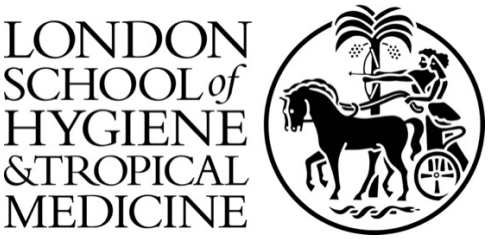

Respiratory pathogen transmission among exposed household contacts

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Declaration

I, Kate Gaskell, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Thesis Outline

Chapter 1

Introduction and Research Question

I describe the evidence base on the transmission of respiratory pathogens within households using SARS-CoV2 and MDR TB as exemplars. I discuss the similarities and differences between the two pathogens, and include exposure, infection, disease, and treatment for both pathogens whilst summarising current gaps in knowledge and differences in practice. The discussion covers how these knowledge gaps translate to problems in clinical care and management, and how respiratory pathogens spread in the household setting even in the context of high community transmission.

Research Question

What drives the increased risk of infection with respiratory pathogens in households and close contacts?

- What is the risk of progression to active SARS-CoV-2 in exposed household contacts?
- What is the risk of progression to active MDR TB in exposed household contacts?
- Are there identifiable predictors of increased or decreased risk to guide future targeting of surveillance and preventive interventions?

Chapter 2

Prospective cohort study in MDR TB exposed household contacts

I completed a prospective cohort study in MDR TB exposed household contacts in Lima, Peru. I followed up contacts for 2 years from infectious MDR TB index case diagnosis. The primary objectives were to calculate cumulative incidence (CI) and temporal incidence of TB in MDR TB contacts, with a denominator of person-time-at-risk. The hypothesis was that almost all cumulative TB will occur within the first year of follow up and there will be limited benefit to follow-up beyond one year. The sample size required was 1500 MDR exposed contacts expecting 5% of household contacts (HHC) developing cumulative (incident and co-prevalent) TB. TB incidence in HHC in published data ranges between 3.3 – 7.8% in high prevalence settings, expecting a margin of error of 3.5-6.5%. Even if 30% were lost to follow-up, a sample size of 1050 would be expected to generate between 36 and 68 cumulative secondary cases.

Cox proportional hazards were used to model incidence rates over time. A survival analysis will be used to identify covariates associated with changes in the hazards of developing TB. I describe the cumulative incidence of MDR TB over 2 years according to time of MDR TB diagnosis and control for possible confounding variables such as crowding, ventilation, and smear status. I investigate confounding caused by increased household exposure and transmission during lockdown and explore whether community influence was less than normal on transmission.

Chapter 3

Transmission of SARS-CoV-2 in a UK Ultra-Orthodox Jewish population

I implemented a community led cross sectional serosurvey of SARS-CoV-2 prevalence in a religious minority with perceived higher rates of SARS-CoV-2 early in 2020. I used detailed data collection on households, their social interactions and serological testing of seasonal CoV and SARS-CoV-2 to describe the seroprevalence of SARS-CoV-2 and the proportion of the population reporting a COVID-19 like illness. Households were randomly selected from a community wide list held by community partners. The sample size assumed a minimum seropositivity of 10%, with a 2% absolute precision and design effect of 2 which is 1730 individuals, the average household size is 6, therefore we aimed for 300 households. The outcomes reported on include seroprevalence by age and gender, and a random effects logistic regression model of factors associated with seroprevalence. I explored household level risk factors for transmission and higher secondary attack rates in households.

Chapter 4

Measuring markers of immune and endothelial activation in previously exposed SARS-CoV-2 community samples with a very high seroprevalence of SARS-CoV-2.

We aimed to understand if markers of immune and endothelial activation vary between symptomatic and asymptomatic SARS-CoV-2 seropositive and seronegative individuals from a community with 64.3% seroprevalence of SARS-CoV-2. Recent published and unpublished data identified markers of interest in severe COVID-19 disease as IL-6, IL-10, and IP-10. Using serology samples from the Ultra-Orthodox household survey we described cytokine profiles in >900 samples comparing profiles in symptomatic, asymptomatic, seropositive, and

seronegative groups and interrogated the data between those with symptoms in wave 1 and wave 2 of the UK pandemic. We compared children's cytokine profiles from groups of SARS-CoV-2 negative, positive symptomatic and positive asymptomatic. Additionally, we explored the relationship between survey data on illness severity and mortality with cytokine profile.

Chapter 5

Community transmission of emerging SARS-CoV-2 variant transmissibility through active contact tracing.

Using a cross-sectional seroprevalence study we described the difference in secondary infection attack rates between households with PCR confirmed wild type (WT) SARS-CoV-2 and households with the Alpha variant of concern (VOC) SARS-CoV-2. All individuals diagnosed with PCR confirmed SARS-CoV-2 at two London Hospitals between November 2020 and January 2021 and their household contacts were eligible. The outcome measured was the prevalence ratio for SARS-CoV-2 seropositivity between household contacts exposed to the alpha VOC SARS-CoV-2 and WT SARS-CoV-2. The sample size was 350 households with a ratio of 4:1 VOC: WT, 3 people per household assuming the seroprevalence in WT households is 0.3 gives a 90% power to detect an increase in seroprevalence to 0.42.

Chapter 6

Discussion and Conclusion

I review the results from all the chapters within this thesis. Reflecting on the similarities and differences in themes, scientific findings, difficulties, and outcomes. I explore the key findings in each chapter, the similarities, differences, and difficulties. I review the methods used in this thesis, in particular the household transmission model and progressive models for developing research in unison with communities. I explore the strengths and weaknesses of transmission studies within households and the importance of the socioeconomic context of this research. I critically appraise the areas of weakness within this research, discuss policy impact and future work.

COVID-19 Impact statement

In early 2019, I set out to investigate and address the evidence gap in the investigation, management and understanding of the natural history of infection and disease in individuals exposed to multidrug-resistant tuberculosis within the household. By December 2019 I had consolidated the research questions and planned the agenda. I initiated the first exposed contacts cohort study in Lima in early January 2020 with plans to run this for 2 years of recruitment and follow-up.

By late March 2020 the SARS-CoV-2 pandemic interrupted all research work and our ability to deliver on the intended objectives. I returned to the UK and was unable to return to Peru for another 2 years. All research work in Lima was interrupted for 18 months due to a strictly enforced and prolonged Peruvian lockdown, high Peruvian mortality rate, a severe impact on the Peruvian economy and specific political scandals surrounding vaccine trials, the government, and the Universidad Peruana Cayetano Heredia where my work was based.

As it became increasingly clear that the possibility to execute this PhD as originally envisaged was very limited by SARS-CoV-2 restrictions, I pivoted the PhD subject matter and took the opportunity to include the closely related investigation of SARS-CoV-2 transmission within household contacts.

As a result of global events, this PhD expanded to explore both pathogens, transmission within exposed households, and risk to exposed household contacts. In this thesis, I will describe the investigation of household transmission of both these respiratory pathogens and the impacts on the understanding of airborne infection transmission risk.

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Definitions

The 2021 WHO definition of a High TB burden country:

> "Countries that collectively accounted for 86% of the estimated global burden (in terms of the estimated number of MDR-TB cases) and that had either >4000 estimated cases each year and/or ≥10% of new TB cases with MDR TB." To meet this definition the minimum number of estimated incident cases/year for MDR TB is 1,000.
>
> The top 20 MDR TB countries by estimated absolute number are:
> Bangladesh, China, DPR Korea, DR Congo, Ethiopia, India, Indonesia, Kazakhstan, Kenya, Mozambique, Myanmar, Nigeria, Pakistan, Philippines, Russian Federation, South Africa, Thailand, Ukraine, Uzbekistan, Vietnam
>
> An additional 10 countries not in the top 20 with an estimated incidence rate >1000 incident cases/year:
> Angola, Azerbaijan, Belarus, Kyrgyzstan, Papua New Guinea, Peru, Republic of Moldova, Somalia, Tajikistan, Zimbabwe

The 2021 WHO definition of a Low TB burden country:

> A country with a TB incidence rate of fewer than 10 cases per 100,000 population per year.

MDR TB:

> Tuberculosis resistant to at least rifampicin and isoniazid, the two main anti-tuberculous agents.

RR TB

Tuberculosis known to be resistant to rifampicin, isoniazid susceptibility often unknown.

Incident TB:

> A new case of TB within a defined time period.

Co-prevalent TB:

> TB presenting with symptoms or diagnosis within two months of the index patient's diagnosis.

Cumulative TB:

> Number of TB cases which includes incident and co-prevalent TB.

Chapter 1

Introduction

1.1 Transmission of respiratory infectious diseases within households

Epidemic spread is determined by three key variables: the duration of infectious contact (duration of infectiousness), the rate of effective contact (average contact rate between susceptible and infected), and the probability of transmission during contact (transmissibility) (1). These three parameters contribute to the basic reproductive rate number or ratio (R_0) which allows an understanding of how many secondary cases each infectious person creates in an entirely susceptible population(2). In most epidemics, the model used to understand the proportions of the population at risk at any one time is the S-E-I-R model or susceptible-exposed-infected-recovered(3).

Respiratory infectious disease transmissibility can be effectively quantified using a household transmission model. First used in the influenza epidemic of 1919, it has been used effectively in many respiratory infectious disease epidemics, and the Office for National Statistics (ONS) has collected community-level household data throughout the COVID-19 epidemic in England and Wales (2,4,5). Given the prolonged, repeated nature and clearly defined number of susceptible and infected contacts, a household is a useful unit for transmission calculation. Prior to the COVID-19 pandemic, there was some existing evidence for influenza that the risk for onward transmission of respiratory infections was correlated to age, numbers of locations and contacts to which at-risk individuals are exposed (6,7).

Outside of the basic transmissibility model within households, it is important to consider the probability that infection exposure has occurred from an outside force, that infectiousness varies for any given pathogen in any setting, that asymptomatic infection and pre-symptomatic periods may lead to secondary infections, that prior immunity alters secondary infection rates, and that groups of individuals may have higher or lower infectiousness (for example children or immunosuppressed individuals). Whilst the epidemiological calculations remain constant across household transmission settings the individual pathogen and human immune response

vary. In this thesis, I explore within household contacts two respiratory pathogens with very different biology, multidrug resistant *Mycobacterium tuberculosis* and SARS-CoV-2.

1.2 Contacts definition

Within tuberculosis (TB) epidemiology a close contact is generally accepted as a susceptible individual who spends at least eight hours/day exposed to an infectious index patient with TB. Despite a close contact being an essential comparator, this interpretation varies and there is no consensus definition. The definition of a household contact varies from a susceptible individual who sleeps overnight within the same household as an infectious index patient with TB for at least 7 consecutive nights within 3 months of index case TB diagnosis (8) to an individual who has close, frequent contact by living in the same house as the index case for at least three days in the three weeks prior to TB diagnosis, the definition used in this thesis (9).

During the SARS-CoV-2 pandemic, the definition of close contacts for COVID-19 has evolved as scientific knowledge developed and varies by individual research studies (10). A household contact has remained anyone living in the same household overnight as an index diagnosed with COVID-19 (10).

1.3 MDR TB literature review

Mycobacterium tuberculosis and MDR TB

Despite global efforts, TB is still an uncontrolled problem with an estimated 1.3 million deaths and 10.6 million (99.9-11.4 95%UI) new cases of TB in 2022, only 6.4 million of which were reported (11). It was the leading infectious cause of death worldwide, causing more mortality than Human Immunodeficiency Virus (HIV) prior to the COVID-19 pandemic (12). This aerobic bacillus is a facultative anaerobe and is mainly transmitted via aerosols. The complete mechanisms of transmission, immune recognition of infection, clearance or persistence of infection and progression to active disease are not fully understood (13). Bacilli are aerosolised in a symptomatic patient with pulmonary TB and then inhaled into the alveoli of the exposed individual allowing recognition and phagocytosis by macrophages. Primary infection is through lymphocyte organisation and granuloma formation around infected macrophages within the lungs. Bacilli survive within macrophages and are transported into the lymphatic system where a cell-mediated immune response both controls the infection and causes the pathology of TB.

In 2022 there were 410,000 (95% UI: 370 000–450 000) estimated incident cases of MDR and RR TB globally [global TB report 2023]. At the outset of this PhD in 2018 there were 483,000 cases of multidrug resistant (MDR) TB and rifampicin resistant (RR) TB worldwide enrolled on treatment, only 32% of the estimated target 1.5 million; and yet an estimated 500,000 incident cases of MDR and RR TB occur each year (12). MDR TB is defined as TB resistant to at least rifampicin and isoniazid, requiring prolonged therapy, which has significant toxicities and a negative impact on quality of life (14). Treatment success for MDR TB is still only 59% globally (12). TB control programs in countries with high TB prevalence rely on passive case finding; requiring patients to seek health care once symptomatic and clearly missing too many people.

COVID-19 pandemic and TB

The WHO global notification data describes a fall in annual TB notifications between 2019 and 2020 from 7.1 million to 5.8 million, following year-on-year increases in TB notifications prior to 2019 (12). These trends have regional variation in the degree of notification reduction, but all reporting regions show a decline and therefore a pandemic impact. There are many reasons for variation in these TB trends, not least: the severity and timeline of the pandemic

impact, the restrictions imposed and their duration, and the capacity and resilience of countries' health systems (12).

The WHO report a 15% reduction in MDR/RR treatment initiation, a reduction in preventative therapy (PT) initiation, reductions in spending on prevention, diagnostics and treatment, and a reduction in BCG coverage (12). In country specific terms for this PhD, diagnostic and treatment delays are likely to have impacted notification data (15). A reduction in income and travel will have altered health-seeking behaviour and reduced notifications (16). Disruptions to treatment and diagnostics provision will have impacted TB incidence (17,18). Patients with active TB will have been impacted first, increasing mortality rates early on in 2020. A delay in diagnosis and treatment start will have increased infectious transmission but since the time between exposure and disease is lengthy the impact on incidence will be spread over many years. However, as the pandemic progresses saturation of household transmission driven by prolonged and mandated household confinement during COVID lockdowns with a reduction of transmission outside the home may affect near-term TB epidemiology(15).

There is a reported link between a country's COVID-19 case fatality rate (CFR) and TB incidence. Shared population-level risk factors including poverty, crowding, and malnutrition, alongside health system factors and health system resilience, are amongst the multifactorial reasons for this (19,20).

TB Clinical Disease and Treatment

TB pneumonia causes the majority of disease, but it can affect any organ system or disseminate throughout the body causing miliary disease. Typically, it presents with prolonged cough (>2 weeks), fevers with profuse sweating, weight loss, anorexia and malaise.

Treatment for drug sensitive pulmonary disease consists of quadruple therapy for two months with rifampicin, isoniazid, ethambutol and pyrazinamide followed by dual therapy for four months (rifampicin and isoniazid). Disease in different body compartments requires treatment for different durations. MDR TB frequently presents with a prolonged illness due to delayed recognition and detection of drug resistance and therefore a delay in appropriate MDR TB treatment (21). Until recently MDR TB treatment consisted of at least 5 drugs for 20 months with 6-7 months of intravenous therapy. This is still the case in most high-burden countries. Since the start of this PhD management of MDR TB has changed dramatically in light of data

from a cohort of patients treated with the so-called "Bangladesh Regimen" and the STREAM trial data. The World Health Organization (WHO) changed its guidance on treatment combinations and duration; WHO treatment guidance now includes oral regimens for 9-12 months alongside the previous 20 months of intravenous combinations (22–24). Recent publications from the Nix-TB and TB-PRACTECAL trials changed WHO guidance again to include shorter 6-month regimens and a BPaL (bedaquiline, pretomanid, linezolid) or BPaL and moxifloxacin (BPaLM) regimen (25–27).

Risk factors for *M.tuberculosis* infection acquisition: individual, environment, pathogen

Individual risk factors

Increasing age increases the risk of a positive test for TB infection (28,29). HIV infection and immunocompromise does not increase the likelihood of latent tuberculosis infection (LTBI) positivity (30).There is some evidence of increased immunogenicity risk in males from older adolescence (28,30). Prior TB, index sputum smear grade and prolonged exposure to an infectious index all increase the risk for TB infection acquisition (31).

Environmental risk factors

Shared sleeping room and overcrowding are risk factors for TB infection. Residing in a moderate or high TB endemic area increases the risk for LTBI test positivity (31,32).

Pathogen risk factors

There is overlap with risk for TB disease however the risks for infection acquisition include the inoculating dose of TB, the inhaled respiratory particle size containing TB and the lineage of TB; Beijing strain exposure in children is more likely to result in infection and Euro-American (31,33).

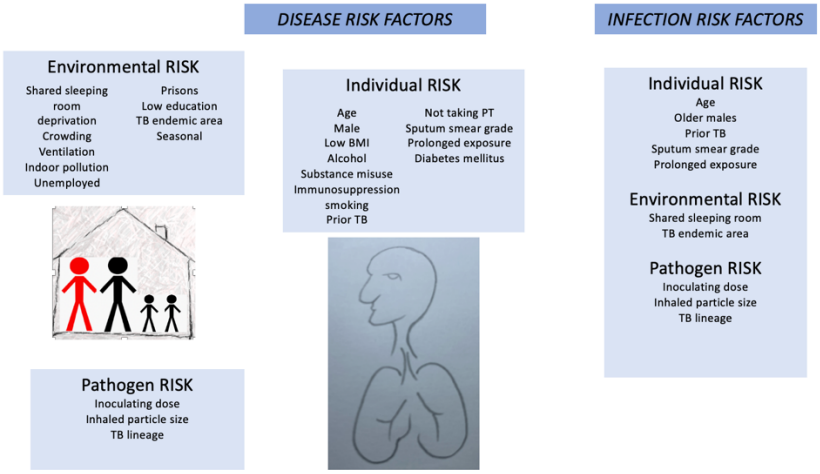

Diagram 1. Risk factors for TB disease and infection

Risk factors for TB disease acquisition: individual, environment, pathogen

Individual risk factors

For all types of TB (DS/MDR and RR) include age (32,34), male sex (31), malnourishment/low BMI (32,35), alcohol and substance misuse (36–38), immunosuppression (medication-induced and HIV infection) (31,39), index sputum smear grade (32), smoking (40,41), not completing preventive therapy (PT) (31), prolonged exposure to infectious index (32) and diabetes mellitus (31,42). Individual risk factors for MDR/RR TB also include prior TB or TB treatment and previous poor compliance with TB treatment (31,32,43).

Environmental risk factors

These include shared sleeping room (31), socioeconomic deprivation (31,32), crowding (44), poor ventilation (32), indoor air pollution (32), employment status (31), imprisonment (45,46), and low educational attainment (32). There is an established and strong evidence for increased TB incidence in Spring (47). The cause for this is not fully understood but could be a combination of co-infection with respiratory viruses, increased indoor TB exposure during the winter season and humidity or temperature changes affecting mycobacterium survival in aerosols or droplets (48–51).

Pathogen risk factors

These include the size of the inoculating dose of TB, the inhaled respiratory particle size containing TB and the lineage of TB; Euro-American and Haarlem spoligotype families are associated with transmission and secondary cases (31,33). MDR TB was thought to be less infectious than TB due to resistance mutations impeding infectivity and growth (8). However recent evidence counters this by suggesting compensatory adaptations occur in wild-type MDR TB restoring MDR TB's ability to grow (52–54). A recent epidemiological study effectively resolved this debate, suggesting that MDR TB is as transmissible as drug susceptible TB (DS-TB) and even if there is a fitness cost it does not impact on transmission (55).

An important overarching consideration is the country-specific epidemiology driving the relative weight of different risk factors. Risk scores for developing LTBI or active TB disease in TB HHC are gaining traction. Difficulties in testing for LTBI and following up HHC systematically in high burden settings mean that alternative solutions are being sought. Mandalakas et al used a principal components analysis (PCA) to develop a LTBI score in South African children based on a 10-question assessment with some success (56), Wang developed a LASSO regression method to model data from Grandjean et al(31,57). Saunders et al have developed a household level assessment of TB contacts (44). All these tools are potentially useful resources but lack the ability to translate their ideas into routine practice and do not validate whether their methods in tool development are robust by comparison with other statistical methods.

Latent infection

Latent tuberculosis infection (LTBI) is the persistence of bacilli within the immune system. A quarter of the global population (1.7 billion people) were estimated to have LTBI (58) and there are high rates of both infection and active disease in exposed close contacts (59,60). In contrast, Behr et al argue that latent infection does not persist indefinitely and is overestimated, that tuberculosis immunoreactivity is a more appropriate term for an immune response to tuberculosis antigens and that global prevalence of infection is much lower than a quarter of the global population (61). In MDR TB exposed contacts an estimated 44-52% develop LTBI (62) but only 10% of latently infected people progress to disease in their lifetime; a way of risk stratifying LTBI enabling effective targeting of prevention strategies at those most at risk of progression to clinically apparent active TB disease is needed. Understanding of the pathway from TB exposure to disease has evolved to include a continuum from exposure to infection to

incipient disease to sub-clinical disease to clinically apparent active TB (63,64). Diagnosis of latency is difficult in TB endemic settings due to programmatic and logistical challenges. The evidence base for risk factors predicting progression from exposure to disease is limited in drug susceptible (DS) TB and there is even less evidence on risk factors for MDR TB (65). Risk factors may be similar in DS and MDR TB but improved clarity is needed to target care. Recent projections of the latent MDR TB infection burden identify an increasing global tread, directly contrasting with DS LTBI (66). Projected peaks in prevalence in 20–35-year-olds and children are particularly concerning, plus children more frequently progress from LTBI to active TB (66,67).

Diagnostic testing for latent infection

There is no direct test for LTBI - existing tests are indirect and measure T cell response to TB. Currently tuberculin skin tests (TST)/interferon gamma release assays (IGRA) are routinely used, though they have a poor predictive value for an individual to progress to TB (68,69). There is some evidence in children that a higher cut off for IGRA positivity is associated with a higher risk of disease progression (70). Global availability of both tests is low. LTBI testing is a well-described barrier to the rollout of isoniazid preventive therapy (IPT) in those infected with Human Immunodeficiency Virus (HIV). Low sensitivity of IGRA tests in high burden settings in both children <5 years and HIV infected persons is another obstacle to their usefulness (71,72). Serial healthcare worker testing using IGRAs shows high rates of reversion and lower rates of conversion, which make interpretation of results and their clinical utility difficult. This may relate to low cut-off points for positivity or a low pre-test PPV in these settings (73,74).

Treatment of latent MDR TB infection

Preventive Therapy (PT) for DS LTBI is highly effective but there are no PT regimens of proven efficacy for presumed latent MDR TB infection (75). Treating patients for latent MDR TB infection with unproven drug therapies for an unclear amount of time could be harmful, drive drug resistance and expose patients to toxic side effects without benefit (76). This is particularly pertinent since we know that adherence to IPT in DS TB over six months is poor (77), some modelling suggests community-wide IPT could increase selective pressure for resistance (78) and the significant operational challenges of PT means that implementation in

high burden settings is patchy (79,80). Two of three randomised controlled trials (RCTs) on preventative therapy in MDR reported preliminary results at the Union conference in Nov 2023 (81–83). Individually the trials did not show an impact of six months of Levofloxacin PT on TB incidence in contacts at 54 weeks of follow up. The small sample size and lack of precision needed to detect an effect in both trials was dealt mid trial with in a pre-defined Bayesian meta-analysis combining both trials' results, HR 0.41 (95% CI 0.18 – 0.95) (83). Prior to this the best evidence for PT in latent MDR TB infection comes from observational data during an outbreak in Chuuk, in the Federated States of Micronesia. Of those who received combination PT (n=104 for 12 months of Fluroquinolone [FQ] based regimens), none went on to develop MDR TB. 3/15 latently infected who refused MDR TB PT developed TB and 13 cases from the community not screened for infection developed TB (84). Both strains of MDR TB circulating in this outbreak were susceptible to FQ. In a prospective cohort in South Africa 6/186 child contacts given PT (FQ, Ethambutol [E], high dose Isoniazid [H] for 6 months) developed TB(85).All MDR TB index strains were susceptible to FQ but the authors report concerns about all 6 children's adherence. A 30 month follow study in South Africa in MDR TB HHC children <5 years old suggested tailored PT might prevent disease (86). A meta-analysis in 2023 estimated PT reduced the risk of incident MDR TB by 66% (95%CI 28-84%) but the 11 studies pooled in this analysis had small numbers of heterogeneous poor-quality data (87).

As the low quality of this observational data is insufficient evidence to inform or change policy on preventative therapy in MDR TB three randomised controlled trials (RCTs) will report in full imminently (81,88,89). In the interim, the WHO recommends close observation of MDR TB exposed contacts for two years but there is a paucity of tools to support the implementation of such monitoring by National Tuberculosis Programmes (NTP), with no details given on how this monitoring should be done. The WHO recommendation that certain high risk contacts may be offered MDR TB PT does not define what drug or drugs the PT regimen should contain and how long it should be given (90). The WHO LTBI guidelines are based on very weak evidence: 10 uncontrolled observational studies on MDR TB HHC with marked heterogeneity consisting of only 256 contacts (90).

The drugs suggested for use in MDR TB PT are fluroquinolones (FQ) in combination with high dose isoniazid, FQ alone, or FQ with Ethambutol or delamanid alone. There are some

concerns around the safety of FQ. The European Medicines Association has released a warning around FQ, relating to the significant risk of joint and tendon side effects (91). In the Chuuk outbreak 52/104 reported adverse events to therapy (84). Current RCTs investigating different combination PT regimens for MDR TB HHC are six months duration based on the evidence in DS TB for IPT durations of 6-9 months. Recent trials indicate one month combination PT in DS TB is non-inferior to longer courses of PT (22,92).

Despite the absence of strong supporting data indicating benefit and the known toxicity of agents used, some frontline clinicians are understandably choosing to offer PT (93). In paediatrics, TB progresses rapidly and is potentially life-threatening whilst waiting for six-monthly review. Furthermore, in resource-limited settings where a repeat follow-up assessment is untenable there is understandable physician pressure to prevent ongoing TB spread in vulnerable groups. The imperative for preventive therapy in MDR TB has always been that the treatment of active disease was long, arduous and of low efficacy thus anything that might prevent active MDR TB was worth trying (93). However, this space has seen significant advances in therapeutic regimens in recent years as described above, diluting the argument that any PT, regardless of strength of evidence of effect, is better than none (22,25,26).

MDR TB Household contact screening

Household transmission is estimated to account for 8-19% of all TB in high burden settings (13). Screening households identifies high rates of infection and there is increasing pressure to improve screening of high-risk groups including household contact screening (94). In MDR TB household contacts (HHC) rates of LTBI are higher than in DS TB HHC, 40.8% vrs 25.8% in a small Vietnamese study and 44.7% vs. 34.3% in a South African study of <5-year-olds (95,96). This may relate to prolonged infectious exposure in MDR TB HHC, multiple previous TB exposures (within the household or the community) or different groups suffering from MDR TB and DS TB. Effective symptom screening and LTBI testing for all high-risk contacts is important. In reality, systematic symptom or LTBI screening of all MDR TB HHC does not happen due to logistical challenges.

The majority of disease in close contacts presents by 12 months, 90% by 2 years and nearly all occurs within three years (62,86,97). 7.8% of MDR TB household contacts develop TB disease within 2 years (62,98). At initial screening visits of MDR TB HHC the rates of co-prevalent

disease are high (95,99). A meta-analysis found a mean of 47.2% of MDR TB household contacts had LTBI, though this varies widely (62). MDR TB HHC are exposed for prolonged periods of time due to delays in diagnosis, delays in effective treatment and subsequently prolonged sputum positivity (31). MDR TB child HHC, particularly under 5-year-olds, are high-risk for infection but do not get MDR TB frequently (96,100). The total numbers of exposed HHC far outweigh the proportion who do develop disease.

Epidemiology of MDR TB in Peru

In 2021, estimated TB incidence in Peru was 130 cases per 100,000 people, an increase from the reported incidence of 116/100,000 in 2020 (101). 4.9% of new cases and 9.6% of retreatment cases have MDR or RR TB. The annual estimated incidence of MDR/RR TB in Peru is 2,800 cases/year over 80% of which occur in Lima (101,102). High transmission is focused in several Lima districts including Callao, Lima Centro and Lima Sur (103). 1,753 of 2,800 MDR/RR TB cases are reported to start DR treatment; access to the newer oral regimens is limited and most patients remain on the previous 20-month regimens (101). In 2019 62% of MDR/RR cases completed treatment successfully. Peru had one of the highest numbers of cumulative COVID-19 deaths globally at 624 deaths/100 000 people(104). The pandemic impacted TB care significantly, there was a 50% reduction in patient assessments for TB, microbiological diagnoses of TB and HHC screening and management. Sputum samples submission for analysis fell to 25% of pre pandemic levels (105).

94% of pulmonary bacteriologically confirmed cases are tested for rifampicin resistance; an impressive feat given the health system challenges in Peru. Barriers include a lack of funding, insufficient health care staff, disjointed care, and no integrated health information system (106). The liquid microscopic observation drug susceptibility (MODS) assay was developed at the Universidad Peruana Cayetano Heredia (UPCH) in Lima, is used countrywide and is a cost-effective, sensitive, and specific method with which to diagnose MDR/RR TB (107–109). A rapid increase in the availability of MODS and Genotype MTBDRplus rapid sensitivity tests has enabled widespread MDR testing.

Peruvian guidance is for all close contacts to be screened at index diagnosis and subsequently three monthly until index treatment completion (110); in reality, this does not systematically happen even at baseline with only some passive case finding occurring resulting in missed

opportunities to identify ongoing transmission (111). All LTBI or high-risk contacts should be treated with isoniazid preventative therapy (IPT) given the high prevalence of DS TB (110). However, only 29% of household contact children <5 years are started on preventive therapy (PT) (101); lack of health staff awareness, training and supervision exacerbate poor access to PT (112).

MDR TB household contacts: Guidance, evidence, and current practice

There is no question that screening household contacts is a productive exercise, but exactly how to manage MDR TB household contacts is unclear. Practice and guidelines vary widely, which should not be surprising given that the clinical management of DS TB contacts differs despite a good evidence base for practice (113). Table 1 summarises the current available guidance on MDR TB Contact management. The table in Appendix 1 summarises the existing published literature on MDR TB HHC, their management, where PT has been given, and TB and LTBI outcomes.

Table 1. Guidance on MDR TB contact management

Guidance	Follow-up duration	LTBI testing?	PT advised	Drugs in PT	PT Duration	Comments
WHO (114)	6 mthly for 2 years	No	No*	-	-	*except in HIV+, <5yrs and individual case-based decision about LTBI testing, PT and drug choice
South African (115)	6 mthly for 2 years	No	Yes in <5yrs HIV + child contacts of infectious case	H 15mg/kg	6 mths	contact screening a priority. Symptom screen plus GeneXpert on sputum
Harvard consensus panel in 2015 (116)	18 mths	No	Yes in all infection	FQ/ FQ plus 2nd	6 mths	majority of panel agreed but not a consensus
American Academy of Paediatrics (117)	-	Yes all contacts	Yes in LTBI +	x2 not specified	-	drugs and duration not specified
CDC & ATS(118)	-	?Yes	Yes in all suspected infection	DST guided/ Z and E/ Z and FQ	6-12 mths HIV 12 mths	individual case management
NICE (119)	6 mthly for 2 years	No	No	-	-	practice differs
European guidance(120)	as per national guidelines	Yes	Neither for or against	-	-	discusses evidence but no new guidance statements

Globally and regionally clinical management of MDR TB contacts varies. UK wide adherence to NICE guidance varies amongst specialist centres; 54% monitor MDR TB contacts without PT for two years and 38% decide on each individual case management (121). The European TBNET consensus statement supports LTBI testing in all close contacts and cautiously supports PT with FQ plus one other drug based on drug susceptibility for 9 months in high-risk contacts (122). 9/30 highest MDR TB burden countries are in Europe with an estimated total 122 000 cases in 2016 (123). A European survey of 72 paediatricians treating MDR TB was conducted in 2016. Practice varies widely; 57% prescribe PT to MDR TB latently infected children. 10% prescribe PT to exposed uninfected, 29% do so if additional risk factors are present and 62% follow up exposed uninfected without treatment (124). In the USA the majority of physicians test for infection and treat it if present or if high risk for disease with 2 drugs to which the index is susceptible (125).

India has one quarter of the world's MDR TB. In 2021 the Indian government published a guideline on the management of MDR TB, including shorter treatment regimens, contact management and decentralisation of care (126). China is the second highest MDR TB burden country globally; in 2017 12% of incident TB was calculated to be MDR TB (127,128). Contact screening and investigation is policy for MDR TB contacts, but PT in latent MDR TB infection is not given (129,130). In Russia very little is published on MDR TB in children or HHC, yet it has the third highest number of MDR TB cases globally (131). However, MDR TB contacts are screened, tested for LTBI with TSTs and closely followed up for symptoms of TB (132,133).

Whilst MDR TB treatment guidance in South Africa was expediently updated in 2018, contact screening practice adheres to their 2014 guidelines listed in the table above (134,135). The PT advised is high dose isoniazid but this differs across regions, in the Western Cape ofloxacin, ethambutol and isoniazid are chosen (136). In South America most of the published evidence base comes from Peru or Brazil where the policy is to screen and follow up contacts for two years but not to test or treat for LTBI, though in reality screening of all contacts and 2 years of follow up does not happen (97,137).

1.4. Significance and knowledge gaps/scientific rationale

There are clear and large knowledge gaps around how to manage MDR TB HHCs.

- It is not clear who to screen for MDR TB, when this should happen, how frequently and how long the screening continues, and with what tools. It is unclear which contacts carry a high enough risk of TB that they are worth investing limited time and money in.
- There is a poverty of evidence around whether PT works in this population, which PT to use, for what duration and the real concerns regarding drug side effects.
- Because it is unclear who would benefit from PT it is also unclear who should be tested for LTBI. It is difficult to know how to proceed when testing for a condition with is poor evidence on it's treatment. To add complexity it is not possible to know if the presence of LTBI relates to MDR TB exposure or to DS TB in high-prevalence settings.
- In summary, it is unclear how MDR TB HHC should be screened; with a regular symptom screen, for how long, tested for LTBI, or PT for all those considered high risk without LTBI testing.

Aside from the knowledge gaps above there are logistical difficulties in screening, following up HHC for two years and testing for LTBI. There are technical problems with procuring tests, financing the NTP, whilst performing TST on patients requires multiple clinic visits, and incurs costs to patients. Two years of follow-up requires a lot of time, money and capacity within the health system and for patients. We see that most high-burden countries simply do not have the infrastructure or capacity to screen and follow up all MDR TB HHC.

I hypothesise that a simpler method with a minimum dataset for screening and following up contacts would be helpful, and more easily implementable. Contact screening is a high yield exercise in DS TB and MDR TB and whilst it is unclear what should be done to mitigate TB risk in MDR TB contacts we should be urgently identifying and treating all new cases of MDR TB. It is essential that we learn as much and as quickly as possible about how to look after this patient group whilst awaiting RCT results. There should be a defined harmonised minimum dataset, which could be incorporated into MDR TB contact screening. We set out to provide this within a tool that is imminently scalable.

The WHO have developed a systematic process to strengthen TB surveillance using a digital platform with District Health Information System 2 (DHIS2) software to enhance reporting of TB cases to WHO (138). This allows country level reporting for core TB indicators; it also enables country, region, and district level review of data via a dashboard for real time analysis. The logical next step is an integrated digital platform to aid management and surveillance of LTBI, something WHO advises (90). With technical support of collaborators at a partner organisation, HISP-India, I developed a DHIS2 module which interacts with the WHO DHIS2 TB case surveillance software and can be deployed as an integration to the WHO system or as a standalone system. The software is secure, free and open-source and aims to improve clinical service, national reporting systems and facilitate research into how to control the spread and manage LTBI, something that is urgently needed (99).

My aim is to improve and systematise MDR TB contact tracing in Lima, Peru as a pilot site using an e-registry with DHIS2 software. Going forward the e-registry offers the opportunity to (a) enhance the capability of NTPs to keep MDR exposed contacts under follow-up and (b) capture the extent of PT use and explore observationally the potential effect upon future disease risk. It delivers a line list of subjects who may be eligible for a future PT regimen.

1.5. Research question (MDR TB)

What is the optimum duration of follow-up for HHC of MDR TB? Do all HHCs require 2 years of follow-up, or can this be shortened to six months or one year? What is the optimal approach to surveillance and management of HHC? Are there identifiable predictors of future progression to active TB disease amongst MDR TB HHC?

1.6 SARS-CoV-2 Literature Review

SARS-CoV-2 biology

A betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, Hubei Province, China in 2019 linked to clusters of pneumonia (139). Six beta coronaviruses were previously known to cause respiratory syndromes in humans, four of which cause 15-30% of common colds: 229E, OC43, NL63, and HKU1 and two which cause severe disease: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) (140,141). Coronaviruses have a spherical shape with four main components of note; spike proteins which project like clubs from the surface, attached via the receptor binding domain (RBD) to the nucleocapsid (NC) within which is the single-stranded RNA genetic envelope. The spike protein and RBD undergo the most genetic variation, they are the source of pathogenesis effectively binding with the human angiotensin-converting enzyme 2 (ACE2) receptor (142,143).

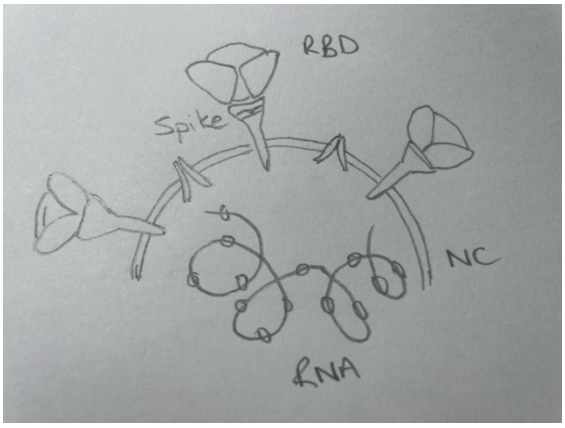

Diagram 2. SARS-CoV-2 structure displaying spike protein with receptor binding domain projecting from the nucleocapsid and internal single stranded RNA.

Epidemiology

SARS-CoV-2 epidemiology has changed rapidly over the past 4 years both in terms of our understanding and its infectious spread across the globe. In this dynamic context, the content of this chapter has changed.

In December 2019 unexplained pneumonia presentations in Wuhan, China were linked to genetic viral clusters of a coronavirus from a zoonotic outbreak through intermediate animal hosts in the Wuhan food market (144,145). By February 2020 the virus then labelled 2019-nCoV had spread across mainland China (146,147). By March 2020 it had spread to major

international travel hubs including Europe, in particular Italy, Spain and the UK. The UK government enforced a national lockdown on 23/03/2020. During this lockdown (between the dates of 9 March – 30 June 2020) 5,330 deaths related to COVID-19 were recorded among 20-65-year-olds. This underestimates the total number of deaths, including those in care homes, those without COVID-19 on the death certificate or those who were unable to access a SARS-CoV-2 test in the 28 days prior to their death. Between 20/03/2020 – 28/05/2020, there were 59,537 more registered deaths than usual in the UK (148). 6,656,601 COVID-19 deaths were reported globally to the WHO as of 23 Dec 2022 with 651,918,402 confirmed cases (149). WHO estimates true excess mortality to be higher, 14.83 million deaths in 2020-2021, with even this an underestimate given limited data reporting systems for most of the world (150). Global SARS-CoV-2 seroprevalence, seropositivity following infection not vaccination, has risen with time and increasing data robustness and availability have made it possible to estimate seroprevalence across continents. Seroprevalence is much higher than reported case numbers; in September 2021 global seroprevalence from infection or vaccination was 59% (151).

As COVID-19 case numbers increased, genetic variation rose leading to escape mutations with greater transmissibility and fitness (142,152). The viral lineages with increased transmission, detrimental change in epidemiology, or increase in virulence or decreased efficacy of public health measures/diagnostics/vaccines/therapeutics were labelled Variants of Concern (VOC) (153). VOC led to sequential pandemic waves with increased case numbers, re-introduced lockdowns with economic impacts, health system strain and excess morbidity and mortality (Table 2).

Clinical Disease, Treatment and Vaccines

COVID-19 presents with a biphasic illness; an initial upper respiratory syndrome which progresses to lower respiratory symptoms whilst the patient is viraemic, and in a small proportion a later immune-mediated inflammatory syndrome involving multiple organ systems.

In the initial UK wave the median age at presentation to hospital was 73 years, 60% were male and 10% of admitted women were pregnant (154). The commonest symptoms included cough, fever, and shortness of breath. However, the range of symptoms varied and included abdominal pain, anosmia, confusion, chest pain, diarrhoea, headache, lymphadenopathy, muscle and joint

ache, sore throat, skin rash, and runny nose (154). Each Variant of Concern (VOC) wave has presented a slightly different preponderance of symptoms (Table 2).

Many large adaptive multicentre RCTs (e.g. RECOVER, DISCOVERY) have generated evidence which has improved treatment and outcomes for COVID-19, including dexamethasone, low molecular weight Heparin, Remdesivir, Anakinra, monoclonal antibodies, interferon (155,156).

A massive international effort produced a pipeline of >150 vaccines, 9 of which have been validated for use by the WHO (157). There are four different types: subunit vaccines eg. NVX-CoV2373 (Novavax, USA) which uses a spike trimer, viral vector vaccines eg. AZD1222 (Oxford/AstraZeneca, UK) which uses adenovirus as the vector, gene vaccines eg mRNA-1273 (Moderna, USA) or BNT162b2 (Pfizer/BioNTech) which both use mRNA sequences to enable host cell antigen production, and whole virus vaccines eg. BBIBP-CorV (Sinopharm, China). Clinical trials validated mRNA vaccines as the most effective and two doses were shown to have excellent efficacy against symptomatic disease and severe disease (158,159).

Table 2. SARS-CoV-2 VOC emergence through the pandemic

Variant	Date of emergence/ VOC	Symptom focus	Transmissibility	Severity	Vaccine Effectiveness (VE)
Wildtype (WT)	China 12/2019	SARS Pneumonia	-	-	-
Alpha B.1.1.7	UK 09/2020 (160) VOC 18/12/2020	No change (161,162)	43-90% more transmissible (162,163) R_0 increased by factor 1.35 (95CI 1.02-1.69) (161)	Higher VL vs WT Likely no increase in severity (164–166)	Reduction in VE after 1 dose, limited change after 2 doses (167)
Beta B.1.351	South Africa 05/2020 VOC 18/12/20	No change	Increase OR 3.3 of Beta vs WT (95CI 1.4-8.2) (168,169)	Increased in hospital mortality vs WT (170)	VE reduced ChAdOx1 – no protection vs symptomatic COVID-19 (171) 2 doses mRNA protect vs severe disease (172)
Gamma P.1	Brazil 11/2020 VOC 11/01/21	No change	Increase 1.7 to 2.4 x more transmissible (173)	Increased mortality vs previous variants (174)	VE reduced (175)
Delta B.1.617.2	India 11/2020 VOC 11/05/2021	No change	Increase OR 1.7 of Delta transmission vs Alpha (95CI 1.48-1.95) (176)	Increased hospitalisation vs Beta (170)	VE reduced (167,177)
Omicron B.1.1.529	Many countries 11/2021 VOC 26/11/2021	URTI symptoms predominate	Increase vs Delta	Reduced hospitalisations vs Delta (170)	VE reduced (178,179)
XBB.1.5	USA 12/2022 (180)	unknown	96% increase (181)	unknown	VE reduced (182)

URTI=upper respiratory tract infection, VL=viral load, VOC=variant of concern, VE=vaccine efficacy, WT=wild type

Testing for SARS-CoV-2

Current WHO guidance emphasises the need for effective public health SARS-CoV-2 testing interventions in all countries. Ensuring timely disease management, early diagnosis interrupting transmission, and accurate epidemiological tracking of incidence and all emergent VOCs.

RT-PCR are available to detect SARS-CoV-2 in nasal swabs, throat swabs, saliva, stool. Upper respiratory tract RT-PCR tests are the most accurate to diagnose early COVID-19 (183–185). Lower respiratory samples or blood are more accurate in severely unwell patients later in the COVID-19 disease course.

Viral culture is slow, requires specialist staff in a biosafety laboratory category 3 (BSL-3), cultures differently in different cell lines and carries risk in a routine diagnostics lab (186,187). For these reasons, it is not recommended for routine diagnostics. Viral neutralisation assays are also unsuitable for routine diagnostic testing and require skilled staff in a BSL-3 but are the gold standard for detecting functioning antibodies (187). There are a range of serological assays which measure antibody binding (total Ig or IgM or IgG or IgA) which all function slightly differently depending on the clinical setting and test. Commercial non-quantitative lateral flow immunoassay tests are useful non-specialist point-of-care (POC) tests providing rapid results to aid diagnosis, infection control and reduction in transmission. However, they have to meet a prespecified sensitivity of >80% and specificity of >97% to comply with WHO guidance and are most useful and reliable where prevalence is >5% (188).

Transmission & transmission prevention

Reducing the amount of virus circulating and the onwards viral transmission reduces the selection pressure and risk of new VOC emerging (189). Thereby reducing the risk of severe disease and risk to vulnerable populations from COVID-19. Early diagnosis and treatment prevent onward transmission and morbidity and mortality from COVID-19. Over the course of the last two years, it has become clear that the main burden of infectious transmission is via respiration. Initially, respiratory droplets and indirect contact from fomites were suspected to cause the burden of transmission rather than respiratory aerosols however the relative contributions of how these respiratory viral particles transferred were not clear until recently. The risk of transmission is determined through networks of respiratory exposed contacts, the frequency, the proximity and the duration of contact (190).

Non-pharmaceutical interventions effectively reduce transmission via social distancing, hand hygiene, personal protective equipment, and ventilation. Mask use is effective at reducing risk of SARS-CoV-2 transmission, prevention of infectious transmission is improved if use is consistent, correctly fitted and of a good quality (191). Testing for SARS-COV-2 and in particular the use of lateral flow immunoassay tests to identify viable culturable virus that could cause onward infections has been an effective transmission

prevention tool (192). Contact tracing and quarantine measures have been implemented throughout the world with different success rates and with subsequent political, economic, and social impacts. Effective implementation of this policy requires massive health system infrastructure, manpower and efficient systems to identify, test and isolate all exposed contacts. In most countries, it also requires public goodwill and supplication. Last updated in March 2023 international WHO guidance is pragmatically to screen contacts for COVID-19 and to quarantine until test negative on day 5 post-index diagnosis. If no SARS-CoV-2 tests are available quarantine should last 10 days in the current environment (Omicron dominant, no new VOC, high population immunity) (193).

Vaccination roll-out is the most important public health intervention in persistently reducing illness severity. There is good evidence that prevention of breakthrough infections falls over time from vaccination particularly rapidly with Delta and Omicron VOC (194,195). The data around transmission interruption is less clear all studies have been observational however on balance fully vaccinated (2 doses) individuals transmit Omicron and Delta VOC less than unvaccinated but still transmit a lot (177,178,196).

Risk factors for transmission: individual, environmental, pathogen

Individual risk factors

Older persons are at a higher risk of severe disease and in the initial UK cohort reports of in-hospital death the median age was 80 years and men fared worse with higher mortality (154,197). At the time of setting up the studies within this PhD children were thought to be less infectious than adults and cause less transmission. This theory has been discredited, in part due to the work within this thesis along with many other published pieces. The comorbidities of cardiac disease, simple diabetes mellitus, chronic pulmonary disease, and chronic kidney disease are all linked to more severe disease and worse outcomes but not to an increased likelihood of contracting SARS-CoV-2 (154,197). Patients with obesity, moderate liver disease, dementia, neurological disorders and malignancy had higher hazard ratios for death (154).

Immunosuppression increases susceptibility to SARS-CoV-2, chronic infection, increased symptomatic disease, hospitalisation and mortality (198). HIV infection is a risk factor for severe disease and increased mortality (199). Different immune deficiency states vary in their vulnerability to SARS-CoV-2; compromised innate immunity vs. impaired B cell immunity vs impaired T cell immunity. Additionally, chronic infection, and here prolonged PCR positivity has been correlated with culturable virus, can lead to SARS-CoV-2 evolution within a host, generating mutations and new variants (200–202). Transmission from persistent viral shedding in immunocompromised PCR persistently positive individuals is not fully understood but is thought unlikely to contribute much to overall transmission (203,204).

Previous SARS-CoV-2 infection does not protect long-term against re-infection or ability to transmit infection but does provide a window of protection which starts to wane after 90 days (193). Likewise, vaccination (with any of the current vaccines) provides some protection from re-infection and therefore from infectiousness for 90 days, however particularly with the Omicron variant this protection from re-infection wanes (205).

Early on during the pandemic reports from several countries highlighted an apparent difference in mortality outcomes between ethnic minorities and non-minority populations (206–209). Over time it became clear this difference was not related to race but to socioeconomic structures, access to care, pre-existing health inequalities and structural health racism (210–212).

Environmental risk factors

Exhaled respiratory particle size impacts transmission; from large droplets (>100μm) thought to cause most short-range transmission (<1-2m) to droplets that evaporate (aerosols) to be as small as 5 μm and transmit over longer distances (213). However, these are all exhaled together in a cloud of particles, and the speed of their travel is impacted by multiple factors including ventilation, wind speed, cough velocity, and UV radiation (214). High relative air humidity reduces the duration that droplets containing viable virus remain in the environment, and environmental surface contamination plays a much smaller role in transmission than previously understood (215,216). High humidity and temperature have been linked to a reduction in COVID-19 cases (217).

Close proximity of persons increases the possibility of transmission. Several settings have been recognised to be venues for transmission, including households, close gatherings, work meetings, restaurants, churches, shared transport (taxis, cars, tubes and trains) health and care settings (218,219). Good evidence now exists that there is little environmental contamination causing onward transmission in school settings (220,221). Plus, effective precautions prevent onward transmission in school and community settings (221). By comparison, households have large amounts of surface contamination with SARS-CoV-2 (221). Indeed, household contact or family contact increases transmission risk (197).

Pathogen risk factors

Viral tropism causing infectiousness is impacted by the spike protein of SARS-CoV-2's ability to attach to the ACE2 receptor. Each mutation in the spike or RBD associated with a new variant allowing improved binding increases the variant's transmissibility. Each VOC has had different propensities to being transmitted. Additionally, the ability of each VOC to spread, and the amount of virus circulating in a community determines the speed of new variant emergence.

A preponderance for the respiratory tract has been clear from the start, initial reports of wild-type SARS-CoV-2 focused on pneumonia, and subsequent research and VOC waves led to an awareness of upper respiratory tract symptoms. The virus actively replicates within the respiratory tract, identified early in 2020 and published in Nature (222). Importantly, the detection of RNA by PCR does not equate to an individual's infectiousness and patients can remain PCR-positive for a prolonged period after a COVID-19 illness (223).

During the first wave of the pandemic, it was established that transmission during the symptomatic period is likely, peaking with viral load at symptom onset and remaining high during days 0-5 of symptoms, falling quickly from day 6 onwards (224,225). Presymptomatic transmission has been confirmed to occur but at lower viral loads (226). Asymptomatic transmission is likely particularly in children though again with lower viral loads (226–228). The post-symptomatic period shows very little evidence of transmission (224). As discussed above incubation, symptomatic and infectious periods vary with each VOC.

Human challenge studies identified viral shedding at upper respiratory sites over different durations. During the course of the COVID-19 illness, the virus is first detected in throat swabs, and later in nasal swabs at higher viral loads which peak by day 5 (192). Post-vaccine work identified an immune response in the upper airways to the virus, possibly preventing virus spread to the lower airways.

A higher salivary or nasal SARS-CoV-2 viral load is associated with an increased risk of transmission to household and community contacts, in both symptomatic and asymptomatic COVID-19 patients (197,229). Higher viral load predicts culturability, and infectiousness, and shortens incubation time but debate still exists around higher viral loads equating to symptomatic disease (192,197,222,223,230).

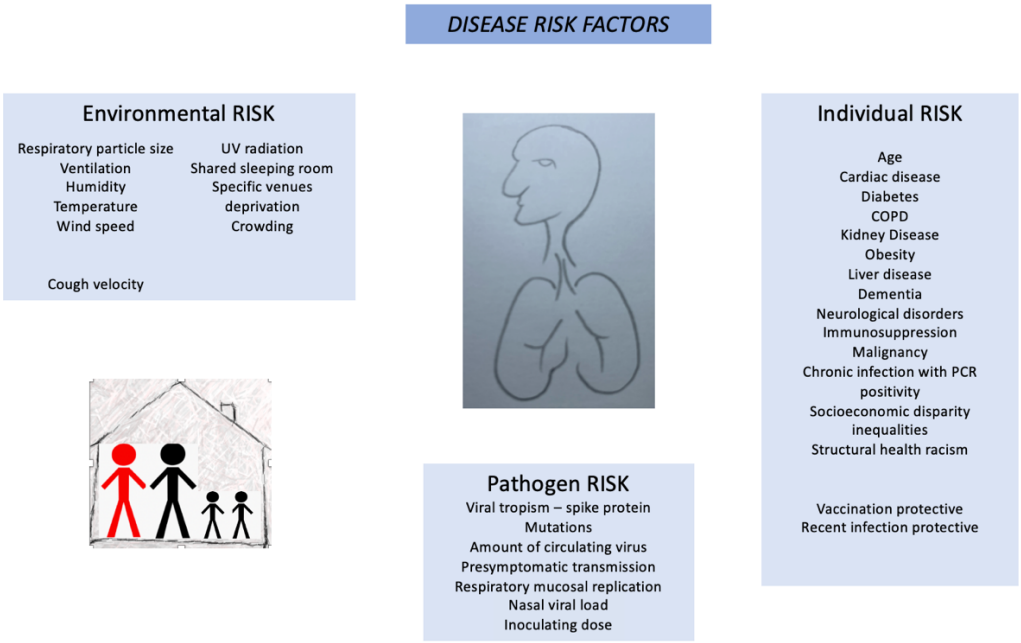

Diagram 3. SARS-CoV-2 disease risk factors

1.7 Significance and knowledge gaps/scientific rationale in 2020-21

In 2020 SARS-CoV-2 transmission routes were unclear, particularly what proportions should be ascribed to respiratory aerosols, touch, and droplet transmission. The likelihood of SARS-CoV-2 transmission between individuals was also unknown; and extended to include the duration of infectiousness, the rate of effective contact, and the probability of transmission during contact.

As diagnostics for SARS-CoV-2 developed, the research community relied on using RT-PCR alone to identify secondary cases (10,231). This depends on a symptomatic secondary case testing positive on PCR within a window of viral shedding, missing both asymptomatic transmission and those who completed a PCR test outside the window of nasal or throat mucosal viral shedding. The rationale for this was clear, as new knowledge was required at speed and due to practicality; the research data largely came from national screening programmes where contact screening and testing were coordinated (231,232). However, it leads to inaccurate underestimations of secondary attack rates for SARS-CoV-2 and as the pandemic progressed these questions arose for each successive VOC.

At the start of the pandemic and hence the start of this PhD the relationships between variables affecting transmission were unknown, the series of studies in this PhD set out to explore these relationships and define which variables carry greater weight within the household setting. This remains a useful setting in which to answer these questions in a way that reduces bias and improves the accuracy of estimations.

Additionally, it was unclear how an individual's immune responses to SARS-CoV-2 varied and how or if this altered infectiousness, symptom development, disease severity, virus transmission or long COVID.

1.8 Research question (SARS-CoV-2)

1. How transmissible is SARS-CoV-2 within the household to household contacts?
   Addressed in chapter 3 and chapter 5.
2. What individual and household factors increase transmissibility?
   Addressed in chapter 3 and chapter 5.
3. What is the difference in transmissibility within the household between circulating SARS-CoV-2 and a new VOC?
   Addressed in chapter 5.
4. Is there an immune response signal within the soluble cytokines that persists in SARS-CoV-2-infected communities?
   Addressed in chapter 4.

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Chapter 2

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Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH212211	**Title**	Dr
First Name(s)	Katherine		
Surname/Family Name	Gaskell (Wilson)		
Thesis Title	Respiratory pathogen transmission among exposed household contacts		
Primary Supervisor	Professor David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	PLOS ONE
Please list the paper's authors in the intended authorship order:	Katherine Gaskell, Sonia Lopez Romero, Jorge Coronel, Tansy Edwards, Chrissy H. Roberts, David AJ Moore
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am the first author. I planned the project with the support of DAJM. I designed, conducted and managed the research. I collated the data, and planned and executed the analysis. I wrote the first paper draft and developed the manuscript in consultation with DAJM and CHR.

SECTION E

Student Signature	Katherine Gaskell
Date	07/02/2024

Supervisor Signature	David AJ Moore
Date	07/02/2024

MDR TB Exposed household contacts – a prospective cohort study

Background

In 2019, before the COVID-19 pandemic, there were an estimated 10.2 million tuberculosis (TB) cases globally, rising to an estimated 10.6 million (99.9-11.4 95%UI) cases in 2023 (1). Prior to the COVID-19 pandemic, it was the leading cause of death by an infectious disease, the WHO (World Health Organization) figures for estimated TB deaths globally fell during the pandemic but returned to 2019 levels in 2023 with 1.3 million deaths (1). WHO surveillance figures show a worsening of global trends in TB during the pandemic, however, the estimated incidence of MDR TB has remained stable at 410 000/year. In Peru, one of 30 MDR TB high-burden countries, the estimated number of incident cases of MDR TB rose from 2,300 in 2019 to 2,800 in 2022, 80% of which occurred in Lima. MDR TB presents as 4.9% of new cases and 9.5% of retreatment cases (1). The WHO 2015 "End TB" strategy aims to reduce 80% of new cases of TB by 2030(1). The first pillar of END TB focuses on prevention, particularly screening in high-risk groups and, identifying and treating all MDR TB. Household contacts (HHC) are a well-described group with a generalised increased risk of TB, but the evidence is inadequate around MDR TB contacts with respect to when TB develops in HHC, which HHC are at risk, with what risk factors, how to prevent incident TB and how to investigate and manage the HHC. Due to the current paucity of evidence, the guidance of WHO (and that of most other advisory bodies) is still to screen all high-risk contacts and to follow them up for 2 years. This is logistically and financially unfeasible in high prevalence and low resource settings (2–4). The nature of this follow-up is not clearly defined; it neither clear which screening tests are needed, nor which time points during follow-up are the most effective for identifying incident TB, nor if in-person screening is required or whether National Tuberculosis Programmes (NTP) utilising light touch remote screening methods could be as effective. Most NTPs struggle to implement MDR TB HHC care. Adherence to NTP MDR TB contact management guidelines (tracing all contacts and following them up for 2 years) is poor, by example even for drug-susceptible TB only 4% of all HHC globally receive PT (2).

Three randomised controlled trials (RCTs) set out to identify effective preventive therapy (PT) for MDR-exposed latent TB infection (LTBI). The V-QUIN and TB-CHAMP trials both reported their findings at the November 2023 Union conference: with not entirely clear outcomes. Both trials experienced slow recruitment and low incidence of TB outcomes, so experienced problems around statistical power. Both trials separately report the effect of 6 months of levofloxacin PT on TB incidence up to 54 weeks in household contacts (HHC) with wide confidence intervals and Hazard Ratios (HR) of 0.44 (0.15 – 1.25 95%CI) and 0.34 (0.09 – 1.25 95%CI) respectively. The lack of precision needed to detect an effect in both trials was dealt with in a pre-defined Bayesian meta-analysis combining both trials' results, HR 0.41 (0.18 – 0.95 95%CI) (3–5). The PHOENIX trial is yet to report (6).

Despite this progress, the potential need to screen and manage all exposed household contacts (HHC), not just those with evidence on TST or IGRA testing of presumed latent infection, is unaddressed. This is most pertinent for exposed children within households in regions with high background TB prevalence and limited resources to screen and follow up well children.

There are clear and large knowledge gaps around how to manage MDR TB HHCs.

- It is not clear **who** to screen for MDR TB, **when** this should happen, **how frequently** and **how long** the screening should continue, and what tools should be used. It is unclear whether a subset of higher risk contacts can be identified to enable targeted screening to optimise return on use of limited financial and human resources.
- There is a poverty of evidence around **whether PT works, or could work**, in this population. It is unclear which PT to use for what duration, and there are concerns regarding drug side effects and resistance (7).
- Because it is unclear who would benefit from PT, it also unclear **who should be tested** for LTBI. It is difficult to know how to proceed when testing for a condition where the treatment has poor evidence for strong efficacy, particularly in populations where the prevalence of drug sensitive TB is also high and a positive LTBI test may reflect community transmission rather than infections acquired in the household.
- It is unclear **how MDR TB HHC** should be screened with a regular symptom screen, for how long, tested for LTBI, or PT for all those considered high risk without LTBI testing.

Aside from the knowledge gaps described above, there are logistical difficulties in screening, following up HHC for two years and testing for LTBI. There are technical problems with procuring tests, financing the NTP, performing Tuberculin skin tests (TST) on patients requires multiple clinic visits and cost to patients. Two years of follow up requires a lot of time, money and capacity within the health system and can be burdensome for patients. We see that most high burden countries simply do not have the infrastructure or capacity to screen and follow up all MDR TB HHC. A simpler method appropriate for low resource settings, with a minimum dataset for screening and following up contacts would be helpful, and more easily implementable for NTP.

Given these knowledge gaps and setting, I set out to understand whether all MDR TB household contacts (HHC) need to be followed up for 2 years from the start of index patient MDR TB treatment. I aimed to observe the cumulative incidence of TB among exposed HHC, with the intention to design a surveillance protocol which balances resources against sensitivity. Furthermore, I aimed to determine if there is a group of identifiable risk factors that allow targeting of surveillance to specific individual or household level groups of HHC who will not develop TB.

Methods

Recruitment

In January 2020 we began recruiting index patients receiving treatment for MDR or rifampicin resistant (RR) TB across two districts of Lima (Lima Centro and Lima Sur). Recruitment was prospective but included index patients who remained on MDR TB treatment who had been diagnosed within the previous 2 years. The Peruvian national TB programme provided a line list of TB centres treating MDR TB patients at three-monthly intervals. Our field team also contacted participating TB centres to identify index patients who had not been included on the line list. In March 2020, the SARS-CoV-2 pandemic interrupted recruitment for 12 months until February 2021. During a staged recommencement of the study, potential participants were contacted by telephone until national guidance permitted a reduction in social distancing

measures in January 2022. At this point we restarted field work and returned to the standard study procedures and protocols.

All eligible index patients were informed about the study and invited to participate, they were screened for eligibility and if consent was given their data was entered into a health system information software module developed in line with the WHO TB surveillance District Health Information System 2 (DHIS2) module. We developed this DHIS2 module as a study specific tool and as a pilot for future work. Tool development was collaborative with the National TB programme (NTP) and developers at the Universidad Peruana Cayetano Heredia (UPCH). DHIS2 software validation was completed by using excel in parallel and twice weekly data checks. All consenting index patients provided detail of their household contacts who were cohabiting when their MDR TB treatment started or during their symptomatic months pre-diagnosis. Household contacts were contacted by telephone and invited to participate.

Participant procedures

I hypothesised that the majority of the burden of cumulative TB in household contacts of MDR TB index patients would present in the first 6 months after the index started treatment and that there would be limited incremental benefit to screening and follow-up of TB in HHCs beyond 12 months. The aims of this work were to generate a large prospective cohort e-registry of MDR TB contacts to improve understanding of exposed contacts and inform programmatic control, monitoring, and surveillance. The primary instrument of analysis took the form of data describing the cumulative incidence of TB in MDR TB HHCs at 0, 3, 6, 12, 18 and 24 months after the date on which the linked index MDR TB patient started treatment. The second goal was to identify MDR TB HHCs at individual or cluster level, who were at such low risk of cumulative (incident or co-prevalent) TB that they would not require two years of surveillance to ensure TB does not develop. We accept that no combination of factors is going to identify all those at low risk but if a combination of factors can confidently identify, a large proportion, say 50% of those who don't develop TB without wrongly including those who do it would be very useful during screening of large numbers of household contacts. Whilst creating the e-registry, I aimed to improve and facilitate routine TB data collection using a software tool. This would lead to the generation of a minimum dataset required for monitoring and surveillance; along with the generation of an up-to-date line-list of people at risk. These people at risk would

be potential beneficiaries once an effective PT for MDR TB exposed contacts is identified and becomes available.

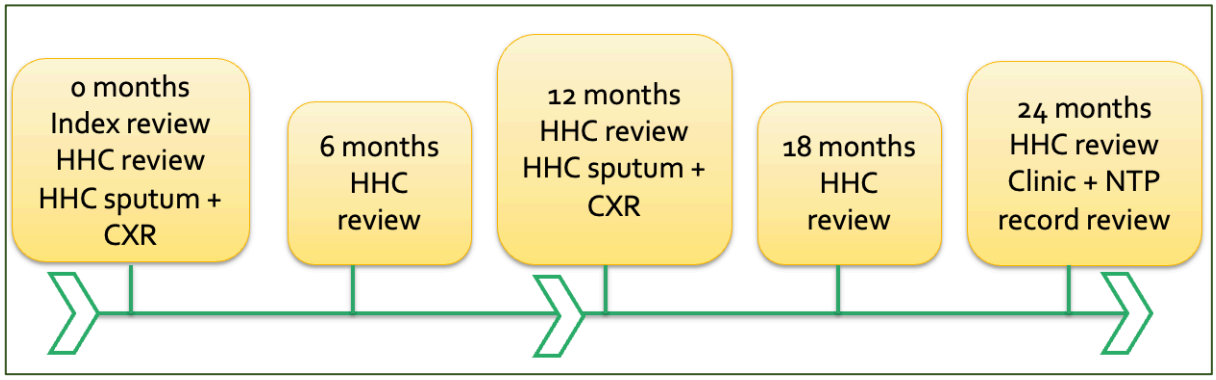

Diagram 1. Planned study procedures and follow-up

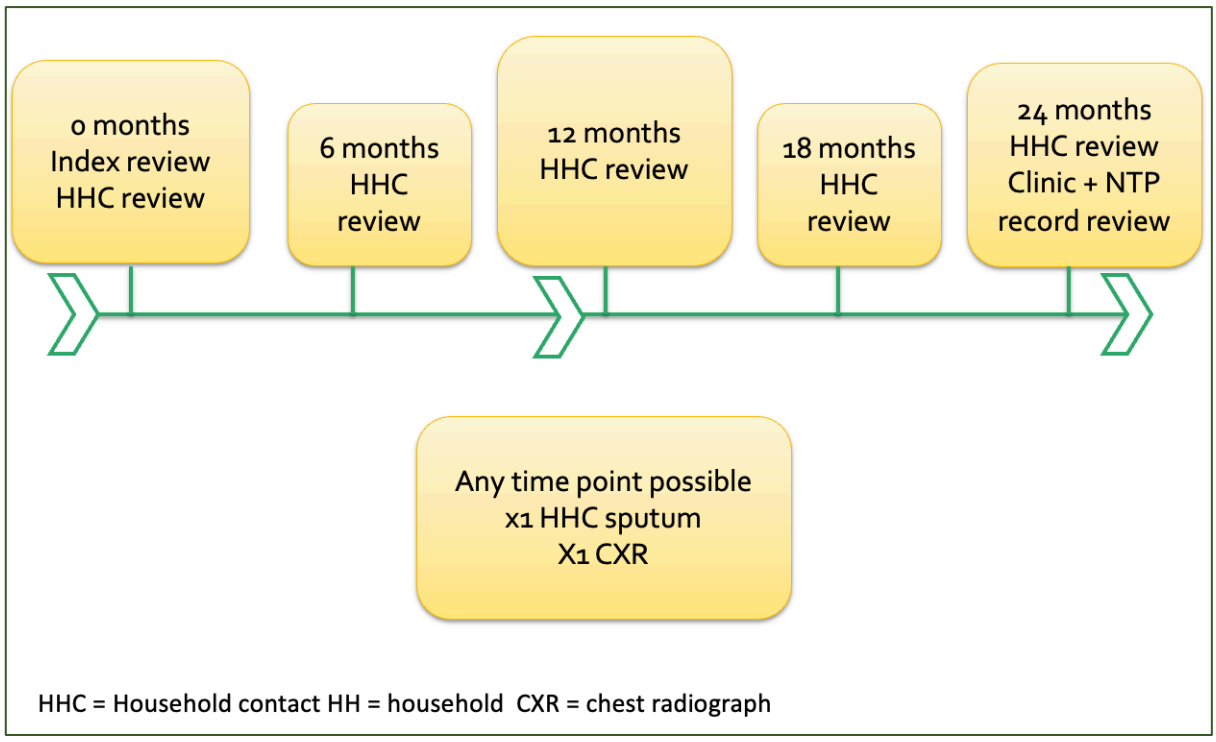

Diagram 2. Study procedures and follow-up in reality

We collected data on index patient age, sex, site of TB, diagnostic features, household size, bedroom number, index risk factors, HHC age, sex, relationship, bedroom sharing, contact risk factors, duration of exposure to an infectious index case, HHC symptoms and diagnostic testing. HHC were reviewed 6 study time points: 0 months after index MDR TB treatment start, 3 months, 6 months, 12 months, 18 months, and 24 months. The study follow-up points were telephone clinical surveys. Prior to pandemic interruptions we planned to test all HHC sputum at initial screening, 12 months and 24 months follow up time points and to obtain chest radiographs at initial screening and 12 months. Following a return to field work in January

2022 we tested all HHC sputum once for *Mycobacterium tuberculosis (M.tb)* and offered one chest radiograph. Participants with abnormal results or a new diagnosis of TB were referred to the National TB programme for management. Participants were followed up to 2 years from Index MDR TB treatment start or were censored at either the last contact point or at the study end point in August 2023.

Microbiology and Radiology analysis

Sputum samples were processed in the Infectious Diseases Laboratory of the Universidad Peruana Cayetano Heredia (UPCH) by study staff. All sputum had an auramine stained sputum smear for acid and alcohol fast bacilli (AAFB), a microscopic-observation drug-susceptibility (MODS) assay and mycobacteriological culture to identify *M.tb* and the presence of rifampicin or isoniazid resistance (8,9). Microbiological diagnosis within the National TB programme included Ziehl-Neelsen sputum smear AAFB, mycobacteriological culture, microscopic-observation drug-susceptibility (MODS) assay, or a Genotype MTBDR on the Hain platform.

Chest radiographs were taken at two Hospitals, both Hospital de la Solidaridad (SISOL) one in Lima Sur (Villa Maria del Triunfo) and one in Lima Centro (San Juan de Lurigancho) and were reported by radiologists at each site. Radiological suspected TB criteria are defined as “abnormal, likely TB”, “abnormal, possible TB”, “abnormal, unlikely TB” and “normal”. The criteria for an “abnormal, likely TB” report that requires further investigation for suspected TB include only: miliary radiology pattern, an active cavitating lesion, upper lobe consolidation with evidence of volume loss and hilar or paratracheal lymphadenopathy.

The clinical criteria for suspected TB requiring investigation included any one of fever, weight loss, persistent cough for over 2 weeks, night sweats or lymphadenopathy. In children, additional clinical criteria include anorexia, unusual fatigue, and failure to thrive.

Case Definition

A microbiological diagnosis was made on microbiological evidence of a tissue specimen with *M.tb* either in our UPCH laboratory or within the NTP. A clinical diagnosis was made on a combination of 2 or more symptoms for a minimum of 2 weeks plus radiological suspected TB criteria in the absence of positive microbiology. Clinical diagnoses were confirmed by the NTP

and reported to the study or identified by the study team as suspicious for TB, requiring further investigation and referred to the NTP for investigation and management. These participants were then reported back to the study as either confirmed clinical TB or under investigation not TB.

Statistical analysis

A primary outcome of confirmed/probable TB was a composite of microbiological and clinical diagnoses. A second outcome of possible TB included participants with clinical symptoms and radiological changes, but without confirmed disease and no treatment was started.

The target sample size was 300 index MDR TB patients with 1800 household contacts. This was based on the expectation to observe a cumulative incidence of (co-prevalent and incident) TB at 5%. In a previous Peruvian cohort study, 3.3% of MDR TB household contacts developed TB (10). A meta-analysis in 2014 identified 7.8% of DRTB HHC developed TB, whilst in MDR TB HHC incident TB was 6.5% (11,12). We expected a margin of error of 3.5-6.5%. In 2018, around 80% of the 3,000 Peruvian patients who started on MDR TB therapy were in Lima, and we expected that if we were able to recruit 25% of these, we would have access to 300 index TB patients, 1,500 HHC and 75 (52-97) cumulative TB diagnoses in HHC. This sample size would provide 90% power to find TB in 5% of HHC with precision of 1.5% around a 95% confidence interval. Loss to follow up (LTFU) was expected to be around 30%. The minimum numbers required to complete this study were 881 HHC which was expected to result in around 41 cumulative cases of TB.

All statistical analyses were completed in R software. We report the proportion of household contacts with a confirmed TB diagnosis, and the cumulative incidence (co-prevalent and incident) of TB in the first 2 years from index patient MDR TB treatment start. Using Cox Proportional Hazards, we report the hazard ratio of TB in Household contacts and a cumulative incidence plot to estimate the person-time-at-risk. Including a second outcome of possible TB in the cumulative incidence plot will allow increased sensitivity to assess how long household contacts need to be followed up to identify the burden of onward household transmission. Finally, we planned a multivariate analysis to investigate a secondary outcome of risk factor effect on cumulative TB using logistic regression to assess the odds of TB in HHC with prolonged exposure, crowding and shared bedrooms, adjusted for household-level clustering.

Variables included in the multivariate model were selected using a directed acyclic graph (DAG) to explore relationships, causality and confounding.

Ethics & Financial disclosure

The study was approved in Peru by UPCH Ethics Committee (104423), the Peruvian National TB Programme and the Ministry of Health Ethics committees for each of Lima Sur and Lima Centro districts. In the UK, the study was approved by the London School of Hygiene & Tropical Medicine (LSHTM) Ethics Committee (14651). The study was funded by a Wellcome Trust fellowship [210830/Z/18/Z].

Results

Recruitment

We recruited a total of 935 household contacts (HHC) from 223 index patients with MDR TB (Figure 1). From an identified 603 index MDR TB patients, 77 were ineligible. Reasons for ineligibility included living alone (no HHCs), incapacity to consent, MDR treatment interruption and XDR identification. 100 index patients declined participation and 203 we were unable to contact, we attempted to call each index between three-five times using their contact phone numbers provided by the TB clinics. Additionally, we attended the TB clinics at the time each index was due to attend for MDR TB treatment. During the pandemic lockdown periods we were unable to visit their households, any travel during this period required an official permission slip. We frequently found that phone numbers were no longer in use and that patients changed the TB treatment centres they received treatment at as their circumstances and home addresses changed during the pandemic. We did not recruit any patients with Rifampicin mono-resistant (RR) TB (without confirmed concurrent isoniazid resistance). 223 index patients agreed to participate. From the 223 index patients, there were a total of 942 HHC of which 7 were found to be diagnosed with TB prior to their index patients' MDR TB treatment start date and were excluded from the analysis making a final total of 935 HHC.

Incident TB

From the remaining participants, 20 of 935 (2.14%) contacts from 17 households were found to have microbiologically or clinically confirmed TB and were started on MDR TB treatment. A further 9 household contacts from 9 households had possible TB (radiological changes and clinical symptoms), these were all in follow-up and had not yet received TB treatment. These 29/935 (3.10%) of participants met the study's criteria requiring monitoring or continued management. Only the confirmed clinical or microbiological TB were included in the Cox proportional hazards analysis. Of the participating HHC 690 (73.80%) underwent sputum microbiological sampling and 317 (33.90%) had a chest radiograph within the study. For the 20 events during follow up the total person-time-at-risk is 95.12 years.

Comparison of characteristics of HHC with and without TB

The baseline characteristics of participants are shown in Table 1, along with comparisons between HHC with TB, with possible TB, total HHC, HHC who had a sputum sample and HHC who had a chest radiograph. Age, sex, familial relationship, median exposure to an infectious index, intensity of exposure and preventive therapy (PT) medication were broadly similar across all groups. Greater numbers of HHC with TB had recorded risk factors. Implementation of PT was low with only 9/105 (8.5%) children aged 5 and younger receiving PT. Overall, during follow up, 70 (7.50%) reported symptoms consistent with possible TB.

Comparison of characteristics of index patients and households with and without a HHC who developed TB

Baseline characteristics of index patients and their household environment are shown in Table 2 comparing households with TB, possible TB, total households, and no TB. Greater numbers of households with TB in contacts had index patients with smear and culture positive TB. There were no other significant differences between index and household level variables.

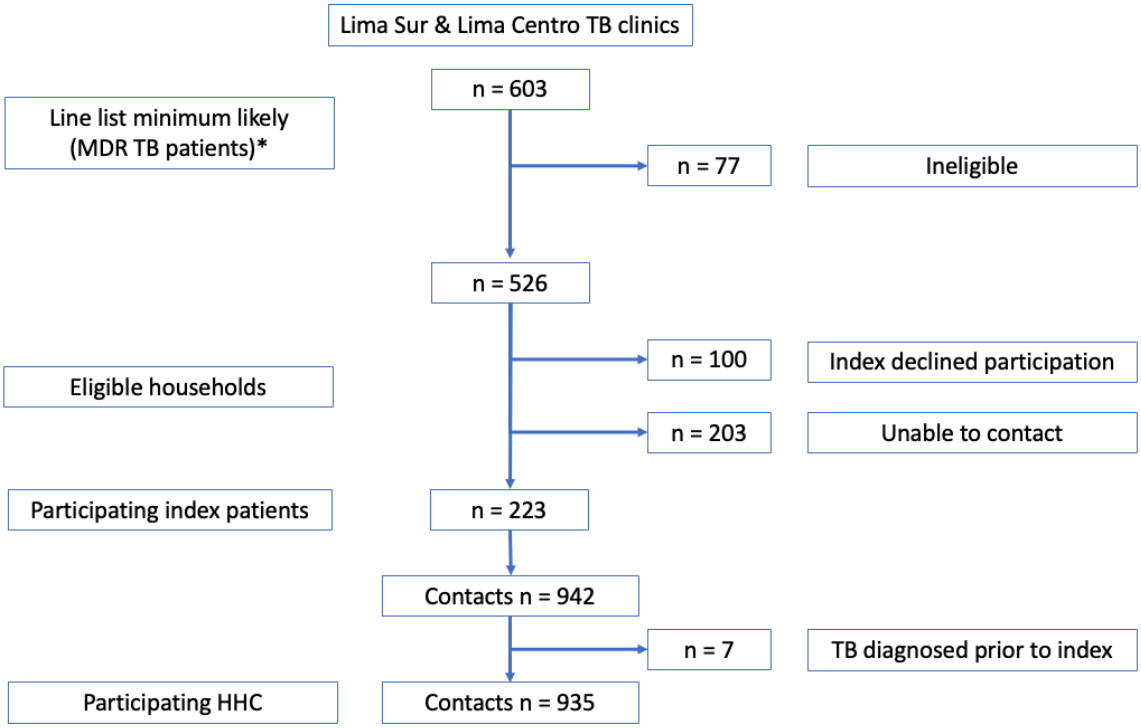

Figure 1. CONSORT Recruitment flow diagram. **Of** ***a known 603 MDR TB patients, 223 consented and had a total of 942 household contacts. Of these 935 were included in the final analysis.***

*Total number of known MDR TB patients, no rifampicin mono-resistant patients were recruited.

Table 1. Baseline characteristics of Household Contacts (HHC) with cumulative confirmed TB, possible TB requiring follow up, total HHC numbers, HHC for whom we have sputum and HHC for whom we have radiology.

Variables (metrics)		HHC with TB n = 20		HHC with possible TB n = 9		Total HHC n = 935		HHC with sputum sample n = 690		HHC with radiology n = 317	
Sex female n, % (CI95*)		12	60 (39 - 81)	4	44 (12 - 77)	510	55 (51 - 58)	382	55 (52 - 59)	183	59 (53 - 64)
Median Age (years, IQR)		30 (21 - 50)		55 (19 - 59)		24 (11 - 45)		27 (13 - 46)		26 (11 - 47)	
Any risk factors† n, % (CI95*)		13	65 (44 - 86)	3	44 (12 - 77)	207	22 (19 - 25)	134	19 (17 - 22)	72	23 (18 - 27)
HHC given PT n, % (CI95*)		2	10 (00 - 23)	1	11 (0 - 32)	20	2 (12 - 31)	14	2 (1 - 3)	9	3 (1 - 5)
Median duration of exposure to an infectious index (weeks, IQR)		8 (4 - 12)		8 (4 - 11)		8 (4 - 12)		8 (4 - 12)		8 (4 - 12)	
Intensity of contact to index^ n, % (CI95*)		2	10 (0 – 23)	2	22 (0 – 49)	135	14 (12 – 17)	86	13 (10 – 15)	43	14 (10 – 17)
Relationship to index n, % (CI95*)	Parent	5	25 (6 – 44)	2	22 (0 – 49)	173	19 (16 – 21)	127	18 (16 – 21)	68	22 (17 – 26)
	Child	3	15 (0 – 31)	2	22 (0 – 49)	218	23 (21 – 26)	145	21 (18 – 24)	74	23 (19 – 28)
	Sibling	9	45 (23 – 67)	2	22 (0 – 49)	187	20 (17 – 23)	139	20 (18 – 23)	65	21 (16 – 25)
	Partner	0	0	1	11 (0 – 32)	65	7 (5 – 9)	45	7 (5 – 8)	19	6 (3 – 8)
	Other relation	3	15 (0 – 31)	2	22 (0 – 49)	291	31 (28 – 34)	233	34 (31 – 37)	87	28 (23 – 33)

*CI95. 95% Confidence interval
†risk factors include diabetes, <5 years, >65years, people living with HIV, immunosuppression, current smoker
^sharing a bedroom with the index

Table 2. Baseline characteristics of index and households with and without a secondary case of TB and in total

Variables (metrics)	Household with TB n = 17		Household with possible TB n = 7		Household without TB n = 199		Total household n = 223	
Index sex male n, % (CI_{95}*)	11	65 (62 - 68)	3	43 (40 - 46)	103	54 (45 - 59)	117	54 (49 - 56)
Index age (years, IQR)	30 (21 – 38)		40 (30 - 51)		32 (25 - 45)		32 (25 - 46)	
Pulmonary TB n, %, (CI_{95}*)	17	100	7	100	178	97 (85 - 94)	202	97 (96 - 98)
Index risk factors† n, %, (CI_{95}*)	6	35 (32 - 38)	3	43 (40 - 46)	55	29 (21 - 34)	64	30 (27 - 33)
Index sputum smear positivity n, %, (CI_{95}*)	12	71 (68 - 74)	3	43 (40 - 46)	118	62 (52 - 66)	133	62 (58 - 65)
Index culture positivity n, %, (CI_{95}*)	12	71 (68 - 74)	1	14 (12 - 17)	73	38 (30 - 43)	86	40 (37 - 43)
Index GeneXpert positive diagnosis n, %, (CI_{95}*)	1	6 (4 - 7)		0	7	4 (1 - 6)	8	4 (2 - 5)
Household crowding^ n, %, (CI_{95}*)	2	17 (14 - 19)	2	29 (26 - 31)	39	26 (14 - 25)	43	26 (21 - 26)
Household size (median number of people, IQR)	5 (3 - 7)		6 (4 - 7.5)		5 (3 - 7)		5 (2 - 20)	

*CI_{95}. 95% Confidence interval
†risk factors include alcohol excess, diabetes, miner, previous TB contact, <5 years, >65years, people living with HIV, immunosuppression, current smoker.
^household overcrowding was calculated using the total number of household residents and the number of bedrooms (13).

Table 3. Baseline characteristics of Household contacts (HHC) lost to follow-up and not lost to follow-up and in total

Variables (metrics)		HHC not lost to follow up n = 352		HHC lost to follow up n = 583		Total HHC n = 935	
Sex female n, % (CI95*)		192	55 (50 - 59)	318	55 (51 - 59)	510	55 (51 - 58)
Median Age (years, IQR)		24 (10 - 45)		24 (12 - 45)		24 (11 - 45)	
Any risk factors† n, % (CI95*)		83	24 (19 - 28)	124	21 (18 - 25)	207	22 (19 - 25)
HHC given PT n, % (CI95*)		2	1 (0 - 1)	18	3 (2 - 4)	20	2 (12 - 31)
Median duration of exposure to an infectious index (weeks, IQR)		8 (4 - 12)		8 (5 - 12)		8 (4 - 12)	
Intensity of contact to index^ n, % (CI95*)		36	10 (7– 13)	99	17 (14 –20)	135	14 (12 – 17)
Relationship to index n, % (CI95*)	Parent	54	15 (12 – 19)	119	20 (17 – 24)	173	19 (16 – 21)
	Child	87	25 (20 – 29)	131	22 (19 – 26)	218	23 (21 – 26)
	Sibling	56	16 (12 – 20)	131	22 (19 – 26)	187	20 (17 – 23)
	Partner	20	6 (3-8)	45	8 (6 – 10)	65	7 (5 – 9)
	Other relation	135	38 (33 – 43)	157	27 (23 – 31)	291	31 (28 – 34)

*CI95. 95% Confidence interval
†risk factors include diabetes, <5 years, >65years, people living with HIV, immunosuppression, current smoker
^sharing a bedroom with the index

Cumulative incidence of TB among HHCs of MDR TB indexes in Peru

The primary analysis looked at cumulative incidence of 20 participants presented with incident TB by 2 years of follow up (Figure 2). Overall, of those who were identified as having developed incident TB during 2 years of follow-up 11 (58%) developed TB by 6 months of follow up and 15 (79%) occurred by 12 months of follow up. A further 3 cases were diagnosed between 12-24 months. Participant loss to follow-up was a substantial problem, with only 45% (95%CI 42-48%) still in follow-up at two years from index case MDR TB treatment start. 73% (95%CI 70-75%) were in follow-up at six months, 68% (95%CI 65-71%) were in follow-up at one year and 55% (95%CI 52-58%) at eighteen months from index case MDR TB treatment start. However, baseline characteristics of HHC are broadly similar amongst those lost to follow-up compared with those in follow-up and overall (Table 3). There was one death unrelated to TB amongst the HHC, too small a number to require a competing risks model as it would have made negligible difference to the model.

The Cox proportional hazards analysis (Table 4) showed no significant effect of any of the covariates in the model except for TB risk factors in a household contact. Amongst the subgroup of HHCs determined to have TB risk factors additional to their household exposure, the hazard of developing TB was increased six-fold (HR 6.56, 95%CI 2.16-21.92, $p<0.001$). The Likelihood Ratio test indicates that TB risk factors are associated with TB events on follow up. Meanwhile the Wald test result of 0.01 suggests the effect of TB risk factors on outcome is statistically significant. The covariate “shared sleeping room” was a confounder, interacting with overcrowding in the initially screened model, and was removed from the final model.

The data set had small numbers of outcomes, limiting the degrees of freedom we were able to use and hence the number of variables included in any model. Using the conservative Bonferroni correction (14) to calculate the corrected p value level to prevent us incorrectly rejecting the null hypothesis or causing a type 1 error. There are 9 degrees of freedom tested in this model, giving a Bonferroni corrected p value of 0.005. We can confidently reject the null hypothesis, there is an association between household contact risk factors and cumulative TB.

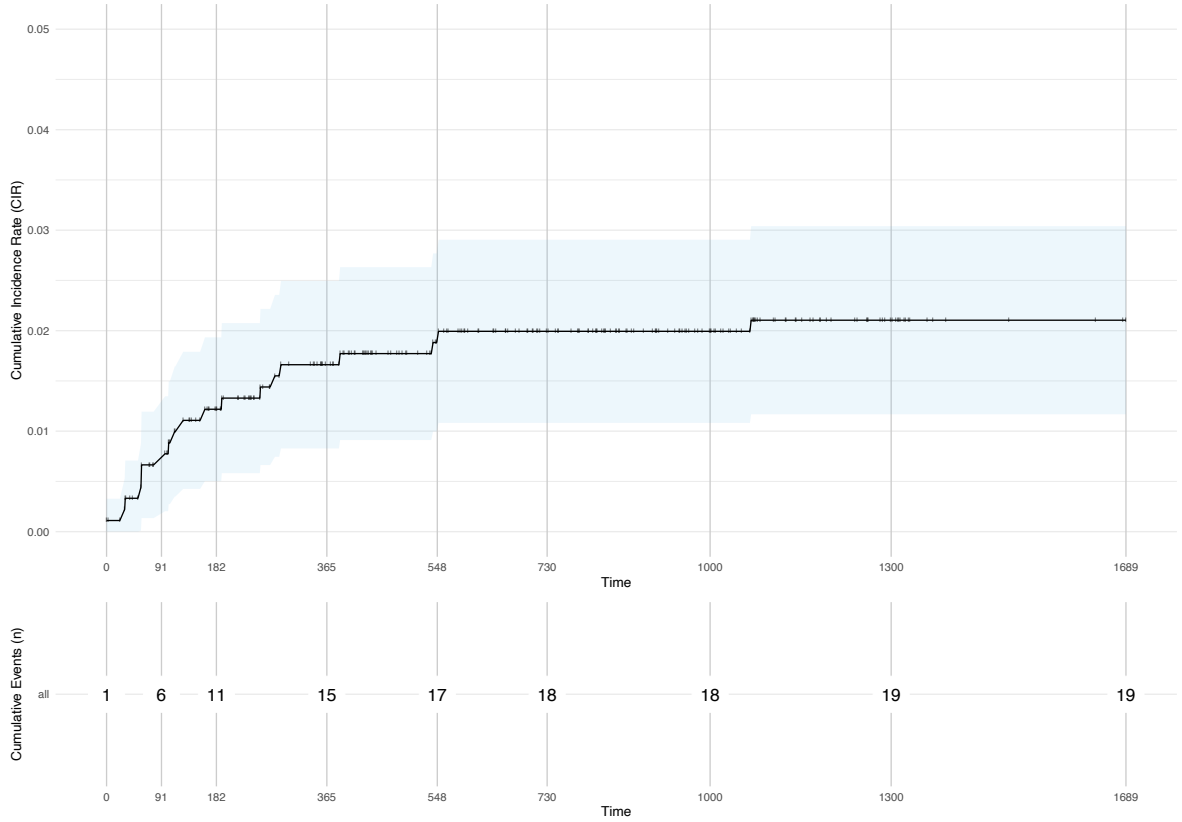

Figure 2. TB disease cumulative incidence at each follow-up time point.
11/19 (58%) cases occur by 6 months of follow up, 4 (21%) more occur by 12 months, 2 (10%) more by 18 months, 1 (5%) final TB case by 24 months.

Table 4. Cox proportional Hazard Ratios of cumulative TB in HHC from MDR index treatment start date. Variables included were HHC age, HHC sex, Overcrowding, any HHC risk factors for TB, and duration of exposure to an HHC.

	HR	Log HR	95% CI	SE	Z score	p-value
HHC Age	1.007	0.994	0.99 - 1.03	0.010	0.625	0.532
MALE Sex	0.634	1.576	0.21 - 1.90	0.559	-0.815	0.415
Overcrowding	0.314	3.179	0.07 - 1.08	0.773	-1.47	0.135
Risk factors in HHC	6.56	0.152	2.16 - 21.92	0.566	3.322	<0.001
Duration of exposure to infectious index (weeks)	0.984	1.016	0.90 - 1.08	0.048	-0.332	0.740
Concordance= 0.787, Likelihood (LR) ratio test 16.47 5df p=0.006, Wald test = 15.42 5df p=0.009						

The cumulative incidence graphs for time to the primary outcome TB in household contacts with 3 different variables of interest are shown below (Figure 3). Figure 3a shows the effect of sex on time to TB incidence, there is no significant difference in time to TB between males

and females in this study with wide overlapping confidence intervals. Figure 3b shows the lack of effect of sharing a sleeping room with their index case on TB incidence in this study. There was no TB in a HHC who shared a sleeping room with their index until 18 months of follow up. Figure 3c shows the effect of any 1 risk factor for TB in a HHC on cumulative TB incidence compared with HHC without any risk factors for TB. There is a clear difference between the two groups with a strong correlation between exposure and disease with non-overlapping confidence intervals. The remaining variables (Table 1 and 2) did not show any significant difference in their cumulative incidence graphs.

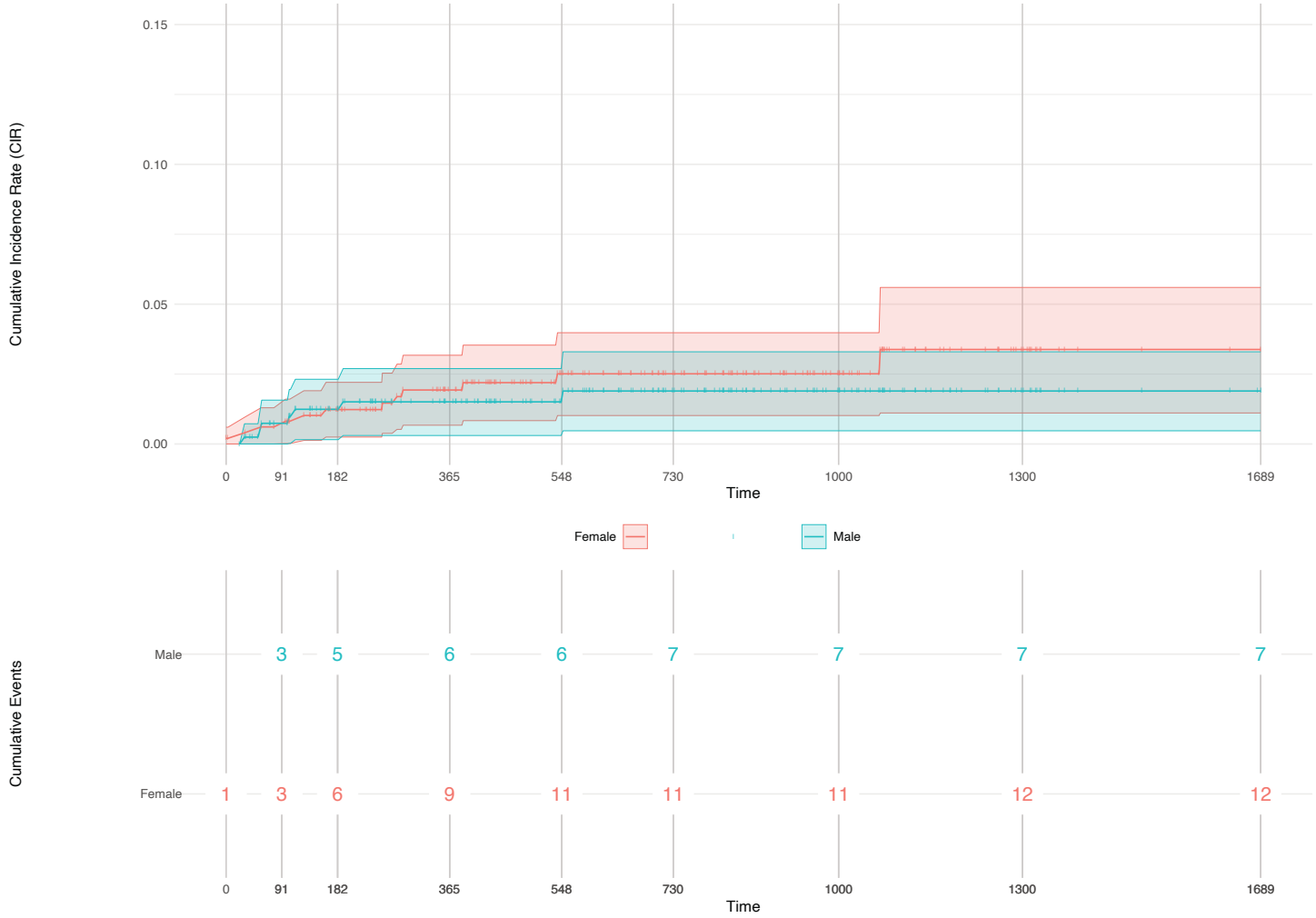

Figure 3a. Cumulative Incidence graph of the effect of sex on temporal incidence of cumulative TB. Colours indicate male and female participants. There is no difference in the cumulative incidence between male and female participants with overlapping confidence intervals.

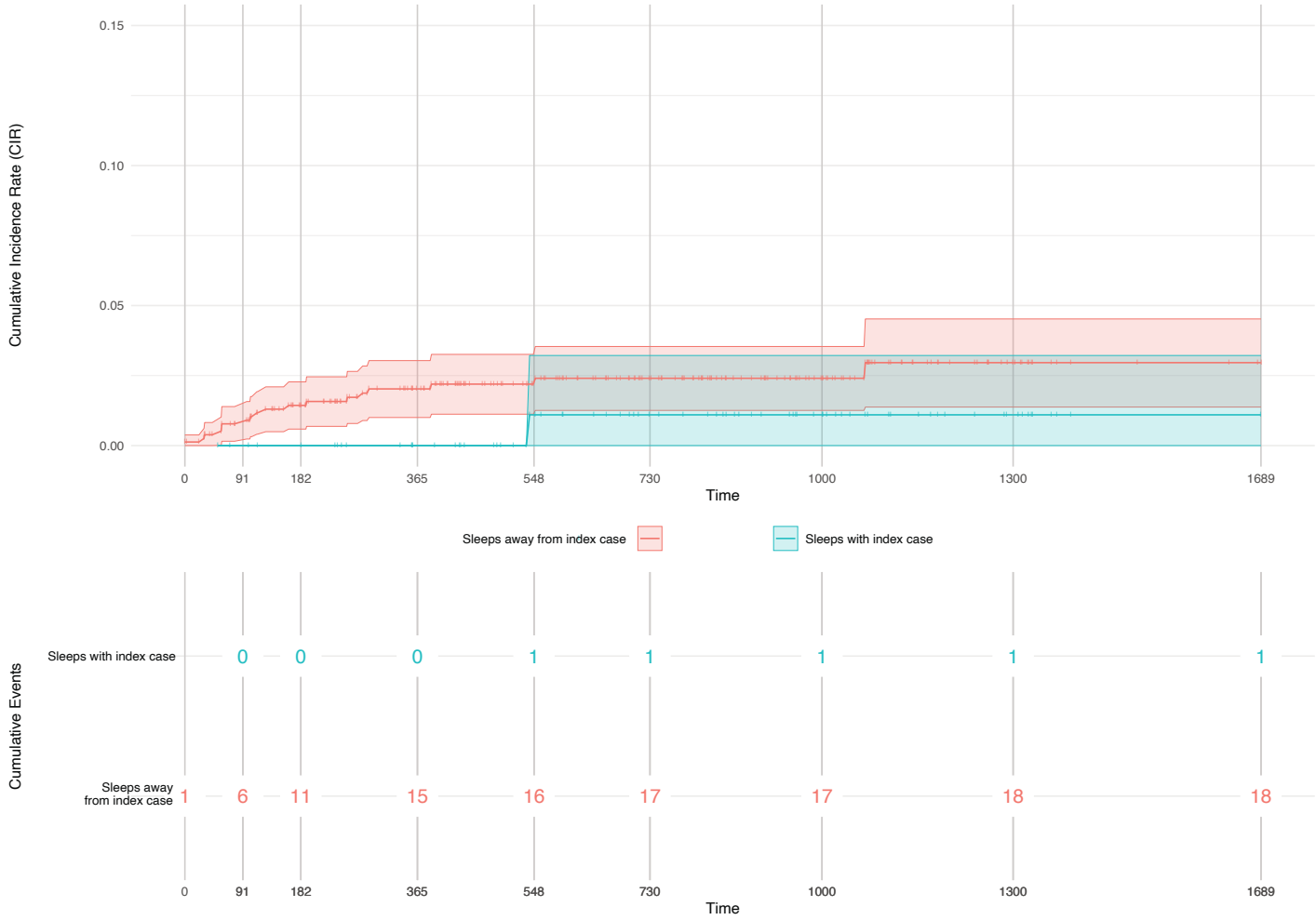

Figure 3b. Cumulative Incidence graph of the effect of sharing a sleeping room on temporal incidence of cumulative TB. Colours indicate if HHC shared a sleeping room with their index, blue = shared a room & red = separate sleeping room. There is no difference in cumulative incidence with overlapping confidence intervals. No HHC who shared a sleeping room with their index developed TB until 18 months of follow up.

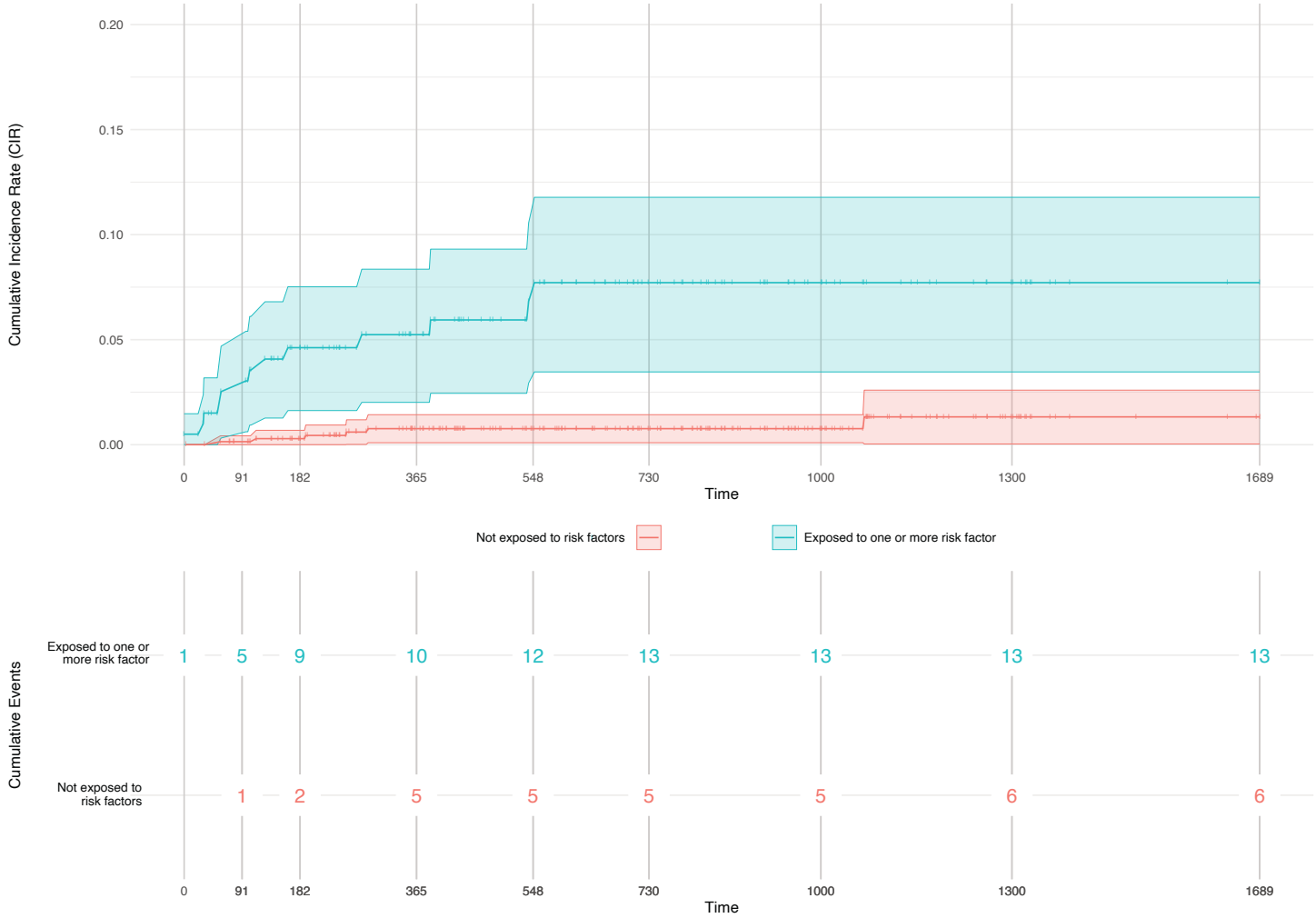

Figure 3c. Cumulative Incidence graph of the effect of any HHC risk factors on temporal incidence of cumulative TB. Colours indicate the presence of any risk factors for developing TB, Any 1 risk factor = blue & no risk factors = red. There was a significant difference in cumulative incidence of TB between HHC who had at least 1 risk factor and HHC who had no risk factors. *risk factors were diabetes, age <5, >65, people living with HIV, current smoker, and immunosuppression

Discussion

Screening household contacts of MDR TB patients at baseline and up to 12 months from the initiation of index MDR TB treatment identified most cumulative TB cases in this cohort. The effort of contact surveillance, investigation and follow up remains constant throughout the 2 years with diminishing gains after the 1-year point. 80% of incident TB was identified by screening and investigation of HHCs up to 1 year after their index case initiated MDR TB treatment. In pragmatic terms for national tuberculosis programmes (NTPs) there was an obvious benefit from screening and managing HHC up to one year, but beyond that point the incremental benefits appeared to be smaller. This finding is tempered by lack of precision within the Cox model for all the parameters measured, driven by difficulty in ascertaining cases and high loss to follow-up. Following HHCs for a second year increased the detection to around 95%, but the extended period of surveillance required to identify the additional 15% of all cases at two years would increase the effective cost-per-case found substantially (with different scaling factors for each of time, effort, and financial costs). In fact, focusing the burden of surveillance and monitoring within the first 6 months of follow up provides the highest returns (57% of all cases were identified by 6 months) in this context. If we are to reach the WHO End TB strategy goals of reducing new cases of TB by 80% by 2030, screening and following up MDR TB HHC for at least six months and preferably for one year must be a priority for NTPs.

HHC risk factors did significantly affect TB incidence, and we observed how the combined risk factor variable correlated significantly with the hazard of developing TB over time. Despite this, there was neither a single variate, nor any set of covariates which could reliably screen HHC at baseline to pragmatically select out those who were unlikely to get TB and whom therefore did not need further follow-up or management. It is not possible from this work to identify (i.e. through genome sequencing) if the TB cases were secondary to the index cases in this study, or whether conversely the high community transmission rate in an endemic setting might have impacted on the cumulative TB incidence. It is however possible to conclude that household screening up to 12 months from MDR TB index treatment initiation is an effective method of identifying ongoing transmission and cumulative disease. Whilst there was no cost analysis component within this work perhaps the next logical step would be to consider whether for an NTP, the costs of following up all household contacts beyond 12 months are potentially too large to justify given the small relative gains in either identifying incident TB or

interrupting transmission. This data is useful to focus NTP attention, time and funds on initial screening and management for 12 months. Early unreported results from both the VQUIN and TB-CHAMP trials support our findings that TB incidence in HHC occurs early, and specifically before the 12-month follow-up point in most cases (3,4).

The data is censored at the two year-follow-up point. Not all participants were enrolled for two years and the loss to follow up rate was high, which impacted right censoring of the data. It is unclear whether the loss to follow up was random or whether HHCs left the study for reasons which might relate to TB infection or disease. Additionally, HHCs were eligible to participate if they had been exposed to an infectious index who remained on treatment but had started treatment up to two years prior to study enrolment. we included any index MDR patients who were still on TB treatment, not only those starting MDR treatment at the time of recruitment. For example, some of the index patients had 3 months of treatment and some had a year at recruitment. Their HHC were entered into the study, had their initial screening consult (a 0-month review) and then had their time appropriate follow up. This caused left censoring, but we do not believe that it has prevented us from reliably capturing all new TB events.

There are several questions that emerge from this study.

Would the implementation of a six- or twelve-month screening programme make it possible to manageably identify and interrupt the ongoing transmission of TB in Peruvian households?

Household transmission does not account for most onward TB infection and disease yet if NTP are able to improve household screening enough is it possible to identify and interrupt the ongoing transmission risk and reduce TB incidence? Identifying 80% of incident cases would allow the Peruvian NTP to incrementally interrupt transmission and prevent new cases. The burden of MDR TB in new cases in Peru is high at 4.9% and in retreatment cases at 9.5%, the resource cost of investigating, treating, and managing MDR is high. Identifying, treating early so therefore preventing 80% of MDR cases alone would have a significant impact on the NTP treatment costs. However, we know that incident cases in HHC are not all MDR TB, meaning there would be a benefit to the generalised drug sensitive (DS) TB prevalence by reducing incidence. This would require a TB nurse at each clinic to contact all HHC by phone at 4 times points over the first year of follow up to enquire about symptoms, arrange sputum testing and

radiology if any symptoms are present. Whilst not perfect screening and follow up it is a pragmatic way of screening and identifying any incident disease in HHC and would improve the current case identification in HHC.

Where should the intensive investigation and follow up of HHC lie within one year of follow up?

Our aims with this work were to carefully describe regular follow up and investigations at routine times to identify where there was efficient and effective TB identification and where investigations and resources were superfluous to need. This was not possible due to the limitations of this cohort during the pandemic. However, it is clear from the cumulative incidence plots that the burden of incident TB occurs in the first 6 months, so it follows that TB screening and investigation during this period have the highest returns. The second six months of follow up identify a further 21% of cases, again this is likely to be a productive investment of resources. Focusing the burden of resources in the first 6 months with sputum and chest radiology during this period seems a pragmatic approach in the light of limited evidence. The final year of follow up returns only another 15% of cases despite the resource expenditure to be the same as the first year of follow up,

Does saturation of transmission occur at the household level?

As hypothesised during the COVID-19 pandemic, there was probably prolonged TB disease duration and longer periods of increased transmissibility, this may have led to a saturation of transmission and incident cases within the household. This could then mean sporadic and non-repeated contacts are at greater risk of infectious spread. Previous modelling work suggests this but there is as yet no good data to support this theory (12). In this study the covariates ‘sharing a bedroom’ and ‘overcrowding’ had HR close to 0 but with wide 95CI% crossing 1. There was clearly no effect from these covariates, intensity of exposure beyond time spent together within a household does not appear to impact likelihood of transmission in this work. This result does fit within the theory of household level saturation. Given the advances in understanding of respiratory pathogen transmission dynamics due to SARS-CoV-2 it is a sound theory that there may have been a saturation of household transmission during the pandemic and increased transmission following. This theory needs further research to describe it accurately in TB.

How should global health actors navigate and mitigate the impacts of pandemics and ecological crises in trials and research?

In a world that is increasingly susceptible to ecological crises and pandemics, it may become necessary for us to adapt our research and clinical trial strategies and protocols. This means embracing flexibility, innovation, and redundancy in our approaches to ensure resilience and sustainability in the face of these growing challenges.

The pandemic affected the study design, the testable hypothesis and interrupted follow-up procedures, recruitment, and outcomes. We were unable to assess the most impactful method to follow up participants, where the focus of investigation and management should lie nor where the effort and cost is redundant. In the future contingency planning would help mitigate some of these impacts. As part of study development, we should plan regular crisis management meetings, mobile radiology, and sputum collection protocols, and regularly reflect on what study measures could be reimplemented at each time point. Identifying light touch research methods would enable research to continue despite pandemic societal lockdowns. During the pandemic disruptions to TB diagnosis and effective treatment initiation occurred in most countries with high TB prevalence due to disruption of health services. The unavoidable delays will have increased within household exposure to infectious index patients and increased the possibility on ongoing transmission.

The pandemic impacted TB diagnoses, with Peruvian data showing lower annual reporting rates to WHO which increased post pandemic and continue to increase (1). Peru had one of the highest COVID-19 adjusted Infection-Fatality Rates (IFR) globally (13). Peruvian TB clinics were converted to COVID-19 immunisation centres, there was a prolonged lockdown period with poor access to health care, and an insecure health system structure which was overwhelmed. Poverty, a lack of capacity, corruption and limited health system funding impacted Peru's national COVID-19 outcomes and patients were reluctant and unable to access care (14–16). We had been interested to look at TB reporting and HH incidence during pandemic lockdown periods, but our data are unable to assess any more than the overall incidence of TB from index MDR TB treatment start. The methods we used resulted in a positive selection bias in those accepting chest radiology. We were unable to assess timely and accurate capture of all sputum *Mycobacterium tuberculosis* positivity because we were unable to take sputum samples at early follow-up points as planned.

Whilst improving understanding of transmission is essential it must be considered with the other main components within the TB risk equation, pathogen biology and host immune response. Inefficient diagnostics, health system barriers, treatment regimens, slow pathogen clearance, and poor host immune clearance due to pre-existing patient variables all impact TB transmission, risk, and outcomes. Individual patient factors also impact MDR TB outcomes and treatment success. For example, undernutrition is associated with mortality and a longer time to sputum culture clearance (17). In this setting during the pandemic, this is an important factor.

Our work adds to the body of growing evidence that two years of follow-up for MDR TB contacts is no longer appropriate for NTP with limited resources (2). Twelve months of light-touch screening and follow-up appears to be enough to identify most of the incident TB cases that will arise among HHCs whilst providing results for the investment of costs. This active 12 months of follow-up could be followed by 12 months of passive follow-up where well-informed HHCs have streamlined access to diagnostic testing if they develop symptoms. Those HHCs who are exposed to or live with additional risk factors for TB (i.e. beyond cohabiting with a TB case) require careful assessment and investigation given their significantly higher HR within this study. That is not to say that those without risk factors should not be closely observed, as this is in fact the larger group and the numerical majority of incident cases is still likely to occur in this group.

Shorter periods of screening and follow up are easier, more likely to be completed by NTPs and likely to identify almost all the cases of household transmission and TB. To have an impact on reducing TB in line with the WHO goal of 80% fewer TB cases by 2030 we must improve the ability of NTPs to screen and manage household and high-risk contacts (18).

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Chapter 3

Extremely high SARS-CoV-2 seroprevalence in a strictly-Orthodox Jewish community in the UK

Impact of work

The staggeringly high seroprevalence in this minority population in London attracted both the Jewish, National and International press. It was reported on in The Times, The Financial Times, the Guardian, BBC, Associated Press and the New York Times (1–4). Highlighting longstanding inequalities in health and healthcare access amongst minorities and faith communities. This process initiated community stakeholder and local government engagement to address the structural causes of these inequalities and identify how to better engage support communities to achieve improved health (5–8). The aim of this ongoing local government work is to improve public health policy for minorities.

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SECTION A – Student Details

Student ID Number	LSH212211	**Title**	Dr
First Name(s)	Katherine		
Surname/Family Name	Gaskell (Wilson)		
Thesis Title	Respiratory pathogen transmission among exposed household contacts		
Primary Supervisor	Professor David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	The Lancet Regional Health - Europe		
When was the work published?	18/05/2021		
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Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am the first author. I assisted in the design, I conducted and managed the research. I prepared the specimens for analysis. I collated the data., I helped plan and execute the analysis with MM, RE, CHR, DG. I wrote the first paper draft and developed the manuscript in consultation with MM, RE, CHR, DG and WW.

SECTION E

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Date	07/02/2024

Supervisor Signature	David AJ Moore
Date	07/02/2024

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Research paper

SARS-CoV-2 seroprevalence in a strictly-Orthodox Jewish community in the UK: A retrospective cohort study

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ABSTRACT

Background: Ethnic and religious minorities have been disproportionately affected by SARS-CoV-2 worldwide. The UK strictly-Orthodox Jewish community has been severely affected by the pandemic. This group shares characteristics with other ethnic minorities including larger family sizes, higher rates of household crowding and relative socioeconomic deprivation. We studied a UK strictly-Orthodox Jewish population to understand transmission of COVID-19 within this community.

Methods: We performed a household-focused cross-sectional SARS-CoV-2 serosurvey between late-October and early December 2020 prior to the third national lockdown. Randomly-selected households completed a standardised questionnaire and underwent serological testing with a multiplex assay for SARS-CoV-2 IgG antibodies. We report clinical illness and testing before the serosurvey, seroprevalence stratified by age and sex. We used random-effects models to identify factors associated with infection and antibody titres.

Findings: A total of 343 households, consisting of 1,759 individuals, were recruited. Serum was available for 1,242 participants. The overall seroprevalence for SARS-CoV-2 was 64.3% (95% CI 61.6-67.0%). The lowest seroprevalence was 27.6% in children under 5 years and rose to 73.8% in secondary school children and 74% in adults. Antibody titres were higher in symptomatic individuals and declined over time since reported COVID-19 symptoms, with the decline more marked for nucleocapsid titres.

Interpretation: In this tight-knit religious minority population in the UK, we report one of the highest SARS-CoV-2 seroprevalence levels in the world to date, which was markedly higher than the reported 10% seroprevalence in London at the time of the study. In the context of this high force of infection, all age groups experienced a high burden of infection. Actions to reduce the burden of disease in this and other minority populations are urgently required.

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1. Introduction

The UK has been severely affected by the COVID-19 pandemic with 66,197 deaths involving COVID-19 up to the 27th November 2020 [1]. In the UK the pandemic has disproportionately affected minority ethnic populations with relatively higher numbers of positive cases and overrepresentation in admissions to hospitals and Intensive Care Units and deaths [2–4]. Similar disparities have been

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Research in context

Evidence before the study

In January 2021, we searched PubMed for articles on rates of SARS-CoV-2 infection amongst ethnic minority groups and amongst the Jewish population. Search teams included "COVID-19", "SARS-CoV-2", seroprevalence, "ethnic minority", and "Jewish" with no language restrictions. We also searched UK government documents on SARS-CoV-2 infection amongst minority groups. By January 2021, a large number of authors had reported that ethnic minority groups experienced higher numbers of cases and increased hospitalisations due to COVID-19. A small number of articles provided evidence that strictly-Orthodox Jewish populations had experienced a high rate of SARS-CoV-2 infection but extremely limited data was available on overall population level rates of infection amongst specific ethnic minority population groups. There was also limited data on rates of infection amongst young children from ethnic minority groups.

Added value of the study

We report findings from a population representative, household survey of SARS-CoV-2 infection amongst a UK strictly-Orthodox Jewish population. We demonstrate an extremely high seroprevalence rate of SARS-CoV-2 in this population which is more than five times the estimated seroprevalence nationally and five times the estimated seroprevalence in London. In addition the large number of children in our survey, reflective of the underlying population structure, allows us to demonstrate that in this setting there is a significant burden of disease in all age groups with secondary school-aged children having an equivalent seroprevalence to adults.

Implications of the available evidence

Our data provide clear evidence of the markedly disproportionate impact of SARS-CoV-2 in minority populations. In this setting infection occurs at high rates across all age groups including pre-school, primary school and secondary school-age children. Contextually appropriate measures to specifically reduce the impact of SARS-CoV-2 amongst minority populations are urgently required.

observed in other settings and amongst hospitalised patients, mortality has been found to be higher in ethnic minority patients in both the UK and USA [5–7]. The reasons for this disparity are unclear but are likely multifactorial, reflecting both higher rates of infection resulting from socio-economic factors including deprivation, less ability to work from home, higher use of public transport and larger household sizes, as well as increased severity of illness due to higher rates of comorbidities or delayed access to care [8].

Most population based studies in the UK have focused on the impact experienced by larger minority ethnic groups and not on smaller groups, such as the Jewish community, who compromise approximately 265,000 individuals or 0.5% of the UK's population [9]. In particular, strictly-Orthodox Jewish communities have anecdotally reported high rates of infection, morbidity and hospitalisation during the first wave of the UK pandemic [10]. These anecdotal reports are supported by findings from Public Health England (PHE) which suggests a higher risk from infection in the UK Jewish population and higher rates of death due to COVID-19 in those self identifying as Jewish with an age standardised mortality rates between March and May for Jewish men over 65 years of 759 per 100,000 population, much higher than in other religious groups [11]. This higher rate of infection amongst the Jewish community might reflect socio-demographic differences compared to the general UK population. Strictly-Orthodox Jewish communities have among the highest total fertility rates in the UK, and experience high rates of overcrowded homes and higher levels of socio-economic deprivation [12,13]. However, after adjusting for socio-economic factors the hazard ratio for death in Jewish men remained double that of Christian men suggesting that routine socio-economic factors alone do not explain all of the increased rate of infection seen in this community [11]. During the first wave of the COVID-19 pandemic in Israel, strictly Orthodox Jewish communities also reported higher incidence rates of COVID-19 than other socio-economically similar communities [14,15], or when compared to households in Arab communities with similar sizes and levels of crowding [15].

In Spring 2020, a tightly-knit strictly-Orthodox Jewish in the UK community became aware that they appeared to be experiencing a high burden of SARS-CoV-2. In view of this, and national data suggesting a high burden of infection in the Jewish community and a possible disproportionately high burden in the strictly-Orthodox community, we co-developed a cross-sectional serological survey to measure the burden of infection and identify factors associated with transmission and to inform local control efforts.

2. Methods

2.1. Recruitment and survey methodology

Within a strictly-Orthodox Jewish community in the UK we conducted a household-focused seroprevalence survey between late-October and early December 2020 prior to the third national lockdown. We obtained a comprehensive list of all resident households within the community, held by our community collaborators. Each household was assigned a unique identifier and we used simple-random sampling to identify households for recruitment in to the study. A second list of households where laboratory confirmed infections were known to have occured (the 'enriched' list) was also included to inform subsequent transmission models. Members of the study team telephoned households to complete a standardised questionnaire, including demographics, comorbidities, report of any previous presumed COVID-19 illness, previous PCR or serological testing for SARS-CoV-2, access to care whilst unwell with a COVID-19 like illness, illness severity, attendance at work, educational or other community locations and travel overseas between February - November 2020. All households were visited within 10 days of completing the questionnaire for collection of a serum sample except where a member of the household reported active symptoms of COVID-19 in which case the visit was scheduled for a minimum of 14 days after symptom onset. All members of the household were eligible to be included without age restrictions.

2.2. Laboratory analysis

Serum samples were analysed for the presence of IgG specific for SARS CoV-2 trimeric spike protein (S), Receptor Binding Domain (RBD) and nucleocapsid (N) antigens using a multiplex chemiluminescence immunoassay (MSD, Rockville, MD) evaluated by our laboratory as previously described [16,17].

2.3. Statistical analysis

For the purpose of this analysis, we considered a positive trimeric-spike response as evidence of a prior SARS-CoV-2 infection as this target was shown to be the most sensitive and specific target in assay validation16. We designed the survey to detect a seroprevalence of

anti-trimeric spike antibodies of 10%. We presumed there would be clustering of infections at the household level and used a design-effect of 2 to increase our sample size to adjust for this. We calculated we would therefore need to recruit 1,730 participants. Assuming an average household size of 6 we estimated this would be approximately 300 households.

To obtain unbiased estimates, we restricted our analysis to individuals from randomly selected households. Results related to the 'enriched' household dataset will be presented in future analyses. We used cut-off values for the SARS-CoV-2 immunoassay from previous validation studies for antibodies against trimeric-spike, nucleocapsid and receptor binding domain.As assay cut-offs are well defined in adults but less well defined in children, we conducted a sensitivity analysis in which we considered thresholds for the trimeric-spike assay of twice the previously defined limit.

We report the proportion of individuals who had a clinical illness consistent with COVID-19 and the proportion of individuals who had accessed testing prior to this survey. We calculated the seroprevalence of SARS-CoV-2 infection stratified by age and sex and adjusted for clustering at the household level. Age was classified as pre-school (0-4 years), primary education (5-10 years), secondary education (11-18 years), adults and retirement (greater than 67 years). We created a proxy variable which accounted for the number of community events attended during the year (hereinafter 'community-gatherings') and a variable reflecting household overcrowding based on the number of household residents and the number of bedrooms [18]. To identify factors associated with SARS-CoV-2 infection we fitted a multivariable random-effects logistic regression model adjusted for clustering at the household level. We hypothesised that age, sex, being unable to work from home, attendance at community events and household overcrowding were *a priori* likely to be associated with an increased risk of infection. We reported p-values for associations based on likelihood ratio-tests. We assessed relationships between titres of antibodies against each of SARS-CoV-2 spike, receptor binding domain and nucleocapsid in relation to individuals age, sex, the presence or absence of a symptomatic illness, and for symptomatic individuals the timing of their illness using a log-linear random effects model. Analysis was performed in R version 4.02 and random-effects models were fitted using lme4 version 1.1.26

2.4. Ethics

The study was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (*Ref 22532*). Verbal informed consent was given during the telephone survey and written consent provided prior to phlebotomy. Parents provided written consent for children.

2.5. Role of the funding source

This work was jointly funded by UKRI and NIHR [COV0335; MR/V027956/1], a donation from the LSHTM Alumni COVID-19 response fund, HDR UK, the MRC and the Wellcome Trust.

The funders had no role in the design, conduct or analysis of the study or the decision to publish.

3. Results

3.1. Enrollment and demographics

A total of 903 randomly selected households were approached, of which 343 households comprising 1,759 individuals were enrolled (Fig. 1). An additional 70 households with known cases of COVID-19 were provided by our community partners (referred to as 'enriched households') were approached to participate, of which 28 households comprising 183 individuals were enrolled (Supplementary Figure 3). Serum samples were collected from 1242 individuals (70.6%) from 283 randomly selected households and 137 individuals (74.9%) from 24 'enriched households.

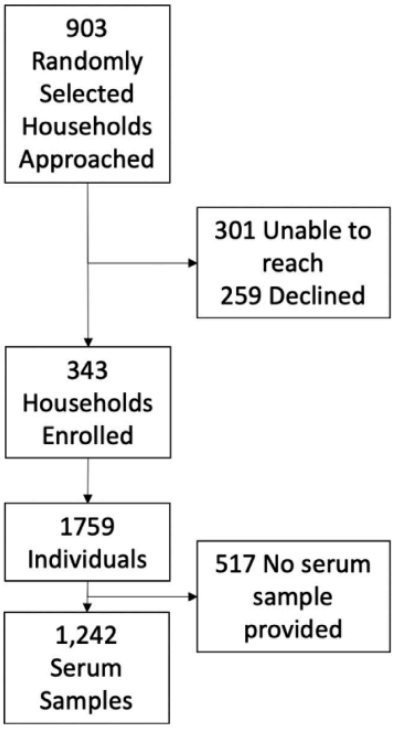

Fig. 1. CONSORT diagram for recruitment into the study.

The median household size was 5 (IQR 3-7, Range 1-14 cf. UK median 2) [19] with a median of 3 bedrooms (IQR 2-5). The median age of survey participants was 14 years (IQR 7-33, cd. UK median age 40 years) and 48.6% of participants were male (Supplementary Table 1). Of individuals who gave a serum sample, 48% were male and the median age was 16 years (IQR 9-37).

3.2. Routine testing for SARS-CoV-2

Amongst randomly selected households, a total of 446 individuals (25.4%) had undertaken either PCR or serological testing for SARS-CoV-2 and 182 individuals (10.3%) reported a positive result on at least one test. Reported reasons for testing were because of symptomatic disease or stated as "for other reasons" (Table 1). Individuals who reported testing were older (median 31 IQR 15-46 vs 12 IQR 5-25) and more likely to be male (52.7% vs 47.1%).

Overall 228 individuals (13%) underwent either PCR and/or serological testing because of symptomatic illness. A total of 191 symptomatic individuals reported providing a sample for PCR with a swab positivity rate of 59% (n = 113). By comparison 20 of 181 PCR tests done for other reasons were positive (11%). Overall 182 individuals (10.3%) reported already having received a positive test for SARS-CoV-2 prior to the survey.

Serology on this cohort revealed an overall seroprevalence for SARS-CoV-2 of 64.3% (95% CI 61.6-67.0%, 799/1,242). The seroprevalence varied by age between 27.6% (95% confidence interval (CI) 20.8 - 35.6%) for children aged under 5 years of age to 73.8% (95% CI 68.2 - 78.8%) amongst secondary school children and 74% (95% CI 70.0 -77.6%) adults (Fig. 2) (Supplementary Table 2). Seroprevalence was significantly higher amongst men (68.8%, 95% CI 64.9 - 72.5%) than women (59.7%, 95% CI 55.8 - 63.5%) ($p = 0.001$). Only three individuals (2%) reported a previous positive PCR result but did not have detectable anti-spike antibodies. All three individuals had a positive PCR in

Table 1
Characteristics of the tests undertaken, stratified by presence of symptoms.

	Tested for any reason		Tested due to symptomatic illness		Tested for any other reasons	
	Tested	Positive Result	Tested	Positive Result	Tested	Positive Result
PCR	364 (20.7%)	133 (36.5%)	191 (10.9%)	113 (59.2%)	181 (10.3%)	20 (11.0%)
Serology	128 (7.3%)	55 (43.0%)	47 (2.7%)	31 (66.0%)	83 (4.7%)	25 (30.1%)
Total	446 (25.4%)	182 (40.8%)	228 (13.0%)	141 (61.8%)	238 (13.5%)	44 (18.5%)

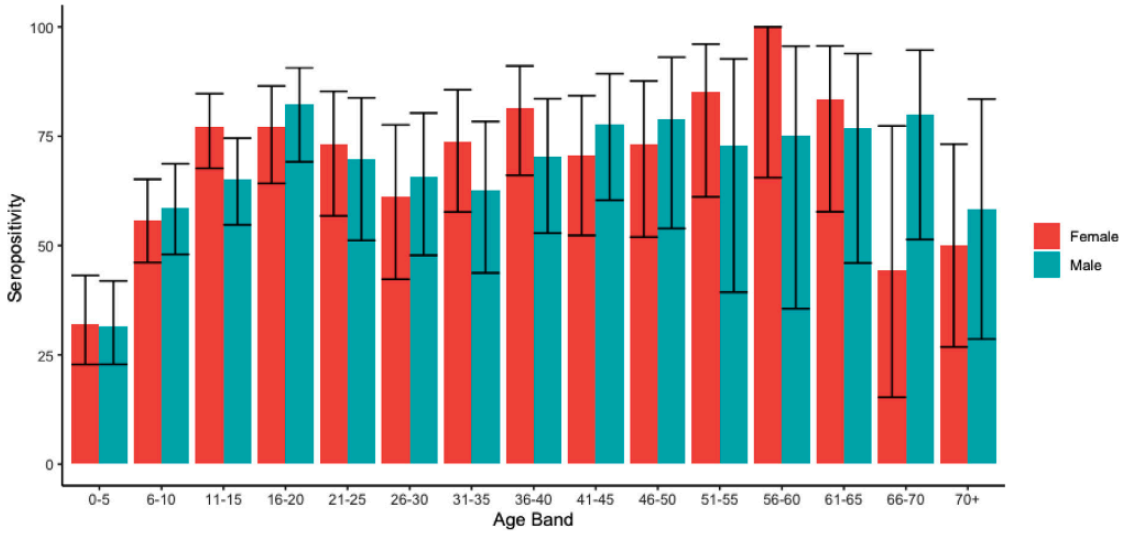

Fig. 2. Age specific seroprevalence in participants in the study. Colours indicate male and female participants.

October and therefore a negative serological test might reflect that they had not yet seroconverted at the time of sample collection. In a multivariable random-effects logistic regression model, seropositivity was associated with increasing age, male sex, household density and whether and individual was was working or in education, but not associated with attendance at community gatherings (Table 2). No pre-existing comorbidities were associated with seropositivity.

Overall, 697 (37.5%) individuals reported an illness they thought was consistent with COVID-19. There were clear peaks in reported illness consistent with the first and second waves of COVID-19 in the UK (Fig. 3). Of individuals reporting a suspected illness 49.8% were male and the median age was 28 (IQR 14-41). A total of 16 (0.9%) individuals reported hospitalisation for COVID-19 and a further three individuals were reported to have died of COVID-19.

Overall 81.9 % of individuals who reported an illness consistent with COVID-19 were sero-positive and 53.7% of asymptomatic individuals were sero-positive. Of cardinal symptoms of COVID-19, self-reported cough (OR 3.0, 95% CI: 2.1-4.2), fever (OR 2.3, 95% CI: 1.6 -3.4) and loss of smell or taste (OR 11.8, 95% CI: 6.8 -20.6) were all associated with seropositivity. In the overall population self-reported loss of smell or taste had a positive

Table 2
Association between seropositivity and demographic and behavioural variables. *Likelihood ratio test

		Unadjusted Analysis			Adjusted Analysis		
		Marginal Probability	OR (95% CI)	p-value*	Marginal Probability	aOR (95% CI)	p-value*
Age	**Young Children (0-4 years)**	29.7%	0.07 (0.04 - 0.12)	<0.001	31.5%	0.10 (0.05-0.20)	<0.001
	Primary Education (5-10 Years)	57.6%	0.36 (0.24-0.54)		64.2%	0.77 (0.37-1.61)	
	Secondary Education (11-18 Years)	73.4%	0.87 (0.58-1.31)		77.4%	1.70 (0.89-3.27)	
	Working age Adults (19-67)	74.9%	1		70.1%	1	
	Retirement age adults (67+ Years)	58.3%	0.32 (0.13-0.80)		54.7%	0.35 (0.14-0.91)	
Sex	**Female**	63.4%	1	0.009	62.1%	1	0.002
	Male	69.2%	1.46 (1.10 - 1.93)		69.0%	1.62 (1.18-2.24)	
Housing	**Not overcrowded**	67.2%	1	0.498	63.9%	1	0.412
	Overcrowded	64.3%	0.86 (0.55 - 1.34)		67.2%	1.23 (0.74-2.06)	
Employment and Education Status	**Neither Working nor in Education**	68.5%	1	<0.001	69.7%	1	0.015
	In Education	56.4%	0.49 (0.35 - 0.67)		58.6%	0.45 (0.25-0.81)	
	Working From Home	77.8%	1.78 (1.1 -2.87)		74.7%	1.40 (0.82-2.38)	
	Working Outside Home	72.1%	1.25 (0.68-2.3)		66.3%	0.81 (0.41-1.58)	
Number of Community Gatherings Attended		1 = 66.2% 3 = 66.1% 5= 66.1%	1.02 (0.98-1.06)	0.666	1 = 65.2% 3 = 65.4% 5 = 65.5%	1.04 (0.84-1.28)	0.702

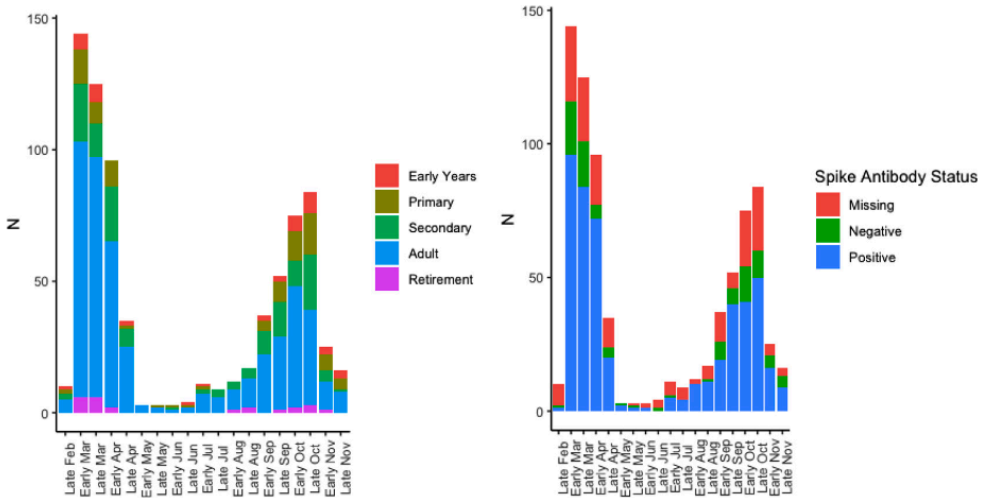

Fig. 3. Self-reported COVID-19-like illness in participants in the study stratified by age and antibody status. The reduced number of self-reported cases in November is an artefact reflecting the timing of the survey. Missing Anti-Spike antibody status occured when participants did not participate in phlebotomy after completing the survey. No phlebotomy samples gave an inconclusive result.

predictive value of 94.5% for positive serology (Supplementary Table 3).

Titres of spike, receptor binding domain and nucleocapsid antibodies were higher amongst individuals who reported a symptomatic illness (Fig. 4) (Table 3). Amongst symptomatic individuals titres declined following time since reported symptomatic illness which was more marked for nucleocapsid than for other targets (Supplementary Figure 1).

4. Discussion

We found an extremely high seroprevalence of SARS-CoV-2 antibodies in a strictly-Orthodox Jewish community in the UK. Our estimate of 65% population seroprevalence is markedly higher than recent estimates of 6.9% (95%CI 6.3-7.4%) nationally and 10.8% (95% CI 9.3-12.5%) in London by random sampling in October by the Office for National Statistics (ONS) [20]. Rapid declines in self-reported illness followed the introduction and adherence to lockdown in March, demonstrating that even in this highly connected community such measures are effective at reducing transmission. However over the course of 2020, the overall seroprevalence in this tightly knit religious community reached levels similar to those seen in Manaus, Brazil where a seroprevalence of more than 65% has been reported in adults [21]. As our survey was completed by early December 2020, prior to the subsequent winter case surge in London, it is likely that the overall burden of infection in this community is now even higher.

Our estimates are amongst the highest sero-prevalence of SARS-CoV-2 described anywhere in the world to date. In the UK other studies have also reported a higher seroprevalence of SARS-CoV-2 amongst ethnic minority individuals. In the nationally-representative REACT-2 study in September 2020, seroprevalence amongst adults from an ethnic minority was 7.9% compared to 3.6% for the white population21. Whilst direct comparison with our study population is limited by the absence of equivalently detailed sampling of other populations, the overall pattern suggests a particularly high seroprevalence in our study population compared with ethnic minority groups across the UK. The precise reasons why the burden of SARS-CoV-2 has been so high in this population are unclear. In Israel, strictly-Orthodox Jewish communities had markedly higher SARS-CoV-2 PCR swab positive incidence rates compared to other socio-economically similar communities during the first wave of the Israeli epidemic [14,15], whilst seroprevalence following the first wave of COVID-19 was above 30% in many strictly-Orthodox communities the United States [22]. Data from other sources suggest that lower socio-economic status, ongoing need to travel to work and a greater burden of pre-existing comorbidities, may all contribute to increased risk of acquiring SARS-CoV-2.

In our study there was high seroprevalence in all age groups, with the highest in working-age adults and older children where it reached 74%. A strength of our study is the extremely high number of children recruited, which reflects the higher total fertility rates amongst strictly-Orthodox Jewish women compared to the UK average [12,13]. Seroprevalence in the youngest children was lower at 27% but rose rapidly to more than 50% amongst primary school-aged children. The seroprevalence found in this group is approximately four times that reported in a UK multicentre study on healthcare worker children aged 2-15 years demonstrating the high rates of infection in all age groups in the current study [23].

Seroprevalence was higher amongst men than women in our study. Higher SARS-CoV-2 attack rates in men compared to women have also been reported in some but not all otherstudies [24,25]. The higher rate of infection amongst men might reflect biological differences, differences in comorbidities, differences in social mixing patterns or other behaviours. As these differences might vary between different ethnic minority groups it is possible that association between sex and infection may not be consistent across all communities. We did not find any significant associations with reported attendance at community events, workplace or household overcrowding in this population which may be because of a true absence of effect, or that due to the extremely high seroprevalence, the ability to detect risk factors is limited. Further modelling work is planned to investigate this in more detail.

The majority of reported symptomatic illness occurred during the first and second waves of the COVID-19 pandemic mirroring case reporting patterns seen across London and the UK. Just over 10% of participants reported at least one swab test due to symptomatic illness since March, with an overall swab positivity rate of 59%. In line with other studies, children were significantly less likely to report a symptomatic illness [26,27] and this likely explains the higher rates of routine testing amongst adults we observed in our study.

We found that antibody titres against spike and nucleocapsid proteins declined at different rates, and in line with other studies [16,28], that anti-nucleocapsid declined more quickly. If we had used only anti-nucleocapsid antibodies as a marker of previous infection

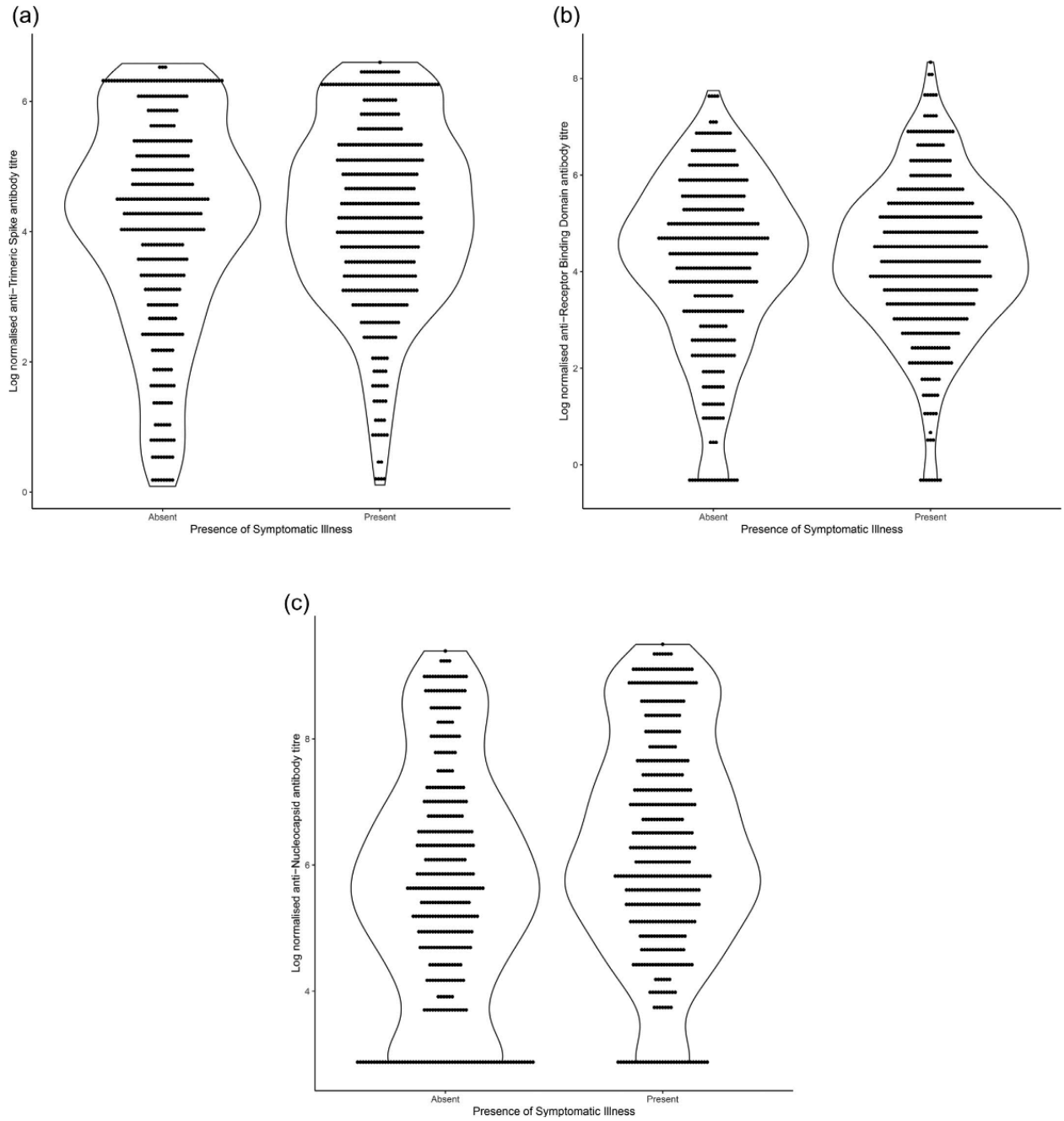

Fig. 4. Log normalised antibody titres for anti-Spike, anti-Receptor Binding Domain and anti-Nucleocapsid. Each panel is stratified by reporting of COVID-19-like symptoms by the participant. The shape shows the density of the distribution of samples.

our estimated seroprevalence would have been 42.8%, significantly under-estimating the likely size of the epidemic that has occurred in this population. Declines in anti-spike antibody titre were less marked over time but it is still possible that we have under-estimated the true seroprevalence in this population. Titres of both spike and nucleocapsid antibodies were higher amongst individuals who reported symptomatic illness.

Our study has a number of limitations. We recruited 38% of households that were approached and obtained serum samples from 70% of study participants. This enrollment rate is similar to other national COVID-19 household surveillance studies, such as the ONS COVD-19 Infection survey [29], suggesting it is unlikely to be a major source of bias. Individuals who gave serum samples were slightly older than those from whom serum was unavailable which may result in an over-estimation of the overall population seroprevalence. We relied on self-report of presumed COVID-19 illness which may be unreliable. However the timings of self reported illness match well to national surveillance data and self-reported illness was strongly associated with the presence of anti-spike antibodies suggesting that in this population this was a reliable metric. Whilst household income

Table 3
Association between log-transformed antibody titres and participant characteristics.

	Anti-Spike Antibody Titre			Anti-Receptor Binding Domain Titre			Anti-Nucleocapsid Antibody Titre		
	Beta	95% CI	p-value	Beta	95% CI	p-value	Beta	95% CI	p-value
All Patients									
Male Sex	0.383	0.142-0.623	0.002	0.412	0.193-0.640	<0.0013	0.314	0.120-0.509	0.002
Age (years)	0.006	-0.001-0.013	0.095	0.008	0.001-0.014	0.031	0.010	0.004-0.0160	0.001
Symptomatic Illness	1.25	0.975 - 1.532	<0.001	1.029	0.772-1.286	<0.001	1.020	0.796 - 1.244	<0.001
Symptomatic Individuals									
Male Sex	0.137	-0.202 - 0.479	0.448	0.1411	-0.173 - 0.457	0.381	0.079	-0.212 - 0.372	0.612
Age (years)	0.023	0.013-0.033	<0.0001	0.021	0.012 - 0.030	<0.001	0.028	0.020 - 0.037	<0.001
Time since reported illness (days)	-0.002	-0.004 - 0.000	0.041	-0.002	-0.004 - 0.000	0.022	0.005	-0.007 - -0.003	<0.001

in strictly-Orthodox Jewish communities is below the national average this is partly offset by the wide network of community charities and support networks [30]. We did not collect detailed data on socio-economic status beyond household size and employment status and so are not able to directly assess the importance of these factors on seroprevalence in the community.

Although our study was conducted in a strictly-Orthodox Jewish community, these communities share many characteristics with other ethnic and religious minority groups including larger family sizes, increased population density, children attending select schools, regular attendance at communal events and gatherings, and English as a second language. As such our findings are likely relevant to other tightly-knit ethnic and religious minority groups in the UK and elsewhere. Our work, conducted in direct collaboration with the community, should be a model for understanding risk in minority populations where there are no similar published data currently.

In conclusion, we found evidence for an extremely high rate of SARS-CoV-2 infection in this specific community affecting individuals of all ages. This provides further evidence that minority communities in the UK and elsewhere are disproportionately affected by the COVID-19 pandemic. The reasons for this remain unclear, although are likely to be a complex interplay of socio-economic and behavioural factors. Further studies to better understand drivers of transmission in ethnic and religious minority populations, conducted wherever possible in partnership with communities themselves, are urgently needed to reduce health disparities and improve outcomes for these populations.

Supplementary data

1. Supplementary Appendix
2. STROBE Checklist

Author Contributions

MM, RME, ChR, DG conceived of the study. KG and DL co-ordinated the survey. KG, MJ, VG conducted lab work. SL, WW, BK, TC, NS contributed to the design of the study. MM, KG, WW, RME verified the underlying data. All authors contributed to the analysis and writing of the manuscript.

Data sharing

Data available on request to the corresponding author:
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Declaration of Competing Interests

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.lanepe.2021.100127.

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Accessory work not in the published paper

Literature review since the publication of this paper

Since the publication of this work, there has been much published on SARS-CoV-2 transmission, disparity of impact in ethnic minorities and adherence to government-enforced guidance around pandemic response.

Published work on ethnic minorities is restricted by limited inclusion of or adjustment for social and structural health factors. Meta-analyses exist which investigate the disproportionate impact on ethnic minorities however, the minority groups assessed are broad, and there is little data on religious minority groups or socioeconomic factors (9,10). Whilst SARS-CoV-2 incidence and hospitalisation were higher amongst minorities, certainly, during the first waves of the pandemic, in-patient mortality and ICU admission are not impacted though there is much heterogeneity amongst published data (10). Health systems are inextricable from the social contexts in which they work, marginalised groups suffering health disparities are the result of complex social structures and interactions (11,12). Population density clearly has an impact on COVID-19 incidence and hospital admissions (13).
Lack of trust in the government, in the health care system and experience of racial or ethnic discrimination, has been linked with poorer adherence to government guidance, in particular to vaccine uptake (14).

Additional Methods not included in published work

LSHTM was approached in June 2020 by a non-governmental organisation (NGO) providing medical and financial support for the strictly Orthodox Jewish community in North London. They recognised the need to identify the causes and source for their perceived high burden of COVID-19. They co-developed the research protocol with a team of infectious disease modellers, social scientists, and clinical epidemiologists. I joined the team once National Institute of Health Research (NIHR) funding had been granted to lead the epidemiological serosurvey. All close-knit community members are included in a telephone book held by the strictly Orthodox community, we randomly generated household identifiers to select households within the community to contact by telephone. Working with the NGO we hired local community members to conduct a telephone survey in Hebrew.

The survey was developed using Open Data Kit (ODK) and captured key indicators listed above alongside culturally appropriate questions on key religious and educational mixing locations and events within the community. Verbal consent was given by telephone by the household lead. We arranged a subsequent single household visit by trained phlebotomists organised by the locally trusted phlebotomy service, and written consent or assent was collected for all. This work was possible given the endorsement and collaboration of key local rabbis, the NGO and a trusted phlebotomy service within the community. The serum samples were held at 4-8^{o}C with the community phlebotomists prior to transfer to LSHTM for serum separation, and aliquot storage at -20^{o}C. Samples were transferred to Professor Goldblatt's laboratory at GOSH.

In addition, antibodies to the four seasonal human coronaviruses that cause pathology were included on the immunoassay panel (HCoV-OC43, HCoV-HL63, HCoV-HKU1, HCoV-229). We assessed the relationship between antibody titres present to human seasonal coronaviruses and SARS-CoV-2 spike. We calculated the risk of seropositivity against reported attendance at community events and calculated the proportions of participants with seropositivity against the number of community events attended.

Additional Results not included in published work

41% (196/473) of households did not have evidence of seropositivity amongst household members, indicating transmission was unlikely to have occurred in these households.

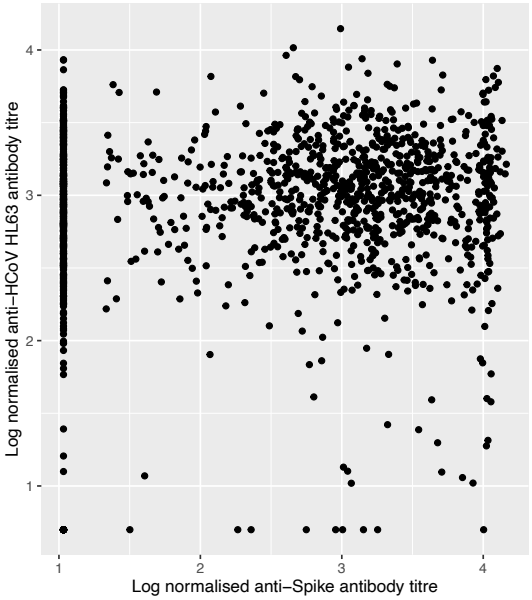
The risk of seropositivity did not increase with increasing community events attended. The proportion of participants with seropositivity fluctuated but did not increase against the number of community events attended (Table 7).

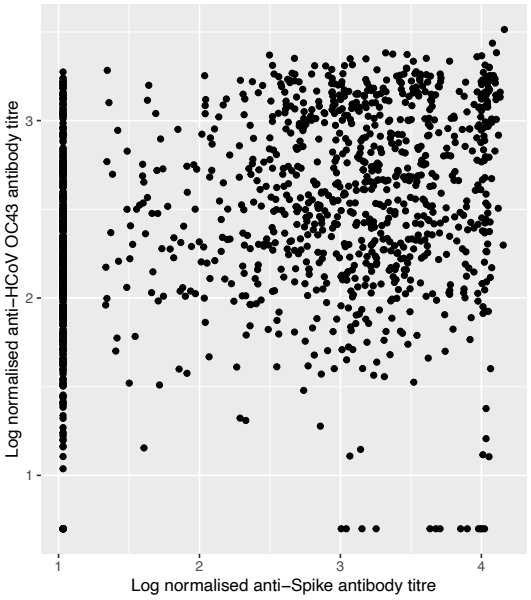
Table 7. Risk of seropositivity in participants reporting attending between 0-7 community events between February – October 2020.

Cumulative Community events attended	Participants	Spike seropositive cases	Proportion % (100/N x seropositive cases)
1	17	13	77
2	69	41	59
3	214	140	65
4	608	413	68
5	352	231	66
6	89	51	57
7	29	21	72

No relationship or trend was seen between measured seasonal human coronavirus antibody titres and SARS-CoV-2 spike antibody titres (Figure 5).

Figure 5. Log normalised antibody titres for anti-Spike against endemic human seasonal coronaviruses (HCoV-OC43, HCoV-HL63, HCoV-HKU1, HCoV-229).


Log normalised anti−HCoV HL63 antibody titre
Log normalised anti−Spike antibody titre
1
2
3
4


Log normalised anti−HCoV OC43 antibody titre
Log normalised anti−Spike antibody titre
1
2
3
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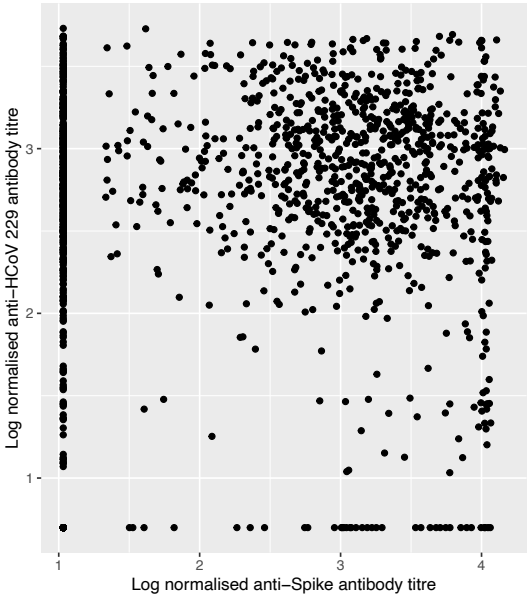
Log normalised anti−HCoV 229 antibody titre
Log normalised anti−Spike antibody titre
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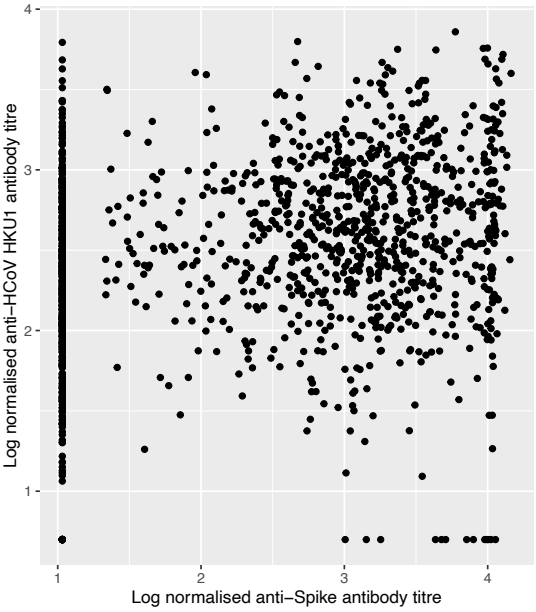
3
4


Log normalised anti−HCoV HKU1 antibody titre
Log normalised anti−Spike antibody titre
1
2
3
4

Discussion not included in published work

The population surveyed, typical for the strictly Orthodox Jewish Community (see population structure Figure 2), has low rates of reported comorbidities and mortality. Whilst we included survey questions on participants no longer residing in households or deceased, there were few within this population. We have limited information on the severity of the SARS-CoV-2 epidemic in this population during the first two waves of the pandemic.

From the survey results, repeated attendance at community events did not confer a greater risk of SARs-COV-2 seropositivity, and thereby infection, the likely cause is population saturation prior to the reported community events. It is not possible to conclude this resolutely from this data. That 41% of households with no evidence of seropositivity does support this hypothesis. Nervousness amongst the community about the illegality of community gatherings during the pandemic may have influenced accuracy of reported data. This was partially mitigated using female community members to collect the survey in Hebrew with assurances of anonymity. Additionally, attendances at community gatherings are viewed as an essential part of their religious practice and therefore their membership of the community. However, this will have had some unmeasurable impact on survey responses.

The community and organisations I collaborated with during this work had a strong sense of identity separate to secular society as a whole and to secular Jewish communities. With hard work over a long period of time the organisations had become a trusted pillar of their community, an essential asset in their work navigating secular health and public health systems to advocate for community members. Their community's opinion of them and wider society's opinion of them carried weight and was guarded closely using public relations officers. It was essential to respect the organisation's privacy and that of its community, this work would not have been possible without this guiding principle.

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Supplementary Information for

Extremely high SARS-CoV-2 seroprevalence in a strictly-Orthodox Jewish community in the UK

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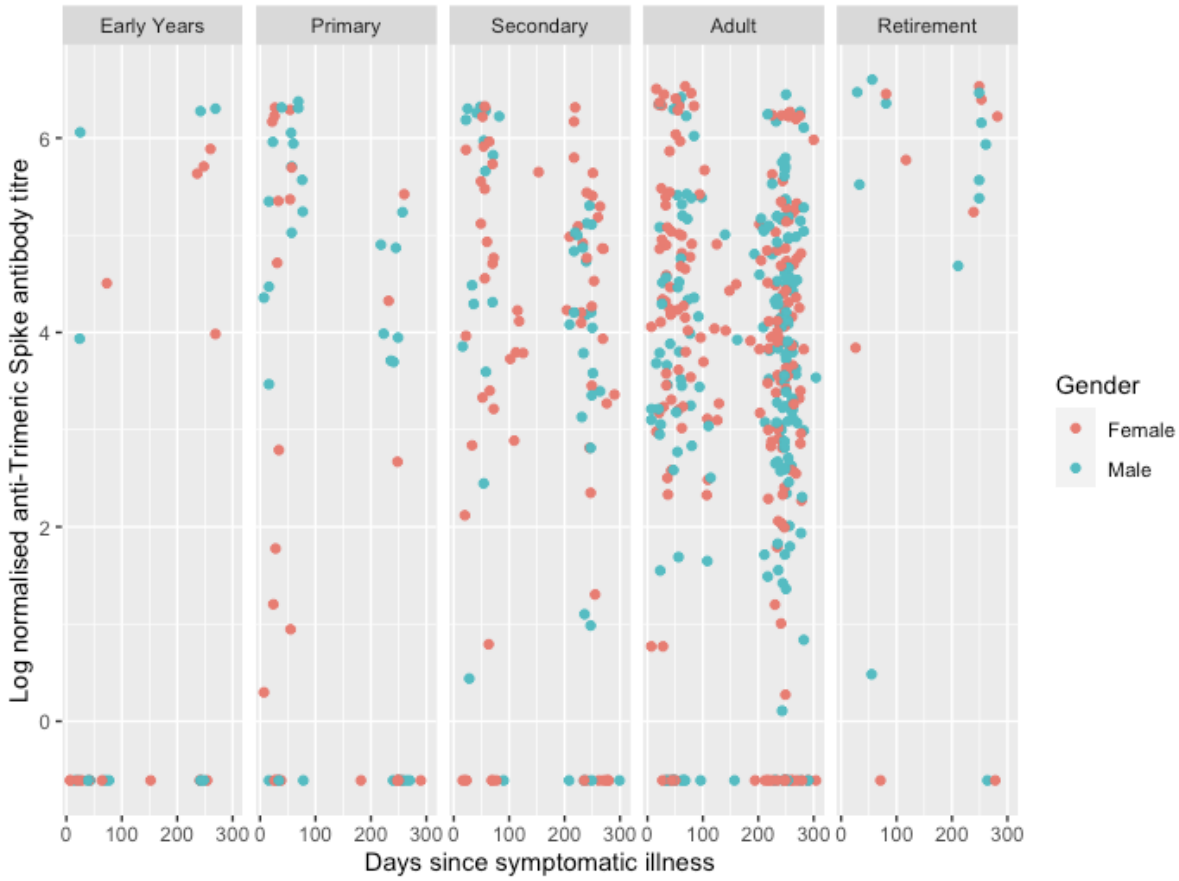
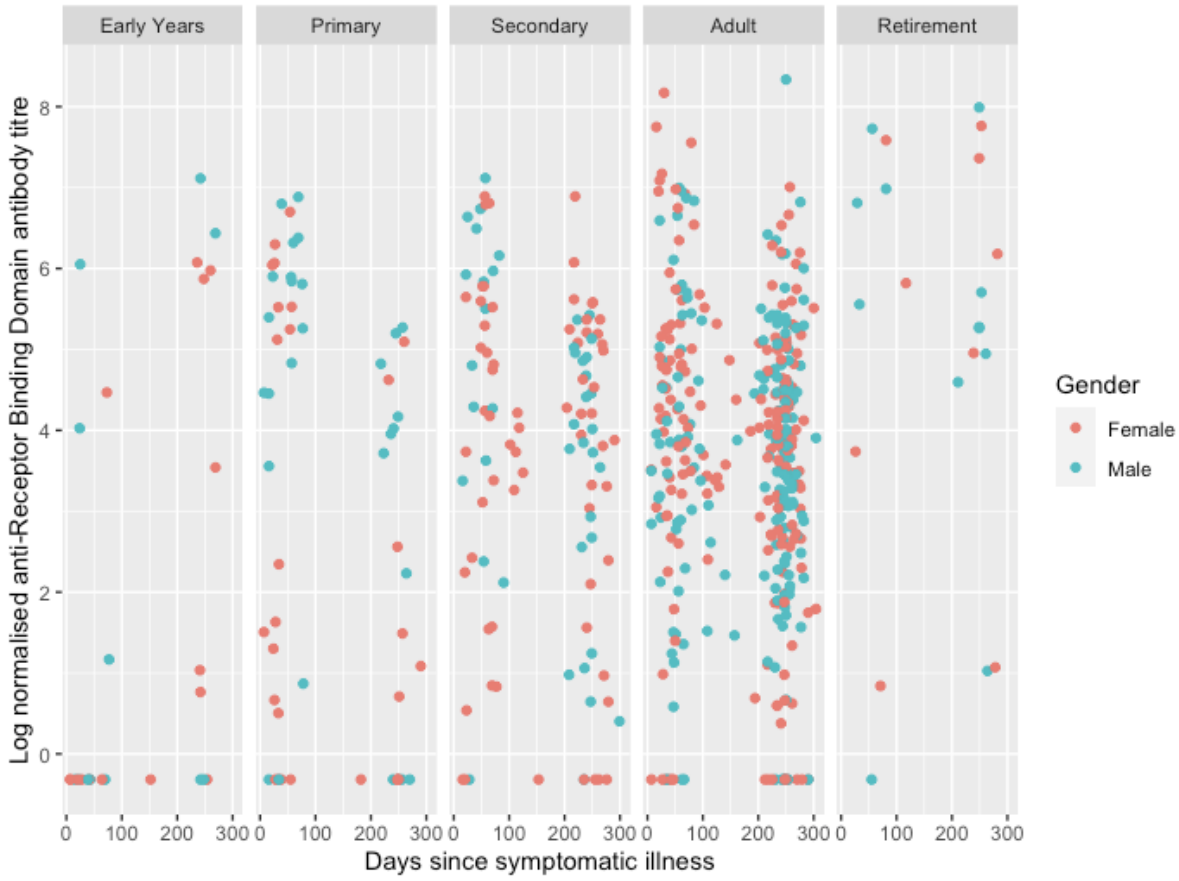
1. Demographics

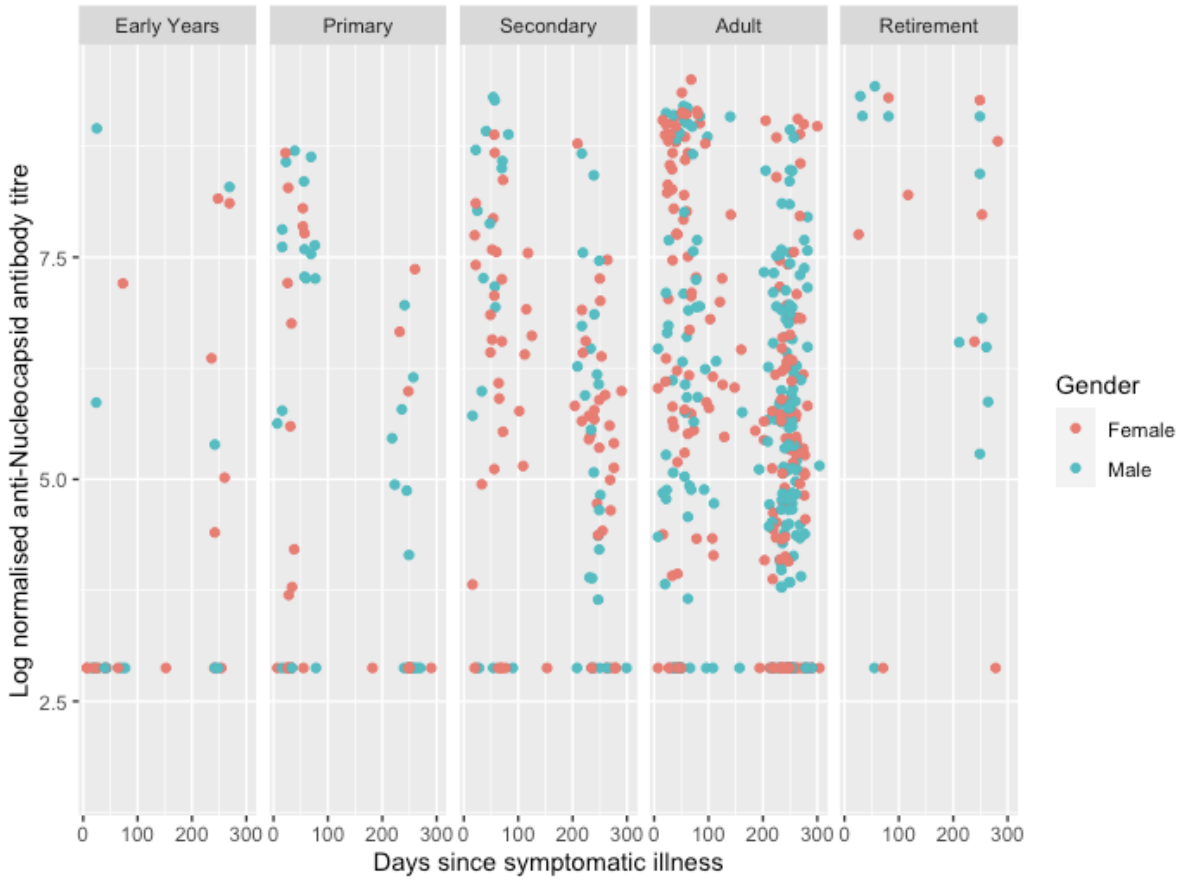
Supplementary Table 1: Survey respondent demographics

Variable		Frequency
Sex	Male	853 (48.5%)
	Female	906 (51.5%)
Age	Median (IQR)	14 years (7-33)
Age Group	Early Years (0-4 years)	307 (17.5%)
	Primary School (5-10 years)	357 (20.3%)
	Secondary School (11-18 years)	360 (20.5%)
	Adults (19-66 years)	684 (38.9%)
	Retirement Age Adults (67+)	51 (2.9%)
Education and Employment	In formal education	776 (44.1%)
	Working from home	238 (13.5%)
	Working outside home	133 (7.6%)
	Neither in education or formal employment	612 (34.8%)
Self Reported Comorbidities	Asthma	11 (0.6%)
	COPD	2 (0.1%)
	Hypertension	31 (1.8%)
	Diabetes	21 (1.2%)
	Cardiovascular Disease	9 (0.5%)
	Chronic Kidney Disease	1 (0.1%)
	Dementia	0 (0%)

2. Antibody Titres and time since self-reported COVID-19-like symptoms




Early Years
Primary
Secondary
Adult
Retirement
Log normalised anti-Receptor Binding Domain antibody titre
0
2
4
6
8
0
100
200
300
Days since symptomatic illness
Gender
Female
Male


Early Years
Primary
Secondary
Adult
Retirement
Log normalised anti-Nucleocapsid antibody titre
2.5
5.0
7.5
0
100
200
300
Days since symptomatic illness
Gender
Female
Male

Supplementary Figure 1. Log normalised antibody titres against spike, receptor binding domain and nucleocapsid antigens by time since self-reported COVID-19 symptoms. Values are shown stratified by age (panels) and sex (colour).

3. Antibody Seroprevalence by antibody target and age group

Supplementary Table 2: Age stratified seroprevalence.

Age Group	anti-Spike SARS-CoV-2 antibodies	anti-Receptor Binding Domain antibodies	anti-Nucleocapsid SARS-CoV-2 antibodies
Early Years (0-4 years)	27.6% (20.8-35.6%)	22.4% (16.2 - 30.0%)	18.4% (12.8 - 25.7%)
Primary School (5-10 years)	56.4% (49.8-62.7%)	43.8% (41.8 - 54.9%)	42.8% (36.4-49.4%)
Secondary School (11-18 years)	73.8% (68.2-78.8%)	65.6% (59.7 - 71.1%)	50.9% (44.9-56.9%)
Adults (19-66 years)	74% (70.0-77.6%)	57.8% (53.5-62.0%)	45.4% (41.1-49.7%)
Retirement Age Adults (67+)	54.8 (38.8-69.8%)	40.5% (26.0-56.7%)	45.2% (30.2-61.2%)

4. Positive and negative predictive value of symptoms by age group

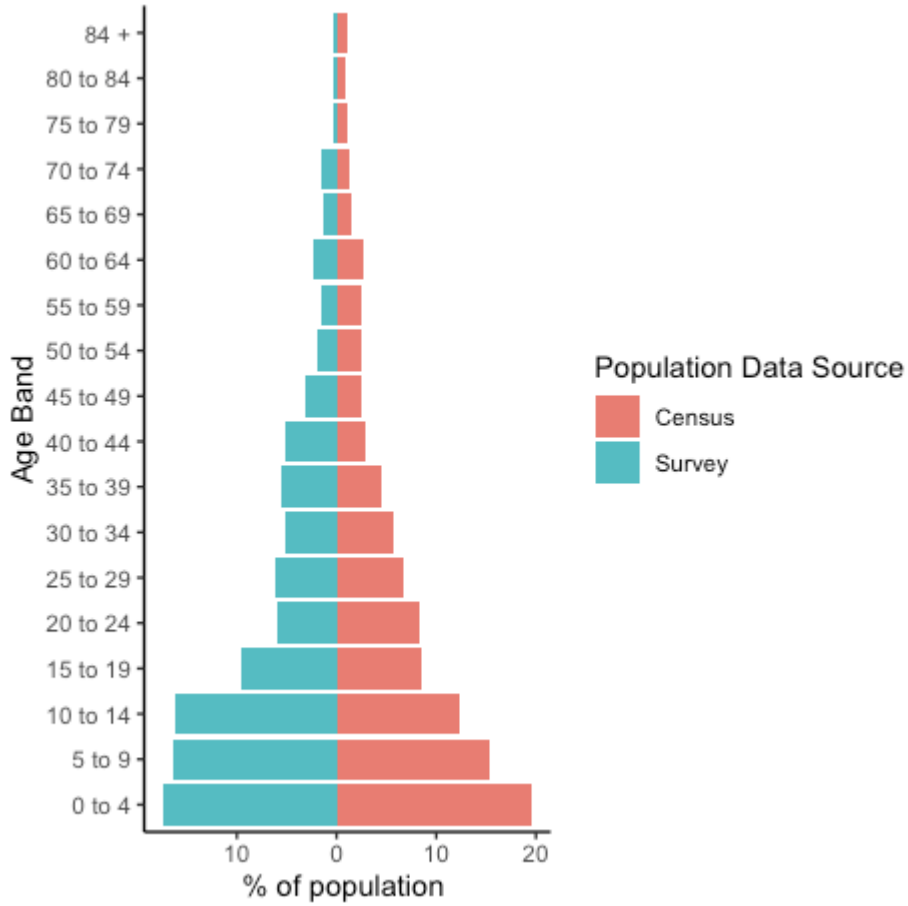
Supplementary Table 3: Positive and negative predictive values stratified by age for symptoms reported as COVID-19-like illness. PPV = positive predictive value, NPV = negative predictive value.

	Fever		Cough		Loss of Smell or Taste	
Age Group	**PPV**	**NPV**	**PPV**	**NPV**	**PPV**	**NPV**
Overall	79.0%	38.3%	81.6%	43.6%	94.1%	42.7%
Early Years (0-4 years)	33.3%	68.2%	36.4%	76.1%	33.3%	68.1%
Primary School (5-10 years)	57.1%	42.9%	65.2%	48.3%	100%	44.6%
Secondary School (11-18 years)	63.0%	25.0%	73.0%	28.0%	88.9%	29.1%
Adults (19-66 years)	90.9%	31.0%	91.4%	35.9%	96.6%	37.1%
Retirement Age Adults (67+)	77.8%	51.5%	90.0%	56.3%	80.0%	48.6%

5. Sensitivity Analysis

In a sensitivity analysis in which the threshold for spike positivity was doubled, seroprevalence was 49.7%.

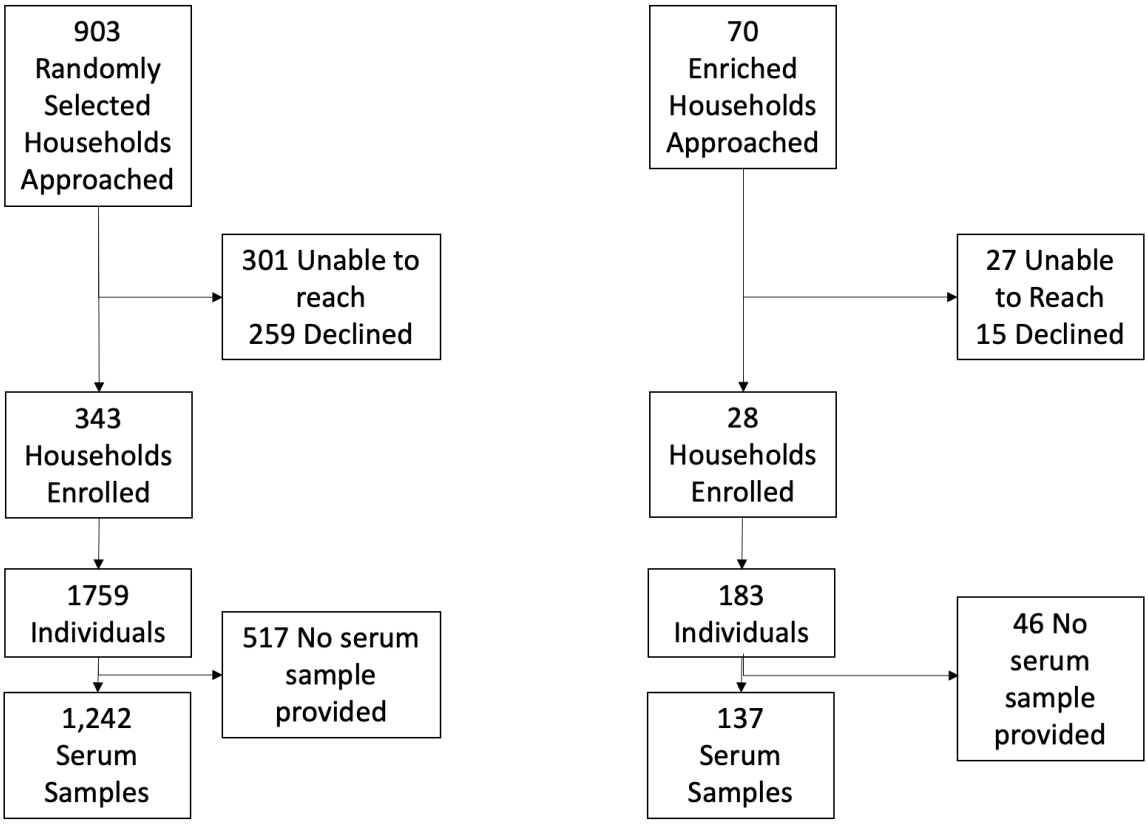
6. Population Structure



Supplementary Figure 2. Population age structure for survey respondents compared to the overall Haredi population. Census data is from 2011*.

*2011 Census - Office for National Statistics. https://www.ons.gov.uk/census/2011census Whilst broadly similar there are larger numbers of 0–4-year group in the census compared with our data. Whilst the birth rate may have been lower in 2021, we cannot exclude lower age group participation in this study. Not this census data is not stratified by sex, which would have been a useful comparison given the study findings.

7. Consort Diagram



Supplementary Figure 3. Consort diagram showing enrolment of both the randomly selected and the enriched households into the study

Chapter 4

Evaluating immune profiles for COVID-19 in the hyperendemic Strictly Orthodox Jewish community in North London

4.1 Introduction

In the previous chapter, I explored the intense epidemic of SARS-COV-2 in the Strictly Orthodox Jewish community of North London (1). This work left unanswered questions about how immune responses to SARS-CoV-2 differ. In particular, whether participants with un-hospitalised symptomatic COVID-19 experienced a different immune response to those without symptoms. And the follow-up question, whether the immune response differences between these groups explain symptom severity or symptom presence. Chapter 4 entitled "Transmission of SARS-CoV-2 in North London Jewish Communities" is the parent project for the piece of work described here.

SARS-CoV-2 spread rapidly throughout this community and affected nearly every household by November 2020, disproportionately affecting this vulnerable ethnic minority population. Complex multigenerational living, large household size (median 5), close-knit interconnected households and low median age mean that this community can help to understand how and why ethnic minorities have been critically affected. The work within the strictly Orthodox community to date focussed on transmission dynamics of SARS-CoV-2 and is informed by sero-epidemiological surveys with mathematical modelling of household and antibody data of participant blood samples.

The high burden of infection and large sample size of this sero-epidemiological work also allows exploration of immune and symptom response. Permitting comparison between household members who do and don't develop disease and whether there are different responses in their immune response profiles. Additionally, as 50% of the sample are children, it allows unique insights into the role of children in transmission, differences in their immune

responses (as compared with adults) and their susceptibility to infection. I aimed to increase the scope of the project and perform further testing on these blood samples.
My aims in the work described in this chapter were:

- To explore whether functionally different immune responses to SARS-CoV-2 could be discerned from one another through characteristic cytokine expression patterns in some sub-populations of the community.
- To determine whether specific immune response patterns/types could be used as prognostic tools, markers of severity or predictors of infection.

To date, most cytokine work in this field has been completed in hospitalised patients with severe disease or those attending accident and emergency services. Published data identified markers of interest in severe hospitalised COVID-19 disease, including IL-6, IL-10 and IP-10 (2), prompting the research question addressed in this work. To date, there is still very little work within communities on the impact of COVID-19 illness (including so-called 'long COVID') on soluble inflammatory markers or T cell response, amongst adults or children. One possible explanation will likely be the technical difficulties in collecting highly granular immune response data from sufficient numbers of well-characterised community members to be statistically powered for robust analysis.

Long-term persistence of T cell immune response post severe viral infection is a recognised and necessary part of immune recognition, subsequent virus susceptibility, and possibly symptom severity (3). When planning this work, little was known about the long-term effects of SARS-CoV-2 on both the cellular and soluble cytokine immune response (4,5). Subsequent published work has identified persistent T cell and soluble cytokine changes in severe hospitalised patients (6). Recently published data on separate cohorts compared ICU-admitted COVID-19 patients to non-hospitalised patients over 21 days from illness onset; but the datasets are small and mainly explore gene signatures rather than soluble bio-markers (7). In children, early work on reduced T cell response (in comparison with adults) propagated the theory that children had milder symptoms and potentially greater re-infection risk (8). Subsequently, soluble cytokine levels of IL-6 and IL-8 were found to be lower in children (9). And finally, IL-6, IL-8, and TNF-alpha were to be found to be independently associated with severe disease in hospitalised patients (9,10).

Immune and endothelial activation in seropositive and seronegative community-level data is both novel and useful in exploring household dynamics of infection transmission and infection response. Cytokine bio-marker panel work has been used in other infectious diseases to identify a series of immune bio-markers potentially useful in determining a likely bacterial cause of febrile illness (11–14). This theory was further explored in the FIEBRE study (15), a large multi-centre investigative prospective cohort of (adult and child) febrile illness cases and in a sub-study which aimed to identify markers of severity for the diagnosis and evaluation of fever (Mos-Def). As part of the Mos-Def study (LEO ref 16160), the research team developed a high throughput and low-cost multiplex Luminex assay which could estimate the expression of a dozen cytokines, including several of those previously linked to COVID-19 immune responses.

Luminex multiplex assays are an effective method of assessing multiple cytokines simultaneously from a small volume of plasma or dried blood spot. Existing non-commercial assays have been developed and validated using similar techniques (16,17). Storage of blood samples prior to separation into serum may increase the concentration of inflammatory markers released within the sample, however, once spun to separation, samples can be stored for prolonged periods without effect on the cytokines within (16,18,19); making the method useful in large-scale sero-epidemiological work. Enzyme-linked immunosorbent assays (ELISA) are the commonest method of analysis for this type of data, using within-test standards to fit calibration curves against which results can be validated. To interpret the results of cytokine data from a multiplex assay to validate their usefulness as a prognostic tool, receiver operating curves (ROC) are a frequently used tool. They better represent the compromise between diagnostic sensitivity and specificity in an interpretable graphic form than multiple box plots allow (20).

Within my overarching aims, I focused on understanding whether markers of immune/endothelial activation vary between participants of a randomised household-based population sample collected from within this community with a very high prevalence of SARS-CoV-2 infection. By screening specimens from participants with differing serostatus and symptomatology, I determined whether:

- Symptomatic disease is associated with an immune profile of soluble cytokines.

    - Does non-severe symptomatic disease provoke a typical cytokine profile in adults or children that is persistent in recent SARS-CoV-2 infection but absent 4-6 months later?
- Or is severe disease related to a greater and specific immune response?
    - Is there a difference in cytokine profiles in community managed severe vs non-severe disease.
- Whether these specific immune response types could be used as prognostic tools, markers of severity or predictors of protection.
    - If a cytokine profile is identified, does it reliably identify different phenotypes with a high enough sensitivity and specificity to be clinically useful?

In a subset analysis, I will compare two groups of profiles to look at both the long-term impact on the immune system and differences in immune response between children and adults:

- Those infected during wave 1 (Feb-April) and wave 2 (Sept-Nov) of the UK COVID-19 epidemic to determine whether there is evidence for long-term perturbation of the immune response.
- Seropositive children compared to symptomatic adults to understand the immunological responses of younger people with symptomatic COVID-19.

I will look for systematic differences within this dataset between these sub-populations of participants firstly within the individual cytokines tested and secondly between multiple cytokines. The methods I use focus on diagnostic accuracy as the primary measure to discriminate between subpopulations exposures' both within individual cytokines or groups of cytokines responses.

4.2 Objectives

1. Comparison of cytokine profiles in SARS-CoV-2 sero-negative and sero-positive groups.
2. Describe the cytokine profiles of symptomatic, asymptomatic, and seronegative participants, with reference to whether their primary infection occurred during wave 1 or wave 2 of the COVID-19 pandemic.
3. Comparison of cytokine profiles from groups of SARS-CoV-2 negative, Positive-symptomatic and positive-asymptomatic children.
4. Exploration of the relationship between survey data on illness severity and mortality with cytokine profile.
5. Determine whether some individuals who were infected during wave 1 have persistent changes to their immune profile several months after infection compared with those in wave 2.

4.3 Methods

From a total of 1,377 stored serum samples collected from 373 households that were screened for SARS-CoV-2 antibodies during the parent study, 'Transmission of SARS- CoV-2 in North London communities' (LEO ref: 22532), I investigated 936 participants' serum who consented to further research.

4.3.01 Sample size and statistical power

1,943 participants were recruited in the parent study in Chapter 3, of which 1,379 gave serum samples and finally 936 who gave consent to further research participation were included in this study.

I compared participants' serum with differing serostatus and symptomatology, by separating serum into four categories. Initially, grouping individuals who are seropositive and those who are seronegative. Within the seropositive individuals, I divided the group into asymptomatic, those with symptoms during wave 1 (Feb- Apr 2020) and those with symptoms in wave 2 (Sep-Nov 2020) of the UK COVID-19 epidemic.

The results of the parent study are summarised in Chapter 3 however the pertinent results to plan this sub-study for the comparator subgroup sample sizes were:

- 696/936 (74%) individuals were seropositive and 240/936 (25%) were seronegative.
- 492/936 (53%) reported symptomatic illness consistent with COVID-19 and 444/936 (47%) did not report symptomatic illness consistent with COVID-19.
- The number of participants who self-reported symptomatic COVID-19 disease (between February and November 2020) peaked in alignment with the national peaks of waves 1 and 2; 272 participants reported symptoms in wave 1 and 277 reported symptoms in wave 2.
- 468/936 (50%) individuals were children under 18 years of age.

If 60%-95% of seropositive individuals are found to have different cytokine profiles to seronegative individuals in each analysis, then the sensitivity and specificity of the assay will have the following 95% Confidence Intervals:

a) Seropositive group vs Seronegative group

Statistic	60%	70%	80%	90%	95%
Sensitivity 95% CI	56.2 – 63.6%	66.4 – 73.3%	69.4 – 75.8%	87.46 – 92.08%	93.0 – 96.4%
Specificity 95% CI	53.5 – 66.2%	63.7 – 75.6%	74.3 – 84.8%	85.49 – 93.49%	91.2 – 97.2%

b) Symptomatic COVID-19 group vs Asymptomatic COVID-19 group

Statistic	60%	70%	80%	90%	95%
Sensitivity 95% CI	55.5 – 64.3%	65.6 – 73.9%	76.2 – 83.5%	87.0 – 92.5%	92.5 – 96.6%
Specificity 95% CI	55.2 – 64.5%	65.3 – 74.0%	75.9 – 83.5%	87.0 – 92.6%	94.0 – 97.8%

c) Symptomatic in Wave 1 vs Symptomatic in Wave 2

Statistic	60%	70%	80%	90%	95%
Sensitivity 95% CI	53.8 – 65.7%	64.0 – 75.2%	74.8 – 84.6%	85.3 – 92.9%	91.8 – 97.3%
Specificity 95% CI	53.9 – 65.7%	64.2 – 75.3%	74.9 – 84.6%	85.6 – 93.1%	91.5 – 97.1%

d) Adults vs Children

Statistic	60%	70%	80%	90%	95%
Sensitivity 95% CI	55.4 – 64.5%	65.7 – 74.2%	75.9 – 83.4%	86.8 – 92.5%	92.6 – 96.8%
Specificity 95% CI	55.4 – 64.5%	65.7 – 74.2%	75.9 – 83.4%	86.8 – 92.5%	92.6 – 96.8%

4.3.02 Luminex Multiplex Assay

The Mos-Def multiplex assay multiplex Luminex assay which measures cytokine expression in a panel of 12 immune response factors (Table 1). The assay uses 15 µL of serum from each sample and provides highly reproducible (figure 1) estimates of cytokine expression levels.

Table 1. Mos Def Response Panel: Markers of immune and endothelial activation

Marker	Name	Function
Ang-1	Angiopoietin 1	Has vascular protective effects, suppresses plasma leakage, inhibits vascular inflammation and precents endothelial death
Ang-2	Angiopoietin 2	Increased levels lead to increased vascular leakage resulting from destabilised vascular endothelium.
AZU1 (aka HBP)	Azurocidin 1 (Heparin Binding Protein)	Elevated levels may indicate severity through prediction of circulatory collapse during sepsis. Mediator/Predictor of multi-organ failure
IL-10	Interleukin 10	Master regulator of cellular immune response. Mediator of compensatory anti-inflammatory response syndrome in sepsis/Systemic Inflammatory Response Syndrome (SIRS)
IL-6	Interleukin 6	Endogenous pyrogen mediating temperature changes leading to fever, procoagulative.
IL-8	Interleukin 8	Macrophage expression as early marker of infection. Chemotactic factor in target cells, triggers histamine release from target cells and causes angiogenesis. Minor pyrogen
IP-10 (aka CXCL 10)	Interferon g-induced protein 10 kDa (C-X-X motif chemokine 10)	Involved in the chemo-attraction of activated T-cells
MxA	Myxovirus resistance protein A	Cytoplasmic GTPase with activity against a wide range of viruses.
CHI3L1 (akaYKL-40)	chitinase 3-like 1	Produced in response to inflammation. Involved in innate immune activation, angiogenesis and endothelial dysfunction.
sTNFR-1	Soluble Tumour Necrosis Factor Receptor 1	Cell death pathway, role in response to tissue damage and subsequent inflammation
STREM-1	Soluble Triggering Receptor Expressed on Myeloid Cells	Soluble form of TREM-1 shed from the membrane of activated phagocytes in response to bacterial and fungal infections
TRAIL	TNF-Related Apoptosis-Inducing Ligand	Viruses may stimulate TRAIL expression in host or immune cells, may also sensitize host cells to TRAIL-medicated induction of apoptosis

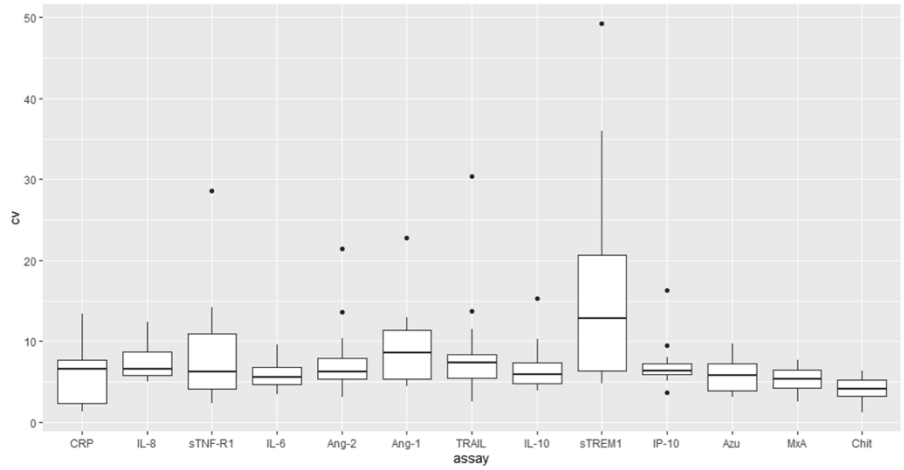


Figure 1: Within-plate coefficients of variance (%) of the Mos-Def Luminex panel indicate that test results are highly reproducible. Taken with permission from the Mos-Def study.

The multiplex assay was run on the Luminex platform for all 936 available samples. These 96 well plate-based assays use antibodies attached to magnetic beads with a fluorescent label added which is read by the MagPix Luminex machine. 8 standards in duplicate are tested against single 15 µL serum samples on each plate. All the work for this multiplex assay was completed in a Level 2 Microbiological safety cabinet (MSC II) for health and safety compliance.

All the antibodies for capture and detection have been assessed for non-specific binding in the absence of an analyte. For each marker, the capture antibody was coated onto MagPlex beads at a single concentration previously defined in the Mos Def research study. A 96-well plate was used with equal numbers of MagPlex beads in each well. Detection antibodies were used at pre-determined concentrations, followed by a fluorescent label detected by a MagPix Luminex machine.

- PBS-TBN-pi dilution buffer was prepared: protease inhibitor tablets were dissolved in phosphate buffer solution (PBS) at a dilution of 25X and added to PBS-TPN buffer solution.

- The pre-prepared standards 1-7 are kept at -70^{o}C and were slowly thawed using wet ice. Standard 8 was PBS-TBN-pi dilution buffer.
- The MagPlex preprepared beads are kept in the dark at 4-8^{o}C, these are vortexed and then sonicated for 30 seconds each. For each plate run, I added 10.9 µL for each bead volume to 5015 mL buffer volume to ensure that each well had at least 1000 beads. This is based on a stock of 9.4x10^{6} beads/mL. Then 50 µL of mixed beads were added to each of the 96 wells. The beads were washed using a magnet, attaching the magnet for 60 seconds prior to flicking out the liquid and then adding 100 µL of phosphate-buffered saline with 0.1% Tween (PBST) to each well.
- All soiled plastic wear was disposed of in Presept within the MSC II.
- 30 µL of each standard were added in duplicate to the first 2 columns of plate wells.
- To each of the sample wells, 15 µL of PBD-TBN and 15 µL of serum sample were added, slowly thawed from -20^{o}C.
- The plates are then covered and incubated on a plate shaker at room temperature in the MSCII for two hours.
- Throughout the process, all beads are kept covered with foil as much as possible to protect them from light degradation.
- Following incubation, the plates are washed using a plate magnet and 100 µL of PBST per well. This was repeated three times.
- The biotin detection antibodies were slowly thawed and recovered using PBS-TPN, with a 30-second vortex and spin down. The Azu and MxA detection antibodies are kept at 4^{o}C and added separately.
- After the washing process, 30uL/well of biotin detection antibody mixture were added. The plates were then covered and incubated for one hour on a plate shaker within the MSCII.
- The plates are washed again three times.
- After this 30 µL/well of streptavidin-PE (SA-PE) was added and the plates were incubated for a final 45 minutes on a plate shaker in the MSCII.
- A final three repeat washes completed the process before adding 100 µL/well of PBST, covering the plate with foil and a lid, and storing overnight at 4^{o}C.

- The following morning, the plates were placed on a plate shaker for 30 minutes whilst the MagPix Luminex machine was set up to read the 13 soluble biomarkers.

Where errors occurred with low bead counts or low median fluorescence the wells were re-run on the MagPix. If an error persisted the serum sample was re-processed and re-run. Once all 12 plates were processed satisfactorily the data was recorded. The first 2 plates were run at 1 in 2, 1in 4 and 1 in 8 dilutions and in duplicate. On review of these initial results, the initial samples were re-run along with all the subsequent plates using 15 µL of serum from each sample diluting the samples to an optimal dilution of 1 in 2 to a total of 30 µL. All subsequent samples were run in single wells. CRP was removed from the multiplex; the results were not interpretable; a much higher dilution was required as compared with the other markers. The biotin antibodies were preprepared in pre-dispensed plate-size aliquots and kept at -20^{o}C.

4.3.03 Data cleaning and normalisation

1) Normalisation approach and data handling

The raw data from all 12 plates were cleaned, and separate set standard results were added and tidied into one set with an analysable format. Low poor values were removed, missing data identified, samples without sample ID were removed, and each sample result was assigned a unique number. The standards were used to set an estimate for standard curves with the highest and lowest values. Standard 8 was used to set the background median fluorescent intensity (MFI) the background fluorescence was not subtracted from this calculation. Standard curves were run for all the assays. Figure 2 shows the raw data prior to normalisation and data cleaning.

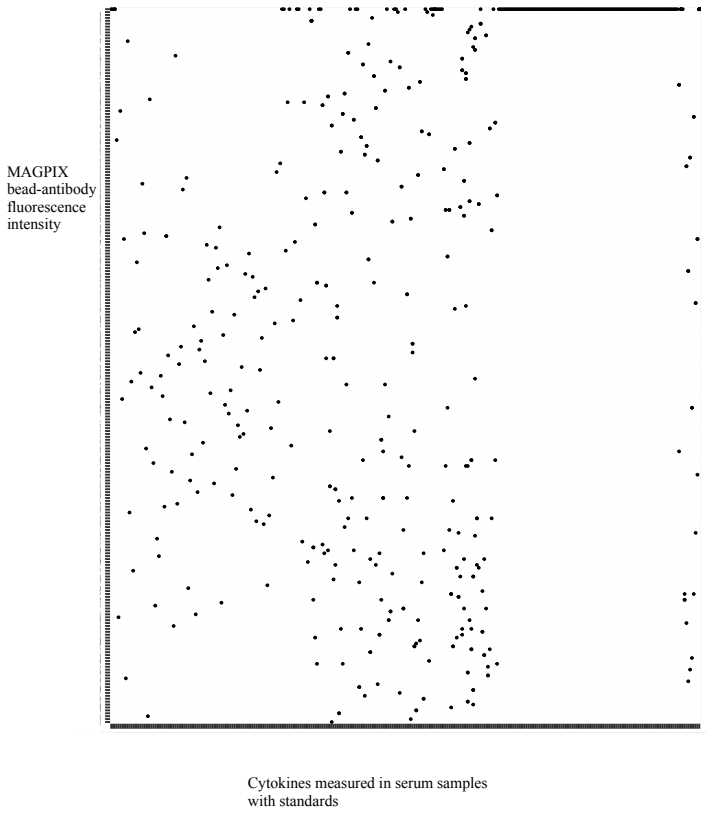


Figure 2. Raw data from plate 1 prior to normalisation and data cleaning.

2) Models used

A dose-response model was run using the *drc* package in R which allows a model to be fitted to the standards assay pre-prescribed parameters allowing estimation of the MFI data (21). The median, highest and lowest MFI per bead count were entered into the model.

4.3.04 Statistical analysis

All analysis was completed in the statistical package R. Outputs from the cytokine panels were matched with symptoms, severity, and outcome data from the parent study. I ran logistic regressions on the meta-data and an analysis of variance between cytokine panels using Principal Components Analyses and ROC curves.

The *stats* package in R was used to run a principal components analysis (PCA) (22). This allows an analysis of the variance of the MFI scores for each cytokine and calculates their correlation. Hence summarising the multivariate data set in summary tables and graphically allowing an assessment of whether the cytokine populations are different. This provides a standard deviation and a proportion of variance for each principal component run. The first PC carries the greatest weight, to visualise this we run a graph showing the proportions of variance within the data set across the 12 PCs.

Table 2. A PCA example showing standard deviation and spread of variance.

PC	standard deviation	proportion of variance	cumulative variance
PC1	2.37	0.469	0.469
PC2	1.47	0.18	0.648
PC3	0.971	0.0786	0.727
PC4	0.865	0.0623	0.789
PC5	0.696	0.0403	0.83
PC6	0.668	0.0371	0.867
PC7	0.616	0.0316	0.898
PC8	0.585	0.0285	0.927
PC9	0.546	0.0248	0.952
PC10	0.479	0.0191	0.971
PC11	0.452	0.017	0.988
PC12	0.384	0.0123	1

Using the output from the PCA we can then run a logistic regression on all the PC against the outcomes of interest to assess which PC associate with the outcome and warrant further analysis. This is run in the *stats* package in R (22).

Table 3. A logistic regression of a PCA example

	Estimate	error	Z value	P value
PC1	0.09432	0.03632	2.597	0.0094
PC2	0.04224	0.05513	0.766	0.4436
PC3	-0.05707	0.08633	-0.661	0.5086
PC4	-0.20122	0.09804	-2.052	0.0401
PC5	-0.11736	0.11954	-0.982	0.3262

The *ROCit* package in R allows the assessment of sensitivity, specificity, negative predictive Value (NPV), positive predictive value (PPV), accuracy and F-score and produces interpretable

Receiver Operating Characteristics (ROC) graphs to aid analysis of binary diagnostic tests (23). ROC curves clearly show where a diagnostic test can discriminate between two populations of cytokines.

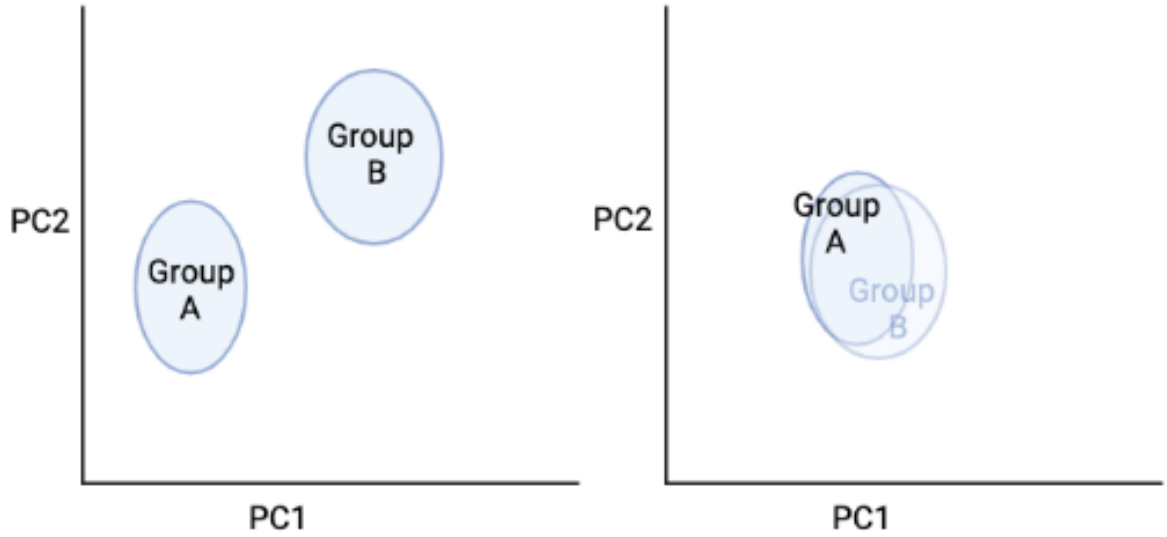


Figure 3 (a) & 3 (b). Modelled PCA results after using a ROC and logistic Regression analysis to identify profile-wide differences in the immune response. Figure 3a. shows evidence found for a systematic difference between two populations of cytokines associated with the outcome. Figure 3b. shows no evidence found for a systematic difference between the two cytokine populations.

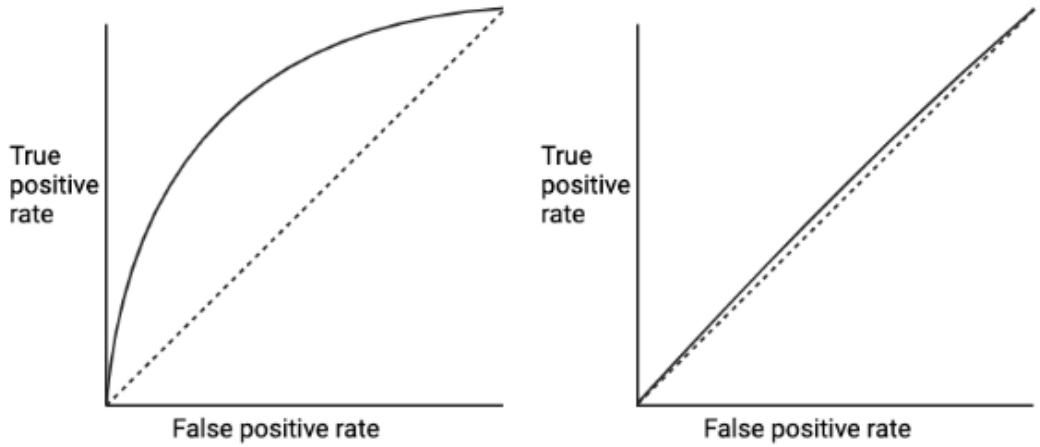


Figure 4 (a) & 4 (b). Modelled PCA results analysed with logistic regression and ROC analysis to assess if two cytokine populations are a diagnostically useful test of the outcome. 4a. identifies the PCA may be diagnostically useful, and 4b. shows that the PCA is not diagnostically useful.

4.3.05 Ethics

The study was approved by the London School of Hygiene & Tropical Medicine (LSHTM) Ethics Committee (LEO ref:25180), the parent project Transmission of SARS-CoV-2 in North London Jewish Communities was approved by LSHTM Ethics Committee (22532), as was the in-house 13-panel cytokine multiplex assay study "Marker of Severity Diagnostics for Evaluating Fever" (MosDef), LSHTM Ethics Committee (16160). All samples were obtained in the parent study with written consent provided prior to phlebotomy, only samples with consent for additional research were included in this study. Parents provided written consent for children under 10 and assent was collected for children over 10 years.

4.4 Results

All 936 serum samples were tested for the full panel of 12 soluble cytokines. Figure 2 shows the range of variance within each cytokine assay, there was more variance for STREM1 and TRAIL than AZU or ANG1 (Figure 5). For validation and inter-plate comparison the cytokines were then compared across all 12 Luminex plates run and compared with the set standards run on each Luminex plate (Figures 6-11). These broadly matched across all plates and standards.

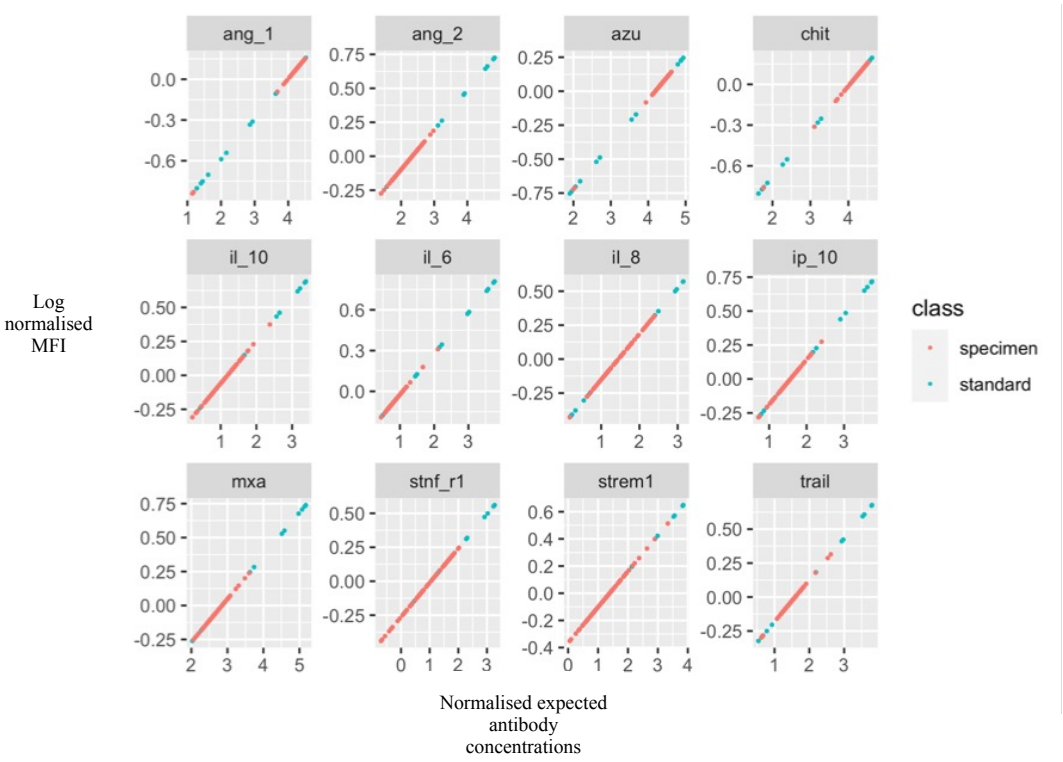


Figure 5. Individual cytokines were measured across all subjects against their standards presented as mean normalised.

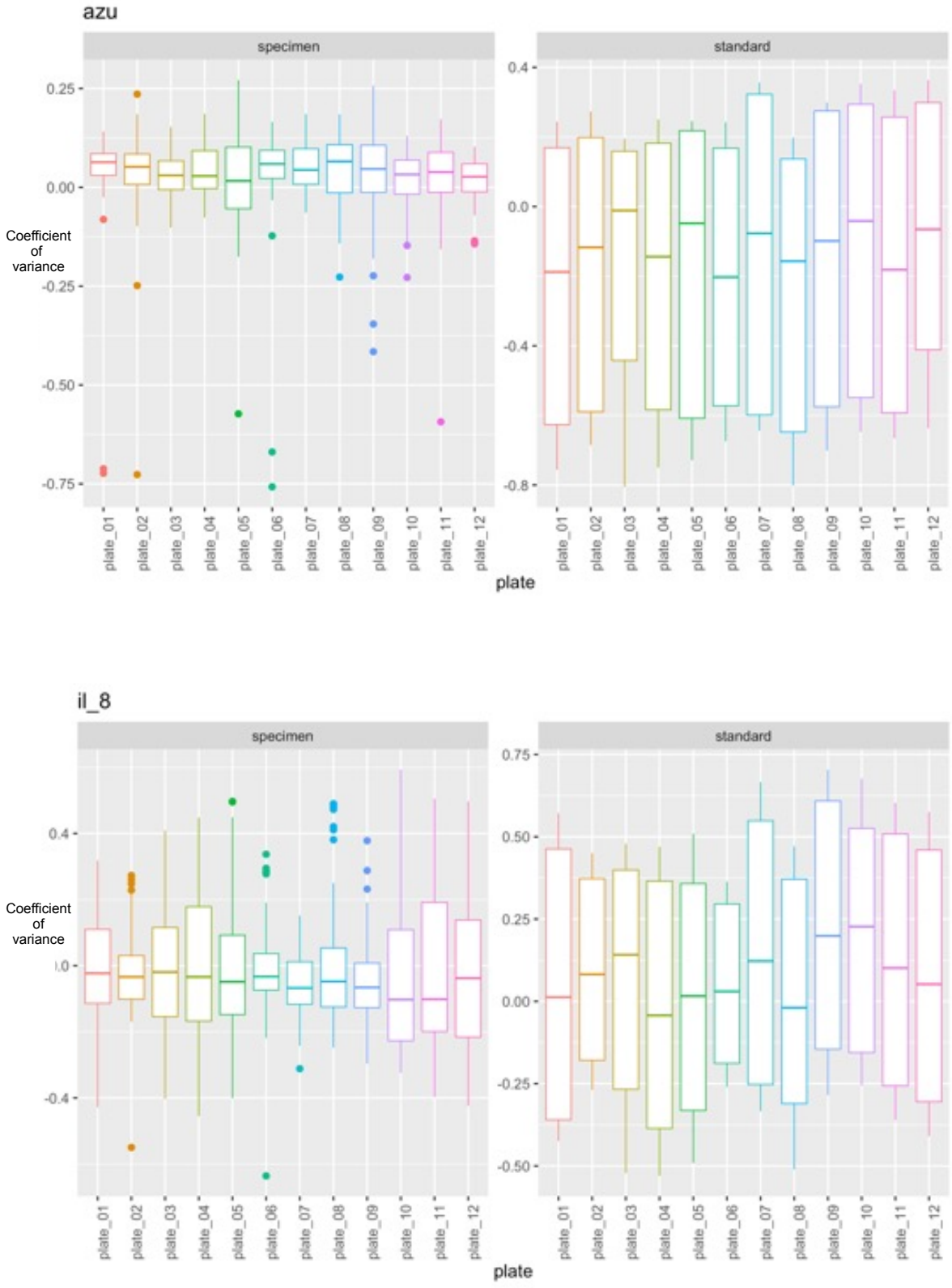


Figure 6. AZU and IL-8 MFI variation by plate against their set standards.

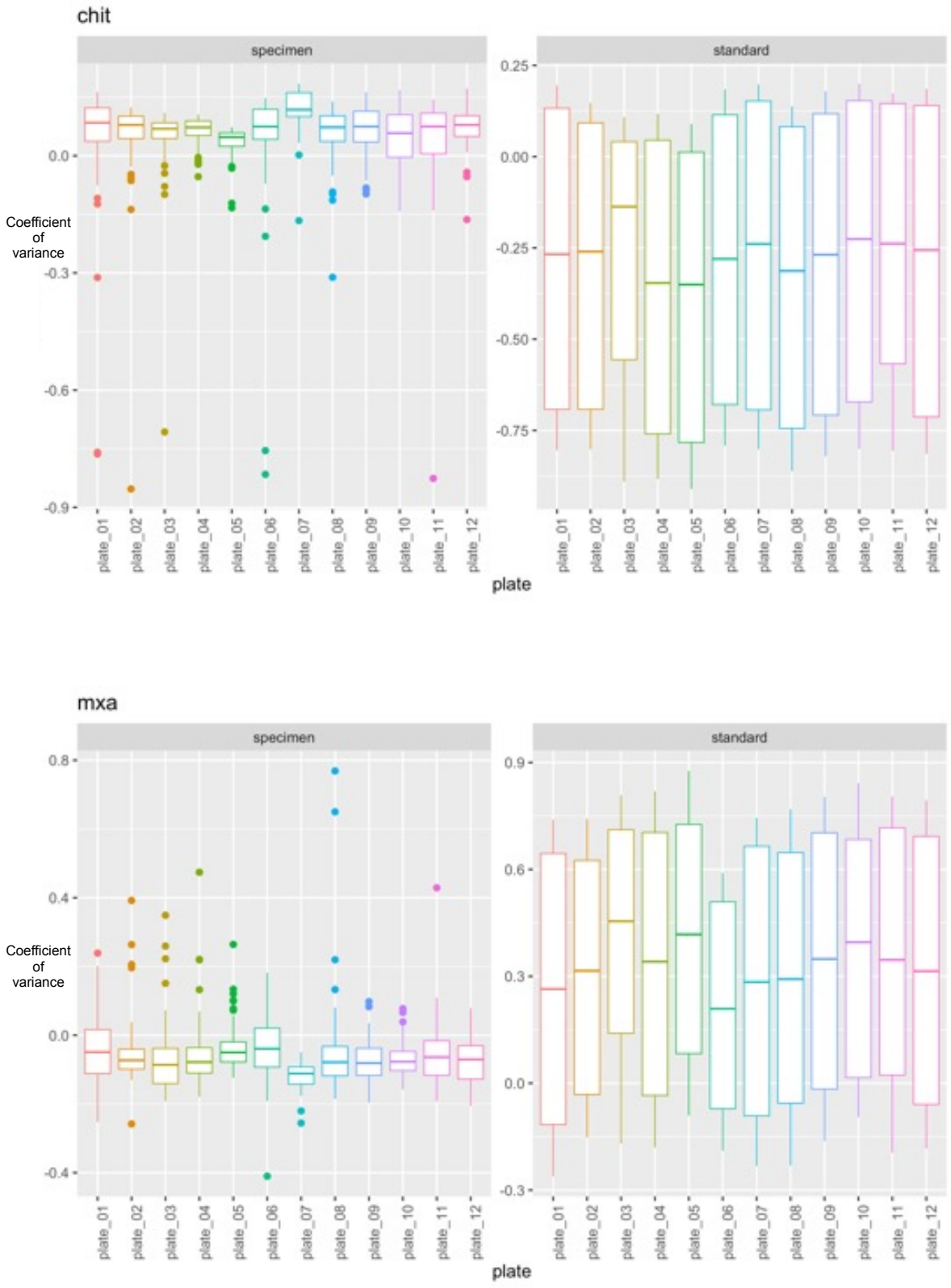


Figure 7. CHIT and MXA MFI variation by plate against their set standards.

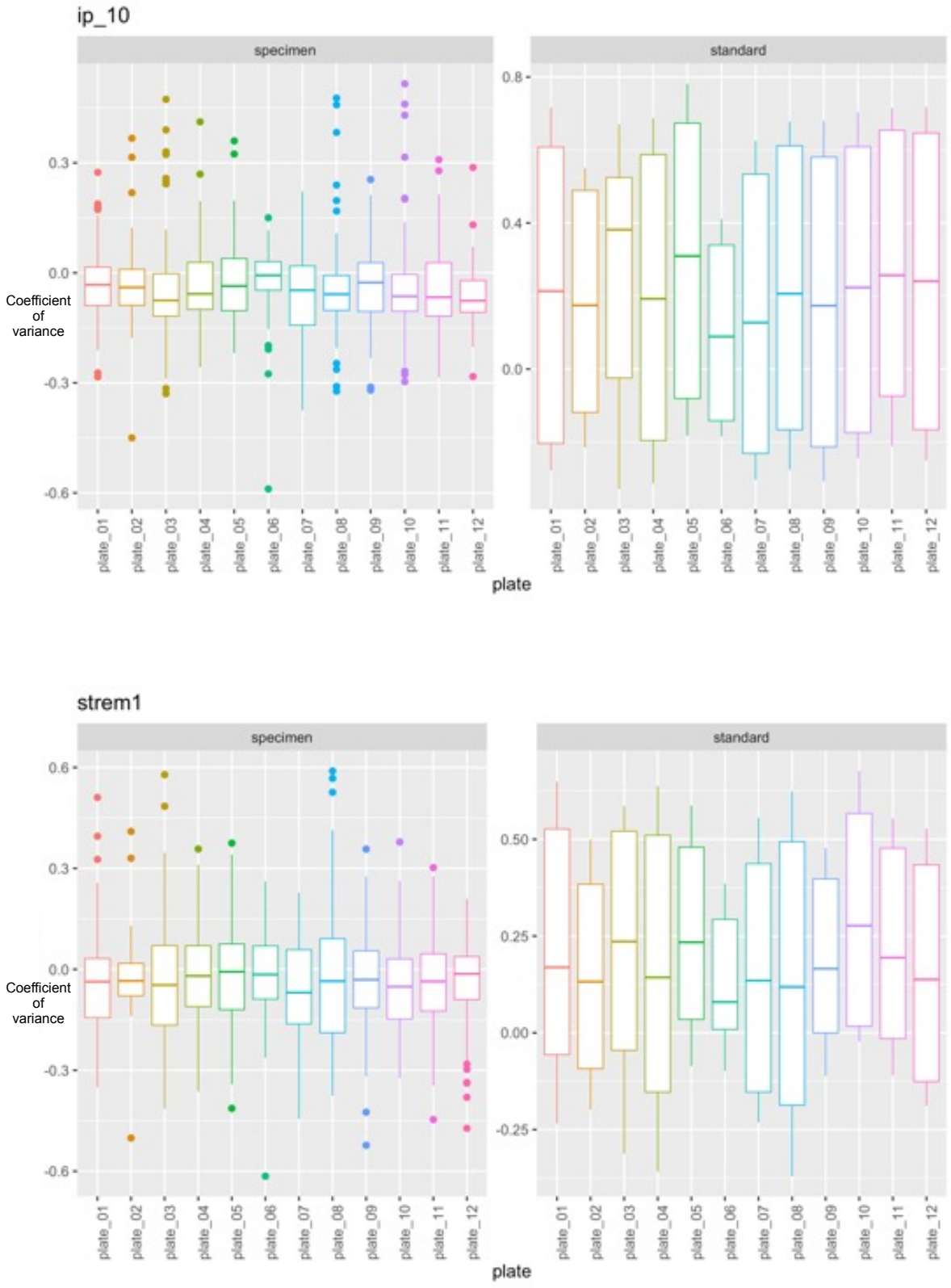


Figure 8. IP1- and STREM1 MFI variation by plate against their set standards.

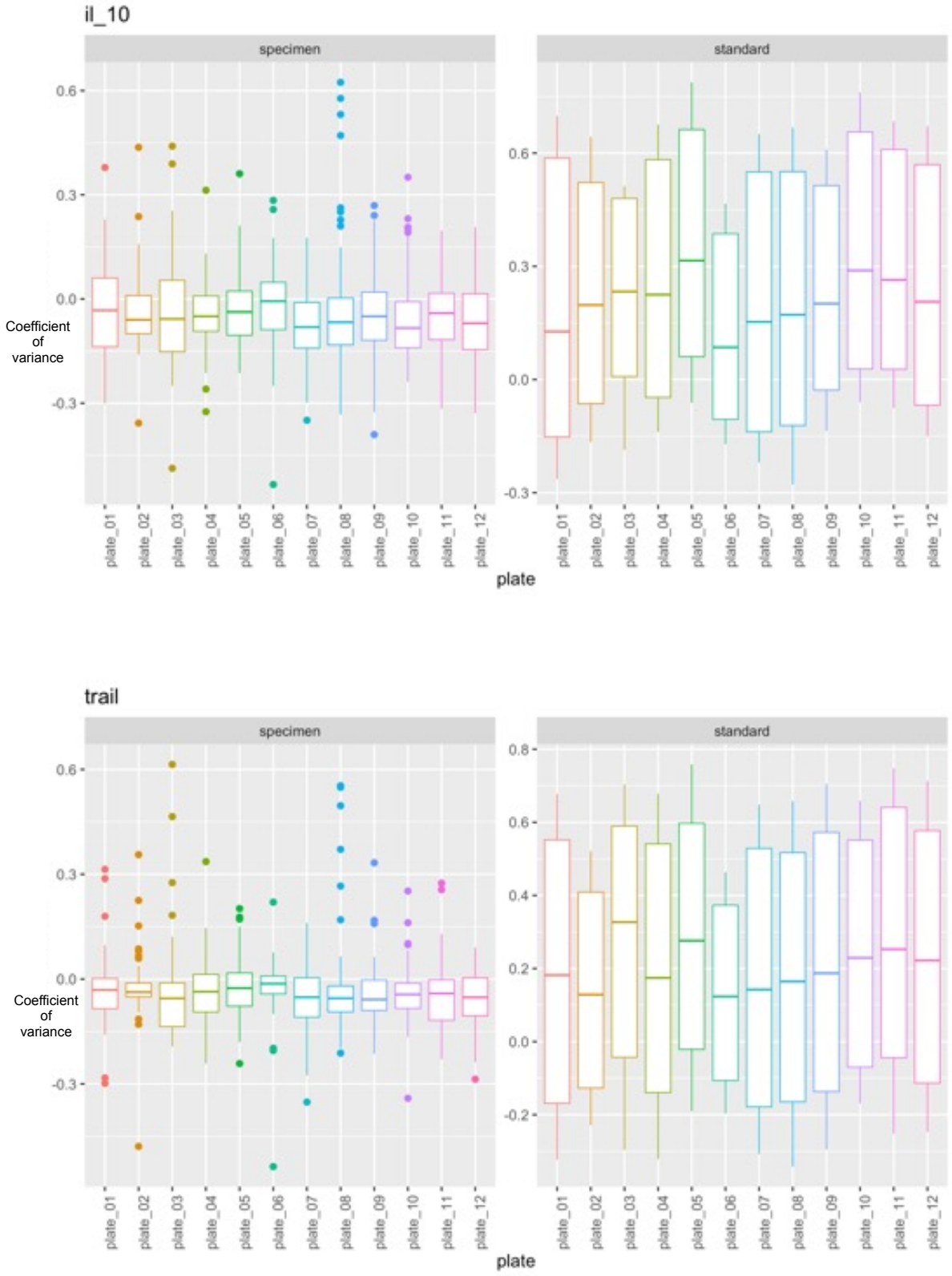


Figure 9. IL-10 and TRAIL MFI variation by plate against their set standards.

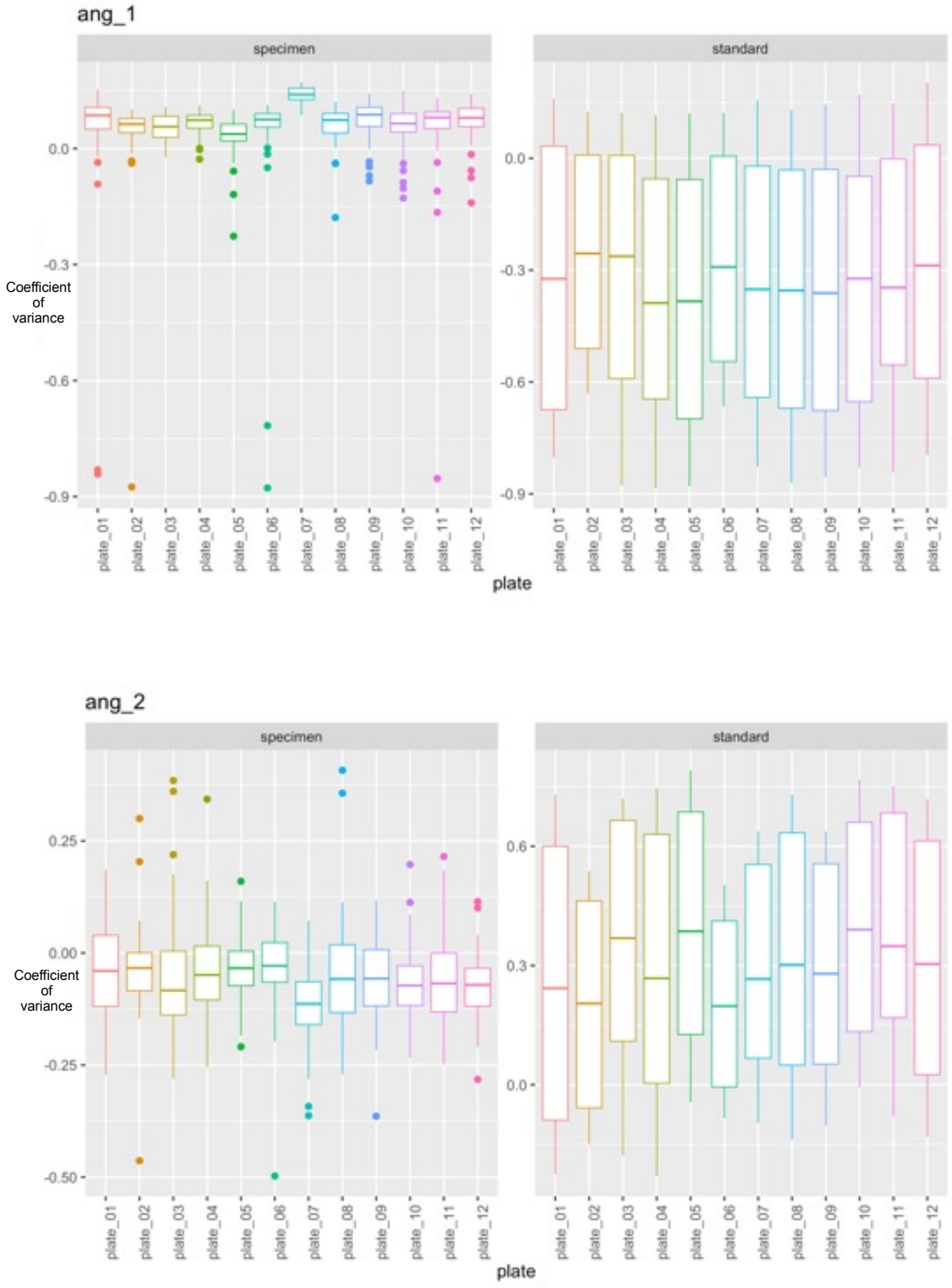


Figure 10. ANG1 and ANG2 MFI variation by plate against their set standards.

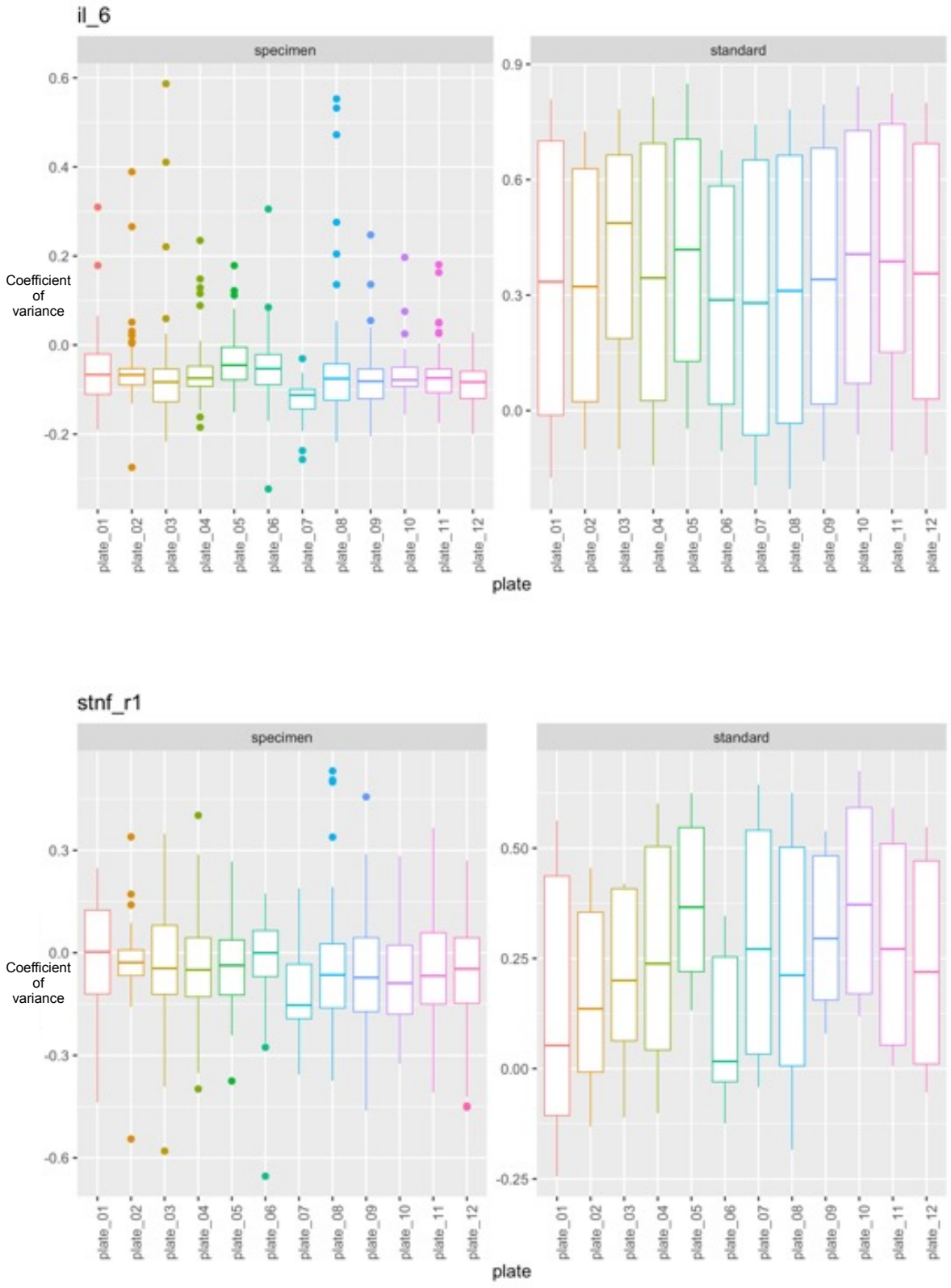


Figure 11. IL-6 and STNF1 MFI variation by plate against their set standards.

4.4.01 Total Cytokine population analysis

I then performed a principal components analysis (PCA) on the cytokine profiles and using the serosurvey data from the parent study compared PCA results across binary outcomes of interest. The analysis ran 12 principal components (PC), and the majority of the variance was accounted for within PC1 (Figures 12a & 12b).

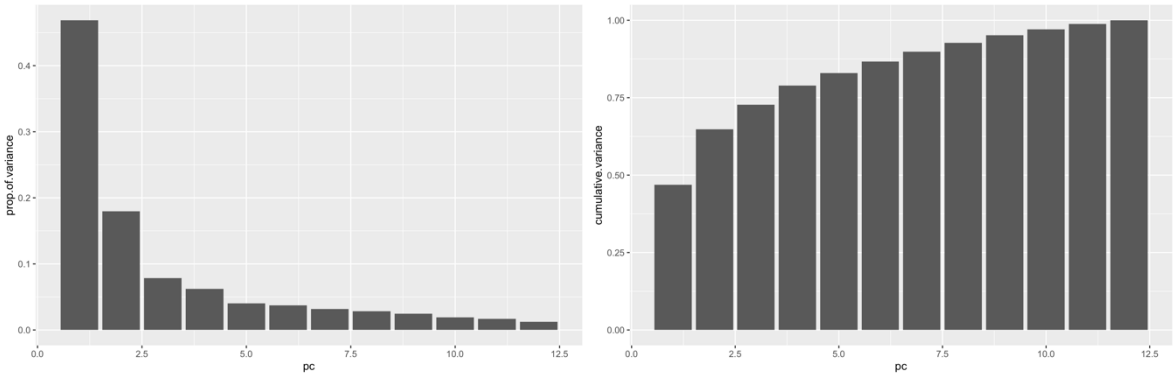


Figure 12 (a) Principal components analysis showing absolute variance. Figure 12 (b) Principal components analysis showing cumulative variance across all 12 PC.

The primary outcome looked for a difference in cytokine profiles between SARS-CoV-2 spike protein seropositive individuals and seronegative individuals. 696/936 (74%) individuals were seropositive and 240/936 (25%) were seronegative. I fit the PCA to a linear model to assess the binary outcome of whether SARS-CoV-2 seropositivity was associated with a cytokine profile.

Of the 12 PCA for this outcome, the PCAs showing the greatest between-group difference were PC1, PC4, PC6, PC9 & PC12 (Table 4). I went on to interrogate these with a Receiver operating characteristic (ROC) analysis to assess the PCA diagnostic ability (Figure 5).

Of the 12 PCA for this outcome PC1, PC4, PC9 and PC12 showed the greatest area under the ROC curve (AUC) of 0.56, 0.55, 0.55 and 0.55 respectively (Figure 13). In summary, there was no real difference in the cytokine profiles between SARS-CoV-2 seropositive and seronegative participants. On comparing the principal components directly, the seropositive and seronegative groups show major overlap with no discrete groups.

Table 4. PCA results from a generalised linear model assessing whether any combination of soluble cytokines differentiates between SARS-CoV-2 seropositive and seronegative participants.

Principal Components	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.79195	0.08490	9.328	<2e-16 ***
PC1	0.09432	0.03632	2.597	0.0094 **
PC2	0.04224	0.05513	0.766	0.4436
PC3	-0.05707	0.08633	-0.661	0.5086
PC4	-0.20122	0.09804	-2.052	0.0401 *
PC5	-0.11736	0.11954	-0.982	0.3262
PC6	-0.26445	0.12980	-2.037	0.0416 *
PC7	-0.20426	0.13644	-1.497	0.1344
PC8	0.09526	0.14214	0.670	0.5027
PC9	-0.35821	0.15981	-2.241	0.0250 *
PC10	-0.12273	0.17583	-0.698	0.4852
PC11	0.26501	0.18670	1.419	0.1558
PC12	0.44854	0.22079	2.032	0.0422 *
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Dispersion parameter for binomial family taken to be 1) Null deviance: 858.51on 684 degrees of freedom Residual deviance: 826.31 on 672 degrees of freedom AIC: 852.31 Number of Fisher Scoring iterations: 4				

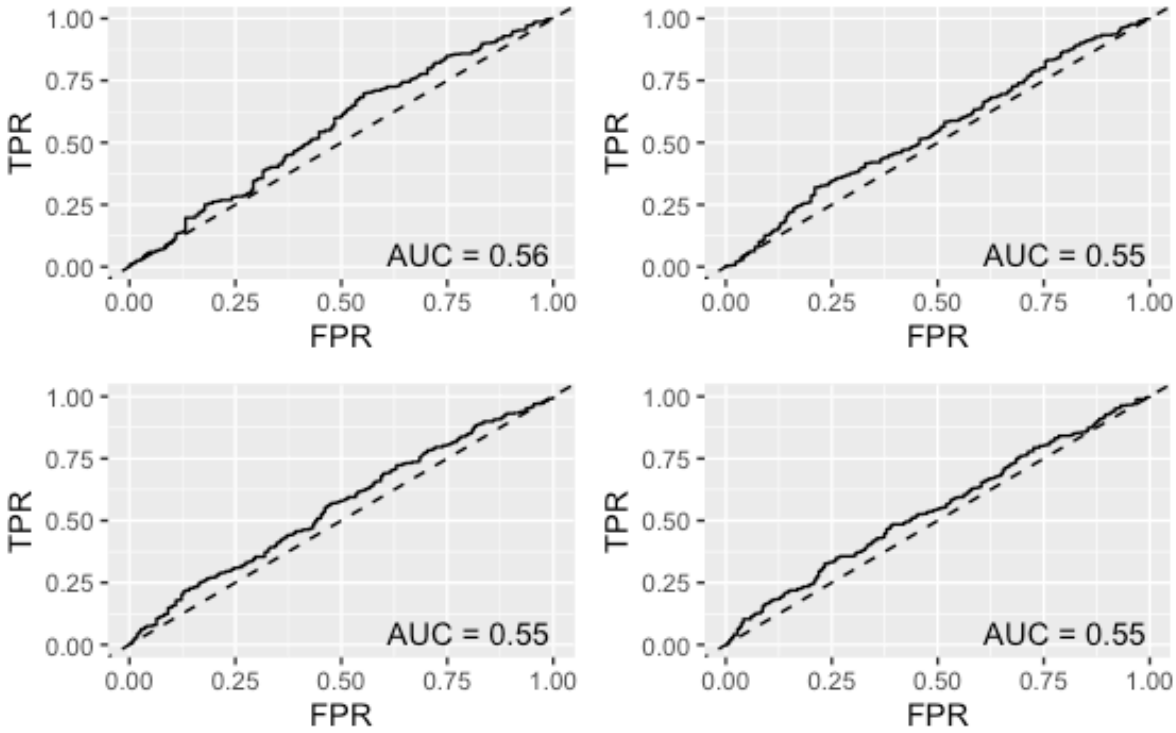


Figure 13. ROC curves with the greatest AUC assessing in
Figure (a) PCA1, Figure (b) PCA4, Figure (c) PCA9 and Figure (d) PCA12 compared seropositive and seronegative participants' cytokine profiles across these 4 principal component analyses.

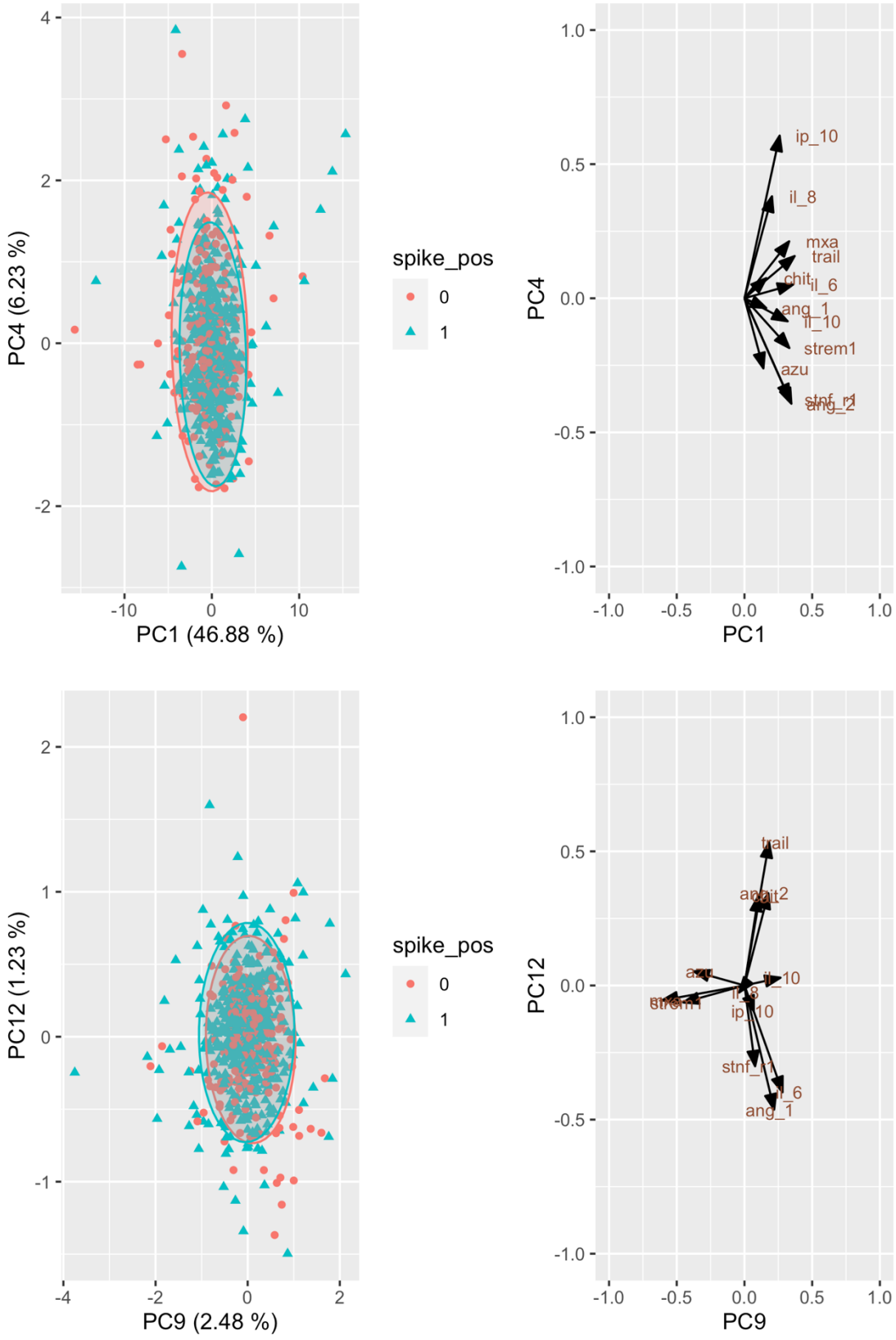


Figure 14 (a) PC 1 & PC4 directly compared across seropositive and seronegative groups.
Figure 14 (b) PC9 & PC12 directly compared across seropositive and seronegative groups.
Figure 14. PC1, PC4, PC9 & PC12 compared seropositive and seronegative participants' cytokine profiles across these 4 principal components.

There is no difference in the primary outcome groups and the null hypothesis cannot be rejected, there is no difference in cytokine profile between SARS-CoV-2 seropositive groups in this community population.

4.4.02 Symptomatic vs asymptomatic infections

Therefore, I further interrogated the data to assess whether there was a difference between seropositive participants reporting symptomatic COVID-19 illness and seropositive participants who did not report COVID-19 illness.

Of the 12 PCA for this outcome, the ones showing the greatest between-group difference were PC5 & PC10 shown in table 5. I interrogated these results with a Receiver operating characteristic (ROC) analysis to assess the PC diagnostic ability. Three PC: PC5, PC10 and PC11 had AUC of 0.55, 0.54 and 0.54 respectively (Figure 15). No population-level combinations of cytokines can clearly discriminate between seropositive participants with and without COVID-19-like illness, symptoms are not discriminated between.

Table 5. PCA results from a generalised linear model assessing whether any combination of soluble cytokines differentiates between SARS-CoV-2 seropositive participants reporting COVID-19-like illness and participants who did not report a COVID-19-like illness.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.20507	0.07830	-2.619	0.00882**
PC1	0.01408	0.03410	0.413	0.67969
PC2	0.10528	0.05928	1.776	0.07573
PC3	0.05462	0.08107	0.674	0.50044
PC4	-0.05954	0.09190	-0.648	0.51707
PC5	-0.24681	0.11636	-2.121	0.03392 *
PC6	-0.09441	0.11863	-0.796	0.42612
PC7	-0.16128	0.13014	-1.239	0.21522
PC8	-0.05334	0.13663	-0.390	0.69624
PC9	-0.22145	0.14519	-1.525	0.12720
PC10	-0.42218	0.16651	-2.535	0.01123 *
PC11	0.31055	0.17761	1.749	0.08038
PC12	-0.28136	0.20916	-1.345	0.17857
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Dispersion parameter for binomial family taken to be 1) Null deviance: 943.05 on 684 degrees of freedom Residual deviance: 918.15 on 672 degrees of freedom AIC: 944.15 Number of Fisher Scoring iterations: 4				

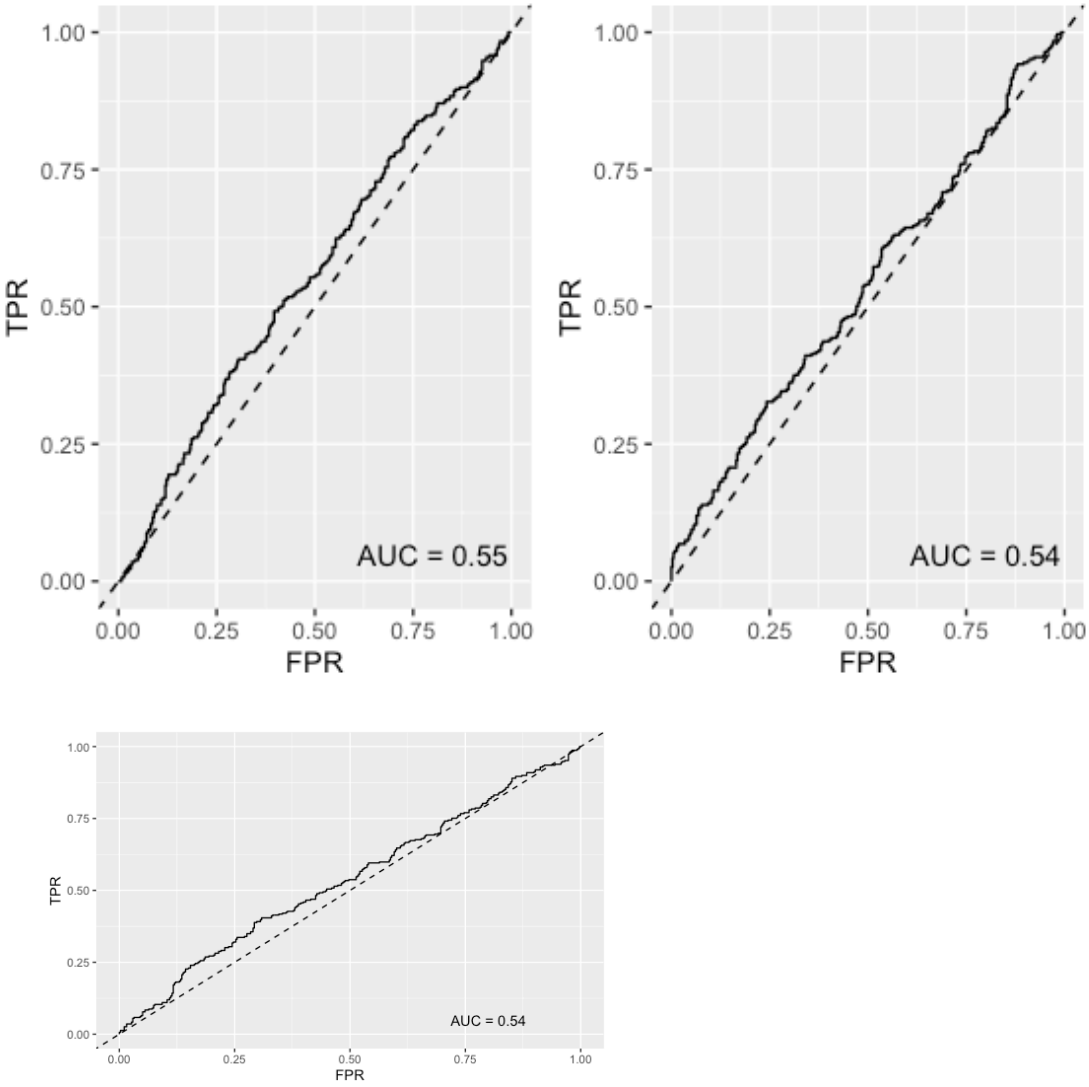


Figure 15 (a). PC5 ROC curve. Figure 15 (b). PC10 ROC curve. Figure 15 (c). PC11 ROC curve.
Figure 15. ROC curves assessing the PC5, PC10 & PC11 respectively of cytokine combinations to assess differences between seropositive participants reporting COVID-19-like illness.

4.4.03 Wave 1 vs Wave 2 – long range effects

I performed a secondary sub-analysis to interrogate whether cytokine profiles were different in these community participants between those with COVID-19 in the first and second waves of the UK pandemic in 2020. In Table 6 the PCA analysis of various cytokine combinations within a linear model to differentiate between seropositive participants reporting COVID-19-like illness across wave1 and wave 2 showed that only one of the PC (PC11) showed any difference between the outcome groups. On performing a ROC analysis, the were four best-performing PC, PC2, PC7, PC8 and PC11 with AUC of 0.56, 0.55, 0.56 and 0.56 respectively. Once again combinations of cytokines do not differentiate well between groups seropositive participants with symptoms in wave 1 or wave 2 of the pandemic (Figure 16).

Table 6. PCA results from a generalised linear model assessing whether any combination of soluble cytokines differentiates between SARS-CoV-2 seropositive participants reporting COVID-19-like illness in wave 1 (Feb-Apr 2020) and seropositive participants reporting a COVID-19-like illness in wave 2 (Sep-Nov 2020)

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.047977	0.104599	-0.459	0.6465
PC1	0.031449	0.046905	0.670	0.5025
PC2	-0.099220	0.083128	-1.194	0.2326
PC3	-0.070636	0.107057	-0.660	0.5094
PC4	-0.073885	0.127327	-0.580	0.5617
PC5	0.005832	0.152903	0.038	0.9696
PC6	-0.185339	0.162210	-1.143	0.2532
PC7	0.259651	0.182008	1.427	0.1537
PC8	-0.346761	0.181985	-1.905	0.0567
PC9	0.006358	0.183025	0.035	0.9723
PC10	-0.036614	0.219406	-0.167	0.8675
PC11	-0.516647	0.252496	-2.046	0.0407 *
PC12	0.190728	0.280473	0.680	0.4965
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Dispersion parameter for binomial family taken to be 1) Null deviance: 551.49 on 397 degrees of freedom Residual deviance: 536.41 on 385 degrees of freedom (68 observations deleted due to missingness) AIC: 562.41 Number of Fisher Scoring iterations: 4				

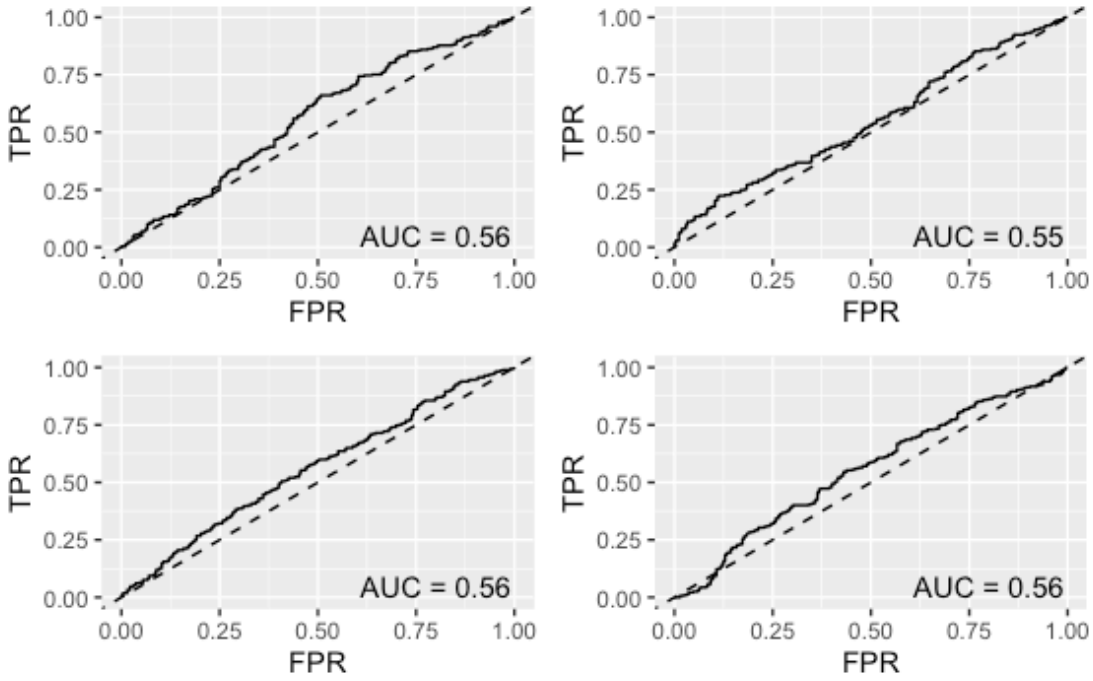


Figure 16 (a). PC2 ROC curve. Figure 16 (b). PC7 ROC curve. Figure 16 (c). PC8 ROC curve. Figure 16 (d). PC11 ROC curve

Figure 16. ROC curves assessing the PC2, PC7, PC8 & PC11 respectively of cytokine combinations to assess differences between seropositive participants reporting COVID-19-like illness in wave 1 and wave 2.

4.4.04 Adults vs Children

In the next sub-analysis, I interrogated the data to answer whether there was a difference in cytokine populations between seropositive adults and seropositive children. Table 7 shows that three of the PC (PC5, PC10, PC12) do contain combinations of cytokines that may differentiate between seropositive adults and children (<18 years of age).

On performing a ROC analysis comparing cytokine profiles in seropositive children and adults, the were three best-performing PCs, PC5, PC10 and PC12 with AUC of 0.58, 0.57, and 0.60 respectively. These combinations of cytokines differentiate slightly better between outcome groups than the earlier sub-analyses however not with enough diagnostic accuracy to be of use discriminating between groups (Figure 17).

Table 7. Results from a generalised linear model assessing whether the PCA results from combinations of soluble cytokines differentiate between SARS-CoV-2 seropositive adults and children (<18 years of age)

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.26103	0.09908	-2.635	0.00842 **
PC1	-0.01244	0.04388	-0.284	0.77674
PC2	-0.09350	0.08068	-1.159	0.24651
PC3	-0.08449	0.10449	-0.809	0.41877
PC4	0.09690	0.11948	0.811	0.41735
PC5	0.37868	0.14559	2.601	0.00930 **
PC6	0.14051	0.15327	0.917	0.35928
PC7	0.32257	0.16572	1.946	0.05160
PC8	- 0.19436	0.17431	-1.115	0.26486
PC9	0.07040	0.17665	0.399	0.69023
PC10	0.55758	0.21364	2.610	0.00906 **
PC11	-0.44879	0.23326	-1.924	0.05436
PC12	1.11687	0.28162	3.966	7.31e-05 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Dispersion parameter for binomial family taken to be 1) Null deviance: 638.27 on 465 degrees of freedom Residual deviance: 599.48 on 453 degrees of freedom AIC: 625.48 Number of Fisher Scoring iterations: 4				

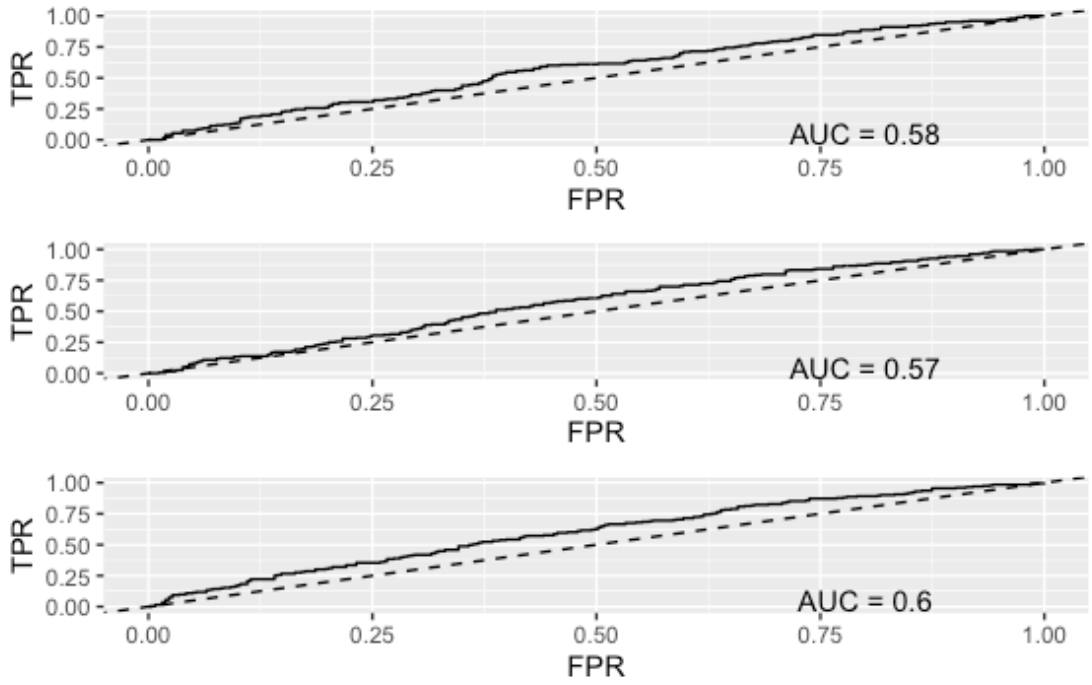


Figure 17 (a). PC5 ROC curve. Figure 17 (b). PC10 ROC curve. Figure 17 (c). PC12 ROC curve.
Figure 17. ROC curves assessing the PC5, PC10 & PC12 respectively of cytokine combinations to assess differences between adult and children (<18 years of age) seropositive participants.

4.4.05 Asymptomatic vs symptomatic children

In the final sub-analysis, I investigated whether there was a difference in total cytokine populations between seropositive children reporting COVID-19 symptoms and seropositive children without COVID-19 symptoms. In table 8 I show that only PC4 displays the potential for a cytokine difference between outcome groups. In the ROC analysis for this subgroup outcome, I identified that (Figure 18).

Table 8. Results from a generalised linear model assessing whether the PCA results from combinations of soluble cytokines differentiate between symptomatic SARS-CoV-2 seropositive children and asymptomatic SARS-CoV-2 seropositive children.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.79924	0.16813	-4.754	2e-06 ***
PC1	0.07644	0.08287	0.922	0.356
PC2	0.14080	0.20985	0.671	0.502
PC3	0.12590	0.17658	0.713	0.476
PC4	-0.41357	0.20632	-2.004	0.045 *
PC5	-0.03601	0.24550	-0.147	0.883
PC6	-0.22735	0.25952	-0.876	0.381
PC7	-0.22356	0.28122	-0.795	0.427
PC8	-0.35759	0.29436	-1.215	0.224
PC9	-0.38703	0.28456	-1.360	0.174
PC10	-0.02497	0.36366	-0.069	0.945
PC11	-0.03811	0.39743	-0.096	0.924
PC12	-0.01083	0.43186	-0.025	0.980
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Dispersion parameter for binomial family taken to be 1) Null deviance: 256.05 on 202 degrees of freedom Residual deviance: 246.28 on 190 degrees of freedom AIC: 272.28 Number of Fisher Scoring iterations: 4				

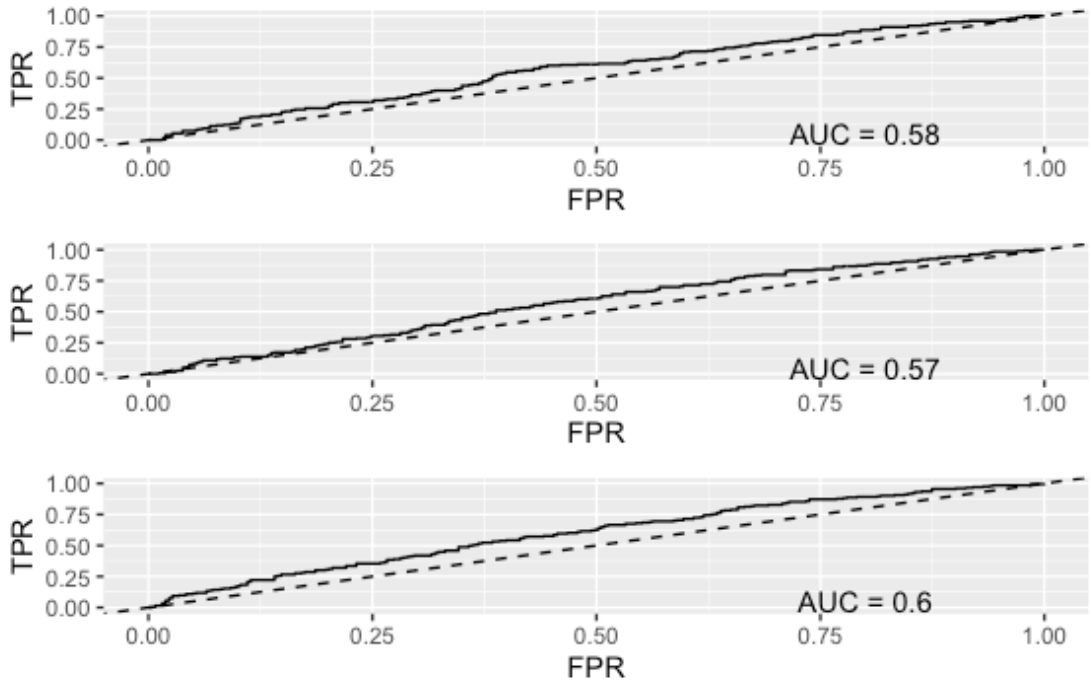


Figure 18 (a). PC5 ROC curve. Figure 18 (b). PC10 ROC curve. Figure 18 (c). PC12 ROC curve.
Figure 18. ROC curves assessing the PC respectively of cytokine combinations to assess differences between seropositive children with and without symptomatic disease.

4.4.06 Individual Cytokine Analysis

I then interrogated each cytokine with a Receiver operating characteristic (ROC) analysis to assess each individual cytokine's diagnostic ability for the primary outcome and the secondary subgroup analyses.

For the primary outcome differentiating between SARS-CoV-2 seropositive and seronegative participants, 5 soluble cytokines performed with AUC > 0.55, these included chitinase 3-like 1 (CHIT) with an AUC of 0.55, Interleukin-6 (IL-6) with an AUC of 0.55, soluble tumour necrosis factor (STNF) with an AUC of 0.58, Angiopoietin 2 (ANG-2) with an AUC of 0.58, and Myxovirus resistance protein A (MxA) with an AUC of 0.56 (Figure 19). None of these results are sufficiently sensitive to accurately discriminate between these outcome groups.

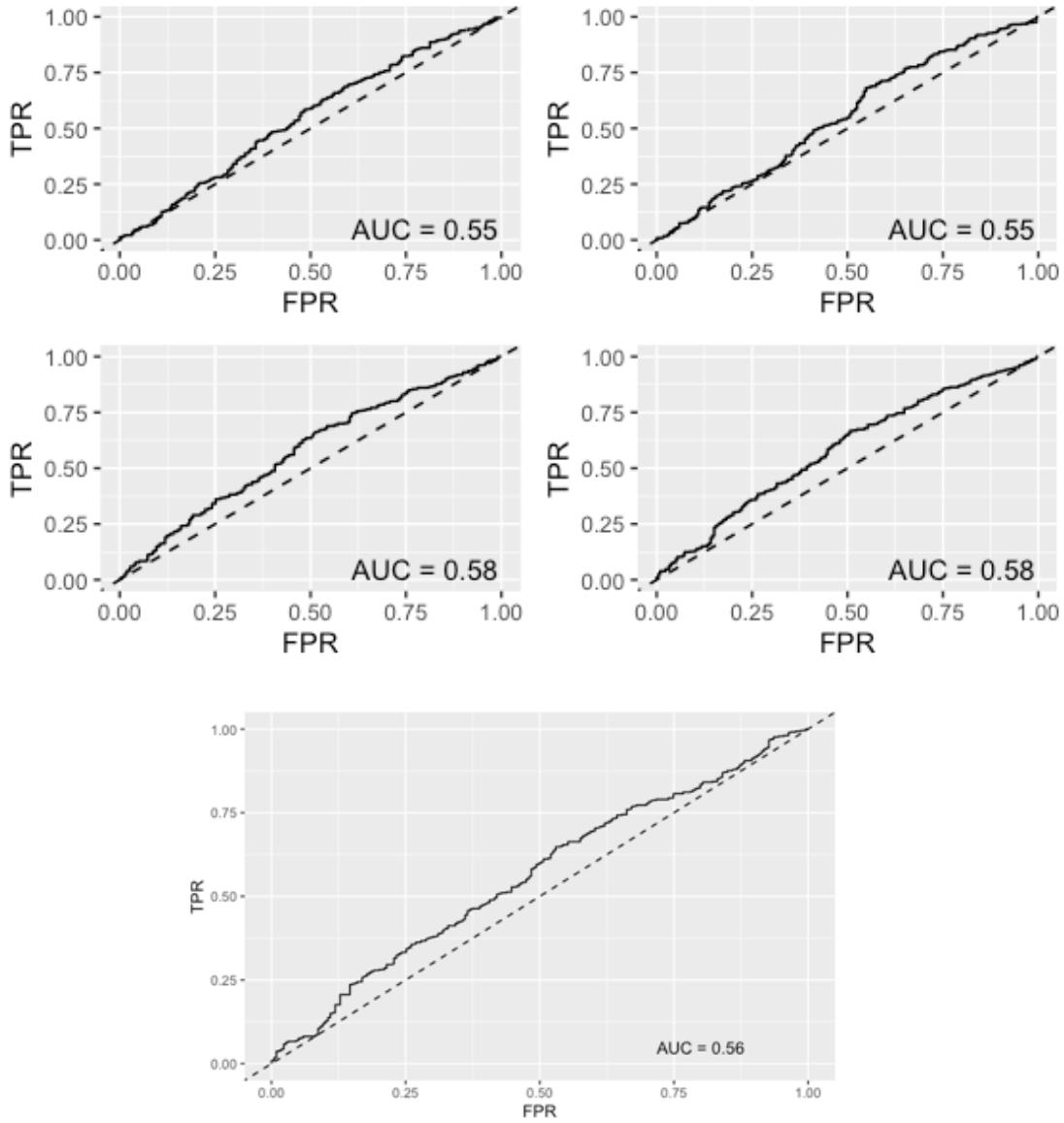


Figure 19 (a). CHIT ROC curve Figure 19 (b). IL-6 ROC curve Figure 19 (c.) STNF ROC curve
Figure 19 (d). ANG-2 ROC curve Figure 19 (e). MxA ROC curve
Figure 19. The cytokines with the strongest performance for differentiating SARS-CoV-2 seropositive form seronegative participants

4.4.07 Symptomatic vs asymptomatic infections

I interrogated the data for the secondary outcomes of interest, firstly to identify if any of the cytokines differentiated between symptomatic and asymptomatic seropositive participants. Three cytokines showed potential, Interlukin-8 with an AUC of 0.55, Interlukin-10 with an AUC of 0.54, and Azurocidin 1 (AZU) (alternatively called Heparin Binding Protein) with an AUC of 0.56, but none with enough precision to discriminate well between outcome groups (Figure 20).

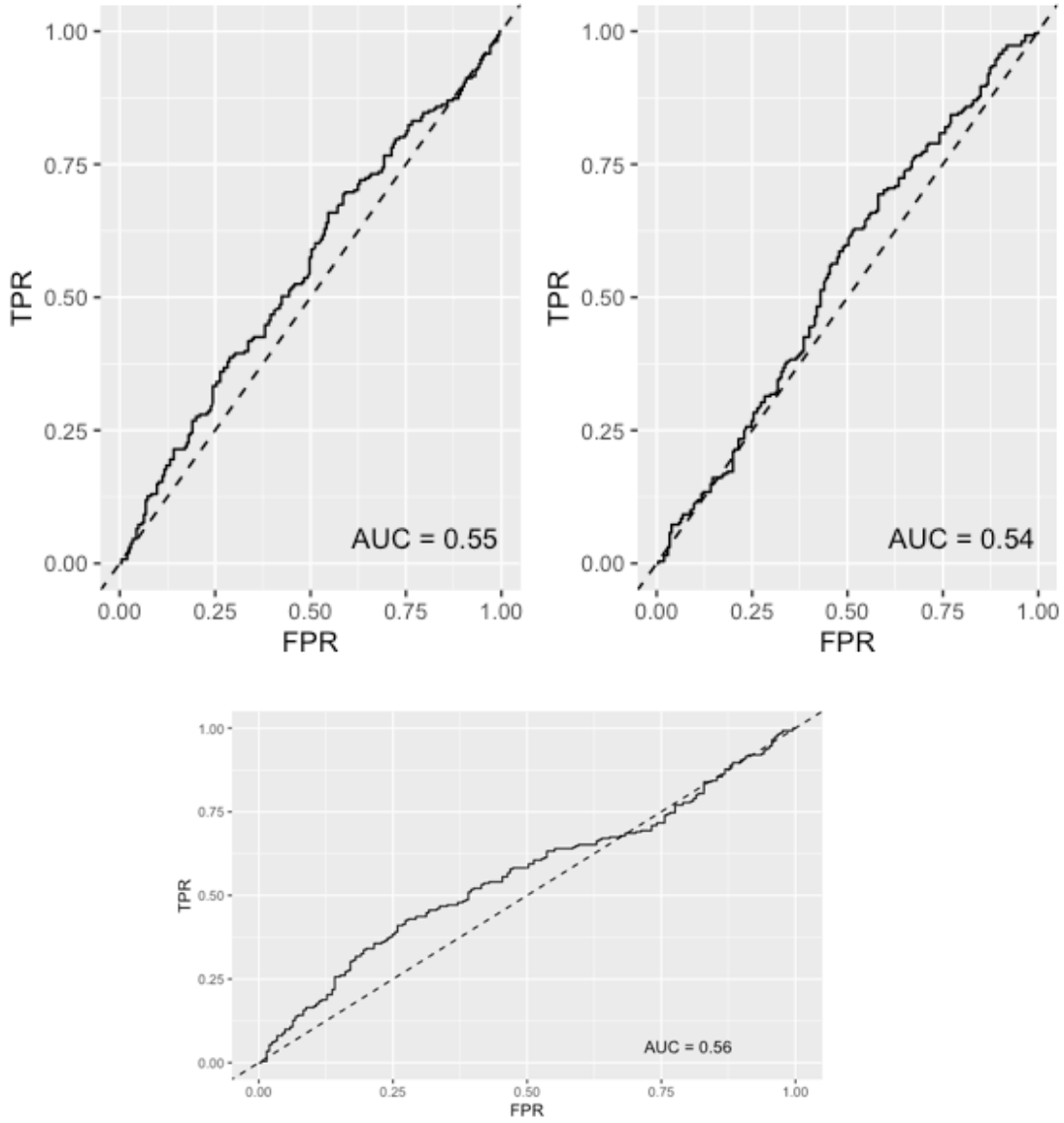


Figure 20 (a). IL-8 ROC curve, Figure 20 (b). IL-10 ROC curve, Figure 20 (c). AZU ROC curve
Figure 20. The cytokines with the strongest performance for differentiating SARS-CoV-2 seropositive symptomatic and asymptomatic participants

4.4.08 Wave 1 vs Wave 2 – long range effects

Secondly to identify if any of the cytokines differentiated between seropositive participants symptomatic in wave 1 and wave 2. Two cytokines showed potential, TNF-Related Apoptosis-Inducing Ligand (TRAIL) with an AUC of 0.56, and Azurocidin 1 (AZU) with an AUC of 0.57, but neither with enough precision to discriminate well between outcome groups (Figure 21).

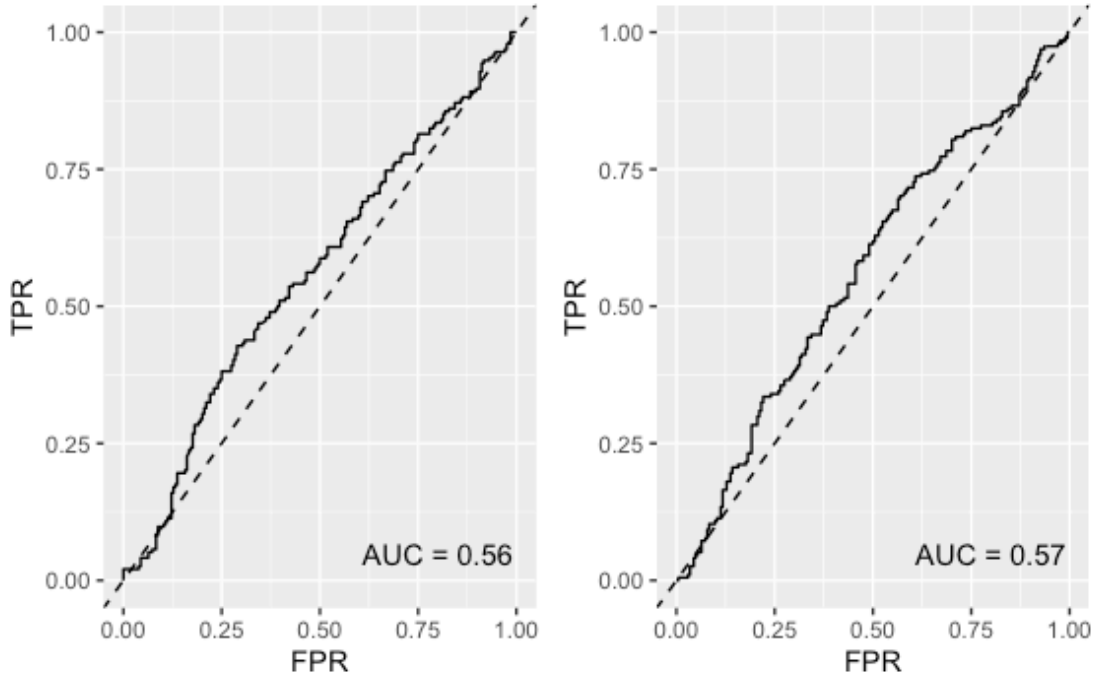


Figure 21(a). TRAIL ROC Curve, Figure 21(b). AZU ROC Curve
Figure 21. The cytokines with the strongest performance for differentiating SARS-CoV-2 seropositive participants with COVID-like disease in wave 1 and wave 2

4.4.09 Adults vs Children

Finally, I ran an analysis to investigate the individual cytokines potential to differentiate between seropositive adults and seropositive children. Four cytokines showed potential (Figure 22), chitinase 3-like 1 (CHIT) with an AUC of 0.55, Interlukin-6 with an AUC of 0.55, soluble Tumour necrosis factor (STNF) with an AUC of 0.56 and Azurocidin 1 (AZU) with an AUC of 0.57, but none with enough sensitivity to discriminate between child and adult groups.

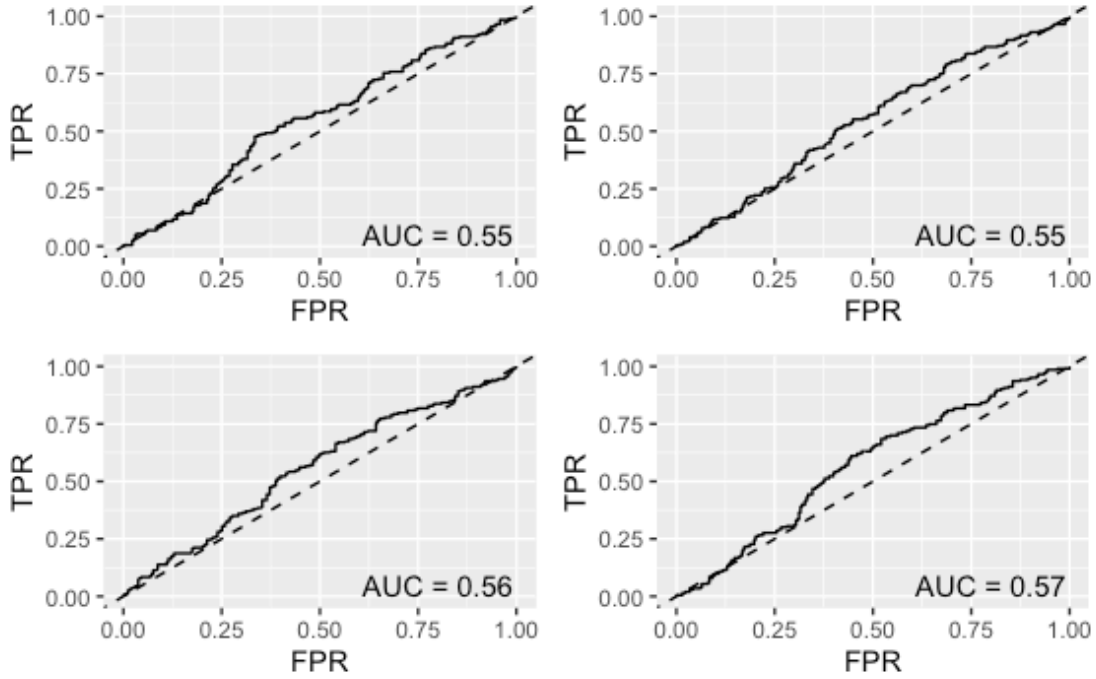


Figure 22 (a). CHIT ROC Curve, Figure 22 (b). IL-6 ROC Curve,
Figure 22 (c). STNF ROC Curve, Figure 22 (d). AZU ROC Curve.
Figure 22. The cytokines with the strongest performance for differentiating SARS-CoV-2 seropositive child (<18 years) and adult participants

The final planned sub-group analysis was to compare the cytokine profiles in participants reporting illness, comorbidity, and death. We could not perform this analysis due to the extremely low proportion of participants reporting serious COVID-19 illness, comorbidity and death which was likely related to the age of the population sampled. We were unable to analyse whether individuals with these conditions had a systematic difference in their cytokine profiles associated with COVID-like illness reporting.

4.5 Discussion

None of the 12 cytokines tested for within our in-house Luminex assay showed differential potential between these SARS-CoV-2 seropositive and seronegative participants, nor within the subgroup analyses comparing symptomatic with asymptomatic, wave 1 and wave 2 COVID-19 like disease or children and adults. The tri-signal shown in SARS-CoV-2 hospitalised patients of IL-6, IL-10, and IP-10 to be associated with severe disease, mortality and long ICU stay does not flag within these community sampled, well individuals with a history of recent COVID-19 illness, only two of whom required hospitalisation (2). Nor are the soluble cytokines IL-6, IL-8, and TNF-alpha independently shown to be associated with poor outcomes in hospitalised patients raised in this population (9,10).

Importantly not only is there no discernible soluble immune response in this large population of community sampled SARS-CoV-2 seropositive symptomatic population but there is no effect of time on the immune markers nor of participant age. The ROC curve analyses of both the PCA and the individual cytokines show no testable difference between true positives and false positives across any of the binary outcomes.

Severe COVID-19 is thought to produce an early large pro-inflammatory T cell and cytokine response with a delayed disproportionate adaptive immune response (24). Soluble cytokines are released temporarily into the bloodstream and can remain within the bloodstream for up to 24 hours after release unless there is continued stimulation or activation. Similarly, T cells which produce these cytokines have been shown to rise transiently during mild SARS-CoV-2 disease before falling at one month from disease onset (25). If T cells fall in the month following mild COVID-19 it would follow that the stimulus for cytokine release also tapers off. The results from my work are perhaps unsurprising given this context. Yet activated memory T cells can be found in the lungs up to 8 months from mild disease onset (24). And are a recognised and important part of the development of long-term immune response. However, in a small sample of 19 children from Shanghai with mild disease IP-10 was seen to rise acutely in the first week of illness before returning to normal levels 27 days after illness onset (26). Yet IL-16, a chemokine possibly related to viral infection (27), and IL-10, an anti-inflammatory

cytokine, rose in the acute phase and remained high in convalescent serum 27 days after disease onset. Overall cytokine levels in these children did not rise as significantly as they are known to in adult SARS-CoV-2 patients (26).

Cytokines of interest in differentiating viral disease from other causes of systemic inflammation have been described in the context of other groups of viruses. A four-marker gene signature has been identified within all seven Baltimore viral classification groups containing Interferon Stimulated Gene 15, Interleukin-16, 2',5'-Oligoadenylate Synthetase Like, and, Adhesion G Protein Coupled Receptor E5 for up to one month after viral infection (27). Additionally, prolonged IL-10 release is thought to potentiate T cell inactivation and hence impact long-term or persistent viral infection (28).

There are several strengths to this study, within the existing literature there are limited pieces of work with small sample sizes, and our study has a large sample size for this type of work. If a signal was to be found, this sample size would be sufficient. The nesting of this study within our parent study ensures that all samples were treated equally and with the same methods, no systemic or individual differences induced by handling, testing, or sampling methods are likely.

This study has several limitations. Sampling serum from a community group who were required to be asymptomatic at the time of sampling excludes any participants with active disease at the point of sampling, removing the possibility of a range of soluble cytokine responses. Additionally, due to the population structure of the sample, there were limited cases of severe disease and no cases diagnosed with "Long COVID". Explorative analyses always have the potential for a negative result. Due to the design of the parent study, this was a single time point analysis, with no sequential sampling or surveying which would have improved this work.

This work has clearly shown that there are no identifiable soluble cytokines that systematically differentiate different populations of community-sampled recovered or convalescent SARS-CoV-2 sera.

Acknowledgement

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Chapter 5

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Student ID Number	LSH212211	**Title**	Dr
First Name(s)	Katherine		
Surname/Family Name	Gaskell (Wilson)		
Thesis Title	Respiratory pathogen transmission among exposed household contacts		
Primary Supervisor	Professor David Moore		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where was the work published?	Plos One		
When was the work published?	24/04/2023		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am the first author. I assisted in the design, I conducted and managed the research. I prepared the specimens for analysis. I collated the data. I helped plan and execute the analysis with KF, DAJM, DG. I wrote the first paper draft and developed the manuscript in consultation with DAJM, KF, CH, TR.

SECTION E

Student Signature	Katherine Gaskell
Date	07/02/2024

Supervisor Signature	David AJ Moore
Date	07/02/2024

Comparison Of New and emerging SARS-CoV-2 variant Transmissibility through Active Contact Testing. A comparative cross-sectional household seroprevalence study

Impact of work

Prior to this work there was no accurately calculated secondary attack rate (SAR) in household transmissibility studies. Neither was there a direct comparison of non-VOC with Alpha variant SARS-CoV-2 transmissibility within households.

PLOS ONE

RESEARCH ARTICLE

Comparison of new and emerging SARS-CoV-2 variant transmissibility through active contact testing. A comparative cross-sectional household seroprevalence study

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Abstract

Historically SARS-CoV-2 secondary attack rates (SAR) have been based on PCR positivity on screening symptomatic contacts; this misses transmission events and identifies only symptomatic contacts who are PCR positive at the time of sampling. We used serology to detect the relative transmissibility of Alpha Variant of Concern (VOC) to non-VOC SARS-CoV-2 to calculate household secondary attack rates. We identified index patients diagnosed with Alpha and non-VOC SARS-CoV-2 across two London Hospitals between November 2020 and January 2021 during a prolonged and well adhered national lockdown. We completed a household seroprevalence survey and found that 61.8% of non-VOC exposed household contacts were seropositive compared to 82.1% of Alpha exposed household contacts. The odds of infection doubled with exposure to an index diagnosed with Alpha. There was evidence of transmission events in almost all households. Our data strongly support that estimates of SAR should include serological data to improve accuracy and understanding.

Introduction

In autumn 2020 the first SARS-CoV-2 variant of concern (VOC) B.1.1.7 was reported in Southeast England. National incidence was tracked by Public Health England through the

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failure of routine SARS-CoV-2 PCRs to detect the spike gene (spike gene target failure, SGTF) in community samples. Initial modelled estimates of the infectiousness of B.1.1.7. (subsequently named Alpha) ranged between 43–100% more transmissible than the previously circulating wild type SARS-CoV-2 virus [1, 2].

One empiric metric of transmissibility for infectious diseases is the secondary attack rate (SAR) in exposed contacts. To date, reporting of SAR in SARS-CoV-2 has mostly defined secondary infection as contemporaneous PCR positivity in a symptomatic contact [3]. This approach significantly underestimates the total number of people infected by only capturing those who are both symptomatic and still have detectable viral RNA at the time of sampling.

Historically, reported SARS-CoV-2 secondary attack rates based upon PCR-positivity were approximately 20% overall, irrespective of variant [4], though this was reported to rise to around 40% for Omicron [4, 5].

Serological testing captures previous infection, whether symptomatic or not, including potentially missed transmission events from which the contact has become PCR-negative by the time of sampling. Comparison of SARs between settings or viral variants should thus incorporate serological responses if the true transmissibility is to be understood. In a study from Singapore, PCR testing of symptomatic household contacts (HHC) found a SAR of 5.9% (95%CI 4.9–7.1), but Bayesian modelling of serological data estimated 62% of infections were missed [6].

In March 2021, four months into a national 'lockdown', we set out to compare the measured transmissibility of the Alpha variant of SARS-CoV-2 relative to other contemporaneously circulating variants (referred to as non-VOC) using serology and aimed to determine household SAR from index patients diagnosed with SARS-CoV-2 across two London Hospitals between November 2020 and January 2021.

Methods

We performed a cross sectional seroprevalence study of HHC of individuals who had SARS-CoV-2 detected and whose virus was successfully whole genome sequenced (WGS) between 9th November 2020 and 31st January 2021. This period was selected as both Alpha and non-VOC SARS-CoV-2 were concurrently circulating within the community. Symptomatic index patients who were diagnosed across two North London hospitals (Hospital #1 and Hospital #2) were invited to participate alongside all their HHC. Potential participants were identified from those who submitted any sample where SARS-CoV-2 was detected by PCR to either laboratory including those submitted from the Emergency Department, out of hours GP services, occupational health services and hospitalised patients. We collected telephone verbal consent and undertook a questionnaire, subsequently visiting households once to collect serum with written consent for serological testing from all consenting household members. Verbal consent was witnessed by CONTACT team members and documented within the questionnaire. All household members of any age were eligible to participate. We initiated recruitment on 26th March 2021 for hospital #1 index cases and on 11th May 2021 for hospital #2. Recruitment was completed by 11th July 2021. The questionnaire included data on the index case, age, gender, ethnicity, and severity of COVID-19 disease, and for each contact on the duration of household exposure to the index patient whilst they were symptomatic, intensity of contact, additional SARS-CoV-2 exposures, vaccination status, and history of COVID-19 diagnosis or SARS-CoV-2 detection.

Patient and public involvement

Patient and public engagement was sought at the local ethical approvals stage. The in-hospital patient advice and liaison service, the palliative care service and patient representatives were

involved in how best to approach patients, relatives, and relatives of the deceased. The research question, study design and outcome measures were not altered by this engagement, but operational research procedures were adapted to incorporate patient and relative priorities and preferences. Patients were not involved in the recruitment or conduct of the study. Participant serology results were sent to all who had a serum sample taken and the final results will be emailed to all participants on publication.

Laboratory analysis

Samples were analysed for presence of IgG to SARS-CoV-2 spike protein (S) and nucleocapsid protein (NC) for both non-VOC and Alpha SARS-CoV-2 using a multiplex chemiluminescence immunoassay (MSD, Rockville, MD) evaluated by our laboratory as previously described [7]. All households were provided with their results.

Statistical analysis

Assuming a conservative 15% seroprevalence in non-VOC households [8, 9] and an average cluster size of 2 (for a household size of 3; it is 2.4 in southeast England) we estimated that 292 Alpha and 292 non-VOC households (1168 participants) would provide 90% power to detect a 50% increase in seropositivity. For the primary outcome of interest, we used IgG reactivity to SARS-CoV-2 NC rather than IgG reactivity to S to confirm previous SARS-CoV-2 infection to avoid any effect of vaccination status. We subsequently undertook a secondary analysis with additional inclusion of unvaccinated IgG SARS-CoV-2 S reactive and IgG NC unreactive individuals to the primary outcome and assigned them as infected contacts (S3 Fig in S1 Appendix). We fitted a multivariable random effects logistic regression model adjusted for clustering at the household level having initially assessed the inclusion of continuous variables using fractional polynomials. We considered IMD rank, interval between index PCR diagnosis and HHC serum sampling and duration, and intensity of exposure to an infectious household index case as potential confounders and adjusted for these in our multivariable model (STATA code included in supplementary material).

Definitions

HHC were defined as individuals living in the same household as the index case at the time of PCR-confirmed SARS-CoV-2. Duration of exposure was defined as the number of days of unquarantined exposure that an HHC had with the index case from the onset of symptoms up to hospital admission or symptom resolution. Whilst this unavoidably excludes pre-symptomatic infectiousness of index cases there is no evidence to suggest that this differs between VOC and non-VOC infections. Symptom duration captured the number of days the index was symptomatic at home. Socioeconomic status was captured through the use of the Index of Multiple Deprivation (IMD) which assigns nationwide ranking by postcode in the UK. Data were analysed by decile and rank. Long COVID was captured if participants had a clinical diagnosis of Long COVID.

Ethics

The study was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (LEO ref:25265), the NHS Health Research authority (IRAS ref:295376), and local hospital review committees. Verbal informed consent was obtained during the telephone survey and written consent provided prior to phlebotomy. Parents provided written consent for

children under 10 and assent was collected for children over 10 years. The study was sponsored by LSHTM.

Results

During the study period a total of 1366 individuals tested positive for SARS-CoV-2 across the two sites and had viral genotyping data available. Of these 354 index participants agreed to take part and ultimately 238 index participants (50 non-VOC and 188 Alpha) and 454 household contacts completed the study questionnaires and had a serum sample available (Fig 1).

We collected blood samples from 102 HHC exposed to an index with non-VOC SARS-CoV-2 infection and 352 HHC exposed to an index with Alpha SARS-CoV-2 infection. The characteristics of the 386 participants who declined a serum sample but provided survey information did not differ significantly from those providing serum (S1 Table in S1 Appendix).

The characteristics of index cases are shown by index case Alpha/non-VOC assignment, in Table 1. 95% of index cases reported having been symptomatic, 76% reported respiratory symptoms and 67% were hospitalised, for a median of 9 days (IQR 4–21). There were no statistically significant differences between index cases with non-VOC and Alpha SARS-CoV-2 infection (Table 1).

Overall, the HHC study population was 56% female with a median age of 42 years (IQR 23–59). Median household size was three (IQR 2–4, range 2–9) with 46% of index cases reporting their ethnicity as white, 18% as black African or black Caribbean and 13% as south Asian. Local demographic data from the 2021 UK Census reported a population which is 52% female, with a median age of 36 years, a median household size of two, and 54% reporting their ethnicity as white, 21% as black African or black Caribbean, and 10% as Asian, indicating that the study participants were representative of the population from which they were drawn. Compared to contacts from non-VOC index households, HHC of Alpha index cases reported a greater average duration of exposure to their infectious index case, and to report a diagnosis of symptomatic COVID-19 around the time of their index case's diagnosis (Table 2).

Spectrum of clinical features in seropositive contacts in Alpha and non-VOC affected households

A diagnosis of SARS-CoV-2 contemporaneous to the index case diagnosis was reported by 47% and 67% of contacts exposed to non-VOC and Alpha index cases within the household

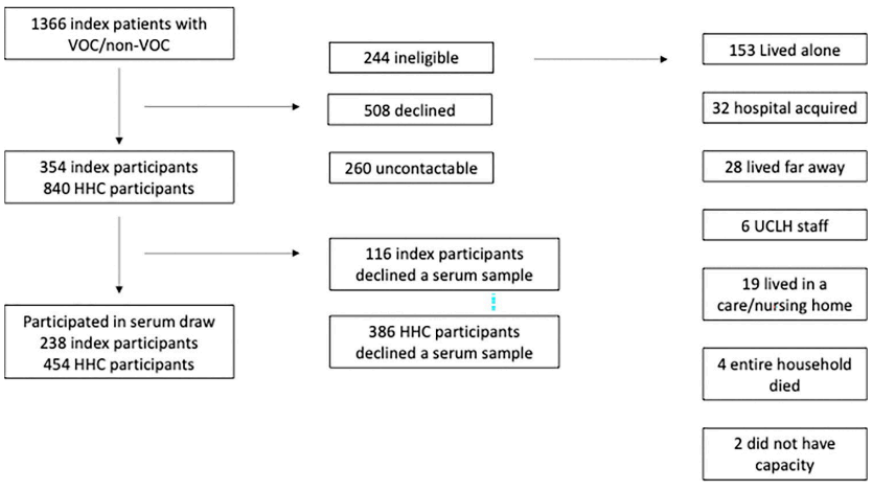

Fig 1. Flow diagram for recruitment into the study.

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Table 1. Baseline characteristics of 238 index cases stratified by index case Alpha or non-VOC SARS-CoV-2 status.

		Non-VOC index case household	Alpha index case household
Number of index—n		50	188
Index characteristics			
index case female—n (%)		26 (52)	111 (59)
Index case age–median (IQR)		56 (45–72)	57 (47–69)
ethnicity	white (%)	27 (54)	96 (51)
	Asian (%)	2 (4)	14 (8)
	Black (%)	7 (14)	34 (18)
	Middle Eastern (%)	5 (10)	10 (5)
	SE Asian (%)	7 (14)	13 (7)
	other	2 (4)	20 (10)
Hospital site	Hospital 1	20 (40)	88 (47)
	Hospital 2	30 (60)	100 (54)
index case respiratory symptoms[1] –n (%)		35 (70)	146 (78)
symptom duration in days–median(IQR)		7 (3–10)	7 (5–14)
index case hospitalisation–n (%)		29 (58)	124 (66)
index case ICU admission–n (%)		5 (17)	35 (29)
index case mortality–n(%)		1 (2)	2 (1)

[1] any of cough, dyspnoea, flu-like symptoms

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respectively (48/102 vs. 235/352, chi squared p value = 0.02). Not all contacts who reported a diagnosis of COVID were symptomatic at the time, however either COVID symptoms or a SARS-CoV-2 diagnosis around the time of index case diagnosis were more frequently reported by contacts of an Alpha index case (OR 4.87, 95%CI 3.67–21.18); anti-nucleocapsid IgG titres were higher in those reporting symptoms (Fig 2).

Seroprevalence in household contacts

61.8% (63/102) of non-VOC SARS-CoV-2 exposed HHC were seropositive compared to 82.1% (289/352) of Alpha SARS-CoV-2 exposed HHC. Household contacts exposed to an index with the Alpha variant had a 3.5-fold increased odds of being nucleocapsid seropositive (OR 3.5, 95%CI 1.7–7.4) when adjusted for household clustering.

The percentage of households in which no contacts had demonstrable SARS-CoV-2 anti-nucleocapsid antibodies, implying a complete absence of household transmission, was 3/50 (6%) and 1/188 (0.5%) in non-VOC and Alpha affected households respectively (Fisher's exact p value = 0.008).

Several covariates were identified as confounders requiring adjustment in logistic regression analyses, including IMD rank, interval between index PCR diagnosis and HHC serum sampling and duration and intensity of exposure to an infectious household index case (Table 3).

Non-VOC strains of SARS-CoV-2 presented in index cases earlier within the study period and earlier within the pandemic wave. Index cases with Alpha SARS-CoV-2 presented later within the pandemic wave and became the dominant strain causing COVID-19. The interval between index PCR diagnosis and HHC serological testing differed between the Alpha and non-VOC groups, (S1 Fig in S1 Appendix, Table 4). The observed decay in anti-nucleocapsid IgG titres over time (S2 Fig in S1 Appendix) highlights the importance of accounting for this differential delay; longer delay increases the risk of decay of anti-NC IgG below the threshold

Table 2. Baseline characteristics of 454 household contacts stratified by index case Alpha or non-VOC SARS-CoV-2 status.

		Non-VOC index case household	Alpha index case household
Number of contacts—n		102	352
Index Characteristics			
HHC exposed to a female index—n (%)		58 (57)	197 (56)
Index age HHC exposed to—median (IQR)		62 (45–76)	56 (47–69)
HHC exposed to index ethnicity	white (%)	47 (46)	163 (46)
	Asian (%)	6 (6)	24 (7)
	Black (%)	11 (11)	72 (21)
	Middle Eastern (%)	14 (14)	19 (5)
	SE Asian (%)	21 (21)	39 (11)
	other	3 (3)	35 (10)
Hospital site	Hospital 1	49 (48)	173 (49)
	Hospital 2	53 (52)	179 (51)
household size–median (IQR)		3 (2–4)	3 (2–4)
HHC exposed to index case respiratory symptoms[1] –n (%)		71 (70)	276 (78)
HHC exposure to symptomatic index in days–median (IQR)		7 (4–12)	7 (4–12)
HHC exposure to a hospitalised index–n (%)		62 (61)	244 (69)
HHC exposure to an index requiring ICU admission–n (%)		11 (19)	86 (35)
HHC exposure to an index who died–n (%)		8 (8)	33 (9)
Household IMD decile (IQR)		3 (2–6)	3 (2–6)
Time since index PCR diagnosis—median (IQR)		146 (125–181)	119 (93–157)
Contact characteristics			
contact female–n (%)		57 (56)	204 (58)
contact age–median (IQR)		44 (22–59)	42 (24–60)
days of exposure to index–median (IQR)		4 (1–10)	7 (3–14)
proximity to index[2] –n (%)	no close contact	19(19)	49(14)
	assisted in personal care	24 (24)	130 (37)
	shared bedroom	16 (16)	59 (17)
	shared bathroom	43 (42)	114 (32)
other COVID exposure (not index)–n (%)		26 (26)	107 (30)
COVID-19 diagnosis–n (%)		48 (47)	235 (67)
Contact symptoms—n (%)		40 (39)	183 (52)
Long COVID[3]—n (%)		4 (4)	8 (2)
vaccination status at time of serum sampling[4]	unvaccinated	49 (48)	169 (48)
	single vaccination	24 (24)	102 (29)
	double vaccination	29 (28)	81 (23)

[1] any of cough, dyspnoea, flu-like symptoms

[2] this variable captures the intensity of contact—level '0' no close contact but still being a household member; level '1' sharing a bathroom but not sharing a bedroom; level '2' sharing a bedroom but not providing physical assistance; level '3' ascribed to those providing physical assistance to the unwell index case including washing, dressing, feeding, assisting with movement

[3] individuals with a clinical diagnosis of 'Long COVID'

[4] no more than 2 vaccinations available at this time

https://doi.org/10.1371/journal.pone.0284372.t002

of detection and consequent misclassification of infected HHCs as seronegative, uninfected subjects.

Table 4 highlights the difference in median follow up time according to detection of IgG to S and NC, stratified by vaccination status (vaccination generates anti-S but not anti-NC IgG antibodies). The falling IgG NC titre over time did impact the odds of seropositivity in HHCs.

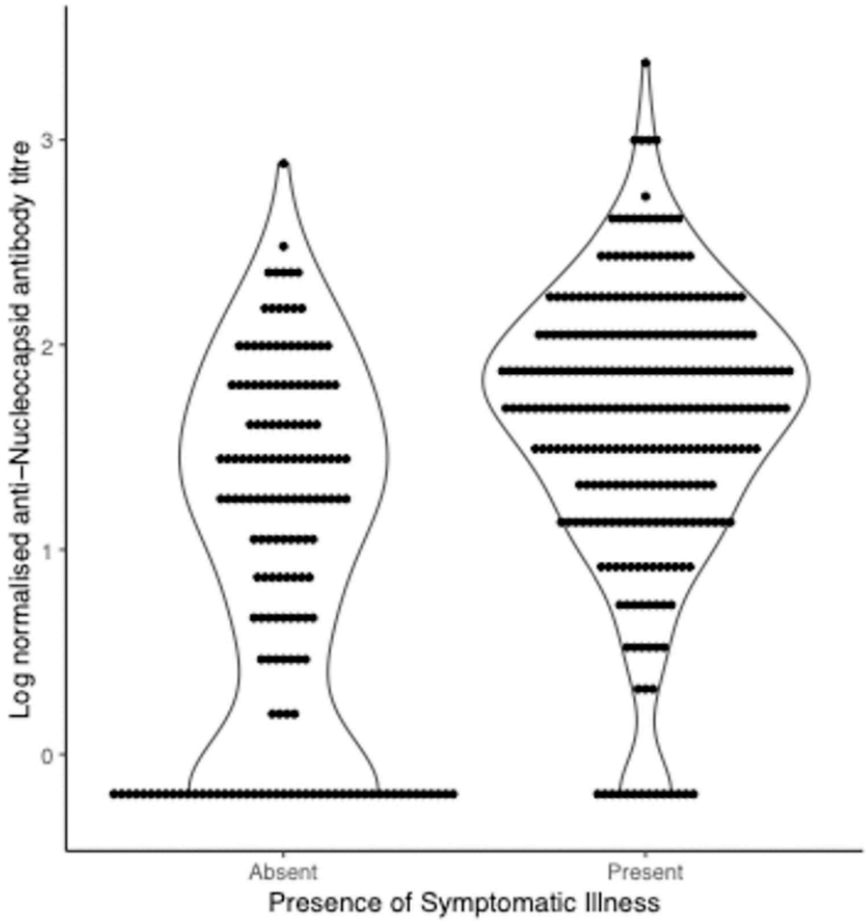

Fig 2. Magnitude of anti-nucleocapsid SARS-CoV-2 antibody response in 454 household contacts with and without reported COVID symptoms.

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This contrasts with previous published work where nucleocapsid titre declines more rapidly over the first 6 months from infection than Spike protein titre but does not impact positivity overall.

Table 3. Unadjusted and adjusted odds ratios for the effect of index case Alpha SARS-CoV-2 on household contact infection (defined as anti-nucleocapsid IgG sero-positivity) compared to non-VOC.

	% NC positive (n/N)	Unadjusted OR (95% CI)	P-value	Adjusted OR*(95% CI)	P-value
Non-VOC	62% (63/102)				
Alpha	82% (289/352)	3.5 (1.7–7.4)	0.001	2.5 (1.2–5.4)	0.02

All OR are adjusted for household level clustering

* adjusted for sex, age (years; linear), time between index PCR date and date of HHC serology (days; linear), duration of contact between infectious index and HHC (days; linear), IMD (rank; linear), intensity of contact (4 levels assuming a linear trend).

NC = nucleocapsid, n = total positive, N = total population

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Table 4. Relationship between vaccination status, antibody positivity and the interval between household exposure (PCR positive test in index case) and serological testing of HHC.

Vaccination status	Serological response	Number of contacts	Interval between household exposure and serological testing	
		Total n = 454	median	IQR
Unvaccinated Total 221	Spike positive	166	117	91–147
	NC positive			
	Spike positive	32	157	121–161
	NC negative			
	Spike negative	3	198	143–198
	NC positive			
	Spike negative	20	124	108–167
	NC negative			
Vaccinated	Spike positive	183	133	108–161
Total 233	NC positive			
	Spike positive	50	137	126–163
	NC negative			
	Spike negative	0		
	NC positive			
	Spike negative	0		
	NC negative			

https://doi.org/10.1371/journal.pone.0284372.t004

To improve the classification of infection and explore the effect of Alpha exposure further we performed a secondary analysis using a combined outcome definition of infection including all NC IgG positive contacts and unvaccinated participants seropositive for anti-S IgG (S3 Fig in S1 Appendix) we found a similar effect to in our main analysis. We performed an unadjusted and an adjusted logistic regression model with this outcome, adjusting for the same confounding exposures, which yielded similar though slightly lower point estimates (aOR 2.1, 95%CI 0.8–5.5) (Table 5).

Discussion

The most striking finding of our study is the very high secondary infection rate amongst household contacts of SARS-CoV-2 irrespective of variant; 62% in non-VOC exposed and 82% in Alpha exposed contacts. The odds of infection in a household contact, already high with the original SARS-CoV-2 virus, doubled with the arrival of Alpha. Very few households were identified in which no transmission had taken place (6% and 0.5% of non-VOC and Alpha households respectively). A recently published human challenge model of pre-Alpha

Table 5. Unadjusted and adjusted odds ratios for the effect of index case Alpha variant SARS-CoV-2 on household contact infection (defined as either anti-nucleocapsid IgG seropositivity or anti-spike IgG seropositivity in an unvaccinated individual) compared to non-VOC SARS-CoV-2.

	% infection (n/N)	Unadjusted OR (95% CI)	P-value	Adjusted OR*(95% CI)	P-value
Non-VOC	75% (77/102)				
Alpha	87% (307/352)	3.1 (1.2–8.3)	0.02	2.1 (0.8–5.5)	0.14

All OR are adjusted for household level clustering

* adjusted for sex, age (years; linear), time between index PCR date and date of HHC serology (days; linear), duration of contact between infectious index and HHC (days; linear), IMD (rank; linear), intensity of contact (4 levels assuming a linear trend)

n = total positive, N = total population

https://doi.org/10.1371/journal.pone.0284372.t005

SARS-CoV-2 identified viral shedding onset within 2 days of infectious exposure continuing up to 12 days post-inoculation [10]. Our data demonstrate a dose-response of exposure to transmission, supporting the suggestion that being able to isolate from household members mitigates against transmission of SARS-COV-2 to some extent, regardless of the strain or variant.

These data, capturing evidence of infection previously overlooked by highly time-sensitive estimates of SAR dependent upon PCR testing of contacts, demonstrate much higher rates of infection than have been previously reported [11]. In addition to the effect of Alpha, household contacts were much more likely to be infected with greater duration and higher intensity of exposure in a dose-response fashion. Whilst Omicron is believed to have outcompeted and cause more infections than previous VOCs, due in part to a high rate of re-infections in the partially immune [11], Danish data reported a household secondary attack rate using RT-PCR of only 31% and 21% in Omicron and Delta SARS-CoV-2 infections respectively [12]. A meta-analysis of household SARs for Alpha and Delta VOC were estimated at 38% and 31% respectively [4], significantly lower than our study findings. Most previous data included secondary cases identified as RT-PCR positive infections only [12]. A previous study assessing a composite secondary attack rate using both serology and PCR results in Los Angeles found a similarly high SAR of 77% to our study [13]. Our data strongly support that estimates of SAR should include serological data to improve accuracy and understanding.

Our original analysis focussed upon anti-nucleocapsid IgG as the marker of infection as earlier data had suggested this should remain detectable within the timeframe that our study was planned for. In the event, recruitment was delayed longer than expected and some anti-NC IgG decay was seen earlier than previously reported [11]. To capture those who we believed to have been infected but in whom anti-NC IgG was undetectable (or no longer detectable at the threshold of the test) we took advantage of the unvaccinated contacts with only anti-S IgG to improve our definition of infection. The lower adjusted odds ratio of 2.1 is thus more likely to be closer to the true value.

Our study has several limitations. Firstly, an assumption of all our analyses is that the most likely explanation for a household contacts' seropositivity is their household exposure. It is plausible that exposure may have occurred during the first wave of infections in England which started in March 2020, or indeed outside the household at any time including to a common source at the same time as the index. Any such effect would tend to exaggerate the odds in Alpha contacts, because Alpha infections came later, allowing for greater exposure-risk time. However, low population prevalence prior to November 2020 and societal lockdown conditions in England will have minimised this effect, although adherence to lockdown was dwindling during the emergence of Alpha. We have no local data for community prevalence during the period studied.

Secondly, the index cases in this study were tested and diagnosed in hospital laboratories so may have been more severely unwell than the general population. Whilst this might bias overall estimates of the SAR, we did not find any evidence of difference in severity between the two exposure groups and so this is unlikely to have impacted our finding of an increased SAR amongst contacts of Alpha infection. The increased severity may have resulted in higher rates of viral shedding and more prolonged viral shedding, however those with more severe illness in this population were admitted to hospital, interrupting domiciliary transmission. We cannot present data on the viral loads or cycle thresholds (CT) for PCR positive index patients as a variety of PCR platforms were used across the laboratories involved, some of which reported relative light units. There are reported differences between variants in viral loads, duration of shedding, and in duration of infectivity [14–16]; our data cannot be directly extrapolated to more recent VOCs.

Finally, we were unable to meet our planned sample size within the available time frame to complete the study prior to relaxation of lockdown regulations, in part due to delays in regulatory and ethics application approvals. Community uptake and participation with the study was lower than anticipated both at initial approach and for the follow up serum sample. Despite this we were still able to demonstrate the increased SAR for Alpha compared to non-VOC infections and that using serology resulted in a markedly higher estimate of the SAR for both VOC and non-VOC infections.

Secondary attack rates amongst household contacts are a direct measure of transmissibility, particularly if community transmission is not high. There is however a risk that saturation—almost all contacts becoming infected—can obscure differences between infecting strains. Careful participant selection minimises the risk of confounding by time, place or person allowing the rates of secondary infection and secondary disease to be directly estimated and compared between different virus variants. Calculating transmissibility for novel and previous VOC(s) is an essential component of the public health response. These data support our argument to include serology in calculating secondary attack rates to assess transmissibility of new SARS-CoV-2 VOCs more accurately. Household contact screening to assess SAR is a useful model to replicate in future variant-driven pandemic waves.

Conclusions

Secondary attack rates (SAR) in SARS-CoV-2 were previously calculated using PCR positive samples only, though it is more accurate to use a household transmission model and screen contacts using serology, as done in this study. SAR should include serological data to improve accuracy and understanding. Almost all households in this study had transmission events. SAR were 61.8% in non-VOC SARS-CoV-2 exposed household contacts compared to 82.1% in Alpha SARS-CoV-2 exposed household contacts.

Supporting information

S1 Appendix.
(DOCX)

S1 File.
(PDF)

S1 Data.
(XLSX)

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Appendix
Supplementary information

STATA code for primary analysis

```
mfp, df(agenew diffpcrsero dayshh imd_rank close_contact:4): xtlogit nc_pos vocnew sexnew
agenew diffpcrsero dayshh imd_rank close_contact, re or nolog

mfp: xtlogit infected vocnew sexnew agenew diffpcrsero dayshh  imd_rank close_contact, re or
nolog
```

Supplementary Table 1. Baseline characteristics in 454 participant contacts with and 385 without serum samples

		Participants without serum samples	Participants with serum samples
Number of index - n Alpha non-VOC total		162 38 200	188 50 238
Number of contacts - n		385	454
Index characteristics[1]			
index case female - n (%)		220 (57.1%)	255 (56.2%)
Index case age – median (IQR)		51 (40- 64)	57 (47-70)
ethnicity	white (%)	27 (54)	96 (51)
	Asian (%)	2 (4)	14 (8)
	Black (%)	7 (14)	34 (18)

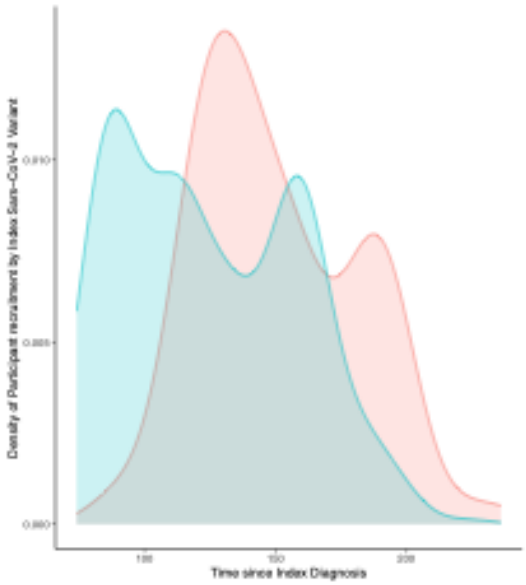
	Middle Eastern (%)	5 (10)	15 (6)
	SE Asian (%)	7 (14)	13 (7)
	other	2 (4)	8 (4.3)
Hospital site	Hospital 1	49 (48)	173 (49)
	Hospital 2	53 (52)	179 (51)
household size[1] – median (IQR)		3 (2-4)	3 (2-4)
index case respiratory symptoms – n (%)		35 (70)	146 (78)
symptom duration in days – median (IQR)		7 (3-10)	7 (4-12)
index case hospitalisation – n (%)		29 (58)	124 (66)
index case ICU admission – n (%)		5 (17)	35 (29)
index case mortality – n(%)		1 (2)	2 (1)
IMD decile (IQR)		3 (2-6)	3 (2-5)
Time since index PCR diagnosis - median (IQR)		139(111-164)	130 (101-158)
Contact characteristics[2]			
contact female – n (%)		172 (45)	204 (58)
contact age – median (IQR)		25 (12-45)	42 (24-60)

days of exposure to index – median (IQR)		5 (2-10)	7 (3-14)
proximity to index – n (%)	no close contact	86(22)	49(14)
	assisted in personal care	167 (43)	130 (37)
	shared bedroom	37 (10)	59 (17)
	shared bathroom	97 (25)	114 (32)
other covid exposure (not index) – n (%)		26 (26)	107 (30)
previous COVID-19 diagnosis – n (%)		48 (47)	235 (67)
Contact symptoms - n (%)		40 (39)	183 (52)
Long COVID - n (%)		4 (8)	8 (4)
vaccination status at time of serum sampling	unvaccinated	260(68)	169 (48)
	single vaccination	68(18)	102 (29)
	double vaccination	57(15)	81 (23)

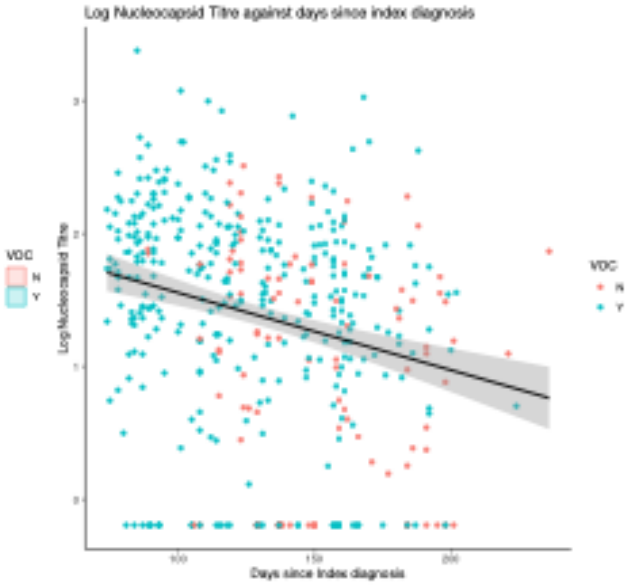
[1] The index characteristic figures in the table refer to the number of household contacts for whom the corresponding index case had each given characteristic

[2] The contact characteristics figures in the table refer to the number of household contacts with each given characteristic

Supplementary Figure 1



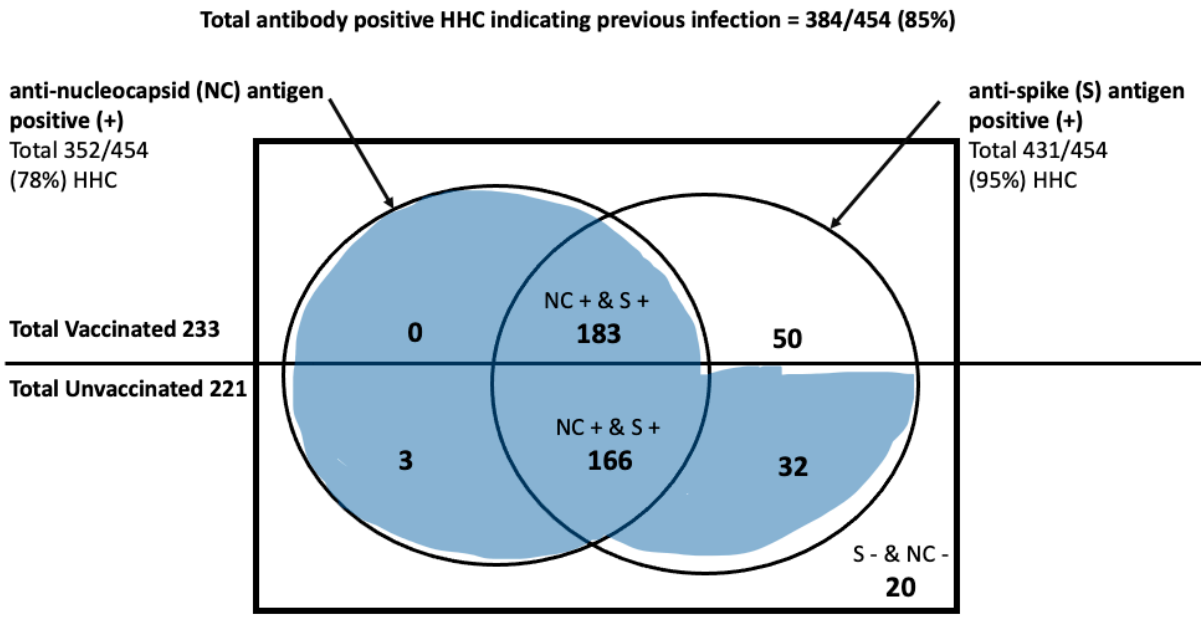
Supplementary Figure 2



Supplementary Figure 1. Density plot showing participant recruitment from time of index PCR diagnosis by SARS-CoV-2 variant of their index case.

Supplementary Figure 2. Log normalised anti-NC SARS-CoV-2 antibody titre in household contact against time since index PCR SARS-CoV-2 diagnosis

Supplementary Figure 3. Calculating the secondary outcome with infection defined as either anti-NC IgG seropositivity in any subject or anti-spike IgG seropositivity in an unvaccinated subject with S positive unvaccinated results. The shaded area displays all the HHC included in the secondary outcome.



Further detailed methodology additional to that in the published work

Recruitment

The Pathology Departments of both hospitals provided a list of all Whole Genome Sequenced (WGS) SARS-CoV-2 positive samples during the investigatory period but did not disclose which participants had Alpha variant and which had non-VOC. I have an honorary contract at both participating hospitals enabling access to patient records to record telephone numbers, home addresses and if the patient was alive or deceased. We recruited a team of ten volunteer doctors from the Diploma of Tropical Medicine and Hygiene and the MSc in Tropical Medicine and International Health to whom we provided a stipend and covered travel expenses. I am three other members of the team telephoned living patients and their relatives or sent study invitation letters to relatives of deceased patients prior to a telephone call. This decision was made following consultation with patient representative groups within the hospitals, who reported that initially approaching bereaved relatives via letter was more respectful. We collected telephone verbal consent and undertook a telephone questionnaire using the Open Data Kit (ODK) software available through the LSHTM London School of Hygiene and Tropical Medicine (LSHTM) secure server.

The remaining team subsequently visited households once to collect serum for serological testing from all consenting household members, taking written consent from all who gave a blood sample. Within the study team paediatricians took blood from children 1 year old and above enabling recruitment and participation of all household members of almost all ages.

The questionnaire included verbal consent, and data on the index case, age, gender, ethnicity, and severity of COVID-19 disease, range of symptoms, hospitalisation and its duration, intensive care admission, the duration of the index patient's COVID-19 symptoms whilst at home prior to admission or death, if the index was able to isolate from their household, vaccination status of the household, and travel history of the household. For each contact, the questionnaire included age, gender, duration of household exposure to the index patient whilst symptomatic, intensity of contact, additional SARS-CoV-2 exposures, and history of COVID-19 diagnosis or SARS-CoV-2 detection. Additionally, we collected data on self-isolation during the index case's illness, the duration of household quarantine, presence of Long-COVID

diagnosis, relationship to the index case, whether the household contact (HHC) left the household during lockdown for work or other reasons, and if so, what transport methods they used.

Laboratory

Samples were kept refrigerated between 4-8^{o}C degrees at each hospital site prior to transfer to LSHTM where they were spun down to separate serum from red blood cells before aliquoting and freezing at -20^{o}C degrees. Samples were then transferred to Professor Goldblatt's laboratory at Great Ormond Street Institute of Child Health to be analysed for presence of IgG to SARS-CoV-2 nucleocapsid protein (NC) for both non-VOC and Alpha SARS-CoV-2, and spike protein (S), and receptor binding domain (RBD) for Alpha, Beta, and Gamma SARS-CoV-2 using a 10 well multiplex chemiluminescence immunoassay (MSD, Rockville, MD) evaluated by(11,12). In order to measure IgG antibodies an MSD Blocker A was used to block the plates, then dilution to 1:500 using a diluent buffer added to the reference standard, controls, and samples. MSD SULFO-TAG™ Anti-Human IgG detection antibody was added to the plate and finally MSD GOLD read Buffer B was added prior to reading.

Analysis

An additional analysis was planned using propensity score matching, this statistical method was chosen because of the over parameterisation of the model due to a large number of variables creating 11 degrees of freedom, co-linear confounders, and a small number of seronegative HHC with small within household cluster numbers. Additionally, there was no within cluster variation of the exposure of interest. Prior to completing the propensity score using fractional polynomials, we assessed if the continuous variables could be entered into the model as linear continuous variables instead of fitting into ranked groups allowing a reduction in the required degrees of freedom within the logistic regression model. The continuous variables included age, time between index PCR result and HHC serology test, days of household exposure to the index, and Index of Multiple Depravation (IMD) rank. There was a limited relative difference of each of the covariates on the outcome of interest when assessed using fractional polynomials either with or without adjustment for close contact (this contains four levels assuming a linear trend) indeed the fitting algorithm converged after one cycle. It was therefore possible to enter all the confounding covariates into the logistic regression model without over parametrisation.

This reduced the need for a propensity score matching (PSM) analysis. A PSM was part of the original analysis plan as it reduces the effects of confounding by accounting for systemic differences in baseline characteristics, and because the seronegative outcome was rare it was explored for completeness. There are four main methods to calculate a propensity score, we selected the propensity score matching method as it has the greatest evidence base. PSM reduces the effects of confounding by accounting for systemic differences in baseline characteristics. There are several steps to PSM; firstly, in the logistic regression dataset in both exposed and unexposed groups match those with similar values (nearest neighbour matching) to allow an estimate of the average exposure effect for the exposed. Secondly, we calculated the variance of the estimated exposure effect and its statistical significance. Finally, we adjusted the results using standard error to compensate for being unable to adjust for clustering within this method. The variables used within the PSM were intensity of contact, time between index PCR result and HHC serology test, days of household exposure to the index, and IMD rank. Despite various iterations of this method the PSM was not correctly specified, and we removed this method from the final results.

Further results in addition to the published paper emerging from my analysis

HHC who reported isolating from their symptomatic index case were more likely to have negative serology results than those who were unable to isolate from their index case. HHC who did not isolate within the household had twice the odds of having been SARS-CoV-2 infected than those who did (OR 2.10, 95%CI 1.2-3.6; p=0.005). However, there was co-linearity between the variables ‘days of household exposure’ and ‘intensity of contact within the household’ and as such reported isolation from index case exposure was excluded from the covariates entered into the multivariate analysis.

HHC exposed to non-VOC index cases reported isolation from their index case more frequently than HHC exposed to Alpha index cases, 41% (41/102) of non-VOC exposed HHC were able

to isolate vs. 25% (89/352) Alpha exposed HHC. We do not have granularity of data on whether the isolation variable reflected ability to isolate or choice. Additionally Alpha exposed HHC were exposed to infectious indices for a longer duration. These confounding variables, captured in 'duration of exposure to an infectious index,' were adjusted for in the multivariate logistic regression.

For the 233 HHC participants for whom we have data on a positive SARS-CoV-2 PCR swab date there is a similar distribution of time intervals between index patient PCR positivity date and HHC PCR positivity date within households with Alpha and non-VOC. Figure 6 shows clearly that our hospital identified index patients were often not the first infections within each household. The term index case in this cross-sectional study relates to our method of household cluster identification through hospital testing, and not to case-zero in each household. A greater number of Alpha HHC tested PCR positive than non-VOC HHC, this result was confounded by availability of PCR testing. However, there is a clear difference in time difference between PCR positivity with Alpha HHC testing positive between 10-0 days prior to Alpha index cases.

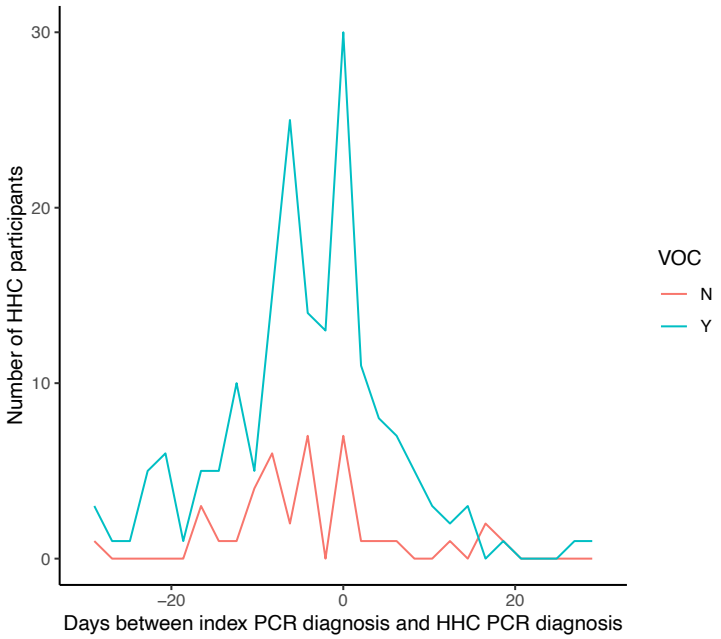


Figure 1. Days between index case PCR positive result and HHC PCR positive result in 233 HHC with a PCR result.

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Chapter 6

Discussion

6.1 Synopsis

Despite its long history and ubiquity as a public health threat, our understanding of MDR TB transmission has remained poorly studied and little understood. Meanwhile, the acute global crisis of the SARS-CoV-2 pandemic led to rapid progression in our understanding of respiratory pathogen transmission, the importance of ventilation dynamics, and both individual & community risk factors. Elements of this progress are now being utilised in TB research. An increased understanding of who is at risk of infection, and subsequently of developing disease, strengthens the medical and research community's ability to prevent onward TB transmission.

The three household contact studies in this thesis have played a part in furthering understanding of secondary attack rates (SAR), airborne infection transmission dynamics indoors, individual and household level risk factors for infection and disease development, and both social & medical risk factors that affect transmission. All the work in this thesis drives towards improving this understanding for TB and SARS-CoV-2 specifically, for respiratory pathogens more generally, and in the context of the emerging threat of future pandemics.

6.2 Summary of key findings

Tuberculosis prospective cohort household contact study

- Screening household contacts of MDR TB patients at baseline and up to 12 months from index MDR TB treatment start identified 80% of cumulative TB.
- These data are useful to focus NTP attention, time and funds on initial screening and management for 12 months.
- Adds to the growing body of evidence that it is no longer appropriate to expect National Tuberculosis Programmes to complete two years of follow-up for MDR TB contacts when resources are limited (1).
- From this cohort, light touch screening appears to be sufficient to identify most of the incident TB cases that will arise among HHCs.
- Those HHCs who are exposed to, or who live with additional risk factors for TB (i.e. beyond cohabiting with a TB case) require careful assessment and investigation given their significantly higher HR within this study.
- There was no one variate nor set of covariates which could reliably screen HHC at baseline to pragmatically select those who were unlikely to get TB and therefore did not need further follow-up or management.
- The remaining HHC need to be closely observed as the population attributable risk remains high and cases of TB will be missed as the numerical majority of incident cases are still likely to occur in this larger group.

SARS-CoV-2 retrospective cohort household seroprevalence survey

- Among members of a strictly-orthodox Jewish community in London, we identified a SARS-CoV-2 seroprevalence of 65% [late 2020]. This was much higher than expected and five times higher than estimated seroprevalence (10.8%, 95% CI 9.3-12.5%, data from ONS) in other population groups nationally.
- Antibodies against SARS-CoV-2 were sero-prevalent among children, which discredits theories of the time that children were relatively protected from, or resistant to infection.
- We observed escalating seroprevalence among preschool, primary, and secondary school children respectively.
- Antibody titres against spike and nucleocapsid proteins declined at different rates over time from reported symptomatic disease. Anti-nucleocapsid antibodies declined more quickly.
- A reliance of the use of nucleocapsid antibodies as a marker of previous infection would have estimated the seroprevalence at 42.8%, markedly underestimating SARS-CoV-2 exposure compared to spike antibodies (65%).
- That this ethnic minority group was disproportionally affected by SARS-CoV-2 potentially relates to sociodemographic factors and relative socioeconomic deprivation.
- This highlights how communities facing barriers inclusion are at higher risk, even when those communities are found in countries with high human development indexes.
- It was essential to work with community actors allowing full community engagement and representative outcomes for the affected population. It would have been impossible to complete this work without community buy in. With community buy in these data inform us about what will be effective interventions in this specific population and provide a platform of future engagement to improve health outcomes for communities facing barriers to inclusion.

SARS-CoV-2 markers of immune activation study

- There were no characteristic cytokine expression patterns that defined disease states, previous infection, age or demographics in the community sub-populations studied.
- Soluble cytokines were not elevated amongst individuals with evidence of prior recent and prior remote community SARS-CoV-2 infections.
- There was no difference in inflammatory soluble cytokines between community SARS-CoV-2 infected adults or children, symptomatic or asymptomatic, recent infection or recent COVID-19 disease, or 3–4-month distant COVID-19 symptomatic disease.
- We did not identify a specific immune response pattern which could be used as a prognostic tool.

SARS-CoV-2 comparative cross-sectional household seroprevalence survey

- Calculating secondary attack rates (SAR) is an essential part of understanding transmission potential in a pandemic response.
- Household studies are an accurate method to calculate SAR.
- Using PCR positivity testing alone to identify all secondary cases captures only currently PCR-positive contacts and misses all those who are no longer PCR-positive or shedding virus.
- Combining PCR positivity with serological positivity captures previous infections and missed transmission events within the household, improving SAR accuracy and understanding.
- Both SARS-CoV-2 variants studied had a very high household secondary infection rate, 61.8% were seropositive in non-variant of concern (VOC) exposed HHC compared to 82.1% of Alpha VOC-exposed HHC.
- The odds of infection doubled with household exposure to an index diagnosed with Alpha.
- There was evidence of transmission events in almost all households, only 6% of non-VOC and 0.5% of Alpha households had no evidence of transmission (seropositivity).
- The ability of an index COVID patient to self-isolate within a household was associated with lower seroconversion rates among their HHCs. Although variable co-linearity precluded inclusion within the logistic regression model our data did demonstrate exposure dose was associated with transmission frequency. This phenomenon is likely regardless of the strain or variant.

6.3 Methodological observations

Household studies at the population level provide useful insights into the dynamics of infection transmission. In particular during outbreaks of respiratory viral pathogens, where sentinel signals of transmission occur early. Despite increasing prevalence of SARS-CoV-2 seropositivity, household transmission models continue to be useful in assessing SAR. A few large-scale public health studies on household contact PCR testing programmes have been published (with lower SARs, estimated in later pandemic waves), but most published work on SARs within households are from smaller studies. The low participant numbers reported in many studies probably reflects how difficult it is to effectively deliver these studies during health emergencies (2,3). Research on SAR in SARS-CoV-2 mostly remains on PCR testing of symptomatic cases and this highlights the ongoing need to use PCR positivity, frequent testing of asymptomatic contacts, closely knitted transmission units (such as households) and serostatus to assess SAR (4–7). Two published European studies report combined PCR and serology SARs and highlight the difference in sensitivity of detection obtained from the two methods. For example, in Finland during the early pandemic, SARs in household contacts were 50% when defined by PCR test results and 62% when neutralising antibodies were tested (8,9). Utilising the correct assay to assess serostatus is important, due to vaccination coverage nucleocapsid (NC) assays will be needed in any future SARS-C-V-2 work but our London data highlights that this will systematically underestimate incident infection and NC seropositivity is more likely to wane more rapidly over time.

Engagement with community actors to collaboratively develop research questions and study designs is essential in addressing topics important to the population studied; as well as to deliver representative and relevant results. The results can then be used to support real-world interventions and positive health impacts for the communities studied. This was evident in the work described in Chapter 5; a study which was made possible only by the involvement of community leaders who co-produced the study design and collaborated with our research team to lead the project from within the community (10). The co-production model used in that study (which took place among the strictly-orthodox North London Jewish community) helped to allay the strong suspicions of secular society and of external figures of authority; whilst enabling important health research to take place in a way which preserved community identities and values. Working with a respected, well-known community charity and phlebotomy service ensured that the community engaged with the research in a way that was appropriate to the

expectations of the community. The successful collaboration enabled several secondary studies which addressed public health issues of concern for the strictly Orthodox population in North London (11,12). In chapter 7, I described elements of the CONTACT study. By comparison to the work of chapter 5, CONTACT was less able to address the specific needs of minority communities. The cross-sectional household sero-survey team of doctors recruited participants from several ethnic minorities and worked hard to ensure that potential participants were approached in appropriate languages, or by a member of the team with experiences from within their communities. The CONTACT research study was however initiated from within hospitals and not from within communities. As such, there was no broad community buy-in to the study and there was reluctance and suspicion across the communities against participation. When CONTACT took place, the COVID-19 pandemic was at a later stage, with widespread fatigue and trauma, which may have substantively affected interest in participation. The MDR TB HHC study in Chapter 4 worked with existing colleagues and collaborators in Lima TB clinics, with both the National TB program and local district buy-in. This enabled support for recruitment that is likely to have been of high value to recruitment and retention rates in that project. There are a wide range of factors that underscore community reluctance to engage with mainstream actors, including social isolation and stigma. Lack of trust in the government, in the health care system and experience of racial or ethnic discrimination, have all been linked with poorer adherence to government guidance (13). All these factors were important dynamics affecting participation across all three household studies.

A common theme throughout this thesis is the impact of external events on research methods. Future pandemics are entirely possible, by conceptualising all potential problems during study development we can plan for research practice in future pandemics. This is essential, usefully we have shown that household studies can be run remotely with technology, with remote problem-solving methods and crisis management teams, but some participant contact is needed. Building and maintaining relationships with community actors is complex and difficult but leads to productive work and if the infrastructure is maintained a platform for reactive responses during future emergency responses or pandemics. Further thoughtful work is needed to plan this systematically and comprehensively during enforced lockdown periods and in advance of future pandemics. Investment in a research infrastructure that allows this reactive work is lacking, both in the UK and Peruvian settings and relies on insecure income and personnel to develop and maintain it. Government and scientific-community led public health

planning of research priorities would allow coordinated research, evaluation, and implementation in the event of future pandemics.

6.4 Learning from transmission studies of respiratory pathogens

The individual, pathogen, and environmental factor approach applies to understanding the transmission of both pathogens. Household-focused studies provide many benefits, enabling accurate measurement of SAR, and transmission factors within a closed network of transmission which during social lockdowns limits external transmission events. This model helps inform control efforts focused at a local level to have the greatest impact. However, it is important to be sensitive to household-level and individual-level interactions with the community within which they are placed. Outside of pandemic lockdown conditions within these household studies presented it is not possible to attribute all incident diseases to within-household transmission. Whole genome sequencing (WGS) of index and contact pathogens would have enabled this directly, though this is difficult to complete. Indirect partitioning of within-household transmission and community transmission has been done previously with limited success (14). This was completed using a Bayesian generalised linear model called a unified probability model where community transmission is assumed to be constant within specified age ranges (15). Given community transmission was unlikely to be constant in any of the settings or pathogens we conducted research in it would not have been an appropriate method here. Despite this transmission evidence gap, households are useful places to identify and interrupt transmission.

Contact tracing in tuberculosis has long been used within a network of experienced specialists. This model was rapidly adapted and learnt from in SARS-CoV-2. TB requires prolonged (>8 hours of exposure time) mainly aerosol transmission, contact tracing identifies latent infection and disease, and treatment of both prevents onward transmission. In SARS-CoV-2 a very short exposure time is required for onward transmission (the likelihood of which increases with duration and proximity). The main route of transmission is now understood to be aerosol, though initially transmission was thought to be largely due to droplets (16,17). Contact tracing identifies current or previous cases, and at a population level isolation of cases reduces transmission. Environmental factors impacting aerosol transmission affect both pathogens.

Ventilation factors within indoor environments are clearly important in aerosolised infections, addressing these factors within the built environment of communities affected by aerosolised infections is much harder (18).

Both infections disproportionately affect populations with socioeconomic deprivation. This relates more to environmental and individual factors. Conditions of lower socioeconomic strata enable respiratory pathogen transmission: household crowding with multigenerational occupancy structures; higher proportions of household members with medical risk factors for more severe disease and therefore prolonged illness with increased index transmission; including malnutrition, smoking, and lung disease; delayed health care access and delayed testing leading to more severe disease and increased transmission. Population density clearly has an impact on COVID-19 incidence and hospital admissions (19). Good evidence exists for all these factors in both SARS-CoV-2 and *M.tb* (20–23). Vulnerable populations are disproportionally affected by the pandemic lockdowns, both in the financially and health-vulnerable lower socioeconomic classes in Lima and the socially isolated minority religious populations in London, impacting all three household studies. Health systems are inextricable from the social contexts in which they work, communities and groups which face barriers to inclusion suffer health disparities which are the result of complex social structures and interactions (24–26).

6.5 Critical evaluation

Despite significant factors interrupting our work we were able to complete carefully thought-through, impactful studies which have and will continue to inform discussion in both TB household transmission and MDR TB contact management and SARS-CoV-2 transmission in minority communities. Developing collaborative work with communities was a strong approach to delivering clear and appropriate understanding of pathogen transmission factors. During outbreak responses, cross-sectional comparative household serosurveys are useful tools to calculate SAR and measure variables affecting respiratory pathogen transmission.

Pandemic preparedness and clear plans to mitigate the impact of political, social, and economic shocks on the ability to complete research would have helped significantly in maintaining the study team's ability to complete their work. The MDR TB cohort study was planned to provide

an evidence base for the investigation and management of household contacts with a clear description of which tests and interventions were useful or accessory-to-need at each follow-up time point. Due to pandemic interruptions, the planned case-control study which was to run alongside the MDR TB cohort did not happen. It would have looked at WGS-linked index-contact pairs and households without infection or secondary disease, providing a strong platform to understand risk factors with which to stratify household contacts. We carefully developed an e-registry platform for MDR TB household contact follow-up and management nested within the WHO DHIS2 TB tracker for surveillance. Developing the module was a time-consuming, lengthy process. However, having this in situ during the pandemic allowed real-time remote management of HHC and research. More widely used, easily implementable tools such as ODK or REDCap would have on balance provided the same functionality. On reflection, there was some redundancy of effort in the tool development process. However, the DHIS tool has been reworked through a Delphi process for use in the UK to standardise and consolidate the management of MDR TB contacts, work which is ongoing. Additionally, remotely managing a prospective cohort study in a second language was a huge challenge.

6.6 Implications for policy & recommendations

The MDR TB HHC cohort study identifies the greatest gains from contact investigation and management in the first year of follow-up. Pragmatically focusing on investigating and screening HHC of MDR TB during that first year will provide NTP with the greatest returns in identifying incident TB and preventing onward transmission. Light touch remote screening is sufficient to identify contacts needing further investigation and management. Allowing well-informed HHCs to self-refer if symptomatic in the second year of follow-up could be explored as a way for NTPs to address the need for ongoing screening and would represent a transition from health system-led screening in year one to passive screening through contact-led self-presentation in year two.

Building collaborative partnerships with all study populations, particularly with minority groups, to develop research priorities and public health intervention strategies is essential to ensure all voices are heard. This collaborative working enabled access to a tightly knit ethnic and religious minority group, enabled a representative assessment of risk that was likely

relevant to other ethnic and religious minority groups and built a foundation of trust for further work.

6.7 The future

High respiratory pathogen incidence and transmission are likely in populations with poor healthcare access, poor vaccine uptake, socioeconomic deprivation, and vulnerable populations. Household-level surveys are a useful tool to focus intervention needs. Calculating SAR should be an essential part of outbreak response, enabled with previously ethically approved, prepared template protocols, which can be rapidly implemented in the event of a future pandemic, these would include household-level studies.

Most research is siloed into diseases and does not ensure that learning from other diseases is capitalised on, developing research work addressing the common themes affecting all respiratory pathogen transmission would be an interesting next step. A package of interventions addressing nutrition, sanitation and ventilation over time and investigating outcomes in a few respiratory pathogens' incidences would be challenging but valuable. The aim of these intervention packages would be to improve socioeconomic situations, focusing on nutrition, sanitation, ventilation, cost-effectiveness, and impact on multiple respiratory pathogen outcomes. Investing funders' money in broad programmes and measuring their impacts on multiple health outcomes seems a pragmatic way to build cost-effective interventions. There is a growing evidence base to support this theory, the RATIONS trial in India proves resoundingly that improved nutrition is associated with reduced TB incidence in HHC (21). Nutrition and overcrowding increased the risk for developing influenza-associated influenza-like illness (ILI) in Malawi (27).

Within MDR TB HHC studies, our previously planned case-control study with case households containing WGS index-contact paired household cases and control households with no secondary infection or cases could provide interesting insights into stratifying risk factors in household contacts.

6.8 Conclusions

Household contact studies are incredibly useful epidemiological tools in studying infectious respiratory pathogens but need to be developed with consideration to the individual, the community, the pathogen, and the environment. Household contact screening to assess SAR is a useful model in infectious disease transmission and should be part of the response to any new pandemic. Co-producing research work alongside affected communities creates inclusive research and whilst hard to implement and maintain, creates productive research and public health partnerships. The lack of infrastructure investment by health systems makes this kind of surveillance research difficult to maintain or prepared to rapidly implement in a health crisis.

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Appendix

Table. Evidence base for TB, LTBI and preventive therapy in MDR TB household contacts

Author	*Pub Year*	*Study design*	*Study site*	*Number of MDR TB HHC*	*Screening practice*	*PT drugs*	*PT Duration*	*follow up period*	*TB in HHC*	*LTBI in HHC*
Teixeira L (233)	2001	CC	Brazil	133	symptoms, exam, TST	-		0	6 (4%)	59 (44%)
Schaaf HS (86)	2002	PC	South Africa	125	symptoms, TST, CXR, Sputum, gastric aspirates x2 (+/-bronch if CXR non diagnostic)	3/4 drug: H 15-20mg/kg/d, P25-35mg/kg, TH 10-15mg/kg (+/or E 15-20mg/kg/d +/or oflox 15mg/kg/d)	6 mths	30mths	26 (21%) at 12mth 29/117 (24%) at 30 mth	66 (53%) 0mth 64/117 (54%) 30 mth
Nitta AT(234)	2002	RC	USA	946	symptoms, TST, CXR	0		0	6 (0.6%)	58 (6%)
Sasaki Y(235)	2005	CS	Japan	659	symptoms, LTBI, PT	18 H 3TH 2 H+RFP+E 1x E+Z+TH 1x Z+TH 1x Z+levo 1x Z+E		0	13 (2%)	58 (8.8%)
Attamna A (236)	2009	RC	Israel	476	symptoms, TST, exam, Dr dep PT	12 got cipro+Z	?	6 years median	0	245 (51.5%)
Miramontes R (237)	2010	RC	USA	189	symptoms, TST +IGRA, PT	-		0	-	97 (51%)
Singla N (238)	2011	CS	India	302	symptoms, TST in 135 symptomatic	-		0	16 (5.3%) 2/16 MDR on DST	109/135 (80%)
Vella V (239)	2011	PC	South Africa Tugela Ferry	1766	symptoms, sputum	-		1 year	14/793 (1.8%) 32 (4%) secondary cases	-
Becerra MC (99)	2011	RC	Peru	4191	symptoms, sputum	-		4 years	117 (2.6%)	-

									co prevalent disease 242 (5%) cumulative	
Grandjean L (240)	2011	RC	Peru	2122	symptoms, sputum	-		3 years	108 (5%)	-
Denholm JT(241)	2012	RC	Australia	570	symptoms, 3 mth TST	11 got PT dependant on DST	2-9 mths	not routine	0 in PT 2 in no PT	49 (8.6%)
Johnston J (242)	2012	RC	Canada	89	TST, sputum	12 got PT	?	123 mths	5 (5.6%)	42 (47%)
Morcillo N (243)	2013	RC	USA	405	symptoms, exam, epi, DST	-		0	33 (8.1%)	-
Becerra MC (97)	2013	RC	Peru	1299	symptoms, sputum	-		4 years	23 (1.8%)	-
Leung ECC (244)	2013	RC	Hong Kong	704	symptoms, sputum	-		17mth median	12 (1.7%) co prevalent cases + 17 (2.4%) secondary TB	-
Williams B (245)	2013	RC	UK	23	symptoms, sputum, LTBI, PT	2 drugs dependant on DST	6-12 mths	3 years min	3 (13%)	12 (52%)
Seddon JA (85)	2013	CS + PC	South Africa	228 186	Symptoms, sputum, TST, CXR	oflox (15-20 mg/kg), E(20-25 mg/kg daily) high-dose H (15-20 mg/kg daily)	6 mths	1 year	15/228 (6.6%) co-prevalent 6/186 (3.2%) secondary in those given PT	102 (44.7%)
Paul D (246)	2014	CS	India	260	Symptom screen, sputum, CXR	-		0	12 (4.6%)	-
Laniado-Laborin R (247)	2014	CS	Mexico	96	symptom screen, TST, IGRA, CXR	-		0	-	76%
Bamrah S (84)	2014	PC	Chuuk State	232	TST, CXR, symptom, exam,	104 got PT	12 mths	36 mths	20 (8.6%)	119 (51.3%)

			Federated States of Micronesia			FQ + (x1 dep on age and DST) Moxi+E or Levo+ E[<12yrs]) or moxi or Levo plus TH			0 in those given PT	
Amanullah F (248)	2014	PC	Pakistan	133	Hx, Ex, TST, CXR, sputum smear+Culture, DST	-		12mths	7 (7.5%)	30 (22.6%)
Adler-Shohet FC (249)	2014	RC	USA	118	TST, repeat at 8-10 weeks. All TST + got CXR.	26/31 got PT Levo+Z	9 mths	TST – at 10wk discharged 2 years if TST +	0	31 (26.3%)
Garcia-Prats AJ (136)	2014	RC	South Africa	32	PT in all <5 yrs contacts	24/32 got PT high dose H, E, oflox	6 mths	12mths	0	8 (25%)
Elmi OS (250)	2014	CS	Malaysia	70	symptoms, TST, IGRA	-		0	-	52.8%
Titiyos A (251)	2015	RC	Ethiopia	155	symptoms, sputum	-		2 years	16 (10.3%)	-
Grandjean L (31)	2015	PC	Peru	1055	symptoms, sputum	-		3 years	35 (3.3%)	-
Trieu L (125)	2015	RC	USA	241	Screening TST, CXR	50 moxi across 2 outbreaks in PLHIV		8 years	5 incident TB without PT 1 case of TB in PT	10% LTBI 30/50 completed PT 3 AE
Javid A (252)	2016	CS	Pakistan	610	Symptom, sputum, CXR	-		0	51 (8.4%) 41 MDR 10 DS	-
Mazahir R (253)	2017	PC	Egypt	80	symptom, sputum, TST, CXR	-		1 year	9 (11.3%) co-prevalent	19 (23.8%)

								1 (1.4%) secondary	
Fox GJ (95)	2017	PC	Viet Nam	147	Symptom screen, TST, CXR	-	0	-	40.8%
Fournier A (254)	2017	PC	France	84	symptom screen, LTBI test	-	0	3 (4%)	23 (27%)
Golla V (96)	2017	CS	South Africa	229	screening, TST	-	0	15 (6.6%)	86 (38.1%)
Qadeer E (255)	2017	CS	Pakistan	1467	Screening, sputum geneXpert, smear micro, CXR	-	0	172 (12%) 54/114 MDR 2/114 DS	-
Huerga H (100)	2018	PC	Armenia	150	screening, TST, IGRA, CXR	-	2 years	3 (2%)	prevalence58% incidence19.9/100/yr first 6 mths highest 33.3/100/yr
Hiruy N (256)	2018	CS	Ethiopia	331	screening + GeneXpert sputum	-	0	20 (6%)	-
Boonthanapa T (257)	2019	RC	Thailand	70	Symptoms, Sputum, TST, CXR, rapid molec testing	-	0	0%	0%
Swindells S (258)	2018	CS	8 countries	1018	screening, CXR, sputum, IGRA/TST	-	0	121 (11.8%)	631/981 (64.3%)
Chatla C (259)	2018	PC	India	4858	screening, sputum	-	0	34/4771 (0.7%)	-
Kimaro GD (260)	2018	CS	Tanzania	210	screening, sputum, GeneXpert IGRA	-	0	41/207 (19.8%)	38/86 (44.1%)
Lu P (261)	2018	PC	China	397	Screening, sputum, TST	-	0		111 (29.3%)
Gupta A (262)	2019	CS	*PHOENIx sites	1016	screening, CXR, sputum, IGRAorTST	-	0	121 (12%)	72%

Becerra MC (263)	2019	PC	Peru	1559	screening, sputum, TST	-		12mths	57 (3.6%)	1041 (75.5%)
Malik AA (264)	2020	PC	Pakistan	792	Symptoms, CXR, sputum, TST	165 received PT Moxi E	6 mths	2 years	8 on TB Tx 3 co-prevalent 2/165 cases of TB on PT	-
Chang V (265)	2021	RC	Australia	247	Disease status, TST, IGRA	18/62 received PT Moxi H+R H+R+Z	6 mths 4/6mth 5/6mths	6 years	1 co-prevalent	105(42.5%) 62 had newly diagnosed LTBI
Guureva T (266)	2022	PC	Russia	72	TST, Diaskintest	58/72 received FQ	9 mths	1 year with PT 2 years without PT	1/14 case of TB from those without PT	51 (71%) 6 AE
Ahmed S (267)	2023	PC	Pakistan	1911	91% Symptoms 16% sputum Xpert 7% CXR	-	-	3 years	20 (1%) MDR/RR TB cases	-
Krishnan S (268)	2023	PC	*PHOENIx sites	1016	Screening, CXR, sputum, IGRA/TST	-	-	1 year	16/742 (2.2%) TB	52/242 (21%) at 1 year

Table 1. A comprehensive list of all published papers reporting incidence and co-prevalence of TB and LTBI in MDR TB HHCs who did or did not receive PT.

CS= Cross-sectional, RC=Retrospective cohort, PC=Prospective cohort, CC=case control study
R=rifampicin, H=Isoniazid, E=ethambutol, Z=pyrazinamide, TH=thionamide, RFP=rifampin, oflox=ofloxacin, levo=levofloxacin, moxi=moxifloxacin, cipro=ciprofloxacin, FQ=flouroquinolone
AE = adverse events Grade 1-2, SAE = Serious Adverse events Grade 3-4
**PHOENIx sites data reported twice*