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Transmission and the evolution of diseases caused by chlamydia trachomatis, SARS-CoV-2, and plasmodium species.

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TRANSMISSION AND THE EVOLUTION OF DISEASES CAUSED BY *CHLAMYDIA TRACHOMATIS*, SARS-COV-2, AND *PLASMODIUM* SPECIES

By

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April 27, 2023

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DEDICATION

I dedicate this dissertation to my parents

Sherese and Randy Steffens

who have provided unwavering encouragement and support at every step of my journey.

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I would first like to thank my advisor, Paul Ewald, for his guidance and insight. I would also like to thank the other members of my committee - Stephen Yanoviak, Holly Ewald, Lee Dugatkin, and Fabian Crespo - for their valuable expertise and project feedback. I am also grateful to Stephen Yanoviak for generously assisting with travel arrangements. Lastly, I would like to show appreciation for Tryphena Sithu, who has motivated me to persist and succeed.

ABSTRACT

TRANSMISSION AND THE EVOLUTION OF DISEASES CAUSED BY *CHLAMYDIA TRACHOMATIS*, SARS-COV-2, AND *PLASMODIUM* SPECIES

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Principles of natural selection have proven valuable for explaining why pathogens cause the diseases that they do. In theory, the evolved level of host exploitation should reflect how dependent a pathogen is on host health for transmission. This dependency is shaped by transmission mode and transmission opportunity, which should therefore be predictors of disease manifestations. In this dissertation, I apply these principles to investigations of depression in *Chlamydia trachomatis* and virulence of SARS-COV-2 and *Plasmodium* species.

This dissertation has five chapters. In chapter I, I describe the theoretical foundation of my dissertation research. I also briefly introduce each study system. In chapter II, I evaluate whether *C. trachomatis* is associated with depression based on a candidate mechanism for within-host persistence. I hypothesize that *C. trachomatis* should be associated with depression independent of urogenital symptoms, and that the effect should be stronger in females than in males. In chapter III, I assess evolutionary trends in SARS-CoV-2's virulence and viral loads by conducting meta-analyses of published results. From the evolutionary perspective that virulence of respiratory pathogens should correlate positively with environmental survivability, I hypothesize a

trend of reduced virulence. In chapter IV, I qualitatively review epidemiological factors that may affect malaria severity and critically evaluate the hypothesis that virulence evolves as a function of transmission intensity. In chapter IV, I summarize the main findings and conclusions from chapters II through IV. I also discuss future research directions and potential applications.

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CHAPTER I

INTRODUCTION

Background and Theoretical Framework

There is tremendous variation in the timing and severity of infectious disease manifestations. One explanation for this variation is that transmission mode and transmission opportunity influence a pathogen's optimal within-host exploitation strategy, thereby affecting disease virulence (Ewald 1983, Ewald 1991, Ewald 1994). Genetic variants that intensely exploit host resources accrue the benefit of producing more progeny over a given time, each with a chance of continuing the lineage through infection of a new host. However, higher rates of multiplication typically cause more severe disease, which can reduce the likelihood of a pathogen contacting a susceptible host. Therefore, when transmission depends on a relatively healthy and mobile host, natural selection is expected to favor relaxed acute exploitation strategies and low virulence. Alternatively, continued transmission from immobilized hosts favors harmful variants because they reap the benefits of rapid propagation while paying minimal costs.

In this dissertation, pathogen virulence is defined as the harmfulness attributable to a particular pathogen in a host population. Much of the variation in acute virulence across human pathogens is explained through transmission mode. Unlike most directly

transmitted pathogens, vector-borne pathogens can readily transmit from hosts experiencing severe disease. A sickened and immobilized child with malaria, for example, is just as likely or more to be bitten by a mosquito as is a healthy child. Accordingly, vector-borne diseases are significantly more lethal on average than directly transmitted diseases (Ewald 1994), though infection in the vector is rarely harmful, probably because transmission depends heavily on its health. Transmission from immobilized hosts also explains the high virulence of some non-vector-borne diseases. By remaining infectious in the external environment for long periods, pathogens can cause severe illness, even death, and still transmit through fomites. The transmission of these environmentally stable pathogens is thus less dependent on host health. Consistent with this "sit and wait" hypothesis, the infection fatality ratio across human respiratory pathogens is positively associated with the amount of time each pathogen remains viable in the external environment (Walther and Ewald 2004).

When transmission opportunity is rare, whether due to transmission mode or environmental conditions, selection favors variants that can persist inside their host. Sexually transmitted pathogens (STPs) are especially dependent on this persistence, as their transmission requires not only sex between hosts but unprotected sex and with new partners. Sexual intercourse is also generally dependent on a healthy host, and aggressive exploitation that causes severe disease soon after infection will hinder transmission prospects. Instead, relaxed exploitation strategies and the ability to continually evade immune detection while causing minor if any acute illness is advantageous. This explains why sexually transmitted pathogens so often cause chronic infections. Although the need for sustained within-host persistence may reduce acute-phase disease severity, persistent

infections can nonetheless cause debilitating and even fatal chronic disease. The symptoms that manifest from a chronic infection are determined by the specific pathogen and the mechanisms utilized for persistence.

Study Systems

Because the evolutionary principles described previously have broad explanatory power, this dissertation is not system specific and does not focus on a particular disease or pathogen. Rather, it applies these principles across pathogen types inclusive of viruses, bacteria, and protozoans. In each chapter I apply the same principles of natural selection – those of transmission, host dependency, and optimal exploitation strategy – to explain the disease manifestation of a different pathogen.

In chapter II, I examine associations that are consistent with the sexually transmitted pathogen *C. trachomatis* contributing to depression as a side effect of mechanisms that afford it prolonged within-host persistence. This chapter analyzes data from University of Louisville Campus Health Services electronic medical records.

In chapter III, I investigate intrinsic changes in viral loads and virulence across SARS-CoV-2 variants. To examine changes in virulence, I conduct meta-analyses on studies that compare the risk of hospitalization and/or mortality between variants. To examine changes in viral load, I conduct meta-analyses on studies that compared qPCR cycle threshold values between variants. I then evaluate whether the observed trends in viral load and virulence are consistent with the optimal virulence predicted by SARS-CoV-2's environmental survivability.

In chapter IV, I qualitatively review literature on the exploitation characteristics and virulence of human *Plasmodium.* The purpose of this chapter is to provide a holistic review and discuss what is known about factors that contribute to the virulence of *Plasmodium* infections. The less-established idea that virulence evolves as a function of transmission intensity is critically evaluated.

CHAPTER II

ASSOCIATIONS OF LOW MOOD WITH *C. TRACHOMATIS* INFECTION IN SUBJECTS WITH AND WITHOUT UROGENITAL MANIFESTATIONS

Summary

Transmission opportunity generally comes rarely for sexually transmitted pathogens. Natural selection consequently favors within-host persistence, which often causes chronic disease. Infection with the sexually transmitted bacteria *Chlamydia trachomatis* has previously been associated with chronic depression. In the current study, I tested several associations that are consistent with *C. trachomatis* contributing to depression as a side effect of mechanisms that afford it prolonged within-host persistence. This study extends previous findings of the association between *C. trachomatis* and low mood as indicated by questionnaire responses. Specifically, it assesses the association in subjects with and without urogenital symptoms, in males relative to females, and across different intensities of low mood. This retrospective chart review was conducted using electronic medical records from the University of Louisville Campus Health Services. Data on depression (mood), *C. trachomatis* and *Neisseria gonorrhoeae* infection, symptoms, age, sex, and the use of alcohol, cigarettes, or illicit drugs were extracted and analyzed. Patient health questionaries in the medical records were used to screen and rank depression. Testing for *C. trachomatis* and depression occurred within the same appointment. *C. trachomatis* infection was significantly associated with depression in

patients who did and did not report urogenital symptoms and in a multivariable analysis that included all patients but adjusted for symptoms and other potential confounders. When considering each sex independently, the association between low mood and *C. trachomatis* was significant for females but not males. The difference between sexes, however, was not statistically significant. In within-subject comparisons, subjects were more likely to report low mood when they were infected relative to when they were uninfected. When subjects were categorized according to an ordered scale of low mood, *C. trachomatis* was associated with greater intensity of depression when all subjects were included, but not when the comparison was restricted to individuals with a score indicating at least some level of low mood. Our results are consistent with *C. trachomatis* contributing to the spectrum of low mood through an immune response independent of urogenital symptoms.

Introduction

Sexually transmitted pathogens (STPs) are not the only cause of persistent infections, but they are disproportionate culprits. An evolutionary explanation for this observation is that infrequent transmission opportunity selects for prolonged survival inside the host, which causes chronic infection. Although chronic depression is a major cause of illness globally, the causes have not yet been resolved. Part of the difficulty undoubtedly arises because depression is an umbrella category encompassing a variety of illnesses which may have different effects on mood. In the present study, I address these concerns by evaluating whether a candidate cause of depression, the bacterium *Chlamydia trachomatis*, is associated with low mood in subjects with and without

reported urogenital symptoms, and in males as well as females. I consider low mood to be inclusive of recognized depressive disorders and less severe depressed states. I also extend previous studies by investigating whether *C. trachomatis* is associated with intensity of low mood and if mood changes when an individual's infection status changes.

C. trachomatis infection has been associated with depression in previous studies (Doyle, Swain et al. 2015, Doyle, Swain et al. 2019). Its interactions with immunological defenses make it a particularly strong candidate for lowering mood. As a response to infection, hosts can temporarily deplete intracellular tryptophan to starve pathogens that are tryptophan auxotrophs, killing or sending them into a quiescent state. This process is carried out through an upregulation of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO). In what is thought to be a coevolutionary response to this defense, genital strains of *C. trachomatis* encode a functional tryptophan synthase, enabling biosynthesis of tryptophan from indole (Fehlner-Gardiner, Roshick et al. 2002, McClarty, Caldwell et al. 2007). Thus, when indole is available as a substrate in the infection microenvironment, genital strains can maintain growth and reproduction despite IDO upregulation and reduced tryptophan concentrations (Caldwell, Wood et al. 2003, Kari, Goheen et al. 2011, Aiyar, Quayle et al. 2014). Doyle et al. hypothesized that persistent *C. trachomatis* infections might cause depression by chronically depleting host tryptophan and reducing serotonin availability to the brain. Because acute tryptophan degradation may fail to control *C. trachomatis*, it might induce sustained but ineffective IDO upregulation, resulting in chronic tryptophan depletion. This depletion may lower mood because tryptophan is a molecular precursor for the mood-enhancing neurotransmitter serotonin. When tryptophan is depleted, serotonin synthesis may

therefore be reduced (Fernstrom 1983, Richard, Dawes et al. 2009). In clinical studies, tryptophan depletion lowers mood in patients with a pre-existing vulnerability to depressive disorders (Smith, Fairburn et al. 1997, Moreno, Gelenberg et al. 1999, Feder, Skipper et al. 2011). Through a related and non-mutually exclusive mechanism, prolonged IDO activity may also contribute to depression through neurotoxic effects. The degradation of tryptophan by IDO creates products whose metabolites cause oxidative stress and neuronal apoptosis (Schwarcz, Whetsell Jr et al. 1983, Okuda, Nishiyama et al. 1998, Moroni 1999, Wichers, Maes et al. 2004) -- effects that are associated with low mood (Sheline, Sanghavi et al. 1999, Bremner, Narayan et al. 2000, Salim 2014, Bhatt, Nagappa et al. 2020). Under conditions of persistent *C. trachomatis* infection, sustained IDO upregulation may continue to break down host tryptophan, causing an accumulation of these neurotoxic metabolites.

Previous studies of the association between *C. trachomatis* and depression have not directly accounted for the presence of urogenital symptoms. Infections that generate urogenital symptoms could be more strongly associated with low mood than asymptomatic infections because of psychological responses to signs of disease, or if symptomatic infections are associated with stronger biochemical effects on mood. However, if depression results from a prolonged immune response to infection, as suggested above, *C. trachomatis* could contribute to low mood even among subjects without urogenital symptoms.

The previous reports of associations between *C. trachomatis* and depression studied female subjects only. Although *C. trachomatis* could contribute to low mood in both sexes, effects on serotonin dysregulation and neurotoxic metabolites might be more

intense in females. One reason is that tryptophan restriction is enhanced during the last half of each menstrual cycle and during pregnancy as an innate defense that avoids damage to sperm cells, the embryo and fetus (Doyle, Swain et al. 2015). A second reason is that females may experience elevated indole concentrations associated with bacterial vaginosis (BV). BV is characterized by a shift in the vaginal microbiome and affects nearly one-third of reproductive age females in the United States (Koumans, Sternberg et al. 2007, Onderdonk, Delaney et al. 2016). Several species associated with the BV microbiome are indole-producers and their presence can rescue *C. trachomatis* from tryptophan starvation (Sasaki-Imamura, Yoshida et al. 2011, Ziklo, Huston et al. 2016). In this study, I evaluate whether the association between *C. trachomatis* and low mood differs between males and females.

Finally, previous studies have not assessed whether changes in the infectious status of individual patients are associated with changes in mood. Such within-subject comparisons are useful because they control for variables that change from patient to patient. In this study I report changes in mood for patients in which infection status changed.

Methods:

Study Design:

This retrospective chart review was conducted using electronic medical records from the University of Louisville Campus Health Services, which provides healthcare for students, faculty, and staff. As standard procedure, all patients are screened for depression at each appointment with patient health questionnaires (PHQs), specifically the PHQ-2

and PHQ-9. Patients who are positive on the PHQ-2 are subsequently given a PHQ-9 to determine depression severity. I consider a positive PHQ-2 screening as depression (any severity).

Patients who completed the patient health questionnaire(s) and were tested for *C. trachomatis* at the same appointment were included in this study. Patients with indeterminate *C. trachomatis* test results were excluded from statistical analysis. All medical records reviewed in this study were generated between January 1,2014 and January 1, 2019. Patients underwent testing for a variety of reasons, including routine checkups, symptoms of sexually transmitted disease, and exposure concerns. This study was approved by the Institutional Review Board at the University of Louisville (RB no. 16.0098).

Data Collection:

Campus Health Services detect *C. trachomatis* using the Aptima® Combo 2 Assay -- a target amplification nucleic acid probe test, which also detects *Neisseria gonorrhoeae*. In this study, a patient was considered infected if a urine sample, cervical swab, or urethral swab gave a positive assay result.

Medical records were also reviewed for PHQ responses, symptoms, age, sex, and the use of alcohol, cigarettes, or illicit drugs. A patient was defined as asymptomatic if the attending healthcare professional explicitly described them as such, or if no typically associated urogenital symptoms of *C. trachomatis* or *N. gonorrhoeae* were documented in the appointment record.

Statistical analyses:

Simple and multivariable logistic regressions were used to assess whether low mood was more prevalent in infected patients. In these analyses, low mood and infection were treated as dichotomous response and explanatory variables, respectively. The multivariable model was used to adjust for potential confounding effects of *N. gonorrhoeae*, age, sex, symptoms, and drugs/alcohol/cigarette use. These variables were chosen based on their availability in the medical records and plausible, potentially spurious associations with depression and infection. The assumption of little to no multicollinearity among explanatory variables was evaluated by calculating variance inflation factors.

Ordinal logistic regression was used to assess differences in the likelihood to report depression on an ordered scale. The model treated depression as an ordinal response variable with six levels that were determined by PHQ responses (none, minimal, mild, moderate, moderately severe, severe).

McNemar's binomial test was used to compare within-subject differences in depression with and without infection. The p-value was calculated with continuity correction.

For descriptive statistics and between-subject inferential analyses (simple, multivariable, and ordinal logistic regressions), if a patient was tested on more than one date for *C. trachomatis*, only data from the first chronological appointment were used. For the within-subject analysis (McNemar's binomial test), each patient contributed data from two appointments – data from the first chronological appointment and data from the

next appointment in which *C. trachomatis* positivity had changed relative to the first appointment.

A two-tailed p-value of < .05 was considered statistically significant for all analyses. Data were analyzed with IBM SPSS Statistics version 28.0.1.1 and GraphPad Prism version 9.4.0.673.

Results

Study Population:

This study included 839 patients, with ages ranging from 17 to 57 years (M = 22.2, SD = 4.04). 56.1% patients were female and 43.9% were male. At the time of their appointment, 16.4% of patients reported depression on the PHQ-2 survey, including 23.2% of infected patients and 14.1% of uninfected patients. 57.3% of infected patients did not report urogenital symptoms, of which 22.2% reported depression (Table 1, Figure 1). 57.2% of patients reported the use of alcohol, cigarettes, and/or illicit drugs.

Symptoms:

Using PHQ-2 responses, I examined associations between *C. trachomatis* and low mood of any severity. In the evaluation of variance inflation factors, the values for all variables were less than 2.5, indicating little to no multicollinearity. Infection with *C. trachomatis* was significantly associated with low mood in patients without urogenital symptoms (p = .021, OR = 1.75, 95% CI 1.07 to 2.80). In a multivariable analysis that included all patients but adjusted for the effects of symptoms (±), age, sex, *N. gonorrhoeae* and drug/alcohol/cigarette use, *C. trachomatis* remained significantly associated with depression (p = .005, OR = 1.79, 95% CI = 1.192 to 2.685) (Table 2).

Sexes:

Infected females were significantly more likely to report depression than uninfected females, p = .005; OR = 2.086 (95% CI = 1.245 to 3.453). However, infected males were not significantly more likely to report depression than uninfected males (p = .097, OR = 1.666; 95% CI = 0.9022 to 3.028). To directly compare the strength of the relationship between infection and depression in females to that in males, I included an interaction term (sex: infection). The coefficients between the two populations did not differ significantly (p = .576).

Matched case-control and antibiotic treatment:

Out of the 839 patients, 111 (13.2%) had both a positive and a negative *C. trachomatis* test result on separate dates (Table 3). A McNemar's binomial test determined the same patient was more likely to report depression while infected than uninfected (p = .016, OR = 3.17, 95% CI =1.22 to 9.69). To assess the effect of antibiotic treatment, I analyzed a subset of patients who were infected at the first appointment, prescribed antibiotics, and then tested negative within 60 days. The sample size was small (N= 29), and infection was not significantly associated with depression, though the odds ratio was above two (p = .34, OR = 2.33, 95% CI=0.533 to 13.98).

Depression severity:

Using PHQ-2 and PHQ-9 scores, low mood was stratified into ordinal responses, ranging from none to severe. For ordinal logistic regression, the assumption of proportional odds (parallel lines) revealed no significant difference in slopes between the null and general model (LL test p = .517). I therefore considered *C. trachomatis* to be similarly associated with the odds of low mood regardless of the threshold, and a single

OR value was used to describe the effect of infection on depression severity. In this ordinal logistic regression analysis, infection was significantly associated with an increased odds of falling into a more severe depression category, $p = .002$; OR = 1.83 (1.251 to 2.694) (Table 4). The lack of significant differences in slopes between the null and general model across depression thresholds indicates that *C. trachomatis* is associated with low mood similarly across the spectrum.

Table 1

Descriptive Statistics of Study Population

	All Patients (%) N	Uninfected (%) N	Infected (%) N	Infected Asymptomatic (%) N	Infected Female (%) N	Uninfected Female (%) N	Infected Male (%) N	Uninfected Male (%) N
Not Depressed	(83.6%) 701	(85.9%) 532	(76.8%) 169	(77.8%) 98	(72.7%) 80	(84.8%) 306	(80.9%) 89	(87.6%) 226
Depressed	(16.4%) 138	(14.1%) 87	(23.2%) 51	(22.2%) 28	(27.3%) 30	(15.2%) 55	(19.1%) 21	(12.4%) 32
Total	839	619	220	126	110	361	110	258

Table 1. ⎪Counts of patients who did or did not report depression by *C. trachomatis* infection status and sex. Percentages refer to the number of patients in the cell (N) divided by the column total

Table 2

Depression and Infection Multivariable Results

Variable	**β-coefficient Estimate**	**SE**	**|Z|**	**P value**	**Odds Ratio**	**95% CI**
C. trachomatis (β1)	0.5844	0.2069	2.825	0.005	1.794	1.192 to 2.685
Age (β2)	-0.0001301	0.02553	0.005098	0.996	0.9999	0.9486 to 1.049
N. gonorrhoeae (β3)	0.3636	0.6074	0.5986	0.549	1.439	0.3842 to 4.426
Sex[F] (β4)	0.3255	0.1944	1.674	0.094	1.385	0.9492 to 2.037
Smoking/drug/alcohol (β5)	0.2059	0.1938	1.063	0.288	1.229	0.8432 to 1.805
Symptoms (β6)	0.2951	0.1996	1.479	0.139	1.343	0.9044 to 1.980

Table 2. │ Coefficient estimates and odds ratios of explanatory variables incorporated into the multivariable logistic regression. Depression was treated as the dichotomous response variable. N = 220 and 15 for *C. trachomatis* and *N. gonorrhoeae* respectively.

Table 3

Depression and Infection Matched-Controlled Results

	Infected: depressed	Infected: not depressed	Row total
Uninfected: depressed	6	6	12
Uninfected: not depressed	19	80	99
Column total	25	86	111

Table 3. | Counts of individuals who completed the PHQ-2 on separate *C. trachomatis* testing dates. Each table entry represents one pair of depression results per patient -- one from an infected appointment and one from an uninfected appointment.

Table 4

Depression and Infection Ordinal Logistic Regression Results

Infected	**None**	**Minimal**	**Mild**	**Moderate**	**Moderately Severe**	**Severe**
Cumulative N infected	220	51	39	24	11	5
Cumulative proportion	1.00	0.232	0.177	0.109	0.050	0.023
Cumulative odds	-	0.302	0.215	0.122	0.053	0.023
Uninfected	**None**	**Minimal**	**Mild**	**Moderate**	**Moderately Severe**	**Severe**
Cumulative N uninfected	619	87	70	36	20	5
Cumulative proportion	1.00	0.141	0.113	0.058	0.032	0.008
Cumulative odds	-	0.164	0.128	0.062	0.033	0.008
Odds ratio (infected/uninfected)	-	1.84	1.68	1.97	1.61	2.88
Location	**β-Estimate**	**SE**	**Wald**	**P value**	**Odds Ratio**	**95% CI**
Infected	.607	.196	9.625	.002**	1.83	1.251 to 2.694
Uninfected	Ref					

Table 4. | The cumulative number, cumulative proportion, and cumulative odds of patients who reported each level of depression or above (no depression is considered the lowest level). The odds ratio at each threshold equals the cumulative odds for infected divided by cumulative odds for uninfected. The relationship between infected and uninfected at any threshold is summarized by a single estimate, OR = 1.83 (1.251 to 2.694), p = .002.

Figure 1

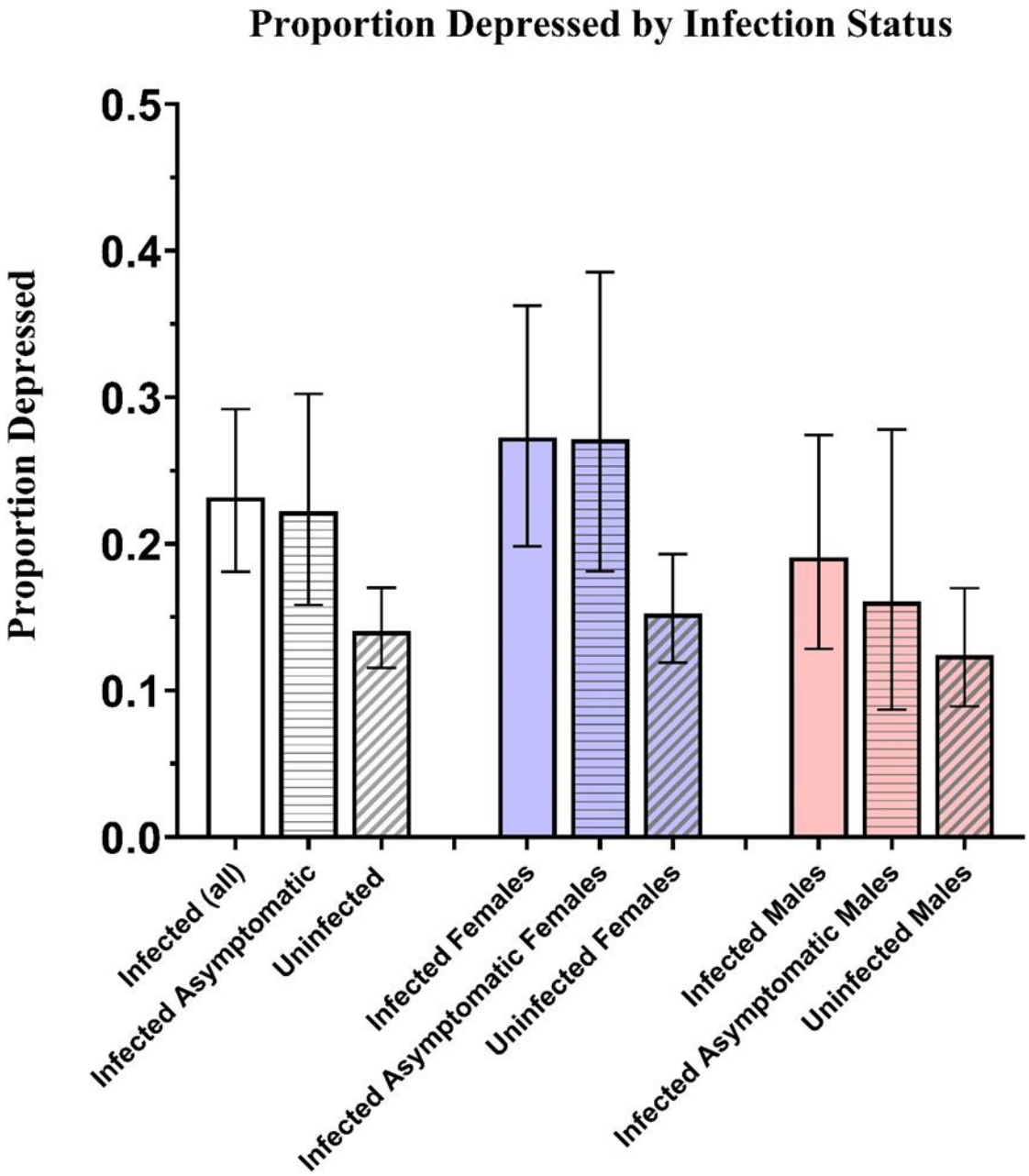

Proportion Depressed by Infection Status
Proportion Depressed
0.5
0.4
0.3
0.2
0.1
0.0
Infected (all)
Infected Asymptomatic
Uninfected
Infected Females
Infected Asymptomatic Females
Uninfected Females
Infected Males
Infected Asymptomatic Males
Uninfected Males

Figure 1. ⎪ The proportion of patients who reported depression of any severity for each *C. trachomatis* infection status. From left to right: All infected = 0.232 (95% CI = 0.181 to 0.292); Infected asymptomatic = 0.222 (95% CI =0.158 to 0.302); Uninfected = 0.141 (95% CI = 0.115 to 0.170). Infected female = 0.273 (95% CI = 0.198 to 0.363); Infected asymptomatic female = 0.271 (95%CI = 0.181 – 0.385); Uninfected female = 0.152 (95% CI = 0.119 to 0.193); Infected male = 0.191 (95% CI = 0.128 to 0.274); Infected asymptomatic male = 0.161 (95%CI = 0.087 to 0.278); Uninfected male = 0.124 (95% CI = 0.089 to 0.170)

Discussion

Associations Between C. trachomatis and Low Mood:

The results confirm the association between *C. trachomatis* and low mood that was reported in previous studies and broaden the previous findings by documenting this association in a predominantly student population. The case control sub-analysis of the present study also strengthens the confidence in the association between *C. trachomatis* by confirming the association within individuals according to changes in their infection status.

C. trachomatis was significantly associated with depression in patients without recognized symptoms and after adjusting for symptoms in the multivariable analysis. These findings indicate that the association of *C. trachomatis* with depression is not simply a consequence of subjects' urogenital symptomology.

In the ordinal analysis, infected patients had nearly twice the odds of falling into a higher (rather than a lower) depression category, regardless of the threshold used. This finding indicates that the effects of infection extend to all PHQ-9 levels of low mood.

These results could arise if existing depression is exacerbated by infection, perhaps through disruption of the serotonergic system and/or neurotoxic effects, as mentioned in the Introduction.

The association of low mood with *C. trachomatis* in males was not significantly lower than the association in females, but it also was not significantly elevated relative to uninfected males. However, the p-value associated with the comparison of infected and uninfected males was closer to statistical significance and would be significant by a one-tailed test (one-tailed p=.049), which would be advocated in this case by some statisticians (because one would not expect *C. trachomatis* to be associated with elevated mood). Additional study with large sample sizes is therefore needed to determine whether the association occurs in males and if so whether it is significantly weaker than the association in females. This study lacked BV data; it would be worthwhile to assess the risk of depression in women who are positive for BV and *C. trachomatis*.

Causal mechanisms:

Only a minority of infected patients (≈ 23%) reported low mood of any severity in our study. This finding is consistent with an interaction between low serotonin induced by *C. trachomatis*. In clinical trials, tryptophan reduction lowers mood in subjects with preexisting risk factor(s) for depression, but those without a history of depression or familiar risk factor are largely unaffected (Smith, Fairburn et al. 1997, Moreno, Gelenberg et al. 1999, Hughes, Gallagher et al. 2003, Booij, Van der Does et al. 2005, Feder, Skipper et al. 2011). *C. trachomatis* might therefore only cause depression in individuals with a preexisting susceptibility to the disease. Nevertheless, elimination of *C. trachomatis* might restore normal mood for some of these patients.

The evidence on mood disorders and unprotected sex is conflicting, but several studies have found associations between the two, including in young populations (Lehrer, Shrier et al. 2006, Mazzaferro, Murray et al. 2006, Tesfaye, Negash et al. 2019). These studies have either used a proxy for increased rates of sexually transmitted infections (i.e., risky sexual behavior) or grouped sexually transmitted infections together as a single outcome variable. If low mood is causing increased rates of sexually transmitted infections through risky sexual behavior, then all or most sexually transmitted infections should be associated with the disease independently. In bivariate analyses, different sexually transmitted infections are associated with depression, but after correcting for correlations between the different pathogens, only *C. trachomatis* and *T. vaginalis* remained significantly associated with depression (Doyle, Swain et al. 2019). In our study, *N. gonorrhoeae* infection was not associated with depression in the multivariable analysis, though it was rare in the population.

Limitations:

This study has several limitations. It relies on the absence of any indication of urogenital manifestations to assign infections to the asymptomatic category. If health personnel failed to record such manifestations the association between asymptomatic infection and low mood might have been artificially strengthened. This quantification of low mood in urogenital asymptomatic infections, however, was essentially the same as that of symptomatic subjects at least for women (compare middle column to first column in first two triplets of Figure 1). This similarity negates concern that the low mood among symptomatic patients resulted from some misidentification of some symptomatic patients as asymptomatic.

The associations tested are consistent with *C. trachomatis* contributing to depression through a prolonged immune response that includes tryptophan degradation, but this mechanism has not been experimentally verified. Also, as with any correlational study, there is a possibility of missing correlates that could influence results. Other than *N. gonorrhoeae*, I did not have data for other sexually transmitted pathogens, and thus was unable to include them as covariates. In this regard however, assessments of other pathogens have not implicated any that are so strongly associated with low mood as is *C. trachomatis* (Doyle, Swain et al. 2015, Doyle, Swain et al. 2019). Also, the within-patient analysis partially remedies this issue by controlling for time-invariant confounders that may have been missed in the between-subject analysis. One limitation of the matched-control design is that infection status order was unaccounted for. Some of the subjects tested positive for *C. trachomatis* on their first appointment, whereas others tested negative on their first appointment. Also, the interval between positive and negative appointments was variable. Only in the subset of patients treated with antibiotics was the order of infection status the same for all patients.

High scores on the PHQ-9 are strongly associated with diagnosis of clinical depression, but low scores in the range of minimal and mild depression often signify the absence of clinical depression (Kroenke, Spitzer et al. 2001). The focus of our study was *C. trachomatis* and mood, whether recognized depressive disorders or less severe but still detectable mood shifts. As such, further studies are needed to assess the relationship between *C. trachomatis* and clinically recognized depressive disorders, including major and/or persistent depression.

Conclusions:

The results suggest that *C. trachomatis* infection contributes to low mood and does so even in subjects without urogenital symptoms. The effect is significant in females only, though the extent to which *C. trachomatis* is associated with low mood in men remains uncertain. Our results also suggest that *C. trachomatis* contributes to low mood across the spectrum from mild to severe. These findings support the overarching idea that within-host persistence is selected for in *C. trachomatis*, likely due to infrequent transmission opportunity, and because of this persistence the host experiences chronic mood reduction. The evidence provided by this study and previous research advocate for further investigation into causal mechanisms. I propose that experimental studies evaluate whether mood can be enhanced by antibiotic treatment of *C. trachomatis* and whether such improvement is related to amelioration of neurotoxic metabolites and serotonin dysregulation.

CHAPTER III

THE EVOLUTION OF SARS-COV-2 VIRULENCE AND VIRAL LOADS

Summary

SARS-COV-2 has moderate survivability in the external environment and transmission depends largely on host mobility. Compared to human respiratory pathogens with similar qualities, the virulence of the ancestral virus was atypically high and perhaps maladaptive. To evaluate if SARS-CoV-2 evolved lower virulence throughout the pandemic, I conducted meta-analyses on studies that compared the risk of hospitalization and/or mortality between variants. I also conducted meta-analyses on studies that compared viral loads between variants, as measured by qPCR cycle threshold. These analyses reveal a trend of increased virulence that continues through the first two years of the pandemic, through the emergence of the Delta variant, followed by a sharp reduction in virulence at the end of 2021 as the Omicron variant emerged. The results show increasing viral loads in the upper respiratory tract (which should facilitate transmission) with the highest loads associated with Delta and Omicron. Together, these trends suggest that the fitness costs of increased virulence were outweighed by the benefits of higher viral loads, initially favoring more exploitative variants. However, the results also demonstrate that Omicron has high viral loads and lower virulence, which probably results from an adaptive change in tissue tropism.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) began infecting humans in late 2019. In less than a year the ancestral virus was displaced by more transmissible variants, most notably Alpha (B.1.1.7). The predominance of Alpha and other early variants was short-lived, however, as the Delta variant (B.1.617.2), which emerged late in 2021, spread globally and displaced the pre-existing variants. Omicron emerged at the end of 2021 with even higher transmissibility. Today all reported SARS-CoV-2 isolates are in Omicron lineages. The rapid and successive emergence of increasingly transmissible variants indicates that the ancestral virus was not well adapted to exploiting humans. As the pandemic progressed, the generation of new viral variants provided the basis for natural selection to favor changes in viral growth rates, tissue tropism, and virulence.

Evolutionary change in a pathogen's exploitation strategy, and hence virulence, should reflect its dependence on host survival and mobility for transmission (Ewald 1991). For respiratory tract pathogens of humans, durability in the external environment appears to be a key characteristic. Durable pathogens depend less on the movement of the infected host to reach susceptible hosts and more on the movement of susceptible hosts to contaminated environments. Natural selection should therefore favor higher levels of exploitation when durability is greater. SARS-CoV-2 has moderate durability in the external environment. In a typical indoor setting (room temperature, 40° relative humidity), where most transmission is thought to occur, the virus remains infectious on most surfaces between a few hours and a week (Van Doremalen, Bushmaker et al. 2020, Geng and Wang 2023). Although SARS-CoV-2 can transmit through fomites, it is thought

to spread primarily through aerosols, in which it remains viable for an estimated 9 hours in indoor conditions. (Van Doremalen, Bushmaker et al. 2020). This durability is approximately the same as that of influenza virus, greater than that of most respiratory viruses, but much less than the most lethal respiratory pathogens. (Walther and Ewald 2004).

One indicator for assessing the virulence of SARS-CoV-2 infections is the infection fatality ratio (IFR), which is typically expressed as the percentage of infections that result in death. The earliest reliable estimate for SARS-CoV-2 IFR came from a Diamond Princess cruise ship outbreak in February 2020, which after age adjustment was approximately 0.6% (Russell, Hellewell et al. 2020). Subsequent studies early during the pandemic generated similar estimates, which were summarized in a meta-analysis that yielded a mean IFR of 0.68% (0.53-0.82) (Meyerowitz-Katz and Merone 2020). In the context of other human respiratory pathogens, the virulence of the ancestral virus appears out of place (Table 5). The highest IFR of any respiratory pathogen with a mean survival time under one week is 0.007%, or approximately 100 times less lethal than the ancestral virus was per infection. It is important to note that mortality estimates from other pathogens are derived from human populations with some level of acquired immunity obtained through vaccination and/or infection. Nonetheless, acquired immunity fails to explain the differences entirely. Even the highest estimates for the protective effects of prior SARS-CoV-2 infection are ≈ 90% relative reduction in mortality (Stein, Nassereldine et al. 2023), explaining approximately $1/10^{th}$ of the observed IFR variation.

Table 5

Virulence and Survivability Table for Human Respiratory Pathogens

Human Respiratory Pathogen	**Estimated IFR (%)**	**Survival Time in External Environment (days)**	**Overall survival tendency**
Variola Virus	10	885.1	Months to years
Mycobacterium Tuberculosis	5	244.3	Weeks to months
SARS-COV-2; 2020	**0.68***	**7.15****	**Hours to weeks**
Corynebacterium diphtheriae	.2	369.8	Weeks to months
Bordetella pertussis	.1	11.6	Hours to weeks
Streptococcus pneumoniae	0.036	28.6	Hours to weeks
SARS-COV-2; predicted	**0.1-0.01%**	**7.15**	**Hours to weeks**
Influenza virus	0.010	13.7	Hours to weeks
Neisseria meningitidis	0.007	1.9	Hours
Rubeola virus	0.007	4.4	Hours
Mumps virus	0.005	0.9	Hours
Parainfluenza virus	0.004	4.6	Hours
Mycoplasma pneumoniae	0.003	1.9	Hours
Respiratory syncytial virus	0.003	1.1	Hours
Varicella-zoster virus	0.003	0.9	Hours
Rubella virus	0.003	0.9	Hours
Haemophilus influenzae	0.002	1.3	Hours
Rhinovirus	0.000	2.3	Hours

Table 5. | Endemic human respiratory pathogens and SARS-COV-2 ranked in order of estimated fatality per infection.

*Ancestral SARS-CoV-2 virulence based on meta-analysis Meyerowitz-Katz and Merone 2020 **Estimated SARS-CoV-2

surface survivability based on (Geng and Wang 2023). All other data in the table taken from (Walther and Ewald 2004). The survival time in the external environment is an average of the high estimate for all tested surfaces. It depends strongly on the number of studies and the conditions of the study. The overall tendency for durability in the external environment is therefore represented in the last column, which takes into account the extent to which the durability of the pathogens listed below influenza viruses cannot be reliably distinguished by the existing data.

Proximate changes in virulence and transmission can occur through mutations that alter viral growth kinetics and tissue tropism. Differences in cellular characteristics across tissues, such as surface receptors, transcription factors, proteases, intracellular conditions influence the tropic range for a given pathogen's genotype (Baron, Fons et al. 1996). Mutations can narrow or broaden this range and thereby alter host symptoms and the overall harmfulness of an infection (Nagai, Klenk et al. 1976, Jnaoui, Minet et al. 2002, Liu, Childs et al. 2010). Natural selection is expected to restrict pathogen replication inside of tissues that maximize the benefits and minimize the costs of the virulence tradeoff described in Chapter I. Directly transmitted respiratory pathogens with limited environmental survivability tend to intensely exploit the upper respiratory tract but remain there. This strategy minimizes the risk of causing incapacitating symptoms that are associated with the lower respiratory tract (LRT) or other systems. Unlike these more benign pathogens, ancestral SARS-CoV-2 had less restricted exploitation -- it frequently invaded deeply in the lungs, causing high rates of pneumonia, dyspnea, and respiratory distress syndrome (Polak, Van Gool et al. 2020, Hu, Guo et al. 2021). Selection for an exploitation strategy that mirrors the less virulent respiratory pathogens, characterized by intense exploitation of the nasal passages, sinuses, or pharynx rather than in the lower respiratory tract, might therefore be favored. Tissues that are susceptible to SARS-CoV-2 infection consist of cells that express transmembrane protein Angiotensin-Converting Enzyme 2 (ACE2). ACE2 is a required binding receptor for the virus prior to endocytosis and therefore a limiting factor for cellular susceptibility to infection (Wang, Li et al. 2020, Zhang, Penninger et al. 2020). It is expressed at variable degrees across many tissues in the body (Hikmet, Méar et al. 2020), however neither severe disease nor broad

tropism are unavoidable outcomes of utilizing this receptor. The human coronavirus NL63 also uses ACE2 but it is substantially milder and typically remains in the URT. This may be because it has had ample time to adapt to humans, as it was first identified in 2004 but phylogenetic evidence indicates it has been spreading in humans for centuries (Pyrc, Dijkman et al. 2006).

The clinical and virological characteristics of SARS-CoV-2 have been studied extensively. Missing, however, is the characterization of an evolutionary trend in these features throughout the pandemic. The aim of this chapter is to quantitatively review differences in viral load and virulence across variants, from the ancestral virus through Omicron. To that end, this chapter includes two separate but complementary systematic reviews of scientific literature. In the first section, I review literature on comparative risks of hospitalization and/or mortality between variants. A separate meta-analysis is conducted for each variant comparison and clinical outcome. In the second section, I review literature on differences in viral load between variants. I use qPCR cycle threshold values taken from clinical samples as a surrogate for viral load. Similar to the first section, a separate meta-analysis is conducted for each variant comparison.

Methods: Virulence Systematic Review

PubMed and Web of Science were searched during January 2022 using a combination of the following terms and operators: (((Sars-cov-2 OR covid-19 OR 2019-nCoV) AND (virulence OR mortality OR hospitalization OR survival OR outcome OR severity OR fatality)) AND (Wuhan-Hu-1 OR "Wuhan-1" OR "Wuhan strain" OR "Ancestral" OR wild-type)) AND/OR (Alpha OR "B.1.1.7" OR D614G) AND/OR (delta

OR "B.1.617.2") OR (Omicron) The search was repeated on 15, March 2022 and again on 19, February 2023 to incorporate recent articles, particularly those including Omicron.

Across both search engines 3,735 articles were identified initially and 1,828 remained after removing duplicates. The titles and/or abstracts were then screened leaving 303 articles. The full texts of the 303 articles were assessed for the following inclusion criteria: 1) The study compared the risk of hospital admission and/or mortality between infection with different variants 2) Outcomes were reported as an adjusted hazard ratio (aHR) which accounted for differences in age, vaccination status (if applicable), and co-morbidities between groups 3) The risk denominator was a positive SARS-CoV-2 RT-qPCR test obtained through community surveillance or out-patient testing

After screening full texts for inclusion criteria, fifteen articles remained and five separate meta-analyses were possible (Table 6). Individual meta-analyses were performed when ≥ 2 studies had results for the same variant comparison and for the same clinical outcome.

Statistical analyses:

Random effects meta-analyses were performed on adjusted HRs for each variant comparison and outcome. Inverse variance was used to weight studies. Tau-squared was used to estimate between-study heterogeneity. Analyses were performed in SPSS version 29.0.0.0

Results: Virulence Systematic Review

Table 6

Summary of Mortality and Hospitalization Results

Reference/Comparison	Number of studies	Outcome	*Mean Effect (95% CI)	Percent change in risk from reference
Pre-VoC/Alpha	3	Hospitalization	1.52 (1.45 – 1.60)	+52%
Pre-VoC/Alpha	5	Mortality	1.50 (1.24 – 1.83)	+50%
Alpha/Delta	3	Hospitalization	2.15 (1.74 – 2.66)	+115%
Delta/Omicron	4	Hospitalization	0.36 (0.27 – 0.47)	-64%
Delta/Omicron	4	Mortality	0.21 (0.12 – 0.37)	-79%

Pre-Variant of Concern (Pre-VoC) represents ancestral strains that existed prior to the designation of the first variant of concern. *Effect size for adjusted hazard ratio values. Null = 1

Pre-Variant of Concerns and Alpha Variant

Three studies compared the hazard ratio risk of hospital admission between pre-variant of concerns and the alpha variant (figure 2). Individually all three studies found a significantly higher risk associated with the alpha variant. The total mean effect for all studies was 1.52 (1.45 – 1.60), $p < .01$. Five studies compared the hazard ratio risk of mortality between pre-variant of concerns and the alpha variant (figure 3). Three of the five studies found a significantly higher risk associated with the Alpha variant and two studies found no significant difference. The total mean effect for all studies was 1.50 (1.24 – 1.83), $p < .01$.

Alpha Variant and Delta Variant

Three studies compared the hazard ratio risk of hospital admission between the alpha and delta variants (figure 4). Individually all three studies found a significantly higher risk associated with the Delta variant. The total mean effect for all studies was 2.15 (1.74 – 2.66), $p < .01$. No studies that compared the hazard ratio risk of mortality between the alpha and delta variants were found.

Alpha Variant and Delta Variant

Four studies compared the hazard ratio risk of hospital admission between the delta and omicron variants (figure 5). Individually all four studies found a significantly lower risk associated with the Omicron variant. The total mean effect for all studies was 0.36 (0.27 – 0.47), $p < .01$. Four studies compared the hazard ratio risk of mortality between the delta and omicron variants (figure 6). Individually all four studies found a significantly lower risk associated with the Omicron variant. The total mean effect for all studies was 0.21 (0.12 – 0.37), $p < .01$.

Figure 2

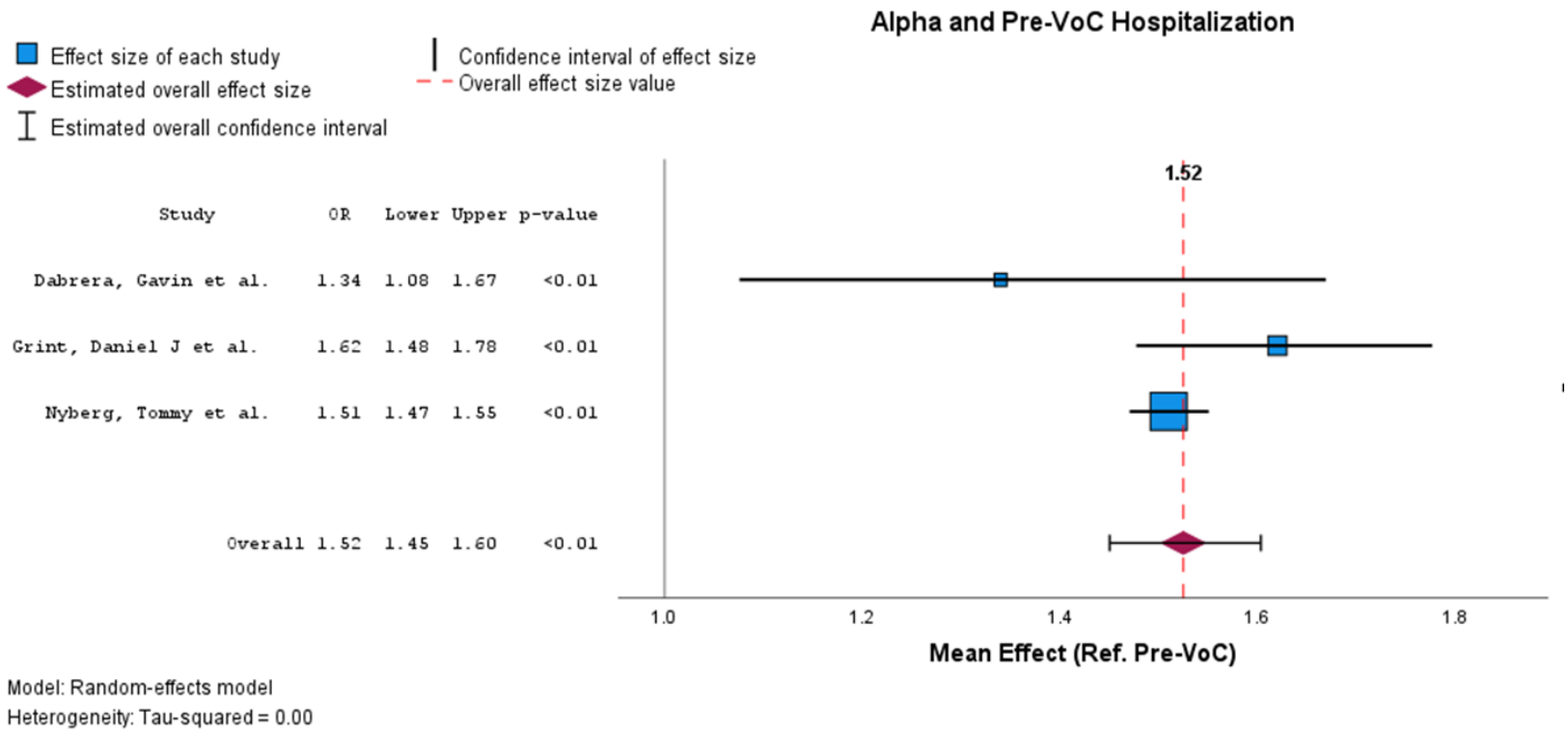
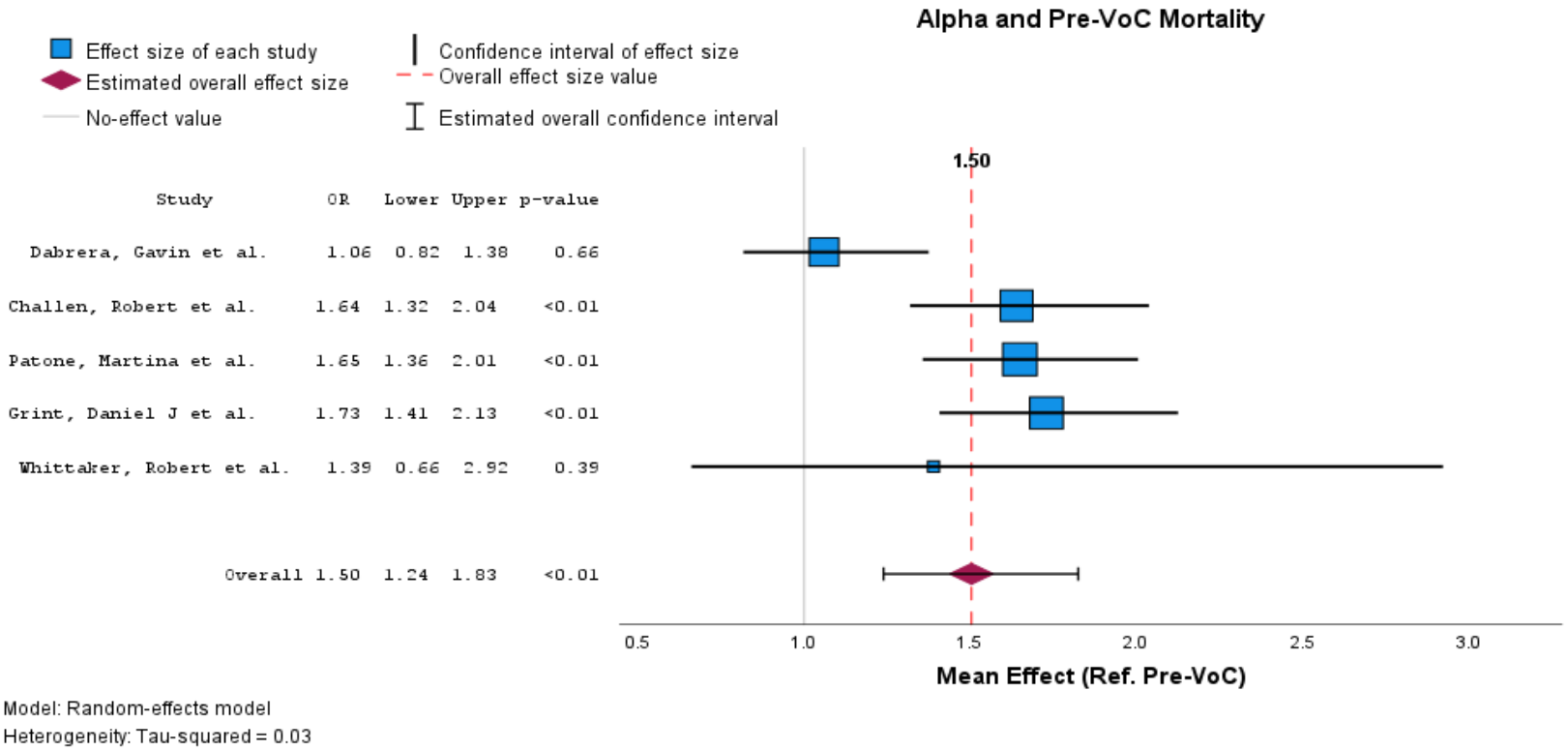

Alpha and Pre-VoC Hospitalization
Effect size of each study
Estimated overall effect size
Estimated overall confidence interval
Confidence interval of effect size
Overall effect size value
Study OR Lower Upper p-value
Dabrera, Gavin et al. 1.34 1.08 1.67 <0.01
Grint, Daniel J et al. 1.62 1.48 1.78 <0.01
Nyberg, Tommy et al. 1.51 1.47 1.55 <0.01
Overall 1.52 1.45 1.60 <0.01
1.52
1.0
1.2
1.4
1.6
1.8
Mean Effect (Ref. Pre-VoC)
Model: Random-effects model
Heterogeneity: Tau-squared = 0.00

Figure 3



Model: Random-effects model
Heterogeneity: Tau-squared = 0.03

Figure 4

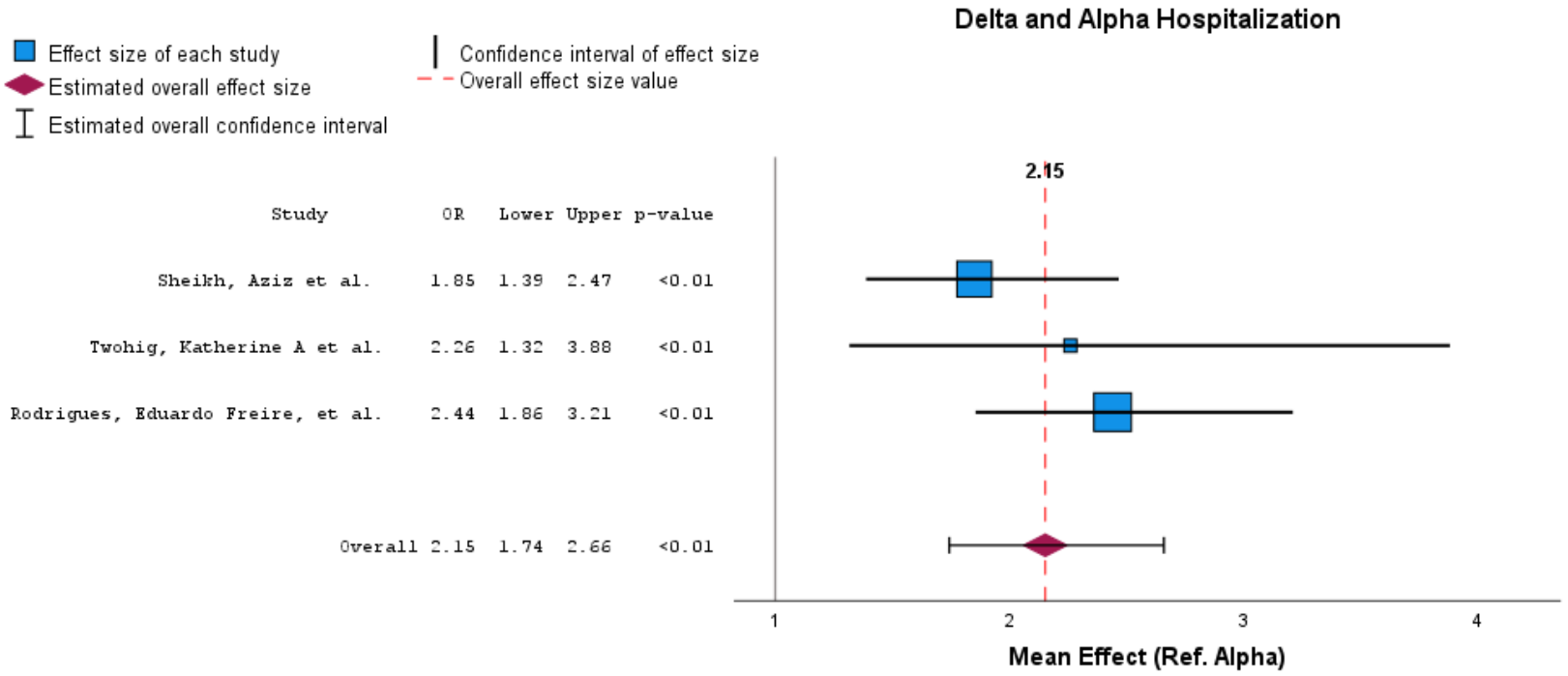

Delta and Alpha Hospitalization
Effect size of each study
Estimated overall effect size
Estimated overall confidence interval
Confidence interval of effect size
Overall effect size value
Study OR Lower Upper p-value
Sheikh, Aziz et al. 1.85 1.39 2.47 <0.01
Twohig, Katherine A et al. 2.26 1.32 3.88 <0.01
Rodrigues, Eduardo Freire, et al. 2.44 1.86 3.21 <0.01
Overall 2.15 1.74 2.66 <0.01
2.15
1
2
3
4
Mean Effect (Ref. Alpha)
Model: Random-effects model
Heterogeneity: Tau-squared = 0.01
Axis is shown using log scale

Figure 5

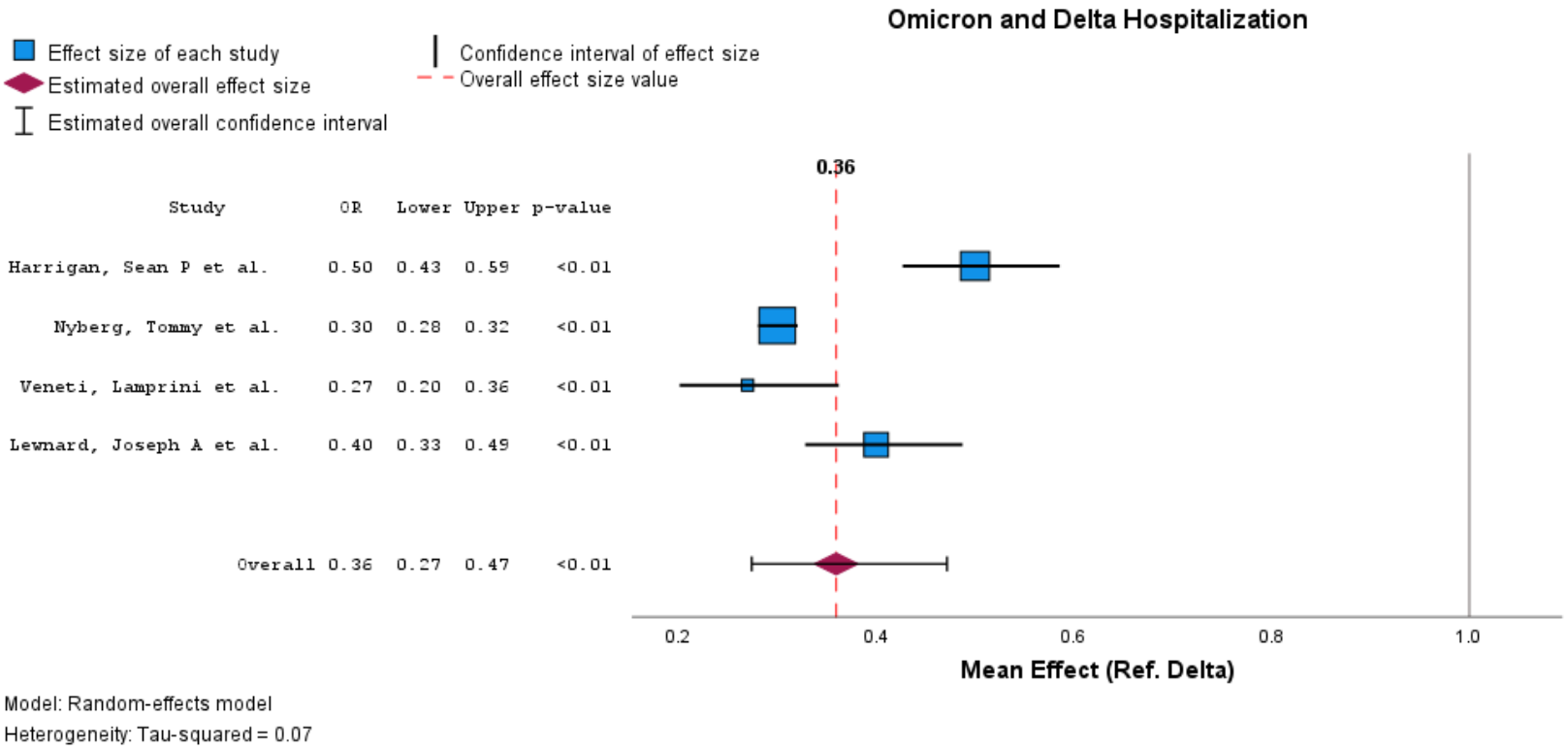

Omicron and Delta Hospitalization
Effect size of each study
Estimated overall effect size
Estimated overall confidence interval
Confidence interval of effect size
Overall effect size value
Study OR Lower Upper p-value
Harrigan, Sean P et al. 0.50 0.43 0.59 <0.01
Nyberg, Tommy et al. 0.30 0.28 0.32 <0.01
Veneti, Lamprini et al. 0.27 0.20 0.36 <0.01
Lewnard, Joseph A et al. 0.40 0.33 0.49 <0.01
Overall 0.36 0.27 0.47 <0.01
0.36
0.2
0.4
0.6
0.8
1.0
Mean Effect (Ref. Delta)
Model: Random-effects model
Heterogeneity: Tau-squared = 0.07

Figure 6

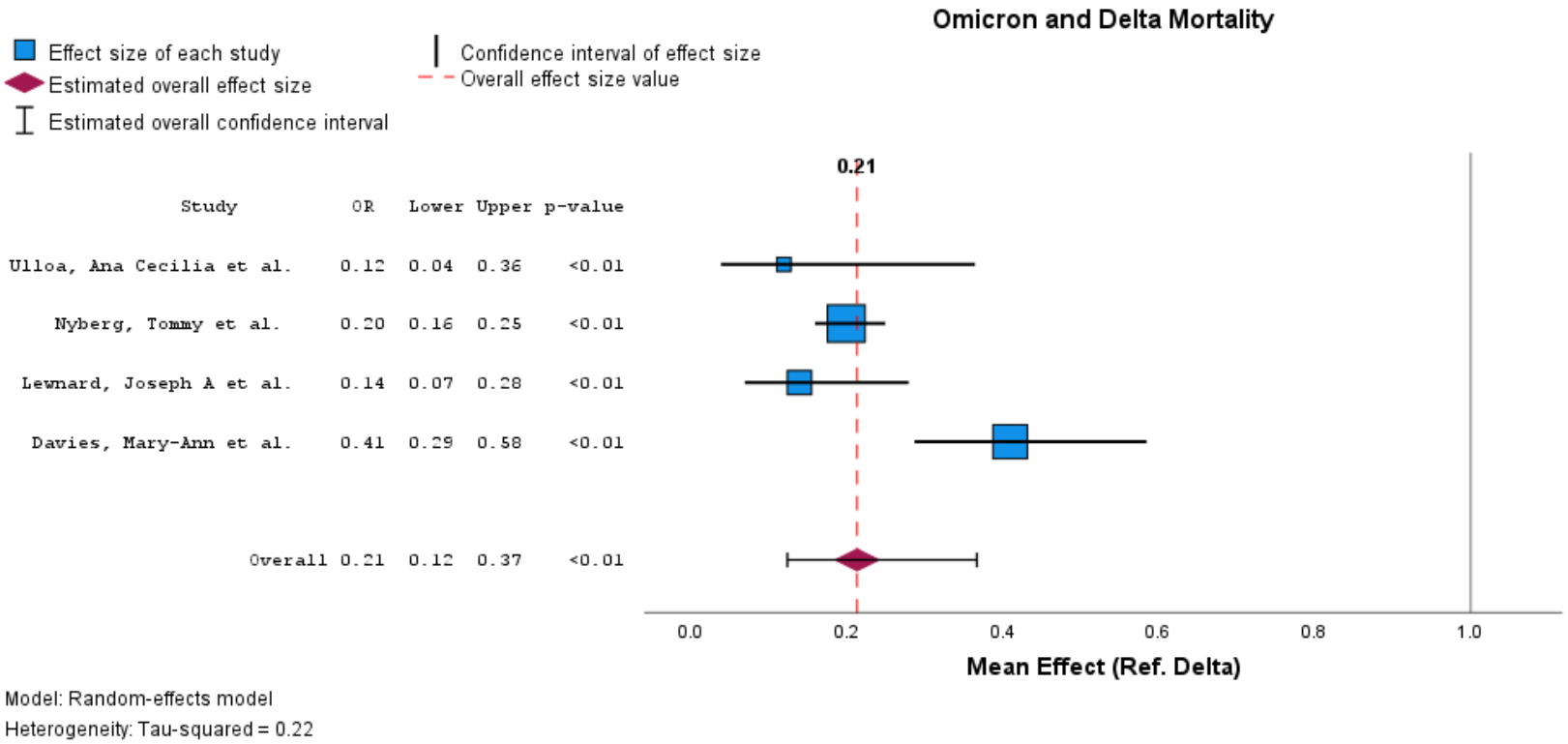

Omicron and Delta Mortality
Effect size of each study
Estimated overall effect size
Estimated overall confidence interval
Confidence interval of effect size
Overall effect size value
Study OR Lower Upper p-value
Ulloa, Ana Cecilia et al. 0.12 0.04 0.36 <0.01
Nyberg, Tommy et al. 0.20 0.16 0.25 <0.01
Lewnard, Joseph A et al. 0.14 0.07 0.28 <0.01
Davies, Mary-Ann et al. 0.41 0.29 0.58 <0.01
Overall 0.21 0.12 0.37 <0.01
0.21
0.0
0.2
0.4
0.6
0.8
1.0
Mean Effect (Ref. Delta)
Model: Random-effects model
Heterogeneity: Tau-squared = 0.22

Table 7

Mortality and Hospitalization Studies

Study	**Variant Comparison and primary outcome**	**Adjusted HR (95% CI)**	**Population inclusion**
(Dabrera, Allen et al. 2022)	Pre-Voc/Alpha; mortality within 28 days of positive test	1.06 (0.82–1.38);	Nationwide routine monitoring Second-Generation Surveillance System
(Challen, Brooks-Pollock et al. 2021)	Pre-Voc/Alpha; mortality within 28 days of positive test	1.64 (1.32 to 2.04)	Community testing of symptomatic >30 years old
(Patone, Thomas et al. 2021)	Pre-Voc/Alpha; mortality within 28 days of positive test	1.65 (1·36–2·01)	Positive community COVID-19 test
(Grint, Wing et al. 2022)	Pre-Voc/Alpha; mortality	1.73 (1.41–2.13)	Positive community COVID-19 test
(Whittaker, Kristofferson et al. 2021)	Pre-Voc/Alpha; mortality within 28 days of positive test	1.39 (0.68 – 3.01)	Variety of central health registries, national clinical registries and other national administrative registries
(Dabrera, Allen et al. 2022)	Pre-Voc/Alpha; hospitalization within 14 days	1.34 (1.07–1.66)	Nationwide routine monitoring Second-Generation Surveillance System
(Grint, Wing et al. 2022)	Pre-Voc/Alpha; hospitalization	1.62 (1.48–1.78)	Positive community COVID-19 test
(Nyberg, Twohig et al. 2021)	Pre-Voc/Alpha; hospitalization within 14 days	1.51 (1.47 to 1.55)	Large scale community originated testing
(Sheikh, McMenamin et al. 2021)	Alpha/Delta hospitalization	1·85 (1·39–2·47)	Nationwide surveillance program
(Twohig, Nyberg et al. 2022)	Alpha/Delta; hospitalization	2.26 (1.32–3.89)	All patients with positive COVID-19 in England
(Rodrigues, Moreno et al. 2022)	Alpha/Delta; hospitalization within 15 days of positive test	2.44 (1.85 - 3.20)	large scale surveillance system >17 years

(Harrigan, Wilton et al. 2023)	Delta/Omicron; hospitalization	0.50 (0.43 - 0.59)	large surveillance platform; laboratory confirmed test
(Nyberg, Ferguson et al. 2022)	Delta/Omicron; hospitalization within 14 days from positive test	0.30 (0.28-0.32)	nationwide laboratory confirmed test
(Veneti, Bøås et al. 2022)	Delta/Omicron; hospitalization within 14 days from positive test	0.27 (0.20–0.36)	Norwegian Preparedness Registry
(Lewnard, Hong et al. 2022)	Delta/Omicron; hospitalization 30 days from outpatient detection	0.40 (0.33–0.49)	Testing in clinical outpatient settings
(Ulloa, Buchan et al. 2022)	Delta/Omicron BA.1; mortality	0.12 (0.04-0.37)	City-wide data set; all diagnosed infections
(Nyberg, Ferguson et al. 2022)	Delta/Omicron; mortality 28 days from positive test	0.20 (0.16-0.25)	nationwide laboratory confirmed test
(Lewnard, Hong et al. 2022)	Delta/Omicron; mortality 60 days from outpatient detection	0.14 (0.07–0.28)	Testing in clinical outpatient settings
(Davies, Kassanjee et al. 2022)	Delta/Omicron; mortality within 14 days of positive test	0.41 (0.29 - 0.59)	Western Cape Provincial Health Data Centre laboratory-confirmed COVID-19 diagnosis

Methods: Viral Load Systematic Review

PubMed and Web of Science were searched December 2022 using a combination of the following terms and operators: ((((Sars-cov-2 OR covid-19 OR 2019-nCoV) AND (viral load OR "viral burden" OR "viral titer" OR “RNA copies”)) AND (Wuhan-Hu-1 OR "Wuhan-1" OR "Wuhan strain" OR "Ancestral" OR wild-type)) AND (Alpha OR "B.1.1.7" OR D614G) AND/OR (delta OR "B.1.617.2”) AND/OR (Omicron) NOT (wastewater OR sewer)

Across both search engines 603 articles were identified initially and 210 remained after removing duplicates. The titles and/or abstracts were then screened leaving 59 articles. The full texts of the 59 articles were assessed for the following inclusion criteria: 1) The study compared the mean difference in cycle threshold (CT) values between subjects infected with different virus variants 2) Within each study, the reported cycle threshold results were generated from the same assay and laboratory* 3) Subjects were unvaccinated or vaccination frequency was presumed negligible by the authors with accompanying rationale. I excluded studies that tested clinical performance of new RT-qPCR assays.

*RT-qPCR provides semi-quantitative measurements. Samples run using the same protocol and assay can be accurately compared for relative differences in cycle threshold (CT). However, CT values are not comparable across different protocols and assays. I therefore excluded studies that consolidated cycle threshold results from different assays and labs.

After screening full texts for inclusion criteria 8 articles (11 studies) remained. Woodbridge, Yonatan et al. was broken down into four individual studies because it

provided separate data and results from four different labs. From the 11 studies, three meta-analyses were conducted, each on a different variant comparison (Table 8). Separate meta-analyses were performed when ≥ 2 studies had results for the same variant.

Statistical analysis:

Random effects meta-analyses were performed on mean differences of CT values. The effect size is expressed as Standardized Mean Difference (SMD) Hedges' g variation. Inverse variance was used to weight studies. Tau-squared was used to estimate between-study heterogeneity. Analyses were performed in SPSS version 29.0.0.0

Results: Viral Load Systematic Review

Table 8

Summary of Viral Load Results

Reference/Comparison	**Number of Studies**	***Mean Effect (95% CI)**	****Approximate* percent change in viral RNA copy concentration from reference**
Pre-VoC/Alpha	3	-0.271 (-0.38 – -0.16)	+20%
Alpha/Delta	3	-0.265 (-0.36 – -0.17)	+20%
Delta/Omicron	5	0.083 (0.00 – 0.16) ns	- 9.1%

Pre-Variant of Concern (Pre-VoC) represents ancestral strains that existed prior to the designation of the first variant of concern. *Effect size for cycle threshold values. Null = 0 **Estimations assume 1 unit cycle threshold corresponds to a two-fold change in RNA copy concentration (inversely related).

Pre-Variant of Concerns and Alpha Variant

Three studies compared the cycle threshold values between pre-variant of concerns and the alpha variant (figure 7). Two studies found a significantly lower CT estimate for the alpha variant and one study found no significant difference ($p = 0.28$). The total mean effect for all studies was -0.271 (-0.38 – -0.16), $p < .01$.

Alpha Variant and Delta Variant

Three studies compared the cycle threshold values between the alpha and delta variants (figure 8). Two studies found a significantly lower CT estimate for the delta variant and one study found no significant difference ($p = 0.18$). The total mean effect for all studies was -0.265 (-0.36 – -0.17), $p < .01$.

Delta Variant and Omicron Variant

Five studies compared the cycle threshold values between the delta and omicron variants (figure 9). Two studies found a significantly higher CT estimate for the omicron variant and three studies found no significant difference between the two variants. The total mean effect for all studies was 0.083 (0.00 – 0.16), $p = .07$

Figure 7

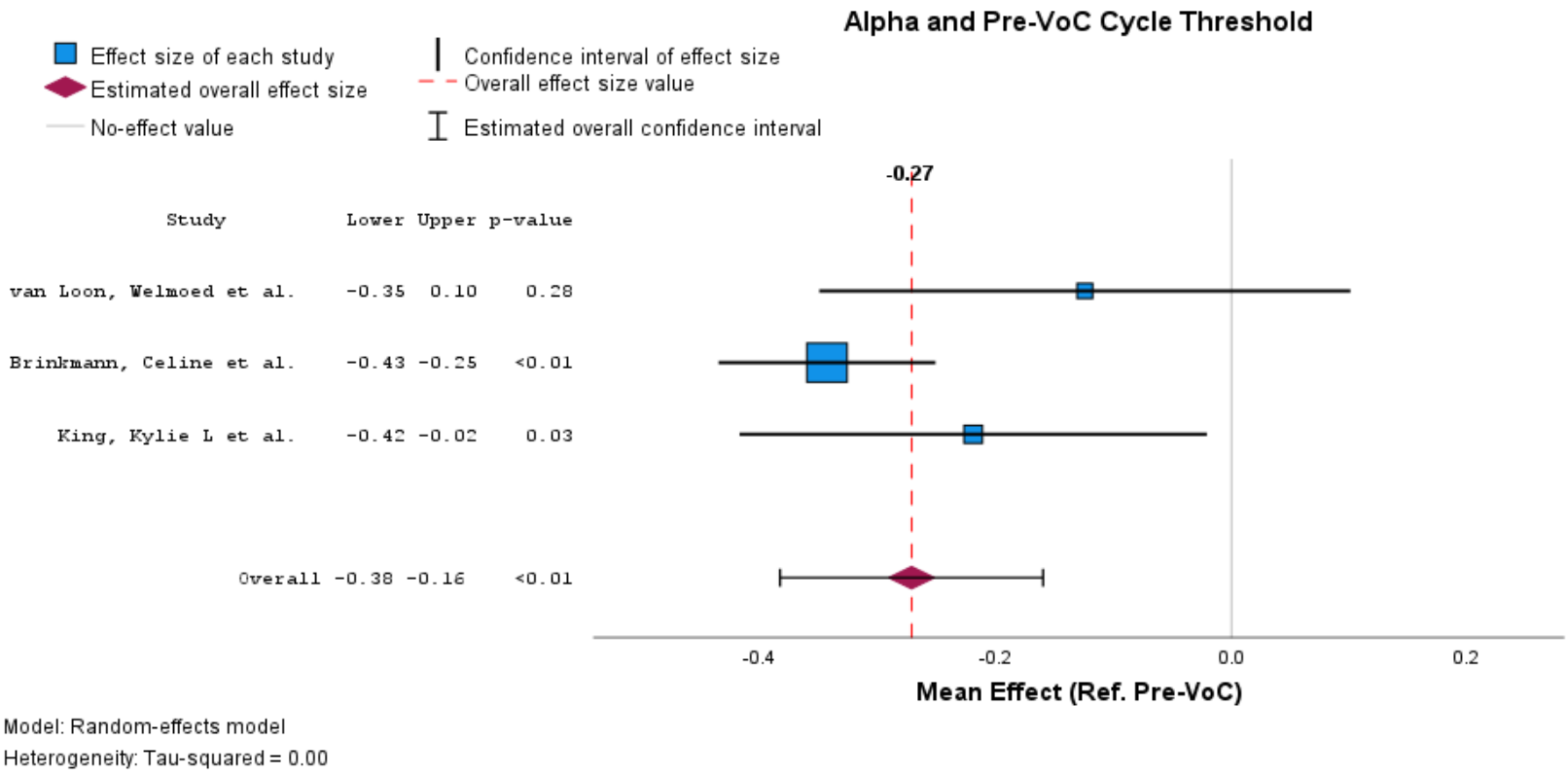
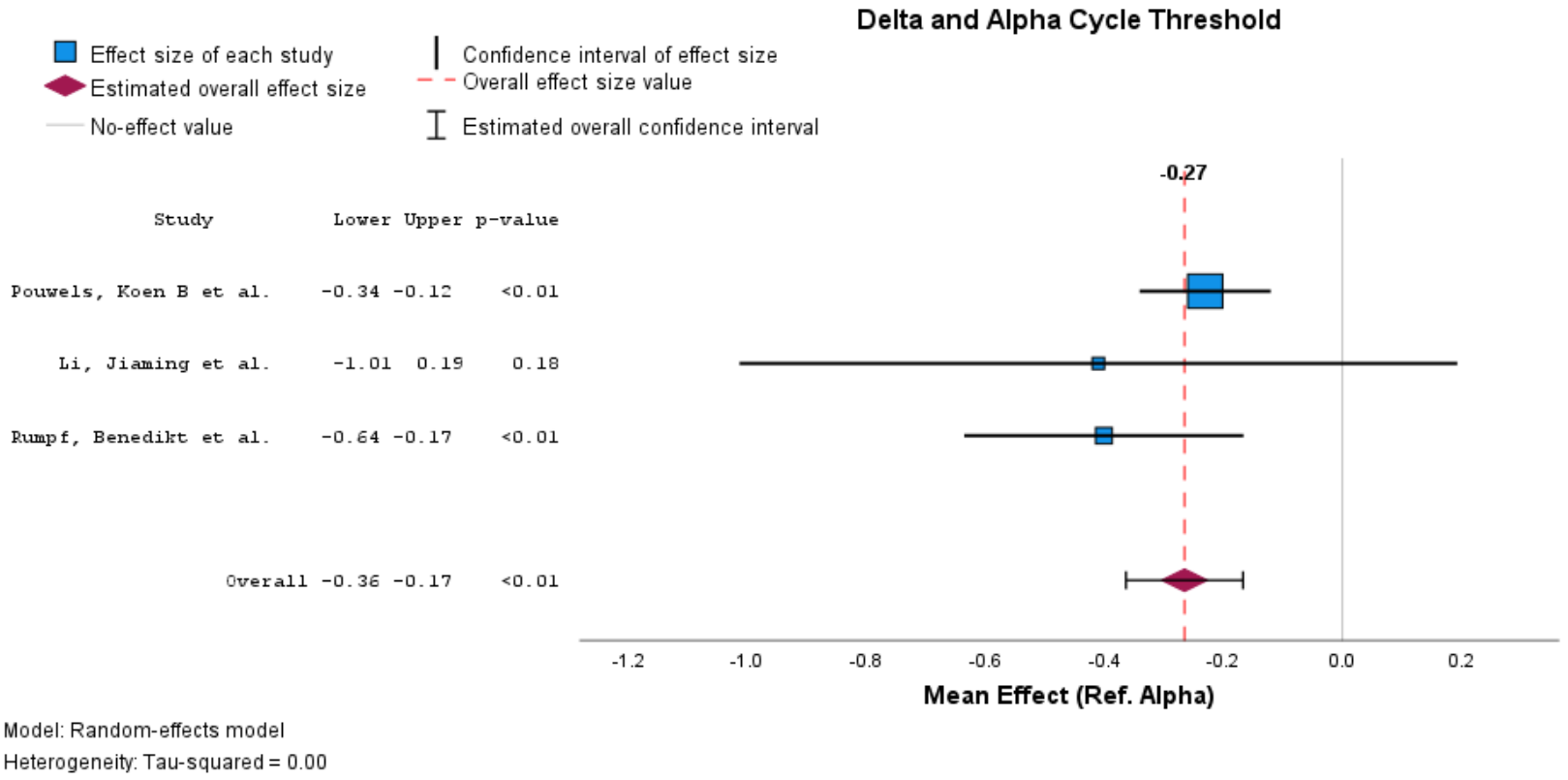
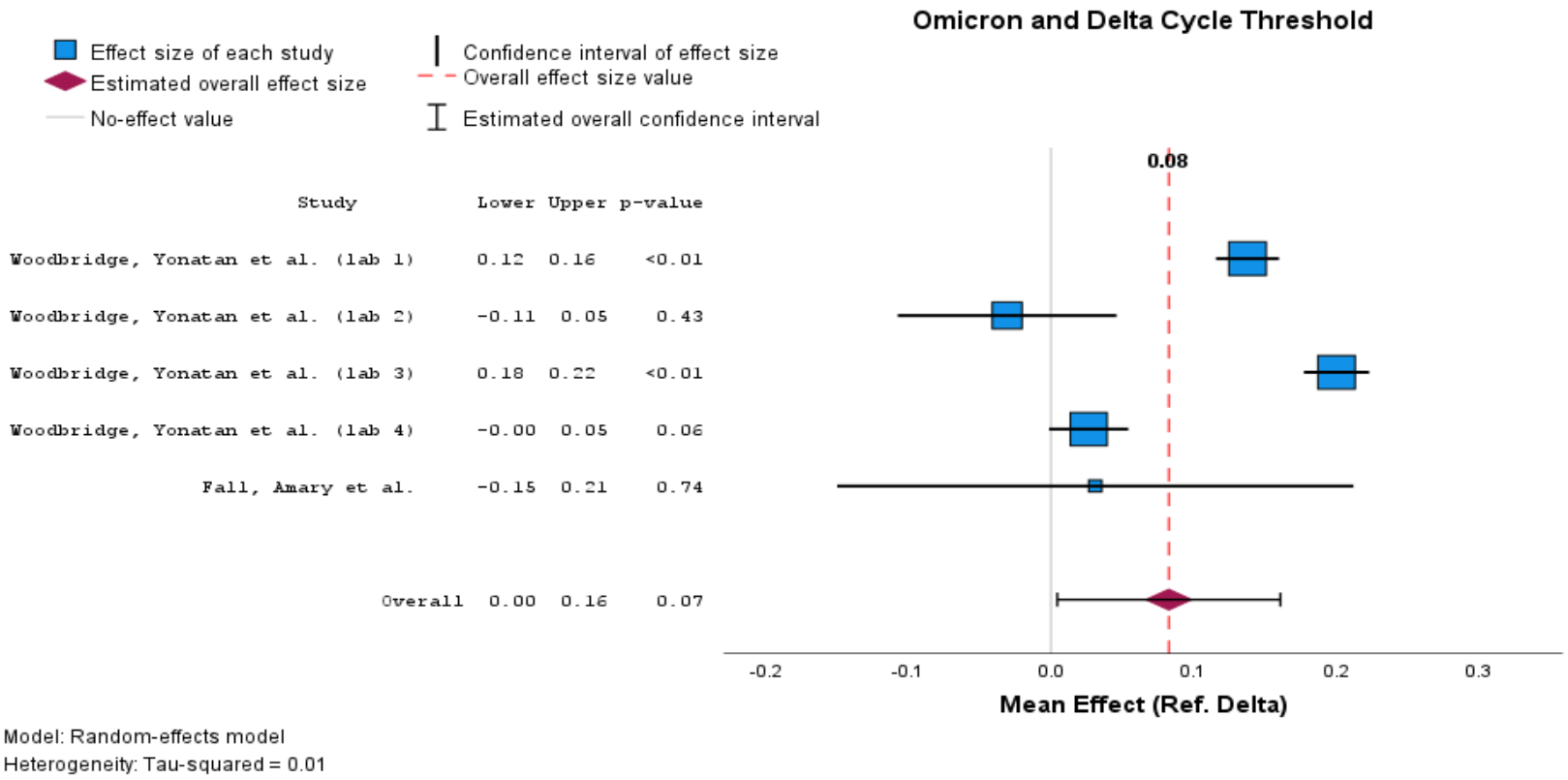

Alpha and Pre-VoC Cycle Threshold
Effect size of each study
Estimated overall effect size
No-effect value
Confidence interval of effect size
Overall effect size value
Estimated overall confidence interval
-0.27
Study Lower Upper p-value
van Loon, Welmoed et al. -0.35 0.10 0.28
Brinkmann, Celine et al. -0.43 -0.25 <0.01
King, Kylie L et al. -0.42 -0.02 0.03
Overall -0.38 -0.16 <0.01
-0.4
-0.2
0.0
0.2
Mean Effect (Ref. Pre-VoC)
Model: Random-effects model
Heterogeneity: Tau-squared = 0.00

Figure 8



Model: Random-effects model
Heterogeneity: Tau-squared = 0.00

Figure 9



Model: Random-effects model
Heterogeneity: Tau-squared = 0.01

Table 9

Cycle Threshold Studies: Region, Sample Size, Inclusion

Study	**Country or region**	**Total sample size**	**Sampling method and inclusion**
(Fall, Eldesouki et al. 2022)	United States	566	Testing through screening or care diagnostics. All ages
(Pouwels, Pritchard et al. 2021)	United Kingdom	11179	Randomly selected households, individuals aged ≥18 years who were experiencing symptoms or required for workplace or school attendance
(Woodbridge, Amit et al. 2022) Lab 1	Israel	33028	Public voluntary screening: individuals aged ≥12 years
(Woodbridge, Amit et al. 2022) Lab 2	Israel	9575	Public voluntary screening: individuals aged ≥12 years
(Woodbridge, Amit et al. 2022) Lab 3	Israel	29737	Public voluntary screening: individuals aged ≥12 years
(Woodbridge, Amit et al. 2022) Lab 4	Israel	20255	Public voluntary screening: individuals aged ≥12 years
(Van Loon, Rössig et al. 2021)	Germany	318	Public testing site all ages
(Brinkmann, Gohl et al. 2022)	Germany	1858	All patients (in and out) in single center
(Li, Zhang et al. 2022)	China	86	Inpatients swabbed on admission
(King, Wilson et al. 2022)	United States	417	Mandatory surveillance testing students and staff
(Rumpf, Lickefett et al. 2022)	Austria	321	Inpatients on admission

Table 10

Cycle Threshold Studies: Specimen Location, Disease Status, Age

Study	**Specimen location**	**Disease Status of Subjects**	**Mean or median age of study cohorts (years)**
(Fall, Eldesouki et al. 2022)	Nasopharyngeal or lateral mid-turbinate nasal swab	Hospital inpatients and outpatients. Asymptomatic and symptomatic, including severe.	Delta median 35 (IQR 17-55); omicron median 32 (IQR 22-46)
(Pouwels, Pritchard et al. 2021)	Naso- or oropharyngeal swab	Asymptomatic and symptomatic; not hospitalized	Delta median 57 (IQR 42,69); Alpha median 56 (IQR 41,68)
(Woodbridge, Amit et al. 2022) Lab 1	Not provided	Asymptomatic and symptomatic; not hospitalized	Not provided
(Woodbridge, Amit et al. 2022) Lab 2	Not provided	Asymptomatic and symptomatic; not hospitalized	Not provided
(Woodbridge, Amit et al. 2022) Lab 3	Not provided	Asymptomatic and symptomatic; not hospitalized	Not provided
(Woodbridge, Amit et al. 2022) Lab 4	Not provided	Asymptomatic and symptomatic; not hospitalized	Not provided
(Van Loon, Rössig et al. 2021)	Oro-nasopharyngeal swab	Mostly symptomatic, not hospitalized	Wild-type mean 36.6 (±13.8); alpha mean 34.8 (±15.9)
(Brinkmann, Gohl et al. 2022)	Naso- or oropharyngeal	In- or outpatient status, asymptomatic or symptomatic	Not provided
(Li, Zhang et al. 2022)	Oropharyngeal swab	Hospitalized	Alpha mean 41.8; delta mean 32.8; omicron mean 34.5
(King, Wilson et al. 2022)	Saliva collection	Asymptomatic and symptomatic; not hospitalized	Pre-VoC mean 30.75; alpha 28.6
(Rumpf, Lickefett et al. 2022)	Nasopharyngeal swab	Hospitalized patients	Alpha median 55 (IQR 45-64); Delta median 52 (IQR 41-65)

Discussion

Summary of findings:

In section one of this chapter, I systematically reviewed SARS-CoV-2 clinical outcome literature. The objective was to characterize an evolutionary trend in virulence from the original strain through Omicron. I used the risk of hospitalization and mortality per infection in unvaccinated persons to assess intrinsic virus virulence. Results from the meta-analyses show that infection with Delta is associated with a greater risk of hospital admission than infection with Alpha, which is associated with a greater risk of hospital admission and mortality than infection with the ancestral virus. Delta is also associated with a greater risk of hospital admission and mortality than Omicron. No studies comparing Omicron to Alpha or the ancestral virus met inclusion criteria.

In section two of this chapter, I systematically reviewed SARS-CoV-2 viral load literature. I conducted meta-analyses on studies that compared qPCR cycle threshold values from URT clinical samples of different variants. Results showed that Delta is associated with higher viral loads than Alpha, which is associated with higher viral loads than the ancestral virus. There was not a significant difference between Delta and Omicron viral loads ($p = 0.07$).

Interpretation:

The results reveal increasing virulence from the ancestral virus through Delta, followed by a sharp reduction in virulence with Omicron. The increased virulence relative to the original virus through Delta suggests that the transmission benefits from higher viral loads outweighed the costs of causing more severe disease. One benefit of higher viral loads for SARS-CoV-2 is increased infectiousness, i.e., a higher chance of

transmission given contact between infected and susceptible hosts (Marc, Kerioui et al. 2021, Marks, Millat-Martinez et al. 2021). Higher growth rates and viral loads can also extend the duration of potential transmission and thereby increase fitness. Within individuals, SARS-CoV-2 growth rates and peak viral loads are negatively associated with viral clearance rates and the duration of viral shedding is considerably longer in Alpha, Delta, and Omicron compared to historical lineages (Wang, Chen et al. 2021, Elie, Roquebert et al. 2022, Puhach, Meyer et al. 2023).

Because lower respiratory tract specimens are tested infrequently, it was not possible to perform a meta-analysis on viral loads within them. This would have allowed for a more complete assessment of adaptive change in replication rates and tissue tropism. However, in vitro and in vivo studies have shown that Alpha and Delta were more exploitative throughout the body and that higher viral loads were not restricted to the URT. In both variants, the mutations that are associated with increased transmissibility do not involve cellular characteristics that would be differentially expressed and upregulated in the upper respiratory tract exclusively. Instead, these mutations mostly involve the viral spike protein and increase binding affinity and entry into cells with the surface receptor ACE-2, which is expressed in a wide range of cell types (Hikmet, Méar et al. 2020). Delta's P681R mutation seems to play a particularly important role not only enhanced cellular infectivity but also pathogenicity. The mutation causes increased viral fusogenicity, the formation of larger syncytia, and distinct lung histopathologies (Saito, Irie et al. 2022).

Despite the previously described benefits associated with higher virulence, the observed evolutionary trend from the ancestral virus through Delta was surprising. Given

the IFR and environmental survivability of other respiratory pathogens, overall selection for lower virulence was expected. One possibility is that a lower optimal virulence may not necessarily imply a downward trend right from the beginning. Omicron is substantially more benign than Delta which suggests that although increased virulence was favored initially, it probably was not optimal. As our results demonstrate, the decreased virulence from Delta to Omicron was not accompanied by a significant reduction in URT viral load. These findings argue that Omicron's transmission advantage over Delta is largely attributable to reduced virulence rather than higher rates or longer durations of viral shedding. With Omicron, natural selection apparently found a way to retain most of the benefits of high viral loads, while removing the cost of severe disease. This win-win for Omicron was associated with a changing tissue tropism and a reduction in the exploitation of the lower but not upper respiratory tract. Omicron replicates less efficiently in lung organoids and lung epithelial cells and has a reduced ability to form syncytia compared to previous variants (Meng, Abdullahi et al. 2022, Peacock, Brown et al. 2022, Willett, Grove et al. 2022). These changes are thought to manifest from multiple mutations in the spike protein which shifted Omicron's cell entry mechanism away from use of TMPRSS2 – a cell surface receptor protein that is expressed at high levels in lung epithelial cells. Is it also expressed in other cells that are outside the lungs and adversely affected in COVID patients, such as cardiovascular, neuronal and intestinal cells (Cao, Feng et al. 2021).

Omicron is the only SARS-CoV-2 variant still reported to be in circulation but has undergone extensive diversification leading to genetically and phenotypically distinct sub lineages. Much of evolutionary change within Omicron results from selection to evade

acquired immunity and it unclear how intrinsic virulence has changed across subvariants. Most infections are now in people with some level of acquired immunity, which allows for a robust comparison in infection virulence between it and other respiratory pathogens. The IFRs for Omicron BA.1 and BA.2 have been estimated at 0.03% and 0.09% respectively (Chen, Yan et al. 2022, Ulloa, Buchan et al. 2022), which places them roughly equal to influenza A but higher than parainfluenza, respiratory syncytial virus, rhinoviruses, and endemic coronaviruses.

Strengths and limitations:

Rather than relying on a single metric for virulence this used two clinical outcomes that involve different aspects of virulence. Hospital admission is more arbitrary than death, however some changes in virulence are sub-lethal and will not manifest in mortality risk. Hospitalization from COVID-19 occurs much more commonly than death does, and thus meaningful (statistically significant) change is easier to detect. The results show that these two outcomes were always in agreement, although more studies on hospitalization risk were included overall. It is also important to note, and as mentioned in the Methods, this study defines hospitalization as being admitted into the hospital as an in-patient with COVID-19. Another strength of this study is that it restricted virulence analyses to literature in which the risk denominator was a positive infection in out-patient or community testing setting. This study excluded publications that examined relative differences in mortality between variants but did so by assessing the risk in patients already hospitalized with COVID-19. This can lead to a strong bias for the null hypothesis if, as I showed, the risk of hospitalization differs between variants to begin with. Because vaccination can influence viral load and clinical disease outcome, both

systematic reviews only included studies with unvaccinated subjects or if vaccination was accounted for in the multivariable model.

One limitation of this study is that viral load results were not statistically adjusted for potential confounders, including age and sex. This is because most studies reported cycle threshold differences using only a p-value and not as an effect size. Unadjusted mean differences are thus the only way to quantitatively combine results from different studies into a meta-analysis. It is unclear to what degree these variables influence viral load, but qualitatively the subject groups within most studies were similar in age and sex ratio (Table 10). Another limitation is that most, but not all the virulence studies accounted for prior infection. This may have disproportionately underestimated Omicron virulence because of its increased ability for re-infection. However, there is no striking difference between studies that did and did not account for history of infection as shown by minimal between study heterogeneity in the Omicron analyses.

In conclusion, the results of this study demonstrate that SARS-CoV-2 evolved to be more exploitative and virulent throughout the first half of this pandemic (ancestral virus through Delta). Then, selection favored the Omicron variant, which is characterized by equal intensity of exploitation in the URT but reduced virulence, explaining its fitness advantage over the Delta variant. The exploitation strategy observed in Omicron is comparable to endemic respiratory pathogens that are similarly dependent on host health and mobility for transmission. I was unable to directly compare the virulence of Omicron to the ancestral virus to see if intrinsic virulence has reduced since the start of the pandemic.

CHAPTER IV

VARIATION IN PLASMODIUM VIRULENCE

Summary

Malaria is caused by infection with *Plasmodium* parasites. It kills an estimated 400,000-500,000 people per year and is the leading cause of child mortality in sub-Saharan Africa (Ho 2019). The harmfulness of malaria varies, spatially and temporally, at the host population level. The focus of this chapter is to review what is known about the factors that contribute to this variation. This chapter is meant to be a holistic review, covering non-mutually exclusive factors, some of which are well-established in their contribution to malaria virulence and others that are not. I review and discuss each factor, explaining why it is expected to affect disease severity and if it is consistent with epidemiological patterns in virulence. Specifically, these factors are intrinsic virulence, reproductive plasticity, host genetics, and naturally acquired immunity. The contributions of intrinsic virulence and reproductive plasticity are less established than the others, and so these sections are partly a critical evaluation of the evidence.

Figure 10

Asexual Life Cycle of Human *Plasmodium*

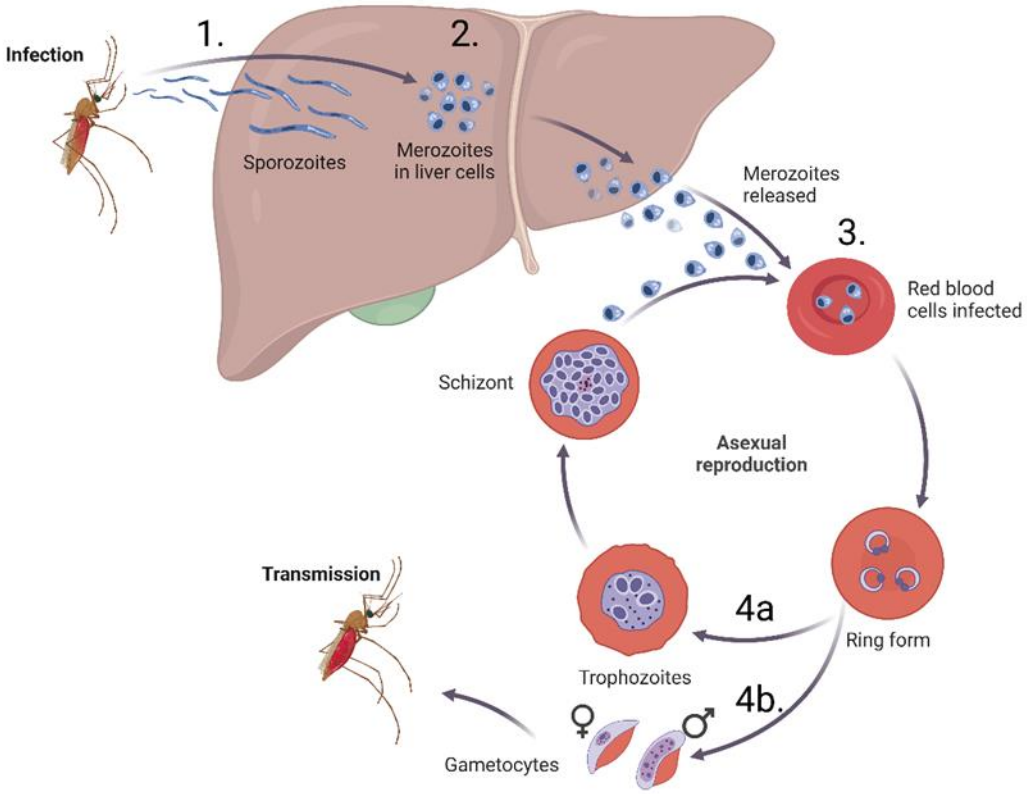


Illustration adapted from Biorender.com with thesis publishing privileges

(1) The sporozoites released from *anopheles* mosquito enter human blood and migrate to the liver to infect hepatocyte cells (cells not shown). (2) Within the hepatocyte, the sporozoite nuclei and organelles replicate leading to multinucleated schizonts

(schizogony). Cytokinesis subdivides the schizont into individual daughter cells called merozoites, which rupture the hepatocyte. (3) The merozoites begin infecting red blood cells (RBC). Inside the RBCs, the merozoites develop into ring-stage immature trophozoites. From there, the immature trophozoites can undergo one of two developmental processes: (4a) Development into mature trophozoites which undergo schizogony and create merozoites. The merozoites rupture the RBC and repeat the cycle. (4b) Differentiation into a male or female gametocytes which are then transmitted to the mosquito during feeding.

Intrinsic Variation in Virulence

Variation in malaria severity could result from differences in intrinsic virulence driven by natural selection. As described in chapter I, horizontally transmitted vector borne pathogens pay a relatively small cost for high virulence because they do not depend on host mobility for transmission. For these pathogens the primary cost to virulence is assumed to be host mortality rather than morbidity. For vector borne pathogens the spatial and temporal abundance of susceptible vectors and thus transmission frequency might also influence optimal virulence. In regions with high year-round densities of susceptible vectors and high transmission rates, there is little evolutionary incentive for within-host persistence. Early and frequent transmission is expected to favor variants that replicate rapidly and become infectious earlier (Cooper, Reiskind et al. 2002, Ewald 2004, Cressler, McLeod et al. 2016). Regarding *Plasmodium*, the assumptions behind this hypothesis are supported by the available evidence. Erythrocytic-stage multiplication rate has a genetic basis and is positively associated with both disease severity and the

likelihood that the sexual stage (gametocyte) is transmitted to the mosquito during feeding. (Mackinnon and Read 1999, Chotivanich, Udomsangpetch et al. 2000, Ferguson, Mackinnon et al. 2003). Also, genetically diverse infections occur more commonly during periods of high transmission (Daubersies, Sallenave-Sales et al. 1996), and within-host competition favors increased merozoite replication rates and higher virulence (Bell, De Roode et al. 2006, Pollitt, Mideo et al. 2011)

Plasmodium falciparum and *Plasmodium vivax* are the most prevalent species of human *Plasmodium*. Although both species cause malaria, the infection fatality ratio of *P. falciparum* is approximately ten times higher than *P. vivax* (Hwang, Cullen et al. 2014). This difference in virulence is attributable to the contrasting exploitation strategies of each species. *Plasmodium falciparum* is characterized by traits that enhance blood-stage propagation. Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is a family of proteins that has a key role in merozoite multiplication and disease severity. Expression of the proteins on the outside of infected red blood cells causes cytoadherence to both vascular epithelial cells and uninfected red blood cells. In doing so, merozoites avoid clearance in the spleen and can spread more easily to uninfected cells. Unfortunately, the adhesion of these cells to the blood vessel can cause microvascular obstruction and other severe pathologies (Buffet, Safeukui et al. 2011). The larger effect of rapid red blood cell destruction also contributes to a higher risk of severe disease outcomes including anemia (Mohandas, An et al. 2012). *Plasmodium vivax* is more benign, often causing chronic infections with sub-clinical parasitemia levels. It also has a distinct hypnozoite stage that can remain latent in the liver for months (Price, Commons et al. 2020, Schäfer, Zanghi et al. 2021).Why the two species differ in reproductive

strategy and virulence is partly explained by their geographical ranges. The range of *P. vivax* extends into temperate regions where anopheles are present only part of the year (Howes, Battle et al. 2016). Lengthy periods with no transmission have probably favored parasites with restrained reproduction and a reduced chance of causing acute host death. Alternatively, *P. falciparum's* geographic range is restricted more to equatorial regions where transmission intensity is typically (although not always) higher throughout the year (Guerra, Snow et al. 2006). In these settings, rapidly multiplying strains that monopolize the limited availability of RBCs and become infectious earlier will have a competitive advantage.

Genetic and epidemiological evidence support the hypothesis that transmission intensity influences the evolution of intraspecific *Plasmodium* virulence. *Plasmodium falciparum* is most common in equatorial regions but nonetheless exists across a range of transmission intensities (Guerra, Snow et al. 2006). A higher proportion of asymptomatic *P. falciparum* infections were found in a region with unstable and seasonal transmission compared to a region with stable levels of transmission (Elhassan, Hviid et al. 1995). Transmission intensity is also associated with higher blood parasitemia levels and an increased risk of anemia and spleen enlargement in children (Guillebaud, Mahamadou et al. 2013, Ardiet, Graz et al. 2014). These observations are consistent with higher multiplication rates and increased virulence evolving in response to transmission frequency, but other hypotheses, including a dosage effect or reproductive plasticity, predict similar patterns.

Genetic evidence supports the idea of intrinsic reproductive differences between strains from low and high transmission settings. A population genomic study found that

loci governing gametocyte development were highly genetically divergent between strains from low and high transmission settings, however, it did not evaluate if the differences were associated with higher reproductive rates or virulence (Mobegi, Duffy et al. 2014). Relatedly, at least one laboratory study has demonstrated that *P. falciparum* strains from high transmission settings have higher in vitro merozoite growth rates than those from low transmission settings (Rono, Nyonda et al. 2018).

The evidence for genetic variation in *P. vivax* exploitation and virulence is less compelling than for *P. falciparum*. The latency period of hypnozoites varies geographically, with frequent relapses in the tropics and prolonged relapses in more temperature regions (Battle, Karhunen et al. 2014). This observation accords with the evolutionary hypothesis that low vector abundance selects for persistent strains with reduced rates of propagation and lower acute virulence. However, there is little evidence that differences in hypnozoite latency are genetically based. The best evidence for intrinsic variation comes from an experimental study in the 1970s (Shute, Lupascu et al. 1976). The authors observed that a temperate strain of *P. vivax* had a shorter prepatent period than a tropical strain, and that the periods could only be equalized with disproportionately higher inoculum loads for the temperate strain. To date, however, no studies have established a relationship between *P. vivax* genotypes and latency duration or disease severity.

Reproductive Plasticity and Plasmodium Virulence

Previously I explained why *Plasmodium* exploitation and virulence are expected to evolve in response to transmission intensity. Evolution might also favor variants with

the ability to facultatively regulate exploitation as a function of transmission opportunity. Specifically, under conditions of variable transmission opportunity, facultative reproduction is predicted to outcompete fixed alternatives (Cornet, Nicot et al. 2014). For malaria parasites in regions with variable *Anopheles* densities due to seasonality, the ability to detect transmission opportunity and adjust the rate of exploitation accordingly would be especially beneficial. In theory, reproductive plasticity allows the parasite to aggressively extract host resources and propagate during periods of high transmission rates and to restrain exploitation in periods of low transmission rates. Species with life cycles that include a true stage of dormancy, such as *P. vivax*, can remain latent in an exoerythrocytic stage, evading immune detection and avoiding the risk of infection-induced host death. Upon a reliable transmission cue, they could exit dormancy and begin erythrocytic-stage replication. Species lacking a true dormancy stage (e.g., *P. falciparum*) could regulate the rate of erythrocytic-stage replication and similarly minimize the risk of host death until transmission opportunity arises again. In either case, the exploitation of the host, and thus virulence, increases in response to the cue indicating transmission potential.

Because of the limitations associated with human studies, the most compelling evidence for *Plasmodium* reproductive plasticity comes from non-human species. Blood densities of *Plasmodium relictum* elevate in response to corticosterone, a host hormone that increases with the spring season (Applegate 1970). Densities of the natural vector for *P. relictum* begin increasing at the start of spring which suggests an indirect environmental cue used by the species to detect transmission opportunity. More direct cues have also been demonstrated. In Cornet, Nicot et al. (2014), *P. relictum* increased

replication rates in response to the avian host being probed by uninfected vectors. *Plasmodium chabaudi* has displayed similar exploitation strategies in response to vector probing in some settings but not others (Billingsley, Snook et al. 2005, Shutler, Reece et al. 2005). Geographic origins of the strains provide a resolution to the conflicting results because plasticity is less likely to evolve in parasites sampled from regions of constant year-round transmission (Cornet, Nicot et al. 2014). It is unresolved if the changes in blood parasitemia levels from these experiments result from the parasites exiting dormancy, increasing blood-stage replication rates, or both.

Most evidence for reproductive plasticity in human *Plasmodium* stems from epidemiological data. In temperate regions *P. vivax* malaria is associated with increasing vector densities that come with warming weather. However, the timing of disease spikes makes new infections an unlikely cause and instead implicates relapsing hypnozoites acquired months before (Gill 1938, Winckel 1955, Venkatesan, Dedicoat et al. 2003, Huldén, Huldén et al. 2008). The probing of uninfected vectors has been put forward as the best explanation for the timing of these events although indirect cues such as hormone levels and sunlight may also play a role (Venkatesan, Dedicoat et al. 2003, Huldén, Huldén et al. 2008). While transmission cues seem to associate with a higher proportion of symptomatic infections, which is an indication of higher virulence, it is unclear if they also associate with a greater risk of severe disease and death.

The facultative reproduction of *P. falciparum* is less studied than that of *P. vivax*, partly because it lacks a dormant hypnozoite stage. However, *P. falciparum* can persist asymptomatically in a host at low parasitemia levels for months, often followed by sharp spikes in blood densities at the beginning of wet seasons (Mayengue, Kouhounina

Batsimba et al. 2020). It is unknown if the cause of these blood-density spikes is due solely to new infections or if increased growth rates contribute as well. The latter is feasible because *P. falciparum* is well known for complex gene regulation and has shown the ability to regulate reproduction in response to environmental changes (Reece, Ali et al. 2010, Leggett, Benmayor et al. 2013).

Overall, the evidence that some species of *Plasmodium* facultatively regulate reproduction in response to transmission cues is strong. In theory we should expect the same from *P. vivax* because its range often extends to temperate regions. Also, like *P. relictum* and *P. chabaudi, P. vivax* has a true dormancy stage which makes the ability to detect transmission especially beneficial. Nonetheless, the evidence that *P. vivax* does so is limited to correlational studies. The evidence that *P. falciparum* regulates replication in response to transmission cues is scarce. However, complex gene regulation is a defining feature of *P. falciparum* and populations of the parasite that have evolved in regions with dry seasons would theoretically benefit from this ability.

For both *P. vivax* and *P. falciparum*, one implication of reproductive plasticity is temporally fluctuating virulence. Thus, predictions made from this hypothesis overlap with the idea that pathogen virulence evolves across seasons. Although these hypotheses are not mutually exclusive, evolution for reproductive plasticity implies a selective advantage over fixed alternatives making the coexistence of both traits in the same population unlikely. From epidemiological data alone it is difficult to determine which process accounts for the observed changes in blood parasitemia, symptomatic ratio, and disease severity across seasons.

Genetic Variation in Host Resistances

Differences in *Plasmodium* virulence may arise because host populations differ genetically in their ability to resist infection or reduce disease. Mechanisms of host protection can come from 1) the host's ability to resist initial infection 2) the host's ability to reduce growth and replication after infection and 3) the host's ability to better tolerate infection. For this section, I use the term "genetic resistance" to encompass all these aspects.

Hemoglobopathies:

Hemoglobopathies are the most common form of genetic resistance against malaria. During the blood-stage of the life cycle, malarial parasites invade red blood cells, replicate, and digest the protein portion of the oxygen transport molecule hemoglobin. Normal adult hemoglobin (HbA) is constructed of four polypeptide chains; two alpha (HAA) and two beta (HBB) globins. Variation in the composition or number of these chains can create intracellular environments that hinder parasite survival and growth. Despite significant reductions in performance compared to normal hemoglobin (HbA), and even lethal effects in some homozygotes, hemoglobin variants are found in high frequencies throughout malaria endemic regions. This presence is a testament to the selection pressures that malaria has placed on humans historically.

The most studied genetic resistance to malaria is sickle-cell trait. It originates from a single nucleotide polymorphism in the β-globin gene that leads to an amino acid substitution of glutamic acid to valine, giving rise to the structural variant hemoglobin S (HbS). The protective properties manifest from numerous immunological and biochemical pathways that collectively help to resist infection, reduce disease

progression, and increase tolerance (Gong, Parikh et al. 2013). Sickle-cell trait refers to those individuals who are heterozygotes (HbAS), possessing one normal allele and one hemoglobin S allele. These individuals gain protection from clinical malaria caused by *P. falciparum* but avoid the severe sickle-cell anemia disease present in homozygotes (HbSS). Sickle-cell trait reduces severe malarial anemia by between 60 and 90 percent in infants and young children (Hill, Allsopp et al. 1991, Aidoo, Terlouw et al. 2002, Piel, Patil et al. 2010)

Hemoglobin C **(**HbC**)** is a genetic variant in the β-globin gene at the same location as HbS, but glutamic acid is replaced by lysine. Like HbS, the single amino acid substitution provides numerous mechanisms that collectively protect against malaria (Fairhurst, Fujioka et al. 2003, Fairhurst, Baruch et al. 2005, Verra, Simpore et al. 2007). However, homozygotes (HbCC) are not at risk of serious disease complications unlike HbSS. Overall, heterozygotes are afforded estimated reductions in risk of clinical *P. falciparum* malaria at near 30 percent and homozygotes at over 90 percent (Modiano, Luoni et al. 2001, Fairhurst, Fujioka et al. 2003, Fairhurst, Baruch et al. 2005, Verra, Simpore et al. 2007).

Hemoglobin E (HbE) is another hemoglobin variant that protects against malaria. Like HbS and HbC, it arises from a mutation in the β-globin gene, but the polymorphism is at a different nucleotide location in the gene. The primary protective property comes from changes in the cellular membrane which reduce susceptibility to merozoite entry (Chotivanich, Udomsangpetch et al. 2002). Hosts with hemoglobin E trait (HbAE) are nearly seven times less likely to develop severe complications from *P. falciparum* infections (Hutagalung, Wilairatana et al. 1999).

Thalassemia

Thalassemia is an umbrella term for describing polymorphisms which lead to an imbalance of hemoglobin α and β polypeptide chains. In humans, two different genes and a possible four alleles are expressed to produce the α -chains of the hemoglobin protein. α+ -thalassemia refers to individuals with either one or two of the four alleles lacking expression, resulting in a reduction in the number of total α-chains and the presence of unpaired β-chains. The exact mechanism(s) for how α+ -thalassemia might protect against malaria are not entirely understood. α+ cells are not resistant to parasite invasion or growth (Luzzi, Torii et al. 1990), nor do they protect against cytoadherence once infected (Luzzi and Pasvol 1990). Infected α + cells do, however, have reduced binding affinity to uninfected cells, i.e., rosetting (Carlson, Nash et al. 1994), a process which can lead to blood clotting and merozoite dissemination into vital organs. Additionally, infected α + cells have increased binding affinities for immunoglobins (Luzzi, Merry et al. 1991). At least one large cohort study has shown that α+ -thalassemia is not associated with a reduction in symptomatic malaria, nor a reduction in blood parasitemia levels, but is associated with a reduction in severe malaria (Wambua, Mwangi et al. 2006). Alternatively, the β -chains in hemoglobin are controlled by only one gene, and lack of expression in one of the two alleles leads to unpaired α -chains, resulting in β -thalassemia. It is considerably less common than α-thalassemia, but is associated with significant reductions in *P. falciparum* blood parasite densities and symptomatic malaria in young children (Willcox, Björkman et al. 1983).

Duffy Negative Phenotype

The human Duffy antigen is a transmembrane glycoprotein that acts as a receptor for *P. Vivax.* Region II of the parasite's Duffy binding protein (DBP) binds to the Duffy antigen and allows for *Plasmodium's* passage into human erythrocytes. A single nucleotide polymorphism in the antigen promoter's GATA box causes a transcriptional interruption and silences the allele coding for the antigen, a process associated with the Duffy-negative phenotype. Total resistance to *P. vivax* is achieved with expression of a homozygous phenotype for the Duffy-negative blood group antigen, while partial resistance has been observed with a heterozygous phenotype (Kasehagen, Mueller et al. 2007). The near absence of *P. vivax* in Central Africa is attributed to Duffy antigen receptor negativity.

Glucose-6-phosphate dehydrogenase deficiency

Glucose 6-phosphate dehydrogenase (G6PD) catalyzes the first reaction of the pentose phosphate pathway and is essential in maintaining redox equilibrium because of its role as sole supplier of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The *G6PD* gene is X-linked and highly polymorphic with over 300 alleles. Different alleles can vary substantially in G6PD activity. More deficient alleles expose RBCs to oxidative stress by lack of G6PD function and therefore lack of NADPH. The resulting oxidative stress creates an intracellular environment unfavorable for pathogen growth and replication (Miller, Golenser et al. 1984, Golenser, Miller et al. 1988, Turrini, Schwarzer et al. 1993). Conflicting epidemiological evidence exists on the protective properties of G6PD deficiency (Ruwende and Hill 1998). Varying observations probably arise for several reasons, including sex-linkage and high heterogeneity in host populations. However, at least one allele that persists at considerable frequency in Africa

(A-) is associated with *P. falciparum* resistance in heterozygous females and hemizygous males (Ruwende, Khoo et al. 1995).

Naturally Acquired Immunity

The body of literature covering acquired immunity to malaria is vast. The goal of this section is not to review in depth the entirety of mechanisms governing these responses, but to focus on broad trends within the field that help explain variation in virulence at the individual and population levels.

Naturally acquired immunity (NAI) provides considerable protection against malaria. Today most immunologically naïve travelers to endemic regions take prophylactics to prevent infection or therapeutics to halt disease progression. However, historical references shed light on the severity of malaria in immunologically naïve hosts who lacked the benefits of modern medicine. Through 1819-1831 naïve French troops stationed in Senegal died from malaria infections between 9-58% of the time (Curtin 1994). In one 19th century expedition, naïve Europeans traveling to malaria endemic regions were reported to die from fevers at rates of 28% (McGregor 1993). Comparatively, the mortality per infection in adults living endemically with *P. falciparum* is <1% (Sani Kalil, Hasen Bedaso et al. 2020). Although host genetics contribute to this discrepancy, they do not explain the disproportionate mortality rates in children. It is estimated that children under 5 years of age account for two in every three malaria deaths (Owusu-Agyei, Koram et al. 2001). Data from infected migrant groups suggest that the virulence discrepancy between adults and children is due to acquired immunity rather than innate defenses. In instances of migration from non-endemic regions into endemic

regions, the severity of malaria was equal or greater in adults compared to children (Baird 1998, Calleri, Lipani et al. 1998, Barcus, Krisin et al. 2003).

Acquired immune responses to malaria can be broken down into two categories: 1) anti-disease immunity and 2) anti-parasite immunity. The former is acquired sooner and involves mechanisms aiding in parasite tolerance, i.e., the ability of the host to reduce disease severity for a given parasitemia level. *Plasmodium* infections harm the host through damage inflicted by the parasite directly and through overreactive inflammatory responses. Overreactive inflammation is tightly coupled with severe malaria outcomes and controlling harmful inflammation is the anti-disease arm of malaria immunity (Artavanis-Tsakonas, Tongren et al. 2003, Ademolue and Awandare 2018). Although inflammation is a generalized fast-acting innate immune response, memory B and T cells play important roles in controlling the mediators of these responses (Cai, Hu et al. 2020). Unlike anti-parasite immunity which requires a high number of exposures, the processes that regulate inflammation need only a few exposures to provide significant protection. The risk of severe malaria drops quickly beginning at 2-5 years of age, however high parasitemia levels persist into adolescence until effective anti-parasite immunity develops (Doolan, Dobaño et al. 2009).

Anti-parasite immunity keeps parasite numbers low during an infection or clears them entirely. The ability to do so against *P. falciparum* takes an unusually long time to develop, often over a decade of living in an endemic region. Although our immune systemS can target all stages of *P. falciparum*'s life cycle, the blood-stage is preferentially targeted. In response, *P. falciparum* has evolved complex gene regulation strategies to evade merozoite destruction. Through antigenic variation – a process by which a single

clonal genotype sequentially varies surface antigens – *P. falciparum* can significantly delay immune-mediated destruction of infected RBCs. To perform the rosetting and sequestration functions, PfEMP1 proteins need to be expressed on the outside of infected red blood cells. Human red blood cells are enucleated and lack MHC-I, which is used by most other cells to indicate to the immune system that they are infected. PfEMP1 expression therefore provides an important opportunity to detect intracellular infection. Unfortunately, *P. falciparum*'s genome has at least sixty different genes (*var* genes) that code different proteins within the PfEMP1 family. Only one of these genes is expressed at a time and each generation expression shifts to another var gene (Deitsch and Dzikowski 2017). This antigenic variation puts the immune system in a continual state of catch up because cross-reactivity between different PfEMP1 antigens is limited (Joergensen, Turner et al. 2006).

At an individual level NAI explains why young children and migrants are at high risks for severe malaria. At the population level it explains why introductions of malaria into new regions or re-introductions after prolonged absences are associated with high disease burden. There is an important implication to these observations. Climate change is already responsible for shifts in the range of *Anopheles* mosquitoes (Carlson, Bannon et al. 2023), and as the warming trend continues it is predicted that vector ranges will more frequently overlap with human populations that have little or no acquired immunity (Ngarakana-Gwasira, Bhunu et al. 2016). Where this happens, we can expect high infection virulence in adults and children alike.

Conclusions:

Factors of the environment, the host, and the parasite contribute to the severity of malaria. Host genetics and naturally acquired immunity play well-established roles in reducing disease severity, although at the individual level genetic factors are less time dependent. Environmental cues that indicate transmission potential appear to increase the proportion of symptomatic *P. vivax* infections, probably due to the parasite exiting dormancy and increasing blood-stage multiplication rates. The contribution of reproductive plasticity to severe disease outcomes is unknown. Finally, intrinsic parasite virulence also appears to influence malaria severity. For *P. falciparum*, high transmission rates likely favors more exploitative strains with higher blood parasitemia levels and increased virulence. Separately, each of the above factors helps to explain certain epidemiological patterns in *Plasmodium* virulence (Table 11). An approach that integrates these different factors may be useful for explaining the total spectrum of variation between populations. Accurately predicting relative differences in virulence will require an understanding of the factors present in each population and their relative contribution to disease.

Table 11

Factors That Contribute to Malaria Virulence

Factor D**escription**	**Evidence**	**Implications**
Reproductive plasticity: The facultative regulation of reproduction in response to direct and indirect transmission cues	• Parasitemia levels of non-human *Plasmodium* increase in response to indirect (corticosterone) and direct (vector probing) transmission cues (Applegate 1970, Billingsley, Snook et al. 2005, Cornet, Nicot et al. 2014) • *P. vivax* relapse in humans is associated with the onset of spring (Huldén, Huldén et al. 2008)	• In regions with variable transmission intensity, vector control should reduce the ratio of symptomatic infections • Seasons with unusually high biting rates should experience more symptomatic infections.
Naturally acquired immunity: The acquired immune system protects against severe malaria (anti-disease immunity) and reduces parasite growth (anti-parasite immunity).	• Adults living in endemic regions experience reduced virulence and parasitemia levels compared to children and naïve travelers (Owusu-Agyei, Koram et al. 2001, Kamau, Mtanje et al. 2020) • Adults experience equal or more severe disease compared to children when both are immunologically naïve (Baird 1998, Barcus, Krisin et al. 2003) • Malaria epidemics are associated with high virulence (Romi,	• If malaria is introduced to a new region or re-introduced after a long absence, the infection virulence will be high. Monitoring changes in the vector's range is important

	Razaiarimanga et al. 2002)	
Host genetic resistances: Innate genetic factors reduce the risk of severe malaria outcomes	• Laboratory and epidemiological studies demonstrating the protective effects of numerous genetic resistances to malaria (see in-text citations)	• The virulence of malaria in populations with high frequencies of genetic resistances is not representative of virulence broadly
Natural selection: Intrinsic virulence evolves as a function of transmission intensity	• Genetic differences in multiplication rates across strains, including higher replication in strains from higher transmission settings(Mobegi, Duffy et al. 2014, Rono, Nyonda et al. 2018) • Positive associations between parasitemia, virulence, and transmission intensity (Elhassan, Hviid et al. 1995, Guillebaud, Mahamadou et al. 2013, Ardiet, Graz et al. 2014)	• Vector control measures should reduce the mean infection virulence in a host population • Interventions that isolate severely disease hosts, such as mosquito-proof dwellings, should select for milder strains

CHAPTER V

SUMMARY AND CONCLUDING REMARKS

In chapter II, I analyzed medical records from University of Louisville Health Services and tested associations that are consistent with *C. trachomatis* contributing to depression as a side effect of within-host persistence. I reasoned that if *C. trachomatis'* IDO upregulation causes chronic tryptophan depletion in patients, then depression should be associated with infection independently of urogenital symptoms. In support of this hypothesis, I found a significant association between infection and depression even after restricting the analysis to patients without urogenital symptoms. I also reasoned that the effect would be greater in females compared to males because of exacerbating effects the menstrual cycle and bacterial vaginosis may have. The results showed a significant association between depression and infection in females but not in males. However, the strength of association (effect size) did not differ significantly between the sexes. These findings technically reject the hypothesis of a sex difference, though it is possible the difference in effect would manifest with a larger sample size.

Experimental studies that evaluate the effect of antibiotic treatment on depression within *C. trachomatis* positive patients are a logical continuation of this work. I ran a sub-group analysis on patients who returned for a checkup after completing antibiotic treatment, but the sample size was small. That analysis was further limited because I could not control for time or if the likelihood of returning for a follow up was itself associated with mood. Prospective experimental studies that control for these variables could provide more definitive conclusions on the effect of antibiotic treatment. A broad underlying implication of this chapter is that sexually transmitted pathogens may be responsible for chronic diseases of unknown etiologies. Research into chronic diseases with poorly understood causes might benefit by considering sexually transmitted pathogens as possible causes or contributors. Also, by considering what is already known about the mechanisms of persistence for each pathogen, the field of potential candidates can be narrowed for a given chronic disease.

In chapter III, I conducted systematic reviews on studies that compared the viral loads and virulence between SARS-CoV-2 variants. The goal of this chapter was to characterize evolutionary trends in these traits throughout the pandemic. From the evolutionary perspective that virulence of directly transmitted pathogens should correlate positively with environmental survivability, I hypothesized a trend of reduced intrinsic virulence. The results indicate increasing virulence through Delta followed by reduced virulence in Omicron, as well as increasing viral loads through Delta which do not change significantly in Omicron. The net effect of virulence is unknown because I could

not directly compare Omicron to the ancestral virus. One explanation for these trends is that increased virulence was favored until the viruses evolved altered tissue tropism, thereby maintaining the benefits of high URT viral loads but removing the costs of high virulence.

In chapter IV I reviewed what is known about the factors that affect malaria severity. The overarching goal of this chapter was to demonstrate that virulence is multifaceted and to better understand why malaria varies in severity at the individual and population levels, effects of the pathogen, host, and environment must be considered. Specifically, I reviewed the effects of acquired immunity, host genetics, reproductive plasticity, and intrinsic virulence. Part of this review was a critical evaluation of the hypothesis that natural selection drives changes in intrinsic Plasmodium virulence. This hypothesis is less established than the others and is given less attention as a possible explanation for variation in disease severity across host populations. Through a qualitative review of literature, it appears likely that *P. falciparum* virulence evolves in response to transmission intensity, but there is limited evidence for the same in *P. vivax*. An absence of evidence is not evidence of absence, and so future studies that evaluate potential genetic differences in virulence between *P. vivax* strains would be useful. An important conclusion of this review is that many factors, such as the timing and intensity of vector availability, contribute to virulence in multiple ways. Thus, interventions that target the vector would not only decrease the number of infections but also the harmfulness of infections.

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CURRICULUM VITAE

Nathan Steffens

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EDUCATION

In progress	Ph.D. Biology, University of Louisville, Advisor: Dr. Paul Ewald
2014	B.S. Veterinary Science, University of Arizona

PROFESSIONAL POSITIONS

2020-pres	Adjunct Faculty, Spalding University, School of Natural Science
2017-pres	Graduate Teaching Assistant, University of Louisville, Dept. of Biology
2015-2017	Research Technician, University of Arizona, School of Animal and Comparative Biomedical Sciences

TEACHING EXPERIENCE

Spalding University

2020-pres Adjunct Faculty

Introductory Microbiology Lecture, BIO 256 (Fa21, Sp21)
Course covering biochemistry, evolution, genetics, pathology, and epidemiology of microbial systems

Introductory Microbiology Lab, BIO 257 (Fa20, Fa21, Sp21)
Lab covering aseptic technique, staining methods, biochemical tests used in identification, and the effects of chemical and environmental factors on microorganism growth

University of Louisville

2020-21 Instructor-of-record

Environmental Biology Lecture and Lab, BIOL 263 (Fa20, Fa21)
Co-instructed course with lecture and lab components covering environmental processes that affect life on earth and how anthropogenic forces influence these processes.

2017-pres Graduate Teaching Assistant

Unity of Life Honors Recitation, BIOL 240 (Fa22)
Recitation sessions for biology honors students, promoting active learning opportunities and real-world applications of lecture topics

Introductory Microbiology Lab, BIOL 258 (Fa19, Sp19-Sp22, Su20-Su22)
*Awarded head teaching assistant position in spring 2020

Human Anatomy & Physiology Lab, BIOL 262 (Fa18)

Principles of Quantitative Biology Lab, BIOL 244 (Sp18)

Introduction to Biological Systems Lab, BIOL 104 (Fa17)

Guest Lectures

2021	*Why durable respiratory pathogens evolve to be more virulent*, Science Information Literacy and Communication (BIOL 203 - online), University of Louisville
2019	*Evolution of Plasmodium virulence with emphasis on human genetic resistances,* Evolutionary Ecology of Disease (BIOL 372), University of Louisville
2017	*An introduction into genetically modified organisms*, Biology: Current Issues and Applications (BIOL 102), University of Louisville

UNDERGRADUATE RESEARCH MENTORSHIP

2022-pres	Madeline Martinez – Undergraduate student, guided on data extraction and statistical analysis for SARS-CoV-2 tissue tropism project
2018-2019	Meghan Schneider – Undergraduate student, guided on project development and writing for independent study on genetic resistances to malaria

PUBLICATIONS

Garces, K.R., Steffens, N.R., Sexton, A.N., Hazelwood, A.C., Christian, N.S., "It takes two: Online and in-person discussions offer complementary learning opportunities for students". *Life Sciences Education* (In peer-review, manuscript available upon request)

Camacho, L.E., Davis, M.A., Kelly, A.C., Steffens, N.R., Anderson, M.J. and Limesand, S.W., 2022. Prenatal Oxygen and Glucose Therapy Normalizes Insulin Secretion and Action in Growth-Restricted Fetal Sheep. *Endocrinology*

Davis, M.A., Camacho, L.E., Pendleton, A.L., Antolic, A.T., Luna-Ramirez, R.I., Kelly, A.C., Steffens, N.R., Anderson, M.J. and Limesand, S.W., 2021. Augmented glucose production is not contingent on high catecholamines in fetal sheep with IUGR. *Journal of Endocrinology*.

Davis, M.A., Camacho, L.E., Anderson, M.J., Steffens, N.R., Pendleton, A.L., Kelly, A.C. and Limesand, S.W., 2020. Chronically elevated norepinephrine concentrations lower glucose uptake in fetal sheep. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*.

Kelly, A.C., Camacho, L.E., Pendarvis, K., Davenport, H.M., Steffens, N.R., Smith, K.E., Weber, C.S., Lynch, R.M., Papas, K.K. and Limesand, S.W., 2018. Adrenergic receptor stimulation suppresses oxidative metabolism in isolated rat islets and Min6 cells. *Molecular and Cellular Endocrinology*.

In Preparation

Steffens, N.R., and Ewald, P., Associations of low mood with *Chlamydia trachomatis* infection in subjects with and without urogenital manifestations. (In preparation to Sexually Transmitted Diseases)

Non-refereed Publications

Steffens, N. and Ewald, P., 2022. An association between *Chlamydia trachomatis* infection and depression in asymptomatic patients. *International Journal of Infectious Diseases*. (abstract)

Brandt, T.J., Stewart, H.S., Cecil, R.E., Steffens, N.R., Yoder-Himes, D.R., (2022). *University of Louisville microbiology laboratory manual.*

GRANTS AND AWARDS

2022	Dissertation Fellowship Award, $9,600
2022	William Furnish Teaching Award, $250
2022	Love of Learning Award, Phi Kappa Phi Honor Society, $500
2022	1st place presentation, Graduate Student Regional Research Conference
2022	Society for the Study of Evolution Travel Award, $500
2022	University of Louisville Graduate Student Council Travel Grant, $350
2022	University of Louisville School of Arts & Sciences Research Grant $250
2021	University of Louisville Biology Graduate Student Association, $200
2021	University of Louisville Graduate Student Council Travel Grant, $350
2021	University of Louisville School of Arts & Sciences Travel Grant, $250
2020	University of Louisville School of Arts & Sciences Research Grant $250
2020	University of Louisville Biology Graduate Student Association, $170
2019	University of Louisville "Faculty Favorites" Nominee (teaching award)

PROFESSIONAL PRESENTATIONS

2022	Steffens, N.R., Sexton, A.N., Garces, K.R., Hazelwood, A.C., Christian, N.S., *Discussion Modality and Exam Performance in Introductory Environmental Biology*. National Association of Biology Teachers Professional Development Conference
2022	Steffens, N., Ewald, P., *An Evolving Pandemic*: *A Systematic Review Of SARS-Cov-2 Virulence.* Society for the Study of Evolution
2022	Steffens, N., Ewald, P., *Chlamydia Trachomatis Infection and Depression In University Students.* Annual Department of Biology Presentations, University of Louisville
2022	Steffens, N., Ewald, P., *The relative virulence of SARS-CoV-2 variants of concern*. Graduate Student Regional Research Conference

2021 Steffens, N., Ewald, P., *An Association Between Chlamydia Trachomatis Infection and Depression In Asymptomatic Patients*. International Meeting on Emerging Diseases and Surveillance

2013 Steffens, N., Patel, R., Birky, W., *Death by Mutations in an Asexual Invertebrate*. Annual Ecology and Evolutionary Biology Presentations, University of Arizona

COMMUNITY AND PROFESSIONAL SERVICE

2022	Graduate student orientation, invited session leader: *Effective teaching in the lab*
2022	West Louisville Math and Science Project volunteer: *Exploring human anatomy*
2022	Beer with a Scientist, invited speaker: *An adaptive shift in the severity of COVID-19*
2021	Manuscript reviewer for The Cardinal Edge (undergraduate research journal)
2021	Kentucky Governor's Scholars Program, invited speaker: *What to Know About Graduate School*
2020-21	Kentucky Regional Science Fair judge
2020	Grant reviewer for Biology Graduate Student Association
2019-21	Peer Mentoring Program – Mentor for first-year graduate students, providing resources for professional development and helping to build community connections
2019	Kentucky Science Center STEMinar Series, invited speaker: *Mind Control: A Parasite and Their Host*
2019	Girls Rule STEM Event volunteer
2019	Manual High School, Mentor for STEM research and careers
2018	University of Louisville Black and Red Event, invited scholar-athlete mentor
2018	Kentucky Science Center STEMinar Series, invited speaker: *Night of the living diseases*
2017-19	Central High School, advisor and mentor for science fair projects

MEMBERSHIPS

National Association of Biology Teachers
Phi Kappa Phi Honors Society
Science Policy and Outreach Group (University of Louisville)
University of Louisville Biology Graduate Student Association
Elected officer: Graduate Committee Representative 2021-22
Elected officer: Outreach Chair 2020-21
International Society for Evolution, Medicine, and Public Health
American College of Epidemiology
International Society for Infectious Diseases
Society for the Study of Evolution
Kentucky Academy of Science
Kentucky Society of Natural History