Rebounding Case Notifications of Chlamydia:

An Epidemiological Game of Clue?

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By

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Abstract

The genus *Chlamydiae* encompasses a unique class of obligate intracellular bacteria that can cause disease in a wide range of animals. In humans, *Chlamydia trachomatis* infections are common and are frequently observed in diseases of the eye, genital and respiratory tracts. Prevalent worldwide, Chlamydia infections can progress to chronic inflammatory sequellae and are the leading cause of curable sexually transmitted disease and preventable blindness. After falling in the face of intensified control efforts, case notifications of sexually transmitted Chlamydia in many countries are rising. In many jurisdictions, this unprecedented rise of Chlamydia case notifications has occurred after the introduction of wide spread control programs, and has been discussed to be a result of either increased testing volume, improvements to testing technologies, changes in sexual behaviour, or increased reinfection rates brought about by deleterious effects of treatment on acquired immunity. This thesis seeks to answer the question of why are observed Chlamydia case notifications rebounding? I have attempted to answer this question using simple dynamical models of Chlamydia transmission framed from immunological and epidemiological perspectives. Model structures are drawn from frameworks previously used for studying sexually transmitted infections, and represent a combination of theoretical and data-oriented formulations, as well as different (hierarchical) ecological scales. The results of this thesis highlight that increased testing volumes, rather than changes in the sensitivity and specificity of testing technologies, sexual behaviour, or truncated immunological responses brought about by treatment can explain the increase in observed chlamydia case notifications, and that simple explanations for these observed rates appear to have been dismissed in favor of an increase to the underlying prevalence. In addition to providing insights into current epidemiological trends, this thesis has also been able to produce significant insights into the natural history of chlamydial infection. In particular, the phenotype of an individual's immunobiology that results from multiple chlamydial infections suggests that longer periods between initial and repeat infection may increase an individual's chlamydial load, their duration of infection, as well as non-intuitively the formation of protective immunity, persistent infection, and the potential for immunopathogenesis. Additional population-scale analyses suggest the existence of a period of immunity that is, on average, much longer lasting than currently discussed in contemporary literature. The results of this research outline a potential way forward through filling several gaps in the immunological and epidemiological understanding of Chlamydia infections that involves both reviewing existing data as well as continued research using "systems science" approaches.

Acknowledgements

I would like to thank my External Examiner (Dr. Hazhir Rahmandad), my supervisor (Dr. Nathaniel Osgood), and my committee members (Drs. Cory Neudorf, Jo-Anne Dillon, Ann Jolly, David Fisman, and Carl D'Arcy) for their guidance, instruction, feedback, and encouragement throughout my thesis research. I have learned a lot, and this completed thesis is a direct result. All remaining mistakes in the thesis are mine.

Offer a cad lung shows. Yuk!

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Ab	Antibody
Ag	Antigen
CTL	Cytotoxic T-Lymphocyte
DC	Dendritic Cell
EB	Elementary Body
IDO	Indoleamine-2,3-dioxygenase
IFN-g	Interferon-gamma
LN	Lymph Node
NAAT	Nucleic Acid Amplification Test
PID	Pelvic Inflammatory Disease
RB	Reticulate Body
SEIRS	Susceptible-Exposed-Infected-Removed-Susceptible
SEIRS	Susceptible-Exposed-Symptomatically-Infected-Asymptomatically-Infected-Removed-Susceptible
SIR	Susceptible-Infected-Removed
SIS	Susceptible-Infected-Susceptible
SITRS	Susceptible-Infected-Treated-Removed
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
Th1	Type-1 T-Helper Cell
Th2	Type-2 T-Helper Cell

A NOTE TO THE READER:

With the exception of Chapters 1 and 6, this thesis is made up of four related manuscripts: two that have been published in peer-reviewed journals:

- "Study 1" (i.e., Chapter 2) is published in *BMC Infectious Diseases* 2010, 10: 70 entitled: "Current crisis or artifact of surveillance: insights into rebound chlamydia rates from dynamic modelling";

- "Study 3" (i.e., Chapter 4) is published in in *PLoS ONE* 2009, 4(9): e6886 entitled: "Immunobiological outcomes of repeated chlamydial infection from two models of within-host population dynamics". It should also be noted that this paper was co-authored by another graduate student of Dr. Osgood. This student (Qian Zhang) was responsible for the analytical study of the equations presented in the paper;

one that has been peer-reviewed, and is at the "revise-resubmit" stage:

- "Study 4" (Chapter 5) with the journal *Theoretical Population Biology* as an Original Article entitled: "Endogenous reinfection and the dynamics of sexually transmitted infections: local effects of treatment in an immunoepidemiological model of transmission" (also currently being revised for a June 2011 resubmission);

and one that is under peer-review:

- "Study 2" (Chapter 3) with the journal *Sexually Transmitted Infections* as an Original Article entitled: "A comparative model-based method for estimating the duration of chlamydial immunity".

It should be noted that the manuscripts presented in the main text of this thesis (Chapters 2-5) were not the only works pursued throughout my PhD training. Included in an Appendix is an account of some additional theoretical work that was published in the *Journal of Theoretical Biology and Medical Modelling 2007, 4: 49.* This initial work, incidentally, formed the basis of the research presented in Chapter 5.

Chapter 1

Introduction: Background and Basic Concepts

It is obvious that the character of a specific disease must be understood thoroughly in order to model it [89]. Because the subject matter of each of the following sections is vast, I have not attempted to provide an exhaustive review of the literature. Instead, I have tried to communicate, at least in my opinion, the most important concepts of chlamydial infections, and how these impact our understanding of its epidemiology and the study of sexually transmitted infections (STIs) in general. Chlamydia incidence, prevalence, transmission, host-pathogen interactions, and pathogenesis are described in sections 1.1 to 1.2. Current control policies, as well as their definitions, are described in section 1.3. Four types of models for studying infectious disease transmission are presented in section 1.4; two of the modelling frameworks are more traditional, while two, for the study of STIs, are relatively novel. Based on my review of previous modelling work, it is evident that different types of models can yield different levels of information suitable for obtaining a thorough understanding chlamydia epidemiology. It should be emphasized that the information obtained from one level of modelling might inform others. Specific remarks are made throughout the chapters of this thesis, and a unifying statement of its content is outlined in Chapter 6.

1.1 The Magnitude of the Problem

Sexually transmitted infections exhibit a large degree of heterogeneity in their transmission dynamics, most of which is likely due to behavioural variability in sexually active populations [120]. In contrast to all other commonly-reported STIs, *Chlamydia trachomatis* represents a unique and important public health concern [29], as prevention efforts are hampered by cryptic infections and delayed diagnosis [146]. This ability to thrive has made it the world's most common cause of curable sexually-transmitted diseases [208]. As seen in figure 1.1 genital chlamydia is the most frequently reported disease in Canada [164]. In 2004, approximately 59,325 cases of genital chlamydia were reported in Canada [164]. Compared to 1991 and 1996, this represents a 29% and a 71% increase in reported cases, respectively (see figure 1.2). Sexually-active youth and young adults (aged 15-24 years) disproportionately account for approximately two-thirds of nationally-reported cases, most of which are amongst females [163]. Risk factors for infection include (unprotected) sexual contact
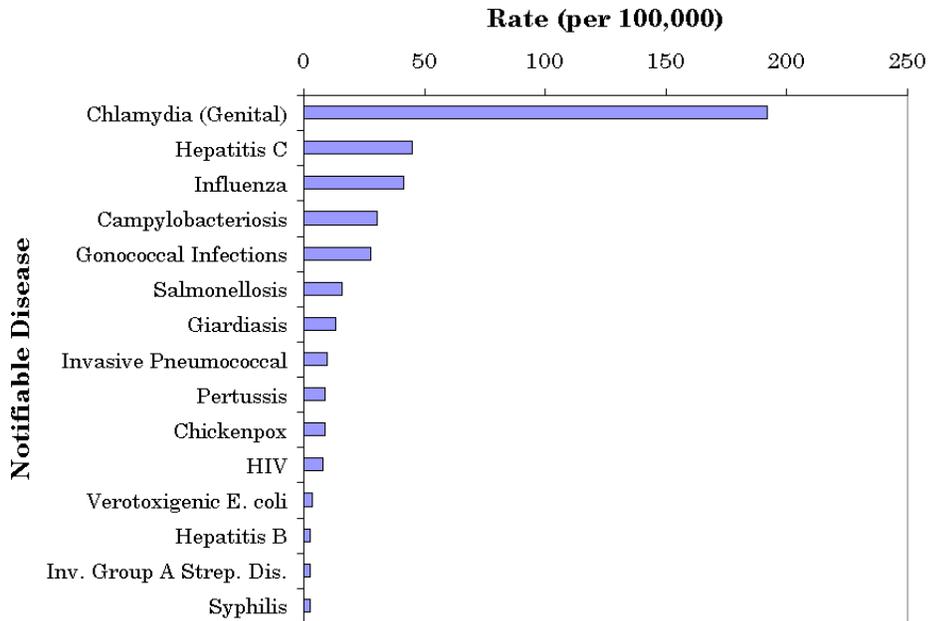
Figure 1.1: Incidence rates of the fifteen-most reported infectious diseases in Canada in 2004. Figure is modified from Notifiable Diseases On-Line [164].

with an infected person, having a new sexual partner or multiple sex partners, previously having an STI, or being a member of a vulnerable population (e.g., injection-drug users, sex-trade workers, or street youth) [164].

Sexually transmitted Chlamydia is an important public health concern largely because of its adverse effects on reproduction [208]. In women, untreated Chlamydia infections can result in Pelvic Inflammatory Disease (PID) and can have long-term consequences such as scarring of the fallopian tubes and ovaries, ectopic pregnancy, chronic pelvic pain, and infertility [29]. In addition, infection with Chlamydia has been demonstrated to facilitate HIV transmission [166] and may likely be an important correlate in human papilloma virus (HPV)-induced cervical neoplasia [7].

Because of the association with other equally serious STIs, as well as the possibility of undesirable sequellae, large-scale chlamydia control programs have been implemented in many developed countries. Generally these include detecting infected individuals through diagnostic testing, followed by antimicrobial treatment and contact-tracing of individuals who might have been exposed through sexual contact with the infected person [29, 163]. Although these control programs might, in part, be managing chlamydia infection, they may fail to reach large proportions of the population [125, 138]. They may also be making matters worse.

Since the initiation of chlamydia control programs, a common and distinct epidemiological pattern in Chlamydia rates has emerged throughout many developed countries: incidence rates are increasing [31], however the mechanistic details involved in this rise may differ across countries. For
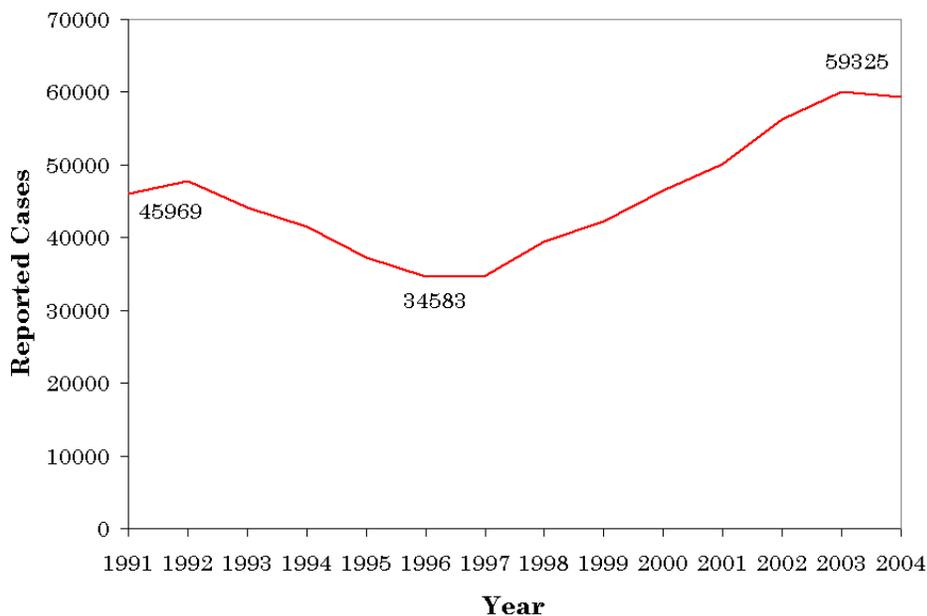
Figure 1.2: Number of reported cases of genital chlamydia in Canada between 1991 and 2004. Case counts within the figure are at the earliest time point available (1991), the year chlamydia rates were at their lowest (1996), and the last year data was available (2004). Figure has been modified from Notifiable Diseases On-Line [165].

example, in Sweden a novel genetic Chlamydia variant has emerged that, in the previous four years accounts for over 65% of the reported cases [88]; in Finland, decreasing population seroprevalence of anti-Chlamydia IgG antibodies is thought to have increased population susceptibility to infection with Chlamydia [127]; in Canada, increasing reinfection rates [29], improved detection [53], and dynamic changes in sexual behaviour with sexual networks [48, 220]. In Norway, Sweden, Finland, and Canada case rates of chlamydia were in decline for nearly a decade following the introduction of mass control programs [31]. Unfortunately, despite enhanced control efforts, these declining trends have recently been upturned by increasing chlamydia case counts that exceed those recorded before large-scale intervention strategies were implemented [31]. In contrast, chlamydial rates in the U.K., the U.S., and Australia appear to have been steadily increasing since implementing their respective programs without an initial decrease [40, 94, 196]. The causal mechanism driving these trends remains to be specifically determined.

To date, the above outlined epidemiological trends have led researchers to advance seven hypotheses as to why these increasing incidence rates may be occurring. A connected statement of these hypotheses can be found in Rekart and Brunham [175], and are reproduced in table 1.1. Of these seven hypotheses highlighted, the first four focus on direct consequences of switching from culture-based testing, to immunofluorescence, and more recently to nucleic acid amplification test

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(NAAT) methods. The first hypothesis (H1 in table 1.1) argues that because of the lower specificity of NAAT methods compared to culture and immunofluorescence methods (94%, 98%, and 100%, respectively), increased rates are due to an increased number of false positives. The second through fourth (H2-H4 in table 1.1) hypotheses base their argument on improved case detection techniques, where, because of higher sensitivity, higher patient-acceptance rates (especially among male patients), and enabled self-collection will lead to higher testing rates and targeted screening among high risk populations. There is no lack of epidemiological evidence to support H2-H4 (e.g., [35, 40, 66, 80, 136]). Hypothesis 1, however, is likely the least defensible cause of increased chlamydia rates, as its overall impact is unlikely to carry any significant weight [175]. The fifth and sixth hypotheses are remarked to have mixed evidence, and may be subject to denial or modification as central causes of rising chlamydia rates [175]: the fifth (H5), focuses on treatment failures as a result of decreased susceptibility of chlamydia bacteria to antimicrobials. Though reported to be a concern for syphilis and gonorrhea infections, treatment failures for chlamydia infections are rarely reported [31] – in B.C. this is reported to occur on the order of two treatment failures per year; the sixth (H6), involves population-wide changes in safe sexual practices. The declining chlamydia rates in the late 1980s and early 1990s are thought to be a subsequent result of a reduction in risky sexual behaviour in response to safe-sex campaigns against the threat of HIV/AIDS. On this basis, the recent increase rates of chlamydia are argued to be a result of a decreased risk perception of HIV infection followed by a decline in safe sex practices [125]. The defensibility of this hypothesis is bolstered by the decline of chlamydia rates, in the 1980s and 1990s, in countries without mass chlamydia control programs [125]. The seventh hypothesis (H7), designated the *arrested immunity hypothesis*, states that treating chlamydia too early in the course of infection disrupts the formation of protective in-host immune responses. Though only previously demonstrated in animal models [190], the tenets of the arrested immunity hypothesis have been largely derived through simulation modelling [29]. Recent research has credited this hypothesis as the source of treatment failures in Vietnam [12] and declining geographic seroprevalence in Finland [127]. However, the degree to which it holds in human immunology remains to be determined [29, 100, 175].

The largest obstacle to determining the merit of each of the above hypotheses lies in the fact that the prevailing epidemiological trends have, in part or entirely, been based on routine surveillance data [125, 138]. Overall, routine surveillance data of notifiable infectious diseases is an excellent source of descriptive information. However, data that are often collected via routine surveillance usually do not contain detailed epidemiological information and therefore will place important limits on the insight that can be gained. Some have argued that changes in the number of people being tested (and re-tested), changes in diagnostic test performance, and the risk profile of the people being tested could have equally likely produced the observed surveillance trends even if the underlying burden of chlamydia has not changed [138]. Determining whether, or not, the control of

H1.	More false positive tests because NAAT methods have lower specificity than culture methods.
H2.	NAAT testing results in increased case detection due to better sensitivity than non-NAAT methods.
H3.	NAAT testing of urine is more acceptable, especially among men, leading to higher testing rates.
H4.	NAAT methods allow female self-collected specimens and targeted screening among persons at high risk.
H5.	Decreased chlamydial susceptibility to antimicrobials.
H6.	Increased rates of unsafe sexual practices due to reduced threat of HIV infection.
H7.	Arrested Immunity because of treatment with antimicrobials.

Table 1.1: Seven Hypotheses of Increased Chlamydia Rates

chlamydia is being fettered by current prevention programs, via the use of surveillance data, will be challenging given many measurement issues [138]. While perfect quantification of the true burden of disease will likely never exist, performing research that makes use of contextual information (e.g., changes in test volume, diagnostic test used) from multiple data sources will help provide meaning into observed trends.

1.2 The Infected Person

The genus *Chlamydia* encompasses a unique class of obligate intracellular bacteria that can cause disease in a wide-range of animals [17]. In humans, Chlamydia can cause ocular trachoma and several sexually transmitted diseases [29]. It has 18 main serovars (or strains), as determined by DNA-sequence analysis and immuno-typing of the major outer-membrane protein (MOMP see table 1.2) [29, 152]. Although inconsistent, some previous studies have found a correlation between associated human disease, clinical symptoms, and particular serovars [149–152]. With respect to human diseases, serovars A, B, Ba, and C cause trachoma a leading cause of blindness worldwide [208] whereas serovars D, Da, E, F, G, H, I, Ia, J, Ja, and K are primarily responsible for sexually transmitted disease [29]. Serovars L1, L2, L2a, and L3 cause lymphogranuloma venereum [142].

Chlamydia normally infects the single-cell columnar layer of the epithelium in the endocervix of women (see figure 1.3) and the urethra of men [29]. At the site of infection, inflammation of the mucosa is characterized by redness, edema and discharge can occur resulting in the clinical syndrome of mucopurulent cervicitis in women [29, 162, 163]. Clinical symptoms of can include urethral discharge, burning on urination, irritation in the distal urethra or meatus, while more serious sequellae (e.g., genital ulcer disease or PID) dysuria, abnormal vaginal discharge or menstrual
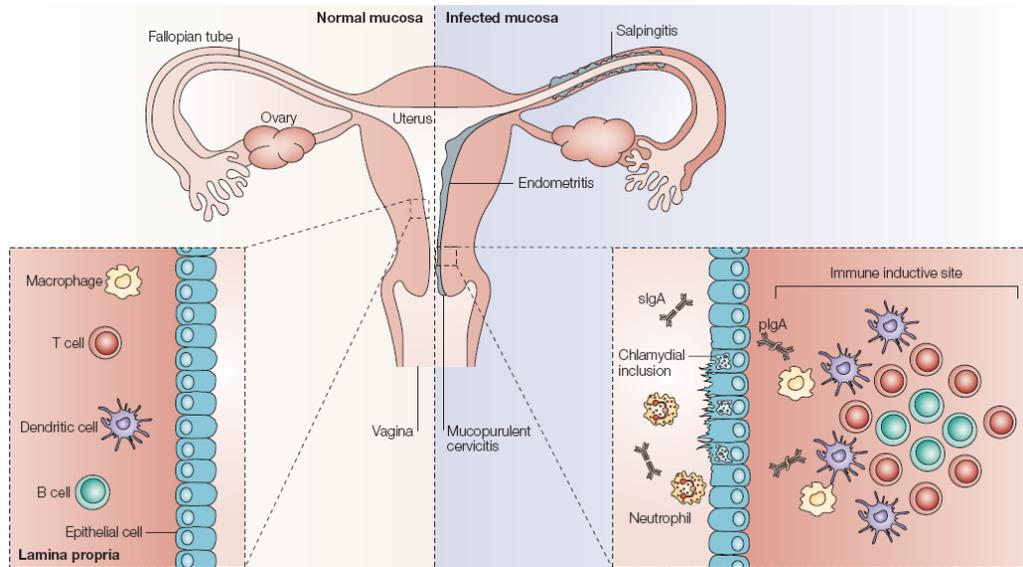
Figure 1.3: Infection of the Female genital tract with *Chlamydia trachomatis*. The inflammatory reaction is characterized by an influx of macrophages and neutrophils, as well as inductive sites that contain B and T cells, dendritic cells, and the deployment of secretory and polymeric IgA antibodies (modified from Brunham and Rey-Ladino [29]).

bleeding, postcoital bleeding and lower abdominal pain are likely to be reported [163]. In 20 to 40% of untreated women, infection ascends the endometrial epithelium to the fallopian tubes, where Chlamydia can establish persistent infection and cause PID [29]. Overall, approximately 11% of women with PID develop tubal factor infertility and 9% develop ectopic pregnancies [41]. The overall rate of these complications has recently been deemed susceptible to measurement biases [61], and a recent survey of the literature demonstrates that the percentages of infertility and ectopic pregnancies resulting from Chlamydia infections may, in fact, be lower than previously thought [202]. Interestingly, this risk seems to be higher for those with PID caused by infection with Chlamydia compared to PID caused by other factors, such as infection with *Neisseria gonorrhea* [28].

Chlamydia spp. undergo a unique development cycle that can infect neighbouring epithelial cells (see figure 1.4). *Chlamydia trachomatis* is an obligate intracellular pathogen that resides within a specialized vacuole and has a biphasic developmental cycle [17]. An infectious, but metabolically inactive, elementary body (EB) is taken up by mucosal epithelial cells. After internalization, the EB is surrounded by an endosomal membrane to form an inclusion a vacuole formed from normal endosomal-trafficking pathways which creates a permissive intracellular niche for the replication of Chlamydia [50]. Within the inclusion, the EB transforms into a larger metabolically active reticulate body (RB), which divides by binary fission. Within 40 to 48 hours, the RBs transform back into infective EBs, which are subsequently released from the inclusion vacuole to infect neighbouring
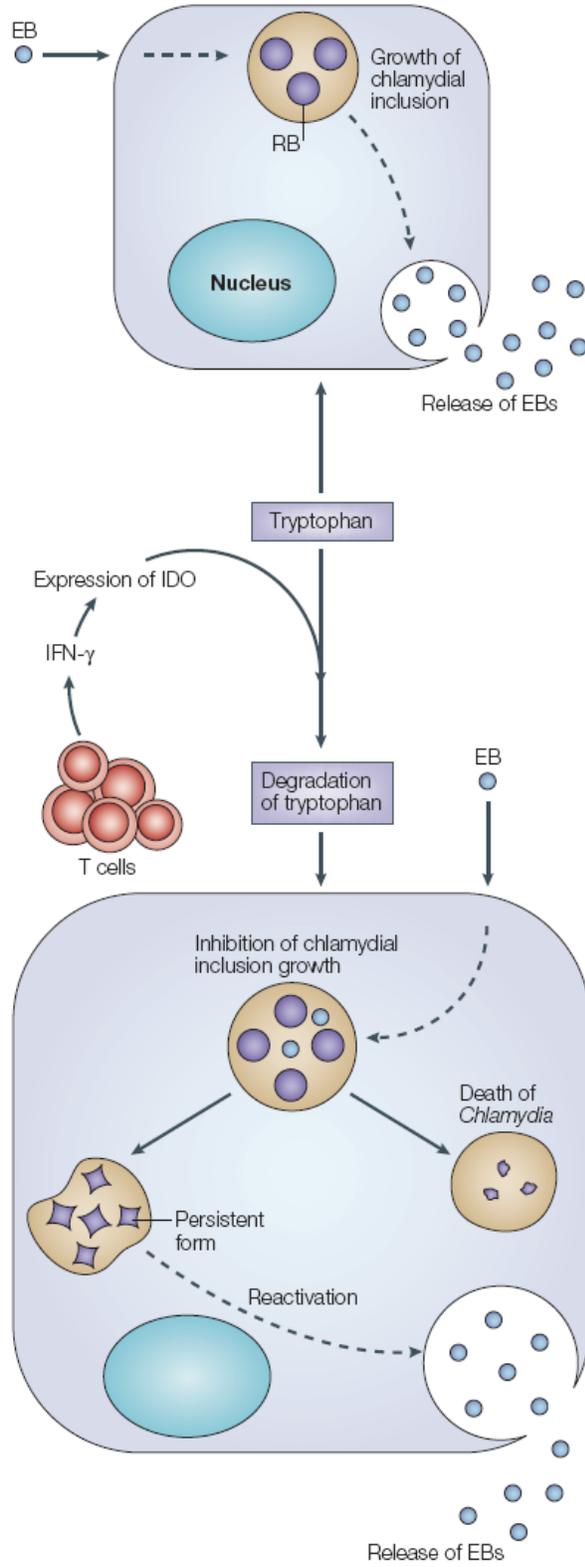
Figure 1.4: The developmental cycle of and T cell response against *Chlamydia trachomatis* (modified from Brunham and Rey-Ladino [29]).

cells. In the presence of growth inhibitors, such as interferon (IFN)-γ, intracellular Chlamydia bacteria acquire a non-replicating, persistent form, and bacteria in this form differentiate back into infectious forms after removal of the inhibitor [121].

The current understanding of chlamydial immunology has recently been thoroughly reviewed elsewhere [29, 56, 146]. These more-detailed collections can be readily accessed by the interested reader. Given recent feedback from my advisory committee, focus on immunological components has become secondary to larger-scale, more appropriate, epidemiological components. Although a more implicit consideration of chlamydia immunology will be carried out for my thesis research, I still, for the purposes of completeness, discuss the current understanding of chlamydial immunology, at a high level.

Inference by analogy with animal models demonstrates that Chlamydia-specific immune responses occur not only at mucosal inductive sites, but also at more distant peripheral lymph nodes [29]. Sampling of microbial antigen (Ag) across epithelia is accomplished by migratory Dendritic Cells (DCs) that carry Ag to peripheral lymph nodes (LNs) and present it to naive T cells [29]. Toll-like receptors (TLRs) expressed on the surface of DCs detect microbial infection and have an essential role in the induction of innate and adaptive immune responses [97]. After infection with *Chlamydia* spp., epithelial cells of the genital tract produce various pro-inflammatory mediators (such as CXC-motif chemokine ligands, CXCL, 1, 8, and 16, granulocyte-monocyte colony-stimulating factor (GMC-SF), interleukin (IL)-1 and -6 and tumor-necrosis factor, (TNF)) [29, 101, 173], up-regulate the expression of specific other chemokines (CC-motif chemokine ligand 5 and CXCL10), as well as secrete cytokines that promote the production of IFN-γ, IFN-α, IFN-β and IL-12 [101, 132]. Together, these chemokines and cytokines have been demonstrated to trigger an inflammation cascade that promotes the recruitment of lymphocytes to the site of infection [139].

Studies from animal models of infection have also clearly established that T cells (CD4+ T_H1 cells, in particular) are crucial for resolving chlamydial infections [146, 147, 189]. IFN-γ produced by T cells induces the expression of indoleamine-2,3-dioxygenase (IDO) which degrades, and therefore depletes, cellular tryptophan [121] (see figure 1.4). The reduced cytosolic tryptophan then leads to the death of chlamydial RBs via starvation. Effector CD8+ T cells (CTLs) have also been demonstrated to be involved in resolving chlamydial infections (see review in [212]). However, the specific role of the effector mechanisms of chlamydia-specific CTLs is somewhat unclear [147, 189].

Although considered important, the role of antibody (Ab) in protective immunity against chlamydial infections has been demonstrated to have more of a secondary role to IFN-γ-related resolution. It has been suggested that B cells and CD4+ T cells may function synergistically in providing immunity against chlamydia [147]. High titres of IgA and IgG of anti-Chlamydia Ab do not appear to correlate with resolution of infection [99], and appear to be more likely linked

to increased severity of secondary sequellae that result from type II or III hypersensitivities [139]. Rather B cells, and by extension Ab, are thought to be important in controlling reinfection through Ab-mediated neutralization, instead of resolving primary infection [29].

Despite generating and mobilizing a vast arsenal of immune mechanisms, infections with Chlamydia can be recurrent or prolonged [121]. This may reflect the abundant mechanisms available to Chlamydia bacteria to evade the immune system [17]. Such intracellular mechanisms include: enhanced survival both outside and inside host cells [27, 49, 50], reduced inflammatory and adaptive immune responses [29], and the ability to persist in alternative intracellular forms [121]. Immune-avoidance mechanisms might also contribute to pathogenesis and tissue damage, by inducing persistent infection and by enhancing susceptibility to re-infection [29].

Because Chlamydia infections can readily be treated with antibiotics, little is known about the proportion of infections that resolve without therapy, the time line until resolution, or the factors that are associated with spontaneous clearance. Only a few prospective and retrospective studies of Chlamydia infections have been performed – most of them with low sample sizes and short follow-up [142]. From what little evidence from human populations there is, resolution of Chlamydia infections appears to be dependent upon the time line for which a population is followed-up. One smaller study of 74 patients attending an STI clinic in the U.S. demonstrated that 32% had negative follow-up cultures within 45 days of initial observation [160]. Two earlier European studies have also demonstrated a clearance rate of 16% in three months [168] up to 44.7% of infected people per year [152]. More recently, a 5-year follow-up study has been able to reproduce, and elaborate upon, previous findings [142]. In a cohort of 82 women, an evident inverse time-positivity pattern can be observed: approximately 53% of patients had resolved infection at 1 year, 81% at 2 years, 90% at 3 years, and 93% at 4 years.

In each of these studies, declining Chlamydia positivity was associated with increasing age of patients [142, 152, 160], age at first intercourse [142], and previous STI infection [142, 152]. When compared to patients without persistent infection, patients who were infected with Chlamydia reported the same number of self-reported complaints (such as abnormal vaginal discharge, intermenstral bleeding, postcoital bleeding, frequent urination, dysuria, and lower abdominal pain) [152]. Resolution of Chlamydia infection has not been associated with civil state (i.e., marital status), use of oral contraceptives [152], the overall and new number of sex partners, and a partner's STI infection status [142, 152]. Where available, serovar analyses have demonstrated that serovars D, E, F, and G are the most abundantly detected among Chlamydia infections [142, 152]. Persistence of infection was most observed with types B, D, and E [142, 152].

Chlamydia Serovar	Disease	Method of Transmission
A-C	Ocular trachoma	Hand to eye, fomites, and flies
D-K	Oculogenital disease	Sexual and perinatal
L1-L3	Lymphogranuloma venereum	Sexual

Table 1.2: Human *Chlamydia trachomatis* (*Chlamydia*) serovars and their respective diseases (reproduced from [29]).

1.3   Control Measures

There are numerous activities that can be considered control measures. Throughout this section, I will largely be referring to primary and secondary prevention efforts such as screening (i.e., detecting and treating), contact tracing, and partner notification. Primary prevention strategies help ensure early and efficient detection; it allows treatment to be administered in a timely manner; it shortens the duration of an individual's infection; and attempts to prevent reinfection. Secondary prevention is a direct consequence of primary prevention; it attempts to reduce the risk of transmission to susceptible sexual contacts in the population through partner notification and treatment of infected sexual contacts [129].

Historically, detection of chlamydia within infected individuals relied on the presence of symptoms, the use of invasive testing methods, and insensitive (though very specific) diagnostic tools [163]. Recent development of more sensitive (though less specific) testing technologies such as immunofluorescence and nucleic acid amplification has produced an appreciation of the asymptomatic reservoir of many commonly reported STIs. These noninvasive diagnostic tools have also appeared to have mitigated the general unwillingness of the population, particularly males, and primary care physicians, to present for and to conduct testing, respectively [19]. As a direct result, this has allowed a larger proportion of the population to be tested, and thus more infected individuals to be detected.

Although screening is epidemiologically well defined, screening programmes, as applied to chlamydia control, has no concise or universal definition [124]. Current designs of screening programmes against chlamydia can be structured around two main definitions: proactive or opportunistic [103, 124] (see table 1.3). Proactive screening – breast cancer screening in Saskatchewan for example – contacts people from a defined registry of people at risk. Those who attend are recorded as uptake and non-attendees are contacted to attend. Opportunistic screening targets those, and only those, who attend a health care facility. As a result, continual and regular re-screening is unlikely to occur. Current proactive screening programmes that have been evaluated have focused their efforts on particular high-risk populations, such as adolescents [20, 51, 118], and have implemented some innovative methods of screening and re-screening to ensure patient acceptance and

adherence [186].

Currently in Canada, there is no dedicated national proactive screening programme to address the important reservoir of asymptomatic Chlamydia infections [125]. Rather, a more-opportunistic set of guidelines have been synthesized as a resource for clinical and public health professionals [19, 163, 164]. Though not to be construed beyond suggested practices, these guidelines place an emphasis on both primary and secondary prevention measures, and are applicable for preventing and managing STIs across diverse patient populations [129]. The remainder of this section is a summary of the proposed guidelines in how they relate to detection, treatment, prevention, reporting, and partner notification of chlamydial infections (see [163, 164] for comprehensive compilation of STI guidelines).

For routine chlamydial infections, laboratory testing is the standard means for detecting Chlamydia infections. Results of laboratory testing are highly dependent upon the type of test available [164]. Due to its noninvasive nature, urine-based NAAT methods are recommended to be the primary source for testing asymptomatic individuals when more invasive procedures, such as pelvic exams, cervical or urethral swabs, are not warranted [164]. Though useful in infants aged less than 3 years, anti-chlamydial serology is not recommended for the diagnosis of acute genital Chlamydia infections.

When indicated (i.e., a positive chlamydial test, presence of symptoms compatible with chlamydial infections prior to return of test results, diagnosis of chlamydia in a sexual partner, or coinfection with gonorrhea), treatment for Ct infections successfully cures infection. Preferred treatment regimens prescribe Doxycycline or Azithromycin [163]. Alternative treatment regimens include Ofloxacin or Erythromycin [163]. Drug resistance is rare, but remains a concern [31, 140, 141, 185]. Because of the high efficacy of primary treatment regimens, as well as their short duration, test-of-a-cure is not routinely needed – especially if signs and symptoms disappear and the patient has adhered to treatment recommendations [164]. However, repeat testing of all individuals with previous Chlamydia infection within 6 months should occur, as the probability of reinfection is high.

Chlamydia trachomatis is a notifiable infection in all Canadian provinces and territories [164]. All partners reported to have had sexual contact with an index case within the previous two months, either prior to onset of symptoms or date of diagnosis (if asymptomatic), should be tested and treated regardless of clinical findings and test results. Partner notification, when deemed suitable by public health professionals, includes appropriate referrals for clinical evaluation, testing, treatment, or health education. In the event of limited public health resources, it is recommended that the priority for partner notification be directed toward high-risk populations (i.e., adolescents and young adults younger than 25 years of age) [164].

Screening	Members of a defined population, who may not know they are at risk of a disease or its complications, are asked a question or are offered a test to identify those who are more likely to be helped by further testing or treatment
Screening Programme	A continual public health service that ensures screening is delivered at regular intervals to a high proportion of the target population to achieve defined levels of benefit at the population level.
Proactive Screening	Population registers are used to invite members of the population at risk to be screened at appropriate intervals.
Opportunistic Screening	A health professional offers a screening test to patients obtaining health care for unrelated reasons. The onus is on the health care professional to repeat the test at appropriate intervals.

Table 1.3: Definitions of Screening and Screening Programmes (Modified from [124]).

1.4   Modelling Infection Transmission

"All models are wrong, but some are useful." – George Box and Norman Draper

Planned experiments are an effective means of obtaining information in many sciences. However, experiments with infectious diseases in human populations are generally not possible for obvious ethical and practical reasons; repeatable experiments and the data they can generate, are for the most part, not available in epidemiology [113]. Mathematical models allow one to perform needed theoretical experiments to better understand the biological and sociological mechanisms that influence infectious disease transmission [4, 5, 58, 89]. They also furnish a means of assessing quantitative conjectures and evaluating control procedures [188]. In this respect, the fundamental role of mathematical models is to develop an understanding of a complex system in a very concise way [57, 113].

A model's structure demonstrates an idealization of the causal mechanisms of a system. While mathematical models can provide significant contribution to the understanding of infectious disease transmission and control, it should be recognized that any given class of models will be unsuitable for answering many important questions [89]. As discussed below, model structure, stratification, and resolution, should ideally depend on the particular questions being asked by the researcher and the function of the proposed model. Often, a law of diminishing returns applies as more detail is added to a model: simpler measures often correlate well with more-detailed levels of information [4].

In some cases, simple models will suffice, while in others, very complex models are required [58]. As a result, the most difficult problem faced by a modeller is finding the right combination of an interesting question, obtaining adequate and relevant sources of available data, and a mathematical model which can lead to an answer [89].

In STI epidemiology, mathematical models allow for a description of individual risks for acquiring and transmitting infection [21, 57, 63, 112, 116], patterns of sexual behaviour and pathogen biology to compare with observed patterns in the population (see review in [57]), as well as consequences of health policy [59, 207]. Mathematical models can serve a number of different purposes. The most important are to: delineate basic principles and processes underlying transmission; to determine what needs to be measured to both interpret epidemiological patterns and assess the impact of defined interventions; to help define the most important determinants of observed patterns; to help design and evaluate different interventions; and to test well-defined hypotheses [4].

Given the different epidemiological, behavioural, and proposed immunological characteristics of chlamydia, there are several convenient ways to model the transmission of chlamydia. Where possible, I have tried focusing the discussion of each around a useful epidemiological concept: the basic reproductive number, R_0. Each of these is discussed in the following subsections.

1.4.1 Population-Level Compartmental Models

The main focus of mathematical models is the incidence of infection at the level of the population [58]. Traditionally, for the description of the transmission dynamics of an infectious disease, it has been convenient to divide the population into mutually exclusive classes, or compartments, with which numbers, or proportions, of the population "flow" through over time [5]. The susceptible class, S, contains those who have not yet become infected, the exposed class, E, contains those who are in period of latent infection, but are not yet infectious, the infectious class, I, and the removed class, R, who have acquired some degree of protective immunity. These compartmental, or population-level models, are often titled by a sequence of letters that describes the movement of individuals between classes: to model infections that confer long-term immunity, SIR or $SEIR$ models have been appropriate, whereas for models for infections that confer short-lived immunity, $SIRS$ or $SEIRS$ models are appropriate, and diseases that do not confer any protective immunity are best described by SIS or $SEIS$ models [89]. For almost all STIs, SIS models have formed the basic and most studied mathematical templates [57]. This is largely because they capture factors that influence the observed epidemiology that are common to all STIs. Under an SIS model, the progression of a generic STI is represented by a generic system of differential equations:

$$\dot{S} = \delta I - \lambda S \tag{1.1}$$

$$\dot{I} = \lambda S - \delta I. \tag{1.2}$$

The other parameters in equations (1.1) to (1.2) are the rate of recovery from infection, δ, and the rate at which susceptible individuals become infected, λ (also called the force of infection or incidence rate) [5, 82]. The force of infection is an aggregate parameter that is determined by product of the fractional prevalence of infection in the population, $\frac{I}{N}$ (where N is the population size $= S + I$), the probability of transmission within a sexual partnership, β, and the number of new sex partners the infected person has per unit of time, c:

$$\lambda = \frac{I\beta c}{N}. \tag{1.3}$$

The effects at the population level are controlled by the behaviours of individuals. Important insights into the transmission of STIs can be gained from an understanding of simple endemic and epidemic thresholds [5]. This includes the notion of an "infectee" or "reproductive" number as a measure of transmission success [89]. This number, often designated R_0, is defined as the average number of secondary infections that are produced by one infectious individual in an entirely susceptible population [5] prior to their recovery. Spread and persistence occurs when the basic reproductive number is greater than or equal to one. For the simplest model of an STI in a homogenous population, the symbolic representation of R_0 is defined as the product of the probability of transmission within a sexual partnership, β, the number of new sex partners the infected person has per unit of time, c, and the average duration of infection, $\frac{1}{\delta}$:

$$R_0 = \frac{\beta c}{\delta}. \tag{1.4}$$

This yields a critical threshold of the minimum average number of contacts an individual can have before disease will spread to increasing numbers of people in the population:

$$c_T \geq \frac{\delta}{\beta}. \tag{1.5}$$

Because of societal sensitivity to the study of what are essentially private behaviours, our knowledge of sexual behaviour has improved during the period after the emergence of AIDS [59]. Not everyone has the same risk of acquiring and passing on STIs to a new partner. Heterogeneity will manifest in many of the factors that influence the likelihood of transmission, and thus will have a significant influence on the magnitude of R_0. For instance, the interpretation of the average values of the simplified behavioural parameter, c, and its effect on R_0 requires elaboration. For instance, skewed rates in the acquisition of new sexual partners will alter the effective rate of sexual partner change, c, such that:

$$c = \mu + \frac{\sigma^2}{\mu}. \tag{1.6}$$

This will change the definition of R_0, where its value will proportionally rise according to the coefficient of variation (ratio of the variance and the mean), to:

$$R_0 = \frac{\beta}{\delta}\left(\mu + \frac{\sigma^2}{\mu}\right). \tag{1.7}$$

A further example of the manifestation of heterogeneity is contained in the distribution of the number of sexual acts per partnership. If we assume that infection either does or does not happen during sexual contact between a susceptible and infected person, then the likelihood of transmission, β, will follow a binomial distribution:

$$\beta = 1 - (1 - \gamma)^{\alpha}, \tag{1.8}$$

where γ is the probability of infection during a single act and α is the average number of sex acts per unit time [4]. Alternatively, if there is a low probability of infection during a single sex act and many sex acts during a given partnership, then (1.8) can be approximated by a Poisson process and β becomes:

$$\beta = 1 - e^{-\gamma\alpha}. \tag{1.9}$$

Accounting for both independent and co-varying degrees of heterogeneity in sexual behaviour can assume other, more complex, distributions [3]. However, the general point illustrated by this example is that heterogeneity in behaviour of individuals will play an important role in the formulation of R_0, and therefore the resulting epidemiological patterns [58].

Studies of random samples of populations [59] and of patients attending STD clinics [60] have informed us about the distribution of many behaviours. Some behaviours, like the number of sex partners over a given period in a random sample of the US population and patients with gonorrhea in Newark, New Jersey [60] are relatively easy to measure. Other relevant behaviours, such as the frequency of sex within partnerships with different characteristics and the pattern of sex partner choice according to risk behaviours, are less straightforward. The number of sex partners reported by the subjects illustrates the existence of great heterogeneity in risk behaviour and shows that those infected with gonorrhea have, on average, more sex partners.

The SIS model has been the predominant structure used to study STIs where repeat infections are common [3,21,57,89]. Many biologically motivated modifications have been made to these simple frameworks to include more heterogeneities to reflect more complex pathogen or host structure [2,64,76,89,105]. Heterogeneity in sexual activity, for example, increases the likelihood of an STD persisting in a defined population [58]. However, the magnitude of the epidemic or endemic state will also be dependent upon the patterns of mixing between individuals who have different activity levels. For example, if a population is stratified according to gender and sexual behaviour, then the pattern of partnerships within and between the various sub-groups of the population can be described by mixing matrices, ρ_{ijk} [58,59,78].

$$\rho_{ijk} = (1 - \epsilon)\,\delta_{jk} + \epsilon \left(\frac{N_{i'k}c_{i'k}}{\sum_{u=1}^{n} N_{i'u}c_{i'u}} \right). \tag{1.10}$$

The elements of the mixing matrix, ρ_{ijk}, represent the probability that when someone of sex i, activity groups j form a sex partnership, it is with someone from activity group k of the opposite

sex i'. In the absence of reliable data that allow for a "real world" representation, an effective option is to examine the influence of a range of mixing patterns that are likely to pertain to a particular community [58]. The mixing parameter determines where mixing occurs on a scale from fully assortative (like with like, $\epsilon = 0$) and random mixing ($\epsilon = 1$) according to the rate of sexual-partner change. Between the two extremes of assortative and random mixing, the probabilities in the matrix are equal to the proportions of the total supply of sexual partnerships provided by each class of the available u activity classes, $\frac{N_{i'k}c_{i'k}}{\sum_{u=1}^{n} N_{i'u}c_{i'u}}$ [59]. While this framework does not capture all possible nuances of the level of mixing in a population, it does allow for an understanding of how different mixing patterns will impact the spread (and thus the epidemiology) of an infectious disease.

It is clear that the patterns of mixing between different sub-groups of the sexually active population can have a key influence on the rate and patterns of infection in a defined community. However, there are many other forms of heterogeneity that are known to be important for STI transmission. These can include heterogeneity in the strain population and infectiousness, immune responses, concurrent partnerships, and health seeking behaviour of infected individuals. Unfortunately, a common feature of all such models is an increased level of complexity in the definition (and formulation) of an overall reproductive number, R_0. As the number of stratifications for the host (or pathogen) population grows, the definition becomes $R_{0(i,j)}$. This represents the number of secondary cases produced in the jth group of susceptibles by an infective in group i [4].

In addition to these general properties of STIs, chlamydia has some very distinct characteristics that make it a particularly interesting disease to model. The first characteristic is that a large proportion of those who are infected do not present symptoms [164]. Secondly, the development of protective immunity is a somewhat contentious issue: though infection is likely to confer protective immunity, its duration and extent within the general population has yet to be understood. However, it is likely to be short-lived. Thus, individuals will become susceptible again after recovering from infection. The third characteristic is the formation of persistent (or chronic) infection, where it has been suggested that, in some instances, apparent reinfection may rather be a re-emergence of persistent infection [29].

These three characteristics of chlamydia require an SIS model (or some modification of this structure such as $SIAS$, $SEIAS$, or $SEIARS$). Numerous models with this preliminary structure have been formulated and analyzed for chlamydia [4,58,89]. With respect to qualitative behaviour, these groups of models do not demonstrate periodic solutions (or oscillatory behaviour) arising from Hopf bifurcations [89,222], but rather have stable asymptotic equilibria. Thus, with constant parameter values the disease will either die-out or approach an endemic state [89].

Several structural variations of the SIS or $SIRS$ models have been developed for studying the movement of endemic equilibrium in response to changing social, biological, or medical conditions.

For example, changes in prevalence as a result of changing parameter values to mimic health policy changes in the population (e.g., screening, healthcare demand and capacity, or vaccination [30, 59, 207], respectively), biological variability in the pathogen (e.g., multiple coexisting strains) [133, 157], cost-effectiveness analyses of intervention programmes [51, 65], as well as emergence of secondary complications [124].

For most directly transmitted viral or bacterial diseases, such as measles or whooping cough, population density plays a major role in the rate and success of transmission, and therefore the value of the reproductive number [5]. However, for STIs, population size or density will not, in general, influence the number of sex partnerships formed per unit of time in a simple proportional manner. Because of the limits on the number of potentially infectious contacts placed by the necessity for sexual intercourse occurring, STIs typically will depend upon high transmission probabilities, long durations of infection, and the existence of asymptomatic carriers [26]. It is usually the case, however, that the number of contacts an individual has is considerably smaller than the population size [106]. Rather, it is more likely that individuals in a population have a set of contacts that are fixed. Networks capture the presence of these interactions.

1.4.2 Network Models

Networks and the epidemiology of directly transmitted diseases are fundamentally linked [106]. While traditional mathematical models that aggregate the population into different compartments have allowed for major advances, they may inappropriate for when contact patterns are heterogeneous [137]. Network analysis has been used as an individual-level alternative tool to describe the evolution and spread of infectious diseases [102, 110, 111, 154, 178, 179, 219] (see figure 1.5). The field of graph theory has provided a wealth of quantitative tools for describing networks, many of which have epidemiological applications [106]. In particular, epidemiological interest is focused on the spread of the disease, in which case the network represents a constraint to the transmission dynamics.

As highlighted by [155, 156], the study of social (and other) networks has three primary areas. *First*, empirical studies of networks probe network structure using a variety of techniques. The goal of such studies is to create a picture of the connections between individuals. Since there are many different kinds of possible connections between people (i.e., social connections versus sexual connections) studies must be designed appropriately to measure the particular connections of interest. Numerous techniques for gathering information about network structure exist, including participant interviews, questionnaires, direct observation, use of archival records, and specialist tools like "snowball sampling" and "ego-centered" studies [155]. However, according to Keeling and Eames [106], three main techniques have been employed to gather network information: infection tracing (i.e., only sources of infection are traced), contact tracing (i.e., a proportion of all contacts
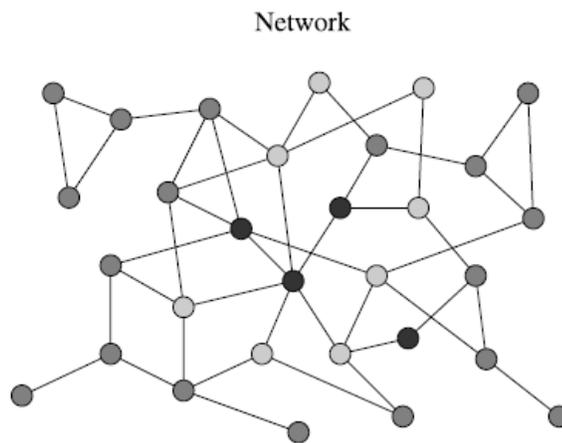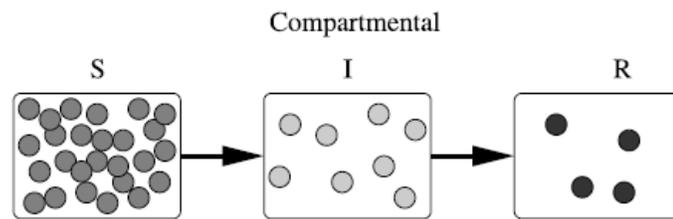
Figure 1.5: A comparison of traditional ("compartmental") and network models. The figure has been modified from [137].
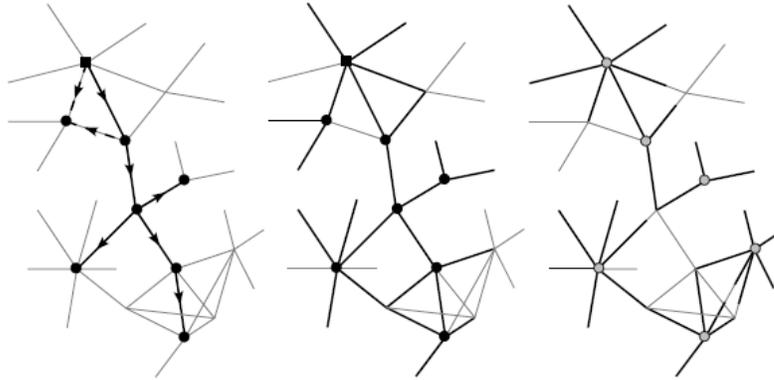
Figure 1.6: For the same simple network (thin grey lines), the type of network information that is gathered using infection tracing (left), contact tracing (middle), and diary-based (right). For the left and middle networks, circles represent infected individuals, and squares (top vertex) represent the primary infected case. The figure has been modified from [106].

from infected individuals are traced), and diary-based studies (i.e., study participants record contacts as they occur, however it is often difficult for the researcher to link the information into a comprehensive network). A depiction of the same network gathered through the three different methods is displayed in figure 1.6.

For the study of STIs, contact tracing is often not applied as a network constructing device [106]. Rather, it has been used as a means of control. However, from the data collected from contact tracing, a subset of a sexual network can be uncovered, usually from populations where disease burden is the highest [219]. Thus, the network obtained is of immediate epidemiological relevance [102].

Second, once empirical data on a network has been collected, one can answer questions about the community the network represents using mathematical or statistical analyses. This is the domain of classical social network analysis, which focuses on issues such as: Who are the most central members of a network and who are the most peripheral? Which people have most influence over others? Does the community break down into smaller groups (or components)? Which connections are most crucial to the functioning of a group? Traditionally, attention has been given to the nature of connections, particularly symmetry (whether a relationship between A and B implies a relationship between B and A), transitivity or clustering (whether the friend of a friend is a friend).

A network can be represented mathematically, and describing connections between individuals (or vertices) is primarily done through constructing an adjacency matrix [106, 155] $\mathbf{A}$; $A_{ij} = 1$ if there is a connection between individuals i and j such that infection could pass from person i to person j; otherwise $A_{ij} = 0$. A number of useful quantities can be derived from the adjacency matrix. These include the number of connections (i.e., centrality measures like degree, the number of neighbours, or eigenvector centrality – which identifies the most influential connections) and

the number of paths between vertices on which an individual lies (i.e., closeness and betweenness centrality) [155].

Third, building on the insights obtained from observational data and its quantitative analysis, one can create mathematical or computer models of processes taking place in the observed networked system. Modelling work of this type allows us to make predictions about the behaviour of a community as a function of the parameters affecting the system. However, in some instances the collection of network data is fraught with difficulties and the use of observed networks lack generalizable epidemiological results [106].

Applied questions for long-term disease spread or the risk of an epidemic over a given mixing network have been derived from many areas of graph theory (e.g., random graphs and percolation theory) [106,155]. Several forms of computer-generated (or idealized) networks have been studied in the context of disease transmission. Briefly, these include Poisson random networks, lattices, small-world networks, spatial networks, exponential random graphs, and scale-free networks. Research using the latter network structure has led to an understanding of some general properties of the distribution of the number of sexual contacts [122], and are thus of great interest to the epidemiology of STIs.

Among many of the idealized network structures, a common concept that is reminiscent of simpler network structures (i.e., random networks), and that is readily applied to epidemiology, involves the number of vertices in a network n, the probability of an edge (or a connection) existing between pairs of vertices p, and the emergence of connected structures (or components).

Considering the spread of an infectious disease, it is likely that each person who has become infected communicates it with independent probability T_{ij} to each of his or her friends (also called the transmissibility). Consider a pair of individuals who are connected, person i (who is infected) and person j (who is susceptible). Suppose that the average rate of disease-causing contacts between them is r_{ij}, and that the infected individual remains infective for a time τ_i [154]. Therefore, the probability that the infection is not transmitted from person i to j is:

$$1 - T_{ij} = e^{-r_{ij}\tau_i}. \tag{1.11}$$

Therefore, the probability of transmission between persons i and j is:

$$T_{ij} = 1 - e^{-r_{ij}\tau_i}. \tag{1.12}$$

The quantity r_{ij} summarizes essential aspects of disease transmission including the likelihood that a given contact will successfully result in transmission [137]. Depending on our assumptions of r_{ij}, such as a higher likelihood of transmission per contact between person i and j, the transmissibility between person i and j can also be estimated by:

$$T_{ij} = 1 - (1 - r_{ij})^{\tau_i}. \tag{1.13}$$

Note the similarity of equations 1.12 and 1.13 with equations 1.9 and 1.8, respectively.

Because r_{ij} is a measure of individual susceptibility, it will vary across individuals, and therefore is assumed to be chosen from a distribution, $P(r)$. Because of this, the transmissibility, too, will vary across partnerships. As a result, the spread of disease, overall, will depend upon the mean probability of transmission between individuals:

$$T = T_{ij} = 1 - \int_0^\infty Q(r)\, dr, \tag{1.14}$$

where $Q(r) = 1 - P(r)(1-r)^\tau$ or $Q(r) = 1 - P(r)e^{-r\tau}$.

To predict the fate of an outbreak, it is important to summarize some useful information about the degree distribution of a network called the average excess degree of a vertex [137]. Essentially, the average excess degree of a given vertex (in a network of size n) is a ratio of its mean-squared degree, $\langle k^2 \rangle = \frac{\sum_i k_i^2}{n}$, and its mean degree, $\langle k \rangle = \frac{\sum_i k_i}{n}$ [155]:

$$\frac{\langle k^2 \rangle}{\langle k \rangle} - 1. \tag{1.15}$$

When a disease is introduced into a network, it will pass through some but not all of the edges according to the average transmissibility T [137]. Calculating the weighted average of the degree distribution of the vertices in a network yields an important (and recurrent) quantity: the basic reproductive number:

$$
\begin{aligned}
R_0 &= T\frac{\sum_i k_i (k_i - 1)}{\sum_i k_i} \\
&= T\frac{\sum_i k_i^2 - \sum_i k_i}{\sum_i k_i} \\
&= T\frac{\langle k^2 \rangle}{\langle k \rangle} - 1
\end{aligned}
\tag{1.16}
$$

where $\langle k \rangle$ is as before, the mean degree of each vertex, and $\langle k^2 \rangle$ is the mean-squared degree of each vertex [155]. Notice that R_0 depends explicitly on the structure (and degree distribution) of the network (that is, $\langle k \rangle$ and $\langle k^2 \rangle$). As with the aggregated population models of the previous section, if R_0 is greater than 1, then the number of people becoming infected grows. If R_0 is less than 1 then the infection will go extinct rather than grow. Recall that the basic reproductive number is the number of secondary infections caused by a single infected person in a completely susceptible population [5]. Thus, in the contact network framework, this is simply the weighted average number of infected neighbours of an initially infected individual multiplied by the average transmissibility (equation 1.14). From this a critical transmissibility, T_c (where $T_c = T$), can be calculated.

Above this threshold, there exists a phase transition, or tipping point, at which a giant component will exist (figure 1.7). This is where the spread of infection (and an epidemic) can occur if and only if:

$$T_c \geq R_0 \frac{\langle k \rangle}{\langle k^2 \rangle - \langle k \rangle}. \tag{1.17}$$
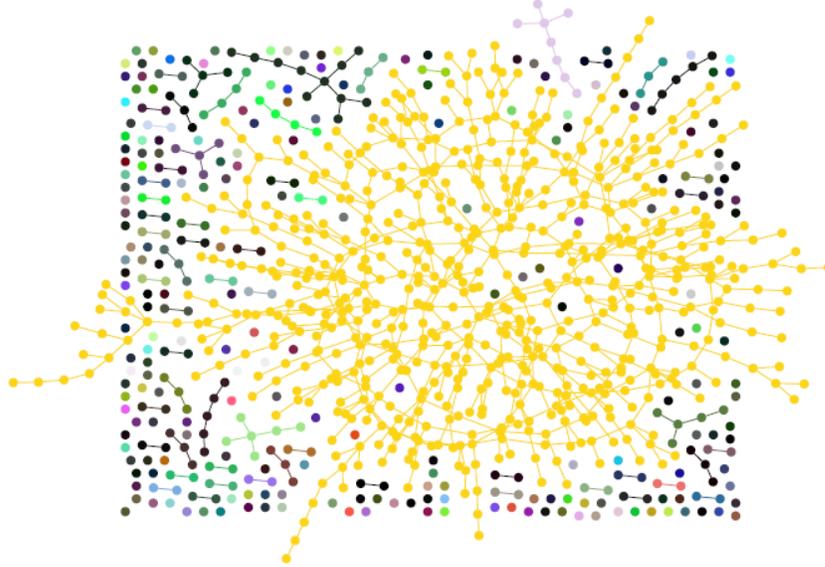
Figure 1.7: A graph with a giant component. The graph is modified from [77].

If T_c is less than the right-hand side of equation (1.17), most vertices will either exist in isolation (figure 1.8) or as smaller groups of connected vertices (figure 1.9), respectively.

Conceptualizing a population as a set of connected individuals to form networks provides an advantage over other population-aggregated methods for understanding the spread of infectious diseases [110]. However, there are other individual-level representations that can add to our understanding to the spread of many infectious diseases.

1.4.3 Within-Host Models and Incorporating Immunological Concepts into Epidemiology

While the insights gained from more traditional modelling techniques (discussed above) has been remarkable, there is evidence in many diseases of important interactions between epidemiology and immunology [47,120]. Both empirical data and mathematical models suggest that epidemiologically-centric variables like frequency and intensity of exposure, can affect immunological outcomes [120]. These include malarial [9,10], and helminth [183,184] infections. Many of potential interactions (e.g., rebounds in the prevalence, antigenic variation and competition, waning immunity, and transient cross-immunity) may have significant consequences for creating optimum prevention strategies (e.g., vaccination or prophylactic chemotherapies) and establishing an adequate level of herd immunity.

Within-host models are similar to conventional epidemiological frameworks, with the individual person as the host being replaced by target cells within the infected individual [4]. As with the population-level models described in section 1.4.1, target populations are divided into several mutually exclusive compartments. However, as the name implies, within-host models study the spread

23

Figure 1.8: A graph with the majority of vertices isolated. The graph is modified from [77].

Figure 1.9: A graph with small groups of connected vertices. The graph is modified from [77].

| Infected Cell | Pathogen release | Basic reproductive number |
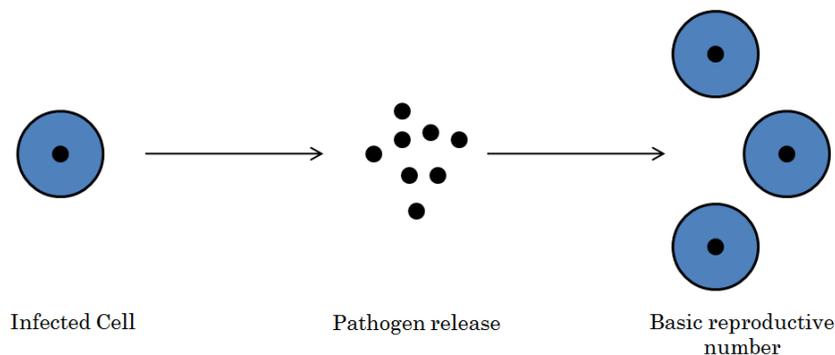
Figure 1.10: The reproductive number of a particular intracellular pathogen is the total number of newly infected cells that arise from any one infected cell when the abundance of uninfected cells is at the disease-free equilibrium (figure was modified from [159].

of infection from cell to cell. For these models, cells within a host are divided up into uninfected cells U, infected cells I, and cells of the pathogen P – in particular intracellular pathogens, and a system of differential equations are produced (see [159] and [5] for thorough examinations of this topic):

$$\dot{U} = \lambda - \beta U P - \delta U \tag{1.18}$$

$$\dot{I} = \beta U P - \alpha I \tag{1.19}$$

$$\dot{P} = \epsilon I - q P. \tag{1.20}$$

Whether or not a pathogen can grow and establish infection is dependent upon a condition that is very similar to a condition outlined in the previous modelling sections. This crucial quantity is the basic reproductive number R_0 and is fundamental to any discussion of the demography of populations of living things, be they humans, frogs, oaks, helminths, or protozoa [159]. Here R_0 is a ratio of the number of individual pathogens (i.e., the burst size)(see figure 1.10) that arise from one infected cell:

$$R_0 = \frac{\beta \lambda \epsilon}{\alpha \delta q}. \tag{1.21}$$

As before, if $R_0 > 1$, then spread of infection will take place, and every infected cell will initially produce more than one newly infected cell on average.

When $R_0 > 1$, the pathogen population will replicate according to equations (1.18)-(1.20), and an immune response will be activated. In the simplest case, effector cells of the immune system, E, are then produced at a rate c and die at a rate γE. It is assumed that c is positive if $I > 0$, (that is, if an infection is present), otherwise $I = 0$. This modifies equations (1.18)-(1.20) to form

a system of equations with four variables:

$$\dot{U} = \lambda - \beta UP - \delta U \tag{1.22}$$

$$\dot{I} = \beta UP - \alpha I - \mu IE \tag{1.23}$$

$$\dot{P} = \epsilon I - qP \tag{1.24}$$

$$\dot{E} = c - \gamma E. \tag{1.25}$$

Whether or not a given pathogen will form a persistent infection or not, depends on its basic reproductive number in the presence of the immune response, R_I. For the model above, this quantity is given by:

$$R_I = \frac{\beta \lambda \epsilon}{\left(\alpha + \frac{c\mu}{\gamma} \right) \delta q}. \tag{1.26}$$

If $R_I < 1$, then the infection will be eliminated. If $R_I > 1$, then the infection will persist at an endemic equilibrium [159].

The concept of "immunoepidemiology" reveals how immunological differences between individual hosts affect pathogen population biology rather than disease incidence, as well as how host variability in immune responses influences the variability in the pathogen population [47, 75, 87]. Progress in immunoepidemiology, and in particular immunoepidemiological modelling, relies on an understanding of within-host differences between infected, recovered, and immunized individuals [87]. Several versions of unified theoretical templates across these biological domains have been developed [33, 34, 47, 75, 79, 83, 86, 115, 130, 167, 193, 199, 215–218]. Recently, the integration of within-host and population-level dynamics has elaborated on the structure of the within-host models described by equations (1.22)-(1.25) [115, 193, 199]. Each individual is then connected in a network such that an individual's pathogen load is linked with the pathogen load of their network contacts. Mathematically, this adds an additional term specifying the rate at which each kth person in the population has an incoming pathogen "flow" that is proportional to the pathogen load of their neighbours:

$$\omega_k \sum_{l \in N} A_{kl} P_l. \tag{1.27}$$

Here, ω_k is the coefficient of connectedness that defines the weights on each of the connections between neighbours and person k; A_{kl} is the adjacency matrix (from section 1.4.2) that describes "who is connected to whom"; the vector, P_l, is the pathogen load of the lth network contact of person k, and N is the population. Incorporating these assumptions into equations (1.22)-(1.25)

produces the following system of ordinary differential equations:

$$\dot{U}_k = \lambda - \beta U_k P_k - \delta U_k \tag{1.28}$$

$$\dot{I}_k = \beta U_k P_k - \alpha I_k - \mu I_k E_k \tag{1.29}$$

$$\dot{P}_k = \epsilon I + \omega_k \sum_{l \in N} A_{kl} P_l - q P_k \tag{1.30}$$

$$\dot{E}_k = c_k - \gamma E_k. \tag{1.31}$$

Whilst there exists explicit combinations of the nonlinear dynamics of both immune reactions and of the interaction between an infection and a population of hosts, when compared to the modelling methods discussed in sections 1.4.1 and 1.4.2, within-host, and by extension immunoepidemiological, models have remained under-explored. Though modelling infection dynamics from this perspective is relatively new, within-host and immunoepidemiological modelling opens up a completely new perspective for understanding infectious diseases, as a whole [159, 199]. In particular, investigating the reciprocal influences between host-pathogen interactions and the prevailing epidemiological environment is well-suited for the study of chlamydia [120, 194]. Study of this nature will require framing hypotheses in a comprehensive ecological context, exploring their implications, and most importantly, identifying strategies to obtain data relevant to test them. However, because of the known complexity and diversity of the immune system, most experimental immunologists tend to possess a strong allergy to mathematical models, despite their recent successes in other areas of infectious diseases, such as HIV-1 pathogenesis [4, 87].

1.5   Rationale for this Thesis

A critical appraisal of the rebound hypotheses outlined in Table 1.1 (above) has not been the focus of contemporary discussion in the Chlamydia literature. Therefore, this thesis seeks to answer an over-arching question: why have observed chlamydia rates rebounded? Providing an adequate explanation is a non-trivial task, and may require integration of several of the hypotheses outline in Table 1.1. To this end, I propose to employ techniques from systems thinking to help expand the boundaries of epidemiological and public health knowledge of chlamydia. My aim will be to create parsimonious models so that I can investigate how the above mentioned rebound hypotheses may be influencing chlamydia transmission both at the individual and population level. The models used throughout this thesis will be a combination of theoretical and empirical (i.e., data-oriented) formulations. Their design will draw on models previously used for studying different STI screening and treatment strategies [59, 89], the effect of treatment strategies on the development of protective immunity [29], modelling within-host pathogen dynamics [159], as well as immunoepidemiology [199].

Chapter 2

Study 1: Insights into Chlamydia Rates from Simple Population-Scale Dynamical Models

2.1 Background

With millions of new cases occurring annually, *Chlamydia trachomatis* is the most common cause of bacterial sexually-transmitted infection (STI) worldwide [29]. Among women, the magnitude of morbidity associated with sexually transmitted chlamydia can be staggering [29, 146]. Chronic and progressive disease due to unresolved chlamydia infections include endometritis, salpingitis, pelvic inflammatory disease, ectopic pregnancy [31, 163, 175], and has also been associated with an increased risk of human immunodeficiency virus infection and cervical dysplasia [29]. Given the detrimental impact that chlamydia infections can have on reproduction, currently observed rates, and how best to reduce them, have been at the forefront of national policy agendas in North America, Europe, and Australia [124, 163, 174].

Collectively, two noticeable epidemiological profiles among reported case rates have emerged throughout many developed countries [31, 175]. The first of these profiles has been observed in Canada, Finland, Norway, and Sweden where, after initially falling in the face of intensified control efforts, reported rates of chlamydia infections have rebounded [31, 175]. The second profile has been observed in the U.S., the U.K., and Australia where incidence rates have been steadily increasing throughout their entire reporting history [31]. Recent hypotheses for these rising trends of chlamydia have focused on the introduction of improved testing technologies, antimicrobial resistance, wide spread changes in the riskiness of sexual practices, and arrested immunity [175] (see Table 1.1). While the evidence supporting these hypotheses has been the subject of recent debate [125,138,175], their validity remains to be a focal point of current chlamydia research [125].

None of the hypotheses in Table 1.1 are mutually exclusive, and several are likely operating simultaneously [175]. Therefore, understanding how these hypotheses are contributing to the dynamics of chlamydia will benefit from (but not entirely depend upon) techniques for modelling dynamic complexity. The primary advantage to using dynamical models is that they depict explicit statements about system structure and how the elements within the system interact [188].

As a result, they enable valuable insights into how certain behaviour has arisen over time [89,188]. We believe that this methodological strength is particularly well suited for elucidating the main drivers behind rebounding case counts of chlamydia infections. In this article, we discuss: one, the methodological approach we used to construct simple mathematical models of chlamydia transmission; two, how we were able to integrate them with observed data; and three, how we used this approach to, in the context of recent rebound hypotheses (Table 1.1), parsimoniously explain the current epidemiological profile of chlamydia infections in the Canadian province of Saskatchewan.

2.2 Models and Methods

2.2.1 Data Sources and Trends

Saskatchewan has a population of approximately 1,014,649 people [84]. Of those who are of sexually-active age groups, approximately 7.7 per cent of its residents are between the ages of 15 and 19 years, 7.5 per cent are between the ages of 20 and 24 years, and 84.8 per cent are ≥ 25 years of age. In Saskatchewan, 13 health regions collect surveillance data on reportable diseases, which are then reported to the Communicable Disease Division of the Saskatchewan Ministry of Health. Of the three above mentioned age groups, those aged between 15 and 24 years comprise 64 to 76 per cent of all reported chlamydia cases in Saskatchewan [163].

Since 1984, chlamydia infections have been a reportable infectious disease in Saskatchewan. During this time, all reported cases of chlamydia have either been diagnosed based on clinical criteria (i.e., urethral discharge, burning on urination, irritation in the distal urethra, dysuria, abnormal vaginal discharge or menstrual bleeding, post-coital bleeding, and lower abdominal pain) [163,175], laboratory methods (i.e., culture, enzyme immunoassay or polymerase chain reaction), or both depending on the year.

Key, aggregated longitudinal data between 1983 and 2007 were assembled from a combination of Provincial Health Reports, records of the Provincial Laboratory, as well as the Public Health Agency of Canada. These data consisted of reported chlamydia case counts, incidence rates (per 100,000 population per year), and testing volume. Case notifications and incidence data is publicly available, and was obtained through a combination of reviewing Public Health Reports of the Saskatchewan Ministry of Health, and from the Public Health Agency of Canadas Notifiable Diseases Online website where data gaps existed for historic years in Provincial records. Data of testing volumes is also publicly available, and was provided to us by the Saskatchewan Ministry of Health upon request. No formal permission was required to use these data.

Between 1983 and 1991, chlamydia test volume data was combined with another category of viral testing. However, we were able to obtain documented viral testing levels for several years before 1983 (i.e., 1979-1982) from public health reports of the Saskatchewan Ministry of Health.

Using the earliest data that separated chlamydia testing volumes (1991-1992) in conjunction with records of "combined" viral testing volumes (1982-1990) we were able to impute testing volumes for chlamydia, between 1983 and 1990. The combined category imposed an upper bound on the level of chlamydia testing that could have occurred. Each of the different imputation strategies was bounded within a narrow defined range of possible values that converged by 1990 (not shown). Because of this asymptotic consistency, the resultant imputed test volume data series did not affect the overall interpretation of the results we report below. From reported chlamydia case counts and testing volume, we were also able to derive a time series for prevalent chlamydia infection among those tested.

Two characteristics of this data gave us a unique advantage over previous studies on chlamydia transmission: the first was a 25-year reporting history of chlamydia. To our knowledge, there are few jurisdictions, worldwide, that have access to similarly broad data; the second was that all testing in the province has been done by one agency (i.e., the Provincial Disease Control Laboratory). This provided reliable testing volume (i.e., denominator) data over the entire reporting history.

Some of the salient trends of reported chlamydia cases are displayed in Figure 2.1. This time series displays rapid growth from 1983 to the late 1980s. A factor that was undoubtedly fueled by the fact that chlamydia had become a reportable infection in 1984. This was followed by pronounced downward trend between 1988 and 1996. Since 1991, the province of Saskatchewan has recorded incidence rates up to two-times higher than national rates, and since 1997, an observable rebound has occurred.

2.2.2   Model Structure and Formulation

The models we examined provided robust frameworks for data analysis. In particular, they were developed to integrate testing volume data, reproduce reported chlamydia case counts, while also seeking to understand the general epidemiological processes underlying them. Throughout this investigation, the above data contributed to adding confidence to model assumptions and structure. Model construction followed an iterative process where various causal hypotheses were translated into systems of differential equations. These equations were then simulated to determine whether they were capable of reproducing historical data using plausible parameter values derived from the chlamydia literature.

Model structures were purposefully kept simple so to focus on broad insights into the processes that have shaped chlamydial patterns over time [58]. Through an extensive process of testing and evaluation (see below), several model structures were investigated. The model presented below emerged from that process as the most parsimonious dynamic hypothesis that adequately captured the historic patterns. However, we should note that the results we describe below remain consistent across the model structures we examined. An overview of the model structures are displayed in
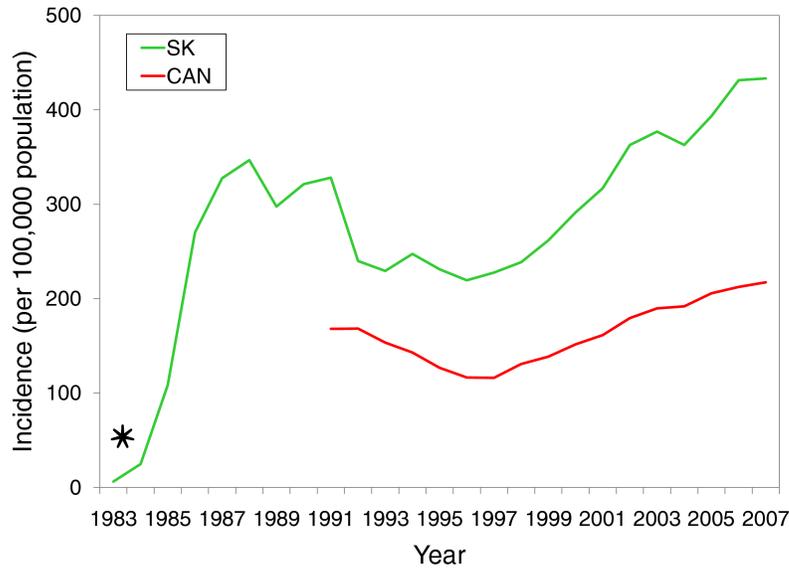
Figure 2.1: Historic trends of Chlamydia trachomatis infections in Saskatchewan and Canada between 1983 and 2007. Asterisk indicates when chlamydia infections became reportable in Saskatchewan (1984). Saskatchewan Incidence rates (per 100,000 population per year) are crude rates and are calculated as the ratio of number of reported cases and the population of Saskatchewan.

Figures 2.2-2.5.

For the description of the transmission dynamics of an infectious disease, it has been traditionally convenient to divide the population into mutually exclusive classes, or compartments, with which numbers, or proportions, of the population "flow" over time [5, 58, 89]. The susceptible class, S, contains those who can become infected, the infectious class, I, and the removed class,R, who have acquired some degree of protective immunity. Much of the preliminary structural assumptions combine aspects of models previously published for studying gonorrhea [59] and chlamydia [26, 30], and were devoted to specifying the interaction of infection spread in the presence of readily-accessible healthcare provision.

A number of the assumptions in the model we presented in the main text have other plausible alternatives. We must therefore demonstrate the extent to which our main results and predictions are robust to different epidemiological assumptions and model structures. To do so, we investigated three alternative models that are described in more detail below. In every case we observed that: one, we can explain the observed data with relatively simple models; and two, all four of the models do not have to appeal to special factors to explain the recent rise in observed cases (i.e., changes in the sensitivity of diagnostic tests, sexual behaviour, or the arrested development of immunity because of treatment). We should note that the structure of Model 2 (below) was our initial dynamic hypothesis (i.e., our true Model 1). However, for the sake of convenience (and hopefully

not confusion) we refer to the model presented in the *BMC Infectious Diseases* article as Model 1.

Model 1: A Simple Susceptible-Infected-Removed Model with Treatment of Infectives

We adopted a deterministic, compartmental, susceptible-infected-treated-removed-susceptible (or $SITRS$) framework [5,197]. The susceptible class, S, contained those who were sexually active and could become infected; the infected class, I, contained those who were infectious; the treated class,T, contained those who either sought health care or were found by contact tracing. Testing sensitivity and specificity were assumed to range between those of cell culture, enzyme immunoassays, and nucleic-acid amplification testing (NAAT). As a result, the treated class T contained both true and false positives; these people were assumed to have been tested, diagnosed as cases, and treated appropriately; these individuals were also assumed to abstain from risky sexual contact according to Public Health Agency of Canada guidelines [163] and remain "quarantined" in the treated class for the duration of treatment. The flow of people from the treated class to the removed class were assumed to occur at the rates $T\sigma'$ and σI, respectively; the removed class, R, contained those who had naturally recovered from infection. This class also contained those who were given a positive diagnosis and temporarily reduced their sexual risk-taking behaviour after being treated. People in the removed class were assumed to eventually exit the sexually active population, or return to the susceptible class as a result of waned immunity or relapse into previous risky sexual behaviour (Figure 2.2).

The fractions of the population that were susceptible, infected, treated, or were removed at time t were denoted by $S(t)$, $I(t)$, $T(t)$, and $R(t)$ respectively. We assumed that the sexually-active population had a constant size N, where $N = S + I + T + R$. The actual or true prevalence of infection in the sexually-active population is represented by $I(t)$. People were assumed to enter the susceptible state at sexual debut at a constant rate, μN, and exited at a rate μS. The probability of chlamydia transmission per year for a given partnership between a susceptible and infected individual is given by βc, hereafter denoted $\hat{\beta}$. Thus, for a given fraction of infected people in the population, $\dfrac{I}{N}$, the number of susceptibles that become infected per year is $S\dfrac{\hat{\beta}I}{N}$. The number of false positives detected for a given test was assumed to occur at a rate $n_S(1 - \phi')$. Here, the number of susceptibles tested is the difference between the recorded testing volume, V, and the number of infectives tested, $n_S = V - n_I$. The number of infectives tested was a function of the fraction of infectives tested, $\beta_T\left(1 - e^{-\alpha(V/I)}\right)$, and the prevalence of infection in the population, I: $\beta_T\left(1 - e^{-\alpha(V/I)}\right)I$. Our testing assumptions here posit a relation that states if testing increases, more cases will be found. However, simply doubling the number of tests does not mean that twice the number of infection will be found. In effect, the model assumes a "law of diminishing returns" where there is a lower and lower incremental benefit with increased testing. Thus, when testing volume, V, approaches zero, the fraction of infectives tested approaches zero at a rate α; as testing

Figure 2.2: Schematic stock and flow diagram of the susceptible-infected-treated-removed Model 1. We adopted a deterministic, compartmental, susceptible-infected-treated-removed-susceptible (or SITRS) framework. The susceptible class, S, contained those who were sexually active and could become infected; the infected class, I, contained those who were infectious; the treated class, T, containing both true and false positives; these people were assumed to have been tested, diagnosed as cases, and treated appropriately; the removed class, R, contained those who had naturally recovered from infection, and those who were given a positive diagnosis and temporarily reduced their sexual risk-taking behaviour after being treated. People in the removed class were assumed to eventually exit the sexually active population, or return to the susceptible class as a result of waned immunity or relapse into previous risky sexual behaviour.

volume increases, the fraction of infectives tested will approach β_T, reflecting that even with great testing effort, it takes a certain amount of time to identify some infectives. The fraction of infected people detected by testing (per year) were treated at a rate $n_I\phi$, recovered naturally at a rate σI, or exited the infected class at a rate μI.

Those deemed infected (i.e., both true and false positives) were treated and returned to the susceptible class at a rate $T\sigma'$ or exited the treated class at a rate μT. While both seeking healthcare services and contact tracing are likely two separate processes responsible for bringing infected individuals to the treated state, we assumed that there is some net time, $n_I\phi$, that will result (that is mathematically equivalent to representing two separate rates). Keeping these as a single parameter helped reduce the risk of overfitting. For simplicity we made an assumption that those being treated follow the recommended clinical guidelines published by the Public Health Agency of Canada that patients abstain from risky sexual contact for 7-14 days whilst being treated [163]. All those in the removed state then were assumed to return to the susceptible class as a result of waned immunity or relapse into previous risky sexual behaviour at a rate δR. Under these assumptions, the flow of individuals between the S, I, T, and R classes is depicted in Figure 2.2 and described by the following system of equations:

$$\dot{S} = \mu N + \delta R - S\frac{\hat{\beta}I}{N} - n_S\phi' \tag{2.1}$$

$$\dot{I} = S\frac{\hat{\beta}I}{N} - I(\mu + \sigma) - n_I\phi \tag{2.2}$$

$$\dot{T} = n_S\phi' + n_I\phi - T(\sigma' + \mu) \tag{2.3}$$

$$\dot{R} = I\sigma + T\sigma' - R(\delta + \mu). \tag{2.4}$$

The primary contribution of this approach is that it brings a unique perspective of integrating data with mathematical models in order to gain a novel understanding of chlamydial patterns that have been observed between 1983 and 2007. Model structure directly incorporated data for recorded test volumes as part of the testing assumptions explicitly captured by the model structure. Models were then calibrated to match case notification data. Integrating testing volume into the models placed a constraint on the model calibrations in order to match observed case counts. As a result of these data constraints, we were then able to triangulate an estimate of the prevalence of chlamydia infections in the population.

Other model structures examined the effect of treatment on the development of acquired immunity in combination with changes in sexual risk-taking behaviour (see Models 3 and 4 below). While acknowledging the importance of intricate biological and epidemiological differences by gender, age, and symptomotology our models followed the assumptions of Hethcote and Yorke [89] and ignored their effect. Instead, the models assume that chlamydia transmission occurs in one uniform homogeneous population of sexually active people. The population represented by these models

therefore consists of those individuals at high risk, those who are efficient transmitters, as well as their sexual contacts.

Model 2: Chlamydia Transmission using a Susceptible-Infected-Treated Model

For this model, a compartmental $SITS$ (susceptible, infected, treated, susceptible) framework was adopted. This model provided a starting theoretical framework to use in drawing simple conclusions about the epidemiology of chlamydia. It made use of the notion that susceptibles become infectious, and then susceptible again. Because chlamydia is a reportable and curable infection, this model also assumed that a fraction of infectives were tested, treated, and then, too, become susceptible again. As with Model 1 (above), Model 2 made the assumptions that chlamydia infection occurs in one uniform homogeneous population, and that there are negligible periods of latency. In contrast to the other models examined, Model 2 made the assumption of negligible periods of protective immunity (i.e., no R state). The population represented by the model consisted of the segment of the sexually-active population who are at high-risk or are efficient transmitters (following the assumptions of Hethcote and Yorke [89]). This model also did not account for differences in sexual activity and it ignored epidemiological differences between men and women.

The movement (or flow) between S, I, and T classes, based on the above assumptions, was described by the following system of ordinary differential equations (see Figure 2.3 for schematic diagram):

$$\dot{S} = \mu N + \sigma I + T\sigma' - S\frac{\hat{\beta}I}{N} - n_S\phi' \tag{2.5}$$

$$\dot{I} = S\frac{\hat{\beta}I}{N} - I\left(\mu + \sigma\right) - n_I\phi \tag{2.6}$$

$$\dot{T} = n_S\phi' + n_I\phi - T\left(\sigma' + \mu\right). \tag{2.7}$$

Model 3: A Model that Assumes Treatment of Prevalent Infection Truncates Transient Acquired Immunity

Here, we made an additional assumption adopted from Brunham et al. [30] that being found to have infection and thus being treated did not impact a person's sexual risk-taking behaviour. In contrast to Model 1, our assumptions in Model 3 were motivated by the observation that being treated may inhibit the development of acquired immunity, and thus returned a person to the susceptible state at a rate $T\sigma'$. As was mentioned above, this assumption jointly accounted for no change in sexual risk behaviour, but also assumed that treatment truncated any benefit of transiently acquired immunity [30, 175]. These additional assumptions produced the following system of equations (see Figure 2.4 for schematic diagram):
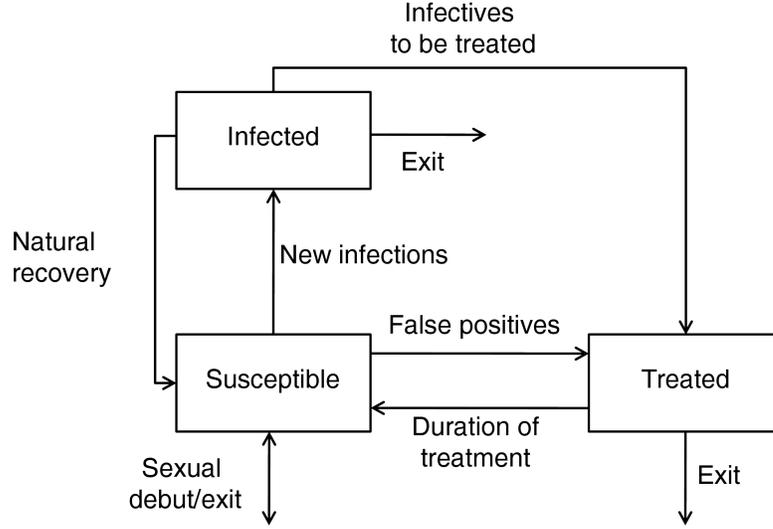
Figure 2.3: Schematic stock and flow diagram of the susceptible-infected-treated-removed Model 2.

$$\dot{S} = \mu N + \delta R + T\sigma' - S\frac{\hat{\beta}I}{N} - n_S\phi' \tag{2.8}$$

$$\dot{I} = S\frac{\hat{\beta}I}{N} - I(\mu + \sigma) - n_I\phi \tag{2.9}$$

$$\dot{T} = n_S\phi' + n_I\phi - T(\sigma' + \mu) \tag{2.10}$$

$$\dot{R} = I\sigma - R(\delta + \mu). \tag{2.11}$$

Model 4: A Model that Combines Models 1 and 3

Here, we combined our assumptions in Models 1 and 3. More specifically, the removed class $R(t)$ was assumed to contain a combination of those who had not presented for treatment and recovered naturally from infection, and a fraction, θ, of those who had temporarily changed their sexual risk taking behaviour after being treated. In addition to this, we also considered a scenario that jointly accounted for the lack of developing acquired immunity and no changes in sexual risk behaviour after being treated. This additional assumption returned the remaining fraction of the population, $1 - \theta$, to the susceptible state at a rate $(1 - \theta)T\sigma'$. These additional assumptions produced the following system of equations as well as the schematic diagram in Figure 2.5:

Figure 2.4: Schematic stock and flow diagram of the susceptible-infected-treated-removed Model 3.

$$\dot{S} = \mu N + \delta R + (1 - \theta) T \sigma' - S \frac{\hat{\beta} I}{N} - n_S \phi' \tag{2.12}$$

$$\dot{I} = S \frac{\hat{\beta} I}{N} - I (\mu + \sigma) - n_I \phi \tag{2.13}$$

$$\dot{T} = n_S \phi' + n_I \phi - T (\sigma' + \mu) \tag{2.14}$$

$$\dot{R} = I \sigma + \theta T \sigma' - R (\delta + \mu). \tag{2.15}$$

2.2.3   Parameter Values and Model Calibration

Initial parameter values were derived from available literature. Their values, ranges, and references are summarized in Table 2. Model calibration, cross-checking, and sensitivity analyses were performed using a four-step process adapted from Van de Velde et al [44]:

1. *Setting initial parameter values*: Each parameter value associated with the natural history of infection or with healthcare provision was estimated from key epidemiological or review articles in the available literature between 1997 and 2007. Where unavailable, estimates of parameter values (i.e., duration of sexual activity) were derived from modelling literature on gonorrhea infections [59, 207]. Given that both gonorrhea and chlamydia are transmitted by similar behaviour, we think using data from other STIs is a reasonable assumption.

2. *Sampling parameter ranges and fitting the model*: Each parameter value was associated with

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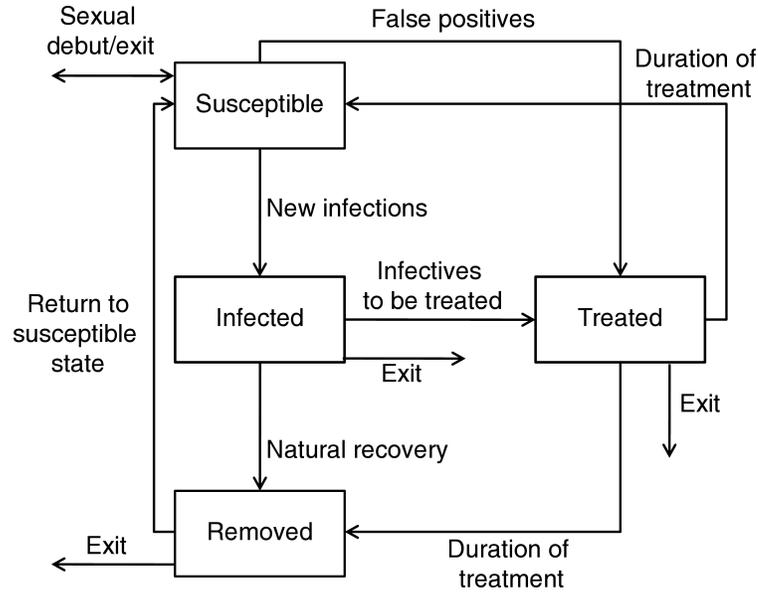
Figure 2.5: Schematic stock and flow diagram of the susceptible-infected-treated-removed Model 4.

a range of values between the maximum and minimum values found in the literature surveyed. Parameter settings that minimized the discrepancy between the historic reported case counts and those output by the model, were determined by a sequence of 50 optimizations using the Powell global optimization algorithm available in Vensim DSS for Windows (version 5.5d). Each optimization used a distinct random number seed, and performed approximately 1.0×10^6 simulations (yielding a total of 5.0×10^7 simulations across all optimizations).

3. *Cross-checking model fit*: To build confidence in the model results, we compared model simulations to observed data series that were not used in step 2. These included the reported incidence rate (per 100,000 population per year) and fraction of positive cases among those tested.

4. *Sensitivity Analysis*: Each of the optimization scenarios identified a different point in parameter space that offered the "best fit" to the historic data. Because of this inherent variability present in each optimization scenario, we performed a sensitivity analysis based on the distribution of parameter vectors produced in step 2.

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Parameter	Description	Value (units)	Reference
$\hat{\beta}$	The mean number of susceptible individuals infected with chlamydia per year by an index case.	0.8-10 (1/year)	[26]
$1/\sigma$	Average duration of natural infection.	1.25 (years)	[26]
$1/\sigma'$	Average duration of infection when treated.	0.038 (years)	[163]
ϕ	Diagnostic test sensitivity.	0.5-0.92	[174]
ϕ'	Diagnostic test specificity.	0.98-1.0	[174]
$1/\mu$	Average duration of sexual activity.	15 (years)	[59, 207]
$1/\delta$	Average duration removed.	0.5-10 (years)	[66, 124]
n_I	Number of infectives tested.	Calculated	
n_S	Number of susceptibles tested.	Calculated	

Table 2.1: Baseline parameter values and ranges used during model calibrations.

2.3 Results and Key Model Insights

2.3.1 Model Fit and Validation

Figure 2.6 compares the observed historic trends in Saskatchewan and calibrated model simulations. In contrast to other seminal work on modelling STI transmission [59, 89, 207], a model that incorporates a removed state best reproduces observed chlamydia trends (see Figures 2.6 and 2.7). As shown, the model was able to accurately mirror observed temporal changes in reported case counts (Figure 2.6A). These trajectories also accurately reproduced the temporal changes in the observed incidence and the proportion positive among those tested without explicit instruction to match these data (Figures 2.6B and C, respectively).

One major advantage of this analysis was that it allowed us to produce a triangulated estimate of the "true" epidemiological state (i.e., the infected class I) that underlies the observed trends. Hereafter this will be referred to as the "actual" prevalence. As shown in Figure 2.6D, the model suggests that actual prevalence reached a maximum shortly after 1984, was in decline between 1991 and 1996, and was followed by an upward rebound between 1996 and 2003. It is interesting to note that while the model suggests that a rebound in the actual prevalence has indeed occurred, the peak of this upward trend lies below the peak attained in the mid 1980s. This behaviour suggests that the prevalence is moving towards an endemic steady state by a series of weakly damped oscillations – a familiar feature of the types of infectious disease models we studied here [5].
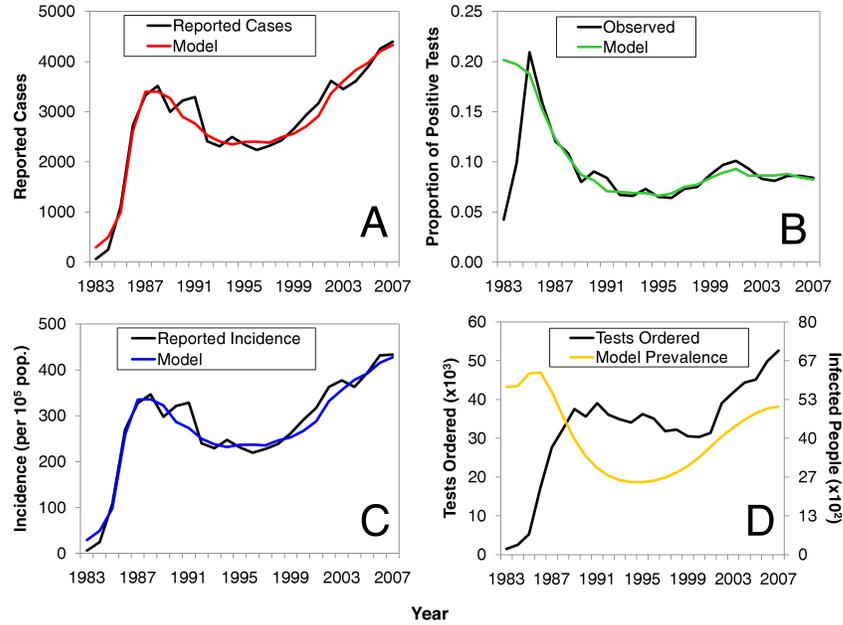
Figure 2.6: Comparison of model calibrations to observed trends. Calibrated numbers of (A) cases from the models compared to reported numbers of cases in Saskatchewan. Parts (B) and (C) cross-check the model to the observed proportion positive among those tested (B), and the reported incidence (per 100,000 population) (C). Model-generated curves in parts (A), (B), and (C) were arbitrarily chosen from 50 million optimization simulations. Part (D) is a visual comparison of testing volume between 1983 and 2007 to the actual prevalence in Saskatchewan generated by the model.

When the actual prevalence is superimposed on the observed trends in testing volumes, an obvious divergence between 2005 and 2007 is demonstrated (Figures 2.6D and 2.8). Specifically, testing volume appears to be steadily increasing, while the actual prevalence has plateaued.

2.3.2 Parameter Uncertainty

The parameter sets that best fit the observed epidemiological data produced a wide range of combinations. When we accounted for variability in the calibrated parameter values, the models trajectories for the actual prevalence of chlamydia exhibited minor change and retained the same basic behaviour over time (Figure 2.9A). Even though this model did not explicitly simulate the efficiency of contact tracing, the model results suggest that the level of healthcare coverage (expressed as the fraction of recovering cases recovering through treatment) rapidly increased in the mid 1980s and has remained quite consistent over time (Figure 2.9B).
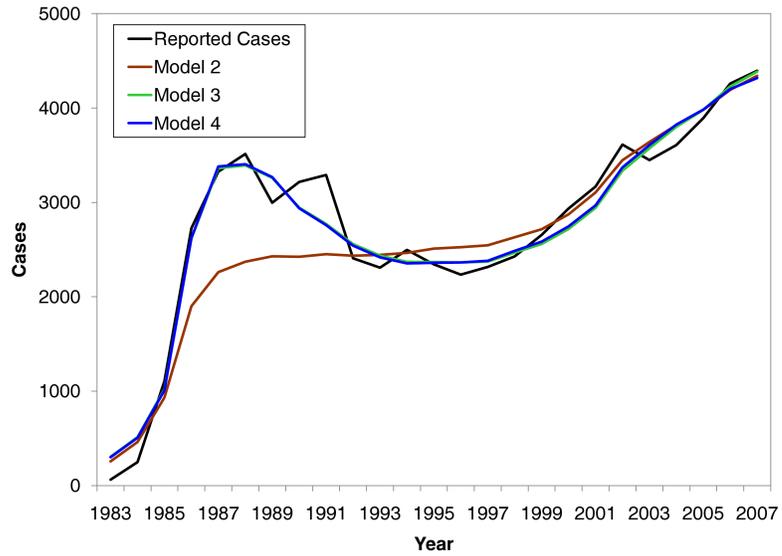
Figure 2.7: A comparison of the model-predicted case notifications from Models 2-4 to the actual case notifications in Saskatchewan between 1983 and 2007.
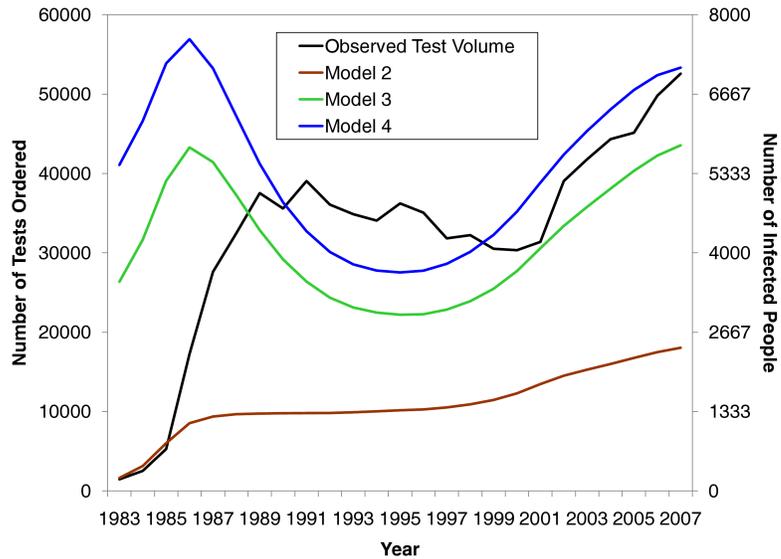
Figure 2.8: A comparison of testing volume between 1983 and 2007 to the actual prevalence in Saskatchewan predicted by Models 2-4.
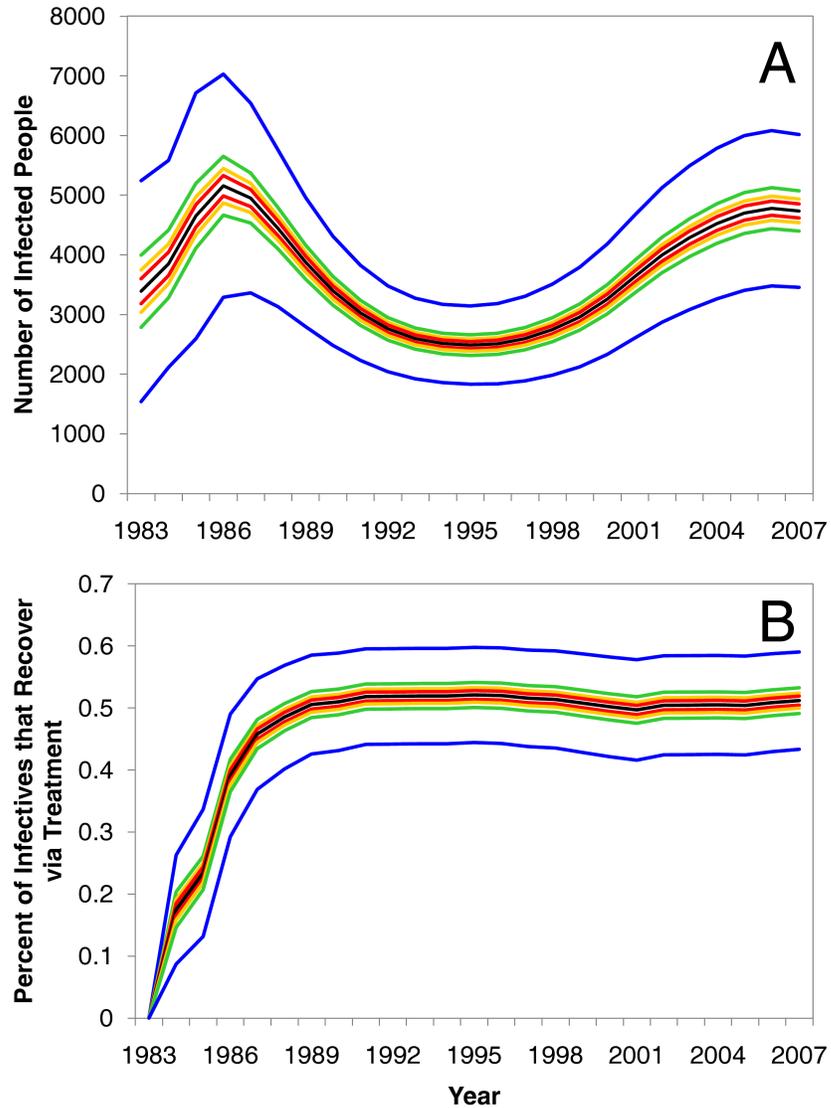
Figure 2.9: Uncertainty and sensitivity analysis of model results. Parts (a) and (b) are the results of a sensitivity analysis on the "actual" prevalence generated by the model and the fraction of infectives that have recovered via treatment, respectively. In both parts (a) and (b), the black line represents the mean value, and the coloured bands represent the 50 (red), 75 (yellow), 95 (green), and 99 per cent (blue) confidence intervals.

2.4 Discussion

Globally increasing chlamydia rates have been widely discussed to be a result of both changes to testing technologies and changes in human sexual and social behaviour near the mid- to late-1990s [125, 138, 175]. Although initially intuitive, the results presented here provide evidence that such observed rebounding trends in chlamydia infections have resulted from a simpler set of oscillating epidemiological processes, in particular a significant delay in replenishing the susceptible population, that have been operating throughout the entire history of this infection. Taken together, our results demonstrate that currently observed rebounding chlamydia notifications are more likely a combined artifact of: one, when chlamydia infections became reportable; and two, the state of the underlying prevalence once surveillance was well-established (shortly after 1987), rather than because of fundamental changes that have arisen because of some of the hypotheses in Table 1.1.

Several pieces of evidence suggest that adopting NAAT technologies have likely had an impact on observed chlamydia case counts. First, testing via NAAT methods is done on urine, which is more acceptable and easier to collect from high-risk youth and male patients [175]; this will, ultimately, allow more tests to be collected. Second, because of an increased sensitivity and decreased specificity profiles compared to non-NAAT methods, NAAT technologies allow for improved case detection [35, 53, 66]. While the improved sensitivity and decreased specificity profiles of NAAT methods are likely to impact observed chlamydia case rates [138], our models were able to reproduce observed trends without having to account for changes in test sensitivity or specificity since their introduction (which in many parts of Canada was between 2000 and 2001). Overall, our analysis is able to reinforce previous statements that higher testing frequency, alone, can have a significant influence on observed rates [66, 138].

Additionally, our analysis suggests the existence of a positive feedback from testing volume that accounts for the recent continued climb in the reported chlamydia case counts between 2002 and 2006: a greater number of positive tests led to more awareness of the infection, which led to more testing being done, and still a larger number of positive tests (Figure 2.6D). However, higher rates of testing will also bring higher rates of treatment. Higher rates of treatment appear to have led to a reduction of the underlying prevalence over time, and thus contributed to the observed damped oscillations in the actual prevalence. Upon reflecting on the behaviour of the actual (model-predicted) prevalence, we would also expect that observed rates will eventually begin to plateau as the prevalence among people tested begins to approach the actual prevalence in the population at risk.

The arrested immunity hypothesis posits that early treatment interrupts the immune response, thereby enhancing population susceptibility to infection as people re-enter the same sexual networks [30, 175]. When we accounted for this phenomenon in two of the alternative model structures, nearly

identical results were observed when compared to a model that did not account for it (i.e., the model represented in Figure 2.2). The fact that the structures of Models 3 and 4 were able to accurately reproduce data, after being constrained by testing volume data, suggests that arrested immunity is very likely an important component of the underlying dynamics of chlamydia transmission. However, additional sensitivity analyses on Models 3 and 4 (not shown) revealed that the impact of arrested immunity at the population level may not be as significant as previously discussed [31]. Overall, this too seems to suggest that adverse immunological impacts of current test-and-treat polices are not required to explain the rebound.

Similarly, previous discussions in the literature have also implicated rebounding chlamydia rates to be a function of increased high-risk sexual behaviour that has, ultimately, resulted in an increase in both on-going and new chlamydia infections [124, 125, 175]. The models we presented here captured two different aspects of behaviour change. These were the mean number of susceptible individuals infected with chlamydia per year by an index case, $\hat{\beta}$, and the average duration a person stayed in the removed class, $\frac{1}{\delta}$. However, as with changes in testing technologies and arrested immunity, our models were able to reproduce observed trends without having to appeal to changes in sexual risk-taking behaviour.

2.4.1 Limitations

There are some limitations to our analysis that need to be discussed. As with any model, the given structure represents a simplification of reality. We chose to develop a population-based model that is appropriate for exploring transmission dynamics in a large population where the infection is endemic. Therefore, we did not evaluate the impact of network structure or the duration of sexual partnership on our results. Although we may have described our results at the level of the individual, models of this type do not capture events that occur at the individual level. As a result, the models we present here will offer poor resolution for investigating network-based interventions. Despite this, these types of models are still capable of providing broad-level insights into historic and current epidemiology at the level of the population [8, 91], and are able to shed light on specific questions in a way that alternative models, including human intuition and traditional epidemiology, do not.

Models are only as useful as the data available to inform them. Although our simple models contain many plausible relations that have some precedent in previous epidemiological observations, they were developed and calibrated in the absence of some important numerical data (e.g., data stratified by age and gender). Despite this simplification of reality, we do not think that stratifying the model by age or gender would have benefited the results presented here. This is largely because of inadequate provincial data to calibrate age and gender specific mixing parameters. In our opinion, calibrating a stratified model in the absence of such data would have suffered from

overfitting. Regardless of these simplifying assumptions, one of the strengths of this analysis is that integrating surveillance data with dynamic models of chlamydia transmission allowed us to provide some underlying epidemiological context to data that has been largely criticized for lacking it [125, 138]. More notably, these analyses have also highlighted how, once a surveillance system is well established (which in Saskatchewan was near 1987), and given a concerted effort to identify as many cases as possible, surveillance data can accurately mimic the underlying prevalence of infection.

Although extant data limitations ruled out constructing a stratified model, two considerations give studying demographic heterogeneity priority for future work. First, having been able to explain the rebound in observed case notifications as a function of testing volume, collection of more detailed data could highlight contextual epidemiological differences among those being tested for chlamydia. Second, for these models to offer value in assessing intervention tradeoffs, it will be important to account for these population heterogeneities in the model structure. We are currently drawing on more-detailed data from a sub-provincial level (e.g., changes in contact rates, gender distribution amongst tested cases, the age distributions of contacts, rates of pelvic inflammatory disease, and changes in the social marketing of healthcare services over time) to expand the insights of the current analyses.

2.4.2 Conclusions

With 25 years of data corresponding to over 804,000 tests and 69,000 cases this is likely one of the largest retrospective analyses on genital chlamydia. To help clarify the dynamic mechanisms underlying observed trends, the models presented here draw upon available indicators within existing data and integrate several cause-and-effect hypotheses. As a result, this has allowed us to critically examine the appropriateness of several key rebound hypotheses.

The primary aim of this study was to parsimoniously explain the recently observed rebound epidemiological profile of chlamydia since it became a reportable infection in the Canadian Province of Saskatchewan. By combining dynamic models with testing volume data to reproduce observed surveillance data, our results highlight the significant impact testing volume can have on observed case counts. The results of this study also illustrate the usefulness of our methods for deriving estimates of infection prevalence from freely available surveillance data. Overall, they provide a viable explanation for reported trends that appears to have been overlooked or dismissed in favor of hypotheses involving large-scale, aberrant changes to the underlying prevalence [66].

The calibrated models presented here offer value as tools for improving our understanding of chlamydia epidemiology. With some structural modification and additional data, they will be useful for examining how current trends might behave into the future under a variety of control scenarios. For example, they may be extended to explore the dynamics of introducing various candidate

control policies, such as a nationally dedicated proactive screening program [124] or expedited partner therapy [68, 182]. It bears emphasizing that while we have presented results from a single province with an exceptionally long record of chlamydia data, our methodological approach is both straightforward and general. Moreover, these methods offer geographic portability that can help inform the public health efforts in other contexts that collect similar information to that used here. Most importantly, these results reassure the public health effort towards monitoring and controlling chlamydia.

CHAPTER 3

STUDY 2: ESTIMATING THE DURATION OF IMMUNITY

USING SIMPLE POPULATION LEVEL MODELS

3.1 Relationship to the previous Study

Nonlinear ordinary differential equations can encompass a wide-range of feedback effects. For example, the models developed in the previous study uniquely captured, not only, the interaction between control efforts and Chlamydia prevalence, but they also provided insight into the natural history of infection. The transitions among the state variables are modelled as expected values in the population, and therefore can also be effectively used to estimate those expected values. One observation that consistently emerged from the previous analysis was that the calibrated estimates of the average duration of immunity were much longer than what was currently discussed in the literature.

This next study focuses on investigating the sensitivity of the models to assumptions about the duration of immunity after infection. It uses the same data and methodological approaches (i.e., calibration techniques) in combination with a subset of the four models used in the previous study. The result was an analytical methodology to estimate this difficult-to-observe natural history parameter by calibrating key parameters and exploring the agreement of the models with 25 years of surveillance data.

3.2 Background

There are clear indications that the normal host immune response against chlamydia bacteria stimulates protective immunity over time [175]. Historically, immunity to chlamydial genital infection has been studied using animal models that evaluate immune responses that develop in naïve animals, either after infection, passively transferring immune cells, or by vaccination and assessing resistance to reinfection [96, 148]. These approaches have confirmed the dominant role of Th1 CD4+ T cells and antibody in resolving and resisting chlamydial genital infection, respectively [22, 95, 96, 148]. These studies have also confirmed that protective immunity in these animals is temporary and

wanes within a matter of months [95, 190].

Among humans, however, descriptive studies explicitly examining the period of infection-acquired immunity are not available [16]. Instead, data that do support the concept of protective immunity against chlamydial reinfection arise from a compilation of indirect evidence from both cross-sectional and longitudinal study designs (clinical [73, 98, 192] vs. natural ecology [12, 24, 74, 104, 142]), different chlamydial diseases (ocular [12] vs. genital [142]), and the contextual epidemiology across different countries [30, 59, 72, 117, 174]. Despite the lack of direct evidence, protective immunity for humans – as for animal models – is widely discussed to be short-lived and partial at best [16].

Immunity plays a key role in the both the natural history of infection and transmission of many infectious diseases [114]. However, because of obvious ethical considerations, defining the natural course of protective immunity after the resolution of chlamydial infection by traditional immunological and epidemiological means is unavailable. Therefore, developing new, robust methods of estimating the natural course of infection and immunity should be the focus of ongoing research. In this respect, mathematical (or dynamical) transmission models provide an effective methodological trajectory. Dynamical models of infectious disease transmission have played important roles in policy analysis [30, 114], and have been deemed appropriate tools for decision making when parameterized to observed data [117, 126]. As theoretical frameworks, these models can extract information from data that may not be accessible by more traditional epidemiological means, which can then be tested by continued study [37, 114]. Their mathematical equations can be solved numerically and matched to observed sets of data to obtain "best fit" estimates for desired parameters [198, 204]. Dynamical models also provide a means to hypothesize how observed trends emerge from causal processes of infection spread [200]. While this approach has proven effective when applied to problems in immunology [37], it has not been their recent function in epidemiology [44, 60, 114].

Producing an estimate of the duration of immunity that is consistent with epidemiological data would both improve our understanding of the natural history of infection, as well as inform the design of control policies such as vaccination. However, the challenges experienced by previous clinical and epidemiological research at obtaining a consensus about the duration of chlamydial immunity [28] highlight the need for developing new approaches for estimating this crucial parameter. Here, we approach this problem by integrating two dynamical models with historic test volume records, and then calibrate them to match uniquely extensive case notification data from the Canadian province of Saskatchewan. Calibrated parameter estimates are then compared with more widely discussed periods of negligible immunity in their ability to match empirical chlamydial time series.

3.3 Models and Methods

The original data sources and model structures have been described in detail elsewhere [200]. Therefore, technical and mathematical details of model construction, the models' integration with available data, the methods of initializing and calibrating model parameters, assessing goodness-of-fit, and sensitivity analyses are extensively outlined in the previous chapter. Only a brief summary of their main characteristics is described below.

3.3.1 Data Sources

The Canadian Province of Saskatchewan has a population of approximately 1 million people. Thirteen health regions collect surveillance data on notifiable infections, which are then reported to the Communicable Disease Division of the Saskatchewan Ministry of Health. Since 1984, Chlamydia has been a reportable infectious disease, and during this time all reported cases have either been diagnosed based on clinical criteria (e.g., urethral discharge, burning on urination, irritation in the distal urethra, dysuria, abnormal vaginal discharge), laboratory methods (i.e., enzyme immunoassay or polymerase chain reaction), or both depending on the year. Nearly 15.2% of Saskatchewan's residents are between the ages of 15 and 24 years of age, however it is this age range that disproportionately comprises the majority of all reported Chlamydia cases (64-76%) [200].

Two data sources provided constraints on model calibration, which allowed for estimation of context-specific model parameters. For the first, Chlamydia case notifications were accumulated over a 25-year reporting history in Saskatchewan (1983-2007). These data were collected from Annual Public Health Reports of the Saskatchewan Ministry of Health, and the Public Health Agency of Canada. For the second, testing volumes were obtained from a combination of Annual Public Health Reports of the Saskatchewan Ministry of Health (1983-2001), and from the Saskatchewan Provincial Laboratory (2001-2007). Overall, these data contained over 804,000 tests and 69,000 Chlamydia cases. Figure 3.1 summarizes 25-year trends of Chlamydia in Saskatchewan.

3.3.2 Models of Chlamydia Immunity and Transmission

To study the impact of waning immunity on the epidemiology of Chlamydia, we adopted a Susceptible, Infected, Treated, Removed, Susceptible (SITRS) modelling paradigm that accounts for reinfection. In all, two versions of this deterministic, compartmental framework were developed. Figure 3.2 illustrates how these compartmental models were set up.

People were divided into four mutually exclusive categories: those who are susceptible, those who are infected (this combines those who are symptomatic and asymptomatic), those who are tested and treated (this combines those who seek medical care, those contact traced, as well as those who are both true and false positives), and those who recovered from infection naturally.
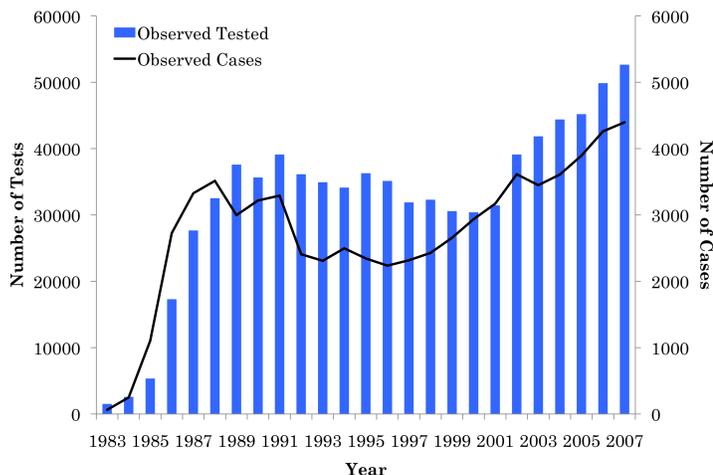
Figure 3.1: Historic time series of case notification and testing volume data in the Canadian Province of Saskatchewan between 1983 and 2007.

Upon natural recovery from infection, individuals are assumed to remain immune before returning to the susceptible class. In both models, immunity resulting from natural recovery of infection was assumed to be truncated by treatment [30, 175, 190].

Both models differ in their post-treatment progression of individuals. In Model 1 (Figure 3.2A), treated individuals are assumed to return to the susceptible state (i.e., recover without changing their sexual behaviour); by contrast, Model 2 (Figure 3.2B) assumes that some treated individuals are temporarily "behaviorally removed" or temporarily immune before returning to the susceptible state. Here, we define the duration of immunity in broad terms as the mean length of time between natural resolution of infection and re-emergence of susceptibility. Each model contains parameters relating to the key components of infection dynamics: transmissibility, sexual contact rate, and duration of infectiousness, as well as the capabilities of testing technologies (i.e., sensitivity and specificity).

These models were developed for the purpose of reproducing reported Chlamydia case notifications while seeking to provide estimates of several relevant epidemiological parameters. Model construction was iterative where various causal hypotheses were translated into systems of differential equations. Data were directly integrated into the models and provided empirical constraints on model behaviour, which allowed us to estimate several model parameters, including the average duration of immunity. Model calibration was performed based on a four-step process outlined below. Briefly, using ranges of parameter values derived from the chlamydia literature (see Table 2.1

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in the previous chapter) model equations were then calibrated to determine whether the models were capable of reproducing historical data. Model fit was first assessed visually (i.e., based on the ability of the model to qualitatively reproduce observed trends) and secondly by calculating the coefficient of determination, r^2, between observed chlamydia case notifications and model-predicted cases from least-squares lines.

Model 1: A Model that Assumes Treatment of Infection Truncates Acquired Immunity

The fractions of the population that were susceptible, infected, treated, or were removed at time t were denoted by $S(t)$, $I(t)$, $T(t)$, and $R(t)$ respectively. We assumed that the sexually-active population had a constant size N, where $N = S + I + T + R$. The actual or true prevalence of infection in the sexually-active population is represented by $I(t)$. People were assumed to enter the susceptible state at sexual debut at a constant rate, μN, and exited at a rate μS. The probability of chlamydia transmission per year for a given partnership between a susceptible and infected individual is given by βc, hereafter denoted $\hat{\beta}$. Thus, for a given fraction of infected people in the population, $\dfrac{I}{N}$, the number of susceptibles that become infected per year is $S\dfrac{\hat{\beta}I}{N}$. The number of false positives detected for a given test was assumed to occur at a rate $n_S (1 - \phi')$. Here, the number of susceptibles tested is the difference between the recorded testing volume, V, and the number of infectives tested, $n_S = V - n_I$. The number of infectives tested was a function of the fraction of infectives tested, $\beta_T \left(1 - e^{-\alpha(V/I)}\right)$, and the prevalence of infection in the population, I: $\beta_T \left(1 - e^{-\alpha(V/I)}\right) I$. Our testing assumptions here posit a relation that states if testing increases, more cases will be found. However, simply doubling the number of tests does not mean that twice the number of infection will be found. In effect, the model assumes a "law of diminishing returns" where there is a lower and lower incremental benefit with increased testing. Thus, when testing volume, V, approaches zero, the fraction of infectives tested approaches zero at a rate α; as testing volume increases, the fraction of infectives tested will approach β_T, reflecting that even with great testing effort, it takes a certain amount of time to identify some infectives. The fraction of infected people detected by testing (per year) were treated at a rate $n_I \phi$, recovered naturally at a rate σI, or exited the infected class at a rate μI.

Those deemed infected (i.e., both true and false positives) were treated and returned to the susceptible class at a rate $T\sigma'$ or exited the treated class at a rate μT. All those in the removed state then were assumed to return to the susceptible class as a result of waned immunity at a rate δR. Here, we made an additional assumption adopted from Brunham et al. [30] that being found to have infection and thus being treated did not impact a person's sexual risk-taking behaviour. Our assumptions for this model were motivated by the observation that being treated may inhibit the development of acquired immunity, and thus returned a person to the susceptible state at a rate $T\sigma'$. As was mentioned in the main text, this assumption jointly accounted for no change in sexual risk
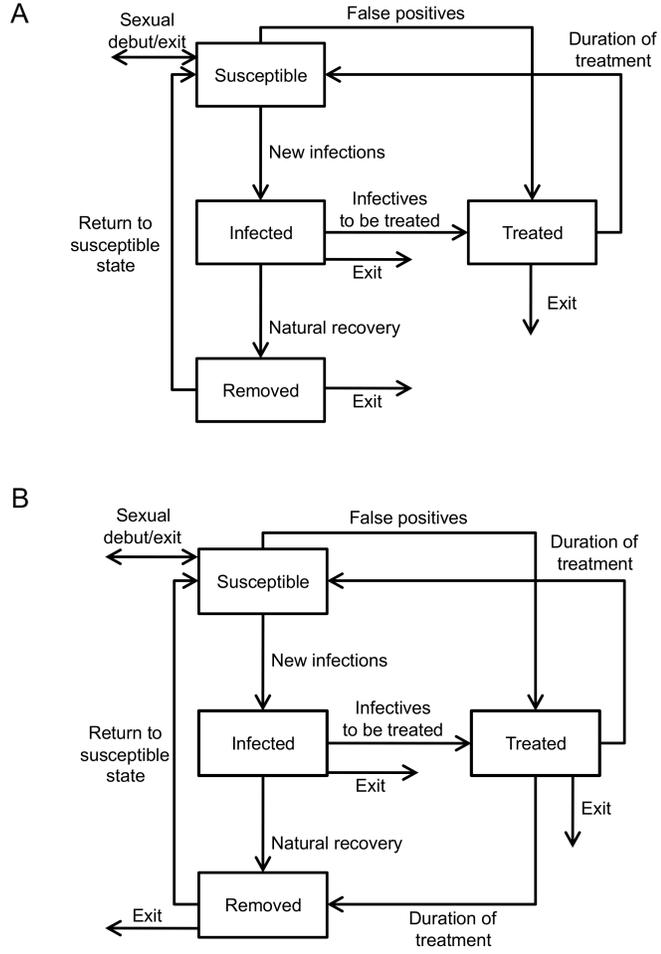
Figure 3.2: Schematic stock-and-flow diagrams of the structure of (A) Model 1 and (B) Model 2.

behaviour, but also assumed that treatment truncated any benefit of acquired immunity [30, 175]. These assumptions produced the following system of equations:

$$\dot{S} = \mu N + \delta R + T\sigma' - S\frac{\hat{\beta}I}{N} - n_S\phi' \tag{3.1}$$

$$\dot{I} = S\frac{\hat{\beta}I}{N} - I\left(\mu + \sigma\right) - n_I\phi \tag{3.2}$$

$$\dot{T} = n_S\phi' + n_I\phi - T\left(\sigma' + \mu\right) \tag{3.3}$$

$$\dot{R} = I\sigma - R\left(\delta + \mu\right). \tag{3.4}$$

Model 2: A Model that Integrates Behaviour Change into Model 1

Here, the removed class $R(t)$ was assumed to contain a combination of those who had not presented for treatment and recovered naturally from infection, and a fraction, θ, of those who had temporarily changed their sexual risk taking behaviour after being treated. In addition to this, we also considered a scenario that jointly accounted for the lack of developing acquired immunity and no changes in sexual risk behaviour after being treated. This additional assumption returned the remaining fraction of the population, $1 - \theta$, to the susceptible state at a rate $(1 - \theta)\,T\sigma'$. These additional assumptions produced the following system of equations as well as the schematic diagram in Figure 3.2:

$$\dot{S} = \mu N + \delta R + (1 - \theta)\,T\sigma' - S\frac{\hat{\beta}I}{N} - n_S\phi' \tag{3.5}$$

$$\dot{I} = S\frac{\hat{\beta}I}{N} - I\,(\mu + \sigma) - n_I\phi \tag{3.6}$$

$$\dot{T} = n_S\phi' + n_I\phi - T\,(\sigma' + \mu) \tag{3.7}$$

$$\dot{R} = I\sigma + \theta T\sigma' - R\,(\delta + \mu)\,. \tag{3.8}$$

3.3.3 Setting Initial Parameters, Model Calibration, and Assessing Goodness-of-fit

Results from simple models (such as the ones described above) can be viewed with suspicion because of their failure to explicitly represent known complexities. However, the ability of these simple models to explain observed epidemiological patterns for chlamydia have not, until recently [198, 200, 204], been exploited in part because little work has been directed at estimating model parameters [44, 60]. Because complete data for all model parameters are often not available, calibration to epidemiological data aids in estimating these lesser-known parameter values. Model calibration, sensitivity analyses, and assessment of model fit were performed using the following four-step process adopted from [200]:

1. *Setting initial parameter values*: Initial parameter estimates were derived from key previously published vaccine trials [73, 98, 192], epidemiological studies [24, 74, 104], and modelling experiments [72, 174]. For the duration of immunity $1/\delta$, values were assumed to range between 6 months and 10 years (see Table 2.1). We based our longer end-point of 10 years on the duration of (a hypothetical) vaccine-based immunity [30], despite other assumptions of life-long immunity in the previous study by Brunham et al. [30]. Though not shown, it should be noted that assuming life-long immunity in the models presented here does not significantly alter our results or interpretations. Where unavailable, parameters – such as duration of sexual activity – were adopted from modelling research of gonorrhea infections [24, 59, 207]. Given

that both gonorrhea and Chlamydia are transmitted by similar behaviour, we believe this is a reasonable assumption. Because certain epidemiological and demographic characteristics such as the average duration of infection, and the average lifetime of sexual activity have been relatively well defined [24, 59], we fixed these parameter values throughout our investigations (see Table 3.1). However, we note that variability in these parameters do not significantly affect our conclusions.

2. *Sampling parameter ranges and fitting the model*: Each parameter value was associated with a range of values found in the literature surveyed. Unique parameter combinations that minimized the discrepancy between the reported case notifications and those "predicted" by the model were determined by a sample of 30 optimizations using the Powell global optimization algorithm available in Vensim DSS for Windows (version 5.5c). Each optimization used a distinct random number seed, and performed approximately 5×10^5 simulations (totalling 15×10^6 simulations across all optimizations).

3. *Goodness-of-fit*: model fit was first assessed visually (i.e., based on the ability of the model to qualitatively reproduce observed trends) and secondly by calculating the coefficient of determination, r^2, between observed chlamydia case notifications and model-predicted cases from least-squares lines (using SPSS for Windows, version 14, Chicago, IL) for different assumptions about the average period of immunity.

4. *Sensitivity Analysis*: Each of the optimization scenarios identified a different point in parameter space that offered the "best fit" to the collected data. Because of the inherent variability in each optimization scenario, sensitivity analyses of the model-predicted duration of immunity were performed based on the distribution of parameter vectors produced in step 2.

3.4 Results

We are interested in the sensitivity of model predictions to key parameters that are difficult to measure empirically. While both models accurately mirrored historic trends (Figure 3.3), this ability was sensitive to assumptions about the period of naturally acquired immunity, but not to other calibrated parameters. In particular for both models, an average period of infection-acquired immunity between 8.4 and 9.0 years (for Model 1, Table 3.1) and 8.6 to 9.3 years (for Model 2, Table 3.1) gives the best fit to the data, both qualitatively (Figure 3.3) and based on least-squares estimates of the coefficient of determination ($r^2 = 0.966$ and 0.967, respectively, Table 3.2 and Figure 3.4). Interestingly, both models also shared an inability to reproduce observed case notification data when negligible or short periods of immunity (Figure 3.3) were assumed, despite yielding statistically significant goodness-of-fit ($r^2 = 0.827$, Table 3.2 and Figure 3.4). While both

Model	Description	$1/\delta$ (SD) (yrs)	95 % CI (yrs)
Model 1	For this model, the removed state contains only those who recover naturally from infection.	8.7 (1.05)	8.4, 9.0
Model 2	Here, the removed state contains a combination of those who recover naturally from infection, as well as the subset of those treated individuals who have temporarily changed their sexual risk taking behaviour.	8.9 (1.28)	8.6, 9.4

Table 3.1: Uncertainty and sensitivity analyses of the calibrated estimates of the average period of immunity (in years), $1/\delta$, from two susceptible-infected-treated-removed dynamic models that were calibrated to reproduce 25-year case data from the Canadian province of Saskatchewan. In each model, the removed state was assumed to represent removal due to different biological and behavioral processes. The "best-fit" values for the mean, standard deviation, and 95% confidence intervals of $1/\delta$ were derived from a sample of 30 calibration experiments. Each calibration experiment was based on 5×10^5 simulations. Therefore, the "best-fit" estimates for each model are a result of 15×10^6 simulations. 95% confidence intervals estimate variability in optimization results. Abbreviations: SD, Standard Deviation; CI, Confidence Interval.

models with calibrated values of $1/\delta$ contain one more degree of freedom than the models with fixed values of $1/\delta$, it could be argued that comparison of r^2 is inappropriate. However, we would like to note that, although not shown, discrete (fixed) values greater than 4 years also demonstrate increased "fit" to observed data.

The removed state in Model 2 contains a combination of people "biologically" removed (by immunity) and those who are "behaviorally" removed (i.e., people who temporarily change their sexual behaviour after being treated for infection). To try and account for the effect of those individuals returning to risky sexual behaviour on the estimate of the average duration of immunity, we compared Model 2's estimate to the estimate of Model 1. When compared, the calibrated estimate of Model 2 suggests an epidemiological picture with two noteworthy features: the first, that the assumption of temporary behaviour change does not significantly change the estimate of infection-acquired immunity between Models 1 and 2 (see Table 3.1); and second, a small percentage of people, approximately 12% (95% confidence interval: 6.8, 18.1), alter their behaviour to temporarily remove themselves after receiving treatment for infection.

Like many other aggregate STI models, the structures of the models presented here assume that the duration of immunity is exponentially distributed [204]. Therefore, there can be substantial variance in this distribution, and many people will lose immunity faster than others [204]. To examine how fast immunity might, on average, wane yet still produce results consistent with observed data, we manually varied our estimate of the mean duration of immunity over fixed, discrete

| | 1/δ fixed | | | 1/δ cal | |
	0.5 years	2 years	4 years	Model 1	Model 2
Initial Susceptibles $S(0)$	0.280	0.000	0.100	0.563	0.581
Initial Infected $I(0)$	0.006	0.025	0.151	0.033	0.016
Initial Removed $R(0)$	0.714	0.975	0.749	0.404	0.403
Able to reproduce data?	No	No	Yes	Yes	Yes
Goodness-of-fit r^2	0.827	0.870	0.963	0.966	0.967

Table 3.2: Displayed are the fractional densities of model-derived initial suscep-
tible, infected, removed populations, as well as the goodness of model fit, r^2, for
(arbitrary) fixed and calibrated periods of immunity (in years). Fixed values of
the average period of immunity, $1/\delta$, are from calibrations of Model 1. Model 2
produced comparable results. Calibrated values are taken from one of the 15000000
simulations used to derive the parameter estimates in Table 3.1. The coefficient
of determination, r^2, measures the goodness of fit between the observed cases and
the model-predicted cases, by least-squares lines, under specific assumptions about
the average period of immunity, $1/\delta$. Scatter plots and fitted lines are displayed in
Figure 3.4. All goodness-of-fit measures were statistically significant, $P < 0.01$.

intervals, beginning with the assumption of negligible periods of immunity. Both Models 1 and 2
were then recalibrated according to the four-step process outlined above to best match the available
data for each discrete estimate. The results in Figure 3.3 demonstrate that an average duration of
immunity of at least 4 years is needed to qualitatively mirror observed case notification records.
One striking result was that a fixed estimate of 4 years generated model-predicted behaviour of
Chlamydia case counts that was almost qualitatively indistinguishable from the estimates produced
through calibration (Figure 3.3).

3.5 Discussion

An important prerequisite in ascertaining the feasibility of an anti-chlamydial vaccine is the ability
of naturally occurring infections to provide protection against reinfection [28,190]. However, little is
known about the average duration of immunity to Chlamydia in human populations or its epidemio-
logical implications [16,28]. Here, we have provided a useful, but by no means exclusive, analytical
methodology to estimate this difficult to observe natural history parameter; this approach inte-
grated two dynamical models with historic test volume records, and then calibrated them to match
case notification data from the Canadian province of Saskatchewan. Overall, the results presented
here highlight the importance of considering natural immunity in the representation of Chlamydia
incidence, and suggest that successful development of an effective vaccine is probably feasible. Most
notably, these results suggest that, for the model structures used here, assuming negligible periods
of immunity strongly disagree with empirical observations, and are robust to variability in other
model parameters. The current analyses found that a mean period of infection-acquired immunity
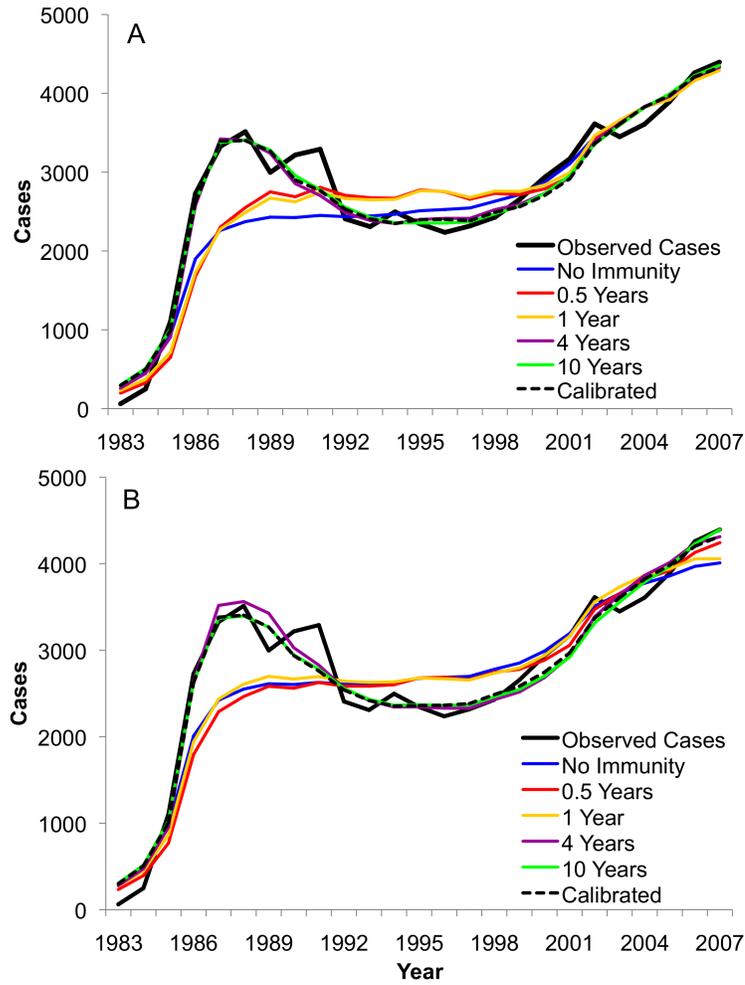
Figure 3.3: Comparison of model output to observed case notification data for different assumptions about the duration of infection-acquired immunity. Model predicted cases for different assumptions about the average duration of immunity are as follows from Model 1 (A) and Model 2 (B). "Calibrated" refers to values of $1/\delta$ displayed in Table 3.1. Model predicted cases are plotted against reported case notifications in Saskatchewan.
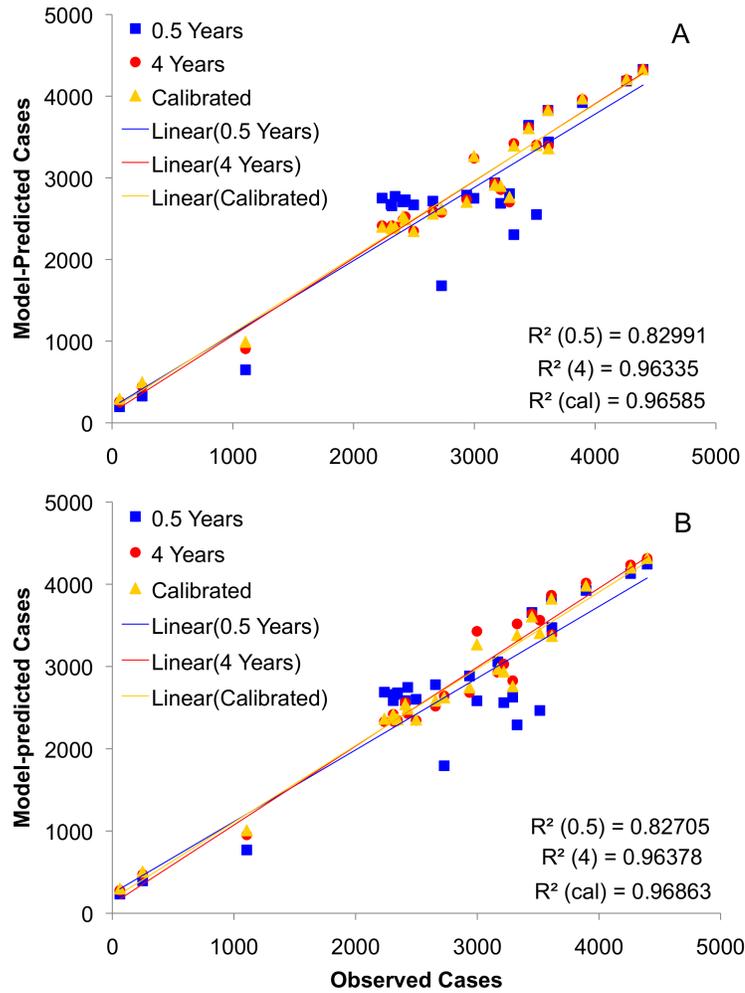
Figure 3.4: Scatter plots used to assess goodness-of-fit between observed case notifications in Saskatchewan and model-predicted case counts for different fixed assumptions of the average duration of immunity, $1/\delta$, for Model 1 (A) and Model 2 (B). The coefficient of determination, r^2, measured the goodness-of-fit between the observed cases and the model-predicted cases, by least-squares lines under specific assumptions for $1/\delta$. "Calibrated" refers to values of $1/\delta$ displayed in Table 1.

between 8.4-9.0 years (for Model 1) and 8.6-9.3 years (for Model 2) is most consistent with the historical records of chlamydia in Saskatchewan.

In these analyses, we attempted to place a lower bound on the mean period of immunity by fixing it over discrete intervals and examining the models' ability to reproduce observed data. This analysis consistently demonstrated that periods of immunity < 4 years could not provide a reasonable fit to observed case notifications. In addition to being unable to qualitatively reproduce observed data, both models posited an interpretation of the underlying epidemiology that initially placed the majority of sexually active people in either the infected or removed state, and few or no people in the susceptible state (Table 3.2). In our opinion, this scenario seems implausible because we could expect most people in the sexually active population to be susceptible; combining larger initial fractions of removed people with shorter periods of immunity will replenish the susceptible population quickly, enable the feedback loop driving infection to become dominant, cause the fraction of infected people to increase rapidly, and result in model-predicted case counts that poorly match observed case counts. Although a lower bound of 4 years allowed for adequate fit to observed data, it required the assumption that approximately 90% of the initial sexually active population was in either the infected or removed states (Table 3.2). Interestingly, these skewed distributions of initial states became less extreme, and more plausible, as the assumed period of immunity increased. The calibrated estimates of 8-9 years assume that approximately 55% of the sexually active population was susceptible in 1983.

Evidence from previous clinical and epidemiological research indicates that genital reinfection with Chlamydia is common and that reinfection occurs soon after eradication of infection by treatment (see [92] for a review). This has led investigators to question whether there is natural protective immunity to chlamydial genital infection in human populations [181]. Our estimates of the average duration of immunity are considerably longer than those in other epidemiological research [12, 24, 30, 59, 72–74, 98, 104, 117, 142, 174, 192]. The disparity between our estimate and those of previous work might simply be a result of significant variability in the duration of immunity in different individuals that was not explicitly captured by the models used here; our estimate of the period of immunity represents a population average of the time until an individual re-enters susceptibility. Conversely, it could also reflect the fact that, unlike the total sexually active population, previous studies of chlamydial reinfection – such as the ones reviewed in [92] – focus on core groups of highly sexually active people or those populations reached by STI clinics, both of which are actively followed-up and have high rates of treatment. For such highly treated populations, the time until reinfection is likely to bear little relationship to the duration of natural immunity. Instead, these types of follow-up studies are better suited to measure the time until reinfection after treatment, where a fundamental entanglement between treatment and impaired development of immune responses [30, 175] are likely to be observed.

The uncertainty about natural immunity to reinfection has had a significant impact on model-based analyses of public health strategies to reduce chlamydial prevalence. When immunity to reinfection has been assumed, previous evaluations of screening and vaccination programmes have had to infer a period of immunity derived from animal models of genital infection, which wanes within a matter of months [72, 174, 201]. In light of the results presented here, two important questions can be raised about the use of data from animal models of genital infection for inference about human genital infection: firstly, how suitably do animal-derived periods of immunity map onto the biology of human chlamydia infections? And secondly, will the projected policy outcomes accurately reflect the behaviour of chlamydial epidemiology in the presence of that intervention programme? We believe these questions extend equally well to model-based policy evaluations that have employed a Susceptible-Infected-Susceptible (or SIS) modelling framework where a negligible period of immunity is assumed [59, 117, 118].

An ideal estimate of the average period of immunity would be derived from active longitudinal follow-up of the time to reinfection in the absence of treatment. However, such study protocols in human populations are neither ethical nor feasible. Yet, if our estimates of the average duration of immunity are accurate, then upon investigating existing cohort studies in populations where other Chlamydia infections are endemic and untreated, we would expect to observe epidemiological signatures of prolonged immune periods such as low rates of reinfections, and slowed decay of population-level immune correlates in previously infected people over many years. Interestingly, the results of other investigators have reported strikingly low reinfection rates for both genital infections in Columbian women [142] and ocular infections in highly endemic regions of Taiwan [11, 74] over 5 and 10-year follow-up periods, respectively. A Finnish maternal cohort has also demonstrated the maintenance of elevated anti-chlamydia IgG titers over a 7-year segment of a 21-year follow-up period [127]. Although not specifically designed to measure the duration of anti-chlamydial immunity, these studies do provide indirect epidemiological, clinical, and serological support for our conclusions of longer-lasting periods of infection-acquired immunity. Ultimately, additional empirical research designed to investigate this issue will help further support or weaken our results here.

There are some important limitations to this analysis that require discussion. As with any model, its structure represents a simplification of reality [21, 57, 169]. The models used here reflect simple dynamic hypotheses of Chlamydia transmission within the sexually active population. While traditional modelling of sexually transmitted infections (STIs) has made use of simple models that abstract away from population heterogeneity [21, 57], many recent STI transmission models feature a more-detailed characterization of the population [29, 59, 60, 72, 117, 126]. Although more complex models offer advantages for investigating certain types of research or policy questions [169], several considerations motivated the use of simpler models for this study. Specifically, because the focus was

on estimating the population-wide average duration of immunity, because of the aggregate nature of case notification counts and testing volumes (which lack explicit information on behavioral heterogeneity or network structure), and to avoid over-fitting that often accompanies high-dimensional parameter spaces, the models presented here did not explicitly represent heterogeneity in sexual risk behaviour. Instead, people were assumed to mix randomly at an average population rate.

Our calibration methods also have several limitations. For one thing, with several parameters being varied simultaneously, parameter space was extensive and it is difficult to know whether it was searched comprehensively. Although other calibration approaches have been described in the literature [38, 60, 108, 169, 180], there is no universal approach that is sufficient for all models, nor is there consensus on the "best" approach [108]. The choice of calibration method will largely depend on model structure and the granularity of available data [169]. For another, the reported variability in $1/\delta$ (as reported in Table 3.1) is best described as the variability in optimization results, not variability of this parameter in the "real world". To obtain the latter, it would be sensible to employ more robust methods of confidence interval estimation, such as bootstrapping [46]. However with this in mind, we note that our initial estimates of the confidence intervals using bootstrapping techniques for dynamic models outlined elsewhere [46], do not yield significantly different results than those presented here.

Calibrating mathematical models to empirical data helps identify reasonable dynamic hypotheses that parsimoniously explain observed data, as well as aid in identifying inconsistencies between observations and model assumptions [117]. While no model can account for all of the intricate complexities of reality, the methods employed here offer significant high-level insights. Using these simple models, we were able to estimate an epidemiologically relevant parameter from two sets of routinely collected data. More importantly, given the model structures we assumed, we demonstrated that to reproduce observed case notifications, the average duration of immunity must be longer than what is widely assumed in epidemiological literature [59, 72, 117, 124, 174]. We are hopeful that the acquisition and analysis of long-term longitudinal data – be they from serological or clinical follow-up studies – combined with detailed model fitting will help elucidate the natural history of chlamydial immunity and its epidemiological consequences.

Chapter 4

Study 3: Natural History of Repeated Chlamydia Infections from Two Within-Host Models

4.1 Relationship to the Previous Study

Though the population-level compartmental models in the previous two studies indicate that treatment might be impacting the development of immunity, they suggest that it might not be significantly driving the currently observed population trends. However, there are different model structures that contain different representations of interacting individuals. Models at different levels of aggregation may lack the structure needed to observe important dynamics, and policy responses across different model structures can be similar or different [169]. To try and get a more complete understanding of how arrested immunity might impact the transmission and prevalence of Chlamydia infection, I want to extend the previous analysis to examine the conditions arrested immunity might have significant consequences for chlamydial prevalence. This way, I can examine the proposed impact of a policy at the appropriate level.

One important question is whether the impact of a policy (such as treatment) will vary significantly in models of different scale. The most obvious difference between the models in the first two chapters and the ones that follow is the explicit representation of the host-pathogen interaction. Capturing any variability in the spread of infection, either due to immunological differences or network heterogeneity requires moving from an aggregate representation of transmission to an individual one. In the remaining set of studies, I chronicle an iterative approach in which I develop an immunoepidemiological model of chlamydial transmission.

However, developing an individual-level model that not only captures an individual's immunobiology, but also their contacts with others required several iterative steps. In this next chapter I have constructed two simple models of within-host Chlamydia infection. I chose to first focus on within-host models for two reasons: firstly, I wanted to build an adequate representation of in-host infection dynamics. I did this by using known immunobiology of Guinea Pig models as the basis of the models presented from here on; and secondly, I wanted to get an idea of the natural history of the immune dynamics within an individual under different exposure histories.

4.2 Background

The genus Chlamydiae encompasses a unique class of obligate intracellular bacteria that can cause disease in a wide range of animals [17]. In comparison to all other commonly-reported sexually-transmitted infections (STIs), *Chlamydia trachomatis* represents a unique and important public health concern, as prevention efforts are hampered by cryptic infections and delayed diagnosis [29, 146]. This ability to thrive, despite considerable advances in the understanding of the immunobiology, pathogenesis, and epidemiology of this parasite [146], has made it the world's most common cause of sexually-transmitted disease [29].

Inference by analogy with animal models has produced no lack of evidence to support the existence of an effective immune response against *Chlamydia* spp [17,29,95,99,145,146,148,171,189,190]. Notable insights into mechanisms of immunity have been achieved through studying primary and secondary infections. Rodent models of Chlamydia genital tract infections have demonstrated that a large proportion of animals resolve primary infection and are temporarily resistant to reinfection [95,99,145,148,171,189,190]. When reinfection does occur, secondary inflammation and disease is significantly shorter, and bacterial load is greatly reduced.

Because of obvious ethical and practical concerns, equally-detailed insights into human immune responses, with the exception of one early vaccine trial [98], have not been extended beyond observational studies. However, elimination of infection is likely immune mediated [28]. Resolution of Chlamydia infections in humans has been documented to occur within months, even years [142,152,160], and previous estimates of the basic reproductive number, in conjunction with epidemiological evidence from "core" groups, suggests that frequent exposure to Chlamydia infections confers some degree of strain-specific protective immunity [25]. Although these observed patterns are consistent with what might produced by heterogeneity in individual immune responses, it is interesting to note that immune responses have not been directly measured [120]. As a result, this leaves some elementary questions unresolved.

With human immunobiology and reinfection rates being the focus of recent debate in STI epidemiological literature [125,138,175], it is evident that conclusions drawn from either shorter-term experimental or observation research as they apply to long-term exposure and immunobiological kinetics should be tentative. With the exception of one study [171], no research, to our knowledge, has examined susceptibility to multiple chlamydial infections, and the resulting implications for the spread of sexually-transmitted Chlamydia infections. Due, in part, to the study protocol of previous analyses of the natural history of Chlamydia infection [142], little research has directly explored the effect exposure of history on an individual's immune repertoire, or investigated any potential consequences for the spread of sexually-transmitted Chlamydia infections [171]. Since complete immunity (i.e., immunity that does not permit reinfection) has been demonstrated to

wane over time [146], it remains unclear, in our opinion, how the severe secondary sequellae related to Chlamydia positivity are modified by both duration of infection and the number of prior infections – particularly when many years may separate reinfections.

Most, if not all, modeling related to chlamydia (and sexually-transmitted infections, in general) is concerned with the population dynamics of these infections. Although very insightful, traditional modeling techniques either disregard the effects of immune system dynamics on the spread of infection, or will abridge chlamydial in-host pathogenesis into a few simple model parameters. However, we argue that the complexity of chlamydial pathogenesis, and its capacity to persist in the presence of mass control programmes, suggests a need for developing novel analytic tools to better understand the within-host behavior of Chlamydia, evaluate interventions, and to identify future research priorities [120,158,188]. Our present objective is to examine anti-chlamydial immunity and multiple reinfections in an individual (here a simulated Guinea Pig) as a starting point for studying the potential impact these factors will have on the spread of Chlamydia infections. To do so, we consider a simple mathematical framework of the dynamic interaction between Chlamydia bacteria and in-host immune responses – using known immunobiology of Guinea Pig models. We explore basic within-host kinetics to help inform our understanding of the potential qualitative impact of frequent exposure to Chlamydia on in-host humoral or cell-mediated adaptive immune responses.

4.3 Models and Methods

Our aim was to consider a highly parsimonious model and explore its implications in the context of chlamydia infection dynamics. To do this, we constructed two simple mathematical models with explicit expressions for chlamydia bacteria, host target cells of the genital tract, and chlamydia-specific immune responses: the first, a "basic" model; and the second, an "extended" model. These two structures represented a combination of dynamical hypotheses about chlamydia immunology that allowed us to iteratively investigate, and build confidence in, the role of both CD4+ T cell, and anti-chlamydia antibody responses under different exposure histories. Each of the models are represented schematically in Figure 4.1.

4.3.1 A Basic Model of Within-Host Chlamydia Replication with CD4+ T cells

We elaborated an established within-host model $[5, 23, 39, 158]$ to include uninfected endothelial cells (ECs) of the genital tract (X), infected ECs (Y), free, infectious, and metabolically inactive elementary bodies (EBs) (E), T_H1 CD4+ T cells (Z) that produce interferon gamma (IFN-g), and induce the expression of indoleamine-2,3-dioxygenase which depletes cellular levels of tryptophan (TRP) [29]. Uninfected ECs were produced at a constant rate, λ, died at a rate δX, and recovered
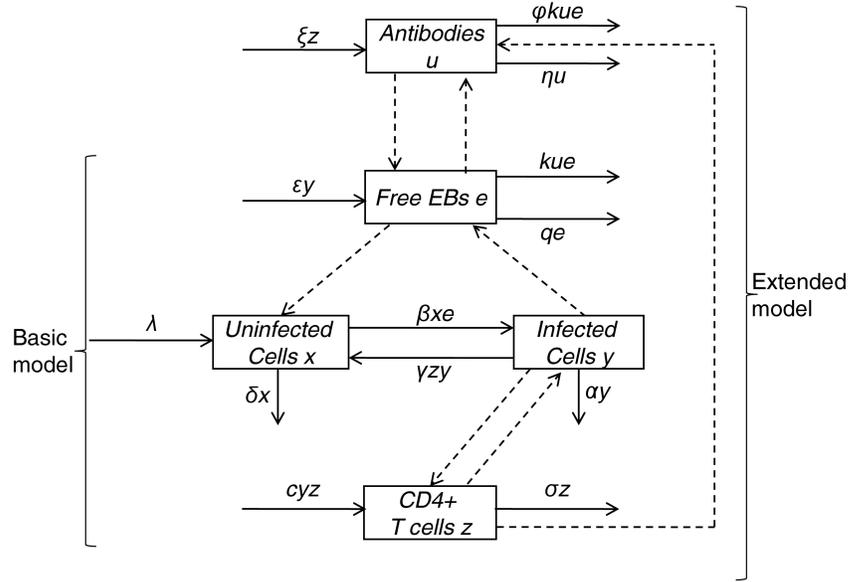
Figure 4.1: Stock and flow diagram illustrating the assumptions about chlamydial dynamics in the models. Displayed is a schematic representation of the models, equations 1-6. Basic and extended models are labeled by curved braces. Dashed arrows between state variables indicate interactions between them.

from infection at a rate γZY. We used mass-action kinetics to model the interactions of uninfected cells with infectious EBs; infected cells were produced at a rate βXE, and died at a rate αY. We further assumed that activated chlamydia-specific CD4+ T cells proliferate and differentiate at a rate cY. In reality, the activation and proliferation of CD4+ T cells is induced by antigen-presenting dendritic cells; however here, we followed Nowak and May [159] in assuming that activation and proliferation is roughly proportional to the number of infected cells. Activated CD4+ cells died at a rate σZ. The main effector mechanism of CD4+ T cells in this model was IFN-g-mediated TRP starvation of chlamydial reticulate bodies (RBs) (i.e., the metabolically active intracellular stage of the chlamydia life cycle). Here, the concentration of cytosolic TRP was modeled implicitly and was assumed to be inversely proportional to the number of CD4+ cells. Because low levels of TRP have been demonstrated to have minimal effect on the viability of host cells [159], infected cells were therefore assumed to "recover" from infection as a result of RB starvation. These assumptions produced the set of ordinary differential equations:

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$$\dot{X} = \lambda - X\left(\delta + \beta E\right) \tag{4.1}$$

$$\dot{Y} = \beta X E - Y\left(\alpha + \gamma Z\right) \tag{4.2}$$

$$\dot{E} = \epsilon Y - qE \tag{4.3}$$

$$\dot{Z} = cYZ - \sigma Z. \tag{4.4}$$

4.3.2 An Extended Model of Within-Host Chlamydia Replication with CD4+ T cell and Antibody Responses

We extended the basic model to include chlamydia-specific antibody (U) that inhibit infection of genital tract ECs. Although antibody-mediated cellular cytotoxicity is also likely to aid the resolution of primary infection, for this study, we ignored it as its significance during chlamydia infection has yet to be determined [121, 148, 221]. We followed Yao et al [221] in assuming the production of antibody, ξ, is proportional to the number of CD4+ cells. In reality, the level of antibody is dependent upon the proportion of plasma cells [139]. Given that antigen-specific B and T cells are activated and proliferate in local lymphoid aggregate tissue in the genital tract by similar mechanisms [29,139,221] we assumed that they were roughly proportional to each other. For chlamydia, this appears to be a safe assumption as it has been demonstrated that anti-chlamydial antibody does not develop in the absence of CD4+ T_H1 cells [148]. Previous studies have also explored this issue in sufficient detail and found negligible impacts on conclusions [221].

$$\dot{E} = \epsilon Y - kUE - qE \tag{4.5}$$

$$\dot{U} = \xi Z + \phi kUE - \eta Z. \tag{4.6}$$

From Yao et al [221], the natural decay rate of the antibody population (in 1/days) was denoted by η, the efficacy of antibody-induced EB neutralization is denoted by k, and the number of antibody particles that are consumed in forming an EB-antibody complex is denoted by ϕ. Because complete protective immunity is likely to wane over time [146], we included no explicit representation of the formation of memory T or B cells in either the basic or extended models. Instead, we implicitly represented immune memory by slow die-off rates of CD4+ and antibody cell populations. These assumptions modified equation 4.4 of the basic model and produced an additional equation for antibody kinetics to form the extended model (equations 4.5 and 4.6). For both basic and extended models, the respective set of ordinary differential equations was solved numerically using the default Euler integration method in the modeling software Vensim DSS for Windows (version 5.5c).

Because these models are deterministic, and have previously been used to study persistent and recurrent viral infections, CD4+ and antibody state variables cannot drive $Y(t)$ or $E(t)$ to

zero. Therefore, following Wodarz et al. [213] we defined a threshold value below which chlamydia infection was considered extinct. Our extinction threshold was arbitrarily chosen to be marginally larger than the endemic equilibrium value of a model lacking a threshold, such that a single infection would eventually be cleared. We included an extinction threshold because we anticipated that this would better match empirical observations of individual infection and immune kinetics than a model without an extinction threshold. Initial conditions for the models were $X(0) = 100$, $Y(0) = 0$, $Z(0) = 1$, $E(0) = 0.01$, for both basic and extended models, and $U(0) = 0.01$, for the extended model.

Clearly, a complete mathematical description of the immune system is neither feasible nor analytically tractable, due to the vast complexity of the immune system. However, recent theoretical work has demonstrated the merit of simple mathematical models in reproduction and explanation of experimental results [5,6,23,39,213]. Our model structures were purposefully kept simple so to focus on broad immunobiological insights. The philosophy behind starting with a simple representation of a complex system designed to address certain well-defined questions is similar to that motivating the methods of experimental scientists [58]. Such simple models can often lead to important insights of a general nature into the factors or processes that shape epidemiological patterns [58].

4.3.3 Parameterizing and Calibrating the Models to Experimental Data

Often, obtaining experimental estimates for many parameters in a model can be difficult [5, 23]. Where possible, however, the model has been parameterized to chlamydia-specific kinetics based on previous research [29]. Where no experimental or observational information could be readily obtained, the remaining parameters of the two within-host models were either taken from similar models [5, 23], or were calibrated to approximate published experimental data from both mouse and guinea pig models [95, 99, 146, 148, 171, 189]. Though not an exact mimic of human infection and disease, animal genital tracts have been frequently-used for studying immunity to Chlamydia. Although the duration of immunity varies between animal species, there is strong evidence to suggest that complete chlamydia-specific immunity to homologous strains will wane with time [28, 95, 171, 190]. Therefore, for the analyses presented below, the duration of immunity is assumed to be short-lived. Here, we assumed that the rate of T cell die-off (in basic and extended models) and the rate of antibody die-off (in the extended model) occurred at a sufficient rate so that the duration of complete immunity remained consistent with empirical studies (i.e., reinfection could occur between approximately 70-80 days post-initial infection) [189]. See table 4.1 for parameters used throughout simulation experiments.

Parameter	Description	Value (1/day)	Reference
λ	Rate uninfected cells replenish.	1.54	[39]
β	Infection rate.	2.31	[95, 99, 146, 148, 171, 189]
δ	Uninfected cell die-off.	0.01	[95, 99, 146, 148, 171, 189]
α	Infected cell die-off.	0.01	[95, 99, 146, 148, 171, 189]
γ	Rate infected cells are cured by CD4+ cells.	6.58	[95, 99, 146, 148, 171, 189]
c	T cell responsiveness rate.	1.86	[121]
σ	T cell die-off.	0.02	[95, 99, 146, 148, 171, 189]
ϵ	EB production rate from infected cells.	2.02	[29]
q	Rate of EB decay.	2.00	[95, 99, 146, 148, 171, 189]
ξ	Antibody production rate.	0.12	[148, 171, 190]
η	Antibody decay rate.	0.05	[148, 171, 190]
ϕ	Number of antibody consume per disabled EB.	5.41×10^{-5}	[148, 171, 190]
k	Rate EBs are disabled per Ab-EB complex.	0.001	[39]

Table 4.1: Parameters for basic and extended models.

4.3.4 Re-exposure Scenarios

Because exposure history to, and subsequent reinfection with, chlamydia is of current epidemiological concern, we examined the long-term within-host dynamics under four different re-exposure scenarios: the first scenario mimicked previous experimental studies with a single re-exposure after the resolution of a primary infection, but where a second infection would occur (after 70 days); the second, investigated single re-exposure at either 100, 200, 300, or 600 days after initial infection; the third scenario studied multiple re-exposures, every 30 days within 300 days of initial infection; the fourth was a combination of scenarios one and three (i.e., frequent re-exposure, though not as frequent as scenario three, within 300 days followed by a single re-exposure at 900 days after initial infection). Re-exposure times, other than in the first scenario, were chosen arbitrarily to mimic assumed differences in sexual re-exposure to chlamydia. Re-exposure was modeled by an instantaneous inflow of EBs at each of the above-described times using a multiple of the initial infectious dose.

4.4 Results and Key Model Insights

4.4.1 Existence and Stability of Fixed Points

This analytic study was performed to support the results of the simulation analyses. For initial infection (i.e., no re-exposure), the basic model has three equilibrium states. These included an

unstable disease-free equilibrium:

$$\hat{X} = X(0) = \frac{\lambda}{\delta} \tag{4.7}$$

$$\hat{Y} = 0 \tag{4.8}$$

$$\hat{E} = 0 \tag{4.9}$$

$$\hat{Z} = 0, \tag{4.10}$$

an unstable defense-free equilibrium:

$$\hat{X} = \frac{\alpha q}{\beta \epsilon} \tag{4.11}$$

$$\hat{Y} = \frac{\alpha q \delta - \lambda \beta \epsilon}{\beta \epsilon \alpha} \tag{4.12}$$

$$\hat{E} = \frac{\alpha q \delta - \lambda \beta \epsilon}{\beta \alpha q} \tag{4.13}$$

$$\hat{Z} = 0, \tag{4.14}$$

as well as a locally stable endemic equilibrium:

$$\hat{X} = \frac{\lambda c - \alpha \sigma}{c \delta} \tag{4.15}$$

$$\hat{Y} = \frac{\sigma}{c} \tag{4.16}$$

$$\hat{E} = \frac{\epsilon \sigma}{cq} \tag{4.17}$$

$$\hat{Z} = \frac{c \beta \epsilon \lambda - c \alpha q \beta - \sigma \beta \epsilon \alpha}{c \delta \gamma q}. \tag{4.18}$$

In the extended model, however, there exist five different fixed points. However, because two of them are non-physical (i.e., producing negative equilibrium values for state variables), and unreachable from initial conditions with non-negative state variables, we will only outline three of them. These included, as demonstrated in the basic model, disease- and "defense-free" equilibria, as well as an endemic equilibrium. However in contrast to the basic model, none of these equilibria are stable and trajectories approach a limit cycle rather than a static equilibrium (see Figure 4.2).

Since the parameter values in each model were calibrated to produce chlamydia-specific results, they were held constant throughout these analyses. This included the number of chlamydia bacteria during re-exposure. However, further investigation of our results suggests that a range of different values for re-exposure do not affect the stability of the fixed points in the basic model. More specifically, based on Routh-Hurwitz criteria we found that any non-negative perturbation to free EBs, e (i.e., chlamydial re-exposure) in the basic model will not change the stability of the endemic equilibrium. A similar analysis in the extended model revealed that the endemic equilibrium will

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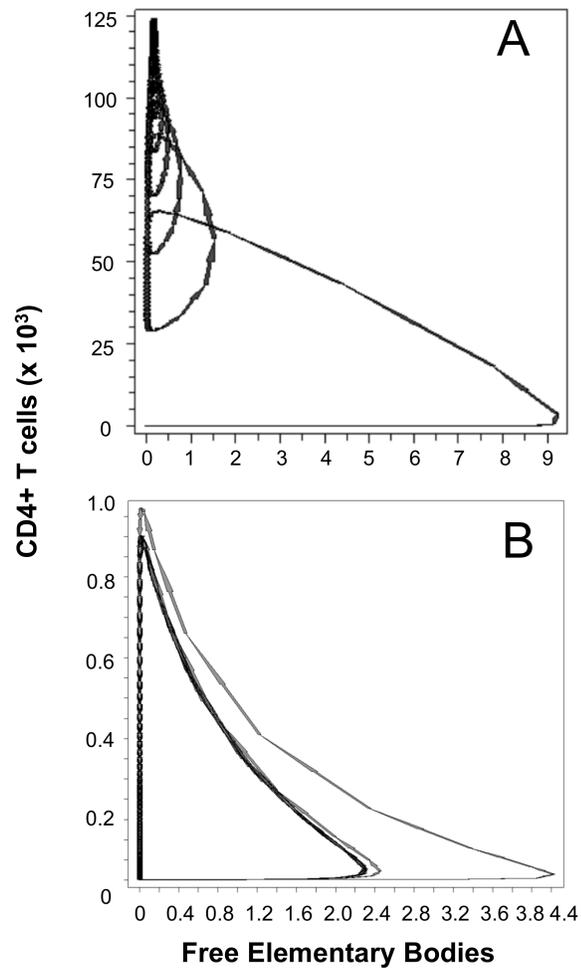
Figure 4.2: Elementary Bodies for the basic and extended models during frequent re-exposure. For the basic model (A), re-exposure results in damped oscillations to an endemic equilibrium and high CD4+ T cell concentrations. However, in the extended model (B) re-exposure will produce a trajectory that approaches a stable limit cycle.

likely remain unstable for shorter-term re-exposure doses similar to that of initial infection. However, an interesting finding was that very large (compared to initial infection), prolonged exposure can render the endemic equilibrium stable.

4.4.2 Both Models Reproduce Experimentally-observed Kinetics of Primary and Secondary Chlamydial Infections

Figure 4.3 illustrates that previously observed kinetics of infected cells and CD4+ T cells (Figure 4.3A) can be approximated by the basic model (Figure 4.3B). Although it may be argued that our parameter values do not exactly reproduce experimental results, we do not feel that our results stray qualitatively from what would be expected in reality. The presence of qualitative, rather than quantitative, similarity does not diminish the value of the insights gained through careful analysis of mathematical models [214]. Because results from rodent models are only approximations of human results, exactly mimicking data from rodent models may add little benefit to understanding human immunity to Chlamydia infection.

Figure 4.4 illustrates the behavior of the basic and extended models when exposure to a second Chlamydia infection occurs. Figure 4.4A demonstrates that the basic model reproduces expected kinetics of free EBs and CD4+ T cell activity during a second infection [146], and Figure 4.4B supports the predominant role of antibody in controlling reinfection [148]. This is in contrast to the basic model where reinfection is not dependent upon the level of antibody, but rather on levels of CD4+ T cells. However, in both models CD4+ T cells are demonstrated to have a decisive role in resolving reinfection.

4.4.3 Increasing the Time between Initial and Second Infection increases Bacterial Load

In Figures 4.5 and 4.6 we examined a single re-exposure at increasingly long intervals from initial infection. In the case of both models, interesting, yet similar dynamical behavior is observed: as the time between initial and secondary infection increases, the severity of that second infection also appears to increase. In the basic model this is associated with an increased magnitude of infected cells (Figure 4.5A). In the extended model, this was illustrated by an increased duration of elevated infected cells (Figures 4.6A).

Immune responses were also affected. For the basic model, reinfection at later times from initial infection appears to produce higher levels of CD4+ cells (Figure 4.5B). In the extended model, however, negative feedbacks associated with both antibody and CD4+ T cells keep immune levels within a range similar to that seen for initial infection (Figure 4.6B and 4.6C).
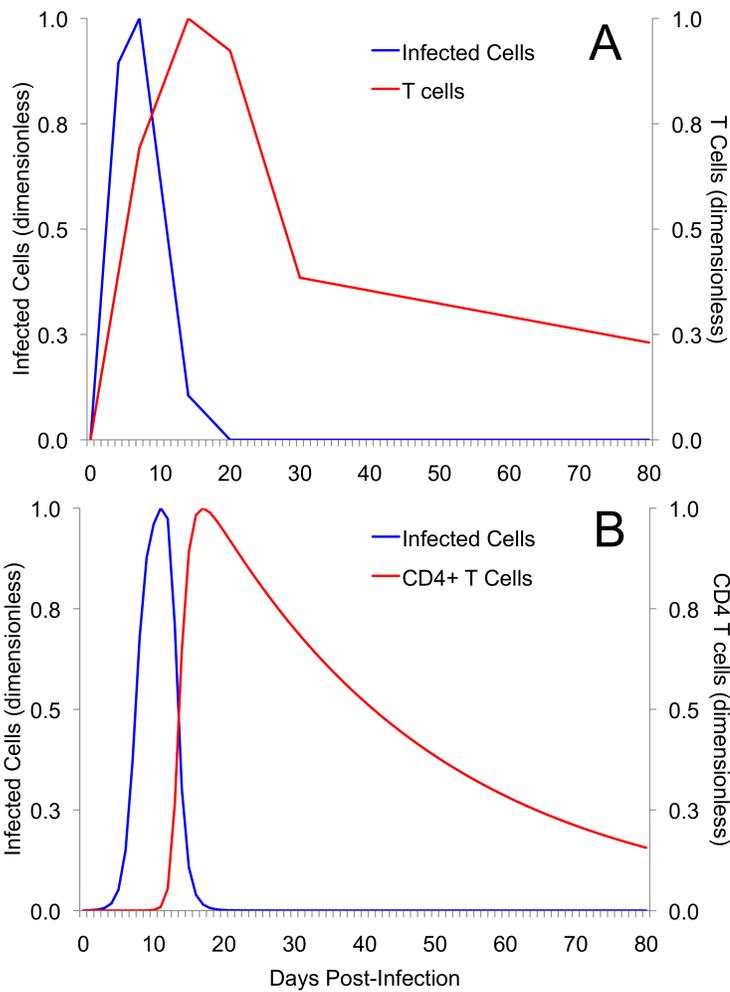
Figure 4.3: A comparison of experimental and simulated kinetics of primary Chlamydia infection and CD4+ T cell responses. Experimental kinetics (A) have been reproduced, from Igietseme and Rank [95]. Simulated behavior (B) was observed when both basic and extended models were calibrated to part (A). Only the results of the basic model are displayed.
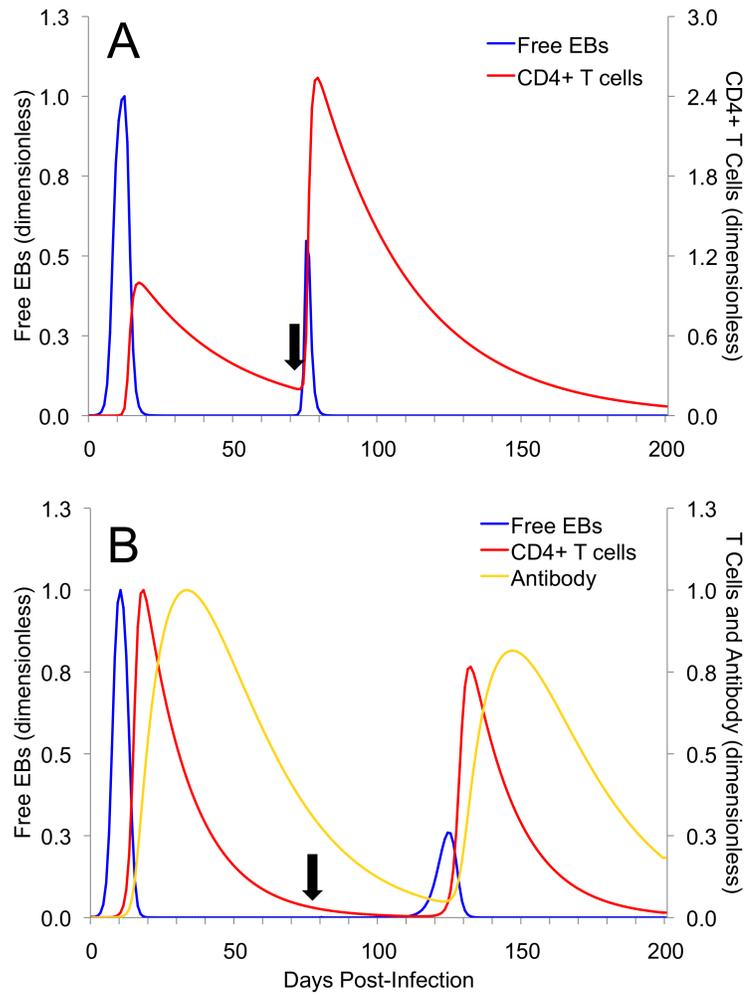
Figure 4.4: Secondary challenge experiments using the basic and extended models. Single re-exposure scenarios using the basic model (A), and the extended model (B) to reproduce expected behavior observed in experimental studies. The magnitude and time evolution of the system of equations in (B) has been uniformly rescaled so to allow for a visual comparison. Black arrow indicates time point of secondary exposure.
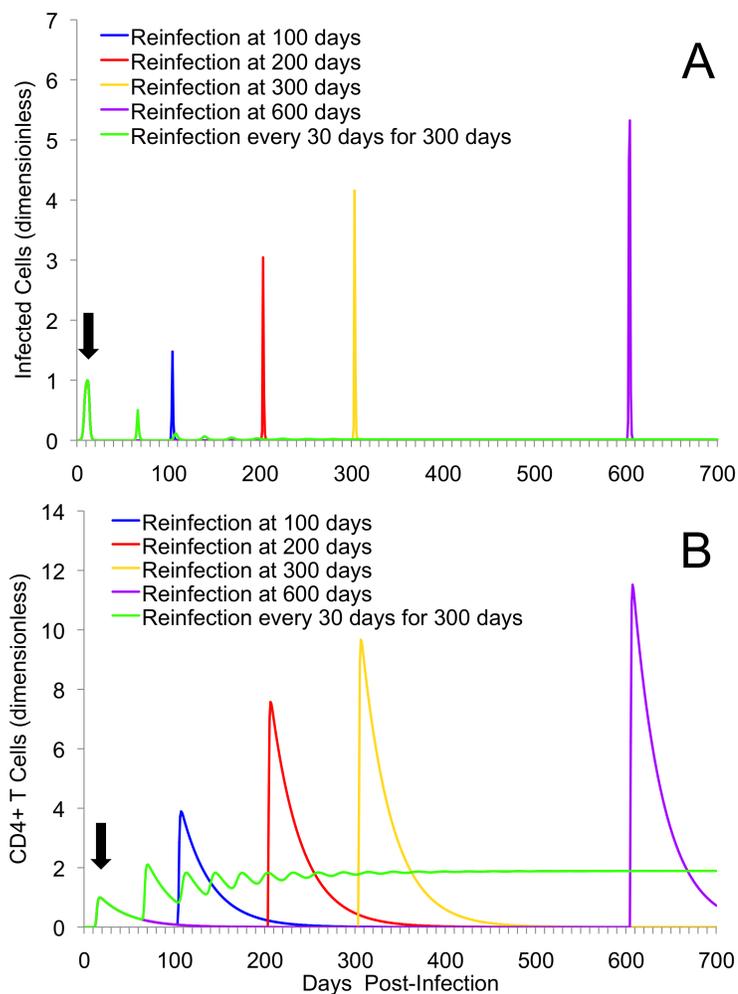
Figure 4.5: Multiple re-exposure experiments using the basic model. Displayed are the kinetics of infected cells (A), and CD4+ T cells (B) for single reinfection at either 100, 200, 300, or 600 days after initial infection in the basic model. Also included is the simulated kinetics of frequent re-exposure every 30 days over a span of 300 days after initial infection. For frequent re-exposure, it should be noted that oscillatory behavior continued after the removal of further infection. Single and multiple re-exposure scenarios are used to represent individuals that have low and high sexual exposure to Chlamydia, respectively. Black arrow indicates point of initial infection (common to all scenarios).
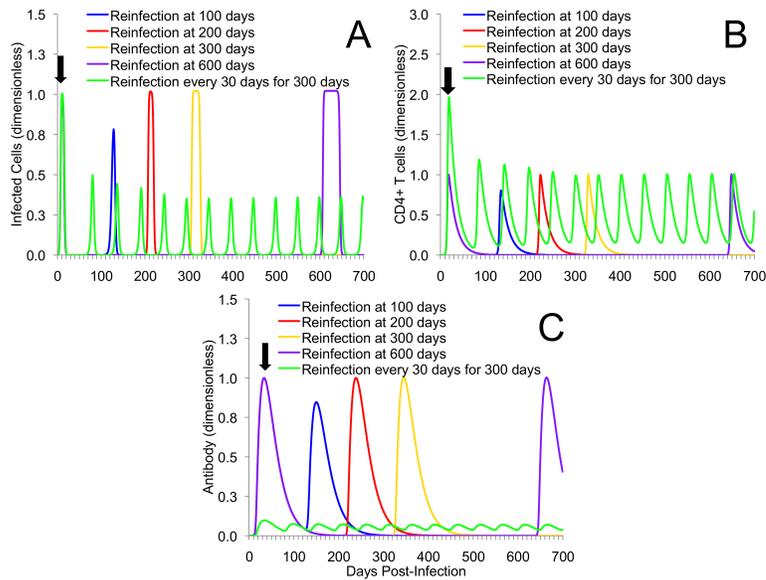
Figure 4.6: Multiple re-exposure experiments using the extended model. Displayed are the kinetics of infected cells (A), CD4+ T cells (B), and Chlamydia-specific antibody (C) for single reinfection at either 100, 200, 300, or 600 days after initial infection in the extended model. Also included is the simulated kinetics of frequent re-exposure every 30 days over 300 days after initial infection under antibody deficiency. It should be noted that, for frequent re-exposure scenarios oscillatory behavior continues once further infection is removed. Black arrow indicates point of initial infection (common to all scenarios). For parts (B) and (C), initial immune cell levels are higher (part B) and lower (part C) at initial infection because of imposed antibody deficiency.

4.4.4 Repeated Exposure Reinforces the Importance of Antibodies and Suggests a Role in Preventing Persistent Infections and Immunopathology

Figures 4.5, 4.6, and 4.7 also contain the simulated outcomes of frequent re-exposure over time: Figures 4.5 and 4.6 examine frequent re-exposure, as might occur amongst "core" members of a sexual network, and Figure 4.7 for someone who is experiencing higher initial exposure, though not as high core members of a network, followed by a long period of no exposure. For EBs and infected cells in the basic model (Figure 4.5), frequent re-exposure every 30 days produced damped oscillations that approach a low endemic equilibrium that persists in the absence of further exposure. Given that high CD4+ T cell responses will be positively correlated with production of IFN-g, the basic model demonstrates that frequent re-exposure is likely to keep CD4+ cell population elevated, and therefore production of IFN-g continual. For baseline values of the extended model, where an individual's antibody response is pronounced, this behavior was not demonstrated (not shown). However, by lowering their antibody response, frequent re-exposure produces similar elevated, oscillating immune responses and persistent infection that continued in the absence of further exposure (Figure 4.6). An analytic study of re-exposure corroborates the simulated results for both basic and extended models (Figure 4.2). Taken together, this suggests that a pronounced antibody response may have an important role in preventing the formation of persistent infection. Figure 4.7 demonstrates that removing higher short-term exposure to Chlamydia allows partial immunity (i.e., immunity that does not prevent infection, but will reduce the duration and severity of second infection) to wane and return to near baseline levels.

4.5 Discussion

A person's history of exposure to STI-causing pathogens has been considered central to shaping their repertoire of effector B and T lymphocytes [30, 120], as well as for driving STI persistence and evolution [120]. Our primary concern in this paper was to illuminate unique immunobiological characteristics that may result from differences throughout an individual host's exposure history. Using two simple models of immune responses to Chlamydia infection has allowed us to qualitatively explore some elementary questions about anti-chlamydial immunity under repeated, long-term exposure. Overall, our results suggest several generalised interpretations that agree well with previously observed experimental studies: one, CD4+ T cell responses impart a marked level of immunity to primary and secondary infections; two, in the presence of CD4+ T cells, antibody contributes in an important way to an individual's immunity against reinfection; and three, when reinfection does occur after being re-exposed in relatively rapid succession (relative to the immuno-
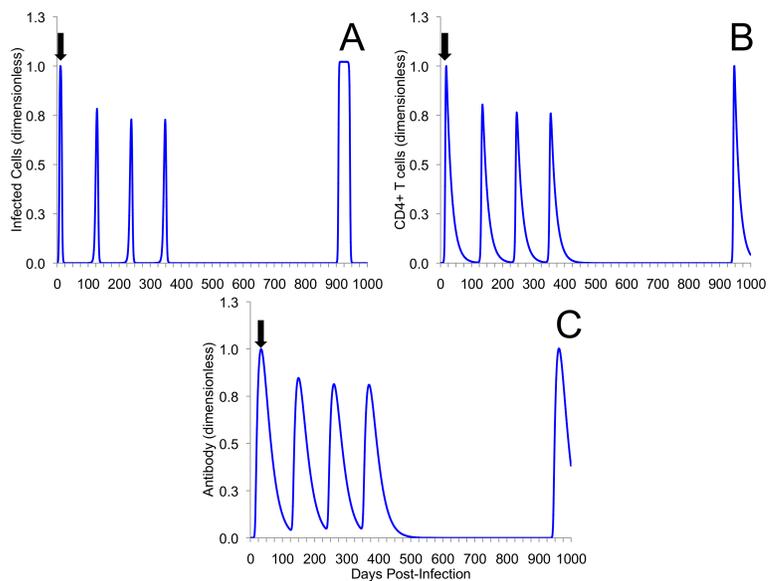
Figure 4.7: Higher initial re-exposure followed by a long period of no exposure. Displayed is the kinetics of infected cells (A), CD4+ T cells (B), and chlamydia-specific antibody (C) for the extended model with re-exposure every 100 days over a span of 300 days followed by single re-exposure 900 days after initial infection. These scenarios were used to represent higher initial rates of exposure, between 100 and 300 days, followed by low exposure between 300 and 900 days. Black arrow indicates point of initial infection.

logical decay time) the resulting infection is less severe and produces a decreased bacterial load. The latter has allowed significant insight to be gained into the timing of reinfection and the severity and duration of disease: as long as some level of pre-existing immunity remains, repeat infections are likely to be of decreased severity and duration when compared to initial infection. However, this does not appear to apply to repeat infections that occur at time points after immunity has waned further (demonstrated in Figures 4.5, 4.6, and 4.7).

One of the major immune mechanisms for controlling Chlamydia infection occurs through depletion of cellular TRP by IFN-g-inducible indoleamine-2,3-dioxygenase: a T_H1-mediated process [29, 45, 176]. A failed or weak T_H1 response will allow *Chlamydiae* bacteria to respond to immune challenge by converting into a nonreplicating but revivable persistent state [45]. In this persistent state, the bacteria have been demonstrated to remain able to direct their own survival and still allow for antigen-presentation to occur [176], and consequently a predominant antibody- or T_H2-mediated hypersensitivity [45]. However, an over-stimulated T_H1 response will lead to delayed-type hypersensitivity, and an increased risk of IFN-g-mediated tissue damage; an effective immune response that successfully clears infection will contain a balance of cell-mediated and humoral immune responses [45]. Our experiments of repeated exposure have highlighted two novel immunobiological outcomes that appear to connect the formation of antibody to the outcome infection and long-term protective immunity.

The in-host models used here suggest that for frequent exposure to Chlamydia, the formation of a proportionate antibody response is not only central for preventing reinfection, but for regulating an individual's effector T cell populations – therefore lessening the risk of inflammatory damage and the likelihood of persistent infection – as well. Specifically, antibody will reduce the population of free EBs, which reduces the levels of infected cells; a lower magnitude of infected cells will regulate the activation and proliferation of CD4+ T cells, and therefore prevent an over-production of IFN-g.

However, a lack of a proportional antibody response during frequent repeated infection allows for more infected cells to be produced and thus requires higher levels of effector T cells to eliminate infection. The higher levels of T_H1 CD4+ responses will be associated with continual production of IFN-g, and possibly an increased likelihood of persistent infection and inflammatory damage. Because fluctuations between acute replication, IFN-g-mediated immune responses, and persistence are believed to the norm with Chlamydia infections [45], these results outline, what may be, a previously unidentified mechanism of chronic infection that is driven by continual disproportionate T_H1 CD4+ cell populations – in conjunction to the known mechanism of persistent infection that is a result of disproportionately high antibody responses (i.e., where it is likely that $T_H2 >> T_H1$) [45]. To our knowledge, research into the link between persistent infection and immune responses has not assigned an important role of prolonged (or elevated) IFN-g-mediated mechanisms.

The higher levels of T_H1 CD4+ responses, the continual production of IFN-g, and the formation

of chronic infection also appear to be associated with the development of complete (life-long) protective immunity. Previous research among humans in developing countries have reported that while there are high prevalence rates of STIs among commercial sex workers [170], there may well be some degree of protective immunity in these highly sexually-active populations [25, 120]. At a general level, the results from both our simple and extended models appear to support these previous observations: frequent re-exposure can yield stable and persistent elevated immune memory (figures 5 and 6). It also appears that individuals experiencing brief periods of higher-than-usual exposure (Figure 4.7) result in, to a lesser extent, partial protective immunity (i.e., immunity that does not prevent infection, but will reduce the duration and severity of secondary infection and disease).

Although the accumulation of immunity through repeated infection has been assumed elsewhere [30], our results demonstrate that for those individuals who would not have levels of exposure comparable to those in a core group, removal of the repeated infection pressure allows levels of immune cells to return to near baseline levels (Figure 4.7). Taken together, our results suggest that the natural development of life-long immunity to Chlamydia may well be the exception rather than the rule. This leads us to conclude that, for all but the most heavily exposed, complete life-long immunity, may simply be an artifact of continued exposure coupled with disproportionate immune responses. At the very least, it appears that the formation of life-long immune memory to Chlamydia infection, among the majority of sexually-active people, may not fit the standard precepts of infection and acquired immunity. This appears to challenge current evidence of chlamydial immunity and suggests the need for novel empirical data.

Re-exposure to Chlamydia that results from behavioral variability is also thought to alter transmission potential [120]. Clearly, many of the insights presented here may have important implications for the spread of Chlamydia. As such, within-host models can provide quantitative predictions of immune status that offer valuable insights into the factors that influence transmission [85]. Incorporating some simple extensions to these models that will account for network spread [199] will, at the very least, begin to lay the foundations for assessing the feasibility of any undesirable interactions between current Chlamydia control programs, a host's immune responses, and any association they might have on the prevalence of infection [31]. Collection of quantitative immunological and epidemiological information will be of great value for informing this work.

Chapter 5

Study 4: The Local Effects of Treatment on Reinfection in an Immunoepidemiological Model of Transmission

5.1 Relationship to the Previous Study

The within-host model of the previous chapter was developed to build an adequate representation of in-host dynamics that would then serve as the foundation of an immunoepidemiology model. The previous model allowed me to build-up an intuition of how the model would behave under different exposure histories. However, the re-exposure/re-infection scenarios (and by extension network structure) were exogenous in this model. Network topologies linking individuals is important in infection transmission. Therefore, I extended a previous, general immunoepidemiological model for a chlamydial context that integrated the "reduced" model of the previous chapter that then connects each individual in small static networks. This not only, allowed reinfection to occur endogenously, but also was able to examine its occurrence when treatment is also introduced into the model structure.

5.2 Background

For commonly reported bacterial sexually transmitted infections (STIs), such as *Chlamydia trachomatis*, effective detection and treatment is vital to reducing the duration of infection and interrupting transmission [163]. For many developed countries with effective mass control programs, such as Norway, Sweden, Finland, and Canada, Chlamydia incidence rates were in decline for almost a decade [31]. However, despite the best efforts of these public health programs, Chlamydia case notifications have risen and now appear to exceed those recorded before large-scale intervention strategies were implemented [31, 175].

Recently discussed reasons for the rebounding of Chlamydia notifications have focused on several hypotheses. Of the seven propositions presented by [175], the first four focus on revised testing technologies, and have been long-supported by epidemiological data [35, 66, 80, 136]. The fifth

and sixth propositions, which respectively focus on antibiotic resistance and changes in sexual behaviour, are remarked to have little or mixed supporting evidence, and may be subject to denial as central causes of rising incidence rates [175]. The seventh proposition, designated the *arrested immunity hypothesis*, argues that treating Chlamydia early in the course of infection disrupts the formation of a protective in-host immune response [31]. Though previously demonstrated in animal models [190], the tenets of the arrested immunity hypothesis, initially developed through simulation modelling [30], have been substantiated by geographical and seroprevalence data [127] as well as randomized clinical approaches [12].

The interaction between antimicrobials, the development of an immune response, and any detrimental effects on population susceptibility has potentially weighty consequences for Chlamydia re-infection rates. Such effects are particularly important for individuals who return to the same or similar sexual networks with the same or similar sexual behaviour [31]. To our knowledge, the observed immunological effects of antibiotic treatment have only been observed in non-interacting individuals. Conversely, the proposed counter-intuitive epidemiological implications of arrested immunity have been observed in the absence of detailed data on immunobiological characteristics. Therefore, questions remain about whether individual-level effects of treatment on immune responses propagate across scales to the level of the population, and if the epidemiological dynamics of infection will simply be the sum of individual immunological effects?

Understanding the impact of public health efforts for infectious disease control is central to evidence-based public health policy [31]. However, because complexity behind disease dynamics hinders our ability to discover the delayed and distal impacts of our actions, the outcome of many public health programs can be poorly understood [188]. Modelling has become a central tool in understanding the epidemiological processes underlying infectious disease transmission and aids the design of effective control strategies. For a wide range of infectious diseases, individual heterogeneity can be modelled effectively. For example, individuals can be represented as vertices in a network, where the connections between individuals represent potentially infectious contact. Models that use explicit network structures enable the study of how behavioural variability will impact diffusion processes, and have proved useful for understanding the spread of SARS, influenza, foot-and-mouth disease, tuberculosis, and quintessentially, STIs [109]. Network models represent an alternative approach to modelling infection spread by aggregate, compartmental models distinguished by their greater ability to capture behavioural heterogeneity and allow for examination of various control strategies that take advantage of a given network structure [120], rather than assuming that contact among susceptible and infected individuals is a random process [93]. However, network models seldom incorporate individual representations of immunological heterogeneity.

Recent developments in immunoepidemiological (IE) modelling frameworks offer a chance to explore and explain heterogeneity in STI transmission dynamics that is linked not only to be-

havioural variability (i.e., network structures), but also to variability in an individual's immuno-biology. A number of previous methodologies have been developed to examine "between-host" infectivity and disease suffered by infected hosts as functions of the disease progression at the within-host level [42, 47, 85, 115, 193, 199, 216, 218]. Though guided by different objectives and model structures, each of these frameworks has yielded important insights. For example, the between-host models by [85] were used to explore the effects of natural boosting and vaccination on the formation of immune memory, while [42] examined the selective pressures exerted from both population and within-host levels on the emergence of mutated pathogen strains. However, these frameworks do not capture the impact of complex population structures, such as networks. In contrast, the models used by [193], [115], and [199] have examined spread over random network structures. However, such studies have been limited to network structures that closely mimic a random mixing assumption (i.e., Poisson networks).

In this paper, we aim to build an introductory link between Chlamydia infection and host immune responses, with an emphasis on how treatment impacts the local spread of chlamydia infection. Here, we explicitly extend our previous analyses [199, 201] to include host heterogeneity represented by varying network structures. We present a general method for examining multiscale feedbacks in a specific IE framework. We do this by "nesting" a model of in-host Chlamydia dynamics into small, idealized sexual networks. This nesting allows the between-host variability in their life history of infection and transmission to be functions of both immune dynamics at the within-host level, as well as their position in a given network structure. Nesting individuals in small networks also allows us to build up an intuition for the dynamic feedbacks amongst these different scales and their impact on infection spread.

5.3 Material and Methods

5.3.1 Chlamydia Infection and the Immune Response

The genus *Chlamydiae* encompasses a unique class of obligate intracellular bacteria that can cause disease in a wide range of animals [17]. Chlamydia bacteria have a biphasic developmental cycle that consists of an extra- and intracellular form [17, 29]. The extracellular form, the elementary body (EB), is infectious and thought to be metabolically static. During infection, the EB is internalized into host epithelial cells via small vacuoles resembling endosomes, most of which avoid fusion with host cell lysosomes [29]. The EB differentiates within the entry vacuole into metabolically active reticulate bodies (RB), which are non-infectious [29]. Infection is propagated further when the RBs differentiate back into EBs, which are released from the host cell by either exocytosis or lysis.

Inference by analogy with rodent models supports the existence of an effective immune response against Chlamydia infection [17, 29, 95, 99, 146, 148, 171, 190]. These animal models of human genital

tract infections demonstrate that a large proportion of animals resolve primary infection and are temporarily resistant to reinfection. When reinfection does occur, secondary inflammation and disease is significantly shorter or non-existent, and bacterial load is greatly reduced [172].

One of the major immune mechanisms for controlling Chlamydia infection occurs through depletion of cellular tryptophan (TRP) by indoleamine-2,3-dioxygenase (IDO) – a Th1 process that is mediated by interferon gamma (IFN-g) [29, 45, 176]. A failed or weak Th1 response will allow Chlamydia RBs to respond to immune challenge by converting into a nonreplicating but revivable persistent state [45, 121]. In this persistent state, Chlamydia bacteria have been demonstrated to remain able to direct their own survival and still allow for antigen-presentation [176]. A direct consequence of this prolonged infection is antibody- or Th2-mediated hypersensitivity [45]. However, an over-stimulated Th1 response will lead to delayed-type hypersensitivity, and an increased risk of IFN-g-mediated tissue damage, that is likely a consequence of an initially dominant Th2 response.

5.3.2   Basic Model of Chlamydia Replication and Immune Responses

In extending our general IE formulation [199], we specify the following: a within host model, the initial state of each host, the network topology over which infection spreads, a representation of control strategies (here, antibiotic treatment), and a formulation of the diffusion of infection from one individual to another.

Expanding epidemiological and public health knowledge will depend upon pragmatic learning through simulation and systems thinking [188]. Our aim is to consider the simplest possible model and explore its implications for Chlamydia infection dynamics. The vehicle for this research is a simple model that includes Chlamydia bacteria, host cells, and cell-mediated immune responses (see Figure 5.1).

Because cellular immunity is crucial for resolution of Chlamydia infections [29,56,146], the basic model combines a representation of host-pathogen interactions [5,158,159] with known Chlamydia immunology in the reproductive tract [29, 56]. This basic model contains uninfected endothelial cells (ECs) of the genital tract (X), infected ECs (Y), free, infectious, and metabolically inactive EBs (E), and Th1 CD4+ cells (Z). Uninfected ECs are produced at a constant rate, λ, and died at a rate δX. We used mass-action kinetics to model the interactions of uninfected cells with infectious EBs; infected cells are produced at a rate βXE, and died at a rate αY. We assumed that chlamydial peptides presented to the immune system are roughly proportional to the number of infected cells [159, 211]; therefore, activated chlamydia-specific CD4+ T cells are assumed to proliferate and differentiate at a rate cY. Activated CD4+ cells died at a rate σZ.

The main effector mechanisms of CD4+ T cells assumed in this model were IFN-g-mediated TRP starvation of chlamydial bacteria and immune-mediated death of infected cells. Here, the concentration of IFN-g and cytosolic TRP were modelled implicitly, and the concentration of each
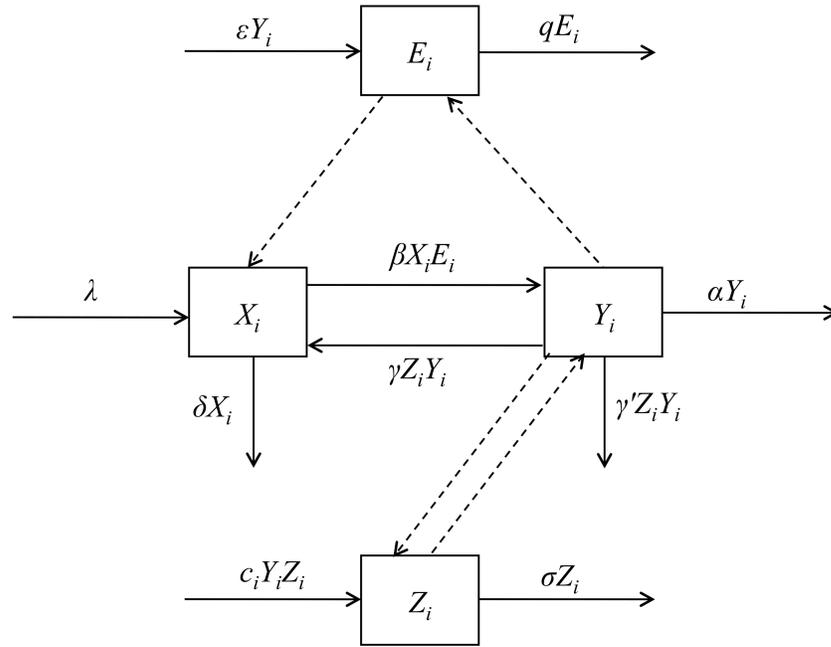
Figure 5.1: Stock and flow diagram illustrating the assumptions about chlamydial dynamics for an ith individual in equations 1-4 in the main text. Uninfected ECs (X_i) are produced at a constant rate, λ, and die at a rate δX_i. Infected cells (Y_i) are produced at a rate $\beta X_i E_i$ by contact with free EBs (E_i), and die at a rate αY_i. CD4+ T cells (Z_i) proliferate and differentiate at a rate $c_i Y_i$ and die at a rate σZ_i. Infected cells are killed at a rate $\gamma' Z_i Y_i$ recover from infection at a rate $\gamma Z_i Y_i$. Dashed arrows between state variables indicate feedbacks between state variables.

was assumed to be directly- and inversely proportional to the number of CD4+ cells, respectively. Because low levels of TRP have been demonstrated to have minimal effect on the viability of host cells [121], infected cells were therefore assumed to recover from infection at a rate γZY. However, because other Th1 immune mechanisms are also driven by IFN-g, infected cells were also assumed to die at a rate $\gamma' ZY$. These assumptions produced the set of ordinary differential equations for an individual i (see also Figure 5.1 for schematic representation):

$$\dot{X}_i = \lambda + \gamma Z_i Y_i - \delta X_i - \beta X_i E_i \tag{5.1}$$

$$\dot{Y}_i = \beta X_i E_i - \alpha Y_i - \gamma Z_i Y_i - \gamma' Z_i Y_i \tag{5.2}$$

$$\dot{E}_i = \epsilon Y_i - q E_i \tag{5.3}$$

$$\dot{Z}_i = c_i Y_i Z_i - \sigma Z_i. \tag{5.4}$$

Specific immune responses are induced for a majority of infections, and the magnitude and quality of these responses varies greatly for different infections [139]. The particular structure of equations 5.1-5.4 are not a comprehensive depiction of immunological processes and other innate and specific immune responses are likely to mediate the spread of a Chlamydia infection in a population. However, explicitly describing the cooperative interactions between other specific immune responses (e.g., B- and CD8+ T cells), and their effect on the transmission of Chlamydia is left for future work.

5.3.3 Initial Infection

Infection begins with the introduction of a small amount of Chlamydia bacteria into a single host whose state variables represent the uninfected state. In the current model, an individuals initial state is specified by:

$$(X_i(0), Y_i(0), E_i(0), Z_i(0)) = (X_0, 0, E_0, Z_0) \tag{5.5}$$

representing the initial numbers (or densities) of uninfected target cells, infected target cells, free, viable *Chlamydia* bacteria, and Th1 CD4+ cells, respectively. At time $t = 0$, we assume that there is a small amount of innoculum of EBs introduced into a host. Based on research for the sexual transmission of Chlamydia in Guinea Pigs [172], we assume that the initial quantity $E_0 = 10^3$ inclusion forming units (IFUs) [172].

5.3.4 Network Structures

The epidemiological literature contains many plausible representations for between-host transmission. These include models based on differential equations [5] and those based on network structure
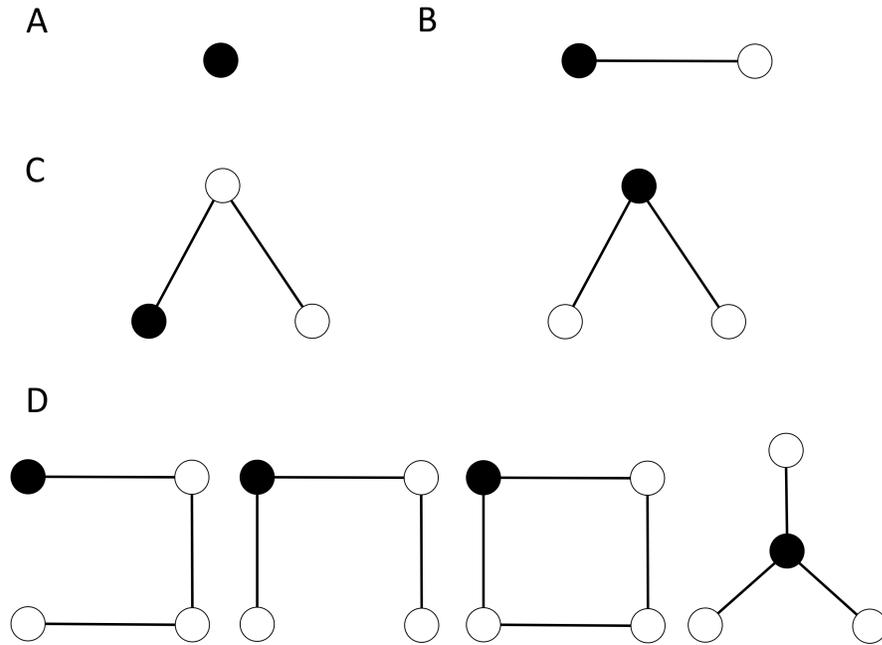
Figure 5.2: Exemplar bipartite network structures representing (A) a single isolated host, (B) a pair of connected hosts, or "dyad", (C) networks of order three: a "path" and a "tree", (D) networks of order four: a "path", a "tree", a "cycle", and a "star". The vertices that are filled-in (solid black) represent the position of the initially infected host, and thus determine the network's descriptor of "path" or "tree" (for example).

(e.g., see review in [106]). Here, we have chosen to represent between-host transmission via particular network structures. To help build our intuition about the implications of treatment on reinfection, we focus our efforts on understanding the resultant infection spread over small, idealized, static, bipartite network structures with fixed populations of size, $|V_T|$, where $1 \leq |V_T| \leq 4$. We focus on bipartite graphs because of an implicit similarity with sexual networks in heterosexual populations. While other network structures (e.g., fully connected graphs or "cliques"), are important social network structures and may likely be important to the spread of STIs when considering same sex partnerships, for simplicity we do not consider them here. Each network is represented by a graph $G = (V_1, V_2, E)$, with vertex sets partitioned into V_1 and V_2 and edges E, where every edge joins a vertex in V_1 with a vertex in V_2 and $|V_T| = |V_1| + |V_2|$. Specific network examples include a single "isolated" individual, $|V_T| = 1$; a "dyad", $G = (1, 1, 1)$; a "tree", $G = (1, 2, 2)$; a "cycle", $G = (2, 2, 4)$; and a "star", $G = (1, 2, 2)$. Please refer to Figure 5.2 for graphical depictions of the network structures.

$$\omega_i \sum_{j \in |V_T|} A_{i,j} E_j$$

$$\varepsilon Y_i \qquad \boxed{E_i} \qquad qE_i$$
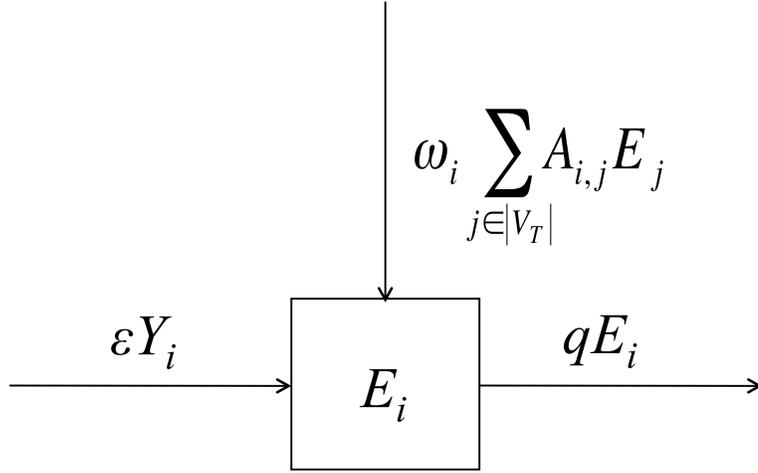
Figure 5.3: A schematic representation of chlamydial transmission between hosts in the immunoepidemiologcal model discussed in the main text. Here, infection is represented by an inflow into Chlamydia bacteria, E_j, from the ith individual's j neighbours. The stock and flow representation focuses on a segment of the equations represented in Figure 5.1 and equations 5.1-5.4 in the main text.

5.3.5 Link of Infection spread to Host State Variables

We need to understand how the internal state of an infected host affects the spread of a pathogen between hosts. Our basic supposition is that the host state variables will govern this process. This model contains immuno- and contact-dependent parameters governing the transmission between hosts that have been outlined previously in [199]. In particular, we suppose that the chlamydial load of the transmitting host affects the rate at which infectious innocula is released (adapted from [42]). For this, we add to the in-host model an additional term specifying that a single individual's incoming flow rate of free chlamydial particles is proportional to the chlamydial load of their neighbours $\omega_i \sum_{i \in |V_T|} \mathbf{A}_{i,j} E_j$. Here, ω_i is the (typically very small) coefficient of connectedness that defines the weights on each of the connections between neighbours. We hereafter refer to ω_i as the connectivity coefficient. The expression $\mathbf{A}_{i,j}$ is a predefined, symmetric, binary $n \times n$ adjacency matrix that describes "who is connected to whom". The vector, E_j, is the chlamydial load of network member j (Figure 5.3).

We further assume that infection does not affect host activity level (i.e., disease is either minimal

or nonexistent). Thus, the transmission rate of an infection was assumed to be an increasing function of the chlamydial load that exceeds an infectious threshold. For an initial innoculum of 10^3 IFU, it has been experimentally observed that after a mean incubation period of approximately 4 days, active shedding of Chlamydia bacteria can be measured from infected Guinea Pigs [172]. Therefore, we used this information to determine the threshold level of chlamydial EBs at which an infected host became infectious.

5.3.6 Individual Heterogeneity

Each individual has two relevant characteristics that can affect contact rate, infectivity, and duration of infection. As mentioned above, these are an individual's immune responsiveness, c_i, and the connectivity coefficient, ω_i. Therefore, we examine the effect of varying the heterogeneity spectrum in these parameters. In the homogeneous condition (H_0) all individuals share identical values for connectivity and immune responsiveness. In the heterogeneous condition (H_E), each individual i has a distinct immune responsiveness, $c_i \sim U(0.007, 0.015)$, and connectivity coefficient, $\omega_i \sim U(0,1) \times 0.01$, where the multiplier term ensured that infection was able to propagate, and $U(a, b)$ denotes a random variable uniformly distributed between a and b.

Because our basic model is deterministic and was originally used to describe chronic infections [158], CD4+ T cell responses cannot reduce $E_i(t) \to 0$. Therefore, following Wodarz and colleagues [213], we defined a threshold value where Chlamydia bacteria, although likely at low levels, was considered extinct, EB_{ext}. Here, our extinction threshold was chosen to be marginally larger than the initial innoculum of 10^3 IFU.

5.3.7 Treatment

Model representation of the removal of infected individuals by contact tracing and subsequent treatment has two relevant characteristics: the time they begin to receive treatment and their behaviour during treatment. For the former, we follow the experimental protocol of Su et al. (1999) [190], and provide treatment to specific individuals at the time of infection (day 0), or at days 3, 7, and 10 days after infection. Here, treatment was assumed to last for approximately 7 days. Treatment of infected hosts (here, simulated Guinea Pigs) within these small sexual networks was administered under 3 different scenarios. Each scenario was fashioned after idealized contact-tracing strategies outlined in [109].

1. *Treatment of "trigger" vertices*: This involved treating the initially infected individual. Here we define the "trigger" vertex as the individual who is initially infected.

2. *Single-step contact tracing*: The neighbours of the trigger vertex are assumed to be contact-traced, treated, and have their infection levels reduced by treatment at a rate of, ρY_i. This

modifies equations (1) and (2) to be:

$$\dot{X}_i = \lambda + \gamma Z_i Y_i + \rho Y_i - \delta X_i - \beta X_i E_i \qquad (5.6)$$

$$\dot{Y}_i = \beta X_i E_i - \alpha Y_i - \gamma Z_i Y_i - \gamma' Z_i Y_i - \rho Y_i. \qquad (5.7)$$

Further contact tracing is not initiated for this strategy.

3. *Multi-step contact tracing*: Because the exposed/infected neighbours of the trigger vertices can themselves spread infection (once infected), we assumed that a second "round" of individuals will be traced and treated. Because of the size of the networks studied here, everyone in the network was treated at this point.

The conditions of heterogeneity were extended to the delivery of treatment as well. For H_0, individuals were assumed to remain in contact for the duration of treatment, while for H_E, $\forall \, \rho Y_i > 0$, $\omega_i = 0$ for the duration of treatment of individual i, and retained its original value, otherwise. As a result of contact tracing, all individuals that were treated were treated at the same time.

5.3.8 Measuring Infection Spread

For each experimental condition, infection spread was gauged through each network structure (outlined in Figure 5.2) via the prevalence of infection (i.e., the number of individuals infected at a particular time point), and the duration of infection. This allowed the overall burden of infection to be compared across networks of different sizes and configurations, as well as for different immune, behavioural, and treatment scenarios.

5.3.9 Calibrating the Within-host Model

Sources of empirical data were not available for all input parameters, and calibration to laboratory data was necessary to estimate unknown parameter values. Where possible, the model was parameterized to chlamydia-specific kinetics based on laboratory studies using Guinea Pigs (see below). Model calibration was performed using a two-step process:

1. *Setting initial parameter values*: Each parameter value associated with the natural history of infection or treatment was estimated from key laboratory studies of chlamydial infections in Guinea Pigs or other modelling articles in the available literature [95, 131, 172, 211]. Where unavailable, parameter values (e.g., susceptible cell production and death rates, as well as immune responsiveness) were taken from our experience with other within-host dynamic models [108, 159, 177, 213]. Given that the model presented here uses similar structure to previous models, we think this is a reasonable assumption (see Table 5.1). However, we should note that these parameter assumptions contribute to simulated output that matches chlamydial infection time series from laboratory research.

Parameter	Description	Value	Units	Notes
λ	Rate uninfected cells replenish.	10	cells/day	fixed
β	Infection rate.	$(10^{-4}, 0.01)$	1/(IFU×day)	calibrated
δ	Uninfected cell die-off.	0.1	1/day	fixed
α	Infected cell die-off.	$(0.33, 2.0)$	1/day	calibrated
γ	Rate infected cells are cured by CD4+ cells.	$(10^{-6}, 0.1)$	1/(T cells×day)	calibrated
γ'	Rate infected cells are killed by CD4+ cells.	γ	1/(T cells×day)	
c_i	T cell responsiveness rate.	$H_0 : 0.01$ $H_E : U(0.007, 0.015)$	1/day	varied
σ	T cell die-off.	0.01	1/day	fixed
ϵ	EB production rate from infected cells.	1.28	IFU/(cells×day)	fixed
q	Rate of EB decay.	$(0.2, 3.0)$	1/day	calibrated

Table 5.1: Parameter ranges used for calibrating the within-host model of *Chlamydia* infection.

2. *Sampling parameter ranges and fitting the model*: Parameters that were calibrated were first assigned a range of values found in the literature surveyed. Parameter settings that minimized the discrepancy between laboratory observations and model outputs were determined by using the Powell global optimization algorithm available in Vensim DSS for Windows (version 5.5c). This enabled different combinations of parameter vectors to be explored.

5.4 Results

5.4.1 Clearance of Chlamydial Infection in Antibiotic Treated Individuals

We first analyze the impact of treatment on infection and immune response within a single infected host. The purpose of examining treatment within a single individual is to ensure that our dynamic hypothesis of within-host processes produces results that are expected and previously observed in laboratory research. Figure 5.4 illustrates that our within-host model can accurately reproduce the natural history of infection in a Guinea Pig model of genital infection.

Infected individuals that did not receive treatment exhibited typical infection curves (Figure 5.5), characterized by the shedding of large numbers of organisms during the first two weeks of infection. Antibiotic treatment had a marked effect on chlamydial shedding and on the duration of infection (Figure 5.5A). Treatment at the time of infection (day 0) completely inhibited infection from taking off. Compared to untreated individuals, those treated at 3 and 7 days after infection
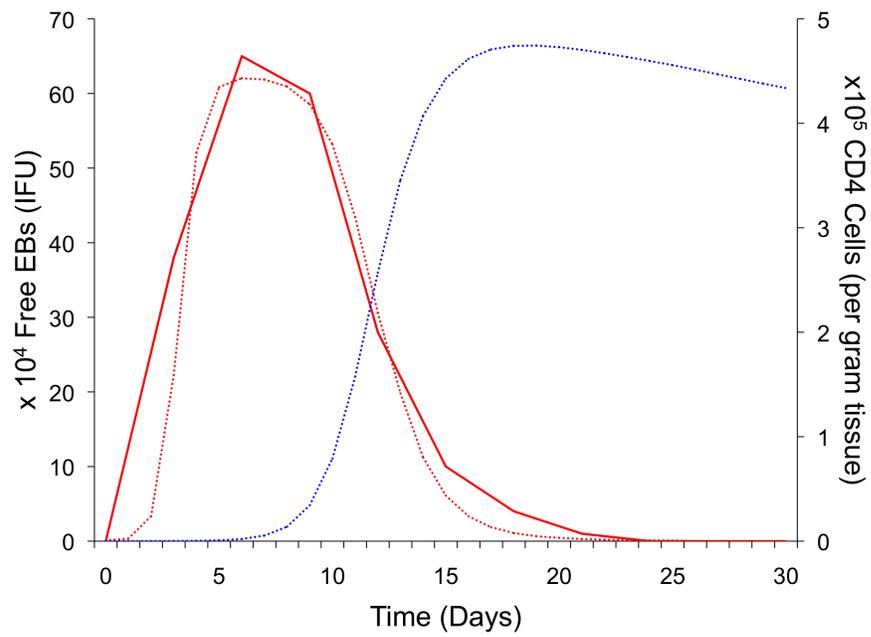
Figure 5.4: A comparison of experimental and simulated kinetics of primary chlamydia infection and CD4+ T cell responses. Experimental data for free elementary bodies (inclusion forming units, IFUs) (solid line), model-derived (round dots) and CD4+ T cells (dash dot) at initial infection. Data are modified from [172].

exhibited a significantly reduced duration and severity of infection, while those treated at 10 days also demonstrated a reduced duration of infection.

To determine whether antibiotic treatment affected the development of protective immunity, we also compared the magnitude of the CD4+ T cells in treated hosts compared to untreated hosts (Figure 5.5B). Despite effectively extinguishing infection, treatment at the time of infection (day 0) or 3 days after infection significantly removes antigen impingement, which consequently removes the stimulus for the development of an immune response. Perhaps the most intriguing finding is that treatment as late as 7 or 10 days after infection reduced the magnitude of the CD4+ response compared to that in an untreated host. Therefore, it appears that maximal protective immunity against chlamydial infection requires that acute infection be allowed to proceed into the late stages of infection [190]. The degree to which these levels of CD4+ cells protect against reinfection, for different treatment times, are examined below.

5.4.2   Transmission of Chlamydial Infection in Antibiotic Treated Pairs

We build upon the above results by connecting individuals in specific network topologies, beginning with a network of order 2. Table 5.2 displays to the chains of infection that were generated for a variety of network structures when one trigger case is introduced into each network. We define a chain of infection over a network as the enumeration of the number of cases in each generation [18]. For example, we will write $1 \to 2$ to denote a chain consisting of the trigger case, and two first generation cases. In general, the chain of infection $i_0 \to i_1 \to i_2 \to \ldots \to i_r$ has i_t infectives in generation t, where $t = 0, 1, 2, \ldots, r$, and no cases thereafter [18]. Dyads that were not treated exhibited an expected chain of infection, where the trigger case infects their neighbour. The observed chains of infection hold for both H_0 and H_E conditions. For the H_0 condition, both infected hosts demonstrated typical baseline infection curves observed to occur in a single infected individual. However, for the H_E condition, at least one of the connected hosts was unable to resolve infection that ultimately damped to a low endemic equilibrium.

As expected, treatment of the trigger case early in the course of infection has a marked effect on transmission of infection (Figure 5.6). Delivering treatment at the time of infection (day 0) and at 3 days after not only shortened the infection, but also prevented the infection from being transmitted to the neighbour of the trigger case; an effect that obviously due to the fact that the trigger case was not infectious before receiving treatment. These results are consistent across the H_0 and H_E conditions and contact tracing strategy (not shown).

Compared to the treatment of the trigger case at 0 and 3 days, treatment at 7 or 10 days post-infection exhibit immune dynamics that are vastly different. In the H_0 scenario, treatment of the trigger case at 7 or 10 days after infection leads to reinfection of the trigger case (Figure 5.6). This behaviour is mirrored in the H_E condition, however the occurrence and severity of reinfection
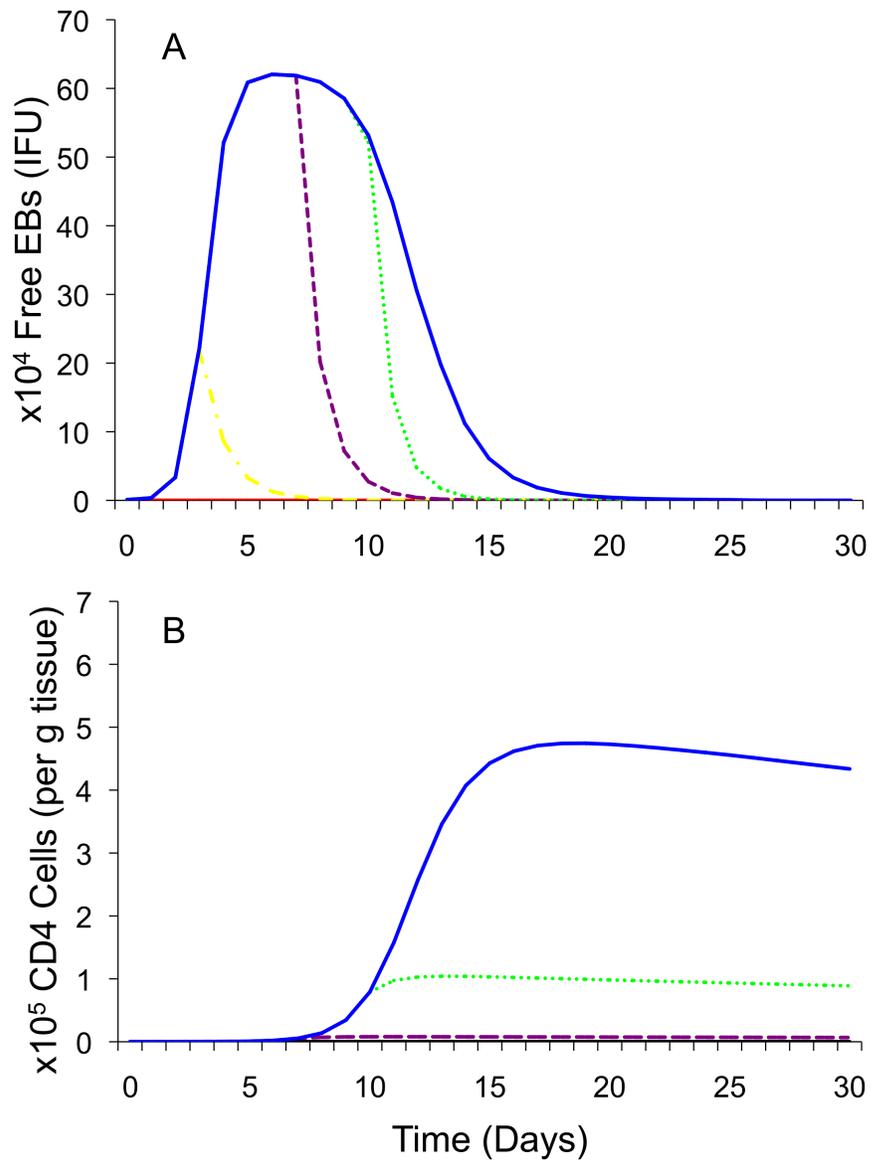
Figure 5.5: Comparison of infection (A) and immune dynamics (B) for untreated (solid line) and treated individuals at: time of infection (not visible), 3 days after infection (short dash dot), 7 days after infection (dash), and 10 days after infection (dot).

Network Exemplar	Chain of Infection
$\lvert V_T \rvert = 1$	1
$G = (1,1,1)$: "dyad"	$1 \to 1$
$G = (1,2,2)$: "path"	$1 \to 1 \to 1$
$G = (1,2,2)$: "tree"	$1 \to 2$
$G = (2,2,3)$: "path"	$1 \to 1 \to 1 \to 1$
$G = (2,2,3)$: "tree"	$1 \to 2 \to 1$
$G = (2,2,4)$: "cycle"	$1 \to 2 \to 1$
$G = (1,3,3)$: "star"	$1 \to 3$

Table 5.2: Chains of infection by network exemplar. Graphical depictions of each exemplar are displayed in Figure 5.2. The descriptor of "path" or "tree" (for example) depicts the location of the trigger vertex and thus the topology of the graph, itself, not necessarily the flow of infection over the network structure.

is limited to treatment delivered at 7 days after infection, and is completely absent when they are treated at 10 days after infection. When treatment at 7 or 10 days after infection extends beyond the trigger case to include contact tracing, both the severity and duration of infection is greatly reduced for both the trigger case and their contact. No reinfection is observed under the H_E scenario. Here, it appears that although infection spreads between the pair, treatment prevents reinfection.

5.4.3   Dynamics of Infection as Neighbourhood Size Increases

The results displayed in Figure 5.6 provide an intuitive guide to explain the results observed for networks $3 \leq \lvert V_T \rvert \leq 4$. For each network structure, we focused our analysis on describing the spread of infection among the population as a whole, under the different scenarios of treatment and individual heterogeneity (Figures 5.7-5.12), rather than describing the infection dynamics of each individual in the network. Despite variation in the particular network structures, and the location of the trigger vertex observable similarities in the magnitude of the prevalence and the duration of infection was observed for treatment ≤ 3 days. For both H_0 and H_E conditions as well as all three contact-tracing strategies, treatment at the time of infection or 3 days post-infection reduces the number of infected individuals, as well as the duration of the spread of infection significantly below baseline values where no treatment was administered. In these specific conditions, infection is limited to the trigger case and thus effectively prevented from spreading throughout the remainder of the network.

For treatment at 7 or 10 days after infection, the timing of treatment and the contact tracing
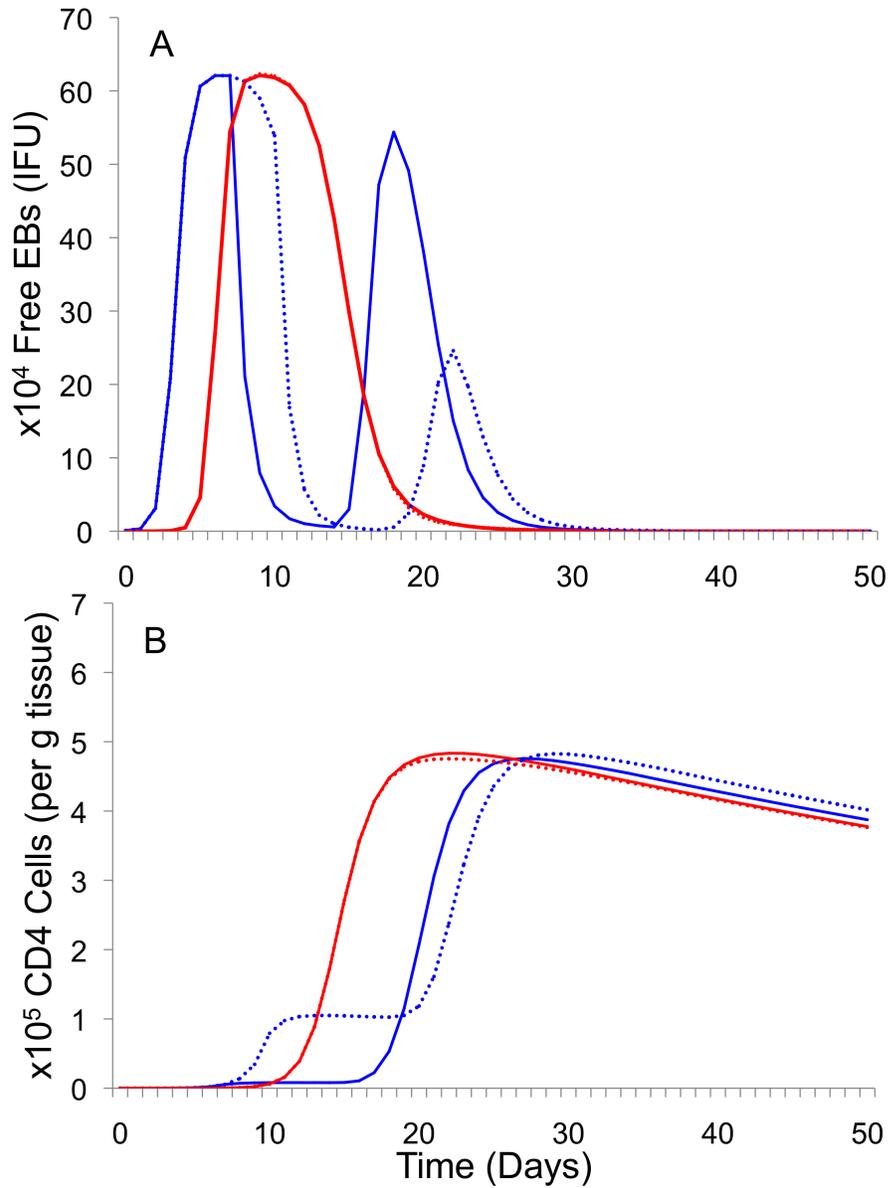
Figure 5.6: Reinfection graphs for chlamydial load (A) and Th1 CD4+ T cells (B). Results are derived from analyses of a "dyad" when trigger case (black lines) is treated at 7 (solid lines) or 10 (dotted lines) days after infection. Grey lines are representations of untreated neighbour of the trigger case.

strategy employed can produce diverse epidemiological outcomes across network configurations. For some of the network-heterogeneity-treatment combinations, chlamydial prevalence is similar to baseline, while for other combinations the prevalence of infection is noticeably different than baseline. Despite the variability in outcomes, these specific simulation experiments begin to provide an outline as to which contact tracing strategy and treatment combinations are most likely to lead to the reinfection of individuals in the network (Figures 5.7B, 5.8(B, C, D), 5.9(B, C, D), 5.10(B, C, D), 5.11B, and 5.12(B, C, D, E)), prolong the spread of infection (Figures 5.7A, 5.8(A, C), 5.9(A, C), 5.10(A, B, C, D), 5.11(A, B), and 5.12(A, B, C, D, E)), as well as maintain chlamydial prevalence at higher endemic levels (Figures 5.7B, 5.8B, and 5.11B). In particular, reinfection appears most likely to occur as a result of "incomplete" contact tracing – represented here by treating only trigger vertices or by single-step contact tracing – or when treatment is administered after an individual has become infectious. That is, trigger vertices infect their neighbours, are then treated, and consequently cured of infection. However, since treatment also prevents the sufficient development of an immune response, these individuals then re-enter (or continue) contact with infected, untreated neighbours, and are promptly re-infected. It is these results that support the predictions of the arrested immunity hypothesis [31]. Although this applies to local spread over small networks, application of these insights to real-world, human contexts would need to be carefully evaluated on a case-by-case basis.

5.5   Discussion

There is evidence among many infectious diseases for important interactions between epidemiology and immunology [47]. The results of the immunological "battle" between host and parasite will also determine the ability of the parasite to spread [47, 86, 199]. Both empirical data and mathematical models suggest that epidemiological variables like frequency and intensity of exposure can affect a host's immunological characteristics [120]. Such effects have been observed in malarial [10], helminth [183], Measles [85, 86], and chlamydial infections [201]. Here, we have expanded here upon a simple, but general mathematical framework for *Chlamydia* replication and in-host immune responses [199, 201]. The techniques demonstrated here provide an intuitive and general framework in which to study the impact of population-level policies on individual immune responses, and how these, in turn, impact the local spread of infection. Our investigations have produced the following four major insights: first, that antibiotic treatment appears to impair the development of an immune response; second, that as the number of people ultimately treated increases, the timing at which treatment is delivered, and the behaviour of those being treated during treatment can significantly alter the burden of infection for an individual as well as among their contacts; third, the effectiveness of antibiotic treatment for individuals in isolation may differ significantly from those obtaining for
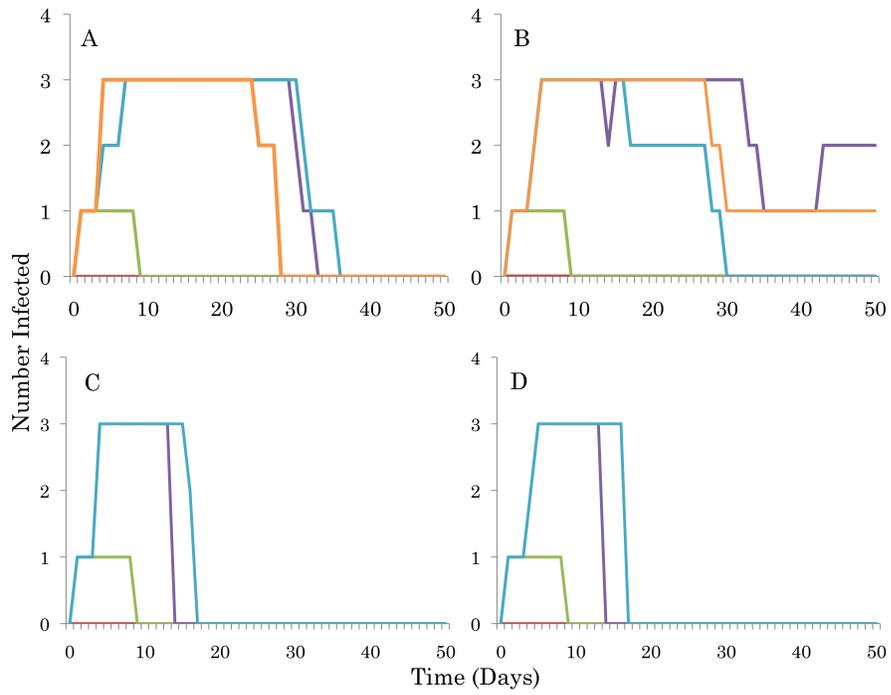
Figure 5.7: The effects of treatment on the prevalence of infection in a "tree" of order 3 for H_0 (A, C) and H_E (B, D) conditions across treatment of the trigger case (A, B), single-step (C, D). Treatment times after infection were: at the time of infection (rose), 3 (green), 7 (purple), and 10 (aqua) days post-infection. These can be compared to no treatment (orange).
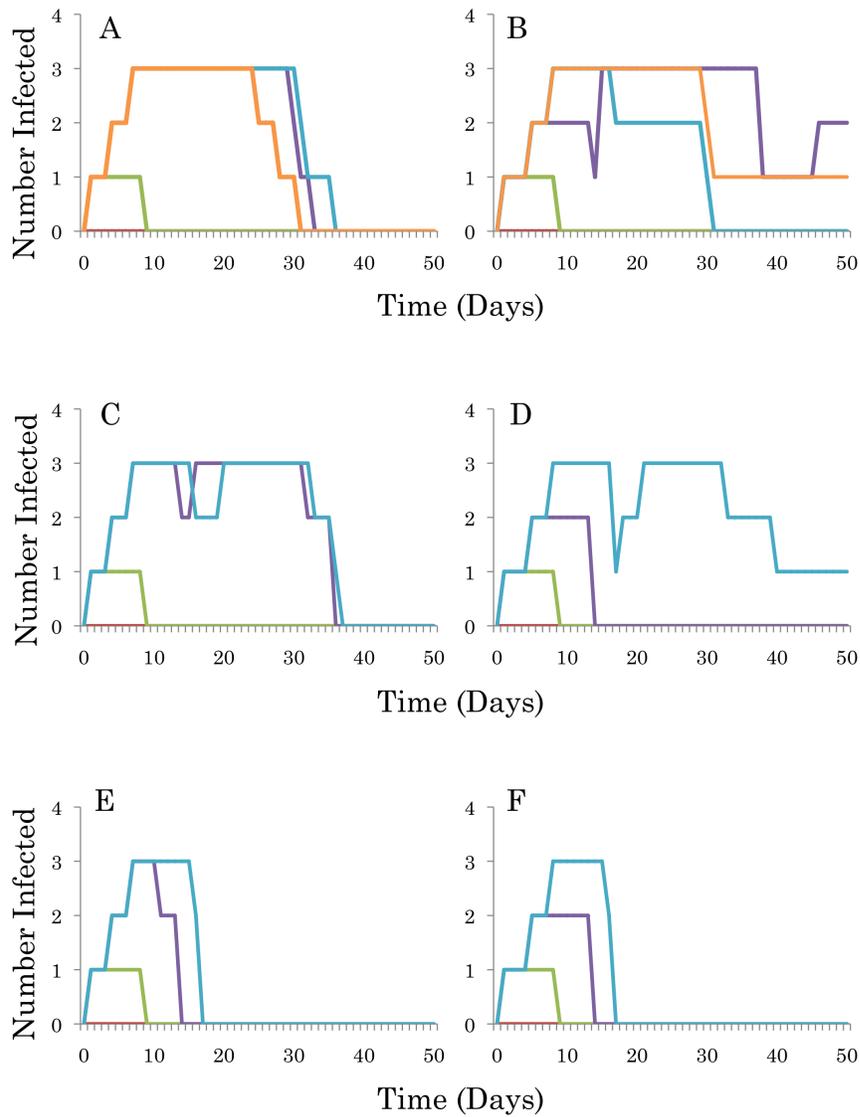
Figure 5.8: The effects of treatment on the prevalence of infection in a "path" of order 3 for H_0 (A, C, E) and H_E (B, D, F) conditions across treatment of the trigger case (A, B), single-step (C, D) and multi-step contact tracing (E, F). Treatment times after infection were: at the time of infection (rose), 3 (green), 7 (purple), and 10 (aqua) days post-infection. These can be compared to no treatment (orange).
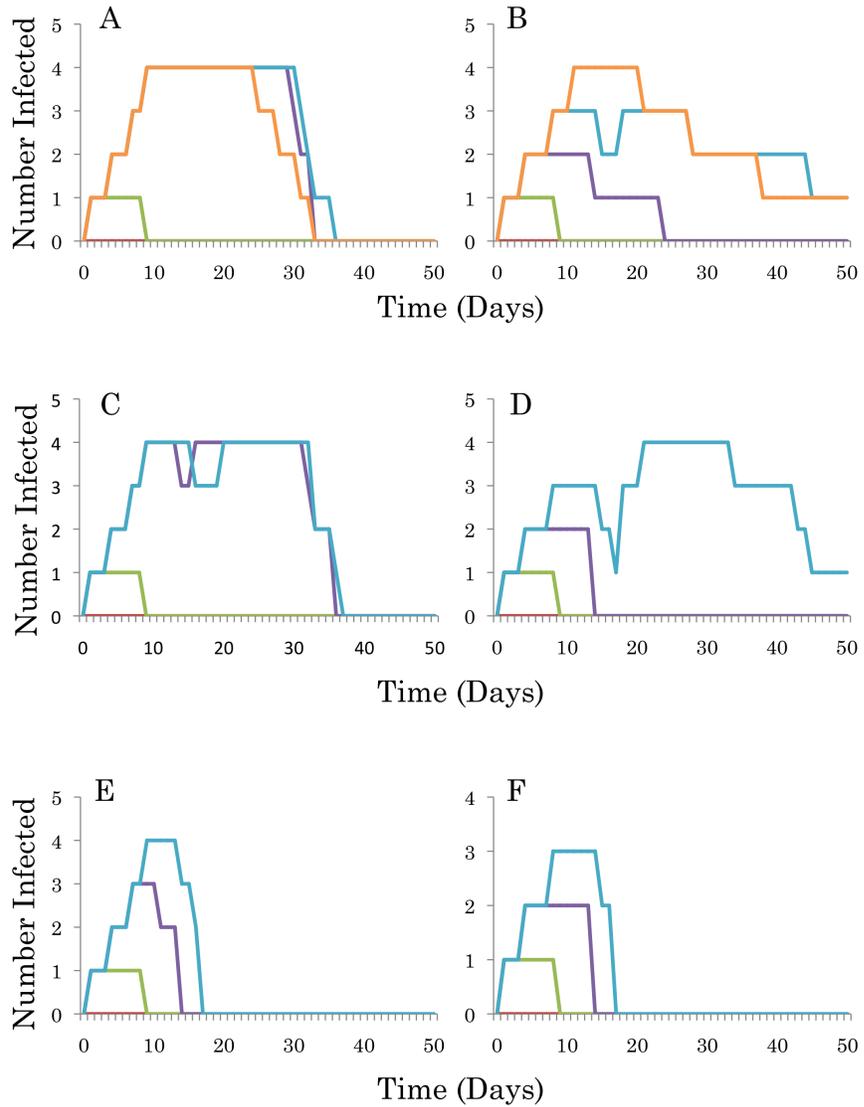
Figure 5.9: The effects of treatment on the prevalence of infection in a "path" of order 4 for H_0 (A, C, E) and H_E (B, D, F) conditions across treatment of the trigger case (A, B), single-step (C, D) and multi-step contact tracing (E, F). Treatment times after infection were: at the time of infection (rose), 3 (green), 7 (purple), and 10 (aqua) days post-infection. These can be compared to no treatment (orange).
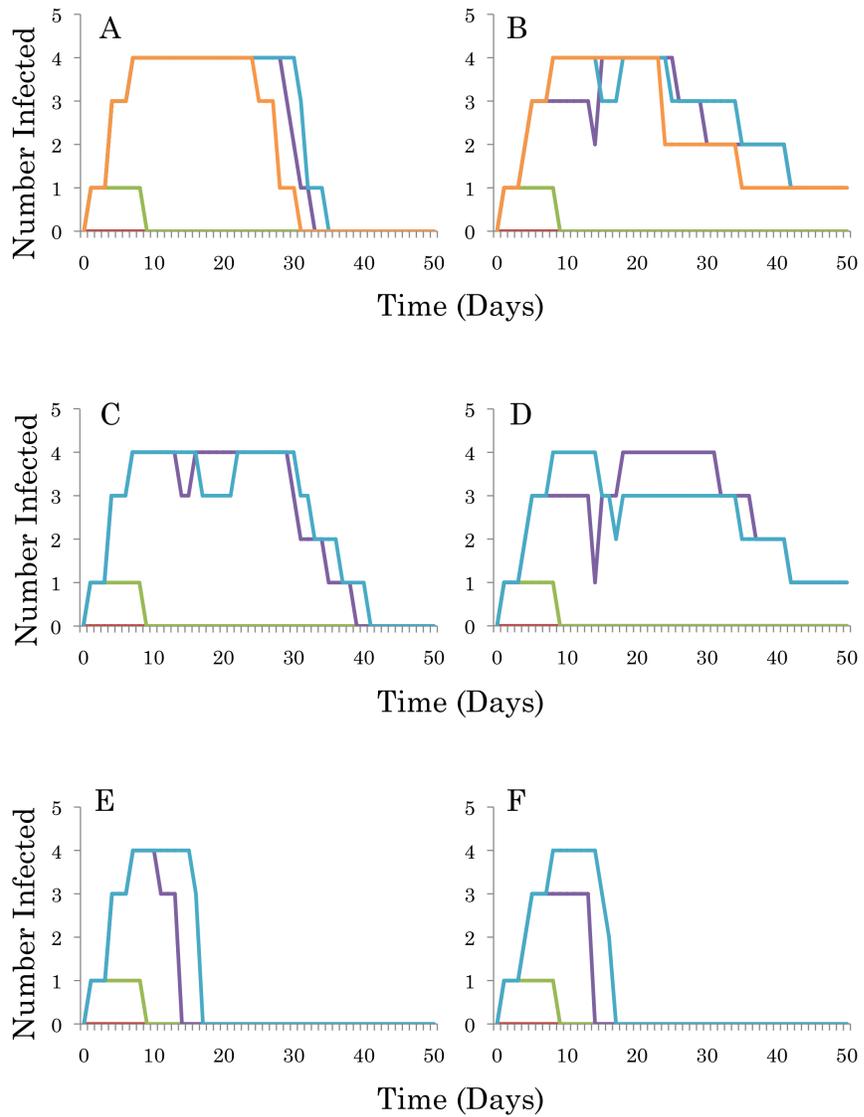
Figure 5.10: The effects of treatment on the prevalence of infection in a "tree" of order 4 for H_0 (A, C, E) and H_E (B, D, F) conditions across treatment of the trigger case (A, B), single-step (C, D) and multi-step contact tracing (E, F). Treatment times after infection were: at the time of infection (rose), 3 (green), 7 (purple), and 10 (aqua) days post-infection. These can be compared to no treatment (orange).
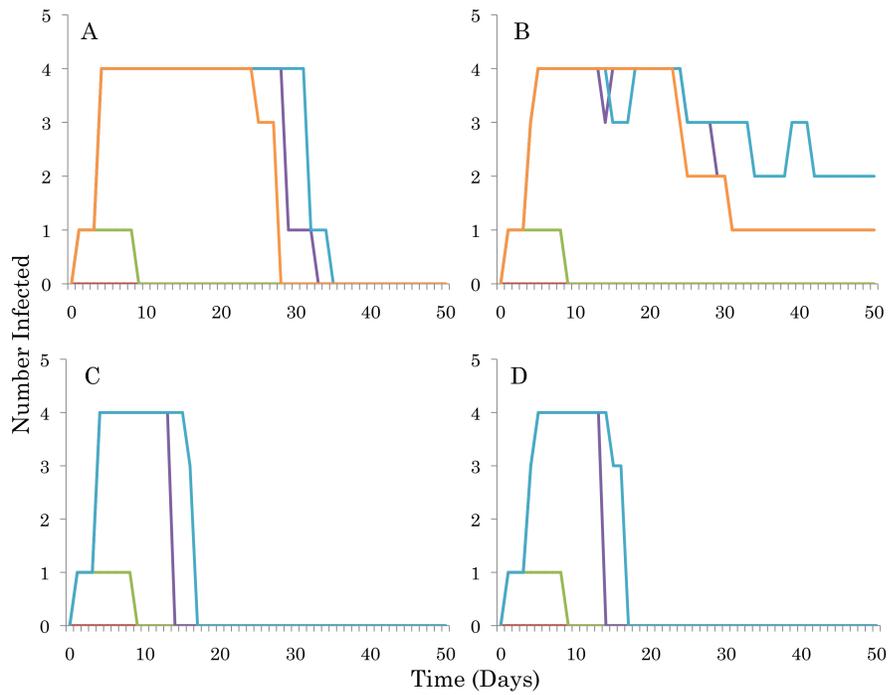
Figure 5.11: The effects of treatment on the prevalence of infection in a "star" of order 4 for H_0 (A, C) and H_E (B, D) conditions across treatment of the trigger case (A, B), single-step (C, D). Treatment times after infection were: at the time of infection (rose), 3 (green), 7 (purple), and 10 (aqua) days post-infection. These can be compared to no treatment (orange).

Figure 5.12: The effects of treatment on the prevalence of infection in a "cycle" of order 4 for H_0 (A, C, E) and H_E (B, D, F) conditions across treatment of the trigger case (A, B), single-step (C, D) and multi-step contact tracing (E, F). Treatment times after infection were: at the time of infection (rose), 3 (green), 7 (purple), and 10 (aqua) days post-infection. These can be compared to no treatment (orange).
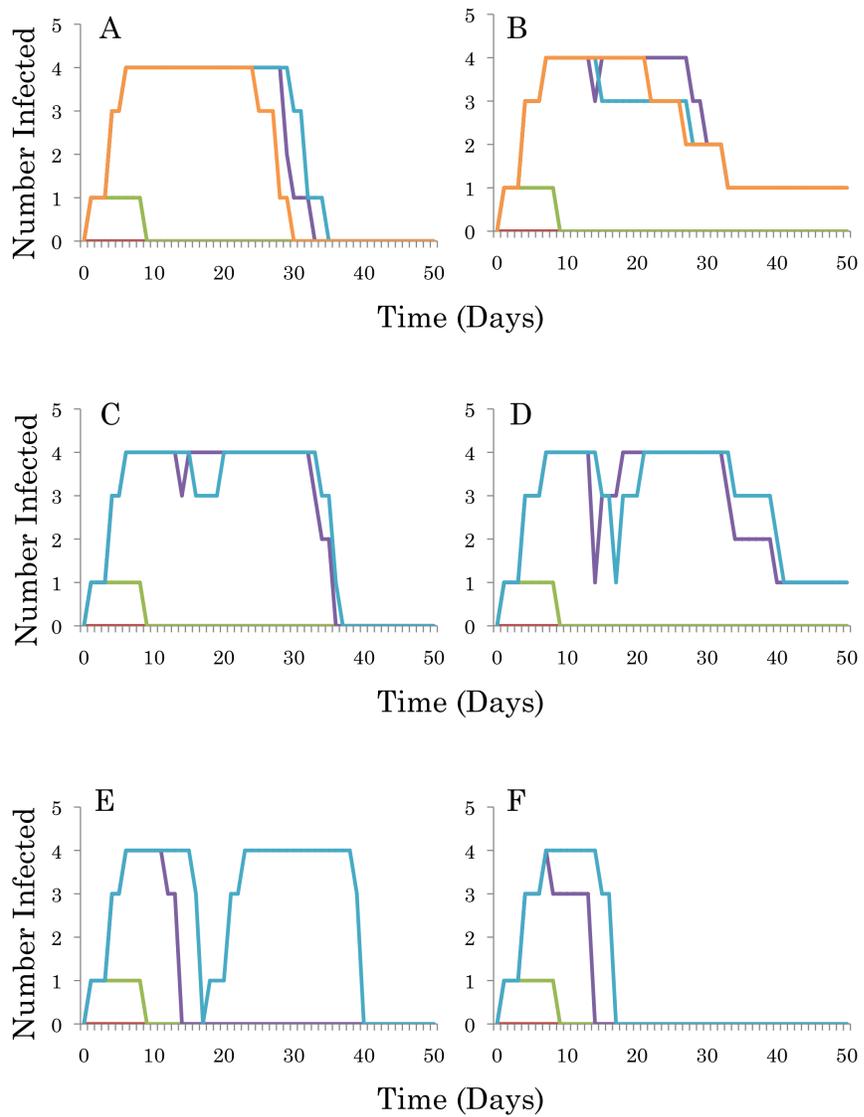
connected networks of individuals; and fourthly, that the effects of treatment leading to increased susceptibility – and as a result, reinfection – are not completely general, and can be prevented by effective surveillance and timely clinical management of infection. This final result demonstrates the need for careful investigation when considering both the beneficial and deleterious effects of treatment on the spread of infection between individuals. Unfortunately, though the analysis here provides clear results, formulating a general "rule of thumb" that scales-up to larger populations, may be challenging.

Because the models here are stylized descriptions of Chlamydia infection and host immune responses, we have erred on the side of starting simple to help gain some initial insights into IE model dynamics. In particular, we chose to develop the within host model (equations 5.1-5.4) under simple assumptions about the spread of infection in the genital tract and the development of immune memory. As a representation of the spread of infection, we assumed a within-host model that describes populations of cells within the genital tract that interact in random mixing process. While this is often assumed for most mathematical models of infectious disease spread, it could be argued that homogeneous mixing is not a realistic assumption for the genital tract, and that our model does not capture events that occur within the genital tract as well as other geometric representations, such as a rectangular lattice with wrapped side boundaries [131].

For the development of immune memory, we chose a heuristic approach over a mechanistic description. Because memory cells are activated at a faster rate than naïve cells, and because they can proliferate before they acquire effector functions, the inclusion of a state variable for immune memory would have altered the time course of and likely the magnitude of reinfection observed. As a result, overall burden of infection may be significantly reduced compared to that shown here.

Despite these simplifications, the most striking result observed was that reinfections arise endogenously from the general model structure. We believe that because of the widespread presence of regulatory feedbacks between hosts and pathogens, treatment for many network-mediated infections will give rise to phenomena similar to that observed here. Upon antigen impingement, a host's immune system is stimulated to react against the infectious agent. As the number of infected cells increase, pathogen production from infected cells will also increase. This increases stimulation to the immune system, which after some delay results in antigen-specific immune responses accumulating. Increased numbers of antigen-specific immune responses increase the clearance of pathogen (or infected cells) until the infectious agent is eradicated or effectively controlled. Treatment, however, also removes pathogen from an infected host. Because the removal of an infectious agent by treatment often occurs at a rate that is faster than the activation of the immune response, effective treatment will result in lower rates of antigen-mediated activation of immune effector cells. This causes the accumulation of immune cells to occur less quickly, leading to a smaller population of immune cells being amassed. Any amount of immune-mediated removal of pathogen that does

occur also reduces pathogen load, further decreasing antigen-mediated activation.

Determining whether this feedback structure is universally detrimental to an infected host has yet to be explored. For Chlamydia infections, we have demonstrated that the timing and extent of treatment can have significant consequences for reinfection. However, when the regulatory feedbacks between host immune responses, pathogen levels, and treatment (described above) are considered in the context of an infection like HIV, the arrested immunity hypothesis provides an interesting perspective as to why effective treatment is able to significantly slow the progression of disease – in a fashion that extends beyond the direct consequences on the virus itself. Decreased antigen impingement, via treatment, reduces immune system stimulation, thus slowing the growth of activated CD4+ T cells for HIV to infect. Fewer infected CD4+ T cells will reduce the loss of these important immune cells to other effector mechanisms (such as Cytotoxic T-Lymphocytes), and therefore will slow an individual's progression to full-blown AIDS.

Our approach has been to consider the impact of treatment on networks with fixed, small neighbourhood sizes in the absence of other population heterogeneities such as risk structure and preferential mixing. The dynamic complexity associated with even the simplified model presented here provides justification for our approach of starting simple. Despite our focus on these simple networks, it is of significant interest to examine the generalizability of these insights as neighbourhood size increases and individual agent behaviour (with respect to contact-tracing and treatment) become less stylized. We emphasize that the current approach can readily accommodate larger and more complex networks (e.g., small world and scale-free), dynamically evolving network structures, as well as other pathogen types and the immune responses against them. However, a comprehensive investigation of these extensions will take us beyond the aims of the present work.

Chapter 6

Conclusions

"I have now in my hands... all the threads that have formed such a tangle. There are of course, details to be filled in, but I am certain of all the main facts... I will give you proof of my knowledge."

– Sherlock Holmes, A Study in Scarlet, Chapter 7.

6.1   A Summary of the Thesis Work and its Significance

Genital *Chlamydia trachomatis* infections are an important public health concern. The most common bacterial STI in Canada and worldwide, human infection with Chlamydia bacteria can elicit an extraordinary range of disease states and observed pathologies [29, 146]. Acute host reactions to sexually transmitted Chlamydia infection range from a subclinical state to erythema, edema, and mucopurulent discharge [163]. Chronic and progressive disease (or sequellae) from human Chlamydia infections includes pelvic inflammatory disease, ectopic pregnancies, and infertility [163].

Since the mid-1990s, many countries with substantial investment in Chlamydia control have observed a rise in case notifications [70]. One important contribution of this thesis was an investigation of current chlamydial trends in the Province of Saskatchewan in the context of several proposed rebound hypotheses [175] (see Table 1.1 in Chapter 1). By combining four simple dynamic models of infection transmission with routinely collected surveillance data, I was able to demonstrate that expanded testing with diminishing returns could effectively account for the observed rebound in case notifications in Saskatchewan (please see Chapter 3). Considering the regulatory feedbacks associated with testing and treatment has also demonstrated that current control policies are, indeed, reducing chlamydial prevalence, contrary to what is indicated by case notifications. However, it is widely discussed that current efforts can be improved [16, 36, 43, 62, 69, 71, 71, 81].

A second important contribution of this thesis is demonstrating the value models of complex systems have to offer our understanding of chlamydial infection dynamics. Using aspects of the systems modelling methodology of system dynamics, the research presented in this thesis has also provided broad insights into both the natural history and immunobiology of Chlamydia infection using models of both the population and individual. Of the six statements given below, the first three apply specifically to the natural history of infection in such a way that they enable insights

that may be difficult to observe among human populations. The last three provide a "systems" account of how wide-scale surveillance of chlamydia infection is affecting chlamydial prevalence. Each proposition is discussed in more detail, and is to serve as a connecting statement of the previous thesis chapters.

1. *Susceptible-Infected-Removed model structures contain essential "natural history" structure needed to understand the governing epidemiological dynamics of sexually transmitted chlamydia infections.*

   This assertion proposes a system structure that is contrary to the traditional Susceptible-Infected-Susceptible (SIS) models of STI transmission. When used in the context of sexually transmitted Chlamydia, more traditional SIS models (and the SIS-like models used in Chapters 2) lack a temporary stay in the recovered state during which they are not susceptible to infection; that is, SIS-like models do not contain the crucial $I \to R \to S$ flow of people that is explicitly represented in SIR and SIR-like models.

2. *The delay from a removed state is caused by a long and effective period of immunity.*

   These observations stem directly from statement 1, and their implication contrasts with the more popular notion of brief and partial immunity currently discussed in the Chlamydia literature [16]. Two elements of this statement should be emphasized: (i) that because of a significant delay introduced by the negative feedback loop, via recovery through a "removed" state, $I \to R \to S$, chlamydial prevalence is oscillating; and (ii), that because of this systemic behaviour, we can immediately identify which variables are responsible for causing the oscillations [187]. Here, the delays arise from the time required for an individual to recover naturally from infection, and then return to the susceptible state through the loss of immunity. Such a postulate as a prolonged immune period also suggests that immunity plays a significant role in both the natural history and transmission of Chlamydia infections.

3. *Frequent re-exposure to chlamydial genital infection may result in unique immunobiological profiles within the host.*

   The unique immunological profiles observed in Chapter 4 support previous observations [30] that complete protective immunity can be obtained through repeated exposure. These observations also appear to non-intuitively link the formation of persistent infection and the potential for immunopathology with the formation of protective immunity. Given that there are several adequate animal models of chlamydial genital infection, further insights into the immunobiological profiles may be won by study of the infection with, and frequent re-exposure to, chlamydial antigens. Of the findings presented here, this statement should be the most amenable to ongoing experimental research.

4. *Antibiotic treatment appears to arrest the development of natural immunity to infection, thus putting an individual at risk for reinfection.*

The function of a public health policy, such as treatment, is to suppress pathogen replication and eliminate the infection from infected cells, therefore rendering an infected host cured. However, since host and pathogen are tightly coupled, policies aimed specifically at the pathogen will ultimately have an effect on the host as well: "you can't do just one thing" [188]. Accordingly, early, effective treatment will not only eliminate the pathogen impingement, but it will also interrupt the natural immune response and the formation of immune memory. Some researchers have argued that this phenomenon is likely to enhance population susceptibility to infection as susceptible patients re-enter the same or similar sexual networks [31].

5. *This deleterious effect of treatment on the development of immunity does not appear to materially affect population level susceptibility, and yet treatment can effectively reduce chlamydial prevalence.*

Note the implication that treatment is able to both effectively reduce population prevalence (as was mentioned above) while also truncating the immune response of infected individuals. Despite evidence that treatment will make individuals susceptible sooner (as compared to becoming susceptible after a period of immunity), it is evident that there are extremely specific conditions in which the deleterious effect of treatment will contribute to increased population prevalence (see Chapter 5). Outside of those conditions, treatment will carry its intended effects at both a population and individual level. The duality of these insights is an indication that while the negative feedback loops discussed in statements 1 and 2 are currently dominant over treatment, treatment is still able to effectively reduce the population prevalence. This lack of dominance may be because the majority of the "infected" population is still recovering naturally.

6. *Treatment of infection can be at the forefront of Chlamydia control programmes, however their effectiveness will likely be best realized in combination with adequate prevention and proactive screening.*

While the results of the study in Chapter 5 may produce more questions than answers with respect to the full population-level impact of "arrested immunity", it is clear from other regions of Canada that current efforts behind Chlamydia control are achieving their public health goal of decreasing the occurrence of Chlamydia-related sequellae [32] (please see Figure 6.1). Therefore, the significant ethical implications notwithstanding, stopping treatment for Chlamydia infection, despite its demonstrable potential to increase population susceptibility, would most likely result in an explosion in Chlamydia prevalence (see Figure 6.2), and
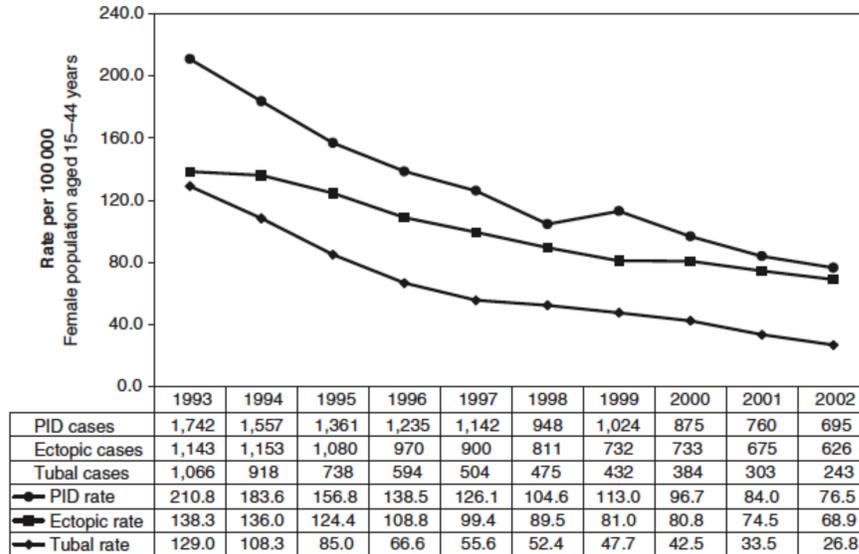
Figure 6.1: Annual reported cases and rates for Chlamydia complications for British Columbia. PID: pelvic inflammatory disease; ectopic: ectopic pregnancy; tubal: tubal infertility. Figure modified from Brunham and Rekart (2009) [32].

therefore is an unlikely policy alternative. More importantly, since current levels of treatment appear insufficient for the deleterious effects of arrested immunity on population prevalence to manifest, the efficacy of treatment to cure infection and significantly decrease the population prevalence still provide promising foci for anti-Chlamydia public health policy. For example, it has been discussed that the introduction of a nationally coordinated enhanced surveillance system among high-risk groups (particularly adolescents and youth) may be an effective advancement [134]. Given that adolescents appear to be the most at-risk sexually active subgroup of the population [54, 153], the introduction of a proactive Chlamydia prevalence monitoring system for Canadian youth could provide effective targeting, prevention, and control strategies to further reduce the population prevalence of Chlamydia infection [134] (see also Figure 6.2 for potential impact of increased screening and prevention on Chlamydia prevalence). Canada does have an extant enhanced surveillance system in place amongst street youth (e.g., the I-Track network), however extending this to a broader collection and dissemination of data may provide a mechanism to guide allocation of resources for Chlamydia prevention and control among the general population [134].
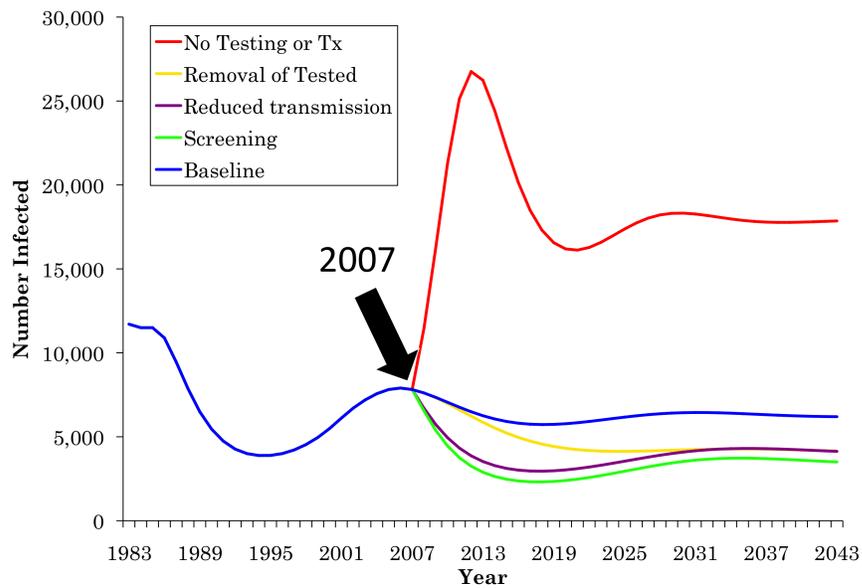
Figure 6.2: Plausible futures of Chlamydia prevalence under hypothetical policies: one, if a screening program is implemented to increase the number of people tested and treated by 35%; two, a safer sex campaign that produces behavioural changes to reduce transmission (by 10% per year) to susceptible people; three, " quarantining" those who test positive, without treatment, for the remainder of their sexual lifetime; and four, no longer testing or treating for this infection.

6.2 Reassessing Contemporary Knowledge though a Systems Lens

Chlamydia is an infection that can cause chronic and progressive disease with serious reproductive tract sequellae. Because of this, many countries worldwide have initiated chlamydia control programs. However, the expected, continuous decrease in observed Chlamydia case notifications has not been witnessed after the introduction of these programs [70]. Contemporary opinion views this current paradox as the result of unforeseen and unpredictable outside forces [71].

The proposed solution is stated to be obvious: diligent adherence to the scientific method through ongoing, novel epidemiological and immunological research [70, 71]. Recent discussion surrounding Chlamydia epidemiology and immunology encourage continued policy-focused research, and carefully planned prospective studies to better understand the natural history of *Chlamydia trachomatis* infections in humans [70]. Innovative translational research is also suggested as essential to determine the degree to which mechanisms of clearance, pathogenesis, and immunity in humans parallels those found in animal and in vitro studies; likewise, refinement of animal models are suggested to more closely parallel human responses [71].

In an ideal world, evidence-based learning should improve our knowledge base and allow for beneficial decision-making [188]. However, learning often fails even when reliable evidence is available [52]. Information generated from distinct parts of the system will often adhere to disciplinary approaches that are static and reductionist. These perspectives lead to reliable and self-confirming inferences that allow narrow (and possibly inappropriate) beliefs to persist [188]. If the endemicity of genital Chlamydia infections truly reflects the combined effects of processes across immunological, behavioural, and sexual network levels, then it would be surprising if infection and disease dynamics across these levels could simply be understood by independently studying a single piece of the system [55]. Because public health policies are embedded in intricate systems that are governed by many physical, ecological, social, behavioural, technical, and economic relationships, generating reliable evidence through the scientific method becomes increasingly difficult, the more complex the phenomenon [91, 187, 188].

Complexity can be marked by the existence of feedbacks, delays in cause and effect, hierarchical organization, nonlinear responses to perturbation, compensatory mechanisms, counterintuitive and emergent behaviour [52, 55, 188]. Complexity hinders the generation of evidence, learning from evidence, and the implementation of policies on the basis of evidence [188]. In such situations, it is difficult to know how, where, and when to intervene [135]. As a result, most well-intended interventions will have little or no effect, or will have unintended consequences and, thus will be undermined because of a failure to address the causal mechanisms driving observed trends [52, 91, 187].

Failure to consider the interactions between other pieces of the system – which may or may not be understood – overlooks the importance of cumulative effects, delays, feedback loops, and nonlinearities [52]. Reassessing existing chlamydial research from a systems perspective has highlighted, what appear to be, misconceptions arising from the application of traditional epidemiological methods that have lead to incomplete interpretations of available data. The unexpected behaviour of chlamydia case notifications in the face of intensified control efforts contains two examples.

For the first, it is apparent that – 25 years after the beginning of the "treatment era" – the currently observed case notifications are not a feature of ineffective control efforts. Rather, they are an indication that the perceived alarm over increased case notifications (in the presence of worldwide control programs) have arisen from mental models of that are too narrow, linear, and based on time horizons that are too short [188]; there is a focus on proximal causes in time and space. Effect is rarely, proportional to cause [52, 188]. Therefore, while testing and treatment levels may have increased in recent years, one should not expect a proportional decline in the prevalence of infection. There are other processes – besides treatment – that are simultaneously influencing the dynamic behaviour of prevalence over time (such as the negative feedback loops and the delay brought about by a prolonged immune state, see summary statements 1 and 2 from the previous section).

A second example of where thinking in systems can be informative involves an observed discrepancy between the trends in Chlamydia incidence and prevalence in Finland [128]: prevalence has been observed to be increasing, while the prevalence is decreasing. Some researchers argue that this discrepancy is discussed to be the result of the "treatment era" decoupling these two measures of chlamydial morbidity [32, 70, 128]; in other words, it is thought that these two related measures are now behaving in a nonintuitive, discordant manner since Chlamydia has become a reportable (and treatable) infection.

From a system dynamics perspective, this behaviour is far from counterintuitive, and when interpreted in the context of stocks and flows the observed discrepancy becomes understandable. Stocks and flows are familiar to all of us: the product inventories of a manufacturing firm its warehouses, the balance in your chequing account, and the number of people infected with Chlamydia are all examples of a stock. Stocks change over time as a net function of their inflows and outflows [187]. A firm's inventory is increased by the flow of production and decreased by the flow of shipments. Your bank account balance increases with deposits and decreases as you spend. Prevalence of infection increases with incidence and decreases by recovery and treatment (and possibly other outflows due to death or migration). However, despite everyday experience of stocks and flows, people fail to distinguish clearly between them [52, 187].

This failure to understand the difference of stocks and flows has produced unclear numeric comparisons between different concepts – such as prevalence and incidence. As a result, this has
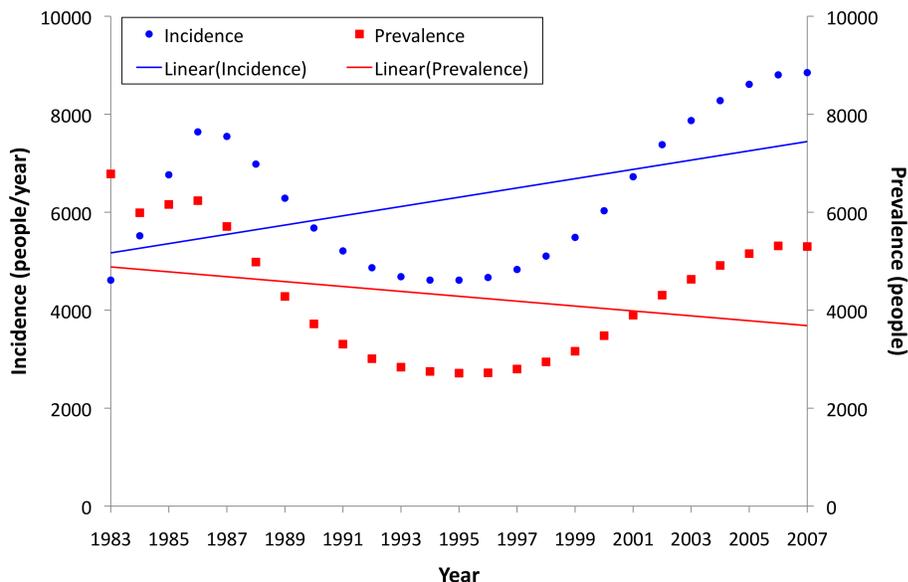
Figure 6.3: A comparison of the different behaviour of model-predicted incidence and prevalence of Chlamydia in Saskatchewan between 1983 and 2007. The displayed trends are from a single calibration of Model 4 in Chapter 2.

led to the generation of perceived (and alarming) discrepancies when, given that Chlamydia is a treatable infection, such discrepancies are to be expected. From these discussions, it appears that previous researchers were most likely thinking statically, where the more traditional relation between prevalence and incidence, $p = i \times d$, holds: given that duration of infection, d, is constant, an increase in the incidence, i, will also increase the prevalence, p. While this is true in equilibrium, it is evident from the oscillatory behaviour of the model-predicted prevalence and incidence that the system is not in equilibrium. The models presented in Chapters 3 and 4 demonstrate that the incidence rate of Chlamydia in 2007 has surpassed the incidence in 1984 (see Figure 6.3). However, they also suggest that the prevalence of infection is damping out to an endemic equilibrium. This is a strong indication that even though the inflow due to incidence might be increasing, the flow out of prevalence through treatment is greater, and thus is lowering the prevalence in the population.

Currently, systems thinking and traditional epidemiology represent two dissimilar methodological paradigms. On the one hand, traditional epidemiology has had a high reliance on methods that produce data and robust estimates of parameter values. These methods are traditionally married with biostatistical techniques and are almost entirely used on data that has already been collected (via an epidemiological study) to document an association present in data [55]. Despite providing

many useful insights in the previous 2-3 decades, these traditional methods, still, represent a reductionist approach that is fundamentally focused on "controlling away" risk factors and potential confounders to identify a linear, one-way cause and effect relationship – all of which, from a systems perspective, might be relevant to understanding the behaviour of that system.

System dynamics modelling, on the other hand, has been – by definition – used for understanding and managing feedback systems. In system dynamics modelling, the emphasis is placed on connecting system structure and observed data [188]. This is particularly well suited to address the challenges of dynamic complexity that currently eludes traditional epidemiological methods: one cannot study the link between X and Y, independently, and predict how the system will behave [55, 91, 188]. Inherently interdisciplinary, system dynamics builds atop a solid mathematical foundation and involves the development of computer simulation models that portray processes of accumulation and feedback which can be tested in a systematic and rigorous fashion to find effective policies for achieving the "best" public health outcomes; these models not only help answer questions of "what if?" but questions of "why?" as well [135]. Only the study of the whole system as a feedback system will lead to a thorough understanding of a system's underlying processes [52]. Sometimes, however, the focus is placed on how the model "works" (i.e., does the model behave in a similar fashion as the system?), and not on whether parameters are tied to observable data [55].

The adoption of systems thinking and modelling in epidemiology will necessitate incorporating appropriate data into systems models that lead to tangible, evidence-based, parameterization for both epidemiological and policy-related insights [55]. However, because of the current disparities related to methodological commitments, two important questions that immediately arise are: is direct integration of data generated by traditional epidemiological approaches into a systems model appropriate? And, are significant modifications to current data collection required to make data compatible for a systems approach? I believe that providing answers to these questions is nontrivial, but attainable. Recent discussion on this matter cautions that, absent a clear conceptualization of how to creatively collect data so that it fits with the assumptions of a systems approach, the ability of these techniques to further our understanding of disease processes will remain a daunting endeavor [55].

6.3 Strengths and Weaknesses of the Thesis Research

Though mentioned previously throughout each of the studies in Chapters 2-5, there are some important limitations to this analysis that require discussion. As with any model, the structure of the models described here represent a simplification of reality [21, 57, 169]. Models at different levels of aggregation may lack the structure needed to observe important dynamics and adequately inform policy makers. The particular scale of the model will depend on the questions asked as well

as the data that is available [169].

The models used throughout this thesis reflect simple dynamic hypotheses of Chlamydia transmission within the sexually active population. While traditional modelling of STIs has made use of models that abstract away from population heterogeneity [21,57], many recent STI transmission models feature a more-detailed characterization of the population [30, 59, 60, 72, 117, 126]. Although more complex models offer advantages for investigating certain types of research or policy questions [169], several considerations motivated the use of simpler models. Because the focus was on trying to understand the rebound in the population, because of the aggregate nature of case notification counts and testing volumes in the Province (which lack explicit information on behavioral heterogeneity or network structure), and to avoid over-fitting that often accompanies high-dimensional parameter spaces, the models presented in Chapters 2 and 3 did not explicitly represent heterogeneity in sexual risk behaviour.

The within-host models presented in Chapters 4 and 5 are limited by similar abstractions. These models are stylized descriptions of Chlamydia infection and host immune responses, and I have erred on the side of starting simple to help gain some initial insights into model dynamics. In particular, I chose to develop a model that describes populations of cells within the genital tract that interact in a mass-action like (or random mixing) process. While this is often assumed for most mathematical models of infectious disease spread, it could be argued that this is not a realistic assumption for the genital tract (i.e., it is not a well-mixed system), and that these models do not capture events that occur within the genital tract. There are likely better representations of an infection in the genital tract than those of mass-action. For example, I could have considered density-dependent, saturation-based, or spatial compartments as a model for infection spread.

Another limitation of these analyses is that the model structures were not stratified by different chlamydial serovars (or strains). As was mentioned in the Introduction (Chapter 1), the genus *Chlamydiae* has 18 major serovars [29,152], and although inconsistently demonstrated, previous research has reported a correlation between clinical symptoms, the resilience of infection, and particular serovars circulating in a population [149–152]. In addition to this, there is evidence to suggest that immunity to specific chlamydial strains is produced within a host who naturally resolves infection [24,28]. Therefore, the introduction of a different strain, or complimentary shifting patterns of strain dominance may likely have a significant impact on reinfection rates in an immunologically-experienced host population [120]. Changes to strain ecology could facilitate reinfection, particularly among groups of highly sexually active individuals – who may already possess a degree of strain-specific immunity. If larger fractions of people are immune for a prolonged period of time (as the analysis in Chapter 3 suggests), then we may expect to see cyclical switching between dominant strains. Obtaining population level data on serotypes in positive cases may reveal some interesting information into chlamydial serovar population ecology.

6.4 Conclusions

Generally, the potential of applying systems thinking approaches to epidemiology appears vast, but the challenges to adopting this way of thinking are commensurably daunting [55]. In particular, overcoming dogmatic commitments related to discipline-specific theoretical paradigms, study methodologies, and language will likely be prominent and recurring issues that need to be overcome. It will require listening with respect to others, then using these systems thinking capabilities to act in consonance with our long-term goals [188]. The primary goal of this thesis was to approach Chlamydia epidemiology a little differently, and to ask questions like "how?" and "why?" Systems thinking is not a better way of seeing the world than traditional scientific methods; it is complementary, and therefore revealing [135]. A systems thinking lens will allows us to reclaim our intuition about whole systems, and therefore hone our abilities to understand parts, see interconnections, ask "what if?" questions about future system behaviour, and ultimately be creative and effective at addressing system problems [52,135,188]. While I do not think that systems thinking and modelling will be a panacea that offers a solution to all the challenges remaining in Chlamydia research, I do think that it offers an effective way forward for both approaching and analyzing research initiatives aimed at understanding disease processes.

Interventions to decrease adverse outcomes of Chlamydia infections need to target causes at cellular, individual, network, and regional levels. This final chapter was intended to serve as both a challenge to contemporary methods as well as to initiate discussion of a way forward. I suggest that epidemiological thinking needs to broaden its conception of disease processes across multiple scales, and that systems approaches provide a promising framework to augment and unify aspects of traditionally columnized disciplines. For the study of genital Chlamydia infections, a systems approach will enable an evaluation of the contemporary perspectives with which research questions are being framed, the assumptions driving these perspectives, and whether useful predictions are likely to result. This will highlight where communication between various clinical, immunological, and epidemiological communities is inadequate, but also outline creative ways for these disciplines to combine their knowledge of a problematic situation, and the development of an explicit and visible *dynamic hypothesis*. Then, using computer simulation, stakeholders can formulate and compare various scenarios for how to navigate change [1]. Learning via simulation will ultimately reveal critical leverage points that take into account a system's counterintuitive tendencies, opening new avenues for fundamental improvement for understanding chlamydial disease processes [52].

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Appendix A

Development of a Unified Framework for Incorporating Immunology into Epidemiological Spread

A.1 Background

Epidemics consist of dynamic processes at multiple biological scales. From host-pathogen interactions to host-host interactions infectious diseases have had a major influence on the development of our immune systems and the evolution of human ecology [5, 159]. In recent decades, remarkable advances in immunology and virology have provided fundamental insights into the detailed mechanisms of infection pathogenesis and immune recognition [158, 213]. Meanwhile epidemiological modelling has enriched our understanding of the properties of infectious disease thus enabling humankind to better control its spread [5].

Within an individual host, a major factor governing infectious disease dynamics is how quickly and effectively the immune system can respond to infection (hereafter referred to as immune responsiveness) [159]. For clearing a viral infection, this is defined as the average rate at which naive CD8+ cells proliferate into cytotoxic T-lymphocytes (CTLs) after encountering a viral antigen for the first time [5, 158, 213]. The CTL responsiveness against a specific viral antigen is likely to vary between individuals, as well as within individuals over time (for example, at successive stages of HIV infection) [159]. The effectiveness of an anti-viral CD8+ response will depend on molecular factors such as the affinity of the T-cell receptor for the viral peptide in the context of Major Histocompatibility Complex (MHC) molecules, as well as MHC polymorphisms that determine which particular viral peptides are presented to the immune system [139, 158, 159].

At epidemiological (or population) levels, the importance of contact structure (or network connectivity) for disease transmission has long been acknowledged [143]. Locally structured networks can qualitatively alter infection dynamics through clustering behaviour with pairs of connected individuals sharing many common neighbours. The effects of population heterogeneity on infection spread are important but complex. Thus, when compared to well-mixed populations, local heterogeneous contact patterns can either slow or accelerate the progression of infection - depending on the structure of the network [8, 15, 107, 119, 123, 143, 156, 161, 203].

There are rich traditions of modelling centered specifically on the dynamics of infections at the cellular level [6, 159], or at the population level [5], that have profoundly advanced our understanding of disease dynamics and control. While the insights gained from these modelling techniques is remarkable, it is becoming evident that there are unique epidemiological processes of infectious diseases that are likely governed by the dynamics of the immune systems of individuals in a population (e.g., rebounds in the prevalence of some infectious diseases, antigenic variation and competition, waning immunity, and transient cross-immunity of sexually transmitted infections) [120]. Many of these may have significant consequences for creating optimum prevention strategies (e.g., vaccination or prophylactic chemotherapies) and establishing an adequate level of herd immunity.

In spite of the focused nature of current modelling applications, the need for integrating an immune system mechanism into epidemiological models has been recognized [47, 75, 87], and unified theoretical templates of these biological domains have been developed [115, 193]. Although these initial immuno-epidemiological frameworks demonstrate innovation and clarity, they lack the representation of certain cellular components and immunological processes needed to characterize the dynamics of some important epidemiological contexts such as antigenic variation, coinfection, and the immunological impact of prevention efforts. As a result, the link between host-pathogen interactions and their impact on the spread of infectious diseases across a population remains underexplored. Here, we present a simple mathematical framework that provides an alternate approach for unifying infection dynamics at the immune system and epidemiological scales. Although the

analyses presented in this paper are almost entirely abstract, in the broadest context we advance the arguments that: one, individual immune response dynamics are important for shaping population-wide disease dynamics; and two, a modelling framework should not only be focused on a linked transmission system that can advance overall theoretical understanding, but also inform infection control decisions.

A.2   Models and Methods

A.2.1   Combined model for infection dynamics

To gain insight into how the basic laws of viral dynamics, within an individual, will eventually affect the spread of a virus throughout a population of connected individuals, we considered a simple integrated model of the immune response and population structure. To this end, we elaborated on a simple, previously described model of the interactions between a replicating virus, host cells, and cells of the immune system specific for infected host cells (namely CD8+ T-lymphocytes) [159,213]. We have modified this framework by placing each individual in the population within a simple randomly-distributed (Poisson) network of 1000 people such that the viral load of a given individual is linked with the viral load of adjacent individuals within the network (described below). This basic model of anti-viral immune responses and population dynamics for each individual contains five variables: uninfected cells X_i, infected cells Y_i, free virus particles V_i, precursor CTLs ($CTLp$) (i.e., CD8+ cells that have recognized a specific antigen but lack specific effector functions) W_i, and $CTLp$ cells that differentiate and inhibit viral replication through cytotoxic effector activity ($CTLe$) Z_i.

Following Nowak and May [159] and Wodarz and colleagues [213], the emergence of uninfected cells occurs at a constant rate λ. Infected cells arise through contact between uninfected cells and free viral particles at a rate $\beta X_i V_i$ and die at a rate aY_i. A person's free virus load is produced by infected cells, at a rate kY_i, and declines at a rate uV_i. The rate of $CTLp$ proliferation for each person in the population in response to antigen is given by $c_i Y_i W_i$. The parameter c_i denotes the $CTLp$ responsiveness, which is defined as the proliferation of specific precursor CTLs cells (i.e., $CTLp$ cells) after their first encounter with a foreign antigen at the site of infection. While antigen is present, $CTLp$ cells differentiate into $CTLe$ cells at a rate $c_i q$. In the absence of antigenic stimulation, each ith person's $CTLp$ population decays at a rate bW_i. Infected cells are killed by $CTLe$ cells at a rate of $pY_i Z_i$. The parameter p specifies the rate at which $CTLe$ cells kill infected cells. Once the infection is brought under control by the immune system, the $CTLe$ population decays at a rate hZ_i.

To this model, we have added an additional term specifying that the rate at which a person's incoming flow of free viral particles is proportional to the viral load of their neighbours, $\omega_i \sum_{j \in P} \mathbf{A}_{ij} V_j$. Here, ω_i is the (typically very small) coefficient of connectedness that defines the weights on each of the connections between neighbours. We hereafter refer to ω_i as the connectivity coefficient. The expression $\mathbf{A}_{ij}$ is a randomly selected, symmetric, binary $n \times n$ adjacency matrix that describes "who is connected to whom". This matrix describes the structure of the Poisson-distributed network and is fixed throughout the period of simulation (once it is selected). The vector, V_j, is the viral load of the jth network contact of person i, and P is the population. These assumptions lead to the following system of ordinary differential equations:

Parameter	Description	Value	(units)
λ	Production rate of uninfected cells	10	(cells/day)
d	Rate of uninfected cell die-off	0.1	(1/day)
β	Infection rate	0.01	(virion/day)
a	Infected cell death rate (due to virus)	0.5	(1/day)
p	Rate that infected cells are killed by CTL_e cells	1.0	(cells/day)
b	Rate that CTL_p cells die	0.001	(1/day)
q	Fraction of CTL_p cells that turn into CTL_e cells	0.1	
h	Rate of CTL_p death	0.1	(1/day)
k	Rate at which free virions are produced from infected cells	3.0	(virion/day)
u	Viral decay rate	3.0	(1/day)

Table A.1: Simulations were based on values used by Wodarz and colleagues [213] and Nowak and May [159]. Immune responsiveness c_i and the connectivity coefficient ω_i were varied throughout this paper. Their specific values for each simulation experiment are described in the Methods section.

$$\dot{X}_i = \lambda - X_i\left(d + \beta V_i\right) \tag{A.1}$$

$$\dot{Y}_i = \beta X_i V_i - Y_i\left(a + pZ_i\right) \tag{A.2}$$

$$\dot{V}_i = kY_i + \omega_i \sum_{j \in P} \mathbf{A}_{ij} V_j - uV_i \tag{A.3}$$

$$\dot{W}_i = c_i Y_i W_i\left(1 - q\right) - bW_i \tag{A.4}$$

$$\dot{Z}_i = c_i q Y_i W_i - hZ_i. \tag{A.5}$$

$$\tag{A.6}$$

We numerically solved the above system of equations for each individual i in the population ($i = 1, \ldots, 1000$). The initial conditions that accompanied this system of equations for viral introduction were:

$$\left(X_i\left(0\right), Y_i\left(0\right), V_i\left(0\right), W_i\left(0\right), Z_i\left(0\right)\right) = \left(X_0, 0, E_0, W_0, 0\right) \tag{A.7}$$

In all simulation experiments, parameter values were based on those presented previously by Wodarz and colleagues [213] (see Table 1). Symbolic equilibrium analyses are presented in the Results section below.

For describing infection spread among the population, we used the mean and accumulated mean viral load as our main measure of infection prevalence. The accumulated mean viral load, $A_V\left(t\right)$, in the population was the integral of the mean viral load from the beginning of a given simulation (time 0) until time t, and was used as a proxy for the final size and severity of an outbreak. It was defined as $A_V\left(t\right) = \int_0^t \bar{V}\left(\tau\right) d\tau$, where $\bar{V}\left(t\right) = \dfrac{\sum_i V_i\left(t\right)}{|P|}$ is the mean viral load in the population at time t, and where $|P|$ is the number of people in the population.

A.2.2 Individual immune responsiveness

For experiments associated with parameter c_i, we examined the effect of assuming specific values (homogenous across the population) on infection spread. However, because individuals are likely to

vary in their ability to respond to infection [139, 213], we also conducted experiments in which the population was divided into two halves with different c_i, and in which each individual's immune responsiveness was drawn from a truncated normal distribution with ($\mu = 0.063$ and $\sigma^2 = 0.0005$) and confined to support over the interval $[0.01, 0.1]$. Variance was estimated from the square of the interval divided by four: $\left[\frac{0.1 - 0.01}{4}\right]^2$. Our mean and range values were derived from the values studied by Wodarz and colleagues [213]. In all cases, values of c_i were set at the beginning of the simulation, and remained static for the duration of that simulation.

A.2.3 Weight of network connectivity between people and infection spread

One of the most obvious features of viruses is their capacity for person-to-person transmission [203]. Contact patterns provide important information for understanding the transmission properties of the pathogens, themselves, as well as where to concentrate prevention efforts [143]. Because exact values for the connectivity coefficient ω_i will often vary over time [203], we assumed that ω_i followed a random uniform distribution with mean, $\frac{\theta_1 + \theta_2}{2} = 0.5$ and variance, $\frac{(\theta_2 - \theta_1)^2}{12} = 0.083$. The value of ω_i was dynamically varied for the majority of our analyses. Just as with immune responsiveness, the circumstances where we focused on the specific effect of a person's connectivity, ω_i was assigned a constant value for the entire population. High and low values of ω_i were arbitrarily assumed to be 1.0×10^{-3}, 1.0×10^{-6}, and 1.0×10^{-9}, respectively.

A.2.4 Time until re-infection and immunological memory

A direct consequence of an individual's ability to respond to and eliminate an infection is the formation of immunological memory. Within the host, memory CD8+ T-cell populations have the ability to rapidly elaborate effector functions to respond quickly and efficiently when re-exposed to infection. These properties of memory cells will not only decrease the duration of subsequent infection within the host, but their presence is considered to increase the level of herd immunity in a population [14, 205]. And yet, the generation of memory T-cells exhibits both antigen-dependent and antigen-independent characteristics [13, 213]. This appears to rely on the time scale of the infection being studied: antigen-independent immunological memory has largely been observed in acute infections, while antigen-dependence has been observed in the context of persistent infections [206].

To examine the effect of re-infection on the accumulated viral load in the population, we considered two different scenarios. Scenario one was after an acute infection that was completely cleared by the immune system and where memory CTLs (here $CTLp$ cells) persist for long periods of time in an antigen-independent environment. Scenario two was for a low-grade persistent infection characterized by a high acute-phase viral load followed by a reduction to very low levels but not complete elimination. Specifically, this involved re-introducing infection at a disease-free equilibrium (see below), where viral antigen has been eliminated (scenario one), and comparing it to re-introducing infection near an endemic equilibrium (see below), where viral antigen has persisted at low levels (scenario two). For all re-infection experiments, both the population and an individual were separately re-infected at time $t = 9000$ days with a viral load that is equal to the initial amount of virus, $V_i(0)$. We also investigated periodically re-infecting the population and an individual at $t = 1000$, 3000, 6000, and 9000 days. For each scenario, the values of c_i (immune responsiveness) and b (rate of $CTLp$ die off) were varied according to Wodarz and colleagues [213] for the comparison of antigenic persistence and elimination. Here, individuals were assumed to be strong responders $c_i = 0.1$, and have a slow rate of $CTLp$ die off $b = 0.001$.

Because our basic model is deterministic and was originally used to describe persistent viral infections [158], $CTLe$ responses cannot reduce both $V_i(t)$ and $A_V(t) \to 0$. Therefore, following Wodarz and colleagues [213], for scenario one (above) we defined a threshold value where virus, although likely at low levels, was considered extinct, v_{ext}. For our simulations of long-term dynamics that assumed that the virus was eliminated, our extinction threshold was chosen (arbitrarily) to be marginally larger than the endemic equilibrium value $\hat{V}_i = 0.013$. Here $V_{ext} = 0.015$.

A.2.5 Varying the infecting dose

The outcome of viral infection, in general, is thought to be related to the size of the infecting dose a person initially receives [14]. Therefore, we also investigated the impact of varying the infecting doses a person received from their network contacts. More specifically, we examined the situation of $\dot{V}_i = kY_i + \omega_i\phi\sum_{j\in P}\mathbf{A}_{ij}V_j - uV_i$, where ϕ is the constant for the infecting dose received by a person from their network contacts, with $\phi = 1$ being the default value. These experiments allowed to us to obtain an initial understanding of the dynamical behaviour of the model under different viral quantities transmitted throughout the population. For these experiments a person's immune responsiveness, c_i, was a static random variable and the network connectivity coefficient, ω_i, was a stochastically-varied random variable.

A.3 Results

A.3.1 Equilibrium analyses

For a single-person where $\mathbf{A}_{1,1} = 0$, the equations in the basic model are associated with three equilibria. The first is a disease-free equilibrium in which free virus, infected cells, $CTLp$, and $CTLe$ cells are all absent, and only uninfected cells are present: $\hat{X} = X(0) = \frac{\lambda}{d}$, $\hat{Y} = \hat{V} = \hat{W} = \hat{Z} = 0$. This equilibrium is unstable for the case in which viral antigen persists, but is locally stable when viral antigen is eliminated. The second equilibrium is a stable endemic equilibrium, in which free viral particles and infected cells are in balance with uninfected, $CTLp$, and $CTLe$ cells:

$$\hat{X} = \frac{\lambda uc(1-q)}{duc(1-q) - \beta kb} \tag{A.8}$$

$$\hat{Y} = \frac{b}{c(1-q)} \tag{A.9}$$

$$\hat{V} = \frac{kb}{uc(1-q)} \tag{A.10}$$

$$\hat{W} = \frac{h\left((1-q)^2(\beta\lambda ck - aduc) - a\beta kb(q-1)\right)}{qbp(duc(q-1) + \beta kb)} \tag{A.11}$$

$$\hat{Z} = -\frac{(aduc - \beta\lambda ck)(q-1) - a\beta kb}{pduc(q-1) - p\beta kb}. \tag{A.12}$$

The final equilibrium is an unstable "defense-free" equilibrium in which free viral particles, uninfected cells, and infected cells are present, but at which $CTLp$ and $CTLe$ cells are absent:

$$\hat{X} = \frac{ua}{\beta k}, \tag{A.13}$$

$$\hat{Y} = \frac{uad - \lambda\beta k}{a\beta k}, \tag{A.14}$$

$$\hat{V} = \frac{uad - \lambda\beta k}{ua\beta}, \tag{A.15}$$

$$\hat{W} = \hat{Z} = 0. \tag{A.16}$$

The equilibria described above for the single-person case have a close relationship with the equilibria for a connected multi-person population. For a multi-person population, the number of equilibria for our basic model rises geometrically with population size. While the count and stability of these equilibria differ significantly for the cases of antigenic persistence and elimination, two equilibria are shared by both scenarios: the first is a unique disease-free equilibrium, in which the values of the state variables for each individual are identical to those obtaining under the

single-person disease-free equilibrium. Compared to the corresponding single-person equilibrium, this multi-person equilibrium is unstable for the case in which viral antigen is assumed to persist, but is locally stable for the case in which a viral antigen is eliminated; the second is a unique stable endemic equilibrium, in which the values of the state variables for each individual are very close to those that would obtain for a single-person endemic equilibrium, but are slightly offset due to the small rate of virions transmitted by neighbours. For example, given a very high coupling coefficient ($\omega_i = 0.001$), the difference of viral levels between the single-person and multi-person endemic equilibrium is only 3 per cent for an individual with 5 neighbours (not shown). The exact formula the equilibria value of each individual will depend on population size and network structure; because of this dependence, and because the equilibria for each individual within a multi-person population are so close in values to the corresponding single-person equilibrium, we do not describe a general formula here.

The number and stability of the remaining equilibria beyond the two just described depend on whether viral antigen is assumed to be eliminated. If antigen persists, and we ignore all non-physical equilibria associated with negative values of state variables for a population of size $|P|$ will be associated with a total of $2^{|P|} + 1$ distinct equilibria. In addition, there is a set of unstable $2^{|P|} - 1$ "combinatorial" equilibria in which some individuals are in a state very close to the defense-free equilibrium for the single person case, and in which other individuals are in a state very close to the endemic equilibrium for the single person case. Thus, each such population-wide unstable equilibrium is very close to being a simple superposition of individual defense-free and endemic equilibria. Because both the disease-free and each of the combinatorial equilibria are unstable (given the absence of a non-zero viral extinction threshold), the endemic equilibrium is the sole stable equilibrium (as it is in the single-person case).

For a model that assumes viral antigen is eliminated, the structure and stability of the equilibria are significantly different. Recall that for a given non-zero virus extinction threshold, the disease free equilibria for each individual in isolation and for the population as a whole are locally stable. Given extinction of a virus within a person, any finite-rate perturbations to the viral load in that individual disease free equilibrium will be insufficient to elevate the viral load above the set viral extinction threshold, and will therefore maintain complete extinction of the virus. A given individual who has undergone viral clearance will therefore remain virus-free even in response to coupling with nearby neighbours. As a result, a population of size $|P|$ will exhibit $3^{|P|}$ equilibria. Specifically, equilibria will be present near any combination of the equilibria for different individuals, including the homogenous $|P|$-person endemic and the disease-free Equilibria already discussed above. These equilibria include both $2^{|P|}$ stable and $3^{|P|} - 2^{|P|}$ unstable equilibria. The stable equilibria include the global endemic and disease-free equilibrium discussed, and include cases in which individuals are close to either their single-person disease-free or endemic equilibria. The remaining (unstable) equilibria are those in which at least one person in the population is at a point very close to their single-person defense-free equilibrium.

A.3.2 Simulation experiments

Immune responsiveness limits viral transmission

The abundance of virus - that is, the viral load - is an important correlate of pathogenicity and disease progression of many viral infections [158]. Our integrated model both reproduced the well-known relationships between an individual's immune responsiveness c_i and their viral load (Figs. 1 and 2) [159, 213], and demonstrated the implications of this relationship to the short-term dynamics of an outbreak (Fig. 3). Overall, a population that possesses a high value for c_i will reduce the scale and overall severity of an outbreak when compared to a population of weaker responders (Fig. 3A and 3B). Interestingly, these results demonstrate a correlation between immune responsiveness and the natural history of infection in the population. For populations of strong responders, infection is eliminated (or at least depleted to very low levels), whereas in a population of weak responders infection is likely to become endemic (Fig. 3A). If we assume that a population is composed of a combination of strong and weak responders, then starting an infection in either a weak (low c_i) or strong (high c_i) responder, interestingly, had no significant impact on the overall severity of
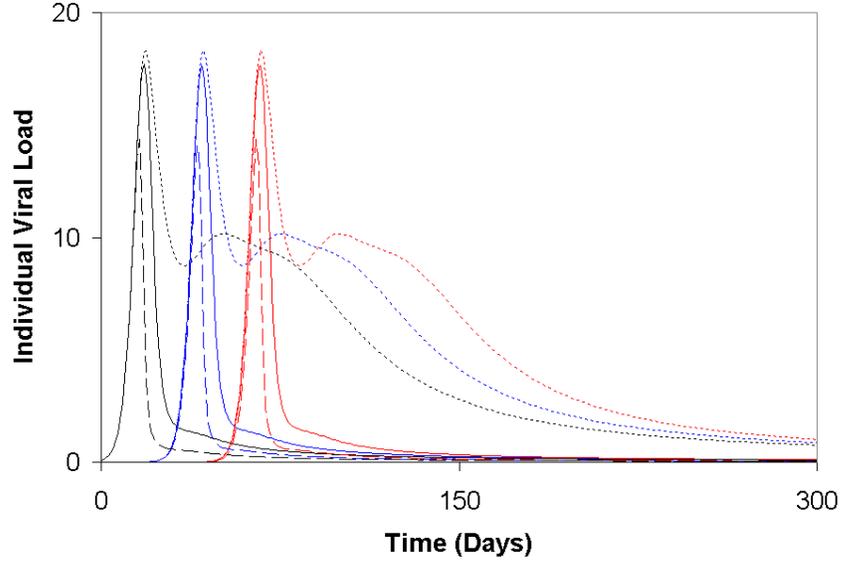
Figure A.1: Evolution of individual viral load of infected cases and their network contacts. For illustrative purposes, results displayed here are for three people in the population. Person 3 (black lines) and Person 1 (blue lines) are connected, and Person 1 and Person 2 (red lines) are connected. Here, $c_i = 0.01$ (dotted lines), 0.05 (solid lines), and 0.1 (dashed lines) (Here $V_{ext} = 0.015$ and ω_i was assumed to be a uniformly distributed random variable).

an outbreak (Fig. 3C). More realistic assumptions of heterogeneity, in which a person's immune responsiveness is drawn from a random normal distribution, resulted in a lower viral load in the population. On the whole, these experiments suggest that increasing the disparity between people's ability to respond to an infection, while maintaining an average rate may worsen the overall impact of an outbreak within that population (Fig. 3A and 3B).

Network connectivity affects the time between peaks in the viral load

Varying the magnitude of peoples' connectivity coefficient ω_i in our model re-produced previously described behaviour of infection spread, and therefore built confidence in our model structure with respect to previous discussions of contact patterns [123, 143, 156, 203] (Fig. 4). High values for ω_i reduced the time until the peak of an outbreak as well as the timing between peak viral levels in neighbouring individuals, while infection spread was delayed among the population when values of ω_i were low (Fig. 4A). Given these particular assumptions regarding the strength of connectivity among individuals, it is also likely that delays in disease progression (demonstrated by an increased period between oscillatory peaks) will be observed. With larger values of ω_i, the numbers of peaks and troughs in the prevalence are reduced, and begin to merge into a more continuous (and more familiar) outbreak pattern (Fig. 5). While changing ω_i changes the rate with which the population-wide viral load accumulates, it has little impact on the asymptotic limit of that viral load (Fig. 4B).

Our present methodology also allowed us to investigate, in the context of different combinations of immune responsiveness, the impact of a person's connectivity coefficient ω_i, on infection spread in a population. These considerations demonstrate, rather intuitively, that the peak mean viral load of an outbreak and the subsequent accumulated viral load in the population will decrease for a combination of low connectivity and high immune responsiveness, while increasing for high connectivity and low immune responsiveness (Fig. 4C and 4D). Furthermore, performing 100 Monte Carlo iterations across randomly varied parameter values for immune responsiveness, the connectivity coefficient, and randomly generated network structures highlighted that the above
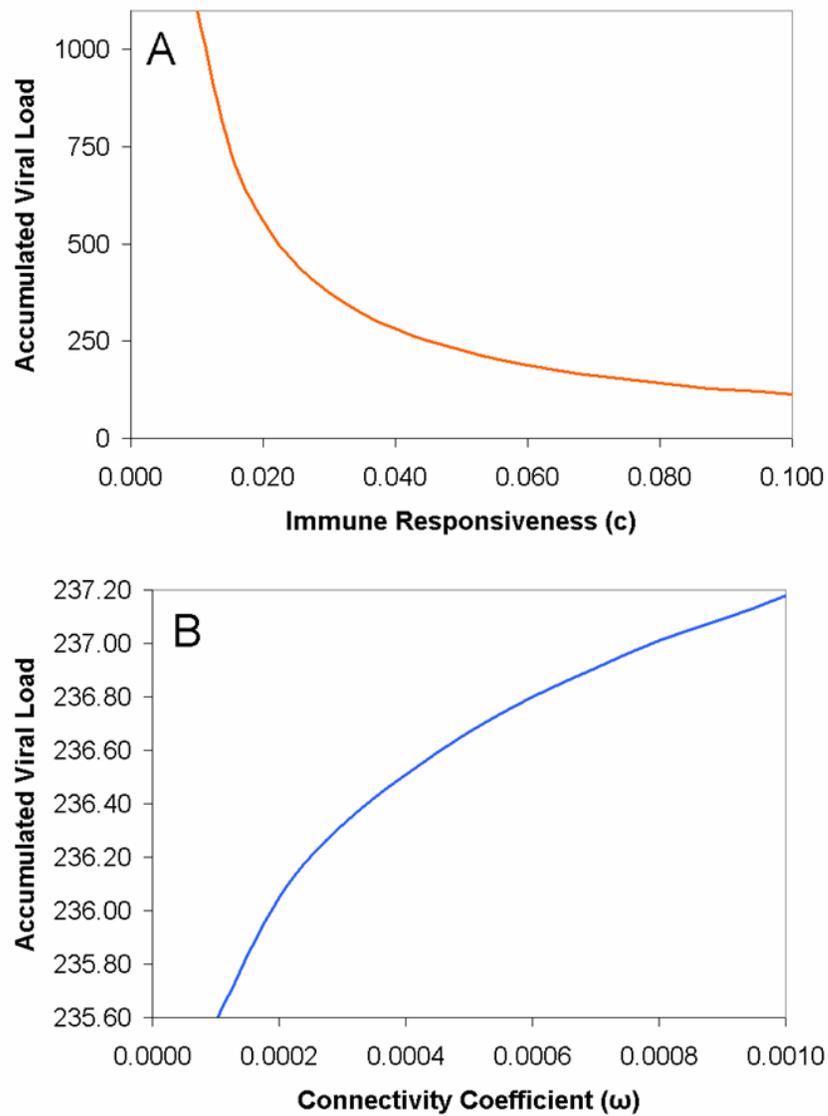
Figure A.2: Variations in parameter values and their effect on the population-wide accumulated viral load. Additional parameter values investigated when studying the effect of (A) immune responsiveness and the connectivity coefficient (B) on the population-wide accumulated viral load.
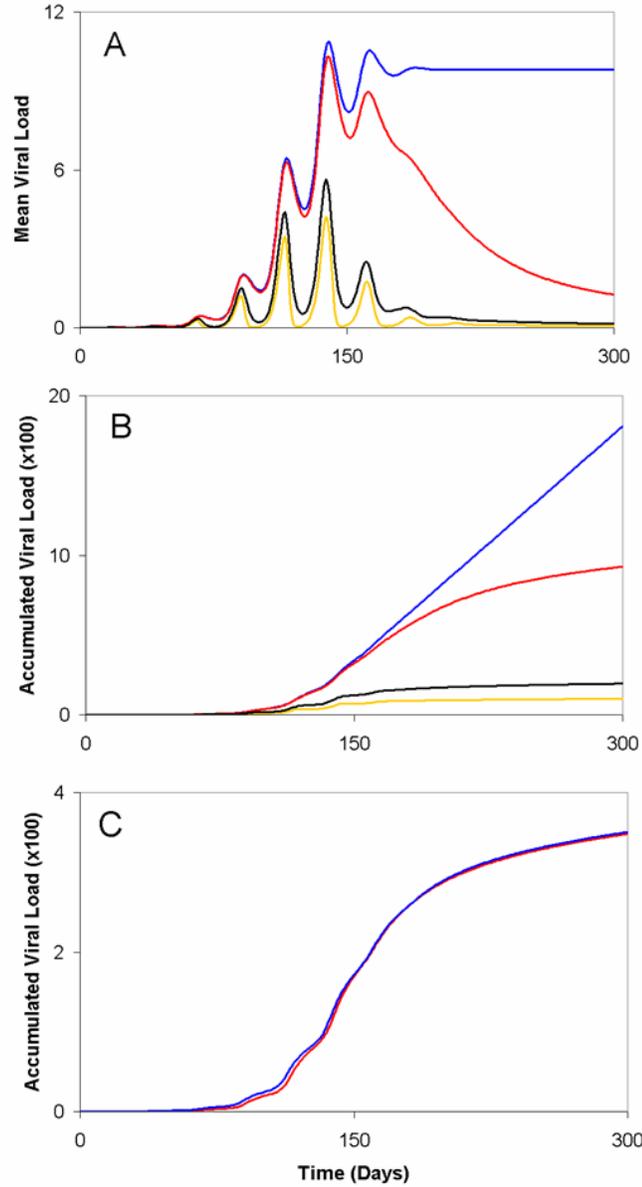
Figure A.3: The impact of a person's immune responsiveness for the short-term dynamics of an outbreak. (A and B) A comparison between the immune responsiveness and the overall behaviour of an outbreak (A), as well as the overall severity an outbreak (B), as measured by the mean and accumulated viral load in the population, respectively. Mean and accumulated viral loads were computed from simulating model 1 for constant values of immune responsiveness: $c_i = 0.001$ (blue line), 0.01 (red line), 0.1 (yellow line), and random uniformly distributed (black line). (C) Assuming that the population is composed of an equal proportion of stronger $c_i = 0.1$ and weaker responders $c_i = 0.016$, the model was simulated to study the effect on the accumulated viral load in the population by starting the infection in the sub-population of stronger responders (red line) and weaker responders (blue line). These experiments demonstrate no clear correlation between viral load and starting an infection in either strong or weak responders. For scenarios (A, B, and C) the connectivity coefficient, ω_i, was a stochastic random variable. All other parameter values were based on values presented by Wodarz and colleagues [213] and are displayed in Table 1.
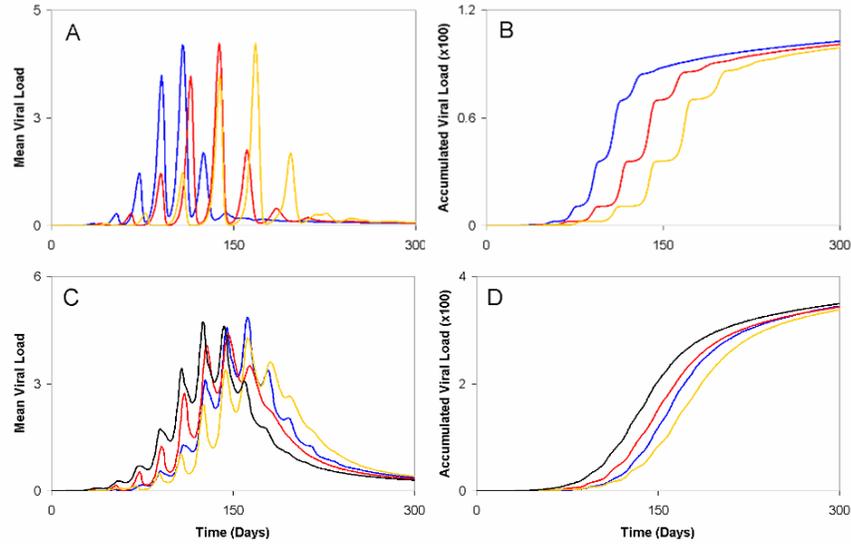
Figure A.4: The transmission of virus across the population differs for variations in the connectivity coefficient, ω_i. (A) Higher values of the connectivity coefficient ($\omega_i = 1.0 \times 10^{-3}$) shortened the time required to spread the disease through the population, as well as the peak of the outbreak (blue line). Lower values of the connectivity coefficient ($\omega_i = 1.0 \times 10^{-4}$ and 1.0×10^{-5}) had the opposite effect (red and yellow lines, respectively). (B) Both high and low values of ω_i demonstrated no apparent sizeable relationship with the accumulated viral load in the population (colour code the same as 3A). For scenarios (A and B) a person's immune responsiveness was randomly determined from a random normal distribution with $\mu = 0.063$ and $\sigma = 0.0225$ (see Methods for further details). For scenarios (C and D), immune responsiveness for fixed values of $c_i = 0.1$ and 0.016 were combined in simulations with different fixed values of $\omega_i = 1.0 \times 10^{-3}$ and 1.0×10^{-5}. The colour code is the same for 3A.

results are likely to be quite robust for many different combinations of parameter values (Fig. 6).

Re-infection, immunological memory, and herd immunity

Figures 7 and 8 present the simulation experiments for re-infection. Under scenario one, our model indicates that the longer the period until re-infection, the larger the post-exposure mean viral load in the population will be (Fig. 7A). This reflects that, as the time prior to re-infection increases, the $CTLp$ populations are likely to decline towards naive levels (0) and approach the disease-free equilibrium. With increasing time until re-infection, an individual will require a longer time to mount an effective immune response to reduce the severity of that re-infection (Fig. 8A). For scenario two (i.e., viral antigen persists after primary exposure), the recovered population does not experience positive viral growth if the virus is reintroduced after the initial outbreak (Fig. 7B). Therefore, any re-infection that is likely to occur will result in immediate inhibition of viral particles, and no considerable infection will take hold. What is interesting is that the asymptotic accumulated viral load from re-infection is essentially the same regardless of antigenic requirements or whether re-infection occurs repeatedly over time or infrequently later in time (Fig. 7B).

Notably, having key core people's immune system primed against re-infection causes them to serve as barriers that prevent that infection from reaching the rest of the population (Fig. 9A). We expect this to be because by time $t = 9000$ days, one person possess an elevated level of virus-specific $CTLp$ cells (Fig. 9B) and will be able to easily increase the abundance of $CTLe$ cells (Fig. 9C). Thus, this person is able to (almost instantaneously) clear the infection when it is reintroduced
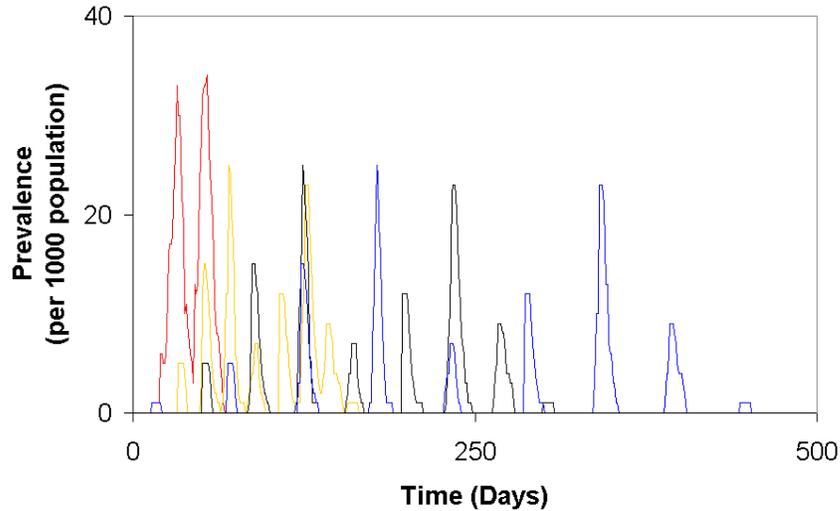
Figure A.5: Prevalence of a disease (per 1000 population) based on different values of ω_i. Here, $\omega_i = 1.0 \times 10^{-1}$ (red curve), 1.0×10^{-3} (yellow curves), 1.0×10^{-6} (black curves), and 1.0×10^{-9} (blue curves).

at $t = 9000$ days. This interestingly implies that, given the assumptions used in the model here, re-infecting key core people can be beneficial to the population.

Variations in the infecting dose

As expected, increases to the constant ϕ resulted in an increase in a person's viral load. It bears noting that, increasing the viral load incoming from a person's neighbour also appeared to have a similar effect on the timing of a person's peak viral load (i.e., larger values of ϕ lead to tighter spacing in time between the peaks in viral load of connected individuals) (Fig. 10A). However, this change in behaviour at the individual level did not appear to have quite the same impact at the population level, as there was no substantial change in the asymptotic behaviour of the accumulated viral load (Fig. 10B).

A.4 Discussion

Future infectious disease research would benefit by striving to not only understand the properties of the invading microbe, or the body's response to infections [139], but also how individual responses affect the propagation of an infection throughout a population. Whilst this is not the first attempt to explicitly combine the nonlinear dynamics of immune reactions within individuals and the overall nonlinear dynamics of the interaction between an infection and a population of hosts, previous frameworks are better adapted to understanding very specific aspects of viral infections, such as re-exposure to viral antigen [193] and the role of memory T-cells in clearing reinfection [115]. In our opinion, our framework complements such previous contributions by incorporating a more detailed representation of the mechanisms of antiviral immune response, and thus will contribute towards improved understanding the immuno-epidemiological dynamics of viruses and other intracellular pathogens.

These initial results reinforce how coupling principles of immunology with epidemiological mixing provide a multi-scaled description of the relational aspects of an ecological system. In the short-term, the immune responsiveness of the population as a whole produces some very well-defined emergent properties and thus is likely to determine the natural history of disease in that
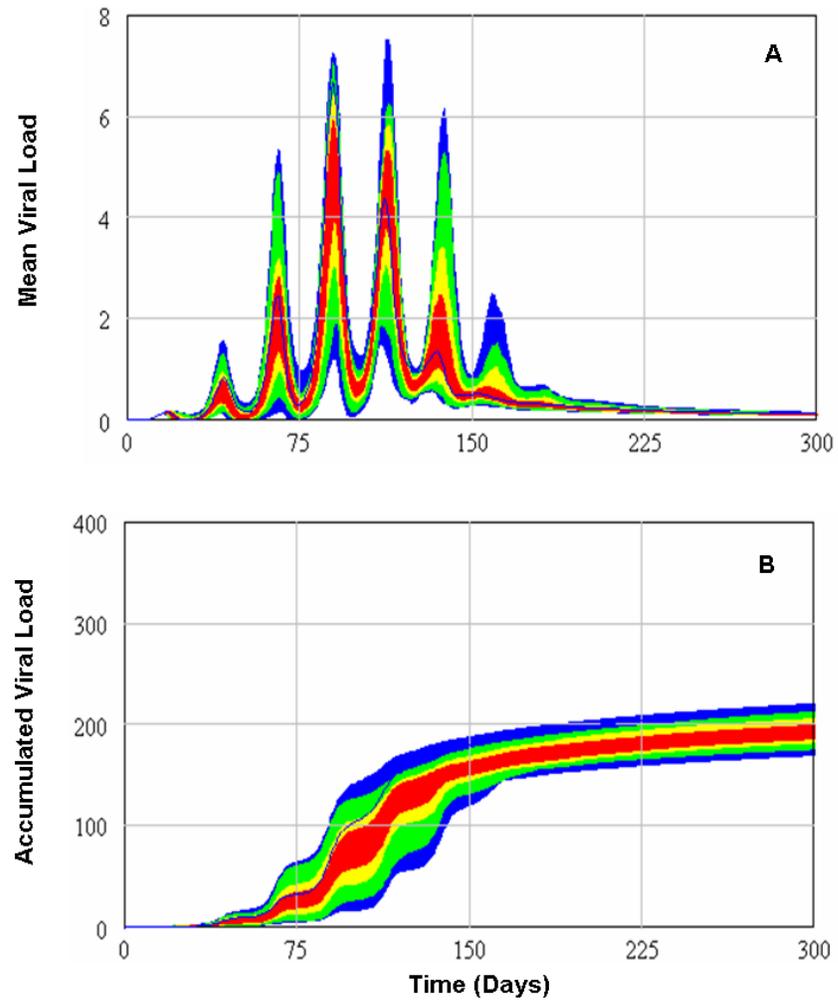
Figure A.6: Mean (A) and Accumulated (B) viral loads in the population after 100 Monte Carlo realizations. Each realization is associated with a randomly selected Poisson network, as well as a randomly selected value of immune responsiveness (drawn from a normal distribution) and distinct stochastic trajectories for network connectivity coefficients (drawn from a uniform distribution).
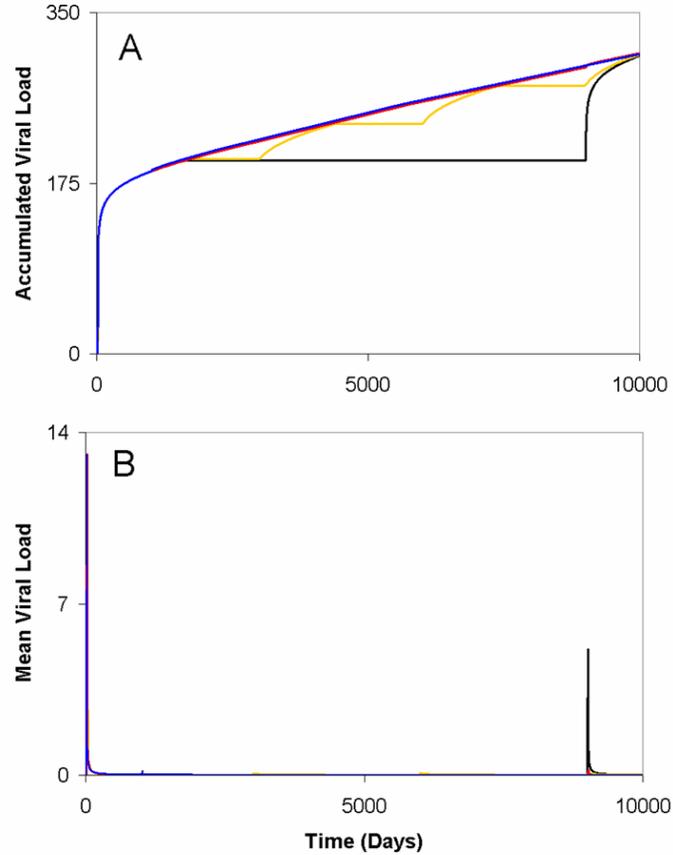
Figure A.7: Viral dynamics for re-infection to antigen when it is eliminated compared to when it persists. Antigen was re-introduced to the whole population, at $t = 1000$, 3000, 6000, and 9000 days (yellow and blue lines), or at a single time step ($t = 9000$ days) (black and red lines) under the assumption of antigenic elimination and antigenic persistence, respectively. Here, $\omega_i = 0.1$, and a $V_{ext} = 0.015$ was used in antigenic elimination simulations. (A) With the exception of antigenic persistence (red and blue lines), re-infection for the population at different intervals produces qualitatively different behaviour than antigenic elimination (yellow and black lines). However, the asymptotic accumulated viral load in the population is similar, regardless of whether or not antigen persists or is eliminated. (B) These qualitative differences are also observable for the mean viral load in the population. Assuming either scenario one or two, a small positive growth in the mean viral load following re-infection at $t = 1000$, 3000, 6000 days (yellow line), and at $t = 9000$ days (black and red lines) occurs.
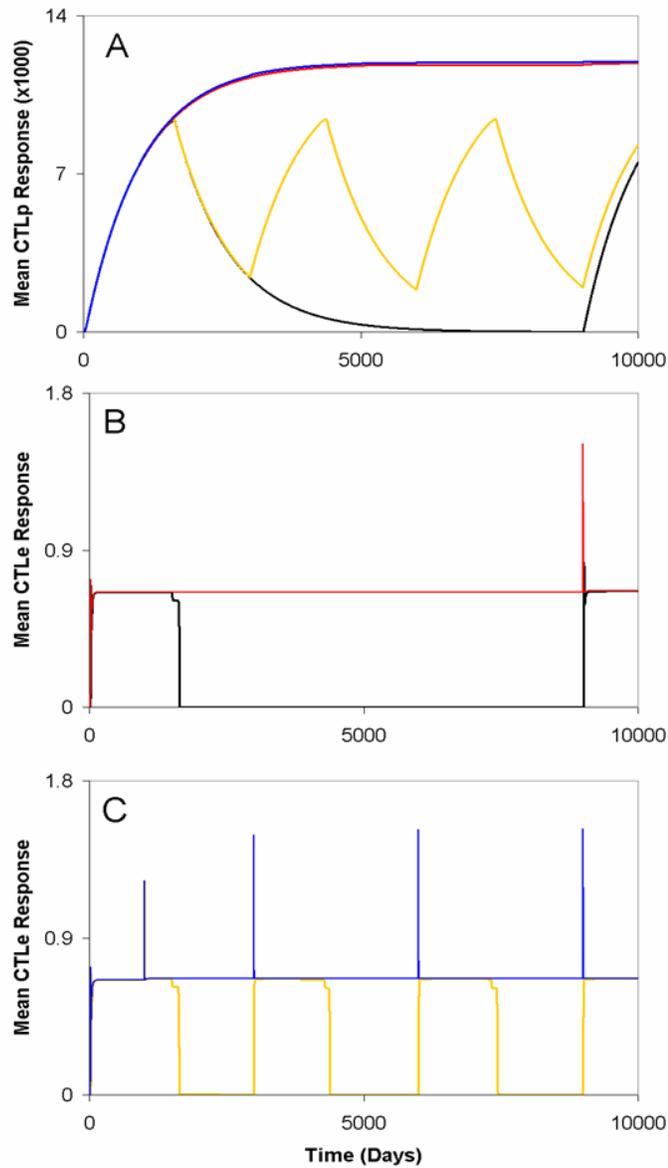
Figure A.8: Immune system dynamics for re-infection when viral antigen is eliminated compared to when it persists. Here, the same re-introduction protocol as for Fig. 5 was followed. (A) Antigenic persistence (red and blue lines) keeps $CTLp$ abundance continually high regardless of when antigen is re-introduced repeatedly at $t = 1000$, 3000, 6000, and 9000 days (blue line) or only once at $t = 9000$ days (red line). Antigenic elimination (with slow rates of $CTLp$ decline, $b = 0.001$ 1/day, high immune responsiveness, $c_i = 0.1$, and assumed $V_{ext} = 0.015$) demonstrates that re-expansion requires time for individuals to mount an effective immune response (yellow and black lines). (B and C) There is also a proportional, positive growth in the abundance of $CTLe$ cells that follows directly from the expansion of $CTLp$ cells after single instance of re-introducing viral antigen (B) assuming antigen is eliminated (black line) or antigen persists (red line), as well as repeated re-introduction (C) assuming antigen persistence (blue line) and antigenic elimination (yellow line).
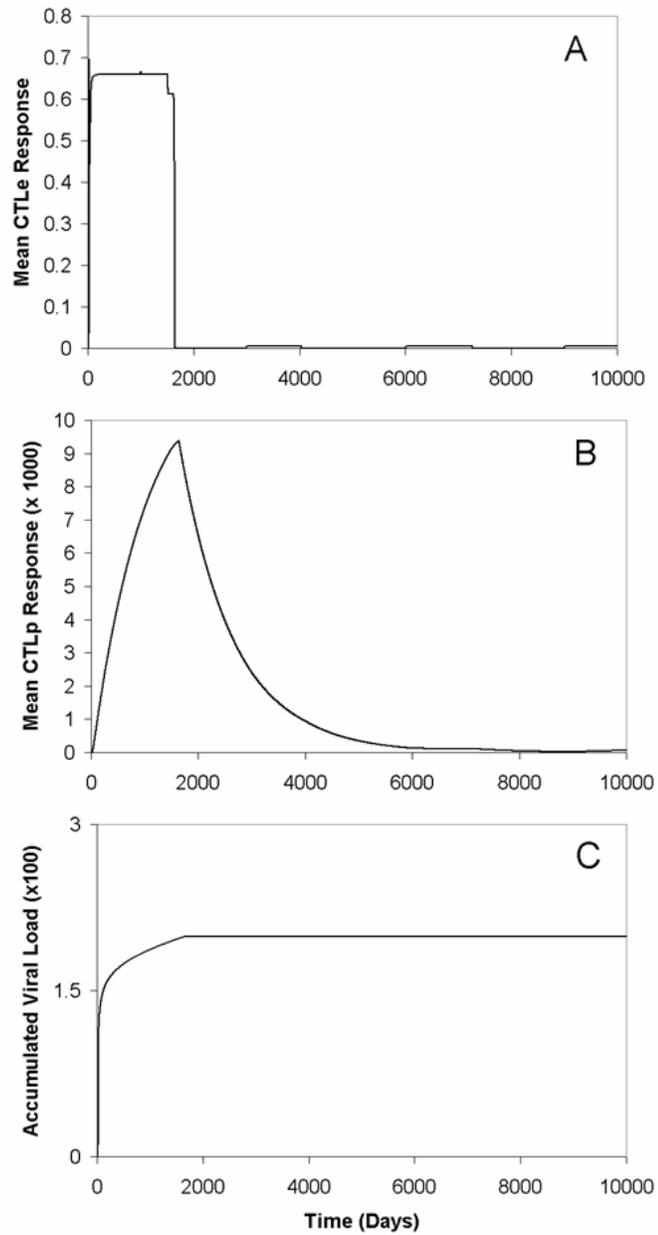
Figure A.9: Having people's immune systems primed through re-infection prevents infection from reaching the rest of the population. Having key core people's immune system primed against re-infection (A and B) causes them to serve as barriers that prevent an outbreak from reaching the rest of the population, as measured by the accumulated viral load (C).
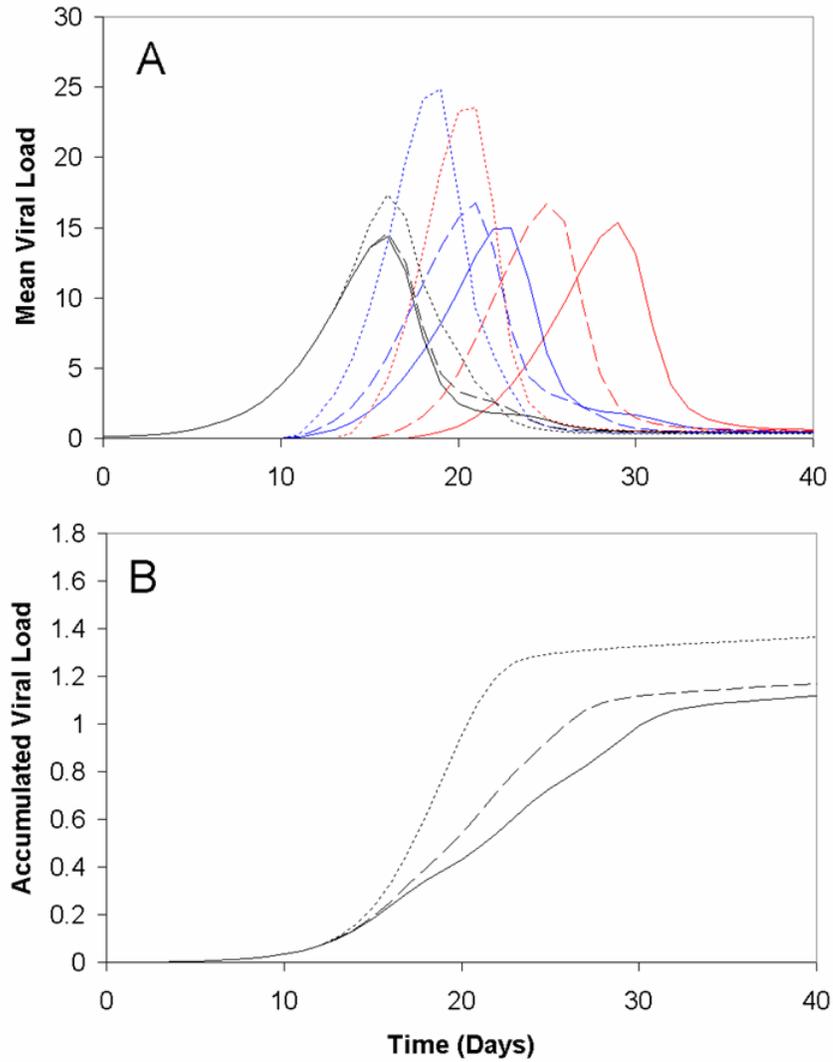
Figure A.10: Simulations of increasing the viral load transmitted to a person from their network contacts. Individual viral loads (A), and accumulated viral load in the population (B) for a two- (dashed curves) and five-fold (dotted curves) increases in the quantity of free viral particles transmitted from a person's neighbour, compared to the simulations of model 1 used in the main text (solid curves). Again for illustrative purposes, the results in (A) are displayed for the same three individuals used in Fig. 1: Person 1 (blue curves), Person 2 (red curves), and Person 3 (black curves).

population [115]. That is, there exist levels of immune responsiveness whereby a population of connected individuals will be able to eliminate a viral infection, while at others, it will likely become endemic. Interestingly, these emergent properties of our model demonstrate consistency with both traditional susceptible-infectious-removed properties (for populations with higher values of immune responsiveness) and susceptible-infectious-susceptible properties (for populations of weaker responders) within the clusters of people in the population even though these compartments were not explicitly defined (see Figs. 3A and 5). They also reproduce well-known dynamics of re-infection in a population after long periods of time [5], as well as intuition-based observations of how host-pathogen interactions influence herd immunity [13, 205]. However, because these population-based results stem from an explicit description of the immune system, hypotheses relating the production of immunological memory to the long-term effects of re-exposure on the population can now be mathematically formulated and studied.

Another interesting result from this particular system is that the asymptotic accumulated viral load after re-infection is essentially conserved regardless of whether the virus is eliminated, if it persists, or whether re-infection occurs repeatedly over time or infrequently later in time. This conservation property reflects the fact that given the same starting point in state space, the value of $Z(t)$ and $W(t)$ depends only on the integral of the count of infected cells Y from 0 to t, and not on the specific trajectory taken by Y within that interval. However, this conservation of morbidity within the population raises a potentially important (and possibly controversial) question when it comes to creating control strategies, particularly for recurrent diseases such as influenza: is preventing population-wide reinfection until later in time that much more effective than having continual population-wide reinfection over time when the end results are likely to be similar?

Our methodology has made several simplifying assumptions that should be investigated. We imposed neither viral load thresholds required for contagion, nor any difference or quantization in the infecting dose people received. Although the outcome of viral infection, in general, is thought to be related to the size of the infecting dose a person initially receives [87], we found that our results were robust against variations in this parameter (see Fig. 10). Investigating the impact of different network structures (e.g., scale-free and small-world networks) is an important area of ongoing work.

Following Nowak and May [159], we have also assumed a basic model for virus dynamics. Because of the known role of CD8+ T-cells in the elimination of virally-infected host cells (e.g., influenza A infections [195, 209, 210], or adenovirus infections [191]), we have focused our discussion of immune responsiveness on CTLs, and thus ignored other types of innate and specific immunity. Our focus on CTL-mediated viral elimination was, largely, an attempt to establish plausibility of the multi-scale methods presented, not necessarily their complete adherence to immunological reality; the cytotoxic properties of activated CD8+ cells for clearing a viral infection are certainly not the whole story, and other immune responses are likely to affect the production of free virus. It should be noted, however, that the effect of other immune responses can be described in terms of this basic model by modifying its existing parameters. For example, production of cytokines by CD4+ T-helper cells are likely to reduce the infectivity parameter β and/or the rate at which infected cells are produced, k, while the role of neutralizing antibody- or complement-mediated responses may also enhance the removal rate of free viral particles, u [159]. Although considering other immune responses is assumed to have an additional influence on the viral dynamics at population levels [67, 90], previous research at the individual level suggest that they are associated with qualitatively similar dynamics to those governing CTLs [158, 159, 213]. However, explicitly describing the cooperative interactions between CTLs and other immune responses, in the form of additional state equations, and their effect on the transmission of specific microparasite infections is also an important area of ongoing study.

A.5 Conclusions

Despite the extensive use of mathematics in epidemiology, many theoretical challenges remain [144]. To improve our understanding of infectious diseases, future research will require theoretical

tools that incorporate immunological and epidemiological features into a unified template [87, 120]. Our goal in this paper was to expand upon the utility of merging aspects of immunology and epidemiology into a single conceptual framework. This analysis has produced some interesting and potentially important conclusions. We anticipate this framework to be a step towards articulating an overall, integrated, and more refined epidemiological theory that simultaneously describes broad categories of diseases dynamics at both cellular and organismal levels. Under a unified framework, continued molecular research on disease pathogenesis and host-pathogen interactions will likely have a reciprocal influence on epidemiological theory. Ideally, improvements to these combined theoretical templates will prove useful for the prediction of future trends in infectious disease epidemiology. Such combined methodologies could also lead to novel insights into understanding microparasite evolution and its role in disease virulence and persistence. Ultimately, these initial findings suggest that there are important immunological consequences to consider when designing effective interventions to control new variations of familiar diseases.