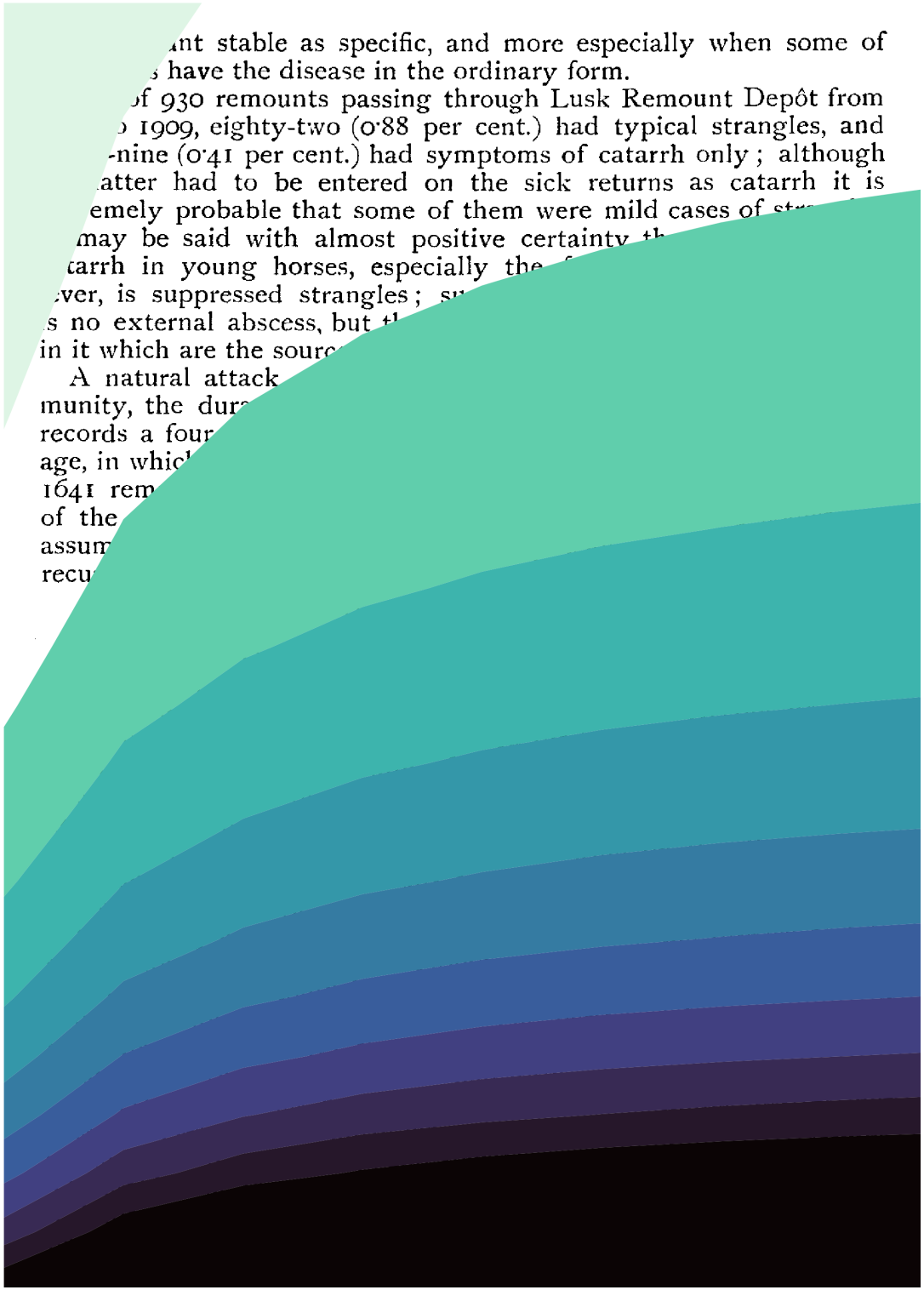
In the future, the estimate for R_0 calculated in this study should be corroborated by analysis of further unpublished outbreak data from sources such as diagnostic laboratories or national disease collating centres. To mitigate the drawbacks of analysing outbreaks with 100% AR where Estimator 1 cannot be applied, more longitudinal early outbreak incidence or peak incidence data of extensive outbreaks are required. Additional data will also be needed to better evaluate the effect of housing type on the reproduction number.

Many papers included in the study did not mention explicitly the immune status of the herd involved and therefore the decision to include outbreaks for the R_0 meta-analysis was open to classification error. In including papers, a decision was made to err on the side of caution as accuracy of the R_0 estimate was prioritised. The first outbreak in (Newton et al. 2000) was included due to the description of the affected horses being in a 'closed herd'; the outbreak described by (Piché 1984) was described to have occurred on a stud farm with a long strangles-free history, therefore the youngsters on the premises were considered to likely be naive. Whether assuming a fully naive population in these outbreaks was correct remains unknown. If the assumption was incorrect, inclusion of these outbreaks could contribute to underestimation of the overall R_0 estimate.

As the focus of the current paper was the reproduction number of outbreaks of strangles, the question of the epidemiological contribution of post-clinical persistent carriers of *S. equi* was not addressed as these carriers mainly become epidemiologically significant in instigating new outbreaks at time-spans exceeding those of the initial outbreaks analysed in this study.

CONCLUSION

The overall estimate for R_0 produced by meta-analysis of outbreak reports was lower than anticipated for *S. equi*, which suggests that even small improvements in biosecurity, screening and disease prevention could have important benefits. The precision and accuracy of the R_0 estimate found in this study may be improved through analysis of more longitudinal outbreak data, and more outbreaks where housing type and herd immunity status is clearly stated. Nonetheless, the R_0 estimate produced in this study can be used to parameterise epidemiological models studying the possible effects of preventive or mitigating interventions, such as changes in husbandry practices, hygiene protocols, and vaccination.



Chapter 3

UNTANGLING THE STRANGLEHOLD: MATHEMATICAL MODELS

Houben RMAC, Newton JR, van Maanen K, Waller AS, Sloet van Oldruitenborgh-Oosterbaan MM, Heesterbeek JAP: Untangling the stranglehold through mathematical modelling of *Streptococcus equi* subspecies *equi* transmission. *Preventive Veterinary Medicine* doi:10.1016/j.prevetmed.2024.106230

Abstract

Strangles, a disease caused by infection with *Streptococccus equi* subspecies *equi* (*S. equi*), is endemic worldwide and one of the most frequently diagnosed infectious diseases of horses. Recent work has improved our knowledge of key parameters of transmission dynamics, but important knowledge gaps remain. Our aim was to apply mathematical modelling of *S. equi* transmission dynamics to prioritise future research areas, and add precision to estimates of transmission parameters thereby improving understanding of *S. equi* epidemiology and quantifying the control effort required. A compartmental deterministic model was constructed. Parameter values were estimated from current literature wherever possible. We assessed the sensitivity of estimates for the basic reproduction number on the population scale to varying assumptions for the unknown or uncertain parameters of: (mean) duration of carriership ($1/\gamma_C$), relative infectiousness of carriers (f), proportion of infections that result in carriership (p), and (mean) duration of immunity after natural infection ($1/\gamma_R$). Available incidence and (sero-)prevalence data were compared to model outputs to improve point estimates and ranges for these currently unknown or uncertain transmission-related parameters. The required vaccination coverage of an ideal vaccine to prevent major outbreaks under a range of control scenarios was estimated, and compared available data on existing vaccines. The relative infectiousness of carriers (as compared to acutely ill horses) and the duration of carriership were identified as key knowledge gaps. Deterministic compartmental simulations, combined with seroprevalence data, suggest that $0.05 < \hat{f} < 0.5$ and that the duration of protective immunity after infection is likely 4–6 years. The presence of carriers alone may suffice to keep *S. equi* endemic in a population implying that carriers cannot be ignored in control efforts. Weekly screening of herds for signs of strangles could be sufficient to ensure $R < 1$, provided all horses are screened for carriership post-infection. In some of worst-case scenarios, vaccination alone would not suffice to prevent major outbreaks from occurring. A stochastic agent-based model was also constructed and validated, and used to simulate a remount depot, to evaluate whether historical incidence data of recurrence of strangles within individuals could be explained without the assumption that one in four horses fail to mount a lasting immune response. These simulations demonstrated that the observed data could have occurred without that assumption.

INTRODUCTION

Strangles, a disease caused by *Streptococcus equi* spp *equi* (*S. equi*), is endemic nearly worldwide with substantial impact on the equine industry and on equine health and welfare (Boyle et al. 2018; Mitchell et al. 2021).
Progression towards quantifying the effort required for the control of *S. equi* has been made by our recent work to find an estimate for the range of the basic reproduction number (R_0) from data of outbreaks in naive populations, in the absence of interventions (Houben et al. 2023). This can provide an estimate for the range of the contact rate (β) between infectious and susceptible horses for use in epidemiological models for strangles exploring intervention scenarios. After an outbreak, a proportion of convalescent animals fail to clear the pathogen from their guttural pouches or sinuses (Newton et al. 1997; Riihimäki et al. 2018; Tscheschlok et al. 2018; Boyle et al. 2018). These animals remain infectious, without themselves displaying clinical signs of disease, and are considered to be "carriers". The *S. equi*-horse-system is complicated by this and related factors in non-naive populations. These aspects will influence infection dynamics and hence most likely also the effectiveness of interventions. Important knowledge gaps remain concerning some of these epidemiological parameters of *S. equi*, such as the duration of carriership, the duration of protective convalescent immunity, and the relative infectiousness of carriers. We can, however, make use of the estimates of R_0 and (derived from that) β in a model to determine how ranges of values for the unknown parameters influence control effort.

The duration of carriership has been described to last anywhere from several months to lifelong (Newton et al. 1999; Gröndahl et al. 2015; Boyle et al. 2018). However, limited data is available on the mean duration of carriership or the distribution of duration thereof, in particular in the absence of interventions. The longest recorded duration of carriership after clinical disease in one study was 56 months (Newton et al. 1999). Another study prospectively followed a group carrier horses after a recorded outbreak, but only up to 45 weeks (Pringle et al. 2019). Prospective long-term follow up data of carriers until self-cure is rare, as many horses undergo treatment upon diagnosis, which is why obtaining sufficient records on the duration of carriership without interventions is difficult.

The probability of becoming a carrier after infection has been reported in several descriptions of outbreaks of strangles, and appears to be 10-40% (Newton et al. 1997; Gröndahl et al. 2015; Riihimäki et al. 2018; de Brauwere and Kirton 2019; Delph et al. 2019), although most reports are in the 10-20% range. In a 1999 study with prospective sampling of equine premises after outbreaks of strangles in the UK, at least one carrier was found in one in four outbreaks (Newton et al. 1999).

The duration of protective immunity after natural infection lacks precise reports of its mean duration or the distribution of its duration. It is likely that local immunity plays an important role independent of the serological response (Galan

and Timoney 1985; Jacobs et al. 2000), making extrapolation of duration of protective immunity from serological data an invalid approach. The current assumption for the duration of protection against reinfection is that it must have a bimodal distribution where approximately 25% of horses fail to develop protective immunity lasting more than a few months, and the remaining 75% are resistant to reinfection for a long duration, usually assumed to be ≈5 years (Waller et al. 2014; Boyle et al. 2018).
This assumption is primarily based on the following text in Todd (1910), transcribing findings at an army horse remount depot where "*...a four years' experience with horses over two years of age, in which 2195 had it once, 543 twice, and 121 three times, and 1641 remained unaffected.*" Although Todd in his 1910 paper does not make the inference that one in four horses do not mount lasting convalescent immunity, this interpretation was made explicitly later (Hamlen et al. 1994) and has since been cited in other work (Sheoran et al. 1997) and has been incorporated in both the first as well as the revised *S. equi* consensus statement (Sweeney et al. 2005; Boyle et al. 2018). In an infection-rechallenge experiment, when six ponies were re-challenged with a 10^{10} CFU intranasal dose 20 days after recovering from disease resulting from a 10^{8} CFU intranasal dose, all six ponies remained disease-free (Galan and Timoney 1985), although it should be noted that 3/6 had received an immunisation course prior to the first intranasal challenge; the immunisation had failed to prevent clinical strangles in 10/10 of the ponies it had been administered to. A 1994 study described an infection experiment where 2/12 foals that had been previously among a herd of foals which experienced a strangles outbreak, were affected with clinical signs of strangles when re-exposed 6 months later (Hamlen et al. 1994). The proportion of "poor responders" in that experiment would be 1/6 or approximately 17%. The main drawback for extrapolation from the study by Hamlen *et al* is that its subjects were exclusively foals aged 5-7 weeks at the time of the initial outbreak, and they cannot be assumed to have had mature immune responses at that time and some may have benefited from protection by maternal antibodies, interfering with the development of acquired immunity. Furthermore, it is not entirely clear from that paper whether the previously exposed foals that demonstrated clinical signs of strangles in the second phase did or did not demonstrate clinical signs of strangles in the initial outbreak.
In another study (Sheoran et al. 1997), local and serological protective antibody was demonstrated to last up to at least 28 weeks in convalescent horses after experimental infection by intranasal inoculation; within that time-span, these authors did not report observing a proportion of horses with markedly poor or rapidly declining immune responses. If such a marked bimodal distribution in the duration of convalescent immunity truly exists, then this affects the suitability of a simple SEIRS model, as possibly the *Recovered* compartment should be split into two, reflecting the long and short convalescent immunity. If a large proportion of animals returns to the *Susceptible* compartment soon after recovering from infection, epidemics might persist indefinitely.

Several options for immmunisation against *S. equi* currently exist. An extract

vaccine (Hoffman et al. 1991), a live-attenuated vaccine (Borst et al. 2011) and a multi-component protein vaccine (Robinson et al. 2020) are commercially available, of which the latter demonstrated the highest clinical efficacy after experimental challenge of 94% after a course of three immunisation doses (Robinson et al. 2020).

The relative infectiousness of carriers compared to horses with typical strangles is a parameter for which no current estimate exists. Infectiousness depends on discharge of infectious material from airways or abscesses (Boyle et al. 2018), and therefore carriers, who produce much less discharge, may be less infectious than horses with strangles.

Compartmental deterministic models can provide insights into the likely range of unknown or uncertain parameters and be used to explore the effect of varying assumptions for a range of parameter values on model outcomes, and thus can highlight key areas of future (field) research. For example, Shi et al. (2023)(2023) recently published a mathematical stability analysis of a compartmental strangles model. As a case study, they applied it to reported disease incidence from an outbreak in an open herd with unknown, but probably variable levels of immunity against *S. equi* described by Christmann and Pink (2017) (2017). Stochastic models can incorporate the effect of random events, to mimic the effect of chance in real world conditions, a feature which is inherently absent from deterministic models. Compartmental deterministic models are best suited to modelling dynamics in very large populations, as in smaller populations chance effects play a greater role. Agent-based (also termed "individual-based") stochastic models are better suited to modelling smaller populations, can track each individual in the simulation and record how the events that occur during the simulation affect each individual in it. Over sufficient repetitions of runs of a stochastic model, a distribution of likely outcomes emerges.

Stochastic models (Glass et al. 2002; Baguelin et al. 2010; Machado et al. 2021) and even agent-based stochastic models (Rua-Domenech et al. 2000) have seen previous applications in the field of equine infectious disease research. The model applied by Rua-Domenech et al. (2000) unfortunately is not publicly available and is written in a proprietary programming language.
A parallel aim for this report was therefore to implement and validate a stochastic agent-based model of disease spread in equine populations in an open-source statistical computing language (R) with which many researchers have prior experience, improving accessibility and repeatability for further work.

Our main aims were to add precision to current estimates of key epidemiological parameters of *S. equi*, provide estimates for epidemiological parameters which previously did not exist, and highlight key knowledge gaps in epidemiological parameters of *S. equi*.

METHODS

A compartmental model for *S. equi* transmission was constructed (Figure 3.1). A review of available literature was carried out to find estimates for parameters required to inform the model, and for any prior descriptions of models for *S. equi* transmission dynamics. In addition, an agent-based stochastic model was constructed. All models were built in R version 4.1.2 (R Core Team 2021). Differential equations were solved numerically using the deSolve (Soetaert et al. 2010) package.

Model parameterisation

Assumptions about parameter values and their sources are collated in Table 3.1. Recovery after carriership, loss of protective immunity and other parameters that occur over time are, in mathematical modelling, considered as rates per unit of time, therefore $1/\gamma$, for example, gives the (mean) number of days it takes for a horse to recover from acute disease and infectiousness.

Compartmental deterministic model of *S. equi*

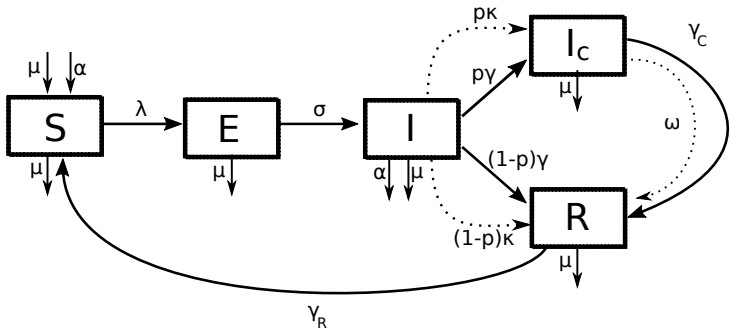


Figure 3.1: *Basic compartmental model of S. equi infection. Parameter descriptions and values are listed in Table 3.1. Interventions are indicated by dashed lines, solid lines represent the natural history of the disease. S: susceptible, E: exposed but not infectious, I: infectious (strangles), I_C: infectious (carrier), R: recovered from infection, convalescent immunity, μ: birth and death (natural causes) rate, α: the disease induced mortality rate, λ: force of infection, σ: rate of transition from E to I, p: probability of becoming a carrier after infection, γ: rate of recovering from acute disease, γ_c: daily rate of horses losing carriership status, γ_R: rate of loss of protective immunity, κ: daily rate of removing horses with clinical disease from the general population, ω: daily rate of removing carriers from the general population.*

The model depicted in Figure 3.1 is described by differential equations 3.1 - 3.5

where S is susceptible, E is exposed but not infectious, I is infectious (strangles), I_C is infectious (carrier), R is recovered from infection, convalescent immunity, μ is the birth and death (natural causes) rate, α is the disease induced mortality rate, λ is the force of infection, σ is the rate of transition from E to I, p is the probability of becoming a carrier after infection, γ is the rate of recovering from acute disease, γ_c is the daily rate of horses losing carriership status, γ_R is rate of loss of convalescent protective immunity, N is the total number of horses. The following interventions are also incorporated into the model: κ which is the daily rate of removing horses with clinical disease from the general population and ω which is the daily rate of removing carriers from the general population.

$$\frac{dS}{dt} = \mu N + \alpha I - \frac{\beta SI}{N} - \frac{f\beta SI_C}{N} + \gamma_R R - \mu S \tag{3.1}$$

$$\frac{dE}{dt} = \frac{\beta SI}{N} + \frac{f\beta SI_C}{N} - \sigma E - \mu E \tag{3.2}$$

$$\frac{dI}{dt} = \sigma E - \gamma I - \kappa I - \mu I - \alpha I \tag{3.3}$$

$$\frac{dR}{dt} = (1-p)\gamma I + \gamma_C I_C + \omega I_C + (1-p)\kappa I - \gamma_R R - \mu R \tag{3.4}$$

$$\frac{dI_c}{dt} = p\gamma I + p\kappa I - \gamma_C I_C - \omega I_C - \mu I_C \tag{3.5}$$

In this model, horses with new infections that are identified through screening for clinical signs (and presumably quarantined) are instantly transferred to their next compartment (R or I_C), skipping whatever remaining time they would have been ill and infectious but not contributing to new infections. This does result in infectious and ill horses no longer having the opportunity to die as a result of the disease during what would have been their remaining time in compartment I, which leads to a decrease in α. We considered the alternative option of adding a compartment Q with a fixed (obligatory) quarantine period with an α similar to the I compartment. However, this would have the effect of artificially inflating mortality due to disease. Given the low overall mortality due to disease, we opted to apply the first approach and let κ be a rate. ω the rate at which convalescent horses are checked for carriership by (e.g. by guttural pouch sampling) is also a rate, which resembles reality as treatment of carriers is swift compared to γ_C.
Based on Equations 3.1 - 3.5, R_0 without interventions can be described as a function of p:

$$R_0(p) = \frac{\sigma}{\sigma+\mu} * \frac{\beta}{\gamma+\mu+\alpha} + p * \frac{\gamma}{\gamma+\mu+\alpha} * \frac{f\beta}{\gamma_c+\mu} \tag{3.6}$$

If the time-span is short enough to assume $p = 0$ (because no horse has had sufficient time passed since infection to be able to be considered a carrier), which was the case for $\hat{R}_0 = 2.7$ (Houben et al. 2023) in new outbreaks, the second

term on the right hand side of Equation 3.6 vanishes, and the contact parameter β can be directly calculated as:

$$\beta = R_0(0) * \frac{\sigma}{\sigma + \mu} * (\gamma + \mu + \alpha) \tag{3.7}$$

The force of infection is described by:

$$\lambda = \frac{\beta I}{N} + \frac{f \beta I_C}{N} \tag{3.8}$$

Dynamics at the population scale

Having calculated a contact parameter β using Equation 3.7, Equation 3.6 can be used to calculate R_0 for time-spans exceeding those of a single outbreak. The relative influence of unknown or uncertain parameters (f, p, γ_C, γ_R) on the final estimate of R_0 was evaluated. The compartmental model was run for 10,000 days (until a steady state was reached), at which point $(R + I_C)/N * 100$ at steady state was taken to be the expected seroprevalence, $(I_C)/N * 100$ the expected prevalence of carriers, and $I/N * 100$ the prevalence of animals with clinical signs of strangles. Results were compared to real-world observational data (Todd 1910; Minai and Araghi-Sooreh 2020).
Finally, the interventions presented in Figure 3.1 were used to construct the following equation for the reproduction number R in a population where control measures are active:

$$R = \frac{\sigma}{\sigma + \mu} * \frac{\beta}{\gamma + \mu + \alpha + \kappa} + p * \frac{\gamma}{\gamma + \alpha + \mu + \omega} * \frac{f\beta}{\gamma_c + \mu + \omega} \tag{3.9}$$

$R = 1$ was then computed for a range of assumptions for ω, κ, p and f. For this paper, vaccinations are not explicitly modelled but vaccination is evaluated in the context of the herd immunity threshold of $1 - \frac{1}{R}$. This implies the assumption of a perfect vaccine, with 100% efficacy for the prevention of both clinical disease and infectiousness. Although data is available on clinical efficacy of *S. equi* vaccines (Jacobs et al. 2000; Robinson et al. 2020), no quantitative data on the effect of vaccination on infectiousness or R is currently available. We therefore use the herd immunity threshold as a means to assess what vaccine efficacy would be required to achieve the effect of vaccination that is being modelled. Any interaction between vaccination, ω and κ was not evaluated.

Table 3.1: *Model parameters.*

Parameter	Description	Deterministic model input	Stochastic model input	Source
$1/\mu$	Birth and death rate	1/5475 per day	Normal distribution 1/3650±365 per day	None
β	Group housing contact parameter	0.199 per day	0.199 per day	Houben et al. (2023) and Equation 3.7
$1/\sigma_L$	Duration of latency (no clinical signs, not infectious)	7 days	Poisson(7)	Boyle et al. (2018)
$1/\sigma_P$	Duration of pre-infectious period (clinical signs, not infectious)	3 days	Poisson(3)	Boyle et al. (2018)
$1/\sigma$	$1/\sigma_L + 1/\sigma_P$	10 days	Poisson(10)	
$1/\gamma$	Duration of / recovery from acute infection (and infectiousness)	14 days	Poisson(14)	Boyle et al. (2018)
$1/\gamma_C$	Recovery from carriership	Varied	1825 days	Newton et al. (1999) and Pringle et al. (2019)
$1/\gamma_R$	Loss of protective immunity after infection	Varied	Normal distribution, mean and sd varied (see text)	Waller et al. (2014) and Boyle et al. (2018)
f	Relative infectiousness of carriers	$f \in (0, 1)$	0.25	None
p	Proportion of infections resulting in carriership	$p \in (0.1, 0.4)$	0.1	Newton et al. (1997), Riihimäki et al. (2018), and Delph et al. (2019)
α	Daily mortality rate as a result of disease	Total mortality as a result of disease 3(0-10)% , set at 0.03*γ	Death with probability of 0.03/14 each day spent in state I	Todd (1910): 0-3%; Piché (1984): 3.6%;Sweeney et al. (1989): 8.1%; Christmann and Pink (2017): 11%
$1/\omega$	Number of days until detection of carriership and treatment/isolation (after resolution of acute disease)	$\omega \in (0, \infty)$ days	Not modelled	
$1/\kappa$	Number of days until detection of acute disease and removal from herd	$\kappa \in (0, \infty)$ days	Not modelled	

Stochastic agent-based model

The stochastic agent-based model algorithm is described in the text box. In the agent-based stochastic model, values for μ, σ, γ_C and γ_R for different individuals are independent and identically distributed and drawn randomly from a Poisson distribution with a rate of $\lambda = Value$. γ_R is randomly and independently distributed with a Normal distribution; γ_R is also independent of the individual's prior infections or prior values for γ_R. Two implementations were trialled: For a model applying the Sellke construction (Diekmann et al. 2013) a horse acquires an infection if $\lambda > Q$ where Q is a an individual's threshold for becoming infected ; Q is randomly drawn from an exponential distribution with a mean rate of 1 (Diekmann et al. 2013) and λ is recalculated for each time-step via Equation 3.8. For the second model, for all daily contacts between an infectious and susceptible individual (occurring at probability $\frac{SI}{N}$) a Bernoulli trial (Lequime et al. 2020) of $\beta > Q; Q \in (0, 1)$ (where Q is randomly drawn from a uniform distribution) determines whether the contact is successful. Discrete-time simulation with time steps of one day were applied for both implementations.

Stochastic model pseudocode
Read disease parameters
Create horses
- assign latency and infectious period (random draw from poisson distribution)
- assign duration of carriership (random draw from poisson distribution)
Start day simulations (repeat until required number of days is reached)
- disease status evolution (latent to infectious, infectious to recovered, etc)
- infect new horses (see text for description) and write to record
- collate daily totals, write daily record

Validation of the stochastic agent-based models

The compartmental deterministic and individual-based stochastic models were run with parameter assumptions from Table 3.1 and compared to longitudinal incidence data from reports of naturally occurring outbreaks (Newton et al. 2000; Tscheschlok et al. 2018). The proportion of simulations resulting in an outbreak and the mean size of major outbreaks were compared to expected values, based on R_0 (Diekmann et al. 2013).

Remount depot dynamics

Since the interpretation that 1 in 4 horses do not mount lasting convalescent immunity, which was taken from Todd's data *a posteriori* has not been substantiated since, the possibility of an alternative interpretation of the data was investigated. The necessity for the assumption of a bimodal distribution of the duration of convalescent immunity was assessed by reproducing the proportions observed in the remount depot described in Todd (1910). We wished to determine this to validate our choice for a single *Recovered* compartment in the compartmental deterministic model, instead of two *R* compartments each representing one of the

fractions. A stable population of horses was assumed; no new horses were added throughout the simulation, and also there were no exits from the population; it is unclear whether horses entered and/or exited the population that is described by Todd (1910). It is also unclear from the report whether any outbreaks occurred in the four year observation period, but since only about a third of the population escaped infection $(1641/(2195 + 543 + 121 + 1641) = 0.36)$ it is likely transmission of *S. equi* was ongoing during the observation period. As the immune status of the horses entering the remount in question is unknown, the proportion of horses that escaped infection throughout the four year period was ignored, as that quantity is dependent on the prior immune status of individuals in the herd at the start of the observation period. Instead, the recorded outcome, for each run of the simulation, was the proportion, out of all horses that presented signs at least once, that presented signs once, twice, or three times.
These distributions were assessed for similarity to the distribution reported by Todd (1910) of 2195/2859 (77%), 543/2859 (19%), and 121/2859 (4%) respectively. An outbreak was seeded on the first day of the simulation in a fully susceptible population of 1000 adult horses without interventions and then run for four years. Parameters $(1/\sigma_L, 1/\sigma_P, \gamma, \gamma_C)$ were identically and independently distributed as described in Table 3.1 and the text section "Model parameterisation". A value for γ_R was assigned randomly for each infection; in other words, the assigned duration of protective immunity in a horse after a repeat infection was independent of the duration of protective immunity after a previous infection. This means that there was no assumption of consistent "poor responders" in the population.

RESULTS

A Kaplan-Meier plot (Figure 3.2) of collated data from two reports of carriership duration (Newton et al. 1999; Pringle et al. 2019) shows that 50% of horses remain carriers between 1-4 years. As can be seen in the Kaplan-Meier plot, a large proportion of horses are censored; most censoring is because the horse is treated before it can self cure. We predominantly use the range of one to five years for the mean duration of carriership as model inputs.

Dynamics at the population scale

Expected range for mean duration of convalescent immunity, carriership, and *f*

The deterministic compartmental models were run until a steady state was reached, for a range of values of f, γ_C and γ_R, and with the value of p fixed at 0.1. The resulting prevalence of horses showing clinical signs of strangles (compartment "I", at steady state) is presented in Figure 3.3. Neither γ_C nor f matters much here and the main driver of the outcome is the duration of convalescent immunity - in a large enough herd, the prevalence of infectious animals is so high that an animal can be expected to become re-infected soon

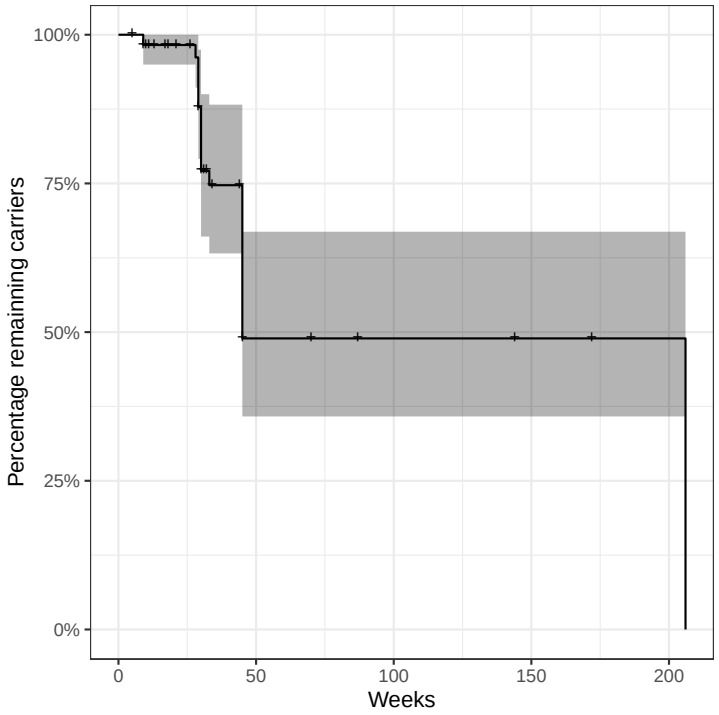


Figure 3.2: *Kaplan-Meier plot of duration of carriership, collated data (Newton et al. 1999; Pringle et al. 2019). Horses that were treated, died, or lost to follow up were censored (+).*

after protective immunity has waned. Assuming a prevalence of clinical disease of 0.88% (Todd 1910), the duration of protective immunity post-infection is estimated to be 4-6 years, depending on both f and γ_C, where the precise value for f is only of influence in the range of $0 < f < 0.5$.

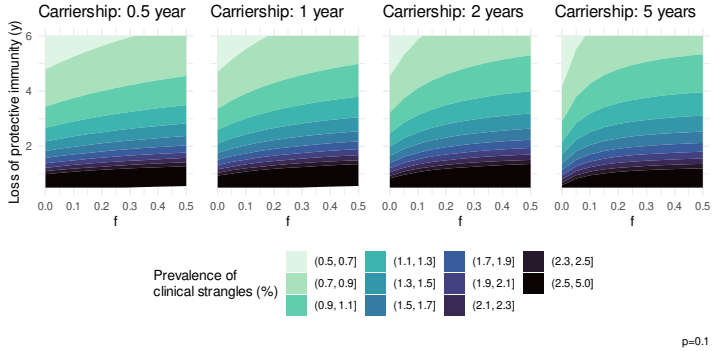


Figure 3.3: *Expected prevalence of strangles cases in a steady-state situation without control measures in the population.*

In Figure 3.4 the expected prevalence of carriers, again in a steady state, is presented. These percentages are only expected to occur in populations where no control effort for *S. equi* is in place. Unfortunately, no suitable real-world data to compare these outcomes to was available; retrieved reports on carrier prevalence do not meet the assumption of no intervention or are not random cross-sectional surveys (Libardoni et al. 2016; Durham and Kemp-Symonds 2020; Jaramillo-Morales et al. 2022).
The expected seroprevalence, as a proxy for convalescent immunity, in populations without control of *S. equi* is presented in Figure 3.5. As in the previous paragraphs, runs of the deterministic compartmental model until steady state were repeated for a range of values for f, γ_C and γ_R, and with $p = 0.1$.
Assuming that mean carrier duration is 0.5-5 years, and that convalescent immunity lasts ≥ 2 years and is measurable serologically (which for bacterial diseases is not always true), $\hat{f} \in (0.05, 0.5)$. This estimate is based on the highest reported seroprevalence in (likely) non-vaccinated horses, which is 74% (Minai and Araghi-Sooreh 2020). The results of repeating this analysis with $p = 0.2$ are presented in the Appendix (Figure B.3). Another noteworthy conclusion from Figure 3.5 is that at least 55% of horses would expected to have convalescent immunity in this scenario of no control effort, regardless of f, γ_C and γ_R.

The basic reproduction number at the population scale

The dependency of population-level R_0 on p and f is visualised in Figure 3.6a and b. Figure 3.6b indicates that variation between the current best guess of $p \in$

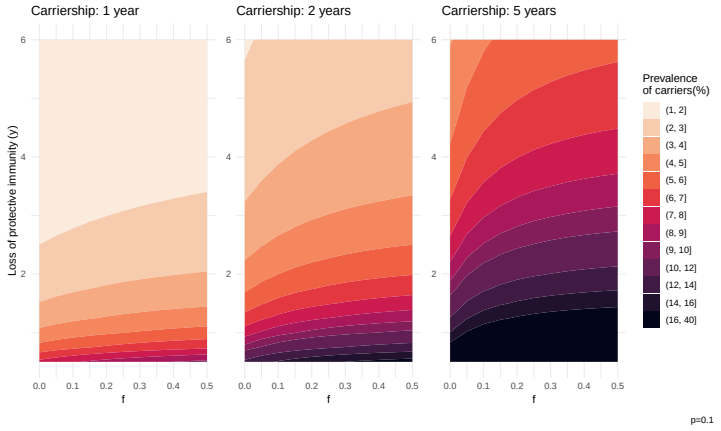


Figure 3.4: *Expected prevalence of apparently healthy horses shedding S. equi (carriers), in a steady-state situation without control measures in the population.*

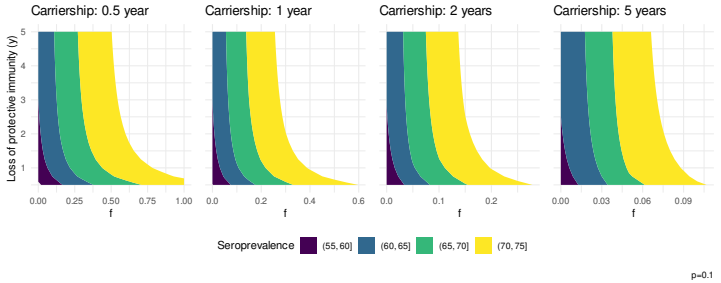


Figure 3.5: *Expected percentage of horses in the population with convalescent immunity, for a range of relative infectiousness of carriers (f) and γ_R. Note that the x-axis is not the same for different panels.*

$(0.1, 0.4)$ is in the flat section of the graph and does not have much effect on R_0 unless f is high. This holds true for alternative assumptions of $1/\gamma_C \in (0.5, 10)$ years (Figure B.2 in the Appendix). As the R_0 of 2.7 (Houben et al. 2023) for horses with acute disease (ignoring carriers altogether) was used to inform the parameters of this model, all estimates of R_0 incorporating infectious animals with acute disease as well as carriers are > 2.7.
A closer look with a smaller range for $f \in (0.05, 0.5)$; p fixed at 0.1; and $1/\gamma_C \in (1, 10)$ is shown in Figure 3.6c.

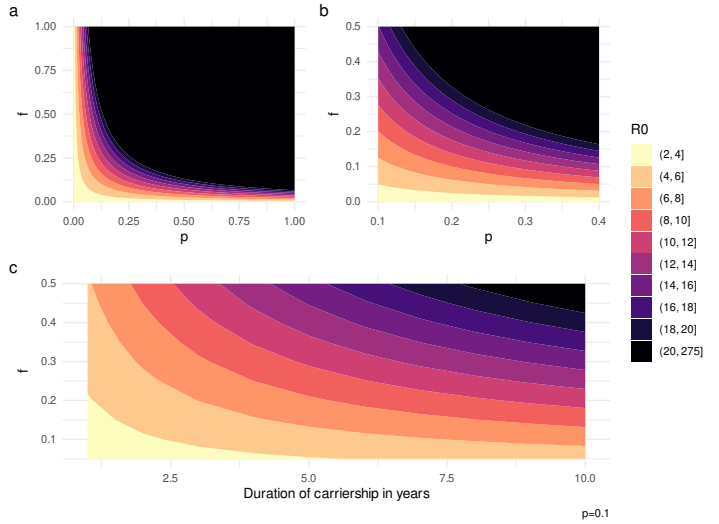


Figure 3.6: *(a) and (b); $R_0(p, f)$ at the population scale; (b) is a subsection of (a) where $p \in (0.1, 0.4)$ (Newton et al. 1997; Riihimäki et al. 2018; Jaramillo-Morales et al. 2022). For (a) and (b), $\gamma_C = 1/1825$ i.e. carriership duration of 5 years. (c): $R_0(\gamma_C, f)$ at the population scale; $p = 0.1, 1/\gamma_C \in (1, 10)$*

Could *S. equi* be eliminated by vaccination alone?

Figure 3.7 demonstrates the parameter values for f, γ_C and p that are required to arrive at a population-level $R_0 > 18$, the point at which the herd immunity threshold is $> 94\%$, which is higher than currently available vaccines can possibly achieve (Robinson et al. 2020). So if $R_0 > 18$, control and/or elimination of *S. equi* by vaccination alone is impossible and additional control measures will always be required besides vaccination. The non-shaded areas of the graph indicate a situation where $f > 1$ (i.e. carriers are *more* infectious than acutely

ill animals) which is highly implausible; we can therefore safely assume that for those combinations of p, f and $1/\gamma_C$, R_0 will never be > 18.

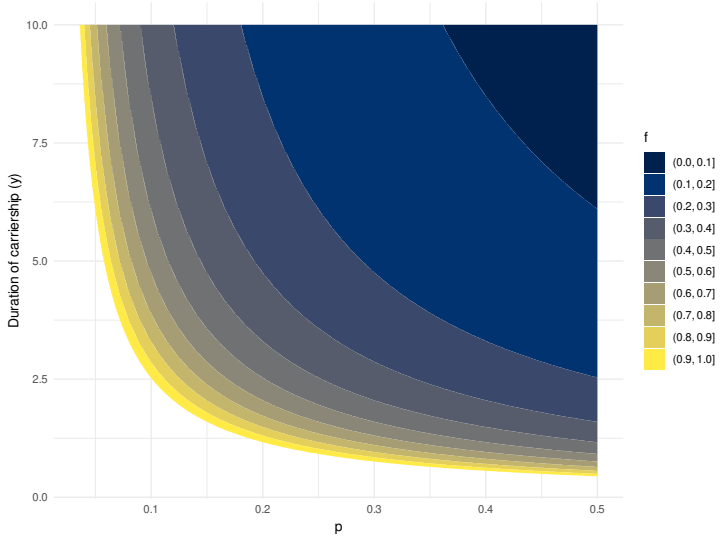


Figure 3.7: *A visualisation of the parameter values of p, γ_C, and f required to create a population-level $R_0 \geq 18$, which would give a herd immunity threshold $\geq 94\%$ (Robinson et al. 2020). The darker the shading, the less infectious carriers need to be to still arrive at $R_0 \geq 18$.*

Can carriers alone sustain endemicity c.q. can *S. equi* be eliminated without addressing carriers?

Figure 3.8 shows R for a range of values for f, γ_C and p, if acutely ill horses are assumed to have no role in epidemiology (due to, for example, immediate identification and isolation of acute cases) and $1/\gamma = 1$. In the non-shaded areas of the graph, $R_0 < 1$ which means that under those parameter assumptions, carriers alone would not suffice to keep *S. equi* endemic.
Figure 3.8 demonstrates that for this question, p is important. With $\hat{p} = 0.4$, $R < 1$ for carriers only for very short (and implausible (Newton et al. 1997; Riihimäki et al. 2018)) assumptions of $1/\gamma_C$ is R from carriers alone < 1. With $p = 0.1$ however, the non-shaded area of the graph covers values of $1/\gamma_C$ and f that may apply for natural infections of *S. equi*.

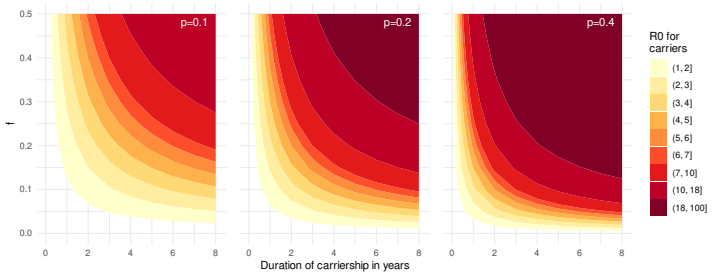


Figure 3.8: *R for carriers only (assuming all acute infections are identified and isolated instantly) for a range of duration of carriership, probability of becoming a carrier after infection (p) and relative infectiousness of carriers (f). In the non-shaded areas of the graph, $R < 1$.*

Incorporating test-and-isolate and vaccination strategies

Results are presented in Figures 3.9 and 3.10. These graphs highlight that if all animals are screened for carriership prior to re-introduction to the herd (i.e. $\omega = 1$) , an interval for screening for acute disease of ≈ 8 days should suffice to prevent the occurrence of major outbreaks. It becomes apparent that the assumption for γ_C matters less for the overall outcomes than those for f and p.

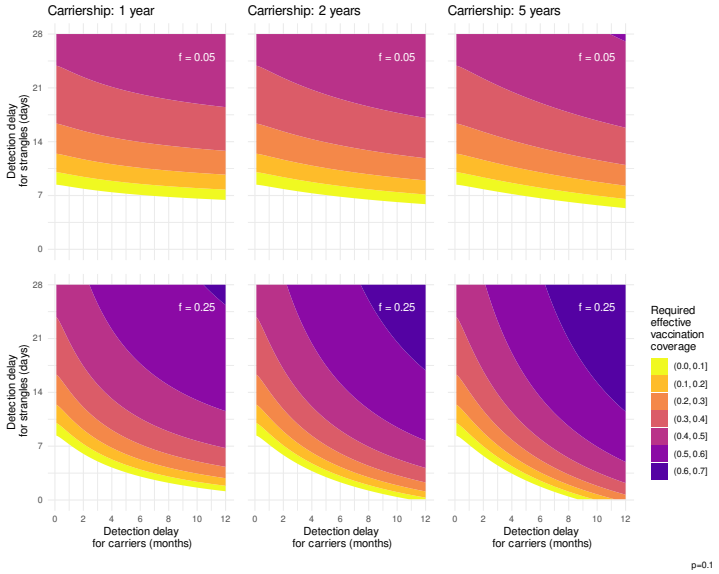


Figure 3.9: *Depiction of combinations of the delay in detection of acute cases ($1/\kappa$), delay in detection of convalescent carriers($1/\omega$), and vaccination coverage, required to achieve $R < 1$. The acute disease detection delay is given in days, the carrier detection delay is given in months. The non-shaded areas of the graph indicate combinations of parameter values for κ and ω that lead to an $R < 1$, indicating that the combination of acute disease and carrier detection is sufficient for $R < 1$. For combinations of parameter values in the shaded area of the graph, the shading colour indicates the level of vaccination required to prevent major outbreaks under those circumstances.*

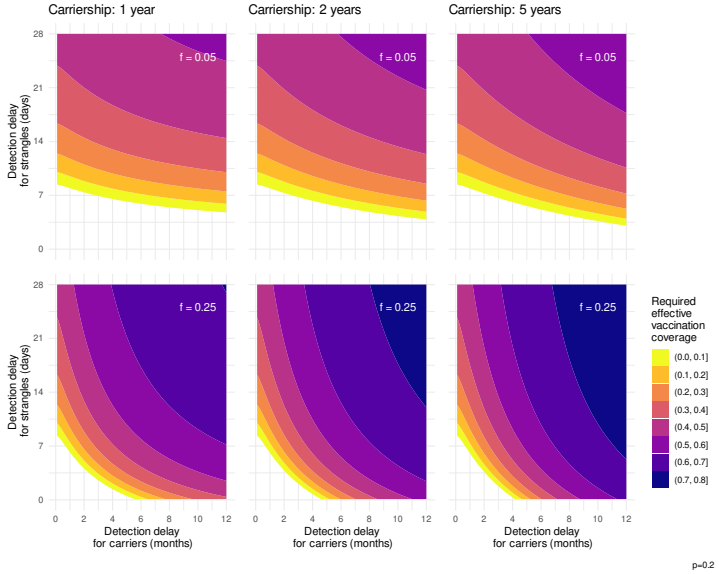


Figure 3.10: *As figure 3.9, but with $p = 0.2$.*

Stochastic model

Model validation

Steps taken to validate the stochastic model are presented in the Appendix.

Stochastic model of remount depot dynamics

The mean proportion of horses infected once, twice, or three or more times in 10 repeated runs of 1000 adult horses for a duration of 4 years are presented for $1/\gamma_{R_C}$ of 1 or 5 years, $1/\gamma_R$ of 1-6 years, and standard deviation for $1/\gamma_R$ of 1 or 2 years, is presented in a supplementary item. Figure 3.11 demonstrates that assuming $p = 0.1, f = 0.25, 1/\gamma_C = 1825$ days,$1/\gamma_R = 1460 \pm 7$ days results in a distribution of horses infected once, twice, or three times over the course of four years which are similar the proportion given by Todd (1910). Variation in assumptions for $p \in (0.1, 0.4)$ and $f \in (0.1, 0.5)$ had minimal effect on the overall outcome (Appendix Figure B.8).

DISCUSSION

Expected range for mean duration of convalescent immunity, carriership, and *f* For the duration of convalescent immunity, the range of 4–6 years drawn from the simulations in Figure 3.3 are similar to existing assumptions on the duration of convalescent immunity (Waller et al. 2014).
It should be noted here that estimates (for f and $1/\gamma_R$) are based on assumptions that in the populations where the highest seroprevalence was recorded, horses were able to mix unhindered and no effort was made to control *S. equi*, which may be incorrect. In addition, carrier horses were assumed to consistently have detectable antibody titers, which also may be incorrect (Gröndahl et al. 2015; Durham and Kemp-Symonds 2020). More data on seroprevalence in stable herds were *S. equi* is uncontrolled could increase the precision of $\hat{f}$ achieved by modelling.
Our estimate of $\beta = 0.199$ was derived from an estimate for R_0 from previous work (Houben et al. 2023) which was a weighted mean of individual R_0 estimates from outbreaks in naive herds. It differs substantially from the estimate by Shi et al. 2023 of $\beta = 4.091 \times 10^{-3}$. However, in the simulation from Shi et al. (2023) it was assumed that all horses present at the breeding farm or newly arrived during the months of the outbreak were fully susceptible, which may not be a correct assumption, as their actual immune status and strangles history were not reported in the original outbreak report. If a substantial proportion of the horses is assumed to be susceptible but is in fact not, the resulting estimate of β may be an underestimation. The model used in the present manuscript was more simple than the model applied by Shi et al. (2023) as our aims were principally to estimate ranges for key epidemiological parameters rather than to fit our model to specific circumstances.

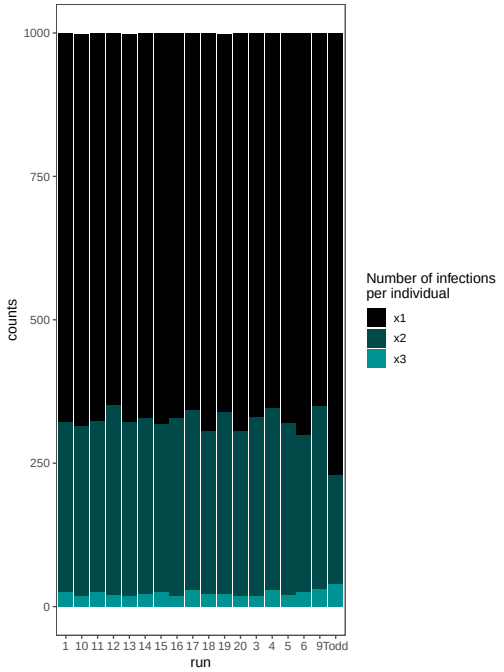


Figure 3.11: *Single four-year run outcomes of a remount depot simulation of 1000 adult horses, with $p = 0.1$, $f = 0.25$, $1/\gamma_R = 4 \pm 2$ years, $1/\gamma_{R_C} = 5$ years. Outcomes of the proportion of horses infected once, twice or three or more times are identified by colour and compared to the outcome reported by Todd (1910), which is provided in the column labelled Todd on the right.*

Our estimated range for $f \in (0.1, 0.5)$ is substantially higher than the 4.86×10^{-4} of Shi et al. (2023). One reason why the estimate by Shi et al. (2023) may be incorrect is their assumption that the total of four horses which during the course of the several-months long outbreak were diagnosed with some form of guttural pouch pathology, were in fact strangles carriers at the start of the outbreak, even though the authors of the outbreak report described that these were sequelae of strangles episodes during the outbreak.
Figure 3.4 can be compared to reports of *S. equi* positives on cross-sectional surveys, in herds where it is likely no control effort for *S. equi* is in place, such as Jaramillo-Morales et al. (2022) and Libardoni et al. (2016) which reported 13.5% and 2.3% respectively. There are important caveats to extrapolating from these studies however. Libardoni et al. (2016) reports PCR results of nasal swabs of 1010 apparently healthy equines from 341 farms in Rio Grande, Brazil and Jaramillo-Morales et al. (2022) reports culture results of endoscopically-guided guttural pouch swabs from 137 horses from 15 farms. Results from both studies were likely affected by clustering of samples within farms, as for *S. equi*, it is likely that the presence of one positive animal on the farm influences the likelihood of other horses on the farm testing positive. Jaramillo-Morales et al. (2022) tested 9 animals per farm, and Libardoni et al. (2016) tested 3 animals per farm, on average, indicating that the prior study is more prone to distortion of results by clustering than the latter. Secondly, the method of diagnosis of shedding influences sensitivity of testing (Pringle et al. 2019). Neither report applies the gold standard of guttural pouch lavage and PCR of the acquired sample, therefore both were affected by decreased sensitivity (compared to the reference standard), and their results were likely underestimations. Considering the issues affecting these two studies, it is difficult to draw meaningful conclusions on the likely values of $1/\gamma_R$ or f based on these two reports. The carrier prevalence of 13.5% from Jaramillo-Morales et al. (2022) does not really fit with any of other estimates derived from the models - a prevalence this high would only be possible with a much shorter duration of convalescent immunity. The report of 2.3% (Libardoni et al. 2016) would fit with our other assumptions if $1/\gamma_R \approx 2$ years, but this prevalence was established using an insensitive detection method and is likely an underestimation. We must therefore conclude that there currently is insufficient real-world data that can be used to compare to the model outcome parameters.

The basic reproduction number at the population scale The expected range for R_0 at the population scale remains wide. When assuming $p = 0.1$, $1/\gamma_C \in (1, 10)$ years and $f \in (0.05, 0.25)$, $4.3 < \hat{R}_0 < 13.2$). It is clear, therefore, that further precision for these estimates is needed to be able to come to a truly usable estimate for the effort required to control (or eradicate) strangles.

Could *S. equi* be eradicated by vaccination alone? If $p = 0.1$, the mean duration of carriership would need to be < 2.5 years for eradication of *S. equi* to be possible by vaccination alone, assuming 100% of animals is vaccinated with a vaccine with an efficacy of 94% (Robinson et al. 2020). If p is actually closer to

0.2, then the mean carrier duration would need to be half that. Of course, it is unlikely that it will ever be possible to achieve a vaccination coverage of 100%, nor is it likely that carriers would remain unaddressed in any eradication effort.

Can carriers alone sustain endemicity? Carriers are assumed to play an important role in endemicity of strangles (Newton et al. 1997; Mitchell et al. 2021), but their importance in *S. equi* epidemiology has so far not been quantified. From Figure 3.8, and with information gained from Figure 3.5 that $0.05 < f < 0.5$, mean carrier duration would need to be ≈3 years or less for $R_0 < 1$. With p=0.2, it is ≈14 months. With higher $\hat{f}$, the tolerance for carriers decreases, but f may be as low as 0.05. In the absence of good data or estimates on the mean duration of carriership, it is not possible to conclude with certainty whether carriers on their own suffice to keep $R_0 > 1$ and to keep *S. equi* endemic. Control efforts which ignore the carrier state may therefore be unsuccessful.

Incorporating test-and-isolate and vaccination strategies The range of combinations of ω and κ which result in $R < 1$ can be considered favourable. For large, extensively managed herds, a weekly check for clinical signs of strangles (including fever) would suffice to prevent outbreaks occurring, as long as all affected horses are screened and treated for carriership after clinical disease, prior to re-introduction into the herd. If for example, checks for strangles are performed daily, there exists a tolerance for a prolonged presence of carriers in the herd at least for the lower ranges of f. This might explain why, for example, the *S. equi* screening process described by de Brauwere and Kirton (2019) were highly successful at preventing strangles outbreaks, despite relying on serological screening for carriers before allowing horses into the herd, which has been demonstrated to be an imperfect predictor of carriership (Durham and Kemp-Symonds 2020). This study did not investigate the effect of vaccination on transmission dynamics and only provided an insight into the vaccine coverage that would be required for a perfect (protection against disease and protection against infectiousness) vaccine. Future work could explicitly explore the effects of vaccines when realistic estimates their effectiveness during field use are available.

For the scenarios with higher p and f, it becomes apparent that currently available vaccines might not suffice to prevent major outbreaks under those circumstances. Vaccine efficacy (both in terms of reduction of clinical signs and infectiousness) would need to be up to 80% which may be difficult to achieve with currently available vaccines, based on data from experimental challenge studies that used a high infectious dose. Future data from the use of vaccination to prevent outbreaks of natural infection are required in order to determine the actual impact of vaccination strategies.

When interpreting the results from Figures 3.9 and 3.10, it is important to note here that an absence of major outbreaks does not mean absence of transmission; if $R < 1$ minor outbreaks (short outbreak chains) are still possible, but the number

of infected individuals will not be in the same order of magnitude as the number of individuals at risk at the start of the outbreak. However, in future work evaluating cost-benefit of prevention strategies, the monetary and reputations costs of even a minor outbreak should be taken into consideration as well.
Our results here are similar to those of Shi et al. (2023) who concluded that screening was the most effective measure.

Remount depot dynamics The results from the agent-based stochastic simulation in Figure 3.11 suggest that the current assumption that there must be a bimodal distribution for the duration of convalescent protective immunity may be incorrect and that future modelling studies do not need to incorporate this assumption.. When assuming a duration of convalescent protective immunity of 4 years with a standard deviation of 2 years, only 6.7% of infections leads to immunity of $<$365 days and 3.9% of infections leads to protective immunity of $<$180 days. The results presented in Figure 3.11 suggests the assumption that 25% of horses fail to mount a protective immune response after natural infection does not need to be true to explain the results cited by Todd (1910) over a four-year period. In our model to replicate Todd's data, we did not account for the possibility that during the observation period, other infectious diseases besides *S. equi* were circulating and may have contributed to some clinical respiratory disease. We cannot know whether or not this was the case, but it is worth pointing out that the interpretation of the data that says 25% of horses do not mount convalescent immunity also does not account for the circulation of other infectious agents causes respiratory disease. Furthermore, the presence of persistently infected carriers within these populations may have re-stimulated the immune responses of recovered horses, such that they may have appeared to have maintained protective immunity for longer. Although these remount depot incidence data may be old, they are reliable and were also acquired under circumstances where control of the disease appears to have been near absent, making them good reference data for this simulation.
This finding should be considered good news; if it were true that a large proportion of the population fails to mount an immune response of substantial duration after natural disease, then there may be concerns as to whether a significant proportion of vaccinated horses are likewise poor responders. Results from vaccine efficacy trials Jacobs et al. (2000) and Robinson et al. (2020) did not indicate such an effect, which is consistent with the findings from this simulation. The findings from our stochastic agent-based model provide clarity for future work in mathematical modelling of *S. equi* on the input parameter of convalescent immunity.

Main limitations

The mean duration of infectiousness chosen as a starting parameter was 14 days in all analyses in this paper, while acknowledging that an assumption of infectiousness of two weeks may seem short when infectiousness can last up to

six weeks (Boyle et al. 2018). However, the period of two weeks was chosen to reflect the observation that infectiousness probably is highest over a shorter period, when the production of infectious discharge is greatest. In this paper, β was calculated directly from $\hat{R}$ and $1/\gamma$; therefore, a longer assumption for γ would have led to a lower $\hat{\beta}$, with little to no change on most of the other findings in this paper. The only deterministic model-based outcomes that would have been obviously affected are those in Figures 3.9 and 3.10, where the intersection with the y-axis would change corresponding to the change in $\hat{\gamma_R}$. To incorporate the assumption of decreasing infectiousness over a longer time, a two-phase approach for $\hat{\beta}$ could have been chosen: higher in the first weeks, lower in later weeks. However, this approach was considered unnecessarily detailed for the current purposes of the model. It can be considered for future implementations.

Some questions were omitted from analyses in the current study. For one, the potential influence of the presence of carriers in the herd on the duration of convalescent immunity in herdmates was not investigated.

CONCLUSION

Through compartmental deterministic modelling, this study made an estimate of the duration for convalescent immunity of 4–6 years, and that carriers may be up to 20 times less infectious than horses with strangles. Through stochastic modelling, we have demonstrated that it is not necessary to assume that $1/4$ convalescent horses fail to mount an effective immune response after infection. Further, this study has highlighted the importance of a more precise estimate for the duration of convalescent immunity, of relative infectiousness of carriers, and, to a lesser extent, the probability of becoming a carrier after infection and the duration of carriership. We have provided predictions as to which combinations of intensity of screening and/or vaccination will result in the prevention of major outbreaks, showing that weekly screenings might suffice in otherwise ideal circumstances. In our worst-case scenarios, we demonstrated that vaccination alone may not be able to prevent the occurrence of major outbreaks.

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Chapter 4

CARRIER PREVALENCE & CARRIERS IN COMPETITIONS

Rosa Houben, Els Broens, Marian Broekhuizen-Stins, Marianne Sloet, Kees van Maanen, Hans Heesterbeek: The prevalence of *S. equi* carriers in the Netherlands, and implications for carrier-susceptible contacts at competitions
Manuscript submitted

Abstract

Background *S. equi* carriers are thought to be important drivers for strangles outbreaks. Limited data is available on the prevalence of carriers in European horse husbandry settings, and no data is available on how frequently carriers contact susceptible horses. **Objectives** To estimate the prevalence of *S. equi* carriers among apparently healthy horses and ponies in the Netherlands, and to estimate the opportunities for contact of carriers with susceptible horses at Dutch competitive events. **Study design** Cross-sectional survey and simulations. **Methods** PCR analyses of three repeated nasopharyngeal lavage at weekly intervals and Bayesian true prevalence estimation. To estimate the annual number of carrier-susceptible contacts at competitions, simulations drew estimates from the Bayesian true prevalence posterior distribution, assigned carrier status to horses in a real-world network based on Dutch sports and racing records, assigned non-susceptible status to a proportion of horses in the network informed by published seroprevalence surveys, and counted the number of direct contacts, defined as presence at the same location on the same day, between carrier and susceptible horses for an entire year. **Results** A full set of three lavages was available for 166 horses on 86 premises. The estimated true prevalence was 3.8% (95% Credible Interval 1.2–7.7%). The median annual number of carrier-susceptible contacts in the simulation runs was $1.0 * 10^6$(IQR $7.3 * 10^5 - 1.4 * 10^6$). **Main Limitations** Our target of 200 participants in the cross-sectional survey was not reached. Seropositivity is an imperfect proxy for resistance to infection for *S. equi*, therefore the simulations may have overestimated the number of susceptible horses. **Conclusions** Our carrier prevalence estimate is similar to a recent report from the UK. A large number of carrier-susceptible contacts at competitions means that even if the probability of transmission per contact in these settings is small, it may still be of epidemiological importance.

INTRODUCTION

Strangles (infection with *Streptococcus equi* subspecies *equi*, or *S. equi*), is a bacterial infectious disease of the upper respiratory tract in horses. The disease is endemic nearly worldwide (Mitchell et al. 2021).
After clinical recovery from infection, a proportion of horses remain infectious (George et al. 1983), because they continue to harbour, and intermittently shed, the infectious agent in their guttural pouches (GP) or possibly their paranasal sinuses, although the site of carriage for the latter remains undetermined (Newton et al. 1997; Newton et al. 2000; Gröndahl et al. 2015; Pringle et al. 2019; Pringle et al. 2022). Carriers are thought to be an important factor in the continuing endemicity of *S. equi* (Waller 2018; Pringle et al. 2019; Mitchell et al. 2021).
The prevalence of apparently healthy, but potentially infectious individuals is important epidemiological information. It can help inform horse owners, horse premises operators, and equestrian event organisers in a risk assessment regarding the introduction of apparently healthy, but potentially infectious horses into contact with their horse or onto their premises. In addition, carrier prevalence data in combination with equine contact patterns and outbreak data can help evaluate the role of these carriers in the epidemiology of *S. equi* and is valuable input to inform epidemiological models for scenario analysis. In this study, we will combine new data on carrier prevalence with preliminary analysis of contact patterns of horses at competitive events, to evaluate the possibilities for transmission of *S. equi* by carriers at equine competitive events.

Carrier status can be be diagnosed by endoscopy-guided GP lavage (GPL) or repeated nasopharyngeal lavage (NPL) (Boyle et al. 2018); nasal or nasopharyngeal swabs or serology are no longer recommended due to their poor sensitivity (Boyle et al. 2017; Pringle et al. 2019; Pringle et al. 2020b; Durham and Kemp-Symonds 2020) and, in the case of serology, poor specificity as non-carrier convalescent horses may be seropositive without being carriers (Durham and Kemp-Symonds 2020; Pringle et al. 2020b). Relying on only endoscopic GP evaluation without lavage PCR or culture is insufficient as optically normal GPs have been reported to be PCR positive for *S. equi* in several studies (Boyle et al. 2017; Durham and Kemp-Symonds 2020).

The probability of becoming a carrier after infection appears to be 10-40% (Newton et al. 1997; Gröndahl et al. 2015; Riihimäki et al. 2018; de Brauwere and Kirton 2019; Delph et al. 2019). Differences in reported probabilities of becoming a carrier after clinical disease can in part be attributed to differences in study setup, and definition and diagnosis of carriership, in particular how much time must have passed since resolution of clinical signs and the method of detection of the pathogen: culture or antigen detection (usually PCR or LAMP). There is no current consensus as to how long after infection a horse should be considered a carrier if it is still infectious. In reports, this threshold has varied from 40 days after the initial strangles diagnosis (Duffee et al. 2015), two to three weeks after recovery (Boyle et al. 2017) to six weeks after recovery (Boyle

et al. 2018; Morris et al. 2021), or up to six months after recovery (Pringle et al. 2020b). Timing of testing matters – horses in the early convalescent phase may no longer show clinical disease, but may also not yet have had sufficient time to clear remaining *S. equi* from their GPs.

The virulence of the strain infecting the horse may influence the probability of becoming a carrier (Pringle et al. 2019) although an extensive genomics and transcriptomics investigation was unable to detect a pathogen-related factor which predicted the development of carriership (Morris et al. 2021).

Reports of the duration of carriership, where carriers were followed-up prospectively, include up to 39 months (Newton et al. 1997) and up to 45 weeks (Pringle et al. 2022). Horses that have been shown on testing to be carriers at six months post clinical disease can revert to non-carriership without intervention (Pringle et al. 2022). Anecdotal reports that the carrier state can persist for many years or even lifelong are numerous (Waller et al. 2014). Carriers are infectious to susceptible horses (Harris et al. 2015), and even culture-negative, PCR-positive horses should be considered infectious. Detection of *S. equi* by PCR is not proof of the presence of viable bacteria, but since Riihimaki *et al.* demonstrated that after instilling non-viable *S. equi* into GPs, *S. equi* DNA became undetectable by PCR within days (Riihimäki et al. 2023), PCR-positive horses probably carry live and potentially infectious bacteria.

The prevalence of *S. equi* carriers among apparently healthy horses and ponies in the Netherlands is currently unknown, and there is little data from which to estimate or extrapolate this prevalence. A limited number of cross-sectional surveys in other countries have been reported, but all had limitations to their suitability for extrapolating the result to the Netherlands. For example, a study in Colombia found 13.5% of 137 horses sampled via GP swab and culture to test positive (Jaramillo-Morales et al. 2022). However, a limitation of that study was that they sampled on only 15 premises, and since the presence of one *S. equi*-positive horse likely influences the probability of other horses on the premises being positive, this study was highly susceptible to the potential clustering effect of carriers. Furthermore, instead of PCR, the more insensitive diagnostic method of culture was used to confirm presence of *S. equi* in that study. A cross-sectional survey in Brazil detected *S. equi* by PCR in 2.3% of 1010 horses on 314 farms, and found that 5.9%of farms had one or more horses with positive swabs (Libardoni et al. 2016). However, that study used the insensitive method of sampling of one single nasal swab, and therefore their outcomes are likely underestimations. Among new arrivals to an equine sanctuary in the UK, 3.1% of horses (screened by serology, GP endoscopy, and GPL PCR) were carriers; however, this was a prevalence estimate from a convenience sample in a population of horses that likely does not represent the British, or Dutch, general horse population (Durham and Kemp-Symonds 2020).
Serology is a poor predictor of carrier status (Durham and Kemp-Symonds 2020; Pringle et al. 2020b), and no clinical measure has been identified to reliably

detect carriers at six or more months after outbreaks (Pringle et al. 2020b). Therefore, although seroprevalence data is available for the Netherlands (van Maanen et al. 2021), it is of little use for the estimation of the prevalence of carriers.

The strength of transmission for *S. equi* in groups of horses with random mixing has been estimated in previous work (**Chapter 2** - Houben et al. (2023) and **Chapter 3**), but individuals in populations do not mix randomly. The contact network structure of a population influences transmission dynamics of infectious diseases (Wallinga et al. 1999; Keeling and Eames 2005), and the WOAH recommends the use of livestock contact network data to understand infectious disease dynamics (Dubé et al. 2011). For this study, in particular, we were interested in transmission parameters of *S. equi* associated with participation in competitive events which we evaluated by constructing a contact network based on competitive event participation data.

A precise estimate for the sensitivity of three consecutive NPLs for the diagnosis of carriership is not currently available. One longitudinal study after an outbreak noted that the four horses that had previously tested positive for strangles but tested negative on NPLs at weeks 28, 28 and 30 after the outbreak, were confirmed to be *S. equi*-free by combined GPL and NPL at 45 weeks, concluding that three repeated weekly NPLs were a reliable method for determining non-carrier status (Pringle et al. 2022). There is no single gold standard test, as on some sampling occasions, horses may not test positive on PCR of a GPL sample yet test positive on an NPL or even nasal swab test (Pringle et al. 2019). However, another study by the same group, including more horses from other outbreaks but analysing only single NPLs against carrier status, over 3 distinct testing moments, reported that $9/15 + 3/12 + 6/14 = 18/41$ of total carriers tested positive on a single NPL, giving an overall sensitivity of a single NPL-PCR of 44%(95% confidence interval 28%-60%) (Pringle et al. 2019).

The veterinary laboratory Royal GD in the Netherlands hosts SEIN (Surveillance of Equine Infectious diseases in the Netherlands), a passive surveillance framework based on voluntary reporting of positive diagnoses of disease caused by *S. equi* (amongst other infectious pathogens). Outbreaks are reported, stripped of identifying data, and collated totals are listed on a publicly available online resource (seinalerts.nl and equinesurveillance.org/iccview).

The aims of this study were 1) to estimate the prevalence of *S. equi* carriers among apparently healthy horses and ponies in the Netherlands of at least 2 years old, and 2) to estimate the number of carrier→susceptible contacts that occur at equestrian competitive events per year using nation-wide contact network data of equine event participation.

METHODS

In the following sub-sections, we discuss the study design for the survey of carrier prevalence, the network census and analysis, and the simulation of transmission by carriers at competitions.
The prevalence study protocol was approved by Utrecht University's institutional Animal Welfare Body (IVD; protocol number 5204-2-05). Informed consent was obtained from each participating horse's owner or caretaker upon enrollment in the study. The protocol for data transfer, storage and analysis of equine event participation records was evaluated by the Ethical Review Board of Utrecht University's Geography Department (DGK S-23016) which ruled that an ethical review of use of personal data was not required for the study protocol.

Study design for carrier prevalence

A cross-sectional survey for *S. equi* carriers status among apparently healthy horses and ponies residing in the Netherlands.

Sample size calculation A prevalence of about 3% was expected (Durham and Kemp-Symonds 2020), with an expected test sensitivity of 90% (Boyle et al. 2017) for the three repeated lavages, see Sampling protocol below. The total size of the population (horses and ponies in the Netherlands) was approximately 300,000 in 2018 (Nielsen et al. 2022). The desired precision of the estimate was to be 95% certain that our seroprevalence estimate confidence interval was within 2.5 absolute percent points of the true population value (Stevenson et al. 2024). This calculation resulted in a target sample size of 200 participants.

To mitigate issues with clustering, as probability of horses on the same premises being carriers cannot be assumed to be fully independent, we chose to test no more than two horses per premises. This was a compromise between the ideal (which would be to test only one horse per premises) and what was thought to be feasible (considering logistics, recruiting and travel costs).

Study population All horses and ponies of age 2 and older, from any type of premises were eligible for inclusion if they were apparently healthy and had not had, in the three months prior to sampling, clinical signs typical of infection with *S. equi*: fever, cough, persistent nasal discharge, lymphadenopathy (Boyle et al. 2018).

The data recorded for each horse were: age, sex, breed, principal use (in training, sports, pleasure, racing, breeding, riding school/lessons, hunting, equine assisted therapy for persons with physical or mental disabilities, retired, or other), how long it had been in the owners possession or care, whether the horse had had strangles in that time and if so, when, and the horses country of origin (from the owner's history or from the animal's microchip). Of the horses' home premises the following details were recorded: size (categorized as small: 2 to 9 equids; medium: 10 to 19; large: 20 or more; or extra large: 100 or more equids), principal function (boarding/training, breeding, racing, private residence, riding school), whether the premises had ever experienced a strangles outbreak in the past (as far as the orse owner/handler or premises operator was aware) and if so, when.
No publicly accessible data exists about the composition of horse herds in the Netherlands, so there were no distributions of age, sex, breed or principal use, *et cetera*, to inform balanced stratified sampling. Also unavailable were characteristics of equine premises in the Netherlands such as premises size, horse use, turnover of horses on the premises, *et cetera*. We therefore relied on equine veterinarians to nominate what in their opinion was a representative sample of the horses under their care.

Recruitment of study participants The authors' informal network, mostly consisting of equine veterinarians who offer primary care to equines in the Netherlands, was asked to nominate premises and owners that they thought were a good representation of their clientele, and who would likely be willing to participate. As the Netherlands is a small country with frequent cross-country transport of horses (Figure 4.3), we did not anticipate significant regional differences in prevalence, nor was our target sample size designed to evaluate regional differences. For these reasons, and to alleviate the logistical challenges of the survey, we did not specifically aim to sample evenly throughout the country.

Sampling protocol A single-use 40 cm 16 French PVC Nelaton catheter (Ratiomed, Medicoplast International, Illingen, Germany) was advanced through the nostril up to the level of the medial canthus of the eye; over the course of three lavages, each nostril was accessed a least once. Fifty ml. of sterile lukewarm isotonic saline (B. Braun, Melsungen, Germany) was administered through the catheter and collected at the level of the muzzle into a cardboard or steel medical emesis basin. If a volume of $<$10 ml was collected, the lavage was repeated.
If a twitch was required for fixation of the animal to enable the lavage, a twitch made out of a cotton rope and a PVC handle was used. The cotton ropes were discarded after every single use and the handles were first cleared of any visible debris and then submerged in an chloramine-T containing solution (Halamid, VEIP Desinfectants, Wijk bij Duurstede, the Netherlands) overnight before being rinsed and prepared for re-use. A note in the participant's documentation was made for each sampling where a twitch was applied.
After publication of results by Riihimäki et al. (2023) the sampling protocol was modified to include a step of cleaning the muzzle with gauze drenched in a 5 mg/ml chloorhexidinedigluconate & 600 mg/ml isopropylalcohol solution, followed by wiping off of the disinfectant with gauze drenched in sterile 0.9% NaCl irrigation solution. This modified protocol was implemented after 44 premises had already been sampled.

Sample analysis The collected fluid was transferred to a 50 ml falcon tube (Cellstar Tubes, Greiner Bio-one, Alphen aan den Rijn, Nederland), chilled within 10 minutes of collection, and transferred to a lab for processing within 24 hours of collection. Sample tubes were centrifuged at 3200x g for 10 minutes and the supernatant was separated and frozen at -20°C. The remaining pellet was re-suspended with $500\mu l$ of FE buffer of which $200\mu l$ was frozen at -20°C until analysis.

DNA isolation was performed using the Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany). The re-suspended pellet ($200\mu l$) was mixed with $20\mu l$ of proteinase K and $200\mu l$ of lysis buffer; $5\mu l$ of PhHV was added as internal control. The mixture was incubated at 56°C for 10 minutes and then $200\mu l$ of molecular grade ethanol 96% was added. Primers and probe targeting the ICESe2 region of *S. equi* as previously described (Båverud et al. 2007) were used in a $20\mu l$ reaction that contained, $10ul$ GoTaq™Probe qPCR mastermix (Promega) $900nM$ forward primer, $900nM$ reverse primer, $250nM$ probe and $5\mu l$ of extracted DNA. Real–time PCR was performed on a LightCycler 96 system (Roche Diagnostics).

Estimation of carrier prevalence from the cross-sectional survey Limited data was available which directly compared the sensitivity of three consecutive NPLs to alternative testing methods, such as the reference standard of GPLs. We assumed that the probability of any sample of a carrier horse being positive was independent of the results from the other two samples, so the probability of a carrier escaping detection over the span of three samples was set as $(1-0.44)^3$ (95%CI $(1-0.28)^3-(1-0.60)^3$) resulting in an estimated sensitivity for the ensemble of three NPL PCRs of 83% (95%CI 63%-94%). We assumed a test specificity of 100% given that a pathogen-specific PCR was used (Båverud et al. 2007). We calculated a Bayesian estimate for the true prevalence with a Beta-PERT distribution of 0.83 as the most likely estimate, 0.63 as the pessimistic (minimum) estimate and 0.94 as the optimistic (maximum) estimate for the test sensitivity. The R (R Core Team 2021) package *prevalence* (Devleesschauwer et al. 2022) was used for the true prevalence estimation.

Contact network through competitive events

The KNHS is the Dutch federation for equestrianism and oversees registration for all official events in the disciplines of dressage and para-dressage, showjumping, driving and para-driving, vaulting, eventing, and endurance. This includes both adult/horse as well as youth/pony competitions. The KNHS oversaw reining competitions up to 2022, which is when reining ceased to be an FEI discipline. The KNHS was approached for access to its database and consented to sharing of the data containing all registered event participation for the year 2022. The KNHS data records comprise: a unique ID for each horse, event location (municipality and geographic), all horses entering a competition class (a horse can compete in more than one class per day, with the same or a different rider) and a date for the event attendance.

Racing in the Netherlands is governed by the foundation *Stichting Nederlandse Draf- en Rensport* (NDR). Standardbred races predominate; these consist of both classical length races on dedicated racetracks as well as short-track street races which are run over a distance of 300 meters, often accompanying village summer fairs. All Dutch horse racing results are published on a website (ndr.nl), and upon our request the NDR provided a collated table of all results for 2022. These data include horse identifier, race date, and location name. Geographic information for the racetracks and short-track street races was found via Google maps. The excel databases containing the KNHS event participation data and NDR racing data were imported into R and network metrics (see Table 4.1) were calculated using the R package *igraph* (Csardi and Nepusz 2006).

As horses that are *S. equi* carriers are expected to remain infectious for a long time (Newton et al. 1999; Boyle et al. 2018), a static representation of the contact network for the entire year was constructed ("annual network"). In the network for this study, horses were nodes, and an edge was present between nodes if they were present at the same event, an event here meaning a competition in one location on one day. Since the same pair of horses can meet each other at an event they both visit more than once throughout the year, more than one edge could exist between pairs of nodes.

Metric	Definition
Node	Unit of interest; for this study, horses were nodes.
Edge	Connection between units of interest; in this study, there was an edge between horses if they were present at the same event.
Network size	Number of nodes in the network.
Connected	Are all nodes reachable from all other nodes?
Edge density	The ratio of the number of edges to the number of possible edges.
Transitivity	Probability that the adjacent nodes of a node are connected (also known as the clustering coefficient).
Diameter	The greatest number of links in the shortest path between any two nodes.
Mean distance	Shortest path between two nodes, averaged over all pairs of nodes in the network.
Degree	The number of connections per node.

Table 4.1: *Definition of network terms (Dubé et al. 2011; Csardi and Nepusz 2006).*

Simulations pseudocode

Input parameters for all iterations:
The posterior distribution of the Bayesian estimate for carrier prevalence.
Network file (static horse network for the entire year of 2022); sports horses and racehorses combined into a single network file of two non-connected components, with N horses(nodes).

Steps per iteration:
Draw one random sample from the carrier prevalence distribution: p.
$p * N =$ number of carriers in simulation: C.
Draw C random carriers from all horses in the combined network.
A number $(0.112-p)*N$ (if > 0) among all horses are designated non-susceptible by random draw.
Determine the immediate neighbours (i.e., horses with a direct connection) from the network for each of the horses that were assigned carrier status and collate into a neighbour-list.
Remove entries from the neighbour-list who were themselves carriers or who were assigned immune status.
The entries remaining in the neighbour-list are the contacts from carriers to susceptible horses in one year. Susceptible horses can be listed more than once if they were contacted more than once, regardless whether it was by the same horse or a different horse.
Record the number of entries in the contacts list.

Repeat for 1,000 iterations of the same simulation.

Report the distribution of number of carrier→susceptible contacts: range, median, IQR, 2.5th-97.5th percentiles.

Opportunities for transmission of *S. equi* by carriers at competitions

We used the newly constructed contact network to estimate the annual number of contacts (contact meaning attending the same event) between carriers and susceptible horses; for a verbal description of the algorithm, see the Box. As input parameters we used the posterior distribution of our Bayesian estimate for carrier prevalence. To estimate the proportion of horses that are not carriers but that are nevertheless not susceptible as a result of convalescent immunity or vaccination, we used as input a report on the seroprevalence of *S. equi* antibodies in the Netherlands (van Maanen et al. 2021). This seroprevalence estimate, when including any results with an OD ≥ 0.3, was 11.2% (van Maanen et al. 2021). The absence of detectable antibodies against *S. equi* on serology does not mean that the horse is susceptible to *S. equi* (Galan and Timoney 1985; Timoney and Eggers 1985; Davidson et al. 2008; Robinson et al. 2020), but it is reasonable to assume that a horse that tests positive on serology likely has protective immunity. In our simulations, we will therefore likely overestimate the number of horses that are susceptible, but this fits our aim of a worst-case estimate.

All analyses and simulations were performed in R (R Core Team 2021) version 4.3.2.

RESULTS

Cross-sectional survey

We sampled 166 horses on 86 premises at least once. Sampling was performed at weekly intervals from November 2022 to January 2024. The interval between consecutive samples was usually one week, but on some occasions, due to logistic constraints, the interval was longer; the longest interval between consecutive samples was 29 days.

One horse tested positive on its first NPL, at which time its owner had insisted on using their own (non-sterilised) twitch. PCR results from the remaining two NPLs, when a study twitch was applied, were negative. The status of this horse was therefore not ascertainable and the horse was removed from the analysis. On one of the premises, one of the sampled animals tested positive on only the first two NPLs and the other horse from that premises tested positive on only the third NPL. This was a location that was visited before the sampling protocol was modified to include disinfection of the muzzles prior to the NPL. These animals at this location were group housed 24/7 in a densely populated paddock, and for this reason we argue that the most likely explanation for our finding here was environmental contamination of the muzzle of one of these animals, such as reported by Riihimäki et al. (2023). We considered that more likely than the alternative interpretations of either both horses being carriers, or of human error during sample processing. Consequently, only one of the horses that tested positive at this location is counted towards the overall prevalence estimate. All other horses that returned one or more positive tests were the only horses at that location to test positive.

Two horses were only sampled twice and one horse was only sampled once, as these horses stopped cooperating on the second or third sampling attempt. One sample of another horse was lost during processing, leaving only two samples available for PCR analysis. These four horses with an incomplete set of samples were excluded from further analysis; none of these horses had a positive PCR result on the samples that were successfully collected and analysed.

Four out of the 161 participants remaining in the study had a positive PCR result at least once. They were split midway between premises sampled before (A and B) and after (C and D) the addition of muzzle disinfection to the sampling protocol.

The geographic distribution of sampled premises is displayed in Figure 4.1. Additional descriptive characteristics of the horses that were included for analysis, and size and location of their home premises are shown in Figure 4.2 and Table 4.2

Sex

Mare	84
Gelding	68
Stallion	10

Country of origin

Netherlands	121
France	8
Germany	6
Ireland	6
UK	4
Spain	2
Switzerland	1
USA	1

Breed

Warmblood	79
Shetland or Miniature	14
Frisian	11
Mixed breed	11
Haflinger, Fjord or Icelandic	11
Welsh A-C	8
Cob	7
Coldblood other	7
Standardbred	6
Pura Raza Española	2
Quarter horse	1
Irish Hunter	1
Arabian	1

Table 4.2: *Description of the sampled horses: Sex, Breed, Country of Origin.*

An overview of PCR results from the horses which tested positive is provided in Table 4.3.

Carrier ID	Cp		
	T1	T2	T3
A	36.88	36.61	Negative
B1	Negative	Negative	36.15
B2	36.93	35.28	Negative
C	Negative	36.95	Negative
D	Negative	Negative	36.78

Table 4.3: *PCR results from positive horses. B1 and B2 indicate the two horses from one paddock where environmental contamination was thought to be the cause of a positive test result in one horse (see text for details). Cp: PCR analysis crossing point (sometimes termed cycle threshold or Ct).*

The Bayesian estimate for true prevalence was 3.8% (95% Credible Interval 1.2-7.7%).

Network

THe KNHS network data for the year 2022 contained 353,883 entries of 41,081 horses at 721 event locations in the Netherlands. The racing data contained 6112 entries of 1089 horses on 33 racetracks. There were eight classical racetracks and 24 short street tracks; 1065 racehorses participated in at least one classical race

and 85 competed in at least one short-track race; 61 horses competed in both categories at least once.
A static network with locations as nodes, and horses as edges is presented in Figure 4.3, to illustrate the geographical distances travelled by horses in the Netherlands for events. These locations-based networks were built solely for illustrative purposes here, and were not used further in the current study.
Next, a static undirected network was constructed with horses as nodes and with participation at the same event as edges. We did this for the sporting and racing events separately. The node degree distribution for the sports horse network is presented in Figure 4.5. Network metrics for each of the networks are summarised in Table 4.4.

Metric	Sports horses	Racehorses
Network size	41,081	1,089
No. edges	10,167,944	122,988
Connected?	Yes	Yes
Edge density	0.012	0.42
Transitivity	0.32	0.49
Diameter	5	3
Mean distance	2.6	1.8
Degree: min	2	28
Degree: median	272	378
Degree: max	4,155	1,714

Table 4.4: *Descriptive statistics of the static horse networks for the entire year of 2022.*

Opportunities for transmission of *S. equi* at competitive events

An overview of the outcomes of the simulation runs is shown in Table 4.5 and Figure 4.6. The number of contacts between carriers and susceptible horses per simulation was right-skewed and not normally distributed.

Outcome measure	Range	Median	IQR	2.5th to 97.5th percentile
No. of carriers in simulation run	335 - 4607	1523	1085 - 2054	525 - 3162
No. of contacts per carrier	1 - 9379	296	104 - 862	18 - 3305
Total carrier→susceptible contacts per simulation run	215,107 - 3,046,751	1,015,126	728,340 - 1,359,538	337,512 - 2,107,048

Table 4.5: *Simulation run outcomes*

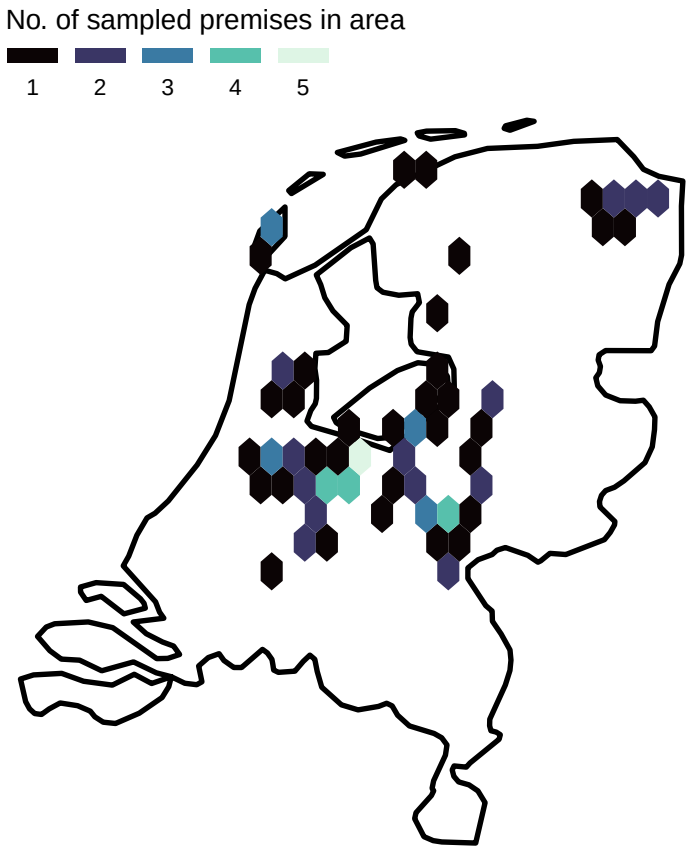


Figure 4.1: *Geographic distribution of sampled premises. The locations are indicated by the geographic centre of the area described by the four digits of the postal code, and therefore do not represent the exact locations.*

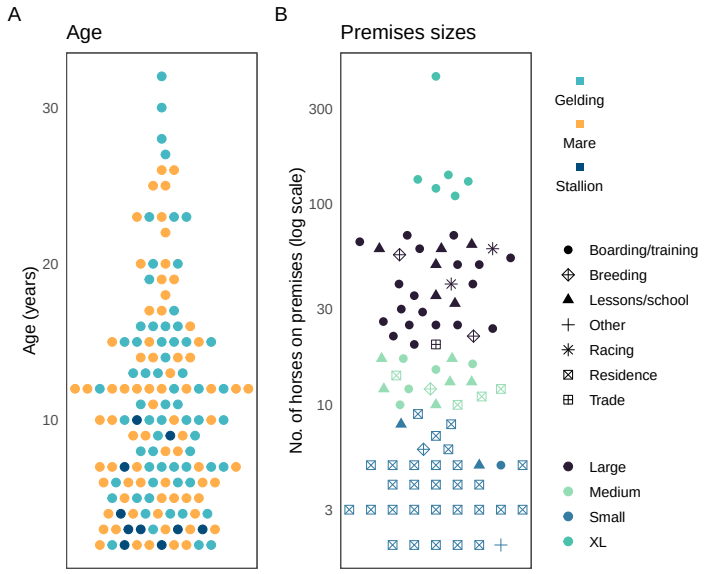


Figure 4.2: *(A) distribution of age and sex of the sampled horses; (B) Distribution of size and main purpose of the sampled premises.*

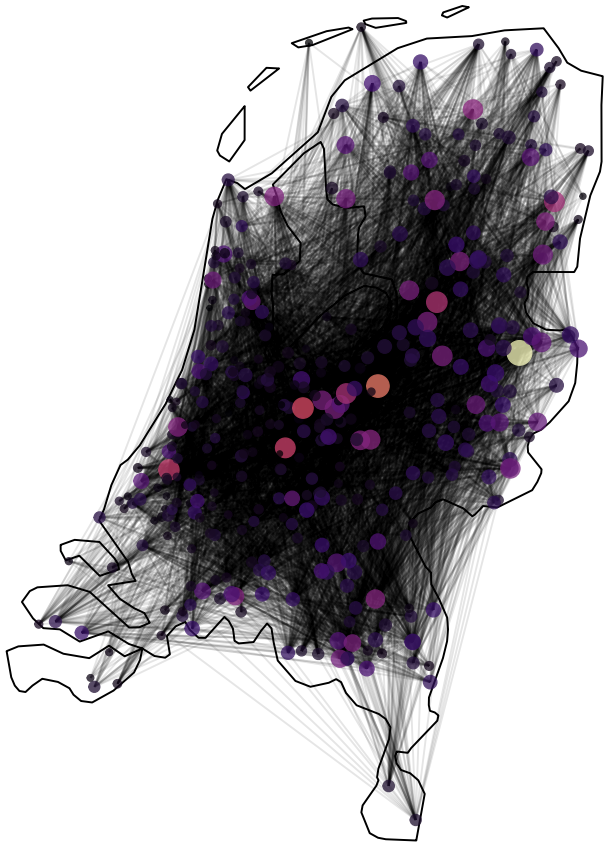


Figure 4.3: *Geographic distribution of sporting event locations for the month of June 2022 (the busiest month), with 19869 horses and 395 event locations. Each node is an event location. An edge exists between the nodes if one or more horses visited both locations. A lighter node color indicates a higher degree centrality, and a larger node size indicates a higher hub score (Csardi and Nepusz 2006).*

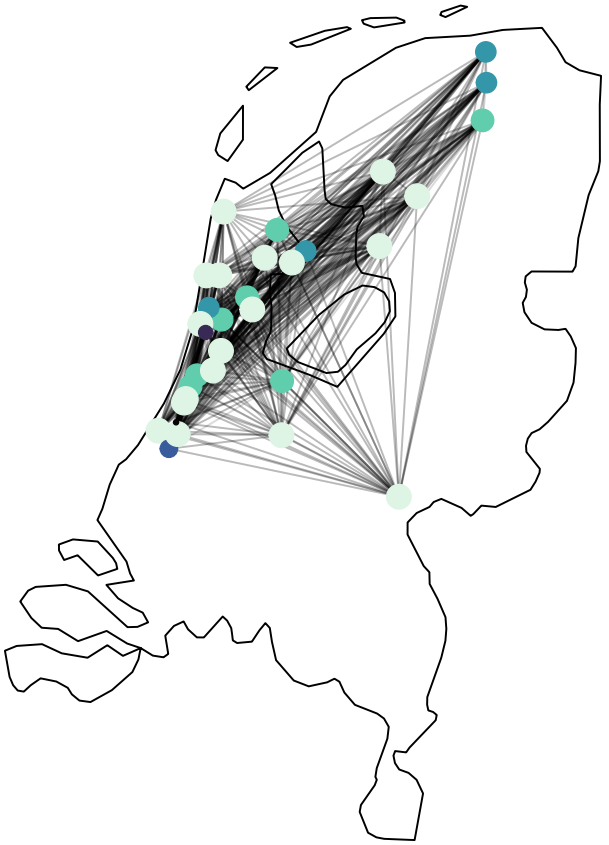
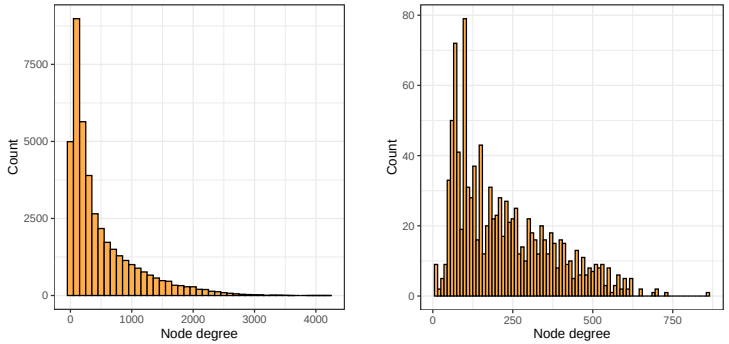


Figure 4.4: *Same as Figure 4.3 but for racing locations, for all of 2022.*



(a) *Sport horses* (b) *Racehorses*

Figure 4.5: *Degree distribution: number of all direct contacts (horses that attended the same event at least once) for all horses for all of 2022.*

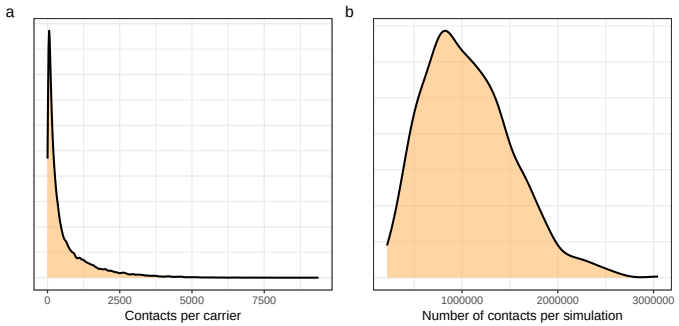


Figure 4.6: *Distribution of outcome measures after simulation runs. a) Annual number of contacts with susceptible horses per carrier. b) Total annual number of contacts between a carrier and a susceptible animal.*

DISCUSSION

Our results in context

Carrier prevalence

The true prevalence from the cross-sectional survey was 3.8 (95% Credible Interval 1.2%-7.7%), which is similar to other reports (Libardoni et al. 2016; Durham and Kemp-Symonds 2020) but lower than the estimate in (Jaramillo-Morales et al. 2022) where carrier prevalence was 13.5%. In the latter cross-sectional survey, on the prevalence of carriers in Colombia, 60% of sampled farms had at least one horse test positive, which is surprisingly high. The authors stated that none of the farms had experienced disease outbreaks in the three months prior to sampling, but no information was provided on events before that time (Jaramillo-Morales et al. 2022).

Cross-sectional survey sample selection

No census data was available to quantitatively inform stratified sampling; presence of all horses residing in the Netherlands on premises is recorded in a national database, but these data do not include information on the primary function of premises nor on the primary use of its resident horses. Some selection bias will have been present in our sample group of premises, which may have influenced results. Although we asked the participating veterinarians to nominate a representative range of the types of premises among their clientele, this inevitably ruled out participation of equines belonging to owners that never seek veterinary care.
Even if we had known the national distribution of the size of premises, we might not have been able to obtain a representative sample. A 2016 USA Census recorded that $2/3$ of premises in their census were small (5-9 equids) yet only $1/3$ of horses reside on small premises. Similarly, a 2023 Royal GD Animal Health census using government Identification & Registration data reported that the mean size of premises was seven horses and that the most prevalent size was two horses. Since we would not sample more than two animals from one location, the sampling may inevitably have been biased towards horses from smaller premises. The USA Census reported that $2/3$ of horses were between 5-20 years old. In our sample, 106 of the 149 horses (71%) for which an age was known were between 5-20 years old, which is a good match with the age distribution of horses in the USA Census (USDA 2016).

A completely random selection and recruitment of participants would have been ideal, but was not practically feasible for a number of reasons; firstly, we did not have access to "Identification and Registration" data to know which horses to draw from and where to find them, and, most importantly, participation was voluntary and required a significant time commitment from horse owners. We intentionally avoided recruitment of participants via (social media) appeals to the general public as selection bias when owners self-selected for participation is

likely more influential than the selection bias which still remains in our sample group. A public appeal would probably not have resolved the main issue in our sampling bias, which is the absence of owners that never seek veterinary care. Since vaccination for equine influenza (a treatment only veterinarians are allowed to perform) is mandatory at competitive gatherings in the Netherlands, this selection bias was appropriate for the current research question. We were unable to find data on the proportion of equine premises in the Netherlands that never seek veterinary care.

Network

Network metrics differed between the racing and sporting contact networks. At first glance, the racing network appears much more connected (and much more vulnerable to disease spread) given the lower diameter and mean distance, and higher edge density and degree. However, there is a size difference of several orders of magnitude between the racing and sporting network. Further comparisons between the racing and sports network, and comparisons to publications of other horse and livestock networks, can be found in **Chapter 7**.

Opportunities for transmission of *S. equi* by carriers at competitions

The ICC reported 57 outbreaks of *S. equi* for the year 2022, and 92 for the year 2023. We do not know whether this difference is due to an actual increase in the number of diagnoses, or because the readiness to report has increased. The SEIN reporting network, which is the source of the ICC data, was introduced in 2019 and awareness was still being built in 2022. Using the higher 2023 number of outbreaks (to establish a worst-case scenario), and assuming that all outbreaks were caused by a carrier→susceptible contact at a competition (which is likely an overestimation), then 1:11,034 [IQR 7,920 – 22,903] carrier→susceptible contacts leads to a new outbreak. The proportion of contacts that is successful at instigating a new outbreak may be low, but given the large numbers of contacts that horses have annually (Table 4.5), even a very low probability of a successful contact still leaves many potentially successful contacts.

Main limitations

Carrier prevalence cross-sectional survey

For various logistical reasons, we were only able to sample 166 premises, making the study of the carrier prevalence underpowered. The planned sample size was therefore not attained which inevitably affected the precision of our final prevalence estimate.

In this study, three nasopharyngeal lavages were used as a detection method for carriers, in lieu of the gold standard of an endoscope-guided guttural pouch lavage. This choice was in part practical (due to higher cost of GP lavages, availability of endoscopy unit and personnel) and also in part driven by the study's source of funding which, as a welfare-promoting organisation, prohibited use of procedures as invasive as GP endoscopy. This limitation of imperfect sensitivity of our testing method was accounted for in our true prevalence calculation.

A sensitivity of three repeat washes of 90% has been mentioned (Boyle et al. 2017) but the supporting data for this estimate could not be traced. Hence, although the point estimate of 90% test sensitivity was used in the sample size calculation prior to the study, in the eventual data analysis we applied estimates for sensitivity for three repeated NPLs from a study designed specifically for that purpose (Pringle et al. 2019). Interestingly, the estimates for sensitivity of single and triple NPLs that we extracted from the 2019 report, are not all that dissimilar to estimates for nasopharyngeal swabs from a 1997 study which put the estimates at 45% and 85%, respectively (Newton et al. 1997).

One advantage of the chosen diagnostic testing method in this study is its increased likelihood of detecting carriers in which the origin of carriership is not in a guttural pouch, but in a paranasal sinus if this is indeed a possibility (Pringle et al. 2019; Pringle et al. 2022). In another study, all positive sample swabs and washes were also accompanied by a positive GPL (Boyle et al. 2017). Past discordant results form GP and NP lavages may in hindsight have been due to temporary colonisation of the muzzles of non-carrier horses co-mingling with carriers (Riihimäki et al. 2023).

Another advantage of repeated NPLs over GPLs was avoiding of false positives from endoscope contamination failing to be cleared by cleaning and desinfection protocols, which has been reported to occur (Svonni et al. 2020; Nadruz et al. 2023).

Network

The horse identifiers in the NDR and KNHS databases were not the same, and therefore we may have missed crossover between the racing and sporting networks. We think that such a connection is unlikely; racing and sporting events are invariably held at different locations. Anecdotally, we are aware that Standardbreds that retire from racing sometimes find new careers in sporting events, usually endurance. However, we suspect that a horse in racing training is unlikely to also be competing at sporting events in the same time period. We therefore do not think the lack of a common horse identifier between the two databases has any significant effect on our findings.
We compressed our time-resolved data to a single static network, which incurs a loss of information (Vernon and Keeling 2009). However, for our specific research question, a whole-year static network was appropriate; we assumed carriers remained equally infectious during the year, and were only interested in counting the number of direct contacts by the infectious horses, so not having information on the timing sequence of chains of contacts was not a true limitation for our specific research question.

Transmission simulations

The nature of the available data also did not allow us to evaluate the relative contribution of carriers at competitive events to the number of annual *S. equi* outbreaks but we were only able to estimate a worst-case scenario for the probability of transmission for these horses in these settings. The relative contribution to the national incidence of outbreaks by carriers at competitive events (which in reality may be small) must be assessed by different means.
At both racing events and sporting events, not all horses that compete on the same day are present at the location at the same time, which is why our transmission parameter in this study is termed "per horse at the same event on the same day". It is likely higher between horses that are in the same class or race, and lower between horses that are not, and timing of the classes likely matters also. Future work could include construction of a mixing matrix for animals participating on the same day.

It is important to note here that the low estimate for transmission should not be interpreted as a reason to become less vigilant to the possibilities of transmission of infectious disease at equestrian events. For one, *S. equi* is not the only infectious agent that can be transmitted at events; other pathogens, especially those of respiratory or gastro-intestinal nature, may be transmitted more readily than *S. equi*, but were not assessed in the current study. Furthermore, and more importantly, the low probability of transmission for *S. equi* that we calculated here, may be a result of current awareness and vigilance about the potential for disease spread; for example, widespread knowledge about the importance of not sharing water sources or stables has likely already impacted the transmission of *S. equi* at equine gatherings.

Conclusion

We report a *S. equi* carrier prevalence of 3.8 (95% Credible Interval 1.2-7.7%) among apparently healthy horses and ponies in the Netherlands, in line with reports from other countries. The one-year contact network of horses participating in equestrian sports events was found to be highly connected with a diameter of five, despite its large size. We estimate that in the setting at a racing or equestrian sports event, only about 1:11,033 (IQR 1:22,903 to 1:7,920) of susceptible horses that attend an event on the same day as a carrier become infected that day, in a worst-case scenario. However, it is important to note that this low number is likely a result of current awareness and vigilance about risk of disease transmission at equestrian events.

SOURCE OF FUNDING

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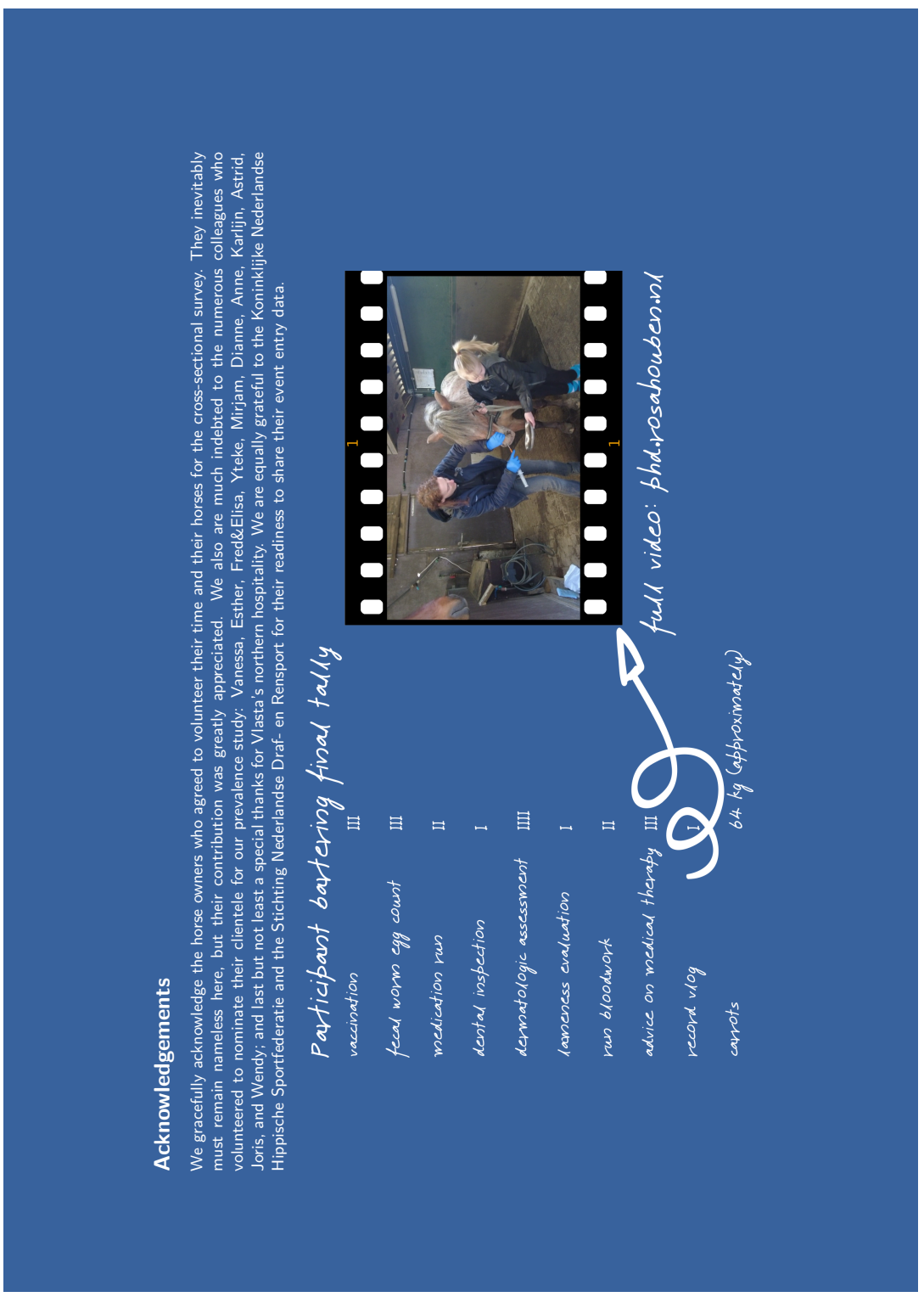
Acknowledgements

We gracefully acknowledge the horse owners who agreed to volunteer their time and their horses for the cross-sectional survey. They inevitably must remain nameless here, but their contribution was greatly appreciated. We also are much indebted to the numerous colleagues who volunteered to nominate their clientele for our prevalence study: Vanessa, Esther, Fred&Elisa, Yteke, Mirjam, Dianne, Anne, Karlijn, Astrid, Joris, and Wendy; and last but not least a special thanks for Vlasta's northern hospitality. We are equally grateful to the Koninklijke Nederlandse Hippische Sportfederatie and the Stichting Nederlandse Draf- en Rensport for their readiness to share their event entry data.



Part III

EHV-1

Chapter 5

EHV-1 BASIC REPRODUCTION NUMBER & EFFECT OF VACCINATION

R.M.A.C. Houben, C. van Maanen, J.R. Newton, J. van den Broek, M.M. Sloet van Oldruitenborgh-Oosterbaan, J.A.P. Heesterbeek: A model-based approach to evaluate the effect of vaccination of the herd on transmission of equine herpesvirus 1 in naturally occurring outbreaks.
Resubmitted after revision

Abstract

Background Equine herpesvirus 1 (EHV-1) infection is the cause of high impact disease syndromes, affecting the global horse industry. The effect of vaccination on transmission dynamics of EHV-1 in naturally occurring outbreaks is not quantified. **Objectives** Estimate R_0 for EHV-1 in equine populations from outbreak data, and evaluate the effect of vaccination status of the herd on R. **Study design** Systematic review, model-based estimations and meta-analysis. **Methods** A literature search for outbreak reports was carried out. Depending on available data, the early epidemic growth rate (GR) or final attack rate (AR) approach was used to estimate the basic reproduction number for that outbreak. Herd vaccination status, as well as virus genotype and use of antivirals were recorded. Only outbreaks in herds where either none or all of the horses had been vaccinated were included. An overall estimate for R_0 (non-vaccinated herds) and R_v (vaccinated herds) was computed by meta-analysis and the two groups were compared using a random effects model. **Results** Twelve outbreaks, in herds of 16-135 horses, met the inclusion criteria, of which six occurred in non-vaccinated herds and six in vaccinated herds. One R_0 calculation from a report describing empirical determination of a herd immunity threshold was also included. We found no evidence for a significant effect of vaccination status of the herd on the effective reproduction number in outbreaks: $\hat{R}_0 = 3.3(2.6 - 4.0)$ and $\hat{R}_v = 2.7(2.1 - 3.2)$, $p = 0.15$. **Main limitations** Insufficient (discordant) data were available to investigate the influence of genotype or antivirals on results. Sensitivity analyses gave volatile p-values. **Conclusions** We found no robust evidence for a significant reduction on transmission of EHV-1 in herds where all horses were vaccinated *vs* non-vaccinated herds. $\hat{R}$ in herds where all horses were vaccinated was substantially > 1 and vaccination as a sole mitigating measure may have limited effect on transmission of EHV-1.

INTRODUCTION

Equine herpesvirus 1 (EHV-1) is an alpha-herpesvirus which affects only equidae, and is endemic worldwide (Lunn et al. 2009; Carvelli et al. 2022). EHV-1 primarily causes respiratory tract disease, the severity of which appears to be related to age and immunological status (Lunn et al. 2009; Carvelli et al. 2022). EHV-1 infections can progress beyond the respiratory mucosa and cause late term abortion, perinatal foal death, or neurological dysfunction in a syndrome called equine herpesvirus myeloencephalopathy (EHM). EHV-1 seroprevalence is usually high (Lunn et al. 2009; Nielsen et al. 2022); 26% in mares on thoroughbred stud farms in Australia (Gilkerson et al. 1999), 39% in horses on farms in China (54% in mares) (XueZhu et al. 2020). EHV-1, like all herpesviruses, causes latent infection and is part of a group of herpesviruses of veterinary importance including gallid herpesvirus 2 (Marek's disease), bovine herpesvirus 1 (bovine respiratory disease) and porcine herpesvirus 1 (pseudorabies; Mahony 2015). It is assumed horses first encounter EHV-1 at a young age, and are presumably infected by their dams (Gilkerson et al. 1999; Gilkerson et al. 2000). After initial infection, most horses develop a persistent latent infection, with the virus often detectable in the lymphoid tissue and the trigeminal ganglia (Edington et al. 1994; Carvalho et al. 2000; Pusterla et al. 2012). "Latent" infection in the context of EHV-1 should be interpreted to be akin to a carrier state, meaning the horse has been infected (and been infectious), is now not infectious, but can revert to infectiousness again at any point in time through reactivation of the latently present virus. Latent infection in the context of EHV-1 therefore has a different meaning than in the context of, for example, tuberculosis, where the (long) time-span between becoming infected and becoming infectious is what is termed "latent" infection.

Introduction of infectious horses to susceptible herds and subsequent lateral spread is a cause of new infections and subsequent outbreaks. Reactivation of latent infection, with recurrence of nasopharyngeal shedding of virus, can also lead to new infections wherever horses with reactivated shedding are in contact with susceptible horses.

Although a single nucleotide polymorphism (N_{752} to D_{752}) has been shown to result on average in greater levels of viraemia (Allen and Breathnach 2006; Franz et al. 2017), results from the field demonstrate that EHM outbreaks can be associated with both the N_{752} as well as D_{752} genotypes. In addition, two outbreaks with a new H_{752} variant have also recently been reported (Sutton et al. 2020; Pusterla et al. 2021). The D_{752} genotype has been shown to cause higher viraemia, and evidence exists that the D_{752} genotype is associated with higher (up to fourfold) nasal shedding, but reports have been inconsistent (Franz et al. 2017; Pusterla et al. 2023a). To date, the N_{752} genotype remains the most prevalent genotype of EHV-1 (Pusterla et al. 2023a).

EHV-1 can have a profound impact on the equine industry, as observed with

the 2011 Ogden, USA EHV-1 outbreak among participants at Western National Championship event (USDA 2013) and the 2021 international outbreak among elite sport horses in Europe, which originated from a competition in Valencia (Termine et al. 2021). Veterinary hospitals can also encounter great difficulty when an outbreak occurs on their premises (Vandenberghe et al. 2021) which is particularly worrying as agent escape from isolation facilities at veterinary hospitals has been described (Goehring et al. 2010a; Burgess et al. 2012). Due to its effects on equine industry and trade, the European Food Safety Authority (EFSA) recently considered whether EHV-1 should be subject to control at the EU regulatory level (Carvelli et al. 2022; Nielsen et al. 2022). One of the issues which were raised was the scarcity of data on the effectiveness of risk-mitigating measures for EHV-1 which, in combination with EHV-1 posing negligible to no risk to human health or food security, led to a negative verdict. The EFSA panel did note that it estimates that the basic reproduction number (R_0) for EHV-1 probably lies within the range of 3-10 (Nielsen et al. 2022) which was based on a report on two outbreaks in the USA, of which one occurred in a partially vaccinated herd (Meade 2012).

EHV-1 infection is notifiable in some countries, and some equine sports governing bodies require an up-to-date EHV-1 vaccination status for participation in events, but currently no universal regulation exists (Pereira et al. 2021; Nielsen et al. 2022). The German equestrian federation made vaccination against EHV-1 compulsory in 2023 for horses participating in sporting events, citing that the main goal of the mandatory vaccination was to reduce virus spread (Herpes-Impfung bei Pferden | FN 2023).

Whether vaccination against EHV-1 should be mandatory, and whether vaccination will reduce the incidence of EHM, have both been subjects of debate (Kydd 2021; Equine Herpesvirus (Rhinopneumonitis) | AAEP 2021). It has been suggested that by lowering the force of infection (the rate at which susceptible individuals become infected, per unit time, Diekmann et al. 2013) through vaccination, fewer animals are eventually exposed to the virus in an outbreak and therefore, the number of affected animals could be decreased (Equine Herpesvirus (Rhinopneumonitis) | AAEP 2021; Lunn et al. 2009). By design and usually because of financial and ethical restrictions on sample sizes, clinical efficacy trials cannot directly investigate the effect of vaccination on transmission, as all participants receive an experimental inoculation, rather than being naturally exposed to the infectious agent.
Several vaccines against EHV-1 are available, and differ in composition: whole inactivated, and modified live virus (MLV) – based vaccines have been marketed.

One study comparing inactivated and an MLV vaccine found a significant reduction in viral loads on nasopharyngeal swabs in the MLV *vs* inactivated vaccine and placebo groups, but relied on virus isolation, rather than the more sensitive diagnostic method of PCR, for their results (Goodman et al. 2006). In a study comparing MLV, inactivated vaccine, and control groups and assessing shedding

by nasal swab PCR (Goehring et al. 2010b), lower total viral loads and number of days shedding were found in the combined vaccine *vs* control group, but no significant difference between the two vaccine groups was demonstrated.
A recent systematic review of vaccine efficacy randomised controlled trials Marenzoni et al. (2022) found overall evidence of a small but favourable effect on epidemiological parameters, such as the number of challenged animals that became infectious, as well as the duration of infectiousness in those animals.

A significant reduction in (nasal) shedding cannot be assumed to result in a similar, let alone linearly correlated, reduction in transmission. Transmission of infection depends not only on an individual being infectious, but also on the contacts it has with susceptible individuals. If, for example, a horses' social contacts are arranged in such a way that it can infect all of its potential susceptible contacts within the first day of becoming infectious, then a reduction of the number of days of infectiousness from 5 to 4 may have no perceivable effect on the transmission rate and therefore R. Whether or not the decreased shedding leads to a significant reduction in R needs to be assessed separately.

The observed overall improvement of clinical parameters in the meta-analysis (Marenzoni et al. 2022) suggests that vaccination carries some benefit for the individual horse, although the eventual outcome of disease is probably multifactorial, as is evidenced by several reports of occurrence of serious illness, sometimes fatal, in herds where all horses were vaccinated (Gryspeerdt et al. 2011; Sutton et al. 2020; Pusterla et al. 2021).
The aim of this study was to estimate R_0 for EHV-1 and determine whether vaccination of the herd leads to a significant reduced reproduction rate (R_V), through a systematic review, model-based estimations and meta-analysis of naturally occurring outbreaks.

METHODS

MOOSE (Meta-analyses Of Observational Studies in Epidemiology) guidelines were followed (Stroup et al. 2000).

Search Strategy

The Pubmed/MEDLINE and CAB Abstracts databases were queried with the search term:

(equine OR horse AND herpesvirus) OR (equine OR horse AND EHV) OR (equine OR horse AND EHM) OR (equine OR horse AND herpesvirus AND myeloencephalopathy)

for the years 1992-2023. The year 1992 was chosen as a cut-off as around this time a PCR (Wagner et al. 1992) became available which could differentiate

EHV-1 from EHV-4. Infection by EHV-1 is not reliably diagnosed based on clinical findings alone and molecular diagnostics are required for confirmation of EHV-1 as the causative agent of outbreaks of disease (Lunn et al. 2009; WOAH 2022). Reference lists of discovered papers as well as key review papers on EHV-1 were also scanned. If no English-language full-text or only a conference abstract was available, the corresponding author was contacted for additional information.

Studies were considered for inclusion if they were in the English language and within the manuscript or abstract there was information on outbreak final size (attack rate) or early outbreak longitudinal incidence data of unmitigated EHV-1 outbreaks. Reports were excluded if from the outbreak description it seemed likely that multiple infectious individuals were introduced to the susceptible group; reports were considered eligible if one point source of the outbreak was detected or if the source of the outbreak was not determined, but likely to be a single source. Because clinical signs of EHV-1 infection (e.g. low-grade fever) are easily missed or absent, outbreaks reporting attack rate data were only included in the meta-analysis if daily systematic records of temperatures and other clinical signs for all at-risk animals directly from the start of the epizootic were available, or if molecular diagnostics were available for all at-risk animals. Judgement on suitability for the inclusion of the meta-analysis for each of the outbreaks was by consensus between RH, CvM and RN. Whenever the contents of the report were not clear as to whether it was suitable for inclusion (i.e. missing information), the corresponding author was contacted by email for further details.

Parameters

Underlying assumptions used for the model-based R_0 estimates are presented in Table 5.1.

Parameter	Description	Value	Source
GT	Generation time	5 ± 2 days 7 ± 2 days 2 ± 0.5 days	Sutton et al. 1998 Friday et al. 2000 Meade 2012
ν	Equi N Tect vaccine efficacy	90%	Bannai et al. 2019

Table 5.1: *Assumptions used in the model-based estimations*

Data extraction and calculation of the basic reproduction number

Data extracted from the reports, when available, were number of animals at risk, number of animals infected, causative EHV-1 genotype (if determined), vaccine used (where applicable and available) and herd immune/exposure status prior to the outbreak. Only reports where either all horses were vaccinated or all horses were explicitly not vaccinated were compared; outbreaks in herds with mixed or unknown vaccination status were not included. Herd immune/exposure status prior to the outbreak was assessed by information on known vaccination or recent disease in the outbreak report, and was recorded as either "none" (horses were stated not to have been vaccinated within the last six months), or "all" (horses were stated to have all been vaccinated within the previous 6 months). A horse was considered infected if presented a raised temperature, seroconversion, or was PCR positive at any point during the outbreak investigation.

An estimate of the basic reproduction number was computed using two common estimators from early epidemic (exponential) growth rate data or from the final attack rate (AR). The latter is the total fraction of the initial population that eventually becomes infected in the outbreak $(1 - AR)$. This is also referred to as the final size of the outbreak, $1 - s(\infty)$, the fraction of the original population that has escaped infection when the outbreak has run its course. For these estimates there are a number of assumptions (Diekmann et al. 2013). We assume that the herds are closed for the duration of the outbreak in the sense that there is no birth, death or migration in that time period. In addition, we assume that mixing inside the herd is homogeneous in the sense that a contact of the type that can potentially lead to transmission is equally likely for any pair of individuals in the herd (the herd is well-mixed). We assume that immunity that arises from infection lasts at least for the duration of a typical outbreak. We assume that all individuals in the herd are equal in their susceptibility, infectivity and contact pattern. Finally, it is assumed that the outbreaks run their initial (exponential phase-) course without mitigation through control measures.

Considering that most adults horses are assumed to have been exposed to EHV-1 early in life, and that up to 88% of adults are latently infected (Carvalho et al. 2000) (although this may differ by geographical region), R_0 in this context is interpreted to refer to "susceptible animals", meaning horses that are susceptible to (re-)infection as they have no current benefit of protection to (re-)infection owing to recent vaccination or recent natural infection. We do not assume that these adult horses are fully naive to EHV-1. The cut-off for absence of recent vaccination or infection was set at 6 months, meaning that any horse that had not been vaccinated or naturally infected > 6 months prior to the outbreak was considered susceptible.

The estimator based on the final size/attack rate is given by

$$\hat{R}_0 = \frac{\ln(s(0)) - \ln(s(\infty))}{s(0) - s(\infty)} \tag{5.1}$$

where $\ln$ is the natural logarithm, $s(0)$ is the fraction of the herd that is susceptible at the start of the outbreak (so $s(0) = 1$ in a fully susceptible herd) and $1 - s(\infty)$ is the final size (Dietz 1993).

The simplest estimator based on early outbreak exponential growth rate, denoted by r, is given by

$$\hat{R}_0 = e^{rT} \tag{5.2}$$

where T is the generation interval of the epidemic (see Roberts and Heesterbeek (2007) and Wallinga and Lipsitch (2007) for this and related estimators). We assumed the mean generation interval, or the time interval between successive cases in a chain of transmission, to be 5±2 days as Sutton *et al.*,Sutton et al. (1998) in an experimental infection study, reported that the first day of nasopharyngeal shedding of virus detectable by immunocytochemistry of respiratory tract samples was at 3 days post-infection and the highest recorded nasopharyngeal shedding was at day 5. In a more recent infection experiment using the H_{752} strain, the first detection by qPCR of nasopharyngeal shedding was at day 1 post-infection and peak shedding was on days 2-6 (Sutton et al. 2020). The assumption of a 5±2 days generation interval was consistent with the early pattern of incidences in the report by Sutton et al. (2020).

Horses housed in individual boxstalls, but within the same airspace (i.e barn) were considered to be equally well-mixed as horses in group housing. There is evidence from outbreaks due to airborne transmission of EHV-1 in enclosed airspaces, in the absence of direct contact (Lunn et al. 2009; Termine et al. 2021). When outbreaks occurred in multiple barns on a premises, only the outbreak in the index barn was used, because in subsequently infected barns barriers to spread could have been installed as the outbreak was occurring, since the index barn had served as a warning to caretakers.

Meta-analysis

For meta-analysis, to evaluate the effect of vaccination on $\hat{R}$, a random effects model with the Restricted Maximum Likelihood estimator was used (Viechtbauer and Cheung 2010; Harrer et al. 2021). A random effects model was considered to be appropriate, assuming that vaccination statuses of the herd (full or none) is a fixed effect and each individual outbreak is a random effect, as outbreaks will differ in herd composition, housing, husbandry practices, and other factors, which are not otherwise captured in the analyses. The between-study heterogeneity variance was assumed to differ between the subgroups. Prior to the final meta-analysis, outliers and influence analyses were carried out using visual inspection of forest plots and Baujat plots, leave-one-out plots analyses for the non-vaccinated and vaccinated study groups were evaluated to decide whether any outbreaks should be removed prior to analysis (Harrer et al. 2021).

Both R estimators, including 95% confidence intervals (CIs) were calculated using the R_0-package (Obadia et al. 2012) in R (R Core Team 2021). The meta-analysis was then performed using the meta-package in R (Balduzzi et al. 2019). As the CIs produced by the R_0-package were derived via a log transformed variable they

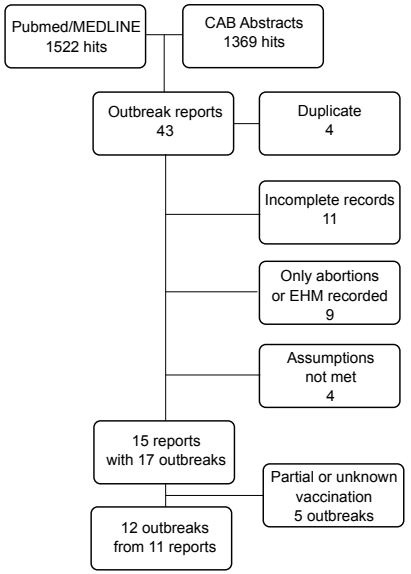


Figure 5.1: *Flow chart of search results as of October 25 2023. Duplicates indicate separate reports on what appeared to be the same outbreak. Assumptions not met was due to horses mixing with mules (1) or hospital outbreak after agent escape from isolation, barrier nursing already in place at the start of transmission (n=1), and ongoing horse movements on/off premises during the outbreak(n=2).*

were not always symmetrical around the mean (but the difference was always in the order of magnitude of 10^{-2}). However, the meta-package assumes a symmetrical estimate and calculates the standard error as $(CImax - CImin)/3.92$. As the deviations from this assumption were minimal, we did not consider this issue problematic.

RESULTS

Outbreak reports retrieved

Search results are presented in Figure 5.1 and Table 5.2. An overview of all assessed outbreaks, and reasons for exclusion form analysis (if applicable) is given in Supplementary item Table B.2.
One additional report, the design of which was not part of our *a priori* search strategy, was considered for inclusion in the meta-analysis, as it contained useful information on empirical discovery of a range for the herd immunity threshold (by

vaccination) in a large transient population of young racehorses in Japan (Bannai et al. 2014). The mean of the reported range of 79.3 to 85.3% was corrected for a vaccine efficacy of 90% (Bannai et al. 2019) and then used to calculate the point estimate for R_0 (via $HIT = 1 - \frac{1}{R_0}$); the efficacy-corrected range was used as the confidence interval. This estimate was included in the meta-analysis.

Table 5.2: *Overview of herd and outbreak characteristics of included reports; n/m: not mentioned in report. Shaded rows indicate vaccinated herds.* [1] *Lower limit estimate, as described in Houben et al. (2023).*

Source	Herd size & Housing	R_0 estimate method	Genotype	Antivirals	Vaccinations	Location
Pusterla et al. 2021	31 Barn	AR	H_{T52}	Valacyclovir all horses "early" (and Heparin if febrile)	All horses q6 months, Vetera 2XP flu/rhino (Boehringer Ingelheim Animal Health, killed/adjuvanted multivalent EHV-1/EHV-4/EIV vaccine) (personal communication). Time from last vaccine to onset of outbreak was 1 to 6 months with a median of 1 month	USA
Sutton et al. 2020	64 One stable block	GR	H_{T52}	n/m	All, no details available	France
Strang and Newton 2017 - Index barn	16 Barn	AR	n/m	n/m	None within a year prior to outbreak	UK
McFadden et al. 2016	21 Paddocks	AR	D_{T52}	n/m	Lapsed: 10 months before outbreak, Pneumequine (inactivated)	New Zealand
Bannai et al. 2014	≈2000	Empirical HIT	n/m	n/n	Not applicable	Japan
Barbic et al. 2012 One horse from outbreak at farm 1 was source for outbreak on farm 2 - Farm 1 - Farm 2	Shared common paddocks/runs and in close contact 69 88	 AR[1] AR[1]	 D_{T52} D_{T52}	 n/m n/m	None, and all seronegative a year before the outbreak	Croatia
Meade 2012 - CDR	37 One barn ("No.38")	AR	D_{T52}	Acyclovir in all horses from 6 days after index case	None	USA
Gryspeerdt et al. 2011 premises 1	96 Riding school	GR	D_{T52}	Valacyclovir in 4 horses in total (unclear whether this was on Premises 1)	All twice yearly, Duvaxyn, inactivated (personal communication).	Belgium
Henninger et al. 2007	135 Three (connected) stall barns	AR	n/m	Acyclovir days after onset of fever in 56/80	96%: All resident horses q3 months, MLV (n=4) and inactivated (n=125)	USA
van Maanen et al. 2001	41 Riding school	AR	n/m		None	Netherlands
Friday et al. 2000	46 Barn and (group) paddocks	AR	n/m	Acyclovir in 7 horses after start of neurologic signs	All q4 months, killed (Fluvac EHV4/1, Fort Dodge)	USA

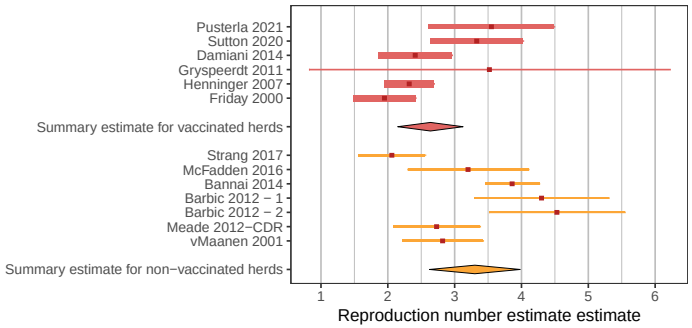


Figure 5.2: *Forest plot of the main results of meta-analysis.*

Main analysis

Results of the meta-analysis are presented in Figure 5.2. $\hat{R}_0$ for outbreaks in non-vaccinated herds was 3.3 (95% CI 2.6-4.0) and $\hat{R}_v$ 2.7 (95% CI 2.1-3.2). There was substantial between-study heterogeneity: ($I^2 85\%$ overall, 71% and 87% for the vaccinated and non-vaccinated subgroup, respectively). We found no evidence for a significant effect of vaccination status of the herd on the effective reproduction number in outbreaks (p=0.15) in our main analysis.
Figure 5.3 demonstrates the effect on the subgroup summary estimate of leaving one study out. The original summary estimates are displayed as well for visual comparison.

Sensitivity analyses

The outbreak described by Strang and Newton (2017) just met the crude criterion of being an outlier. Our first sensitivity analysis was to repeat our original analysis, but with that outbreak removed, which led to estimates of $\hat{R}_0 = 3.5$ (95% CI 3.0-4.1), $\hat{R}_v = 2.7$ (95% CI 2.1-3.2), p=0.038.
As expected, the per-outbreak R estimates were dependent on the assumption of the generation time (see Supplementary materials Figure B.9). The sensitivity of the summary $\hat{R}$ estimates produced by estimator (2) to alternative assumptions of the generation time could not be meaningfully assessed, as a growth-based estimate was only available for outbreaks that occurred in vaccinated herds (n=2; (Gryspeerdt et al. 2011; Sutton et al. 2020)) which meant that this sensitivity analysis would affect one arm of the meta-analysis only. Instead, the analysis was re-run with only attack rate-based estimates. This led to summary estimates of $\hat{R}_0 = 3.2$ (95%CI 2.4-4.0), $\hat{R}_v = 2.8$ (95% CI 2.0-3.6), p=0.48. In this sub-selection of studies, outbreak Strang and Newton (2017) no longer qualified as an outlier, however, the attack rate-based only analysis was repeated without Strang and Newton (2017) which gave $\hat{R}_0 = 3.4$ (95% CI 2.7-4.2), $\hat{R}_v = 2.8$ (95% CI

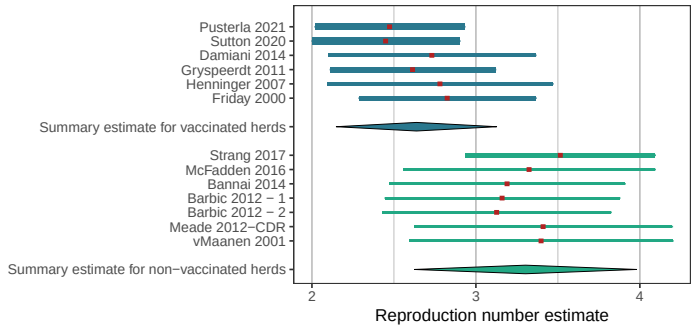


Figure 5.3: *Leave-One-Out analysis.*

2.0-3.6), p=0.24.
Insufficient discordant data were available to assess for the effects of genotype, vaccine product or antiviral treatment on the outcome.

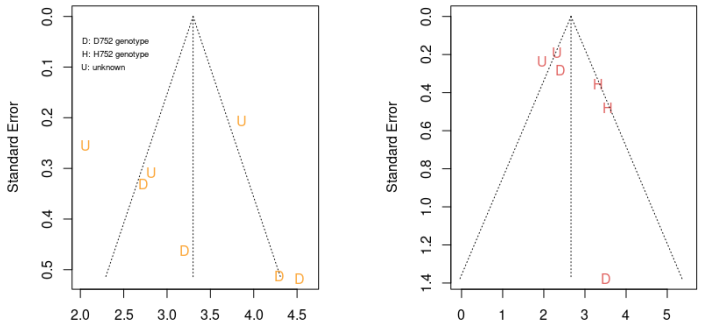
Publication bias assessment

The funnel plot from the meta-analysis is presented in Figure 5.4. A formal test for a bias to publish outbreaks in vaccinated herds or publication bias towards genotype is not available. An overview of all assessed outbreaks and considerations for exclusion is presented in the Supplementary materials.

DISCUSSION

Our estimate for $\hat{R}_0$ of EHV-1 was 3.3, which is similar to the lower limit of but within the range of 3-10 that was suggested by the EFSA panel (Nielsen et al. 2022), higher than the current estimates for strangles outbreaks of 2.1-2.7 (Houben et al. 2023) and similar to the lower limit of the estimate for equine influenza (2-5 and 10) (Satou and Nishiura 2006; Daly et al. 2013).

The finding that even in a herd where all horses are vaccinated transmission continues at a rate sufficient for major outbreaks to occur is not unique to EHV-1. For example, an experimental vaccine field trial on cattle farms, conducted as part of a national bovine herpesvirus 1 eradication program, compared vaccinated and non-vaccinated herds. A significant reduction in viral transmission was found with use of a modified-live virus vaccine *vs* a placebo, but the study also concluded that even in the vaccinated herds the reproduction rate is greater than 1, and that additional measures besides vaccination were essential to reduce virus introduction and transmission (Mars et al. 2001).



(a) *Herds where no horses were vaccinated* (b) *Herds where all horses were vaccinated*

Figure 5.4: *Funnel plot from meta-analysis. Colour legend: orange, non-vaccinated; red, vaccinated. Symbol legend: D, D_{752} genotype; H, H_{752} genotype; U, genotype unknown.*

The sensitivity analyses led to volatile p-values, most likely due to the limited number of outbreaks available for inclusion. As a result we failed to find robust evidence for an effect of vaccination on R, but a small effect may actually exist. From the available data it seems unlikely that the true effect of vaccination will be an R_v of < 1, so vaccination may not lead to a level of herd immunity where major outbreaks no longer occur.
Assuming that the difference in the summary estimates of R_0 and R_V from the present study were true differences, would vaccination still be worthwhile? To quantify the effect of vaccination of the herd on the probability of a major outbreak occurring, we can assume a hypothetical situation where the distribution of infectiousness among individuals in the population is exponentially distributed, in which case the probability of a major outbreak following the introduction of one infectious individual into the herd, is $p = 1 - 1/R$ (for $R > 1$ and when the length of the infectious period follows an exponential distribution. (Diekmann et al. 2013) This means that, assuming this so-called general stochastic epidemic model, in non-vaccinated herds a major outbreak will occur with a probability of 0.70 for each incursion, and in herds where all horses were vaccinated this probability is 0.63. In reality, EHV-1 likely behaves differently as the infectious period may follow a distribution that is not exponential, nevertheless this example provides an indication of the expected impact of vaccination of the herd on the probability of a major outbreak after the introduction of an infectious horse.

Main limitations

The many assumptions that were made, as described in the methods section, may in some cases not be completely consistent with reality. These assumptions were necessary to be able to obtain an estimate of the epidemiological parameters of interest, for which no current data-driven estimate was available. It is possible, if not likely, that some of the assumptions, notably those on homogeneous mixing, were potentially not entirely valid (Milwid et al. 2019b); however, there is no reason to assume that the violations differed between the two subgroups. Future work could evaluate the effect of violations of these assumptions to produce a more precise estimate for the effective R under a variety of circumstances. Such analyses would either require the availability of more detailed clinical data, or could be estimated in experimental settings.

In the outbreak reports which explicitly mentioned that horses in the affected herd were vaccinated, detailed information on the vaccination history beyond the most recent vaccination was sometimes lacking, as shown in Table 5.2; however, to be included in the vaccinated subgroup, the authors of the report needed to state explicitly (either in the manuscript or on request after having been contacted) that all the animals had been appropriately vaccinated; detailed information on what the report authors considered "vaccinated" was not available in 1/6 of the outbreaks in the vaccinated group.

Vaccines may vary in their inherent efficacy (Goehring et al. 2010b) and the vaccination schedule likely also further influences efficacy. As the events of interest in this study were naturally occurring outbreaks, variation in vaccine products and schedules within the vaccinated subgroup was unavoidable, but does represent the reality of the situation in the field. With the available data we were unable to assess the relative efficacy of one vaccine product or schedule over another on the reproduction number, but there is some evidence that some vaccines may have better efficacy against shedding than others (Goodman et al. 2006). In the real world, horses often are vaccinated with a variety of different products and have varying lifetime vaccination histories, not least because official requirements for vaccinations vary (Pereira et al. 2021). There is currently no evidence that either vaccination or natural infection confers long-term protective benefits. The possible heterogeneity in vaccination history in this study therefore, in our opinion, does not invalidate the applicability of our findings. As the relative efficacy of different vaccine types could not be assessed in this study due to the sparsity of suitable outbreak records, further research is necessary to determine whether any individual or group of vaccines demonstrates better efficacy against transmission.

We were also unable to assess the effect of genotype, antiviral use, and prevailing disease syndrome in the outbreak on the results.

Reporting bias may have influenced the results. An incursion of an infectious

animal that does not lead to an outbreak, may not get detected and will be unlikely to get reported, whether or not this incursion occurred in a vaccinated herd. Authors could be more motivated to publish details of outbreaks that occurred in vaccinated herds as this could be considered a more noteworthy event than an outbreak occurring in a non-vaccinated herd.

Only outbreaks where daily temperature checks were carried out from the start, or where blanket molecular testing was performed, were included in the AR estimates to avoid the pitfall of underestimatimg $\hat{R}$ due to missed cases. However, even in outbreak reports which met these inclusion criteria unrecorded transmission may have taken place; in some reports, molecular diagnostics (nasal swab / blood PCR and serology) were only performed once and in most reports, clinical and molecular monitoring began after the outbreak was already underway. It is therefore possible that both the $\hat{R}_0$ and $\hat{R}_v$ reported here are underestimations.

Reports, mainly anecdotal, on outbreaks of EHV-1 associated neurological disease in herds where many horses were already vaccinated may discourage owners, as well as veterinarians, from introducing vaccination as a mitigating strategy, as they consider vaccination to have little impact on the likelihood of an outbreak occurring or on the severity if an outbreak occurs. We were unable with our modelling approach, to provide evidence substantiating the current assumption that vaccination of horses will result in decreased transmission of EHV-1. Improved knowledge on the magnitude of the effect, if any, of vaccination of the herd, derived from the current study, will help future modelling studies where the impact of vaccination is to be considered.

Future prospects

EHV-1 infection usually establishes latency, which is difficult to definitively confirm or rule out (especially ante–mortem) in individual horses, and these animals can revert back to infectiousness under a range of circumstances. No treatment currently exists which can resolve latency, and so a test–and–treat or test–and–eliminate strategy of apparently healthy infected animals is likely to be an ineffective as well as inappropriate approach for the long-term control of EHV-1.

It is important to note that the current manuscript describes just one of many approaches to evaluate the efficacy of EHV-1 vaccinations. The advantage of the approach that is presented in this manuscript is that it can be performed using already existing data derived from naturally occurring field outbreaks. As we were unable to demonstrate a significant effect using this approach, the efficacy of any EHV-1 vaccine in reducing R could, in the future, be more reliably be assessed by transmission experiments of EHV-1 which incorporate groups of vaccinated animals and which are designed wherever possible to control for likely confounding factors. To the best of the authors' knowledge, no transmission experiments for EHV-1, with or without vaccination, have been reported to date.

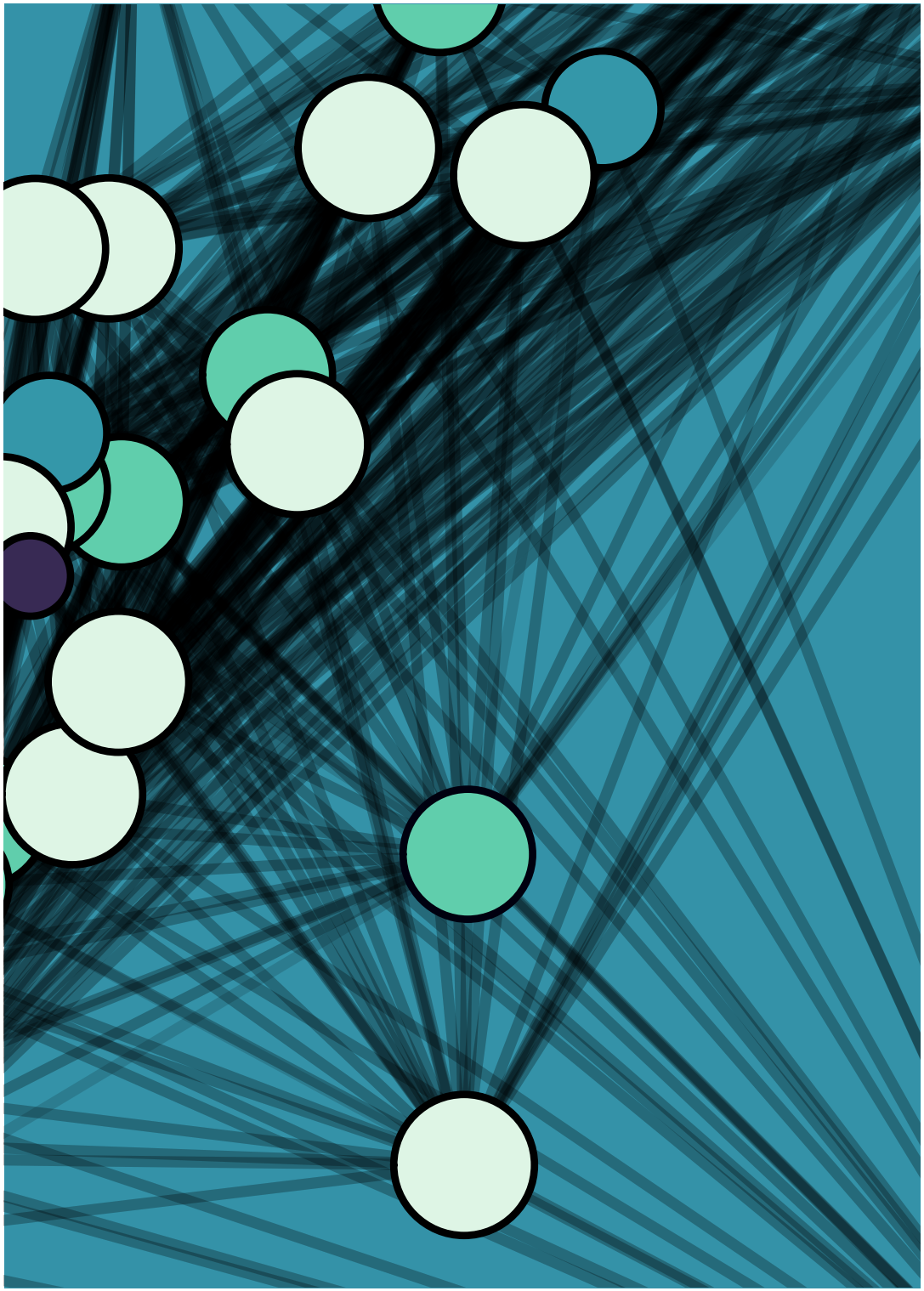
This study has highlighted the need for high-quality evidence for the effect of vaccines on transmission of EHV-1. If transmission experiments are not feasible in the foreseeable future due to the budget and ethical constraints, field studies could provide an alternative avenue for a more precise quantification of the effect of vaccines than could be provided by the current study.

CONCLUSION

The estimate of R_0 for outbreaks of EHV-1 found in this study was within expected estimates for a highly endemic viral respiratory tract pathogen. We were unable to find a statistically significant decrease in R in herds where all horses were vaccinated, compared to non-vaccinated herds. EHV-1 genotype, vaccine type and antiviral use were possible confounding factors we were unable to meaningfully assess in the current study. In herds where all horses were vaccinated, in all sensitivity analyses, the estimated reproduction number remained greater than 1, the threshold value under which only minor outbreaks are expected. This finding indicates that vaccination against EHV-1 with currently available vaccines as a solitary measure may be insufficient to prevent the occurrence of major outbreaks. Appropriately designed experimental studies evaluating the effect of vaccines on the transmission of EHV-1 should be conducted to better quantify its benefits.

Part IV

OPPORTUNITIES FOR TRANSMISSION



Chapter 6

BIOSECURITY ON EQUINE PREMISES

Rosa Houben, Kees van Maanen, Marianne Sloet, Hans Heesterbeek: A limited survey of biosecurity practices in a convenience sample of equine premises in the Netherlands

Pilot study

Abstract

For the transmission of infectious disease, contact (direct or indirect) between an infectious and a susceptible individual is necessary. Biosecurity measures can be implemented at the premises level to avoid such contacts and decrease the risk of outbreaks of infectious disease. Reports from other countries suggest that the majority of equine premises implement sub-optimal biosecurity practices. To investigate biosecurity practices on Dutch equine premises, a cross-sectional questionnaire survey was carried out among a convenience sample of horse owners and equine premises operators in the Netherlands. Premises which exclusively housed non-adult horses were not included in the sample, and horse trading premises were underrepresented. The survey questions were focused on bio-exclusion (external biosecurity) measures. There were 86 respondents in the survey, for 33 of those, one or more questions were unanswered. Most premises were private residential premises (38%), boarding and/or training premises (36%) or riding schools (16%). The remaining premises main purpose was breeding, racing, or trade. Most (92%) of respondents had horses that were housed in groups for at least part of the day. Half of the respondents never implemented quarantine measures for new arrivals. Only 6% always implemented quarantine of new arrivals, and the remaining quarantined new arrivals sometimes, or incompletely. None of the respondents required testing of infectious disease prior to taking in new arrivals, so none of the premises followed consensus recommendations for the control of *Streptococcus equi* subsp. *equi*. None of the respondents implemented bio-security measures for resident horses returning from short single-day trips such as for training or competition, even though 54/65 respondents had horses on the premises which took trips to locations with other horses. The responses in this limited survey were similar to surveys in the UK, New Zealand, Australia, and the USA. A follow-up survey on a larger and more inclusive sample of horse premises, which also investigates motivations behind management choices, is needed.

INTRODUCTION

For an infectious agent to cause an outbreak, it needs to be transmitted to a group of susceptible individuals. Transmission can occur, for example, via direct contact, faecal-oral contact, droplets, aerosols (airborne), vectors, and fomites. Exclusively airborne transmission, between *premises* in geographical proximity, has not been recorded for any equine infectious disease to date. Most equine infectious respiratory diseases require either direct, vector, or fomite contact, or proximity that is sufficiently close for droplet transmission between horses.
This suggests that for all but the vector-borne equine infectious agents, to cause an outbreak, a horse or a fomite carrying an infectious agent from outside of the premises must come into direct or close contact with a resident horse. For equine infectious diseases in the Netherlands, this opportunity arises when horses change home premises, when they visit other locations for training, veterinary care, or breeding purposes, or when large numbers of horses gather temporarily at markets or to participate in competitive events. These are also the routes along which infectious agents can travel long distances between premises.

Many horse movements cannot be avoided in the pursuit of the horses' function to their owners (Weese 2014). Social contact is essential for horse welfare (Hartmann et al. 2012; Yarnell et al. 2015), therefore allowing mixing of horses, at least eventually, is inevitable. Consequently, the main ways to avoid introduction of infection onto a premises are to minimise contact with infectious horses (whenever travel to other locations is inevitable), and to minimise the probability of an infectious horse coming into contact with the premises' resident herd.

For the latter purpose, horse owners can apply biosecurity measures at their premises. *Biosecurity* refers to measures used to prevent the entry of pathogens into a population (Weese 2014; USDA 2018), and can be split into *bio-exclusion*: preventing pathogen entry, and *bio-containment*: limiting pathogen transmission within the resident population and preventing onward spread from an infected population (FAO and United Nations 2010).

The importance of observing hygiene measures to address the impact of strangles on herds of horses was noted by Todd (1910) more than a century ago (Figure 6.1). Todd also noted at the time that implementation of measures to prevent disease transmission was often sub-optimal, and blamed a lack of motivation.

In the present day, outreach programs exist aiming to make horse owners aware of recommended practices to mitigate the risk of infectious disease to their horse or premises. Examples include the HBLB Codes of Practice (HBLB Code of Practice: Strangles n.d.), which are geared towards racehorse breeders in the UK, but are applicable for most horse owners, and "Strangles Awareness Week", a recurring annual international event aimed specifically at raising awareness among horse owners of steps they can take to avoid strangles, caused by *S. equi*.

Preventive Measures.

Owing to the facility with which horses recover from strangles, and the slow and uncertain manner in which it spreads in the ordinary way, preventive measures are rarely adopted. The breeder does not look upon it seriously, because very few die from it, and most animals on farms recover with no after effects. The sufferers are those who keep large numbers of young horses together at the time when they are first put into work. Here the loss is more from depreciation of value from unsoundness in the wind following on the disease than from actual mortality. Serious as this loss is, it is nearly always accepted as inevitable.

Figure 6.1: *An excerpt from Todd (1910).*

A number of surveys of biosecurity practices on equine premises have been reported in the last two decades: from New Zealand (Rogers and Cogger 2010; Rosanowski et al. 2012; Rosanowski et al. 2013a), Australia (Schemann et al. 2011; Schemann et al. 2013), the UK (Spence et al. n.d.), and the USA (Kirby et al. 2010; USDA 2016; USDA 2018). Outcomes vary, but the prevailing conclusion from these surveys, as was summarised in a recent narrative review (Crew et al. 2023), is that the measures are deficient. Most premises had no measures in place to reduce the infectious disease risk posed by the introduction of new horses, and of those that did, the measures described were insufficient for the prevention of the transmission of infectious disease. For more detail on biosecurity measures on equine premises around the globe, the reader is referred to (Crew et al. 2023). Whether the implementation of biosecurity at Dutch equine premises is similar to that in other countries is not currently known.

The aim of this study was to investigate current implementation of biosecurity practices aimed at bio-exclusion at equine premises in the Netherlands. This was a pilot study in a convenience sample of premises that were already participating in another cross-sectional survey study.

METHODS

We performed an in-person questionnaire survey of a cross-section of equine premises in the Netherlands and a descriptive analysis.
Horse owners or handlers who had agreed to participate in a study to investigate the prevalence of *S. equi* carriers (described in **Chapter 4**) were asked to answer a series of questions relating to their premises, on herd composition, activities, horse movements on and off premises, and measures implemented to prevent incursion of infectious agents. All questionnaires were taken in person as questions posed by one interviewer during a visit for the sampling described in **Chapter 4**. The questionnaire form is available in the Supplementary materials to the current Chapter (**6**), as Figure B.10. The questions in the questionnaire included

specifically: the number of horses on the premises, the premises' primary purpose (Residential, Boarding/training, Lessons/School, Breeding, Racing, Trading, or Other), the housing type (Group, Individual & Group, meaning turnout in groups for a substantial part of the day, Boxstalls in a common main barn, or Individual with no shared airspace), the travel of resident horses to single-day and multi-day equestrian events, and the measures that are in place when they return, the number of new residents that the premises takes in annually and the measures that are in place for them, and lastly, whether the premises had ever experienced a strangles outbreak, and if so, when. Responses were copied from the paper forms onto an Excel file within a week of the interview. Responses were tabulated, numeric responses assessed for normality by a Shapiro-Wilk test, and visualised using R (R Core Team 2021).

RESULTS

We visited 86 premises and a complete set of responses was available for 53 premises. Reasons for missing responses were either because of the horse handler's time constraints during the sampling visit, or the horse handler not being certain of the yard's history and/or policies. There were no instances where the horse handler declined to answer when queried.
An overview of the sizes and main purposes of the premises that were visited was presented in Figure 4.2 in **Chapter 4**. An overview of premises' main purposes, housing types, and quarantine policy for new arrivals is given in Table 6.1 and Figure 6.2.
The annual number of new arrivals, weekly number of single-day trips and annual number of multi-day trips were not normally distributed, due to the large number of "0" responses. The data remained non-normally distributed after log transformation.

Of the premises, two-thirds (58) had not experienced a strangles outbreak as far as the horse's handler was aware; no answer could be given for six premises. Twelve premises (14%) reported that an outbreak had occurred. These outbreaks had happened 6-240 (median: 60) months prior to the sampling visit.

Respondents for four (6%) of the premises visited reported routinely quarantining new arrivals for two weeks. None of these required ancillary testing (for example, for *S. equi* non-carrier status) before entry onto the general population at the end of quarantine. For at least one of these four, the isolation box was a boxstall in a main barn, which allowed for droplet transfer of infectious disease, and possibly fomite contamination and direct contact as well. In another, the isolation was in a pasture separate from resident horses (distance unknown). The isolation units for the remaining two respondents who reported routine isolation of new arrivals could not be assessed. There were ten additional premises (without routinely quarantining horses) who did have a quarantine unit; the appropriateness for purpose of these quarantine units was not evaluated.

Premises main purpose	Count	Percentage
Residence	33	38%
Boarding/training	31	36%
Lessons/school	14	16%
Breeding	4	5%
Racing	2	2%
Trade	1	1%
Other	1	1%
Total responses	86	100%

Housing	Count	Percentage
Individual and group	69	80%
Group	10	12%
Boxstall in barn	7	8%
Individual	0	0%
Total responses	86	100%

Quarantine of new arrivals	Count	Percentage
Yes	4	6%
Partial	10	14%
Sometimes	11	16%
None	34	49%
No arrivals; n.a.	11	16%
Total responses	67	81%

Table 6.1: *Premises' main purpose, housing type, and quarantine policy for new arrivals. Partial quarantine: horses are routinely quarantined, but the quarantine itself is sub-optimal. Sometimes quarantine: decision to quarantine new arrivals is ad hoc. n.a.: not applicable because the premises does not take in new arrivals.*

On 11 (16%) of the premises visited, routine measures for new arrivals were in place, but these were largely insufficient to prevent entry of infectious individuals onto the herd: examples include routinely avoiding direct contact (co-grazing) of new arrivals with pregnant mares but no quarantine of any kind; requiring quarantine, but only for horses brought on as riding school horses but not boarding clients, requiring clinical evaluation on the day of arrival (directly into the resident population) or shortly thereafter; requiring an up to date vaccination (usually only for equine influenza).

Eight of the visited premises did not routinely quarantine new arrivals, but did so if they felt they had reason to; if the premises of origin was suspicious (examples given were if they were boarded at a horse trader or if an infectious disease outbreak was in ongoing in the vicinity), or if they observed the horse to have clinical signs suggestive of infectious disease, such as nasal discharge, cough, or diarrhea.

None of the premises in the survey had routine measures in place for resident horses returning from events, neither for single-day nor multi-day trips. Some premises had custom hygiene guidelines, such as instructing clients not to let their horses share buckets with non-residents at event locations; or not allowing overnight stays at event locations.

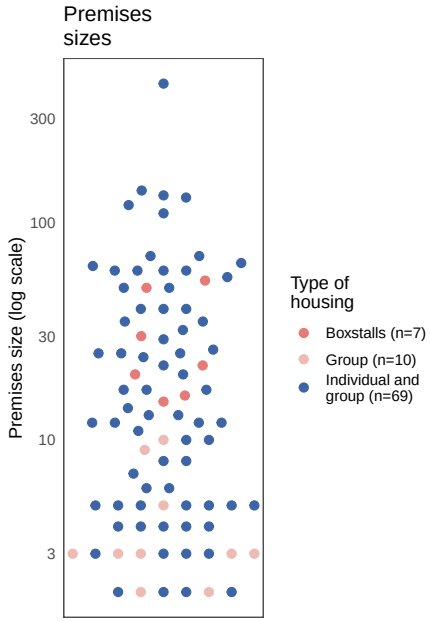


Figure 6.2: *Housing type and premises size.*

DISCUSSION

In our study, we found a limited adherence to biosecurity recommendations. The percentage of premises in our survey that had a quarantine unit was low (16%) and not all quarantine units were adequately located or built. In a USDA survey, two-thirds of premises had an isolation unit, but only just over half of those quarantine stalls were placed and built such that aerosol or droplet transmission was prevented (USDA 2018). In a 2018 UK postal survey, 52% of 708 respondents reported having a dedicated isolation facility, usually a designated paddock

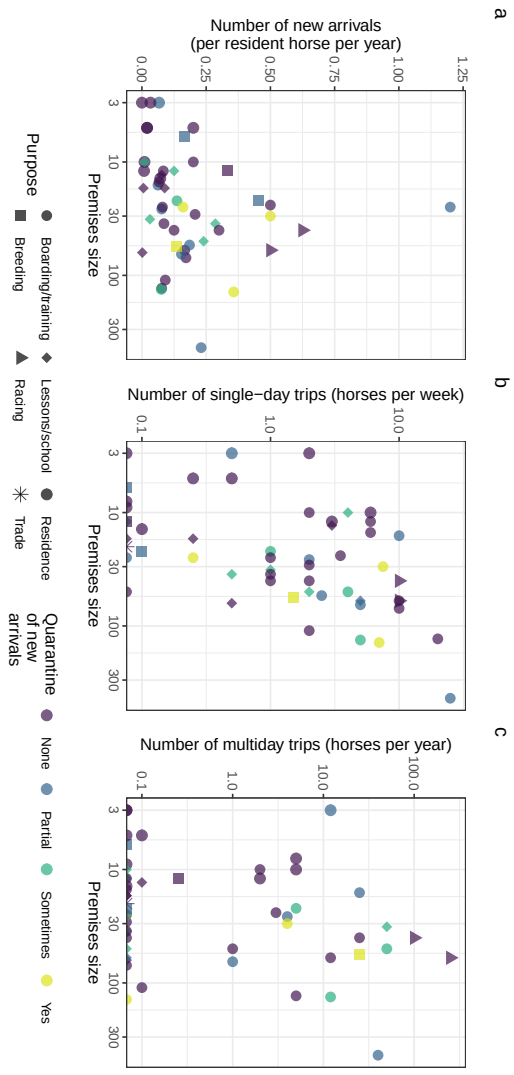


Figure 6.3: *Scatterplots of the sizes of premises and the annual number of new arrivals, normalised to the number of resident horses on the premises (a), weekly number of single-day trips (b), annual number of multi-day trips (c).*

or box, but as this was a postal survey, no information on the appropriateness of these facilities was available (Hodgkinson et al. 2018)

None of the respondents in our study implemented bio-exclusion measures sufficient to avoid incursion of *S. equi* as described in the Strangles Consensus Statement (Boyle et al. 2018). Only four respondents implemented a routine quarantine for new arrivals, which would serve as a barrier to entry of at least some infectious agents. None of our respondents implemented measures preventing outbreaks caused by a resident horse becoming infected after visiting a location with non-resident horses.

This limited adherence to biosecurity guidelines is not unique to Dutch premises. A USDA survey noted that one in five premises had a strategy for new arrivals categorised as "optimal"; this percentage differed between type of premises and was lowest (7.7%) in "boarding/training" premises (7.7%)(USDA 2018). Spence et al. (n.d.) described a "social norm" of lax biosecurity in their 2024 UK survey of 23 horse owners. Most premises where their respondents housed their horses had low-level or absent biosecurity measures, which they attributed to "complacency" on the one hand, and on the other hand a reluctance to harm horses' welfare by limiting opportunities for social contact, even temporarily. Weese (2014) described a high acceptance of infectious disease among young racehorse traders, citing a non-published survey among purchasers at a yearling auction where respondents indicated that they expected 80-100% of yearlings to fall ill after visiting an auction. In a 2015 US survey, 86% of premises never requested testing for carrier-free status, and 78% never implemented quarantine of new arrivals prior to contact with resident horses (USDA 2016). A survey of Australian horse owners revealed a strong correlation in the owner's believes in the effectiveness of their biosecurity measures and the quality of their biosecurity measures, suggesting that owners were aware that their measures were insufficient, but lacked the means or motivation to improve them (Schemann et al. 2011). A survey of stud farms in New Zealand identified the main reasons for not implementing measures: limitations of time and space, staff workload, and communication between staff (Rogers and Cogger 2010).

The number of trips which included multi-day stays outside of the home premises was small among our respondents. This is perhaps unsurprising, as the country is small enough to be able to travel back to the home premises for the night, even if the horse is participating in classes spread over more than one day. The two racing yards in the survey stood out, with a large number of multi-day trips. Combined with a high annual turnover of horses on the two racing premises in our sample, and a high number of single-day trips, this suggests that the risks of introduction of infectious disease onto racing stables may differ from the other premises types. Interestingly, a survey of threat perception (to the vulnerability of an outbreak) among horse owners in Australia found that operators of premises who were involved in horse racing perceived themselves to have particularly low levels of vulnerability (Schemann et al. 2013). However, due to the small number

of racing yards in our sample, the only conclusion that can be drawn here is that the movement patterns on and off racing stables in the Netherlands deserve separate attention as they may differ substantially from other types of premises.

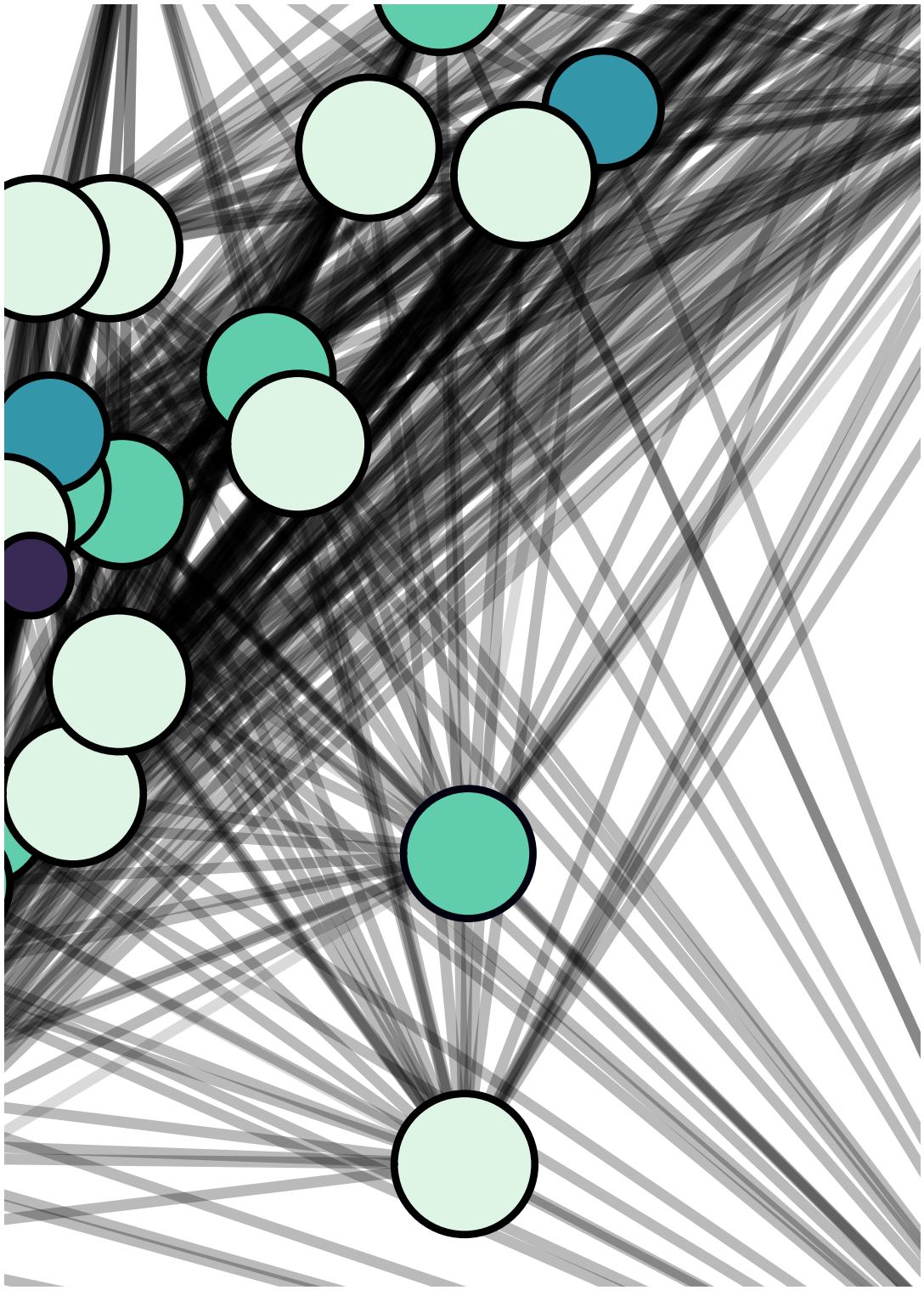
Multi-day events, where horses are stabled at the event site, are of particular interest, as being stabled in close proximity, or different horses occupying the same stable without disinfection in between, likely increases the potential for the transmission of infectious disease, compared to attendance at single-day events. Infectious agent shedding and transmission at multi-day events has received considerable research interest. In a study performed in the summer on one such event location over the span of several weeks, researchers found *S. equi* in 1/62 nasal swabs and 5/132 stall swabs; EHV-1 was not detected in that study (Pusterla et al. 2023a). A higher proportion of stall swabs was positive for equine respiratory pathogens during winter months, seemingly confirming that circulation of respiratory pathogens increases in the colder months; in this study, both *S. equi* and EHV-1 were detected on stall samples (Lawton et al. 2023). This apparent seasonality would imply that the risk of disease incursion onto a premises after participation in competition is higher in winter. Of note, the event at which this study was performed had already implemented reasonably rigorous biosecurity protocols to decrease transmission of infectious disease at the site, following an outbreak of EHM a year before. Overall, the number of stall swabs testing positive to a true pathogen were low. A common theme among these and other investigations (Pusterla et al. 2022b) is that the proportion of positive samples is higher for the environmental samples than for the horses. As it is usually only a subset of horses that undergo testing in these studies, a possible conclusion would be that the environmental swabbing detects shedding by horses that were not in the study sample.
Infectious agent transmission at single-day equestrian events has received less research attention, but it is not reasonable to assume that no transmission will take place there. Even if horses are not stabled together, they do share arenas and preparation spaces, may contaminate and touch surfaces, may come into direct contact, and may share tack, feed troughs and water buckets. Anecdotal evidence suggests that most equine owners are aware that sharing water buckets with non-familiar horses is not recommended, but awareness of other opportunities for the transmission of infectious agents may not be as pervasive, although no data on this currently exists for the Netherlands.

This was a survey in a convenience sample of horse owners and handlers who had already agreed to participate in another cross-sectional prevalence study (**Chapter 4**) for which most participants had been nominated by their veterinarian. Moreover, the survey required intrinsic motivation and time commitment from the owners. As such, the study sample was biased towards owners who seek veterinary care, at least occasionally, and who probably also have an active interest in equine health issues. Since only adult horses were eligible for the prevalence study, premises with predominantly or exclusively young horses were not included. The sample size for this questionnaire survey was dictated by the sample size required

for the research question of the prevalence study. The survey questionnaire was not trialled and validated prior to application in this study; a short set of relevant questions was prioritised due to time constraints imposed by the prevalence study. Respondents in this survey were not prompted to explain why they did not implement (more stringent) measures to prevent infectious agent incursion, so motivations and attitudes towards biosecurity remain unknown. Since this was part of a project focusing mainly on respiratory infections, de-worming requirements prior to entry into the herd and insect control measures were not assessed, therefore these results provide no information on infectious diseases caused by or transmitted by macro-parasites.
Future work could be undertaken with a larger sample size, a sample which was more inclusive of all types of premises, including those that were absent or nearly absent here, such as horse traders and young-stock raising ("opfok") premises. Ideally, a follow-up study should be designed to examine correlations between type of premises, size of premises, frequency trips, and implementation of biosecurity, to better estimate which premises are most at risk, and where the most can be gained through behaviour changes.
Future work could focus on the quality and completeness of the survey questionnaire and follow guidelines proposed by (Kirby et al. 2010), and in addition pilot test and validate the questionnaire prior to deployment, and include an investigation into the beliefs and motivations underlying the implementation of biosecurity practices.

CONCLUSION

Most premises had no policy for bio-exclusion, and of those that did implement quarantine, none had a quarantining strategy that could be considered optimal; either because the layout of the quarantining area would not prevent droplet or even nose-to-nose transmission, or because the selection of horses that required quarantine before being (re-)introduced to the resident herd was too narrow. None of the respondents had any policy for horses returning from events where they had mixed with non-resident horses. The results from this questionnaire survey of a cross-sectional sample of equine premises suggest that implementation of biosecurity measures at Dutch equine premises is limited, which is on par with reports from other countries. This pilot study has also highlighted key areas for future investigations, such as focus on specific high-risk premises types and on motivational factors.



Chapter 7

FIVE DEGREES OF SEPARATION

Rosa Houben, Kees van Maanen, Marianne Sloet, Hans Heesterbeek: Five degrees of separation – the contact network of horses in competitions in the Netherlands
Work in progress

Abstract

Background Dynamics of infectious diseases are influenced by population contact structure. Limited data is available on horse contact networks. **Objectives** To describe the contact network of horses participating in sports or racing competitions in the Netherlands, and to compare static and dynamic representations. **Study design** Network census. **Methods** Participation records from the Royal Dutch Equestrian Sports Organisation and Dutch racing records for all of 2022 were made available upon request. Four networks were analysed: sport horses and racehorses, with horses as nodes and presence at the same event as edges; and sports locations and racing locations, with locations as nodes and travel of horses from one event to the next as directed edges. Annual static and temporal network metrics were calculated. **Results** The sport horse network was the largest network, with 41018 nodes, its diameter (highest number of steps in the shortest path between any two nodes) was five, and the network had "small world" properties, a topology that is favourable for spreading of infectious disease. All static annual networks were fully (strongly) connected. The connectedness of the networks was robust to targeted node removal, except for the racing locations network. The temporal reach distribution of nodes suggested that static representations of the networks overestimated the network connectedness. **Main Limitations** Lack of information on contacts on the horses' home premises. **Conclusions** The Dutch equestrian competition network is highly connected. Since 4/5 Dutch premises house at least one horse that participates in competitions at least occasionally, this connectedness affects most if not all Dutch horses. Targeting high-risk horses or locations for preventive measures may not be equally effective in all networks.

INTRODUCTION

In **Chapters 2**, **3** and **5**, we focused on disease transmission in herds or populations where individual horses were assumed to mix randomly. In reality, horses will experience a combination of a local contact structure in their own herd and contacts in a network structure with individuals from other herds during sporting and other events. This chapter will focus on the other extreme: real-world contact networks. Insight into the contact network contributes to understanding of observed dynamics of disease spread by outlining who is most at risk of infection, can inform who should be targeted for interventions, and can help evaluate the expected efficacy of mitigating measures (Wallinga et al. 1999). The structure of a network has great influence on the tipping point when infectiousness of a disease becomes sufficient to infect the majority of a population (Watts 2003). The use of network modelling for infectious diseases of veterinary importance was encouraged by the WOAH (Dubé et al. 2011).

Preventive measures that are attainable in farm animal husbandry, such as closed herds, all-in-all-out systems, and one-directional production chains (Lentz et al. 2016), when applied to equine premises may cause too much disruption to the purpose for which an owner wants to use their horse. As we demonstrated in **Chapter 6**, there are few barriers to entry of infectious animals at most premises, in the Netherlands as well as elsewhere. Although it is certainly worthwhile to try to initiate a behavioural change to combat some of the observed high-risk bio-exclusion policies, the equine sector will never be amenable to measures as stringent as those that are in place in the livestock sector (Weese 2014). If there is no or little barrier to entry of horses onto a premises, the contact network of horses outside of their home premises is highly relevant, as infections acquired outside can easily enter the home premises through unimpeded entry of horses. Because the contact network structure of horses likely differs substantially from that of livestock, measures that are effective in livestock husbandry may not be as effective in horse husbandry settings, or are simply not possible (Weese 2014). An understanding of the movement patterns of horses can improve predictions on the effectiveness of various mitigating measures for equine infectious diseases.

Contact networks based on animal movements have received substantial interest (Danon et al. 2011); a recent overview of the use of contact network modelling in livestock species is available (Leung et al. 2022).
Several options for establishing animal contact networks have been applied to investigate equine contact patterns. The option most often used in equine research has been to construct the networks from questionnaires or diary-based data, typically on contacts outside of the home premises (Hayama et al. 2012; Rosanowski et al. 2013a; Rosanowski et al. 2013b; Rosanowski et al. 2015; Spence et al. 2017; Spence et al. 2018a). A limitation of this so-called egocentric sampling is that it is impossible to know which unknown individuals are links between the sampled individuals, as there is no common identifier (Figure 7.1). These links then need to be randomly assigned (Danon et al. 2011).

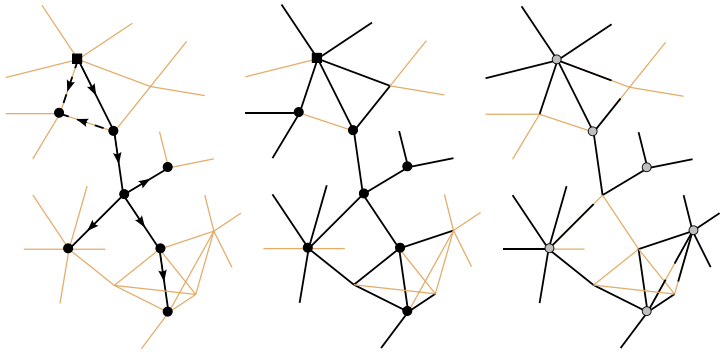


Figure 7.1: *An illustration of methods for determining a network. The underlying network (orange lines) is the same, the black lines indicate which links are known. Left: Infection tracing. Middle: contact tracing. Right: Diary-based (egocentric) sampling. Note in particular, in the right figure, the difficulty in connecting the different diarists due to the lack of a common identifier for the nodes. From: Keeling and Eames (2005).*

Nevertheless, questionnaire-based contact data has been used successfully to model infectious disease dynamics in humans (Mossong et al. 2008; Kretzschmar and Mikolajczyk 2009; Dekker et al. 2024).

Another option is the use of proximity sensors, which has the advantage that it provides a high level of detail of individual behaviour and contact patterns (Danon et al. 2011). This method has been applied to analyse the contacts of horses within medium-sized equine premises (Milwid et al. 2019b). A limitation of this method of establishing a contact network is that for larger groups of subjects, cost and logistic constraints become prohibitive.

The third option that has been employed for animal networks is the use of movement data, when a database of recorded movements exists, for example because it is routinely being collected by government or other regulatory bodies (Danon et al. 2011). In horses, this method has been implemented by using Standardbred racing starts (Brown et al. 2022), and recorded horse movements from Identification & Registration databases for regions in Brazil (Cárdenas et al. 2019) and Spain (Sánchez-Matamoros et al. 2013). This approach resolves the issue of the missing common identifier from the questionnaire method, but is mostly applied to species and settings where a tracking system is already in place. A limitation of this method is that non-recorded movements (those that do not require reporting, such as short trips or stays), or those that are omitted from reporting, either by mistake or intentionally, are missing from the resulting

network. A full overview of published equine contact network studies is presented in Table 7.1.

Table 7.1: *Equine contact network publications*

Reference	Location	Methods and outcome metrics
Hayama et al. 2010	Japan	Questionnaire survey in non-racing horse premises operators, descriptives of replies.
Hayama et al. 2012	Japan	Scenarios of EIA surveillance with individual-based stochastic modelling, with contact patterns from (Hayama et al. 2010).
Sánchez-Matamoros et al. 2013	Leon and Castille region, Spain	Livestock tracing scheme entries for horse movements, sliding window approach with static network slices.
Rosanowski et al. 2013b; Rosanowski et al. 2013a	New Zealand	Questionnaire survey of movements on racing and non-commercial premises, descriptives of replies.
Rosanowski et al. 2015	New Zealand	Racing records sample, descriptives of replies.
Spence et al. 2017	Canada	Questionnaire survey of 55/69 horses after single equestrian sporting event in Ontario. Static two-mode (horses and locations) and single-mode (horses) network statistics.
Spence et al. 2018b	Canada	Agent-based stochastic simulation. Using network described in (Spence et al. 2017); predicting number of influenza infections.
Spence et al. 2018a & (Spence et al. 2019)	Canada	Diary survey of 330 horse owners ; tally of movements and purpose of movement. Construction of network from diary entries with locations as nodes.
Milwid et al. 2019a; Milwid et al. 2019b	Canada	Within-yard contact patterns using proximity sensors, within yard outbreak simulations (influenza). Static slices of one day and of one week, then turned into dynamic network of one week with time-steps of one day.
Brown et al. 2022	North America	Standardbred racing starts census. Monthly and annual static networks statistics, with locations as nodes.
Cárdenas et al. 2019; Cardenas et al. 2022	Rio Grande do Sul, Brazil	Livestock tracing scheme entries for horse movements. Spatiotemporal network of locations and municipalities as nodes, but focus on spatial, monthly and annual network statistics. Also contact tracing networks of glanders cases.

An important aspect of the study of contact networks, in particular when used to simulate the spread of an infectious disease, is whether to use a static network representation, or to incorporate the timing of contacts (if such information is available) and establish a temporal (or dynamic) instead of a static network representation. When the timing of events is taken into account in a temporal network instead of "collapsing" the network into a static network, only the pathways that are truly possible remain (Figure 7.2; Dekker et al. 2022).

Simulations of the spread of foot-and-mouth disease over both a static and a temporal network demonstrated that a static model, where the sequence of

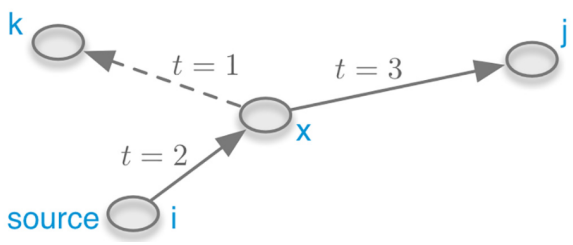


Figure 7.2: *Causal path between nodes in a temporal network. Suppose node i is the source of transmission to node x. Because the contact between node x and node k happens at t=1, so before the contact between i and x at t=2, there is no path $i \to k$ in the temporal network and hence x cannot infect node k. However, if the temporal network is collapsed to a static network and the sequence of events is ignored, then the path $i \to k$ does become available and x can infect k. From: Lentz et al. (2016)*

(contact) events is not taken into account, gives different results compared to a temporal model. Vernon and Keeling (2009) evaluated fully dynamic, static, as well as intermediate representations of the cattle movement network in the UK, and found that none of the alternative network implementations correctly captured epidemic behaviour under a range of assumptions for infectious period duration and transmission probability; for example, for shorter infectious period assumptions, the outbreaks simulated over the temporal network were smaller than the collapsed static networks, but as the infectious period increased, the effect was reversed.

Simulations on a static and temporal representation of the pig trade network in Germany similarly concluded that the temporal model was preferable. Lentz et al. (2016) calculated that 26% of the pathways that existed in a collapsed static network did not exist in the temporal network, and further concluded that static representations overestimated outbreak sizes in their network simulations by 35%.

Others have argued that using a static projection of a temporal network can be appropriate, if the duration of incubation and of infectivity is clearly larger than the time period that is being collapsed, and as long as it is accepted that a worst-case scenario is being evaluated by the overestimated connectivity of the static network (Eubank et al. 2004). Static networks do have the advantage that calculation of node centrality measures is straightforward, and centrality measures can help identify high-risk nodes. When nodes with a high centrality score are targeted, fragmentation of the network may be achieved with the removal of a much smaller number of nodes, compared to when nodes are randomly removed (Lentz et al. 2016).

Apart from the work done by Milwid et al. (2019a), who examined a temporal

network of within-yard contacts, temporal contact networks of equine populations have not been applied in equine infectious disease research, as far as we are aware.

Our study aims to provide an initial descriptive exploration of the contact network of horses participating in competitions in the Netherlands, by using event participation data of all sports and racing competitive events in 2022. In future studies we aim to investigate the spread of infectious diseases across such networks.

METHODS

The network census data that were described in **Chapter 4**, are further analysed and described here. In addition to the metrics calculated for the horse-based annual networks provided in **Chapter 4**, we will calculate metrics for the static monthly and annual horse-based networks, similar to previous equine-focused publications (Brown et al. 2022; Spence et al. 2019). Monthly slices of the horse-based static networks were constructed, and network metrics were calculated with the package *igraph* (Csardi and Nepusz 2006) in R (R Core Team 2021). For the dynamic horse-based networks, the day of the event where the edge occurred was recorded. In this chapter we will also analyse locations-based networks for both competition categories. A directed edge between two locations was defined as a horse attending an event at one location (origin node) first, and then the next event attendance of that horse was the target location node. For the dynamic location-based networks, the day the horse attended the target location was taken as the day of contact. This network was simplified to remove loops (caused by a horse attending an event at the same location consecutively) prior to analysis.

The static network metrics first described in Table 4.1 are extended with additional metrics (Table 7.2).
In addition to the aforementioned metrics, for the locations networks, which are directed networks, mean In- and Out-Degree , whether they were strongly and weakly connected, and the size of the giant strong and weak component was recorded. A network is strongly connected if all nodes can be reached from all other nodes while observing the direction of the edges; in a weakly connected network, all nodes can be reached from any node in the network only if the direction of the edges is ignored.

Metrics were also calculated per month, as monthly static "slices", and their change over time was visualised. For the locations networks, the monthly static statistics were not calculated on isolated slices (as with the horse-based networks), but for 28-day rolling aggregates, with time-steps of one day, using the R (R Core Team 2021) package tsna (Bender-deMoll and Morris 2021).

The node Degree distributions for the annual horse-based and locations-based

Term	Definition
Node	Unit of interest; horses and event locations.
Edge	Connection between units of interest.
	Graph level metrics
Network size	Number of nodes in the network.
Connected	Are all nodes reachable from all other nodes? In a directed network, a distinction is made: "strong" (connected when the direction of the connection is respected) *vs* "weak" (only connected if the direction is ignored) .
Edge density	The ratio of the number of edges to the number of possible edges.
Transitivity	Probability that the adjacent nodes of a node are connected (also known as the clustering coefficient)
Diameter	The greatest number of links in the shortest path between any two nodes.
Mean distance	Shortest path between two nodes, averaged over all pairs of nodes in the network. In the directed locations networks, the direction of the connection is respected.
Centralisation	Graph-level centrality score based on node-level degree centrality
Degree assortativity	Network homophyly - do nodes preferentially connect to nodes with a similar degree? If positive, vertices with similar degrees tend to connect to each other, and vice versa.
Power-law fit test	Does the distribution of node degrees fit a power-law distribution? A network in which the out-degree and in-degree distributions fit a power law distribution is a scale-free network. Tested with MLE with detection of the optimal lower bound (*xmin*): the value for which the p-value of a Kolmogorov-Smirnov test between the fitted distribution and the original sample is the largest.
Giant component	A connected subsection of nodes that form a connected component of the order of magnitude of the network size. For directed graphs, a distinction between a strong and weak giant component is made.
	Node level metrics
Betweennes	The number of shortest paths going through a node.
Degree	The number of connections per node. For the directed network, the distinction between in-degree and out-degree is made.
Reach	Temporal network only: the number of nodes that can be reached from each node, when respecting both the timing and the direction (if the graph is directed) of edges.

Table 7.2: *Definition of network terms (Dubé et al. 2011; Csardi and Nepusz 2006; Martínez-López et al. 2009).*

networks were evaluated for the probability of following a power-law distribution (a requirement for scale-free networks). Erdös-Rényi random graphs with the same number of nodes and edges (Erdös and Rényi 1959) were constructed for each of the networks, and the Degree distribution, transitivity, mean distance and diameter of the random graphs was recorded and compared to the real-world graphs.

The robustness of the four annual static networks to targeted node removal was evaluated by calculating the node Betweenness score for the full network, followed by stepwise removal of the node with the highest Betweenness centrality, and recalculating the remaining network's giant (strong) component (Albert et al. 2000; Dezs and Barabási 2002). For the sport horse network, instead of node-by-node removal, the top one percent of nodes of the original network size was removed incrementally.
Finally, networks were analysed as temporal networks with discrete time-steps of one day. For the horse-based temporal network, the time of contact was recorded as the day that the two horses were present at the same event. For the locations network, the day of arrival from another location was recorded as the day of contact. For the temporal networks, the distribution of Reach, or the forward reachable set; i.e., the number of nodes that can be reached from each node taken as a starting point, when respecting the sequence of contacts, was recorded (R package tsna Bender-deMoll and Morris 2021.

RESULTS

7

The network input data

An overview of the input data was provided in **Chapter 4**. Table 7.3 summarizes the number of horses, locations, days, and entries in the two datasets.
An overview of the distribution of event participation throughout the year is provided in Figure 7.3.
Based on the limited data spanning only one year, the data show some features. Sporting events take place on most days of the week, but clearly much more on the weekends and national holidays. The sporting calendar shows a more year-round activity with higher intensity in the periods May-June and November-December. There are overall dips in sports event participation around the new year and during the summer school holidays in August. From the data of the racing calendar, we see that most days are without races; racing events took place on 104 days in 2022. The horse racing calendar suggests a more obvious one-peak seasonal pattern than the sports events, with the majority of races happening in May-September, and without the dip in events participation in August that occurs in the sports calendar.

	Sports	Racing
Locations	721	33
Classic		8
Street		25
Horses	41,081	1,089[1]
Classic		1,065
Street		85
Entries	353,883	6112
Classic		5709
Street		403
Event days	340	104[2]
Classic		81
Street		25

Table 7.3: *Number of horses, locations, entries, and event dates.* [1] *61 horses were entered in both classic and street races.* [2] *There were two days where both a classic and a street race event took place on the same day.*

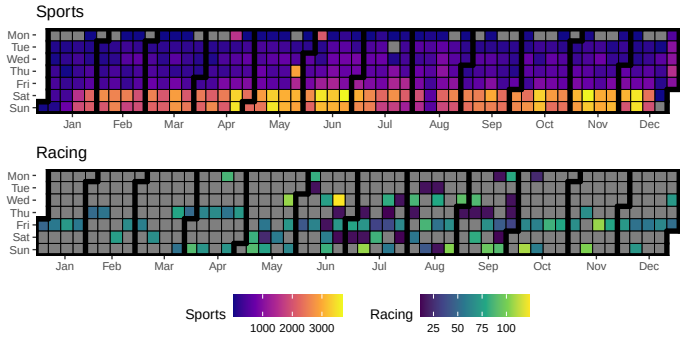


Figure 7.3: *Calendar heatmap for number of starts per day for the sports and racing networks. Note that each calendar has its own colour scale, because the difference in number of starts per day is several orders of magnitude. Grey block: no events that day.*

Static network analysis

Annual network The locations-based annual static metrics are presented in Table 7.4. In Table 7.5, metrics from same-sized random graphs are provided.

Metric	Sports locations	Racing locations
Network size	721	33
No. edges	312,832, simplified: 37,740	5023, simplified: 211
Connected?	Yes, Weak & Strong	Yes, Weak & Strong
Edge density	0.073	0.20
Transitivity	0.37	0.51
Diameter	4	3
Mean distance	2.2	2.1
Degree: median (min – max)	80 (4-796	10 (6-32)
In-degree: median (min – max)	40 (1-332)	5 (3-15)
Out-degree: median (min – max)	40 (2-464)	5 (2-17)
Centralisation	0.48	0.31
Degree assortativity	-0.029	-0.081
Power-law fit	$xmin = 291$	$xmin$ =9
	$\alpha = 5.6$	α =3.3
	$KSp = 0.99$	KSp =0.97
Metric	**Sport horses**	**Racehorses**
Network size	41,081	1,089
No. edges	10,167,944	122,988
Connected?	Yes	Yes
Edge density	0.012	0.42
Transitivity	0.32	0.49
Diameter	5	3
Mean distance	2.6	1.8
Degree: median (min, max)	272 (2 - 4,155)	378 (28 - 1,714)
Centralisation	0.18	1.16
Degree assortativity	0.35	-0.095
Power-law fit	$xmin = 2533$	$xmin = 498$
	$\alpha = 10.1$	$\alpha = 9.3$
	$KSp = 0.33$	$KSp = 0.85$

Table 7.4: *Descriptive statistics of the static networks for the entire year of 2022. KSp: Kolmogorov-Smirnov-test p-value*

The node Degree distributions for the annual networks, and the distributions for their companion random graphs, are shown in Figure 7.4. Locations In- and Out-Degrees are presented in Figure 7.5.

According to the criteria by Broido and Clauset (2019), for the racing locations network there is "weakest" category evidence for being a scale-free network, and for the remaining three networks there is "weak" evidence; the racing locations network is too small to meet the criterion that the power-law region contains ≥ 50 nodes. None of the network Degree distributions resembled that of an Erdös-Rényi random graph of the same number of nodes and edges (Figure 7.4)

The distribution of node Betweenness for the locations networks is displayed in Figure 7.6. The distributions seem largely similar-shaped for all four networks,

Metric	Sport horses random graph	Racehorses random graph	Sports locations random graph	Racing locations random graph
Transitivity	0.012	0.21	0.14	0.37
Mean distance	2.0	1.8	1.9	2.0
Diameter	3	2	3	4

Table 7.5: *Graph metrics for Erdös-Rényi random graphs with the same number of nodes and edges as the observed networks.*

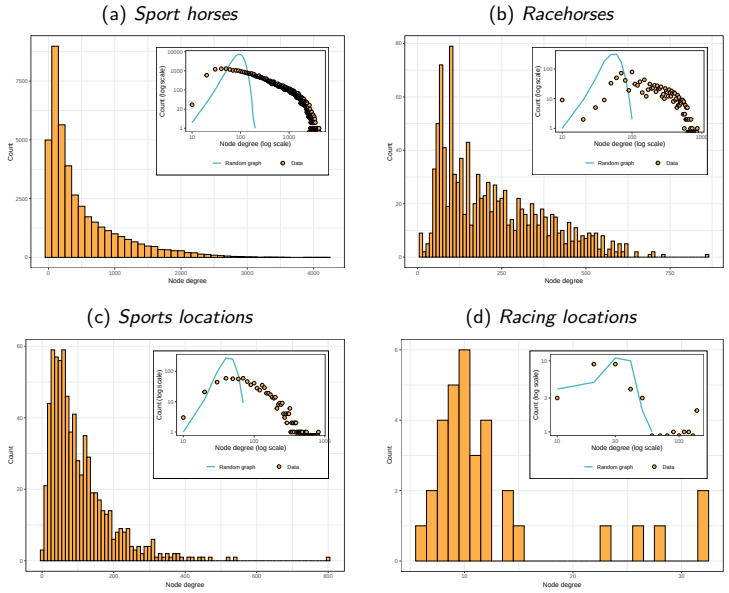


Figure 7.4: *Main: Annual network node Degree distributions. Inset: Log-log plot of the same distributions (dots) overlaying the node distribution from an Erdös-Rényi random graph with the same number of nodes and edges (teal line). NB: The Main images from (a) and (b) were previously shown in* ***Chapter 4*** *as Figure 4.5.*

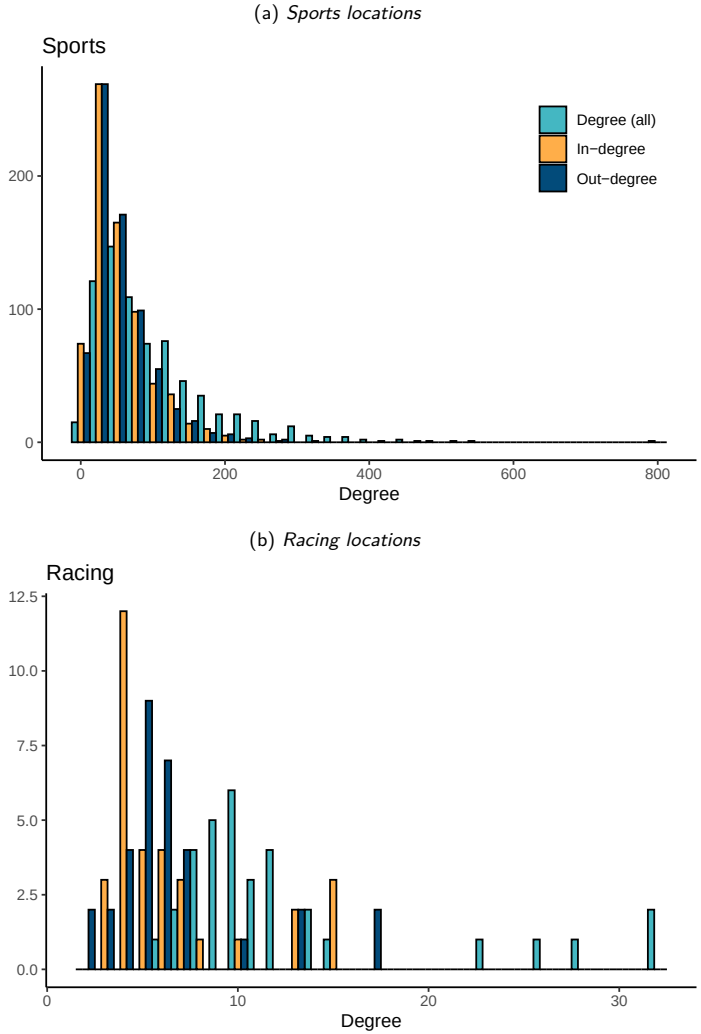


Figure 7.5: *Degree distribution (in, out, and overall) of the sports (a) and of the racing (b) static locations networks.*

although the horse networks have a more left-tailed shape, indicating that a subset of nodes with very low betweenness occurs in the horse-based, but not in the locations-based networks. Horses owners may choose to attend only few and maybe smaller events, and therefore have a very low Betweennes score. It makes little sense, however, to purposely organise an event with very little attendees (who can then move onto other events), which explains why there would be very few locations with an exceptionally low Betweennes score.

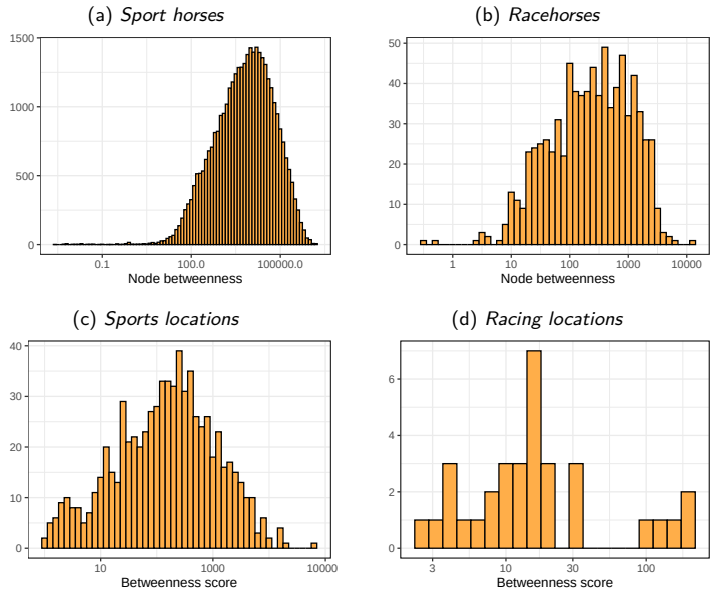


Figure 7.6: *Distribution of node Betweenness for the sport horse (a), the racehorse (b), and for the sports (c) and racing (d) locations static annual networks.*

Figure 7.7 demonstrates how many of the network nodes would need to be removed from the network before fragmentation is achieved and a giant component is no longer present, if nodes are targeted for removal by their Betweenness centrality score. To achieve fragmentation, $2/3$ to $3/4$ of all nodes in the network would need to be removed, with the exception of the racing locations network which fragments as soon as the four main racetracks are removed.

Monthly networks The monthly static metrics are presented in Figures 7.8, 7.9, 7.10 and 7.11.
The median Degree for the horse-based networks does not change much during the year, and is similar for racehorses and sport horses; the range of node Degrees

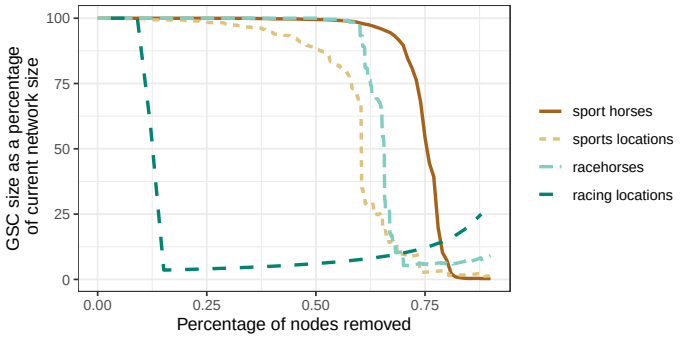


Figure 7.7: *Robustness of the annual static networks to targeted node removal. Nodes with the highest Betweenness score were removed incrementally. The y–axis shows the size of the giant (strong) component (GSC) in the remaining network as a percentage of the total remaining network size.*

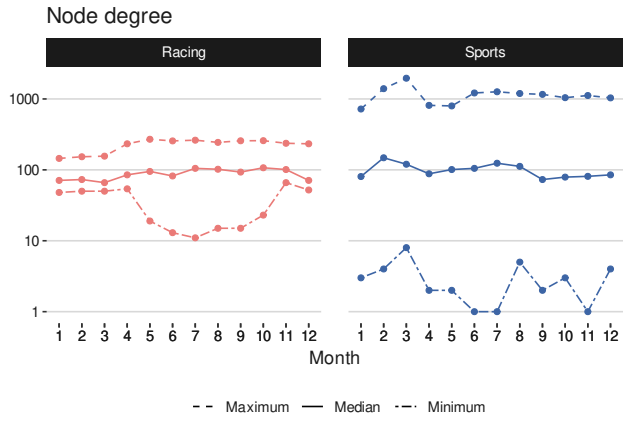


Figure 7.8: *Monthly static Degrees for the horse-based networks.*

is much larger for the sport horse network however. The minimum Degree for the racehorses decreases during the summer season, coincident with the timing of the short-track street races in which only small number of horses participate on any given day.
Overall, the racehorse network is smaller than the sport horse network by several orders of magnitude in both size and number of edges, and seems more connected, with a lower diameter and mean distance, and higher density and transitivity. During the summer months, the direction of change for the diameter and mean distance is opposite for the racehorses *vs* the sport horses, despite both having more nodes and more edges during that time. This can likely be ascribed again to the timing of the short track street races, as described above, as well as the fact that during the summer months, more sports events take place that are held outdoors, which can accommodate larger numbers of participants per day.
The locations edge counts over the year follow the pattern encountered in Figure 7.3 and Figure 7.9; a single peak over the summer months for racing, and two peaks on either side of august for the sports networks. a similar pattern is observed in Figure 7.11 where the 28-day aggregate static measures for the locations networks are displayed.

Temporal network analysis

The distribution of Reach distributions for the temporal networks are displayed in Figure 7.12a&b.

Locations The median Reach for the locations-based networks was 706 (range: 29-721) for the sports locations, and 22 (range: 4-33) for the racing locations. The one sports location with the singularly low Reach of 29 was a smaller event (60 participants) held mid-December.

Horses The median Reach for the horse-based racing network was 939 (range: 53 - 1,089). The distribution is presented in Figure 7.12c.

Figures 7.12a&c suggest a highly connected temporal network, where the majority of other nodes can be reached from most starting nodes. however, for all three networks displayed in Figure 7.12, nodes exists from which only a minor subsection of the full network is reachable.
The Reach distribution as well as the aggregated 28-day network metrics (Figure 7.12 and) for the racehorse network look different from the remaining three networks, but this is explainable. Only one track ("Wolvega") is open the entire year, four tracks in total are open for a large part of the year. Of the total 1,089 horses in the racing dataset, 864 visited "Wolvega" at least once. Four additional summer-only classic tracks, are opened during the favourable weather seasons. The street races are likewise held from May until the beginning of October. Significantly, the 27 street tracks are each open only one day for one single event, with 16-24 horses attending each event. This explains how the

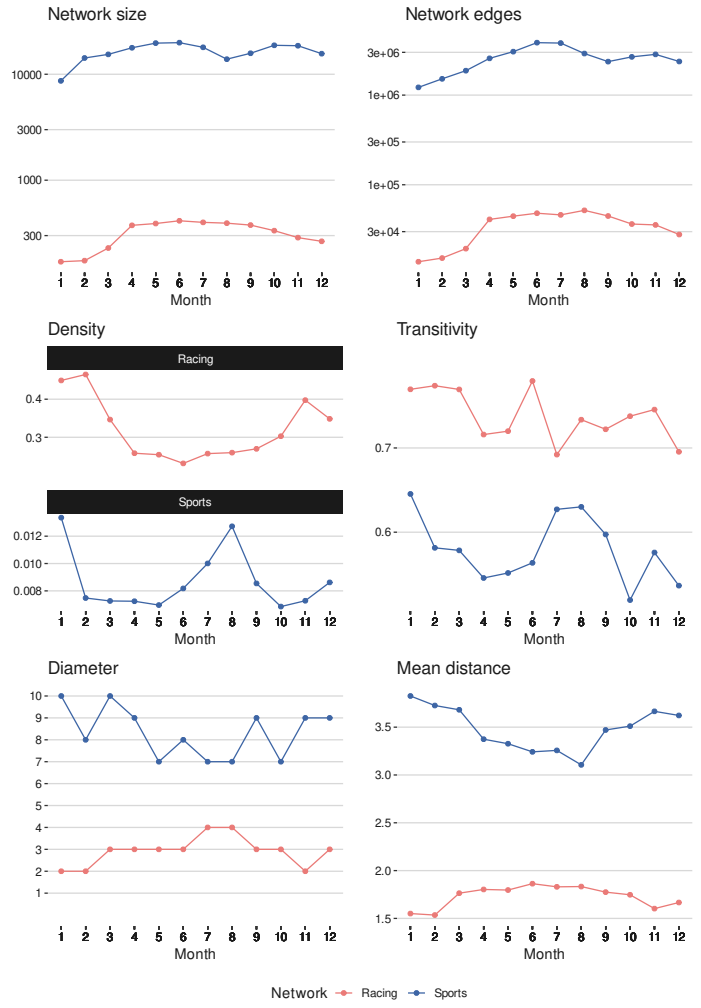


Figure 7.9: *Monthly static metrics for the horse-based networks.*

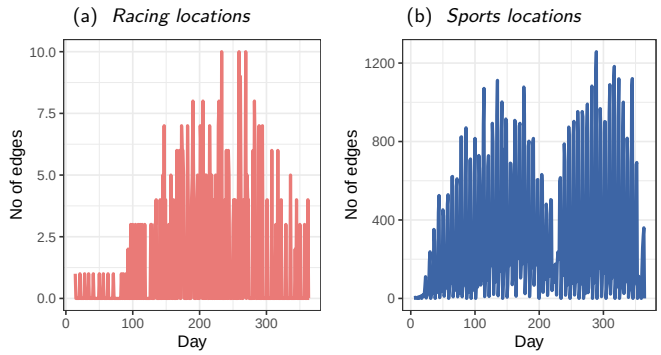


Figure 7.10: *28-day aggregate edge counts for the locations networks.*

Reach distribution shows four locations that reach the entire network, which are likely the four racetracks that are open all year. Then there are sets of one or two smaller locations that have incrementally greater Reach, which are likely the smaller tracks that host only one event each; tracks that have events early in the year likely having great Reach than those late in the year, as horses have less opportunities left to travel to other locations. Nodes to the left of the graph are likely the sites with events late in the year.

DISCUSSION

We explored the contact networks of horses in the Netherlands at events. We have demonstrated that the contact networks of horses participating in sports or racing competitions in the Netherlands are highly connected. In the static annual networks, there were no isolates and both the racing and the sports network consisted of a single strong component. In addition to the short diameters that were calculated for the static annual networks in **Chapter 4** (five for the sport horses, three for the racehorses), the diameters for the locations-based static networks calculated here were equally short (four and three, respectively).

In their 2019 review, Broido and Clauset (2019) concluded that most social networks are at best "weakly" scale-free. Of the four networks that were examined in this study (racehorses, racing locations, sport horses, and sports locations), all but the racing locations network fitted in this category. Networks with a scale-free node distribution typically are not robust to selective targeting of nodes of high centrality for removal (Dezs and Barabási 2002). Most social networks are Degree assortative (Newman 2003). In our study, the sport horse network was Degree assortative, indicating that horses with a high number of contacts tend to connect to other horses with a high number of contacts. A high assortativity influences

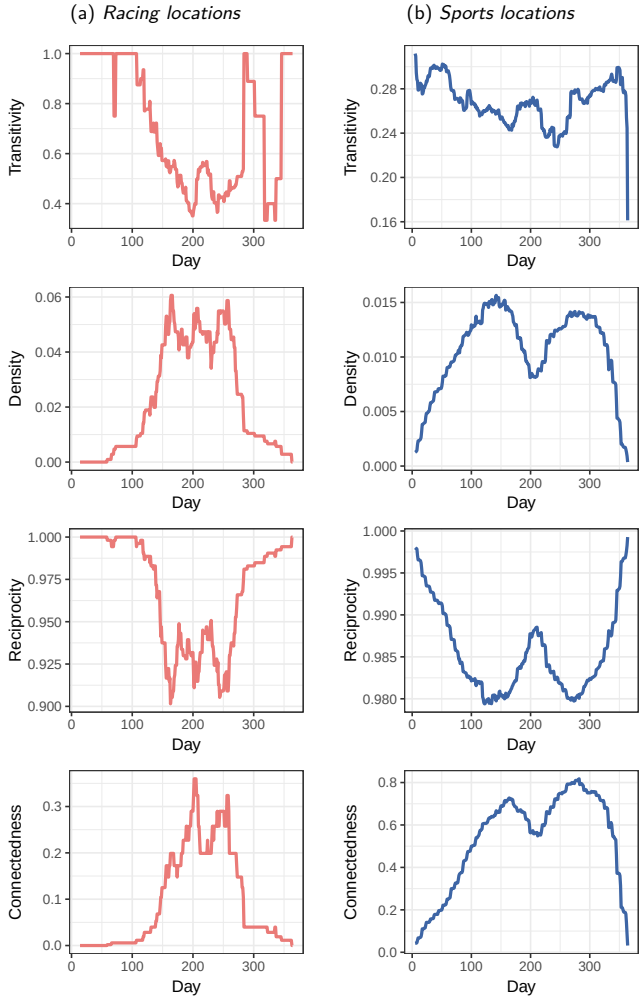
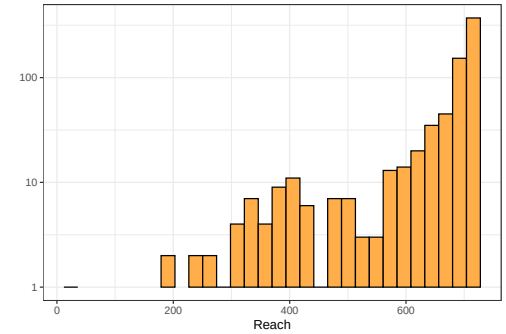
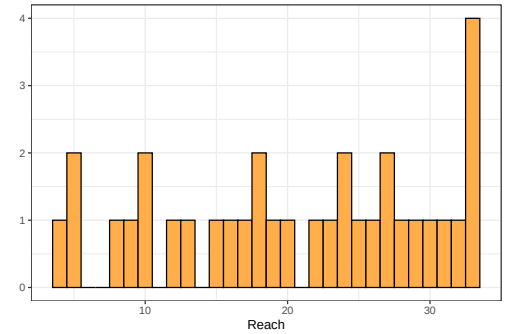


Figure 7.11: *28-day aggregate metrics for the locations networks.*

(a) *Sports locations Reach distribution*



(b) *Racing locations Reach distribution*



(c) *Racehorses Reach distribution*

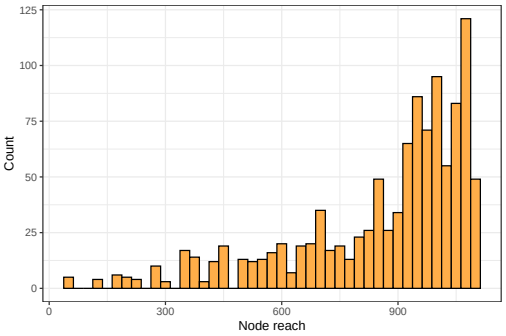


Figure 7.12: *Distribution of Reach (the number of other nodes that can be reached, per node), for the sports (a) and for the racing (b) temporal location-based networks, and for the racing horse-based temporal network (c). NB: Graph (a) has a log-transformed y scale.*

the resilience of the network; more assortatively mixed networks are more robust against the targeted removal of high-centrality nodes (Newman 2003).
The robustness of the Dutch equine competition networks to targeted node removal is apparent in Figure 7.7. To fragment the sport horse network, $\approx 3/4$ of nodes need to be removed. For the racehorse and sport locations network it is $\approx 2/3$. The distribution of node Betweenness scores (Figure 7.6a–c) partly predicted this: these were not at all right-skewed distributions, indicating that it is not only a few nodes with high Betweenness holding the giant component together for the sport horses, sports locations, and racehorse networks. For the racing locations (Figure 7.6d), it is evident that removal of the four permanent racetracks suffices to fragment the network.

All networks had a mean distance similar to the mean distance of a random network of equal size. Clustering in the observed graph was an order of magnitude higher than in the random graph for the sport horse network, and larger by a factor ≈2 for the racehorse and sports locations network. The sport horse network in particular can be said to have small world properties, which can have facilitated the spread of infectious diseases (Watts 2003).

The racehorse network has a greater edge density than the sport horse network and had a shorter diameter and mean distance. That, in combination with the relatively frequent trips that racing premises operators reported in our biosecurity survey sample in **Chapter 6**, suggests that the racehorse network could be more vulnerable to spread of infectious disease than the sport horse network. Anecdotally, the mean age of racehorses in active competition is significantly lower than that of sport horses, which would imply a larger proportion of susceptible horses in racing. Differences in the nature of the competition might also contribute to increased chances of disease spread in racehorses. In sports competitions, a horse is usually in the competition arena on its own, and may share the warm-up arena with a handful of other horses, whereas racing is done in groups of horses in close proximity at the same time.
Data on the mixing patterns of horses attending the same event on the same day is not available and could be a future research aim.

Given the relatively small size of the racehorse network, it is questionable whether racehorses have much impact on infectious disease dynamics in the Netherlands. As discussed in **Chapter 4**, we were unable to test for overlap or communication between the two networks.

Previously, a census-based network of horse movements in a part of Spain (Sánchez-Matamoros et al. 2013) and Brazil (Cárdenas et al. 2019), and a questionnaire survey-based network of sport horses have been described (Spence et al. 2019), but these reports mostly used locations, rather than horses, as nodes. One Canadian questionnaire survey-based report on 779 horses attending one dressage event and their contacts included metrics of the horse network; Spence et al. 2017 reported a diameter of five, a mean distance of 3.3, a median

degree of 33 and a density of 0.05. The density in our sports horse network was lower, as was our mean distance. The diameter of five in our network of 41,081 horses was the same as for the 799 horse network reported by Spence et al. 2017. However, since this was a report from a single show and the questionnaire response was $< 100\%$, their results cannot be directly compared to ours.
A three-year static farm-based (n=38,263) contact network from Rio Grande do Sul, Brazil had a density of $4 * 10^{-5}$, a transitivity of 0.0041 and a diameter of 26; the diameter in the monthly static slices mostly varied between 5-15 (Cárdenas et al. 2019). The North American Standardbred racing locations one-year static network described by Brown *et al.* consisted of 254 nodes, had a density of 0.06, a diameter of 6, a mean distance of 2.5 and a transitivity of 0.4, was both strongly and weakly connected, and had a median In-Degree of 9 and a median out-Degree of 9.5. Size-wise, the network by Brown *et al.* sits between our racing and sports location networks; the density is similar to our racing network, the diameter and mean distance are larger than for both our racing and the sports locations networks.

In a 2022 tracing-built network of 244,004 farms from Brazil, the total static network of all livestock species combined had a giant strong component of 44%, and when the species were analysed separately, the giant strong components were 14-40% of the total network size. This is a clear difference with our static networks, which were fully connected and therefore had giant strong component sizes of 100% of the total network size.

A 2019 study of pig transports from one major transporting company in two Canadian provinces (Augusta et al. 2019) calculated monthly static metrics, with locations as nodes, and in their monthly network slices they reported a median of 207 nodes and 205 edges, a median density of 0.0014, an average clustering coefficient of 0.003, a mean distance of 2.3, a diameter of 7, and a node Degree distribution that suggested power-law characteristics. An average "outgoing infection chain" was also reported, which is somewhat akin to a static version of the Reach in our temporal networks. This pig shipment network was a smaller locations network than our sports locations network, yet the Canadian pig transport network diameter was longer, and the mean distance similar. The median Reach for both the Racing and the sports locations networks far exceeded the average outgoing infection chain in the Canadian pig transport network.

The German pig trade network described by Lentz et al. (2016) had 97,980 location nodes, a giant strong component of 28% of the total network size, and a diameter and mean distance of 18 and 5.5 respectively. Again, this differs from our horse networks which all consisted of one single strong component containing all nodes. The German pig trade network was concluded to have small world properties, like our sport horse network, but was not a fully connected network. A 2018 nationwide cross-sectional survey in Bangladesh among 849 poultry traders who visited 138 live bird markets was used to construct a static network of a period of one week. The resulting network of 445 nodes (farms or markets) had a

giant weak component of 97% of the total size of the network, but a much smaller giant strong component of only two nodes, reflective of the one-directional flow through the network of these birds (Moyen et al. 2018).
Although we have not made a comprehensive or systematic comparison here, the above examples support our hypothesis that horse contact networks are more connected than most livestock contact networks.

For this study we calculated monthly static networks of both horses and locations to facilitate comparison of our metrics with previous reports. Eventually, these static slices are of limited value, as arbitrary monthly cutoffs for what is essentially a continuous process introduces artifact: the shorter the "slice", the less connected the network appears, and nodes at the early and tail end of the month have less opportunity for contacts than do those in the middle of the month. A "sliding window" approach resolves this latter issue, but still does not address the problem of ignoring the sequence of events within the window, and ignores the paths that take part over a longer timeline than the window size.

The difference in connectedness between the static and temporal networks in this study is in line with prior findings that temporal networks can be preferable over static representations of the same network (Vernon and Keeling 2009). For all three networks for which we were able to calculate the time-respecting Reach, the static network analysis indicated that the networks were fully connected (both weakly and strongly), yet the temporal networks demonstrated the presence of nodes from which only a minority of the entire network is reachable. These nodes were relatively few, most nodes had a Reach of the majority of the network, as was demonstrated in Figure 7.12. Still, this suggests that the connectivity of the network was overestimated by a static representation which ignores the sequence of events, as previously described by Dekker et al. (2022). When using an over-connected static representation for outbreak simulations, the size of an outbreak can be overestimated (Lentz et al. 2016).

As described by Vernon and Keeling (2009), the direction of the error in outbreak size prediction can go either way depending on infection transmission metrics, which could be taken to mean that the direction of the error in simulations of the impact of interventions on static networks may be difficult to predict. Our results suggest that for future outbreak and control measure simulations, application of the temporal network may be preferable. Eubank et al. (2004) indicated that for a disease like measles, for which incubation and infectivity duration are several days each, slice periods of one day were appropriately short for network simulations. Several globally important equine infectious diseases such as EHV-1, *S. equi*, and influenza, similarly have generation intervals in the order of magnitude of days duration (**Chapter 2**, **Chapter 5** Glass et al. 2002), so for these diseases, it may be prudent to pay attention to the timescale and consider temporal networks, if network-based modelling is undertaken.

If the contact network of horses in competitions is highly connected, as our results

suggest, then the same is likely true for the entire Dutch equine population. In our biosecurity survey in **Chapter 6**, 4/5 respondents had horses leave and return on the same day at least occasionally. In most cases, these trips are taken to participate at competitive events. Although the network of horses that attend competitive events consisted of only ≈41,000 individuals, their direct contacts, when including horses at their home premises, may encompass 4/5 of all ≈300,000 horses in the Netherlands. Any infectious disease for which transmission under the settings of a competitive event is likely, is able to reach the majority of the Dutch equine population with few steps, thanks to the small-world properties of the sport horse network.

We were only able to include movements associated with participation at competitive events, and therefore our current results can only be used to answer research questions concerning those contacts. We do not know the home premises contacts of these horses. Also missing are non-KNHS sporting events (reining, non-competitive events), permanent re-locations of horses, trips for training and recreation, hunting, or trips for medical or breeding purposes. Additional data sources should be explored to fill the gaps, such as Identification & Registration databases. Better insight into the connections between competing horses and the Dutch equine population at large, through the distribution of competition horses over Dutch equine premises could be achieved by combining the competition data with local Identification & Registration data, which holds information about the home place of residence of all Dutch horses, as well as any stays elsewhere that are more than 30 days long. A mixing matrix for the intensity of contacts between horses at the same competitive event would add further precision to this contact network.

The size of the sport horse network exceeded R's capabilities for temporal analysis and an alternative framework for analysis will need to be sought.

CONCLUSION

The contact network for horses participating in competitive events is highly connected, and differs from livestock contact networks in Europe and Canada. The sport horse network has small world properties. We compared static and temporal representations of these networks and conclude that the static network overestimates the connectivity of the network, as previously reported for livestock veterinary species, and advise that for the purpose of modelling equine infectious disease outbreaks and the effects of mitigating measures, use of a temporal contact network of an appropriate time resolution is considered. We also demonstrated that with the exception of the racing locations network, the equine competition contact networks are highly robust to targeted node removal.

Part V

Chapter 8

GENERAL DISCUSSION

At the start of this thesis, my intention was to take the strangles eradication scheme proposed by Prescott and Timoney (2007) (Figure 1.6) and use modelling to predict what would happen while the world tried to implement it. However, you cannot do meaningful modelling without a solid knowledge base with which to parameterise models. And (perhaps surprisingly) despite their obvious and well-recognised impact on equine health and welfare worldwide, important parts of that base were still missing, both for *S. equi*, my original focus, as well as for EHV-1, which was included later, following the international outbreak that originated in Valencia in 2021 (Couroucé et al. 2023).

So for this thesis, I had to take a step back and focus on relevant basic aspects and data that are essential to understand and have before one can meaningfully give actual practical advice for horse owners, equine veterinarians or policy-makers. Hence, work for this thesis has included the calculation of epidemiologically relevant parameters such as R_0, transmission rates, the duration of convalescent immunity for *S. equi*, the effect of vaccination for EHV-1, prevalence of *S. equi* carriers, and contact patterns. This work has helped shape a more solid foundation on which future studies can hopefully build and start providing evidence-based practical insights that can help future prevention and control efforts.

CONTROL OF STRANGLES AND EHV-1: THE CURRENT STATUS

The past decades have brought many advances in understanding behaviour of *S. equi* and EHV-1 in the host, and host-to-host (and host-environment) transmission. Likewise, a wealth of information on disease outbreaks and environmental surveillance has become available, with more undoubtedly to come as (international) surveillance initiatives like SEIN, EIDS, and the ICC continue to encourage and normalise reporting of equine infectious disease outbreaks.

While this surveillance data is valuable, there are limits to how well surveillance alone can lead to control of an infectious disease, or inform best control practices. Surveillance, in particular when passive and on an *ad hoc* basis (i.e. testing is only done if a suspicion of disease has arisen, and reporting is voluntary), can help reduce the spread and size of outbreaks, but without further information beyond that which can be provided by passive surveillance, it is difficult to quantify the total burden of disease, or the impact of surveillance and other interventions on that burden. Data-driven modelling scenarios could be an effective way to examine the cost-effectiveness of control strategies, and evaluate if there are strategies that are a more (cost-)efficient approach for the control of EHV-1 and *S. equi* than a game of Whac-A-Mole.

S. equi

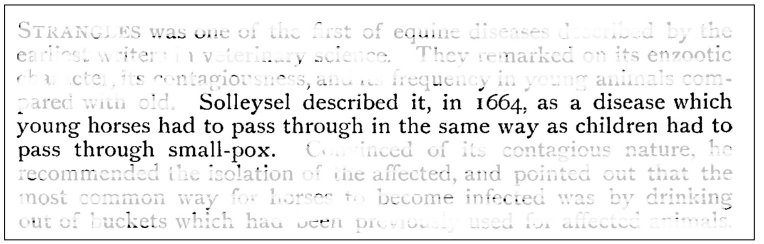

STRANGLES was one of the first of equine diseases described by the earliest writers on veterinary science. They remarked on its enzootic character, its contagiousness, and its frequency in young animals compared with old. Solleysel described it, in 1664, as a disease which young horses had to pass through in the same way as children had to pass through small-pox. Convinced of its contagious nature, he recommended the isolation of the affected, and pointed out that the most common way for horses to become infected was by drinking out of buckets which had been previously used for affected animals.

Figure 8.1: *An excerpt from Todd (1910), describing historical attitudes towards strangles.*

It remains to be seen whether strangles really is a "childhood disease" (Figure 8.1). As discussed in **Chapter 1**, numerous outbreaks have involved predominantly older horses. Strangles may very well be a "childhood disease" in the same sense that rubella, for example, was once considered a childhood disease. The reason for the historically high disease burden of rubella in children can be attributed not to inherently decreased susceptibility in adults, but to the high prevalence and high contagiousness of the pathogen, which meant that most children were likely to first encounter the virus at a young age, after which they were protected against infection at a later age by convalescent immunity (Panagiotopoulos et al. 1999; Heesterbeek et al. 2015). The observed predisposition for strangles in younger horses may be at least partially explained by the same phenomenon.

The attitude of UK owners of semi-feral ponies (for whom biosecurity measures are admittedly difficult to implement) towards strangles has been described as a belief that the disease is "*ever present (and therefore unavoidable) yet also of minor consequence*" (de Brauwere 2023). In my personal experience, many horse owners seem to think that strangles is an inconvenience at most, until they experience an outbreak.
Anecdotally, vaccination against strangles is employed at some premises, including premises where young animals are gathered; yet on the other hand, some horse breeders in the Netherlands still prefer to let their horses get strangles at a young age, believing that as a result, the disease will not cause problems later in the horse's life. Todd (1910) had the following to say about that approach: "*The above system is conducive to most horses passing through the disease before their issue to regiments, but whether it is an advantage or not is doubtful. The high percentage of malignant cases, the mortality, and the number which are left unsound in the wind after the disease are not in its favour.*" The century-old attitude from Todd's description may no longer prevail, but has certainly not yet fully disappeared.

EHV-1

An EHV-1 outbreak generally is considered by all to be a big problem, which is a step up from attitudes towards *S. equi*. Unfortunately, control for EHV-1 will be a lot less straightforward than for *S. equi*. It is probably no coincidence that the possibility of eradication of *S. equi* has been raised on multiple occasions (Prescott and Timoney 2007; Waller 2013) yet this has not occurred for EHV-1. An infectious agent that is so prevalent that hardly any horse escapes infection in its lifetime, that causes latent infection in many, if not the majority of horses – a latent infection for which no reliable ante-mortem test nor effective treatment exists, and which can revert to infectiousness at any time – is not amenable to eradication. There is a great desire in the equine community to reduce the impact of EHV-1, but unfortunately, how this can be effectively achieved remains elusive.

Competitions and regulations

Many equestrian oversight organisations, e.g. KNHS (www.knhs.nl/reglementen, Art. 47), The British Racing Authority (codes.hblb.org.uk), and the FEI (FEI Horse Health Requirements), require up-to-date vaccination against only Equine Influenza for event entry. For competitions at the regional and national level in the Netherlands, there are no requirements regarding *S. equi* or EHV-1. Vaccination against EHV-1 is mandatory for competition entry for French racehorses and for young French sport horses (Couroucé et al. 2023), and was temporarily mandatory for all German sport horses (Herpes-Impfung bei Pferden | FN 2023). Proof of vaccination against *S. equi* or of non-carrier status is not required for event entry in the Netherlands or for the FEI. Premises operators or horse owners are advised to notify the KNHS of ongoing outbreaks of infectious disease at their home premises, as are premises hosting events, but none are required to do so.

The FEI, through its Horse Health Requirements, has introduced self-certification (by the horse owner or handler) which requires submitting twice daily rectal temperature measurements for three days preceding arrival at events, and declaring that the horse has not been in contact with horses with confirmed or suspected disease caused by EHV-1. Sanctions are in place for those presenting their horse for competition and failing to meet the requirements. This is commendable progress, but it is not yet certain how much impact it has had on the probability of an outbreak occurring. The eventual size and impact of an outbreak may be reduced by this measure, provided that the owner-reported rectal temperature recordings are completed diligently and truthfully. However, since nasal shedding of EHV-1 can occur without detectable clinical signs, clinical monitoring alone may not prevent outbreaks from occurring.

There are currently no national regulations for the control of *S. equi* or EHV-1 in the Netherlands. Neither infectious agent is notifiable by law in the EU. Import regulations are not currently in place for *S. equi* or EHV-1 in the Netherlands or the EU. WOAH guidance on EHV-1 entails recommendations for pre-transport verification of absence of clinical disease or recent exposure to animals with confirmed or suspected clinical disease, but as was outlined in **Chapter 1**, this will not fully prevent entry of infectious horses.

TOWARDS AN EVIDENCE-BASED CONTROL EFFORT

Opportunities

In **Chapter 3**, we demonstrated that the persistent belief that 1/4 horses fail to develop lasting convalescent immunity after infection (Boyle et al. 2018) is likely based on an incorrect interpretation of historical reports of strangles incidence. Future modelling work on *S. equi* does not need to account for a significant proportion of the population being unable to mount convalescent immunity, which is positive news since having a large proportion of the population being almost constantly susceptible could make control more difficult.

In **Chapter 3** we also demonstrated that at the herd or premises level, *S. equi* likely could be contained through rigorous and regular clinical monitoring, provided that this is a practically viable option for the premises. Additionally, vaccination can be used strategically where regular checks are not feasible.

Only eight percent of UK horse owners regularly check their horse's rectal temperature, and two-thirds only do so if their horse seems unwell or if their veterinarian advises them to do so (McPherson 2023). On some premises, regular temperature checks may not be feasible, especially those with large number of untrained youngstock and few personnel, but only a minority of horses is housed under such conditions. Better uptake of regular temperature and clinical checks (followed by immediate isolation), as part of internal biosecurity, will have a significant effect of the average size of strangles outbreaks in case of incursions into the herd. In the future, the cost-benefit profile of the use of implantable temperature monitoring devices in various husbandry settings could be explored. Eventually, fewer horses affected by clinical strangles should also result in less carriers and therefore less infectious horses circulating in the general population.

In **Chapter 4**, we determined the prevalence of *S. equi* carriers among apparently healthy adult horses and ponies in the Netherlands to be 3.8%, which means that about 1 in 26 horses that are brought onto a new premises, or that are encountered at an equestrian event, is a carrier. This is helpful information for premises' operators when they are considering admitting new horses. Future research would benefit from a consensus on the definition of a carrier *vs* a convalescent strangles case (Pringle et al. 2022).

Insights from equine contact network analysis

From an epidemiological viewpoint, and in a first brief analysis, the competition horse contact network in the Netherlands seems disastrously well-connected (**Chapter 7**), and few barriers to entry of infection onto premises are imposed (**Chapter 6**).

In **Chapter 4**, we demonstrated that transmission of infection of *S. equi* from carriers at equestrian competitive events is relatively rare, at least in the Netherlands, where multi-day competitions are rare. Very few contacts (i.e. horses attending the same event on the same day) presumably lead to a new outbreak, even in a worst-case scenario. This information can be used in future modelling scenarios evaluating the impact of interventions. However, the relative contribution of apparently healthy infectious horses in maintaining the endemic state of *S. equi* remains unknown, and should be a focus of future work.

Future work should also evaluate how the challenges posed by the equine contact structure can be mitigated. For example, by evaluating on the one hand the impact of the targeted removal of nodes or edges from the network on the total burden of disease, and on the other hand how removal of these nodes and edges is best achieved. Examples of measures that can be evaluated are changes in participant routing at event sites to minimise contacts, environmental sampling at event sites, and stall-side rapid testing of infectiousness. For *S. equi*, the impact of testing of carrier-free status as a prerequisite for event entry could also be evaluated. The effect of targeted interventions that can reduce re-activation and nasal shedding (for EHV-1) or mandatory testing of non-carrier status (for *S. equi*) in high centrality horses or at high centrality events should be quantified in future work. Results from **Chapter 7** suggests that targeting horses or locations with high centrality scores could be an effective approach for racing locations, but possibly less so for racehorses, sport horses, or sports event locations, since the latter three networks were much more robust to targeted removal of nodes based on Betweenness centrality (Figure 7.7).

A large proportion of premises have horses that leave the premises temporarily to take part in equestrian competitive events (**Chapter 6**). The connectedness of the contact network of horses participating in competitions extends beyond just the Netherlands (Figure 8.2).
Re-activation of latent EHV-1 is often referred to as a rare event (Kydd 2021; Lunn et al. 2024), but rare is not the same as not important; this rarity should be given further context. Extrapolating from nasal shedding of 1.9 per 100 horses per month (Doubli-Bounoua et al. 2016), we can infer that the prevalence of nasal shedding on any given day is 1 in 1,605 horses. For context, in 2022 there were 353,883+6,112 (Table 7.3) entries (i.e a horse attending an event on one day). These figures suggest that in 2022, that a horse with nasal shedding may have attended a Dutch competitive event approximately 224 times.

Figure 8.2: *International travel for competitions in 2022 of sport horses with a KNHS registration.*

The relative contribution of equestrian competitions to total EHV-1-related disease incidence is not known, but several recent high-profile outbreaks reportedly originated from competitions. These outbreaks receive much attention perhaps due to the large geographic spread caused by horses returning home after being exposed at the event site, or to the notoriety of the horses and riders involved in the outbreaks. These high-profile competition-origin outbreaks of EHV-1 have occurred mostly at multi-day events, where horses were stabled together, such as in Ogden, USA (USDA 2013); Valencia, Spain (Couroucé et al. 2023); Oliva, Spain (inside.fei.org/media-updates/ehv-1-update-cases-mainland-europe--all-remaining-isolated-horses-oliva-cleared-leave); and California, USA (usef.org/media/press-releases/faq-california-ehv-1-outbreak). A large multi-centre EHV-1 outbreak occurring after a single-day event has also been described (Gryspeerdt et al. 2011).
The course of the outbreak in Oliva in 2023, which occurred after the introduction of the Horse Health Requirements, suggests that the goal of reduction of the impact of the outbreak on horses not attending the event was achieved (although nine exposed horses still left the premises prior to the diagnosis of a EHV-1 outbreak on the event site, and one of the exposed horses made it as far as Qatar before being quarantined). It is debatable whether the goal of preventing transmission among horses at the event site was fully achieved.
Targeted regular monitoring of nasal shedding of EHV-1 could be a valuable addition to current protocols, but its substantial cost will need to be weighed against its realistically expected benefits.

Challenges: missing data

Who Infects Whom? A vital piece of information needed to evaluate the relative efficacy of intervention schemes, is: where do new infections come from? The question of which age groups or categories of horses are the most important

cause for new EHV-1 and *S. equi* outbreaks remains mostly unanswered. EHV-1 has two distinct paths to infectiousness: a new infection, or a re-activation of latent infection. *S. equi* has two distinct types of infectious horse: the horse with recent infection (strangles, including early and convalescent cases) and the carrier. Once an outbreak of EHV-1 or *S. equi* is underway, most new infections are probably caused by contact with herdmates that were themselves recently infected, but how did the index case become infectious?

For EHV-1, there is evidence that first infection occurs at a young age, that mares at stud farms are the source of this infection (Gilkerson et al. 1998; Gilkerson et al. 1999; Gilkerson et al. 2000), and that vaccination of the mares does not prevent this (Foote et al. 2002; Foote et al. 2003; Foote et al. 2004; Marenzoni et al. 2008). These stud farm investigations were performed prior to whole genome sequencing becoming widely available and affordable, and therefore it remains unknown whether single or multiple EHV-1 strains were circulating during these investigations, and many questions remain. For example, is it usually one mare with re-activation from latency that infects everybody else, or are multiple (or even most) post-partum mares reverting to shedding around the same time, and are perhaps multiple strains circulating concurrently?
In none of the EHV-1 outbreaks included in **Chapter 5** were the authors able to say whether the index case was a recent infection, or a re-activation in a latently infected horse. Phylogenetic outbreak investigations of EHV-1 are rare; a report on phylogenetic analysis of nasal swabs from five Belgian and French horses with a link to the 2021 Valencia outbreak (Vereecke et al. 2021) concluded that these were all from the same clade and likely one single strain; an isolate from a Swiss horse that had also attended Valencia in 2021 was highly similar to the isolates from these five horses (Kubacki et al. n.d.). When all 43 isolates after an outbreak of abortions in China were sequenced, researchers recovered multiple distinct clusters from that one outbreak, but unfortunately did not explore or discuss this finding further (Tong et al. 2022). A small study of EHV-1 infections in captive zebras after transport and mixing of new groups, resulted in a phylogenetic tree that could have resulted from lateral transmission, but could also indicate that all shedding zebras were shedding strains they were known to already be latently infected with (Seeber et al. 2018).

None of the reports evaluated for **Chapter 5** described an outbreak in which multiple genotypes (N_{752}, D_{752}, or H_{752}) were detected simultaneously; however, it is not always clear from the outbreak reports how many swabs from how many horses were genotyped or sequenced, so it is possible that concurrently circulating strains were overlooked. Different EHV-1 isolates can be recovered from the same horse (Allen et al. 2008; Pusterla et al. 2010b; Pusterla et al. 2012; Bryant et al. 2018), demonstrating that horses can be infected by multiple EHV-1 strains at the same time. With multiple locus typing of stored historical Irish isolates, more than one isolate was recovered from 29/220 premises (Garvey et al. 2019). There is also evidence of recombination occurring for EHV-1, suggesting that infection of the same cell by different strains can occur (Bryant et al. 2018; Emelogu et al. 2023).

It is currently not known if and how concurrent latent and new EHV-1 infections interact within a horse, and if and how they contribute to nasal shedding loads or to disease severity. The opportunities provided by the availability of whole genome sequencing and phylodynamic techniques should be embraced to improve understanding of the behaviour of EHV-1 between and within equine hosts.

For *S. equi*, carriers are assumed to be the driving force of endemicity and an important cause of new outbreaks (Waller 2014; Mitchell et al. 2021), but this assumption has not yet been confirmed. Genotyping of surveillance data has been performed by sequencing the SeM allele region (Lindahl et al. 2011) and by whole-genome sequencing (Mitchell et al. 2021), but this work has not yet resulted in quantifiable attribution of the origins of outbreaks to specific categories of horses.

It is difficult to decide who to target for interventions to control spread, without knowing where new outbreaks originate. Systematic sampling and sequencing would make phylodynamic investigations possible, which can help answer the important question of Who Infects Whom (Grenfell et al. 2004). Future work, including but not exclusively phylodynamic outbreak investigations, should be undertaken, as knowing where new outbreaks come from will help accurately target high-risk individuals or conditions.

The role in the Netherlands of youngstock (so-called "opfok") premises, where young horses are gathered from different sources and group housed for a period of 1-3 years, remains unexplored in this thesis. Typically, horses aged ≈6 to ≈36 months are housed in such facilities: from the time of weaning until the time of starting training, for which they are usually relocated back to their premises of birth or to a training-oriented premises. In a pilot study carried out by Royal GD in 2021 with longitudinal sampling (upon the formation of the newly mixed groups and 2-3 months later) by nasal swab and serology on six youngstock premises, *S. equi* was detected on pooled nasal swabs by PCR at least once on 3/6 premises and EHV-1 was detected once on 1/6 premises (Royal GD / C. van Maanen, unpublished data). Two premises had multiple groups return *S. equi* PCR-positive pooled nasal swab samples. Interestingly, these two premises vaccinated their foals for *S. equi*, either prior to the start of the study or between first and last samples, yet at the end of the study period all or nearly all group pooled nasal samples were PCR positive, indicating ongoing shedding despite vaccination. Unfortunately, a detailed vaccination history was not available for the foals on these premises.
Anecdotally, some premises with youngstock currently choose to vaccinate only the youngest horses, aiming to let slightly older youngsters become infected, resulting in – such is the hope – less severe disease, and future protection against disease for years to come. Young horses, in particular those housed in large groups on dedicated premises, may be an important reservoir for EHV-1 and *S. equi*, which is then transported to other premises types when they enter training

as young adults. Quantifying the role of the "opfok" premises in epidemiology of *S. equi* and EHV-1 should be a future research priority for equine infectious disease research in the Netherlands.

Challenges: prevention and control

Eradication of *S. equi* may theoretically be feasible (Table 1.1 and **Chapter 3**), but the presence of pockets of feral horse herds worldwide, some with protected or conservation status, will be a complicating factor if such an effort were ever undertaken.
In practice, it is unlikely that an organised global control effort and attribution of resources of the magnitude that is required for such an enterprise will ever be raised. For such kinds of resources to materialise, an infectious disease would need to be a serious threat to global human health, food security or (regional) economy.

While *S. equi* could at least in theory be eradicated, there is no such luck for EHV-1; even with daily clinical checks, the minor temperature hike that can occur around the time nasal shedding starts is easily missed, if it occurs at all (Slater 2014; Pusterla et al. 2022a). Often, by the time anyone on the premises realises something is wrong, the outbreak has, for the most part, already happened. Clinical monitoring alone probably will not remedy this. For EHV-1, regular clinical checks, especially temperature taking, will likely help reduce the size of outbreaks, as some infectious horses will demonstrate fever and/or upper respiratory signs at the onset of infectivity. However, as was seen in the 2023 Oliva outbreak at a multi-day FEI event, it will not stop all transmission. In the absence of clinical markers for infectivity, ancillary (laboratory) test of shedding, nasal or otherwise, will likely be needed. Whether sampling of the environment, rather than horses (Pusterla et al. 2022b) is a more sensitive and cost-efficient means of monitoring is an area worthy of further investigation. Finding practical, rapid and (importantly) reliable ways to detect infectiousness will be essential to reduce the probability of the development of major outbreaks of EHV-1 in the future.

Efficacy of available control measures

Effective measures to prevent infection with *S. equi* exist; it is clear how to prevent transmission effectively. The problem is that this collection of control measures at full force is incompatible with equine husbandry practices (**Chapter 6**). For *S. equi*, the difficult question is not how to effectively prevent infection or disease, but how to design and implement a set of control measures that will actually see significant uptake and actually lead to a decrease in the disease burden.

For EHV-1 on the other hand, it is unclear what control measures, if any, can effectively prevent re-activation and/or transmission of EHV-1 and subsequent disease.

EHV-1 vaccination The currently available EHV-1 vaccines have proven clinical efficacy against respiratory disease, but respiratory disease is the least concern of horse owners in outbreaks of EHV-1, by a considerable distance. Owners of broodmares worry about loss of their unborn foals (for which EHV-1 vaccines offer incomplete protection), and everybody worries about horses becoming ataxic and remaining unfit for work for the rest of their lives, or even experiencing paralysis so severe that euthanasia is the only humane option. When control of EHV-1 is discussed, it is predominantly with the intention of reducing the incidence of these latter two disease syndromes, not the respiratory disease.

The effect of vaccination on transmission of EHV-1 remains uncertain. There is some evidence for a detectable reduction in the incidence of nasal shedding after vaccination, although the results vary with the type of vaccine given and the method by which shedding is detected (Marenzoni et al. 2022; Osterrieder et al. 2023). In the most methodologically sound of the two vaccine trial meta-analyses, Marenzoni et al. (2022) found a small, and not statistically significant effect on the number of animals shedding virus after vaccination with commercially available products, compared to placebo. Some authors have commented that the setting of vaccine trials does not sufficiently mimic field conditions, and therefore continue to recommend vaccination as a means to reduce transmission (Lunn et al. 2024).

The outcome of reduced shedding however, is a proxy outcome with an unknown relation to the outcome of interest, which is reduced transmission. A detectable decrease in shedding does not guarantee a similar or even a detectable decrease in pathogen transmission. In **Chapter 5**, we were unable to demonstrate a statistically significant effect of vaccination of the herd on transmission, measured directly as the outbreak reproduction number R, instead of as a proxy outcome (i.e. nasal shedding). Since **Chapter 5** was a study examining naturally occurring outbreaks rather than experimental settings, our input data was far from perfect and the number of studies available for inclusion was limited. It is therefore possible that a true effect exists but was undetected in the available outbreak data. It does however appear unlikely that vaccination alone will lead to $R < 1$ (Figure 5.2).

Proof of reduced transmission with vaccination has now been attempted by two distinct approaches, both of which failed to demonstrate a significant effect, but neither of which was an ideal way of detecting such an effect, if it does exist in reality. Ideally, transmission experiments in vaccinated, non-vaccinated and partially vaccinated herds should be conducted so that the true outcome of transmission can directly be measured in an experimental setting.

The 2024 updated EHV-1 Consensus Statement (Lunn et al. 2024) features a paragraph on the expected effect of vaccination on reduced shedding, taken as a proxy for transmission, and includes the recommendation to "maximize herd

immunity through vaccination". This recommendation echoes similar statements in a variety of recent EHV-1 reviews (Kydd 2021; Pusterla et al. 2022a). Vaccination will improve the protective immunity of horses in the sense that it will protect them from respiratory disease and reduce the probability of abortion. Herd immunity in the typical sense of $R_e < 1$ (to prevent major outbreaks) requires a vaccine to effectively reduce transmission, not just to reduce clinical signs. In our study in **Chapter 5**, in the vaccinated subgroup, the effective (vaccinated) reproduction rate remained above 1 (Figure 5.2). It is of vital importance that further work is undertaken to quantify the effect of vaccination on EHV-1 transmission, and that until such time, caution is taken when stating the expected benefits of vaccination.

Antiviral drugs There is moderate quality evidence that treatment with valacyclovir can decrease viral loads in nasal shedding after infection with EHV-1, and poor quality evidence for an effect on clinical signs, including EHM (Goehring et al. 2024). Valganciclovir was recently shown to decrease nasal swab loads of infectious virus particles after experimental infection with EHV-1 (Thieulent et al. 2022). Further investigation of the effect of these antiviral drugs, on their own or in combination with an up to date vaccination status, on transmission of EHV-1 should have a high priority in the field of EHV-1 research.

Stall-side testing Commercially available rapid stall-side tests for equine infectious agents are a relatively new development, and aim to circumvent the logistic challenges in infectious disease control associated with wait times for traditional reference lab PCR results. Reports on accuracy of equine pathogen-specific stall-side tests are currently sparse, but two publications suggest that LAMP-based methods have decreased (approximately 85%) sensitivity compared to reference PCR (Tsujimura et al. 2019; Jelocnik et al. 2021).

Challenges: Horses are a different kind of livestock

Most equine premises, in the Netherlands and elsewhere, do not routinely implement bio-exclusion measures that will prevent incursion of infectious disease (**Chapter 6**). However, it would be unfair to simply point a finger at horse owners and premises operators and accuse them of negligence or lack of motivation.

With the exception of breeding, and niche functions such as pharmaceutics or food production, a horse's value (economical or emotional) typically does not lie in what it produces but in what it can provide, for example companionship, leisurely rides, participation in amateur competitions, or even monetary winnings in elite competitions or racing. The type of measures that are effectively applied to production animal settings are, for the most part, incompatible with the activities that give horses their value.

We cannot implement the level of biosecurity any modern poultry or pig farm does, because this would largely void the purpose the horse had to us to

begin with – for sport or recreation. Breeding-oriented (non-Thoroughbred) premises are the only type of equine operations that could implement the type of biosecurity that is currently reserved for livestock. Given the value of their bloodstock and abundance of susceptible animals present, these types of premises in particular should consider implementing more stringent biosecurity measures than what is reportedly currently in place. Some breeding farms may already do this; in a 2015 USDA survey, a larger proportion of breeding premises required *S. equi* screening and other health certificates for new arrivals, compared to ranch or residence premises (USDA 2016).

For everyone else however, an optimal combination of biosecurity and other measures will need to be sought that will keep the risk and consequences of incursion of infectious agents onto the premises manageable. In addition to the work on epidemiological parameters of *S. equi* and EHV-1 in this PhD thesis, for an assessment of the risk of incursion, information on the contacts between premises was necessary. **Chapter 7** has shed light on where horses go and who they might meet there, and these insights will be expanded in a future more in-depth analysis of the data with network tools.

If quarantine measures as stringent as advised in the *S. equi* Consensus Statement (Boyle et al. 2018) were to be imposed, it is possible that many equestrians might no longer find their hobby appealing, nor would equine professionals be able to make a profitable living. One could therefore wonder whether stringent implementations of these measures is something the equine industry should really wish for, if it wants to remain a economically viable industry. But then, what should it wish for? Is continuing the current status quo, where transmission risks and occasional outbreaks are accepted in order to allow for the equine industry to function, a desirable strategy? As outlined in **Chapter 1**, *S. equi* and EHV-1 currently pose a significant health, economic and welfare burden. We could accept the current economic burden and thus accept the current status quo, and resign ourselves to the inevitability of the continued impact of frequent outbreaks, but in the context of increased scrutiny of the welfare consequences of being a domesticated horse, simply accepting that sometimes the horses we keep get potentially lethally ill as a result of their need to travel and socialise with strangers, may not be an advisable course of action.

However, control strategies that rely solely or heavily on stringent biosecurity are unlikely to be taken up widely, and thus doomed to be ineffective. This should not be taken to mean that promoting implementation of biosecurity should be abandoned completely, but control programs will need to take into account the mobility requirements for horses that are held for their performance, not for their products. Ideally, control programs account for routes of transmission between premises and the feasibility and cost-benefit profile of control strategies.

WHAT WOULD HUMANS DO?

Perhaps, if we cannot deploy many of the infection control tools that are possible in the livestock industry, can we use the tools that are available to humans? The contact structure of horses likely resembles that of humans at least as much as it does that of livestock. Horses are (mostly) kept and valued as individuals, rather than groups, and protection against (or treatment of) disease for the individual animal, rather than for groups of animals, is usually the ultimate goal.
Many horses are seen as pets by their human owners, and if a human has been to a sports match, or moves to a different city, they do not go into quarantine, so why should horses? Ignoring for the moment that most humans, in Europe at least, do not typically live in households of up to dozens or even hundreds of herdmates, which adds a layer of difficulty to infectious disease control to horses compared to humans, what would we do about *S. equi* and EHV-1 if we ourselves had to deal with diseases as unpleasant as strangles and EHM?

Imagine, if you can, that a bacterial disease existed that caused humans horrible and potentially life-threatening throat infections, or a viral infection that could leave you paralysed if you happen to be unlucky, and both those diseases were so endemic that dozens to hundreds of outbreaks occurred per year, and almost everyone would get sick from them at one point in their lifetime? What would we, humans, do about it?
The answer is not difficult, because such diseases exist. The polio virus was controlled to the point of near-eradication by worldwide vaccination programs (www.cdc.gov/polio/why-are-we-involved); mortality due to diphtheria dropped from 200 per year in the Netherlands to close to zero in a little over a decade after a national vaccination program was instituted (www.rivm.nl/difterie). These infections caused frequent disease globally until about two generations ago, but following a collective decision to not accept the status quo, because of the suffering these infections caused, (global) control program were implemented, with great success.

The fact that this has not happened for *S. equi* or EHV-1 is not exclusively due to a lack of motivation or willingness to invest by the equine community. The impact of diseases such as polio and diphtheria, measles, rubella, etc. on humans worldwide has successfully been minimised, but the same cannot be said for other infections such as tuberculosis, malaria, or HIV/AIDS, which despite great advances made, continue to have a significant impact on global health to this day (Childs et al. 2015). The causes for the continuing impact of these diseases are too varied and multi-factorial to be fully reviewed here, but one thing they have in common is that effective vaccines are not currently available.

The currently available EHV-1 vaccines have limited efficacy, both for clinical protection against the most severe disease syndromes, as well as for infectivity (**Chapter 5**). For *S. equi*, vaccines with good protection against clinical disease are available, but their effect on transmission is currently not quantified. In addition, these vaccines need to be boostered every six or every twelve months, which requires a substantial amount of ongoing motivation and financial commitment from horse owners. Some owners will happily and voluntarily make this commitment, whereas other will be hesitant about the cost-benefit ratio of vaccination for *S. equi*.

If we cannot or will not vaccinate our horses like we do our humans, and cannot or will not quarantine our horses like we do our livestock, then what do we about EHV-1 and *S. equi*?

Again, we can look towards human health challenges for guidance. Another aspect that infectious diseases like malaria have in common with EHV-1 and *S. equi*, is that resources to fund the control effort are limited. In addition, for malaria and many others, the current control effort is data- and modelling-driven, with design of nationally or even locally tailored intervention strategies (Heesterbeek et al. 2015; WHO 2021). The control effort is supported by real-world data that is continuously updated, such as What interventions are available, What is their effectiveness, Where is the highest disease burden, Who is most at risk, What is the expected impact of interventions? Importantly, when resources are limited, as is the case for the equine community, a modelling approach can help determine whether "not all interventions are necessary everywhere" (WHO 2021).

FINAL NOTE

For the effort to control EHV-1 and *S. equi*, outside help may not be forthcoming, since neither infectious agent is a serious threat to human health or food supplies, nor are they of sufficient economical impact for the world as a whole. The onus is on the equine community – globally, regionally, or nationally – to design, implement and evaluate its own control programs. Also, one of the reasons for the EFSA to decline listing of EHV-1 was the lack of proven control options. Perhaps if equine scientists can demonstrate courses of action that are proven to be effective, the decision could pivot more favourably in the future.

The most prioritised future research directions, out of those discussed here, should be Who Infects Whom investigations, as well as determining the efficacy of the most promising interventions on pathogen transmission: of vaccination for EHV-1 and *S. equi*, and of antivirals for EHV-1. The development and evaluation of reliable, rapid, stable-side testing for EHV-1 infection would also be a valuable tool in control of EHV-1. These are all currently missing yet are required for realistic, data-driven modelling of control scenarios for these infectious diseases of global importance to the equine community.

Part VI

APPENDIX

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Appendix B

Chapter Supplements

SUPPLEMENTS TO CHAPTER 2

Meta-analysis results for $\hat{R}_0$ for *S. equi* in individually housed horses are shown in figure B.1.

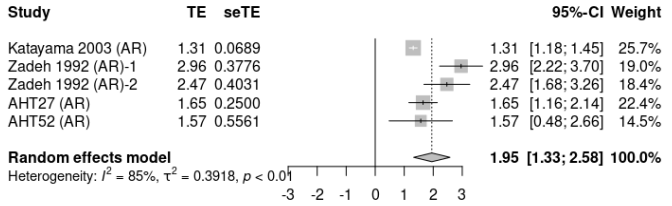


Figure B.1: *Overall estimate by meta-analysis of the basic reproduction number (R_0) for S. equi outbreaks in individually housed horse herds.*

SUPPLEMENTS TO CHAPTER 3

Additional deterministic model output figures

Figures B.2 - B.3 show additional model outputs not included in the main text.

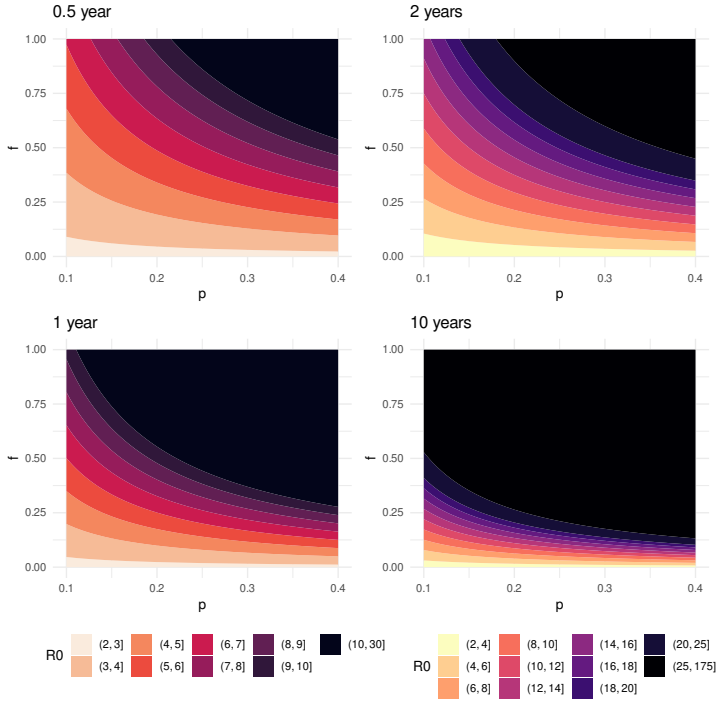


Figure B.2: *$R_0(p, f)$ at the population scale, for a range of $1/\gamma_C \in (0.5 - 10$ years.*

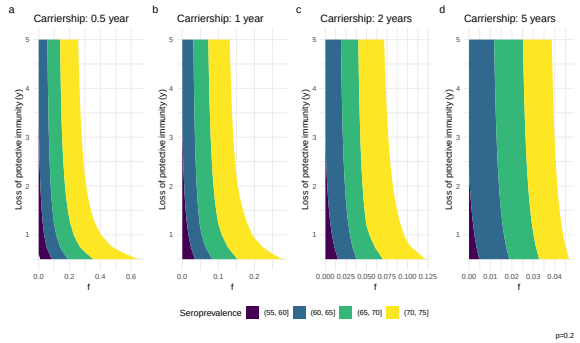
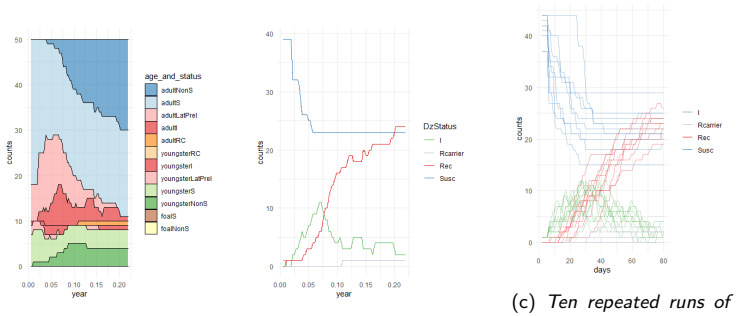


Figure B.3: *Expected percentage of horses in the population with convalescent immunity, for a range of relative infectiousness of carriers (f) and γ_C for $p = 0.2$.*

Stochastic model validation

Example simulation outputs are shown in Figure B.4



(a) *Plot detailing disease stages and age groups for 1 single run.*

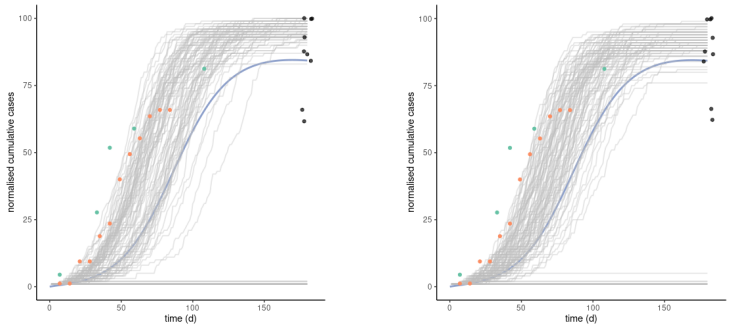
(b) *Same outbreak as a) but summarized by infection status.*

(c) *Ten repeated runs of the model with the same starting conditions as (a) and (b).*

Figure B.4: *Runs of the individual-based stochastic model with 10 young horses and 40 adults.*

Predictions from the deterministic compartmental and repeated runs of stochastic models are displayed in Figure B.6, and compared to to two naturally occurring outbreaks(Newton et al. 2000; Tscheschlok et al. 2018) in Figure B.5. Table B.1 lists model metrics of performance. Figure B.7 demonstrates the expected bimodal distribution of outbreak final sizes is present in both versions of the stochastic model.

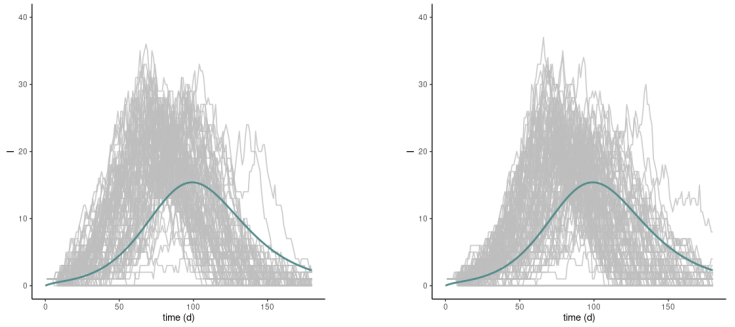
Because of the threshold properties of the prototype stochastic model, on which the current stochastic model is based, a two-point distribution of outbreak final sizes in individual simulation runs is expected, with either outbreaks where only a handful of individuals get infected, and larger outbreaks where the number of individuals that become infected over the course of the epidemic is in the order of magnitude of the population size at the start. The probability and eventual size of a major outbreak can be predicted based on R_0(Diekmann et al. 2013). For sufficiently large populations, the probability of only a minor outbreak occurring can be calculated as the smallest solution to $\theta = e^{-R_0(1-\theta)}$ and the final size of major outbreaks as the positive solution to $x = 1 - e^{-R_0 x}$ (Diekmann et al. 2013). The results for both implementations of the model are provided in Table B.1



(a) *Sellke construction*

(b) *Bernoulli trial*

Figure B.5: *Deterministic compartmental model prediction (blue line), repeated runs of the stochastic model in a herd of 100 group housed adults (grey lines) and two naturally occurring outbreaks in green(Tscheschlok et al. 2018) and orange(Newton et al. 2000) dots (with number of animals normalised to 100), showing the cumulative number of cases for the exponential part of the outbreak, for both the model applying the Sellke construction (a) and the model applying a Bernoulli trial to determine successful contacts (b).*



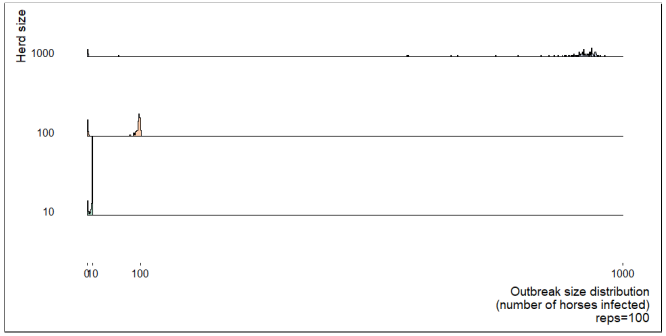
(a) *Sellke construction*

(b) *Bernoulli trial*

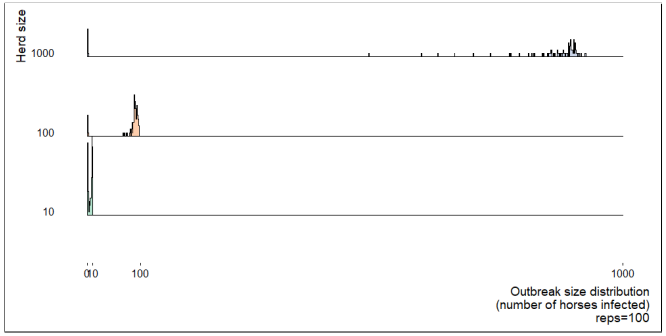
Figure B.6: *Deterministic compartmental model prediction (green line), repeated runs of the stochastic model in a herd of 100 group housed adults (grey lines), for both the model applying the Sellke construction (a) and the model applying a Bernoulli trial to determine successful contacts (b).*

Method	Bernoulli	Sellke	Expected
Proportion of major outbreaks	0.87	0.90	0.915* or 0.63*
Mean size of major outbreaks	863	916	915
Runtime (s)	7971	8657	N/a

Table B.1: *Performance of the stochastic models; 100 runs in herds of 1000 horses for 150 days. *For Reed-Frost model or **general stochastic epidemic (Diekmann et al. 2013).*



(a) *Sellke construction*



(b) *Bernoulli trial*

Figure B.7: *Plots demonstrating the binomial distribution of outbreak size of (a) the Sellke construction and (b) the Bernoulli trial method.*

Results from remount depot simulations

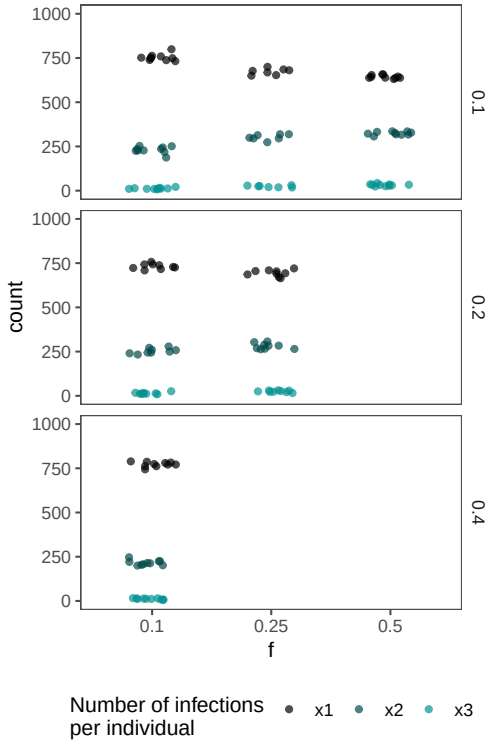


Figure B.8: *Number of horses infected once, twice, or three times when simulating a remount depot of 1000 horses over a period of 4 years, effect of f (columns) and p (rows) on outcome. Each dot represents the final tally of one run. γ_R is fixed at 4±2 years and γ_{R_C} at 5 years.*

SUPPLEMENTS TO CHAPTER 5

Sensitivity analysis

Alternative Generation Time assumptions Figure B.9 displays the effect of alternative assumption of the generation time on $\hat{R}$.

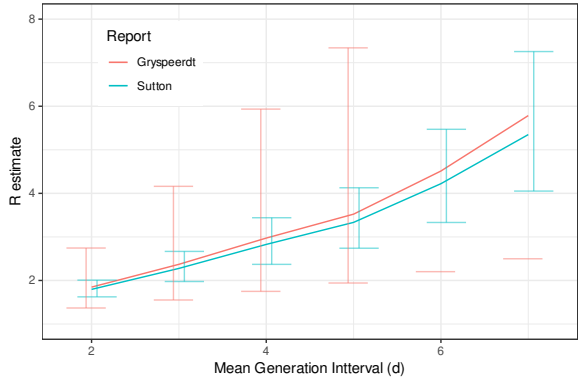


Figure B.9: *Sensitivity analysis for per-outbreak $\hat{R}_0$ to assumptions of Generation Time. Longer generation interval → higher $\hat{R}_0$ (Obadia et al. 2012).*

Outbreak reports: assessment for inclusion

An overview of all assessed outbreaks and reason for exclusion (if applicable) is given in Table B.2.

Table B.2: *Overview of outbreaks for suitability for inclusion the EHV-1 R_O meta-analysis.*

Outbreak	Year	Genotype	Focus	Vaccination	Source	Decision	Reason	Authors contacted
Klouth_A	2022	D752	EHM	Unknown	DOI 10.3390/v14112576	Not included	Incomplete records	No
Klouth_B	2022	D752	EHM	None	DOI 10.3390/v14112576	Not included	Incomplete records	No
Klouth_D	2022	Unknown	EHM	Unknown	DOI 10.3390/v14112576	Not included	Incomplete records	No
Klouth_E	2022	Unknown	EHM	Part	DOI 10.3390/v14112576	Not included	Incomplete records; 1/141 vaccinated	No
Klouth_F	2022	Unknown	EHM	Unknown	DOI 10.3390/v14112576	Not included	Incomplete records	No
Klouth_G	2022	D752	EHM	Part	DOI 10.3390/v14112576	Not included	Incomplete records; 29/54 vaccinated	No
Klouth_H	2022	Unknown	EHM	Part	DOI 10.3390/v14112576	Not included	Incomplete records; 5/23 vaccinated	No
Klouth_I	2022	D752	EHM	None	DOI 10.3390/v14112576	Not included	Incomplete records	No
Klouth_J	2022	D752	EHM	Part	DOI 10.3390/v14112576	Not included	Incomplete records; 1/59 vaccinated	No
Klouth_K	2022	D752	EHM	Part	DOI 10.3390/v14112576	Not included	Incomplete records; 1/40 vaccinated	No
Klouth_L	2022	N752	EHM	Part	DOI 10.3390/v14112576	Not included	Incomplete records; 8/41 vaccinated	No
Klouth_M	2022	Unknown	EHM	None	DOI 10.3390/v14112576	Not included	Incomplete records	No
Tong	2022	D752	Abortions	None	DOI 10.1186/s12917-022-03171-1	Not included	Only reports abortions	Yes, no response

Valencia 2021	2021	N752	EHM	Part	DOI 10.1002/ vetr.905	Not included	Incomplete records, partial vaccination, horse movement during outbreak	No
Vandenberghe	2021	Unknown	No specific focus	Unknown	DOI 10.3390/ v13081429	Not included	Horse movement during outbreak	No
Pusterla	2021	H752	No specific focus	All	DOI 10.3390/ pathogens10060747	Included		Yes, answered query re vaccine type
Gomez	2021	D752	No specific focus	Unknown	Canadian Journal of Veterinary Research 85-1	Not included	Vaccination status unknown	Yes, could not indicate whether horses were vaccinated
Sutton	2020	H752	No specific focus	All	DOI 10.3390/ v12101160	Included		No
Stasiak	2020	N752	Abortions	None	DOI 10.1186/ s12917-020- 02586-y	Not included	Reports only abortions; vaccination lapsed after prior strict vaccination	No
Sutton	2019	D752	No specific focus	Part	DOI 10.3390/ v11100916	Not included	Incomplete records, partial vaccination	Yes, no response
Moore	2019	Unknown	Abortions	All	DOI 10.1136/ vetreccr- 2018- 00799	Not included	Incomplete records	Yes, however records still incomplete after additional information
Strang	2016	Unknown	No specific focus	None	DOI 10.1136/ vr.j5906	Included (index barn only)		Yes, provided additional records
McFadden	2016	D752	EHM	None	DOI 10.1080/ 00480169. 2015. 1096853	Included (high-risk area)		No
Damiani	2014	D752	Abortions	All	DOI 10.1016/ j.vetmic. 2014. 06. 023	Not included	Incomplete records (serology for mares only)	No

Schulman	2015	N752	Abortions	None	DOI 10.1111/ evj.12264	Not included	Incomplete records (reports abortions only)	Yes, no additional information
Walter	2013	D752	No specific focus	Part	DOI 10.1186/ 1751- 0147- 55- 19	Not included	32/79 vaccinated	No
Ogden	2013	D752	No specific focus	Part	USDA-APHIS & Traub-Dargatz et al. 2013	Not included	Partial vaccination, incomplete records	No
Pusterla	2012	D752	No specific focus	None	DOI 10.1136/ vr.100598	Not included	Assumptions not met (mixing with mules)	No
Burgess	2012	D752	No specific focus	Unknown	DOI 10.1111/ j.1939- 1676.2012.00885.x	Not included	Unknown vaccination status	Yes, no additional information
Barbic	2012	D752	No specific focus	None	DOI 10.1136/ vr.100150	Included		No
Pronost	2012	D752	EHM	Part	doi 10.1111/ j.1865-1682. 2011. 01263.x	Not included	Partial vaccination	No
Meade CDR	2012	D752	No specific focus	None	PhD Thesis Gluck University	Included		No
Meade MSU	2012	N752	No specific focus	Part	PhD Thesis Gluck University	Not included	Partial vaccination	No
Gryspeerdt	2011	D752	No specific focus	Part	ISSN 0303-9021 2011 80(2) p147-153	Included (premises 1 only)	Incomplete records and possibly more than 1 simulataneous incursion in the other outbreaks	Yes, answered query re vaccine type on premises 1
Canelli	2010	D752	EHM	Unknown	PMID: 20173342477	Not included	Incomplete records in abstract, unknown vaccination status	Yes, no response

Goehring	2010	Unknown	EHM	Unknown	doi 10.1111/j.1939-1676.2010.0558.x	Not included	Assumptions not met (hospital outbreak, barriers to spread from the onset)	No
Pusterla	2009	D752	EHM	Part	doi 10.1016/j.tvjl.2007.09.018	Not included	Partial vaccination	No
Irwin	2007	Unknown	Abortions	None	doi 10.1136/vr.160.11.378	Not included	Incomplete records	Yes, no additional information
Henninger	2007	Unknown	No specific focus	Part	doi 10.1111/j.1939-1676.2007.tb02942.x	Not included	Partial vaccination	No
Goehring	2006	Unknown	No specific focus	Part	doi 10.1111/j.1939-1676.2006.tb02903.x	Not included	Incomplete records	No
van der Meulen	2003	Unknown	EHM	Part	ISSN 0303-9021 2003 72(5)	Not included	Partial vaccination, incomplete records	No
Studdert	2003	Unknown	Abortions	None	doi 10.1136/vr.153.14.417	Not included	Incomplete records, vaccination during outbreak	No
Barrandeguy	2002	Unknown	Abortions	All	doi 10.1111/j.2042-3292.2002.tb00156.x	Not included	Incomplete records, horse movements during outbreak	No
van Maanen	2001	Unknown	No specific focus	None	doi 10.1111/j.2042-3306.2001.tb00600.x	Included		No
van Maanen	2000	Unknown	Abortions	Unknown	doi 10.1080/01652176.2000.9695030	Not included	incomplete records, focuses on abortions only	No
Friday	2000	Unknown	No specific focus	All	doi 10.1111/j.1939-1676.2000.tb02236.x	Included		No

SUPPLEMENTS TO CHAPTER 6

The questionnaire used in the survey is depicted in Figure B.10.

Horse's name: mare/gelding/stallion Code in study:

_____ _____

Microchip number: Age & Breed:

_____ _____

Premises main purpose, type of housing, number of animals on the premises:

_____

How long have you owned or known this horse?

_____

Did the horse ever have strangles? Has strangles ever been diagnosed on the premises?
(As far as you are aware) If yes, when?
If yes, when?

_____ _____

What is the horse's primary purpose?

_____

How often do horses leave the premises for events (single day trips)? In horses/week.

_____

How often do horses leave the premises for events (multi day trips)? In horses/year.

_____

What measures are in place for horses returning from single day events?
And from multi day events?

_____

How many new residents arrive annually?
And what measures are in place when they arrive?

_____

Figure B.10: *The questionnaire used in the biosecurity survey.*

Appendix C

Summaries

ENGLISH SCIENTIFIC SUMMARY

Streptococcus equi subspecies *equi* (*S. equi*, the cause of strangles) and equine herpesvirus 1 (EHV-1, the cause of rhinopneumonitis, abortion, and equine herpesvirus myelopathy) are infectious agents that are endemic in the Netherlands and most of the rest of the world. Outbreaks of EHV-1 and *S. equi* typically have high morbidity and low mortality. Nevertheless, both infectious agents have significant impact on horse welfare and on the global equine community. Prevention of incursion onto equine premises of *S. equi* and of strangles outbreaks is achievable, but requires stringent preventive measures that are difficult to implement practically on equine premises. Therefore, the current control of *S. equi* remains sub-optimal, with frequent outbreaks continuing to occur. For EHV-1, it is currently much less certain how outbreaks can effectively be prevented. The global control effort for these two infectious agents does not currently include the use of (mathematical) models to evaluate the outcomes of various control scenarios to find the most effective (or most cost-effective) approach to reduce the impact of these infectious diseases. This thesis contains a number of investigations that aim to address this omission.

In **Chapter 2** the basic reproduction number (R_0) for *S. equi* outbreaks was calculated by meta-analysis of R_0 estimates we calculated from reports of naturally occurring outbreaks. A conservative estimate for R_0 was 2.2 (95% CI 1.9- 2.5). A less conservative estimate (including outbreaks with a 100% AR for which a lower limit R_0 was estimated) was 2.7 (95% CI 2.1- 3.3).

In **Chapter 3** the R_0 estimate from **Chapter 2** was used as input for mathematical models which, combined with real-world epidemiological data, were used to estimate additional epidemiologically relevant parameters for *S. equi*. Key findings include the discovery that the currently held belief that $1/4$ convalescent horses fail to mount lasting protective immunity after strangles is likely incorrect, that convalescent horses are protected against re-infection for a period of 4-6

years, that the control effort for *S. equi* can probably not be successful without addressing carriers, that weekly clinical screening of herds may suffice to prevent major strangles outbreaks on equine premises, and that vaccination alone, without any other preventive measure, may not suffice to control *S. equi*.

S. equi carriers are thought to be important drivers for strangles outbreaks. Limited data is available on the prevalence of carriers in European horse husbandry settings, and no data is available on how frequently carriers contact susceptible horses. In **Chapter 4**, we estimated the prevalence of *S. equi* carriers among apparently healthy horses and ponies in the Netherlands, and estimated the opportunities for contact of carriers with susceptible horses at Dutch competitive events.
First, a cross-sectional survey was carried out among 166 apparently healthy horses and ponies from 86 premises in the Netherlands. Participants underwent three repeated nasopharyngeal lavages at weekly intervals. Samples were analysed by PCR, followed by a Bayesian true prevalence estimation. The resulting estimate for true prevalence was 3.8% (95% Credible Interval 1.2-7.7%).
Next, to estimate the annual number of carrier-susceptible contacts at competitions, simulations drew a random single sample from the Bayesian true prevalence estimate posterior distribution, assigned carrier status to horses in a real–world network based on Dutch sports and racing records, assigned non–susceptible status to a proportion of horses in the network informed by published seroprevalence surveys, and counted the number of direct contacts, defined as presence at the same location on the same day, between carrier and susceptible horses for an entire year. The median annual number of carrier–susceptible contacts in the simulation runs was $1.0 * 10^6$(IQR $7.3 * 10^5 - 1,4 * 10^6$). A large number of carrier–susceptible contacts at competitions means that even if the probability of transmission per contact in these settings is small, it may still be of epidemiological importance.

In **Chapter 5**, R_0 for EHV-1 was estimated by the same methods as in **Chapter 2**. In addition to R_0, the reproduction number in fully vaccinated herds (R_V) was estimated, and the two groups (outbreaks in non-vaccinated herds and in fully vaccinated herds) were compared using a random effects model. Twelve outbreaks, in herds of 16–135 horses, met the inclusion criteria, of which six occurred in non-vaccinated herds and six in vaccinated herds. One R_0 calculation from a report describing empirical determination of a herd immunity threshold was also included. We found no evidence for a significant effect of vaccination status of the herd on the effective reproduction number in outbreaks: $\hat{R}_0 = 3.3(2.6 - 4.0)$ and $\hat{R}_V = 2.7(2.1 - 3.2)$, $p = 0.15$. However, insufficient data were available to investigate the influence of genotype or use of antivirals on these results. Sensitivity analyses gave volatile p-values. We concluded that we were unable to detect robust evidence for a significant reduction on transmission of EHV-1 in herds where all horses were vaccinated *vs* non-vaccinated herds. $\hat{R}$ in herds where all horses were vaccinated was substantially > 1 and vaccination with currently available vaccines as a sole mitigating measure may have limited effect

on transmission of EHV-1, and prevention of major outbreaks by vaccination alone with these vaccines does not appear to be possible.

For the transmission of infectious disease, contact (direct or indirect) between an infectious and a susceptible individual is necessary. Biosecurity measures can be implemented at the premises level to avoid such contacts and to reduce the risk of outbreaks of infectious disease. Reports from other countries suggest that the majority of equine premises implement sub–optimal biosecurity practices. To investigate biosecurity practices on Dutch equine premises, in **Chapter 6** a cross-sectional questionnaire survey was carried out among a convenience sample of horse owners and equine premises operators in the Netherlands. Premises which exclusively housed non-adult horses were not included in the sample, and horse trading premises were underrepresented. The survey questions were mostly focused on bio-exclusion (external biosecurity) measures. There were 86 respondents in the survey. Most premises were private residential premises (38%), boarding and/or training premises (36%) or riding schools (16%). The remaining premises‘ main purpose was breeding, racing, or trade. Most (92%) respondents had horses that were housed in groups for at least part of the day. Half of the respondents never implemented quarantine measures for new arrivals. Only 6% of respondents always implemented quarantine of new arrivals, and the remaining respondents quarantined new arrivals sometimes, or incompletely. None of the respondents required testing of infectious disease prior to taking in new arrivals, so none of the premises followed consensus recommendations for the control of *S. equi*. None of the respondents implemented biosecurity measures for resident horses returning from short single-day trips such as for training or competition, while 54/65 respondents housed horses which took trips, to other locations with other horses, on the premises.

Dynamics of infectious diseases are influenced by population contact structure. Limited data is currently available on horse contact networks. In **Chapter 7**, a brief literature review of equine contact network research was presented. Next, the contact network of horses participating in sports or racing competitions in the Netherlands was described, and static and temporal representations of these networks were compared. Participation records from the Royal Dutch Equestrian Sports Organisation and Dutch racing records for 2022 were made available upon request. Four networks were analysed: sport horses and racehorses, with horses as nodes and presence at the same event as undirected edges; and sports locations and racing locations, with locations as nodes and travel of horses from one event to the next as directed edges. Annual static and temporal network metrics were calculated.
The sport horse network was the largest network, with 41018 nodes, its diameter (highest number of steps in the shortest path between any two nodes) was five, and the network had “small world” properties, a topology that is favourable for spreading of infectious disease. All static annual networks were fully (strongly) connected. The connectedness of the networks was robust to targeted removal of nodes with a hub function. The only exception to this was the racing locations

network. The temporal “Reach” distribution of nodes suggested that static representations of the networks overestimated the network connectedness. An important limitation of this work was the absence of information of non–recorded movements, such as for training or due to permanent relocations, and of contacts at the horses‘ home premises. We concluded that the Dutch equestrian competition network is highly connected. Since approximately 4/5 Dutch premises have horses that temporarily leave to participate in competitions, this connectedness likely affects most if not all Dutch horses. The robustness of most networks suggests that targeting high–risk horses or locations for preventive measures may not be equally effective in all networks.

The control of equine infectious diseases poses unique challenges. Horses are typically housed in large groups for practical and welfare reasons, like livestock. However, equine travel and contact patterns likely more closely resemble that of humans. Travel on and off novel premises for competition, training, or trade is common for most horses. Infection control measures that are effectively used for livestock, such as one-directional movement through the production stream and stringent quarantine measures, are not compatible with the purpose for which most horses are kept. Control of infectious diseases of horses therefore will require an approach that differs from that of livestock infectious disease control. The effective control of many infectious diseases of importance to *humans* in the Netherlands leans heavily on vaccination campaigns. This also not an approach that is easily copied to all equine infectious diseases, as no sufficiently effective vaccine against EHV-1 currently exists, and the vaccines against strangles, although clinically effective, require frequent re-vaccination and are therefore cumbersome and costly. The control of EHV-1 and *S. equi* will require tailor-made approaches, and the information gained through the work in this thesis will enable evidence-based evaluations of the (cost-)effectiveness of control scenarios.

NEDERLANDSE SAMENVATTING

Droes (infectie met *Streptococcus equi* subspecies *equi*, in het kort *S. equi*) en rhinopneumonie (infectie met equine herpesvirus 1, EHV-1) zijn in Nederland en wereldwijd veel voorkomende infectieziekten. Behalve rhinopneumonie kan infectie met EHV-1 ook leiden tot abortus en tot neurologische verschijnselen. Uitbraken veroorzaakt door *S. equi* en EHV-1 kenmerken zich doorgaans door een hoge morbiditeit maar lage mortaliteit. Desondanks hebben deze beide pathogenen een grote impact op de paardensector, zowel in Nederland als daarbuiten. Voor droes is bekend hoe besmetting en uitbraken kunnen worden voorkomen, maar omdat het volledig implementeren van die maatregelen in de praktijk lastig is, is de bestrijding van droes op dit moment suboptimaal en blijft het een endemische ziekte met jaarlijks tientallen uitbraken in Nederland. Voor EHV-1 is minder goed duidelijk hoe transmissie en uitbraken het beste voorkomen kunnen worden. In de wereldwijde bestrijding van deze beide infectieziekten wordt tot op heden nog geen gebruik gemaakt van (mathematische) modellen als hulpmiddel

om te onderzoeken wat de optimale (meest effectieve, of meest kosten-efficiente) maatregelen zijn. Deze thesis bevat een aantal onderzoeken die zijn uitgevoerd om bij te dragen aan het dichten van dit gat.

Een belangrijke epidemiologische parameter voor elke infectieziekte is het basale reproductie getal. Voor *S. equi* was nog niet eerder een schatting of berekening van R_0 gedaan. In **Hoofdstuk 2** hebben we daarom de (R_0) van *S. equi* uitbraken berekend. Hiervoor hebben we de gegevens van 10 gepubliceerde droes–uitbraken geanalyseerd, per uitbraak een R_0 berekend, en vervolgens met behulp van meta-analyse van de afzonderlijke R_0's een gewogen gemiddelde R_0 berekend. Onze minst conservatieve schatting op basis van die uitbraken was dat R_0 voor droes 2.7 is (95% CI 2.1- 3.3).

In **Hoofdstuk 3** is de R_0 die **Hoofdstuk 2** berekend is, gebruikt als input voor (mathematische) modellen om meer te weten te komen over epidemiologische kenmerken van droes. Hierbij werden de uitkomsten van de modellen vergeleken met epidemiologische gegevens over *S. equi*. Voorheen werd gedacht dat 1/4 paarden na het doormaken van droes geen beschermende immuniteit opbouwt, maar dat blijkt een waarschijnlijk incorrecte aanname. Vermoedelijk zijn de meeste paarden 4-6 jaar beschermd tegen herinfectie nadat ze droes hebben doorgemaakt. Andere conclusies uit dit hoofdstuk zijn dat eliminatie van *S. equi* waarschijnlijk niet mogelijk is zonder ook dragers aan te pakken, en dat wekelijkse controle van alle dieren op een bedrijf voldoende kan zijn om grote uitbraken van droes te voorkomen. Droes elimineren door uitsluitend vaccinatie, zonder enige andere preventieve maatregel is mogelijk niet haalbaar.

Van droes dragers wordt gedacht dat ze een belangrijke motor zijn voor de voortdurende endemiciteit van droes en een belangrijke oorzaak voor nieuwe uitbraken. Er is weinig bekend over de prevalentie van droes dragers, en niets bekend over hoe vaak droes dragers in contact komen met niet-immune paarden. In **Hoofdstuk 4** is het aantal droes dragers onder gezonde paarden en pony's in Nederland onderzocht, door bij 166 paarden en pony's van 86 bedrijven drie wekelijks herhaalde nasopharyngeale lavages uit te voeren en met een PCR voor *S. equi* te analyseren. Dit resulteerde in een Bayesiaanse schatting dat 3.8% (95% Credible Interval 1.2-7.7%), oftewel ongeveer 1 op de 26, van ogenschijnlijk gezonde paarden en pony's in Nederland een droes drager is.
Die schatting van het aantal dragers is vervolgens als input gecombineerd met startgegevens van sport- en renwedstrijden in Nederland. Hiermee werd, door middel van simulaties, de jaarlijkse frequentie van contact tussen een (infectieuze) droes drager en een niet-immuun paard bepaald. Als "contact" gold in deze context het aanwezig zijn op dezelfde wedstrijd. De frequentie van deze contacten blijkt tussen 730.000 en 1.400.000 keer per jaar te zijn. Omdat er zoveel van zulke contacten zijn, zijn deze zelfs bij een lage kans op overdracht per contact waarschijnlijk toch relevant voor de verspreiding van *S. equi* in Nederland.

In **Hoofdstuk 5** hebben we het basale reproductie getal voor EHV-1 geschat

met dezelfde methodologie (meta-analyse van R_0–berekeningen van individuele uitbraken) zoals reeds beschreven in **Hoofdstuk 2**. Daarnaast is hetzelfde gedaan voor uitbraken in volledig gevaccineerde kuddes (R_V), en de twee categorieën zijn met elkaar vergeleken. Er waren per categorie zes uitbraken, in kuddes van 16-135 paarden. Deze aanpak leidde tot de schattingen $\hat{R}_0 = 3.3(2.6 - 4.0)$ en $\hat{R}_v = 2.7(2.1 - 3.2)$. Hierbij werd geen statistisch significant verschil tussen R_0 en R_V aangetoond ($p = 0.15$). Het is wel belangrijk om bij deze conclusie rekening te houden met de beperkingen van de gebruikte methode. Doordat deze gebaseerd is op data van natuurlijke uitbraken uit het verleden, waren er veel onderlinge verschillen tussen uitbraken, ook binnen de gevaccineerde en niet-gevaccineerde subgroepen. Het is niet uit te sluiten dat vaccinatie wel degelijk een effect heeft op transmissie. De uitkomsten van de meta-analyse suggereren echter ook dat het onwaarschijnlijk is dat vaccinatie met de heden beschikbare vaccins tegen EHV-1 het R-getal onder 1 zal brengen. Voorkomen van grote uitbraken van EHV-1 door middel van uitsluitend vaccinatie lijkt dus niet mogelijk onder de huidige omstandigheden.

Voor de verspreiding van infectieziekten is contact (direct of indirect) tussen paarden of tussen kuddes nodig. Paardenhouderijen kunnen biosecurity maatregelen implementeren om zulke contacten te vermijden en de risico's op uitbraak van een infectieziekte beperken. Uit onderzoek in het buitenland blijkt doorgaans dat de implementatie van biosecurity maatregelen op paardenbedrijven suboptimaal is. **Hoofdstuk 6** bevat een eerste beperkte inventarisatie van de implementatie van biosecurity maatregelen op Nederlandse paardenbedrijven, inclusief privé-stallen. De deelnemers aan de prevalentie-studie uit **Hoofdstuk 4** beantwoordden een korte vragenlijst over biosecurity (voornamelijk over externe biosecurity) op het bedrijf, en over het komen en gaan van paarden op het bedrijf. Er waren 86 respondenten. De meeste paardenhouderijen in deze enquete waren privé-stallen op eigen terrein (38%), pension/trainingstallen (36%), of maneges (16%). De overige paardenhouderijen waren fokkerij, renstal, of handelsstal. Paardenhouderijen waar voornamelijk niet-volwassen paarden gehuisvest worden, waren niet meegenomen in deze enquete, en handelsstallen waren ondervertegenwoordigd.
Op de meeste (92%) van de ondervraagde bedrijven werden de paarden een deel van of de gehele dag in groepen gehuisvest. Ongeveer de helft van de ondervraagden voerde geen quarantaine uit voor nieuwe paarden op het terrein. Zes procent van de ondervraagden hadden wel een standaard quarantaineperiode voor alle nieuwe binnenkomers. De overige ondervraagden voerden sporadisch of incomplete quarantaine uit. Geen van de ondervraagden vroegen om bewijs van ziekte–vrij status van nieuwe binnenkomers. Geen van de ondervraagde paardenhouderijen voerde dus de aanwijzingen uit zoals beschreven in een internationale leidraad over droes. Geen van de ondervraagde paardenhouderijen had maatregelen voor paarden die terugkomen op het terrein na tijdelijk op een ander terrein met paarden geweest te zijn (bijvoorbeeld op wedstrijd of voor training), terwijl 54/65 van de bedrijven die deze vraag beantwoordden, aangaven dat er regelmatig paarden voor zulke doeleinden tijdelijk van het terrein af gingen.

De dynamiek van de verspreiding van infectieziekten wordt beïnvloed door de structuur van de populatie. Er is nog weinig bekend over de contactstructuur van paardenpopulaties. **Hoofdstuk 7** begint met een overzicht van alle publicaties over contact netwerken van paarden tot nu toe. Vervolgens is het contact netwerk van Nederlandse wedstrijdpaarden onderzocht. Zowel statische als dynamische (temporele) representatie van het netwerk zijn geanalyseerd. Er werd daarnaast onderscheid gemaakt tussen netwerken gevormd door locaties, en netwerken gevormd door paarden. Hierbij werden paarden geacht met elkaar in "contact" te zijn geweest als zij op dezelfde wedstrijddag aanwezig waren, en wedstrijdlocaties waren in "contact" wanneer er tenminste één paard van de ene naar de andere locatie was gegaan. De contacten tussen de locaties hadden dus een richting, de contacten tussen paarden niet. Netwerken op basis van KNHS-gegevens (sport) en op basis van NDR-gegevens (race) zijn apart geanalyseerd, beide voor het kalenderjaar 2022. Alle netwerken waren volledig verbonden in de statische representatie van het netwerk over het gehele jaar – er waren geen losstaande clusters van paarden of locaties. De diameter (de langste van alle kortste afstanden tussen twee knopen uit het netwerk) van het statische netwerk van paarden dat deelneemt aan KNHS-wedstrijden was vijf stappen. Dit netwerk heeft ook "small world" kenmerken, hetgeen verspreiding van infectieziekten vergemakkelijkt. De statische netwerken waren goed bestand tegen het gericht weghalen van paarden of locaties met een "hub"–functie in het netwerk. De uitzondering hierop was het netwerk van renbanen. Dat betekent dat bij het wegnemen van de voornaamste knooppunten in een netwerk, bijvoorbeeld door gericht bij bepaalde paarden of op bepaalde locaties preventieve maatregelen toe te passen, het effect daarvan op verspreiding van infectieziekten niet in alle netwerken hetzelfde zal zijn.
Bij inachtneming van het relatieve tijdstip van de contacten (temporale netwerk analyse) bleek dat de statische representatie van het netwerk over het gehele jaar de connectiviteit van het netwerk enigszins overschat, zoals dat ook beschreven is voor bijvoorbeeld contact netwerken in de veehouderij.
Een belangrijke beperking van dit onderzoek is dat (voor nu) contacten op de thuisstal van de paarden, contacten ten gevolge van verhuizing, en contacten ten gevolge van trainingen op andere locaties, nog niet zijn meegenomen in deze netwerkanalyses. Desondanks kunnen we concluderen dat met name het sportpaarden netwerk in Nederland zeer sterk onderling verbonden is. Aangezien ongeveer $4/5$ paardenhouderijen paarden huisvest die wel eens op wedstrijd gaan, kunnen we dus ook concluderen dat het contact netwerk van alle paarden in Nederland sterk verbonden is.

De bestrijding van infectieziekten bij paarden zoals *S. equi* en EHV-1 heeft uitdagingen die uniek zijn voor de paardensector. Paarden worden, deels uit welzijnsoverwegingen, gehuisvest in grote groepen, net als vee. Het reis- en contactpatroon van paarden lijkt juist meer op dat van mensen; in de paardensector is heen en weer reizen van en naar locaties gebruikelijk: voor wedstrijden en voor training, of ten gevolge van handel en verkoop. De maatregelen die in de veehouderij effectief zijn, zoals het inrichten van de gehele sector op éénrichtingsverkeer en stricte quarantaine, zijn niet te verenigen met het doel waarvoor de meeste paarden gehouden worden. Het is dus duidelijk dat voor de bestrijding van infectieziekten bij paarden in het algemeen een andere aanpak nodig zal zijn dan in de veehouderij. De (successvolle) bestrijding van in veel belangrijke *humane* infectieziekten leunt voor een groot deel op landelijk gecoördineerde vaccinatieprogramma's. Enerzijds ontbreekt in de paardenwereld een dergelijke gecoördineerde aanpak van EHV-1 en *S. equi* (en veel andere infectieziekten). Anderzijds ontbreekt voor EHV-1 momenteel een vaccin dat effectief de ernstigste klinische verschijnselen verminderd, of de transmissie voldoende reduceert. Voor *S. equi* zijn wel klinisch effectieve vaccins beschikbaar, maar deze vaccinaties dienen frequent herhaald te worden voor blijvende effectiviteit. Vaccineren is daardoor kostbaar en bewerkelijk. De aanpak van infecieziektebestrijding zoals die in Nederland onder mensen wordt uitgevoerd, is dus ook niet zomaar te kopiëren. Voor bestrijding van infectieziekten bij paarden zal dus een eigen, sector–specifieke aanpak nodig zijn. De hippische wereld zal daarvoor zelf moeten zorgen.

De bevindingen en data die uit deze thesis zijn voortgekomen kunnen worden gebruikt als input voor (mathematische) modellen van scenario's voor interventies. Hiermee is een voorzet gegeven voor het toepassen van op data gebaseerde modellen van de bestrijding van EHV-1 en *S. equi*. Dit zal er hopelijk toe leiden dat de aanpak van deze belangrijke infectieziekten in de toekomst nog meer op onderbouwde feiten, en minder op aannames gebaseerd kunnen worden.

PLAIN LANGUAGE SUMMARIES

English

The infectious agents *Streptococcus equi* subspecies *equi* (*S. equi*, the cause of strangles) and EHV-1 (a cause of mild cold-like symptoms, but also of abortion and neurological disease) occur frequently in horses in the Netherlands, as in most of the rest of the world. Research into the best control strategies for these diseases does not currently include (mathematical) models of transmission. This thesis contains several building blocks which will help apply such models to the control effort of these diseases.

The basic reproduction number (R_0) for *S. equi* was estimated from real-world outbreaks, and for EHV-1, we attempted to demonstrate an effect of herd vaccination on transmission potential of the virus, but were unable to show a significant effect. By comparing model outputs to real-world epidemiological data on *S. equi*, we were able to demonstrate that a long-held belief that one in four horses do not mount lasting immunity after strangles may be incorrect.

We surveyed apparently healthy horses and ponies from throughout the Netherlands and found that around 1 in 26 of these were strangles carriers. Combining

this information with 2022 competition records and disease surveillance reports, simulations showed that carriers and susceptible horses very frequently meet at competitions (hundreds of thousands of times per year), but that very few of these contacts lead to infection and disease in the susceptible horses.

A questionnaire survey revealed that very few equine premises in the Netherlands implement effective biosecurity measures, which is similar to reports from other countries. Approximately 4/5 of surveyed premises reported horses temporarily leaving the premises to participate in competitions or for training.

Network analysis using sports and racing competition records revealed that the "social network" of sport horses and of racehorses are each highly connected. The Dutch sport horse network has five degrees of separation, meaning there is a connection between any two horses (that participate in sports competitions) of no more than five steps of horses that have attended the same competitive event at some time during the year.

Horses are group housed like livestock, but travel and socialise like humans. Therefore, the control of equine infectious diseases poses challenges unique to the species. The information gathered in this thesis can be carried forward into data–driven models to evaluate the most (cost-)efficient control strategies for EHV-1 and *S. equi*.

Nederlands

De ziekteverwekkers *Streptococcus equi* subspecies *equi* (*S. equi*, de veroorzaker van droes) en EHV-1 (een veroorzaker van verkoudheidssymptomen, maar ook van abortus en van neurologische klachten) komen regelmatig voor bij paarden in Nederland, en ook in de rest van de wereld. Momenteel wordt er in het onderzoek naar de beste wijze om deze infectieziekten te bestrijden nog geen gebruik gemaakt van mathematische modellen van ziekteverspreiding. Deze thesis bevat enkele bouwstenen die zullen helpen om zulke modellen te gaan gebruiken om infectieziekten bij paarden in de toekomst beter onder controle te krijgen.

Allereerst is het basale reproductie getal (R_0) voor EHV-1 en *S. equi* berekend, op basis van spontane uitbraken. Voor EHV-1 is daarnaast onderzocht of vaccineren van de kudde een significant effect heeft op transmissie van EHV-1, maar we hebben zo'n effect niet met zekerheid kunnen aantonen. We hebben vervolgens voor *S. equi* epidemiologische gegevens vergeleken met resultaten van modellen gebaseerd op de eerder berekende R_0, en concludeerden dat de huidige aanname dat 1 op 4 paarden na droes niet immuun wordt, waarschijnlijk onjuist is.

Onderzoek onder ogenschijnlijk gezonde paarden en pony's in Nederland wees uit dat ongeveer 1 op 26 van hen drager is van droes. Uit simulaties op basis van

gegevens van wedstrijd deelnames in 2022 en gegevens uit droes-surveillance, concluderen we dat er honderdduizenden keren per jaar in Nederland een drager en een niet-immuun paard elkaar treffen op een wedstrijd, maar dat slechts weinig van die contacten ook leidt tot een nieuwe uitbraak.

Met een vragenlijst is een doorsnede van paardenhouderijen ondervraagd over hun biosecurity maatregelen. Slechts een kleine minderheid implementeerde een effectief infectie–controle beleid, iets wat bij verglijkbare onderzoeken in het buitenland ook geconstateerd wordt. Op ongeveer 4/5 van de ondervraagde paardenhouderijen gaan er wel eens paarden tijdelijk van het terrein, bijvoorbeeld om deel te nemen aan wedstrijden of voor training.

Analyse van het contact netwerk van wedstrijdpaarden op basis van deelname aan sportwedstrijd en harddraverijen wijst uit dat deze netwerken zeer sterk verbonden zijn. Voor het netwerk van sportpaarden vonden we dat twee willekeurige paarden nooit meer dan vijf stappen (paarden die op enig moment in het jaar op dezelfde wedstrijd aanwezig waren) van elkaar verwijderd zijn – het sportpaarden-netwerk kent dus vijf "degrees of separation".

De bestrijding van infectieziekten bij paarden zoals *S. equi* en EHV-1 heeft uitdagingen die uniek zijn voor de paardensector. Paarden worden de huisvest in groepen – zoals vee – maar gaan ook vaak op stap en hebben dan nieuwe contacten – zoals mensen. De in deze thesis verzamelde informatie en methoden kunnen gebruikt worden om in de toekomst realistische inschattingen te maken van het effect van verschillende scenario's voor de bestrijding van infectieziekten.

Appendix D

Publications / Acknowledgements / CV

LIST OF PUBLICATIONS IN PEER REVIEWED JOURNALS

Related to this thesis

- Houben RMAC, Newton JR, van Maanen K, Waller AS, Sloet van Oldruitenborgh-Oosterbaan MM, Heesterbeek JAP (2024): Untangling the stranglehold through mathematical modelling of *Streptococcus equi* subspecies *equi* transmission. *Preventive Veterinary Medicine* Epub ahead of publication doi:10.1016/j.prevetmed.2024.106230
- Houben RMAC, van Maanen K, Kemp-Symonds JG, Waller AS, Sloet van Oldruitenborgh-Oosterbaan MM, Heesterbeek H (2023): Estimation of the basic reproduction number for *Streptococcus equi* spp. *equi* outbreaks by meta-analysis of strangles outbreak reports. *Equine Veterinary Journal* 55(3):506-514. doi:10.1111/evj.13865.

Not related to this thesis

- van Eijk NMHA, Houben RMAC, Tellegen AR, Hagen F, Ankringa N (2024): Pulmonary cryptococcoma in a Friesian horse in the Netherlands. *Veterinary Record Case Reports*. 2024;e829. doi:10.1002/vrc2.829
- Houben, Rosa; Vernooij, Hans; Sloet van Oldruitenborgh-Oosterbaan, Marianne (2021): Effect of recording length and posture on the reliability of heart rate variability in horses. *Pferdeheilkunde* 37(6);577-587 doi:10.21836/PEM20210603

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- Samadi S, Wouters IM, Houben R, Jamshidifard AR, Van Eerdenburg F, Heederik DJ (2009): Exposure to inhalable dust, endotoxins, beta(1->3)-glucans, and airborne microorganisms in horse stables. *The Annals of Occupational Hygiene* Aug;53(6):595-603. doi:10.1093/annhyg/mep040.

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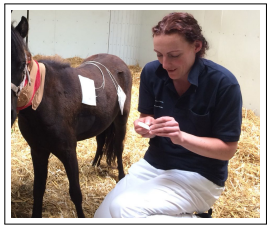
Erik. "*I don't mind you coming here ... and wasting all my time.*"

ABOUT THE AUTHOR



After her VWO Diploma at Stedelijk Gymnasium Nijmegen in 2001, Rosa went on to study Veterinary Medicine at Utrecht University, graduating in 2008. After graduation, she worked as a first opinion ambulatory equine veterinarian in Wales, as a hospital intern in private practice in Emmeloord, as an in-house stud veterinarian in Scotland, and again as a hospital intern, this time in an academic setting, at Liverpool University's Philip Leverhulme Equine Hospital. In 2011 she enrolled in European College of Equine Internal Medicine (ECEIM) residency training at the Pôle Equin – Clinique Vétérinaire Universitaire at Liège University, Belgium. She returned to the equine hospital of her *alma mater* in 2015 and became an ECEIM Diplomate in 2016. Her work has since been predominantly as a senior clinician at the equine internal medicine section of the Clinical Sciences Department of the faculty of Veterinary Medicine of Utrecht University.

Parallel to the clinical training and work, Rosa has kept a keen interest in computer science and in data analysis. In 2007, during a prolonged break between clinical rotations as a student, she started a bachelor's degree in Artificial Intelligence at the computer science department of the Vrije Universiteit in Amsterdam. After finishing her veterinary degree, she has followed courses in R programming, data analysis, statistics & medical epidemiology, data visualisation, and more statistics. After attending a summer course on infectious disease modelling at the London School of Hygiene and Tropical Medicine in 2015 (and winning a prize in the process!), she started noticing the scarcity of modelling–informed policy–making in equine infectious disease control efforts. In 2019, she was offered the opportunity to undertake a part–time PhD alongside her clinical and teaching duties. She chose to focus on mathematical modelling of the equine infectious diseases that had the most impact on her day–to–day clinical work: *S. equi* and EHV-1.