IgG Antibody Responses to SARS-CoV-2 Vaccination in Manitoba, Canada

by

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Abstract

Understanding variables that influence antibody responses to SARS-CoV-2 vaccination within a population can provide valuable information on future vaccination strategies. In this study, I examined the antibody responses to SARS-CoV-2 vaccination in Manitoba, Canada using serum specimens collected by Cadham Provincial Laboratory between March 2020 and March 2023. Specimens were tested for spike and nucleocapsid IgG against SARS-CoV-2 using clinically validated assays. I assessed the impacts of multiple factors on post-vaccination antibody titres including type of vaccine, age, sex, geographic location, number of doses received, timing of vaccination, and influence of previous infections. My investigation demonstrated that vaccination with one dose of Moderna mRNA-1273 elicited higher anti-spike IgG titres overall compared to Pfizer BNT162b2 vaccination, while one dose of Pfizer BNT162b2 followed by a second dose of Moderna mRNA-1273 exhibited higher titres than two doses of Pfizer BNT162b2 or Moderna mRNA-1273, irrespective of age. Age had the greatest effects on antibody responses, with older age groups exhibiting consistently lower anti-spike IgG titres of than younger ages. Antibody titres did not appear to be affected by sex or geographic location. My results identify how factors such as age and type of vaccine can influence antibody responses to vaccination. This information highlights the importance of tailoring vaccine regimens to those at increased risk of severe COVID-19 and can be used to inform future vaccination strategies, scheduling of booster doses and public health measures.

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Dedication

This thesis is dedicated to all the frontline workers who risked their lives to keep their communities safe during the COVID-19 pandemic.

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Abbreviations

2019-nCoV	2019 novel coronavirus
ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
ANCOVA	Analysis of covariance
Ang I	Angiotensin I
Ang II	Angiotensin II
APC	Antigen-presenting cell
ARDS	Acute respiratory distress syndrome
BALF	Bronchoalveolar lavage fluid
CAF	Canadian Armed Forces
CBC	Complete blood count
CDC	Center for Disease Control
CERB	Canadian Emergency Response Benefit
ChAd	Chimpanzee Adenovirus
CMIA	Chemiluminescent Microparticle Immunoassay
COVAX	COVID-19 Vaccines Global Access
COVID-19	Coronavirus infectious disease 2019
CPL	Cadham Provincial Laboratory
CTD1	C-terminal domain 1
CTD2	C-terminal domain 2
CTL	Cytotoxic T lymphocyte
dpso	Days post symptom onset
DSPC	1,2-distearoyl-sn-glycero-3- phosphocholine
E	Envelope
EBOV	Ebola virus
gRNA	Genomic RNA
hACE2	Human angiotensin-converting enzyme 2
HCoV-229E	Human coronavirus-229E
HCoV-HKU1	Human coronavirus-HKU1
HCoV-NL63	Human coronavirus-NL63
HCoV-OC43	Human coronavirus-OC43
HCoVs	Human coronaviruses
HICWM	Hubei Integrated Chinese and Western Medicine
ICTV	International Committee on the Taxonomy of Viruses
IERHA	Interlake-Eastern Regional Health Authority
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHR	International Health Regulations
IL-6	Interleukin 6
IL-8	Interleukin 8
LMICs	Low- and middle-income countries
LNP	Lipid nanoparticle
LRT	Lower respiratory tract
LTC	Long-term care
M	Matrix
MERS	Middle East respiratory syndrome
MERS-CoV	Middle East respiratory syndrome coronavirus
MHC	Major histocompatibility class
MVA	Modified vaccinia virus Ankara
N	Nucleocapsid
NAb	Neutralizing antibody
NET	Neutrophil extracellular trap
NHR	Northern Health Region
nsp	Non-structural protein
NTD	N-terminal domain
ORF	Open reading frame
PAMP	Pathogen-associated molecular pattern
PEG	Polyethylene glycol
PHAC	Public Health Agency of Canada
PHIMS	Public Health Information Management System
PMH	Prairie Mountain Health
PPE	Personal protective equipment
PRR	Pattern recognition receptor
RAS	Renin-angiotensin system
RBD	Receptor-binding domain
RdRp	RNA-dependent RNA polymerase
RHA	Regional Health Authority
RNP	Ribonucleoprotein
RT-PCR	Reverse transcriptase polymerase chain reaction
RTC	Replication and transcription complex
S	Spike
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SARSr-CoV	Severe Acute Respiratory Syndrome-related Coronavirus

sg	Subgenomic
sgRNA	subgenomic RNA
SHSS	Southern Health-Santé Sud
ssRNA	Single stranded RNA
TAG-VE	Technical Advisory Group on Virus Evolution
TMPRSS2	Type II transmembrane serine protease
TNF-α	Tumor necrosis factor α
URT	Upper respiratory tract
VOC	Variant of concern
VOI	Variant of interest
VSV	Vesicular stomatitis virus
WHA	World Health Assembly
WHO	World Health Organization
WIV	Wuhan Institute of Virology
WRHA	Winnipeg Regional Health Authority

1 INTRODUCTION

1.1 SARS-CoV-2

1.1.1 The Emergence of SARS-CoV-2

In December 2019, several health facilities in Wuhan, Hubei Province, China began reporting clusters of patients with pneumonia of unknown etiology that responded poorly to conventional treatments[1–3]. The first patient was hospitalized on December 12, 2019[4,5]. However, it was not until December 26, 2019, that Dr. Jixian Zhang noticed that four atypical cases of pneumonia were admitted to Hubei Integrated Chinese and Western Medicine (HICWM) Hospital, three of which were from the same family. The cases were reported to the local Chinese Center for Disease Control (CDC) by Zhang on December 27. Three more cases of pneumonia of unknown etiology were identified in the same hospital over the following two days[6].

It has been reported that Chinese government records suggest the first confirmed case of COVID-19 can be traced back to a 55 year-old resident of Hubei on November 17, 2019[7,8]. A study attempting to date the first cases of COVID-19 found that November 17 was the most likely timing of the first case of COVID-19[9]. The earliest date of symptom onset among laboratory-confirmed cases was December 1, 2019[10], indicating that this patient was also likely infected in mid-late November 2019.

The Wuhan Municipal Health Commission notified the World Health Organization (WHO) and the public of a pneumonia outbreak of unknown cause on December 31, 2019[2,6,10]. That same day, 59 suspected cases were transferred to Jin Yintan Hospital in Wuhan and placed in isolation. Patients underwent extensive testing, including a complete blood count (CBC), coagulation profile, and serum, biochemical testing. Respiratory specimens including nasal and pharyngeal swabs, sputum, bronchial lavage fluid (BALF), and bronchial aspirates were also collected and tested for common respiratory viruses, as well as SARS-CoV and MERS-CoV using real-time RT-PCR. Routine bacterial and fungal tests were also conducted[10].

The epidemiological investigation by the Wuhan Health Commission revealed that the majority of patients had been linked to the Huanan Seafood Market, which was known to sell seafood and a

variety of live animals, including poultry, bats, marmots, and other wild animals[2,11,12]. The market was closed on January 1, 2020 due to fears of a repeat of the 2002-2003 severe acute respiratory syndrome (SARS) outbreak[1]. Twenty-seven (66%) of the initial patients reported direct exposure to the market; however the first patient identified did not have any previous exposure to the market, and no epidemiological link between this patient and later cases was identified[10].

The etiologic agent was identified on January 7, 2020 by the Wuhan Institute of Virology and tentatively named novel coronavirus 2019 (2019-nCoV)[5,6]. A pan-CoV PCR primers was used to test the samples, since the outbreak occurred in the same type of environment as the SARS outbreak. Metagenomic analysis of a sample collected from BALF was used to identify potential etiological agents. The genome was constructed using de novo assembly and targeted PCR, revealing a SARS-related coronavirus (SARSr-CoV). This sequence was compared to sequences obtained from four other patients and found to be over 99.9% identical to one another, sharing 79.6% sequence identity to SARS-CoV. Pairwise protein analysis confirmed that the unknown virus was a SARS-rCoV[5]. The first sequences of 2019-nCoV were published on January 12, 2020, and by the next day diagnostic testing kits were already available[2,13].

Cases of pneumonia resulting from 2019-nCoV continued to grow, causing the city of Wuhan to shut down on January 23, 2020. Within 1 month the virus had spread to all 34 provinces in China. The first laboratory-confirmed case outside of China was identified on January 13, 2020 in Thailand[1]. By late January thousands of new cases were being diagnosed daily, prompting the WHO to declare the novel coronavirus outbreak a public health emergency of international concern on January 30, 2020[2,6]. 2019-nCoV was renamed SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV), and the WHO named the disease COVID-19 on February 11, 2020[1,2]. The outbreak was officially declared a pandemic by the WHO on March 11, 2020[2,4,14]. As of February 15, 2024 there have been over 774 million reported cases of COVID-19, and over 7 million deaths[15].

1.1.2 SARS-CoV-2 Origins

The topic of how SARS-CoV-2 emerged in humans has been the subject of much debate and controversy, in part due to political involvement and widespread misinformation. The majority of

debates have been centred on whether SARS-CoV-2 emerged zoonotically or through a "laboratory escape" scenario, due to the close proximity of the Wuhan Institute of Virology (WIV) to the Huanan Market, where most of the initial cases were linked to. While the lab escape hypothesis gained popularity amongst some groups, the scientific consensus remains that the most likely scenario is a zoonotic origin of SARS-CoV-2.

All other human coronaviruses (HCoVs) have zoonotic origins, as do the majority of other human viruses. There are a number of similarities between how SARS-CoV-2 emerged, and how SARS-CoV emerged in 2002 and 2003. Both viruses were associated with markets selling live wild animals, and the closest known relatives to both SARS-CoV and SARS-CoV-2 are from bats in the Chinese province of Yunnan[16–18]. SARS-CoV-2 also shares similarities with the four endemic human coronaviruses: human coronavirus-OC43 (HCoV-OC43), human coronavirus-HKU1 (HCoV-HKU1), human coronavirus-229E (HCoV-229E), and human coronavirus NL63 (HCoV-NL63), all of which have zoonotic origins, but how exactly they emerged remains unknown[16]. HCoV-HKU1 specifically has a highly similar origin story to that of SARS-CoV-2. HCoV-HKU1 was first discovered in Shenzhen, Guangdong, another large Chinese city, in the winter of 2004. Like SARS-CoV-2, it has an unknown animal origin, a furin cleavage site in its spike protein, and causes human pneumonia[16,19]. The way in which SARS-CoV-2 emerged is not unique, having followed similar patterns of emergence to numerous viruses seen before.

The closest relative of SARS-CoV-2 is the bat coronavirus RaTG13, which has 96% genetic similarity. The largest difference between SARS-CoV-2 and RaTG13 is in the receptor-binding domain (RBD) of the S protein. Other pangolin coronaviruses exhibit much higher similarity in the RBD to SARS-CoV-2 than RaTG13, indicating that the ability of the SARS-CoV-2 S protein to bind human ACE2 (hACE2) was a result of natural selection[20,21]. Pangolins are a family of terrestrial mammals with scaly armour that closely resemble anteaters[22]. In addition, RaTG13 and other highly similar bat coronaviruses were not collected by the WIV and were not discovered until after the pandemic began[5,23,24]. Currently, no natural reservoir or intermediate host for SARS-CoV-2 has been found. This may be because the right animal population has not been sampled yet, or because any ancestral virus may be circulating at a low level. Spillover events from animals to humans are rarely capable of sustained transmission, and an even smaller portion result in major outbreaks. Many human pathogens that have been circulating for

decades still do not have a confirmed natural reservoir, including Ebola virus, hepatitis C virus, and poliovirus[16].

A notable feature of SARS-CoV-2 that has been the subject of scrutiny over the virus' origins is the polybasic furin cleavage site at the junction of S1 and S2. As mentioned previously, this site is associated with better fusogenicity and transmissibility of SARS-CoV-2 and has not been observed in other 'lineage B' betacoronaviruses[20,25]. However, there are other human betacoronaviruses, including HCoV-HKU1, HCoV-OC43, and MERS-CoV that have this site, and the high genetic variability of the S protein indicates that this feature evolved naturally, and will likely be discovered in other species in the future[16,20,26–28].

What I do know about how SARS-CoV-2 emerged is that it likely originated from bats and may have had an intermediate host before jumping to humans. The most likely reservoir continues to be *Rhinolophus* spp. (horseshoe bats). A recent study provided additional evidence supporting the zoonotic hypothesis that wildlife trade at the Huanan market is the most likely source of SARS-CoV-2. They also detected the DNA of several possible intermediate hosts in a wildlife stall that had increased SARS-CoV-2 positivity near and within the stall. This included the DNA of raccoon dogs, civets, and bamboo rats among others[29]. While the study does not solve the origin of SARS-CoV-2, it does further the body of evidence in favour of a zoonotic origin.

1.1.3 The SARS-CoV-2 Genome, Structure and Replication

Coronaviruses are enveloped positive-sense (+) single-stranded RNA (ssRNA) viruses belonging to the family *Coronaviridae* in the order *Nidovirales.* The *Coronaviridae* family is divided into the subfamilies *Letovirinae* and *Orthocoronavirinae*. *Orthocoronavirinae* contains the four genera *Alpha-, Beta-, Gamma, and Deltacoronavirus*, which are frequently referred to by the common name coronavirus[30–35]. The *Coronaviridae* family have some of the largest RNA genomes, at approximately 30 kb. Most coronaviruses, including SARS-CoV-2, encode two large polyproteins in the first half of their genome (ORF1a and ORF1b), and multiple smaller structural proteins in the second half. The proteins produced by ORF1a and ORF1b undergo processing into several non-structural proteins including the RNA-dependent RNA polymerase (RdRp)[31]. Genes are transcribed using a mechanism called

"discontinuous extension" of minus strands that produces a nested set of subgenomic (sg) mRNAs[31,36,37]. SARS-CoV-2 contains eight sg mRNAs that encode 13 open reading frames (ORFs)[31].

In SARS-CoV-2, ORF1a and ORF1b produce the two polyproteins, pp1a and pp1ab respectively. pp1ab is the result of a programmed -1 frameshift that causes pp1a to be expressed roughly twice as much as pp1ab[38–40]. The non-structural proteins (nsp) nsp1-11 are co-translationally and post-translationally released from pp1a, and nsp1-10 and nsp12-16 are released from pp1ab via proteolytic cleavage by two cysteine proteases found in nsp3 and nsp5. Nsp1 targets host translation machinery, while nsp2-11 support the viral replication and transcription complex (RTC)[38,40–44]. Nsp12-16 contain the core functions for RNA synthesis, proofreading, and modification[38,41,45,46]. Nsp12 contains the RdRp, which is supported by its two cofactors nsp7 and nsp8 during RNA synthesis[38,41,40,47,48]. Nsp14 has 3'-5' endonuclease activity that provides proofreading during RNA synthesis[38,49]. Nsp10 and nsp13-16 serve as the capping machinery to form the 5' cap[38,41,50–52].

Figure 1 depicts the SARS-CoV-2 genome structure. The structural proteins spike (S), membrane (M), envelope (E), and nucleocapsid (N), along with the accessory proteins 3a, 6, 7a, 7b, 8, and 10 are encoded in the second half of the genome[25,53]. The S protein is embedded in the viral envelope and initiates entry into the host cell by binding to the host receptor angiotensin-converting enzyme 2 (ACE2). The S protein is a homotrimer with two major domains, S1 and S2, with a polybasic cleavage site in between[25,54,55]. S1 contains the RBD, an N-terminal domain (NTD), and two subdomains (SD1 and SD2) that make up the C-terminal domain. The RBD and NTD are the major targets of neutralizing antibodies, and prone to mutations that affect ACE2 binding, immune evasion, and species-specificity[25,56–59]. S2 contains S2', an additional cleavage site that exposes a fusion peptide that anchors the virus to the host cell when activated[25,55]. S2 also mediates host membrane fusion and release of viral RNA into the cytoplasm. A polybasic cleavage site is located at the S1-S2 junction and can be cleaved by the furin protease during virion maturation, and is associated with higher fusogenicity and transmissibility of SARS-CoV-2 by exposing S1 to better interact with ACE2[25,54].

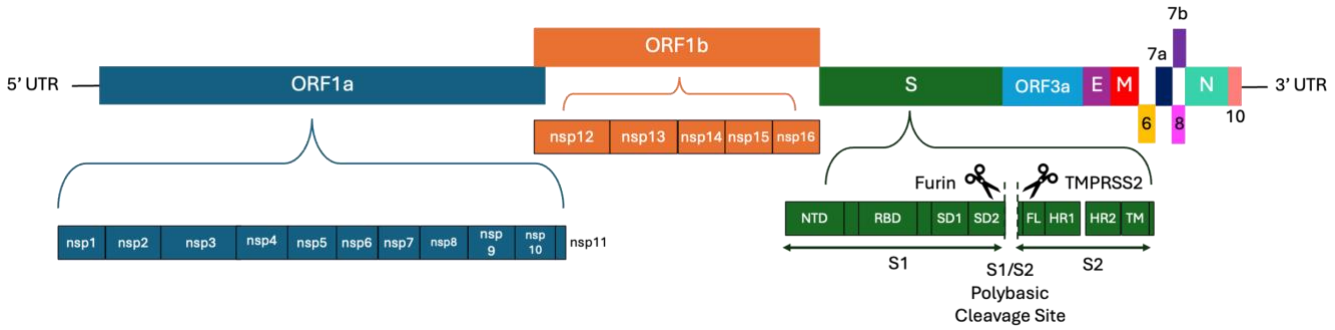

Figure 1. The SARS-CoV-2 genome. ORF1a and ORF1b encode 16 non-structural proteins (nsp1–nsp16) primarily responsible for genome replication. The four structural proteins consist of the spike (S), envelope (E), matrix (M), and nucleocapsid (N) proteins. The S protein mediates host cell binding and is divided into two subunits, S1 and S2, which are separated by a polybasic cleavage site. S1 contains an amino-terminal domain (NTD), the receptor-binding domain (RBD), and two subdomains (SD1 and SD2). S2 contains a fusion loop (FL), two heptad repeats (HR1 and HR2), and a transmembrane domain (TM)[60–62].

In order to enter the host cell after binding to ACE2, the S protein requires proteolytic cleavage of S2' by either the type II transmembrane serine protease TMPRSS2, or the endosomal-lysosomal cysteine protease cathepsin L or cathepsin B, in order to expose the fusion peptide and undergo membrane fusion[25,63,64]. Entry via TMPRSS2 is associated with cell surface entry (early entry pathway), while entry via cathepsin L or cathepsin B is associated with endosomal entry (late entry pathway)[25,64]. The pathway used is dependent on the host cell type and protease expression. Following successful entry and membrane fusion, the SARS-CoV-2 genome is uncoated and released into the cytoplasm[25].

Following viral entry, ORF1a and ORF1b are translated in the cytoplasm to form pp1a and pp1ab, which are then proteolytically processed by nsp3 and nsp5 into the 16 non-structural proteins that go on to form the replication and transcription complex (RTC) that facilitates RNA synthesis[25,65,66]. The RTC replicates the (+) viral genomic RNA (gRNA) to produce full-length negative-sense (-) RNA that then acts as the template to produce additional copies of (+) gRNA that are either packaged into progeny virions or undergo further translation. During (-) strand synthesis, the RTC also synthesizes a nested set of (-) sgRNA strands by discontinuous extension, which then serve as templates to for (+) sgRNAs, which are then translated to produce the structural and accessory proteins[25,37].

The N protein and gRNA assemble into ribonucleoprotein (RNP) complexes that are incorporated into progeny virions[25]. The M protein coordinates the majority of viral packaging[25,67]. The M and E proteins work together to incorporate the S protein and mediate virion maturation[25,67–69]. Viral particles are trafficked to the golgi and trans-golgi network for post-translational modifications, and are then shuttled to the cell surface by lysosomal trafficking, where the lysosomes fuse with the cell membrane and the mature virions bud out[25,70].

1.1.4 Pathogenesis & Symptomology

SARS-CoV-2 infections can range from asymptomatic to life threatening. Whether or not infection progresses to severe disease depends on numerous virus- and host-related factors. Symptoms of COVID-19 typically include cough, sore throat, runny nose, fever, fatigue, headache, body aches, shortness of breath, and loss of taste or smell. Symptoms generally appear within 3–7 days of exposure but can occur anywhere from 1 to 14 days. Symptoms of severe disease can include pneumonia, acute respiratory distress syndrome (ARDS), systemic inflammation and multi-organ dysfunction[71].

SARS-Cov-2 initially targets the multi-ciliated cells of the respiratory tract, due to their expression of ACE2 and TMPRSS2. ACE2 is expressed on the surface of numerous tissues including the lungs, heart, arteries, kidneys and intestines. It's role is to regulate blood pressure as part of the renin-angiotensin system (RAS) by converting angiotensin-II into angiotensin (1–7). RAS is responsible for regulating the cardiac, renal, and vascular systems by controlling blood pressure and fluid balance through a series of reactions beginning with the conversion of angiotensin to angiotensin I (Ang I) by renin. Angiotensin converting enzyme (ACE) then converts Ang I to angiotensin II (Ang II), which is a vasoconstrictor responsible for increasing blood pressure. ACE2 negatively regulates RAS by converting Ang I to Ang1–9, an inactive form of angiotensin. ACE2 also converts Ang II to Ang1–7, a vasodilator that counteracts Ang II by decreasing blood pressure[72,73]. Ang1-7 also exerts anti-inflammatory, anti-apoptosis, and anti-oxidative effects through downstream pathways. SARS-CoV-2 binding in entry results in the cleavage and shedding of ACE2, leading to increased levels of Ang II which promotes inflammation, vasoconstriction, and increased vascular permeability among other effects[72].

In cases of severe COVID-19, the majority of damage is the result of the immune response to infection. Pattern recognition receptors (PRRs) expressed on immune cells recognize pathogen-associated molecular patterns (PAMPs) from SARS-CoV-2, initiating the innate immune response, resulting in the expression of interferons and pro-inflammatory cytokines such as interleukins 6 (IL-6) and 8 (IL-8), and tumour necrosis factor α (TNF-α) among other immune signalling molecules[72,74,75]. Neutrophils and macrophages are recruited by cytokine signalling and contribute to excessive inflammation by forming neutrophil extracellular traps (NETs) and releasing additional pro-inflammatory cytokines[72,76,77]. Over-expression of pro-inflammatory cytokines can trigger a cytokine storm characterized by systemic inflammation, coagulopathy, and other effects that can result in multiorgan failure[72,78].

The adaptive immune response to severe COVID-19 begins with antigen presentation by antigen-presenting cells (APCs), which activate CD4+ and CD8+ T cells. CD4+ cells differentiate into Th1 and Th2 effector cells, which secrete cytokines and interferons in response to infection[72,79]. They also activate CD8+ cytotoxic T lymphocytes (CTLs), trigger B cell differentiation and stimulate antibody production[72,80]. CTLs can target and destroy infected cells by releasing perforin and granzymes. In cases of severe disease, CD4+ CD8+, and regulatory T cell populations are greatly reduced[72,81]. T cell dysfunction and exhaustion, and downregulation of MHC II on B cells are also associated with severe disease[72].

1.2 Transmission Dynamics and Variants of Concern

1.2.1 Modes of SARS-CoV-2 Transmission

The primary route of transmission for HCoVs is through respiratory droplets, however some HCoVs such as SARS have reportedly been transmitted through aerosols, fomites, and by the fecal-oral route[82–85]. In the case of SARS-CoV-2, transmission through respiratory droplets and vertical transmission has been documented. Airborne transmission of SARS-CoV-2 has been confirmed, despite the WHO initially denying airborne transmission at the beginning of the pandemic[86–88]. There have been some reports of possible transmission through direct contact or fomites, however confounding variables make it challenging to determine whether transmission did actually occur through direct contact, or through

respiratory droplets. There is currently no evidence to suggest that SARS-CoV-2 has been transmitted through the fecal-oral route, or by sexual or bloodborne means[89].

Respiratory transmission is the primary mode of SARS-CoV-2 transmission. SARS-CoV-2 replicates in both the upper (URT) and lower respiratory tract (LRT) and can be expelled through normal breathing, coughing, and sneezing[86,89]. Virions are suspended on droplets larger than 5 μm that fall to the ground within about 6 feet, and aerosols under 5 μm that can remain suspended in the air for extended periods of time[89–92]. However, proximity is one of the most important factors in the risk of transmission. Case investigations of densely packed spaces have found that those who were in closer proximity to index cases were more frequently infected than those farther away. This indicates that droplet transmission is more important than aerosol transmission of SARS-CoV-2[89,93–96].

1.2.2 Factors Affecting Transmission

Transmissibility of SARS-CoV-2 is affected by numerous viral, host and environmental factors. The ssRNA genome of SARS-CoV-2 makes it prone to frequent mutations, which can help or hinder transmission. For example, the D614G mutation appeared early on in the pandemic, and increased in prevalence over time. It has been demonstrated that variants with this mutation infect hACE2 cell lines more efficiently than the original Wuhan Hu-1 virus[89,97]. The D614G mutation causes a conformational change in the S protein that increases binding affinity to ACE2, thereby increasing infectivity[98–101]. This higher infectivity, along with decreased mortality seen in this mutation has driven positive selection of the D614G mutation and enabled variants carrying this mutation to become more prevalent than the original Wuhan Hu-1 virus and early variants lacking this mutation[98,100,101].

Not all hosts are equally susceptible to infection and disease by SARS-CoV-2. Factors such as age, sex, lifestyle, comorbidities and immune status can affect susceptibility to disease. SARS-CoV-2 is dependent on host ACE2 for viral entry and infection. This means that viral tropism is determined by the distribution of host ACE2[63,89,102]. When SARS-CoV-2 enters the host respiratory tract, its first targets are the alveolar epithelial cells, vascular endothelial cells, and alveolar macrophages. These cells serve as the starting point for infection and viral replication due to their high levels of ACE2 expression[82,103–105]. In the early stages of disease, viral load is highest in the nasopharynx and oropharynx (URT), whereas in

the later stages of infection viral load is highest in the LRT, indicating that viral replication is initiated in the URT and progresses downward during the course of disease[89,106].

It has been established that susceptibility to infection increases with age, with adults being roughly twice as susceptible to infection than children under the age of 10[89,107–110]. In terms of viral load and shedding dynamics, some studies have found children experience slightly lower viral loads and faster clearance of viral RNA than adults, however other studies have found no differences in viral load between children and adults[111]. Lower levels of ACE2 expression in children than adults may be a partial explanation for decreased susceptibility in children[89,112,113]. However, it remains unclear whether adults are more likely to transmit SARS-CoV-2 than children on the basis of viral load and shedding alone.

Those with compromised immune systems are at increased risk of infection and may be more likely to transmit SARS-CoV-2[89,114]. In immunocompetent individuals, viral loads tend to peak around 5 days post symptom onset (dpso), followed by gradual decline for the remainder of the disease course, with low or undetectable levels of viral RNA 2 weeks after symptoms begin[106,111,115–119]. On some occasions immunocompetent individuals have had detectable levels of viral RNA for up to 28 dpso. In contrast, one study recovered infectious virus up to 61 dpso from nasopharyngeal swabs of immunocompromised patients, while a case report demonstrated isolation of infectious virus up to 78 dpso, indicating that immunocompromised individuals may be able to transmit SARS-CoV-2 longer than immunocompetent individuals[111,120,121].

Environmental factors such as ventilation have also been shown to play an important role in respiratory transmission of SARS-CoV-2. Poor ventilation has been implicated in transmission clusters at numerous locations[89,122–124]. In contrast, events that have occurred outside have rarely been implicated in transmission clusters, with transmission clusters only occurring from crowded events[89,125–127]. Steps can be taken to reduce the risk of respiratory transmission, such as masking, social distancing, and improving ventilation. Even opening windows to improve airflow has been shown to reduce household transmission[89,128].

Geographic location can also impact the transmission dynamics of SARS-CoV-2. There are notable differences in how SARS-CoV-2 is transmitted between rural and urban communities. One study examined the transmission dynamics of a rural outbreak in Hebei, China compared to an urban outbreak

in Tianjin, China. Household contacts were the cause of the majority of infections in both rural and urban communities, however community contacts were also responsible for a large number of infections in rural communities, but not urban communities, who instead saw a greater proportion of cases resulting from subways and office buildings. Rural areas also had a larger proportion of older cases (>65 years old) and younger cases (<20 years old) compared to urban areas. Urban residents were more likely to engage in infection prevention behaviours, and rural residents were less likely to seek medical attention. This may be partly because rural medical professionals were found to be less capable of diagnosing and treating infections than their urban counterparts[129]. This highlights the need to tailor pandemic response plans to specific communities, as opposed to a "one size fits all" approach.

1.2.3 SARS-CoV-2 Variants of Concern

SARS-CoV-2 is constantly evolving due to its ssRNA genome, which is prone to frequent mutations during replication. Mutations that confer an advantage to the virus, such as those that increase transmissibility, become more prominent over time due to natural selection. As the virus evolved over time, different variants emerged with altered transmissibility, severity, and immune escape[130]. Some of these variants were classified as variants of interest (VOIs) or variants of concern (VOCs) due to the advantages conferred by their acquired mutations. The WHO currently defines VOIs as "A SARS-CoV-2 variant with genetic changes that are predicted or known to affect virus characteristics such as transmissibility, virulence, antibody evasion, susceptibility to therapeutics and detectability and identified to have a growth advantage over other circulating variants in more than one WHO region with increasing relative prevalence alongside increasing number of cases over time, or other apparent epidemiological impacts to suggest an emerging risk to global public health". The current definition for VOCs is "A SARS-CoV-2 variant that meets the definition of a VOI and, through a risk assessment, conducted by WHO TAG-VE (Technical Advisory Group on Virus Evolution), and determined to be associated with a moderate or high level of confidence." In addition, a VOC must also have at least one of the following criteria in comparison with other variants: "a) detrimental change in clinical disease severity; OR b) change in COVID-19 epidemiology causing substantial impact on the ability of health systems to provide care to patients with COVID-19 or other illnesses and therefore requiring major public health interventions; OR c) significant decrease in the effectiveness of available vaccines in protecting

against severe disease". These working definitions were updated by the WHO in October 2023, meaning that any variants that emerged before then were classified according to the previous working definitions[131]. As of April 2024, the circulating variants of interest all belong to the Omicron lineage and include XBB.1.5, EG.5, BA.2.86, and JN.1. There are no VOCs currently circulating in Canada as Omicron was de-escalated in August 2023[132].

Prior to the emergence of any VOCs, a single-spike substitution (D614G) evolved early on in the pandemic and became the defining trait of the B.1 lineage[130]. Variants carrying this mutation have glycine at residue 614 (G614) instead of the aspartic acid (D614) found in the original Wuhan Hu-1 strain[133]. The D614G mutation has been found to exhibit increased infectivity and higher viral loads than the original virus, but is not associated with increased disease severity[133–135]. This is thought to be due to a weakened interaction at the S1-S2 interface, leading to a greater proportion of spike trimers in the "open" conformation, allowing the RBD to bind ACE2 more easily[135,136]. Over the course of several months, strains containing the D614G mutation replaced the original Wuhan Hu-1 strain, and by June 2020 it became the dominant circulating strain worldwide[137]. The spike protein of all VOCs to date have contained the D614G mutation[138], which emphasizes the evolutionary advantage it has over D614 found in the original Wuhan Hu-1 strain.

The Alpha variant (B.1.1.7) was identified in the United Kingdom in September 2020, and was the first variant to be designated as a VOC by the WHO[138,139]. This variant was found to have 9 mutations in the spike protein compared to the original Wuhan Hu-1 strain, most notably of which was the N501Y mutation, which was found to enhance binding to the ACE2 receptor and increase viral proliferation and infectivity[140,141]. The N501Y gene was also responsible for some commercial SARS-CoV-2 RT-PCR testing kits to failing to detect the S protein[142,143]. Other notable spike mutations in the Alpha variant include a ΔH69/V70 deletion, which increases infectivity and incorporation of spike into virions, and promotes rapid syncytium formation[144]. The P681H mutation confers resistance to Type I interferon in lung epithelial cells, while N439K and Y453F increase ACE2 binding affinity and enable immune escape[141,144]. As a result of these mutations, the Alpha variant was found to exhibit greater transmission, with a 43-90% higher reproduction number (R_0) than previous variants[142], and an estimated 35% (12-64%) greater risk of death[145]. Fortunately, this variant also had little to no effect on vaccine efficacy[145–159]. Due to the

emergence of other more transmissible VOCs, the Alpha variant has not been seen since September 2021[141,144].

The Beta variant (B.1.351) emerged around the same time as the Alpha variant, and was identified in South Africa in September 2020[160,161]. The three most notable mutations in the RBD of the S protein are K417N, E484K, and N501Y[160]. The K417N mutation works in conjunction with the D614G mutation to promote the open conformation of the spike trimer, enabling ACE2 binding. The K417N and E484K mutations have both been shown to lower susceptibility to neutralizing antibodies[141,162], with E484K greatly enhancing immune escape[135,163–165]. The Beta variant was associated with increased transmissibility, hospitalization, and death[160,166]. While the Alpha and Beta variants had a number of similarities, vaccine efficacy was considerably reduced against the Beta variant compared to the Alpha variant and its predecessors. The Pfizer BNT162b2 vaccine was found to be only 75% effective against infection with Beta[141,167], while the Novavax vaccine had 86% efficacy against the UK variant and 60% efficacy against the South African variant[141,146]. Interestingly, the Moderna mRNA-1273 vaccine had minimal loss of efficacy, and was found to be 96.4% effective against infection by the Beta variant[168]. Fortunately, Pfizer BNT162b2 and Moderna mRNA-1273 still provided over 90% and 95.7% protection against severe disease and death respectively[141,167,168]. The Beta variant was replaced by more transmissible VOCs and is no longer circulating[139].

The Gamma variant (P.1) was reported in Brazil in December 2020, although the earliest sample dates back to September 2020[169]. The variant shares a number of mutations with its predecessors including E484K and N501Y. Gamma also has a K417T mutation in the S protein. These three mutations enhance ACE2 binding and have enabled the Gamma variant to escape neutralizing antibodies at a similar level as the Alpha variant, although Gamma is less resistant to neutralization than Beta[138,141,151,170]. Vaccine efficacy against the Gamma variant was also reduced, with Pfizer BNT162b2 or Oxford-AstraZeneca vaccines only having around 50% efficacy[160,171]. Moderna mRNA-1273 also had reduced protection but maintained a slightly higher efficacy of 61% after two doses[160,172]. Similar to its predecessors, the Gamma variant is no longer circulating due to the emergence of the more transmissible Delta and Omicron variants[139,141,173].

The Delta variant (B.1.617.2) was first identified in India in October 2020, and became the dominant variant globally by June 2021[160]. Similar to previous variants, Delta has the D614G mutation, however it lacks the E484K and N501Y mutations seen in previous variants[141,160]. Delta also has a L452R substitution, which improves the binding to ACE2 and fusogenicity of the viral S2 subunit to host cells[138,174–177]. L452R, along with E484Q and P681R mutations, increase the transmissibility and immune evasion of the Delta variant[138,178,179]. The L452R and T478K mutations are located in the antigenic site I of the RBD, and are associated with decreased vaccine efficacy as they reduce the binding of neutralizing antibodies to the S protein[160,180–182]. Vaccine efficacy appears to vary between studies, with one study reporting two doses of Pfizer BNT162b2 having an efficacy of 88%, while another reported its efficacy had dropped to 51.9% protection against infection[183,184]. However, Pfizer BNT162b2 was found to still be 93.4% protective against severe disease. Moderna mRNA-1273 was found to be 73.1% protective against infection, and 96.1% protective against severe disease[185]. One study only looking at vaccine effectiveness against severe disease reported an efficacy of 93% for mRNA vaccines (Pfizer or Moderna) 20 weeks after a second dose[186]. This indicates that although vaccine efficacy against Delta infection appeared to decline, protection against severe disease was still intact. The Delta variant remained the dominant VOC until late 2021 when it was overtaken by the highly-transmissible Omicron variant[160,187].

The Omicron variant (B.1.1.529/BA.1 and sub-lineages) was first identified in Botswana and South Africa in November 2021[160,187]. Omicron became known for its high transmissibility compared to previous variants, and its ability to subvert the immune responses of those who had been fully vaccinated against SARS-CoV-2. Omicron also had far more mutations than previous variants, and many overlapping mutations with previous variants including D614G, N501Y, K417N and T478K[160]. The mutations in Omicron gave it a higher affinity for ACE2 than Delta, but reduced virulence than its predecessor as Omicron had decreased replication capacity in the lungs due to increased dependence on cathepsins instead of TMPRSS2 for viral entry, and less efficient cleavage of the S protein[188–190]. Vaccine efficacy decreased greatly with the emergence of Omicron, with one study finding that two doses of Pfizer BNT162b2 had a reported effectiveness of 65.5% two to four weeks post-vaccination, which dropped to 8.8% after 25 weeks. Similarly, Moderna mRNA-1273 was 75.1% effective after two to four weeks but dropped to 14.9% after 25 weeks. The same study also found that two doses of ChAdOx1 had no effect against Omicron. While vaccine efficacy increased with the administration of a third booster dose,

efficacy still remained below 75% for all three vaccines[191]. The emergence of increasingly transmissible Omicron-sub-lineages including BA.2, BA.4, BA.5, XBB.1.5, and JN.1, along with further decreases in vaccine efficacy with the emergence of each sub-lineage prompted vaccine manufacturers to release updated formulations targeting Omicron's sub-lineages[192–194].

1.3 Epidemiology and Responses to SARS-CoV-2

1.3.1 Global Response

The global response to the COVID-19 pandemic was one of both groundbreaking successes and profound failures. International cooperation and collaboration led to the fastest development of vaccines in world history, while competition and delayed government responses contributed to excess morbidity and mortality, and inequitable distribution of resources between countries.

Failure to respond in a timely and robust fashion was a common trend by governments and the WHO during the COVID-19 pandemic. Initial delays by the WHO at the start of the pandemic likely contributed to the initial spread of SARS-CoV-2 in China and across the globe. The WHO is governed by the World Health Assembly (WHA), which consists of the health ministers from each member state[195]. The WHA has the authority to implement the International Health Regulations (IHR) in the event of a pandemic, which are "designed to prevent the international spread of disease"[195,196]. These regulations are intended to ensure rapid responses to disease outbreaks by both the WHO and member states, and enables the WHO to declare a Public Health Emergency of International Concern[195,197]. However, since health ministers do not have the authority to make government-wide decisions within their own governments, they also lack the authority to make major decisions for the WHO in public health emergencies. Additionally, insufficient financial backing and convening power makes it difficult for the WHO to enforce regulations in its member states, especially in a timely manner. As a result, the WHO hesitated to make important decisions and announcements in the early stages of the pandemic. The Lancet COVID-19 Commission published an article highlighting some of the key successes and failures by the WHO and governments worldwide during the COVID-19 pandemic. They highlighted five areas where the WHO acted too slowly: (1) the declaration of a Public Health Emergency of International Concern, (2) recognizing human-to-human transmission, (3) recommending travel precautions, (4) recommending

the use of face masks, and (5) acknowledging airborne transmission of SARS-CoV-2[195]. These delays allowed the virus to disseminate worldwide and spread nearly uninhibited for months before the proper prevention measures were implemented.

Overall lack of preparedness and cooperation between governments was another failure to control the COVID-19 pandemic at an international level. Shortfalls in international cooperation disproportionately affected low- and middle-income countries (LMICs), who could not compete in the bid for limited resources such as personal protective equipment (PPE), medical equipment, and vaccines, and were often reliant on aid from high income countries[195]. The COVAX (COVID-19 Vaccines Global Access) initiative was a major failure in international cooperation that primarily impacted LMICs. COVAX was formed to ensure equitable access to COVID-19 vaccines for all countries. While COVAX sounded like a tremendous success for global collaboration, in reality it was frequently unable to deliver sufficient vaccines to LMICs because higher-income governments made independent contracts with vaccine manufacturers at higher prices than what COVAX was able to pay, resulting in vaccines being distributed to high income countries before those who were reliant on COVAX[195]. Many vaccine manufacturing countries also imposed export bans on vaccines in order to ensure sufficient supplies for their own countries, further preventing equitable vaccine distribution through COVAX. This resulted in LMICs being consistently behind higher income countries in vaccine distribution and uptake. Many African countries were the hardest hit by these shortages. By January 2022, high income countries in North America and Europe had vaccinated 60-75% of their population, while the average vaccination rate in Africa was 10%, with some countries such as Nigeria only having a 2% vaccination rate[195,198].

Major differences in the number of cases and deaths were seen between countries. **Table 1** summarizes the five countries with the highest and lowest rates of cases and deaths per 100,000 population. The United States had the highest total number of cases and deaths worldwide. However, Brunei Darussalam had the highest rate of cases per 100,000, while Peru had the highest death rate. At the other end of the spectrum, Niger had the lowest rate of reported cases and deaths per 100,000, at 39 and 1 respectively[199]. That being said, these numbers are most likely highly underreported, as serosurveys in Africa have shown high rates of infection and major underreporting of cases. The Institute

for Health Metrics and Evaluation (IHME) estimated that the total death rate for the WHO African region is over ten times higher than the reported death rate[195].

Table 1. Countries with the highest and lowest reported rates of COVID-19 cases and deaths per 100,000 population[199].

Rank	Country	WHO Region	Population Size[200]	Total Cases	Cases per 100,000	Total Deaths	Deaths per 100,000
Highest Reported Cases per 100,000 Population							
1	Brunei Darussalam	Western Pacific	449,002	345,096	78,883	178	41
2	Cyprus	Europe	1,251,488	691,252	77,843	1,445	163
3	San Marino	Europe	33,660	25,292	74,524	126	371
4	Faroe Islands	N/A	53,090	34,658	70,926	28	57
5	Austria	Europe	9,041,851	6,082,356	68,333	22,534	253
Highest Reported Deaths per 100,000 Population							
1	Peru	Americas	34,049,588	4,524,748	13,723	220,831	670
2	Bulgaria	Europe	6,465,097	1,329,405	19,124	38,700	557
3	Hungary	Europe	9,643,048	2,230,453	22,831	49,051	502
4	Bosnia and Herzegovina	Europe	3,233,526	403,644	12,303	16,388	500
5	Slovenia	Europe	2,111,986	1,356,013	64,700	10,062	480
Lowest Reported Cases per 100,000 Population							
Rank	**Country**	**WHO Region**	**Population Size**	**Total Cases**	**Cases per 100,000**	**Total Deaths**	**Deaths per 100,000**
1	Niger	Africa	26,207,977	9,518	39	315	1
2	Yemen	Eastern Mediterranean	33,696,614	11,945	40	2,159	7
3	Chad	Africa	17,723,315	7,702	47	194	1
4	United Republic of Tanzania	Africa	65,497,748	43,226	72	846	1
5	Sierra Leone	Africa	8,605,718	7,836	98	125	2
Lowest Reported Deaths per 100,000 Population							
1	Niger	Africa	26,207,977	9,518	39	315	1
2	Chad	Africa	17,723,315	7,702	47	194	1
3	United Republic of Tanzania	Africa	65,497,748	43,226	72	846	1
4	South Sudan	Africa	10,913,164	18,823	168	147	1
5	Tajikistan	Europe	9,952,787	17,786	186	125	1

N/A, not applicable.

Countries differed in their use of non-pharmaceutical interventions (NPIs) to control the spread of SARS-CoV-2. Countries in the Western Pacific region implemented suppression strategies to reduce virus transmission. China was especially aggressive with their suppression measures as part of their zero-COVID strategy, which included a strict lockdown of Hubei Province, extensive contact tracing, large-

scale testing, mandatory isolation of known cases and close contacts, and using QR codes to track the movement of residents[195,201]. Other countries in the Western Pacific region adopted similar suppression strategies based on the Asia-Pacific Strategy for Emerging Diseases and Public Health Emergencies used by the WHO after the 2003 SARS epidemic[195,202]. Most of these countries relaxed their suppression strategies with the arrival of the Omicron variant, as it was no longer feasible to maintain such stringent measures, and vaccination coverage was high in most Western Pacific countries. Although cases and deaths did increase with the Omicron wave, they were still much lower than other regions of the world[195].

Prior to the COVID-19 pandemic, the United States and UK were predicted to be the most prepared for future pandemics and epidemics and have the strongest pandemic response capabilities by the Global Security Health Index[195,203]. Despite the optimism, the US and UK were severely underprepared for the COVID-19 pandemic and failed to adequately control the spread of the virus. Most countries in the Americas and Europe implemented a "flatten-the-curve" approach to mitigate virus transmission as opposed to a suppression strategy. These strategies typically included temporary lockdowns to try and reduce the burden on hospitals and slow transmission, but lacked the aggressive testing, contact tracing, and isolation policies used in the Western Pacific[195]. Many countries in the Americas and Europe lifted most restrictions during times when cases were low, only to have to reimplement them during the following wave. Premature lifting of restrictions, especially during times when vaccination coverage was low allowed the virus to spread relatively uninhibited, leading to excess morbidity and mortality[195]. This was particularly problematic during the Omicron wave, which caused the greatest surge in cases and infected over 50% of the global population in the span of five months[195,204].

1.3.2 Canada's Response

Canada fared better than most other G10 countries in their response to the COVID-19 pandemic[205]; however the country's decentralized approach to healthcare led to differences in responses between provinces and territories, and a lack of coordination between the federal and provincial governments resulted in public confusion and a slow vaccine rollout[205–207]. The first recorded case of COVID-19 in Canada occurred on January 25, 2020, when a traveler returned to Toronto from

Wuhan, China[208,209]. Evidence of community transmission was first identified on March 5, 2020, in Vancouver, British Columbia. Several other provinces began to identify more cases of community transmission, leading them to subsequently declare public health emergencies beginning on March 14, 2020[206,207]. All provinces and territories declared a state of emergency during the pandemic, however the federal government did not[210], with the one exception that from February 14 to 23, 2022, the Emergencies Act was invoked by the federal government and a public order emergency was declared due to the illegal blockades and protests against pandemic restrictions and vaccine mandates in Ottawa[211]. Instead, the federal government was responsible for implementing international border restrictions, additional healthcare funding, economic supports, and vaccine and drug procurement and approval[207,212]. The *Quarantine Act* was invoked on March 25, 2020, requiring all incoming international travellers to follow a mandatory 14-day unsupervised quarantine[213]. Economic supports included the Canada Emergency Response Benefit (CERB), which provided $2000 a month to those who experienced a loss of income due to the pandemic, along with various other benefits for caregivers, students, and small businesses[209,214,215].

The Public Health Agency of Canada (PHAC), which was created in 2004 in response to Canada's poor handling of the SARS epidemic, is responsible for developing national clinical and public health guidelines, preventing and controlling infectious diseases, and preparing for and responding to public health emergencies[206,216]. Despite this, PHAC does not have the power to actually require provincial and territorial governments to implement its guidelines and recommendations. Instead, PHAC provided guidance on infection prevention and control measures and the National Advisory Committee on Immunizations (NACI) provided national vaccine guidelines for COVID-19 vaccinations. The responsibility for implementing pandemic restrictions, reopening plans, and creating vaccination eligibility and distribution plans then fell on individual provinces and territories[206]. As a result, cases, hospitalizations, and deaths varied widely across the country. One of the biggest failures by PHAC and the federal government was the lack of a national public health surveillance system that could track cases, hospitalizations, deaths, and vaccinations Canada-wide. PHAC did not have a formal data sharing agreement with provinces and territories prior to the pandemic, meaning that provinces provided epidemiological data on a voluntary basis and the data shared varied by province, making it difficult or impossible to compare how provinces and territories were faring. It also meant that local and provincial

decision-making, contact tracing, and case management efforts were hampered by the lack of national data[206,209].

The decentralization of the Canadian healthcare system means that the majority of pandemic preparedness and response, and public health duties are the responsibility of provincial and territorial governments[217]. Pandemic restrictions varied widely between provinces and territories for this reason. Most provinces implemented similar interventions to curb the spread of SARS-CoV-2 but differed in how and when these interventions were implemented. All provinces and territories declared a state of emergency, introduced the closure of schools and non-essential businesses, restricted gathering sizes, and required masks to be worn in public. COVID-19 interventions differed in regard to travel restrictions, with some provinces restricting travel to northern regions to protect remote Indigenous communities, while other provinces required those traveling out of province to self-isolate upon return. Most provinces and territories required residents to be fully vaccinated in order to visit restaurants and businesses, however Saskatchewan, the Northwest Territories, and Nunavut only required healthcare workers to be fully vaccinated in order to work[217]. These differences in restrictions between provinces also meant that cases and death rates varied as well.

As of July 2024 Canada has had over 4.9 million cases of COVID-19 and nearly 60,000 deaths[218]. Compared to other G10 countries, Canada initially had the second-lowest death rate at 919 per million, with Japan having the lowest (156 per million) in 2022[205]. However, as of October 2024, the death rate climbed to 1,424 per million, greater than the global average of 885 per million, but still considerably lower than the US (3,528 per million) and UK (3,404 per million)[219]. The Canadian government stopped reporting the number of cases by province/territory in June 2024. **Table 2** summarizes the number and rate of cases and deaths in each province/territory up until that point. It is unsurprising that the provinces with the highest case and death counts are those with the largest populations, however when accounting for population size, Prince Edward Island and the Northwest Territories had considerably higher case rates, while Quebec and Manitoba had the highest death rates. The high case rates in PEI and NWT may be at least partially due to their small population sizes compared to other provinces and territories. Quebec and Ontario had particularly high numbers of deaths occurring in long-term care (LTC) facilities[209]. By November 2020, 75% of COVID-19 deaths were LTC residents, with 56% occurring in

Quebec alone, prompting the Canadian Armed Forces (CAF) to send support staff to aid in infection control. A report released by the CAF detailed serious deficits in infection prevention, safety, staffing, and patient care[209,220]. Budget constraints, worker shortages, and increased privatization with insufficient oversight regarding safety were cited as reasons for the poor conditions and outbreak response in these LTC facilities[209,221]. In contrast, LTC facilities in British Columbia fared considerably better due to more funding, better coordination, higher care standards, and a greater number of non-profit facilities compared to private ownership[209,222,223].

Table 2. COVID-19 cases and deaths in Canadian provinces and territories as of June 2024[224].

Province/ Territory	Total Cases	Total Deaths	Cases per 100,000 population	Deaths per 100,000 population
BC	420,303	6953	7616	126
AB	654,678	6419	13,943	137
SK	163,860	2060	13,552	170
MB	161,892	2571	11,127	177
ON	1,719,315	18,649	11,015	119
QC	1,457,500	20,046	16,423	226
NB	95,152	1041	11,400	125
NL	58,755	410	10,909	76
NS	153,081	1099	14,459	104
PEI	58,593	124	33,715	71
YT	4989	32	11,093	71
NWT	11,511	22	25,696	49
NU	3531	7	8,681	17
Canada	**4,963,173**	**59,433**	**12,378**	**148**

Initially, Canada was one of the slowest G10 countries to begin vaccinating its population[205]. The initial rollout of vaccines was marked by supply shortages, delays, and poor coordination between federal and provincial governments. Vaccinations began on December 13, 2020, but only 4% of the population had been vaccinated by March 2021[207,225]. However, by February 2022 Canada had the highest proportion of fully vaccinated people compared to other G10 countries[205]. The Canadian government stopped updating the number of vaccines administered in June 2024, at which point over 107 million doses had been administered. **Table 3** summarizes the number and type of vaccines

administered by province/territory. Pfizer BNT162b2 was the most commonly administered vaccine in the country at 66.1%, followed by Moderna mRNA-1273 at 31.1%, and AstraZeneca ChAdOx1-S at 2.6%. Other vaccines made up less than 1% of all those administered[226]. Over 80% of Canadians have received at least one dose of a SARS-CoV-2 vaccine. The Canadian government has stopped reporting the number of individuals who completed the initial two-dose regimen and switched to reporting only those who are vaccinated per the current recommendations, which is one dose of a JN.1 or KP.2 COVID-19 vaccine for those not previously vaccinated or if it has been at least three to six months since one's last vaccination[226,227]. As of June 2024, only 3.9% of Canadians were vaccinated per recommendations[226]. **Table 4** summarizes the number of individuals vaccinated in each province by vaccination status.

Table 3. Number of vaccinations by province/territory and vaccine type as of June 2024[226].

P/T	Pfizer		Moderna		AstraZeneca		Janssen		Novavax		Medicago		Total	
	n	%[a]	n	%[a]	n	%[a]	n	%[a]	n	%[a]	n	%[a]	n	%[b]
BC	10,016,734	61.5	5,866,960	36.0	388,557	2.4	6,161	0.04	11,424	0.07	0	0	16,289,845	15.2
AB	7,988,168	73.1	2,568,251	23.5	315,311	2.9	8,827	0.08	3,799	0.03	0	0	10,929,713	10.2
SK	2,214,743	71.4	793,344	25.6	92,414	3.0	1,977	0.06	372	0.01	0	0	3,103,871	2.9
MB	2,462,695	66.4	1,155,320	31.2	86,143	2.3	1,565	0.04	807	0.02	0	0	3,706,554	3.5
ON	27,778,630	68.3	11,752,809	28.9	1,087,709	2.7	4,002	0.01	20,881	0.05	0	0	40,645,214	37.9
QC	15,705,685	63.1	8,495,817	34.1	697,743	2.8	188	0.00	5,373	0.02	863	0	24,909,380	23.2
NB	1,531,544	67.6	678,840	30.0	50,974	2.3	466	0.02	671	0.03	0	0	2,264,470	2.1
NL	857,460	53.9	705,130	44.3	27,387	1.7	77	0.01	418	0.03	0	0	1,590,472	1.5
NS	1,937,803	65.5	944,262	31.9	59,855	2.0	461	0.02	649	0.02	0	0	2,958,522	2.8
PEI	329,739	67.2	155,126	31.6	5,962	1.2	130	0.03	86	0.02	0	0	491,047	0.5
YT	17,521	14.4	103,822	85.5	0	0	20	0.02	0	0	0	0	121,363	0.1
NWT	19,963	16.9	98,479	83.1	0	0	0	0	0	0	0	0	118,442	0.1
NU	18,242	20.7	69,670	79.2	8	0.01	0	0	0	0	0	0	87,927	0.1
Canada	70,879,931	66.1	33,387,400	31.1	2,812,063	2.6	23,874	0.02	44,480	0.04	863	0	107,216,820	100

[a]Percent calculated out of total vaccines administered by province. [b]Percent calculated out of total vaccines administered in Canada. P/T, Province/Territory.

Table 4. Number and proportion of individuals vaccinated in Canada by vaccination status as of June 2024[228].

Province/Territory	Received 1+ Dose		Vaccinated per Recommendations	
	n	%	n	%
BC	4,642,396	84.1	457,333	8.3
AB	3,637,825[a]	81.9[a]	103,340	2.2

SK	987,977	81.7	27,641	2.3
MB	1,148,660	79	38,407	2.6
ON	12,622,208	80.9	450,016	2.9
QC	6,998,107	78.9	125, 996[b]	1.5[b]
NB	712,603	85.4	32,179	3.9
NL	504,508	93.7	15,942	3
NS	838,784	79.2	81,184	7.7
PEI	155,143	89.3	9,505	5.5
YT	37,294	82.6	1,682	3.7
NWT	34,250	81.2	1,156	2.7
NU	34,727	85.4	540	1.3
Canada	**28,716,557**	**81.1**	**1,218,925**	**3.9**

[a]Data obtained from COVID-19 Tracker Canada[229]. [b]Data from September 10, 2023 (no data available after).

1.3.3 Manitoba Response

The first case of COVID-19 in Manitoba was identified on March 12, 2020 in a traveller returning from the Philippines[230]. The Manitoba government immediately began implementing measures to mitigate the spread, including restricting public gatherings to 250 people, and limiting visitor access to hospitals. By March 20, the provincial government declared a state of emergency under the *Emergency Measures Act*, reduced gathering sizes to 50 people[231,232]. Additional restrictions were added in the following days including further decreasing gathering size limits to 10 people, closing schools and non-essential businesses, cancelling elective medical procedures, and requiring returning travellers to isolate for 14 days[233–237]. The first death due to COVID-19 in Manitoba was a Winnipeg woman in her 60s who passed away on March 27, 2020[238]. Cases began to stabilize and declined between May and July 2020, prompting the provincial government to lift some restrictions. However, the emergence of the beta variant in late July and early August caused case rates to jump and additional restrictions to be implemented until January 2021, including the requirement that face masks must be worn in all indoor public places[232]. The first 975 doses of the Pfizer BNT162b2 vaccine arrived in Manitoba on December 15, 2020[237,239]. Vaccinations began the following day, starting with high-risk healthcare workers[237,240]. The first doses of Moderna mRNA-1273 were received in early January 2021[237,241].

By mid-February case rates had again declined enough to allow easing of restrictions with the exception that face masks were still required. Case counts remained low until the arrival of the gamma variant in April, which drove up case rates throughout early May. Vaccination rates were still low due to supply shortages, so restrictions were re-introduced until late June. By June 26, 2021, 31% of Manitobans 12 years and older had received both doses of a SARS-CoV-2 vaccine, with that number doubling by August 7[232]. The province once again lifted restrictions, however only vaccinated residents were allowed to visit restaurants, gyms, or other indoor activities such as concerts and sporting events. Residents were provided with proof of vaccination cards that were required to enter restaurants and venues[232,242]. Those who were not fully vaccinated or did not have proof of vaccination were not allowed to enter the premises[232].

In early October 2021 over 70% of eligible Manitobans were fully vaccinated, but the arrival of the Delta variant once again drove up case rates throughout November and December, and further restrictions were implemented. The Omicron variant also arrived soon after, with the first case being reported on December 7, and cases peaking in early January 2022. Cases began to decline mid-January, and positivity rates were low enough by mid-February to allow restrictions to be eased for those with proof of vaccination. At this point in time booster doses were already being administered, and over 38% of Manitobans had received a third dose by February 2022[232]. **Table 5** summarizes the major vaccine eligibility changes that occurred. On March 15, 2022 all remaining pandemic restrictions were lifted in Manitoba[243]. As of June 2024, Manitoba had over 161,000 cases and 2500 deaths, after which the federal and provincial governments stopped updating case and death counts[224].

Table 5. Summary of COVID-19 vaccine eligibility changes in Manitoba. PCH, personal care home. Front line healthcare workers, older ages, Indigenous peoples, and immunocompromised individuals were prioritized for vaccination in Manitoba. NA, not applicable.

Date	Eligibility Changes	Organization	Dose
20/12/09	Pfizer BNT162b2 approved for age 16+	Health Canada	NA
20/12/23	Moderna mRNA-1273 authorized for people 18+	Health Canada	NA
21/02/24	Age 95+; First Nations age 75+	Government of Manitoba	1
21/02/26	Authorized AstraZeneca vaccine for people 18+	Health Canada	NA
21/03/08	Age 80+; First Nation people age 60+	Government of Manitoba	1

Date	Eligibility Changes	Organization	Dose
21/03/15	Age 50-64; First Nations age 30-64 eligible for AstraZeneca vaccine	Government of Manitoba	1
21/03/17	Age 73+; First Nations age 53+	Government of Manitoba	1
21/03/22	Age 65+; First Nations age 45+	Government of Manitoba	1
21/03/29	Age 64+; First Nations age 44+	Government of Manitoba	1
21/04/07	Age 62+; First Nations age 42+	Government of Manitoba	1
21/04/14	Age 59+; First Nations age 39+	Government of Manitoba	1
21/04/21	50+ and First Nations 30+; front-line police and firefighters	Government of Manitoba	1
21/05/03	All Indigenous people 18+	Government of Manitoba	1
21/05/05	Age 45+	Government of Manitoba	1
21/05/05	Authorized Pfizer BNT162b2 vaccine in age 12-15	Health Canada	NA
21/05/07	Age 40+	Government of Manitoba	1
21/05/10	Age 30+	Government of Manitoba	1
21/05/12	Age 18+	Government of Manitoba	1
21/05/14	Ages 12-17 eligible for Pfizer BNT162b2	Government of Manitoba	1
21/05/21	Priority health conditions	Government of Manitoba	2
21/05/24	Indigenous people	Government of Manitoba	2
21/05/25	All Indigenous people; individuals with specific health conditions	Government of Manitoba	2
21/06/25	All 18+	Government of Manitoba	2
21/08/16	All 12+	Government of Manitoba	2
21/09/20	All residents and staff of First Nation PCHs	Government of Manitoba	3
21/10/06	Viral vector vaccine recipients; First Nation health care workers and residents	Government of Manitoba	3
21/10/18	All First Nations living on reserves	Government of Manitoba	3
21/10/27	All PCH residents	Government of Manitoba	3
21/11/03	Age 70+; First Nation, Inuit and Métis age 18+	Government of Manitoba	3
21/11/15	All 18+	Government of Manitoba	3
21/11/19	Age 5-11 eligible for Pfizer vaccine	Government of Manitoba	1
21/12/24	Age 50+	Government of Manitoba	3
22/04/06	Age 70+; PCH residents; First Nations, Inuit and Métis age 50+	Government of Manitoba	4
22/04/13	Age 12 to 17 with medical conditions who belong to racialized/marginalized communities or who live in shelters/group homes	Government of Manitoba	3

Date	Eligibility Changes	Organization	Dose
22/05/20	All 18+	Government of Manitoba	4

The COVID-19 vaccination campaign in Manitoba was not without its share of pushbacks. Misinformation and government distrust fueled opposition to vaccination, particularly in the southern portion of Manitoba. The many residents of the municipalities of Stanley and Hanover, and the cities of Winkler and Steinbach were especially opposed to getting vaccinated, and these areas consistently had the lowest vaccination rates in Manitoba[244–246]. These regions are home to large Mennonite and conservative Christian populations with a historical distrust of governments[245–247]. Some residents went to the extent of protesting vaccine mandates, holding rallies and "freedom convoys"[248–251]. Multiple businesses refused to abide by provincial restrictions, resulting in numerous fines[252]. Several churches challenged the provincial government in court as they believed the pandemic restrictions, which involved the closure of churches, violated the Charter of Rights and Freedoms. The court ruled against their claims and deemed the restrictions reasonable given the circumstances[253]. The opposition to the vaccines was significant enough that local and provincial leaders including pastors, Winkler's chief of police and mayor, and Dr. Joss Reimer, the medical lead of Manitoba's vaccine task force, made statements and created targeted communication campaigns to address vaccine hesitancy[245,246,254]. The majority of Manitobans were unopposed to vaccination, and over 84% have received the initial series of vaccines[255]. **Table 6** summarizes the vaccination rates in Manitoba by health region.

Table 6. COVID-19 vaccination rates in Manitoba by regional health authority. Data obtained from COVID-19 Tracker Canada[255]. WRHA, Winnipeg Regional Health Authority; PMH, Prairie Mountain Health; IERHA, Interlake-Eastern Regional Health Authority; NHR, Northern Health Region; SHSS, Southern Health-Santé Sud.

	Total Doses Administered	% with 1+ Doses[a]	% with 2 Doses[a]	% with 3 Doses[a]
WRHA	1,850,735	93.2	90.1	51.9
PMH	360,133	85.3	81.7	44.4
IERHA	284,248	84.9	81.7	45.4
NHR	143,974	90.8	84.8	33.6
SHSS	357,399	71.5	68.5	34.4
Total	**3,001,252**	**99.3**	**84.6**	**46.7**

[a] Percent of eligible population.

1.4 Vaccines

The rate at which vaccines for SARS-CoV-2 were developed were unprecedented. Prior to the SARS-CoV-2 pandemic, the fastest vaccine to be developed was the mumps vaccine, which took 4 years from development to approval. In contrast, Pfizer BNT162b2 and Moderna mRNA-1273 took 11 months, 8 of which were clinical trials, making them the fastest vaccines ever developed and approved in humans. This accomplishment was the result of decades of previous research on mRNA vaccines and coronaviruses, major financial investments, and the hard work of dedicated scientists, regulatory agencies, and medical personnel[256].

Currently the only SARS-CoV-2 vaccines available in Canada are the Moderna mRNA-1273 (Spikevax), Pfizer BNT162b2 (Comirnaty), and Novavax (Nuxovid). Vaccines that were previously available include AstraZeneca ChAdOx1-S (Vaxzevria), Janssen AD26.COV2.S (Jcovden), and Medicago (Covifenz), which were discontinued in Canada by the manufacturers between 2021 and 2023[194]. **Table 7** summarizes the different vaccines that are currently authorized in Canada, as well as those that were previously authorized.

Table 7. Vaccines currently and previously authorized for use against SARS-CoV-2 in Canada. The vaccines currently authorized for use in Canada are Moderna mRNA-1273 (Spikevax), Pfizer BNT162b2 (Comirnaty), and Novavax (Nuxovid). Vaccines that were previously available include AstraZeneca ChAdOx1-S (Vaxzevria), Janssen AD26.COV2.S (Jcovden), and Medicago (Covifenz)[194,258].

Vaccine	Vaccine Platform	Antigenic Target	Dosage (Primary Series)	Route of Administration	Approval Status	Authorization Date	Updated Booster Available
Pfizer BNT162b2 (Comirnaty)	mRNA	Pre-fusion spike	12+ years: 2 x 30 µg, 21 days apart 5–11 years: 2 x 10 µg, 21 days apart 6 months–4 years: 2 x 3 µg, 21 days apart; then 1 x 3 µg 8+ weeks after	Intramuscular	Approved	2020-12-09	Yes

Moderna mRNA-1273 (Spikevax)	mRNA	Pre-fusion spike	12+ years: 2 x 100 µg, 1 month apart 6–11 years: 2 x 50 µg, 1 month apart 6 months–5 years: 2 x 25 µg, 1 month apart	Intramuscular	Approved	2020-12-23	Yes
AstraZeneca ChAdOx1-S (Vaxzevria)	Viral Vector	Pre-fusion spike	18+ years: 1 x $5x10^{10}$ viral particles (0.5 mL)	Intramuscular	Cancelled by sponsor	2021-02-26	No
Janssen AD26.COV2.S (Jcovden)	Viral Vector	Pre-fusion spike	18+ years: 1 x $5x10^{10}$ viral particles (0.5 mL)	Intramuscular	Cancelled by sponsor	2021-03-05	No
Novavax (Nuvaxovid)	Protein Subunit	Pre-fusion spike	12+ years: 2 x 5 µg, 21 days apart	Intramuscular	Approved	2022-02-17	Yes
Medicago (Covifenz)	Plant-based VLP	Pre-fusion spike	18+ years: 2 x 3.75 µg, 21 days apart	Intramuscular	Cancelled by sponsor	2022-02-24	No

VLP, virus-like particle.

1.4.1 mRNA Vaccines

The SARS-CoV-2 pandemic marks the first use of mRNA vaccines in humans, although the technology behind mRNA vaccines has been in development for over three decades[256,258]. The approval of Pfizer BNT162b2 and Moderna mRNA-1273 have laid the foundation for future mRNA vaccines against numerous infectious diseases and have proven that vaccines can be rapidly developed to combat emerging pandemics.

The formulations of the Pfizer BNT162b2 and Moderna mRNA-1273 vaccines are highly similar. Both vaccines consist of nucleoside modified mRNA encoding the full pre-fusion S protein of SARS-CoV-2, encapsulated by lipid nanoparticles (LNPs)[259,260]. These vaccines both have two nucleoside modifications at residues 986 and 987, in which the original amino acids were replaced with proline (K986P and V987P) to stabilize the pre-fusion conformation of the S protein[261,262]. Additionally, in both vaccines, all uridines have been replaced with N1-methylpseudouridine in order to improve mRNA translation and reduce immune activation[102,256,263,264]. The main difference between the two vaccine formulations are the lipids used. Each vaccine contains proprietary ionizable lipids that are used to improve the conjugation of the mRNA with the lipids and facilitate release of the mRNA into the cytosol. The mRNA is conjugated to the lipids in an acidic environment, giving the lipids a positive charge and

enabling the conjugation. When the vaccine is administered, the lipids are not charged due to the pH of arterial blood being ~ 7.5. Once the vaccine is taken up by endosomes, the pH drops and the lipids return to a positively charged state, releasing the mRNA into the cytosol for translation[265,266].

Pegylated lipids are also used to improve the stability of both vaccines. These are lipids containing polyethylene glycol (PEG), a non-ionic molecule located on the surface of the LNPs to add stability and provide protection against the mononuclear phagocyte system, thereby reducing the clearance of the LNPs. Both vaccines also contain DSPC (1,2-distearoyl-sn-glycero-3- phosphocholine), an auxiliary lipid that adds bulk, and cholesterol to maintain membrane fluidity[265,266]. The vaccines also contain several buffers to maintain pH, and sucrose is used to help the LNPs remain intact during lyophilization or freezing[265]. A complete list of vaccine ingredients and their purpose are shown in **Table 8**. Another difference between Pfizer BNT162b2 and Moderna mRNA-1273 is the dosage. The dosage in ages 12 and up for Pfizer BNT162b2 is two doses of 30 μg, 21 days apart[267]. The dosage for Moderna mRNA-1273 is two doses of 100 μg, 28 days apart[268]. Both vaccines are administered intramuscularly[267,268].

Table 8. Ingredients of SARS-CoV-2 mRNA vaccines.

Pfizer BNT162b2[269]	**Moderna mRNA-1273[270]**	**Purpose[265]**
Tozinameran (mRNA) encodes for the viral spike (S) protein of SARS-CoV-2	Elasomeran (mRNA), encoding the pre-fusion stabilized Spike glycoprotein of 2019 novel Coronavirus (SARS-CoV-2)	Active ingredient; elicits immune response
ALC-0315 [((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)]	SM-102 (Heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecoxyhexyl)amino]octanoate)	Ionizable lipids; improve mRNA conjugation with lipids and release into cytosol
ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide)	PEG2000-DMG (1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000)	Pegylated lipids; improve stability
DSPC (1,2-distearoyl-sn-glycero-3- phosphocholine)	DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine)	Auxiliary lipid; bulking agent
Cholesterol	Cholesterol	Lipid; regulates membrane fluidity
Potassium chloride	Trometamol	Buffers
Disodium hydrogen phosphate	Trometamol hydrochloride	Buffers

Potassium dihydrogen phosphate	Acetic acid	Buffers
Sodium chloride	Sodium acetate trihydrate	Buffers
Sucrose	Sucrose	Cryoprotectant
Water for injections	Water for injections	Dispersant

Pfizer BNT162b2 and Moderna mRNA-1273 were both shown to be highly efficacious in clinical trials, with a vaccine efficacy of 95% and 94.1%, respectively[260,271]. Both vaccines induced S- and RBD-binding antibodies after the first dose, but little to no neutralizing antibodies were present until after the second dose[259,272–274]. Increases in $CD4^+$ T cells secreting T_H1 type cytokines, as well as low levels of $CD8^+$ T cells were also seen after second doses of each vaccine[272,274,275].

Following administration, mRNA vaccines are taken up by antigen-presenting cells into endosomes. The mRNA then escapes the endosome and is translated by ribosomes into protein, forming the antigen. The antigen is degraded by the proteasome complex, and MHC I proteins display the fragments on the cell surface to activate cytotoxic T cells. Once activated, cytotoxic T cells secrete perforin and granzyme to kill infected cells. Secreted antigens can also be taken up by cells, degraded, and displayed on MHC II proteins to activate helper T cells. Helper T cells stimulate B cells to produce neutralizing antibodies and activate phagocytes by releasing inflammatory cytokines in order to clear circulating pathogens[256].

1.4.2 Viral Vector Vaccines

Several vaccine platforms were used to develop vaccines against SARS-CoV-2, including viral vector vaccines. There were two viral vector vaccines approved for use in Canada during the SARS-CoV-2 pandemic: ChAdOx1 (Vaxzevria) made by AstraZeneca, and Ad26.COV2-S (Jcovden) made by Janssen/Johnson & Johnson[194]. The technology behind these vaccines has been in use for over forty years, primarily in veterinary medicine. Only five viral vector vaccines had been approved for human use prior to 2020, one of which was the highly successful vesicular stomatitis virus (VSV) vaccine developed against the Ebola virus (EBOV)[276]. The majority of viral vector vaccines use VSV, modified vaccinia virus Ankara (MVA), or adenovirus platforms. The ChAdOx1 and Ad26.COV2-S vaccines both use a recombinant, replication-deficient adenoviral vector to deliver the full-length SARS-CoV-2 S

protein[274,277,278]. The key difference between the two vaccines is the adenovirus used, ChAdOx1 uses chimpanzee adenovirus (ChAd), while Ad26.COV2-S uses adenovirus type 26[279].

One of the key advantages to using a viral vector platform is the stimulation of the innate immune response without the need for adjuvants, as well as eliciting cellular immune responses on top of antibody responses[276,279]. However, this can sometimes be a hindrance. One of the main issues with adenoviral vector vaccines is pre-existing immunity to adenoviruses can reduce the immunogenicity of the vaccine. This has been especially problematic in adenovirus type 5 vaccines as up to 90% of the population has pre-existing immunity in some countries, which is why the ChAdOx1 and Ad26.COV2-S vaccines contain different adenoviruses with lower rates of pre-existing immunity in the human population[279–283].

The ChAdOx1 and Ad26.COV2-S vaccines were not without their own share of problems. While both vaccines were fairly well tolerated during clinical trials, once they were approved for emergency use authorization and administered to masses, several cases of venous thrombosis and thrombocytopenia were reported, primarily in individuals under the age of 60[279,284–286]. These side effects were highly scrutinized by the public and regulatory officials, and the authorization for use in individuals under the age of 55 was halted[287]. Additionally, the vaccines were less protective than their mRNA-based counterparts, with vaccine efficacy ranging from 62-76% in clinical trials[274,277,288,289]. Many individuals were hesitant to receive a viral vector vaccine due to the inferior efficacy compared to mRNA vaccines and fears over the severe side effects, leading AstraZeneca and Janssen to eventually pull their vaccines from the Canadian market[194].

1.4.3 Other Vaccines

Only two vaccines have been approved for use in Canada that were not mRNA- or viral vector-based, Nuvaxovid from Novavax, and Covifenz from Medicago[194]. Nuvaxovid is a recombinant S protein-subunit vaccine, while Covifenz is a plant-based virus-like particle (VLP) vaccine[257]. Nuvaxovid was found to have an efficacy of around 90% in clinical trials, while Covifenz had an efficacy of 71%[290–292].

Nuxovid contains the full-length pre-fusion S protein, which cloned into a baculovirus and expressed in *Spodoptera frugiperda* Sf9 cells. The vaccine also contains a Matrix-M adjuvant, which

consists of saponin, cholesterol, and phospholipids[293,294]. The vaccine has been advertised as an alternative to mRNA vaccines for those who are unable or unwilling to receive an mRNA vaccine[295]. Nuxovid is still available for use in Canada and has been updated to include the XBB.1.5 variant[194]. As of December 2024 the company is waiting for Health Canada to approve a further updated formulation that protects against the KP.2, and KP.3 variants[296,297].

Covifenz is the first Canadian-made SARS-CoV-2 vaccine to be approved by Health Canada. The vaccine is unique in that it is derived from plants. The full-length pre-fusion S protein is expressed in tobacco plants (*Nicotiana benthamania*). The VLPs assemble spontaneously and accumulate in the space between the plasma membrane and cell wall. The S trimers are anchored to the lipid envelope of the VLP, which is derived from the plant cell membrane. The aerial portions of the plant are then harvested, and a proprietary method is used to extract the VLPs[298]. The vaccine also contains the adjuvant AS03 made by GlaxoSmithKline, which is an oil-in-water emulsion containing squalene and DL-α-tocopherol[293,298,299]. While the vaccine was approved for use in Canada, the WHO rejected the vaccine due to tobacco giant Philip Morris being a Medicago shareholder[300]. Medicago's owners, the Mitsubishi Chemical Group later shut down the company and pulled Covifenz from the Canadian market[301].

1.5 Antibody Responses

1.5.1 Types of Antibody Responses

Antibodies are divided into five classes, immunoglobulin A, D, E, G, and M (IgA, IgD, IgE, IgG, IgM). Each class of antibody has a different role in the adaptive immune response. IgM is the first antibody produced in response to infection. It is primarily located in the blood, and at lower levels in the lymph. IgM tends to have low binding affinity for pathogens but makes up for this by readily forming pentamers that activate the complement system and enable multipoint binding of pathogens, resulting in high avidity[302]. The function of IgD is poorly understood, but is thought to be involved in regulating protective B cell responses and may play a role in mucosal immunity[303]. IgE is responsible for mediating allergic reactions and responses to some parasitic infections[304,305].

IgA is the main driver of mucosal immunity, and is found primarily in mucus membranes, along with the mucosal epithelium of the intestinal and respiratory tracts. It primarily functions as a

neutralizing antibody, as the epithelial surfaces typically lack complement and phagocytes, although it can weakly activate complement[305,306]. IgA can be divided into two subclasses, IgA1 and IgA2. IgA1 is the predominate subclass found in the serum and exists as a monomer, while IgA2 is primarily in mucosal secretions forms a dimer[305].

IgG is the major antibody found in the blood and extracellular fluid, and has the longest serum half-life, making it the most prevalent isotype in the body. It has four subclasses, IgG1 through IgG4, which vary in their serum levels and functions. IgG1 and IgG3 are produced in response to protein antigens, while IgG2 and IgG4 mainly respond to polysaccharide antigens. The major subclass produced may also vary by disease[305]. IgG is capable of activating the complement system, opsonizing pathogens to be engulfed by phagocytes, and function as a neutralizing antibody[305,306].

1.5.2 Antibody Responses to SARS-CoV-2 Infection

Antibody responses to SARS-CoV-2 appear to follow patterns similar to other viral infections, in that IgM tends to appear first, followed by IgA, and then IgG[307–309]. On average, the time to seroconversion for IgM ranges from around four to 14 dpso however there have been cases where IgM against the N protein have been detected as early as one dpso[307,308,310–318]. The time to seroconversion for IgA was similar, ranging from four to 11 dpso on average[310,314,319,320]. The average time to seroconversion for IgG ranges from 12-15 days, but has been reported to take up to 73 dpso[310–315,317,318,321]. IgM titres tend to peak around two to five weeks post symptom onset (PSO), IgA peaks from 16–22 days, and IgG peaks between three and seven weeks PSO. Titres begin to decline after three to five weeks in the case of IgM but have been shown to persist for up to eight weeks before declining. The decline of IgA appears to be rather variable, with studies reporting serum IgA titres to remain steady for at least six months, and mucosal IgA persisting in nasal fluids for four months, although neutralizing IgA appears to wane below detectable limits as early as 70 days PSO[322–327]. IgG persists the longest, with titres remaining detectable for at least 400 days[328].

Neutralizing antibodies (NAbs) are a key part of the antibody response to both SARS-CoV-2 infection and vaccination. IgM, IgA, and IgG are all capable of neutralization, that is, bind to the receptor-binding site on pathogens and preventing binding to host cells[306]. In the case of SARS-CoV-2, NAbs block

the viral S protein from binding ACE2, with the majority of NAbs targeting the RBD, although NAbs targeting the NTD and S2 have also been found[182,329–341]. There are several mechanisms through which neutralization can be achieved. The first, and likely most well-known mechanism is through directly competing with ACE2 by binding to the RBD. Other mechanisms include mimicking receptor interaction and triggering premature S1 shedding, locking the RBD in the closed position which prevents ACE2 binding, using steric hindrance to prevent receptor binding, and targeting conserved distal epitopes which result in premature S1 shedding[56,57,59,182,330,331,333,342–351].

NAbs resulting from SARS-CoV-2 infection typically appear within a few days of symptom onset and peak around four weeks post-infection in most cases. It has been established that NAbs tend to follow a biphasic decline with an initial drop in titres shortly after infection followed by a slow decline over months to years[330,352–358]. However, NAb responses to infection appear to be highly variable. A study by Chia et al. found NAb responses of COVID-19 patients could be grouped into five patterns: negative, rapid waning, slow waning, persistent, and delayed response. Individuals who were negative did not develop NAbs, rapid waning individuals seroreverted within 180 days, slow waning individuals still had NAbs 180 dpso, persistent individuals experienced minimal decline in NAbs, and individuals with a delayed response had an increase in NAbs 90 to 180 dpso. Rates of NAb waning also varied dramatically, with predictions ranging from 40 days to decades, making it difficult to establish a universal duration of protection[352].

There is a general consensus that antibody titres are higher in individuals who experience severe disease compared to those with a mild or asymptomatic infection[329,330,352,359–363]. Individuals with mild or asymptomatic infections were found to have lower levels of IgM, IgA, IgG, and NAbs than those with severe disease[58,310,311,329,352,359,364–374]. Additionally, antibody kinetics also appear to vary between those with asymptomatic or mild versus severe disease. Individuals with mild or asymptomatic infection were more likely to not develop NAbs or in those that did mount a response, NAbs waned rapidly[58,329,330,352,353,360,363,375–377]. In contrast, those who experienced severe disease were more likely to have persistent NAb responses[352]. Delayed NAb responses have also been correlated with severe disease and death[330,352,360–363]. This suggests that early antibody kinetics may provide better insight into disease

outcomes rather than just looking at overall titres. The antibody kinetics in response to SARS-CoV-2 infection are summarized in **Figure 2A**.

1.5.3 Antibody Responses to SARS-CoV-2 Vaccination

The majority of currently available SARS-CoV-2 vaccines, including all approved vaccines in Canada target the pre-fusion S protein[259,260,274,277,278,293,298]. This means that all antibodies produced in response to vaccination will exclusively target components of the S protein. While the majority of antibodies produced in response to SARS-CoV-2 infection also target the S protein, natural infection also induces the production of antibodies targeting other regions of SARS-CoV-2 including the N and M proteins. One of the caveats of current SARS-CoV-2 vaccines is that they do not elicit antibodies against these other antigenic targets. That being said, it has been clearly established that these vaccines still protect against severe disease and death.

The antibodies produced in response to SARS-CoV-2 vaccination differ between vaccines. **Table 9** summarizes the antibodies elicited by different vaccines. All available vaccines in Canada have been shown to induce IgG and NAbs against the viral S protein, but IgM and IgA titres vary and are typically expressed at lower levels than IgG[310,328,329]. In general, antibody titres after one dose of a vaccine appear to be similar to those produced in response to a mild SARS-CoV-2 infection, while titres after two doses of a vaccine are roughly equivalent to antibodies produced after severe infection[378–380]. The antibody kinetics following SARS-CoV-2 vaccination are shown in **Figure 2B**.

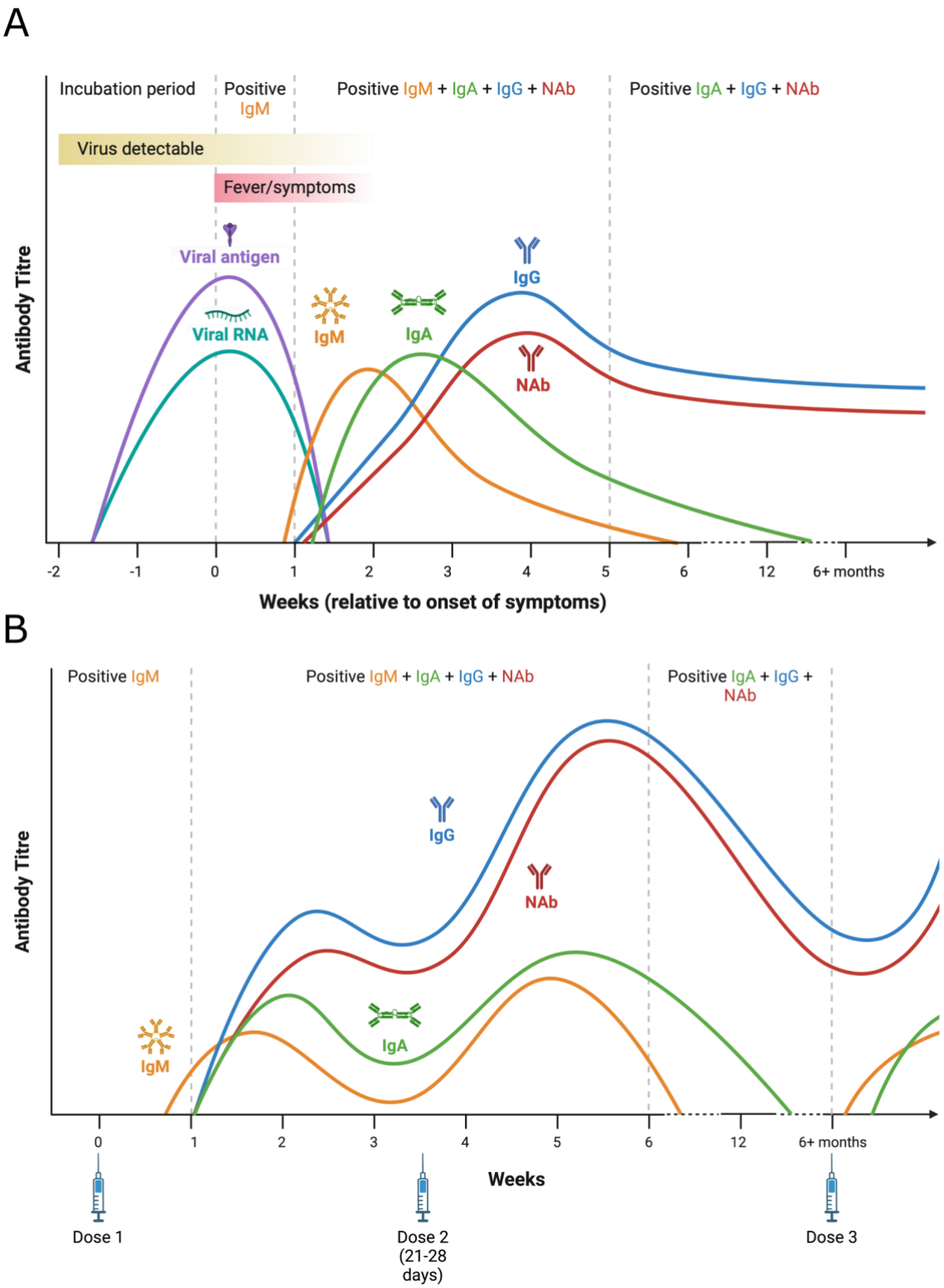

Figure 2. Antibody kinetics following SARS-CoV-2 infection and vaccination. A) Antibody kinetics following SARS-CoV-2 infection. B) Antibody kinetics following SARS-CoV-2 vaccination. NAb, neutralizing antibody. Figure made using BioRender.

Table 9. Antibodies induced by SARS-CoV-2 vaccines after one or two doses.

Vaccine	Dose 1/Dose 2				References
	IgM	IgA	IgG	NAb	
Pfizer BNT162b2	+/+	-/++	+/++	-/++	259,274,381–383
Moderna mRNA-1273	+/+	+/++	+/++	+/++	272–274,383–385
AstraZeneca ChAdOx1-S	+/+	+/+	+/++	++/++	274,277,386,387
Janssen AD26.COV2.S	-/NA	+/NA	++/NA	++/NA	274,289,387–389
Novavax Nuvaxovid	-/?	+/?	+/++	+/++	274,390,391
Medicago Covifenz	+*/++	+*/++	+/++	++/++	299,386,392,393

-, no or non-significant response; +, weak response; ++, strong response; *, results from non-human primate study; NA, not applicable; ?, no results found; NAb, neutralizing antibodies.

1.5.4 Factors Influencing Antibody Responses

Antibody responses to SARS-CoV-2 vaccination and infection are not uniform, and various demographic and lifestyle factors may influence antibody responses at an individual level. In both cases, age seems to have the greatest impact on titres and disease outcomes[166,394–398]. Certain comorbidities have also been associated with reduced antibody responses to vaccination including cancer, pulmonary disease, and organ transplants[394,399]. Other factors such as sex and body mass index (BMI) have conflicting findings. Some studies have found an association between sex and antibody titres, while others have found no relationship[398–400]. Similarly some have found an association between obesity and reduced antibody titres, while others have found no relationship between titres and BMI[398,401]. Further research is needed to clarify the role of these ambiguous factors in influencing antibody responses.

In terms of vaccine-specific factors that may influence antibody responses, the timing of doses and type of vaccines administered can potentially influence antibody responses. Due to supply shortages during the initial vaccine rollout, several countries including Canada extended the interval between first and second doses so more people could receive a first dose[402]. While some were worried about the extended interval between doses potentially having a negative impact on antibody titres, the opposite occurred and titres were found to be higher among those who had an extended interval[403]. The type of vaccines administered can also impact antibody responses, particularly in regard to homologous versus heterologous vaccination regimens. Several studies have shown that using a heterologous (i.e., two

different vaccine types/brands) vaccination regimen may be advantageous and elicit superior antibody responses compared to homologous (i.e., two of the same vaccine type/brand) vaccination[404,405]. For example, a study found that those vaccinated with a combination of Pfizer BNT162b2 and AstraZeneca ChAdOx1-S had higher titres than those vaccinated with two doses of Pfizer BNT162b2[405]. Most studies looking at heterologous SARS-CoV-2 vaccination used two different vaccine types (e.g., mRNA and viral vector), and primarily focused on safety and overall efficacy. Fewer studies have been conducted focusing on heterologous vaccination with different vaccines of the same type, such as with Pfizer BNT162b2 and Moderna mRNA-1273, and there is little information regarding how demographic and other vaccine-related factors such as age, sex, and timing of vaccination affect antibody titres of those who received heterologous vaccines.

1.5.5 Antibody Waning and Breakthrough Infections

For most individuals, antibody titres from SARS-CoV-2 infections and vaccinations will wane over time. One of the potential consequences of this is contracting a breakthrough infection. While antibody titres are not the sole determinant of whether or not someone will experience a breakthrough infection, they can provide an estimate of protection. The factors that impact overall antibody responses to vaccination also impact an individual's risk of breakthrough infection. Factors such as age, comorbidities, the type of vaccine(s) received, previous infection, and the emergence of new variants can have an impact on overall titres and the rate of antibody decay[357,406–410]. In general, COVID-19 vaccines appear to be protective for at least six months post-vaccination in healthy individuals[357,409,410]. However, it is important that a duration of protection is established for high-risk groups such as older individuals and those with immunocompromising conditions so that scheduling of booster doses can be tailored to ensure adequate protection from disease.

1.6 Study Aims

Aim 1: Examine the impacts of demographic factors on post-vaccination serum IgG antibody levels. Variables of interest will include sex, age, and region.

Aim 2: Examine the impacts of vaccine-related factors on post-vaccination serum IgG antibody levels. Variables of interest will include type of vaccine(s), number of doses received, timing of vaccination, and pattern of vaccine administration.

Aim 3: Compare post-vaccination IgG antibody responses between those with and without previous SARS-CoV-2 infection(s).

Hypothesis: Demographic and vaccine-related factors will have an effect on post-vaccination COVID-19 antibody titres.

2 METHODS

2.1 Study Population

Routinely collected serological surveillance data that was collected and stored by Cadham Provincial Laboratory (CPL) was used for the Manitoba COVID Seroprevalence (MCS) study. The MCS study was a population-representative serial cross-sectional study run by CPL to determine the seroprevalence of anti-SARS-CoV-2 antibodies in Manitoba. This study is a secondary data analysis of data from the MCS study. CPL is the only public health diagnostic laboratory in Manitoba and is responsible for nearly all prenatal screening and infectious disease testing in the province. Approximately 16,000 serum specimens are tested by CPL on a monthly basis. These tests include prenatal screening for infectious diseases that can be passed from mother to child, serodiagnostic tests for various infectious diseases, and viral load monitoring for HIV and Hepatitis among others. Since CPL regularly conducts seroprevalence studies for infectious diseases in Manitoba, excess serum specimens that were collected for these routine tests but not used, are often frozen and stored at CPL for future research use (CPL activities ref). Specimens were held in storage for a maximum of two years.

Specimens were de-identified, and basic demographic information including age, sex, and regional health authority were collected. COVID-19 vaccination records in Manitoba are securely stored in the Public Health Information Management System (PHIMS), an electronic public health record that is used to record and store vaccination records across the province. PHIMS contains date of vaccination and vaccine type for each vaccine administered. In Manitoba, every resident has a unique identifier called a

PHIN (personal health identification number), which is required to access public healthcare in Manitoba. All medical records are linked to an individual's PHIN. COVID-19 vaccination records from PHIMS were linked to collected demographic data by PHINs. These steps were done at CPL, and the data was stored on password-protected PHIA-compliant computers at CPL. Specimens were stripped of all identifying information before data were received. Since all identifying information was removed, one of the limitations of this study is there is no way of knowing whether any individuals contributed multiple specimens.

2.2 Specimen Selection

For the MCS study, approximately 1000 serum specimens each month from March 27, 2020, to March 30, 2023, were randomly selected proportionally by percentage for age group (0–9, 10–19, 20–39, 30–49, 40–59, 60+; 20%:20%:20%:20%), sex (50%:50%), and regional health authority (RHA) (60%:10%:10%:10%:10%). Manitoba has five RHAs: Winnipeg Regional Health Authority (WRHA), Prairie Mountain Health (PMH), Southern Health-Santé Sud (SHSS), Interlake-Eastern Regional Health Authority (IERHA), and the Northern Health Region (NHR). The proportions for RHA were based on the population size of each region, with WRHA being the largest. Specimens were randomly selected within these criteria. A subset of the specimens from the MCS study were selected for this study. The proportions used for specimen selection in the MCS study were maintained in this study (i.e., the same ratios for age group, sex and RHA were used when selecting specimens for this study[411]).

Demographic information collected for each specimen included age, sex, and RHA. Serological information for each specimen included dates of specimen collection and testing, anti-N and anti-S IgG titres, and whether the specimen was positive or negative for anti-N and anti-S. Vaccination data collected from PHIMS included dates of each COVID-19 vaccination, type/brand and dose of vaccines received, and whether or not the individual was vaccinated at the time of specimen collection. Vaccination records for up to eight doses were included, however the highest number of vaccines anyone had received at the time of data collection was six.

2.3 Serological Assays

The anti-S and anti-N IgG titres of the selected specimens were tested by CPL using two Health Canada-approved assays. The DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG assay was used to measure anti-spike (anti-S) IgG titres with a positive signal cutoff of ≥ 15 AU/mL[411,412]. The Abbott Architect SARS-CoV-2 IgG assay was used to measure anti-nucleocapsid (anti-N) IgG titres with a positive signal cutoff of ≥ 0.7 S/CO[411,413].

DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG assay used to detect anti-S IgG is an automated indirect chemiluminescent microparticle immunoassay (CMIA). The assay kit contains magnetic particles coated in recombinant S1 and S2 antigens, a diluent, and mouse monoclonal anti-human IgG antibodies are linked to an isoluminol derivative to form the conjugate. The magnetic particles, diluent, conjugate, and specimens and/or calibrators are loaded into the LIAISON® analyzer. Since the assay is automated, all steps are controlled and run by the analyzer. The specimens and/or calibrator, magnetic particles, and diluent are dispensed into reaction cuvettes, followed by an incubation step. During this incubation step, anti-S IgG present in the specimens or calibrators bind to the magnetic particles via the S1 and S2 antigens. A washing step follows the first incubation. Conjugate is then added to the reaction mixture followed by a second incubation. During the second incubation the conjugate reacts with the anti-S IgG antibodies bound to the magnetic particles. Any unbound material is then removed in a second wash. Starter reagents are then added to induce the chemiluminescence reaction with the isoluminol-linked conjugate. The output is measured in relative light units (RLU). The analyzer automatically calculates the anti-S IgG concentrations as arbitrary units per mL (AU/mL)[412].

The Abbott Architect assay used to detect anti-N IgG is a two-step automated CMIA, much like the DiaSorin assay. Frozen serum specimens were thawed and vortexed to ensure homogeneity. Specimens were then recentrifuged and transferred to new tubes for testing. Specimens were combined with SARS-CoV-2 antigen-coated paramagnetic particles and assay diluent consisting of TRIS buffer and a detergent, then incubated. The mixture is then washed, and the monoclonal mouse anti-human IgG acridinium-labeled conjugate was added. The reaction mixture was incubated again, followed by another wash cycle, after which pre-trigger and trigger solutions were added. The results of the reaction were measured in RLU. The antibody levels were quantified by comparing the reaction RLU to the calibrator

RLU, which is calculated by the instrument as an index (S/C). Specimens were not heat-inactivated prior to the assay as it could interfere with the results[413].

2.4 Data Analysis

Data were received from CPL via USB from Dr. Derek Stein. All identifying information had been removed at this point. Data was stored on university OneDrive accounts that were password protected and required two-factor authentication to login. The data was accessed using a password-protected laptop that was only used by the study PI (BM). Data analysis was done using R Studio (v. 2023.12.1+402). The ggplot2, ggh4x, and ggbeeswarm packages were used to generate figures[414–416]. Tables were made using the gtsummary package[417]. The rstatix, stats, and emmeans packages were used to conduct the statistical analyses[418–420].

Fully vaccinated specimens were defined as having received two or more doses of a COVID-19 vaccine at least 14 days prior to specimen collection date(?). Partially vaccinated specimens were defined as having one dose of a vaccine at least 14 days before collection. Unvaccinated specimens were defined as having no doses of a COVID-19 vaccine prior to specimen collection. The initial dataset of 20,365 specimens from CPL included individuals who were vaccinated and unvaccinated for SARS-CoV-2. Unvaccinated individuals were excluded from the analyses since this study focused on post-vaccination antibody titres. Only specimens aged 12 and older were included in the analyses, since children under the age of 12 received a pediatric dose of a COVID-19 vaccine. Age groups used in this study were based off the groups used in the MCS study paper by Duong *et al*., with the exception that the 10–19 age group had to be changed to 12–19, since children <12 were excluded from this study[411].

For the analyses examining the impacts of demographic and vaccine-related factors on post-vaccination IgG titres, specimens were grouped by dose, and vaccine type. Only specimens with proof of vaccination 14+ days prior to specimen collection were included. Doses received were determined by identifying the last vaccine received 14+ days prior to specimen collection. Doses received after specimen collection were not counted. Although the intention was to analyze all the vaccine types that were available to Manitobans during the COVID-19 pandemic, there were not enough specimens that had received each vaccine type with the exception of Pfizer BNT162b2 and Moderna mRNA-1273. Other

vaccines such as Janssen and Novavax had less than five specimens among those with one dose of a vaccine. Additionally, while there were over 200 eligible specimens who had received one dose of the AstraZeneca vaccine, there were only 16 specimens who had received two doses. Thus, only Pfizer BNT162b2 and Moderna mRNA-1273 were included in the study. For the analyses comparing vaccine types, specimens that had received the same number of doses were used to compare the vaccine types. Sex differences were compared used specimens that had received the same vaccine type and number of doses. Similarly, specimens who had received the same dose and vaccine type were used to compare RHAs, as well as age groups.

For the analyses comparing post-vaccination titres between uninfected and previously infected specimens, a modified version of the algorithm used in the MCS study was used to identify specimens with evidence of a previous COVID-19 infection, as COVID-19 testing data were not available [411]. Uninfected specimens were defined as those with a negative anti-N result, while previously infected were defined as those with a positive anti-N response. Since the specimens were previously vaccinated, they would likely already have a positive anti-S response due to the vaccine. Those with positive anti-N IgG were excluded from the analysis of antibody responses to vaccination due to previous COVID-19 infection. One of the limitations of using this method was that there is a possibility that some specimens labelled as uninfected had actually been previously infected, but their antibody titres had waned enough to be below the level of detection. Additionally, COVID-19 testing data were not available for specimens and thus it is unknown whether they were infected before or after vaccination, so in this case "previous infection" would also be defined as infected prior to specimen collection, not vaccination.

The analyses comparing the titres of those with <75 days between their last vaccine dose and specimen collection to those with ≥75 days, the same procedure used for the analyses of demographic and vaccine-related factors was used (i.e., specimens were grouped by dose and vaccine type). I found that there were no specimens from male participants after 150 days post-vaccination, so I only included individuals who had been vaccinated ≤150 days before specimen collection. The 150-day period was divided in half and specimens were grouped by whether they'd been vaccinated <75 days before specimen collection or if it had been ≥75 days since their last vaccination prior to specimen collection.

For all figures, antibody titres were log-transformed to improve data visibility in the graphs. The median titres are reported in their original units of AU/mL in the tables containing the results of statistical testing in the appendix. Statistical significance was determined using the Kruskal-Wallis test followed by Dunn's Multiple Comparison Test using the Benjamini-Hochberg method for multiple comparisons. The Kruskal-Wallis test was chosen since the data was non-parametric and multiple comparisons were made. The Dunn's test is a post-hoc test that was used to make pairwise comparisons between groups. *P* values of <0.05 were considered statistically significant.

2.5 Ethics

This study was reviewed by the University of Manitoba Research Ethics board as routine surveillance and exempt under the Canadian Tri-Council Policy Statement: Ethical conduct for research involving humans, TCSP2. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because this was a retrospective study of anonymized public health surveillance data.

3 RESULTS

3.1 Specimen Demographics

A total of 20,365 residual serum specimens from CPL were included in the MCS study. It is important to note that since the specimens were stripped of all identifying information there is no way to know whether any specimens came from the same individual. Of these specimens, 10,057 (49.4%) were male, and 10,308 (50.6%) were female. The demographics of study participants are summarized in **Table 11**. The median age was 34 (IQR, 17-56 years). At the time of specimen collection, 10,422 (51%) individuals had received at least one dose of a COVID-19 vaccine, with the maximum number of doses received by any individual being six (n = 129). A total of 47,082 doses were administered. The most common vaccine received was Pfizer BNT162b2, accounting for 58% (n = 27,288) of all vaccines administered, while Moderna mRNA-1273 accounted for 22.6% (n = 10,661), and other various vaccines including AstraZeneca ChAdOx1-S, Janssen AD26.COV2.S, and Novavax Nuxovid accounting for the remaining 19.4%. The majority of those who received fifth and sixth doses were given bivalent

formulations of Moderna mRNA-1273 or Pfizer BNT162b2. The median number of weeks between receiving a first dose and specimen collection was 38 (IQR, 20-55). The median number of weeks between first and second doses was 7 (IQR, 5-10), while the median number of weeks between receiving a second dose and specimen collection was 34 (IQR, 19-50). All specimens were tested for SARS-CoV-2 anti-N IgG, with 7,552 (37%) being positive, indicating previous COVID-19 infection either before or after vaccination.

Table 10. Demographics of serum specimens used in this study.

Characteristic	**N**	**Female**, N = 10,308[1]	**Male**, N = 10,057[1]	**Overall**[1]
Age Group	20,365			
1-11		1,443 (14%)	1,483 (15%)	2,926 (14%)
12-19		2,040 (20%)	1,843 (18%)	3,883 (19%)
20-39		2,409 (23%)	2,180 (22%)	4,589 (23%)
40-59		2,248 (22%)	2,298 (23%)	4,546 (22%)
60+		2,168 (21%)	2,253 (22%)	4,421 (22%)
Age	20,365			
Median (IQR)		33 (17, 56)	36 (17, 58)	34 (17, 57)
Range		1, 102	1, 101	1, 102
Vaccinated before Specimen Collection	20,365	5,194 (50%)	5,228 (52%)	10,422 (51%)
First Dose	16,558			
AstraZeneca		420 (5.0%)	598 (7.3%)	1,018 (6.1%)
Moderna		1,715 (20%)	1,911 (23%)	3,626 (22%)
Pfizer		5,599 (67%)	4,973 (61%)	10,572 (64%)
Other		657 (7.8%)	685 (8.4%)	1,342 (8.1%)
Second Dose	15,840			
Moderna		2,392 (30%)	2,737 (35%)	5,129 (32%)
Pfizer		5,110 (63%)	4,480 (58%)	9,590 (61%)
Other		551 (6.8%)	570 (7.3%)	1,121 (7.1%)
Third Dose	8,701			
Moderna		761 (18%)	884 (20%)	1,645 (19%)
Pfizer		2,728 (63%)	2,625 (60%)	5,353 (62%)
Moderna Bivalent		65 (1.5%)	72 (1.6%)	137 (1.6%)
Pfizer Bivalent		115 (2.7%)	139 (3.2%)	254 (2.9%)

Other		631 (15%)	681 (15%)	1,312 (15%)
Fourth Dose	4,236			
Moderna		103 (5.0%)	149 (6.9%)	252 (5.9%)
Pfizer		842 (41%)	909 (42%)	1,751 (41%)
Moderna Bivalent		445 (21%)	502 (23%)	947 (22%)
Pfizer Bivalent		529 (26%)	418 (19%)	947 (22%)
Other		154 (7.4%)	185 (8.6%)	339 (8.0%)
Fifth Dose	1,618			
Moderna		3 (0.4%)	5 (0.6%)	8 (0.5%)
Pfizer		8 (1.0%)	14 (1.6%)	22 (1.4%)
Moderna Bivalent		257 (34%)	310 (36%)	567 (35%)
Pfizer Bivalent		489 (64%)	501 (59%)	990 (61%)
Other		8 (1.0%)	23 (2.7%)	31 (1.9%)
Sixth Dose	129			
Moderna		0 (0%)	1 (1.4%)	1 (0.8%)
Pfizer		0 (0%)	0 (0%)	0 (0%)
Moderna Bivalent		2 (3.3%)	3 (4.3%)	5 (3.9%)
Pfizer Bivalent		52 (87%)	55 (80%)	107 (83%)
Other		6 (10%)	10 (14%)	16 (12%)
Total Doses Received	20,365			
0		1,917 (19%)	1,890 (19%)	3,807 (19%)
1		338 (3.3%)	380 (3.8%)	718 (3.5%)
2		3,753 (36%)	3,386 (34%)	7,139 (35%)
3		2,227 (22%)	2,238 (22%)	4,465 (22%)
4		1,308 (13%)	1,310 (13%)	2,618 (13%)
5		705 (6.8%)	784 (7.8%)	1,489 (7.3%)
6		60 (0.6%)	69 (0.7%)	129 (0.6%)
RHA	20,365			
IEHR		976 (9.5%)	956 (9.5%)	1,932 (9.5%)
NHR		847 (8.2%)	802 (8.0%)	1,649 (8.1%)
PMHR		1,230 (12%)	1,177 (12%)	2,407 (12%)
SHSS		1,447 (14%)	1,376 (14%)	2,823 (14%)
WRHA		5,808 (56%)	5,746 (57%)	11,554 (57%)
Anti-S IgG	14,089			
Negative		2,382 (34%)	2,385 (34%)	4,767 (34%)
Positive		4,667 (66%)	4,655 (66%)	9,322 (66%)

Anti-N IgG	20,365			
Negative		6,567 (64%)	6,246 (62%)	12,813 (63%)
Positive		3,741 (36%)	3,811 (38%)	7,552 (37%)
Positive for Anti-S & Anti-N IgG	20,365	2,176 (21%)	2,167 (22%)	4,343 (21%)
Positive for Anti-S IgG Only	20,365	2,491 (24%)	2,488 (25%)	4,979 (24%)
Negative for Anti-S & Anti-N IgG	20,365	2,062 (20%)	1,998 (20%)	4,060 (20%)
Weeks between First Dose and Specimen Collection	10,561			
Median (IQR)		38 (20, 56)	37 (20, 54)	38 (20, 55)
Range		0, 117	0, 116	0, 117
Weeks between First and Second Dose	15,840			
Median (IQR)		7.0 (5.0, 10.0)	7.0 (5.0, 10.0)	7.0 (5.0, 10.0)
Range		3.0, 65.0	3.0, 78.0	3.0, 78.0
Weeks between Second Dose and Specimen Collection	9,147			
Median (IQR)		35 (19, 51)	34 (19, 49)	34 (19, 50)
Range		0, 114	0, 113	0, 114
Weeks between Second and Third Dose	8,701			
Median (IQR)		28 (27, 33)	28 (26, 32)	28 (26, 33)
Range		4, 109	4, 98	4, 109
Weeks between Third Dose and Specimen Collection	3,432			
Median (IQR)		19 (9, 36)	18 (9, 36)	19 (9, 36)
Range		0, 78	0, 78	0, 78

[1]n (%)

From all the specimens included in the study, 1794 met the inclusion criteria for the vaccine analyses. The demographics of these specimens are in **Table 11**.

Table 11. Demographics of specimens included in vaccine analyses. Only specimens aged 12+ who had received at least one dose of Moderna mRNA-1273 and/or Pfizer BNT162b2 14+ days prior to specimen collection were included in the analyses.

Characteristic	**N**	**Female** N = 936[1]	**Male** N = 858[1]	**Overall**[1]

Age Group	1,794			
12-19		146 (16%)	133 (16%)	279 (16%)
20-39		166 (18%)	142 (17%)	308 (17%)
40-59		233 (25%)	212 (25%)	445 (25%)
60+		391 (42%)	371 (43%)	762 (42%)
Age	1,794			
Median (Q1, Q3)		55 (32, 70)	55 (33, 71)	55 (32, 71)
Min, Max		12, 96	12, 96	12, 96
First Dose	1,794			
Moderna		208 (22%)	225 (26%)	433 (24%)
Pfizer		728 (78%)	633 (74%)	1,361 (76%)
Second Dose	1,794			
Moderna		208 (22%)	225 (26%)	433 (24%)
Pfizer		728 (78%)	633 (74%)	1,361 (76%)
RHA	1,794			
IEHR		83 (8.9%)	80 (9.3%)	163 (9.1%)
NHR		49 (5.2%)	51 (5.9%)	100 (5.6%)
PMH		119 (13%)	115 (13%)	234 (13%)
SHSS		107 (11%)	109 (13%)	216 (12%)
WRHA		578 (62%)	503 (59%)	1,081 (60%)
Anti-Spike IgG	1,794			
Negative		133 (14%)	146 (17%)	279 (16%)
Positive		803 (86%)	712 (83%)	1,515 (84%)
Weeks between First Dose and Specimen Collection	1,794			
Median (Q1, Q3)		15 (6, 29)	15 (6, 28)	15 (6, 29)

Min, Max		0, 60	0, 58	0, 60
Weeks between First and Second Dose	1,794			
Median (Q1, Q3)		8.0 (5.0, 11.0)	8.0 (6.0, 10.0)	8.0 (5.0, 11.0)
Min, Max		3.0, 59.0	3.0, 62.0	3.0, 62.0
Weeks between Second Dose and Specimen Collection	1,273			
Median (Q1, Q3)		17 (6, 24)	17 (5, 23)	17 (6, 23)
Min, Max		0, 57	0, 49	0, 57

[1]n (%)

3.2 Vaccine Rollout and Timeline

To see whether vaccination rates corresponded to changes in eligibility, I looked at the number of COVID-19 vaccines being administered over time amongst study participants and the dates of major vaccine eligibility changes in Manitoba (**Figure 3**). Most individuals in the study were vaccinated shortly after doses were made available. For first doses, the number of vaccinations received had a small spike from December 2020 to February 2021, when only specific groups of healthcare workers were eligible for vaccination[240]. The number of first doses peaked shortly after the general public became eligible in May 2021[421]. A small spike in second doses occurred from January till March 2021, when that first group of healthcare workers became eligible for second doses[422]. After this first spike in second doses, the number of second doses received dropped off as the interval between first and second doses was extended due to limited vaccine supply[402]. The number of second doses received peaked between June and July 2021, when the general adult population became eligible[423]. Third doses were the highest between December 2021 and January 2022, shortly after all adults became eligible once again[424]. The peak in third doses was less prominent than first and second doses, likely because individuals had to wait six months after receiving a second dose before they were eligible for a third, as well as fewer individuals

overall getting a third dose compared to first and second doses. Fourth doses peaked around June 2022, again shortly after all adults became eligible in May[425].

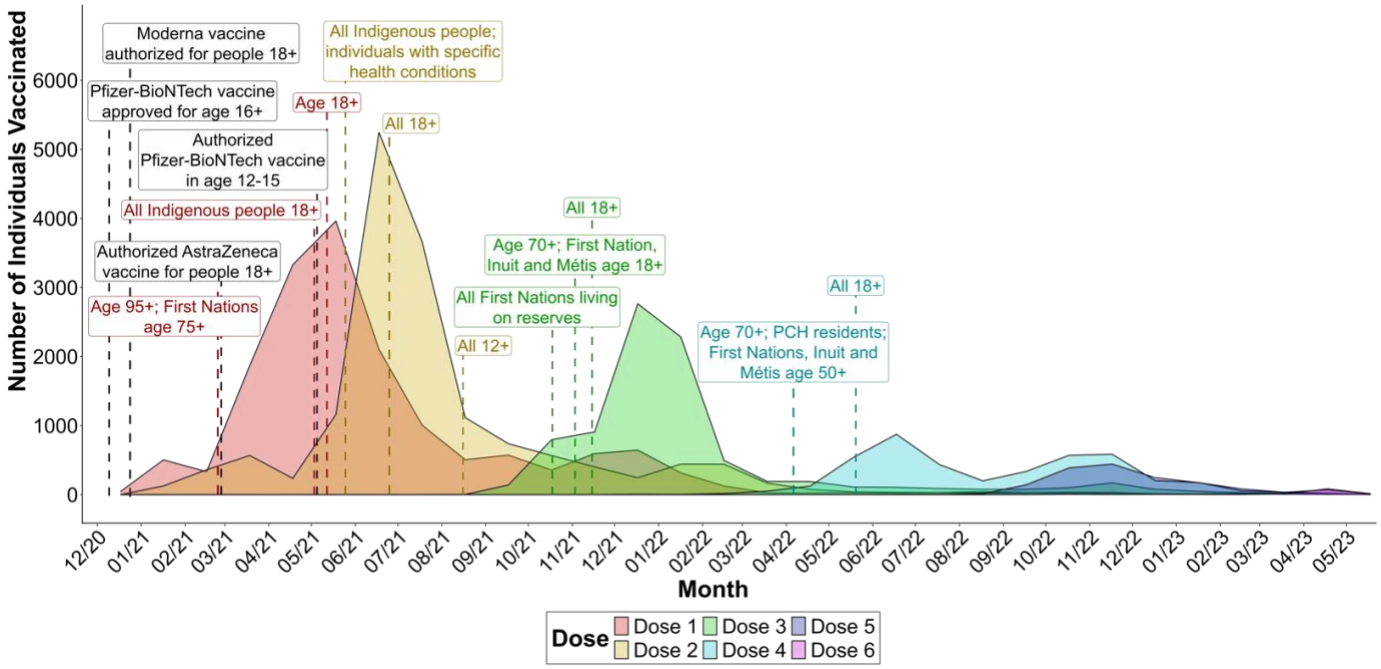

Figure 3. Number of COVID-19 vaccines administered by dose over time for the specimens used in this study, and the major changes in vaccine eligibility in Manitoba. Specimens were collected as part of the Manitoba COVID Seroprevalence study (n = 20,365). PCH, personal care home.

3.3 IgG Titres Vary by Vaccine Type

A total of 14,089 specimens were tested for anti-S IgG, with 9,322 (66%) being positive, suggesting either previous COVID-19 infection or vaccination. From those, I selected the specimens that were negative for anti-N IgG in order to analyze anti-S IgG responses to vaccination alone. Factors such as age, sex, vaccine type, geographic location, and time post-vaccination were examined to determine whether they had any impact on IgG titres. Neither sex nor geographic location appeared to be significantly associated with overall titres (**Figures 4 and 5**), while vaccine type was significantly associated with anti-S IgG responses (**Figure 6**). Results of the statistical tests used for figures 4–6 are located in tables S1–S3 in the appendix.

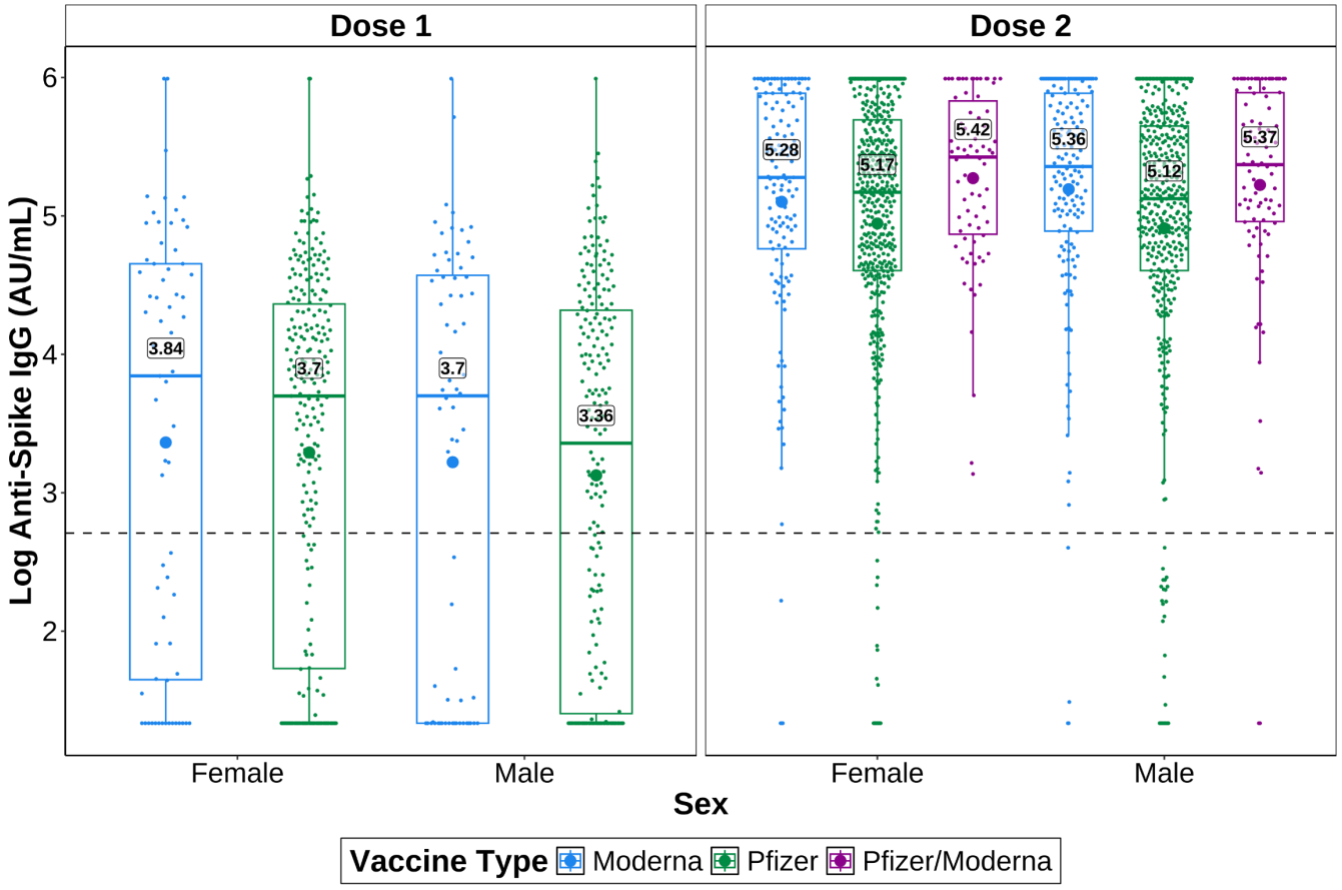

Figure 4. Sex differences in SARS-CoV-2 anti-spike IgG among those who received either one or two doses of a COVID-19 vaccine. Only samples negative for anti-nucleocapsid IgG were included. Specimens were vaccinated with either one dose of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (red), or with two doses of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (red), or one dose of each (Pfizer/Moderna) (blue). Sexes were compared by dose and vaccine type using Dunn's test for pairwise comparisons, correcting for multiple hypothesis testing using the Benjamini-Hochberg method. No statistically significant differences were found at the $p<0.05$ level. Median values are written above the median lines. Larger dots indicate the means. Smaller dots represent individual samples. Error bars correspond to the smallest and largest values within 1.5x the inter-quartile range. ns, not significant.

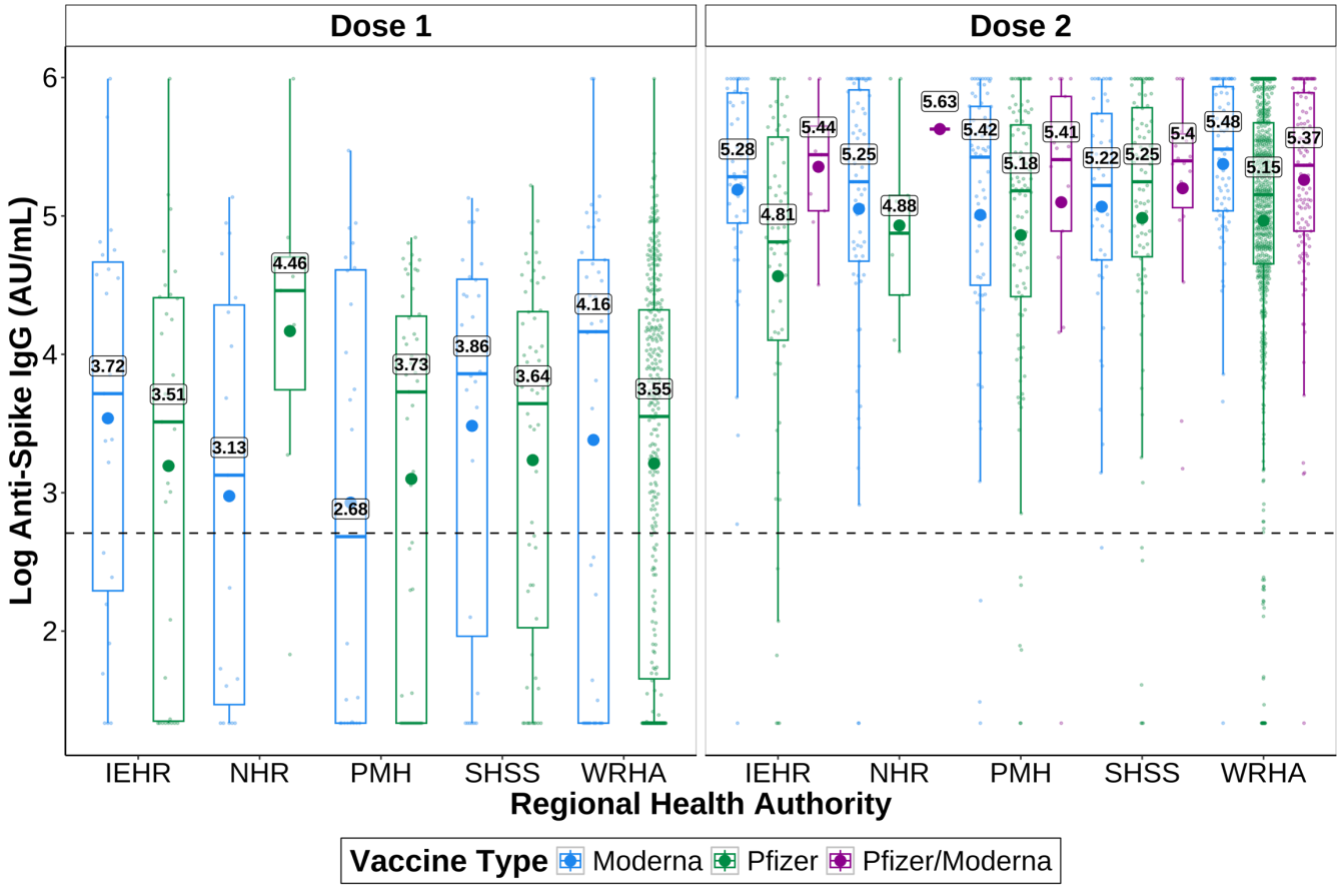

Figure 5. Differences in SARS-CoV-2 anti-spike IgG titres between regional health authorities. Specimens had received either one or two doses of a COVID-19 vaccine. Only specimens negative for anti-nucleocapsid IgG were included. Specimens were vaccinated with either one dose of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (red), or with two doses of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (red), or one dose of each (Pfizer/Moderna) (blue). Dunn's test for pairwise comparisons corrected for multiple hypothesis testing using the Benjamini-Hochberg method was used to compare groups. No significant differences were found. Median values are written above the median lines. Larger dots indicate the means. Smaller dots represent individual samples. Error bars correspond to the smallest and largest values within 1.5x the inter-quartile range. IEHR, Interlake-Eastern Health Region; NHR, Northern Health Region; PMH, Prairie Mountain Health; SHSS, Southern Health Santé Sud; WRHA, Winnipeg Regional Health Authority.

To determine if anti-S IgG titres differed between the different vaccine types, specimens were grouped by whether they received Moderna mRNA-1273 or Pfizer BNT162b2, and the number of doses

they had received at the time of specimen collection. There were no significant differences in titres between the vaccines among those who had received one dose. However among those who had received two doses of a vaccine, those who had received two Moderna mRNA-1273 ($p = 2.91 \times 10^{-4}$) or one dose of Pfizer BNT162b2 followed by one dose of Moderna mRNA-1273 (Pfizer/Moderna; $p = 2.91 \times 10^{-4}$) had significantly higher anti-S IgG titres than those who received two doses of Pfizer BNT162b2 (**Figure 6**).

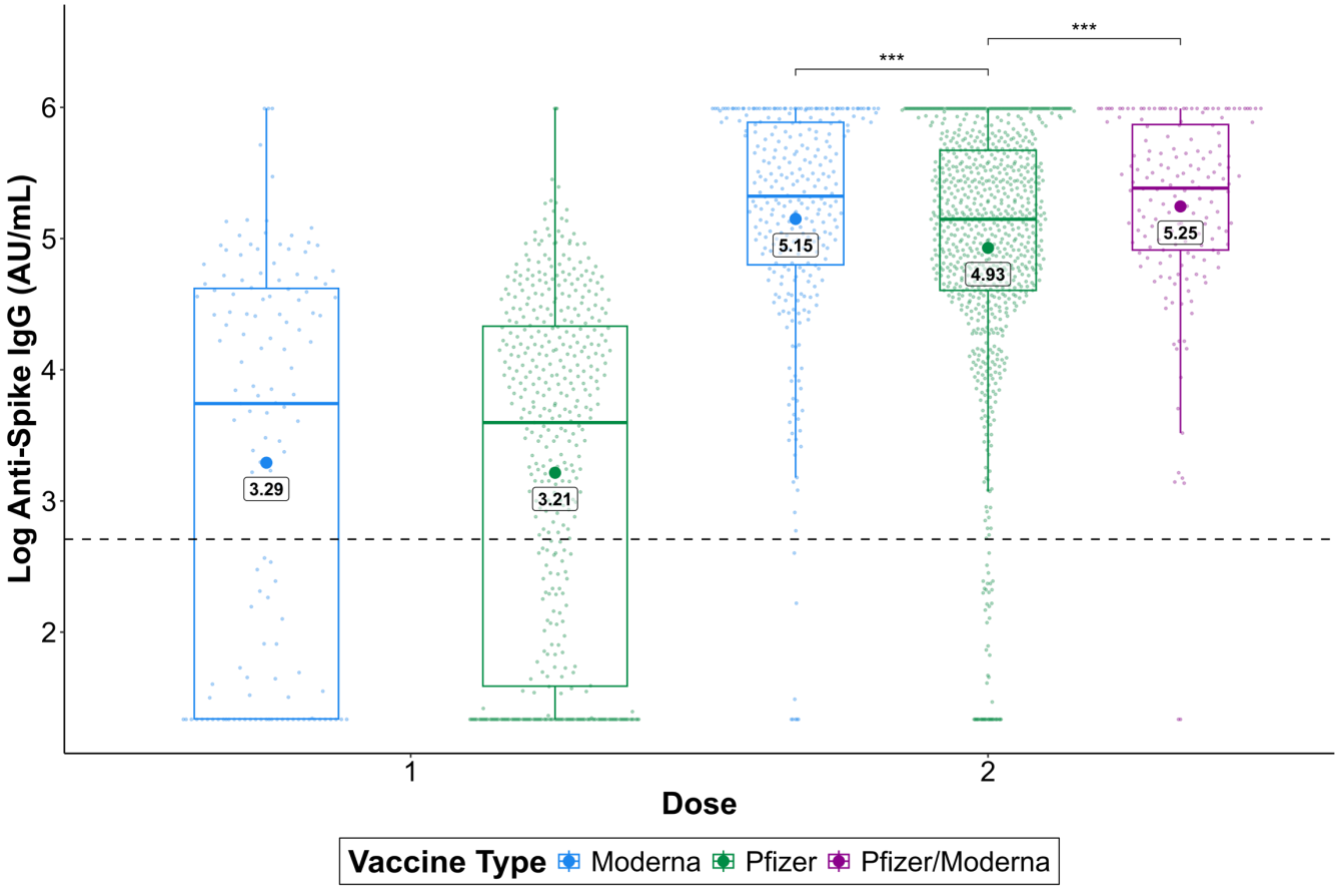

Figure 6. SARS-CoV-2 anti-spike IgG titres of those who received either one or two doses of a COVID-19 vaccine. Specimens were vaccinated with either one dose of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (blue), or with two doses of Pfizer BNT162b2 or Moderna mRNA-1273 or one dose of each (Pfizer/Moderna; purple). Only specimens negative for anti-nucleocapsid IgG were included. Median values are written above the median lines. Larger dots indicate the means. Smaller dots represent

individual samples. Dashed line indicates positive signal cut-off of 15 AU/mL. Error bars correspond to the smallest and largest values within 1.5x the inter-quartile range. *** $p \leq 0.001$.

3.4 IgG Titres Vary by Age

To investigate whether a correlation between age and anti-S IgG exists, titres of different age groups were measured 14+ days after receiving a first or second dose, and before receiving a second or third dose respectively (**Figure 7**). Results of the statistical tests used to compare age groups are found in **Table S3** in the appendix.

Anti-S IgG titres tended to decline with increasing age. Those in the 12–19-year-old age group with one dose of a COVID-19 vaccine had the highest titres overall, regardless of vaccine. From those who received Pfizer BNT162b2, the 12–19-year-olds had significantly higher titres than all other age groups with a median titre of 67.85 AU/mL (20–39 years, median = 49.1 AU/mL, $p = 4.44 \times 10^{-2}$; 40–59 years, median = 36.5 AU/mL, $p = 1.14 \times 10^{-2}$; 60+ years, median = 18.05 AU/mL, $p = 9.79 \times 10^{-9}$). The 20–39 ($p = 2.02. \times 10^{-2}$) and 40–59-year-olds ($p = 1.14 \times 10^{-2}$) also had higher titres than the 60+ year-olds with one dose of Pfizer BNT162b2. Of those who received a single dose of Moderna mRNA-1273, both the 12–19 (median = 141 AU/mL, $p = 4.77 \times 10^{-2}$), and 20–39-year-olds (median = 74 AU/mL, $p = 4.77 \times 10^{-2}$) had higher titres than the 60+ years group (median = 10.5 AU/mL), but the 40–59-year-olds (median = 42.2 AU/mL) did not.

From those who had two doses of a vaccine, the 12–19-year-olds continued to have the highest anti-S IgG titres overall. Of those who received two doses of Pfizer-BNT162b2, the 12–19-year-olds again had higher titres than all other age groups with a median of 258 AU/mL (20–39 years, median = 196 AU/mL, $p = 3.81 \times 10^{-4}$; 40–59 years, median = 145 AU/mL, $p = 1.87 \times 10^{-9}$; 60+ years, median = 141.5 AU/mL, $p = 2.80 \times 10^{-17}$). The 20–39-year-olds also had higher titres than the 40–59 ($p = 3.07 \times 10^{-2}$) and 60+ year-olds ($p = 3.78 \times 10^{-5}$). Additionally, the 40–59-year-olds also had higher titres than the 60+ year-olds ($p = 4.25 \times 10^{-2}$), meaning that the 60+ years group had the lowest titres overall among those with two doses of Pfizer BNT162b2.

Of those who had two doses of Moderna mRNA-1273, the 12–19-year-olds once again had the highest titres among all the age groups with a median of 359 AU/mL, however they were only

significantly higher than the 60+ year-olds (median = 179.5 AU/mL, p = 3.40 x 10^{-2}). The 20–39-year-olds' titres were significantly higher than both the 40–59 (p = 3.28 x 10^{-2}) and 60+ year-olds (p = 1.03 x 10^{-4}). In this case, the 40–59-year-olds did not have significantly higher titres than the 60+ year-olds with two doses of Moderna mRNA-1273.

In the case of those who received Pfizer/Moderna, the 12–19-year-olds had significantly higher titres than all other age groups with a median of 400 AU/mL (20-39 years, median = 230 AU/mL, p = 4.21 x 10^{-6}; 40–59 years, median = 187 AU/mL, p = 2.89 x 10^{-4}; 60+ years, median = 167 AU/mL, p = 4.21 x 10^{-6}). The 20–39-year-olds also had higher titres than the 60+ year-olds (p = 4.67 x 10^{-2}), but not the 40–59-year-olds.

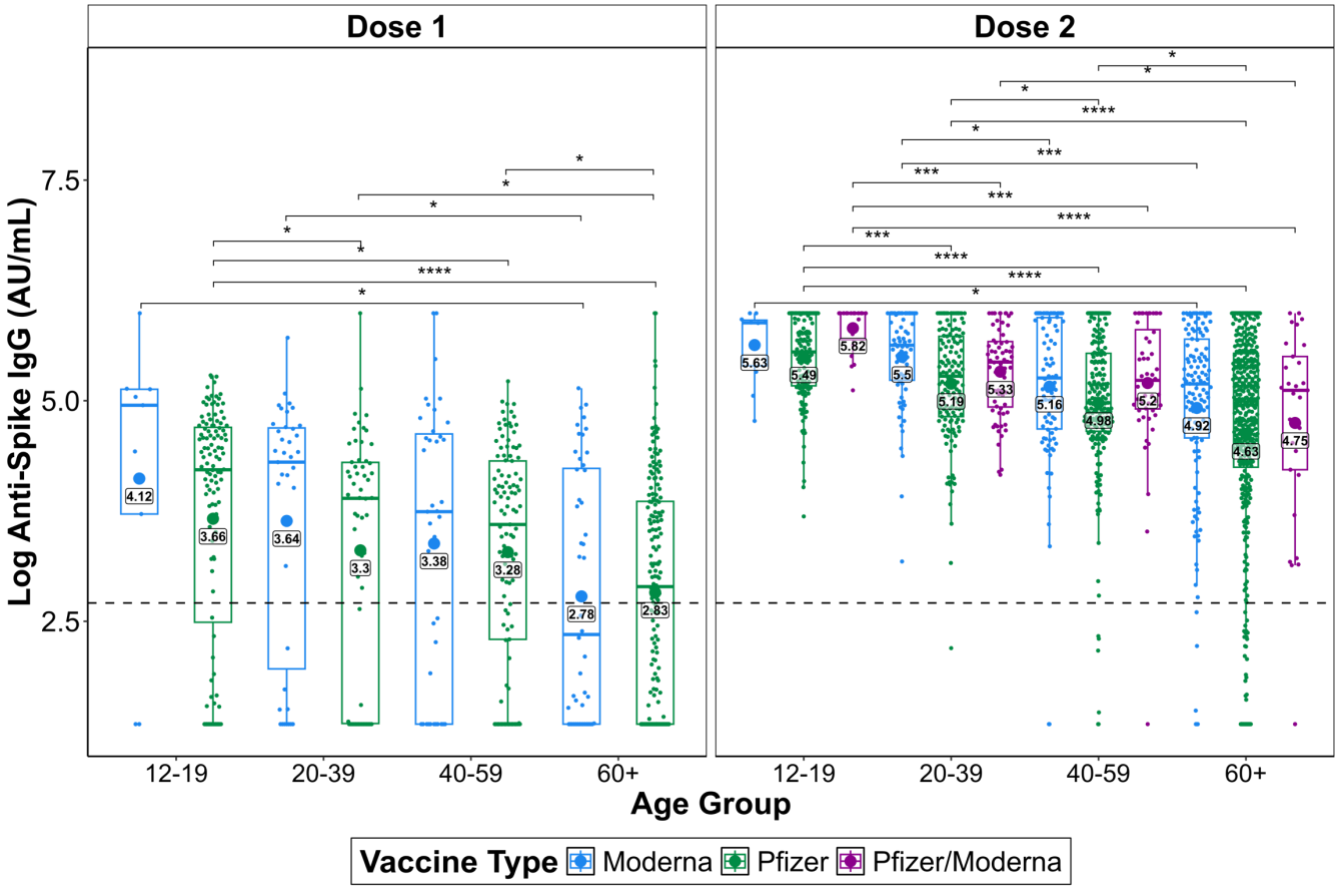

Figure 7. Age differences in SARS-CoV-2 anti-spike IgG among those who had one or two doses of a COVID-19 vaccine. Only samples negative for anti-nucleocapsid IgG were included. Specimens were vaccinated with either one dose of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (blue), or with two doses of Pfizer BNT162b2 or Moderna mRNA-1273, or one dose of each (Pfizer/Moderna; purple).

Dunn's test for pairwise comparisons corrected for multiple hypothesis testing using the Benjamini-Hochberg method was used to compare groups. Median values are written above the median lines. Larger dots are the mean. Smaller dots represent individual samples. Error bars correspond to the smallest and largest values within 1.5x the inter-quartile range. Dashed line indicates positive signal cut-off of 15 AU/mL. ****$p \leq 0.0001$, ***$p \leq 0.001$, $p \leq 0.01$, *$p \leq 0.05$.

Since the 40–59-year-olds only had significantly higher titres than the 60+ year-olds among those who had received Pfizer BNT162b2, I decided to compare anti-S IgG titres between the different vaccines within the same age group (**Figure 8**). There were no statistically significant differences between those who had one or two doses of Moderna mRNA-1273 or Pfizer BNT162b2 in the 49–59 year old age group. However, I did find slightly higher titres between one dose of Moderna mRNA-1273 and Pfizer BNT162b2 in the 20–39 years group ($p = 0.05$). From those who had two doses of a vaccine, the 12–19 years group who received heterologous Pfizer/Moderna had significantly higher titres than those who had Pfizer BNT162b2 ($p = 3.74 \times 10^{-3}$). Within the 20–39-year-olds who had two doses of a vaccine, those who received Moderna mRNA-1273 had higher titres than those who received Pfizer BNT162b2 ($p = 4.36 \times 10^{-4}$) or Pfizer/Moderna ($p = 0.031$). There were no significant differences in the 60+ age group.

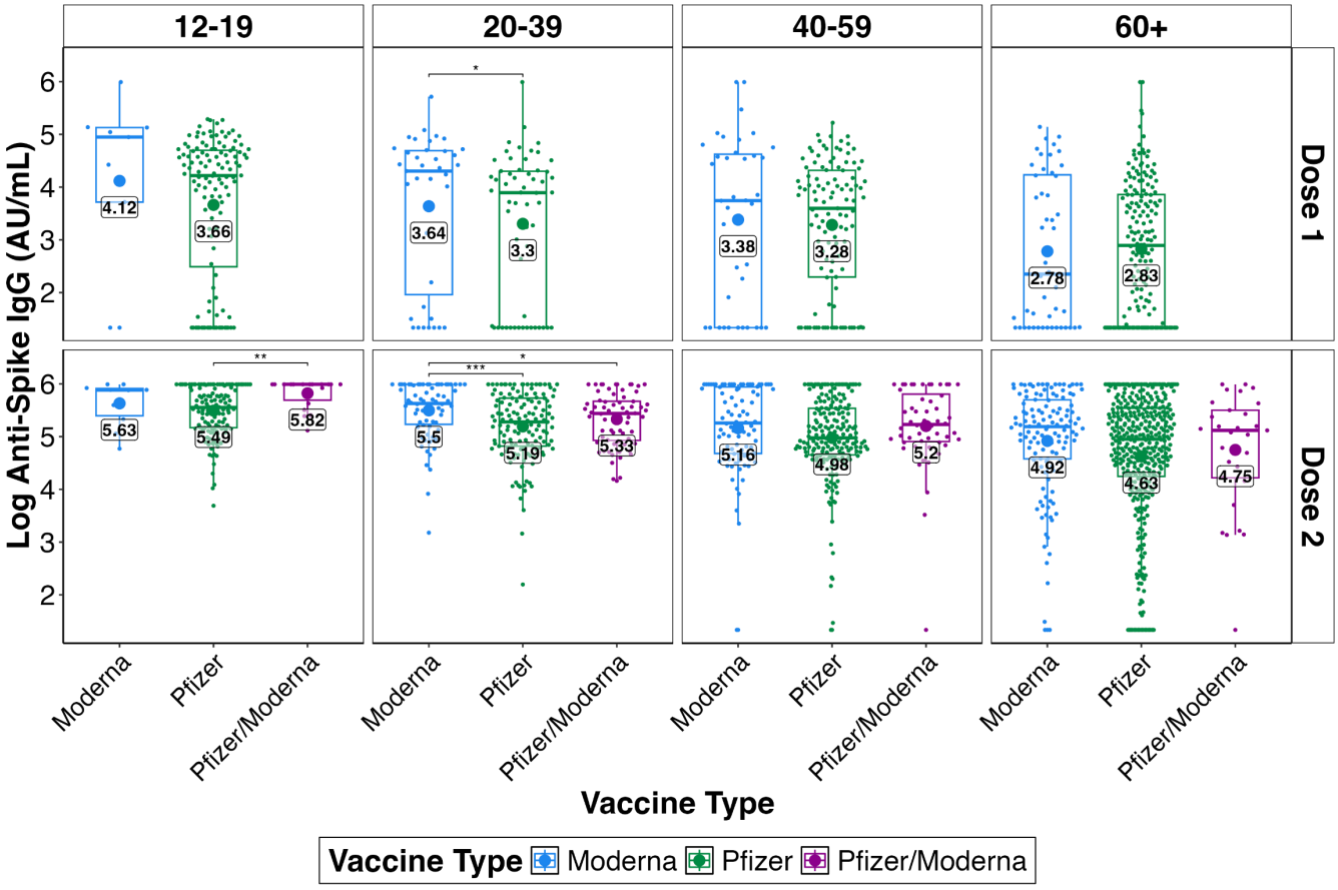

Figure 8. Differences in anti-spike IgG titres between COVID-19 vaccines within the same age group. Only samples negative for anti-nucleocapsid IgG were included. Specimens had been vaccinated with either one dose of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (blue), or with two doses of Pfizer BNT162b2 or Moderna mRNA-1273, or one dose of each (Pfizer/Moderna; purple). Dunn's test for pairwise comparisons corrected for multiple hypothesis testing using the Benjamini-Hochberg method was used to compare groups. Median values are written above the median lines. Larger dots indicate the mean. Smaller dots represent individual samples. Error bars correspond to the smallest and largest values within 1.5x the inter-quartile range. Dashed line indicates positive signal cut-off of 15 AU/mL. ***p ≤ 0.001, p ≤ 0.01, *p ≤ 0.05.

3.5 Previous COVID-19 Infection Boosts IgG Titres

To compare the anti-S IgG responses of vaccinated individuals who were previously infected to those with no known history of SARS-CoV-2 infection, I compared specimens with positive anti-N titres to those with negative anti-N titres (**Figure 12**). Specimens positive for anti-N are presumed to have had

a previous SARS-CoV-2 infection; all available vaccines in Canada only induce anti-S antibodies. Specimens negative for anti-N were presumed to be uninfected. Specimens were also separated by age group, dose, and vaccine(s) received. Specimens were stratified by age group to also examine whether the titres of those previously infected would differ by age. Sample sizes and results from the statistical tests can be found in Table S5 in the appendix.

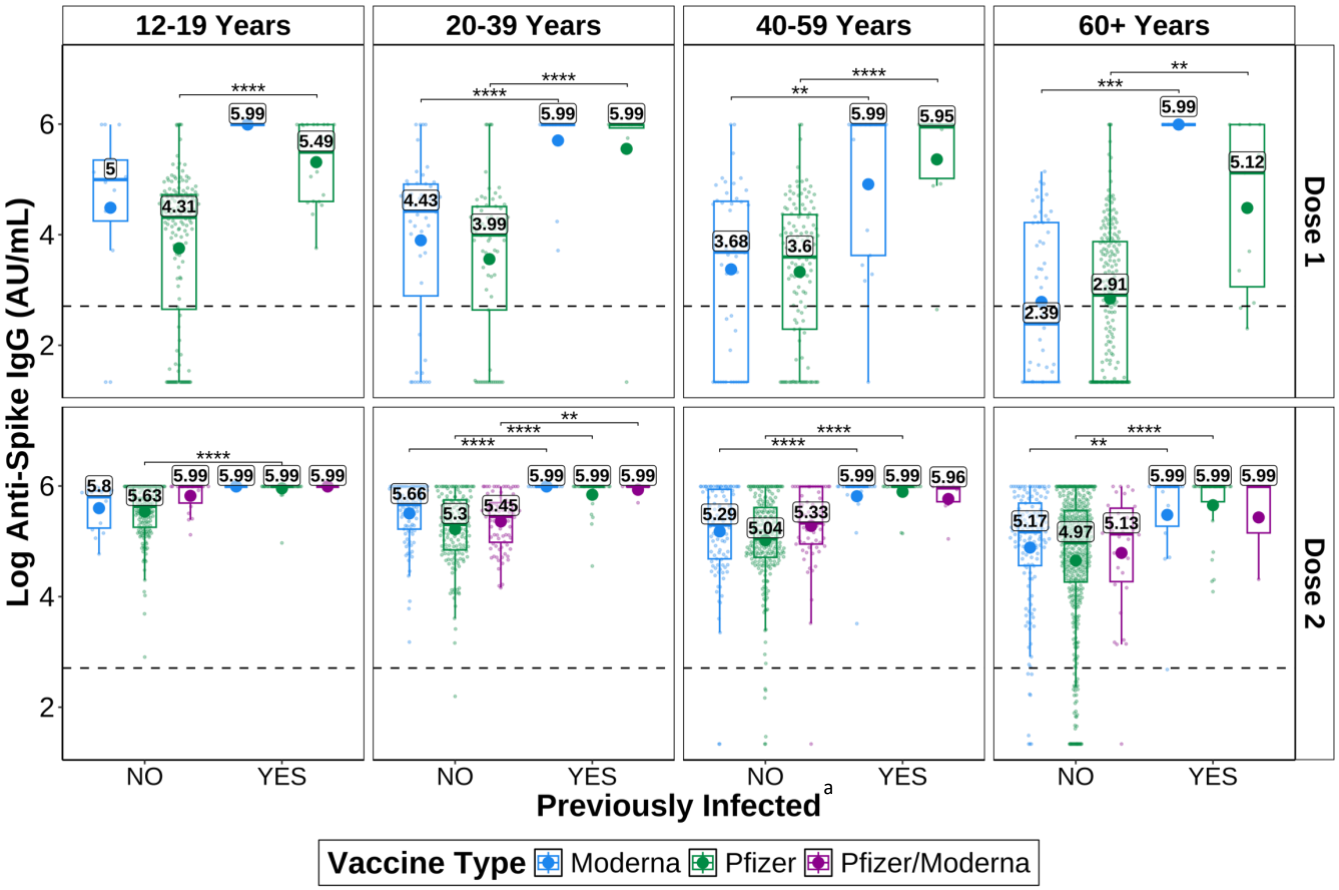

Figure 9. SARS-CoV-2 anti-spike IgG titres of vaccinated individuals who were uninfected or previously infected with SARS-CoV-2. Specimens were vaccinated with either one or two doses of Pfizer BNT162b2 (green) or Moderna mRNA-1273 (blue), or one dose of each (Pfizer/Moderna; purple). Dunn's test for pairwise comparisons corrected for multiple hypothesis testing using the Benjamini-Hochberg method was used to compare groups. Larger dots are the mean. Smaller dots represent individual samples. Error bars correspond to the smallest and largest values within 1.5x the inter-quartile range. Dashed line indicates positive signal cut-off of 15 AU/mL. [a]Specimens positive for anti-N IgG were defined as

"previously infected", specimens negative for anti-N were deemed "uninfected". ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

In most cases, the anti-S IgG titres of those who were previously infected with SARS-CoV-2 were significantly higher than those who were uninfected. After one dose of a COVID-19 vaccine, all age groups who received Pfizer BNT162b2 and were previously infected had significantly higher titres than those who were uninfected (12-19 years, $p = 1.02 \times 10^{-6}$; 20-39 years, $p = 1.85 \times 10^{-5}$; 40-59 years, $p = 1.47 \times 10^{-5}$; 60+ years, $p = 2.05 \times 10^{-3}$).

This association continued for those who received one dose of Moderna mRNA-1273, with the exception of the 12-19-year-olds, who did not have significantly different titres between the uninfected and previously infected due to their small sample size (n=1) (20-39 years, $p = 2.14 \times 10^{-5}$; 40-59 years, $p = 1.37 \times 10^{-3}$; 60+ years, $p = 7.87 \times 10^{-4}$).

Following two doses of Pfizer BNT162b2, once again those who were previously infected had higher titres than those uninfected, for all age groups (12-19 years, $p = 6.85 \times 10^{-11}$; 20-39 years, $p = 8.37 \times 10^{-8}$; 40-59 years, $p = 3.33 \times 10^{-8}$; 60+ years, $p = 1.94 \times 10^{-7}$). The titres of those who received two doses of Moderna mRNA-1273 follow the same pattern as those who received one dose, with all ages except the 12-19-year-olds having significantly higher titres if they were previously infected than those who were uninfected (20-39 years, $p = 2.76 \times 10^{-5}$; 40-59 years, $p = 9.94 \times 10^{-6}$; 60+ years, $p = 4.18 \times 10^{-3}$). Interestingly, from those who received Pfizer/Moderna, only the 20-39-year-olds who were previously infected had higher titres than those who were uninfected ($p = 5.71 \times 10^{-3}$).

4 DISCUSSION

4.1 IgG Titres Vary by Vaccine Type

I compared the anti-S IgG titres of individuals vaccinated with different mRNA COVID-19 vaccines and found that after one dose of Moderna mRNA-1273 elicited slightly higher titres overall compared to the Pfizer BNT162b2 vaccine, although not significantly so (at the p<.05 level). Those who received either two doses of Moderna mRNA-1273 or heterologous Pfizer/Moderna did have significantly higher titres than those who received two doses of Pfizer BNT162b2. This suggests that Moderna mRNA-1273

produces only a slightly stronger antibody response compared to Pfizer BNT162b2. Similar results were seen in other studies comparing the two vaccines. Both Montoya et al. and Steensels et al. found that two doses of Moderna mRNA-1273 elicited higher anti-S IgG titres than Pfizer BNT162b2, although they did not look at heterologous vaccine regimens[426,427]. However, another study did find that heterologous vaccination with Pfizer BNT162b2 followed by Moderna mRNA-1273 did produce a stronger antibody response when compared to two doses of Pfizer BNT162b2[428]. Together this demonstrates that using a vaccine combination that includes Moderna mRNA-1273 may be advantageous over vaccinating with Pfizer BNT162b2 alone.

4.2 Sex Differences

I next looked at how sex differences impact anti-S IgG responses to COVID-19 vaccination. Previous studies have established that females tend to develop higher antibody responses to vaccination than males, such as with influenza and measles, mumps, and rubella (MMR) vaccines[429]. For this reason, I wanted to know whether this trend would continue with mRNA vaccines for SARS-CoV-2. However, I did not find any differences between sexes in regard to overall antibody responses. This study could not detect a statistically significant difference in antibody responses to SARS-CoV-2 vaccination by sex. Other literature on sex differences in antibody responses to COVID-19 vaccination appears to be inconclusive. There are some studies that report higher antibody responses in females vaccinated against SARS-CoV-2, while other studies found no differences between sexes[398,399,430]. One study did not find a difference between sexes specifically, but did find that sex-specific comorbidities influenced antibody responses[430]. Further studies addressing how sex impacts antibody responses to vaccination should ensure that variables such as sex-specific comorbidities are accounted for.

4.3 Regional Differences

Manitoba's population is highly diverse and the populations in each RHA differ from one another in terms of race and ethnicity, lifestyle, education levels, and access to healthcare. I therefore decided to examine whether differences in geographic location/RHA affected antibody responses to COVID-19 vaccination. The NHR has a much higher proportion of Indigenous residents and communities than other health regions in Manitoba. This region also experiences greater difficulty accessing healthcare and other

essential resources including affordable food and transportation due to the remote locations of many communities in the region[431]. The WRHA encompasses the city of Winnipeg, the capital of Manitoba. This region has a higher population density than the rest of the province, and a largely heterogeneous population when it comes to race and ethnicity, socioeconomic status, and lifestyle.

SHSS is a highly diverse region in regard to race, ethnicity, and culture as it is home to large Mennonite, Hutterite, Francophone, and immigrant populations. The region has the highest rate of population growth and migrant mobility in the province[432]. Areas of SHSS also have markedly lower education levels than the provincial average, such as the city of Winkler and surrounding Rural Municipality of Stanley, where over 40% of residents do not have a high school diploma despite plenty of access to schools and educational resources[433,434]. These areas also had the lowest vaccination rate in Manitoba, and possibly Canada[247,435,436]. By examining whether these regions had an impact on antibody responses to COVID-19 vaccination I could infer that some of the factors unique to each region may have impacted the antibody responses of their residents. While I did not find any differences in antibody responses between the regions, factors such as race and ethnicity, lifestyle and culture, education, and healthcare access should be examined independently to determine their effect on antibody responses to COVID-19 vaccination.

4.4 IgG Titres Vary by Age

When comparing the antibody responses between the vaccines within the same age group, I found that 20-39-year-olds who received Moderna mRNA-1273 or Pfizer/Moderna had higher titres after two doses than those who received Pfizer BNT162b2. No statistically significant differences were found between the vaccines with any other age groups, and there were no differences after only one dose. This suggests that antibody responses in younger individuals are stronger in those who receive Moderna mRNA-1273 compared to Pfizer BNT162b2, but middle-aged and older individuals respond similarly to the two vaccines. This information could help inform patients and practitioners on which COVID-19 vaccine may provide the most protection based on age.

The antibody responses of different age groups who had the same vaccination regimens were also compared. Overall, younger age groups had higher antibody titres than older age groups, regardless

of vaccine. Advanced age is commonly associated with diminished antibody responses to both COVID-19 infection and vaccination[357,437–439]. Given that the immune system's ability to respond to antigens declines with age, this result is unsurprising[440]. Another study conducted in British Columbia found older age to be the strongest predictor of hospital admission for COVID-19[395]. Individuals aged 60+ also had the highest infection fatality ratio (IFR) in Manitoba when compared to other age groups[411]. The tailoring of vaccination strategies and public health measures to protect vulnerable populations such as the elderly is key to reducing the burden of disease.

4.5 Previous COVID-19 Infection Boosts IgG Titres

Finally, I compared the anti-S IgG titres of vaccinated specimens who were previously infected to those that were uninfected. Those who were previously infected had higher titres than those uninfected. Of note, among those who received Pfizer/Moderna, none of the age groups who were previously infected had significantly higher titres than those uninfected. There are a few possible explanations for why this may have occurred. First, it is possible that the sample sizes were too small to generate enough statistical power for significance; second, because the assay used has a maximal limit, the majority of specimens that were previously infected may have had titres that hit the maximal limit of the assay, and the difference from uninfected specimens may have not been large enough to warrant significance. The third possibility is that the titres induced by the combination of Pfizer/Moderna in uninfected specimens were nearly as high as those who were previously infected, suggesting that Pfizer/Moderna elicits a superior antibody response than two doses of Moderna mRNA-1273 or Pfizer BNT162b2. Without rerunning the samples using an assay without a maximal limit it is difficult to determine which scenario is true. Numerous studies have established that previously infected individuals develop higher antibody titres to mRNA vaccines than infection-naïve individuals[399,441–443]. Previously infected individuals have also been shown to mount a faster antibody response to vaccination[399,441]. While I may not have been able to fully quantify the antibody responses of previously infected specimens, it is clear that previous infection boosts anti-S IgG titres in response to SARS-CoV-2 vaccination.

My results indicate that Moderna mRNA-1273 and the Pfizer/Moderna combination elicited higher anti-S IgG titres than Pfizer BNT162b2. These findings have been seen in other studies, with Moderna mRNA-1273 producing a superior antibody response than Pfizer BNT162b2[426,427,437,444–446]. One

possible explanation for the superior response produced by Moderna mRNA-1273 is the higher mRNA content than Pfizer BNT162b2 (100 mcg vs. 30 mcg per dose for ages 12+)[227]. In addition to this, heterologous vaccination against SARS-CoV-2 has been shown to elicit equivalent or superior antibody responses compared to homologous vaccination[428]. One study found that priming with Pfizer BNT162b2, followed by a dose of Moderna mRNA-1273 produced superior immunogenicity than two doses of Pfizer BNT162b2, suggesting that this superior immunogenicity may be the result of Moderna mRNA-1273 being more immunogenic, rather than a specific result of mixing vaccines[428]. While there may be some benefit to mixing Pfizer/Moderna, the difference in anti-S IgG titres appears to be minimal compared to homologous vaccination with Moderna mRNA-1273.

4.6 Limitations

The study had a number of strengths, including the large sample size, the distribution of specimens from different ages, sexes, and regions, and the availability of vaccination data. However, there were several limitations. First, the DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG assay I used has a maximal limit of 400 AU/mL, meaning that any specimens with titres above that limit could not be fully quantified. This included the titres of any specimens that received three or more doses, and likely some of the specimens who were previously infected. I also did not have access to PCR test results for any of the specimens to rule out active infection. Specimens were presumed to be negative for previous SARS-CoV-2 infection if they were negative for anti-N IgG. There is a possibility that some specimens may have been previously infected and had anti-N IgG titres that were below the cut-off for a positive result or had waned at the time of sampling. I would have liked to have conducted plaque reduction neutralization assays to obtain neutralizing antibody titres for the specimens, however I did not have access to a containment level 3 laboratory, which is needed to work with live SARS-CoV-2 in Canada. I attempted to perform neutralization assays using pseudotyped VSV and lentivirus systems carrying the S protein, however I was unable to get them to work, and the results generated were unreliable.

I had also wanted to study the titres of those first vaccinated with Moderna mRNA-1273 followed by Pfizer BNT162b2, but there were not enough specimens to make a proper comparison to other vaccination regimens. Additionally, longitudinal data was not collected, and I was unable to track an individual's response over time. Using longitudinal data would likely give a more accurate representation

of how antibody titres change over time. I would have liked to examine how other demographic factors like race and ethnicity, lifestyle, education, comorbidities, and access to healthcare independently impact antibody responses to vaccination, however I did not have access to this information.

One of the biggest limitations of this study was that it is a secondary analysis of previously collected public health data. The data used was not collected specifically for this study. As a result, some data that would have been useful was not collected, such as SARS-CoV-2 PCR testing data, longitudinal data, and whether any study participants contributed multiple specimens. This is also the reason why some vaccine types could not be included in the study, as the sample sizes were too small. It is also why there is a large difference in sample sizes when comparing some groups, as the sample sizes had been previously determined in the MCS study. Thus, the conclusions drawn from comparisons where there is a large difference in sample sizes should be considered carefully. This study sampling method could have also introduced potential bias, as all specimens were from individuals who sought healthcare during the COVID-19 pandemic, and are not necessarily representative of the entire Manitoba population.

Finally, while I did not look at cellular immune responses to vaccination, they remain an important defense mechanism against SARS-CoV-2 infection and further studies examining the impact of COVID-19 vaccination on cellular immune responses at the population level would be beneficial.

5 CONCLUSIONS

Vaccines for SARS-CoV-2 saved millions of lives throughout the COVID-19 pandemic and have set a new precedent for future vaccine development. The COVID-19 pandemic documents the first use of mRNA vaccines in humans, marking a tremendous accomplishment that paves the way for the use of these vaccines against other diseases. This study found that Moderna mRNA-1273 and the combination of Pfizer/Moderna elicited higher anti-S IgG titres than Pfizer BNT162b2 among an analysis of over 14,000 serum specimens acquired within Manitoba. This indicates that Moderna mRNA-1273's efficacy is superior to Pfizer BNT162b2, although the difference is relatively small and both vaccines induced a strong antibody response against SARS-CoV-2. This slight difference in efficacy may be due to differences in formulations or because the dosage for Moderna mRNA-1273 is slightly higher than Pfizer BNT162b2. Further studies are needed to determine the exact cause of this difference.

When examining the impacts of various demographic factors on post-vaccination titres I found that there were no notable differences between sexes or RHAs. Furthermore, advanced age was associated with lower antibody titres overall, especially when second or third doses were delayed. That being said, this study found some evidence that antibody titres were lower among those who delayed getting a second or third dose, regardless of age group. This highlights the importance of regular booster doses, especially among high-risk groups such as the elderly, who may need more frequent boosters than the current recommendation of every six months. Further studies should be conducted on how to both improve the immune response of elderly individuals to the COVID-19 vaccine, as well as how to optimize the vaccination schedule to best prevent SARS-CoV-2 infection. There is a need to improve vaccination scheduling in a way that ensures that immunity to SARS-CoV-2 is maintained, without requiring doses so frequently that individuals become too inconvenienced to receive regular booster doses.

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7 APPENDIX

Table S12. Results of Dunn's test for pairwise comparisons that compared differences in anti-S IgG titres between sexes. Sexes were compared within the same dose and vaccine type. The Benjamini-Hochberg method was used to correct for multiple hypothesis testing. Group 1 and Group 2 refer to the two groups compared. n1 and n2 are the sample sizes for those respective groups.

	Vaccine Type	Group 1	Group 2	n1	n2	Median 1 (AU/mL)	Median 2 (AU/mL)	Statistic	p	Significance
Dose 1	Moderna	Female	Male	67	68	46.7	40.45	-7.83×10^{-1}	4.34×10^{-1}	ns
	Pfizer	Female	Male	255	216	40.4	28.8	-1.09	2.77×10^{-1}	ns
Dose 2	Moderna	Female	Male	141	157	196	212	8.70×10^{-1}	3.84×10^{-1}	ns
	Pfizer	Female	Male	473	417	176	168	-4.45×10^{-1}	6.57×10^{-1}	ns
	Pfizer/ Moderna	Female	Male	74	94	227	215	2.57×10^{-1}	7.97×10^{-1}	ns

ns, not significant.

Table 13. Results of Dunn's test for pairwise comparisons that compared differences in anti-S IgG titres between age groups. Age groups were compared within the same dose and vaccine type. The Benjamini-Hochberg method was used to correct for multiple hypothesis testing. Age Group 1 and Age Group 2 refer to the two groups compared. n1 and n2 are the sample sizes for those respective groups.

	Vaccine Type	Age Group 1	Age Group 2	n1	n2	Median 1 (AU/mL)	Median 2 (AU/mL)	Statistic	p	Significance
Dose 1	Moderna	12-19	20-39	9	39	141	74	-1.12	3.16×10^{-1}	ns
	Moderna	12-19	40-59	9	39	141	42.2	-1.58	1.71×10^{-1}	ns
	Moderna	12-19	60+	9	48	141	10.5	-2.57	4.77×10^{-2}	*
	Moderna	20-39	40-59	39	39	74	42.2	-7.53×10^{-1}	4.52×10^{-1}	ns
	Moderna	20-39	60+	39	48	74	10.5	-2.41	4.77×10^{-2}	*
	Moderna	40-59	60+	39	48	42.2	10.5	-1.62	1.71×10^{-1}	ns
	Pfizer	12-19	20-39	124	58	67.85	49.1	-2.20	3.37×10^{-2}	*
	Pfizer	12-19	40-59	124	113	67.85	36.5	-2.90	7.94×10^{-3}	**

	Pfizer	12-19	60+	124	176	67.85	18.05	-6.18	3.94×10^{-9}	****
	Pfizer	20-39	40-59	58	113	49.1	36.5	-1.71×10^{-1}	8.64×10^{-1}	ns
	Pfizer	20-39	60+	58	176	49.1	18.05	-2.48	1.99×10^{-2}	*
	Pfizer	40-59	60+	113	176	36.5	18.05	-2.88	7.94×10^{-3}	**
Dose 2	Moderna	12-19	20-39	10	70	359	278.5	-4.31×10^{-1}	6.67×10^{-1}	ns
	Moderna	12-19	40-59	10	88	359	192	-1.66	1.17×10^{-1}	ns
	Moderna	12-19	60+	10	130	359	179.5	-2.39	3.40×10^{-2}	*
	Moderna	20-39	40-59	70	88	278.5	192	-2.54	3.28×10^{-2}	*
	Moderna	20-39	60+	70	130	278.5	179.5	-4.30	1.03×10^{-4}	***
	Moderna	40-59	60+	88	130	192	179.5	-1.67	1.17×10^{-1}	ns
	Pfizer	12-19	20-39	136	141	258	196	-3.66	3.81×10^{-4}	***
	Pfizer	12-19	40-59	136	205	258	145	-6.18	1.87×10^{-9}	****
	Pfizer	12-19	60+	136	408	258	141.5	-8.66	2.80×10^{-17}	****
	Pfizer	20-39	40-59	141	205	196	145	-2.23	3.07×10^{-2}	*
	Pfizer	20-39	60+	141	408	196	141.5	-4.28	3.78×10^{-5}	****
	Pfizer	40-59	60+	205	408	145	141.5	-2.03	4.25×10^{-2}	*
	Pfizer/ Moderna	12-19	20-39	19	68	400	230	-3.80	2.89×10^{-4}	***
	Pfizer/ Moderna	12-19	40-59	19	52	400	187	-3.98	2.08×10^{-4}	***
	Pfizer/ Moderna	12-19	60+	19	29	400	167	-4.96	4.21×10^{-6}	****
	Pfizer/ Moderna	20-39	40-59	68	52	230	187	-4.37×10^{-1}	6.62×10^{-1}	ns
	Pfizer/ Moderna	20-39	60+	68	29	230	167	-2.16	4.67×10^{-2}	*
	Pfizer/ Moderna	40-59	60+	52	29	187	167	-1.72	1.04×10^{-1}	ns

****$p \leq 0.0001$; ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; ns, not significant.

Table 14. Results of Dunn's test for pairwise comparisons that compared differences in anti-S IgG titres between vaccine types. Vaccine types were compared within the same dose. The Benjamini-Hochberg method was used to correct for multiple hypothesis testing. Group 1 and Group 2 refer to the two groups compared. n1 and n2 are the sample sizes for those respective groups.

	Group 1	Group 2	n1	n2	Median 1 (AU/mL)	Median 2 (AU/mL)	Statistic	p	Significance
Dose 1	Moderna	Pfizer	135	472	42.2	36.5	-1.21	2.28×10^{-1}	ns
Dose 2	Moderna	Pfizer	298	890	205	172	-3.73	2.91×10^{-4}	***
	Moderna	Pfizer/ Moderna	298	168	205	218	6.87×10^{-1}	4.92×10^{-1}	ns
	Pfizer	Pfizer/ Moderna	890	168	172	218	3.75	2.91×10^{-4}	***

***p ≤ 0.001; ns, not significant.

Table 15. Results of Dunn's multiple comparisons test that compared differences in anti-S IgG titres between regional health authorities (RHAs). RHAs were compared within the same dose and vaccine type. The Benjamini-Hochberg method was used to correct for multiple hypothesis testing. Group 1 and Group 2 refer to the two groups compared. n1 and n2 are the sample sizes for those respective groups.

	Vaccine Type	Group 1	Group 2	n1	n2	Median 1 (AU/mL)	Median 2 (AU/mL)	Statistic	p	Significance
Dose 1	Moderna	IEHR	NHR	23	19	41.1	22.8	-1.10	6.55×10^{-1}	ns
	Moderna	IEHR	PMH	23	24	41.1	19.23	-1.27	6.55×10^{-1}	ns
	Moderna	IEHR	SHSS	23	28	41.1	47.45	-3.14×10^{-1}	9.42×10^{-1}	ns
	Moderna	IEHR	WRHA	23	41	41.1	64.3	-3.73×10^{-1}	9.42×10^{-1}	ns
	Moderna	NHR	PMH	19	24	22.8	19.23	-9.27×10^{-2}	9.71×10^{-1}	ns
	Moderna	NHR	SHSS	19	28	22.8	47.45	8.55×10^{-1}	6.55×10^{-1}	ns
	Moderna	NHR	WRHA	19	41	22.8	64.3	8.83×10^{-1}	6.55×10^{-1}	ns
	Moderna	PMH	SHSS	24	28	19.23	47.45	1.02	6.55×10^{-1}	ns
	Moderna	PMH	WRHA	24	41	19.23	64.3	1.06	6.55×10^{-1}	ns
	Moderna	SHSS	WRHA	28	41	47.45	64.3	-3.63×10^{-2}	9.71×10^{-1}	ns
	Pfizer	IEHR	NHR	31	7	33.5	86.5	1.77	1.93×10^{-1}	ns
	Pfizer	IEHR	PMH	31	51	33.5	41.6	-2.88×10^{-1}	9.90×10^{-1}	ns
	Pfizer	IEHR	SHSS	31	57	33.5	38.35	-1.25×10^{-2}	9.90×10^{-1}	ns
	Pfizer	IEHR	WRHA	31	326	33.5	34.85	3.60×10^{-2}	9.90×10^{-1}	ns
	Pfizer	NHR	PMH	7	51	86.5	41.6	-2.00	1.93×10^{-1}	ns
	Pfizer	NHR	SHSS	7	57	86.5	38.35	-1.85	1.93×10^{-1}	ns
	Pfizer	NHR	WRHA	7	326	86.5	34.85	-1.92	1.93×10^{-1}	ns

	Pfizer	PMH	SHSS	51	57	41.6	38.35	3.26×10^{-1}	9.90×10^{-1}	ns
	Pfizer	PMH	WRHA	51	326	41.6	34.85	4.81×10^{-1}	9.90×10^{-1}	ns
	Pfizer	SHSS	WRHA	57	326	38.35	34.85	6.65×10^{-2}	9.90×10^{-1}	ns
Dose 2	Moderna	IEHR	NHR	49	65	197	190	-5.15×10^{-1}	7.62×10^{-1}	ns
	Moderna	IEHR	PMH	49	63	197	227	-8.87×10^{-1}	6.25×10^{-1}	ns
	Moderna	IEHR	SHSS	49	45	197	185	-9.26×10^{-1}	6.25×10^{-1}	ns
	Moderna	IEHR	WRHA	49	76	197	240.5	1.03	6.25×10^{-1}	ns
	Moderna	NHR	PMH	65	63	190	227	-4.04×10^{-1}	7.62×10^{-1}	ns
	Moderna	NHR	SHSS	65	45	190	185	-4.83×10^{-1}	7.62×10^{-1}	ns
	Moderna	NHR	WRHA	65	76	190	240.5	1.70	3.00×10^{-1}	ns
	Moderna	PMH	SHSS	63	45	227	185	-1.14×10^{-1}	9.09×10^{-1}	ns
	Moderna	PMH	WRHA	63	76	227	240.5	2.10	2.16×10^{-1}	ns
	Moderna	SHSS	WRHA	45	76	185	240.5	2.02	2.16×10^{-1}	ns
	Pfizer	IEHR	NHR	60	9	123	131	3.30×10^{-1}	7.41×10^{-1}	ns
	Pfizer	IEHR	PMH	60	96	123	178	1.57	3.91×10^{-1}	ns
	Pfizer	IEHR	SHSS	60	87	123	190	2.56	5.33×10^{-2}	ns
	Pfizer	IEHR	WRHA	60	638	123	173	2.55	5.33×10^{-2}	ns
	Pfizer	NHR	PMH	9	96	131	178	4.01×10^{-1}	7.41×10^{-1}	ns
	Pfizer	NHR	SHSS	9	87	131	190	8.90×10^{-1}	6.24×10^{-1}	ns
	Pfizer	NHR	WRHA	9	638	131	173	6.76×10^{-1}	6.24×10^{-1}	ns
	Pfizer	PMH	SHSS	96	87	178	190	1.16	6.14×10^{-1}	ns
	Pfizer	PMH	WRHA	96	638	178	173	7.96×10^{-1}	6.24×10^{-1}	ns
	Pfizer	SHSS	WRHA	87	638	190	173	-7.42×10^{-1}	6.24×10^{-1}	ns
	Pfizer/ Moderna	IEHR	NHR	9	1	231	278	4.72×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	IEHR	PMH	9	17	231	223	-2.01×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	IEHR	SHSS	9	18	231	221	-1.90×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	IEHR	WRHA	9	123	231	214	-1.41×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	NHR	PMH	1	17	278	223	-5.64×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	NHR	SHSS	1	18	278	221	-5.60×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	NHR	WRHA	1	123	278	214	-5.44×10^{-1}	9.88×10^{-1}	ns

	Pfizer/ Moderna	PMH	SHSS	17	18	223	221	1.57×10^{-2}	9.88×10^{-1}	ns
	Pfizer/ Moderna	PMH	WRHA	17	123	223	214	1.32×10^{-1}	9.88×10^{-1}	ns
	Pfizer/ Moderna	SHSS	WRHA	18	123	221	214	1.14×10^{-1}	9.88×10^{-1}	ns

IEHR, Interlake-Eastern Health Region; NHR, Northern Health Region; PMH, Prairie Mountain Health; SHSS, Southern Health Santé Sud; WRHA, Winnipeg Regional Health Authority; ns, not significant.

Table 16. Results of Dunn's multiple comparisons test that compared differences in anti-S IgG titres between individuals who had a COVID-19 infection prior to specimen collection and those with no known previous infection. Titres were compared within the same dose and vaccine type. The Benjamini-Hochberg method was used to correct for multiple hypothesis testing. For Group 1 and Group 2, "NO" corresponds to no known previous infection, while "YES" indicates previous infection. n1 and n2 are the sample sizes for those respective groups.

	Vaccine Type	Age Group	Group 1	Group 2	n1	n2	Median 1 (AU/mL)	Median 2 (AU/mL)	Statistic	p	Significance
Dose 1	Moderna	12-19	NO	YES	12	1	400	148	1.22	2.22×10^{-1}	ns
	Moderna	20-39	NO	YES	48	14	400	83.55	4.25	2.14×10^{-5}	****
	Moderna	40-59	NO	YES	47	17	400	39.8	3.20	1.37×10^{-3}	**
	Moderna	60+	NO	YES	55	4	400	10.9	3.36	7.87×10^{-4}	***
	Pfizer	12-19	NO	YES	153	20	250.5	72.9	4.89	1.02×10^{-6}	****
	Pfizer	20-39	NO	YES	69	12	400	54.3	4.28	1.85×10^{-5}	****
	Pfizer	40-59	NO	YES	127	10	383.5	36.5	4.33	1.47×10^{-5}	****
	Pfizer	60+	NO	YES	197	11	167	18.3	3.08	2.05×10^{-3}	**
Dose 2	Moderna	12-19	NO	YES	14	2	400	332.5	1.77	7.60×10^{-2}	ns
	Moderna	20-39	NO	YES	93	13	400	288	4.19	2.76×10^{-5}	****
	Moderna	40-59	NO	YES	101	22	400	199	4.42	9.94×10^{-6}	****
	Moderna	60+	NO	YES	141	14	400	176	2.86	4.18×10^{-3}	**
	Pfizer	12-19	NO	YES	174	41	400	278.5	6.52	6.85×10^{-11}	****
	Pfizer	20-39	NO	YES	163	25	400	201	5.36	8.37×10^{-8}	****

	Pfizer	40-59	NO	YES	235	19	400	155	5.52	3.33×10^{-8}	****
	Pfizer	60+	NO	YES	462	26	400	144.5	5.20	1.94×10^{-7}	****
	P/M	12-19	NO	YES	19	1	400	400	7.12×10^{-1}	4.76×10^{-1}	ns
	P/M	20-39	NO	YES	80	5	400	232.5	2.76	5.71×10^{-3}	**
	P/M	40-59	NO	YES	62	6	389.5	206.5	1.77	7.66×10^{-2}	ns
	P/M	60+	NO	YES	30	3	400	169.5	1.25	2.10×10^{-1}	ns

****p ≤ 0.0001; ***p ≤ 0.001; **p ≤ 0.01; *p ≤ 0.05; ns, not significant.