Title: The Community Burden of Influenza

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PhD Epidemiology

I, Ellen Fragaszy 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.

Abstract

Background: Influenza causes substantial morbidity and mortality. Novel strains from animals can infect humans, but such transmission is poorly understood. Serosurveillance estimates levels of influenza population immunity and infection but obtaining representative sera is challenging. Health-related quality of life (HRQoL) and absenteeism inform cost-effectiveness models of influenza interventions but these parameters are poorly understood. The National Pandemic Flu Service (NPFS) aimed to treat community cases. Little is known about the scheme's coverage or effectiveness.

Objectives: 1) Investigate whether occupational exposure to pigs increases risk of seasonal, pandemic and zoonotic influenza infection. 2) Describe population-level patterns of influenza infection and immunity in England during 2012/13. 3) Quantify work and school absences and HRQoL from community influenza illnesses. 4) Evaluate the success of the NPFS and propose algorithm changes to improve antiviral targeting.

Methods: Flu Watch is a prospective community cohort of influenza and included recruitment of pig workers during the 2009 pandemic. The Pandemic Immunity and Population Spread study (PIPS) is a novel, population-level, cross-sectional, pandemic serosurveillance system utilizing the Health Survey for England.

Results: Pig workers had increased odds of seropositivity to seasonal, pandemic, and zoonotic influenza compared to the general population. A(H1N1)pdm09 and A(H3N2) infected 40% and 25% of the population in 2012/13. HRQoL loss and absenteeism is low for individual community-level influenza cases. NPFS consultation was low and the case definition specificity was 51%.

Conclusions: Influenza spreads readily from pigs to pig workers, posing risks for novel virus emergence and pandemics. Representative, population-level serology show that, before COVID-19, a large proportion of the population was infected each winter. Most community influenza cases take little time off work and school and this has implications for transmission. The coverage and impact of NPFS was low. Community-based surveys are needed to inform the control of seasonal and pandemic respiratory infections.

Impact Statement

My PhD research has impacted UK pandemic influenza response and planning assumptions, the UK response to COVID-19 and the WHO's COVID adaptations to its sentinel influenza surveillance case definition.

During my work on the National Pandemic Flu Service (NPFS), I recommended changes that were subsequently incorporated into the underlying algorithm related to the use of clinical case definitions to determine antiviral prescriptions. My research on absenteeism due to community influenza infection was requested by and informed the review of the UK government's planning assumptions relevant to workforce absenteeism during an influenza pandemic.

The STATA scripts to generate and analyse daily data on Flu Watch illnesses that I developed became instrumental in some of my early COVID-19 rapid response work. My and my colleagues' work more generally on the Flu Watch study and the subsequent spin-off study which I led (Bug Watch) were also critical to the development of our current Virus Watch community cohort study of SARS-CoV-2 infection and illnesses.

My supervisor and I built on my NPFS case definition work and used my illness dataset to compare non-COVID illnesses from Flu Watch with the earliest known UK cases of COVID-19 from PHE's First Few Hundred Study. We rapidly developed a symptomatic case definition for suspected COVID-19 infection for the purposes of community contact tracing. This work was presented to the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG), the UK Government's Scientific Advisory Group for Emergencies (SAGE) and the UK Senior Clinicians Group which consists of the Chief Medical Officers (CMOs) and deputy CMOs from the four nations. The work was influential in shaping the case definition used for the UK Test, Trace and Isolate (TTI) system. I later expanded on this work as part of a PHE roundtable on the potential updating of the TTI case definition. This entailed the evaluation and comparison of the performance of various case definitions using data from four major UK community studies (ONS infection survey, Zoe App and REACT). I conducted and presented the Virus Watch estimates at the PHE roundtable, contributed to the harmonization of estimates from the four studies and then led the comparison of estimates that were presented to the UK Chief

Medical Officers. In related work, I also evaluated the performance of various case definitions for identification of Influenza and COVID-19 to inform WHO's e-Consultation to Adapt Influenza Sentinel Surveillance Systems for Including COVID-19 and their subsequent case definition recommendations.

Another example of COVID-related impact was the direct and rapid adaptation of my Flu Watch protocols, STATA scripts and analytical frameworks which generated and analysed Flu Watch illness data. Building on this work I converted Virus Watch follow-up data rapidly into the core illness dataset which has since fed into a number of influential analyses. I conducted rapid and regularly updated analyses of the symptom profiles and case definition performance for COVID-19 and non-COVID-19 illnesses and compared illnesses caused by Wild-type SARS-CoV-2 and the Alpha and Delta variants of concern when they began circulating in the UK. My findings were presented to NERVTAG and SAGE, were incorporated into an official NERVTAG report on the severity of the B.1.1.7 strain.

My work developing methodologies of conducting representative population-based community studies of serology through PIPS as part of the NIHR pandemic preparedness portfolio highlighted the need for large scale population studies that were subsequently commissioned through the COVID-19 Infection Survey and the REACT studies. The UK Health Security Agency took over the PIPS study after it went untriggered during the COVID-19 pandemic and now may be a good time to restart it given concerns over waning influenza immunity in the community after unprecedently low levels of influenza during the COVID-19 pandemic.

Contribution/Attribution

This PhD thesis presents work I have conducted on two epidemiological studies, The Flu Watch Study and the PIPS study. I began working as a statistical epidemiologist on the Flu Watch study in November 2008, at the start of its third year. In the first six months of my role, I led the data management of the study and began conducting preliminary statistical analyses. My main contribution was the design of data management protocols and automated scripts that combined and processed Flu Watch data from multiple sources and formats, into final topic-specific and analysis-specific datasets.

When the 2009 influenza pandemic arose, the study was extended and expanded from one cohort of approximately 650 participants to three separate cohorts (with differing follow-up) which aimed to recruit a total of 10,000 participants. To cope with the dramatic increase in participant numbers, the study surveys needed to move from nurse-assisted interviews (a rate-limiting step in terms of time and cost) to primarily self-completed online surveys. I led this transition, working in collaboration with AMT, the company that built the Flu Watch website and web-based surveys. Recognising the problems of the current data collection methods and the additional issues that would arise with multiple cohorts going forward, I adapted the surveys and data collection methods to be as simple, efficient and comparable as possible in the new online system. I extensively tested surveys and the subsequent data downloads during the system development. In addition to leading the move to online data collection, I continued to lead the study's overall data management and the production of final topic-specific and analysis-specific datasets. I also contributed to new survey questions including the addition of the Quality of Life questions in the final year of the study which I have analysed as part of this PhD. I contributed to the development of the overarching analytical strategies for the Flu Watch study. I led the Flu Watch analyses presented in this thesis, some of which are already published. I also contributed to other Flu Watch analyses and publications either used as background in this thesis or not included at all.

My work on the PIPS study began in 2011 when I co-designed the project with my supervisor for a grant application. I later led the revision of the grant application to respond to funder requests and led the study once it was funded. I was the project manager, data manager and statistician. The study had four phases: Phase one was a data analysis from

HSE 2010; phase two was the pre-pandemic research phase from HSE 2012/13 data and specimens; phase three was the hibernation phase where the study awaits re-activation; phase four was a reactivation of the phase two research in the event of a pandemic. In the summer of 2018, during the hibernation phase, I led the week-long mock re-activation of the study. Although we knew this exercise would take place, we did not know the exact dates of the exercise. I contributed to the evolution of study design for the hibernation and pandemic phase (phases 3 and 4) by suggesting data items and analyses that could be dropped and some that could be added. I conducted and published the phase one analysis, part of which is presented in the thesis. The serology data from phase two were not finalised before I went on maternity leave in September 2015. Before I went on leave, I contributed to the development of the analytical plans and developed "do files" in preparation for the serological analysis. Ruth Blackburn, my maternity leave cover, in conjunction with my supervisor conducted the initial analysis. They also wrote a 33-page report to the funder outlining the results of the phase two. When I returned from maternity leave, I conducted additional literature review on the topic, added additional methodological explanation and discussion points and rewrote their analysis in the format of a journal article which I present in this thesis.

I led (and unless otherwise stated) conducted all the analyses in this thesis, and I did so in consultation with my PhD supervisor and wider co-authors. In this PhD thesis I have included work which I have done in collaboration with others. In chapter 7.1, I include a piece of modelling work that I co-designed to complement my analysis of quality of life lost and absenteeism, which was executed by a modelling colleague. In order to attribute the work accordingly, prior to each section of primary research, I present a description of the work undertaken and what my specific contributions were.

Just prior to the completion of this thesis in late 2019, the SARS-CoV-2 virus emerged in China and shortly afterwards initiated the first severe pandemic since the 1918 Spanish flu. As a result, I put all work on this thesis on hold until 2021 as I pivoted my work to respond to the COVID-19 pandemic. As the PhD thesis was largely written before the current pandemic – I have kept the scope of the thesis on influenza. However, because so much of my work is applicable to the COVID-19 pandemic and because my research group and I built on this work in our subsequent COVID-19 research, I have included brief sections,

usually at the end of chapters, to discuss how the work presented in the thesis relates to COVID-19.

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Chapter 1 Background

1.1 Influenza overview

Influenza is a common, highly contagious acute respiratory virus which infects all age groups, causing a range of outcomes from asymptomatic infection and mild respiratory disease to severe respiratory disease and death [1]. Annually, it is estimated to cause 3 to 5 million severe cases and between 250,000 and 500,000 deaths globally [2].

1.1.1 Virology and Immunology

1.1.1.1 *Virus*

Influenza viruses are single-stranded, negative-sense RNA viruses belonging to the *Orthomyxoviridae* family [3]. They are divided into four genera: influenzavirus A, B, C and D [4]. Influenza A viruses infect multiple species including humans, are associated with regular epidemics and are the only influenza genus that causes pandemics [1,3,5]. Their natural reservoir is thought to be aquatic birds although other reservoirs may exist [6]. Influenza B viruses also cause regular epidemics but do not cause pandemics and generally do not infect other species although there have been reported infections in seals [3,5]. Influenza C viruses are one of the many viruses causing the 'common cold' and can infect pigs and dogs[7]. The newly discovered influenza D virus has a primary reservoir in cattle but can spill over and infect swine and other mammalian species including potentially humans [3,8,9]. Influenza A in humans is the focus of this thesis given its severity and pandemic potential.

Influenza A viruses are sub-typed on the basis of antigenic and genetic differences in the two surface proteins haemagglutinin (HA) and neuraminidase (NA) [1]. As of early 2019 there were 18 known HA subtypes (H1-H18) and 11 known NA subtypes (N1-N11) [10,11]. In the last century HA subtypes H1, H2 and H3 and NA subtypes N1 and N2 have been found in seasonal and pandemic influenza viruses circulating in humans [1].

1.1.1.2 *Immune response*

When exposed to this virus, the body's adaptive immune system produces an humoral (antibody) response targeting the HA and NA viral proteins in an effort to neutralise the infection[3]. At the same time it also generates an immunological memory, facilitating a

more rapid antibody response following re-exposure which protects the individual against reinfection[3]. Current influenza vaccines use humoral immunity (the priming of rapid antibody response) to protect against infection, although live attenuated influenza vaccines may additionally provide protection through cellular immunity [12].

1.1.1.3 *Antigenic variation*

Influenza A surface proteins exhibit a great deal of antigenic variation, allowing the virus to evade the host immune response. Antigenic variation occurs in two ways. Influenza viral replication is prone to errors resulting in the build-up of mutations over time in a process known as antigenic drift [5]. This gradual evolutionary process produces slightly different 'seasonal' or more accurately 'interpandemic' strains circulating each year. These strains give rise to regular seasonal epidemics. Population level antibody immunity to recent seasonal strains builds up over time and provides individuals some protection against antigenically similar strains. This build-up, however, exerts an immunological selective pressure on the circulating viruses and this gives antigenically 'drifted' viruses an evolutionary advantage [5]. Occasionally, influenza A viruses evolve rapidly through a process known as antigenic shift by exchanging genes (a process known as reassortment) with an influenza strain or subtype (usually those circulating in animals) that has different antigenic properties to the subtypes currently in circulation among humans [5]. This process creates an immunologically distinct virus to which the population may have little to no antibody immunity. The virus can cause a pandemic if a large portion of the global population is susceptible and the virus is easily spread from person to person [1].

1.1.2 **Epidemiology**

1.1.2.1 *Transmission and natural history*

Influenza viruses spread from person to person though contract transmission, droplets and aerosols [13]. Influenza infections are often asymptomatic (subclinical) but can lead to acute respiratory illness, often with sudden onset, following a 1 to 2 day incubation period. Illnesses are usually self-limiting and mild but can be severe [3,5,14,15]. Clinical presentation is indistinguishable from many other respiratory viral infections, making it difficult to determine aetiology on the basis of symptoms alone [5,16].

1.1.2.2 *Age and risk groups*

Influenza infects all age groups but rates of infection and disease are typically highest in children [15]. Age-specific mortality typically follows a 'U-shaped' curve with the highest mortality rates in the very young and the very old. Other groups at higher risk of severe disease and death include the immunocompromised, pregnant women, and individuals who are morbidly obese or have underlying chronic illnesses [5,15,17].

1.1.2.3 *Seasonality*

In the temperate zones of the northern and southern hemispheres, influenza causes a single seasonal epidemic in winter months. In contrast, influenza seasonality in the tropics and equatorial zones can have multiple annual peaks and is highly variable [18].

1.1.3 Prevention and control

1.1.3.1 *Pharmaceutical interventions*

There are two types of influenza-specific pharmaceutical interventions to prevent infection and/or treat disease: vaccination and antivirals. Vaccination protects individuals by reducing their risk of infection (and thus disease). Antivirals, if given early enough, modestly reduce the duration of symptoms and severity of disease [5]. They may also help prevent severe outcomes and death even if given later in the course of disease [19,20]. Antivirals are also used to prevent illness in people recently exposed to influenza [21]. This is known as post-exposure prophylaxis (PEP). Some people with influenza disease go on to develop secondary bacterial co-infections which can lead to complications and poorer outcomes [11]. Therefore, antibiotic treatment may also be appropriate for some influenza cases even though antibiotics do not target influenza per se.

In addition to the direct benefit antivirals and vaccines in particular confer to individuals who receive them, they can also provide indirect benefit to non-vaccinated/treated individuals by reducing transmission. For example, randomised controlled trials provide evidence that vaccinating school-aged children reduces influenza in communities, vaccinating mothers protects infants and vaccinating health care worker protects nursing home residents [22–25].

1.1.3.2 *Non-pharmaceutical interventions*

In addition to vaccines and drugs, there are a number of non-pharmaceutical interventions aimed at reducing influenza transmission in the community. These include travel restrictions (advice, screening, restrictions and border closure), personal protective measures (i.e. hand hygiene, face masks, self-isolation and quarantine), and social distancing measures (school and workplace closures, cancellation of public gatherings and working from home) [26].

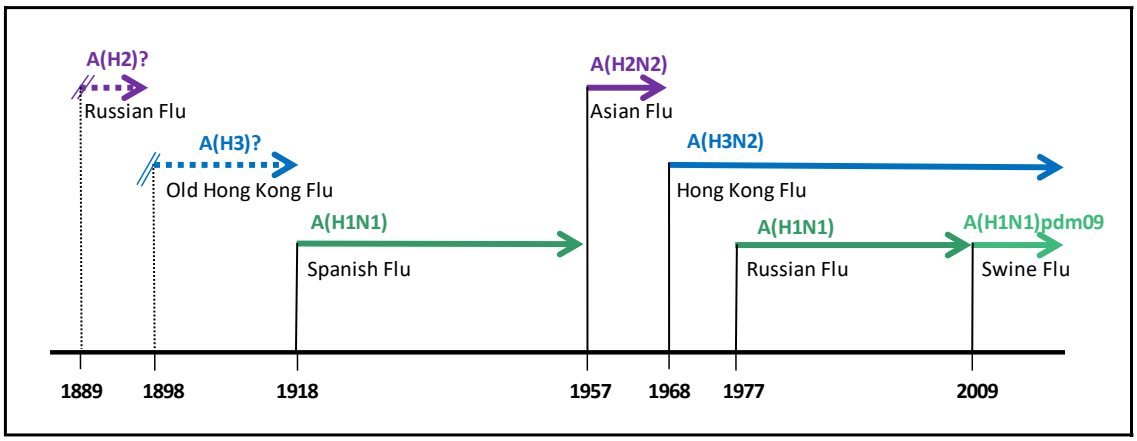
1.1.3.3 *Choice of public health measures*

By far, the most effective public health measure is vaccination and accordingly it is the mainstay of seasonal influenza prevention and control. In a pandemic, vaccination would almost certainly be the most effective public health measure but with current technology it takes 6 months or so to develop and deploy a pandemic-specific influenza vaccine. In those early months of a pandemic before vaccination begins, the available pharmaceutical and non-pharmaceutical interventions would not be expected to stop or prevent spread of infection. Recent modelling efforts have, however, suggested that if a number of interventions could be effectively applied at the same time, a strategy known as 'defence in depth', their combined effects could be enough to slow community transmission down, thus delaying and flattening the peak of the epidemic [27,28]. This would have two main benefits. Firstly, it would reduce the maximum burden on medical services during the peak of the epidemic by distributing cases (and subsequent burden on medical services) over a longer period of time. Secondly, it would 'buy time' for the development and distribution of pandemic vaccine.

1.2 Influenza circulation in humans

Influenza in humans not a new phenomenon, but most of what we know about it comes from the last century when modern laboratory techniques have been able to identify influenza as the causative agent. A summary of influenza A subtype circulation in humans since the late 1800s is presented in Figure 1-1.

Figure 1-1: Timeline of Influenza A subtype circulation in humans since 1889



1.2.1 Pandemic influenza in the late 1800s

The first well documented pandemic was the so-called 'Russian Flu' in 1889-1892 and although the pandemic virus was never isolated, there is serological evidence that it was caused by an H2 virus [5,29]. There is also historical and serological evidence to suggest that there may have been a relatively mild pandemic in 1898-1901, probably caused by an H3 virus [5,29].

1.2.2 1918 Spanish Flu Pandemic (H1N1)

The 1918 Spanish Flu was most devastating pandemic in modern times and caused an estimated 50 million deaths and infected a third of the global population [30]. A unique and defining characteristic of the 1918 pandemic was its unusually high mortality in the young adult age group which transformed the traditional 'U-shaped' mortality by age curve into a 'W-shaped' curve [31,32]. The virus itself was highly virulent but the high mortality is thought to have been primarily driven by the unusually high incidence of secondary bacterial pneumonia which, in the pre-antibiotic era, could often lead to death [30,33,34]. The pandemic occurred in 3 distinct and rapid waves and lasted approximately a year [35].

The full legacy of the 1918 H1N1 virus however goes well beyond the pandemic itself and some have even described the subsequent century as the ongoing 1918 'pandemic era' [36,37].

The 1918 avian-like virus is believed to have been completely novel to humans, a *new* viral introduction rather than a reassortant strain evolved from viruses already circulating in humans [35,37]. It completely displaced whatever subtypes were circulating prior to 1918 (presumably H3 and/or H2 viruses) and has continued to circulate and evolve ever since. All major influenza viruses circulating in humans since 1918, and most of the major influenza lineages circulating in domestic pigs have been evolutionary descendants of the 1918 virus [35,37,38]. The H1N1 subtype continued to circulate and evolve through antigenic drift until one or more reassortant events led to an antigenic shift to H2N2 in 1957 [37].

1.2.3 **1957 Asian Flu Pandemic (H2N2)**

The 1957 Asian Flu (H2N2) pandemic virus was a descendant of the 1918 H1N1 virus but had acquired 3 new avian gene segments through viral reassortment including the H2 HA and N2 NA [37]. The antigenic shift to H2N2 precipitated a pandemic which was less severe than the 1918 pandemic although it was an estimated 10 times more severe than the recent 2009 'swine flu' pandemic [39]. Mortality was highest in the very young and very old [5,40]. Deaths were often due bacterial pneumonia (particularly *Staphylococcus aureus*) however primary influenza viral pneumonia (pneumonia caused by influenza rather than a secondary bacterial infection) was also feature of this pandemic and those with underlying chronic lung or heart disease and pregnant women in their third trimester appeared to be at greater risk [5,40,41]. Rates of illness in the elderly were lower than in other age groups, perhaps as a result to exposure to previously circulating H2 strains in the late 1800s [5]. Although influenza vaccines had been developed in the early 1940s, this was the first time a vaccine for a pandemic virus was produced [3]. In the United States 49 million doses had been made available by the peak of the pandemic in early November 1957 [3,42]. After the pandemic the H2N2 subtype continued to circulate and drift until another antigenic shift occurred in 1968.

1.2.4 **1968 Hong Kong Flu Pandemic (H3N2)**

The 1968 H3N2 pandemic virus was a descendant of the 1957 H2N2 virus and while it retained the N2 NA gene segment it had acquired 2 new avian gene segments including the H3 HA gene [37]. The 1968 pandemic was slightly less severe than the 1957 pandemic [3,5]. Again the elderly were relatively protected, perhaps from previous exposure to an H3 virus

in the late 1800s [5,29]. The 1968 pandemic occurred over two years and has been described by some as 'smouldering' because the most severe wave of disease occurred in the first year for the United States followed by a less severe second season, whereas in Europe and Asia the opposite occurred [43] . There is evidence that the delayed mortality in Europe and Asia may have been due to high levels of pre-existing immunity to the N2 following a particularly severe pre-pandemic H2N2 season compared to North America as well as antigenic drift in the N2 gene between the first and second year of circulation [43].

1.2.5 **1976 Swine Flu Outbreak (H1N1)**

In January 1957 there was an outbreak of influenza at a military training camp at Fort Dix, New Jersey, USA [44]. The outbreak lasted less than a month but during that time an estimated 230 people became infected resulting in 13 hospitalisations and one death [5,44]. Initial outbreak investigations yielded H3N2 and an unknown virus, determined later in the outbreak to be a swine-origin H1N1 virus [44]. The identification of human-to-human transmission of a swine H1N1 virus (a decedent of the 1918 virus) among humans caused a great deal of concern that another pandemic was beginning. This was because it was understood at the time that pandemic influenzas viruses were derived from animal influenza viruses that had acquired the ability to transmit between humans [3]. Additionally, the Haemagglutinin recycling theory (which hypothesised that haemagglutinin subtypes are cyclically and sequentially reintroduced back into the population once population-level immunity to those haemagglutinin has lowered sufficiently with time) predicted that the next pandemic would occur relatively soon and be caused by an H1N1 virus [3,5,29]. As a result of these concerns, the United States quickly developed a vaccine and initiated a mass vaccination programme in October that year despite not having identified any further cases after the outbreak ended [3,5,44]. After vaccinating 43 million people, the programme was terminated early when cases of Guillain-Barre syndrome were associated with the vaccine [5,41,44].

1.2.6 **1977 Reintroduction of H1N1**

The H1N1 subtype returned to circulation in 1977 and has co-circulated with H3N2 viruses until 2009 [5]. Due to the high level of genetic similarity between the 1977 H1N1 viruses and H1N1 viruses that circulated in the early 1950s, it is generally believed that the 1977

re-emergence of H1N1 was not a natural event, but an accidental release from a laboratory or a live-vaccine trial escape virus [45,46]. The reintroduction of the virus caused what could be considered an age-restricted pandemic among those aged 25 and under, most of whom has not been exposed to the H1N1 viruses which disappeared from circulation in 1957 [41].

1.2.7 **1997 and 2003 H5N1 Avian Influenza**

In 1997 Hong Kong experienced an outbreak of H5N1 in poultry at farms and live bird markets [47,48]. The strain caused severe disease and death in poultry and was thus classified as a highly pathogenic avian influenza virus (HPAIV). Zoonotic transmission (transmission between animals and humans) led to 18 human cases, six of which were fatal [48]. In response to the outbreak, all poultry in Hong Kong were culled and the live bird trade was halted for 7 weeks [48]. When trade in live birds recommenced new policies were introduced to lower the risk of H5N1 reintroduction [48]. These efforts appeared to have eradicated the virus from Hong Kong but it reappeared in poultry across South-East Asia in 2003 with human cases in China and Vietnam [49]. By the end of 2005, there were 148 laboratory confirmed cases resulting in 79 deaths [50]. Infections were caused by exposure to animals or contaminated environments (such as live bird markets) and although there were a few potential instances of human-to-human transmission, the virus had not evolved the capacity for sustained transmission among humans [51]. Nevertheless, there was international concern that this novel avian virus would acquire the ability to transmit more efficiently among humans and spark a 1918-like pandemic [52]. The increasing number of human H5N1 cases coupled with the international outbreak of Severe Acute Respiratory Syndrome (SARS) in 2003 prompted national and international organisations and agencies to revisit and expand their pandemic planning and preparedness activities [5,53]. Since 2003, H5N1 has continued to spread and as of the 27th of September 2017, there were 860 laboratory-confirmed cases causing 454 deaths in 16 different countries across 3 continents [50]. Fortunately the virus still has not evolved the ability to transmit efficiently among humans [51].

1.2.8 **2009 H1N1pdm09 ('Swine Flu') Pandemic**

In April 2009 the US Centers for Disease Control (CDC) identified a novel H1N1 virus in two children in California, a virus which would go on to produce the first influenza

pandemic of the twenty-first Century [54,55]. The triple reassortant virus, containing gene segments from avian, swine and human influenzas was sufficiently novel that a large section of the population was immunologically naïve (i.e. susceptible) to the new strain [56]. Individuals born before 1957 were less susceptible to the virus as result of their previous exposure to, and long-term immunological memory from, previous H1N1 viruses circulating between 1918 – 1957 [5,56]. In general the pandemic virus was relatively mild and had a similar severity to seasonal influenza apart from specific groups, including children and pregnant women, which experienced higher mortality than would be expected with seasonal influenza [15,57]. Although overall mortality rates were similar to seasonal influenza, most deaths occurred in the younger age groups (in contrast to seasonal influenza where most deaths occur in the oldest age groups), leading to a substantial number of years of life lost [57,58]. The virus replaced the previously circulating H1N1 strain and has continued to co-circulate in humans with seasonal H3N2 [5,59].

1.2.9 **Post 2009 Zoonotic Influenza**

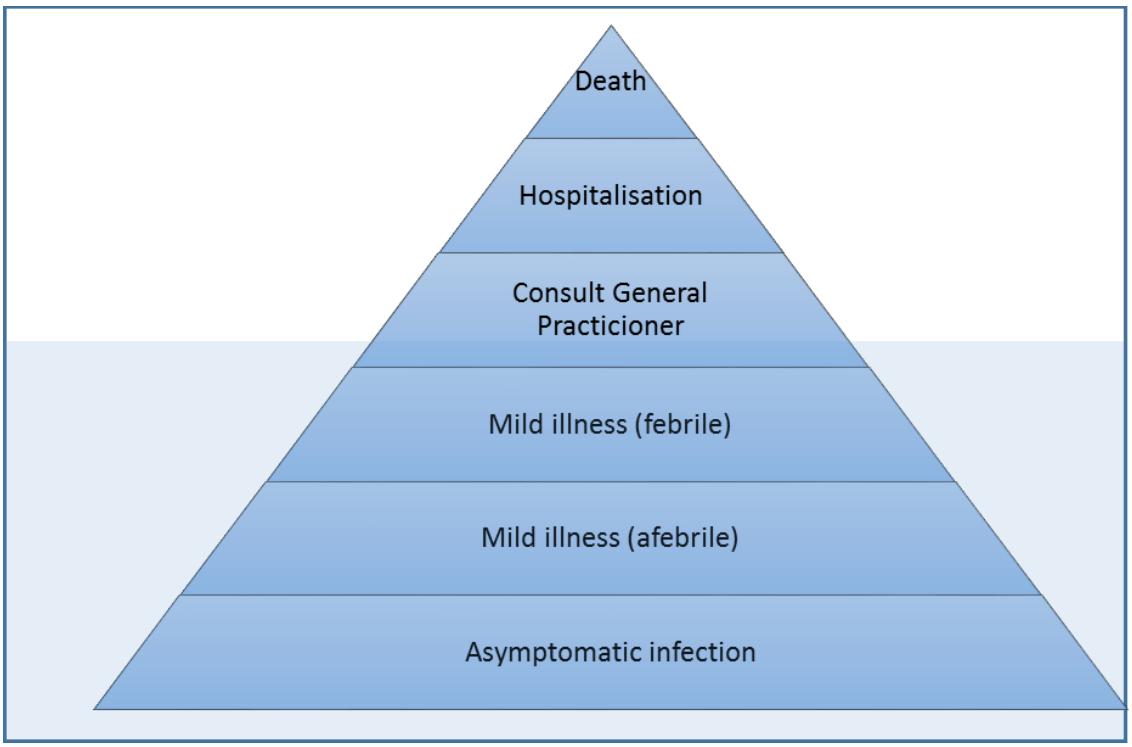
In recent years the number of influenza subtypes crossing the species barrier from animals into humans has increased [60]. Of the 20 known influenza A reassortant viruses that have crossed over from animals into humans since 1918, eight of them have crossed over since the 2009 pandemic, three from swine and six from avian sources [60,61]. None of these newly crossed over reassortants have been able to transmit efficiently between humans and most only cause sporadic human cases with the exception of two viruses [51,60]. In the US, outbreaks of human cases of swine origin H3N2v started to appear in 2011 and have since caused 425 cases, 26 hospitalisations and one death [62]. Although infections are usually traced back to exposure to pigs, often at agricultural fairs, there have been a few instances of probable limited human-to-human transmission [63]. In China, human infections with avian H7N9 were first identified in 2013 and since then there have been regular waves of cases resulting in 1564 reported cases and at least 612 deaths [51,64,65]. Infections in humans are often severe and most can be traced back to exposure to live poultry or contaminated environments such as live bird markets [66].

1.3 Influenza Severity

1.3.1 Iceberg of infection and disease

International influenza surveillance and research is typically based upon cases seeking medical care [67–69]. However this focus greatly underestimates the true community burden of seasonal (and pandemic) influenza: the majority of cases are mild and self-limiting with asymptomatic infections accounting for 25% to 77% of all infections [14,15,70]. The wide range of influenza outcomes and how visible those outcomes are to surveillance systems and research studies can be conceptualised as an iceberg (Figure 1-2). The tip of the iceberg represents the more severe cases who have contact with medical services and thus lie above the water line, 'visible' to surveillance systems. The majority of influenza cases however are mild or asymptomatic and do not seek medical attention and are thus underwater and 'invisible' to most surveillance systems and research studies.

Figure 1-2: Influenza Iceberg of Infection and Disease



The fact that the majority of influenza infections and illnesses are not captured by surveillance or research studies is a serious problem. In order to prepare for and respond

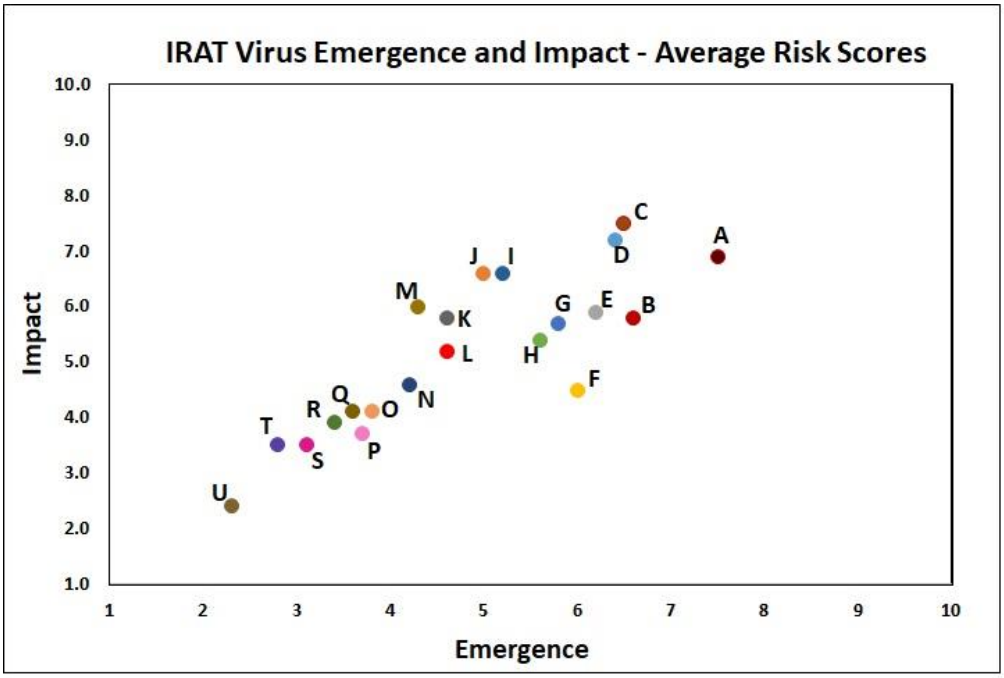
effectively to a seasonal or pandemic influenza virus, it is essential to have an early (and regularly revised) assessment of the virus's overall severity. Unfortunately, many of the parameters used to assess severity are prone to bias because they are derived from data sources that are not representative of the overall population, the overall population of infections (regardless of disease status) and/or the overall population of illnesses (regardless of how serious they are). In the next section I will describe the factors affecting influenza severity and how it can be assessed, how this information is used for public health response, and how the lack of representative, community-level data can bias crucial estimates and negatively impact that response.

1.3.2 Assessing Severity

The term 'severity' has different meanings in different contexts. In this thesis I will discuss severity in terms of 'clinical severity' and 'population-level severity'. Clinical severity refers to how severe the disease is (e.g. the proportion of infected individuals who die). Population-level severity refers to the disease burden (i.e. total number of cases) and the wider impacts of influenza on a population (e.g. the number of individuals who die, the strain on essential services and economic costs). Population-level severity is influenced by the clinical severity of a strain, but it is also influenced by other characteristics of the virus, the population and the environment, so a strain that is clinically severe is not necessarily severe on a population-level. For example, A(H5N1) infection is clinically very severe. However, as the virus strain cannot easily transmit between people, only sporadic infections occur and the total number of deaths on a population-level remains low. In contrast, seasonal A(H1N1) is clinically much less severe than A(H5N1); however, because it infects such a large proportion of the population, the total number of deaths is much higher.

This thesis focuses on assessing population-level severity of influenza viruses that exhibit sustained human-to-human transmission. In contrast, population-level severity assessments for zoonotic influenza viruses that do not transmit easily between people are usually incorporated into a virus-specific risk assessment. These risk assessments compare the likelihood that a virus will become transmissible between humans against the impact of that transmission. The methodological details of zoonotic influenza risk assessments are outside the scope of this thesis. Figure 1-3 gives an example of recent CDC risk assessments of the pandemic potential for zoonotic influenza viruses using their Influenza Risk Assessment Tool (IRAT) [71].

Figure 1-3: CDC risk assessments of zoonotic influenza viruses, figure taken from the CDC*[71]



·	Influenza Virus	Emergence Score	Impact Score
A	A(H1N1) [A/swine/Shandong/1207/2016]	7.5	6.9
B	A(H3N2) variant [A/Ohio/13/2017]	6.6	5.8
C	A(H7N9) [A/Hong Kong/125/2017]	6.5	7.5
D	A(H7N9) [A/Shanghai/02/2013]	6.4	7.2
E	A(H9N2) Y280 lineage [A/Anhui-Lujiang/13/2018]	6.2	5.9
F	A(H3N2) variant [A/Indiana/08/2011]	6.0	4.5
G	A(H1N2) variant [A/California/62/2018]	5.8	5.7
H	A(H9N2) G1 lineage [A/Bangladesh/0994/2011]	5.6	5.4
I	A(H5N1) Clade 1 [A/Vietnam/1203/2004]	5.2	6.6
J	A(H5N6) [A/Yunnan/14564/2015] – like	5.0	6.6
K	A(H7N7) [A/Netherlands/219/2003]	4.6	5.8
L	A(H5N8) clade 2.3.4.4b [A/Astrakhan/3212/2020]	4.6	5.2
M	A(H10N8) [A/Jiangxi-Donghu/346/2013]	4.3	6.0
N	A(H5N8) [A/gyrfalcon/Washington/41088/2014]	4.2	4.6
O	A(H5N2) [A/Northern pintail/Washington/40964/2014]	3.8	4.1
P	A(H3N2) [A/canine/Illinois/12191/2015]	3.7	3.7
Q	A(H5N1) [A/American green-winged teal/Washington/1957050/2014]	3.6	4.1
R	A(H7N8) [A/turkey/Indiana/1573-2/2016]	3.4	3.9
S	A(H7N9) [A/chicken/Tennessee/17-007431-3/2017]	3.1	3.5
T	A(H7N9) [A/chicken/Tennessee/17-007147-2/2017]	2.8	3.5
U	A(H1N1) [A/duck/New York/1996]	2.3	2.4

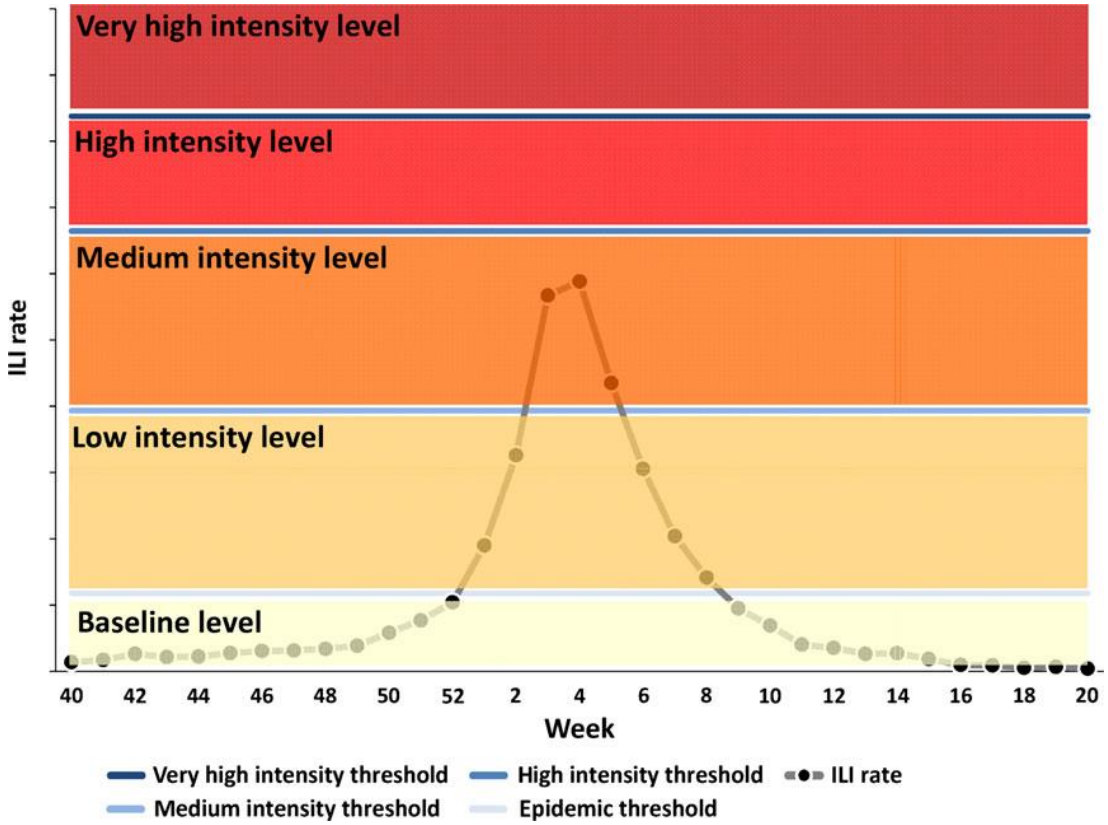
*Materials developed by the CDC. IRAT material is otherwise available on the agency website for no charge (https://www.cdc.gov/flu/pandemic-resources/monitoring/irat-virus-summaries.htm). Reference to specific commercial products, manufacturers, companies, or trademarks does not constitute its endorsement or recommendation by the U.S. Government, Department of Health and Human Services, or Centers for Disease Control and Prevention.

1.3.2.1 *Conceptual frameworks for assessing population-level severity*

The population-level severity of an influenza virus is determined to a large extent by the combination of how easily the virus spreads between people (transmissibility) and the clinical severity of the virus. Traditionally, population-level severity of an influenza virus has been primarily measured with the overall case fatality risk or 'CFR' (proportion of cases that die). Whilst this is an important measure of clinical severity, it is a crude measure that does not fully capture the range of severity (such as cases who consult their general practitioner or are hospitalised), nor the variation felt within and between populations [72]. Additionally, it can be challenging to measure, especially during the initial spread of a novel virus[73]. The over-reliance on the CFR and the challenges in measuring it were acutely felt during the 2009 pandemic. A WHO review on the response to the pandemic with respect to the International Health Regulations (IHR), described the "absence of a consistent, measurable and understandable depiction of severity of the pandemic" as a major problem [74] (p.15). It recommended that the WHO and member states develop and apply measures to assess population-level influenza severity and then apply those measures routinely to seasonal influenza. This would inform the response to current influenza activity (seasonal or pandemic) and also put the data into context through comparison with historical data from the same system. Additionally, if the necessary data collection and assessments are routine, then they would already be in place when a pandemic arises, enabling early estimates of severity [74].

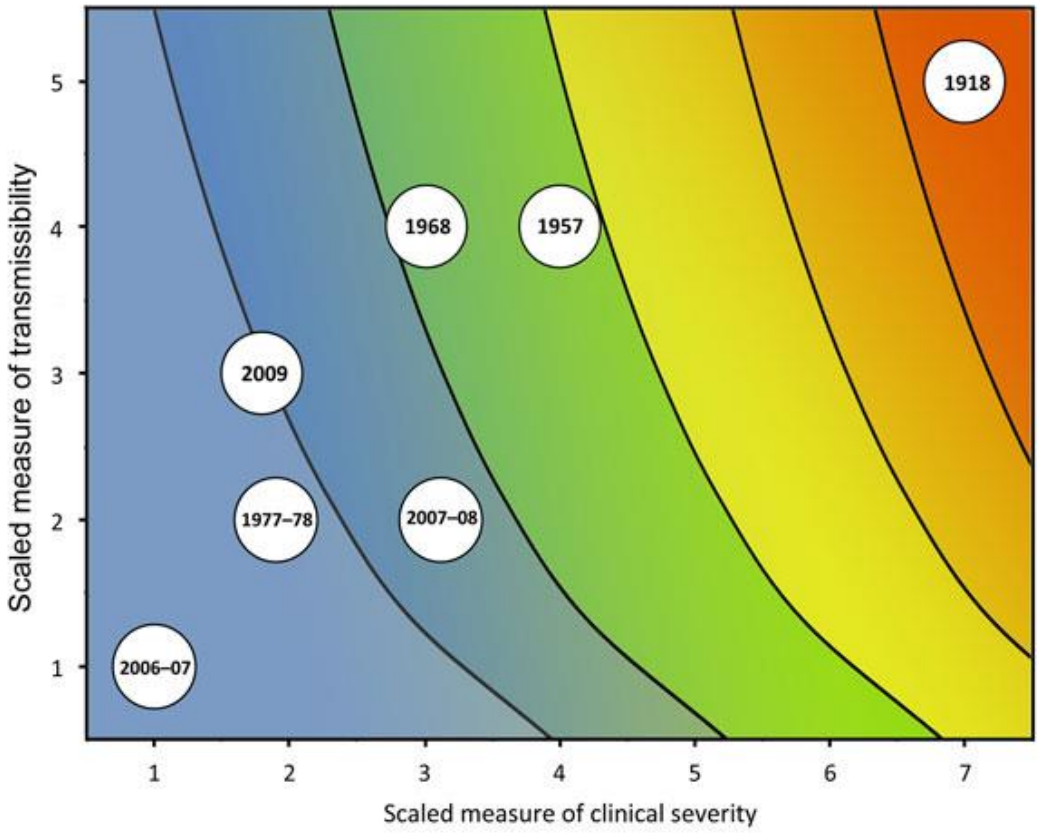
As a result of this recommendation the WHO developed the Pandemic Influenza Severity Assessment (PISA) guidance, which developed a framework for assessing population-level severity using three broad indicators: transmission, seriousness of disease (i.e., clinical severity), and impact. Each indicator is assessed using a combination of key parameters. These parameters are measured against a series of thresholds (e.g. baseline, low, moderate, high, extraordinary) which were set using historical data from the same data collection systems [75]. The moving epidemic method (MEM), used by the European Centres for Disease Control (ECDC) is one approach for setting thresholds [76]. Figure 1-4, taken from Vega et al [77] provides an example of the MEM threshold approach applied to a transmission parameter of ILI incidence.

Figure 1-4: MEM graph model with epidemic and intensity thresholds, intensity levels, and the weekly ILI/ARI rate, taken from Vega et al[77]



The US CDC developed a similar conceptual framework where the population-level severity of an influenza virus is measured using two of the three PISA indicators: clinical severity and transmissibility [72]. Similar to the PISA framework, the transmissibility and clinical severity indicators are measured using a number of parameters which are measured against either an initial assessment scale (low, medium high) or a refined scale once more data becomes available (1-5 for transmissibility and 1-7 for clinical severity). Figure 1-5, taken from Reed et al, shows the framework for the refined assessment with examples of past influenza seasons and pandemics [72]

Figure 1-5: CDC Framework for the refined assessment of the effects of an influenza pandemic, taken from Reed et al[72]



Common themes in the PISA and CDC frameworks include identification of overarching severity indicators (transmissibility, clinical severity and (for PISA) impact) that are measured using multiple parameters, each of which are scaled against historical data to provide context. For the purposes of this discussion, I will focus on the CDC indicators (transmissibility and clinical severity) as umbrellas with which to group key parameters that drive population-level severity (e.g. disease burden and wider impact) although I will include a brief discussion of some of the wider societal and economic impacts.

1.3.2.2 *Transmissibility*

The transmissibility indicator describes how easily and quickly a virus is transmitted between people in a population. There are many interacting factors which affect the way influenza viruses spread through human populations, but three overarching factors stand out in their ability to help us simplify and describe this complex system: the immunity profile of a population, the transmissibility of the virus and contact patterns within the population.

1.3.2.2.1 Immunity profile of population

The immunity profile of the population is ideally measured by the age-stratified estimates of the number and proportion of susceptible individuals, individuals with pre-existing immunity (from previous exposure to one or more similar viruses), individuals who have been or are currently infected (irrespective of whether they became ill) and when a pandemic-vaccine becomes available, individuals with vaccine-derived immunity.

1.3.2.2.2 Virus transmissibility

A critical summary parameter of a virus' ability to transmit between people is known as the 'Basic Reproduction Number' (R_0). It is the average number of secondary cases arising from a primary case in an entirely susceptible population. If a virus has an R_0 greater than one it has the ability to spread, but if the R_0 is less than one then it will not lead to sustained transmission. As the epidemic moves on and the population is no longer fully susceptible this parameter becomes known as simply the 'Reproduction Number' (R). Reproduction numbers can be measured using secondary attack rates, the exponential growth rate and by averaging transmission chains [78]. An R greater than one indicates the spread of the virus is increasing and the epidemic curve is rising whereas an R less than one denotes a slowing in the spread and a downward epidemic curve.

The serial interval is another important parameter which measures the mean time between homologous stages of infection in successive cases. The homologous stages could be the moment of infection or symptom onset, but the resulting mean interval would be the same regardless. Modelers sometimes use the term 'generation interval' or 'generation time' to specifically denote mean interval between successive infections. The serial interval is used widely for the purposes of modelling, contract tracing and determination of transmission trees.

The climate, particularly temperature and humidity also affect transmission. This is one of the reasons that seasonal influenza epidemics occur during winter months in non-tropical areas. As a result, a novel virus entering a population just before or during winter months is likely to transmit more efficiently than the same virus introduced in the same population during summer months.

1.3.2.2.3 Contact Patterns

The third major factor which impacts a virus' spread through a population is the behaviour of the population, particularly the contact patterns among individuals within that population. Influenza is spread via respiratory or close contact and therefore a population's contact patterns will to an extent drive transmission patterns [79–81]. One of the ways contact patterns have been measured is through the use of community-level surveys which ask participants to record the number, age group, nature and duration of contacts throughout the day. The resulting data can be summarised into a contact matrix which plots the number and age group of contacts by participant age group [79]. Measures of contact patterns, including the aforementioned contact matrices are used to parameterise influenza transmission models [81].

1.3.2.2.4 Summary measures

The interplay between population susceptibility, the transmissibility of the virus and the contact patterns that facilitate transmission determines to a large extent the transmissibility of a virus in a population and more specifically, the timing, scale, speed and geographic spread of the epidemic. Summary transmission measures (disease burden measures) of the epidemic include the total numbers and the incidence rates of infection and disease. These could be measured in a variety of settings such as schools, communities and in GP practices. The spatial distribution of a virus (both macro and micro scale) is an important parameter in its own right but may provide insights into modes and routes of transmission (e.g. public transportation routes, building ventilation systems). These types of summary parameter measures can be estimated from surveillance and/or epidemiological studies. They can also be thought of as summary measures of clinical severity and measures of impact in their own right.

1.3.2.3 *Clinical Severity*

The clinical severity indicator describes the extent to which an infected individual becomes ill[75]. This concept is also referred to as the 'seriousness of disease' or the 'severity of infection'[75]. Influenza infection has the widest possible spectrum of outcomes ranging from no symptoms to death. The clinical severity of an influenza strain is measured by the distribution of these outcomes in a population of infections. Knowledge of the overall distribution among all infections is an important summary parameter but equally important is how clinical severity varies by subgroup. The identification of groups at higher risk of

severe outcomes compared to the general population (i.e 'high risk' or 'at risk' groups) is of particular importance. As described previously, clinical severity varies most notably by factors related to the virus (e.g. subtype) by host factors (primarily age group, pregnancy, and underlying health status) and by environmental factors (e.g. health seeking behaviour, access to and availability of health care).

1.3.2.3.1 Case Fatality risk and similar measures

A common way to assess clinical severity is to calculate the proportion of cases with a specific outcome, usually the more severe outcomes like hospitalisation or death. One of the most important and widely used measurements is the case fatality risk (proportion of cases who die). The case fatality risk (CFR) is also referred to as the case fatality rate or the case fatality ratio although it is neither a rate nor a ratio. In its purest form, the CFR would define cases (i.e. the denominator) as the total number of infections in the population. The underlying number or rate of infections is often unknown, however, so many estimate the CFR using alternative denominators that are easier to assess such as laboratory-confirmed and/or hospitalised cases, which as I will explain later, can be a highly biased denominator. Another important measure of the severity of infection, and one that requires infections as the denominator, is the proportion of infections that are asymptomatic. This measure is known as the asymptomatic proportion.

1.3.2.3.2 Summary measures of clinical severity

Population-level burden estimates of the severe cases and deaths are common summary measures of clinical severity. Direct measures of influenza hospitalisation and mortality (although likely to be underestimates) include the total number and rates of lab-confirmed influenza hospitalisations and deaths. Indirect methods includes statistical modelling of influenza-attributable hospitalisation and mortality as well as excess all-cause mortality [82,83]. Population-level measures of absenteeism and quality of life lost due to influenza could also be considered summary measures of clinical severity.

1.3.2.4 *Impact*

The impact of an epidemic or pandemic on a population is multidimensional and is determined in part by the combination of the transmissibility and severity of the epidemic as well as the public and public health response to that epidemic. Impact can be measured in terms the overall number of illnesses and deaths in a population (i.e. disease burden) and

the strain on health care systems and health care workers (e.g. increased rates of GP consultation, hospitalisation and ICU admissions and resulting bed pressures, and increased staff absenteeism due to influenza illness). The impact of an epidemic can also be measured in terms of its wider societal and economic impact. For example, the public health response to an epidemic has direct and indirect costs; public health interventions can cause disruption (e.g. school closures); increased absenteeism has productivity and economic costs and, in the worse-case scenario, can lead to disruption of critical services and infrastructure. The impact of an epidemic on a population can be modified by implementation of public health interventions and by public concern and behaviours such as propensity to consult, uptake of vaccination, compliance with interventions, and contact and travel patterns.

1.3.2.5 *Putting it all together with dynamic transmission modelling*

An increasingly common and important method of combining many of the parameters described above in order to estimate past, current and to some extent, forecast the future trajectory of an epidemic is through the use of dynamic transmission models. The basic forms of these models are built using a few key transmission parameters (immunity profile of the population, R_0, serial interval and contact patterns). Measures of clinical severity can be incorporated into these models, enabling estimates of the scale and timing of specific types of outcomes such as GP consultations, hospitalisations and deaths. This can inform planning and allocation of public health resources. These basic transmission models can also be modified to investigate the potential effectiveness and cost-effectiveness of public health interventions such as vaccination and the use of layered non-pharmaceutical interventions.

1.3.3 **Severity assessments and public health response**

Ideally, Population-level severity assessments should inform, either on their own or in combination with modelling, decisions around the initiation, implementation, targeting and scaling and communication of public health actions in a way that balances the effectiveness of those actions with economic and societal costs as well as political considerations.

Good examples of how severity assessments can inform public health response comes from seasonal and pandemic influenza vaccination. For seasonal influenza, modellers have used severity assessments (in particular assessments of transmissibility and clinical severity by age group) along with economic costings to develop novel vaccination strategies. One such

strategy, recently implemented in some countries including the UK, is universal childhood vaccination[84–86]. These programmes are based on the theory that by routinely vaccinating children (who have high rates of infection and are thought to be the primary drivers of transmission in the community) you not only provide direct benefits to the vaccinated children but also indirect benefits to the wider community through reduced transmission from children [87–90]. This in turn results in fewer hospitalisations and deaths in the older age groups who not only at higher risk of severe outcomes but are also harder to protect through vaccination given the low vaccine efficacy in those age groups. Severity assessments were also used to inform the purchase of pandemic influenza vaccine in 2009. In the following section I will describe the main severity assessments used in the run up to the UK's vaccine purchase decisions and how the lack of community data biased these assessments.

1.3.3.1 *Clinical severity during the 2009 pandemic*

During the 2009 pandemic, early measures of clinical severity were worryingly high. As the number of underlying infections or symptomatic illnesses was unknown, severity measures typically relied on hospitalised laboratory confirmed cases as a denominator to estimate risk of various outcomes such as critical illness, intensive care unit (ICU) admission and death [91]. According to data that the UK government had access to on 27 April 2009 (a mere 4 days after outbreaks in Mexico had been identified), the case count in Mexico was 878 of which 149 had died (although only 18 deaths were confirmed as H1N1 at this point). This leads to a rough CFR estimate of 17% among cases identified primarily in hospital [91,92]. Two days later, on 29 April 2009, the UK announced it would enlarge its stockpile of antiviral doses from 35.5 million to 50 million and a day after that the US followed suit with the purchase of an additional 13 million to add to its already 73 million dose stockpile [92,93]. In the following days, as more was learned about the virus and updates from outbreaks in Mexico and the US became available, it was becoming clearer that 1) severity assessments from outside of Mexico were lower than initial estimates from Mexico (where case finding was focused on people seeking hospital care) and 2) epidemiological and laboratory data suggested that the virus was not as severe as 1918-like virus but the population-level severity could be worse than the 1957 and 1968 pandemics [91–95]. The virus began circulating in the UK in early May and on 11 May 2009 the UK government triggered its advance-purchase agreements for pandemic vaccine, buying enough to cover 45% of the UK population [92].

A 2013 systematic review of CFR estimates from the 2009 pandemic demonstrated a number of important observations surrounding these calculations [73]. They found a great deal of variation in estimates, a large part of which was due to the choice of denominators and numerators. CFR estimates that had been based on laboratory-confirmed cases were more severe (and available earlier) than estimates based on different denominators, with most CFR estimates falling between 0.1% to 5%. CFRs based on symptomatic cases typically had a CFR between 0.005% and 0.05% and those based on estimated numbers of infections (generally published much later) were even smaller with most CFRs falling between 0.001% and 0.01%. Choice of numerators was also important. Most estimates were based on laboratory confirmed deaths, despite the fact that many pandemic deaths would not have been laboratory-confirmed. The studies that used excess deaths as a numerator generated more severe estimates. Additionally, the choice of laboratory-confirmed denominators made them not directly comparable to seasonal influenza mortality estimates as these are typically calculated using excess mortality methods in an effort to estimate total influenza mortality, not just the proportion that are laboratory confirmed [73].

The situation described above highlights two major issues that affect the accuracy of population-level severity assessments and how impactful those assessments are in terms of informing policy decisions. Those issues are 1) the choice of numerators and denominators and 2) the timeliness of the severity assessments.

1.3.3.1.1 Choice of numerators and denominators

Firstly, estimates using different combinations of denominators (lab-confirmed cases, symptomatic case definition or infections) and numerators (lab-confirmed deaths, excess deaths, etc) are calculating different quantities and are not directly comparable. Secondly, even estimates using the same denominator and numerator combination may not be directly comparable. A case fatality estimate using the total number of infections as the denominator would provide the most direct and unbiased way of comparing severity of infection within and between populations, influenza seasons and strains [73,96]. Alternate denominators such as laboratory confirmed cases or hospitalised cases (i.e. cases that are higher up in the clinical iceberg) are less comparable and prone to biases because of differences between populations in factors such as how these cases are identified, health seeking behaviours, symptomatic case definitions, laboratory testing regimes and health

service capacity [73]. Unfortunately, in 2009 many important public health decisions such as the initial purchase of antivirals and vaccine were made at a time when the available estimates were based on cases and deaths primarily identified in hospitals. This leads to the second cross-cutting theme: timelines of public health actions and severity assessments.

1.3.3.1.2 Timelines of public health actions and severity assessments

The timelines for some public health decisions and robust severity estimates are not always aligned. For example, the long lead in times for pandemic influenza vaccine production and the potential for international competition over a limited supply of vaccines and antivirals can force early decisions on the purchase of these products based on incomplete data. During the 2009 pandemic, the UK decided within 3 weeks of the novel virus being identified to purchase vaccine for 45% of the population and increase the national antiviral stockpile by 16.5 million doses [92]. Those decisions were made when the available severity assessments were largely based on cases identified in hospitals and thus biased towards those with the most severe disease. The more robust estimates which followed shortly thereafter were largely based on laboratory-confirmed cases and although they were generally less severe than those first estimates, they were still more severe than later estimates which used less bias denominators [73,97]. There are a few reasons why the least biased estimates only became available much later. Firstly, community level data, needed for more encompassing denominators (i.e. denominators from lower down the iceberg), is not routinely collected and initiating that type of data collection at short notice in the wake of the pandemic is challenging and time consuming [98]. Secondly, it takes time to develop a serological assay needed to identify underlying influenza infections (regardless of symptoms). Therefore, any estimates with serological infections as the denominator will be delayed until an assay is developed and the testing of a large number of samples can be completed.

It is now generally recognised that prior to 2009 pandemic influenza preparedness plans were geared toward a severe pandemic and were not suitably flexible to deal with a more mild pandemic or to adapt easily as the epidemic progresses and new information is available [92].

1.3.3.2 *Clinical severity estimates of less severe outcomes*

Other estimates of severity measuring the less severe spectrum of disease such as the proportion of infections with mild disease and the proportion of infections that consult also inform public health actions. For example, the proportion of cases that have only mild disease (particularly afebrile cases) will influence how effective some of the non-pharmaceutical interventions would be at reducing transmission. For example, border screening relying on fever measurements to identify cases will not be effective if most cases are mild and do not develop fever. Similarly, cases that have only mild disease will probably not feel ill enough to stay home from work or school. Even if the public health advice advocates self-quarantine, these mildly symptomatic individuals are unlikely to realise that they are infected and thus unlikely to stay at home. If a large proportion of cases are only mildly symptomatic then self-quarantine advice is unlikely to reduce community transmission. Finally, cases with only mild disease are unlikely to seek medical advice. In such a scenario, even universal treatment of all those with symptoms who seek medical care is unlikely to reach most of the cases and thus will have little impact on community transmission.

1.3.3.3 *Transmission*

Transmission and economic models heavily influence key government decisions on public health response such as the purchase, targeting and use of vaccines and antivirals. These models rely on age-specific measurements of susceptibility and immunity which can only be reliably obtained from population-level serological studies [99]. Other population-level data such as age-specific contact patterns, risk of infection and R0 are used to parameterise these models and can have large effects on model output [80,100]. Given the importance and potential impact of these models on public health action, it is crucial that accurate, population-level estimates are used to parameterise them. Unfortunately, generating such estimates is challenging for both typical surveillance systems and research studies. In the absence of reliable population-based estimates models may need to rely on surveillance data that do not fully capture the underlying community burden of infection and disease. Such models are likely to be biased toward more severe cases and may produce misleading results.

In the following sections I will describe from where data on the community burden of influenza could be derived, identify information gaps, and explain how modern community studies can fill this gap.

1.4 Overview of available data and information gaps

Community-level data on influenza infection and disease can come from surveillance, routinely collected data such as electronic health records and death certificates and from research studies. Section 1.4 will give an overview of the available data, identify information gaps and describe how new community-level studies such as those used in this thesis are needed to fill those gaps.

1.4.1 Influenza Surveillance

> *Health surveillance: the ongoing systematic collection, analysis and interpretation of data essential for planning, implementing and evaluating public health activities" [101] (p.127)*

Influenza surveillance is designed to collect the information necessary to inform public health responses to influenza. It has a number of functions which can be broadly characterised into 1) providing an early warning system for influenza activity and novel virus variants, 2) generating estimates of the severity and spread of influenza and communicating the findings to relevant stakeholders (primarily medical professionals and public) and 3) informing prevention, treatment and control activities [5].

Comprehensive influenza surveillance systems integrate a number of different surveillance activities (both epidemiological and virological) as no single activity can provide all the necessary data. Surveillance can be described as active, passive or sentinel. Influenza surveillance differs across countries ranging from no surveillance to sophisticated systems like ones that have been developed in the UK. Data and viral isolates generated from national influenza surveillance systems often feed into regional and global surveillance systems such as the European Centres for Disease Control (ECDC) and the World Health Organization's Global Influenza Surveillance and Response System (GISRS).

1.4.1.1 *UK Surveillance systems*

In the UK, influenza surveillance is coordinated and collated by Respiratory Disease Department of Public Health England (PHE) and activities can be categorised as clinical surveillance, virological surveillance or both.

1.4.1.1.1 Clinical Surveillance

1.4.1.1.1.1 Syndromic Surveillance

In England, there are a number of real-time syndromic surveillance systems which include GP consultations (both in hours and out-of hours), emergency department visits and NHS 111 calls[102,103]. These systems monitor various indicators of influenza (i.e. NHS 111 calls for cold/flu or GP consultations for ILI)[102,103].

1.4.1.1.1.2 Internet-based Surveillance

Since the 2009 pandemic the UK has run an internet-based participatory surveillance system known as FluSurvey which estimates community-level incidence rates of influenza-like illnesses[104,105]. Currently the system does not collect respiratory specimens to confirm the aetiology of reported illnesses. There is no sampling frame used to recruit participants. Instead recruitment is achieved through advertising, media coverage and word-of-mouth. Basic demographic, socio-economic, postcode, health, vaccination and risk factor information collected from participants enables analysis of ILI incidence and risk factors[106]. Flu survey utilizes the influenzanet surveillance platform which runs in 10 different European countries[105]. Adaptations of influenza net and similar, but independent systems run in a number of other countries around the world. FluSurvey community ILI rates feed into seasonal and (if relevant) pandemic modelling[107].

The UK also recently introduced a web-based syndromic surveillance system based on google search queries[103]. The system estimates real-time rates of ILI using natural language processing and machine learning techniques[103,108,109].

1.4.1.1.1.3 National Pandemic Flu Service

The UK's National Pandemic Flu Service (NPFS) is a programme designed to supplement GP services during an influenza pandemic. The service was rolled out during the 2009 pandemic and remains a component of national pandemic response plans. The programme was comprised of both a phone-based and web-based service that enabled ill individuals to

do a self-assessment that would lead, depending on their reported symptoms, to self-care advice, an automated antiviral prescription, or referral to other services (e.g. to the GP or emergency services) [92,110]. The programme also included a self-swabbing component for virological surveillance but limited it to illnesses with met the influenza-like-illness case definition use in the NPFS algorithm [110]. In 2009/10 the service helped reduce pressure on primary care and enabled timely collection of antivirals [92].

1.4.1.1.1.4 Primary Care

In addition to the syndromic surveillance at the GP level described above (also referred to as Q-Research), the UK has an additional embedded sentinel surveillance scheme within a networks of general practitioners' (GP) surgeries[111]. In England, the scheme is operated by the Royal College of General Practitioners (RCGP) which collects consultation data and virological specimens from approximately 200 GP practices. Their weekly returns service provides nationally representative ILI consultation rates, using the number registered patients in these practices as the denominator[103,111].

The ILI consultation rates are presented using the Moving Epidemic Method (MEM) which uses data from the previous 10 years to characterise levels of influenza activity as either baseline, low, medium, high and very high [76]. Other data sources and countries using the MEM method of reporting and will have their own thresholds for each level due to variations in consultation and recording practices. In the 2017/18 winter season, the RCGP rates of ILI consultation (per 100,000 people) were considered to be 'baseline' if <13.1, 'low' between 13.1 to 24.1, 'moderate' between 24.2 to 68.6, 'high' between 68.7 to 108.7 and 'very high' at 108.9 and above [111]

1.4.1.1.1.5 Institutions

Certain boarding schools send information on student numbers and the number of ILI cases to PHE where ILI incidence rates are then calculated.

PHE also investigates outbreaks of acute respiratory illness in institutions such as schools, care homes and hospitals which are reported to them. If sampling is done then microbiological investigations can take place.

1.4.1.1.1.6 Secondary Care

Information on influenza in secondary care is collected through The UK Severe Influenza Surveillance Scheme (USISS). USISS has two components: mandatory and sentinel. The mandatory scheme requires all UK hospitals to report laboratory-confirmed influenza cases and laboratory-confirmed influenza deaths among patients admitted to intensive care units (ICU) and high dependency units (HDU). The sentinel scheme is comprised of selected NHS trusts in England which report all laboratory-confirmed influenza cases admitted to hospital (not just those in ICU or HDU).

The MEM method has recently been applied to both of the USISS data streams to establish baseline, low, moderate, high and very high influenza activity levels in hospitals.

1.4.1.1.1.7 Mortality

Apart from the USISS scheme described above, the only information on influenza-associated deaths is from death certificates. Influenza can lead to death indirectly through secondary infections, cardiovascular events and exacerbation of underlying disease. If influenza is not the obvious cause of hospitalisation or death, it is unlikely that the individual was tested for it and as a result, influenza is systematically under-reported on death certificates. As a result, the UK estimates influenza-associated deaths indirectly from all-cause mortality data. This is done in collaboration with the European Mortality Monitoring Project (EuroMoMo) which collates data from 26 European countries to estimate weekly excess mortality (i.e. mortality above the levels expected for a given time of year) by age group and country.

1.4.1.1.2 Virological Surveillance

Virological surveillance informs and prompts a number of public health actions. For example, in the UK, influenza antiviral prescribing in primary care is only triggered when the influenza is confirmed (through virological surveillance) to be circulating in the community[112]. On a global level, genetic and antigenic data collected in the UK through virological surveillance is submitted to the WHO to help guide annual influenza vaccine formulation [113].

The main virological surveillance systems in the UK are described below. However, one-off virological surveillance in the community was also conducted as part of the NPFS

service during the 2009 pandemic and piloted in the FluSurvey community cohort in the 2014/15 winter season[114,115].

1.4.1.1.2.1 Primary Care

In England, Influenza isolates are collected in primary care through two schemes: The RCGP scheme described above in the clinical surveillance section and the Specialist Microbiological Network scheme (SMN). For both schemes, participating GP practices obtain clinical specimens from patients presenting with ILI. These specimens are tested for influenza and respiratory syncytial virus (RSV) using reverse-transcription polymerase chain reaction (RT-PCR). The proportion of specimens testing positive for influenza provides an estimate of influenza activity. Similar schemes are run in Scotland, Wales and Northern Ireland.

1.4.1.1.2.2 Respiratory DataMart System

The Respiratory DataMart System (RDMS) is a virological sentinel surveillance scheme run by PHE and the National Health Service (NHS) laboratories in England[113,116]. It was initiated during the 2009 pandemic to collate influenza RT-PCR testing results (both positive and negative) for routinely collected clinical specimens from a network of laboratories serving primary and secondary care. In addition to influenza it also collates data on other common respiratory viruses including respiratory syncytial virus (RSV), human metapneumo virus (hMPV), adenovirus (AdV), parainfluenza virus (PIV), rhinovirus (hRhV)[116]. The systems runs throughout the year and electronic data (including patient's name, date of birth, sex, specimen date and test results) are transmitted on a weekly basis to PHE[116]. The proportion of specimens testing positive for each virus indicates what viruses are in circulation as well as their seasonality and impact. The MEM method described above has been recently applied to proportion testing positive for the 2017-18 season. The baseline threshold was 8.6%[103]. A limitation of the DataMart system is the lack of clinical data and case definitions. Additionally, the majority of laboratories do not report the source of the sample (primary versus secondary care)[116]. In the few laboratories that report this information, the majority of the specimens come from secondary care[116].

1.4.1.1.3 Serological Surveillance

Serological assays can identify infections and baseline immunity to various influenza strains. There is currently no UK-based serological surveillance system for influenza. During the 2009 pandemic however, PHE conducted a rapid one-off cross-sectional serological study to estimates age-specific prevalence of immunity to and infection with the pandemic virus for the purposes of parameterising disease transmission models [56].

1.4.1.2 *International Surveillance*

The Global Influenza Surveillance and Response System (GISRS)[117] is virological surveillance network which monitors the evolution of influenza viruses for the purposes of providing recommendations on diagnosis, vaccine composition, antiviral use and risk assessments. It also functions as an early warning system for identifying viruses with pandemic potential.

1.4.2 **Electronic Health Records**

Large and representative databases of electronic health records from both primary care (CPRD, THIN) and secondary care (hospital episodes statistics – HES) settings in the UK are available for analysis. They have several advantages, particularly representativeness, statistical power and information on influenza vaccination and risk factors disease and/or complications, in particular pregnancy and various co-morbidities. The systems however were not designed primarily for research and therefore the data contained in these systems are not always ideal for answering research questions.

1.4.2.1 *Primary Care*

The primary care databases have relevant information on vaccination, consultation, diagnosis, testing and treatment as well as patient characteristics such as age, sex and chronic illness. Identifying consultations that either were or may have been attributable to influenza is challenging. Apart from RCGP practices, primary care patients consulting for a respiratory illness are rarely tested for respiratory viruses so virological confirmation of influenza would be rare. Identifying consultations meeting a specific symptomatic case definition is also difficult as there are many codes used to record respiratory illnesses but no systematic collection of individual symptoms with which to verify case definitions.

Finally, a diagnosis of respiratory illnesses may not even be recorded in the medical records at all [15].

1.4.2.2 *Hospital Episodes Statistics*

Hospital Episodes Statistics (HES) contains ICD-10 coded information on all hospital admissions, A&E attendances and outpatient appointments to NHS hospitals in England. Despite the dataset's completeness on all patients, it does not identify all patients with influenza for the same reasons that it is difficult to count influenza deaths in death certificates. Hospitalised influenza patients will be classified by a range of ICD-10 codes and most will not have been tested (and thus not recorded) for influenza specifically, particularly when their admission is related to secondary infections, cardiovascular events or exacerbation of underlying disease[118]. As a result of this under-ascertainment, a range of modelling and statistical methods have been used to estimate influenza-attributable hospitalisation using a combination of HES and non-HES data sources[118].

1.4.3 **Research Studies**

Surveillance is not the only source of data on influenza burden and specific parameters necessary to measure influenza severity. Research studies on influenza both in the UK and worldwide has provided a great deal of information. Below is a description of the various study designs that generate relevant data.

1.4.3.1 *Household based cohort studies*

Until recently, most of the information on the community burden of influenza was generated from household-based cohort studies, primarily in the United States, from the 1940s through the early 1980s [119]. These studies followed up entire households during periods of influenza circulation to prospectively identify episodes of respiratory illnesses, collect specimens during those illnesses for virus confirmation and determine infection through serological analysis of paired pre- and post-season blood samples. This study design is considered the 'gold standard' for measuring community burden for a number of reasons. The active prospective follow-up of households limits recall bias and enables accurate incidence rates of community illness to be calculated across age groups. Serological analysis allows calculation of the proportion infected each season and the proportion of those infections which remain asymptomatic. By identifying respiratory

illnesses regardless of consultation, these studies are not biased towards the more severe, medically-attended illnesses. Additionally, the timing, duration, symptoms and other characteristics of these illnesses can be determined and inferences about secondary spread of infection within households can be made.

Although the household-based cohort study design is considered the gold standard, few such studies are conducted as they are highly resource intensive. After the early 1980s it wasn't until the mid-2000s that new household-based cohort studies started.

1.4.3.2 *Trials*

An alternative method of collecting comparable data to the household-based community cohort study is through the placebo arm of household and/or community randomized control trial of influenza interventions such as vaccination. These studies are even more resource intensive and have different aims than a cohort study, but there are examples of household and community based trials which have provided information on community burden [22,120].

1.4.3.3 *Case ascertained studies*

A variation on the household-based community cohort design is the case-ascertained study whereby households are enrolled and followed up once an 'index' case is identified, typically through medical services [121,122]. This design is aimed at estimating within-household transmission but does not directly measure incidence of infection or disease in the community. It is also possible the secondary cases identified may be biased towards more severe disease given that the household index case was severe-enough to consult for their illness.

1.4.3.4 *Online Cohort*

Another recent variation on the household cohort approach is the online cohort, also referred to as participatory surveillance [123–126]. These studies prospectively follow up individual participants and sometimes their household members with a baseline survey followed by subsequent weekly illness surveys in order to identify episodes of respiratory illnesses and potential risk factors. They aim to generate near real-time estimates of the incidence and distribution influenza-like illness (ILI) in the community at high geographic resolution, and thus complement traditional surveillance systems. Some participatory

surveillance systems also aim to estimate risk factors of ILI, track vaccine effectiveness and assess health-seeking behaviour in the same way as a traditional cohort study [123,124,127]. These studies have many advantages over traditional surveillance systems and cohort studies in that they detect changes in rates of illness more quickly and at greater spatial resolution than typical surveillance systems and can flexibly monitor a much larger population at far less cost than a traditional cohort study [124,127,128]. One of the main limitations of these studies is specificity. Their lack of specimen collection to confirm influenza infection or disease limits their outcomes to all-cause respiratory illness. Other limitations include potential participation bias resulting from crowdsourcing participants, difficulties in adjusting for confounders and maintaining consistent participation which makes it difficult to determine cohort size at any given time and also leads to attrition [128].

1.4.3.5 *Serosurveillance*

Serological analysis of single, cross-sectional blood samples cannot confirm recent infection that paired blood samples can confirm (except when dealing with novel or pandemic strains) but they can provide a snapshot of individuals' past strain-specific influenza exposures and current stain-specific susceptibility/immunity [3]. Population-level, cross-sectional serological surveys (serosurveys) typically rely on blood samples taken for other purposes such as blood banks or residual samples from clinical investigations [129,130]. Such samples are more convenient and inexpensive to obtain compared to samples from prospective studies, but conversely they can be biased towards individuals that are more or less healthy than the general public making them unrepresentative [129,130]. Additionally, they often do not have corresponding vaccination data which is needed for interpretation of past exposure/infection [129,130].

1.4.3.6 *Human Challenge Studies*

Studies in which researchers experimentally infect healthy volunteers with influenza virus and then monitor the results of those infections are known as human challenge studies. These studies can provide detailed data on the proportion of experimental infections which lead to disease as well as the timeline, natural history and severity of infection and disease but cannot estimate incidence of influenza infection and disease in the population. How well these artificial infections mimic natural infections can be hard to determine and will depend on pathogenicity of the viruses (challenge viruses may be less virulent than commonly circulating wild-type viruses), host status (challenge study participants are all

healthy and also typically seronegative/susceptible to infection and thus not representative of the population) and how well experimental exposure to the virus (droplet/mucus induced infections) reflects natural exposure which includes infection acquired through aerosol transmission [14,131].

1.4.3.7 *Internet and Social Media*

In recent years researchers have developed a number of novel approaches to monitor influenza activity in the community using non-traditional data sources such as internet search queries, social media and Wikipedia [132,133]. These 'Big Data' sources share many of the same advantages as participatory surveillance systems such as timeliness, temporal and geographical resolution [133]. They also have the added advantage of leveraging existing data streams and thus do not need to recruit and follow-up participants. They have a number of important limitations however including lack of specificity (there is no biological sampling to confirm influenza infection or disease) and changing user behaviour which can distort results [132,133]. Without data on individuals, it is also difficult to investigate individual risk factors for illness as would be possible in a cohort study. Many of the advantages and challenges for Big Data surveillance were highlighted by Google Flu Trends (GFT) which analysed Google search query data in the United States to estimate influenza-like illness (ILI) in the community [134]. GFT was initially quite successful and provided estimates of ILI a full week before traditional CDC surveillance could and at a finer geographical level [132,134]. However, the original GFT algorithm was found lacking in 2009 when it missed the first wave of pandemic influenza in the United States. Later, an updated algorithm overestimated the severity of the 2012-13 influenza season. These issues were largely due to changes in internet search behaviour as well as changes in the seasonality, age distribution and geographical heterogeneity of seasonal and pandemic influenza [132,135].

In an effort to generate more accurate surveillance of influenza activity and forecasting, novel systems have been developed that combine and analyse a combination of data streams such as Big Data, participatory surveillance, meteorological data, electronic health records and traditional clinical and laboratory based surveillance [132,136–138]. While these methods may enable a more robust assessment of influenza-like-illness in the community using big data, they do not solve the issue of specificity. Without laboratory-confirmed cases of influenza infection and illness in the community, they are unable to assess 1) the

levels of infection and immunity in the community, 2) the levels of influenza illnesses in the community (not just all-cause ILI), 3) the individual-level risk factors for infection and disease and 4) the symptom profiles and economic impact of influenza illnesses in the community.

1.4.4 **Information Gaps**

Prior to 2006 when the first epidemiological study used in this thesis was initiated, there were two major information gaps on the community level. Firstly, there were few data from modern times on underlying influenza infections (both symptomatic and asymptomatic) in the community (i.e. regardless of whether or not patients sought medical attention). It was not well understood what proportion of the population was susceptible each season, what proportion were infected each season and what the risk factors for those infections were. Even after the Flu Watch study shed light on some of these information gaps there were still challenges for ongoing collection of similar data, particularly during a pandemic, due to the timelines, complexity, and cost of setting up community cohort studies and a new need arose to design studies that could be quickly and efficiently initiated in a pandemic. The second major information gap was on community cases of influenza illness. Almost all data on laboratory-confirmed influenza illness came from (and continues to come from) cases with more moderate or severe disease who sought medical attention. In 2009 community data on influenza-like-illnesses were obtained from FluSurvey but without virological confirmation it was difficult to tease out the effect of influenza versus other causes of ILI. The community-level burden, consultation and treatment patterns, and the impact of influenza illnesses were not well understood. How these community influenza cases compared to cases of other acute respiratory viruses was also not well understood. The two studies I used for this PhD (Flu Watch Community Cohort study and the Health Survey for England Pandemic Influenza study) were designed to answer these questions by collecting prospective, representative, community-level data on influenza infection and illness regardless of whether they led to medical consultation.

1.5 The Research Question

The aim of this PhD is to inform control of seasonal and pandemic influenza through analysis of data from community surveys of influenza immunity, infection, and disease.

Specific objectives are to:

1. Investigate whether occupational exposure to pigs increases risk of seasonal, pandemic and zoonotic influenza infection
2. Describe the population-level patterns of influenza infection and immunity in England during the 2012/13 winter season
3. Quantify the work and school absences and health-related quality of life loss due to community influenza illnesses
4. Evaluate the success of the 2009 National Pandemic Flu Service Algorithm against its two primary aims and propose changes to the algorithm to better target community-level antiviral treatment

The four objectives reflect research questions based at different levels (primarily the lower levels) of the influenza iceberg of infection and disease. They can also be viewed as representing different chronological points along the pandemic path – beginning with sporadic zoonotic infections that can lead to the introduction and spread of a novel infection in the community at which point initial assessments of disease severity and impact will be made and pharmaceutical interventions will be initiated and then evaluated in hopes to improve them for the next pandemic.

1.6 Thesis structure

The remainder of this thesis is divided into 7 chapters (2-8). The next chapter (Chapter 2) describes the epidemiological studies that provided the data for this PhD. Chapter 3 describes the tools needed to measure influenza infection and immunity. These methods are then used in analytical chapters 4 and 5 concerning zoonotic influenza transmission (objective 1) and monitoring the spread of influenza during an epidemic or pandemic (objective 2) respectively. Chapter 6 describes the tools needed to measure influenza disease which prepare the reader for the two analyses in chapter 7 which focus on assessing the severity and impact of influenza disease (objective 3) and on the evaluation of a pharmaceutical intervention (objective 4). Chapter 8 concludes the thesis. It provides a summary of the main findings, outlines the strengths and weaknesses of the research, discusses the significance, implications and contributions of the work and provides

recommendations for further research and applications. Table 1-1 lists each objective, where it can be found in the thesis and the main methodologies used.

Table 1-1: Objectives, their location within the thesis and the main methodologies used

Thesis Chapter	Objective	Methods
Chapter 4	1. Investigate whether occupational exposure to pigs increases risk of seasonal, pandemic, and zoonotic influenza infection	• Serological analysis of a selection of human and swine Influenza A viruses in pig industry workers, a sample of pigs those workers were in contact with and a general population sample • Multivariable logistic regression models for each virus strain to estimate the association of occupational exposure to pigs and infection. Series of sub-analyses exploring pig farm workers sero-positivity to positivity status of their farms pig herd
Chapter 5	2. Describe the population-level patterns of influenza infection and immunity in England during the 2012/13 winter season	• Nationally representative cross-sectional serological surveys of currently circulating Influenza A strains in winter of 2012/13. • Descriptive analyses of the proportions of individuals with detectable and protective levels of antibodies and how they vary by demographics, vaccination status, over time and place and by virus strain
Chapter 7	3. Quantify the work and school absences and health-related quality of life loss due to community influenza illnesses	• Descriptive analyses of influenza A and B illness duration, percent of illnesses with reported time off work or education and mean time off • Mean and median quality-adjusted life days lost during influenza illness calculated by illness outcome and stratified by age group and whether or not cases were medically attended. • Total quality-adjusted life years lost and total number of days off work or education due to influenza A and B in England during 2010/11 influenza season estimated using Monte Carlo samples from the distributions of incidence of illness and QALD losses, or days off work, as appropriate, for each age-group

Chapter 7	4. Evaluate the success of the 2009 National Pandemic Flu Service Algorithm against its two primary aims and propose changes to the algorithm to better target community-level antiviral treatment	• Respiratory illnesses from Flu Watch classified according to the national pandemic flu service's (NPFS) clinical case definition and an alternative case definition. • Descriptive analyses of the percent of illnesses consulting medical services, and the percentage taking antibiotics or antivirals. • Subject all respiratory illnesses to the NPFS algorithm using the main and alternate case definition to assess performance. • Test characteristics (sensitivity and specificity) of clinical case definitions calculated among illnesses with PCR results.

Chapter 2 **Data sources**

The PhD uses data from two main sources: The Flu Watch study and the Health Survey for England Pandemic Influenza Study (PIPS study). The Flu Watch study was a modern version of the classic, gold-standard, household-based community cohort study [139,140]. The recruitment and follow up of participants was similar to the original community cohort studies done in the 1940s – 1970s [140], but the study utilized modern technology to collect illness data and modern laboratory methods to explore both humoral and (for the first time) cellular immunity on a large, population-based sample. The study was designed with both seasonal and pandemic influenza data collection in mind and the timing was such that it collected 5 consecutive years' worth of data (2006-2011) including pre-pandemic, pandemic and post-pandemic periods. Even though the study was running when the pandemic arose, there were substantial delays in the necessary pandemic scale-up because of delays in funding and the need to recruit, train and obtain relevant approvals for each practice before participants could be recruited.

The PIPS study was specifically designed to overcome the challenges experienced by the Flu Watch study by piggybacking on existing infrastructure provided by the Health Survey for England (HSE), an annual, nationally representative, household survey which collects health information and blood specimens throughout the year [98]. By adding questions and an additional blood sample to the HSE survey, the study established a streamlined system of conducting population-level serological surveys. It also benefits from having a pre-agreed pandemic reactivation mechanism, ethical approval and budget.

2.1 The Flu Watch Study

2.1.1 Attribution

The following work has been adapted from my first-author paper published in the International Journal of Epidemiology [139]. I wrote the paper with my colleague Dr Charlotte Warren-Gash and we did so in consultation with my PhD supervisor (PI of the Flu Watch Study) and the wider co-authors. I conducted the analysis and developed the tables and figures presented in section 2.1.

The Flu Watch study was a community-level, household-based cohort study of influenza infection and disease in England from 2006 to 2011.

2.1.2 **Participants**

Households were recruited from registers of 146 volunteer general practices (GP) across England who formed part of the MRC GPRF or (from the 2009 pandemic onwards) the Primary Care Research Network. Participants were selected from GP lists by computer-based random number generation. GPs sent invitation letters inviting the randomly selected person and their household to participate. Although it was recognised that this would bias invitations towards larger households, such as those with children, this was accepted as the role of children in influenza transmission was an important research question. Weighting by the inverse of household size in analyses was planned to account for this sampling design.

To be eligible to participate, the whole household had to agree to take part in follow-up over the coming winter with adults aged ≥16 years agreeing to have blood samples. Exclusion criteria included household size >6 people, individuals with terminal illness, severe mental illness or incapacity, and heavy involvement in other on-going research. GPs reviewed invitation lists and removed anyone meeting these criteria prior to sending letters. Cohorts were recruited to allow follow-up of participants over six influenza seasons – the 2006/07, 2007/08, 2008/09 periods of seasonal (interpandemic) influenza circulation and the first three waves of 2009 pandemic (summer 2009, autumn-winter 2009/10, and winter 2010/11). The Winter 2010/11 season could also be considered a return to the interpandemic circulation. From season 3 (2008/09) onwards, previous participants were invited to take part again.

In season 1 invitation letters were sent to 2300 households from 42 practices and 602 individuals from 243 households agreed to participate. In subsequent seasons the response rate was not monitored as practices (rather than the university study team) sent the invitation letters and not all returned data on numbers sent. Compared to the English population, young adults, non-white ethnic groups, people living in socially deprived areas and those living in the North of England, West Midlands and London were under-represented in the Flu Watch cohort, as reported in Table 2-1.

Table 2-1: Characteristics of responders by season compared to national averages

No. GP practices/Households / Persons	National	Nov 06 - Mar 07			Nov 07 - Mar 08			Nov 08 - Mar 09			May 09 - Sep 09			Oct 09 - Feb 10			Nov 10 - Mar 11		
		42/243/602			43/310/779			37/309/729			41/332/797			127/1460/3552			51/361/901		
	%	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Age group 0 to 4	6%	38	6.3	(4.5- 8.6)	42	5.4	(3.9- 7.2)	37	5.1	(3.6- 6.9)	36	4.5	(3.2- 6.2)	179	5	(4.3- 5.8)	45	5	(3.7- 6.6)
5 to 15	11%	87	14.5	(11.7-17.5)	110	14.1	(11.8-16.8)	99	13.6	(11.2-16.3)	109	13.7	(11.4-16.3)	501	14.1	(13.0-15.3)	131	14.5	(12.3-17.0)
16 to 44	42%	151	25.1	(21.7-28.7)	258	33.1	(29.8-36.5)	172	23.6	(20.6-26.8)	192	24.1	(21.2-27.2)	848	23.9	(22.5-25.3)	206	22.9	(20.2-25.7)
45 to 64	25%	203	33.7	(29.9-37.7)	272	34.9	(31.6-38.4)	267	36.6	(33.1-40.2)	293	36.8	(33.4-40.2)	1225	34.5	(32.9-36.1)	344	38.2	(35.0-41.4)
65+	16%	123	20.4	(17.3-23.9)	97	12.5	(10.2-15.0)	154	21.1	(18.2-24.3)	167	21	(18.2-23.9)	799	22.5	(21.1-23.9)	175	19.4	(16.9-22.2)
Gender Male	49%	281	46.7	(42.6-50.8)	366	47	(43.4-50.6)	340	46.6	(43.0-50.3)	377	47.3	(43.8-50.8)	1740	49	(47.3-50.6)	455	50.5	(47.2-53.8)
Female	51%	321	53.3	(49.2-57.4)	413	53	(49.4-56.6)	389	53.4	(49.7-57.0)	420	52.7	(49.2-56.2)	1812	51	(49.4-52.7)	446	49.5	(46.2-52.8)
Region North	28%	99	16.4	(13.6-19.7)	89	11.4	(9.3-13.9)	100	13.7	(11.3-16.4)	106	13.3	(11.0-15.9)	320	9	(8.1-10.0)	115	12.8	(10.7-15.1)
West Midlands	11%	42	7	(5.1- 9.3)	96	12.3	(10.1-14.8)	46	6.3	(4.7- 8.3)	53	6.6	(5.0- 8.6)	179	5	(4.3- 5.8)	53	5.9	(4.4- 7.6)
East & East Midlands	20%	122	20.3	(17.1-23.7)	120	15.4	(12.9-18.1)	124	17	(14.4-19.9)	118	14.8	(12.4-17.5)	1456	41	(39.4-42.6)	321	35.6	(32.5-38.9)
London	15%	28	4.7	(3.1- 6.7)	77	9.9	(7.9-12.2)	26	3.6	(2.3- 5.2)	28	3.5	(2.3- 5.0)	270	7.6	(6.7- 8.5)	65	7.2	(5.6- 9.1)
South East	16%	100	16.6	(13.7-19.8)	117	15	(12.6-17.7)	107	14.7	(12.2-17.5)	155	19.4	(16.8-22.4)	319	9	(8.1-10.0)	110	12.2	(10.1-14.5)
South West	10%	211	35	(31.2-39.0)	280	35.9	(32.6-39.4)	326	44.7	(41.1-48.4)	337	42.3	(38.8-45.8)	1008	28.4	(26.9-29.9)	237	26.3	(23.5-29.3)
Vaccine Vaccinated*	--	115	19.1	(16.0-22.5)	130	16.7	(14.1-19.5)	169	23.2	(20.2-26.4)	0	0	(0.0- 0.5)	157	4.4	(3.8- 5.1)	186	20.6	(18.0-23.4)
Unvaccinated	--	462	76.7	(73.2-80.1)	632	81.1	(78.2-83.8)	527	72.3	(68.9-75.5)	797	100	(99.5-100.0)	3159	88.9	(87.9-89.9)	715	79.4	(76.6-82.0)
Unknown	--	25	4.2	(2.7- 6.1)	17	2.2	(1.3- 3.5)	33	4.5	(3.1- 6.3)	18	2.3	(1.3- 3.5)	236	6.6	(5.8- 7.5)	0	0	(0.0- 0.4)
IMD quintile* **1** (most deprived)	20%	37	6.1	(4.4- 8.4)	39	5	(3.6- 6.8)	28	3.5	(2.3- 5.0)	18	2.3	(1.3- 3.6)	98	2.8	(2.2- 3.4)	29	3.2	(2.2- 4.6)
2	20%	88	14.6	(11.9-17.7)	126	16.2	(13.7-19.0)	91	12.5	(10.2-15.1)	62	7.8	(6.0- 9.9)	310	8.7	(7.8- 9.7)	82	9.1	(7.3-11.2)
3	20%	164	27.2	(23.7-31.0)	235	30.2	(27.0-33.5)	238	32.6	(29.3-36.2)	146	18.3	(15.7-21.2)	915	25.8	(24.3-27.2)	221	24.5	(21.8-27.5)
4	20%	162	26.9	(23.4-30.6)	250	32.1	(28.8-35.5)	187	25.7	(22.5-29.0)	146	18.3	(15.7-21.2)	938	26.4	(25.0-27.9)	280	31.1	(28.1-34.2)
5 (least deprived)	20%	151	25.1	(21.7-28.7)	129	16.6	(14.0-19.4)	185	25.4	(22.3-28.7)	425	53.3	(49.8-56.8)	1291	56.4	(54.3-58.4)	289	32.1	(29.0-35.2)
Ethnicity White	75%	553	97.9	(96.3-98.9)	733	95.4	(93.7-96.8)	666	99.1	(98.1-99.7)	730	99.1	(98.1-99.6)	3306	97.7	(97.1-98.2)	846	97.8	(96.6-98.7)
Non White	25%	12	2.1	(1.1- 3.7)	35	4.6	(3.2- 6.3)	6	0.9	(0.3- 1.9)	7	0.9	(0.4- 1.9)	78	2.3	(1.8- 2.9)	19	2.2	(1.3- 3.4)

*Vaccinated for that influenza season (before or during follow-up)

** Index of Multiple Deprivation (IMD)

Figure adapted from Fragaszy et al, 2016 [139].

2.1.3 The Basic Cohort Design

2.1.3.1 *Baseline/pre-season phase*

A baseline visit was made to the household at enrolment, during which a research nurse collected blood samples for serological and T cell analysis from all adults 16 years or older. Blood sampling was optional for those aged 5-15 years and not done in those under 5 years of age. Visits occurred in the evenings as blood specimens had to be couriered overnight to Oxford for early morning analysis of T cells. The serum samples collected were centrifuged, frozen and later batch-tested for influenza antibodies by the HPA. Nurses assisted families with a series of laptop-based surveys collecting information on basic demographics, health and chronic illness, respiratory hygiene, household structure and relationships, accommodation, contacts and activities. Households received participant packs containing paper illness diaries, thermometers and nasal swab kits including instructions on their use and viral transport medium to be stored in the refrigerator.

2.1.3.2 *Active Follow-up during influenza season*

In order to obtain reliable measures of the number of illnesses we actively contacted participants every week with automated telephone calls to assess the presence or absence of respiratory illness in each household member. For each respiratory illness, participants were reminded to fill in a prospective paper illness diary. These collected the following information: illness onset date, temperature, presence and severity of symptoms such as feeling feverish, headache, muscle aches, cough and sore throat. Diaries also collected data on contact patterns and activities before and during illness. Participants took a nasal swab on day two of any respiratory illness for RT-PCR analysis of influenza, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), rhinovirus, coronavirus, adenovirus and parainfluenza virus. During the first season, swabbing was limited to periods of influenza circulation. The Sanger Institute genetically sequenced some of the viral isolates from the summer and winter waves of the pandemic (seasons 4-5).

In addition, all participants completed one-off activity and contact paper diaries on at least one pre-determined weekday and one weekend day during the active follow-up period. These diaries collected information on where participants were (i.e. at home, at work, etc.), whether they had contact with crowds and the number, duration and age groups of personal contacts throughout the day.

2.1.3.3 *Post-season phase*

At the end of follow-up, nurses made a final household visit to take a follow-up blood sample (for paired serology) and assist participants with an exit survey. Nurses also checked participants' medical records for information on chronic illnesses, influenza and pneumococcal vaccinations, prescriptions, GP consultations, hospitalisations and deaths.

2.1.4 Evolution of data collection

The cohort evolved over time to maximise system reliability, minimise the number of data sources and allow increased recruitment during the pandemic. In season 3 we offered participants the option of moving from paper illness diaries with weekly automated phone calls to weekly emailed surveys, with or without optional SMS reminders. For the pandemic and post-pandemic cohort most surveys moved to a custom-built website for self-completion. In order to achieve real time monitoring of illnesses during the pandemic, participants were emailed a link to a retrospective online weekly survey and provided with laminated wipe-clean charts at home to record daily symptoms as a memory aid.

In season 3 there were additional one-off surveys collecting data on indoor and outdoor temperature and humidity, travel patterns and non-response to weekly surveys. During seasons 5 and 6 we added questions to existing surveys on attitudes towards influenza vaccination and antivirals. In season 6 we included quality of life questions [141].

2.1.5 Evolution of Cohort Design

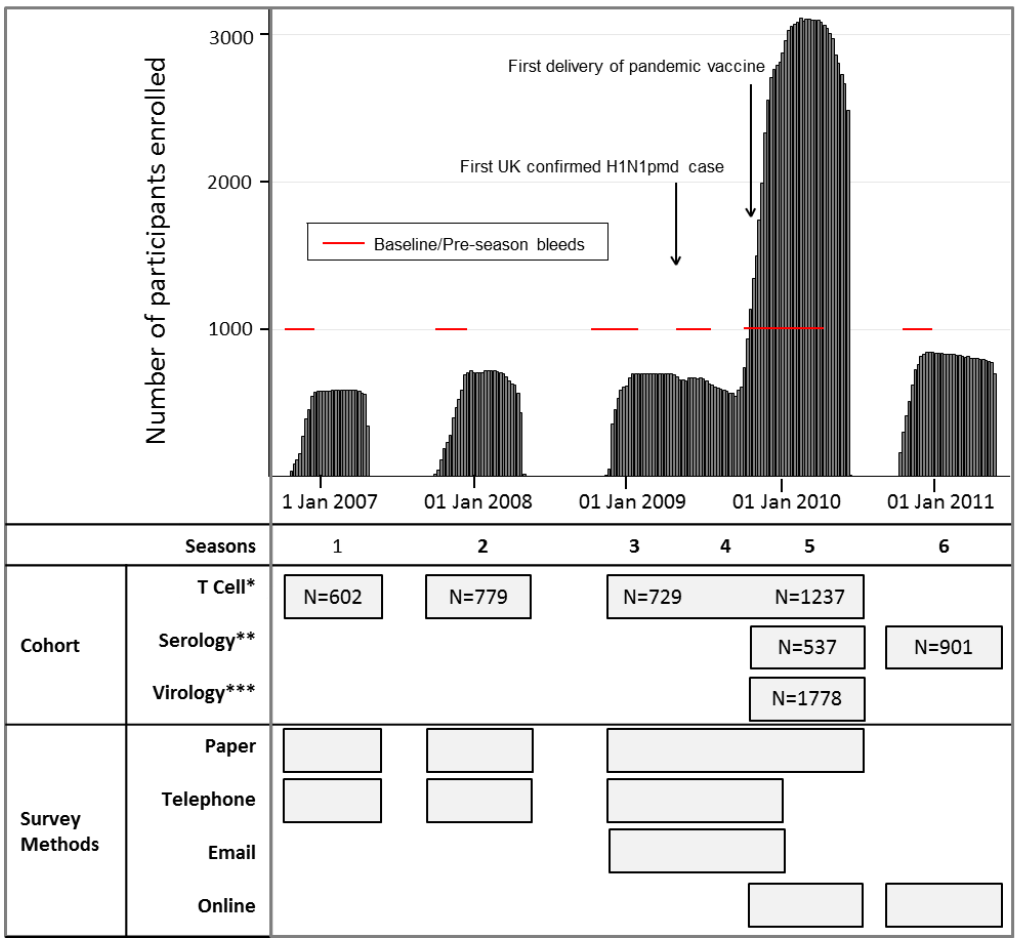
The cohort design evolved with the emergence of the novel H1N1 pandemic strain during season 3. We continued active follow-up through the UK summer wave of the pandemic (season 4). For the UK winter wave of the pandemic (season 5) the study split into 3 separate cohorts: T cell (comprising both previous and newly recruited participants), Serology and Virology (both comprising new participants). For the T cell cohort continuing participants used the spring blood sample from season 3 as a baseline sample. They also gave a pre-vaccination blood sample to allow distinction of antibody rises caused by infection rather than vaccination. This was particularly important for the winter wave of the pandemic as we anticipated widespread vaccination. The Serology cohort was identical but lacked T cell samples. For the Virology cohort, no blood samples were taken. This allowed for rapid recruitment of a large number of participants (n=1,778) to increase

the accuracy of weekly estimates of illness rates during the pandemic with minimal nurse time required. While all nasal swabs were tested for Influenza A and B, RSV and hMPV, due to the large number of samples generated during the pandemic, only a selection of swabs seasons 5 and 6 were tested for other viruses.

2.1.6 Loss to follow-up and missing data

Retention of enrolled participants throughout the cohorts was good. Figure 2-1 displays the number of enrolled participants each week with arrows pointing out the staggered starts and exits of the cohorts along with other important dates. Loss to follow-up came in two main varieties; non-response to weekly contact and loss to follow-up for paired blood samples.

Figure 2-1: Number of enrolled participants, baseline/pre-season bleed periods and different cohorts and data collection methods over time.



* T cell cohorts included T cell, serological and virological (RT-PCR) measurements.
** Serology cohorts included serological and virological (RT-PCR) measurements.
*** Virology cohort only included virological (RT-PCR) measurements.
Figure adapted from Fragaszy et al, 2016 [139].

We obtained weekly responses from 87.3% (95% CI: 86.5% - 88.8%) of follow-up weeks overall, which increased to 88.4% if we exclude periods when there were technical difficulties with our automated phone calls (one week in season 1 and four weeks in season 2). Response completeness generally increased after the introduction of email and online surveys in season 4 (Table 2-2). Only 12.4% (95% CI: 11.2% – 13.5%) of households were classified as poor responders (responding to <70% of follow up weeks). Poor response appeared to be more common as deprivation increased.

2.1.7 **Data Collected and Outcomes**

The three main clinical outcomes were 1) influenza-like illness (ILI), defined as a respiratory illness with cough and/or sore throat and fever >37.8°C, 2) RT-PCR-confirmed influenza illness and 3) influenza seroconversion, defined as a four-fold titre rise in strain specific antibody titres in unvaccinated individuals.

Table 2-3 summarises the data and biological samples collected during baseline, active follow-up and post-season phases. We additionally linked participants' data to small area statistics such as the Index of Multiple Deprivation (IMD) and rural/urban indicators [142,143]. Details of the T cell methodology have been described previously [144–146].

Table 2-2: Characteristics of Non-responding Households (≥30% missing weeks)

Household Characteristics		Good Responders (<30% missing wks)			(≥30% missing wks)			Total
		n	%	95% CI	n	%	95% CI	N
Overall		2640	87.6	(86.5-88.8)	372	12.4	(11.2-13.5)	3012
Season	Nov 2006 – Mar 2007 (1)	199	81.9	(77.1-86.7)	44	18.1	(13.3-22.9)	243
	Nov 2007 – Mar 2008 (2)	202	65.8	(60.5-71.1)	105	34.2	(28.9-39.5)	307
	Nov 2008 – Mar 2009 (3)	287	92.9	(90.0-95.7)	22	7.1	(4.3-10.0)	309
	May 2009 – Sep 2010 (4)	246	74.1	(69.4-78.8)	86	25.9	(21.2-30.6)	332
	Oct 2009 – Feb 2010 (5)	1370	93.8	(92.6-95.1)	90	6.2	(4.9-7.4)	1460
	Nov 2010 – Mar 2011 (6)	336	93.1	(90.5-95.7)	25	6.9	(4.3-9.5)	361
Social Class	Managerial & Professionals	712	87.6	(85.3-89.8)	101	12.4	(10.2-14.7)	813
	Intermediate Occupations	362	87.9	(84.7-91.0)	50	12.1	(9.0-15.3)	412
	Small Employers & Own Account Workers	209	85.3	(80.9-89.7)	36	14.7	(10.3-19.1)	245
	Lower Supervisory and Technical Occupations	111	84.1	(77.9-90.3)	21	15.9	(9.7-22.1)	132
	Semi-routine and Routine Occupations	441	86.5	(83.5-89.4)	69	13.5	(10.6-16.5)	510
	Retired	497	94	(91.9-96.0)	32	6	(4.0-8.1)	529
	Student	109	84.5	(78.3-90.7)	20	15.5	(9.3-21.7)	129
	missing	199	82.2	(77.4-87.0)	43	17.8	(13.0-22.6)	242
IMD quintile	1 (most deprived)	85	81	(73.4-88.5)	20	19	(11.5-26.6)	105
	2	255	84.7	(80.7-88.8)	46	15.3	(11.2-19.3)	301
	3	704	86.9	(84.6-89.2)	106	13.1	(10.8-15.4)	810
	4	732	89.6	(87.5-91.7)	85	10.4	(8.3-12.5)	817
	5 (least deprived)	864	88.3	(86.2-90.3)	115	11.7	(9.7-13.8)	979
Rural/Urban	Urban >10k	1505	86.7	(85.1-88.3)	230	13.3	(11.7-14.9)	1735
	Town & Fringe	373	90.3	(87.5-93.2)	40	9.7	(6.8-12.5)	413
	Village, Hamlet & Isolated Dwellings	643	89.9	(87.7-92.1)	72	10.1	(7.9-12.3)	715
	missing	119	79.9	(73.4-86.3)	30	20.1	(13.7-26.6)	149
Household size	1	354	84.5	(81.0-88.0)	65	15.5	(12.0-19.0)	419
	2	1405	89.7	(88.2-91.2)	162	10.3	(8.8-11.8)	1567
	3	344	85.1	(81.7-88.6)	60	14.9	(11.4-18.3)	404
	4	407	87.3	(84.3-90.4)	59	12.7	(9.6-15.7)	466
	5	109	84.5	(78.3-90.7)	20	15.5	(9.3-21.7)	129
	6	21	77.8	(62.1-93.5)	6	22.2	(6.5-3.9)	27
Number of Children in the Household	0	1932	89.1	(87.8-90.4)	236	10.9	(9.6-12.2)	2168
	1	247	81.8	(77.4-86.1)	55	18.2	(11.5-18.3)	302
	2	360	85.1	(81.7-88.5)	63	14.9	(11.5-18.3)	423
	3	83	86.5	(79.6-93.3)	13	13.5	(6.7-20.4)	96
	4	18	78.3	(61.4-95.1)	5	21.7	(4.9-38.6)	23
Region	North	305	87.9	(84.5-91.3)	42	12.1	(8.7-15.5)	347
	West Midlands	164	84.1	(79.0-89.2)	31	15.9	(10.8-21.0)	195
	East & East Midlands	828	90.5	(88.6-92.4)	87	9.5	(7.6-11.4)	915
	London	164	84.5	(79.4-89.6)	30	15.5	(10.4-20.6)	194
	South East	314	83.5	(79.8-87.3)	62	16.5	(12.7-20.2)	376
	South West	865	87.8	(85.8-89.9)	120	12.2	(10.1-14.2)	985

Figure adapted from Fragaszy et al, 2016 [139].

Table 2-3: Questionnaire data and biological samples collected in three data collection periods.

Phase	Data Type	Data and Samples	Season					
			1	2	3	4	5	6
Baseline / Pre-season	**Self-reported surveys**	Basic demographic, socioeconomic, health, vaccination and potential risk factors for influenza	X	X	X	X	X	X
		Quality of life (EQ5D)						X
	Blood samples	H1N1, H3N2 and Flu B Serology*	X	X	X			
		H1N1pdm09 Serological Serology*				X	X	X
		T cell analysis**	X	X	X		X	
Active Follow-up	**Self-reported surveys**	Respiratory illness timing and characteristics (if ill)	X	X	X	X	X	X
		Risk Factors in previous week (if ill)	X	X	X			
		Time off work/education (if ill)	X	X	X	X		
		Health seeking behaviour and medicines taken (if ill)					X	X
		Full Contact and activity diaries (if ill)	X	X				
		Basic contact and activities (if ill)				X		
		Influenza vaccination that week				X	X	X
		Full Contact and activity diaries (one-off survey)	X	X	X	X	X	X
		Indoor/outdoor temperature & humidity (one-off surveys)	X	X	X			
		Detailed travel survey (one off survey)			X			
	Self-administered nasal swabs	RT-PCR Influenza A (H1 & H3 subtypes), Influenza B, RSV and human metapneumovirus	X	X	X	X	X	X
		RT-PCR Influenza A H1N1pdm09				X	X	X
		RT-PCR Rhinovirus, Coronavirus, Adenovirus and Para-influenza virus***	X	X	X	X	X	X
		Selected viral samples genetically sequenced	X	X	X	X	X	X
	Bloods†	H1N1pdm09 Serological Serology					X	
Post-Season	**Self-reported surveys**	Changed household composition, pregnancy, vaccination, hospitalisation, death & flights	X	X	X	X	X	X
		illness reporting behavior during follow-up			X		X	X
		Attitudes towards vaccination and antivirals					X	X
	Medical records††	chronic illness, vaccination, prescriptions, GP & hospital consultations and death	X	X	X	X	X	X
	Bloods	H1N1, H3N2 and Flu B Serology*	X	X	X			
		H1N1pdm09 Serological Serology*					X	X
		T cell analysis**	X		X			
	Saliva Samples††††	Genetic analysis	X	X	X	X	X	X

* Haemagglutination-inhibition assay

**Peripheral blood mononuclear cells (PBMC) separated, part of the sample was immediately tested against pools of peptides representing each of the virus proteins in an exvivo IFN-γ elispot assay [144,145] and the rest of the sample was frozen for more detailed peptide mapping studies using IFN-γ elispots and/or in vitro culture and testing by intracellular cytokine staining to determine CD8/4 restriction. Post-season T cell analysis was only conducted in seasons 1 and 3.

***Only a selection of nasal swab samples were tested for these viruses in seasons 5 & 6.

† Only taken from participants in T cell and Serology Cohorts prior to influenza vaccination.

†† Medical record checks were requested for all participants except those in the Virology Cohort.

††† Saliva samples were collected in 2011 -2012 from selected participants participating from all seasons and cohorts.

Figure taken from Fragaszy et al, 2016 [139].

2.1.8 **Strength and Weaknesses**

Flu watch is a large community cohort study broadly representative of the population of England. It is the first modern-day household study of influenza transmission in a temperate climate comparable to the landmark Tecumseh studies of the 1960's and 70's [147]. A major strength is the inclusion of different household types (rather than just households with children as in earlier studies) which allows influenza infections to be explored across the whole of society. We actively followed up cohorts for influenza and other respiratory viruses, utilising a range of IT-based technologies including automated telephone surveys, email, internet, and text messages. Broadly similar methods of follow-up were used across six influenza seasons, allowing accurate comparisons of disease burden estimates between pandemic and interpandemic periods despite external factors (such as media reporting during the pandemic) that may have affected consultation behaviour. Robust definitions of influenza were based on a range of diagnostic methods including real-time symptom reporting, RT-PCR and serology, allowing the emergence of the A(H1N1)pdm09 pandemic strain to be tracked. Serological and virological data from previous pandemics are either unavailable (1918 H1N1 pandemic), from small samples sizes (1957 H2N2 pandemic) [148], or from populations with high vaccination rates which greatly limits interpretation (1968 H3N2 pandemic) [147]. Historical data on laboratory-confirmed rates of seasonal influenza mainly come from historical community studies of families in the United States between 1948 and 1981 [140,147,149,150]. Flu Watch is a good example of collaboration between disciplines (epidemiology, immunology, virology and primary care) and partners. The study provides a rich source of data on social, behavioural and biological factors affecting influenza transmission enabling exploration of many research questions.

Limitations include major delays in obtaining funding, ethics and R&D approval across multiple sites, resulting in delayed recruitment during the pandemic and fewer participants overall. Although the initial response to invitation letters was low, it is unclear if this would bias results. Ideally, cohorts would have had pre- and post-influenza season bleeds but recruitment periods were not perfectly streamlined with influenza seasons so adjustments for bleed timings were made during analysis. The study design and data collection methods evolved in response to experience and changing questions. While this optimised and streamlined methods, it also increased complexity of data management.

2.2 The Health Survey for England Serosurvey (Pandemic Immunity and Population Spread – PIPS study)

2.2.1 Attribution

The following work has been adapted from my first author paper published in Public Health Research [98]. I led the overall implementation and analysis study of the PIPS study. For the work presented in section 2.2, I conducted the analysis, developed the tables and wrote the published paper.

2.2.2 History of the PIPS project

In 2009, before the PIPS project was funded, Dr Andrew Hayward worked with colleagues at the Health Survey for England to include pandemic related questions into the upcoming 2010 HSE. This collaboration fostered the idea for using the HSE for real-time serosurveillance and when the NIHR commissioned research projects to pilot pandemic influenza research, the PIPS project was developed. The core objective of the PIPS project was to develop the HSE as a means of conducting real-time population-level serological surveys. However the project also utilised the questionnaire data previously collected in the 2010 HSE survey.

2.2.3 Background

Assessing the severity and spread of a novel influenza strain at the start of a pandemic is critical for informing a targeted and proportional response. It requires understanding of the community burden of infection and disease which can be determined only through community level studies. However, rapidly initiating such studies at the start of a pandemic is difficult.

Our experience from running the Flu Watch study during the 2009 pandemic demonstrated that recruiting community cohorts through primary care introduced substantial delays. These delays related to the need to recruit and train multiple GP practices and the need to obtain appropriate ethical and research & development approvals for each of these sites. The research project described here was specifically designed to overcome these barriers so that we could measure the key parameters rapidly in the event of a pandemic.

2.2.4 Aims & Objectives

The study aimed to establish an efficient system allowing real-time assessment of population susceptibility, spread of infection and clinical attack rates in the event of a pandemic. Specific objectives were to:

- Develop the Health Survey for England (HSE) as a tool for rapid population-based surveys of influenza infection and influenza-like illness rates.
- Provide monthly measures of numbers of cases infected and weekly updates on numbers of influenza-like illnesses during the first two infection waves of a pandemic, to act as denominators for national estimates of case fatality and hospitalisation rates.
- To assess spread of the novel influenza strain geographically, by age, and through time.

2.2.5 Methods

2.2.5.1 *Project overview*

This research project had two components: a pre-pandemic component designed to develop and assess a system to monitor population susceptibility, severity and spread of pandemic influenza and a pandemic component designed to be triggered rapidly in the event of an influenza pandemic.

The monitoring system was designed as a series of cross-sectional serological prevalence studies with retrospective ascertainment of vaccination and respiratory illness history in conjunction with the HSE.

2.2.5.2 *HSE study design*

The HSE is a series of annual surveys which have monitored the nation's health since 1991. All HSE surveys involve a stratified random probability sample of private households in England. There are two parts to the HSE surveys: a household interview visit where a trained interviewer collects information on participants' health and health-related behaviours and measures height and weight, and later a nurse visit where additional

information, measurements and biological samples (including blood for those aged ≥ 16) are collected. The HSE is a rolling survey meaning that the household interviews are spread throughout the calendar year. The main interview schedule is designed so that each quarter is nationally-representative of all private households in England[151].

HSE Blood specimens and data are made available for analysis only after the end of each annual survey, meaning that, without the adaptation agreed for this project, the survey could not normally be used for real-time research.

2.2.5.3 *PIPS project phases and HSE adaptations*

The serological surveillance system we have designed simply adds a small number of additional pandemic-related questions for all participants to answer and an extra blood sample (for those aged 16 years and over) for serological testing to the nurse visit.

The project phases and analyses are broken down into four phases:

- Phase 1 (HSE 2010) - Retrospective validation of population-level influenza-like illness rates derived from the 2010 HSE survey by comparison with illness rates from the concurrently running Flu Watch study.
- Phase 2 (HSE 2012/2013) - Pilot of specimen and data collection alongside the 2012/13 HSE surveys and development of automated analysis and reporting of real-time, monthly measures of the immunity profile (proportion susceptible, immune and vaccinated) and vaccine uptake in the population.
- Phase 3 (ongoing since HSE 2014) - Holding or 'hibernation' phase during which the project is prepared for and ethically approved each year but only triggered in the event of a pandemic.
- Phase 4 (future pandemic) - Real time monitoring through first two infection waves of a pandemic using the methods developed in phase 2 in order to

provide rapid estimates of severity and spread and to monitor any changes to these estimates through the pandemic.

2.2.5.4 *Phase 1*

The main details of the HSE survey methodology are described above and details specific to the 2010 HSE survey methodology have been published previously[152]. An important aspect of the 2010 HSE is that it included a child boost sample in order to increase the number of participants in the age group, thereby increasing accuracy of estimates for this age group. In response to the 2009 pandemic, we included a special "swine flu" section in the 2010 HSE survey main interview where participants were asked about influenza vaccination uptake and timing, and influenza-like illness including timing, duration of sick leave, and treatment. Specifically, participants were asked if they had experienced a 'flu-like illness, where you felt feverish and had a cough or sore throat?' since May 2009. If they had, then the month and year of that illness was also recorded. Since the interviews for the 2010 HSE took place over the 2010 calendar year, the participants would have been recalling illnesses and/or vaccinations anywhere from the past 9 months (those interviewed in January 2010) to the past 21 months (those interviewed in the December 2010). If the participant had more than one illness during the time period, they chose which one to report.

2.2.5.5 *Phase 2*

The only changes made to the 2012/2013 surveys were at the nurse interview stage (not the main interview) and these changes were only in place during a six-month period (Oct 2012 through March 2013). For the purposes of this project, the HSE nurses added an additional 5ml blood sample (one extra bottle) to the existing HSE blood collection process. These specimens were transferred along with the rest of the HSE specimens to the Newcastle General Hospital Microbiological laboratory where they were centrifuged and frozen. The serum samples were later transferred to University College London Hospital (UCLH) microbiology laboratory for serological analysis. We also collected basic demographic data (age and sex) and information on recent influenza vaccination and ILI from all HSE participants at the nurse interview stage, not just those contributing blood samples. These data were collected on a separate form which accompanied the project blood samples to UCLH. This enabled real-time access to these data which otherwise would not be possible.

Serological protocols and assays were developed by the laboratory team for phase two and adapted as a template for use in the pandemic phase. Automated analysis and reporting of age–specific rates of influenza-like illness and monthly estimates of the age-specific proportion of the population with protective antibodies accounting for vaccination (for those aged 16 years and over) and the proportion vaccinated. Using these data we can estimate the number of infections nationally which can be used as a denominator by Public Health England and the research community in calculations of the case fatality proportion and rates of hospitalisation (both measures of severity).

2.2.5.6 *Phase 3*

Included within each annual HSE planning round and ethics application is the ability to trigger, in the event of a pandemic threat, the collection of an additional 5ml blood specimen and data on vaccination and recent respiratory illness history. This enabled, should the study be triggered, the rapid roll out of the system previously described.

2.2.5.7 *Phase 4*

In the event of a pandemic, the collection and transfer of specimens and data could be triggered within one working week. We would use the automated analytical routines developed in phase 2 to continually either directly estimate or provide the denominators necessary to estimate pandemic severity, susceptibility and spread throughout the course of the pandemic. Reports would be sent fortnightly to Public Health England and findings would be presented to key decision makers through the researchers' positions on government advisory committees and links with key policy makers.

Chapter 3 Tools to measure Infection and Immunity

This chapter will describe the methods used to identify and evaluate influenza infection and antibody-mediated immunity. The methods described in this chapter are utilized in the analyses presented in chapters 4 and 5. This chapter will cover the main laboratory assays and how they are incorporated into epidemiological studies. It concludes with a brief overview of the burden of infection in the community.

3.1 Introduction to Serology

Serological methods are used to quantify influenza antibodies that are produced by the immune system in response to natural infection and/or influenza vaccination[3,153]. Depending on the context, strain-specific serological assays can retrospectively identify infections (including those that were asymptomatic), assess an individual's past exposure and response to influenza viruses and vaccines and, if an assay has an immunological correlate of protection, results can help predict the likelihood that that individual is protected against infection with that strain in the future[154,155]. Serological methods are used in a variety of settings including surveillance, epidemiological studies, vaccine evaluation and occasionally for the purpose of diagnosis[154]. The strain-specific serological assays most commonly used are the Hemagglutination inhibition (HI), virus neutralization (now commonly conducted as microneutralization (MN)) and single radial hemolysis (SRH)[156]. The three assays detect different, but overlapping groups of antibodies, primarily against the HA protein, and their results are strongly correlated[156,157].

3.2 Laboratory Methods

3.2.1 Hemagglutination Inhibition

The most widely used serological assay used for the detection of strain-specific influenza antibodies is the Hemagglutination Inhibition (HI) assay[154].

The HI assay measures the ability of an individual's serum antibodies to block (i.e. inhibit) influenza's HA surface protein from binding to the sialic acid on red blood cells, which has the effect of clumping (i.e. agglutinating) the red blood cells together. This is meant to mirror the more relevant process of the influenza HA binding to the sialic acid on target cells in the respiratory track in order to initiate infection[158]. In the HI assay, twofold serial

dilutions of an individual's sera are mixed with a standard concentration of virus. The dilutions are left to react for a certain amount of time, allowing the anti-HA serum antibodies time to bind to the viruses' HA surface proteins. This is followed by the addition of a standard concentration of red blood cells[5,154]. If there are sufficient anti-HA antibodies in the dilution, these antibodies will inhibit agglutination. Conversely, if there are no anti-HA antibodies or too few of them, the virus will agglutinate the red blood cells. The outcome of the assay is the strain-specific HI titre, measured as the reciprocal of the highest dilution which fully inhibits agglutination[154].

The HI assay is inexpensive and relatively easy to perform[155]. Disadvantages include the need to remove non-specific inhibitors, a low sensitivity to influenza B and avian influenza strains and issues with variability between laboratories[154,155]. The HI assay is currently considered to be the best immunological correlate of protection (i.e. the best parameter for predicting whether an individual is protected against infection)[155]. As HI titre increases, so does protection against infection, although there appears to be diminishing returns for increases in tires above 150 or so[159]. An HI titre of 40 is generally considered to be 'protective' in that it corresponding to a 50% reduction of risk of infection in a susceptible population, although some have argued that a higher cut-off would be more appropriate in certain populations such as children and the elderly[154,160,161].

3.2.2 **Virus Neutralization**

Virus neutralization is an assay that identifies predominantly strain-specific functional antibodies against both HA and NA proteins that neutralize the viruses' ability to infect, and be released from mammalian cells in vitro [154,155]. The assay begins by mixing virus with dilutions of serum for a specified period of time. The virus and serum mixture is then inoculated into a host system for a period of time after which levels of virus or viral antigens are measured. Modern virus neutralization assays are commonly done in the microneutralization (MN) format. The MN titre outcome is measured as the reciprocal serum dilution which inhibits at least 50% of cytopathic effect in mammalian cell culture[156]. Results from virus neutralization assays show a high degree of correlation with HI assays[156]. They identify a wider range of antibodies involved in protection against infection, including some cross-reactive antibodies. However, this also means they can be less strain-specific than HI assays[154,155]. In contrast they are more sensitive than HI assays, particularly for influenza B and for seroconversions occurring at low titres[154,155]. The

assays require live virus which in the case of wild-type highly pathogenic viruses, require the use of high-level biocontainment laboratories. Other disadvantages include variation between laboratories and the lack of an established correlate of protection[155].

3.2.3 Single radial hemolysis

The single radial hemolysis (SRH) assay detects mainly complement activating antibodies which target HA and NA proteins and internal antigens[155]. The assay is performed using red blood cells bound with virus and complement in an agarose gel. Undiluted serum is added to wells in the agarose which diffuses overnight. In the presence of anti-influenza antibodies, the overnight diffusion will leave zones of complement-mediated hemolysis of the red blood cells which will be proportional to the amount of antibodies present [154–156]. The assay has an established correlate of protection (hemolysis area of 25mm corresponds to 50% protection). In comparison to HI, it is more sensitive to influenza B, better able to distinguish between similar strains and better able to detect small differences in the levels of antibodies. It also has the additional advantages of being rapid, simple, reliable, reproducible, scalable and unbiased.

3.3 Identifying infections

Influenza viruses are constantly evolving, and individuals are likely to be infected with- and potentially vaccinated against multiple influenza strains during their lifetime. As a result, individuals build up a collection of antibodies reflecting those previous exposures to different strains[154].

In the context of seasonal influenza, even the strain-specific serological assays described here may detect cross-reactive antibodies produced from previous infection or vaccination against a closely related strain[154]. Therefore, in order to identify recent infection with a specific seasonal strain, one needs to evaluate the change in strain-specific antibody titres between consecutive (also known as ‘paired’ or ‘bracketed’) serological samples from the same individual. A four-fold rise in strain-specific antibody titre between the paired samples is considered a seroconversion and is taken as evidence that the individual was infected (either symptomatically or asymptomatically) with that strain between the two blood samples[154]. Two-fold titre rises have not traditionally been considered robust evidence of infection given the possibility of measurement error. However recent work

suggests that some infections may only lead to a two-fold titre rise, particularly in those with already high baseline antibody titres[154,162]. In order to rule out the possibility that a titre rise was due to vaccination and not natural infection, the recent influenza vaccination history of the participant must also be known.

In the context of a virus with which has not widely circulated in the population such as a zoonotic or new pandemic virus, it may be possible to identify infection through a high titre result from a single serological sample provided that the prevalence of pre-existing, cross-reactive antibodies to that virus is very low in the population. Once vaccination to that virus has been introduced, the vaccination status of the individual must be known to confirm that a high titre is the result of natural infection and not vaccination.

3.4 Serology in Epidemiological Studies

Serological methods can be incorporated into cohort or cross-sectional studies in order to measure cumulative incidence (i.e risk) or incidence rates of infection.

In cohort studies, participants are prospectively followed up over time and provide individually paired serum samples at given time points, typically pre- and post- influenza season or epidemic. By assessing participants' seroconversion status, it is possible to directly calculate cumulative incidence and/or incidence rates of influenza infection.

In serial-cross sectional studies, serum specimens are collected at two or more time points (ideally before and just after an outbreak or epidemic) from different samples of people. The cumulative incidence is then indirectly calculated by comparing the proportion of sampled individuals with antibody titres above a specified threshold between the two time points. In the context of an emerging pandemic where there is little to no pre-existing immunity to a strain, post-epidemic serum samples alone can be used to calculate cumulative incidence.

Alternatively, in the context of identifying sporadic zoonotic influenza infections in an individual, a single serum sample can be used to identify previous exposure and/or infection with strains that do not circulate among humans.

3.5 Burden of Infection in the community

Influenza infection is relatively common. In the Flu Watch study (based on 4 fold rises in antibody titres) we found that around 18% (95% CI: 16% - 22%) of the unvaccinated population became infected each winter season with the highest rates in children[15]. The estimates were similar in the pre-pandemic, pandemic and post-pandemic periods covered by the Flu Watch study. This finding is similar to those found in historical and other contemporary community cohort studies[140,147,163].

Chapter 4 **Infection at the Human/Animal Interface**

This work focuses on a population sub-group at the human/animal interface, UK pig industry workers. It investigates whether pig industry workers have greater odds of infection for several human- and swine-adapted influenza strains compared to a general population cohort (the cohort studied in Flu Watch).

4.1 Attribution

The work presented in Chapter 4 has been adapted from my first author paper published in Influenza and Other Respiratory Viruses [164]. I co-led this work with Dr Ishoa. He led the literature review and wrote the first draft of the introduction and conclusions. I developed the overall analytical strategy, managed and collated the data, conducted the analyses and produced the tables. I wrote the methods and results section and contributed to the editing of the other sections. I, along with my co-authors, contributed to the overall interpretation of findings. More information on attribution can be found in the original paper. For the purposes of this thesis, I have modified the original manuscript to flow logically within this thesis.

4.2 Abstract

Background: Pigs are mixing vessels for influenza viral reassortment, but the extent of influenza transmission between swine and humans is not well understood.

Objectives: To assess whether occupational exposure to pigs is a risk factor for human infection with human and swine-adapted influenza viruses.

Methods: UK pig industry workers were frequency-matched on age, region, sampling month, and gender with a community-based comparison group from the Flu Watch study. HI assays quantified antibodies for swine and human A(H1) and A(H3) influenza viruses (titres ≥ 40 considered seropositive and indicative of infection). Virus-specific associations between seropositivity and occupational pig exposure were examined using multivariable regression models adjusted for vaccination. Pigs on the same farms were also tested for seropositivity.

Results: Forty-two percent of pigs were seropositive to A(H1N1)pdm09. Pig industry workers showed evidence of increased odds of A(H1N1)pdm09 seropositivity compared to the comparison group, albeit with wide confidence intervals (CIs), adjusted odds ratio after accounting for possible cross-reactivity with other swine A(H1) viruses (aOR) 20.44, 95% CI: 2.24–186.4), p=0.007.

Conclusion: The results indicate that A(H1N1)pdm09 virus was common in UK pigs during the pandemic and subsequent period of human A(H1N1)pdm09 circulation, and occupational exposure to pigs was a risk factor for human infection. Influenza immunisation of pig industry workers may reduce transmission and the potential for virus reassortment.

4.3 Introduction

Influenza A viruses can cause significant morbidity and mortality in humans and other animal species and show a high degree of genomic variability and adaptability. They are categorised by subtype based on their main surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), which determine a range of key properties including antigenicity. Human-adapted viruses in the past century have been those expressing HA subtypes 1, 2, and 3 and NA subtypes 1 and 2. Since 1968, only the A(H1N1) and A(H3N2) subtypes have circulated widely in humans [165]. Observations over the past 40 to 50 years have documented subtypes of viruses A(H1N1), A(H1N2) and A(H3N2) circulating in pigs worldwide and strain variations between Europe, North America and Asia have been noted [166]. In the UK, A(H1N2) was the most commonly observed swine subtype in a large pig serosurvey conducted between 2008-2009 [167]. Between 1998 and 2009, an avian-like H1N1 strain most commonly and an H1N2 strain were regularly detected in UK pigs [168,169]. The A(H1N1)pdm09 virus was detected in pig herds from autumn 2009, [170] although it may have been first transmitted to pigs from humans several months earlier [171].

Influenza viruses bind to host cell surface receptors with a terminal sialic acid (SA), different versions of which are present in different animal species forming the basis of host-strain specificity [172,173]. Avian strains preferentially bind to SA α2,3-Gal (prevalent in avian species) while human virus strains require SA α2,6-Gal receptors (dominant in humans). The relatively poor fit of avian viruses to human and other non-avian hosts is thought to limit the potential emergence of novel strains [174]. Pigs (and many other species) express

both types of receptors such that could be potentially susceptible to both avian and human viruses.

Co-infection of a single host with two different strains of the influenza virus provides an opportunity for genetic reassortment (rearrangements and altered combinations of genome segments), which could lead to sudden and marked changes (antigenic shift) and the emergence of novel strains or subtypes expressing new surface antigen proteins that the host might have little or no immunity against. Should the newly acquired properties of such a novel strain make it transmissible in humans, then it would have the potential to start a pandemic. Pigs are a particularly important species in this regard as the occurrence of both types of SA receptors permits binding of human and avian influenza viruses making them an efficient "mixing vessel" [175–178]. Inter-species transmission (in both directions) of swine and human influenza viruses is well recognised, evidenced by the isolation of human influenza virus in swine [179,180] and evidence of swine influenza virus (SIV) infection in people with close occupational [181–183] and/or residential proximity to pigs [184,185] or prolonged exposure at an agricultural fair [186]. Transmission between pigs and bird species is exemplified by various reports of isolation of SIV from turkeys [187–189]. The 2009 pandemic virus A(H1N1)pdm09 comprised genetic components from the swine-adapted North American triple reassortant H3N2 viruses and a Eurasian swine virus [190].

There is an increasing need for monitoring transmission between pigs and humans, but data on the extent of such transmission events remains limited. Previous studies attempting to assess serological evidence of swine influenza in people with occupational exposure to pigs all recruited their non-pig-exposed comparison groups from restricted groups such as blood donors [182,191–193], students, teachers, or university or hospital personnel, [194–196] or in some cases, they used serum bank samples [181,197]. This study focused on assessing SIV infection in pig industry workers in England during the emergence of A(H1N1)pdm09 virus. Serological data on SIV infection in pig veterinarians and pig farm workers was compared with a sample from the general population and related to serology from sampled pigs in contact with the pig farm workers.

4.4 Methods

4.4.1 Recruitment and specimen collection

We recruited pig industry workers including pig farm workers and specialist pig veterinarians (each veterinarian typically attended a number of different farms across an area, and some also worked in other settings such as abattoirs). Pig veterinarians were recruited at November 2009 and May 2010 meetings of the Pig Veterinary Society, a species-specialist group of the British Veterinary Association. Pig farm workers were recruited from 17 farms in September-December 2010 from a large group of farrow-to-finish pig farms that participated in a related study of SIV infection in English pigs [167]. Farms came from two main clusters in North Yorkshire and East Anglia, both regions with higher densities of the pig population [198]. Farm owners were first asked for permission to approach their staff, including everyone with direct pig contact such as farm hands, on-site managers, and field maintenance workers. At the farms where owners granted permission, pig farm workers were invited to join the study. At the same time blood samples were collected from pigs from each of the worker's farms.

Participants from the concurrent Flu Watch study - a community-level, household-based cohort study of influenza in England [15] - formed the population comparison group. Flu Watch participants were frequency-matched to pig industry workers on age group, geographic region, calendar month of blood sample, and gender (in decreasing priority order).

All participants gave individual written informed consent and completed a questionnaire including information on demographic characteristics and their history of influenza vaccination for that season (2009 for pig veterinarians or 2010 for pig farm workers). Blood samples were collected from all participants for serological analysis.

In order to examine the association between SIV infection among pig farm workers and SIV infection among the pigs they worked with, blood specimens were obtained from a sample of pigs on their farms as part of the aforementioned SIV infection study [167]. Blood specimens were taken from pigs during the same season as the pig farm workers (autumn 2010).

4.4.2 **Influenza Virus Panel and laboratory methods**

Serum samples from pig industry workers and the Flu Watch population comparison group were tested for the presence of antibodies using an AHVLA standard panel of SIVs representative of contemporary viruses detected through routine SIV surveillance in UK pigs, and known human viruses [168] (See Table 4-1). The SIVs in the panel were A/sw/England/117316/86 classical H1N1 [classical swine H1N1]; A/sw/England/195852/92 avian-like H1N1 [swine avian-like H1N1]; A/sw/England/163266/87 H3N2 [swine H3N2 87]; and A/sw/England/438207/94 H1N2 [swine H1N2]. The human viruses were A/England/195/09 pH1N1 [A(H1N1)pdm09]); A/Brisbane/59/07 H1N1 [H1N1 07]; and A/Perth/16/09 H3N2 [H3N2 Perth]). Standard haemagglutination inhibition (HI) assays [199] were used. A reciprocal antibody titre of greater than or equal to 40 (1:40 from serial dilution) was considered seropositive and taken as indicative of putative previous infection with the corresponding virus in humans.

Table 4-1: Description of influenza Strain names, typical host and whether antibodies were tested in humans and pigs

Typical Host	Virus	Abbreviated name	Antibodies tested	
			Humans	Pigs
Swine	A/sw/England/117316/86 classical H1N1	classical swine H1N1	x	x
Swine	A/sw/England/195852/92 avian-like H1N1	swine avian-like H1N1	x	x
Swine	A/sw/England/163266/87 H3N2	swine H3N2 87	x	x
Swine	A/sw/England/438207/94 H1N2	swine H1N2	x	
Swine & Human	A/England/195/09 pH1N1	A(H1N1)pdm09	x	x
Human	A/Brisbane/59/07 H1N1	H1N1 07	x	
Human	A/Perth/16/09 H3N2	H3N2 Perth	x	

Sera from unvaccinated pigs were tested for a smaller subset of viruses (classical swine H1N1, swine H1N2, swine H3N2 87, swine avian-like H1N1, and A(H1N1)pdm09). It is recognised that in HAI tests with pig sera, the profile against the range of viruses used needs to be analysed and interpreted with care, since homosubtypic cross-reactive antibodies to the HA may be detected without inferring exposure to a particular strain. Difficulties in swine HI serology interpretation can be compounded further by anti NA (especially N2) antibodies interfering in the HI test. Our approach was to evaluate the titres to determine those of the greatest magnitude correlating with the most probable virus subtype to which an individual animal had been exposed to. Within a subtype, if the highest titre was greater or equal to 40 then the pig was considered seropositive for that strain. If

two strains within a subtype shared the highest titre (greater or equal to 40) then the pig was considered seropositive for both. It should also be noted that a single animal may have been exposed to more than one influenza virus.

Most farms had 12-16 pigs tested. We considered a farm positive for a given strain if it had at least three pigs seropositive for that strain.

4.4.3 **Statistical Analysis**

We explored whether occupational exposure to pigs was associated with infection with each virus strain through univariable analysis using Chi-squared (X^2) and Fisher's exact tests. We then built separate multivariable logistic regression models for each virus strain to estimate the association of occupational exposure to pigs and infection. These models accounted for clustering for repeated measurements as some participants contributed more than one sample from different time periods. In each model we investigated the potential confounding effects of vaccination status, age, season (winter 2009, spring 2010, autumn/winter 2010), geographic region and gender. A variable was retained in the model if it was associated with occupational pig exposure, associated with infection, and either independently predicted the outcome or else made an appreciable difference on the effect of occupational pig exposure on infection. We hypothesised, a priori, that the season of the blood sample may modify the effect of occupational pig exposure on infection, and this was explored by testing for interaction terms in the models.

Where an influenza strain was found to be associated with occupational pig exposure, we investigated the possibility of cross-reactivity between that strain and other swine viruses sharing the same haemagglutinin using cross tabulations with X^2 or Fisher's exact test as appropriate. Where there was evidence of association, these strains were forced into regression models to account for possible cross reactivity.

We conducted sub-analyses among pig veterinarians providing more than one blood sample (November 2009 and May 2010) to calculate the risk of seroconversion to each virus strain, as determined by a four-fold rise in antibody titre.

In a series of sub-analyses (one for each strain of SIV tested in both pigs and humans), we explored whether pig farm workers' SIV seropositivity status was associated with the positivity status of their farm's pig herd using X^2 and Fisher's exact tests.

4.4.4 Ethics

This study was approved by the Cambridgeshire-1 Research Ethics Committee (REC) Reference 10/H0304/4. The Flu Watch study, from which the population comparison group was drawn, was approved by the Oxfordshire REC Reference 06/Q104/103. Participants received full information about the study and if interested and eligible, they were enrolled after providing fully informed written consent.

4.5 Results

4.5.1 Participants and blood samples

The characteristics of participants and number of blood samples are described in Table 4-2. A total of 26 pig veterinarians participated in the study, providing 42 separate blood samples, with 16 veterinarians contributing 2 samples (one from November 2009 and one from May 2010). An additional 29 pig farmers from 17 different pig farms participated in the study, each contributing one blood sample. A total of 68 Flu Watch participants provided 71 blood samples which were frequency matched to the samples from the pig industry workers as described in the methods. Sixty-five of the Flu Watch participants contributed only one blood sample but three contributed two blood samples from two of the three possible seasons (winter 2009, spring 2010 or winter 2010). Most pig industry workers were male. The median age for pig industry workers and the frequency matched Flu Watch participants was 44 and 47 respectively. At the time the blood sample was taken, 93% of participants were unvaccinated. Only five Flu Watch participants and four pig farmers had received the currently available pandemic vaccine.

Table 4-2: Participant Characteristics and number of samples

		Flu watch						Pig Worker					
		Participants			Blood Specimens			Participants			Blood Specimens		
		Number (N=68)*	%	95% CI	Number (N=71)*	%	95% CI	Number (N=55)*	%	95% CI	Number (N=71)*	%	95% CI
age group	<45	29	43%	(31-54)	30	42%	(31-54)	28	51%	(38-64)	36	51%	(39-62)
	45-64	34	50%	(38-62)	36	51%	(39-62)	23	42%	(29-55)	31	44%	(32-55)
	65+	5	7%	(1-14)	5	7%	(1-13)	4	7%	(0-14)	4	6%	(0-11)
gender	male	55	81%	(72-90)	57	80%	(71-90)	45	82%	(72-92)	57	80%	(71-90)
	female	13	19%	(10-28)	14	20%	(10-29)	10	18%	(8-28)	14	20%	(10-29)
region	East Midlands	30	44%	(32-56)	31	44%	(32-55)	21	38%	(25-51)	31	44%	(32-55)
	North East	17	25%	(15-35)	18	25%	(15-35)	16	29%	(17-41)	18	25%	(15-35)
	London & SE	14	21%	(11-30)	14	20%	(10-29)	11	20%	(9-31)	14	20%	(10-29)
	West	7	10%	(3-18)	8	11%	(4-19)	7	13%	(4-22)	8	11%	(4-19)
flu vaccination season of blood sample	no	63	93%	(86-99)	65	92%	(85-98)	51	93%	(86-100)	67	94%	(89-100)
	yes	5	7%	(1-14)	6	8%	(2-15)	4	7%	(0-14)	4	6%	(0-11)
pig worker type	Veterinarian	N/A	-	-	N/A	-	-	26	47%	(34-60)	42	59%	(48-71)
	Farmer**	N/A	-	-	N/A	-	-	29	53%	(40-66)	29	41%	(29-52)

*number of people differ from number of blood samples as individuals would occassionally provide blood sample for more than one

** farmers come from 21 pig farms

4.5.2 **Risk of Infection in relation to occupational exposure to pigs**

In the univariable analysis (Table 4-3) there was evidence that antibodies to three out of the eight influenza strains were more common in pig industry workers than the population comparison group: A(H1N1)pdm09 (23% versus 4%, Fisher's exact test p=0.002); swine H1N2 (24% versus 11%, Chi^2 p=0.047) and H3N2 Perth (37% versus 20%, Chi^2 p=0.025).

There was no evidence of swine avian-like H1N1 antibodies in the population comparison group in contrast to three seropositive pig industry workers (4%, 95% CI [0.9% – 11.9%]). Although 10% (95% CI [4.0% – 19.3%]) of pig industry workers and 4% (95% CI [0.0% - 11.9%]) of the comparison group had antibodies to classical swine H1N1, these reactions were most probably due to cross reactive antibodies from an A(H1N1)pdm09 infection as the classical swine H1N1 strain had not circulated in the UK for decades and 70% (95% CI [34.8% – 93.3%]) of those seropositive for the virus were also seropositive for A(H1N1)pdm09. Antibodies to swine A(H1N2 or H3N2) strains were relatively common in both groups (range 11-64%).

In the multivariable analysis (Table 4-3) after adjusting for confounders, there was strong evidence that pig industry workers had elevated odds of A(H1N1)pdm09 seropositivity (Adjusted Odd Ratio (aOR)=20.4, 95% CI [2.2-186.4], Wald test p=0.007) compared to the Flu watch comparator population. We found strong evidence that A(H1N1)pdm09

seropositivity in humans was associated with seropositivity to swine H1N2 (Chi^2 p=0.003), classical swine H1N1 (Fisher's exact test p<0.001) and swine avian-like H1N1 (Fisher's exact test p=0.002). The association between A(H1N1)pdm09 seropositivity and occupational swine exposure remained strong after controlling for the possible effect of cross-reactivity with these strains (aOR=15.11, 95% CI [1.64-139.75], Wald test p=0.017).

Pig industry workers had an increased odds of swine H1N2 seropositivity (aOR = 4.3 [95% CI 1.4-13.5], Wald test p=0.012) compared to the population group. There was strong evidence that seropositivity was associated with A(H1N1)pdm09 (Chi^2 p=0.003) and classical swine H1N1 (Fisher's exact test p<0.001) but less evidence of an association with avian-like swine H1N1 (Fisher's exact test p=0.080). The odds ratio remained elevated after controlling for the possible effect of cross-reactivity with classical swine H1N1, swine avian-like H1N1 and A(H1N1)pdm09 (aOR=3.91, 95% CI [1.19-12.87), Wald test p= 0.025).

Pig industry workers also had an increased odds of H3N2 Perth seropositivity (aOR =3.77, 95% CI [1.52-9.35], Wald test p=0.004) compared to Flu Watch participants. We found limited evidence of an association between the Perth and the swine H3N2 87 strain (Chi^2 test p=0.087). After controlling for possible cross-reactivity with the swine H3N2 87 strain the odds ratio remained elevated (aOR = 4.75, 95% CI [1.48-15.32], Wald test p=0.009).

There was no evidence to suggest that occupational pig exposure increased the odds of seropositivity to the other influenza strains tested.

There was no evidence that season modified the association between occupational exposure to pigs and seropositivity to any of the remaining viruses tested.

Table 4-3: Crude Risk and adjusted odds of influenza infection comparing pig industry workers to a sample from a general population cohort (Flu Watch)

Typical Host	Strain	Univariable Analysis									Multivariable Regression Analysis		
		Flu watch				Pig Industry Worker				P-value			
		N	No. positive (No. of these who were vaccinated)	% pos	95% CI	N	No. positive (No. of these who were vaccinated)	% pos	95% CI	Chi^2	Adjusted OR (95% CI) pig industry worker vs Flu Watch	p-value ŧ	Model covariates***
Swine	A/sw/England/117316/86 classical H1N1 [classical swine H1N1]	71	3 (2)	4	(0.9-11.9)	71	7 (3)	10	(4.0-19.3)	0.326**	4.7 (0.81-27.90)	0.085	vaccination
	A/sw/England/195852/92 avian-like H1N1 [swine avian-like H1N1]	71	0 (0)	0	(0.0 -5.1)	71	3 (1)	4	(0.9-11.9)	0.245**	---		
	A/sw/England/163266/87 H3N2* [swine H3N2 87]	53	34 (3)	64	(49.8-76.9)	53	28 (4)	53	(38.6-66.7)	0.237	0.76 (0.33-1.77)	0.522	sex, season, age group
	A/sw/England/438207/94 H1N2 [swine H1N2]	71	8 (2)	11	(5.0-21.0)	71	17 (4)	24	(14.6-35.5)	0.047	4.32 (1.39-13.46)	0.012	vaccination, season
	Controlled for possible cross-reactivity†										3.91 (1.19-12.87)	0.025	As above +†
Swine & Human	A/England/195/09 pH1N1 [A(H1N1)pdm09]	71	3 (2)	4	(0.9-11.9)	71	16 (4)	23	(13.4-34.0)	0.002**	20.44 (2.24-186.40)	0.007	vaccination
	Controlled for possible cross-reactivity††										15.11 (1.64-139.75)	0.017	As above +†††
Human	A/Brisbane/59/07 H1N1 [H1N1 07]	71	15 (4)	21	(12.3-32.4)	71	15 (3)	21	(12.3-32.4)	1	1.11 (0.45-2.74)	0.22	vaccination
	A/Perth/16/09 H3N2 [H3N2 Perth]	71	14 (3)	20	(11.2-30.9)	71	26 (4)	37	(25.5-48.9)	0.025	3.77 (1.52-9.35)	0.004	vaccination, season
	Controlled for possible cross-reactivity†††	37	9 (2)	24	(11.8-41.1)	30	5 (0)	17	(5.6-34.7)	0.443	4.22 (1.28-13.94)	0.018	As above + †††

*Limited to 106 samples with H3N2 87 readings

**Fishers exact test p-value

*** Possible covariates include age group, gender, region, season and vaccination status

† Controlled for seropositivity to for classical swine H1N1, swine avian-like H1N1, A(H1N1)pdm09

†† Controlled for seropositivity to classical swine H1N1, swine avian-like H1N1 and swine H1N2

††† Controlled for seropositivity to H3N2 87 and swine H1N1

ŧ Wald test

4.5.3 Seroconversion among pig veterinarians

Five of the 16 pig veterinarians with repeat samples seroconverted to one or more strains tested and none had received influenza vaccination between blood samples. One veterinarian seroconverted to four different viruses (human H1N1 07, A(H1N1)pdm09 and swine H3N2 87) while another veterinarian seroconverted to both human H1N1 07 and A(H1N1)pdm09. The other three veterinarians either converted to human H3N2 Perth or swine H1N2.

4.5.4 Pig Serology and Farm-level Seroprevalence

Serology results for pigs were linked for 14 of 17 farms (corresponding to 214 pigs in contact with 25 pig farm workers). Pig- and Farm-level seroprevalence is reported in Table 4-4. Farm-level positivity for a strain meant at least three seropositive pigs for that strain on the farm. After accounting for possible homosubtypic cross-reactive antibodies in the three A(H1) strains tested in pigs, we found that 41% (95% CI [34% - 48%]) of pigs were seropositive to A(H1N1)pdm09 and 79% (95% CI [49% - 95%]) of farms were considered positive for the strain. In contrast, only 3-5% of pigs were positive for classical swine H1N1, swine avian-like H1N1 and swine H3N2 87. No farms were positive for either swine H1N1 strains and only one farm was positive for swine H3N2 87.

Table 4-4: Seroprevalence of SIV infection among pigs on farms linked to one or more pig farmers

Farm	No. pigs tested	Classical swine H1N1				Swine H3N2 87				Swine Avian-like H1N1				A(H1N1)pdm09			
		No. positive pigs	% positive pigs	95% CI	Farm considered positive	No. positive pigs	% positive pigs	95% CI	Farm considered positive	No. positive pigs	% positive pigs	95% CI	Farm considered positive	No. positive pigs	% positive pigs	95% CI	Farm considered positive
1	16	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	8	50	(24.7-75.3)	Yes
2	12	2	16.7	(2.1-48.4)	No	0	0	(0.0-26.5)	No	1	8.3	(0.2-38.5)	No	3	25	(5.5-57.2)	Yes
3	16	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	8	50	(24.7-75.3)	Yes
4	12	0	0	(0.0-26.5)	No	0	0	(0.0-26.5)	No	0	0	(0.0-26.5)	No	5	41.7	(15.2-72.3)	Yes
5	12	0	0	(0.0-26.5)	No	8	66.7	(34.9-90.1)	Yes	0	0	(0.0-26.5)	No	7	58.3	(27.7-84.8)	Yes
6	16	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	15	93.8	(69.8-99.8)	Yes
7	10	0	0	(0.0-30.8)	No	0	0	(0.0-30.8)	No	0	0	(0.0-30.8)	No	0	0	(0.0-30.8)	No
8	16	0	0	(0.0-20.6)	No	1	6.3	(0.2-30.2)	No	2	12.5	(1.6-38.3)	No	4	25	(7.3-52.4)	Yes
9	12	2	16.7	(2.1-48.4)	No	0	0	(0.0-26.5)	No	0	0	(0.0-26.5)	No	0	0	(0.0-26.5)	No
10	16	1	6.3	(0.2-30.2)	No	0	0	(0.0-20.6)	No	2	12.5	(1.6-38.3)	No	3	18.8	(4.0-45.6)	Yes
11	12	0	0	(0.0-26.5)	No	0	0	(0.0-26.5)	No	1	8.3	(0.2-38.5)	No	1	8.3	(0.2-38.5)	No
12	16	2	12.5	(1.6-38.3)	No	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	10	62.5	(35.4-84.8)	Yes
13	16	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	0	0	(0.0-20.6)	No	8	50	(24.7-75.3)	Yes
14	32	2	6.3	(0.8-20.8)	No	1	3.1	(0.1-16.2)	No	0	0	(0.0-10.9)	No	15	46.9	(29.1-65.3)	Yes
total	214	9	4.2	(1.9- 7.8)	0 of 14	10	4.7	(2.3- 8.4)	1 of 14	6	2.8	(1.0- 6.0)	0 of 14	87	40.7	(34.0-47.6)	11 of 14

* Farms were considered positive if 3 or more animals in that herd tested positive (titres ≥40 and highest titre within HA subtype).

4.5.5 Farm-level Seroprevalence and human infection

There was no evidence of an association between farm positivity and risk of infection among pig farm workers for any of the strains tested. All pig farm workers infected with the pandemic virus worked on a farm positive for the same strain. No pig farm workers were infected with swine avian-like H1N1 (Table 4-5).

Table 4-5: Association between pig farm workers' infection status and the positivity status of the pig herd they work with

Strain	Pig farmers working on positive farm	Pig Farm workers						
		Seronegative			Seropositive			p-value*
		N	Column %	95% CI	N	Column %	95% CI	
classical swine H1N1	No	23	100%	(85.2 - 1)	2	100%	(15.8 - 1)	n/a
	Yes	0	0%	(0 - 14.8)	0	0%	(0 - 84.2)	
swine H3N2 87	No	12	100%	(73.5 - 1)	10	77%	(46.2 - 95.0)	0.22
	Yes	0	0%	(0 - 26.5)	3	23%	(5.0 - 53.8)	
swine avian-like H1N1	No	25	100%	(86.3 - 1)	0	n/a	n/a	n/a
	Yes	0	0%	(0 - 13.7)	0	n/a	n/a	
A(H1N1)pdm09	No	6	29%	(11.3 - 52.2)	0	0%	(0 - 60.2)	0.54
	Yes	15	71%	(47.8 - 88.7)	4	100%	(39.8 - 1)	

*Fisher's Exact test

4.6 Discussion

This study improves our understanding of swine influenza transmission to humans by comparing the serological evidence of SIV seropositivity in pig industry workers in England with a general population-based comparison group at the time of the A(H1N1)pdm09 influenza pandemic.

The key finding is that, in the period of this study, pig industry workers had increased odds of influenza A(H1N1)pdm09 seropositivity compared to the general population. Evidence of the association remained after controlling for seropositivity to other swine H1 viruses and is thus unlikely to be the result of cross-reactivity. We also found evidence that pig industry workers had elevated odds of swine H1N2 and H3N2 Perth seropositivity which remained after controlling for sero-positivity to other measured, potentially cross-reactive strains.

The increased risk of A(H1N1)pdm09 in pig industry workers is compatible with the concurrent emergence of infection with A(H1N1)pdm09 in pigs in England, which was first observed in November 2009 [170] and confirmed by the serological results in our study. As there was minimal trade of live pigs between North America and Europe during the period of the study and no reports of the pandemic strain in European pigs prior to human cases, [200] it is likely that pigs were initially infected by humans during the early stages of the 2009 pandemic, and infection then transmitted efficiently within and between pig herds but also through reverse zoonoses events following contact of pigs with infected humans. Phylogenetic analysis has subsequently demonstrated that H1N1pdm2009 has been repeatedly transmitted from humans to swine since the pandemic [201]. Pig industry workers naïve to A(H1N1)pdm09 would be susceptible to zoonotic infection from pig herds undergoing active infection, with exposure to sometimes large groups of pigs simultaneously undergoing acute infection and shedding virus favouring transmission from pigs to pig industry workers. Further bi-directional transmission may have led to an amplification effect leading to high levels of infection in both pigs and pig industry workers. This is important in that it shows that dense populations of pigs can serve as an amplifying reservoir for influenza virus, increasing the risk of novel virus transmission to both pigs and to humans. This has been illustrated during an outbreak of H1N1pdm2009 on a research farm in Canada [202] and explored in mathematical models of the potential amplifying impact of such bi-directional transmission [203].

Our findings overall are consistent with other work identifying increased risk of influenza A(H1N1)pdm09 in pig industry workers compared to others with no occupational pig exposure. However, they could not exclude cross reactivity between other SIVs and influenza A(H1N1)pdm09 as the cause [193,197]; and others have reported no increased risk [194,204]. We found evidence of an increased risk of the A(H1N1)pdm09 strain which is known to affect both pigs and humans in pig industry workers even after controlling for potential cross-reactivity and the effect was not due to confounding by age, region, and time of sample or vaccination status.

With regard to other SIV strains other than A(H1N1)pdm09, previous studies found an increased risk of seropositivity to at least one SIV in pig workers, including H1N1

[181,182,191,193,195–197,205,206], H1N2 [182,192,195], and H3N2 [192–194] strains. In our study, we found increased risk of seropositivity both to swine H1N2 and H3N2 Perth. This increased risk remained after controlling for potential cross reactivity with measured strains. The increased risk of seropositivity to swine H1N2 is consistent with occupational exposure. The increased risk of H3N2 Perth (a human strain) was not explained by cross reactivity to swine H3N2 87. Others have found H3N2 Perth strain assays to cross react strongly with swine H3N2 [207]. Thus, it is plausible that the increased risk of H3N2 seropositivity in pig workers in our study was due to cross-reactivity with an unmeasured H3N2 swine strain. Similarly, the high levels of the historical swine H3N2 87 in the general population in our study could be due to cross reactivity with unmeasured human H3N2 strains.

In contrast to all the previous studies which compared pig workers to highly selective groups, our work has the advantage of using a general population comparison group, frequency matched for age, region, month of bleed and gender. Although we could not exclude pig exposure in the control group, such exposure is likely to be rare in the general UK population. The work is challenged by limited ability of laboratory tests to exclude cross-reactivity between all viral strains, a common issue with studies of this nature. Future work using micro-neutralisation assays would reduce concerns over cross-reactivity.

It is generally considered that influenza virus reassortment with significant pandemic potential is most likely to occur in developing country "hotspots"[208], where the demographic, cultural and economic circumstances and animal husbandry practices together result in settings of dense overlaps between humans and animal populations and opportunities for cross-species transmission. However, given my findings, and observations of new reassortant strains elsewhere in Europe[209,210], there should be no assumption that reassortment with possible zoonotic risk does not occur in industrialised settings.

The study was unable to examine whether there was also an increased risk of clinical disease in pig industry workers, but the work suggests the need for coordinated enhanced surveillance in both pigs and pig industry workers. Observations from this study also offer strong supporting evidence that pig industry workers should be among the occupational groups offered annual seasonal influenza vaccination. Preventing influenza infection in people who work with pigs would seem to be a logical option to minimise the risk of

transmission of human variants into pigs, and by extension to reduce the possibilities for reassortment in pigs.

4.7 Relevance to COVID-19

Despite being entirely different species of respiratory viruses there are many similarities between SARS-CoV-2 (the virus which causes COVID-19) and influenza viruses. Focusing on the broad similarities at the human/animal interface, the zoonotic origins of the SARS-CoV-2 virus, the fact that the initial outbreak was linked to a live wet market and even its geographic location are all reminiscent of avian influenza outbreaks in birds and subsequent sporadic zoonotic cases in humans.

The research I describe above highlights two cross-cutting issues. Firstly, sporadic zoonotic human infections with (in this case) swine-adapted influenza viruses are common. Secondly, animals (in this case pigs) can become reservoirs of human-adapted viruses which not only increases opportunities for viral evolution but also increases the likelihood of human infections from that reservoir. These same issues (frequent zoonotic infection and animal populations maintaining viral strains which can infect humans and drive the evolution of new, potentially dangerous strains) are also relevant to SARS-CoV-2.

The SARS-COV-2 virus comes from the Coronaviridae family of RNA viruses which, like influenza viruses, have a wide range of host species including humans and other mammals and birds[211,212]. Coronaviruses have a number of molecular mechanisms that can lead to changes in the viruses' ability to infect various types of cells, tissues and species. Two important mechanisms include mutation, and homologus recombination which are analogous to influenza viruses' genetic drift and viral reassortment, respectively[213]. As a result of these and other mechanisms, coronaviruses frequently host-shift and these shifts have resulted in the establishment of novel animal and human diseases[211,213]. A great deal of work has been done to identify species that are known to be or are likely to be susceptible to the SARS-CoV-2 and related viruses[214–219]. This has been in the service of identifying the existence of an assumed primary and intermediary reservoir host species for SARS-CoV-2 as well as other species which have the potential to become new reservoir hosts. There is also work being done to predict species that can host multiple coronaviruses and thus enable homologous recombination events[211]. Such events are a concern as they could introduce new phenotypes into the SARS-CoV-2 virus infecting humans and lead to

generation of new strains that could then become a threat to humans. It is believed that homologous recombination events lead to the emergence of not only SARS-CoV-2 but also previous SARS-CoV and MERS-CoV, two virulent coronaviruses that have recently emerged in humans[220].

Although modelling has suggested there are many potential species that are susceptible to SARS-CoV-2 and related viruses, natural infections have mostly been limited to carnivores including domesticated dogs and cats, large cat species (lions, tigers, pumas and leopards), gorillas and mustelids (mink and ferret)[211,216]. Among the species susceptible to human-transmitted SARS-C0V-2, only mink has demonstrated the ability to infect humans[215,219]. Similar to the dynamics we described with pandemic H1N1 and pigs in the UK, the Netherlands and Denmark saw explosive outbreaks of SARS-CoV-2 on mink farms caused by multiple introductions of the virus from humans to mink as well as transmission from mink to human [215,219,221–223]. Even more concerningly, once the virus was circulating among mink, new mink-related variants evolved which not only readily spread among mink, but also spilled over into mink workers who then spread the mink-variant within the community[219,221,223]. Some of the mutations in the mink-related variants were on the spike, the protein that enables entry into cells and is the primary target for antibodies[221]. Concerns that transmission between mink and humans could lead to changes in viral fitness, transmissibility, antigenicity and virulence, prompted a number of control measures including the nation-wide culling of all mink in Denmark and the Netherlands [219,222,222].

In general, options for response to mink infections as suggested by the European Centres for Disease Control (ECDC) include[221]:

1) human testing, sequencing and characterisation of antigenic properties and virus infectivity
2) infection prevention and control measures for mink farm workers and visitors
3) animal testing and prevention of spread from animals
4) development of One Health preparedness and response strategies (e.g. co-ordination and between human, animal and environmental health sectors)

Although influenza and SARS-CoV-2 are different viruses, the underlying epidemiology and response to infection and transmission of the viruses in farmed animals and the people who work with them are very similar. The list of response options above for infections with mink are similar to what you might expect, for example, in the UK to avian influenza [224,225] although perhaps to a lesser degree as cases in animals are relatively uncommon, zoonotic human cases even less so and currently no human-to-human transmission. In the swine influenza research presented in this chapter, I advocated for both increased and coordinated surveillance in animals and the people that work with them. I also suggested that we consider those occupationally exposed to pigs as a high-risk group who should be offered vaccination in order to prevent transmission to or from pigs. These recommendations are in line with the broad response options listed above for SARS-CoV-2.

While the culling of millions of minks is tragic, we are lucky that common livestock animals such as poultry, pigs and cattle do not appear to be susceptible to SARS-CoV-2 infection and at the moment appear unlikely to play a role in the epidemiology of the virus, apart from outbreaks of Covid-19 in meat-packing facilities which is due to the environment and lack of social distancing within those facilities rather than the meat itself [218,226,227]. It seems sensible however to try to limit interactions between infected and/or humans and animals (and visa-versa) and potentially consider SARS-CoV-2 as one possible source of infection in investigations of disease outbreaks in livestock in order to confirm the virus has not jumped species. More generally, I would also recommend increased surveillance as well as epidemiological and evolutionary research on viruses at the human/animal interface as I believe there is underappreciation of the scale of the problem and there is still much to learn about it.

Chapter 5 Measuring Infection and Immunity during a pandemic using serial, cross-sectional seroprevalence surveys

This analysis describes the serological results of the PIPS serological survey. Sections of this chapter have been adapted from my first author paper published in Public Health Research [98]. It presents the population-level immunity and infection (as determined by strain-specific seroprevalence at particular antibody titres) to pandemic H1N1 and H3N2 strains over time and by other characteristics such as age group, sex and vaccination status.

5.1 Attribution

I managed the PIPS study, data collection and data management. I developed the overall analytical strategy as well as the initial statistical programmes for this analysis which was then built on by Dr Ruth Blackburn (my maternity leave cover) and my supervisor Prof Hayward. They finished the analysis and wrote the initial report of findings for the funder (which focused on results and discussion) while I was on maternity leave. I have since rewritten the report in the format of a journal article for the purposes of publishing and for inclusion in this thesis. More specifically, I conducted the literature review and wrote the introduction and methods section and restructured and expanded the discussion and conclusion sections to a large extent.

5.2 Abstract

Background: Assessing severity and spread of a novel influenza strain at the start of a pandemic is critical for informing a targeted and proportional response. It requires community-level studies to estimate the burden of infection and disease. We developed and piloted an efficient, real-time, serosurveillance system that could theoretically be rolled out quickly by linking with the Health Survey for England (HSE), a large, annual, nationally representative rolling survey.

Objectives: To estimate the proportion of the population with detectable and protective (titres ≥1:40) levels of antibodies against A(H1N1)pdm09 and A(H3N2) and how these levels vary by age group, sex, vaccination status and calendar month.

Methods: We added additional questions and blood specimen collection for adults aged 16 and over to HSE between October 2012 and March 2013. Data and sera samples were sent

to University College London Hospital and tested using Haemagluttinin Inhibition Assays to A(H1N1)pdm09 and A(H3N2).

Results: There were 1870 participants, 577 of whom were vaccinated that season. Over the course of the 2012/13 influenza season, the proportion of the unvaccinated population who had detectable antibodies to A(H1N1)pdm09 rose by 42% (95% CI [33% - 51%]). The corresponding figure for A(H3N2) was 24% (95% CI [12%-37%]). Using a higher threshold titre of 1:40 the cumulative increase was 38% (95% CI [29% - 46%] for H1N1pdm2009 and 27% (95% CI [16%-37%]) for A(H3N2). Prior to the A(H1N1)pdm09 epidemic wave, approximately 50% of adults have detectable and 30% protective antibodies against A(H1N1)pdm09. For A(H3N2) these figures were 50% detectable and 35% protective. Immediately following the epidemic peak, levels of detectable antibodies against A(H1N1)pdm09 were approximately 80% and protective levels 50-60%. For A(H3N2) these figures were 60-70% and approximately 55% respectively.

Conclusion: A high proportion of the population are infected over the course of a few months. This estimate is much higher than estimates based on virologically confirmed cases and also higher than the number of serologically confirmed cases in community studies which rely on 4-fold titre rises. Our results may also shed light on the level of strain-specific antibodies in the English population that permit and curtail epidemic spread of influenza.

5.3 Introduction

At the beginning and throughout an influenza pandemic it is critical to have accurate and regularly updated estimates of the proportion of the population that is likely to be susceptible to the pandemic virus and the frequency and distribution of infections that have already occurred[92]. These estimates are essential for many public health decisions as they provide insight into the potential and actual spread of infection, denominators for severity estimates and parameters for transmission models which can estimate the current and near-future trajectory of the epidemic curve as well as the likely impact and cost-effectiveness of public health interventions[5]. Assessing population-level immunity, incidence and spread of infection requires representative serological surveys which quantify individuals' cross-reactive antibody titres.

Prior to the COVID-19 pandemic most serological surveys relied on convenience serum samples, i.e. samples that have been previously collected for other purposes such as residual clinical samples or blood banks. While these samples have their advantages in terms of availability and low cost, they often introduce bias and lack relevant, linked, individual-level data such as age and sex and almost always lack vaccination history which is necessary for determining whether a high antibody titre was caused by natural infection or whether it could have been caused by influenza vaccination[129]. Another challenge of serological surveys is the lead-time and resource needed to set up such studies. In order to have the most impact on public health response to a pandemic, serological findings need to be available as soon as possible and the timelines during a pandemic typically do not lend themselves for the initiation of new research studies within the first wave of infection, particularly serological surveys[98,228]

The PIPS study, a previously described[98], cross-sectional serological survey, was designed to overcome the aforementioned challenges and establish an efficient system allowing the real-time assessment of population susceptibility, spread of infection and clinical attack rates in the event of a pandemic[98]. It aimed to avoid the challenges presented by long lead-times for serological studies by leveraging the data collection already being done as part of the Health Survey for England (HSE), an annual, nationally representative, rolling survey. The HSE collects health related data and serum samples from individuals throughout each calendar year. Our study simply adds a few extra influenza-related questions and an additional blood sample to their regular data collection process. The PIPS study is incorporated into the yearly HSE ethics and other necessary approval and maintains its readiness to activate within two weeks of triggering. Another advantage of collecting data through the HSE is the ability to collect linked, individual-level data on age, sex and crucially, vaccination status. A limitation of the study design is that it relied on the continuation of specimen collection during the pandemic. Although the HSE continued as normal during the 2009 influenza pandemic, there is a risk that in a more severe pandemic the HSE would cease household visits. As part of the PIPS project, we initiated a full-scale pilot of the study during the 2012-13 interpandemic winter season.

According to national surveillance, influenza activity in England during the 2012-13 winter season was a relatively low but unusually long with elevated activity from week 50 to 16[229,230]. There was a mix of influenza viruses circulating with influenza B predominating,

followed by A(H3N2) and smaller amounts of A(H1N1)pdm09 (Figure 5-1, taken from national surveillance report)[230]. It was also an unusual season in that influenza B peaked earlier in the season than influenza A, a pattern that is usually switched. A relatively large influenza B wave began around week 46 and peaked in week 52. Circulation of A(H3N2) and unsubtyped influenza A increased around week 48 and peaked in week 8 but continued to circulate for a prolonged period, with highest activity in the 45+ age group. A smaller A(H1N1)pdm09 wave started later around week 52 but also peaked in week 8[230]. Vaccine efficacy was estimated to be moderate against influenza B (51%), good against A(H1N1) (73%) but was poor against A(H3N2) (26%) with some evidence in intra-seasonal waning of vaccine efficacy for A(H3N2) over the course of the long influenza season[229]. Although influenza activity was relatively low in the 2012-13 season, it was higher than the previous winter season in 2011-12 which was particularly low and late[230,231]. This season was dominated by A(H3N2) although small amounts of influenza B were also detected[231]. That year vaccine efficacy against A(H3N2) was poor at 23% and some have argued that this may partly be due to intra-seasonal waning of immunity[232]. Prior to this the influenza landscape was dominated by the then-new A(H1N1)pdm09 which circulated in a summer wave in 2009, a larger winter wave in 2009-10 and an intense third wave in winter 2010-11 which also included some influenza B and peaked in week 52. Other influenza subtypes (A(H3N2) and the previously circulating A(H1N1)) were largely absent in those two years [15,233].

Figure 5-1: Weekly number of all age influenza positive samples by subtype through the Datamart system and proportion positive by influenza type, England*

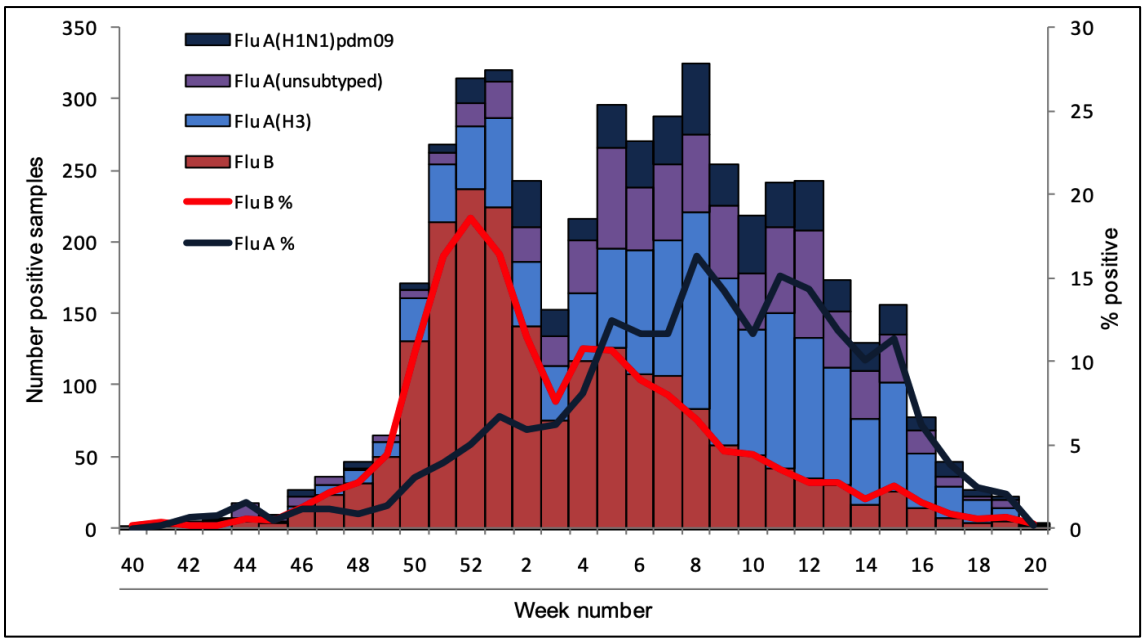


*Figure taken from "Surveillance of influenza and other respiratory viruses, including novel respiratory viruses, in the United Kingdom: Winter 2012/13" [230]

5.4 Methods

5.4.1 Health Survey for England

The Health Survey for England (HSE) is a series of annual surveys that have been documenting the health of the population since 1991. The surveys are designed to be representative of private households in England and employ a stratified random probability sampling design. The HSE is a rolling survey and collects data and samples throughout the calendar year. Households that agree to participate are visited twice. At the first visit, a trained interviewer collects information from household members about their health and behaviours that may affect their health. The second visit is conducted by a nurse who collects additional information, body measurements and biological samples (including blood samples from those aged 16 years and older).

5.4.2 PIPS adaptations and data collection

Between the months of Oct 2012 – March 2013, the PIPS project added an extra 5ml blood sample (one extra vial) to the existing phlebotomy process at the nurse interview. These blood specimens were sent alongside the rest of the HSE specimens to the Newcastle General Hospital Microbiological laboratory. On arrival, the specimens were centrifuged, split into two aliquots of serum and frozen at -80C. They were later transferred to the University College London Hospital (UCLH) microbiology laboratory for serological analysis. The nurses additionally collected information from all household participants. These data included participants' self-reported experience of a 'respiratory illness' in the last month, whether they have had an influenza vaccination and if so the month and year of the most recent one. For those who contributed blood samples, this information, along with age, sex, region, date of blood sample and participant ID were transferred onto a project-specific despatch form which accompanied the associated blood samples.

5.4.3 Serological Analysis

Frozen serum samples were thawed and tested using Haemagglutinin Inhibition Assays (HAI) to H1N1pdm2009 and Influenza A(H3N2) with 2-fold serial dilutions from 1:20 to 1:1280. Antibody titres were calculated as the reciprocal of the highest dilution which prevented hemagglutination.

We had two primary serological outcomes: 'detectable antibody' which corresponded with a titre of 20 (the minimum detection level) and 'protective antibodies' which corresponded with antibodies levels at or greater than 40. We also had two secondary outcomes; influenza vaccination status for the 2012/13 season (to evaluate vaccine uptake) and respiratory illness in the month prior to specimen.

5.4.4 Statistical Analysis

We present simple descriptive analyses, separately for each virus. Using the binomial formula, we calculated the proportion and 95% confidence intervals of the population with detectable and the proportion with protective antibody levels in the overall sample and stratified separately by age group, sex, region, month of specimen, vaccination status and respiratory illness in the month prior to specimen. We explored associations among these factors and our serological outcomes for each virus using logistic regression. We then

repeated these calculations for each virus separately among individuals vaccinated to the 2012/13 influenza season and those not vaccinated for that season. We calculated seroprevalence and cumulative incidence of infection at detectable and protective threshold antibody levels by month and age group both overall and by vaccination status. We also calculated cumulative vaccine uptake by age and month and proportion of participants with a respiratory illness by month and age group.

5.5 Results

5.5.1 Overall Characteristics

The characteristics and serological outcomes of the 1870 participants are presented in Table 5-1. There were 594 participants aged 16-44 (32%), 734 aged 46-64 (39%) and 542 aged 65 and over (29%). There were 1008 females (54%) and 845 males (46%). The regional distribution of participants was close to that of the adult population of England (10% North East, 13% North West, 8% Yorkshire and Humber, 9% East Midlands, 10% West Midlands, 12% East, 29% in London and the South East, 10% South West). Apart from December when only 168 specimens were collected, the number of specimens collected each month ranged from 299 in October to 371 in February. 1123 participants were unvaccinated (60%, 95% CI [58-62]), 190 had been vaccinated for previous seasons (10%, 95% CI [9-12]) and 557 had been vaccinated for the 2012/13 season (30%, 95% CI [8-32]). 383 participants (21%, 95% CI [19% - 23%]) reported experiencing a respiratory illness in the last month. 1195 samples had detectable antibody titres to H1N1pdm2009 (64%, 95% CI [62% - 66%]) including 731 (39%, 95% CI [37% – 41%]) with titres of greater than or equal to forty (the cut off commonly used to indicate protective antibody levels). Similarly, 1087 samples had detectable antibody titres to A(H3N2) (58%, 95% CI [56% - 60%]) including 797 (43%, 95% CI [40% - 45%]) with titres of greater than or equal to forty.

5.5.2 Risk factors for detectable and protective antibodies

For both A(H1N1)pdm09 and A(H3N2), vaccine status and later month within the winter flu season had a marked influence on detectable and protective antibody levels (Table 5-1). For A(H3N2), but not A(H1N1)pdm09 the 65+ year age group had higher titres. When focusing on the unvaccinated, the higher A(H3Ns) titres seen in the 65+ age group were no longer seen.

5.5.2.1 *A(H1N1)pdm09*

Individuals vaccinated for the 2012/13 influenza season had the highest levels of detectable antibodies (79%, 95% CI [75% - 82%]) and protective antibody titre levels (56%, 95% CI [52% - 60%]). In the overall population there was no association between age and detectable or protective antibody levels. In the unvaccinated population (Figure 5-2a) the proportion with detectable and protective antibodies declined with age group (Chi^2 p=0.003 and p=0.007, respectively) with oldest age groups having the lowest levels of detectable (43%, 95% CI [34-53]) and protective (22%, 95% CI [15-31]) antibodies. The percentage of vaccinated participants (Figure 5-3a) with protective antibody titres declined with age from 64% (95% CI [53-73]) in those aged 16-44 to 48% (95% CI [43-53]) in those aged 65+ (Chi^2 p=0.003). There was no evidence of an association between age and detectable antibody titres (Chi^2 p=0.16). There were no significant differences in proportions with detectable or protective antibodies between men and women, between those who did or did not report a respiratory illness in the month prior to the bleed or between regions.

5.5.2.2 *A(H3N2)*

Individuals vaccinated for the 2012/13 influenza season had the highest levels of detectable antibodies (77%, 95% CI [74-81]) and protective antibody titre levels (67%, (95% CI [63-71]). In the overall population, the oldest age group (65+) had the highest levels of both detectable and protective A(H3N2) antibody levels. In the unvaccinated population (Figure 5-2b) there was no evidence of an association between age and detectable antibody titre (Chi^2 p=0.09). However, protective antibody titres were less common among participants aged 45-64 years (24%, 95% CI [21-28]) than people aged 16-44 years (32%, 95% CI [28-36]) (Wald test p=0.008). The percentage of vaccinated participants (Figure 5-3b) with protective antibody titres did not vary with age (Chi^2 p=0.3). However, a greater proportion of the vaccinated over 65s had detectable antibody titres (79% [95% CI: 75-3] versus 68% [95% CI: 58-77] in 16-44 years, Wald test p=0.02). There were no significant differences in proportions with detectable or protective antibodies between men and women, or between those who did or did not report a respiratory illness in the month prior to the bleed. However, the proportion with protective antibody titres was significantly lower - amongst both vaccinated (54% [95% CI: 41-67] versus 65% [95% CI: 61-68]) and unvaccinated participants (21% [95% CI: 13-29] versus 29% [95% CI: 26-31]) - for the North East than other regions (Wald p=0.01 in both instances).

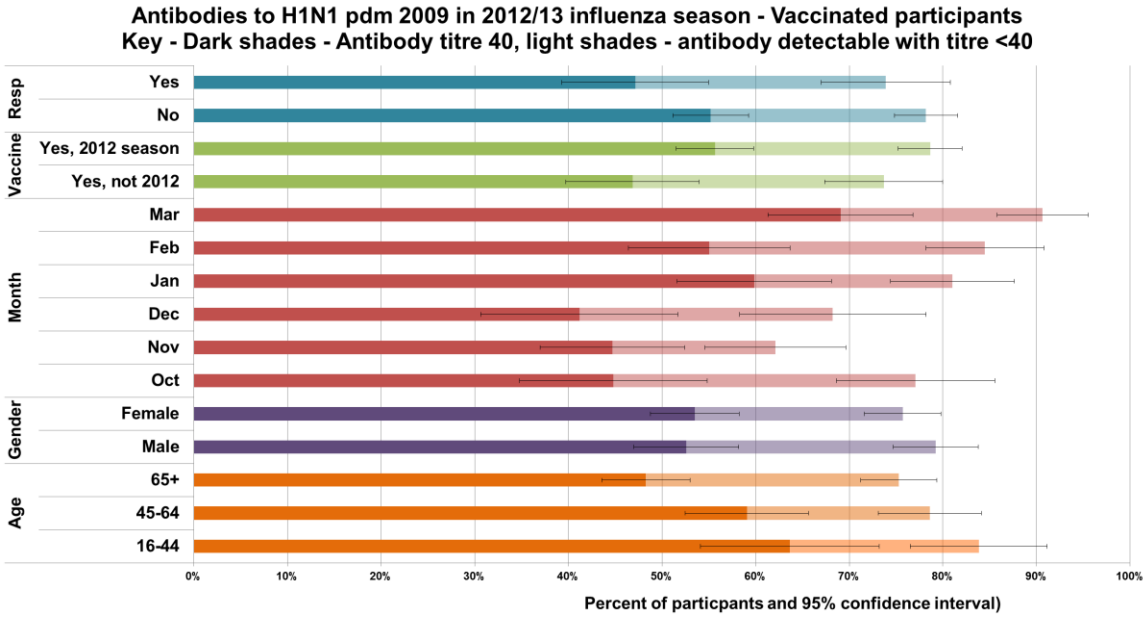
Table 5-1: Serology Results for A(H1N1)pdm09 and A(H3N2) for all participants

All participants		All Participants		A(H1N1)pdm09				A(H3N2)			
				Detectable antibodies		Titre >=40		Detectable antibodies		Titre >=40	
Characteristic	Category	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
Age (years)	16-44	594	32 (30-34)	379	64 (60-68)	232	39 (35-43)	305	51 (47-55)	215	36 (32-40)
	45-64	734	39 (37-42)	443	60 (57-64)	266	36 (33-40)	384	52 (49-56)	270	37 (33-40)
	65+	542	29 (27-31)	373	69 (65-73)	233	43 (39-47)	398	73 (70-77)	312	58 (53-62)
Gender*	Male	845	46 (43-48)	540	64 (61-67)	311	37 (34-40)	473	56 (53-59)	337	40 (37-43)
	Female	1008	54 (52-57)	641	64 (61-67)	410	41 (38-44)	603	60 (57-63)	450	45 (42-48)
Month	Oct	299	16 (14-18)	147	49 (43-55)	72	24 (19-29)	172	58 (52-63)	121	40 (35-46)
	Nov	361	19 (18-21)	172	48 (42-53)	102	28 (24-33)	213	59 (54-64)	146	40 (35-46)
	Dec	168	9 (8-10)	92	55 (47-62)	51	30 (23-37)	80	48 (40-55)	58	35 (27-42)
	Jan	336	18 (16-20)	215	64 (59-69)	136	40 (35-46)	165	49 (44-54)	125	37 (32-42)
	Feb	371	20 (18-22)	290	78 (74-82)	172	46 (41-51)	230	62 (57-67)	167	45 (40-50)
	Mar	335	18 (16-20)	279	83 (79-87)	198	59 (54-64)	227	68 (63-73)	180	54 (48-59)
Vaccine	No	1123	60 (58-62)	617	55 (52-58)	332	30 (27-32)	517	46 (43-49)	317	28 (26-31)
	Yes, not 2012	190	10 (9-12)	140	74 (67-80)	89	47 (40-54)	141	74 (68-80)	106	56 (49-63)
	Yes, 2012 season	557	30 (28-32)	438	79 (75-82)	310	56 (52-60)	429	77 (74-81)	374	67 (63-71)
Respiratory illness in last month**	No	1436	79 (77-81)	927	65 (62-67)	569	40 (37-42)	840	58 (56-61)	606	42 (40-45)
	Yes	383	21 (19-23)	231	60 (55-65)	141	37 (32-42)	221	58 (53-63)	174	45 (40-50)

***missing 17 observations**
****missing 51 observations**

Figure 5-2: Antibodies to A(H1N1)pdm09 (a) and A(H3N2) (b) in the 2012/13 influenza season in vaccinated participants

a)



b)

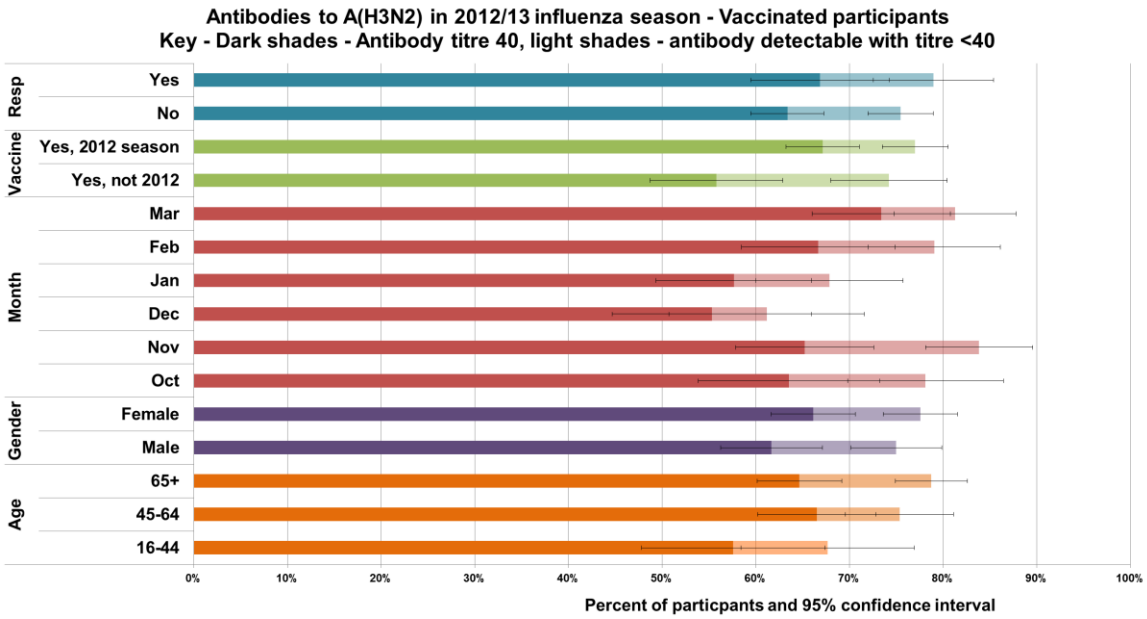
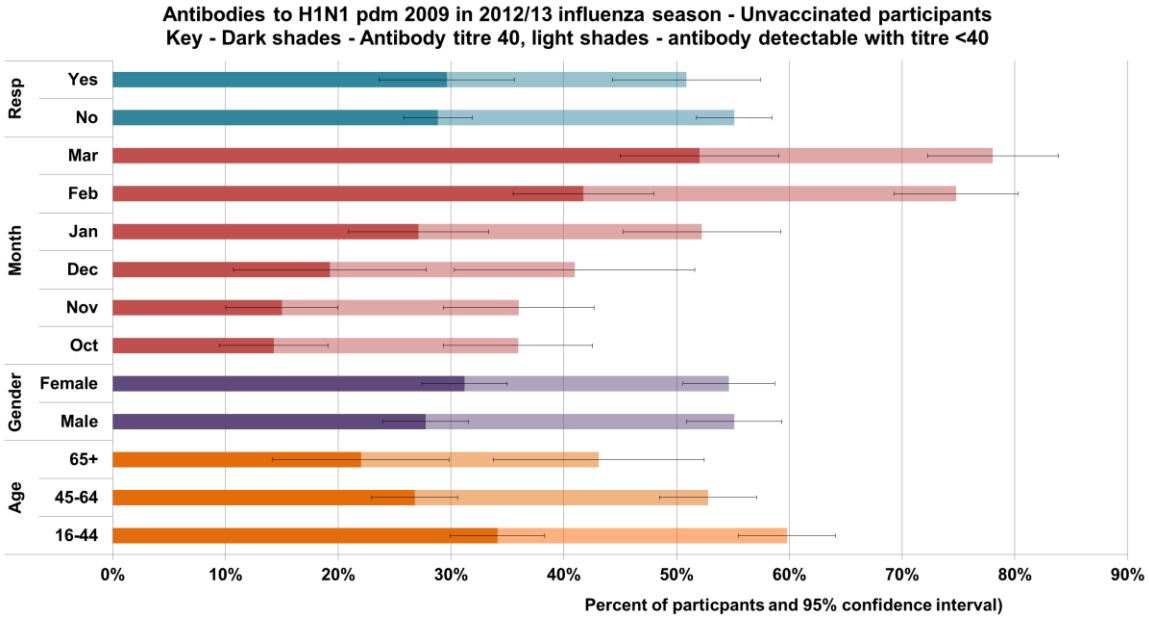
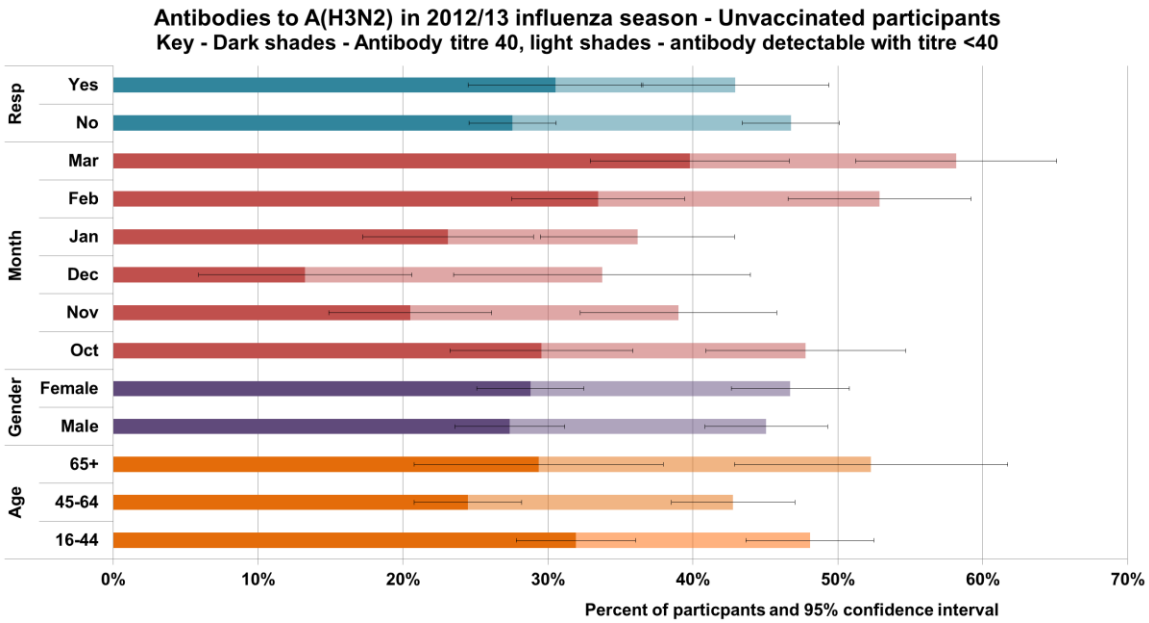


Figure 5-3: Antibodies to A(H1N1)pdm09 (a) and A(H3N2) (b) in the 2012/13 influenza season in unvaccinated participants

a)



b)



5.5.3 Serological changes over the influenza season

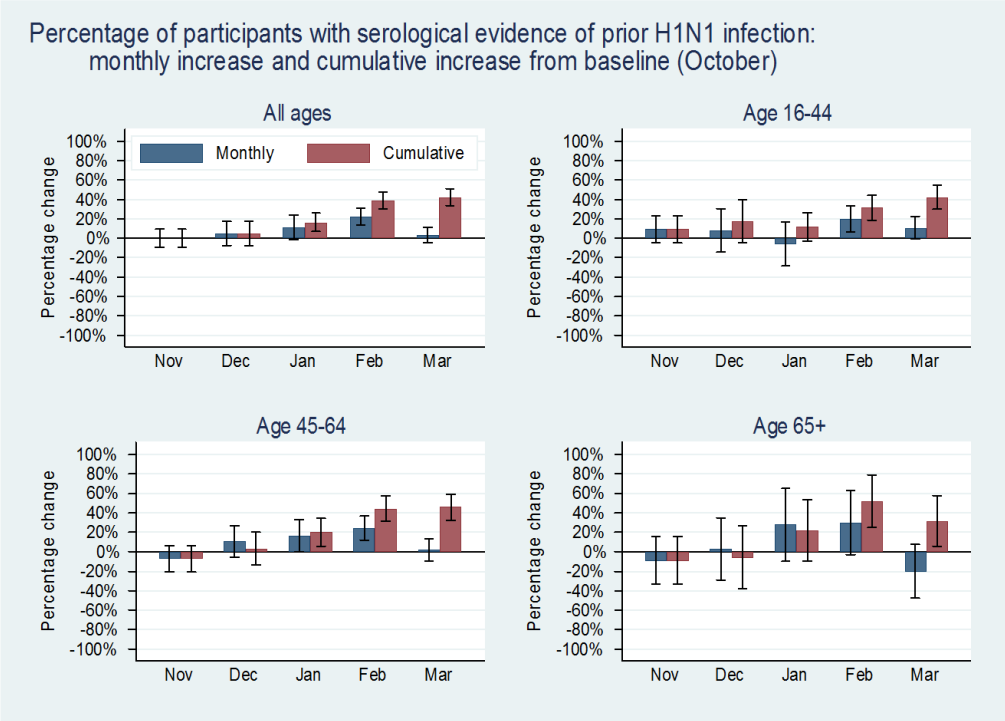
5.5.3.1 *A(H1N1)pdm09*

Overall half (49%, 95% CI [43-55]) of participants had detectable A(H1N1)pdm09 antibodies in October rising to 83% (95% CI [79%-87%]) in March. The proportion with protective titres rose from 24% (95% CI [19%-29%]) in October to 59% (95% CI [54%-64%]) in March (Table 5-1). In the unvaccinated, 36% (95% CI [29%-43%]) had detectable antibodies in October rising to 78% (95% CI [72%-84%]) in March (Chi^2 $p<0.001$). The proportion of unvaccinated participants with protective titres rose from 14% (95% CI [9%-19%]) in October to 52% (95% CI [45%-59%]) in March (Chi^2 $p<0.001$) (Figure 5-2a). Thus, compared to the beginning of the influenza season an additional 42% (95% CI [33% - 51%]) of the unvaccinated population had detectable H1N1pdm2009 antibodies by the end of the season and an additional 38% (95% CI [29% - 46%] had protective antibody titres.

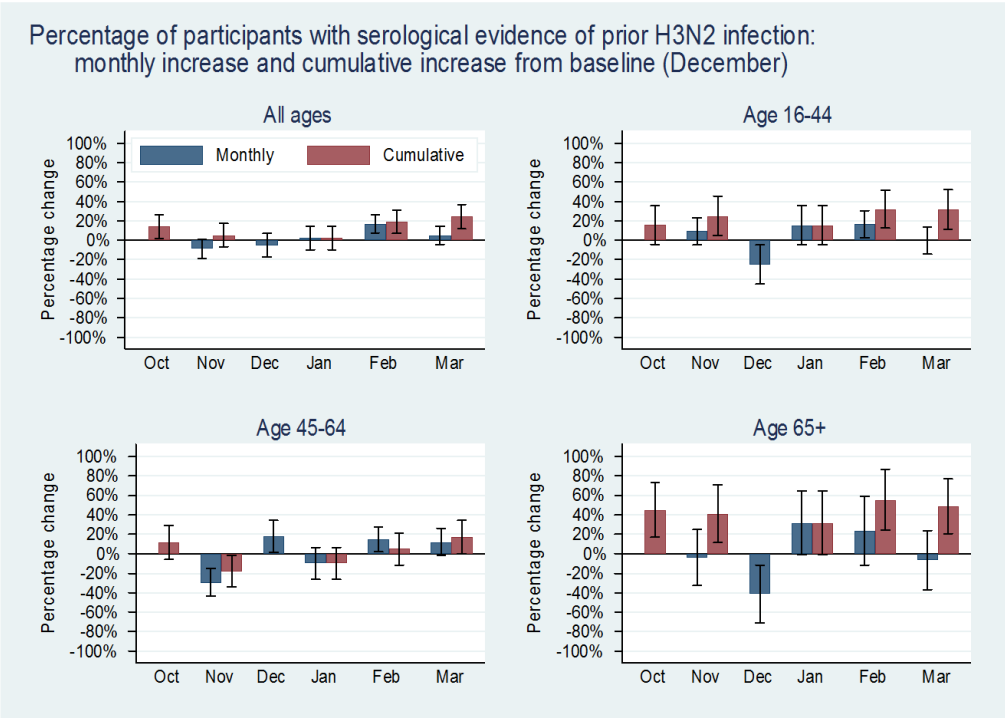
Figure 5-4a displays the increase in the percentage of the unvaccinated population with detectable A(H1N1)pdm09 antibody titres compared to the previous month (monthly increase) and the increase since October for each month (cumulative increase). For all age groups combined, the greatest monthly increase in detectable titres was seen when comparing February to January (52%, 95% CI [45%-49%] to 75%, 95% CI [69%-80%] respectively). Compared to January an additional 23% (95% CI [14%-31%], Chi^2 $p<0.001$) of the unvaccinated population had detectable H1N1pdm2009 antibodies, indicating peak circulation in the January to February period. The timing of this peak is the same in the national virological surveillance data (Figure 5-1). Patterns were similar for all age groups although the numbers of unvaccinated participants per month in those aged > 65 are low, resulting in less clear patterns. The cumulative increase from October through March was 42% (95% CI [30%-55%], Chi^2 $p<0.001$) in those aged 16-44 and 46% (95% CI [33%-59%], $p<0.001$) in those aged 45-64. In those aged 65+ the cumulative increase from October to February was 52% (95% CI [25%-79%], Fisher's exact $p=0.003$) but then there was a decline in March to 32% (95% CI [6%-58%], Fishers exact $p=0.045$), which may reflect precision of estimates due to modest sample size and low incidence during this time period, or instability of antibody response in the elderly.

Figure 5-4: Monthly and cumulative (October baseline) increases in proportion of individuals with a) detectable A(H1N1)pdm09 and b) A(H3N2) (December baseline) antibody titres in unvaccinated participants

a)



b)



5.5.3.2 *A(H3N2)*

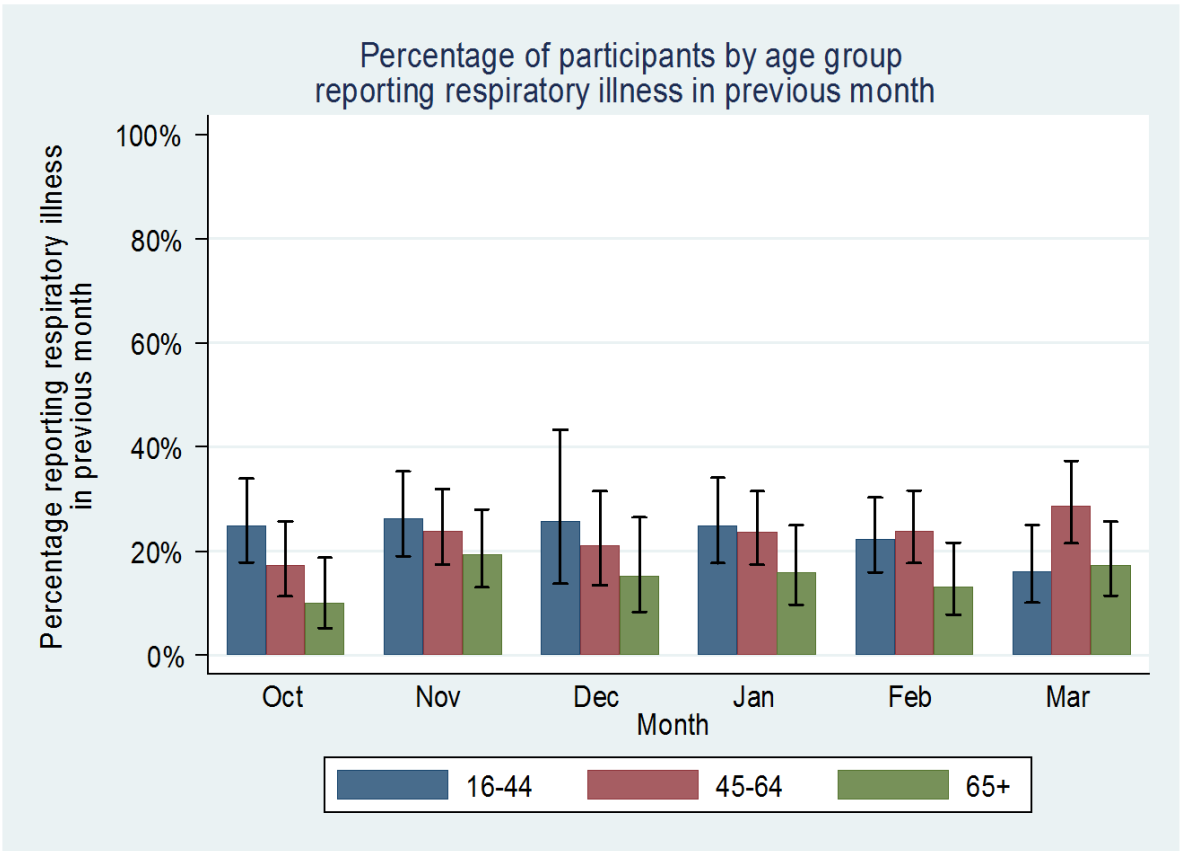
In contrast to A(H1N1)pdm09, overall levels of A(H3N2) antibodies declined between October and December, with increased levels thereafter. Between 48% (95% CI [40%-55%]) in December and 68% (95% CI [63%-73%]) in March of participants had detectable A(H3N2) antibodies. The proportion with protective titres reported from a given month fluctuated between 35% (95% CI [27%-42%]) in December to 54% (95% CI [48%-59%]) in March (Table 5-1). In the unvaccinated 34% (95% CI [23%-44%]) had detectable antibodies in December rising to 58% (95% CI [51%-65%]) in March (Chi^2 $p<0.001$). The proportion of unvaccinated participants with protective titres rose from 13% (95% CI [6%-21%]) in December to 40% (95% CI [33%-47%]) in March (Chi^2 $p=0.01$) (Figure 5-2b). This finding equates to an additional 24% (95% CI [12%-37%]) of the unvaccinated population having detectable A(H3N2) antibodies between December and March and a similar proportionate increase at 27% (95% CI [16%-37%]) in protective antibody titres.

Figure 5-4b shows the increase in the percentage of the unvaccinated population with detectable A(H3N2) titres compared to the previous month (monthly increase) and the increase relative to December for each month (cumulative increase). Across all age groups the greatest monthly increase in detectable titres was seen when comparing February to January (36%, 95% CI [30%-43%] to 53% (95% CI [46%-59%] respectively). Compared to January an additional 17% (95% CI [8%-26%], Chi^2 $p<0.001$) of the population had detectable A(H3N2) antibodies, indicating peak circulation in the January to February period. Patterns were broadly similar for all age groups. This broadly corresponds with national surveillance data (Figure 5-1), which shows H3N2 peaking in week 8. The cumulative increase from December through March was 32% (95% CI [11%-52%], Fisher's exact $p=0.01$) in those aged 16-44 and 17% (95% CI [0%-34%], Chi^2 $p=0.05$) in those aged 45-64. In those aged 65+ the cumulative increase from December to February was 56% (95% CI [24%-87%], Fisher's exact $p=0.013$) but then there was a decline in March to 49% (95% CI [21%-77%], Fisher's exact $p=0.019$), which is similar to the pattern observed for A(H1N1)pdm09.

5.5.4 **Secondary outcomes**

Cumulative vaccine uptake plateaued by December, reaching 75% (95% CI; 62-84%) in those aged 65+, 28% (19-39%) in those aged 45-64 and 16% (7-33%) in those aged 16-44. The percent reporting a respiratory illness in the month prior to recruitment (Figure 5-5) peaked in November at 26% (95% CI; 19-35%) in those aged 15-44. In those aged 45-64 the peak was in March (29%; 21-32%). In those aged 45-64 the peak was in November (19%; 13-28%). Rates of respiratory illness were consistently lower in those aged 65+ than younger age groups (Wald p=0.001).

Figure 5-5: Reported respiratory illness in the previous month



5.6 Discussion

5.6.1 Summary and relevance of key findings

Our results show that a high proportion of the English population is infected with influenza over the course of a few months. Infection with A(H1N1)pdm09 was common with 42% (95% CI [33% - 51%]) of the unvaccinated population moving from undetectable to detectable H1N1pdm2009 antibodies; the equivalent figure for A(H3N2) was 24% (95% CI [12%-37%]). Using a higher threshold titre of 40 the cumulative increase was 38% (95% CI [29% - 46%] for H1N1pdm2009 and 27% for A(H3N2) (95% CI [16%-37%]). These serological estimates of the cumulative incidence of infection will invariably be far higher than those based on virological surveillance of cases seeking medical care since the majority of cases are mild and self-limiting. Additionally, asymptomatic infections account for 25% to 77% of all infections [14,15,70]. Our cumulative incidence estimates are also higher than those typically estimated from community cohort studies (10-20%) which identify infections based on a four-fold rise in antibody titre from pre- and post-season serological samples [15,163,234]. Some have argued, however, that many infections have a lower than 4-fold increase in antibody titre so cumulative incidence estimates based on 4-fold rises may be underestimates [162]. Regardless, both methods indicate that a substantial proportion of the population is infected with circulating strains each year. We also found evidence of A(H3N2) antibody waning in the autumn of 2012, albeit with wide confidence intervals. These findings are consistent with the fact that the previous influenza season was relatively late in the spring and A(H3N2) was the dominant circulating strain[231].

This study may shed light on the levels of antibodies in a population that enable and curtail epidemic spread. Community transmission shapes the rise and fall of epidemics. Many factors related to the virus, the host and the environment influence transmission including host population mixing patterns and immunity, probability of infection per contact, and climatic factors[235]. In temperate zones of the Northern and Southern hemispheres, influenza typically causes annual epidemics in winter months[18]. This seasonal pattern is thought to be partly driven by climatic factors including temperature and humidity levels[236]. The seasonality patterns in temperate zones may also be driven in part by the changing levels of immunity in the population over the course of a year. The annual wave of

infections and vaccinations stimulates population-level antibodies and increases corresponding immunity. As population level immunity builds, the number of susceptible individuals is depleted, reducing community transmission and the corresponding reproduction number (R). The reproduction number is the average number of secondary infections arising from one infection. When R>1 the rate of infections and the corresponding epidemic curve rise, when R=1 the rate of infection is stable and the epidemic curve is flat and when R<1 the rates of infection and the epidemic curve fall. Over time individuals' antibody responses and corresponding immunity wane and this leads to a replenishing of the number of susceptibles in the population, allowing the cycle to begin again.

In this study we found that at a time when up to approximately 50% of adults have detectable antibodies against A(H1N1)pdm09 and up to approximately 30% of adults have protective antibodies, A(H1N1)pdm09 was able to spread epidemically. For A(H3N2) epidemic spread began when approximately 50% of adults had detectable antibodies against A(H3N2) and approximately 35% had protective levels. These low levels may correspond with an immune environment whereby spread is feasible given other conditions that favour transmission such as colder weather. We also found that when detectable antibodies against A(H1N1)pdm09 reached approximately 80% in adults and protective levels around 50-60%, the incidence and the epidemic curve began to fall. Likewise, for A(H3N2) the epidemic began resolving when detectable titres reached 60-70% and protective titres reached approximately 55%. These higher levels of antibodies, captured at a time that represents the peak of transmission (e.g. a few weeks prior to date of specimen collection) may correspond with an immune environment that restrains transmission. These findings may give us insight into the herd immunity threshold which is the proportion of the population that would need to be immune for incidence to decline. It is calculated as $1-1/R_0$, where R_0 is the Basic Reproduction, a special case of R, representing the average number of secondary cases arising from one typical case in a *fully susceptible* population. Therefore, knowledge of herd immunity threshold would also give insights to strain-specific R_0.

The herd immunity threshold is often discussed in terms of vaccination programmes and the desire to reduce or even eliminate vaccine preventable diseases by vaccinating populations to the extent that they reach herd immunity[237]. R_0 and R are important measures of transmissibility and are critical parameters used in dynamic transmission modelling. While the herd immunity threshold is a very useful concept for some well-understood infections in well-characterised populations, a herd immunity threshold for influenza brings challenges both in terms of calculation and interpretation. R_0 and by extension the herd immunity threshold are not static values as they depend on factors affecting transmission which vary by population and virus strain and over time (i.e. seasonal weather patterns). Additionally, defining the proportion of the population immune to influenza is difficult because influenza serology thresholds do not produce a dichotomous outcome of immune or susceptible. Although titres of 1:40 and over are often equated to immunity, in fact they correspond to 50% protection against infection[159,238]. For this reason, methods which map each titre to its corresponding level of protection and then summarizing these values across the population[234,239] may be a better approximation of the proportion that is immune.

To further investigate whether the population-level antibody levels we found represent true immune environments which enable and curtail epidemic spread in England, it would be useful to repeat similar cross-sectional sero-surveys to see if the strain-specific antibody levels at the start and the peak of epidemics remain consistent over multiple seasons. Crucially, these surveys would also need to include children as they are thought to be key drivers in seasonal influenza epidemics due to higher levels of infection and contact patterns. Their immune status as measured by antibody levels may be more impactful on the propagation of seasonal epidemics than levels in adults[240]. Despite these caveats however, it is interesting to note that many previous estimates of seasonal influenza epidemics place R (combinations of R_0 and R) consistently around 1.28 and pandemic R values between 1.46 – 1.8[235]. These numbers give crude herd immunity thresholds at 22% and 32% to 44%.

5.6.2 **Severity of influenza**

By comparing serological evidence on population incidence of infection to national surveillance data on ILI consultations, viral isolations, hospitalisations and deaths we can infer a broad assessment of the severity of influenza infections.

The fact that there were high levels of infection with H1N1pdm2009 and A(H3N2) but lower levels of ILI consultations and of virological isolation of these strains (particularly H1N1pdm2009)[230] suggests that only a very small proportion of infections result in ILI consultation or virological confirmation. This confirms previous finding of the low pathogenicity of the H1N1pdm2009 strain [15,241] and may be related to the finding that cross reactive T cell based immunity can prevent illness in those infected with influenza [242], and that most symptomatic influenza illnesses do not lead to a consultation [15]. The high levels of infection found in the current study but low levels of ILI consultation are consistent with these findings. The fact that A(H1N1)pdm09 and A(H3N2) accounted for almost half of ICU admissions with confirmed influenza[230] demonstrates the A(H1N1)pdm09 strain can, on rare occasions, still cause severe illness. National surveillance showed that excess mortality was higher in this year than in recent years and was mainly due to respiratory illness in the elderly. These reports also show that excess mortality peaked in January and February and that excess mortality coincided with peaks of H3N2, influenza B and RSV as well as with a period of unusually cold weather. Our data show that the peak of excess mortality also coincided with the peak of A(H1N1)pdm09 infection suggesting infection with A(H1N1)pdm09 may also have made a contribution to excess mortality.

We did not conduct influenza B serology, primarily because this study was focused on piloting pandemic influenza sero-surveillance and influenza B does not cause pandemics. However, it would have been interesting to compare the cumulative incidence of influenza B infection relative to surveillance estimates of virological confirmed cases and hospitalization, particularly as influenza B was widely circulating and accounted for approximately half of the influenza ICU admissions[230].

5.6.3 **Design considerations**

The PIPS study overcame the limitations of using convenience serum samples (which are typically unrepresentative of the population and lack individual-level data such as vaccination) by simply adding an additional blood specimen and a few additional survey questions to the existing data collection of an annual, rolling, nationally representative survey (The HSE). Another benefit of participating with the HSE was that it collected data and specimens throughout the year. This enabled our PIPS study to overcome the challenge of specimen timing as serum collection could be initiated quickly (important in a pandemic) and continue throughout the influenza season, enabling the monitoring of changes over time. These study design elements enabled nationally representative analyses of infection and immunity broken down by key characteristics such age, sex, time, and region and, importantly, by vaccination status. Knowledge of vaccination status enabled us to distinguish antibodies generated from natural infection from antibodies generated from vaccination. Knowing the source of various antibodies (from vaccination or from infection) leads to more interpretable serology as well as the ability to answer even more questions about immunity and infection through sub-group analysis of vaccinated and unvaccinated individuals. In comparison, a major serological survey[56] conducted in England during the 2009 pandemic used residual serum samples from routine microbiological and chemical pathology testing which lacked individual-level vaccination data. As a result, interpretation of results was more difficult following the roll-out of pandemic vaccination in Autumn of 2009. The PIPS study was also designed to overcome the challenges that our group and many others experienced when conducting pandemic research generally and specifically collecting serological data. Many of these challenges were in the realm of logistics (i.e., very short time scales, costs, resources, pre-agreed ethics and approvals, etc).

A key limitation to our study is the lack of prospectively collected clinical and virological data. The cross-sectional nature of the data collection only enables retrospective reporting of respiratory illnesses which suffers from recall bias[98,243,244]. Unlike prospectively collected data on influenza-like illness[230], our symptomatic data did not vary by month, making this symptomatic data an ineffective tool for approximating influenza illness.

Ideally study participants would be invited into a prospective cohort study of respiratory illness and virological confirmation.

Another limitation of the study is the fixed sample size of approximately 300 specimens per month. The monthly sample sizes enable monthly estimates of cumulative incidence. However, the age-specific estimates have relatively wide confidence intervals. This is in contrast to the large sero-survey conducted in England during the 2009 pandemic[130,56] which, through the use of residual clinical specimens, had access to many more specimens and was thus better powered for monthly age-specific rates of cumulative incidence than our study.

Aside from power constraints, our statistical analysis has three main limitations[130,245]. Firstly, by analysing serological data by calendar month, our analysis cannot account for changing incidence within a given month, and incidence can change rapidly during an epidemic peak[130,245]. Secondly, the time it takes individuals to develop antibodies following infection is variable and even if all samples were taken on the same day, each result would represent incidence at different previous time points[130,245]. Finally, our estimation of incidence based on comparison of prevalence between time points reduces precision and this can cause negative point estimates in groups with low levels of incidence[56,130,245,246]. The latter may be the reason we estimated negative monthly change in cumulative incidence of H1N1pdom09 and H3N2 between February and March in the oldest age group, although this could also be due in part to antibody waning. These limitations could be overcome to some extent in the future using likelihood-based methods which incorporate information on the changing clinical incidence over the course of the epidemic (as measured through surveillance) and the distribution of the time to seroconversion in conjunction with the exact specimen dates [130,245].

Developing assays for new strains and conducting high throughput serological assays takes significant expertise and time. Failure to maintain the expertise gained through this pilot is a threat to our study's ability to provide timely information in the event of a pandemic. PHE is the only organization in England that currently maintains influenza serological expertise and capacity. Given that PHE respiratory infection teams become extremely busy

during the influenza season and particularly during pandemics we feel there would be national value in maintaining the serological expertise developed through this project to enable ongoing serosurveillance through HSE.

Whilst the PIPS study design of repeated cross-sectional surveys has solved many of the logistical issues and ensured representative samples, collected at the right times with the necessary associated individual-level data, there is still the question of whether cross-sectional serological surveys are the best study design for estimating population immunity and rates of infection or whether longitudinal studies with paired or repeated serological samples are better.

Researchers have investigated how estimates of the cumulative incidence from cross-sectional seroprevalence studies compare with estimates from longitudinal cohort designs with paired sera. Studies from the 2009 pandemic which compared estimates from cross-sectional and longitudinal paired sera data collected in the same city during the same period found that the two study designs produced similar estimates, particularly in sub-groups with high incidence [246] and if baseline cross-reactive antibody levels were accounted for [247]. Further simulation studies based on existing data have concluded that background levels of immunity and level of antibody boosting can affect the accuracy of seroprevalence estimates [248]. If background immunity is high then cross-sectional studies are more prone to bias than longitudinal paired designs[248]. If background immunity is not high however the two designs produce similar results[248]. In situations where background immunity is high and/or post-infection boosting is low, then the ability to detect infections with low-level boosting in paired longitudinal sera would substantially increase the accuracy of estimations [248]. Further research on identifying and classifying infections due to low-level boosting is warranted.

Apart from potentially less biased estimates of incidence, longitudinal paired serological studies have other benefits such as the ability to investigate antibody waning directly and, when coupled with prospective clinical follow-up, the ability to generate other important estimates such as the proportion of infections that remain asymptomatic, proportion of illnesses meeting various case definitions (such as those used in surveillance), and the

proportion of illnesses that consult [247]. In longitudinal studies however, serum samples are typically collected at two defined points and often try to bracket epidemics which may be hard to predict in advance. More frequent sampling of individuals may not be feasible due to cost and participant follow-up fatigue so real-time estimates are unlikely to be as easy to generate in a longitudinal design compared to cross-sectional studies [247]. Another limitation of longitudinal studies, especially if they include clinical follow-up is that they are heavily resource intensive, making them more difficult to fund and run generally and especially difficult to fund on a continuous basis. Continuing surveillance is ideal to provide comparable pre-pandemic baseline measurements.

The best choice of study design will invariably depend on factors which may be known in advance (e.g. the aims of the study, the resources available) but also on unknown factors such as the background level of immunity and incidence rates of novel pandemic virus. Given the uncertainty of pandemics and the need to respond quickly to changing circumstances, it is worth considering whether scalable adaptable or hybrid study designs can be developed in advance and continuously run, ensuring continuity of data and collection of the most suitable data.

To maximise the value and the information generated by the PIPS study, we recommend that the study is run as an ongoing serosurveillance programme to provide information for both seasonal and pandemic influenza. Ideally, our serosurveillance would also be linked to Flu Survey (or similar study) for prospective illness reporting and Flu Survey would be enhanced to include virological surveillance (virological testing was successfully piloted in 2014/15). Results of such a system combining community serological, clinical and virological surveillance could be incorporated into regular national situation reports and "now casting" models to inform the national response to seasonal and pandemic influenza.

Other future modifications could be the incorporation of likelihood-based methods and the extension of the methodology to other endemic or emerging infections of public health importance. Additionally, the study could be used as a base to develop and assess the accuracy of minimally invasive serological tests allowing the incorporation of children into serosurveillance.

5.7 Relevance to COVID-19

The key risk of the PIPS study design was its vulnerability to interruption of sample collection by the HSE in the event of a severe pandemic. In the spring of 2020, this is exactly what happened with COVID-19. The HSE paused its household visits and as a result the PIPS study was not triggered. Just prior to the COVID-19 pandemic, the study team had been in the process of moving the PIPS serology to Public Health England for long term viability. After it was clear the study would not be triggered, we coordinated with the HSE and Public Health England to hand the study over to them so that they could run it as part of national surveillance when the HSE resumed, should they choose to do so.

The need for data on population antibodies and infection remained and early in the pandemic, three large studies were initiated in the UK – the Coronavirus (COVID-19) Infection Survey[249], the Real-Time Assessment of Community Transmission (REACT) study and the Virus Watch study.

The COVID-19 Infection Survey was designed to estimate prevalence and incidence of symptomatic and asymptomatic SARS-CoV-2 infection in general population and how this varies over time using PCR analysis of nose and throat swabs. It also aimed to estimate immunity to SARS-CoV-2 in the general adult population and how this varies over time, as reflected by immunoglobins and neutralising antibodies. The study is a series of repeated cross-sectional surveys of representative households across the UK, with nested serial sampling of a subset of participants. Household members aged 2 and over were invited. Different levels of follow up provided from a one home visit only, weekly visits for one month, or weekly visits for a month plus monthly visits for a year. Initial sample size target of 11,000 households. Visits collected health and socio-economic data, self-administered nasal swabs, blood specimens and self-reported symptoms.

The REACT study is divided into four main programmes of work with REACT-2 being the most relevant for this discussion. The REACT-2 study aimed to measure prevalence of SARS-CoV-2 antibodies in the community using home testing with lateral flow immunoassays (LFIA). After testing various aspects of validity, feasibility, usability and application of LFIAs in different populations, the main study design employed repeated

cross-sectional surveys of age-stratified population samples of 100,000 to 200,000 adults in England[250]. It was designed and powered to provide precise estimates of seroprevalence at the lower-tier local authority level for the purposes of public health response[250]. Between June 2020 and May 2021 it collected data from over 900,000 adults[251]. A sister programme of work, the REACT-1 study employed very similar design and methods except it collected self-administered nasal swabs to identify active infections (asymptomatic and symptomatic)[250]. It recruits over 150,000 participants in England each month and has tested almost 2 million people[252].

The Virus Watch study was a household-based, prospective community cohort study of COVID-19 in England. The study collected bracketed serological samples in autumn 2020 and spring 2021 from a subset of 10,000 participants aged five years and over. In the spring of 2021, monthly finger-prick blood specimens were collected from consenting adults from the wider cohort for serological testing of anti-N and anti-S antibodies.

The PIPS study was designed and piloted during the interpandemic years following the relatively mild 2009 influenza pandemic. Limited funding was available for this type of pandemic research and since serological surveys are expensive endeavors, the study was designed to maximize data on a minimal budget (less than half a million pounds was available for both pilot and pandemic reactivation). The COVID-19 Infection Survey, REACT studies and Virus Watch were implemented at the start of a severe pandemic at much higher levels of funding (e.g., hundreds of millions of pounds annually for the COVID-19 Infection Survey[253] and a few million for Virus Watch). The enormous number of participants of REACT and the COVID-19 infection survey enabled very precise estimate of seroprevalence and transmission by fine resolutions of age, geographic location and over time. The COVID-19 infection survey, REACT studies and Virus Watch also incorporated swabbing and antigen testing as well as varying degrees of follow up of individuals as part of their studies, increasing the number of research questions they could answer. They also benefited from new technologies. These included SARS-CoV-2 serological assays that required only small amounts of capilliary blood collected from fingerpricks rather than traditional venous blood specimens. This eliminated the need for

phlebotomists and home visits. Additionally, LFIA tests could be self-administered at home, eliminating the need for specialist laboratories.

Currently, the REACT-2 study has completed data collection and Virus Watch is due to finish shortly. The COVID-19 Infection Survey is ongoing, but due to its immense cost it is likely unsustainable in the long-term. Ongoing community-level serosurveillance will be needed for SARS-CoV-2 and therefore more cost-effective studies will be needed.

During the COVID-19 pandemic, influenza circulation has been suppressed across the globe to unprecedented levels, likely the result of the non-pharmaceutical interventions and general social distancing in response to the pandemic[254–256]. Population level immunity to influenza is likely to be at an all-time low, prompting fears of a strong resurgence in influenza when it does begin circulating widely again[257]. Now would be an excellent time to start investigating levels of influenza immunity in the population, possibly using residual specimens from the population-level studies of COVID-19 (specimen volume permitting). Additionally, the Health Security Agency could re-initiate specimen collection through the HSE using the PIPS study design. Collection of such data may help us predict what the resurgence of influenza might look like and give us a greater understanding of influenza antibody dynamics in this unusual natural experiment presented by the COVID-19 pandemic.

Chapter 6 **Tools to Measure Mild Disease**

Chapters 3-5 focus on infection and immunity and more specifically the proportion of the population that becomes infected each season and identification of groups at greater risk of infection. This chapter and the following chapter move up a level on the influenza iceberg to focus on the subset of infections that lead to illness. This chapter will introduce the epidemiological considerations and laboratory methods used to identify acute influenza illness. It will conclude with a brief overview of the burden of influenza illness in the community, focusing on the results from the Flu Watch study. The methods presented here are used in the two Flu Watch analyses on influenza disease in chapter 7.

6.1 Epidemiological study design

As discussed in previous chapters, longitudinal epidemiological study designs (e.g. cohort studies) with active follow-up are the most appropriate for identifying acute respiratory illnesses. Active follow-up not only helps ensure the estimates of the rates of acute illness are accurate[258], but also helps remind participants to take specimens (nasal swabs in the case of Flu Watch) shortly after illness onset. As influenza illnesses can be quite mild it is important to collect data on all respiratory illnesses, not just those of a certain severity or symptom profile (e.g. traditional influenza-like-illness case definition). The two data analyses presented in this chapter use data from the Flu Watch study. Previously published Flu Watch analyses, which I contributed to, have primarily focused on measuring the frequency and risk factors of infection and illness[15,242]. In contrast, the analyses presented here focus on quantifying the severity and impact of those illnesses and therefore required a new Flu Watch dataset which focused on illnesses. Prior to analysis I created this dataset by merging daily level data on the type and severity of symptoms, PCR outcome, health seeking behavior, absences from work and school and health-related quality of life for each illness reported in the Flu Watch cohort.

6.2 Choice of Diagnostic Assay

Because community-level studies aim to identify all influenza illnesses in the community, not just those that consult, any technique used to diagnose influenza illness should ideally

only require information and/or specimens which can be easily obtained and/or reported by the participant in their own home. If specimens are taken in the home, they need to be stable enough to withstand the transport to the laboratory conducting the analysis. The technique additionally needs to be scalable to large cohorts or populations, particularly in terms of transport, laboratory infrastructure, staff requirements and costs. Finally, an influenza diagnostic would ideally be capable of typing and subtyping viruses.

Some laboratory diagnostic techniques require specific types of clinical specimens (e.g. nasopharyngeal swabbing or wash/aspirate procedures) but RT-PCR (which was used in the Flu Watch study) can use any type of respiratory specimen[3]. While nasopharyngeal aspirates are considered the gold standard specimen, nasal swabs are the most suitable for self-administration as they are easier and more comfortable to collect, show similar sensitivity to nasopharyngeal aspirates and additionally show similar levels of viral load and swab positivity regardless of whether they were self-administered or collected by a healthcare worker[110,259,260] Additionally, the positivity rate of nasal swabs stored in viral transport medium does not appear to be affected by length of time in post and thus hold up well in transport[261]. Specimen collection should ideally coincide with peak viral shedding (day 2 of illness for seasonal influenza) although virus is often detectable for 5-7 days after illness onset and sometimes longer, especially in children[3,14].

The accuracy of the diagnostic assay is another critical component when it comes to choosing a diagnostic test, particularly if the diagnostic test is not considered a reference or 'gold-standard' test. The accuracy of a non-gold-standard diagnostic assay can be determined through direct comparison with a gold standard test. If subjects or specimens are tested by both assays, then a 2 x 2 contingency table can be constructed (Figure 6-1) from which test characteristics can be calculated. The test characteristics used as part of this chapter are sensitivity, specificity, the predictive value of a positive test and the predictive value of a negative test. Taking the gold standard as the truth, the sensitivity of the diagnostic test is the proportion of true positives (in this context - influenza illnesses) correctly identified by the test and the specificity is the proportion of true negatives (non-influenza illnesses) correctly identified by the test. The predictive value of a positive test is the probability that an illness classified as positive by the test truly is an influenza illness

and the predictive value of a negative test is the probability that an illness classified as negative truly is not an influenza illness.

Figure 6-1: Test characteristics of diagnostic test compared to gold standard

		Gold standard test		Totals
		Positive	Negative	
Diagnostic Test	Positive	a	b	a+b
	Negative	c	d	c+d
Totals		a+c	b+d	a+b+c+d

True Positives = a	False Positives = b
True Negatives = d	False Negatives = c

Using the 2 x 2 contingency table (Figure 6-1) we use the following formulas:

$$True\ prevalence = \frac{a+c}{a+b+c+d}$$

$$Sensitivity = \frac{a}{a+c}$$

$$Specificity = \frac{d}{b+d}$$

$$Predictive\ value\ of\ a\ positive\ test = \frac{a}{a+b}$$

$$Predictive\ value\ of\ a\ negative\ test = \frac{d}{c+d}$$

The predictive values of a positive and negative test are affected by the underlying prevalence of disease, but the sensitivity and specificity are unaffected by prevalence.

6.3 Determining Etiology of illness

There are a number of techniques used to identify acute influenza illnesses ranging from symptomatic case definitions to laboratory diagnostics. For the purposes of this chapter, I will focus on the techniques most used and/or most suited for identifying acute influenza illnesses in large community-level studies or surveillance.

6.3.1 Clinical Case Definitions

Diagnosing acute influenza illness on the basis of symptoms alone is challenging. While self-reported symptoms are easier to collect than are the specimens, the accuracy of symptomatic case definitions is low. The specificity of clinical case definitions is generally quite low as influenza disease presents in the same way as many common acute respiratory infections [16,262,263]. The sensitivity of most clinical case definitions is also low as many symptomatic influenza cases do not develop the more discriminating symptoms (e.g. fever) that are often included in these case definitions [15]. However, if a case definition is made more inclusive and thus more sensitive, then the specificity of that case definition is reduced. Some of the issues surrounding the use of influenza clinical case definitions are explored later in this chapter (section 7.2).

6.3.2 Laboratory Diagnosis

Influenza infection can be detected using laboratory techniques during the acute phase of illness by identification of virus particles or components, typically found in respiratory secretions in seasonal influenza cases or sometimes additionally in the blood or feces in zoonotic cases [3]. Viral replication for seasonal influenza typically peaks in the first few days of illness and remains at detectable levels for 5-7 days in adults and longer in children[3]. Ideally, specimens should be collected as soon as possible during illness, to coincide with peak viral shedding [3].

There are a number of diagnostic techniques to detect, identify and characterize influenza virus infections, each providing different information with its own set of advantages and disadvantages [3].

6.3.2.1 *Virus isolation using cell cultures*

Virus culture, a technique developed in the 1940s has, and continues to be, a gold-standard test for influenza. It involves the propagation of influenza virus in mammalian cells or embryonated eggs followed by virus isolation using various techniques. Depending on the methods, it can take anywhere from 24 hours to 10 days for propagation and confirmation of influenza [3,264,265]. Virus culture generates a large quantity of virus which is necessary for advanced antigenic and genetic characterization of the virus as well as drug susceptibility testing. For these reasons virus isolation and culture are mainstays of advanced surveillance programmes and necessary for vaccine development. Disadvantages of the technique include the need for highly specialized laboratories and staff and the multi-day turnaround times[3].

6.3.2.2 *Immunofluorescence*

Immunofluorescent assays, including direct (DFA) and indirect fluorescent antibody (IFA) techniques, detect viral antigen and usually require specialized equipment. Although they can discriminate influenza A and B they are not able to further subtype influenza A. Turnaround time is 2-4 hours and although they have high specificity, they have relatively poor sensitivity [3,265].

6.3.2.3 *Molecular assays and RT-PCR*

Molecular assays include rapid molecular assays, reverse-transcription polymerase chain reaction (RT-PCR) and other nucleic acid amplification techniques (NAATs). RT-PCR methods are considered gold-standard techniques and have largely replaced virus culture as a diagnostic assay given their high sensitivity and specificity and faster turnaround times [3,266–268]. In RT-PCR, viral RNA is extracted from a clinical specimen, reverse transcribed into single-stranded complementary DNA (cDNA) and amplified in a series of cycles [264]. The resulting DNA fragments are then identified and in some cases quantified, depending on the type of RT-PCR [267,268]. RT-PCR techniques can identify influenza types, sub-types

and lineages [267,268]. The specimens for RT-PCR usually come from the upper respiratory tract. They are typically collected from deep within nostrils using a nasal swab, from the throat using an oropharyngeal swab or from the nasopharynx using a nasopharyngeal swab [267,268]. Recently rapid molecular tests have been developed with an aim to provide point of care testing and these are described further in rapid tests section (6.3.2.4).

6.3.2.4 *Rapid tests*

In recent years there have been many advances in rapid diagnostic tests with the aim for these technologies to be used for point of care testing. These tests, which yield results within 30 minutes, fall into three major categories: traditional rapid influenza diagnostic tests (RIDTs), automated immunochromatographic antigen detection tests (digital immunoassays), and rapid molecular tests [269]. Recent meta-analyses have estimated that traditional RIDTs have low sensitivity, digital immunoassays have much better sensitivity but rapid molecular tests have the highest levels of sensitivity (typically >90%) [269,270].

6.3.2.5 *Serological Testing*

Described in more detail in section 3.3, the diagnosis of a seasonal influenza illness using serological techniques requires paired sera, preferably an acute and convalescent sera taken two weeks apart, in order to determine seroconversion. Given the timeframes and the need for a second, convalescent sample, serological techniques are not usually used to diagnose acute influenza illness [3].

6.4 Burden of influenza illness in the community

Influenza infection, as discussed in the previous chapter, is relatively common. In the Flu Watch study we found that around 18% of the unvaccinated population became infected each winter season with the highest rates in children [15]. This finding is similar to those of historical and other contemporary community cohort studies [140,147,163]. We also estimated that around a quarter of these infections were symptomatic, which again was similar to estimates from other community cohort studies. For seasonal influenza, the Tecumseh study estimated 15-25% of H3N2 infections and 19-34% of Influenza B infections lead to illness [271]. A contemporary community-level, household-based cohort study in Vietnam (the Ha Nam Study) found that 14% of seasonal H1N1, 16% of pandemic H1N1, 11% of

H3N2, and 15% of influenza B infections led to influenza-like-illness [163]. These estimates are lower than the Tecumseh and Flu Watch Studies but are likely to be underestimates as they would have missed out on the milder influenza illnesses which did not develop fever and therefore would not have met the Ha Nam influenza-like-illness case definition.

Chapter 7 **Mild Disease**

This chapter presents two pieces of primary research using data from the Flu Watch study. The first is an analysis of the health-related quality of life lost and absences to work and school due to influenza illness. The second piece of work is an evaluation of the UK's National Pandemic Flu Service, an online and telephone service which assessed illnesses and distributed antivirals to symptomatic community cases during the 2009 pandemic.

7.1 Health Related Quality of Life and Absenteeism

7.1.1 Attribution

The work presented in this section has been adapted from my first author publication in Influenza and Other Respiratory Viruses [272]. I led this work with literature review and writing contributions from my colleague Dr Charlotte Warren-Gash and modelling contributions from Dr Peter White. I developed the research question and the overall analytical framework. I conducted the main statistical analysis and developed and produced the figures and tables. To complement my analysis of quality of life lost and absenteeism, I co-developed with Dr Peter White a modelling component to the analysis which projected my results to a population level. I produced additional population-level estimates which Dr White used in this modelling analysis. My colleague Dr Warren-Gash conducted the initial literature review for the manuscript and wrote the first draft of the introduction and conclusions after discussions on the interpretation of the literature and my analysis findings with myself and my supervisor Dr Hayward. I wrote the methods and results section apart from the sections on the modelling which Dr White provided. In response to peer-review comments, I expanded the literature review and re-wrote the discussion.

7.1.2 Abstract

Background: Estimates of health-related quality of life (HRQoL) and work/school absences for influenza are typically based on medically attended cases or those meeting influenza-like-illness (ILI) case definitions and thus biased towards severe disease.

Although community influenza cases are more common, estimates of their effects on HRQoL and absences are limited.

Objectives: To measure quality-adjusted life days and years (QALDs and QALYs) lost and work/school absences among community cases of acute respiratory infections (ARI), ILI and influenza A and B and to estimate community burden of QALY loss and absences from influenza.

Methods: Flu Watch was a community cohort in England from 2006 to 2011. Participants were followed up weekly. During respiratory illness, they prospectively recorded daily symptoms, work/school absences and EQ-5D-3L data and submitted nasal swabs for RT-PCR influenza testing.

Results: Average QALD lost was 0.26, 0.93, 1.61 and 1.84 for ARI, ILI, H1N1pdm09 and influenza B cases, respectively. 40% of influenza A cases and 24% of influenza B cases took time off work/school with an average duration of 3.6 and 2.4 days, respectively. In England, community influenza cases lost 24 300 QALYs in 2010/11 and had an estimated 2.9 million absences per season based on data from 2006/07 to 2009/10.

Conclusions: Our QALDs and QALYs lost and work and school absence estimates are lower than previous estimates because we focus on community cases, most of which are mild, may not meet ILI definitions and do not result in healthcare consultations. Nevertheless, they contribute a substantial loss of HRQoL on a population level. Work presenteeism during periods of respiratory infection is likely to have an important influence on transmission dynamics.

7.1.3 Introduction

Seasonal influenza has a major social and economic impact. As well as direct healthcare costs, influenza may lead to other indirect effects including school absenteeism, loss of workplace productivity and effects on health-related quality of life (HRQoL) [273].

Presenteeism (i.e. going to work when ill) can lead to non-household transmission and outbreaks in workplaces[274]. The quality of life of both patients and their families may be affected, especially when the patient is a child [275]. Quantifying indirect effects accurately is essential to inform cost utility analyses (CUA) of interventions to mitigate the population impact of influenza, including extension of seasonal vaccination policies.

In the United Kingdom, the National Institute for Health and Care Excellence (NICE) recommends that health effects of interventions are expressed in terms of Quality-Adjusted Life Years (QALYs) as this generic measure of health benefits incorporates both mortality and HRQoL [276]. The standardised validated tool EQ-5D [277] is NICE's preferred measure of HRQoL [276]. NICE use a cost utility threshold of £20,000-30,000 per QALY to judge whether or not interventions are deemed cost effective.

A systematic review of HRQoL in influenza showed a paucity of studies that used standardised, well-validated methods to generate estimates of the Quality-Adjusted Life Days (QALDs) lost [278]. It identified four previous estimates of QALDs lost due to influenza, which varied from 1.57 to 10.69 depending on the population sampled and method of HRQoL measurement used [279–282]. Many of these studies did not measure HRQoL throughout the duration of illness. They tended to measure HRQoL once at baseline and once on the worst day of illness, which required assumptions to be made about the shape of the QALY loss over the course of an illness [278].

Studies that measure HRQoL and work and school absence from influenza cases seeking medical attention may overestimate the indirect cost per case. A systematic review of studies of children's absences from school and day care due to influenza showed a gradient of days lost, with the longest absences reported by cases attending hospital emergency departments, then those in physician office-based studies followed by community cases [283]. Additionally, studies that estimate the population-level burden of HRQoL and absences from only severe cases miss the majority of influenza illnesses which, despite their mild nature, are likely to contribute substantially to the overall burden [278,284]. Although household-based studies are more likely to capture these milder illnesses that do not result

in a medical consultation, and therefore provide less biased estimates, their specificity is often limited by a lack of laboratory-confirmed outcomes.

There is therefore a need for robust estimates of the indirect effects of influenza from community studies identifying illnesses through prospective follow-up of all respiratory illnesses with PCR-confirmation of aetiology. In previously published work, my co-authors and I have described the community burden of influenza, ILI and acute respiratory infections not meeting the definition of ILI from multiple influenza seasons from the Flu Watch Study, a large community-based, household-level cohort in England [15]. Here I present the impacts of these illnesses on HRQoL and work/school absences using the same cohort as well as the population-level burden of these outcomes among community influenza illnesses.

7.1.4 Methods

7.1.4.1 *Study Design*

As described in in Section 2.1, the Flu Watch study is a household-based, community cohort study of acute respiratory disease and influenza infection in England [15,139]. In brief, the study followed up cohorts during six influenza seasons including 3 periods of seasonal influenza (winters 2006-07, 2007-08 and 2008-09) and the first three waves of the 2009 influenza pandemic (summer 2009, autumn-winter 2009/10 and winter 2010/11). In total 5484 participants were followed up for 118,158 person-weeks. Individuals were randomly recruited through primary care practices and their households invited to participate. Participants gave written informed consent and parents/guardians gave proxy consent for children. The Flu Watch study was approved by the Oxford MultiCentre Research Ethics committee (06/Q1604/103).

Baseline surveys collected demographic, socio-economic and occupation data. Participants were categorised into 'working' (employed full-time, part-time or self-employed), 'students' (self-classified, aged 5-15) and 'not in work/education'. Participants were contacted weekly and asked to record any "cough, cold, sore throat, or flu-like illness" which I define as an acute respiratory illness. During these illnesses, participants reported daily symptoms and temperature measurements using prospective illness diaries.

Parents/guardians completed surveys on behalf of their children as needed. Self-administered nasal swabs were requested on day two of any illness. Participants submitted the swabs by mail to be tested for circulating influenza A viruses (H1N1, H3N2 and from 2009 onwards H1N1pdm09), and influenza B viruses using RT-PCR [285,286].

7.1.4.2 *HRQoL Outcomes*

Between 2006/07 and 2009/10 illness diaries included daily questions on whether the ill individual had taken time off work/school. In 2006/07 through 2008/09 and for a subset of participants in 2009/10, illness diaries also asked whether someone else took time off on that day to care for them. During 2009/10 time off was quantified as ≤4 hours or >4 hours. In 2010/11 QALDs and QALYs were measured using the EQ-5D-3L instrument [141,287,288], which was completed at baseline and daily throughout illness. Designed for self-completion, EQ-5D-3L has two components. The first describes health across five domains: mobility, self-care, usual activities, pain and anxiety. Participants rate each domain as 'no problems', 'some problems' or 'extreme problems'. Participants also record their overall health status on a visual analogue scale (EQ-VAS) from 0 (Worst imaginable health state) to 100 (Best imaginable health state). The online EQ-VAS question used in Flu Watch however asked participants to rate their health without the visual scale. The three possible responses for each of the five EQ-5D-3L domains results in 35 possible health states. These health states were mapped to an index value (representing a QALD weight) using a validated UK value set [288]. The QALD weights range between 1 (full health) to 0 (dead).

7.1.4.3 *Illness Outcomes*

All acute respiratory illnesses, regardless of swabbing or RT-PCR result, were classified into two symptomatic outcomes. Those with confirmed fever (≥37.8°C) or symptoms of 'feeling feverish' and either a cough or sore throat at any point were classified as Influenza-like-illnesses (ILI). All other acute respiratory illnesses were classified as acute respiratory infections (ARI). Among the subset of illnesses that had an accompanying swab, some were confirmed as RT-PCR positive influenza cases and these were grouped into influenza A and influenza B viruses. In 2010/11 when the EQ-5D-3L data was collected, all influenza A illnesses were H1N1pdm09, apart from one H3N2 case. The individual-level results

report QALD loss for H1N1pdm09 cases only but the population projections include H3N2.

7.1.4.4 *Statistical Analysis*

7.1.4.4.1 Time of work/education

The duration of illness, percentages of illnesses with time off and mean number of days taken off were calculated for each illness outcome and stratified by age group and employment status. The latter two estimates were done separately for time off taken by the ill person, by someone caring for the ill person and a combination of both.

7.1.4.4.2 HRQoL

Within each illness, the worst day of illness within each domain was identified. The percentage of respondents reporting no, some or extreme problems on their worst day in each domain was compared to the corresponding baseline responses, stratified by illness outcome.

Within each illness, the worst day for EQ-VAS and the worst day for QALD weight were identified. For each illness outcome, mean and median worst day EQ-VAS scores and QALD weights were calculated and compared to baseline measurements.

Total QALD loss for each illness was calculated by subtracting the daily QALD weights taken during illness from the participant's baseline QALD weight and summing these differences up over the course of the illness. Mean and median total QALD and QALY losses per illness were calculated by illness outcome and stratified by age group and whether or not cases were medically-attended.

A sensitivity analysis was also conducted using the respondents' highest reported QALD weight as the comparison (baseline) group, regardless of when it was measured.

7.1.4.4.3 Missing Data

If a participant's baseline questionnaire was missing, then QALDs and QALYs could not be estimated for their subsequent illnesses. All illnesses with daily EQ-5D-3L

measurements were included in the duration of illness, worst day EQ-VAS and QALD weight estimates. If a participant failed to complete illness diaries throughout their illness, then their illness duration would be truncated after the last day of symptom reporting. I also investigated whether influenza cases actively reported no illness in the week following the last reported day of illness, or whether this weekly report was missing.

7.1.4.4.4 Population Impact

The total QALY loss experienced by community cases in the population and the number of days they took time off work/school due to influenza were calculated. Estimates were obtained from Flu Watch data by taking 25,000 Monte Carlo samples from the distributions of incidence of illness and QALD losses, or days off work, as appropriate, for each age-group. The incidence of illness and HRQoL outcomes for the QALY analysis were derived from 2010/11 data while estimates for the absence analysis came from 2006/07-2009/10. The mid-2011 population size and age-distribution for England was used [289].

7.1.5 **Results**

2919 participants reported 4818 illnesses (2805 ARI and 2013 ILI) (Table 5-1). Of the 3161 illnesses with nasal swabs, 177 tested positive for influenza A and 45 for influenza B. 75% (95% CI: 68%-81%) of influenza A cases meet the ILI case definition however only 49% (95% CI: 42%-57%) reported fever (a symptom required for many ILI definitions). For influenza B, 80% (95% CI: 65%-90%) of cases met the ILI definition but only 62% (95% CI: 47%-76%) reported fever. Most influenza B cases were in children whereas most influenza A cases were in adults. 16% (95% CI: 11% - 22%) of influenza A cases and 9% (95% CI: 2%-21%) of influenza B cases were medically-attended either through the government-run pandemic influenza web or phone service which ran during 2009/10 (the National Pandemic Flu Service), the NHS Direct telephone service, or contact with a GP, accident and emergency department or hospital.

Table 7-1: Baseline Characteristics of ill participants

	All People			All Illnesses			All Illnesses (N=4818)						Illnesses tested for Flu A & B (N=3161)					
							ARI			ILI			Influenza A PCR+			Influenza B PCR+		
	n	col %	95% CI	n	col %	95% CI	n	col %	95% CI	n	col %	95% CI	n	col %	95% CI	n	col %	95% CI
Overall	**2919**	**100%**	-	**4818**	**100%**	-	**2805**	**100%**	-	**2013**	**100%**	-	**177**	**100%**	-	**45**	**100%**	-
By influenza season																		
Winter 2006/07	270	9%	(8-10)	399	8%	(8- 9)	146	5%	(4- 6)	253	13%	(11-14)	14	8%	(4-13)	0	0%	(0- 8)
Winter 2007/08	363	12%	(11-14)	539	11%	(10-12)	188	7%	(6- 8)	351	17%	(16-19)	10	6%	(3-10)	4	9%	(2-21)
Winter 2008/09	219	8%	(7- 9)	410	9%	(8- 9)	123	4%	(4- 5)	287	14%	(13-16)	40	23%	(17-29)	13	29%	(16-44)
Summer 2009	33	1%	(1- 2)	110	2%	(2- 3)	42	2%	(1- 2)	68	3%	(3- 4)	2	1%	(0- 4)	0	0%	(0- 8)
Winter 2009/10	1644	56%	(54-58)	2690	56%	(54-57)	1893	68%	(66-69)	797	40%	(37-42)	75	42%	(35-50)	5	11%	(4-24)
Winter 2010/11	390	13%	(12-15)	670	14%	(13-15)	413	15%	(13-16)	257	13%	(11-14)	36	20%	(15-27)	23	51%	(36-66)
By age group																		
0-15 years	647	22%	(21-24)	1203	25%	(24-26)	648	23%	(22-25)	555	28%	(26-30)	68	39%	(32-47)	26	58%	(42-72)
16-65 years	1806	63%	(61-64)	2892	61%	(59-62)	1723	62%	(60-64)	1169	59%	(56-61)	99	57%	(49-64)	15	33%	(20-49)
65 years and over	431	15%	(14-16)	679	14%	(13-15)	409	15%	(13-16)	270	14%	(12-15)	8	5%	(2- 9)	4	9%	(2-21)
By IMD quartile*																		
1 (most deprived)	141	5%	(4- 6)	238	5%	(4- 6)	132	5%	(4- 6)	106	5%	(4- 6)	12	7%	(4-12)	3	7%	(1-18)
2	606	21%	(20-23)	1032	22%	(21-23)	544	20%	(18-21)	488	25%	(23-27)	49	28%	(21-35)	12	27%	(15-42)
3	1010	35%	(34-37)	1715	36%	(35-38)	1012	37%	(35-39)	703	36%	(33-38)	55	31%	(25-39)	14	31%	(18-47)
4 (least deprived)	1099	38%	(37-40)	1750	37%	(36-38)	1065	39%	(37-41)	685	35%	(32-37)	59	34%	(27-41)	16	36%	(22-51)
By occupation																		
In work	1288	51%	(49-53)	2052	46%	(45-48)	1267	51%	(49-53)	785	47%	(45-49)	62	41%	(33-49)	9	22%	(11-38)
Student	533	21%	(19-23)	932	21%	(20-22)	510	21%	(19-22)	422	25%	(23-27)	59	39%	(31-47)	25	61%	(45-76)
Not in work/school	724	28%	(27-30)	1172	26%	(25-28)	708	29%	(27-30)	464	28%	(26-30)	30	20%	(14-27)	7	17%	(7-32)
By sex																		
Female	1513	53%	(51-55)	2574	54%	(53-56)	1491	54%	(52-56)	1083	55%	(52-57)	89	51%	(43-58)	23	51%	(36-66)
Male	1343	47%	(45-49)	2161	46%	(44-47)	1262	46%	(44-48)	899	45%	(43-48)	86	49%	(42-57)	22	49%	(34-64)

***English Indices of Multiple Deprivations 2007**

7.1.5.1 *Time of work/education*

Average illness duration, percentages of illnesses with time off, and the symptom number of days per illness with time off were broadly comparable between influenza A and B cases although influenza A appeared slightly more severe (Table 7-2). Illness duration was 9.6 and 10.7 days for influenza A and B respectively. Among cases where absence data were available for both the ill participant and those caring for them, 50% (95% CI: 37%-63%) of Influenza A and 41% (95% CI: 18%-67%) of Influenza B cases required at least one person to take time off for a combined average of 5.0 and 3.4 days respectively. Among ill children, 56% (95% CI: 43%-68%) and 31% (95% CI: 14-52%) took time off school or childcare for an average duration of 3.5 and 2.1 days for influenza A and B respectively. Among the subset of data where information was available, 70% (95% CI: 46%-88%) and 42% (95% CI: 15%-72%) of children's illnesses required someone else to take time off to care for them for influenza A and B respectively. Ill adults were less likely to take time off (31% [95% CI: 22%-44%] and 20% [95% CI: 4%-48%] for influenza A and B respectively) but took more time off (3.8 and 3.0 days for influenza A and B respectively). Estimates remained similar when limited to working adults aged 16 and over. ILI cases were broadly comparable with influenza cases although more severe than the ARI cases. For the 142 influenza illnesses where the amount of time taken off per day was measured, 83% (95% CI: 76%-88%) of days had more than 4 hours off.

Table 7-2: Illness duration and time off work, education or childcare (Autumn 2006 – Spring 2010)

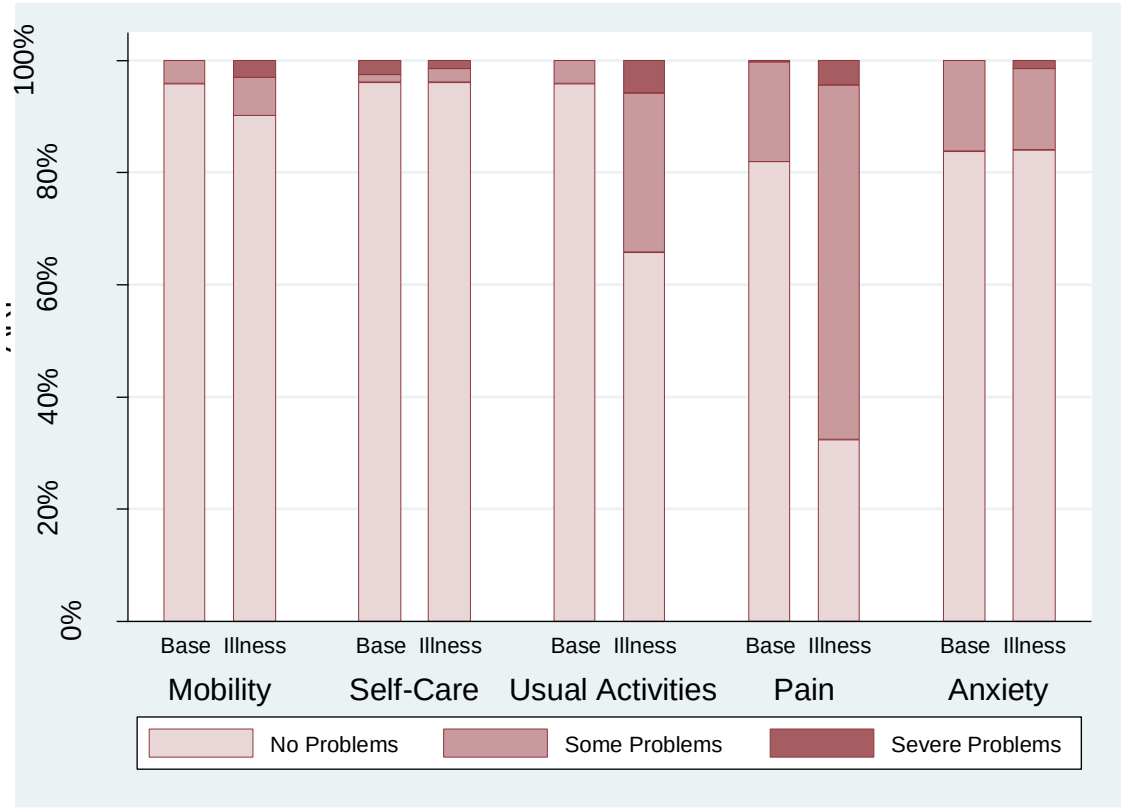
		ARI		ILI		Flu A PCR+		Flu B PCR+	
		N	Estimate	N	Estimate	N	Estimate	N	Estimate
Overall	Duration of symptoms, average (min, max)	2805	6.9 (1, 48)	2013	9.0 (1, 82)	177	9.6 (1, 82)	45	10.7 (1, 65)
	% of illnesses where the ill person and/or someone caring for them takes time off, % (95% CI)*	458	11% (8 - 14)	897	30% (27 - 33)	64	50% (37 - 63)	17	41% (18 - 67)
	Among illnesses with anyone's time off: average number of days someone takes time off, (min, max)*	51	2.5 (1, 6)	269	3.8 (1, 18)	32	5.0 (2, 11)	7	3.4 (2, 6)
	Percent of ill persons taking time off , % (95% CI)	2805	11% (9 - 12)	2013	27% (25 - 29)	177	40% (33 - 48)	45	24% (13 - 40)
	Among ill persons taking time off: Average number of days they take time off (min, max)	296	2.5 (1, 14)	545	3.2 (1, 18)	71	3.6 (1, 13)	11	2.4 (1, 4)
	Percent of illnesses where someone else takes time off to care for ill person, % (95% CI)*	458	4% (3 - 6)	897	11% (9 - 14)	64	28% (18 - 41)	17	29% (10 - 56)
	max)*	19	1.4 (1, 3)	102	2.0 (1, 7)	18	2.7 (1, 6)	5	1.6 (1, 2)
Ill Children (0-15 yrs)**	Percent of ill children taking time off school/childcare for their illness, % (95% CI)	648	14% (12 - 17)	555	39% (35 - 43)	68	56% (43 - 68)	26	31% (14 - 52)
	Among ill children taking time off: Average number of days they take time off school/childcare (min, max)	93	2.3 (1, 12)	218	2.9 (1, 13)	38	3.5 (1, 13)	8	2.1 (1, 4)
	Percent of illnesses where someone else takes time off to care for ill child, % (95% CI)*	78	10% (5 -19)	256	24% (19 - 30)	20	70% (46 - 88)	12	42% (15 - 72)
	max)*	8	1.6 (1, 3)	61	2.2 (1, 7)	14	2.9 (1, 6)	5	1.6 (1, 2)
Ill Adults (16-64 yrs)**	Percent of ill adults taking time off work/education for their illness, % (95% CI)	1723	11% (9 - 12)	1169	26% (23 - 29)	99	31% (22 - 41)	15	20% (4 - 48)
	Among ill adults taking time off: Average number of days they take time off work/education(min, max)	184	2.6 (1, 14)	303	3.3 (1, 18)	31	3.8 (1, 9)	3	3.0 (2, 4)
	Percent of illnesses where someone else takes time off to care for ill adult, % (95% CI)*	319	3% (2 - 6)	535	7% (5 - 9)	39	10% (3 - 24)	5	0% (0 - 52)
	max)*	11	1.2 (1, 2)	35	1.5 (1, 5)	4	2.0 (1, 3)	0	N/A
Ill Older Adults (65+ yrs)**	Percent of ill older adults taking time off work/education for their illness, % (95% CI)	409	5% (3 - 7)	270	9% (5 - 13)	8	13% (0 - 53)	4	0% (0 - 60)
	Among ill older adults taking time off: Average number of days they take time off (min, max)	19	3.4 (1, 7)	23	5.3 (1, 14)	1	3.0 (3, 3)	0	N/A
	Percent of illnesses where someone else takes time off to care for ill older adult, % (95% CI)*	61	0% (0 - 6)	105	6% (2 - 12)	4	0% (0 - 60)	0	N/A
	Among illnesses where someone else takes time off: Average number of days they take time off to care for ill older adult (min, max)*	0	N/A	6	2.5 (1, 5)	0	N/A	0	N/A
Ill Working Adults (16+ yrs)**	Percent of ill working adults taking time off work/education for their illness, % (95% CI)	1267	12% (10 - 14)	785	30% (27 - 33)	62	34% (22 - 47)	9	3%3 (7 - 70)
	Among ill working adults taking time off: Average number of days they take time off (min, max)	155	2.6 (1, 14)	233	3.3 (1, 18)	21	4.0 (1, 9)	3	3.0 (2, 4)
	Percent of illnesses where someone else takes time off to care for ill working adult, % (95% CI)*	235	4% (2 - 8)	361	6% (4 - 9)	24	13% (3 - 32)	2	0% (0 - 84)
	Among illnesses where someone else takes time off: Average number of days they take time off to care for ill working adult (min, max)*	10	1.2 (1, 2)	23	1.2 (1, 3)	3	2.3 (2, 3)	0	N/A

* Estimates limited to subset of data where time off work/education information was collected for both ill person and anyone caring for them

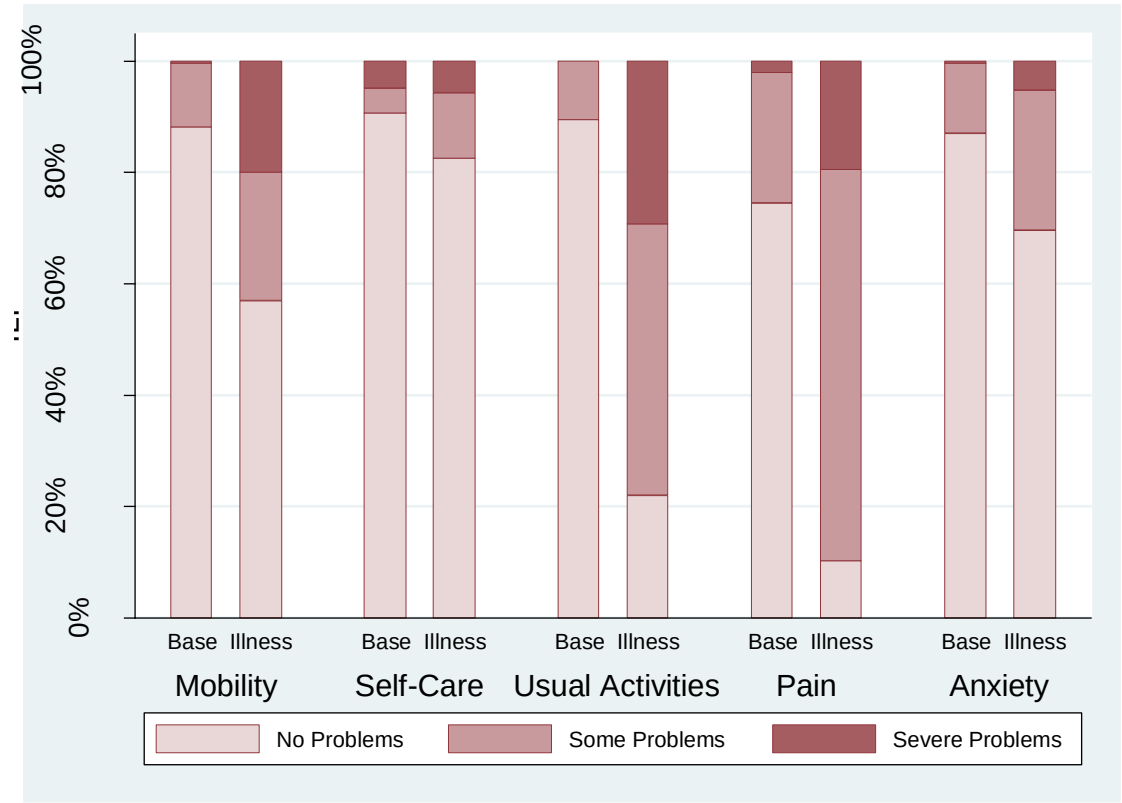
** Age group missing for 2 Influenza A cases, 7 ILI cases and 25 ARI cases

Figures 7-1 a-d: EQ-5D-3L domains comparing baseline and worst day of illness (for the respective domain) for (a) ARI (b) ILI, (c) H1N1pdm09 and (d) Influenza B illnesses.

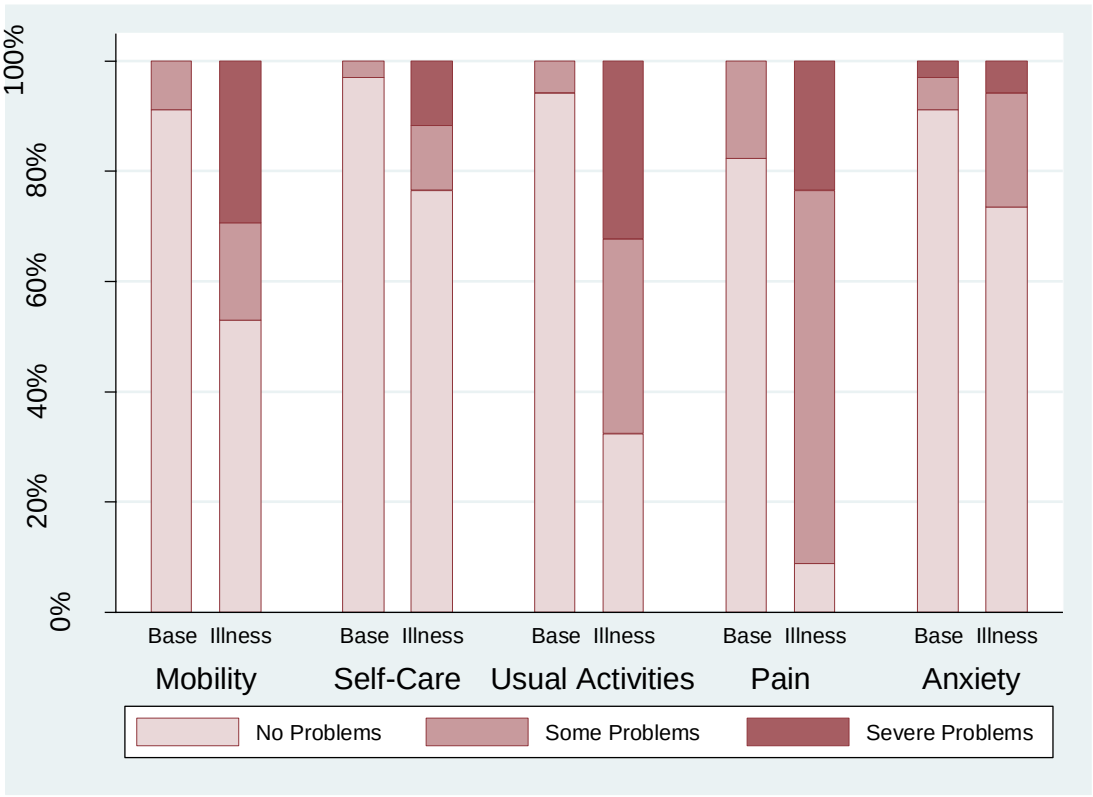
a)



b)



c)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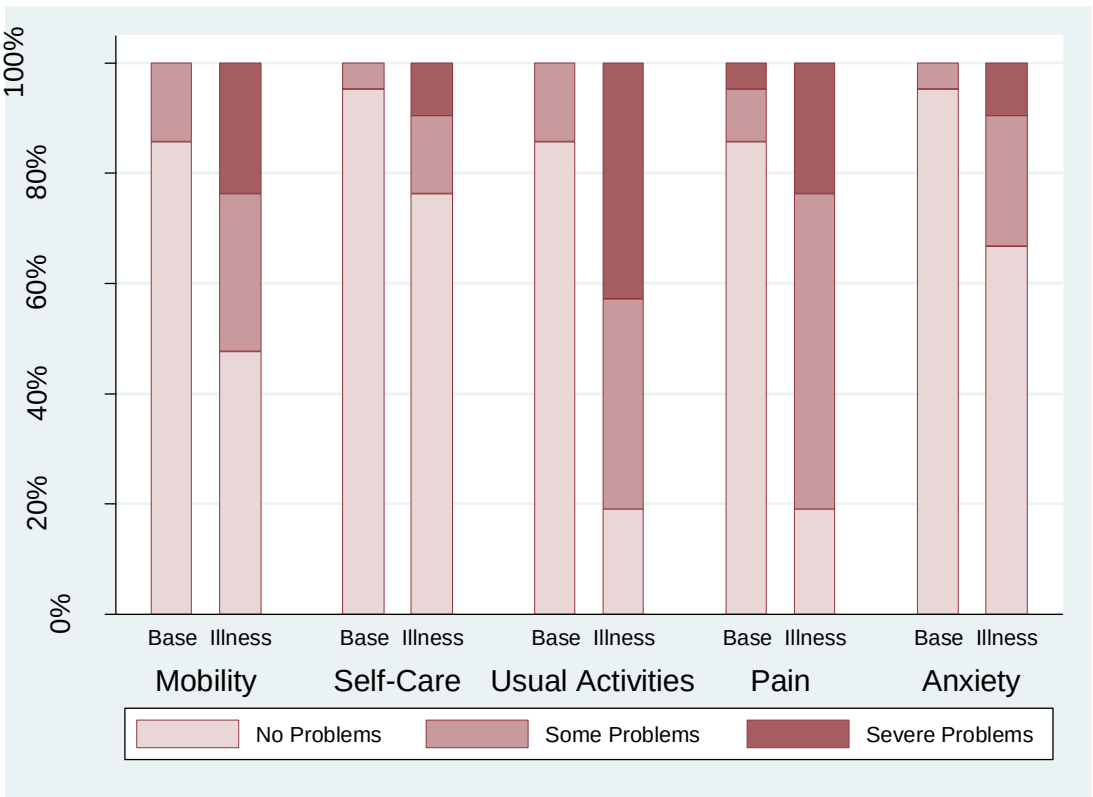
Illness
Mobility
Self-Care
Usual Activities
Pain
Anxiety
No Problems
Some Problems
Severe Problems

d)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Self-Care
Usual Activities
Pain
Anxiety
No Problems
Some Problems
Severe Problems

The median and mean EQ-VAS background scores were between 84-90 for H1N1pdm09, influenza B and ILI, but dropped to between 40-50 on the worst day of illness (Figure 7-2 and Table 7-3). Mean QALD weights were 0.93 and 0.92 at baseline for H1N1pdmo09 and influenza B respectively but dropped to 0.44 and 0.36 on the worst day of illness (Table 7-3). Median QALD weight for H1N1pdm09 (0.73) was much higher than the corresponding mean (0.44) suggesting that a few severe illnesses were greatly contributing to the mean (Figure 7-2 and Table 7-3).

Figure 7-2: EQ-VAS and EQ-5D QALD weights comparing background and worst day of illness by illness outcome

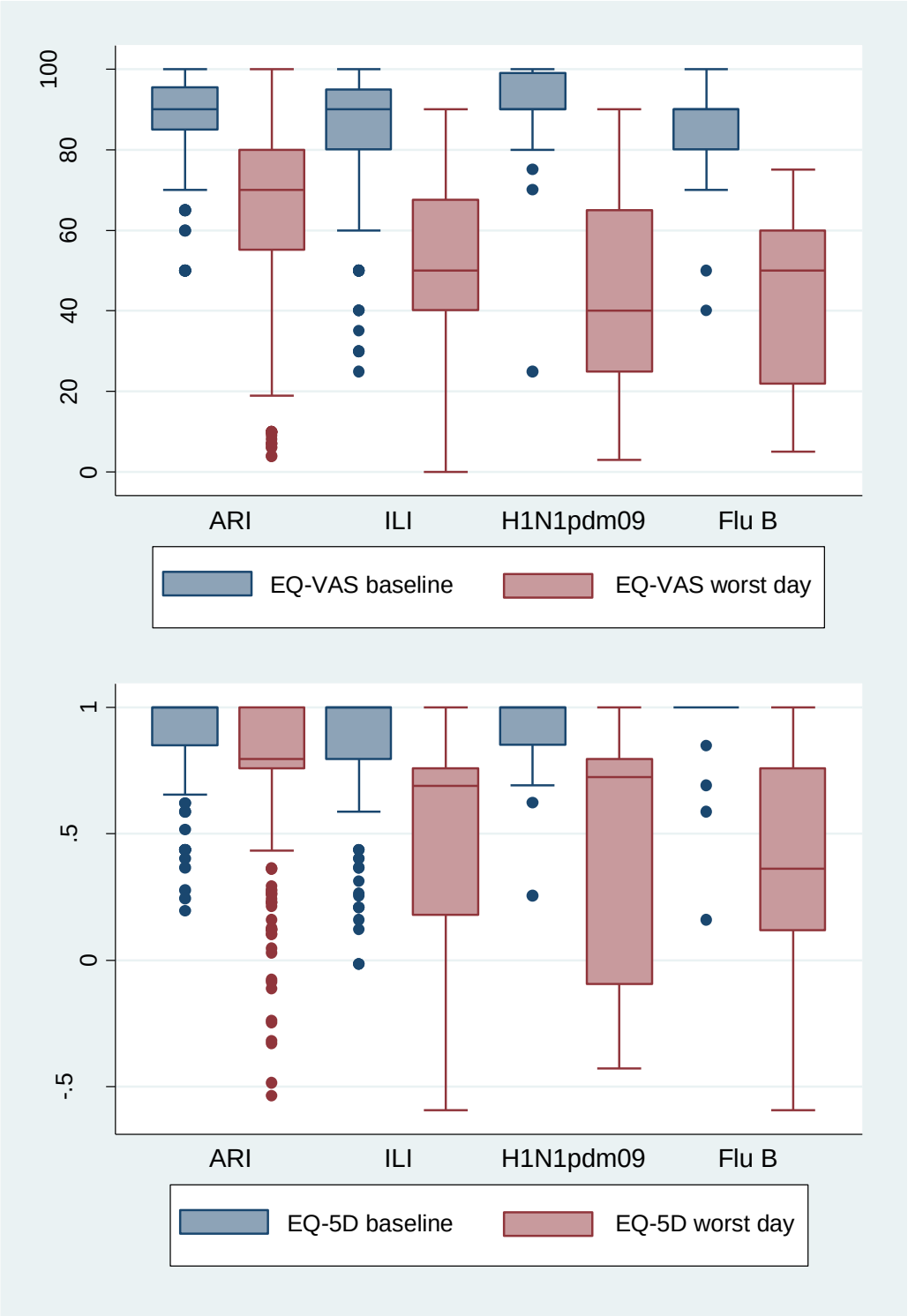


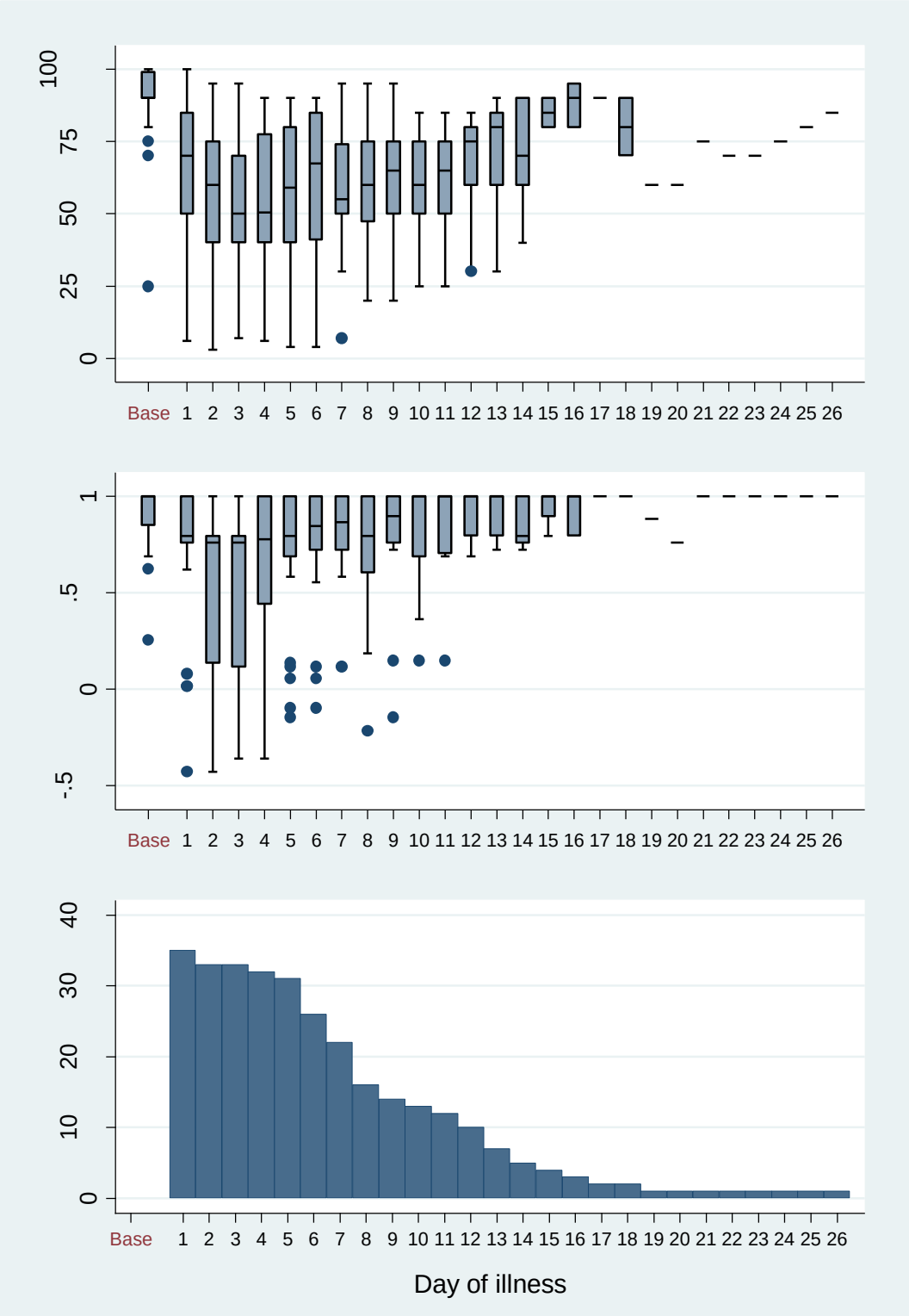
Table 7-3: Impact on Health-Related Quality of Life (Winter 2010/11)

	ARI		ILI		H1N1pdm09 PCR+		Flu B PCR+	
	N	Estimate	N	Estimate	N	Estimate	N	Estimate
Duration of symptoms, mean (min-max, median)	413	7.5 (1-42, 6.0)	256	9.9 (1-65, 7.0)	35	8.8 (1-26, 7.0)	23	11.9 (1-65, 7.0)
VAS background, mean (min-max, median)	408	89.0 (50-100, 90.0)	248	85.0 (25-100, 90.0)	34	89.8 (25-100, 90.0)	22	84.1 (40-100, 90.0)
VAS worst day of illness, mean (min-max, median)	413	66 (4-100, 70)	256	51 (0-90, 50)	35	43 (3-90, 40)	23	43 (5-75, 50)
EQ-5D weight background, mean (min-max, median)	406	0.92 (0.20-1.00, 1.00)	246	0.87 (-0.02-1.00, 1.00)	34	0.93 (0.25-1.00, 1.00)	21	0.92 (0.16-1.00, 1.00)
EQ-5D weight day of illness, mean (min-max, median)	413	0.77 (-0.54-1.00, 0.80)	256	0.48 (-0.59-1.00, 0.69)	35	0.44 (-0.43-1.00, 0.73)	23	0.36 (-0.59-1.00, 0.36)
QALDs lost, mean (min-max, median)	405	0.26 (-5.32-11.47, 0.20)	246	0.93 (-25.28-14.48, 0.74)	34	1.61 (-0.92-6.66, 1.00)	21	1.84 (-2.72-10.83, 1.14)
By Age group								
0-15	84	0.24 (-2.72-7.22, 0.00)	71	0.20 (-25.28-4.65, 0.66)	7	1.08 (0.00-4.27, 0.20)	10	1.82 (0.58-3.27, 1.86)
16-65	257	0.34 (-5.32-11.47, 0.20)	137	1.30 (-4.72-14.48, 0.82)	23	1.74 (-0.92-6.66, 1.15)	7	2.37 (-0.84-10.83, 1.02)
65+	64	-0.03 (-5.02-3.37, 0.00)	38	0.99 (-3.58-7.96, 0.74)	4	1.75 (-0.78-5.47, 1.15)	4	0.95 (-2.72-3.15, 1.68)
QALYs lost, mean (min-max, median)	405	0.0007 (-0.0146-0.0314, 0.0006)	246	0.0026 (-0.0692-0.0397, 0.0020)	34	0.0044 (-0.0025-0.0182, 0.0027)	21	0.0050 (-0.0074-0.0296, 0.0031)
By Age group								
0-15	84	0.0007 (-0.0075-0.0198, 0.0000)	71	0.0005 (-0.0692-0.0127, 0.0018)	7	0.0029 (0.0000-0.0117, 0.0006)	10	0.0050 (0.0016-0.0090, 0.0051)
16-65	257	0.0009 (-0.0146-0.0314, 0.0006)	137	0.0035 (-0.0129-0.0397, 0.0022)	23	0.0048 (-0.0025-0.0182, 0.0032)	7	0.0065 (-0.0023-0.0296, 0.0028)
65+	64	-0.0001 (-0.0138-0.0092, 0.0000)	38	0.0027 (-0.0098-0.0218, 0.0020)	4	0.0048 (-0.0021-0.0150, 0.0032)	4	0.0026 (-0.0074-0.0086, 0.0046)
QALDs lost (sensitivity analysis), mean (min-max,	405	0.72 (0.00-11.47, 0.41)	246	1.97 (0.00-16.33, 1.15)	34	1.89 (0.00-7.12, 1.09)	21	2.64 (0.00-10.83, 1.46)
By Age group								
0-15	84	0.54 (0.00-7.22, 0.20)	71	1.68 (0.00-7.98, 1.03)	7	1.08 (0.00-4.27, 0.20)	10	1.82 (0.58-3.27, 1.86)
16-65	257	0.77 (0.00-11.47, 0.47)	137	2.02 (0.00-14.48, 1.27)	23	1.98 (0.20-6.66, 1.22)	7	2.80 (0.00-10.83, 1.02)
65+	64	0.74 (0.00-7.17, 0.41)	38	2.37 (0.00-16.33, 1.04)	4	2.81 (0.19-7.12, 1.97)	4	4.41 (0.84-7.17, 4.81)
QALYs lost (sensitivity analysis), mean (min-max,	405	0.0020 (0.0000-0.0314, 0.0011)	246	0.0054 (0.0000-0.0447, 0.0031)	34	0.0052 (0.0000-0.0195, 0.0030)	21	0.0072 (0.0000-0.0296, 0.0040)
By Age group								
0-15	84	0.0015 (0.0000-0.0198, 0.0006)	71	0.0046 (0.0000-0.0218, 0.0028)	7	0.0029 (0.0000-0.0117, 0.0006)	10	0.0050 (0.0016-0.0090, 0.0051)
16-65	257	0.0021 (0.0000-0.0314, 0.0013)	137	0.0055 (0.0000-0.0397, 0.0035)	23	0.0054 (0.0006-0.0182, 0.0034)	7	0.0077 (0.0000-0.0296, 0.0028)
65+	64	0.0020 (0.0000-0.0196, 0.0011)	38	0.0065 (0.0000-0.0447, 0.0028)	4	0.0077 (0.0005-0.0195, 0.0054)	4	0.0121 (0.0023-0.0196, 0.0132)

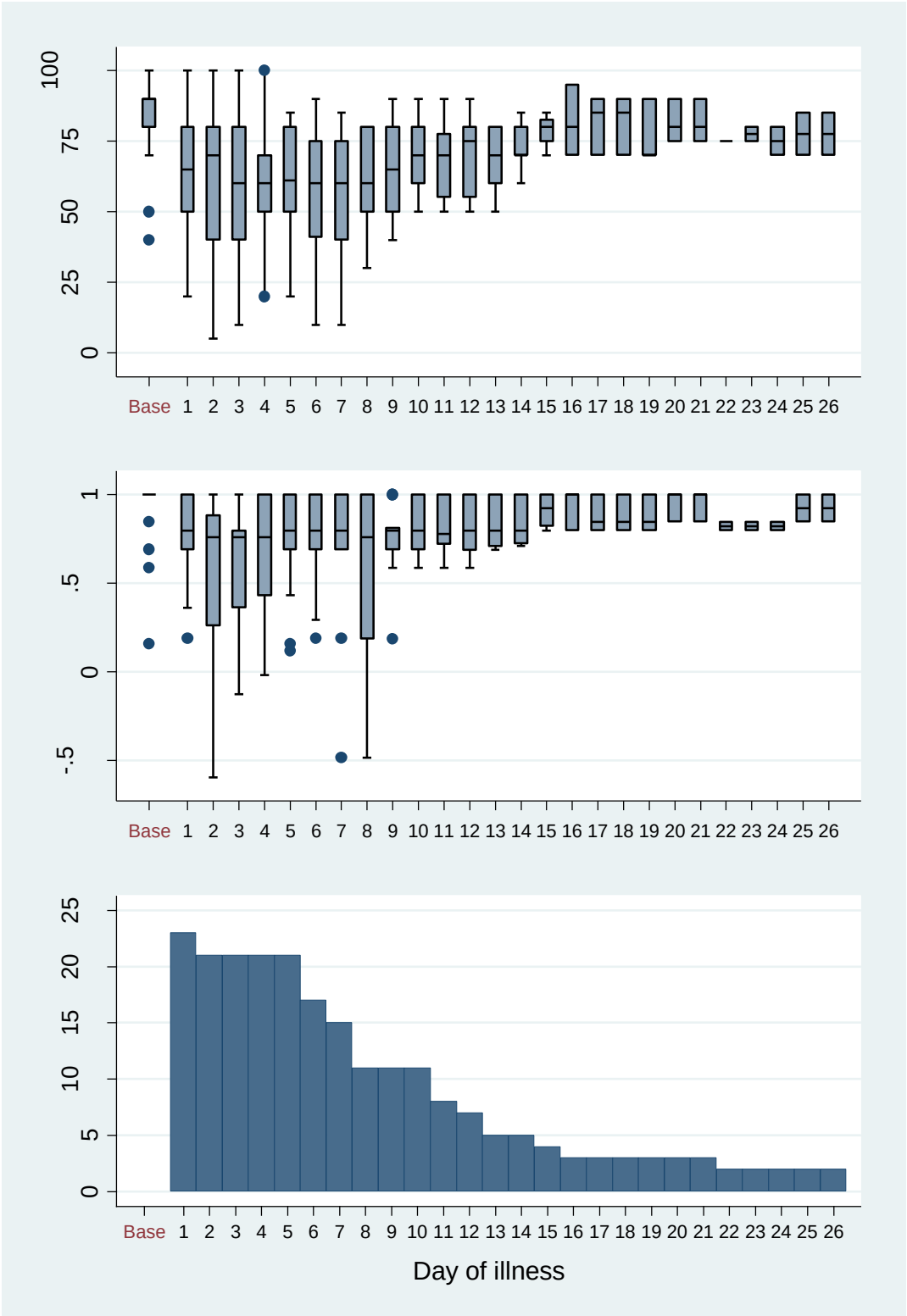
For H1N1pdm09 and influenza B, daily EQ-VAS and QALD weights varied throughout illness, with a rapid decline in the first 2 days (Figures 7-3). The lag time between symptom onset and the most severe day of illness appeared longer for H1N1pdm09 than for influenza B. Although the medians remain relatively low for the first week, over time these estimates reflected fewer illnesses, i.e. those with the longest duration (see bottom panels, Figures 7-3).

Figures 7-3a-b: VAS and EQ-5D-3L QALD weight at baseline and by day of illness for (a) H1N1pdm09 illnesses and (b) Influenza B illnesses over the number of cases reporting symptoms on that day

a)



b)


100
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Base 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
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Base 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
25
20
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10
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Base 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Day of illness

Average illness duration for H1N1pdm09 and influenza B cases with QALD data was 8.8 and 11.9 days respectively, with 3% (95% CI: 0%-15%) and 9% (95% CI: 1%-28%) of illnesses respectively lasting over three weeks. Overall 1.61 QALDs were lost during H1N1pdm09 illnesses. QALD loss increased with age from 1.08 in children, to 1.74 and 1.75 in adults and the older adults respectively. Influenza B illnesses lost more QALDs at 1.84 with age-specific estimates of 1.82, 2.37 and 0.95 for children, adults and older adults respectively. QALD loss during ILI and ARI illnesses were lower (0.26 and 0.93 respectively). Median QALD loss was typically lower than the mean for all illness outcomes, indicating that a small proportion of severe illnesses contributed greatly to the mean. 20% (95% CI: 8%-37%) of H1N1pdm09 and 17% (95% CI: 5%-39%) of influenza B cases with QALD/QALY data were medically-attended. Mean QALD loss was 3.63 for medical-attended H1N1pmd09 cases and 1.08 for non-medically-attended cases. Corresponding figures for influenza B were 5.48 and 1.23.

In sensitivity analysis, overall QALDs lost were higher at 1.89 for H1N1pdm09 and 2.64 for influenza B. Age-specific sensitivity estimates were similar to the main analysis except in the oldest age group where the sensitivity analysis reports higher QALD losses.

7.1.5.2 *Missing Data*

One H1N1pdm09 and two influenza B illnesses were missing baseline EQ-5D-3L measurements. Among the 57 influenza cases with QALD data, all but two reported no illness in the week following their illness.

7.1.5.3 *Population Impact*

The estimated number of QALYs lost due to influenza A and B in England was 24,300 (95%CI: 16,600–34,700), of which two-thirds occurred in the 16-64 years age-group (Table 7-4). The estimated number of days off school in individuals aged 5-15 years with influenza was 1.12 million (95% CI: 0.661–1.78 million) per winter, of which 85% was associated with influenza A. The estimated number of days off work or education in individuals aged 16-64 years with influenza was 1.79 million (95%CI: 1.16 – 2.78 million), almost all of which (>98%) was due to influenza A.

Table 7-4: Population-level burden of HRQoL lost and work/education absences due to community cases of influenza

Outcome	Age group	Flu Type	Estimate	95% CI
QALY loss	Overall	A+B	24,300	16,600 – 34,700
	By age group			
	0-15	A+B	6,410	3,640 – 10,900
	16-64	A+B	16,200	9,710 – 25,800
	65+	A+B	1,660	490 – 4,860
Days off work/education	Overall	A+B	2,910,000	2,090,000 - 3,930,000
	By age group and flu type			
	5-15	A	949,000	528,000 - 1,580,000
		B	1,760,000	1,140,000 - 2,610,000
	16-65	A	170,000	52,300 - 414,000
		B	27,600	4,720 - 89,100

7.1.6 Discussion

7.1.6.1 *Summary of Results*

Community cases of ARI, ILI, H1N1pdm09 and influenza B lost 0.25, 0.93, 1.61 and 1.84 QALDs from their illnesses respectively. The estimated QALDs lost increased with age which is consistent with previous findings [281]. Mean QALD loss was much greater in medically-attended H1N1pdm09 and influenza B cases (3.63 and 5.48 respectively) compared to non-medically-attended cases (1.08 and 1.23 respectively). I found 50% of influenza A illnesses and 41% of influenza B illness required someone (ill participant and/or their carer) to take time off work/education for a combined average of 5.0 and 3.4 days. Compared with adults, children with influenza were more likely to take time off education/childcare and to require someone else to take time off to care for them. Around a third of working adults required time off work for both influenza A and B illnesses with an average of 4 and 3 days off respectively. Illness duration and time off estimates for ILI were comparable to influenza but higher than ARI. In England, community influenza cases lost 24,300 QALYs (8.87 million QALDs) in 2010/11 and had an estimated 2.9 million absences per season based on data from 2006/07 – 2009/10.

7.1.6.2 *Comparison to other studies*

Previous studies show substantial variation in the HRQoL associated with influenza. This reflects differences in subjects' ages, definitions and severity of illness as well as the methods used to estimate HRQoL. Several estimates have been derived from cases seeking medical attention. In a population-based study conducted in England during the 2009 pandemic using EQ-5D-3L, 2.92 QALDs were lost for confirmed cases of H1N1pdm09 and 2.74 for ILI controls [278]. Another study reported a QALD loss of 1.68 for ILI due to confirmed influenza and 1.57 for non-influenza ILI in adult patients [282]. This was calculated by subtracting VAS scores presented by O'Brien et al [290] from pooled oseltamivir trial data in nearly 640 ILI patients who received placebo, from a baseline quality of life weight. A study used data from the same trials to estimate the QALD loss associated with ILI as 5.33 in people aged 0-19 years, 6.35 in people aged 20-64 years and 10.69 for people aged 65 years and over by combining the published QALY weights with unpublished data on disease duration [281]. Finally, a study of patients from hospitals and primary care centres with confirmed H1N1pdm09 in Spain showed individual QALD losses of 3.29 for primary care patients and 11.3 for hospitalised in-patients [284].

There are fewer studies of community influenza cases that may not consult healthcare professionals. Nevertheless, a survey in England of caregivers of children in primary school reporting ILI outbreaks that used EQ-5D-3L showed a mean loss of 2.1 QALDs [273]. In Belgium, a household telephone survey including 2,250 individuals with self-reported ILI used SF-12 to calculate QALDs lost: for an average episode of illness in the community, 1.83 QALDs were lost [291].

In general, the Flu Watch estimates for individual-level QALDs lost due to influenza were lower than earlier findings. This is unsurprising, as the study captured mild illnesses including cases of confirmed influenza that neither consulted for their illness nor met the symptom definition of ILI. Additionally, the Flu Watch study included children who typically have less severe disease as well as a large number H1N1pdm09 cases which in the Flu Watch cohort were generally less severe than H3N2 cases [15]. This work and previous studies have shown that more QALDs are lost when estimates are derived from medically-attended cases, and in particular hospitalised cases. The estimates for work and

school absences from the Flu Watch study were also generally lower than previous estimates; for most illnesses, people did not take time off, although there were differences by age and illness definition. This study shows however, that illness in a household member caused a substantial proportion of people take time off work to care for unwell household members. A study in the US on school and parental absenteeism showed that for every three days a child took off school a parent missed on average one day of work [292].

The aforementioned British and Spanish studies are not directly comparable as they estimated the population-level burden of QALY loss due to influenza for more severe cases in a different season (2009/10) [278,284]. They do however contextualise my findings as they report burden of QALY loss due to hospitalisations and deaths, which when combined with my results for community cases provides an indication of the scale of QALYs lost in a given season and the proportion attributable for different levels of disease severity. For example, the British study estimated that 40% (approximately 11,000 QALYs) of their total QALYs lost came from 337 reported influenza deaths [278]. Similarly, the Spanish study estimated their 318 deaths lost 12,000 QALYs [284]. It also estimated burden of QALY loss for influenza in-patients and primary care patients, demonstrating that less severe yet more numerous primary care patients lost far more QALYs (6,778) than the more severe but less common in-patients (94 QALYs). Given these findings it seems that at least for these two seasons, the biggest contributors of population-level QALY loss are community cases (medically- and non-medically attended) and deaths. The true burden and contribution by level of severity is likely to vary substantially between seasons and populations as it depends on population size and age-specific rates of illness and death. The estimated burden is also highly dependent on severity of cases included in the model.

7.1.6.3 *Strengths and weaknesses*

The estimates of HRQoL and work and school absence presented here were derived from a large community cohort study using active molecular and symptom surveillance to identify episodes of influenza, ILI and ARI. They captured a broad spectrum of illnesses including mild cases of laboratory-confirmed influenza that did not meet the syndromic definition of ILI and/or did not consult a healthcare professional, which gave less biased estimates of the overall HRQoL and absences associated with influenza. A key strength

was that participants completed the EQ-5D-3L daily over the course of an illness. This directly measures HRQoL throughout illness, so unlike other studies that used a single estimate of HRQoL during illness, this analysis required no assumptions about the shape of the QALY loss over time. A further strength is that the population projections were based on incidence estimates derived from the same data source.

Although work and school absences were measured over multiple years, HRQoL was only measured in 2010/11 when influenza A H1N1pdm09 and influenza B strains circulated. I expect that, as H3N2 was associated with more severe symptoms than H1N1, its effects on HRQoL might have been greater [15]. Despite the large cohort size, the numbers with confirmed influenza and EQ-5D were relatively low (N=58) and not sufficient to draw conclusions on differences in HRQoL by strain. The uncertainty in my QALD and QALY estimates is reflected in the 95% confidence intervals for my population projections. My colleagues and I have previously showed that the majority of influenza infections are asymptomatic [15]. Although asymptomatic cases would have no associated QALD loss, it is possible the study failed to capture very mild cases that did not shed sufficient virus for RT-PCR detection and thus slightly overestimated individual-level QALD loss associated with confirmed influenza. Conversely, my population-level estimates of both QALD loss and absences should be considered minimum estimates because if cases were missed (for example from low viral shedding due to mild illness or late swabbing) this would reduce the estimated disease rates and thus overall burden estimates. The population level estimates of absences due to influenza only included absences of the ill individual as data on the absences of those caring for these cases was not consistently collected over the course of the study. Therefore, the population level burden of absences is missing a substantial proportion of the total absences due to influenza.

The Flu Watch study was not fully representative of the general population. However I accounted for this as best I could by calculating age and region adjusted incidence of disease. I found some people reported worse HRQoL at baseline than during illness and my sensitivity analysis showed that when I took the participants' best reported measure of HRQoL as the comparison group, regardless of its timing, the oldest age group had much higher estimates of QALY loss. A further limitation is that children's HRQoL was reported

by their parents. Previous studies show significant differences when both parents and adolescent measure children's quality of life [293]. Instruments such as EQ-5D-3L have not been validated for use in infants and very young children, which is a challenge of assessing HRQoL in this age group [294].

7.1.6.4 *Implications*

Estimates of QALDs lost and work and school absences associated with influenza differ depending on the setting in which cases are identified; community illnesses result in smaller effects but contribute substantially to the population-level burden. Accurate assessment of both the number of expected cases and their QALDs/QALYs is essential to inform CUAs for decision-making bodies such as NICE. While for some interventions, such as antiviral treatments of severe influenza cases, it is appropriate to use utility estimates derived from medically-attended cases, I believe that my estimates are more appropriate for assessing cost utility of community preventive interventions such as vaccines.

Knowledge of presentism informs our understanding of non-household transmission of acute respiratory viruses. These estimates also provide a useful sense check on assumptions in non-pharmaceutical interventions of voluntary self-isolation when ill.

7.1.6.5 *Conclusions*

I present new estimates of individual- and population-level QALDs and QALYs lost and work and school absences due to community cases of influenza to inform CUAs of community interventions to prevent influenza.

7.2 Evaluation of the National Pandemic Flu Service

7.2.1 Attribution

This work was conducted in order to inform the 2019 review and subsequent update of the National Pandemic Flu Service (NPFS) Algorithm. I led all aspects of the work including conducting the literature review, managing the data and creating the necessary dataset, developing and conducting the statistical analysis, interpretation and writing up.

7.2.2 Abstract

Background: During the 2009 pandemic the UK's National Pandemic Flu Service (NPFS) operated an internet and telephone-based service that assessed respiratory illnesses and authorised antiviral prescriptions to those meeting the symptomatic case definition. Its primary purposes were to reduce burden in primary care and to enable timely dissemination of stockpiled antivirals.

Objectives: To assess the success of the NPFS in achieving its primary aims and to inform an NPFS algorithm update by applying it to contemporaneous UK community cases (many of whom did not consult the NPFS) using the original case definition and an alternative afebrile version.

Methods: Flu Watch was a UK community cohort study (2006-2011). Participants completed weekly surveys on symptoms, health-seeking behaviour and treatment of respiratory illnesses and submitted nasal swabs for PCR analysis. During NPFS operation, we calculated the proportion and timing of community illnesses that consulted NPFS and/or GPs, the proportion and timing treated with antivirals, and the test characteristics of the two case definitions among illnesses with PCR data.

Results: Overall 2% (95% CI: 1%-3%) of illnesses consulted NPFS and 11% (95% CI: 9%-12%) a GP. NPFS consultations occurred earlier in illness than GP consultations (median day 2 versus 4 respectively). Among consulting illnesses, 91% (95% CI: 87%-95%) consulted a GP and 17% (95% CI: 12%-22%) consulted the NPFS, although over half of these also consulted a GP. Among consulting illnesses in the not at-risk group, 6%

(95% CI: 2% - 12%) consulted the NPFS and not a GP. 1% (95% CI: 1% – 2%) of community illnesses were treated with antivirals, half within two days of onset. The NPFS and afebrile case definitions classified 15% (95% CI: 13% - 17%) and 90% (95% CI: 88% - 92%) of all illnesses with PCR data as influenza-like-illness (ILI) respectively. NPFS case definition's sensitivity was lower than the afebrile version (51% [95% CI: 38%-63%] versus 96% [95% CI: 88%-99%] respectively) but the specificity was higher (87% [95% CI: 85%-89%] versus 10% [95% CI: 9%-12%] respectively).

Conclusion: Most community-level illnesses do not consult medical services and although it was a faster route to antivirals, low uptake of the NPFS severely limited its population-level impact on both primary care burden and mass antiviral treatment. Targeting antiviral treatment on the basis of symptoms is challenging given the trade-offs in sensitivity and specificity. We recommend using different symptomatic case definitions for high-risk and low-risk cases to simultaneously target antiviral treatment to those who would most benefit from it whilst also preserving antiviral stockpiles.

7.2.3 Introduction

During the 2009 pandemic the UK's National Pandemic Flu Service (NPFS) operated an internet and telephone-based service in England that assessed respiratory illnesses occurring in the community [92,295,296]. The outcomes of the automated assessment were health advice and in some cases a referral to other medical services and/or and an antiviral prescription. The NPFS had two primary purposes. Firstly, it was designed to alleviate the burden of excess consultations among the 'worried well' on general practices, which could otherwise overwhelm primary care services. Secondly it enabled dissemination of the stockpiled antiviral treatments directly to ill patients more quickly than traditional primary care services would have been able to do [92]. The timeliness of influenza antiviral treatment is particularly important as they are more effective the earlier they are taken [19].

A decade has now passed since the NPFS was created and a review is currently underway to assess and update the NPFS algorithm in order to ensure it reflects up-to-date knowledge and guidelines. In order to support the algorithm update, I undertook an evaluation of the success of the NPFS in achieving its primary two aims through analyses of data from the

Flu Watch study, a contemporaneous community cohort study of influenza. Specifically, I sought to describe the consultation behavior for community cases of respiratory illness (to put the NPFS into the wider context of the community burden) and the percent of GP consultations potentially diverted by the NPFS. I also quantified how well the NPFS antiviral prescriptions were targeted to influenza cases. Finally, I explored the impact of an alternative case definition on targeting antivirals.

Although the details of the 2009 algorithm and the proposed 2019 update are not publicly available, a general description of the 2009 algorithm has been published [295]. A key aspect of that algorithm was the symptomatic case definition used to determine whether or not an individual had a 'flu-like' illness. This case definition formed a critical juncture in the algorithm by streaming 'flu-like' illnesses down a path where antivirals could have been authorised and streaming non-flu-like illnesses down a path where antivirals would not have been authorised. Symptomatic case definitions for influenza are not particularly good at discriminating influenza from non-influenza illnesses [297–299]. This is because influenza has a symptomatic presentation that is similar to many other respiratory viruses and colds. Given the imprecise nature of symptomatic case definitions for influenza and the importance of the NPFS case definition in the subsequent determination of antiviral authorisation, it seemed prudent to review the effects of the 2009 case definition in order to inform the 2019 algorithm update.

In order to review how effectively a case definition discriminates true cases from non-cases, it must be compared with a gold-standard test, in this case with influenza RT-PCR-testing of illnesses. While the NPFS had a scheme for RT-PCR-testing among a subset of illnesses consulting the service, these tests were only conducted on illnesses that met the case definition and were authorised to receive antivirals [110]. Without comparable RT-PCR-testing among illnesses not meeting the case definition it is impossible to use the data from the NPFS to calculate the sensitivity and specificity of their case definition. In contrast, the Flu Watch study, a community cohort study of influenza in England (2006 -2011) has the necessary data for this analysis among the full spectrum of respiratory illnesses in the community, included those that did and did not consult the NPFS. In this paper, I have sought to inform the 2019 NPFS algorithm update through an analysis of Flu Watch data.

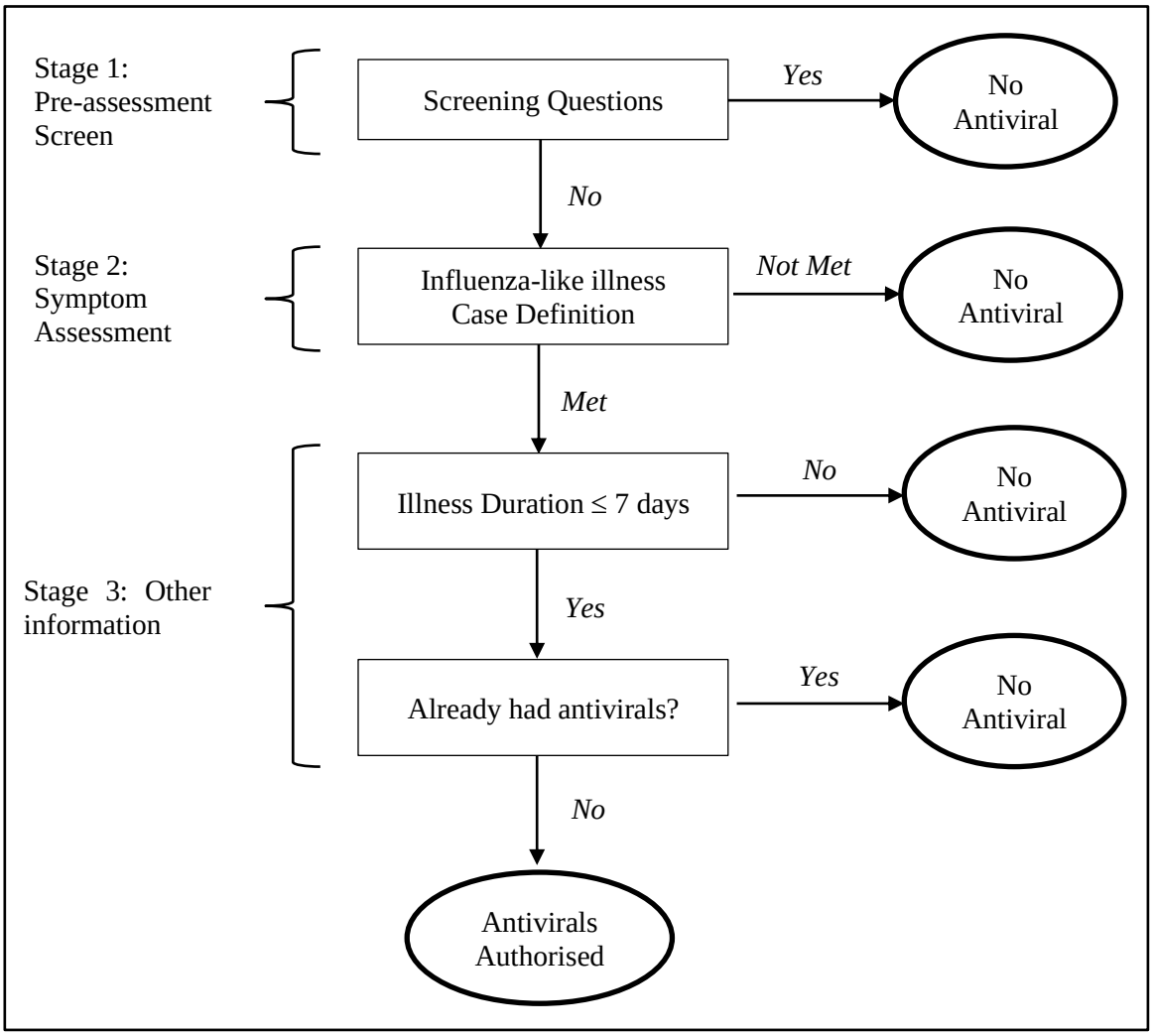
I evaluated the sensitivity and specificity of the 2009 NPFS case definition and an alternative, more inclusive hypothetical case definition among contemporaneous community cases of respiratory illness. As a result of these findings, I propose an alternative approach to the use of case definitions in the UK's NPFS algorithm which would simultaneously target antiviral treatment to those who would most benefit from it whilst also preserving national antiviral stockpiles.

7.2.4 **Methods**

7.2.4.1 *NPFS Algorithm*

The NPFS was an internet and telephone-based service that members of the public with influenza-like symptoms could contact in order to have their illnesses assessed. Outcomes of the service included health advice and in some cases referral to other medical services and/or an antiviral prescription[295]. When a patient contacted the service, they would answer a series of yes or no questions about themselves and their illness (Figure 7-4). These questions followed a pre-determined algorithm with three stages. The first stage was a pre-assessment screen aimed to identify emergency or very high-risk patients and divert them to emergency services or an urgent GP appointment. The second stage assessed whether the patient's symptoms met the algorithm's case definition for a 'flu-like' illness. If the case definition was not met, the patient was reassured, no antivirals were authorised, and the assessment would end. If the case definition was met then the patient would continue to the third stage of the algorithm where information was collected on the duration of illness, whether antivirals had already been taken, whether the patient was at high risk of severe disease (i.e. member of an at-risk group) and whether the patient had symptoms of severe disease. The answers to these questions determined the health advice given, directed patients to other medical services (if appropriate) and determined whether an antiviral was authorised or not [295]. Figure 7-4: Algorithm pathways to antiviral authorisationFigure 7-4 is a simplified depiction of the algorithm, focusing solely on the pathways to antiviral authorisation.

Figure 7-4: Algorithm pathways to antiviral authorisation



7.2.4.2 *Flu Watch Study Design*

The data used in this analysis was generated by the Flu Watch Study, a community-level, household-based cohort study of influenza conducted in England between 2006 and 2011. The Flu Watch study was described in detail in section 2.1. In brief, prospective participants were selected at random from general practice lists and their entire households were invited to join the study. Upon entry into the cohort, participants completed a baseline survey which collected demographic, health and chronic illness information (in order to determine membership of at-risk groups). Households also received participation packs which included thermometers and nasal swab kits. Participants were then prospectively followed up each week. When they had symptoms of respiratory illness, they were asked to fill in daily diaries of symptoms, health-seeking behaviour and treatment. They were

also asked to self-administer a nasal swab on day two of any respiratory illness which was subsequently tested for a panel of influenza viruses (seasonal H1N1 and H3N2 and 2009 pandemic H1N1) using RT-PCR.

When the 2009 pandemic arose, the study increased in size and newly recruited participants had slightly different follow-up compared to participants that were already involved in the study. While all participants (existing and newly recruited) had the follow-up described above, the existing Flu Watch participants additionally had a medical record review at the end of the follow-up season. This survey, completed by a research nurse based in the participant's practice, recorded GP consultations and treatment for any respiratory illness occurring during study follow-up.

7.2.4.3 *Outcomes*

I used three illness outcomes and two consultation outcomes in my analyses.

All respiratory illnesses identified in the Flu Watch study were classified according to whether or not they would have met the 2009 NPFS algorithm case definition for influenza-like illness. The 2009 NPFS case definition, as described by Rutter and colleagues, was "Does the patient have a high temperature and at least two of the following symptoms?—Widespread muscle and joint aches, a cough, headache, blocked or runny nose, sore throat, vomiting, watery diarrhea, cannot stop crying (only children)" [295]. Given the data collected by the Flu Watch study, I was able to approximate this case definition as follows: 'high temperature and at least two of the following symptoms – muscle and joint aches, a cough, headache, blocked or runny nose, sore throat, vomiting, diarrhea'. The Flu Watch study did not collect data on children's crying.

I also classified all illnesses by a second, more sensitive version of the 2009 case definition which omitted fever but was otherwise identical: 'at least two of the following symptoms – muscle and joint aches, a cough, headache, blocked or runny nose, sore throat, vomiting, diarrhea'.

Among the subset of illnesses which had an associated nasal swab, I created an additional outcome of the illness being either RT-PCR-confirmed influenza or not.

In addition to the illness outcomes there were also two consultation outcomes which classified whether illnesses had an associated GP consultation (over the phone or in person) and whether or not they had an associated consultation with the NPFS (via website or phone).

7.2.4.4 *At Risk Groups*

Certain underlying health conditions can increase an individual's risk of serious illness or death if they develop influenza illness. At-risk groups for the 2009 pandemic strain were defined in June 2009 by the Scientific Advisory Group for Emergencies (SAGE). These groups were: 1) people aged six months or older with specific chronic diseases, 2) People who had received any medical treatment for asthma in the last three years, 3) pregnant women, 4) children under the age of 5 years and 5) people over the age of 65 years [92]. Using the health data collected by the baseline survey I classified individuals as members or non-members of an at-risk group.

7.2.4.5 *Statistical Analysis*

I began by limiting the Flu Watch dataset to illnesses which occurred during the NPFS operational period. I excluded illnesses with an end date before the launch of NPFS (23 July 2009) or an onset date after the last day of its operation (10 Feb 2010).

7.2.4.5.1 Consultation

I calculated the percent of illnesses which consulted overall and stratified by age group, sex, whether or not they were a member of an at-risk group and by RT-PCR outcome. Among those who consulted I calculated the percent who consulted each type of service and how quickly they consulted that service. In this group I also calculated the mean number of consultations per illness and the mean number of services consulted per illness. Among those who consulted a GP and/or the NPFS, I calculated the proportion of illnesses that only consulted a GP, only consulted NPFS or consulted both overall and stratified by at-risk group, age group, sex and RT-PCR outcome.

7.2.4.5.2 Treatment

I calculated the percentage of illnesses that reported taking antivirals overall and by age group, sex, at-risk group and by RT-PCR-outcome. I also calculated and the percentage of these illnesses which took their antivirals within the first two days of illness.

7.2.4.5.3 Impact of NPFS algorithm streaming and Case Definitions

In this part of the analysis I subjected all illnesses to the 2009 NPFS algorithm as approximated using Flu Watch data. To mirror the algorithm as closely as possible, I excluded any illnesses which would have been screened out in stage 1 of the emergency assessment. This included those aged under 1 year and women who reported being pregnant at the baseline survey or during follow-up. Flu Watch did not have comparable data on the remaining NPFS screening criteria (i.e. severe illness, meningococcal like symptoms or travel to countries with risk of malaria) so illnesses with these characteristics, while rare, would not have been caught in my pre-assessment stage. The remaining illnesses were then subjected to stage 2 of the algorithm, the assessment of symptoms. Among these illnesses I calculated the proportion meeting each symptomatic case definition within the first week of illness both overall and stratified whether or not they were a member of an at-risk group. Among the subset of illnesses with RT-PCR outcome data, I repeated these calculations and additionally calculated the sensitivity, specificity, and positive- and negative predictive values of the two symptomatic case definitions when compared to the RT-PCR outcome.

7.2.5 **Results**

7.2.5.1 ***Baseline characteristics***

During the period that NPFS was operating, Flu Watch followed a total of 3612 participants. Approximately 17% of these participants were already in the study when the NPFS was initiated but the rest were recruited over the autumn of 2009. In total there were 1864 illnesses reported by 1447 participants, 1244 (67%) of which were swabbed and had RT-PCR data (Table 7-5). The percentage of swabbed illnesses were highly comparable to all illnesses in terms of age group, sex, at-risk group (data not shown). Among illnesses

with RT-PCR data, 72 (5.8%, 95% CI: 5-7%) were positive for Influenza, one with Influenza B and the rest with the 2009 H1N1 pandemic strain.

Table 7-5: Baseline Characteristics of ill participants and the outcome of their illnesses

	Ill People	Illnesses	Consultations*			Antivirals**		
			n	% of illnesses	95% CI	n	% of illnesses	95% CI
Overall	1447	1864	217	11.6%	(10.2 - 13.2)	24	1.3%	(0.8 - 1.9)
By agegp5								
0-4 years	106	170	44	25.9%	(19.5 - 33.1)	1	0.6%	(0.01 - 3.2)
5-15 years	234	319	31	9.7%	(6.7 - 13.5)	6	1.9%	(0.7 - 4.9)
16-44 years	393	502	44	8.8%	(6.4 - 11.6)	4	0.8%	(0.2- 2.0)
45-64 years	484	598	66	11.0%	(8.6 - 13.8)	9	1.5%	(0.7 - 2.8)
65+ years	209	248	28	11.3%	(7.6 - 15.9)	4	1.6%	(0.4 - 4.1)
By sex								
Female	762	1001	120	12.0%	(10.0 - 14.2)	11	1.1%	(0.5 - 2.0)
Male	664	836	93	11.1%	(9.1 - 13.4)	13	1.6%	(0.8 - 2.6)
By at-risk grouping								
not at-risk group	991	1250	109	8.7%	(7.2 - 10.4)	18	1.4%	(0.9 - 2.3)
at-risk group	456	614	108	17.6%	(14.7 - 20.8)	6	1.0%	(0.4 - 2.1)
By RT-PCR outcome								
RT-PCR negative	1015	1172	120	10.2%	(8.6 - 12.1)	12	1.0%	(0.05 - 1.8)
RT-PCR positive	71	72	20	27.8%	(17.9 - 39.6)	4	5.6%	(1.5 - 13.6)
RT-PCR unavailable	547	620	77	12.4%	(9.9 - 15.3)	8	1.3%	(0.6 - 2.5)

* Consultations include GP, NPFS, NHS Direct, Accident and Emergency, and Hospitalisation

** Some individuals reporting antiviral use do not report a consultation

7.2.5.2 *Consultation Behavior*

Overall, 12% (95% CI: 10%-13%) of illnesses led to a consultation at one or more of the following services: GP (in person or over the phone), NPFS, NHS direct, Accident and Emergency (A&E) or Hospital (Table 7-5). When limited to those meeting the 2009 NPFS ILI case definition, the proportion consulting was 32% (95% CI: 26%-39%, data not shown). The percent of illnesses with at least one consultation were highest among children under the age of five (26%, 95% CI: 19%-34%), members of at-risk groups (18%, 95% CI: 15%-21%) and among RT-PCR-confirmed influenza cases (28%, 95% CI: 18%-40%).

The majority (91%, 95% CI: 87%-95%) of consulting illnesses had a GP consultation, either over the phone or in person while only 17% (95% CI: 12%-22%) reported NPFS use (Table 7-6) This equates to 11% (95% CI: 9%-12%) of all illnesses consulting a GP and 2% (95% CI: 1%-3%) of all illnesses consulting NPFS. Most consulting illnesses (81%, 95% CI: 75-86%) had only one consultation although some reported multiple consultations.

The mean number of consultations per consulting illness was 1.6 and the mean number of services consulted per consulting illness was 1.3. The median delay between illness onset and first consultation was 4 days overall but varied by service with a median 4-day delay for GP consultations but only 2 days for other types of services.

Table 7-6: Consultation type and timing

	People			Illnesses			Day of first consultation
	n	%	95% CI	n	%	95% CI	Median (range, mean)
All Consulting Cases	203	100%		217	100%		4.0 (1-38, 5.7)
Type of Consultation*							
GP	185	91%	(86 - 95)	197	91%	(87 - 95)	4.0 (1-38, 6.0)
NPFS	36	18%	(13 - 24)	36	17%	(12 - 22)	2.0 (1-10, 2.6)
NHS Direct	19	9%	(6 - 14)	19	9%	(5 - 13)	2.0 (1- 5, 2.4)
A&E	10	5%	(2 - 9)	10	5%	(2 - 8)	2.0 (1-16, 3.3)
Hospital Admission	11	5%	(3 - 9)	11	5%	(3 - 9)	2.0 (1-16, 4.3)

*Some illnesses have more than one type of consult

Among the 217 consulting illnesses, 211 (97%, 95% CI: 94%-99%) consulted either a GP and/or the NPFS (Table 7-6). Among these consults, 83% (95% CI: 77%-88%) consulted their GP but not NPFS, 10% (95% CI: 7%-15%) contacted both services and 7% (95% CI: 4%-11%) consulted the NFPS but not a GP. This distribution between GP and NPFS consultations did not vary greatly by subgroup (at-risk group, age group, or sex) although small numbers in the subgroups limits the accuracy of these estimates. Among illnesses which did not fall in an at-risk group, 6% (95% CI: 2% - 12%) of consultations were NPFS only, 15% (95% CI: 9%-23%) were both NPFS and GP and 79% (95% CI: 71%-87%) were with the GP only.

Table 7-7: GP and NPFS consultations by age group, sex and at-risk group

	N illnesses	Among Illnesses Consulting a GP or NPFS						
		N Consulting Illnesses	NPFS only		NPFS & GP		GP only	
			n	row % (95% CI)	n	row % (95% CI)	n	row % (95% CI)
Overall	1864	211	14	7% (4 -11)	22	10% (7 -15)	175	83% (77 -88)
By at-risk group								
Not in at-risk group	1250	107	6	6% (2 -12)	16	15% (9 -23)	85	79% (71 -87)
Any at-risk group	614	104	8	8% (3 -15)	6	6% (2 -12)	90	87% (78 -92)
Chronic Illness	263	46	2	4% (1 -15)	1	2% (0 -12)	43	93% (82 -99)
Pregnancy	11	1	0	0% (0 -98)	0	0% (0 -98)	1	100% (3 -100)
By agegp5								
0-4 years	170	41	2	5% (1 -17)	4	10% (3 -23)	35	85% (71 -94)
5-15 years	319	31	3	10% (2 -26)	5	16% (5 -34)	23	74% (55 -88)
16-44 years	502	44	1	2% (0 -12)	4	9% (3 -22)	39	89% (75 -96)
45-64 years	598	64	4	6% (2 -15)	8	13% (6 -23)	52	81% (70 -90)
65+ years	248	27	4	15% (4 -34)	1	4% (0 -19)	22	81% (62 -94)
By sex								
Female	1001	116	10	9% (4 -15)	9	8% (4 -14)	97	84% (76 -90)
Male	836	91	4	4% (1 -11)	13	14% (8 -23)	74	81% (72 -89)
By RT-PCR outcome								
RT-PCR negative	1172	118	9	8% (4 -14)	12	10% (5 -17)	97	82% (74 -89)
RT-PCR positive	72	20	2	10% (1 -32)	4	20% (6 -44)	14	70% (46 -88)
RT-PCR unavailable	620	73	3	4% (1 -12)	6	8% (3 -17)	64	88% (78 -94)

* At-risk groups appear in bold italics

7.2.5.3 *Treatment Outcome*

Among the 1864 illnesses, 24 (1.3%, 95% CI: 0.8% - 1.9%) were treated with antivirals, 13 of which (54%, 95% CI: 32.8% – 74.4%) were taken on the first or second day of reported illness (Table 7-8). The percent of illnesses treated with antivirals was lowest among the youngest age group (0.6%, 95% CI: 0.01% - 3.2%) and highest among RT-PCR-confirmed influenza illnesses (5.6%, 95% CI: 1.5% – 13.6%). More illnesses were treated with antibiotics than with antivirals (n=135, 7.2% of all illnesses, 95% CI: 6.1% – 8.5%). Antibiotic treatment usually occurred later in the illnesses (median first day of treatment was on day 4 of illness).

Table 7-8: Antiviral and antibiotic treatment and timing

	People			Illnesses			First day of treatment
	n	%	95% CI	n	%	95% CI	Median (range, mean)
Antivirals*	24	1.7%	(1.0% - 2.5%)	24	1.3%	(0.8% - 1.9%)	2.0 (1- 5, 2.4)
Antibiotics*	128	8.8%	(7.4% - 10.4%)	135	7.2%	(6.1% - 8.5%)	4.0 (1-37, 5.5)

*Three illnesses reported both Antiviral and Antibiotic use

7.2.5.4 *Impact of ILI Case Definitions*

The flow of illnesses through the NPFS algorithm using both case definitions and focusing on illnesses with PCR outcome data is summarized in Figure 7-5. All 1864 illnesses in the dataset were subject to the stage 1 pre-assessment screen. During this stage I excluded 11 illnesses occurring in pregnant or recently pregnant women and 43 illnesses occurring in infants under 1 year of age.

After the stage 1 exclusions a total of 1810 illnesses from 1425 participants entered into the clinical assessment stage of the algorithm. 560 illnesses (30.9%, 95% CI: 28.8% - 33.1%) were among at-risk individuals. 1208 of the illnesses (66.7%, 95% CI: 64.5%-68.9%) had accompanying RT-PCR data, 69 (5.7%, 95% CI: 4.5-7.2%) of which were RT-PCR-positive for influenza.

7.2.5.4.1 2009 Case Definition

Overall, 226 of the 1810 illnesses (12.5%, 95% CI: 11.0%-14.1%) met the 2009 ILI case definition within the first seven days of symptoms. This figure was 13.4% (95% CI: 10.7% – 16.5%) among members of an at-risk group and 12.1% (95% CI: 10.3%-14.0%) for those not in an at-risk group.

In the subset of 1208 illnesses with RT-PCR data, 15% (95% CI: 13.0% - 17.1%) met the 2009 case definition within seven days (Table 7-9). The sensitivity of the case definition (i.e. the proportion of RT-PCR+ influenza cases correctly identified by the case definition)

was 50.7% (95% CI: 38.4% - 63.0%). The specificity (i.e. the proportion of RT-PCR-cases, correctly identified by the case definition) was 87.2% (95% CI: 85.1% - 89.1%).

7.2.5.4.2 Afebrile Case Definition

For the alternative and more inclusive hypothetical case definition, 1539 of the 1810 illnesses (85.0%, 95% CI: 83.3% - 86.6%) met the case definition, a figure that did not vary when stratified by at-risk group or not.

In the subset of 1208 illnesses with RT-PCR data, the percent meeting the case definition was 90.0% (95% CI: 88.2% - 91.6%) (Table 7-9). As most illnesses met the case definition, the sensitivity was high at 95.7% (95% CI: 87.8% - 99.1%) but that inclusivity meant the specificity was low (10.4%, 95% CI: 8.7% - 12.3%).

Figure 7-5: NPFS algorithm assessment of Flu Watch Illnesses focusing on illnesses with PCR data using a) the 2009 NPFS ILI case definition and b) the afebrile case definition

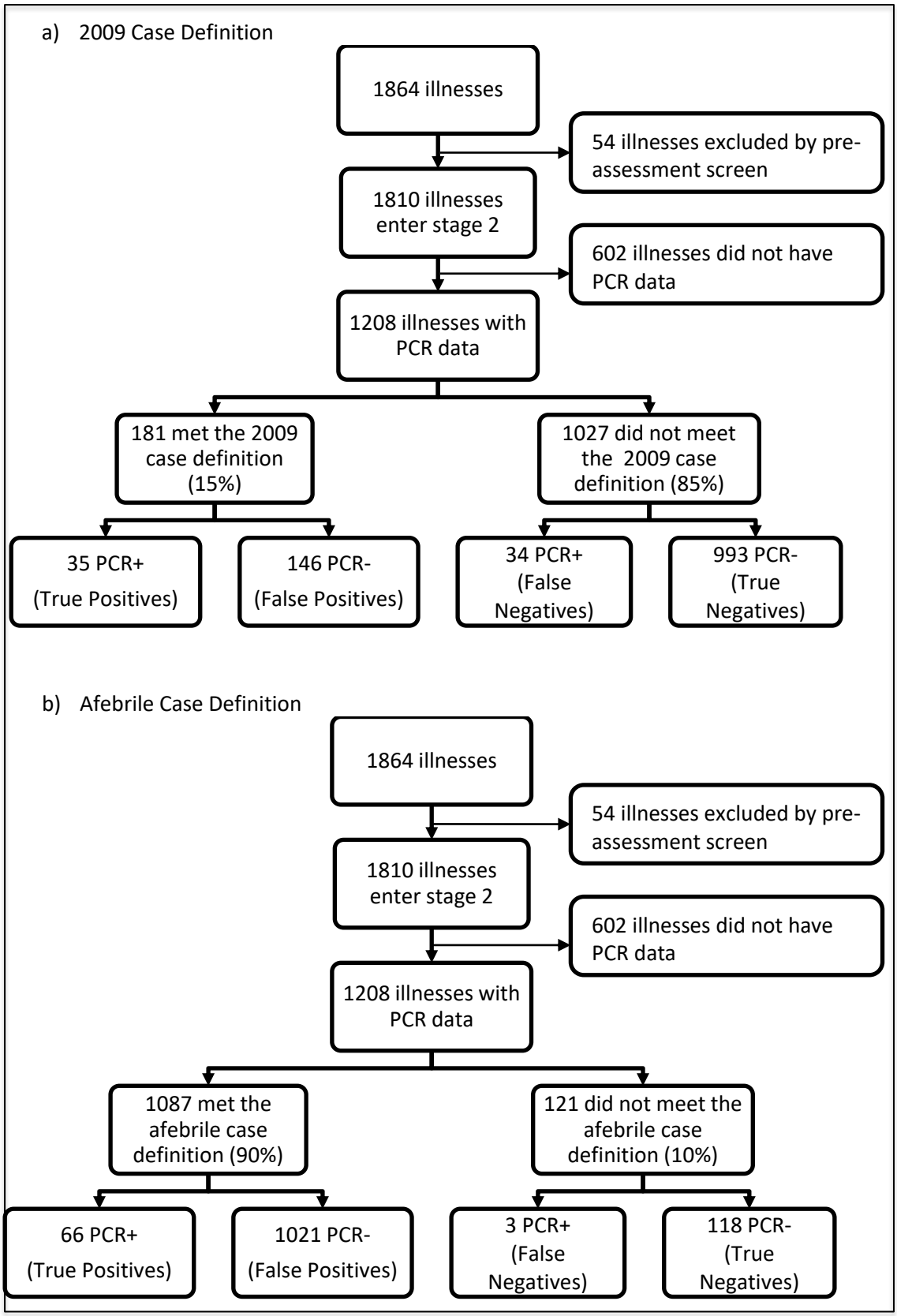


Table 7-9: Test Characteristics for two Case Definitions among illnesses with RT-PCR data

	2009 Case Definition (more specific)		Afebrile Case Definition (more sensitive)	
	%	95% CI	%	95% CI
% of illnesses meeting case definition in 7 days	15.0%	(13.0 - 17.1)	90.0%	(88.2 - 91.6)
Sensitivity	50.7%	(38.4 - 63.0)	95.7%	(87.8 - 99.1)
Specificity	87.2%	(85.1 - 89.1)	10.4%	(8.7 - 12.3)
Positive Predictive Value	19.3%	(13.9 - 25.9)	6.1%	(4.7 - 7.7)
Negative Predictive Value	96.7%	(95.4 - 97.7)	97.5%	(92.9 - 99.5)

7.2.5.4.3 Visualizing the impact of symptomatic case definitions

The sensitivity and specificity of different symptomatic case definitions within the NPFS algorithm have large impacts on the population in terms of the total number of antivirals authorized and how well they are targeted to individuals who have influenza.

Figure 7-6 which was created using an freely available online application [300], visualizes the impact of the sensitivity and specificity each case definition in a theoretical population of 1000 using the influenza prevalence estimated from Flu Watch during the NPFS. The figures display the numbers of correctly identified influenza illnesses (true positives and correctly targeted antivirals), correctly identified non-influenza illnesses (true negatives and appropriately withheld antivirals) as well as the mis-identified true influenza illnesses (false negatives and a missed opportunity to treat with antivirals) and mis-identified true non-influenza illnesses (false positives and mis-directed antivirals). The top panel displays the 2009 case definition, and the bottom displays the afebrile version of the 2009 case definition. The 2009 case definition misses about half of the true influenza illnesses who could have benefited from antiviral treatment and but correctly identifies most true negatives and thus does not mis-direct too many antivirals on non-influenza illnesses. To put another way, approximately 21% (95% CI: 15%-28%) of antivirals are being correctly offered to influenza cases although half of all influenza cases are missed. In contrast, the afebrile case definition identifies almost all influenza cases for treatment but also mis-classifies most non-influenza illnesses (which make up the vast majority of illnesses). This would mean many more antivirals would be prescribed (potentially putting pressure on national stockpiles) and only 6.7% (95% CI: 5.1% - 8.5%) of those antivirals would be correctly targeted at influenza cases.

Figure 7-6: Impact of Sensitivity and Specificity on a theoretical population of 1,000 for the 2009 NPFS case definitions (top panel) and the afebrile version of that case definition (bottom panel).

7.2.6 **Discussion**

7.2.6.1 *Summary of Results*

Only a small proportion of all community cases of respiratory illness (11%) consulted a GP and even fewer (2%) consulted the NPFS. NPFS consultations typically occurred earlier in illness than GP consultations (median 2 days versus 4 days respectively). Among all consulting illnesses, 91% had a GP consultation and only 17% consulted the NPFS, although more than half of these NPFS users also consulted a GP. Among consulting illnesses in the not at-risk group (the group that includes the 'worried well'), only 6% consulted the NPFS and not a GP, indicating only a small potential diversion of GP workload. Approximately 1% of all community illnesses were treated with antivirals, just over half of which took them within two days of illness onset. More community cases were treated with antibiotics (7.2%). The 2009 NPFS case definition classified 15% of all community illnesses as positive for an 'influenza-like illness' whereas the afebrile version of the case definition classified 90% of community illnesses as positive. The sensitivity of the 2009 case definition was lower than the afebrile version (51% versus 96% respectively) but the specificity in turn was higher (87% versus 10% respectively).

7.2.6.2 *Comparison to other studies*

The very low proportion of community cases seeking medical attention (11%) is consistent with seasonal influenza estimates from FluSurvey, a UK online cohort which found that among a combined group of community acute respiratory illnesses (ARI) and ILI illnesses, 9-13% consulted during periods of seasonal influenza [301]. Most other estimates of the proportion of community cases seeking medical care however are limited to illnesses meeting an ILI case definition. When we restricted our community cases to those meeting the 2009 NPFS ILI case definition, we found 32% of them consulted. This again is consistent with comparable estimates from the FluSurvey study during the 2009 pandemic (43% consulted in July 2009 and 25% between August and December 2009) [302]. It also fits with the broader picture of seasonal influenza estimates from other comparable online cohorts in Australia and across Europe [105,123,303]. The European studies show high variability between countries, but generally a low proportion of consultations in Northern Europe which typically take place 5-7 days after illness onset [105,123].

I was unable to find published estimates, drawn from individual data, of the overlap between NPFS and GP consultation. Some researchers report the individual rates of GP and NPFS consultations but without individual-level data it is impossible to know what proportion of those consultations consulted both services [295]. The NPFS was introduced just after the peak of the summer wave of the pandemic (as measured by virological surveillance) and therefore it is difficult to directly estimate how much of the decline in GP consultation rates following the introduction of the NPFS was due to NPFS and how much was due to declining incidence [295,296]. I was also unable to find estimates from the UK of the proportion of all community-level illnesses that received antivirals and/or antibiotics.

I was unable to find any estimates of the sensitivity and specificity of the NPFS clinical case definition either from community-level cases of respiratory illness or from the subset of those cases who consulted the NPFS. My finding that the clinical case definitions were either sensitive or specific but not both is consistent with other studies [16,262,298,304–306]. Estimates of sensitivity and specificity of case definitions are often limited to cases that consult and/or already meet a version of the ILI case definition [16,262,298,304–306]. However, even those that evaluate community-level illnesses still face the same trade-off in sensitivity or specificity [297].

7.2.6.3 *Strengths and Weaknesses*

A key strength of the study comes from the community-level, prospective follow up of all respiratory illnesses coupled with RT-PCR testing for influenza. This design enables accurate identification and characterisation of both the denominator (all illnesses in the community regardless of regardless of severity or RT-PCR status) and numerators (i.e. the symptomatic and PCR outcomes, consultation and treatment). This means my results are less likely to be biased by illness severity or by consultation. It also shed light on the scale of the community burden of respiratory illness which, in a future pandemic, could potentially consult the NPFS system. The RT-PCR testing allows the accuracy of the symptomatic case definitions to be calculated against a gold-standard diagnostic. The NPFS only conducted RT-PCR tests on illnesses that met their symptomatic case definition. By excluding the comparison group of illnesses that did not meet their case definition, they were unable to assess the accuracy of that case definition. The wide range of symptom data

available in the Flu Watch study allowed me to evaluate the accuracy of various case definitions which is a critical component of the NPFS algorithm and one that could be revised in future iterations of the algorithm.

The fact that the symptomatic outcomes are based on self-reported symptoms could be seen as a limitation but the NPFS assessment also relies on self-reported symptoms, so this makes Flu Watch data all the more comparable to the NPFS. A limitation of the study is that not all participants had the full medical record review at the end of follow-up. In terms of this analysis, the medical record review collected the same information that was self-reported in the weekly follow-up surveys, however in rare instances, the medical record review identified consultations and treatments which were not reported by the participants. The fact that the study did not have the medical records data for all participants may mean that some consultations and treatments were missed and this would have led to a slight underestimation the proportion of illness which had consultations and treatments.

It is possible there may be misclassification bias if PCR testing did not identify all influenza cases (e.g. due to the timing or adequacy of the specimen). This could affect the sensitivity and specificity calculations but without knowing the case definition status of those missed influenza cases it is impossible to say how those estimates would be biased.

Due to the rapid increase in study size during the autumn/winter wave of the pandemic, the majority of data and illnesses comes from that period and there are relatively fewer data from the summer wave of the pandemic. There is evidence that the propensity to consult was higher during the summer than the winter wave, possibly due to the fear of the new virus and the intense media coverage in the UK during the summer wave[302]. Therefore, my estimates of the overall proportion of illnesses consulting and the overlap between NPFS and GP consultations may be biased towards a time period when the general public were less likely to consult and possibly less concerned about the pandemic.

7.2.6.4 *Implications*

The fact that less than 2% of community illnesses consulted the NPFS during the 2009 pandemic highlights that this service only assessed the tip of the iceberg of all community

cases, an even smaller proportion of the iceberg than primary care. In a future pandemic, if there was an increase in illness rates, severity of illnesses, public concern, or a combination of these, one might expect many more ill people consulting the service and thus potentially greater drawdown on the antiviral stockpiles.

Despite the NPFS's aim to divert the 'worried well' from the GP to the NPFS, I found that among the consulting illnesses in the not-at-risk group (the group that the 'worried well' would fall into), only 6% of GP appointments were potentially diverted to the NPFS. It is plausible that diverted GP appointments may have been clustered in location and time (e.g. when rates of illness are highest or during a local outbreak) and therefore may have had meaningful effects on keeping services running at particularly busy times. It is also worth considering that if the estimate of 6% of GP appointments diverted were applied to a scenario with much higher consultation rates, the overall impact would be greater as a much larger number of GP appointments would be diverted. The other primary aim of the NPFS was to distribute antivirals to the public more quickly than would be achievable in primary care. I found that the median day of consultation for the NPFS was day two of illness, two days earlier than GP consultations. This could be due to people choosing to contact the NPFS service earlier than they would contact a GP. It could also be partly due to delays in accessing a GP compared with the NPFS. Even if the NPFS did not divert many GP appointments, it would have provided a faster route to antivirals than the traditional route of going through a GP.

The NPFS has been designed to support two different population-level antiviral treatment strategies; a 'treat all' approach whereby all those meeting the case definition could potentially receive antivirals and a 'targeted treatment' approach whereby only members of at-risk groups would potentially receive antiviral treatment [92]. Therefore, the choice of the NPFS case definition not only has to balance the relative importance of correctly identifying and treating as many influenza cases as possible (high sensitivity) while not misdirecting too many treatments to non-influenza cases which could put pressure on the antiviral stockpiles (high specificity), but it also must consider this balance for two different populations: the entire population and the subset of the population belonging to an at-risk group. Clinical case definitions for influenza cannot be both highly specific and sensitive

and therefore the best choice of case definition for the entire population under the 'treat all' policy many not be the best choice of case definition for the at-risk population under the 'targeted' treatment policy. Instead of choosing one case definition which would apply in both policy scenarios, it may be beneficial to have a third hybrid policy option which streams those at higher risk of severe outcomes and those at lower risk and applied a different case definition to each group. For example, for those at higher risk of severe outcomes (i.e. people with moderate to severe illness and / or a member of an at-risk group) one could apply a highly sensitive case definition since identifying and treating influenza cases would be the priority. In contrast, among the non-severe illnesses who are not in an at-risk group (the majority of illnesses), a more specific case definition would reduce the number of mis-directed antiviral treatments to non-influenza cases, while still providing treatment to some influenza cases. By streaming cases into high-risk and low-risk groups and applying different case definitions to each group, the NPFS would simultaneously target antiviral treatment to those who would need and benefit from it the most whilst also preserving antiviral stockpiles.

7.2.6.5 *Conclusions*

Only a small proportion of community-level respiratory illness consulted medical services, only a small proportion of these consulted the NPFS and only a small proportion of NPFS consults consulted the NPFS and no other services. Thus, in the relatively mild 2009 pandemic a modest percent of GP consultations may have been avoided but these may have had a meaningful effect on keeping primary care services running during particularly busy times. A hybrid population-level antiviral treatment policy which applies different symptomatic case definitions for high-risk and low-risk cases should be considered as it could simultaneously target antiviral treatment to those who would most benefit from it whilst also preserving national antiviral stockpiles.

7.3 Chapter Conclusions

The analyses in this chapter, in combination with previous FluWatch findings, demonstrate that most symptomatic seasonal and 2009 pandemic influenza cases are mild, do not meet a traditional ILI case definition, do not stay home when they are ill, do not consult medical services[15], and are rarely treated with antivirals even when the NPFS made them directly

available to the public. Understanding the spectrum of illnesses in the community and how people react to these illnesses is essential for designing impactful and cost-effective public health interventions, for it is likely that the combined effects of the mild (and far more numerous) illnesses have the biggest impact on the effectiveness and cost-effectiveness of population-level interventions.

Vaccines and antivirals are mainstay pharmaceutical interventions for seasonal and pandemic influenza. Such interventions are designed to prevent and treat influenza illnesses and, depending on how they are deployed, dampen wider community transmission. My estimates of absences and QALYs lost due to community-level influenza illness can help improve the accuracy of transmission and economic modeling of vaccination and other interventions by more fully reflecting the full range of influenza outcomes in a population. My evaluation of proportion of community respiratory illnesses which contacted the NPFS and met various case definitions clarifies the potential impact and cost effectiveness of the overall service as well as the impact of different case definitions and combination of case definitions on the community distribution of antivirals. It makes the tradeoffs associated with different case definitions more explicit, which would be helpful when tailoring the service to be proportionate to the threat. My suggested use of multiple case definitions would increase the overall flexibility of the service. Proportionality and flexibility are two of the three key principles underpinning the current UK pandemic preparedness strategy[307]. My finding that only a small fraction of community influenza cases consulted the NPFS during the pandemic and then only some of them would have been eligible for antivirals makes it unlikely that the NPFS antivirals had a wider impact on community transmission.

The design of non-pharmaceutical interventions which aim to reduce community transmission can also be informed by some of the findings presented in this chapter. For example, early self-isolation during illness is a method designed to keep infectious people from mixing with the wider community in an effort to reduce onward transmission. The fact that 1) people may be infectious before symptoms appear, 2) most illnesses are mild and many will not realize they have influenza, 3) people are used to going to work and school even when they have symptoms and 4) even if they did take time off they may still

be infectious when they return – all this taken together implies it would be unrealistic to assume that a policy of advised early self-isolation of cases would have a substantial effect on reducing community transmission. Similarly, entry screening alone is unlikely to be very effective in keeping cases out of a country as it would miss cases who are infectious but not yet displaying symptoms and miss mild cases that do not meet their case definition (e.g. afebrile cases).

In summary, this chapter presents findings on the impact that influenza symptoms have on individuals, the reaction people have to these illnesses in terms of absenteeism, consultation, and treatment, and how this community-level information can be used to improve both our understanding and our public health responses to these illnesses.

7.4 Relevance to COVID-19

Many of the epidemiological issues discussed above that inform public health responses are similar for Influenza and COVID-19 (see Table 7-10 for a summary). For example, although COVID-19 is more severe than influenza strains circulating in the last century, most cases of COVID-19 are mild to moderate severity and do not require hospitalisation[308], many symptomatic cases do not meet the government's case definition[309] and many people do not know what that case definition is[310]. Among those meeting the case definition most do not request a test and despite being required by law to self-isolate, most do not fully adhere to the self-isolation rules[310]. SARS-CoV-2 infection, like influenza, can also lead to asymptomatic and pre-symptomatic viral shedding[311,312].

As with pandemic influenza, when the COVID-19 pandemic arose, there was no vaccine but unlike influenza, there was also no generic antiviral. In the absence of pharmaceutical interventions and specific treatments, societies had to turn to non-pharmaceutical interventions to control spread, flattening and delaying the epidemic curve in order to reduce mortality, prevent hospitals from becoming overwhelmed, and to buy time for COVID-19 vaccines and treatments to be developed and deployed. The choice of non-pharmaceutical interventions for acute respiratory infections like influenza and SARS-

CoV-2 are the same, but the interventions chosen in the UK and elsewhere for the 2009 influenza pandemic and the COVID-19 pandemic were very different. This was due to the differences in severity between the two pandemics.

In the 2009 influenza pandemic the main public health interventions in the UK were largely confined to two pharmaceutical interventions which were not initiated immediately and intended to mitigate rather than suppress the pandemic. These interventions were: a large vaccination programme for children, older adults and elderly and certain clinical risk groups and 2) the NPFS which triaged community cases of respiratory illness in order to ease burden on primary care and treat community cases with stockpiled influenza antivirals[92]. This approach aimed to prevent infection and reduce transmission through vaccination and to treat community-level cases with antivirals.

In contrast, for COVID-19, the UK government aimed for early and strong suppression of the epidemic and employed almost all possible non-pharmaceutical interventions in their most restrictive forms[313–315]. This was due to the severity of COVID-19, the usual pandemic concerns around the lack of vaccines, antivirals and other specific treatments but also a recognition of the importance of asymptomatic and pre-symptomatic transmission[316] which lessens the effectiveness of control measures centered around identification and isolation of symptomatic cases. The non-pharmaceutical interventions used in the UK included stay-at-home orders or 'lockdowns', social distancing, working from home and government backed furlough schemes, school and class closures, travel restrictions and mask wearing[317]. The UK also built-up mass testing and contact tracing capacity as part of the Test, Trace and Isolate system (TTI)[318,319]. In contrast to the NPFS which focused on treating individuals, the TTI focused on identifying, testing and isolating all suspected cases and their contacts in order to suppress community transmission. The non-pharmaceutical interventions and restrictions imposed during the COVID-19 pandemic affected everyone, not just cases and their contacts.

Although the public health response to the two pandemics were very different, they shared some of the same challenges described above including individuals not realizing they are infectious; individuals not contacting NPFS/TTI when they are symptomatic; individuals

not always staying at home when ill; difficulties in choosing the best set of symptoms for the case definition, the importance of asymptomatic infection and transmission. All of these issues lessen the effect of the relevant public health intervention but the simultaneous use of multiple pharmaceutical and non-pharmaceutical interventions (the so-called swiss cheese approach[320]) limits impact. The many types of restrictions, strict social distancing and particularly the stay-at-home orders helped reduce transmission[321].

Similar to influenza, it was difficult to choose which symptoms to include in the TTI case definition as apart from the high frequency of loss or change to sense of smell or taste, the symptoms were similar to other acute respiratory infections[309]. Very early in the pandemic, my supervisor and I developed and evaluated the test characteristics of a COVID-19 clinical case definition using data from the UK COVID-19 first few hundred study and symptom data from non-COVID-19 illnesses from the Flu Watch study (same dataset and similar methods to the work presented in this chapter). We proposed a range of case definitions with a diffing balance between sensitivity, specificity and simplicity. Since the UK CMOs valued sensitivity over specificity and also placed a premium on simplicity the triad of Cough OR Fever OR Loss of Sense of Taste or Smell was chosen (A. Haywards, personal communication, 27 February 2022). Additional symptoms such as headache and fatigue were not included due to large drops in specificity and marginal gains in sensitivity. Later, there were discussions among a group of academics representing the large community studies and Public Health England, debating the advantages and disadvantages of expanding the TTI case definition to increase the sensitivity by including additional constitutional symptoms as case numbers fell, but this would have the knock-on effects of increasing the number of tests needed and the number of people needing to self-isolate while they awaited their test results[309]. So far, the case definition has not been changed although it should be kept under review as the symptom profiles may change with new variants.

In terms of health-related quality of life (HRQoL), one could argue that this was reduced for everyone, cases and non-cases, particularly during lockdowns[322–324]. Among COVID-19 cases, the total HRQoL lost due to illness will be extensive, not only because of the high level of morbidity and mortality, but also because a large number cases suffer from

extended periods of illness known as ‘long Covid’[325–328]. It will take many years to understand the true cost of this pandemic, both in terms of morbidity but also in terms of the effects of the interventions.

The Flu Watch data on attendance at work during periods of influenza illness is relevant to discussions about lifting of legal restrictions requiring isolation of COVID-19 cases as it suggests that unless there is a marked culture change compared to before the pandemic, then a high proportion of those with respiratory infections will attend work promoting transmission.

Table 7-10: Comparison of COVID-19 and Influenza

	Similarities	**Differences**
Clinical Iceberg and disease severity	• Large asymptomatic proportion • Large proportion of illnesses mild to moderate severity • Many people don't stay home when symptomatic or positive	• COVID-19 illness much more severe, particularly in older age groups • Long Covid – no equivalent in influenza • More awareness of the need to stay home during periods of illness
Transmission	• Acute Respiratory Infection spread via aersosol, droplets and fomites • Pre-symptomatic and asymptomatic shedding of virus • Somewhat similar R0 and serial intervals • Antibody waning and viral evolution mean people can be re-infected	• Children aren't as susceptible as adults and don't drive COVID-19 epidemics as much as influenza
Diagnostics & Laboratory assays	• PCR assays detect active infection • Rapid antigen testing now exists for both viruses • Serological Assays identify antibodies	• COVID-19 rapid antigen testing widely available for at home testing with relatively good sensitivity among symptomatic cases; influenza rapid tests limited to clinical settings and not widely available. • At-home fingerprick test available for SARS-CoV-2 antibodies (e.g. lateral flow immunoassays) • SARS-CoV-2 serology can be conducted on small amounts of capilliary blood, self-collected with fingerpricks

		• Anti-N SARS-CoV antibodies are a marker of natural infection and not vaccine derived. No equivalent antibody marker for natural influenza infection.
Pharmaceutical Interventions	• Effective vaccines now available • Ongoing/booster vaccination needed; Vaccine updates likely needed as immune escape variants appear	• SARS-CoV-2 vaccines use new technology (e.g. MRNA vaccines) • COVID-19 specific treatments at first didn't exist • COVID-19 antivirals only recently introduced and not widely available yet
Non-Pharmacuetical interventions	• List of potential non-pharmaceutical interventions for acute respiratory infections are the same (i.e. they are not virus-specific) • Asymptomatic and pre-symptomatic individuals can unwittingly spread virus • Symptomatic people don't always stay home even when they are asked to	• **Test. Trace and Isolate (TTI)** aimed to REDUCE TRANSMISSION of SARS-CoV-2 through identification and isolation of cases and contacts so that medical services (particularly hospitals) weren't overwhelmed. Potential cases told to contact TTI, not GPs. Not aimed to treat individuals as antivirals not available; **NPFS** aimed to treat individuals & reduce burden on primary care • **Lockdowns, social distancing, working from home, school closures, travel restrictions and mask wearing** during Covid-19. Although non-pharmaceutical interventions for acute respiratory viruses are not-virus specific, the severity of covid-19 has meant much larger scale and more restrictive public health interventions (e.g. lockdowns) have been employed in the UK and many other countries around the world
Seleted public health challenges	• Asymptomatic/presymptomatic shedding (people can be infectious without realising it)	

	• Not everyone recognises they might be infected (not knowing the case definition symptoms or assuming symptoms are too mild to be COVID/Flu) • Not everyone seeks out testing (COVID-19) or assessment (NPFS) • Choosing a set of symptoms for case definition is challenging and has differ trade-offs in different circumstances • Not everyone stays at home / self-isolates when ill or when required	

Chapter 8 Conclusion

The aim of this PhD was to inform seasonal and pandemic influenza preparedness, severity assessments and response through the production of empirical estimates on community-level influenza immunity, infection and disease. There were four objectives, one for each main analysis. In the following section I will describe how I met these objectives by summarizing my main findings for each. The emergence of the COVID-19 pandemic arose following the main analyses but I have also attempted to reflect on the significance of the work for the COVID-19 pandemic.

8.1 Summary of main findings

8.1.1 Investigate whether occupational exposure to pigs increases risk of seasonal, pandemic and zoonotic influenza infection

By comparing serological evidence of infection with a selection of human and swine H1 and H3 viruses among pig industry workers and a general population comparison group, I found that pig industry workers showed evidence of increased odds of A(H1N1)pdm09 seropositivity compared to the comparison group, albeit with wide confidence intervals (CIs), adjusted odds ratio after accounting for possible cross-reactivity with other swine A(H1) viruses [(aOR) 15.1, (95% CI: 1.6–140), p=0.017]. I also found evidence that pig industry workers had elevated odds of swine H1N2 seropositivity [a(OR) 4.32 (95% CI: 1.39–13.46), p=0.012] and H3N2 Perth seropositivity [a(OR) 4.22 (95% CI: 1.28–13.94), p=0.018], after controlling for vaccination, season and seropositivity to other measured, potentially cross-reactive strains.

8.1.2 Describe the population-level patterns of influenza infection and immunity in England during the 2012/13 winter season

Using data from a population-level, repeated cross-sectional survey, I found evidence that a high proportion of the English population is infected with influenza over the course of a few months. Infection with A(H1N1)pdm09 was common with 42% (95% CI [33% - 51%]) of the unvaccinated population moving from undetectable to detectable H1N1pdm2009

antibodies; the equivalent figure for A(H3N2) was 24% (95% CI [12%-37%]). Using a higher threshold titre of 1:40 the cumulative increase was 38% (95% CI: 29% - 46%) for H1N1pdm2009 and 27% (95% CI: 16%-37%) for A(H3N2). These proportions are a great deal higher than estimates based on virologically confirmed cases and also higher than those typically estimated from community cohort studies which identify infections based on a four-fold rise in antibody titre from pre- and post-season serological samples [15,163,234]. This suggests these other types of data may underestimate the true infection rate. My results may also shed light on the level of strain-specific antibodies in the English population that permit and curtail epidemic spread of influenza. Prior to the A(H1N1)pdm09 epidemic wave, approximately 50% of adults have detectable and 30% protect antibodies against A(H1N1)pdm09. For A(H3N2) these figures were 50% detectable and 35% protective. Immediately following the epidemic peak, levels of detectable antibodies against A(H1N1)pdm09 were approximately 80% and protective levels 50-60%. For A(H3N2) these figures were 60-70% and approximately 55% respectively. These pre-season low levels and immediately post-peak antibody levels may represent an immune environment where epidemic spread of influenza is feasible and curtailed respectively.

8.1.3 Quantify the work and school absences and health-related quality of life loss due to community influenza illnesses

Using data from Flu Watch, a community cohort study, I found that the average quality adjusted life days (QALD) lost among community cases of acute respiratory infections (ARI), Influenza-like-illness (ILI), H1N1pdm09 and influenza B cases was 0.26, 0.93, 1.61 and 1.84 respectively. Among virologically confirmed cases (who are likely to be infectious) I also found that overall 40% (95% CI: 33%-48%) of influenza A cases and 24% (95% CI: 13%-40%) of influenza B cases took time off work/school with an average duration of 3.6 and 2.4 days, respectively. Among ill children, 56% (95% CI: 42%-68%) and 31% (95% CI: 14%-52%) took time off school or childcare for an average duration of 3.5 and 2.1 days for influenza A and B respectively. Ill adults were less likely to take time off (31% [95% CI: 22%-41%] and 20% [95% CI: 4%-48%] for influenza A and B respectively) but, when they did so, took more time off (3.8 and 3.0 days for influenza A and B respectively). ILI cases were broadly comparable with influenza cases although

more severe than the ARI cases. When scaled up to the 2010/11 English population, we estimated that community influenza cases lost 24,300 quality adjusted life years (QALYs) and had an estimated 2.9 million absences per season based on data from 2006/07 to 2009/10.

8.1.4 Evaluate the success of the 2009 National Pandemic Flu Service Algorithm against its two primary aims and propose changes to the algorithm to better target community-level antiviral treatment

To inform a review of the NPFS treatment algorithm I assessed the 2009 algorithm on contemporaneous UK community cases from the Flu Watch study, many of whom did not consult the NPFS. Only a small proportion of all community cases of respiratory illness (11%) consulted a GP and even fewer (2%) (95% CI: 1%-3%) consulted the NPFS. NPFS consultations typically occurred earlier in illness than GP consultations (median 2 days versus 4 days respectively). Among all consulting illnesses, 91% (95% CI: 87%-95%) had a GP consultation and only 17% (95% CI: 12%-22%) consulted the NPFS, although more than half of these NPFS users also consulted a GP. Among consulting illnesses in the not at-risk group (the group that includes the 'worried well'), only 6% (95% CI: 2% - 12%) consulted the NPFS and not a GP, indicating only a small potential diversion of GP workload. Approximately 1% (95% CI: 1% – 2%) of all community illnesses were treated with antivirals, just over half of which took them within two days of illness onset. More community cases were treated with antibiotics (7.2%, 95% CI: 6.1% – 8.5%). The 2009 NPFS ILI case definition classified 15% (95% CI: 13% - 17%) of all community illnesses as positive for an 'influenza-like illness' whereas the afebrile version of the case definition classified 90% (95% CI: 88% - 92%) of community illnesses as positive. The sensitivity of the 2009 case definition was lower than the afebrile version (51% [95% CI: 38%-63%] versus 96% [95% CI: 88%-99%] respectively) but the specificity in turn was higher (87% [95% CI: 85%-89%] versus 10% [95% CI: 9%-12%] respectively).

8.2 Strengths and weaknesses of the research

Table 8-1 outlines some of the main strengths and weaknesses of the two studies – some generic in nature and some particular to the research questions that these studies were meant to address.

The Flu Watch study was a large, multi-year, community cohort study providing comparable data during periods of interpandemic, pandemic and post-pandemic influenza. It collected extensive data on potential risk factors and outcomes including laboratory confirmed outcomes covering the lower three levels of the clinical iceberg (infection, illness, consultation) and enabled research into many different questions on influenza immunity, burden and transmission. Although the initial participation rate was low, retention was high and the cohort was fairly representative of the population. Weekly prospective follow-up for all respiratory illnesses (regardless of consultation), daily recording of symptoms, treatment and behaviours and self-swabbing illnesses for PCR-analysis of influenza and other common acute respiratory viruses provides a holistic and exquisitely detailed picture of the respiratory illnesses experienced in the community which primary care surveillance almost entirely misses. The study benefits from the generic advantages of cohort studies (timing of exposures and outcomes typically known, lower level of biases, multiple exposures and outcomes collected, ability to directly calculate incidence rates) but also suffers from the many of the generic disadvantages of cohort studies such as time (both set up time and follow-up time can be long), high costs and complicated logistics.

The PIPS study was designed as a much-slimmed down version of Flu Watch, aiming to capture the most critical infection and immunity data at the start of and in real-time throughout a pandemic without the cost and logistical challenges of a full cohort. These advantages would also mean the study would be easier to fund and run on a continuous basis (i.e. as surveillance) rather than a one-off research study. This had the benefit of providing comparable pre-pandemic data and could easily be a platform for research on other acute viruses in the community. The main study limitations were the fact that this particular study design had a fixed sample size, may be more biased when estimating cumulative incidence of infection when compared to a cohort with paired sera, is unable to estimate asymptomatic infection rates and, perhaps most dramatically, data collection could be (and was) shut down in the event a severe pandemic. As a cross-sectional study it lacked prospective follow-up by definition and this limited the additional research questions that could be addressed with a cohort study.

Table 8-1: Strength and weaknesses of the Flu Watch and PIPS studies

Study	Strengths	Weaknesses
Flu Watch	• Community-level & fairly representative of population • Designed for seasonal and pandemic research • Most (possibly all) aspects of study adaptable to other existing or novel pathogens • Many different risk factors and outcomes collected • Relative timing of outcome and exposures typically known • Symptomatic and laboratory-confirmed outcomes for influenza infection and disease • PCR-confirmation of influenza A and B and other common acute respiratory infections including seasonal coronaviruses, RSV and rhinoviruses • Methods were adaptable over time • Laboratory assay conducted by expert team at PHE • Includes review of medical records by research nurse • Multiple levels of iceberg covered (infections, illness, consultation) • Data on humoral and T-cell immunity • Able to directly calculate rates of infection, disease & consultation • Paired antibody data enables less biased estimates of cumulative incidence/incidence rates and also evaluation of antibody waning	• Expensive • Resource Intensive • Difficult and time consuming to set up • Major delays in obtaining further pandemic funding, ethics and R&D approval across multiple sites, resulting in delayed recruitment during the pandemic and fewer participants overall • Delays in obtaining serological results as PHE laboratory extremely busy during pandemic with many competing priorities • Hard to maintain funding during interpandemic periods • Low initial participation rate – can lead to bias • Potential for participant survey fatigue • Potential loss of follow-up • Harder to generate real-time serological data • Sample sizes too small to get accurate rates in small divisions of geography, time or age grouping.

	• Detailed, daily prospective clinical and behavioural follow-up enabling many research questions to be addressed with a high level of accuracy • Able to generate real-time data on clinical disease and behaviour • Data from multiple seasons covering interpandemic, pandemic and post pandemic influenza circulation	
PIPS	• Community-level and highly representative of population • Designed for seasonal and pandemic research • Most (possibly all) aspects of study adaptable to other existing or novel pathogens • Key individual-level variables (including vaccination) collected • Pre-agreed funding, ethics & approvals • Hibernating yet triggerable in 2 weeks in event of pandemic • Cost-effective • Would be easier to run continuously during interpandemic periods than a cohort • Could be applied to other existing and novel viruses (depending on laboratory techniques) • No loss to follow-up / survey fatigue • Easier to generate real-time serological data • Builds serological capacity	• Limited risk factor data & timing relative to outcome not always known • Lack of prospective follow up means no good data on illnesses. • Limited sample size & not able to scale up size • Methods not particularly adaptable • May be more prone to biased estimates of cumulative incidence • Study at risk of shutting down during a severe pandemic • Pilot ran only one influenza season • Difficult to recruit and train laboratory staff • Difficult to maintain laboratory expertise during hibernation periods

8.3 Contribution of the work

8.3.1 Occupational Exposure to Pigs

This study improved our understanding of swine influenza transmission to humans, by comparing the serological evidence of SIV seropositivity in pig industry workers. The increased risk of A(H1N1)pdm09 in pig industry workers is compatible with the concurrent emergence of infection with A(H1N1)pdm09 in pigs in England, which was first observed in November 2009 [170] and confirmed by the serological results in our study. As there was minimal trade of live pigs between North America and Europe during the period of the study and no reports of the pandemic strain in European pigs prior to human cases, [200] it is likely that pigs were initially infected by humans during the early stages of the 2009 pandemic, and infection then transmitted efficiently within and between pig herds but also through reverse zoonoses events following contact of pigs with infected humans. Phylogenetic analysis has subsequently demonstrated that H1N1pdm2009 has been repeatedly transmitted from humans to swine since the pandemic [201]. Pig industry workers naïve to A(H1N1)pdm09 would be susceptible to zoonotic infection from pig herds undergoing active infection, with exposure to, sometimes large, groups of pigs simultaneously undergoing acute infection and shedding virus favouring transmission from pigs to pig industry workers. Further bi-directional transmission may have led to an amplification effect leading to high levels of infection in both pigs and pig industry workers. This is important in that it shows that dense populations of pigs can serve as an amplifying reservoir for influenza virus, increasing the risk of novel virus transmission to both pigs and to humans.

It is generally considered that influenza virus reassortment with significant pandemic potential is most likely to occur in developing country "hotspots"[208], where the demographic, cultural and economic circumstances and animal husbandry practices together result in settings of dense overlaps between humans and animal populations and opportunities for cross-species transmission. However, given my findings, and observations of new reassortant strains elsewhere in Europe [209,210], there should be no assumption that reassortment with possible zoonotic risk could not also occur in industrialised settings.

Observations from this study also offer strong supporting evidence that pig industry workers should be among the occupational groups offered annual seasonal influenza vaccination. Preventing influenza infection in people who work with pigs would seem to be a logical option to minimise the risk of transmission of human variants into pigs, and by extension to reduce the possibilities for reassortment in pigs. Unfortunately, this recommendation to make pig industry workers an occupation that is routinely offered influenza vaccination has not been taken up, partly due to poor uptake of influenza vaccine in poultry workers (A. Hayward, personal communication related to NERVTAG discussions, 03 October 2021)[329].

8.3.2 **PIPS serosurvey**

The PIPS pilot study demonstrated that by appending influenza data and specimen collection to a nationally representative ongoing data collection system (in this case the HSE study), real-time serosurveillance can be done in a cost-effective and continuous manner and produce burden and severity estimates necessary for effective and proportionate response to seasonal and pandemic influenza. It also confirmed that in a cross-sectional study, retrospective reporting of respiratory illness in the previous month suffers from high levels of recall bias. This bias would likely be improved if the recall period was reduced to one or two weeks prior to data collection but this would capture fewer illnesses and the study would suffer from low power to detect trends in illness unless the sample size was increased. Ideally prospective study designs would be used

Unfortunately, the 2020 COVID-19 pandemic demonstrated the importance for pandemic research studies and surveillance systems to be robust to severe pandemics and the major societal disruptions they can cause. In early 2020 whilst I was adapting the PIPS study for SARS-CoV-2 and reactivation, the HSE study made a decision to halt all home visits (and thus all data and specimen collection) out of safety concerns for its staff and participants. This decision made our study unfeasible and as a result it was not triggered during the pandemic. Other major studies were developed that captured the relevant data on population-level antibodies but due to their size and costs they are unsustainable in the long term. There is an ongoing need for influenza and SARS-CoV-2 serosurveillance and this

HSE surveillance platform could still provide an efficient mechanism to collect venous blood specimens, albeit at a much smaller scale than the systems used during the pandemic.

8.3.3 Health Related Quality of Life and Absenteeism

My research on absenteeism due to community influenza infection was requested by and informed the review of the UK government's planning assumptions relevant to workforce absenteeism during an influenza pandemic. This work was also requested ahead of publication by staff at the US CDC.

The finding that among community cases of influenza A illnesses, only 56% of children took time off school or childcare and only 31% of adults took time off work or education highlights the fact that people are used to continuing with their daily activities even when ill. It is important that seasonal and pandemic planning assumptions reflect realistic assumptions about absenteeism and self-isolation and anticipate that these behaviours are likely to vary depending on the severity of illness. These findings also have implications for transmission models incorporating contact patterns of symptomatic community cases. Interestingly, despite the more virulent nature of the SARS-CoV-2 virus, we still see that individuals often do not adhere to self-isolation policies despite being legally required to do so [310].

Estimates of QALDs lost and work and school absences associated with influenza differ depending on the setting in which cases are identified; community illnesses, being less severe than medically attended cases, result in smaller effects but contribute substantially to the population-level burden. Accurate assessment of both the number of expected cases and their QALDs/QALYs is essential to inform CUAs for decision-making bodies such as NICE. For some interventions, such as antiviral medically-attended cases, I believe that my estimates are more appropriate for assessing cost utility of community preventive interventions such as vaccines.

8.3.4 **Evaluation of the National Pandemic Flu Service**

I conducted this work in 2019 at the request of the committee tasked with the job of reviewing and updating the 2009 NPFS algorithm. I was asked to evaluate the sensitivity and specificity of the NPFS ILI case definition which determined antiviral prescriptions to suspected community cases of pandemic influenza. I expanded on this analysis to additionally evaluate how all the respiratory illnesses reported in the Flu Watch study flowed through the algorithm and I presented my findings to the committee in mid-2019 at a meeting in London.

The NPFS has been designed to support two different population-level antiviral treatment strategies; a 'treat all' approach whereby all those meeting the case definition could potentially receive antivirals and a 'targeted treatment' approach whereby only members of at-risk groups would potentially receive antiviral treatment [92]. Therefore, the choice of the NPFS case definition not only has to balance the relative importance of correctly identifying and treating as many influenza cases as possible (high sensitivity) while not misdirecting too many treatments to non-influenza cases which could put pressure on the antiviral stockpiles (high specificity), but it also has to consider this balance for two different populations: the entire population and the subset of the population belonging to an at-risk group. Clinical case definitions for influenza cannot be both highly specific and sensitive and therefore the best choice of case definition for the entire population under the 'treat all' policy many not be the best choice of case definition for the at-risk population under the 'targeted' treatment policy. Instead of choosing one case definition which would apply in both policy scenarios, I proposed to the committee that it may be beneficial to have a third hybrid policy option which streams those at higher risk of severe outcomes and those at lower risk and applies a different case definition to each group. For example, for those at higher risk of severe outcomes (i.e. people with moderate to severe illness and / or a member of an at-risk group) one could apply a highly sensitive case definition since identifying and treating influenza cases would be the priority. In contrast, among the non-severe illnesses who are not in an at-risk group (the majority of illnesses), a more specific case definition would reduce the number of mis-directed antiviral treatments to non-influenza cases, while still providing treatment to some influenza cases. By streaming cases into high-risk and low-risk groups and applying different case definitions to each

group, the NPFS would simultaneously target antiviral treatment to those who would need and benefit from it the most whilst also preserving antiviral stockpiles.

My proposal to alter the algorithm by streaming high- and low-risk individuals and applying different case definitions to these groups was accepted by the committee and was incorporated into the updated NPFS algorithm. The algorithm now has the ability to turn the streaming function on and off and it provides a third policy option for the treatment of community cases of influenza.

There is currently a massive effort underway to develop treatments to COVID-19 and although a few treatments have been licensed their availability is low and their cost is high. If the NPFS were to be adapted as a distribution mechanism for COVID-19 antivirals at the community level, then the functionality of the clinical case definition would likely be replaced by the results of widely available rapid antigen tests. Although these rapid tests may have similar sensitivities to clinical case definitions among symptomatic COVID-19 cases in the first week [309,330], they have much higher specificities. In settings where rapid tests are unavailable and clinical case definitions are needed, the concept of applying different case definitions to high and low risk individuals remains relevant. In a future pandemic with a novel pathogen (influenza or otherwise) clinical case definitions are likely to still be needed until accurate rapid tests are widely available. For this reason, the NPFS (or a similar system), with all the nuances of its use of clinical case definitions, may still remain relevant even if it is not used during the current COVID-19 pandemic.

There is an ongoing debate about the current TTI COVID-19 case definition and whether it should be replaced with an alternative, more inclusive case definition with additional symptoms. I and others have shown such alternative case definitions to be more sensitive however it would increase the number of tests needed and the number of people isolating whilst waiting for their test results[309,331]. For this reason, a change to a more sensitive case definition would be least likely to overwhelm testing capacity if it occurred during periods of low incidence rates. It is periods of low incidence however when finding every case is even more important if you want to impact community transmission.

8.3.5 **Generic contributions of this work to the COVID-19 pandemic**

As part of my PhD work I developed STATA scripts to generate, manipulate and analyse detailed daily data on Flu Watch illnesses. This dataset and those scripts became instrumental in some of my early COVID rapid response work. My and my colleagues' work more generally on the Flu Watch study and the subsequent spin-off Bug Watch study [332] for which I led the development, was also critical to the development, funding and success of our currently running Virus Watch community cohort study of SARS-CoV-2 infection and illnesses [333].

For example, my supervisor and I built on my NPFS case definition work and used my previously mentioned illness dataset to compare non-COVID illnesses from Flu Watch with the earliest known UK cases of COVID-19 from PHE's First Few Hundred Study. We rapidly developed a symptomatic case definition for suspected COVID-19 infection for the purposes of community contact tracing. This work was presented to the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) and the UK Government's Scientific Advisory Group for Emergencies (SAGE) and the UK Senior Clinicians Group comprising CMO's and deputy CMOs from all four nations. We presented a range of case definitions balancing sensitivity, specificity and complexity which was instrumental in helping the CMOs choose the case definition used for the UK Test, Trace and Isolate (TTI) system. I later expanded on this work as part of a PHE roundtable on the potential updating of the TTI case definition. This entailed the evaluation and comparison of the performance of various case definitions using data from four major UK community studies (Virus Watch, the ONS infection survey [334], Zoe App [335] and REACT [336]). I conducted and presented the Virus Watch estimates at the PHE roundtable, contributed to the harmonization of estimates from the four studies and then led the comparison of estimates that were presented to the UK Chief Medical Officers. In related work, I also evaluated the performance of various case definitions for identification of Influenza and COVID-19 to inform WHO's e-Consultation to Adapt Influenza Sentinel Surveillance Systems for Including COVID-19 and their subsequent case definition recommendations [337].

Another example of COVID-related impact arising from my PhD work was the direct and rapid adaptation of my protocols and STATA script which generated and analysed Flu Watch illness data. This enabled me to rapidly convert Virus Watch follow-up data into an illness dataset which has since fed into a number of influential analyses [338,339] It also enabled me to conduct rapid and regularly updated analyses of the symptom profiles and case definition performance for COVID-19 and non-COVID-19 illnesses and enabled comparisons between illnesses caused by Wild-type SARS-CoV-2 and the Alpha and Delta variants of concern (VOC) when they began circulating in the UK. My findings were presented to NERVTAG and SAGE, were incorporated into an official NERVTAG report on the B.1.1.7 VOC and are currently under consideration for publication [309]. Findings related to workplace attendance during periods of influenza are also incorporated into a NervTag paper (yet to be published) on the likelihood of co-circulation of COVID-19 and other respiratory infections in future winter seasons.

8.4 Recommendations for further research and applications

Much has changed in the world of influenza and pandemic research and response generally since the appearance of SARS-CoV-2 but the importance of obtaining continuous, high-quality community-level data during interpandemic and pandemic periods has not. Globally, influenza levels are at historic lows since the COVID-19 pandemic began [340]. Although it seems likely that a combination of non-pharmaceutical interventions, national and international travel restrictions as well as intensive seasonal influenza vaccination campaigns helped reduce influenza transmission [255,340], it has also been hypothesized that viral interference may have caused the SARS-CoV-2 and influenza viruses to compete with one another and further reduced influenza circulation [340]. In the coming years it will be important to monitor how SARS-CoV-2 circulation settles into a post-pandemic circulation pattern and how this affects the circulation of influenza and other acute respiratory viruses.

There has been a great deal of technological advancements made in a very short time period in order to combat the COVID-19 pandemic, including vaccines, treatments, diagnostics and laboratory assays. It would be wonderful if some of the advances made in SARS-CoV-2 serological and virological assays could inform similar advancements for influenza. The rapid development of SARS-CoV-2 serological assays that only require small amounts of

capilliary blood which can be self-collected with a fingerprick; the at home antibody tests and the widespread at-home lateral flow rapid antigen test gives me hope that such assays may soon be developed for influenza. These advances would make sero-surveillance and virological surveillance of influenza and COVID-19 much easier in a practical sense and more cost effective and thus more likely to be funded.

In terms of further research, I think it would be useful to conduct a cohort study or some sort of ongoing co-ordinated surveillance of pig-workers and their pig herds with an aim to further explore the frequency of and risk factors for zoonotic transmission of influenza viruses and whether these zoonotic transmissions lead to clinical illness.

I would also recommend that the UK and other public health agencies consider making pig-industry workers a priority group for seasonal influenza vaccination to help prevent transmission of human influenza viruses into pigs and the potential reassortment events that could follow such transmissions.

I think that it would be useful to conduct further analyses of Flu Watch and Virus Watch data in order to compare and contrast the symptom profiles, consultation patterns and impact of community cases of all the common acute respiratory infections. It would be helpful to know the performance of common surveillance case definitions for all of these viruses, among both community cases and consulting cases in order to gain a better understanding of what surveillance systems do capture and could capture. Although throughout most of the COVID-19 pandemic people have been encouraged in the UK to undertake PCR testing when symptomatic, this level of testing is unsustainable in the long run. Therefore, the UK may need to return to syndromic surveillance, possibly supplemented with rapid antigen tests.

I also think further research on the relative importance of asymptomatic and pre-symptomatic transmission of influenza is warranted[341], given the large role it has played in the effectiveness of non-pharmaceutical interventions for COVID-19.

My recommendation for the UK surveillance systems would be to develop and continuously run a slimmed-down community cohort study as part of national surveillance

efforts. Ideally it would have regular, repeated serological samples of individuals to examine immunity and infection to SARS-CoV-2, seasonal coronaviruses and influenza as well as active prospective follow-up of all respiratory illnesses and whether they consult including swabbing with PCR (and if available rapid tests) for SARS-CoV-2, influenza and other common acute respiratory viruses. This could perhaps be embedded or linked with the RCGP primary care sentinel surveillance as a way to enhance the value of both the RCGP surveillance and the community cohort study. I would recommend that there be rolling waves of ongoing recruitment, similar to the BugWatch study [332] to reduce the length of time any one person is asked to participate. At the time of writing, with the removal of most free testing for COVID-19 the long-term future of community surveillance of respiratory infections is a very active debate. Future sustainable, timely and informative surveillance needs to be informed by the experience of pre-pandemic and pandemic studies including Flu Watch, Virus Watch, Flu Survey, REACT, The COVID-19 infection survey and RCGP sentinel surveillance scheme.

In addition to measuring frequency of infection, symptom profiles, levels of population immunity, risk factors and the importance of symptomatic and asymptomatic infection such systems are also useful for understanding behaviours that influence transmission and the success of control measures.

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