The Impact of Post-Vaccination Fever and Antipyretic Use Upon Quadrivalent Influenza Vaccine Immunogenicity in Children

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Statement of Originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all assistance received in preparing this thesis and sources have been acknowledged.

Signature .....

Ellen Sabrina Mowbray

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Author Note

An independent analysis of this same dataset was performed by Karen Elias in completion of her requirements for a Master of Biostatistics. There was collaboration at the beginning of this project in determining an agreed included study population, and the decision to use a mixed methods linear regression model for the analysis. All statistical analysis, coding, interpretation of results and writing in this thesis was performed independently of Ms Elias's analysis and is my own work.

Abstract

Background

Annual vaccination is the most effective method of preventing serious illness from influenza, however the evidence for the impact of fever and antipyretic use in the post-vaccination period on immunogenicity in children receiving quadrivalent influenza vaccination (QIV) is not well described. This thesis examines the effects of fever and antipyretic use in the post-vaccination period on measures of immunological responses to QIV in children.

Methods

Individual-level data from paediatric clinical trials of QIV performed by GlaxoSmithKline (GSK) were obtained. Post-vaccination immunogenicity for four influenza subtypes (A/H1N1, A/H3N2, B/Victoria and B/Yamagata) was measured by haemagglutination inhibition titres. The effects of fever and antipyretic use upon post-vaccination immunogenicity were assessed using a linear mixed effects regression model. Estimates of the effect of fever, antipyretic use and the combination of both were calculated considering days 0-1, 0-3 and 0-6 post-vaccination.

Results

Data from 4,935 individuals (mean age 4.1 years) was pooled from 8 clinical trials of whom 47% were female and 36% were considered 'primed'. Post-vaccination fever was reported by 8.8% of individuals in the first 6 days post vaccination, with fever rates highest in the youngest children (6 months to < 2 years: 13.3%, 2 to <9 years: 8.1% and 9 years and older: 1.3%). Antipyretic use within the first 6 days was 15.6% overall and was similarly more common in younger cohorts (6 months to < 2 years: 23.4%, 2 to <9 years: 9.7% and 9 years and older: 9.7%).

For the combined all-ages analysis, fever and antipyretic use had a statistically significant effect upon HI titres across all four influenza strains at days 0-6 ($p<0.005$). Fever without antipyretic use was associated with a 26% increase in post-vaccination

geometric mean HI titres for the A/H3N2 strain. Antipyretic use without fever was associated with a decrease in post-vaccination HI titres for the A/H1N1, A/H3N2 and B/Yamagata strains (GMT ratios 0.80-0.82). The combination of both fever and antipyretic use was associated with an increase in post-vaccination HI titres for the A/H3N2 and B/Victoria strains (GMT ratios 1.26 – 1.80). Similar results were found across other studied time periods (days 0-1 and 0-3) and cohorts (6 months to 2 years, 2 years to < 9 years), however the small number of participants aged ≥9 years reporting fever limited this study's power in the 9 years and older cohort.

Conclusions

This thesis demonstrates important associations between fever, antipyretic use and post-influenza vaccination serological measures of immunogenicity in paediatric patients. The findings are suggestive of important clinical implications of these associations, particularly with respect to antipyretic medications, however further prospective studies are required before clinical recommendations can be made.

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List of Abbreviations

AIC	Akike Information Criterion
APCs	Antigen-Presenting Cells
COX2	Cyclooxigenase-2
DTaP	Diphtheria, Tetanus, Pertussis Vaccine
ELISA	Enzyme Linked Immunosorbent Assay
Flu	Influenza
G-CSF	Cytokine Granulocyte-Colony Stimulating Factor
GMT	Geometric Mean Titre
GSK	GlaxoSmithKline
H	Haemagglutinin
HI	Haemagglutination Inhibition
HB	Hepatitis B Vaccine
Hib	*Haemophilus Influenzae* type b vaccine
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IIV	Inactivated Influenza Vaccine
IIV3	Trivalent Inactivated Influenza Vaccine
IIV4	Quadrivalent Inactivated Influenza Vaccine
IPV	Inactivated Poliovirus Vaccine
LAIV	Live Attenuated Influenza Vaccine
N	Neuraminidase
NSAID	Non-Steroidal Anti-Inflammatory Drugs
NT	Neutralising Antibody Titre
PCV13	13-valent Pneumococcal Conjugate Vaccine
QIV	Quadrivalent Influenza Vaccine
RCT	Randomised controlled trial

TIV	Trivalent Influenza Vaccine
VE	Vaccine effectiveness
WHO	World Health Organisation
WIV	Whole Virion Inactivated Vaccine

CHAPTER 1: BACKGROUND TO THE PROJECT

1.1 Burden of Disease

Influenza is an acute viral respiratory infection which occurs in annual epidemics and outbreaks, infecting approximately 5-15% of the world's population (1). Whilst influenza is usually mild and self-limiting, it can cause serious or life-threatening illness. Annually, between 3 and 5 million cases of severe illness are caused by seasonal influenza virus around the globe (2), resulting in an estimated 290 000 – 650 000 deaths (3,4). Symptoms associated with influenza infection vary from mild disease limited to the upper respiratory tract including a sore throat, coryza and cough, to more severe disease including pneumonia, which can be life-threatening (2). Other commonly reported symptoms include fever, headache, and myalgia (2). Influenza virus infection can result in a wide range of non-respiratory medical complications that affect the body's major organs and organ systems including the heart, central nervous system and musculoskeletal system (5). Pregnant women, people with underlying medical conditions and children are at the highest risk of contracting influenza, and experience higher rates of influenza-related morbidity (6). Older adults are particularly at risk of more severe illness, hospitalisation and disease complications due to co-morbid chronic illness, the gradual deterioration of the immune system (immunosenescence) or a combination of the two (7,8).

1.2 The Influenza Virus

Of the four types of influenza viruses within the Orthomyxoviridae family – Influenza A, B, C and D- three types (A, B, and C) infect and cause disease in humans, though type C causes only mild disease (9,10). Type A and B viruses are responsible for global annual seasonal epidemics (2) and thus, are referred to as *seasonal influenza viruses*. These two influenza types are further divided into subtypes depending on the glycoprotein on

the surface of the virus- either hemagglutinin (H) or neuraminidase (N) (2), of which there are 18 known H antigens subtypes and 11 N antigen subtypes. Influenza B viruses are classified into two lineages: B/Yamagata and B/Victoria (11). Until very recently, Influenzas A/H1N1, A/H3N2, B/Victoria and B/Yamagata viruses circulated among humans worldwide (9,12), however, the B/Yamagata lineage has not been detected globally since March 2020- a likely result of COVID-19 mitigation strategies (13). Seasonal influenza virus outbreaks typically occur during the winter months when the temperature and conditions favour transmission. There are two 'flu seasons' each year- one in the Northern Hemisphere and one in the Southern Hemisphere (2) .

1.2.1 Epidemic and pandemic strains of influenza

Annual seasonal epidemics of influenza A and B are driven by waning population immunity or by the virus' evolved ability to escape antibodies induced through previous infections, vaccinations or both, through a process known as *antigenic drift* (9). Pandemic influenza, however, occurs when a novel strain of influenza A emerges, through a process known as *antigenic shift* (9). Antigenic shift occurs when animal hosts are infected with multiple strains of influenza virus, and this co-infection results in gene reassortment between strains, producing novel variants (14). This was the case for the A/H1N1 pandemic of 2009, which was derived from several influenza A viruses that were previously circulating in pigs (14). The introduction of a new, antigenically different influenza A virus strain leading to an influenza pandemic occurs approximately every 10-50 years, and a lack of pre-existing immunity in humans is often associated with an increased severity of the infection, and mortality (2).

1.3 Influenza in Children

Influenza has a significant impact on young children who are often naïve to infection and have a reduced immune response to the virus compared to adults, due to the immaturity of their immune systems (15).

Among children, influenza A more commonly leads to hospitalisation (16), however Influenza B is more likely to cause certain health complications including myocarditis and acute renal failure (17). Children aged less than 5 years (particularly those under 2 years) are at increased risk of serious disease following influenza infection (18). Children infected with influenza virus typically experience a higher fever, more severe symptoms and shed viral particles for a longer period of time than adults (2,19).

Studies of the incidence of annual influenza infection in children demonstrate a significant proportion are infected annually, and at a higher rate than adults. A 25-year-long prospective cohort study found influenza virus attack rates of between 1 and 19% in children aged ≤5 years on viral culture result, however when including serological test results for markers of infection this figure was deemed much higher at 15-42% (20). Similarly, another study found that rates of seasonal influenza virus infections in children aged ≤18 years were > 4 times higher than in adults (15.2% compared to 3.5%) (21), and another study suggested the annual influenza attack rate in paediatric populations was between 20-30% (10). As such, children play a significant role in the transmission of influenza to their household and other close contacts (19,22) and the most effective method for preventing serious influenza-related disease and complications in children and in adults is through annual influenza vaccination (3,23,24).

1.4 Influenza Vaccines

Most influenza vaccines contain vaccine viruses that are isolated and propagated in chicken eggs, known as 'egg-based vaccines' (25). There are also cell-based influenza vaccines which are produced from influenza viruses that have been grown in cultured mammalian cells, and recombinant vaccines which are created synthetically and do not require the use of a candidate vaccine virus (11).

1.4.1 Influenza vaccine composition

Current influenza vaccines include inactivated (IIVs), live-attenuated (LAIVs) and recombinant vaccines (26) and are manufactured in three compositions- whole-virus vaccine, split-virus vaccine and subunit vaccines (27). IIVs are the most commonly administered influenza vaccines, and these come in a trivalent (3 strains) or quadrivalent (4 strains) formulation and contain inactivated or 'killed' influenza viruses. They are administered to children from 6 months of age whereas LAIVs contain weakened live influenza viruses and are given to children over 2 years of age who have no contraindicating medical conditions (28).

Split and subunit formulations of IIVs are used more commonly, and preferred over whole virus formulations, as they are less reactogenic (27,29). LAIVs are administered intranasally, and simulate natural-influenza virus infection (29), however they were found to cause wheezing in infants and therefore are not approved for use in children less than 2 years (27). In Australia, there are currently 5 seasonal inactivated influenza vaccines registered and available for use in individuals aged 6 months and older, one for individuals aged over 5 years, a high-dose vaccine and an adjuvanted vaccine for individuals aged 65+ (25). The high dose preparations contain 4-times the antigen dose of standard influenza vaccines, which are designed for adults over the age of 65 years to enhance the immune response (30). Furthermore, adjuvanted influenza vaccines are used to boost the immune responses in certain populations, most notably the elderly and immunocompromised people (31) and are routinely recommended for use in adults over the age of 65 (32).

1.4.2 Current influenza vaccines

Trivalent influenza vaccines (TIV) contain two influenza A strains and one influenza B strain, whilst quadrivalent influenza vaccines (QIV) contain two A and two B strains. Additionally, there are some single A-strain pandemic vaccines available on the market (27). The effectiveness of influenza vaccines is dependent on the degree of matching between the vaccine strain and the strain of virus circulating in the community (33), and therefore the composition of the influenza vaccine changes each year based upon global influenza surveillance undertaken by the World Health Organization (WHO) (34). Significant mismatch between strains included in vaccines and circulating influenza strains can contribute to influenza morbidity, as was the case for B-strains included in the TIV in the decade leading up to the 2010/2011 influenza season in the United States and Europe (6,17). To prevent B strain mismatch, QIV were developed, and were recommended for seasonal immunisation by the WHO from 2012 through to 2023 to cover both anticipated B strains. As a result of COVID-19 mitigation strategies commencing in March 2020, there has been no circulation of the B/Yamagata strain of influenza at present (December 2024) and as such, The WHO has recommended the use of TIV over QIV for the 2024/2025 Northern Hemisphere influenza season (35). Several countries, including Australia, are switching to TIV for immunising their populations (13) based on this advice.

1.4.3 Influenza vaccination in children

Whilst vaccination is the best means for reducing the morbidity and mortality associated with influenza infection, influenza vaccines are known to be temporarily associated with common, minor adverse events in children, of which fever is the most frequently reported, as well as severe adverse events such as febrile convulsions (12). Though generally thought to have no long-term consequences, post-vaccination fever and febrile seizures can be very concerning for parents (36,37) and can affect vaccine confidence and willingness to vaccinate again (38,39). An unexpected increase in the number of febrile seizures following influenza vaccination from a single manufacturer in children <5 years in Australia during 2010 (40,41) lead to a national pause of influenza vaccination in children (42).

In response to concerns around adverse events following vaccination, analgesics and antipyretics such as paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used to manage acute side effects following vaccination by parents and health professionals. However, some studies have suggested that antipyretics used to manage reactogenicity might reduce the immune response to some vaccine antigens (37,43–51).

1.5 Measuring Influenza Vaccine Immunogenicity

The haemagglutination inhibition (HI) assay is the most commonly used assay in influenza vaccine clinical trials (52,53). Other, less common immunological assays include single-radial haemolysis, virus neutralization (NT) and enzyme-linked immunosorbent assays (ELISA) (54), however the HI assay remains the 'gold standard' for measuring seropositivity (55).

The HI assay is a laboratory test used to detect and quantify serum antibodies to influenza viruses (56). The HI titre is a measure of the amount of antibody in the serum of an individual and is determined by identifying the highest dilution of the serum that completely inhibits the virus from causing haemagglutination (52,54,56,57). The HI titre provides a quantitative assessment of serum antibodies to influenza viruses (57) and thresholds of HI titres representing clinical protection against influenza infection have been estimated in the literature. These thresholds however, may vary across populations, and children in particular may require higher HI thresholds to achieve equivalent protection against influenza infection (58). Furthermore, these cut-offs are only tested with a limited number of virus strains, and would likely not apply to pandemic strains of influenza (59).

1.5.1 Haemagglutination inhibition antibody titres as a correlate of protection against influenza infection

Studies investigating the relationship between HI antibody titres and influenza vaccine effectiveness have consistently demonstrated that HI titres are a useful correlate of protection, though their role varies by age group, influenza strain and vaccine type. The seminal study by Hobson et al. demonstrated that HI titres of 18-36 were associated with 50% protection in adult participants, a threshold widely accepted in the literature (60–66).

A threshold of HI titres of 1:40 correlating with 50% protection has subsequently been validated in a meta-analysis of adult populations (62). This study further demonstrated increasing protection with HI titres above this threshold, plateauing beyond titres of 1:150 (62). Elderly adults >65 years have been reported to require higher HI titres (1:203-437) to achieve equivalent protection against influenza infection, thought to be due to immunosenescence resulting in decreased protection from mismatched influenza viral strains (67).

Research focusing on children has highlighted the correlation between HI titres and protection from influenza, though the degree of protection conferred varied between strains and is often complemented by non-HI immune mechanisms, such as T-cell immunity. Similar to the HI threshold for adults discussed above, an RCT in children aged 6-17 years found that an HI titre of 1:40 provided 48% protection against A/H1N1 and 55% against B/Victoria, with protection maintained above 50% for up to 9 months post-vaccination (63). In younger children aged 6-35 months, a strong correlation between HI titres and reduced illness risk has been shown, with titres above 1:40 conferring 49.7% protection, while optimal protection (88%) required titres above 1:320 (64). Interestingly, studies of QIV in children have found that HI titres only mediate a proportion of a vaccine's protective effect, and that the effect of HI titres varies across A and B strains. For influenza A, HI titres account for a minority of the protection against influenza infection, with non-HI immune responses accounting for the majority of

vaccine protection (65,66). In contrast, HI mechanisms account for a larger proportion of protection against influenza B strains (65,66). These studies highlight that both children and adults benefit from higher HI titres, but differences in immunity and prior exposure to influenza viruses result in varying degrees of reliance on HI versus non-HI immune responses. Children, particularly those with lower baseline HI titres may benefit from booster vaccination to achieve sufficient protection.

1.6 Influenza Vaccine Effectiveness in Children

The effectiveness of influenza vaccination for preventing infection and hospitalisation in children has been extensively studied. A 2021 systematic review evaluating influenza vaccine effectiveness in preventing hospitalisation due to influenza in children found that the pooled vaccine effectiveness across 37 test-negative studies was 53.3% (68). Children aged under 5 years had better protection than those aged 6-17 years (67.1% compared to 51.7%) and vaccine efficacy was higher during seasons when vaccine strains matched circulating strains (59.3%). The authors conclude that annual vaccination is still supported by these findings, despite showing influenza vaccines only provide moderate protection against hospitalisation.

A large RCT involving 12,018 children aged 6-35 months reported QIV vaccine effectiveness (VE) of 63% against moderate to severe influenza illness and this was increased to 78% for vaccine-like strains (69,70). Another RCT found QIV VE of 51% against all influenza types, and 68.4% for vaccine-like strains in infants aged 6 months to <3 years (70,71), and a further RCT published in 2020 reported VE of QIV ranging from 30.3% to 73.4% in children aged 6-35 months (72). In an Australian context, Shrestha et al. (73) investigated QIV effectiveness for preventing laboratory confirmed influenza and hospitalisation following the introduction of a funded influenza vaccine for residents in Queensland, and found that VE against both infection and hospitalisation was highest in children aged 30 months to <5 years (61% and 84%).

1.7 Factors Impacting Vaccine Immunogenicity

1.7.1 Age and sex as effect modifiers in influenza vaccine immunogenicity in children

Studies investigating the effect of age upon immune responses to influenza vaccination have found that whilst children may demonstrate increased immune responses compared to adults (74), older children mount stronger immune responses to influenza vaccination than younger children (75). A study of vaccine-naïve children aged 6-23 months found that children aged 18-23 months were 4.3 times more likely to achieve protective antibodies for the A/H3N2 antigen than were children aged 6-11 months. Similarly, responses to A/H1N1 and B antigens were 2.75 and 1.52 times higher in older children, respectively (75). Further, QIV immunogenicity was found to be higher in the 18-35 month age groups than in the 6-17 month age group in a 2018 RCT (69). It has been suggested that increased immune responses to influenza vaccination in older children is due to the development of antigen-presenting cells (APCs) and memory B cells (32). As children grow, their APCs become more efficient and because older children have encountered more viruses over time than younger children, they have broader memory B cells. These factors in turn result in a more robust and longer-lasting antibody response (32).

Unlike age, the influence of sex on influenza vaccine immunogenicity in children remains unclear and has not been widely reported upon in the literature. In their RCT, Walter et al. found no significant association between sex and vaccine immunogenicity in children aged 6-23 months (75). It has been noted that sex differences in children's vaccine responses are rarely studied as primary endpoints, and argued they may not have any clinical significance if protective antibody levels are achieved in both males and females (76).

1.7.2 The impact of prior vaccination on immune responses to influenza vaccination in children

Studies show that children who have previously received influenza vaccines often produce higher HI antibody titres following subsequent vaccination, compared to vaccine naïve children (77–80). Thus, the 2-dose initial vaccine regimen is recommended for children aged 6 months to <9 years, to provide sufficient immune priming and adequate protection from influenza infection (18). There is, however, some documentation in the literature that repeat annual vaccination with the same antigen across influenza seasons can sometimes lead to a reduced immune response (81–92) however this effect varies by influenza strain as well as individual immune history.

1.7.3 The impact of antipyretic medications upon the immune response to influenza vaccines

Early experimental animal studies examining the impact of antipyretic medications upon the immune response found that salicylate drugs adversely affected antibody formation following exposure to bacterial antigens including diphtheria toxoid and streptococcus pneumoniae (45,46,48). The first RCT involving healthy human participants came in 1978 and examined the impact of antipyretic medications on antibody titres following bivalent influenza vaccination (45). This study found that participants who were given indomethacin orally for 12 days (commencing 1 day pre-vaccination) had no difference in their 3-week post-vaccination antibody titres to the control group for the A/New Jersey strain, but their antibody titres to the A/Victoria were increased. However, baseline serology samples showed that approximately 90% of participants in this study had evidence of antibody titres to A/Victoria but none had serological evidence of the novel A/New Jersey infection (45,47). This increased immune response could be explained by the concept of original antigenic sin, where the immune system utilises its memory immune responses to antigens it has previously encountered (14,92–94), or may suggest that indomethacin has some impact upon antibody production, however the exact mechanism is unclear.

There have been few randomised studies since that examine this effect, and even fewer for influenza vaccines, and they report mixed findings. An early RCT in a population of healthy 5-month-old children randomised participants to receive either a single dose of paracetamol or a placebo 4 hours post-vaccination with a Diphtheria, Tetanus, Pertussis (DTaP) or DTP-inactivated polio vaccine found that 6 weeks post-vaccination, antibody titres to diphtheria and tetanus toxoids and pertussis antigens did not significantly differ between the two groups (44). Another study assessed the effect of antipyretic medications upon the immune response in infants following vaccination with the 13-valent pneumococcal conjugate vaccine (PCV13), and co-administered hexavalent vaccine (diphtheria, tetanus, pertussis (DTaP), Hepatitis B (HB), Polio (IPV) and *Haemophilus influenzae* type b (Hib). This study divided participants into 5 groups- a control group, 2 groups receiving paracetamol (one at the time of vaccination and one later) and 2 groups receiving ibuprofen (also concurrent or delayed administration). The authors found that overall, most participants reached the target antibody levels for all 13 PCV antigens, and all 6 DTaP/HB/IPV/Hib antigens after completing the vaccine series, although the use of paracetamol and ibuprofen showed some impact upon specific immune responses (50). For example, pneumococcal antibodies were lower in the paracetamol groups compared to the control group but there was no statistically significant difference between the ibuprofen and control groups following the initial vaccinations, however, one month following booster dose, participants who received ibuprofen at the time of vaccination showed lower levels of pertussis and tetanus antibodies compared to the control group (50).

Building on these findings, an RCT enrolled 459 healthy infant participants aged 9-16 weeks undergoing their initial routine vaccinations and then booster vaccinations at 12-15 months of age (37). These participants were randomised to receive prophylactic paracetamol at the time of vaccination and then 6-8 hourly over the following 24-hour period, or to receive no intervention. The authors measured fevers and serum antibody titres and reported that fever was much less common in children who received paracetamol after both their initial and booster vaccination, and that children who received paracetamol had slightly lower antibody titres to each of the ten

pneumococcal conjugate vaccine serotypes and the Hib polysaccharide, diphtheria, tetanus and pertactin vaccine antigens (37). The authors concluded that despite demonstrating a slightly reduced immune response to vaccination, children who received prophylactic paracetamol still achieved long-term protection that did not differ from those who did not receive the intervention (37,51). Furthermore, A 2014 systematic review investigating the effect of prophylactic antipyretics upon post-vaccination adverse events and antibody responses in children <6 years found a statistically significant decrease in post-vaccination antibody titres in children who received paracetamol either alone or in combination with ibuprofen at the time of vaccination with a diphtheria containing vaccine (either administered alone or in combination with a pneumococcal-containing vaccine and haemophilus influenza type B vaccine)(43).

Although limited studies have investigated the effect of antipyretic medications upon children's immune response to influenza vaccination, the findings of those that do are mixed, with no clear consensus on whether these medications impact immunogenicity. A UK RCT evaluating reactogenicity and immunogenicity of the A/H1N1(2009) pandemic influenza vaccine in a paediatric study population found that the use of antipyretics did not significantly impact antibody titres or an individual's immune response to vaccination (95). Another study also found no significant differences in seroconversion and post-vaccination seroprotection between children in the oral paracetamol/oral ibuprofen and placebo groups of their RCT (36). However, a 2018 study found antipyretic use in the post-vaccination period (days 0-3) appeared to decrease a child's immune response to trivalent vaccine in an analysis of 3 GSK TIV paediatric clinical trials (41).

1.7.4 The impact of fever upon the immune response to influenza vaccines

Post-vaccination fever in children is a common occurrence, and The Brighton collaboration Fever Working Group has defined fever to be an "endogenous elevation of at least one measured body temperature of ≥38C" (96). Fever induced by inactivated influenza vaccines is a result of the immune response to vaccine antigens on

inflammatory cytokine-releasing cells including monocytes and macrophages (97,98). For inactivated influenza viruses, fever onset is generally within the first 24 hours post-vaccination, but may occur up to 48 hours after the vaccine has been administered (97). Amongst studies assessing fever post-vaccination, there are several inconsistencies in the methodologies for measuring fever. Clinical trials and epidemiological studies generally use longer study timeframes- up to 30 days post-vaccination- which often include fevers caused by factors other than influenza vaccination (97). Fevers measured in the post-vaccination period may instead be related to infection with a viral or bacterial antigen, and readings of a body temperature ≥38C may come from user error or improper positioning of thermometers, or from factors such as mouth breathing during oral measurement (96,99). Furthermore, influenza vaccines in children are often co-administered with other vaccines, making it impossible to determine the cause of the fever (97).

Investigations for the impact of post-vaccination fever upon antibody titres/immunogenicity in the literature is limited. A clinical trial of the A/H5N1 pandemic influenza virus vaccine reported a close association between febrile reactions to vaccination and immunogenicity in children (100). The authors found that younger children had higher rates of fever ≥38C, especially those aged ≤3 years (57%) following the first dose of vaccine, and the comparison of neutralising antibody (NT) titres between participants revealed significantly higher titres in children who experienced fever compared to those who did not (100).

A study investigating cytokine production in response to diphtheria-tetanus-pertussis (DTP), haemophilus influenzae type b (Hib) and pneumococcal vaccines found that children who developed a fever 24 hours post-vaccination had higher levels of the cytokine granulocyte-colony stimulating factor (G-CSF) than children who did not have a fever, however other cytokine levels were similar across participants, regardless of whether fever was present (101).

The UK study evaluating reactogenicity and immunogenicity of the A/H1N1(2009) pandemic influenza vaccine in children found that fever post-vaccination positively correlated with higher antibody titres, particularly after the second dose (95). Furthermore, pooled analysis of 3 RCTs evaluating TIV immunogenicity found evidence of a positive association between post-vaccination fever and increased immune titres for all 3 included influenza strains. The authors suggest this association demonstrates a connection between fever and the body's immune response to antigens in the vaccine, although they note that the influence is not well understood (41).

1.8 Thesis Objective

This thesis will aim to investigate previously demonstrated findings of associations between fever and antipyretic use in the post vaccination period in trivalent influenza vaccines, in QIVs which, until recently, were widely administered. It is hypothesised that we will find similar associations to Li-Kim-Moy et al.'s study; of increased GMTs in children who experienced any fever in the post-vaccination period, and a decrease in children who used antipyretics in the post-vaccination period.

To investigate for these associations, a retrospective analysis of eight randomised clinical trials of GSK's quadrivalent non-adjuvanted, inactivated influenza vaccines involving children aged 6 months-17 years was performed. Patterns of post-vaccination fever and antipyretic use are described, and vaccine immunogenicity was analysed using multivariable regression analysis to assess the impact of fever and antipyretic use on a child's immune response to QIV.

CHAPTER 2: METHODS

2.1 Design and Subjects

Data access was obtained through clinicalstudydatarequest.com in June 2018; a platform set up by a consortium of clinical study sponsors to facilitate independent researchers' access to individual-level data from completed trials. Available randomised controlled trials conducted by GSK of QIV in children aged 6 months to 17 years, with immunogenicity and safety datasets were included in the analysis. Individual level data was obtained from eight separate paediatric QIV trials completed by GSK between 2009 and 2015, under a data sharing agreement. The eight trials included both phase 2 and phase 3 trials of GSK developed QIV, the details of which are summarised in table 1 below.

All studies randomised participants between QIV and another active vaccine; either TIV or other various control vaccines (detailed in Table 1 below). Each QIV targeted four common influenza subtypes (A/H1N1, A/H3N2, B/Victoria and B/Yamagata), however the specific antigens for each subtype included in the QIVs varied across years. Participants were allocated to either a 1- or 2-dose vaccination schedule, depending on if they were considered 'primed' or 'unprimed'. Specific definitions of priming varied across studies, however, to be considered primed, all studies required participants to have a) received an influenza vaccination in a previous season, or to b) be aged 9 years or older.

Primed participants received a single dose of QIV, whilst unprimed participants received 2-doses, scheduled 4 weeks apart. Serum antibodies to the four influenza strains were measured using HI titres, which were measured at baseline prior to the first dose of QIV, and 28 days post final vaccination. Adverse events in all trials were obtained for the day of vaccination, and the following 6 days through diary cards provided to caregivers, prompting them to record any medications taken, and the

presence or absence of common post-vaccination symptoms, including fever. In accordance with the Brighton Collaboration Working Group definition, fever was defined as a temperature ≥38°C measured by any route (96). In our study, medications were considered an antipyretic if they included paracetamol or an NSAID, with topical preparations excluded.

This study included all trial participants aged 6 months to 17 years who underwent vaccination with a GSK QIV. Subjects were included in the analysis if they had HI titre results available at both baseline and 28 days post their final QIV for at least a single influenza strain and had adverse event outcomes reported for the first 6 days post-vaccination, where day 0 is the day of vaccination. Subjects were excluded from the analysis if they did not complete the QIV course according to protocol, did not have HI titre results available at either baseline or 28 days post final vaccination, or if they did not have adverse event outcomes available.

2.2 Statistical Analyses

Serology results for each of the four influenza subtypes were aggregated across studies, and analysis was undertaken separately for each subtype. For participants who were considered 'unprimed' and received the 2-dose schedule, fever and antipyretic use rates were also pooled across the two doses. Results were calculated for all included participants and were additionally broken down into three age cohorts: 6 months to < 2 years, 2 years to <9 years and 9 to 17 years.

Baseline characteristics of age, sex and priming status were calculated for included participants across each of the 8 studies. Rates of first reported fever and antipyretic medication administration were calculated for each day post-vaccination, with results broken down by dose and age cohort. Continuous data are reported as either mean (standard deviation) if normally distributed, or median (interquartile range) if not

normally distributed. Baseline characteristics presented as categorical variables were compared using Chi-squared tests.

The effects of fever, antipyretic use or the combination of both, upon post-vaccination HI titres for each of the 4 influenza strains were assessed during the time periods of 0-1, 0-3 and 0-6 days following vaccination. Because the titres reported from the HI assay are exponentially distributed, analysis was performed using the $\log_{10}$ transformed baseline and post-vaccination HI titres to allow for a linear regression model to be used. Age, sex, priming status and pre-vaccination HI titre were considered and included as covariates in the model.

A linear mixed effects model was constructed for each strain and age group, with age, sex, priming and pre-vaccination titre category included as fixed effects, and the individual trial included as a random effect in the model. A linear mixed effects model with trial as a random effect was chosen as it increases statistical power by pooling data across trials (compared to analysing each trial separately), whilst accounting for variability which may be present between trials (such as differences in unmeasured population characteristics e.g. ethnicity)(102,103).

Results from the linear mixed effects models were estimated for each strain, age cohort and time-period post-vaccination (0-1, 0-3 and 0-6 days). Estimates of the effect of fever alone, antipyretic use alone, or the combination of both fever and antipyretic use compared with a base case of neither fever nor antipyretic use were calculated as a single categorical variable. Age in months, and $\log_{10}$ pre-vaccination HI titre were included as linear covariates, and sex and priming status as categorical variables. A level of significance of α=0.05 was applied to determine statistical significance. Statistical significance of the fever and antipyretic categorical variable was tested using an F-test, followed by analysis of the 95% confidence intervals, to determine which categories were statistically different from the base case of *no fever or antipyretic use*.

Other covariates were assessed using t-tests with p-values and 95% confidence intervals reported. Estimates of effect were backtransformed by antilogging the parameter values, giving GMT ratios which can be interpreted as 'fold' effects per unit change in the variable.

For each strain and age group, a comparison of the model estimates generated using the day 0-1, 0-3 and 0-6 fever and antipyretic outcomes was performed using the Akike Information Criterion (AIC). The AIC measures model fit as well as complexity, allowing for relative measures of competing models, where the model with the lowest AIC is preferred (104). The more parsimonious model was assessed as where the AIV was lower by more than 2 (105). Following assessment of the results of the above models, a post-hoc analysis of the correlation between the highest fever recorded, and whether or not participants used antipyretics was performed. The mean maximum temperatures in those reporting a fever was compared between participants who used antipyretics, and those who did not. The differences between these two groups were tested using a student's t-test, with a level of significance of α=0.05 applied to determine statistical significance. This comparison was performed for each age group for days 0-1, 0-3 and 0-6 post-vaccination.

Data processing and statistical analyses were undertaken using Microsoft Excel for Microsoft 365 MSO and SAS Version 9.4 (SAS Institute, Cary NC), with charts generated using R Version 4.2.1.

CHAPTER 3: RESULTS

3.1 Study Population

Eight clinical trials of QIV performed in paediatric populations were identified and included in this thesis. The details of these studies are summarised in Table 1 below. Ages included in the studies ranged from 6 months to 17 years. Trials were completed between 2009 and 2015, and performed in a variety of countries across Asia, Europe, Central and North America. Study populations ranged from 316 to 12,046 participants (although not all participants were eligible for inclusion in this study).

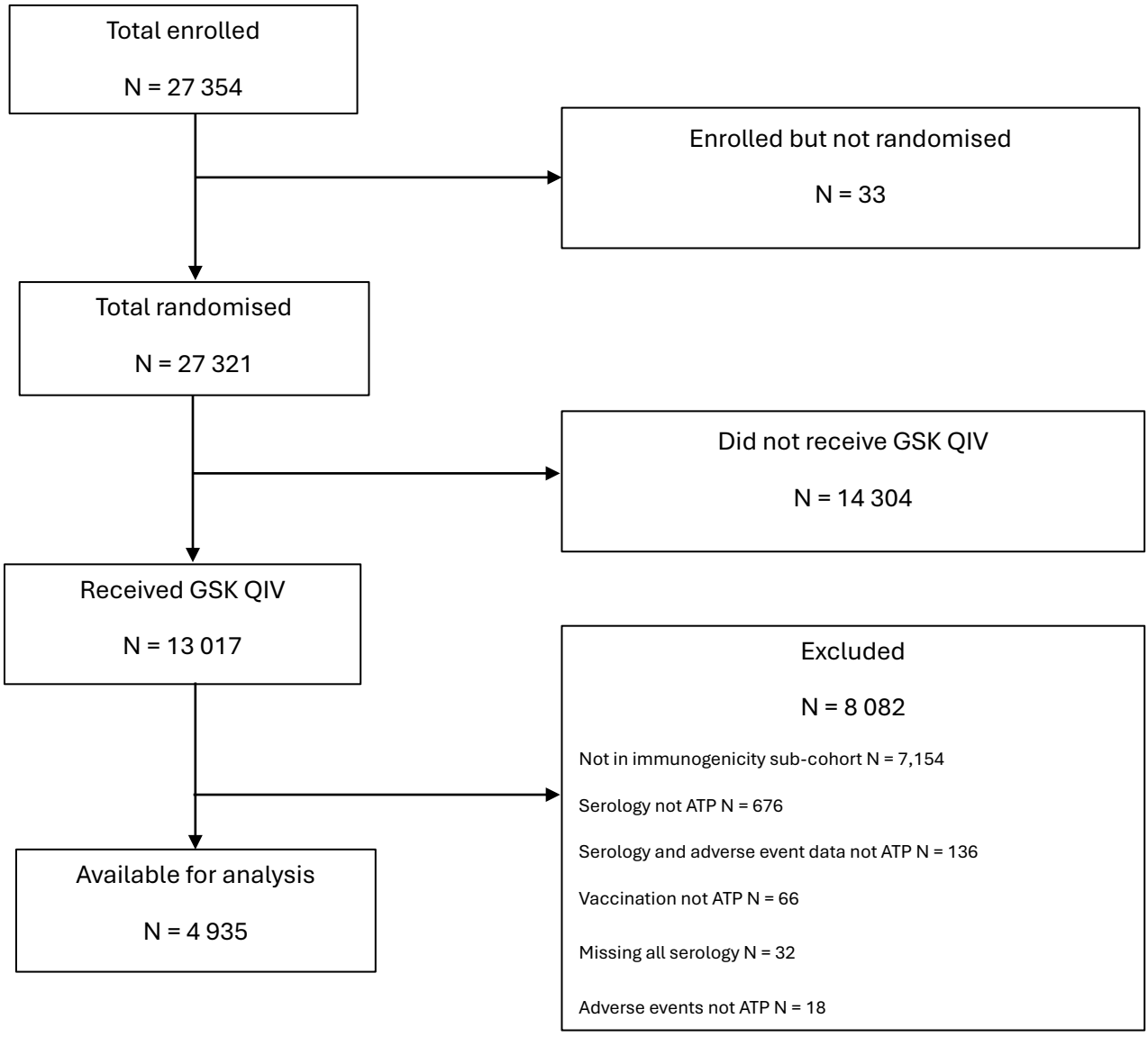
Table 1: Summary of Included Studies

Author of main published paper	ID number 1. OUR IDENTIFIER 2. NCT NUMBER 3. GSK IDENTIFIER	Age ranges	Treatment Groups	Total number enrolled subjects	Number randomised to QIV group	Number included in study population	Geographic locations
Rodrigues Weber [106]	Trial 1 NCT00985790 113237	18 to 47 months	1. QIV 2. TIV	599	298	191	Mexico
Domachowske [17]	Trial 2 NCT01196988 113275	6 months to 17 years	1. QIV 2. TIV-Vic 3. TIV-Yam 4. Open label QIV	3027	1192	1018	Czech Republic, France, Germany, Philippines, USA
Langley [107]	Trial 3 NCT01198756 113314	6 months to 17 years	1. QIV 2. TIV-Vic 3. TIV-Yam 4. Open label QIV	3109	1234	1125	Canada, USA, Mexico, Spain, Taiwan
Jain [108]	Trial 4 NCT02242643 201234	6 to 35 months	1. GSK QIV (FluLaval) 2. Sanofi QIV (Fluzone)	2430	2429	974	USA, Mexico
Wang [109]	Trial 5 NCT01974895 200806	6 to 35 months	1. GSK QIV (FluLaval) 2. Sanofi TIV (Fluzone)	316	158	140	USA
Langley [110]	Trial 6 NCT01711736 116926	6 to 35 months	1. QIV 2. TIV	607	300	284	Canada, Dominican Republic, Honduras

Dbaibo [72]	Trial 7 NCT01439360 115345	6 to 35 months	1. QIV 2. Various non-influenza control vaccines	12046	6022	744	Belgium, Czech Republic, Lebanon, Poland, Spain, Turkey, UK, Bangladesh, Dominican Republic, Honduras, India, Philippines, Thailand
Jain [111]	Trial 8 NCT01218308 114541	3 to 8 years	1. QIV 2. Hep A vaccine	5220	2609	459	Bangladesh, Dominican Republic, Honduras, Lebanon, Panama, Philippines, Thailand, Turkey

A total of 27,354 subjects were enrolled in the eight trials, of which 13,017 were in intervention groups receiving a GSK QIV. Of the 13,017 participants who received a QIV, 8,802 were excluded as they were either a) not part of a cohort undergoing immunogenicity testing (7,154), or b) did not have serology results (pre- and post-vaccination) or adverse event data according to protocol (911). The remaining 4,935 participants were included in the final analysis. The details of participant inclusion and exclusion are summarised in the participant flow diagram in Figure 1 below.

Figure 1: Participant flow diagram



3.1.1 Baseline characteristics

The baseline characteristics for the 4,935 included participants are reported in Table 2 below. The mean age was 4.1 years (s.d. 4.2) across all studies, with 1,574 (32%) in the 6 months to <2 years cohort, 2,608 (53%) in the 2 to <9 years cohort and 753 (15%) in the 9 years and older cohort. There were 2,336 (47%) female participants, and 1,757 (36%) of all participants were considered primed, receiving the single dose schedule. Whilst the number of female participants was similar across studies (p=0.53), there was significant variation in the distribution of participant age (p<0.001), age cohort (p < 0.001) and priming status (p < 0.001).

Table 2: Participant Baseline Characteristics by Study

	All Studies	**Study 1**	**Study 2**	**Study 3**	**Study 4**	**Study 5**	**Study 6**	**Study 7**	**Study 8**	**p-value**
N	4935	191	1018	1125	974	140	284	744	459	
Age (years) mean (sd)	4.1 (4.2)	2.1 (0.8)	6.5 (4.3)	7.3 (5.0)	1.2 (0.8)	1.3 (0.8)	1.0 (0.78)	1.4 (0.7)	5.4 (1.6)	<0.001
Age cohort n (%)										<0.001
6 months to < 2years	1574 (32%)	59 (31%)	116 (11%)	146 (13%)	596 (61%)	72 (51%)	201 (71%)	384 (52%)	0 (0%)	
2 to <9 years	2608 (53%)	132 (69%)	602 (59%)	526 (47%)	378 (39%)	68 (49%)	83 (29%)	360 (48%)	459 (100%)	
9 years and older	753 (15%)	0 (0%)	300 (29%)	453 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Sex N (%)										0.533
Male	2599 (53%)	101 (53%)	529 (52%)	601 (53%)	530 (54%)	75 (54%)	135 (48%)	379 (51%)	249 (54%)	
Female	2336 (47%)	90 (47%)	489 (48%)	524 (47%)	444 (46%)	65 (46%)	149 (52%)	365 (49%)	210 (46%)	
Priming Status n (%)										<0.001
Primed	1757 (36%)	94 (49%)	390 (38%)	621 (55%)	572 (59%)	56 (40%)	12 (4%)	9 (1%)	3 (1%)	
Unprimed	3178 (64%)	97 (51%)	628 (62%)	504 (45%)	402 (41%)	84 (60%)	272 (96%)	735 (99%)	456 (99%)	

3.1.2 Fever rates

Figure 2 below displays fever and antipyretic use by age cohort and priming status for each day post-vaccination, and the findings are further detailed in Table 3. Overall, 6.1% of individuals reported experiencing a fever in the first three days after vaccination, with 8.8% of individuals reporting fever in the first six days after vaccination. Fever rates were highest amongst the 6 months to <2 years cohort (9.7% days 0-3, 13.3% days 0-6), compared to the 2 to <9 years cohort (5.5% and 8.1%) and were lowest amongst those aged over 9 years (0.9% and 1.3%). Fever rates were also higher in the unprimed cohort after any dose (8.3% and 11.9%), compared to those who were primed with a previous dose of vaccine (2.2% and 3.1%), potentially reflecting both the younger age of this cohort, and that those who were unprimed received 2-doses of QIV. Fever rates were highest on the day of vaccination after dose 1, independent of age and priming status, and were highest in the first 48hrs after dose 2.

3.1.3 Rates of antipyretic use

Antipyretic use by age and priming status is also displayed in Figure 2 and summarised in Table 3 below. In the first 3 days post-vaccination, 637 (12.9%) participants reported taking an antipyretic medication, compared to 772 (15.6%) in the first 6 days post-vaccination. Like fever, antipyretic use was highest in the 6 months to <2 years cohort (20.4% days 0-3 and 23.4% days 0-6) compared to the 2 to <9 years cohort (9.9% and 12.7%) and 9-17 years cohort (7.8% and 9.7%). Rates of antipyretic commencement were highest on day 0 after both doses 1 and 2, with rates rapidly declining during the follow-up period (Figure 2). Patterns of antipyretic use were similar for both primed and unprimed participants after the first dose of vaccination.

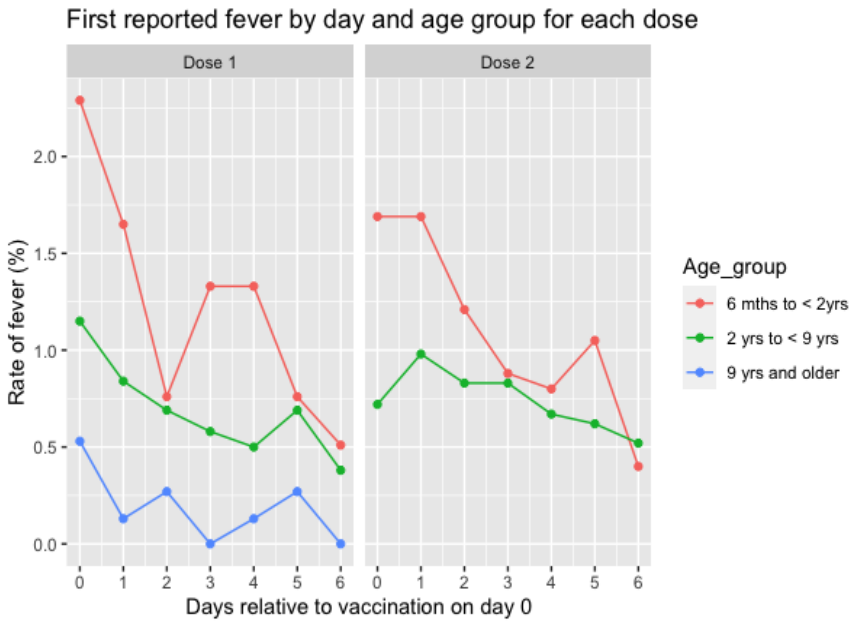
3.1.4 Rates of combined fever and antipyretic use

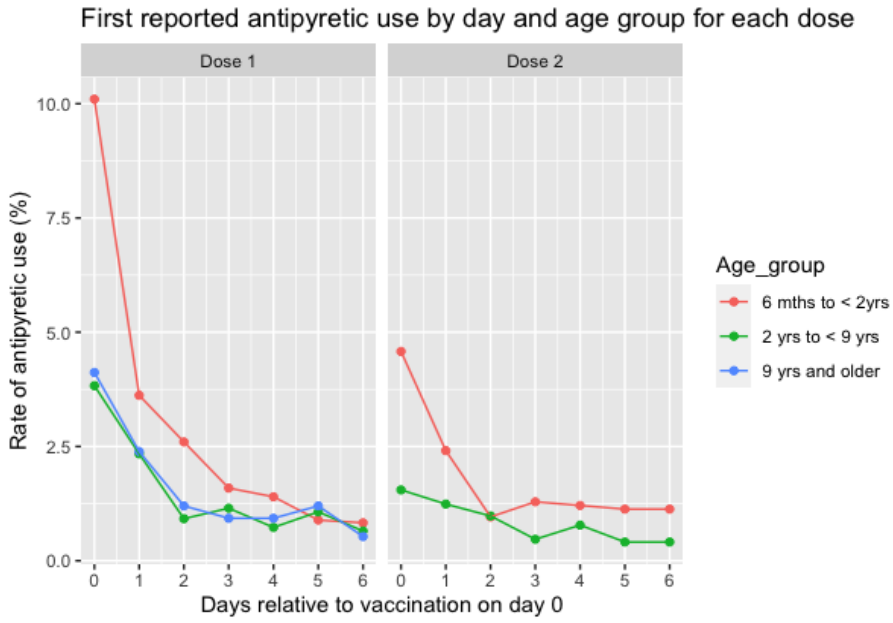
There were 185 (3.7%) individuals that experienced both a fever and received antipyretic medications in the first 3 days post-vaccination, and 276 (5.6%) in the first 6 days post vaccination. Combined fever and antipyretic use was most common in the 6 months to <2 years cohort (6.2% day 0-3, 9% day 0-6), compared to the 2 to < 9 years (3.1% and 4.9%) and 9 years and older cohorts (0.7% and 0.8%). Participants who reported antipyretic use were more likely to also report a fever compared to those who did not, with 36% of the all-age cohort who used antipyretics reporting a fever during days 0-6 compared to 10% of those who did not report antipyretic use (see fever and antipyretic use rates reported in Table 3 below).

Table 3: Rates of Fever, Antipyretic use, and Fever *and* Antipyretic Use by Age Group and Primed Status

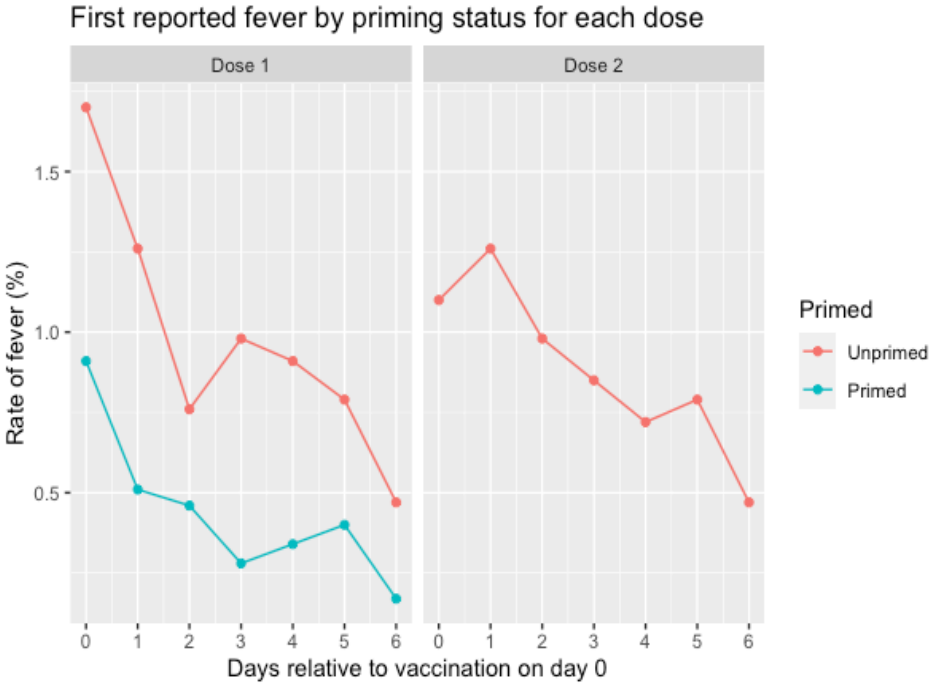
		Day 0-1	**Day 0-3**	**Day 0-6**
Overall		n (%)	n (%)	n (%)
(N=4935)	All fever	149 (3.0%)	303 (6.1%)	432 (8.8%)
	All antipyretic use	497 (10.1%)	637 (12.9%)	772 (15.6%)
	Fever & antipyretic use	85 (1.7%)	185 (3.7%)	276 (5.6%)
By age group				
6 months to <2 yrs	All fever	81 (5.2%)	153 (9.7%)	210 (13.3%)
(N=1574)	All antipyretic use	254 (16.1%)	321 (20.4%)	368 (23.4%)
	Fever & antipyretic use	50 (3.2%)	98 (6.2%)	141 (9%)
2 to <9 yrs	All fever	63 (2.4%)	143 (5.5%)	212 (8.1%)
(N=2608)	All antipyretic use	198 (7.6%)	257 (9.9%)	331 (12.7%)
	Fever & antipyretic use	31 (1.2%)	82 (3.1%)	129 (4.9%)
9 years and older	All fever	5 (0.7%)	7 (0.9%)	10 (1.3%)
(N = 753)	All antipyretic use	45 (6.0%)	59 (7.8%)	73 (9.7%)
	Fever & antipyretic use	4 (0.5%)	5 (0.7%)	6 (0.8%)
By priming status				
Unprimed	All fever	124 (3.9%)	265 (8.3%)	378 (11.9%)
(N=3178)	All antipyretic use	342 (10.8%)	448 (14.1%)	550 (17.3%)
	Fever & antipyretic use	66 (2.1%)	157 (4.9%)	236 (7.4%)
Primed	All fever	25 (1.4%)	38 (2.2%)	54 (3.1%)
(N=1757)	All antipyretic use	155 (8.8%)	189 (10.8%)	222 (12.6%)
	Fever & antipyretic use	19 (1.1%)	27 (1.5%)	39 (2.2%)

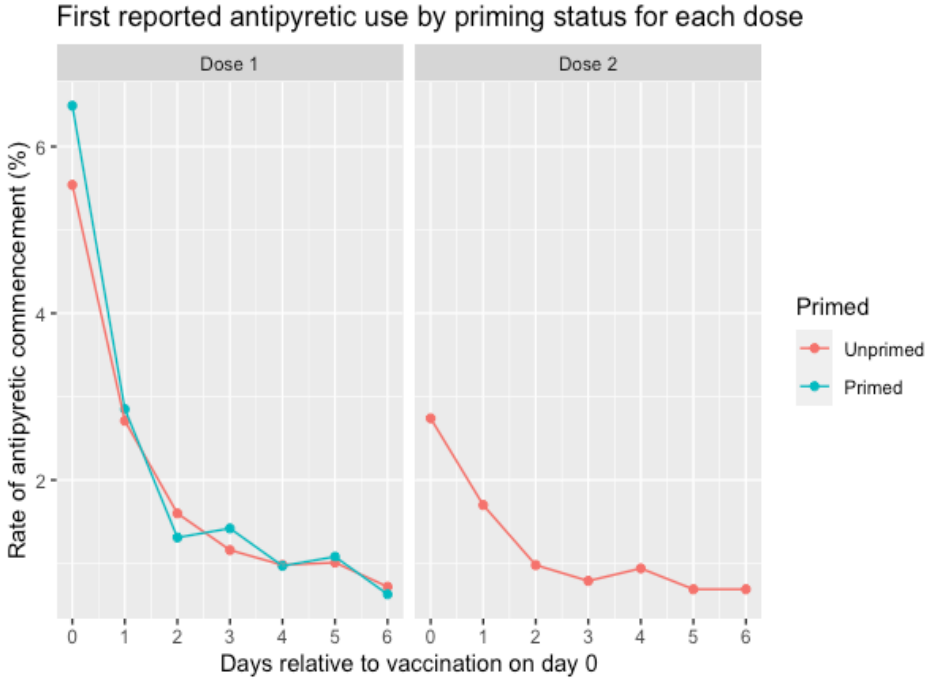
Figure 2: First reported fever and antipyretic use by day and age group





Note: No participants aged >9 years received a 2-dose schedule


First reported fever by priming status for each dose
Dose 1
Dose 2
Rate of fever (%)
1.5
1.0
0.5
0
1
2
3
4
5
6
Days relative to vaccination on day 0
Primed
Unprimed
Primed


First reported antipyretic use by priming status for each dose
Dose 1
Dose 2
Rate of antipyretic commencement (%)
6
4
2
0
1
2
3
4
5
6
Days relative to vaccination on day 0
Primed
Unprimed
Primed

3.2 The Effect of Fever and Antipyretic Use on Post-Vaccination Haemagglutination Inhibition Titres

3.2.1 All age cohorts

Estimates of the effect of fever and antipyretic use upon the post-vaccination HI titres for all participants are summarised in Table 4 below. Results are reported as GMT ratios and 95% confidence intervals, where a ratio of 1 represents no effect, <1 a reduction in post-vaccination immune titre and >1 an increase in the immune titre. P-values are also reported for the F-tests, assessing for a statistical effect of fever, antipyretic use or the combination of both upon post-vaccination immune titres. Results for the effect of fever and antipyretic use on days 0-1, 0-3 and 0-6 are reported.

Fever and antipyretic use have a statistically significant effect for all considered time-periods for the A/H1N1, A/H3N2 and B/Yamagata strains. For the B/Victoria strain, fever and antipyretic use were only statistically significant when the time-period considered included days 0-6 post vaccination.

Fever alone (without antipyretic use in the considered time-period) was associated with a 26% (95% CI 1.073 - 1.481) increase in post-vaccination HI titres for the A/H3N2 strain at days 0-6. Antipyretic use alone was associated with a decrease in post-vaccination HI titres for the A/H1N1, A/H3N2 and B/Yamagata strains across all time-periods, with estimates of effect ranging from a 22.1% decrease for the A/H3N2 strain (days 0-3, 95% CI 0.705 - 0.860) to a 15.7% decrease for the A/H1N1 strain (days 0-1, 95% CI 0.757 – 0.939).

The combination of fever and antipyretic use was associated with an 18.8% increase in immune titres for the A/H3N2 strain at days 0-3 (95% CI 1.024 – 1.378), a 17.9% increase for days 0-6 (95% CI 1.042 – 1.334), and a 25.5% (95% CI 1.097 – 1.435) increase for the B/Victoria strain for days 0-6.

Table 4: Estimates of GMT Ratio for Fever and Antipyretic Use: All participants

Strain	Fever without antipyretic use GMT Ratio (95% CI)	Antipyretic use without fever GMT Ratio (95% CI)	Both fever and antipyretic use GMT Ratio (95% CI)	p-value
		Days 0-1		
A/H1N1	0.874 (0.674, 1.133)	**0.843 (0.757, 0.939)**	0.925 (0.738, 1.159)	**0.014**
A/H3N2	1.272 (0.993, 1.630)	**0.793 (0.715, 0.879)**	1.106 (0.892, 1.372)	**<.0001**
B/Victoria	1.018 (0.776, 1.335)	0.955 (0.854, 1.068)	1.311 (1.035, 1.661)	0.118
B/Yamagata	0.837 (0.659, 1.063)	**0.815 (0.738, 0.900)**	1.038 (0.842, 1.280)	**<.001**
		Days 0-3		
A/H1N1	0.992 (0.818, 1.203)	**0.836 (0.754, 0.927)**	1.016 (0.87, 1.187)	**0.008**
A/H3N2	1.155 (0.960, 1.388)	**0.779 (0.705, 0.860)**	**1.188 (1.024, 1.378)**	**<.0001**
B/Victoria	0.972 (0.795, 1.189)	0.954 (0.857, 1.062)	1.197 (1.018, 1.408)	0.117
B/Yamagata	0.908 (0.759, 1.085)	**0.819 (0.744, 0.901)**	1.019 (0.882, 1.177)	**0.001**
		Days 0-6		
A/H1N1	1.103 (0.932, 1.306)	**0.820 (0.743, 0.905)**	1.084 (0.953, 1.233)	**<.001**
A/H3N2	**1.260 (1.073, 1.481)**	**0.800 (0.727, 0.879)**	**1.179 (1.042, 1.334)**	**<.0001**
B/Victoria	1.103 (0.925, 1.315)	0.948 (0.856, 1.051)	**1.255 (1.097, 1.435)**	**0.004**
B/Yamagata	1.053 (0.901, 1.231)	**0.821 (0.749, 0.900)**	1.096 (0.972, 1.235)	**<.0001**

Results from the linear mixed effects models including study as a random effect, and age, sex, priming status and log_{10} pre-vaccination HI titres as fixed effects.

***Bold** text indicates statistical significance (p-value <0.05 or 95% CI not including 1).*

Results are reported as GMT ratios and 95% confidence intervals compared to the no fever or antipyretic use cohort, where a ratio of 1 represents no effect, <1 a reduction in post-vaccination immune titre and >1 an increase in the immune titre.

P-values are reported for the F-tests assessing the statistical significance of the fever and/or antipyretic use categorical variable.

3.2.2 6 months to <2 years cohort

The estimates of the effect of fever and antipyretic use for the 6 months to <2 years age cohort are presented in Table 5 below. The effect of fever and antipyretic use was only statistically significant for the A/H3N2 strain, with the effect being significant across all 3 time periods. Fever alone was associated with a 39.3% increase in post-vaccination titres for the A/H3N2 strain at days 0-6 (95% CI 1.072 – 1.812). Antipyretic use alone was associated with a decrease in HI titres for the A/H3N2 strain across all time periods, with estimates of effect ranging from a 17.0% decrease at days 0-1 (95% CI 0.705 – 0.978) to a 14.7% decrease considering days 0-6 (95% CI 0.729 – 0.998).

Table 5: Estimates of GMT Ratio for Fever and Antipyretic Use in 6 months to <2 Years Cohort

Strain	Fever without antipyretic use GMT Ratio (95% CI)	Antipyretic use without fever GMT Ratio (95% CI)	Both fever and antipyretic use GMT Ratio (95% CI)	p-value
		Days 0-1		
A/H1N1	0.926 (0.625, 1.372)	0.917 (0.776, 1.086)	0.992 (0.726, 1.356)	0.774
A/H3N2	1.385 (0.945, 2.032)	**0.830 (0.705, 0.978)**	1.001 (0.738, 1.358)	**0.042**
B/Victoria	1.015 (0.685, 1.503)	0.984 (0.832, 1.163)	1.187 (0.866, 1.627)	0.751
B/Yamagata	0.892 (0.612, 1.302)	0.875 (0.744, 1.028)	1.049 (0.777, 1.417)	0.378
		Days 0-3		
A/H1N1	1.112 (0.823, 1.501)	0.954 (0.811, 1.123)	0.986 (0.784, 1.24)	0.831
A/H3N2	1.322 (0.987, 1.772)	**0.835 (0.713, 0.978)**	1.125 (0.900, 1.407)	**0.015**
B/Victoria	1.024 (0.758, 1.382)	1.003 (0.853, 1.18)	1.100 (0.874, 1.385)	0.880
B/Yamagata	0.947 (0.710, 1.264)	0.876 (0.750, 1.024)	1.030 (0.826, 1.284)	0.386
		Days 0-6		
A/H1N1	1.154 (0.881, 1.511)	0.928(0.790, 1.090)	1.077 (0.886, 1.308)	0.440
A/H3N2	**1.393 (1.072, 1.812)**	**0.853 (0.729, 0.998)**	1.110 (0.918, 1.342)	**0.007**
B/Victoria	1.128 (0.861, 1.477)	0.971 (0.827, 1.141)	1.153 (0.948, 1.402)	0.397
B/Yamagata	1.045 (0.806, 1.354)	0.864 (0.740, 1.008)	1.098 (0.910, 1.324)	0.161

Results from the linear mixed effects models including study as a random effect, and age, sex, priming status and log_{10} pre-vaccination HI titres as fixed effects.

3.2.3 2 years to < 9 years cohort

Table 6 below summarises the estimates of the effect of fever and antipyretic use in the 2 years to <9 years cohort. Fever and antipyretic use had a statistically significant effect on post-vaccination HI titres for the A/H3N2 and A/H1N1 strains at days 0-1, A/H1N1, A/H3N2 and B/Victoria at days 0-3 and for all four strains when considering fever and antipyretic use across days 0-6.

Fever alone was associated with an increase in HI titres for the A/H3N2 strain at days 0-6, with fever associated with a 23.3% (95% CI 1.016 – 1.498) increase in post-vaccination HI titres. Although antipyretic use trended towards a decrease in HI titres for all strains across all considered time periods, this did not reach statistical significance with all of the 95% confidence intervals crossing 1.

Both fever and antipyretic use was associated with an increase in HI titres for A/H3N2 and A/H1N1 strains at days 0-1, A/H1N1, A/H3N2 and B/Victoria at days 0-3 and for all four strains when considering days 0-6. Estimates of effect of the combination of fever and antipyretic use ranged from a 29.2% increase for A/H1N1 at days 0-3 (95% CI 1.066 – 1.564), to a 96.2% increase for B/Victoria considering days 0-1 (95% CI 1.371 – 2.807).

Table 6: Estimates of GMT Ratio for Fever and Antipyretic Use in 2 Years to <9 Years Cohort

Strain	Fever without antipyretic use GMT Ratio (95% CI)	Antipyretic use without fever GMT Ratio (95% CI)	Both fever and antipyretic use GMT Ratio (95% CI)	p-value
		Days 0-1		
A/H1N1	0.964 (0.710, 1.310)	1.004 (0.875, 1.151)	1.283 (0.940, 1.751)	0.468
A/H3N2	1.280 (0.940, 1.743)	0.934 (0.812, 1.074)	**1.690 (1.236, 2.312)**	**0.003**
B/Victoria	1.030 (0.724, 1.467)	0.964 (0.823, 1.128)	**1.962 (1.371, 2.807)**	**0.003**
B/Yamagata	0.850 (0.628, 1.148)	0.909 (0.793, 1.042)	1.392 (1.020, 1.899)	0.056
		Days 0-3		
A/H1N1	1.074 (0.860, 1.342)	0.949 (0.830, 1.084)	**1.292 (1.066, 1.564)**	**0.046**
A/H3N2	1.173 (0.936, 1.470)	0.903 (0.788, 1.035)	**1.502 (1.236, 1.826)**	**<.0001**
B/Victoria	1.009 (0.781, 1.304)	0.952 (0.816, 1.111)	**1.369 (1.097, 1.708)**	**0.039**
B/Yamagata	0.978 (0.785, 1.220)	0.917 (0.802, 1.048)	1.168 (0.964, 1.415)	0.219
		Days 0-6		
A/H1N1	1.128 (0.932, 1.366)	0.917 (0.809, 1.039)	**1.309 (1.122, 1.527)**	**0.001**
A/H3N2	**1.233 (1.016, 1.498)**	0.891 (0.784, 1.013)	**1.424 (1.217, 1.666)**	**<.0001**
B/Victoria	1.148 (0.920, 1.431)	0.944 (0.817, 1.091)	**1.428 (1.195, 1.706)**	**0.001**
B/Yamagata	1.125 (0.930, 1.360)	0.896 (0.791, 1.016)	**1.259 (1.079, 1.468)**	**0.004**

Results from the linear mixed effects models including study as a random effect, and age, sex, priming status and log_{10} pre-vaccination HI titres as fixed effects.

3.2.4 9 years to 17 years cohort

Results for the 9 to 17 years cohort are summarised in Table 7 below. The effect of fever and antipyretic use was only statistically significant for the A/H1N1 strain at the day 0-1 time-period, where fever alone was associated with a significant decrease in HI titre (GMT ratio 0.137, 95% CI 0.019 – 0.981). Care must be taken in interpreting this result however, as there were very few participants reporting fever in this cohort on days 0-1 (n=5), and the result is therefore highly susceptible to the effects of outliers and is driven by a small number of participants results.

Table 7: Estimates of GMT Ratio for Fever and Antipyretic Use: 9 years and older

Strain	Fever without antipyretic use GMT Ratio (95% CI)	Antipyretic use without fever GMT Ratio (95% CI)	Both fever and antipyretic use GMT Ratio (95% CI)	p-value
		Days 0-1		
A/H1N1	**0.137 (0.019, 0.981)**	0.799 (0.583, 1.097)	0.448 (0.167, 1.201)	**0.042**
A/H3N2	0.674 (0.125, 3.637)	0.784 (0.598, 1.029)	0.821 (0.353, 1.910)	0.327
B/Victoria	1.374 (0.191, 9.872)	1.080 (0.786, 1.484)	1.192 (0.444, 3.199)	0.932
B/Yamagata	0.442 (0.092, 2.112)	0.917 (0.714, 1.177)	1.004 (0.459, 2.193)	0.683
		Days 0-3		
A/H1N1	0.584 (0.144, 2.363)	0.873 (0.661, 1.154)	0.569 (0.235, 1.380)	0.398
A/H3N2	0.793 (0.241, 2.615)	0.774 (0.610, 0.982)	0.682 (0.320, 1.451)	0.142
B/Victoria	1.126 (0.278, 4.564)	1.078 (0.815, 1.424)	1.032 (0.426, 2.498)	0.959
B/Yamagata	0.731 (0.242, 2.21)	0.952 (0.764, 1.186)	1.153 (0.573, 2.323)	0.881
		Days 0-6		
A/H1N1	1.342 (0.500, 3.602)	0.759 (0.590, 0.978)	0.805 (0.358, 1.806)	0.160
A/H3N2	1.252 (0.539, 2.912)	0.809 (0.651, 1.004)	0.697 (0.349, 1.392)	0.175
B/Victoria	1.177 (0.439, 3.157)	1.139 (0.884, 1.467)	0.937 (0.417, 2.103)	0.767
B/Yamagata	0.993 (0.454, 2.171)	0.942 (0.772, 1.150)	1.000 (0.527, 1.897)	0.952

Results from the linear mixed effects models including study as a random effect, and age, sex, priming status and log_{10} pre-vaccination HI titres as fixed effects.

3.3 Comparison of Maximum Recorded Fever in Children Reporting Fever ≥ 38°C

Supplemental analysis comparing the maximum recorded post-vaccination fever was performed for all participants reporting a fever ≥ 38°C, with results reported in Table 8 below. For the combined all-age and 6 months to <2 years cohorts, participants who reported a fever and used antipyretics recorded a higher magnitude of fever compared to those who did not use antipyretics across all time periods. For the 2 to <9 years cohort, this association was only significant for the day 0-3 and 0-6 time periods. This association was not seen in the 9 years and older cohort, however as previously noted, the sample of participants reporting fever in this cohort was very small.

Table 8: Mean maximum reported fever for participants reporting a fever (≥ 38°C) over days 0-3

	N	All (95% CI)	No antipyretics (95% CI)	Antipyretics (95% CI)	Difference (95% CI)	p-value*
			Day 0-1			
All-ages	149	38.56 (38.47, 38.65)	38.43 (38.30, 38.55)	38.67 (38.54, 38.79)	0.24 (0.06, 0.42)	**0.0104**
6mths to <2yrs	81	38.59 (38.45, 38.73)	38.37 (38.20, 38.55)	38.72 (38.53, 38.91)	0.34 (0.06, 0.62)	**0.0172**
2yrs to <9yrs	63	38.53 (38.41, 38.65)	38.46 (38.28, 38.64)	38.60 (38.43, 38.78)	0.14 (-0.10, 0.39)	0.3891
9 years and older	5	38.56 (38.16, 38.96)	38.90 (N/A)	38.48 (38.00, 38.95)	-0.43 (-1.49, 0.64)	0.6374
			Day 0-3			
All participants	303	38.67 (38.60,38.74)	38.50 (38.40, 38.59)	38.78 (38.68, 38.88)	0.28 (0.14, 0.42)	**0.0001**
6mths to <2yrs	153	38.67 (38.57, 38.77)	38.45 (38.32, 38.58)	38.79 (38.66, 38.66)	0.34 (0.14, 0.55)	**0.0011**
2yrs to <9yrs	143	38.67 (38.57, 38.78)	38.54 (38.40, 38.68)	38.77 (38.61, 38.92)	0.23 (0.02, 0.44)	**0.0332**
9 years and older	7	38.56 (38.18, 38.94)	38.45 (32.73, 44.17)	38.60 (38.13, 39.07)	0.15 (-0.80, 1.10)	0.703
			Day 0-6			
All participants	432	38.73 (38.66, 38.79)	38.53 (38.44, 38.62)	38.84 (38.75, 38.92)	0.30 (0.18, 0.43)	**<0.0001**
6mths to <2yrs	210	38.74 (38.65, 38.83)	38.47 (38.35, 38.58)	38.88 (38.76, 39.00)	0.41 (0.23, 0.60)	**<0.0001**
2yrs to <9yrs	212	38.71 (38.62, 38.80)	38.58 (38.45, 37.70)	38.79 (38.67, 38.91)	0.22 (0.04, 0.40)	**0.0187**
9 years and older	10	38.75 (38.32, 39.18)	38.78 (37.44, 40.11)	38.79 (38.67, 38.91)	-0.04 (-0.99, 0.90)	0.9215

*Comparing difference between mean maximum reported fever with student's t-test

3.4 Comparison of Models Across Time-Periods

The fit of the model estimates discussed above were compared using the AIC, with the results presented in Table 9 below. The fit of the model for days 0-1, 0-3 and 0-6 was compared for each strain and age group. For the all age cohort, the day 0-6 estimates provided the most accurate model fit, although B/Yamagata did not reach the ΔAIC>2 threshold compared to the day 0-1 estimated. Similar results were obtained for the 2 years to <9 years age cohort, with improved model fit for the day 0-6 estimates, reaching the threshold of ΔAIC>2 for all strains. In the 6 months to < 2 years cohort, model fit was similar across the time-periods for all strains, whilst for the 9 years and older cohort, assessing fever and antipyretic use on days 0-1 appeared to have better model fit for the A/H1N1 and B/Yamagata strains.

Table 9: Assessment of Model Fit Using Akike Information Criterion (AIC)

	All			6mths – <2 years			2 years - <9 years			9 years and older		
	AIC	**ΔAIC**	**Likelihood**	**AIC**	**ΔAIC**	**Likelihood**	**AIC**	**ΔAIC**	**Likelihood**	**AIC**	**ΔAIC**	**Likelihood**
A/H1N1												
D0-1	6233.5	6.8	0.033	**2194**			2324.6	10.4	0.006	**912.1**		
D0-3	6233.9	7.2	0.027	2195.4	1.4	0.497	2320.8	6.6	0.037	918.6	6.5	0.039
D0-6	**6226.7**			2194.2	0.2	0.905	**2314.2**			917.4	5.3	0.071
A/H3N2												
D0-1	5832.1	12.1	0.002	2115.2	2.2	0.333	2446.5	10.7	0.005	682.9	0.8	0.670
D0-3	5824.1	4.1	0.129	2114.1	1.1	0.577	2441.2	5.4	0.067	**682.1**		
D0-6	**5820**			**2113**			**2435.8**			683.7	1.6	0.449
B/Victoria												
D0-1	6637.8	5.5	0.064	**2193.9**			3060.9	1.2	0.549	**917**		
D0-3	6639.2	6.9	0.032	2195.7	1.8	0.407	3068.1	8.4	0.015	918.3	1.3	0.522
D0-6	**6632.3**			2194	0.1	0.951	**3059.7**			918.5	1.5	0.472
B/Yamagata												
D0-1	5496	1.6	0.449	**2073.2**			2323.5	3.4	0.183	**564**		
D0-3	5497.7	3.3	0.192	2074.5	1.3	0.522	2328.3	8.2	0.017	566	2	0.368
D0-6	**5494.4**			2073	0.2	0.905	**2320.1**			567.4	3.4	0.183

AIC – Akike Index Criterion, $\Delta AIC = AIC_{min} - AIC_i$, Likelihood – likelihood of obtaining this model, compared to the model with the minimum AIC, ***AIC*** *– minimum AIC for that strain and cohort*

3.5 The Effect of Age, Sex, Priming and Pre-Vaccination HI Titres on Post-Vaccination Haemagglutination Inhibition Titres

The effects of age, sex, priming status and pre-vaccination HI titres were included in the model estimates discussed above to attempt to control for confounding. The inclusion of these confounders allows for an opportunistic assessment of the effects of each of these factors upon post-vaccination HI titres. The estimates of the confounders are detailed in both the full model estimates included in Supplementary Tables 1 through 12 and estimates from the models with the best fit associated (determined by lowest AIC discussed above) are collated in Supplementary Tables 13 to 16.

3.5.1 Age

Age (in months) was included as a linear term in the estimates for all age cohorts. In the all-ages combined analysis, an increase in age was associated with a small but statistically significant increase in post-vaccination HI titres for all strains, with an increase in age by one month resulting in increases ranging from 0.3% - 0.5%. For the 6 months to < 2 years cohort, an increase of a larger magnitude was demonstrated compared to the all-age cohort, with age associated with statistically significant increases associated with a one month increase in age ranging from 6.2% to 12.1%. For the 2 years to < 9yrs cohort, a statistically significant association between age and post-vaccination HI titres was only seen for the A/H1N1 cohort, with a one month increase in age associated with a 0.3% increase in post-vaccination HI titre (95% CI 1.002-1.005, $p<0.001$). For the 9 years and older cohort, the only statistically significant association was demonstrated in the B/Victoria strain where an increase in age by one month was associated with a decrease in post-vaccination Hi titres of 0.004% (95% CI 0.004 – 0.999, $p=0.004$).

3.5.2 Sex

Sex was included as a categorical variable in all models, with female sex used as the base case scenario. The effects of male sex (in comparison to female) are summarised in Supplementary Table 14. For the all ages cohort, male sex was associated with a trend towards decrease post-vaccination HI titres, however this only achieved statistical significant for the B/Yamagata strain where male sex was associated with a 6.3% decrease in post-vaccination HI titres (95% CI 0.887-0.989, p=0.017). This trend was consistent across the 6m to < 2years and 2 years to < 9yrs cohorts (without reaching statistical significance in all cases), however the estimates in the 9 years and older cohort trended to male sex being associated with an increase in HI titres compared to females, however this failed to reach statistical significance for all strains.

For the 6 months to < 2 years cohorts, male sex was associated with a statistically significant decrease in post-vaccination HI titres for the A/H3N2 (GMT 0.863, 95% CI 0.776-0.959, P=0.007) and B/Victoria strains (GMT 0.891, 0.798 – 0.993, p=0.038). For the 2 yrs to < 9yrs, statistically significant association only seen for the B/Yamagata strain where male sex was associated with a 7.3% decrease in post-vaccination HI titres (95% CI 0.868 – 0.991, p-0.025). For the 9 years and older cohort male sex trended towards an opposite association of an increase in HI titres related to male sex, however this failed to reach statistical significance for any of the four strains.

3.5.3 Priming

The effect of priming (and therefore receiving a single dose schedule), whilst controlling for the effect of pre-vaccination HI titre, was assessed for the 6 month to < 2 years and 2 years to < 9 years cohorts and is summarised in Supplementary Table 15. There were no participants in the 9 years and older cohort who were considered unprimed. When controlling for pre-vaccination HI titres, priming was associated with a statistically significant decrease in post-vaccination HI titre for all strains for both cohorts. For the 6m to < 2 years cohort, the effects ranged from a 19.8% to 76.6% decrease in HI titres, and for the 2 years to < 9 years cohort ranged from 22.6% to 42.5% decrease. These results must be interpreted in the context of it being an estimate of the effect of priming,

and therefore receiving a single dose schedule, when the other factors, most importantly pre-vaccination HI titre, have also been controlled for.

3.5.4 Pre-vaccination Log_{10} Hi Titre

Increased pre-vaccination log_{10} HI titres was associated with significant increases in post-vaccination HI titres across all age cohorts, strains and considered time-periods. Results are reported on the effect of a 1 unit increase in pre-vaccination Hi titre on the log_{10} scale. Estimates of effect ranged from a 42.0% increase (95% CI 1.257 to 1.603) for the A/H3N2 estimate for the 9 years and older cohort, to a 7.54-fold increase (95% CI 6.494 to 8.815) for the B/Victoria estimate for the 6 months to < 2 years cohort.

CHAPTER 4: DISCUSSION

4.1 What This Thesis Contributes

This thesis examines patterns of fever and antipyretic medication use and their impact upon post-vaccination HI-titres in paediatric participants from eight clinical trials of QIV performed across Asia, Europe, Central and North America. The analysis pooled a large sample of 4,935 participants with recorded adverse event outcomes and pre- and post-vaccination HI titre serology results. The analysis included participants ranging in age from 6 months to 17 years. The thesis provides further evidence of the influence of fever and antipyretic use independently on post-vaccination HI-titres, and an assessment of the combined impact of fever and antipyretic use.

4.2 Discussing Rates and Patterns of Fever and Antipyretic Use

Our study observed notable patterns in fever and antipyretic medication use among children during the 6 days following influenza vaccination, highlighting age-related and priming-status-related differences. Fever was most common in the youngest cohort (6 months to <2 years), with 13.3% experiencing fever during days 0-6 post-vaccination. This was higher than the 8.1% reported in the 2 years to <9 years cohort and 1.3% in children aged 9 years and older. Rates of fever were also elevated in the unprimed cohort (11.9%) compared to those who were primed (3.1%). These differences likely reflect the younger age of the unprimed cohort and the recording of adverse events across two post-vaccination time periods for this group. Fever was most frequently reported on the day of vaccination (day 0) after dose 1, independent of age or priming status, and were highest within the first 48 hours after dose 2.

Our findings are consistent with observations by Nakayama et al., who reported high rates of fever >38˚C in younger children, following immunisation with an alum-adjuvanted A/H5N1 whole virion inactivated vaccine (WIV)(100). Fever was most prevalent in infants aged below 1 year (100%, though there were only 5 participants in this cohort) and progressively declined with age, to 6% in adolescents aged 13-19 years(100). In contrast to our study which found similar rates of fever post dose 1 and 2, a 2011 RCT found higher fever rates after the second dose of 2009 A/H1N1 pandemic vaccines, particularly for children who received the adjuvanted vaccine (95), with fever rates of 22.4% after the second dose compared to 8.9% after dose one (112).

Similar to our study, fever was less frequent in older children, with fever rates of 7.7% after the first dose and 6.3% following the second dose of the adjuvanted vaccine in children over 5 years of age (95). A 2014 systematic review found fever rates were highest in children aged 6-35 months, especially among those immunised with reactogenic vaccines such as bioCSL's TIV, which was also associated with an increase in febrile convulsions (113). Langley et al. reported identical fever rates of 14.5% in children receiving QIV or TIV after dose 1, with slightly lower rates following dose 2 (10.3% QIV, 9.1% TIV) (110). Comparatively, Stockwell et al. observed low fever rates in children aged 24-59 months receiving LAIV or IIV, with no significant differences between age groups or vaccine types (114). Though similar patterns in fever rates can be observed between these vaccine studies and our study, it should be noted that the QIV vaccine tested in our study is unadjuvanted and therefore not directly comparable to the adjuvanted H5N1 or LAIV vaccines.

In our study, antipyretic medication use was more commonly reported than fever, with 15.5% of participants reporting antipyretic administration during the first 6 days post-vaccination. Like fever, antipyretic use was highest among the youngest cohort (23.4%), followed by the 2 to <9 years (12.7%) and 9 years and older (9.7%) cohorts. First reports of antipyretic use were most common on the day of vaccination (day 0), regardless of age, priming status or vaccine dose. These findings align with previous results which found that antipyretic use was most common on the day of vaccination (day 0) and that rates declined with each subsequent day post-vaccination (41). This pattern is also

evidenced by other published studies; a longitudinal study comparing the safety and immunogenicity of a 3 or 4 dose DTP vaccination schedule in children reported that 73.5% of participants received paracetamol at least once in the first 48 hours following vaccination to treat either fever, pain or local reactions (115). Furthermore, a 2017 survey of 150 parents or caregivers of 6- and 15-month-old children who recently received a vaccine revealed that 64% administered antipyretic medications in the first 48 hours post-vaccination to trat fever or pain, and 11% administered an antipyretic medication prophylactically (116). The authors noted that paracetamol was administered by parents or caregivers 2.6 times more frequently than ibuprofen, and overall, ibuprofen was administered less to 6-month-old cohort than the 15-month-old cohort (7.4% vs 28% respectively) (116). A 2019 study that aimed to investigate post-childhood-vaccination fever patterns from mobile app data found that from 4448 vaccination records, 77% recorded antipyretic medication use, including 669 children who used antipyretics following influenza vaccination (117). 50% of children who received an antipyretic were administered the medication within the first 10 hours post-vaccination (117).

The combination of fever and antipyretic use was reported in 5.6% of participants, following a similar pattern to fever and antipyretic use individually. The youngest cohort had the highest combined rate (9%), compared to the 2 to <9 years cohort (4.9%) and the over 9 years cohort (0.8%). These findings demonstrate that younger children are consistently more likely to experience reactogenicity and receive antipyretic medications following influenza immunisation.

4.3 Immunological Effects of Fever and Antipyretic Use

4.3.1 Immunological effects of fever

Our study found that fever without antipyretic use was associated with increased HI titres, however this effect was only found to be significant for the A/H3N2 strain and was limited to analysis including fever and antipyretic use for day 0-6 for the combined all-age, 6 months to < 2 years and 2 years to < 9 years cohorts. There was a consistent trend

towards increased HI titres in children who experienced fever without antipyretic use for the remaining strains when considering days 0-6, however these trends did not reach statistical significance. In a similar study analysing the impact of day 0-3 fever and antipyretic use on post-vaccination HI titres from 3 pooled TIV RCTs, the authors found that fever was associated with increased HI titres across all included influenza strains (41).

In children receiving an alum-adjuvanted WIV, one study reported significantly higher neutralising antibody titres (NT) in participants who experienced a fever following vaccination compared to those who did not (100). Similarly, an RCT found that HI titres in children vaccinated with either an AS03B-adjuvanted split virion or a non-adjuvanted whole virion H1N1 (2009) vaccine were 50-60% higher in children who experienced post-vaccination fever (95). These results were consistent with findings from another study of the same trial population, who reported that children who experienced a fever post-vaccination had consistently higher immune titres, by both microneutralisation and HI assays in the same trial population (112). Our study potentially did not demonstrate as consistent a relationship between fever and increased HI titres because of the consideration of the combination of fever and antipyretic use. This decreased the sample size of those with fever, reducing the power to detect a statistically significant association. It also potentially removed participants who experienced greater reactogenicity from this group, as demonstrated by the increased magnitude of fever of participants in the *fever and antipyretic* group, compared to those with fever alone.

4.3.2 Immunological effects of antipyretic use

In our study, antipyretic use was associated with a decrease in HI titres for the A/H1N1, A/H3N2 and B/Yamagata strains in the combined, all-age cohort. When broken down by age cohort this negative association only reached statistical significance in the 6 months to <2 years cohort, However, a general trend toward reduced titres with antipyretic use was observed across all age cohorts and strains.

Our study's findings of an association between antipyretic use and reduced immunogenicity are consistent with those of Li-Kim-Moy et al., who reported reductions in post-vaccination HI titres of 13 – 20% amongst those receiving antipyretics day 0-3 post-TIV (41). Prymula et al. also reported a reduction in immunogenicity in their open-label RCT investigating the use of prophylactic paracetamol post coadministration of 10-valent pneumococcal conjugate vaccine and hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus types 1, 2 and 3-H influenzae type b vaccine (37).

There are, however, several studies that have not demonstrated this same association between antipyretic use and reduced post-vaccination immunogenicity. A UK study evaluating A/H1N1 (2009) pandemic influenza vaccine in children found that early antipyretic use (day 0-1) did not significantly affect antibody titres or immune responses (95). Similarly, an RCT involving children aged 6-47 months receiving IIV found no significant differences in seroconversion or post-vaccination seroprotection between children receiving paracetamol, ibuprofen or placebo (36).

Several mechanisms have been posited to explain the causal relationship between antipyretic use and reduced post-vaccination immunogenicity. Bancos et al. demonstrated that ibuprofen reduced IgM production by up to 97% and IgG production by up to 70%, particularly when administered soon after immune activation in in vitro studies. Whilst paracetamol had less impact, it still measurably reduced antibody production, likely by interfering with B cell functionality (118). Our findings add further evidence to the literature of the potential for antipyretics to blunt influenza vaccine induced immune responses, particularly among young children.

4.3.3 Immunological effects of the combination of fever and antipyretic use

To our knowledge, our study is the first to specifically investigate the relationship between the combination of fever and antipyretic use, and post-vaccination immunogenicity in a paediatric cohort receiving quadrivalent influenza vaccination. The combination of fever and antipyretic use was associated with an increase in post-vaccination HI titres, with the strongest evidence in the 2 years to < 9 years cohort where this relationship was statistically significant for all four strains. Supplementary analysis comparing the maximum temperature by those reporting a fever demonstrated that (with the exception of the 9 years and older cohort) antipyretic use was associated with a greater magnitude of fever. We suggest that a potential explanation for this positive association of the combination of fever and antipyretic use and increased immunogenicity may be that those experiencing higher fevers, are more likely to be administered antipyretic medications, as well as mounting a greater immunological response, resulting in higher immune titres.

4.3.4 Time-periods of fever and antipyretic use

Our study explores the associations between fever, antipyretic use and post-vaccination immunogenicity across several time periods, with fever and antipyretic use in days 0-1, 0-3 and 0-6 considered. Our study found increased associations between HI titres and fever and antipyretic use the longer the time post-vaccination was considered, with p-values for these associations consistently lowest at days 0-6. Furthermore, comparisons of model fit using the AIC demonstrated that models considering day 0-6 fever and antipyretic use post-vaccination provided the best fit to the underlying data for the combined all-ages and the 2 years to < 9 years cohorts. Interestingly, this was not the case for the 6 months to <2 years age cohort where there was minimal difference between the different time-period models, however p-values of the associations were still lowest at days 0-6.

These results suggest that the immunological effects of fever and antipyretic use extend beyond the initial 24-48hrs post-vaccination, when these events are likely to be vaccine-related (97). This is in contrast to previous work by Prymula et al. who suggested that the immunologic effects of prophylactic paracetamol are mediated by interference in dendritic, B and T cell interactions, possibly by reducing inflammatory signals at the site of injection thereby disrupting early innate and adaptive immune responses (37). This results in changes in germinal centre induced plasma-cell or memory-cell differentiation, involved in antibody production (37). This would therefore suggest that the effect of paracetamol should only be seen if administered early in the post-vaccination period, during the generation of local inflammatory signals.

Alternative hypotheses relate to the role of cyclooxygenase 2 (COX2) and prostaglandin synthesis in the process of B cell differentiation to plasmablasts and subsequent antibody production, a process which takes place over several days (59,119,120). COX-2 is a critical part of the inflammatory pathway resulting in fever and interference in the COX-2 mediated production of prostaglandins is responsible for the fever-reducing properties of antipyretic medications. NSAIDs exert their antipyretic effects through inhibition of COX-2, interfering with prostaglandin synthesis and thus reducing febrile responses (121–123). Despite its widespread use, the exact mechanism of action of paracetamol's antipyretic effects is an ongoing area of research. Paracetamol is noted to inhibit prostaglandin synthesis and has been shown to be a weak COX-2 inhibitor (121–123). If COX-2 is also involved in the process of B-cell differentiation and antibody production, then there is a potential that fever or antipyretic use may influence this process through this pathway (119,120,124,125). This hypothesis more closely aligns with our findings with the effects of fever and antipyretics extending beyond the initial post-vaccination period.

4.4 Immunological Effects of Age, Sex, Priming and Pre-Vaccination Titres

Age, sex, priming status and pre-vaccination HI titres were included as covariates in the model, to attempt to account for confounding attributable to these factors. The models provide estimates of the effect of these factors upon post-vaccination HI titres; however, these results were not primary endpoints and were not examined in detail.

Increasing age was associated with increased post-vaccination HI titres across all strains in the combined all-age and 6 months to <2 years cohorts, however for the 2 to <9 years cohort, a statistically significant increase was only seen for the A/H1N1 strain. These findings of increasing immune response with increasing age have previously been demonstrated in children (32,75,91,95,112), likely due to the increased efficiency of APCs and broader memory cells in older children (32). In the 9 years and older cohort, increasing age was associated with a decrease in HI titres for the B/Victoria strain, and the A/H3N2 strain for the days 0-1 and 0-6 time periods.

Male sex was associated with decreased HI titres for the B/Yamagata strains in the combined all-age and 2 years to < 9 years cohorts, and the A/H3N2 and B/Victoria strains for the 6 months to < 2 years cohort. Data for the influence of sex upon vaccine immunogenicity in paediatric populations is varied and conflicting, due to the limited number of studies that report their results by sex (76). For example, when considering the measles vaccine, some studies report higher antibody responses in infant females than in males, some report no difference between sexes and others report higher antibody responses in males (126). For diphtheria, pertussis, hepatitis A and B, pneumococcal, rabies, human papillomavirus and rubella vaccines, the literature describes a higher antibody response in female children than in males (76). Though there is limited evidence for sex differences in antibody titres following immunisation with influenza vaccines in paediatric populations, among adults aged 18-49 years who

received either a half or full dose of seasonal TIV, HAI titres were reported to be two times higher in females than in males (127).

Given these mixed findings, it has been argued that sex differences in children's vaccine responses may not have any clinical significance if protective antibody levels are achieved in both males and females (76). This result was not explored further, as the relationship between sex and immunogenicity was not a predefined endpoint for this study, however this is a suggested area for future research to determine if these findings can be replicated.

The inverse association between priming and HI titres potentially reflects that primed participants received a single-dose vaccination schedule, compared to unprimed participants who received two, with post-vaccination HI titre measured 28 days post final vaccination. There are however, several studies which report a negative association between repeat influenza vaccination and measures of immunogenicity (83,84,86,87,92,128). Potential mechanisms for this negative association are the **antigenic distance hypothesis** and **original antigenic sin** (92). The antigenic distance hypothesis, first proposed by Smith et al. in 1999, suggests that if vaccine strains are closely related, the immune system may focus on the first strain, potentially reducing the effectiveness of the vaccine against the second strain. The concept of original antigenic sin, first proposed by Davenport in 1953, suggests that when the immune system is exposed to a new but related strain, it preferentially relies on memory from its first encounter with that particular strain, producing antibodies that target shared features of both the original and novel strains (92,93,129).

Pre-vaccination $\log_{10}$ HI titre was associated with significant increases in post-vaccination HI titres across all age cohorts, strains and considered time-periods. Estimates of effect ranged from a 42.0% increase (95% CI 1.257 to 1.603) for the A/H3N2 day 0-1 estimate for the 9 years and older cohort, to a 656.7% increase (95% CI 6.494 to 8.815) for the B/Victoria day 0-1 estimate for the 6 months to < 2 years age

cohort from a 1 unit increase in pre-vaccination HI titre on the $\log_{10}$ scale. This result is consistent with previous findings of positive associations between pre- and post-vaccination HI titres (41,91).

4.5 Clinical Implications, Limitations and Strengths of This Thesis and Recommendations for Future Research

4.5.1 Clinical Implications

This thesis suggests evidence of a positive association between post-vaccination HI titre and fever alone, and for fever and antipyretic use, but found a negative association between post-vaccination titres and antipyretic use. These findings may suggest that prophylactic antipyretic administration in the absence of fever may be detrimental to post-vaccination immunity, an effect which appears to persist to 6 days post-vaccination. This finding may be particularly pertinent given influenza vaccination is only moderately effective, and children may require a higher HI titre to achieve adequate protection (56, 66-70). However, the findings of increased HI titres in those experiencing fever and antipyretic use are reassuring that antipyretic use in the context of post-vaccination fever do not compromise immune responses to vaccination. This analysis was however limited to serological measures of immunogenicity, and whether these findings translate to clinically significant effects is yet to be established.

4.5.2 Limitations

Our study has some limitations; our analysis relies upon the robustness of the underlying trial data, which included self-measurement and reporting of fever and antipyretic use. As previously discussed, inaccuracies in readings of body temperature may come from user error or improper positioning of thermometers, or from factors such as mouth breathing during oral measurement (96,99). Our analysis has also been limited to a single vaccine manufacturer, which may affect the generalisability of these

results. Investigation of QIVs by other manufacturers may be warranted. The limited number of patients reporting fever in the 9 years and older cohort limited the power of this study to find significant associations in this cohort. Finally, we are unable to assess the clinical implications (such as impacts on vaccine efficacy) of the associations found in our paper and this would be an important area for future studies, particularly given that influenza is one of the few diseases for which there is data regarding correlates of protection, whereby a certain level of immunogenicity may translate to protection (18).

4.5.3 Strengths

This study included a large number of participants from eight separate clinical trials. The inclusion of participants from multiple trials performed in different countries increases the generalisability of the study. The model controls for confounders including age, sex, priming and pre-vaccination titre, and the inclusion of trial as a random effect allows for variation in the immunogenicity and reactogenicity of the vaccines due to trial specific effects without biasing the results (such as vaccine formulations, viral strains, variations in trial populations, rates of circulating influenza, and ethnic differences). The main strength of this study is the novel exploration of the combination of fever and antipyretic use in this patient cohort, which have previously only been explored individually.

4.5.4 Recommended areas for future research

Further research into the clinical implications of fever and antipyretic use post-influenza vaccination is needed to provide guidance to both parents and clinicians on the use of antipyretics in the post-vaccination period. The inclusion of prospective analyses of fever and antipyretic use and immunogenicity as part of manufacturers' randomised controlled trials would help in informing this guidance. If efficacy outcomes were included in these trials, insights could be gained as to the clinical significance of potential differences to HI titres. Uncertainties remain from our research as to whether

peak levels of fever correlate with greater effects on HI titres, and whether differences exist in prophylactic versus therapeutic patterns of antipyretic use in various age groups, and how this may impact immunogenicity. An investigation of differences between classes of antipyretics, such as NSAIDs and paracetamol, upon vaccine immunogenicity would also expand knowledge about this subject. Fever was the only adverse event reported in this study, however other common post-vaccination adverse events such as lumps, pain, rashes and irritability are often collated within trials and could undergo similar investigation. Antipyretics are also commonly used in the context of other childhood vaccines, and similar research into other childhood vaccines would also improve the knowledge base of this interaction.

4.6 Conclusion

This retrospective analysis of the impact of fever and antipyretics upon the immune response of children receiving a QIV adds to previous studies suggesting an increase in immunogenicity following influenza vaccination in children who experience fever, and a decrease in those using antipyretics in the post-vaccination period. The thesis contributes to the existing evidence base by finding that the combination of fever and antipyretic use is associated with an increase in HI titres compared to those who experience neither, and that the effects of fever and antipyretic use appear to be influential to day 6 post-vaccination. Influenza vaccination in children provides moderate protection against infection and changes in immunogenicity may have an impact on the protection afforded by influenza vaccines. Further understanding of these associations and the impact of prophylactic antipyretics will be valuable for future vaccine recommendations, as parts of the world shift back from quadrivalent to trivalent influenza vaccination for their populations.

THESIS REFERENCES

1. Jané M, Vidal MJ, Soldevila N, Romero A, Martínez A, Torner N, et al. Epidemiological and clinical characteristics of children hospitalized due to influenza A and B in the south of Europe, 2010–2016. Sci Rep. 2019;9(1):12853–7.

2. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, et al. Influenza. Nat Rev Dis Primer. 2018 Jun 28;4(1):3.

3. Dos Santos G, Nguyen BY, Damaso S, Godderis L, Martínez-Gómez X, Eckermann T, et al. Brand-Specific Enhanced Safety Surveillance of GSK's Quadrivalent Seasonal Influenza Vaccine in Belgium, Germany and Spain for the 2018/2019 Season. Drug Saf. 2020 Mar;43(3):265–79.

4. Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. The Lancet. 2018 Mar;391(10127):1285–300.

5. Sellers SA, Hagan RS, Hayden FG, Fischer WA. The hidden burden of influenza: A review of the extra-pulmonary complications of influenza infection. Influenza Other Respir Viruses. 2017 Sep;11(5):372–93.

6. Huang C, Fu X, Zhou Y, Mi F, Tian G, Liu X, et al. Comparison of the immunogenicity and safety of quadrivalent and tetravalent influenza vaccines in children and adolescents. Vaccine. 2020 Feb;38(6):1332–44.

7. Thompson WW. Influenza-Associated Hospitalizations in the United States. JAMA. 2004 Sep 15;292(11):1333.

8. Langer J, Welch VL, Moran MM, Cane A, Lopez SMC, Srivastava A, et al. High Clinical Burden of Influenza Disease in Adults Aged ≥ 65 Years: Can We Do Better? A Systematic Literature Review. Adv Ther. 2023;40(4):1601–27.

9. Uyeki TM, Hui DS, Zambon M, Wentworth DE, Monto AS. Influenza. The Lancet. 2022 Aug;400(10353):693–706.

10. Fraaij PLA, Heikkinen T. Seasonal influenza: The burden of disease in children. Vaccine. 2011 Oct;29(43):7524–8.

11. U.S. Centers for Disease Control and Prevention. How Influenza (Flu) Vaccines Are Made. 2022 [cited 2024 Dec 8]. How Influenza (Flu) Vaccines Are Made. Available from: https://www.cdc.gov/flu/vaccine-process/index.html

12. Cross JW, Joy M, McGee C, Akinyemi O, Gatenby P, De Lusignan S. Adverse events of interest vary by influenza vaccine type and brand: Sentinel network study of eight seasons (2010–2018). Vaccine. 2020 May;38(22):3869–80.

13. Australian Technical Advisory Group on Immunisation (ATAGI). Statement on the transition from quadrivalent to trivalent seasonal influenza vaccines in Australia [Internet]. Australian Government Department of Health and Aged Care; 2024 [cited 2024 Dec 8]. Available from: https://www.health.gov.au/sites/default/files/2024-08/atagi-statement-on-the-transition-from-quadrivalent-to-trivalent-seasonal-influenza-vaccines-in-australia.pdf

14. Smith GJD, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature. 2009 Jun;459(7250):1122–5.

15. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc R Soc B Biol Sci. 2015;282(1821):20143085–20143085.

16. Bhat N, Wright JG, Broder KR, Murray EL, Greenberg ME, Glover MJ, et al. Influenza-Associated Deaths among Children in the United States, 2003–2004. N Engl J Med. 2005 Dec 15;353(24):2559–67.

17. Domachowske JB, Pankow-Culot H, Bautista M, Feng Y, Claeys C, Peeters M, et al. A Randomized Trial of Candidate Inactivated Quadrivalent Influenza Vaccine versus Trivalent Influenza Vaccines in Children Aged 3–17 Years. J Infect Dis. 2013 Jun 15;207(12):1878–87.

18. Campbell AJP, Grohskopf LA. Updates on Influenza Vaccination in Children. Infect Dis Clin North Am. 2018 Mar;32(1):75–89.

19. Morris SE, Nguyen HQ, Grijalva CG, Hanson KE, Zhu Y, Biddle JE, et al. Influenza virus shedding and symptoms: Dynamics and implications from a multiseason household transmission study. Glymour M, editor. PNAS Nexus. 2024 Sep 2;3(9):page338.

20. Neuzil KM, Zhu Y, Griffin MR, Edwards KM, Thompson JM, Tollefson SJ, et al. Burden of Interpandemic Influenza in Children Younger than 5 years: A 25-Year Prospective Study. J Infect Dis. 2002 Jan 15;185(2):147–52.

21. Jayasundara K, Soobiah C, Thommes E, Tricco AC, Chit A. Natural attack rate of influenza in unvaccinated children and adults: a meta-regression analysis. BMC Infect Dis. 2014 Dec;14(1):670.

22. O'Leary ST, Campbell JD, Ardura MI, Banerjee R, Bryant KA, Caserta MT, et al. Recommendations for Prevention and Control of Influenza in Children, 2023–2024. Pediatr Evanst. 2023;152(4).

23. Spila Alegiani S, Alfonsi V, Appelgren EC, Ferrara L, Gallo T, Alicino C, et al. Active surveillance for safety monitoring of seasonal influenza vaccines in Italy, 2015/2016 season. BMC Public Health. 2018 Dec;18(1):1401.

24. Cohen SA, Chui KKH, Naumova EN. Influenza Vaccination in Young Children Reduces Influenza-Associated Hospitalizations in Older Adults, 2002-2006:

INFLUENZA VACCINATION IN YOUNG CHILDREN. J Am Geriatr Soc. 2011 Feb;59(2):327–32.

25. Therapeutic Goods Administration. 2024 Seasonal influenza vaccines information for consumers and health professionals [Internet]. 2024 [cited 2024 Dec 8]. Available from: https://www.tga.gov.au/resources/publication/publications/2024-seasonal-influenza-vaccines

26. Aoun T, Borrow R, Arkwright PD. Immunogenicity and safety of seasonal influenza vaccines in children under 3 years of age. Expert Rev Vaccines. 2023 Dec 31;22(1):226–42.

27. Soema PC, Kompier R, Amorij JP, Kersten GFA. Current and next generation influenza vaccines: Formulation and production strategies. Eur J Pharm Biopharm. 2015 Aug;94:251–63.

28. Albalawi ARS, Alhassun JAS, Almarshud RK, Almejali HA, Alharbi SM, Shaybah AM, et al. Unlocking the Power of Influenza Vaccines for Pediatric Population: A Narrative Review. Cureus [Internet]. 2024 Feb 28 [cited 2024 Dec 22]; Available from: https://www.cureus.com/articles/231108-unlocking-the-power-of-influenza-vaccines-for-pediatric-population-a-narrative-review

29. Kumar A, Meldgaard TS, Bertholet S. Novel Platforms for the Development of a Universal Influenza Vaccine. Front Immunol. 2018 Mar 23;9:600.

30. Diaco M, Chang LJ, Seet B, Robertson CA, Chit A, Mercer M, et al. Introductory paper: High-dose influenza vaccine. Vaccine. 2021 Mar;39:A1–5.

31. Tregoning JS, Russell RF, Kinnear E. Adjuvanted influenza vaccines. Hum Vaccines Immunother. 2018 Mar 4;14(3):550–64.

32. Wen S, Wu Z, Zhong S, Li M, Shu Y. Factors influencing the immunogenicity of influenza vaccines. Hum Vaccines Immunother. 2021 Aug 3;17(8):2706–18.

33. Tisa V, Barberis I, Faccio V, Paganino C, Trucchi C, Martini M, et al. Quadrivalent influenza vaccine: a new opportunity to reduce the influenza burden. J Prev Med Hyg. 2016 Mar;57(1):28–33.

34. World Health Organisation. Recommended composition of influenza virus vaccines for use in the 2023-2024 northern hemisphere influenza season [Internet]. 2023 Feb [cited 2023 Nov 23] p. 1–11. Available from: https://cdn.who.int/media/docs/default-source/influenza/who-influenza-recommendations/vcm-northern-hemisphere-recommendation-2023-2024/202302_seasonal_recommendation_a.pdf?sfvrsn=42612ae5_3&download=true

35. Grohskopf LA, Ferdinands JM, Blanton LH, Broder KR, Loehr J. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory

Committee on Immunization Practices — United States, 2024–25 Influenza Season. MMWR Recomm Rep. 2024 Aug 29;73(5):1–25.

36. Walter EB, Hornik CP, Grohskopf L, McGee CE, Todd CA, Museru OI, et al. The effect of antipyretics on immune response and fever following receipt of inactivated influenza vaccine in young children. Vaccine. 2017 Dec;35(48):6664–71.

37. Prymula R, Siegrist CA, Chlibek R, Zemlickova H, Vackova M, Smetana J, et al. Effect of prophylactic paracetamol administration at time of vaccination on febrile reactions and antibody responses in children: two open-label, randomised controlled trials.

38. Cooper Robbins SC, Leask J, Booy R. Parents' attitudes towards the influenza vaccine and influencing factors. J Paediatr Child Health. 2011 Jul;47(7):419–22.

39. King JP, McLean HQ, Meece JK, Levine MZ, Spencer SM, Flannery B, et al. Vaccine failure and serologic response to live attenuated and inactivated influenza vaccines in children during the 2013–2014 season. Vaccine. 2018 Feb;36(9):1214–9.

40. Statler VA, Albano FR, Airey J, Sawlwin DC, Graves Jones A, Matassa V, et al. Immunogenicity and safety of a quadrivalent inactivated influenza vaccine in children 6–59 months of age: A phase 3, randomized, noninferiority study. Vaccine. 2019 Jan;37(2):343–51.

41. Li-Kim-Moy J, Wood N, Jones C, Macartney K, Booy R. Impact of Fever and Antipyretic Use on Influenza Vaccine Immune Reponses in Children. Pediatr Infect Dis J. 2018 Oct;37(10):971–5.

42. Blyth CC, Currie AJ, Wiertsema SP, Conway N, Kirkham LAS, Fuery A, et al. Trivalent influenza vaccine and febrile adverse events in Australia, 2010: Clinical features and potential mechanisms. Vaccine. 2011 Jul;29(32):5107–13.

43. Das RR, Panigrahi I, Naik SS. The Effect of Prophylactic Antipyretic Administration on Post-Vaccination Adverse Reactions and Antibody Response in Children: A Systematic Review. Quach C, editor. PLoS ONE. 2014 Sep 2;9(9):e106629.

44. Uhari M, Hietala J, Viljanen MK. Effect of Prophylactic Acetaminophen Administration on Reaction to DTP Vaccination. Acta Paediatr. 1988 Sep;77(5):747–51.

45. Saleh E, Moody MA, Walter EB. Effect of antipyretic analgesics on immune responses to vaccination. Hum Vaccines Immunother. 2016 Sep 1;12(9):2391–402.

46. Ambrose CT. Inhibition of the secondary antibody response in vitro by salicylate and gentisate. J Exp Med. 1966;124(3):461–82.

47. Goodwin JS, Selinger DS, Messner RP, Reed WP. Effect of indomethacin in vivo on humoral and cellular immunity in humans. Infect Immun. 1978 Feb;19(2):430–3.

48. Swift HF. THE ACTION OF SODIUM SALICYLATE UPON THE FORMATION OF IMMUNE BODIES. J Exp Med. 1922;36(6):735–60.

49. Ooi EE, Dhar A, Petruschke R, Locht C, Buchy P, Low JGH. Use of analgesics/antipyretics in the management of symptoms associated with COVID-19 vaccination. Npj Vaccines. 2022 Mar 2;7(1):31.

50. Wysocki J, Center KJ, Brzostek J, Majda-Stanislawska E, Szymanski H, Szenborn L, et al. A randomized study of fever prophylaxis and the immunogenicity of routine pediatric vaccinations. Vaccine. 2017 Apr;35(15):1926–35.

51. Homme JH, Fischer PR. Prophylactic paracetamol at the time of infant vaccination reduces the risk of fever but also reduces antibody response. Evid Based Med. 2010 Apr;15(2):50–1.

52. Huang Y, Anderson SA, Forshee RA, Yang H. A modified dose-response model that describes the relationship between haemagglutination inhibition titre and protection against influenza infection. J Appl Microbiol. 2018 Jan;124(1):294–301.

53. Domnich A, Manini I, Calabrò GE, De Waure C, Montomoli E. Mapping Host-Related Correlates of Influenza Vaccine-Induced Immune Response: An Umbrella Review of the Available Systematic Reviews and Meta-Analyses. Vaccines. 2019 Dec 13;7(4):215.

54. Trombetta CM, Montomoli E. Influenza immunology evaluation and correlates of protection: a focus on vaccines. Expert Rev Vaccines. 2016 Aug 2;15(8):967–76.

55. Otani N, Nakajima K, Ishikawa K, Ichiki K, Yoda Y, Ueda T, et al. Comparison of the Hemagglutination Inhibition Titers against Influenza Vaccine Strains in Japan from the 2017/2018 to 2021/2022 Seasons Using a Single Set of Serum Samples. Viruses. 2022 Jun 30;14(7):1455.

56. El-Husseiny MH, Pushko P, Tretyakova I, Hagag NM, Abdel-Mawgod S, Shabaan A, et al. A Novel Application of Virus Like Particles in the Hemagglutination Inhibition Assay. Int J Mol Sci. 2024 Aug 11;25(16):8746.

57. Bresee JS, Fry AM, Sambhara S, Cox NJ. Inactivated Influenza Vaccines.

58. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination Inhibition Antibody Titers as a Correlate of Protection for Inactivated Influenza Vaccines in Children. Pediatr Infect Dis J. 2011 Dec;30(12):1081–5.

59. Krammer F. The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol. 2019 Jun;19(6):383–97.

60. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. Epidemiol Infect. 1972 Dec;70(4):767–77.

61. Cox R. Correlates of protection to influenza virus, where do we go from here? Hum Vaccines Immunother. 2013 Feb;9(2):405–8.

62. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Res Methodol. 2010 Dec;10(1):18.

63. Ng S, Fang VJ, Ip DKM, Chan KH, Leung GM, Peiris JSM, et al. Estimation of the Association Between Antibody Titers and Protection Against Confirmed Influenza Virus Infection in Children. J Infect Dis. 2013 Oct 15;208(8):1320–4.

64. Danier J, Callegaro A, Soni J, Carmona A, Kosalaraska P, Rivera L, et al. Association Between Hemagglutination Inhibition Antibody Titers and Protection Against Reverse-Transcription Polymerase Chain Reaction–Confirmed Influenza Illness in Children 6–35 Months of Age: Statistical Evaluation of a Correlate of Protection. Open Forum Infect Dis. 2022 Feb 1;9(2):ofab477.

65. Motaghi S, Pullenayegum E, Morgan RL, Loeb M. The role of influenza Hemagglutination-Inhibition antibody as a vaccine mediator in children. Vaccine. 2024 Aug;42(21):126122.

66. Lim WW, Feng S, Wong SS, Sullivan SG, Cowling BJ. Hemagglutination Inhibition Antibody Titers as Mediators of Influenza Vaccine Efficacy Against Symptomatic Influenza A(H1N1), A(H3N2), and B/Victoria Virus Infections. J Infect Dis. 2024 Jul 25;230(1):152–60.

67. Dunning AJ, DiazGranados CA, Voloshen T, Hu B, Landolfi VA, Talbot HK. Correlates of Protection against Influenza in the Elderly: Results from an Influenza Vaccine Efficacy Trial. Staats HF, editor. Clin Vaccine Immunol. 2016 Mar;23(3):228–35.

68. Boddington NL, Pearson I, Whitaker H, Mangtani P, Pebody RG. Effectiveness of Influenza Vaccination in Preventing Hospitalization Due to Influenza in Children: A Systematic Review and Meta-analysis. Clin Infect Dis. 2021 Nov 2;73(9):1722–32.

69. Claeys C, Zaman K, Dbaibo G, Li P, Izu A, Kosalaraksa P, et al. Prevention of vaccine-matched and mismatched influenza in children aged 6–35 months: a multinational randomised trial across five influenza seasons. Lancet Child Adolesc Health. 2018 May;2(5):338–49.

70. Buchy P, Badur S. Who and when to vaccinate against influenza. Int J Infect Dis. 2020 Apr;93:375–87.

71. Pepin S, Dupuy M, Borja-Tabora CFC, Montellano M, Bravo L, Santos J, et al. Efficacy, immunogenicity, and safety of a quadrivalent inactivated influenza vaccine in children aged 6–35 months: A multi-season randomised placebo-controlled trial in the Northern and Southern Hemispheres. Vaccine. 2019 Mar;37(13):1876–84.

72. Dbaibo G, Amanullah A, Claeys C, Izu A, Jain VK, Kosalaraksa P, et al. Quadrivalent Influenza Vaccine Prevents Illness and Reduces Healthcare Utilization Across Diverse Geographic Regions During Five Influenza Seasons: A Randomized Clinical Trial. Pediatr Infect Dis J. 2020 Jan;39(1):e1–10.

73. Shrestha AC, Field E, Thangarajah D, Andrews R, Ware RS, Lambert SB. Influenza Epidemiology and Vaccine Effectiveness Following Funded Influenza Vaccine in Queensland, Australia, 2022. Influenza Other Respir Viruses. 2024 Sep;18(9):e70007.

74. Guo J, Chen X, Guo Y, Liu M, Li P, Tao Y, et al. Real-world effectiveness of seasonal influenza vaccination and age as effect modifier: A systematic review, meta-analysis and meta-regression of test-negative design studies. Vaccine. 2024 Mar;42(8):1883–91.

75. Walter E.B., Rajagopal S., Zhu Y., Neuzil K.M., Fairchok M.P., Englund J.A. Trivalent inactivated influenza vaccine (TIV) immunogenicity in children 6 through 23 months of age: Do children of all ages respond equally? Vaccine. 2010;28(27):4376 EP – 4383.

76. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and Gender Differences in the Outcomes of Vaccination over the Life Course. Annu Rev Cell Dev Biol. 2017 Oct 6;33(1):577–99.

77. Claeys C, Chandrasekaran V, García-Sicilia J, Prymula R, Díez-Domingo J, Brzostek J, et al. Anamnestic Immune Response and Safety of an Inactivated Quadrivalent Influenza Vaccine in Primed Versus Vaccine-Naïve Children. Pediatr Infect Dis J. 2019 Feb;38(2):203–10.

78. Vesikari T, Forstén A, Arora A, Tsai T, Clemens R. Influenza vaccination in children primed with MF59® -adjuvanted or non-adjuvanted seasonal influenza vaccine. Hum Vaccines Immunother. 2015 Aug 3;11(8):2102–12.

79. Thompson MG, Clippard J, Petrie JG, Jackson ML, McLean HQ, Gaglani M, et al. Influenza Vaccine Effectiveness for Fully and Partially Vaccinated Children 6 Months to 8 Years Old During 2011–2012 and 2012–2013: The Importance of Two Priming Doses. Pediatr Infect Dis J. 2016 Mar;35(3):299–308.

80. Patel MM, Davis W, Beacham L, Spencer S, Campbell AP, Lafond K, et al. Priming with MF59 adjuvanted versus nonadjuvanted seasonal influenza vaccines in children – A systematic review and a meta-analysis. Vaccine. 2020 Jan;38(3):608–19.

81. Belongia EA, Skowronski DM, McLean HQ, Chambers C, Sundaram ME, De Serres G. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. Expert Rev Vaccines. 2017 Jul 3;16(7):723–36.

82. Zelner J, Petrie JG, Trangucci R, Martin ET, Monto AS. Effects of Sequential Influenza A(H1N1)pdm09 Vaccination on Antibody Waning. J Infect Dis. 2019 Jun 5;220(1):12–9.

83. Thompson MG, Naleway A, Fry AM, Ball S, Spencer SM, Reynolds S, et al. Effects of Repeated Annual Inactivated Influenza Vaccination among Healthcare Personnel on Serum Hemagglutinin Inhibition Antibody Response to A/Perth/16/2009 (H3N2)-like virus during 2010-11. Vaccine. 2016 Feb;34(7):981–8.

84. McLean HQ, Thompson MG, Sundaram ME, Meece JK, McClure DL, Friedrich TC, et al. Impact of Repeated Vaccination on Vaccine Effectiveness Against Influenza A(H3N2) and B During 8 Seasons. Clin Infect Dis. 2014 Nov 15;59(10):1375–85.

85. Plant EP, Fredell LJ, Hatcher BA, Li X, Chiang MJ, Kosikova M, et al. Different Repeat Annual Influenza Vaccinations Improve the Antibody Response to Drifted Influenza Strains. Sci Rep. 2017 Jul 12;7(1):5258.

86. Xu Q, Wei H, Wen S, Chen J, Lei Y, Cheng Y, et al. Factors affecting the immunogenicity of influenza vaccines in human. BMC Infect Dis. 2023 Apr 6;23(1):211.

87. Gaglani M, Spencer S, Ball S, Song J, Naleway A, Henkle E, et al. Antibody Response to Influenza A(H1N1)pdm09 Among Healthcare Personnel Receiving Trivalent Inactivated Vaccine: Effect of Prior Monovalent Inactivated Vaccine. J Infect Dis. 2014 Jun 1;209(11):1705–14.

88. Khurana S, Hahn M, Coyle EM, King LR, Lin TL, Treanor J, et al. Repeat vaccination reduces antibody affinity maturation across different influenza vaccine platforms in humans. Nat Commun. 2019 Jul 26;10(1):3338.

89. Andrews SF, Kaur K, Pauli NT, Huang M, Huang Y, Wilson PC. High Preexisting Serological Antibody Levels Correlate with Diversification of the Influenza Vaccine Response. Sandri-Goldin RM, editor. J Virol. 2015 Mar 15;89(6):3308–17.

90. Wall D.J., Patel M.M., Chung J.R., Lee B., Dawood F.S. Antibody response and protection after receipt of inactivated influenza vaccine: A systematic review. Pediatrics. 2021;147(6):e2020019901.

91. Ito K, Mugitani A, Irie S, Ishibashi M, Takasaki Y, Shindo S, et al. Prior vaccinations improve immunogenicity of inactivated influenza vaccine in young children aged 6 months to 3 years: A cohort study. Medicine (Baltimore). 2018 Jul;97(29):e11551.

92. Jones-Gray E, Robinson EJ, Kucharski AJ, Fox A, Sullivan SG. Does repeated influenza vaccination attenuate effectiveness? A systematic review and meta-analysis. Lancet Respir Med. 2023 Jan;11(1):27–44.

93. Davenport FM, Hennessy AV, Francis T, With the Technical Assistance of Phyllis Fabisch. EPIDEMIOLOGIC AND IMMUNOLOGIC SIGNIFICANCE OF AGE DISTRIBUTION OF ANTIBODY TO ANTIGENIC VARIANTS OF INFLUENZA VIRUS. J Exp Med. 1953 Dec 1;98(6):641–56.

94. Skowronski DM, Chambers C, De Serres G, Sabaiduc S, Winter AL, Dickinson JA, et al. Serial Vaccination and the Antigenic Distance Hypothesis: Effects on Influenza

Vaccine Effectiveness During A(H3N2) Epidemics in Canada, 2010–2011 to 2014–2015. J Infect Dis. 2017 Apr 1;215(7):1059–99.

95. Andrews NJ, Walker WT, Finn A, Heath PT, Collinson AC, Pollard AJ, et al. Predictors of immune response and reactogenicity to AS03B-adjuvanted split virion and non-adjuvanted whole virion H1N1 (2009) pandemic influenza vaccines. Vaccine. 2011 Oct;29(45):7913–9.

96. Michael Marcy S, Kohl KS, Dagan R, Nalin D, Blum M, Jones MC, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine. 2004 Jan;22(5–6):551–6.

97. Halsey NA, Talaat KR, Greenbaum A, Mensah E, Dudley MZ, Proveaux T, et al. The safety of influenza vaccines in children: An Institute for Vaccine Safety white paper. Vaccine. 2015 Dec;33:F1–67.

98. Hervé C, Laupèze B, Del Giudice G, Didierlaurent AM, Tavares Da Silva F. The how's and what's of vaccine reactogenicity. Npj Vaccines. 2019 Sep 24;4(1):39.

99. Kohl KS, Marcy SM, Blum M, Jones MC, Dagan R, Rothstein E. Fever after mmunization: Current Concep Future Scientific Understanding.

100. Nakayama T, Kashiwagi Y, Kawashima H, Kumagai T, Ishii KJ, Ihara T. Alum-adjuvanted H5N1 whole virion inactivated vaccine (WIV) enhanced inflammatory cytokine productions. Vaccine. 2012 Jun;30(26):3885–90.

101. Kashiwagi Y, Miyata A, Kumagai T, Maehara K, Suzuki E, Nagai T, et al. Production of inflammatory cytokines in response to diphtheria-pertussis-tetanus (DPT), *haemophilus influenzae* type b (Hib), and 7-valent pneumococcal (PCV7) vaccines. Hum Vaccines Immunother. 2014 Mar;10(3):677–85.

102. Brown H, Prescott R. Multi-Centre trials and meta-analyses. In: Applied mixed models in medicine. Third edition. Chichester, West Sussex ; Hoboken: John Wiley & Sons Inc; 2015. p. 197–230.

103. Schober P, Vetter TR. Linear Mixed-Effects Models in Medical Research. Anesth Analg. 2021 Jun;132(6):1592–3.

104. Sutherland C, Hare D, Johnson PJ, Linden DW, Montgomery RA, Droge E. Practical advice on variable selection and reporting using Akaike information criterion. Proc R Soc B Biol Sci. 2023 Sep 27;290(2007):20231261.

105. Burnham KP, Anderson DR. Multimodel Inference: Understanding AIC and BIC in Model Selection. Sociol Methods Res. 2004 Nov;33(2):261–304.

106. Rodriguez Weber MA, Claeys C, Doniz CA, Feng Y, Innis BL, Jain VK, et al. Immunogenicity and Safety of Inactivated Quadrivalent and Trivalent Influenza Vaccines in Children 18–47 Months of Age. Pediatr Infect Dis J. 2014 Dec;33(12):1262–9.

107. Langley JM, Carmona Martinez A, Chatterjee A, Halperin SA, McNeil S, Reisinger KS, et al. Immunogenicity and Safety of an Inactivated Quadrivalent Influenza Vaccine Candidate: A Phase III Randomized Controlled Trial in Children. J Infect Dis. 2013 Aug 15;208(4):544–53.

108. Jain VK, Domachowske JB, Wang L, Ofori-Anyinam O, Rodríguez-Weber MA, Leonardi ML, et al. Time to Change Dosing of Inactivated Quadrivalent Influenza Vaccine in Young Children: Evidence From a Phase III, Randomized, Controlled Trial. J Pediatr Infect Dis Soc. 2017 Jan 6;piw068.

109. Wang L, Chandrasekaran V, Domachowske JB, Li P, Innis BL, Jain VK. Immunogenicity and Safety of an Inactivated Quadrivalent Influenza Vaccine in US Children 6–35 Months of Age During 2013–2014: Results From A Phase II Randomized Trial. J Pediatr Infect Dis Soc. 2016 Jun;5(2):170–9.

110. Langley JM, Wang L, Aggarwal N, Bueso A, Chandrasekaran V, Cousin L, et al. Immunogenicity and Reactogenicity of an Inactivated Quadrivalent Influenza Vaccine Administered Intramuscularly to Children 6 to 35 Months of Age in 2012–2013: A Randomized, Double-Blind, Controlled, Multicenter, Multicountry, Clinical Trial. J Pediatr Infect Dis Soc. 2015 Sep;4(3):242–51.

111. Jain VK, Rivera L, Zaman K, Espos RA, Sirivichayakul C, Quiambao BP, et al. Vaccine for Prevention of Mild and Moderate-to-Severe Influenza in Children. N Engl J Med. 2013 Dec 26;369(26):2481–91.

112. Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ. 2010 May 27;340(may27 1):c2649–c2649.

113. Li-Kim-Moy J, Yin JK, Rashid H, Khandaker G, King C, Wood N, et al. Systematic review of fever, febrile convulsions and serious adverse events following administration of inactivated trivalent influenza vaccines in children. Eurosurveillance [Internet]. 2015 Jun 18 [cited 2024 Dec 31];20(24). Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES2015.20.24.21159

114. Stockwell MS, Broder KR, Lewis P, Jakob K, Iqbal S, Fernandez N, et al. Assessing Fever Frequency After Pediatric Live Attenuated Versus Inactivated Influenza Vaccination. J Pediatr Infect Dis Soc. 2016 Jun 14;piw028.

115. Long SS, Deforest A, Smith DG, Lazaro C, Wassilak SGF, Pennridge Pediatric Associates. Longitudinal Study of Adverse Reactions Following Diphtheria-Tetanus-Pertussis Vaccine in Infancy. Pediatrics. 1990 Mar 1;85(3):294–302.

116. Saleh E, Swamy GK, Moody MA, Walter EB. Parental Approach to the Prevention and Management of Fever and Pain Following Childhood Immunizations: A Survey Study. Clin Pediatr (Phila). 2017 May;56(5):435–42.

117. Ahn SH, Zhiang J, Kim H, Chang S, Shin J, Kim M, et al. Postvaccination Fever Response Rates in Children Derived Using the Fever Coach Mobile App: A Retrospective Observational Study. JMIR MHealth UHealth. 2019 Apr 22;7(4):e12223.

118. Bancos S, Bernard MP, Topham DJ, Phipps RP. Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. Cell Immunol. 2009;258(1):18–28.

119. Ryan EP, Pollack SJ, Murant TI, Bernstein SH, Felgar RE, Phipps RP. Activated Human B Lymphocytes Express Cyclooxygenase-2 and Cyclooxygenase Inhibitors Attenuate Antibody Production. J Immunol. 2005 Mar 1;174(5):2619–26.

120. Bernard MP, Phipps RP. Inhibition of cyclooxygenase-2 impairs the expression of essential plasma cell transcription factors and human B-lymphocyte differentiation. Immunology. 2010 Jan;129(1):87–96.

121. Ayoub SS. Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. Temperature. 2021 Oct 2;8(4):351–71.

122. Graham GG, Kieran F. Scott. Mechanism of Action of Paracetamol. Am J Ther. 2005;12(1):46–55.

123. Lee JJ, Simmons DL. Antipyretic therapy: clinical pharmacology. In: Handbook of Clinical Neurology. 2018. p. 869–81. (3; vol. 57).

124. Sharma CV, Mehta V. Paracetamol: mechanisms and updates. Contin Educ Anaesth Crit Care Pain. 2014 Aug;14(4):153–8.

125. Steiner AA, Hunter JC, Phipps SM, Nucci TB, Oliveira DL, Roberts JL, et al. Cyclooxygenase-1 or -2—which one mediates lipopolysaccharide-induced hypothermia? Am J Physiol-Regul Integr Comp Physiol. 2009 Aug;297(2):R485–94.

126. Flanagan KL, Jensen KJ. Sex Differences in Outcomes of Infections and Vaccinations in Under Five-Year-Old Children. In: Sex and Gender Differences in Infection and Treatments for Infectious Diseases [Internet]. Springer International Publishing; 2015. p. 273–312. Available from: https://doi.org/10.1007/978-3-319-16438-0_10

127. Engler RJM. Half- vs Full-Dose Trivalent Inactivated Influenza Vaccine (2004-2005): Age, Dose, and Sex Effects on Immune Responses. Arch Intern Med. 2008 Dec 8;168(22):2405.

128. Petrie JG, Ohmit SE, Johnson E, Truscon R, Monto AS. Persistence of Antibodies to Influenza Hemagglutinin and Neuraminidase Following One or Two Years of Influenza Vaccination. J Infect Dis. 2015 Dec 15;212(12):1914–22.

129. Fonville JM, Wilks SH, James SL, Fox A, Ventresca M, Aban M, et al. Antibody landscapes after influenza virus infection or vaccination.

APPENDIX

Supplementary Results Tables

Supplementary Table 1: All ages (6 months to 17 years) day 0-1 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	0.874 (0.674,1.133)	**0.014**	1.272 (0.993, 1.63)	**<.0001**	1.018 (0.776, 1.335)	0.118	0.837 (0.659, 1.063)	**<.001**
Antipyretic	0.843 (0.757, 0.939)		0.793 (0.715, 0.879)		0.955 (0.854, 1.068)		0.815 (0.738, 0.9)	
Both	0.925 (0.738, 1.159)		1.106 (0.892, 1.372)		1.311 (1.035, 1.661)		1.038 (0.842, 1.28)	
Age	1.003 (1.003, 1.004)	**<.0001**	1.003 (1.002, 1.004)	**<.0001**	1.005 (1.004, 1.006)	**<.0001**	1.004 (1.003, 1.005)	**<.0001**
Male	0.954 (0.9, 1.012)	0.117	0.949 (0.898, 1.004)	0.068	0.959 (0.902, 1.019)	0.174	0.937 (0.887, 0.988)	**0.017**
Primed	1.059 (0.973, 1.152)	0.182	0.834 (0.77, 0.903)	**<.0001**	0.523 (0.479, 0.57)	**<.0001**	0.89(0.823, 0.963)	**0.004**
Pre-vaccination Log HI titre	3.834 (3.652 , 4.025)	**<.0001**	3.334 (3.173, 3.503)	**<.0001**	4.361 (4.112, 4.625)	**<.0001**	2.394 (2.272, 2.522)	**<.0001**
N	4928		4932		4928		4932	
AIC	6233.5		5832.1		6637.8		5496	

Results from the linear mixed effects models including study as a random effect, and age, sex, priming status and log_{10} pre-vaccination HI titres as fixed effects. Bolded: statistical significance (P-value < 0.05 or 95% CI not including 1). Results are reported as GMT ratios

and 95% confidence intervals, where a ratio of 1 represents no effect, <1 a reduction in post-vaccination immune titre and >1 an increase in the immune titre. P-values are reported for the F-tests assessing the statistical significance of the fever and/or antipyretic use categorical variable. Estimates are for the effect compared to the base case of a female, unprimed participant experiencing neither fever or antipyretic use, and the effect of a 1 month increase in age and 1 unit increase in the reported pre-vaccination log-HI titre.

Supplementary Table 2: All ages (6 months to 17 years) day 0-3 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	0.992 (0.818, 1.203)	**0.008**	1.155 (0.96, 1.388)	**<.0001**	0.972 (0.795, 1.189)	0.117	0.908 (0.759, 1.085)	**0.001**
Antipyretic	0.836 (0.754, 0.927)		0.779 (0.705, 0.86)		0.954 (0.857, 1.062)		0.819 (0.744, 0.901)	
Both	1.016 (0.87, 1.187)		1.188 (1.024, 1.378)		1.197 (1.018, 1.408)		1.019 (0.882, 1.177)	
Age	1.004 (1.003, 1.004)	**<.0001**	1.003 (1.002, 1.004)	**<.0001**	1.005 (1.004, 1.006)	**<.0001**	1.004 (1.003, 1.005)	**<.0001**
Male	0.954 (0.9, 1.011)	0.114	0.95 (0.898, 1.004)	0.071	0.959 (0.902, 1.019)	0.172	0.936 (0.887, 0.988)	**0.017**
Primed	1.062 (0.976, 1.155)	0.164	0.838 (0.774, 0.907)	**<.0001**	0.524 (0.481, 0.572)	**<.0001**	0.892 (0.825, 0.965)	**0.004**
Pre-vaccination Log HI titre	3.832 (3.649, 4.023)	**<.0001**	3.327 (3.167, 3.495)	**<.0001**	4.353 (4.105, 4.616)	**<.0001**	2.393 (2.271, 2.521)	**<.0001**
N	4928		4932		4928		4932	
AIC	6233.9		5824.1		6639.2		5497.7	

Reference group: no fever or antipyretic use reported D0-3
Linear mixed effects model

Supplementary Table 3: All ages (6 months to 17 years) day 0-6 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	1.103 (0.932, 1.306)	**<.001**	1.260 (1.073, 1.481)	**<.0001**	1.103 (0.925, 1.315)	**0.004**	1.053 (0.901, 1.231)	**<.0001**
Antipyretic	0.820 (0.743, 0.905)		0.800 (0.727, 0.879)		0.948 (0.856, 1.051)		0.821 (0.749, 0.900)	
Both	1.084 (0.953, 1.233)		1.179 (1.042, 1.334)		1.255 (1.097, 1.435)		1.096 (0.972, 1.235)	
Age	1.004 (1.003, 1.005)	**<.0001**	1.003 (1.002, 1.004)	**<.0001**	1.005 (1.004, 1.006)	**<.0001**	1.004 (1.003, 1.005)	**<.0001**
Male	0.954 (0.900, 1.011)	0.115	0.951 (0.899, 1.006)	0.078	0.959 (0.902, 1.019)	0.172	0.937 (0.887, 0.989)	**0.017**
Primed	1.066 (0.980, 1.160)	0.136	0.843 (0.779, 0.913)	**<.0001**	0.528 (0.484, 0.576)	**<.0001**	0.897 (0.83, 0.971)	**0.007**
Pre-vaccination Log HI titre	3.828 (3.647, 4.019)	**<.0001**	3.33 (3.17, 3.499)	**<.0001**	4.355 (4.108, 4.618)	**<.0001**	2.395 (2.273, 2.523)	**<.0001**
N	4928		4932		4928		4932	
AIC	6226.7		5820		6632.3		5494.4	

Reference group: no fever or antipyretic use reported D0-6

Linear mixed effects model

Supplementary Table 4: 6 months to <2 years cohort day 0-1 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	0.926 (0.625, 1.372)	0.774	1.385 (0.945, 2.032)	**0.042**	1.015 (0.685, 1.503)	0.751	0.892 (0.612, 1.302)	0.378
Antipyretic	0.917 (0.776, 1.086)		0.830 (0.705, 0.978)		0.984 (0.832, 1.163)		0.875 (0.744, 1.028)	
Both	0.992 (0.726, 1.356)		1.001 (0.738, 1.358)		1.187 (0.866, 1.627)		1.049 (0.777, 1.417)	
Age	1.121 (1.107, 1.135)	**<.0001**	1.085 (1.071, 1.098)	**<.0001**	1.062 (1.049, 1.076)	**<.0001**	1.097 (1.083, 1.11)	**<.0001**
Male	0.899 (0.806, 1.003)	0.058	0.863 (0.776, 0.96)	**0.007**	0.891 (0.798, 0.993)	**0.038**	0.918 (0.827, 1.02)	0.111
Primed	0.800 (0.671, 0.954)	**0.013**	0.596 (0.502, 0.707)	**<.0001**	0.234 (0.196, 0.279)	**<.0001**	0.560 (0.471, 0.665)	**<.0001**
Pre-vaccination Log HI titre	4.56 (4.103, 5.07)	**<.0001**	4.634 (4.132, 5.198)	**<.0001**	7.567 (6.494, 8.815)	**<.0001**	1.980 (1.736, 2.258)	**<.0001**
N	1571		1573		1571		1574	
AIC	2194		2115.2		2193.9		2073.2	

Reference group: no fever or antipyretic use reported D0-1

Linear mixed effects model

Supplementary Table 5: 6 months to <2 years cohort day 0-3 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	1.112 (0.823, 1.501)	0.831	1.322 (0.987, 1.772)	**0.015**	1.024 (0.758, 1.382)	0.88	0.947 (0.71, 1.264)	0.386
Antipyretic	0.954 (0.811, 1.123)		0.835 (0.713, 0.978)		1.003 (0.853, 1.18)		0.876 (0.75, 1.024)	
Both	0.986 (0.784, 1.24)		1.125 (0.9, 1.407)		1.100 (0.874, 1.385)		1.030 (0.826, 1.284)	
Age	1.121 (1.107, 1.136)	**<.0001**	1.085 (1.072, 1.098)	**<.0001**	1.062 (1.049, 1.076)	**<.0001**	1.096 (1.083, 1.11)	**<.0001**
Male	0.899 (0.806, 1.003)	0.057	0.862 (0.775, 0.959)	**0.006**	0.890 (0.798, 0.993)	**0.037**	0.917 (0.825, 1.018)	0.104
Primed	0.802 (0.673, 0.957)	**0.015**	0.598 (0.504, 0.71)	**<.0001**	0.234 (0.196, 0.28)	**<.0001**	0.562 (0.473, 0.667)	**<.0001**
Pre-vaccination Log HI titre	4.555 (4.098, 5.064)	**<.0001**	4.645 (4.142, 5.21)	**<.0001**	7.54 (6.471, 8.786)	**<.0001**	1.974 (1.731, 2.252)	**<.0001**
N	1571		1573		1571		1574	
AIC	2195.4		2114.1		2195.7		2074.5	

Reference group: no fever or antipyretic use reported D0-3

Linear mixed effects model

Supplementary Table 6: 6 months to <2 years cohort day 0-6 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	1.154 (0.881, 1.511)	0.440	1.393 (1.072, 1.812)	**0.007**	1.128 (0.861, 1.477)	0.397	1.045 (0.806, 1.354)	0.161
Antipyretic	0.928(0.79, 1.09)		0.853 (0.729, 0.998)		0.971 (0.827, 1.141)		0.864 (0.74, 1.008)	
Both	1.077 (0.886, 1.308)		1.11 (0.918, 1.342)		1.153 (0.948, 1.402)		1.098 (0.91, 1.324)	
Age	1.121 (1.107, 1.135)	**<.0001**	1.085 (1.072, 1.099)	**<.0001**	1.062 (1.049, 1.076)	**<.0001**	1.097 (1.083, 1.11)	**<.0001**
Sex	0.899 (0.805, 1.002)	0.055	0.863 (0.776, 0.959)	**0.007**	0.889 (0.797, 0.992)	**0.036**	0.917 (0.826, 1.018)	0.105
Primed	0.807 (0.676, 0.963)	**0.017**	0.601 (0.507, 0.714)	**<.0001**	0.235 (0.197, 0.281)	**<.0001**	0.564 (0.474, 0.669)	**<.0001**
Pre-vaccination Log HI titre	4.557 (4.1, 5.065)	**<.0001**	4.626 (4.125, 5.187)	**<.0001**	7.528 (6.461, 8.77)	**<.0001**	1.971 (1.727, 2.248)	**<.0001**
N	1571		1573		1571		1574	
AIC	2194.2		2113		2194		2073	

Reference group: no fever or antipyretic use reported D0-6

Linear mixed effects model

Supplementary Table 7: 2 years to <9 years cohort day 0-1 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	0.964 (0.71, 1.31)	0.468	1.28 (0.94, 1.743)	**0.003**	1.030 (0.724, 1.467)	**0.003**	0.850 (0.628, 1.148)	0.056
Antipyretic	1.004 (0.875, 1.151)		0.934 (0.812, 1.074)		0.964 (0.823, 1.128)		0.909 (0.793, 1.042)	
Both	1.283 (0.94, 1.751)		1.69 (1.236, 2.312)		1.962 (1.371, 2.807)		1.392 (1.02, 1.899)	
Age	1.003 (1.001, 1.005)	**0.001**	1.00 (0.998, 1.002)	0.758	1.001 (0.999, 1.003)	0.473	1.000 (0.999, 1.002)	0.657
Sex	0.985 (0.921, 1.052)	0.647	0.979 (0.915, 1.048)	0.550	0.948 (0.878, 1.023)	**0.169**	0.926 (0.867, 0.99)	**0.023**
Primed	0.754 (0.685, 0.831)	**<.0001**	0.696 (0.632, 0.766)	**<.0001**	0.565 (0.507, 0.631)	**<.0001**	0.764 (0.693, 0.842)	**<.0001**
Pre-vaccination Log HI titre	3.569 (3.386, 3.762)	**<.0001**	3.077 (2.907, 3.257)	**<.0001**	4.642 (4.34, 4.966)	**<.0001**	2.61 (2.456, 2.773)	**<.0001**
N	2604		2606		2604		2605	
AIC	2324.6		2446.5		3060.9		2323.5	

Reference group: no fever or antipyretic use reported D0-1

Linear mixed effects model

Supplementary Table 8: 2 years to <9 years cohort day 0-3 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	1.074 (0.86, 1.342)	**0.046**	1.173 (0.936, 1.47)	**<.0001**	1.009 (0.781, 1.304)	**0.039**	0.978 (0.785, 1.22)	0.219
Antipyretic	0.949 (0.83, 1.084)		0.903 (0.788, 1.035)		0.952 (0.816, 1.111)		0.917 (0.802, 1.048)	
Both	1.292 (1.066, 1.564)		1.502 (1.236, 1.826)		1.369 (1.097, 1.708)		1.168 (0.964, 1.415)	
Age	1.003 (1.001, 1.005)	**<.001**	1.000 (0.999, 1.002)	0.621	1.001 (0.999, 1.003)	0.419	1.000 (0.999, 1.002)	0.620
Sex	0.984 (0.921, 1.051)	0.626	0.981 (0.916, 1.049)	0.571	0.948 (0.878, 1.023)	0.170	0.926 (0.867, 0.99)	**0.023**
Primed	0.758 (0.689, 0.835)	**<.0001**	0.702 (0.637, 0.773)	**<.0001**	0.568 (0.509, 0.634)	**<.0001**	0.767 (0.696, 0.846)	**<.0001**
Pre-vaccination Log HI titre	3.571 (3.388, 3.764)	**<.0001**	3.065 (2.895, 3.243)	**<.0001**	4.632 (4.33, 4.955)	**<.0001**	2.613 (2.459, 2.777)	**<.0001**
N	2604		2606		2604		2605	
AIC	2320.8		2441.2		3068.1		2328.3	

Reference group: no fever or antipyretic use reported D0-3

Linear mixed effects model

Supplementary Table 9: 2 years to <9 years cohort day 0-6 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	1.128 (0.932, 1.366)	**0.001**	1.233 (1.016, 1.498)	**<.0001**	1.148 (0.92, 1.431)	**0.001**	1.125 (0.93, 1.36)	**0.004**
Antipyretic	0.917 (0.809, 1.039)		0.891 (0.784, 1.013)		0.944 (0.817, 1.091)		0.896 (0.791, 1.016)	
Both	1.309 (1.122, 1.527)		1.424 (1.217, 1.666)		1.428 (1.195, 1.706)		1.259 (1.079, 1.468)	
Age	1.003 (1.002, 1.005)	**<.001**	1.001 (0.999, 1.002)	0.549	1.001 (0.999, 1.003)	0.320	1.001 (0.999, 1.003)	0.493
Sex	0.985 (0.921, 1.052)	0.644	0.982 (0.918, 1.051)	0.605	0.949 (0.879, 1.024)	0.179	0.927 (0.868, 0.991)	**0.025**
Primed	0.764 (0.694, 0.841)	**<.0001**	0.707 (0.642, 0.779)	**<.0001**	0.575 (0.515, 0.642)	**<.0001**	0.774 (0.702, 0.854)	**<.0001**
Pre-vaccination Log HI titre	3.560 (3.377, 3.751)	**<.0001**	3.070 (2.901, 3.249)	**<.0001**	4.633 (4.332, 4.956)	**<.0001**	2.614 (2.46, 2.777)	**<.0001**
N	2604		2606		2604		2605	
AIC	2314.2		2435.8		3059.7		2320.1	

Reference group: no fever or antipyretic use reported D0-6

Linear mixed effects model

Supplementary Table 10: 9 years to 17 years cohort day 0-1 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	0.137 (0.019, 0.981)	**0.042**	0.674 (0.125, 3.637)	0.327	1.374 (0.191, 9.872)	0.932	0.442 (0.092, 2.112)	0.683
Antipyretic	0.7990 (0.583, 1.097)		0.784 (0.598, 1.029)		1.080 (0.786, 1.484)		0.917 (0.714, 1.177)	
Both	0.448 (0.167, 1.201)		0.821 (0.353, 1.91)		1.192 (0.444, 3.199)		1.004 (0.459, 2.193)	
Age	0.999 (0.997, 1.001)	0.41	0.998 (0.996, 1)	**0.049**	0.996 (0.994, 0.999)	**0.004**	1.00 (0.998, 1.002)	0.717
Sex	1.085 (0.94, 1.252)	0.262	1.067 (0.944, 1.207)	0.299	1.070 (0.927, 1.236)	0.356	1.072 (0.957, 1.201)	0.229
Pre-vaccination Log HI titre	2.092 (1.842, 2.376)	**<.0001**	1.420 (1.257, 1.603)	**<.0001**	1.76 (1.550, 1.998)	**<.0001**	2.154 (1.95, 2.38)	**<.0001**
N	753		753		753		753	
AIC	912.1		682.9		917		564	

Reference group: no fever or antipyretic use reported D0-1

Linear mixed effects model

Supplementary Table 11: 9 years to 17 years cohort day 0-3 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	0.584 (0.144, 2.363)	0.398	0.793 (0.241, 2.615)	0.142	1.126 (0.278, 4.564)	0.959	0.731 (0.242, 2.21)	0.881
Antipyretic	0.873 (0.661, 1.154)		0.774 (0.61, 0.982)		1.078 (0.815, 1.424)		0.952 (0.764, 1.186)	
Both	0.569 (0.235, 1.38)		0.682 (0.32, 1.451)		1.032 (0.426, 2.498)		1.153 (0.573, 2.323)	
Age	0.999 (0.997, 1.002)	0.455	0.998 (0.996, 1)	0.051	0.996 (0.994, 0.999)	**0.004**	1.000 (0.998, 1.002)	0.711
Sex	1.081 (0.936, 1.248)	0.289	1.07 (0.947, 1.21)	0.278	1.071 (0.927, 1.237)	0.349	1.069 (0.954, 1.198)	0.251
Pre-vaccination Log HI titre	2.104 (1.851, 2.39)	**<.0001**	1.422 (1.26, 1.605)	**<.0001**	1.761 (1.551, 2)	**<.0001**	2.163 (1.958, 2.389)	**<.0001**
N	753		753		753		753	
AIC	918.6		682.1		918.3		566	

Reference group: no fever or antipyretic use reported D0-3

Linear mixed effects model

Supplementary Table 12: 9 years to 17 years cohort day 0-6 fever and antipyretic use

	A/H1N1		**A/H3N2**		**B/Victoria**		**B/Yamagata**	
	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value	GMT (95% CI)	p-value
Fever	1.342 (0.5, 3.602)	0.160	1.252 (0.539, 2.912)	0.175	1.177 (0.439, 3.157)	0.767	0.993 (0.454, 2.171)	0.952
Antipyretic	0.759 (0.59, 0.978)		0.809 (0.651, 1.004)		1.139 (0.884, 1.467)		0.942 (0.772, 1.15)	
Both	0.805 (0.358, 1.806)		0.697 (0.349, 1.392)		0.937 (0.417, 2.103)		1.000 (0.527, 1.897)	
Age	0.999 (0.997, 1.001)	0.445	0.998 (0.996, 1)	**0.047**	0.996 (0.994, 0.999)	**0.004**	1.000 (0.998, 1.002)	0.709
Sex	1.077 (0.933, 1.243)	0.312	1.068 (0.945, 1.208)	0.291	1.073 (0.929, 1.239)	0.337	1.070 (0.955, 1.199)	0.246
Pre-vaccination Log HI titre	2.098 (1.846, 2.383)	**<.0001**	1.421 (1.258, 1.605)	**<.0001**	1.758 (1.549, 1.996)	**<.0001**	2.164 (1.959, 2.39)	**<.0001**
N	753		753		753		753	
AIC	917.4		683.7		918.5		567.4	

Reference group: no fever or antipyretic use reported D0-6

Linear mixed effects model

Supplementary Table 13: Estimates of the effect of age (months) upon post-vaccination geometric mean tires

	A/H1N1		A/H3N2		B/VICTORIA		B/YAMAGATA	
All ages (6m – 17yrs)	1.004 (1.003, 1.005)	**<.0001**	1.003 (1.002, 1.004)	**<.0001**	1.005 (1.004, 1.006)	**<.0001**	1.004 (1.003, 1.005)	**<.0001**
6m - <2yrs	1.121 (1.107, 1.135)	**<.0001**	1.085 (1.072, 1.099)	**<.0001**	1.062 (1.049, 1.076)	**<.0001**	1.097 (1.083, 1.11)	**<.0001**
2yrs - <9yrs	1.003 (1.002, 1.005)	**<.001**	1.001 (0.999, 1.002)	0.549	1.001 (0.999, 1.003)	0.320	1.001 (0.999, 1.003)	0.493
9 years and older	0.999 (0.997, 1.001)	0.41	0.998 (0.996, 1)	0.051	0.996 (0.994, 0.999)	**0.004**	1.00 (0.998, 1.002)	0.717

Estimates of effect extracted from the linear mixed effects models including study as a random effect, and age, sex, priming status and log_{10}pre-vaccination HI titres as fixed effects. Estimates included from the time-period model with the lowest AIC for each strain/age cohort. Bolded: statistical significance (P-value < 0.05 or 95% CI not including 1). Results are reported as GMT ratios and 95% confidence intervals for the effect of a 1 month increase in age, where a ratio of 1 represents no effect, <1 a reduction in post-vaccination immune titre and >1 an increase in the immune titre. P-values are reported for the F-tests assessing the statistical significance of the fever and/or antipyretic use categorical variable.

Supplementary Table 14: Estimates of the effect of male sex upon post-vaccination geometric mean tires

	A/H1N1		A/H3N2		B/VICTORIA		B/YAMAGATA	
All ages (6m – 17yrs)	0.954 (0.900, 1.011)	0.115	0.951 (0.899, 1.006)	0.078	0.959 (0.902, 1.019)	0.172	0.937 (0.887, 0.989)	**0.017**
6m - <2yrs	0.899 (0.806, 1.003)	0.058	0.863 (0.776, 0.959)	**0.007**	0.891 (0.798, 0.993)	**0.038**	0.918 (0.827, 1.02)	0.111
2yrs - <9yrs	0.985 (0.921, 1.052)	0.644	0.982 (0.918, 1.051)	0.605	0.949 (0.879, 1.024)	0.179	0.927 (0.868, 0.991)	**0.025**
9 years and older	1.085 (0.94, 1.252)	0.262	1.07 (0.947, 1.21)	0.278	1.070 (0.927, 1.236)	0.356	1.072 (0.957, 1.201)	0.229

Supplementary Table 15: Estimates of difference between the unprimed and primed cohort upon post-vaccination geometric mean tires

	A/H1N1		A/H3N2		B/VICTORIA		B/YAMAGATA	
ALL AGES (6M – 17YRS)	1.066 (0.980, 1.160)	0.136	0.843 (0.779, 0.913)	**<.0001**	0.528 (0.484, 0.576)	**<.0001**	0.897 (0.83, 0.971)	**0.007**
6M - <2YRS	0.802 (0.673, 0.957)	**0.015**	0.601 (0.507, 0.714)	**<.0001**	0.234 (0.196, 0.28)	**<.0001**	0.562 (0.473, 0.667)	**<.0001**
2YRS - <9YRS	0.764 (0.694, 0.841)	**<.0001**	0.707 (0.642, 0.779)	**<.0001**	0.575 (0.515, 0.642)	**<.0001**	0.774 (0.702, 0.854)	**<.0001**
9 YEARS AND OLDER	N/A		N/A		N/A		N/A	

Supplementary Table 16: Estimates of the effect of pre-vaccination Log_{10} HI titre upon post-vaccination geometric mean tires

	A/H1N1		A/H3N2		B/VICTORIA		B/YAMAGATA	
ALL AGES (6M – 17YRS)	3.828 (3.647, 4.019)	**<.0001**	3.33 (3.17, 3.499)	**<.0001**	4.355 (4.108, 4.618)	**<.0001**	2.395 (2.273, 2.523)	**<.0001**
6M - <2YRS	4.555 (4.098, 5.064)	**<.0001**	4.626 (4.125, 5.187)	**<.0001**	7.54 (6.471, 8.786)	**<.0001**	1.974 (1.731, 2.252)	**<.0001**
2YRS - <9YRS	3.560 (3.377, 3.751)	**<.0001**	3.070 (2.901, 3.249)	**<.0001**	4.633 (4.332, 4.956)	**<.0001**	2.614 (2.46, 2.777)	**<.0001**
9 YEARS AND OLDER	2.092 (1.842, 2.376)	**<.0001**	1.422 (1.26, 1.605)	**<.0001**	1.76 (1.550, 1.998)	**<.0001**	2.154 (1.95, 2.38)	**<.0001**