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IMPACT OF MATERNAL ANTIBODY-MEDIATED PROTECTION AGAINST COXSACKIE B INFECTIONS IN EARLY LIFE

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ABSTRACT

Nanna Kangasmäki: Impact of maternal antibody-mediated protection against Coxsackie B infections in early life

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Tampere University

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Enterovirus (EV), and especially coxsackievirus B (CVB), infections can be severe or even fatal for newborns and infants. CVB infections have also been proposed to be one of the main environmental triggers of type 1 diabetes (T1D). To address the relationship between the increasing T1D incidence and the declined EV infection rates in Finland, the "polio hypothesis" was proposed in 2000, based on the observation that higher paralytic poliomyelitis frequency was associated with lower rate of polio infections in the population.

With an analogy, the hypothesis proposes that as mothers have encountered less EV infections in Finland, and there is no EV vaccine available, they cannot provide maternal antibodies against EVs for the offspring. Furthermore, children have infections later in life and are not protected by maternal antibodies anymore at the time of infection as the maternal antibody protection wanes during the first year of life. Thus, this could lead to increased T1D incidence in the population through impaired protection against CVB infections.

This thesis aimed to study the frequency of CVB infections and maternal neutralizing antibodies against five CVB serotypes (CVB1-CVB5) in Finland. Also, the aim was to investigate if, and for how long, the maternal antibodies protect the child from CVB infections. In that regard, it was also important to determine the age of first CVB infections. Serum samples from 528 children at 4 different timepoints (cord blood, 3, 6, 12, months) were tested for neutralizing antibodies. The blood samples and clinical health records utilized in this thesis have been collected as part of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study. The longitudinal dataset from DIPP study provided a unique opportunity to study the kinetics of eliminating maternal antibodies and determine how long maternal antibodies are present in child's blood circulation. Thus, the waning of maternal antibodies and the antibody responses in natural infections, that occurred during the first year of life, were analyzed.

The key findings of this thesis implied that in Finland, among the children that have genetic risk for T1D, around 50-75% of the children did not have the maternal antibody protection against CVB at the time of birth. Maternal antibodies against CVB1 were less common than against CVB2-CVB5. CVB1 and CVB2 infections were frequent already during the first year of life, in contrast, CVB3, CVB4, and CVB5 infections were less frequent and acquired later, closer to the age of 12 months. Maternal CVB antibodies seemed to provide effective protection against early life CVB infections. The results of the kinetics of maternal antibody waning provided further evidence that transplacental passive immunity wanes during the first year of life, thus, the maternal antibodies alone are insufficient for sustained protection.

These findings support the rationale for developing a vaccine that can induce neutralizing antibodies against CVB and administering the vaccine early in infancy to prevent the first CVB infections.

Keywords: Coxsackievirus B, Enterovirus, type 1 diabetes, neutralizing antibody, vaccine, Bayesian statistics

The originality of this thesis has been checked using the Turnitin OriginalityCheck service.

TIIVISTELMÄ

Nanna Kangasmäki: Äidiltä saadun vasta-ainevälitteisen suojan vaikutus varhaislapsuuden
Coxsackie B - virusinfektioihin
Pro Gradu
Tampereen yliopisto
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Enteroviruksista (EV) erityisesti coxsackievirus B (CVB)-infektiot voivat olla vaarallisia tai jopa henkeä uhkaavia vastasyntyneille ja imeväisikäisille. CVB-infektioiden on esitetty myös olevan tärkeimpiä ympäristötekijöitä tyypin 1 diabeteksen (T1D) synnyssä. Vuonna 2000 esitettiin ”poliohypoteesi”, jonka tavoitteena oli selittää T1D ilmaantuvuuden kasvua Suomessa viime vuosikymmeninä, vaikka samaan aikaan EV infektioiden määrä vähentyi. Hypoteesi perustui havaintoon, että matalampi polioinfektioiden määrä populaatiossa yhdistyi korkeampaan paralyyttisen poliomyeliitin ilmaantuvuuteen. Hypoteesi sovelsi polioinfektioissa nähtyä ilmiötä: kun äidit kohtaavat vähemmän EV-infektioita Suomessa ja EV-rokotetta ei ole saatavilla, he eivät kykene välittämään raskauden aikana istukan kautta EV-vasta-aineita jälkeläisilleen. Lisäksi lapset sairastuvat infektioihin vasta myöhemmin eivätkä sairastuttuaan ole enää äidiltä saatujen vasta-aineiden suojaamia sillä kyseinen vasta-ainesuoja hiipuu ensimmäisen ikävuoden aikana. Näin ollen puutteellinen CVB-vasta-ainesuoja voisi johtaa korkeampaan T1D ilmaantuvuuteen väestössä.

Tämän pro gradututkielman tavoitteena oli tutkia CVB-infektioiden ja äidiltä saadun vasta-ainesuojan yleisyyttä viittä eri CVB serotyyppiä (CVB1-CVB5) vastaan Suomessa. Lisäksi tarkoituksena oli tutkia tämän suojan kestoa, mikäli äidiltä saadut vasta-aineet suojaisivat infektiolta. Kyseistä tarkoitusta varten oli tärkeää myös määrittää minkä ikäisenä ensimmäiset CVB-infektiot saatiin. Seeruminäytteet kerättiin neljässä eri aikapisteessä (napaveri, 3,6,12 kuukautta) 528 lapselta ja näytteistä tutkittiin neutralisoivien vasta-aineiden taso. Verinäytteet ja kliiniset terveystiedot, joita tässä tutkielmassa käytettiin, on kerätty osana Finnish Type 1 Diabetes Prediction and Prevention (DIPP) tutkimusta. Kyseinen pitkittäisaineisto tarjosi ainutkertaisen tilaisuuden tutkia äidiltä saadun immuunisuojan hiipumista ja määrittää kuinka kauan äidiltä saadut vasta-aineet säilyvät elimistössä, joten sekä äidiltä saatujen vasta-aineiden hiipuminen että ensimmäisen elinvuoden aikana sairastettujen CVB infektioiden aiheuttama vaste analysoitiin tässä työssä.

Tärkeimmät tulokset osoittivat, että Suomessa niistä lapsista, joilla on geneettinen alttius sairastua T1D:een, noin 50–75 %:lla ei ollut syntyessä äidiltä saatua CVB-vasta-ainesuojaa. Äidiltä saatu CVB1-vasta-ainesuoja ei ollut yhtä yleinen kuin vasta-ainesuoja CVB2-CVB5 serotyyppijä kohtaan. CVB1- ja CVB2-infektiot olivat yleisiä jo ensimmäisen elinvuoden aikana, mutta CVB3-, CVB4- ja CVB5-infektiot eivät olleet yhtä yleisiä ja niitä esiintyi vasta lähempänä yhden vuoden ikää. Äidiltä saadut vasta-aineet suojaivat tehokkaasti varhaislapsuuden CVB-infektioilta. Tulokset äidiltä saatujen vasta-aineiden hiipumisen kinetiikasta vahvistivat aiempaa käsitystä siitä, että istukan kautta saatu passiivinen immuniteetti hiipuu ensimmäisen ikävuoden aikana. Näin ollen voidaan todeta, että äidiltä saadut vasta-aineet eivät yksinään tarjoa riittävää suojaa.

Tämän tutkielman tulokset tukevat perusteita ehkäistä varhaislapsuuden ensimmäisiä CVB-infektioita CVB-rokotteella, joka annettaisiin jo imeväisiässä.

Avainsanat: Coxsackievirus B, Enterovirus, tyypin 1 diabetes, neutralisoiva vasta-aine, rokote, Bayesiläinen tilastotiede

Tämän julkaisun alkuperäisyys on tarkastettu Turnitin OriginalityCheck –ohjelmalla.

USE OF AI IN THESIS

The AI tools utilized in this thesis, and the purpose of their use have been described below:

Microsoft Copilot, powered by GPT-4

During the analysis process in this thesis, I used Microsoft Copilot to generate example R codes for some of the analysis steps in a way of quick and targeted search engine or interactive user's manual. Such example codes were generated for example to try different visualization options with ggplot2 R package.

Also, when using brms R package for Bayesian mixed effects modelling, I used Copilot as an interactive user's manual to find information about the different settings options for the very specific type of modelling as the one in this thesis.

However, the analyzed data was not passed to any AI tool at any point of the thesis process, and all the analysis work was done and interpreted by me. The example codes provided by the Copilot were used to learn new things and applied in the analysis upon consideration.

During the writing process, Copilot was used as a tool to find synonyms and inspiration for rephrasing. However, all the text is written by me, reflecting my knowledge, understanding, and capabilities.

I acknowledge that I am fully responsible for the entire content of my thesis, including the parts generated by AI, and accept accountability for any violations of ethical standards in publications.

PREFACE

This Master's thesis was conducted in Prof. Heikki Hyöty's Virology research group at the Faculty of Medicine and Health Technology at Tampere University as part of my studies in Master's Degree Programme in Biotechnology and Biomedical Engineering. I would like to express my heartfelt thanks for Prof. Heikki Hyöty for providing me the opportunity to be part of his research group and learn about the fascinating world of virus and type 1 diabetes research. My deepest thanks go to my supervisors, Jutta Laiho and Amirbabak Sioofy Khojine, for their sustained support and guidance during the entire thesis project. I want to thank Jussi Lehtonen and Niila Jouppila for their support and guidance in the analysis process. My warm thanks also go to the laboratory technicians, Maria Ovaskainen and Eveliina Paloniemi, for their guidance in the virus laboratory when I was familiarizing myself with the plaque neutralization assay. To the entire Virology Group, thank you for the fun and energetic environment – it is a pleasure to work with you.

I want to express my gratitude for the support I have received from my family, especially from you Ville and my parents. Achieving this would not have been possible without you. I also want to thank my children Aino, Armi, and Eino for the lively and busy home that we have. With you, things are in the right perspective. Finally, I want to thank all my friends and my sisters for the support, inspiration, and fun moments you bring to my life.

“Kaikkeaa muuta, kunhan ei vaan nukkuvaa, puolikuollutta elämää.”

-Minna Canth

Tampere, 31 October 2025

Nanna Kangasmäki

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LIST OF SYMBOLS AND ABBREVIATIONS

CAR	Coxsackie and Adenovirus Receptor
CB	Cordblood
CVB	Coxsackievirus B
DAF/CD55	Decay Accelerating Factor
DIPP	Finnish Type 1 Diabetes Prediction and Prevention Study
EV	Enterovirus
Fc	Fragment crystallizable
FcRn	Neonatal Fc-receptors
GADA	Glutamic Acid Decarboxylase Autoantibody
GMK	Green Monkey Kidney (cell line)
GMT	Geometric Mean Titer
GSD	Geometric Standard Deviation factor
HBSS	Hanks' Balanced Salt Solution
HLA	Human Leukocyte Antigen
IAA	Insulin Autoantibody
IAb	Islet Autoantibody
IgG	Type G Immunoglobulin
IgA	Type A Immunoglobulin
IgM	Type M Immunoglobulin
PFU	Plaque-forming Unit
RSV	Respiratory Syncytial Virus
RT	Room temperature
+ssRNA	Positive sense single stranded RNA
SARS-CoV-2	Coronavirus SARS-CoV-2
T1D	Type 1 Diabetes
95%-CrI	95% Credible Interval

1. INTRODUCTION

Finland is one of the leading countries in type 1 diabetes (T1D) incidence (Vanderniet et al., 2022). While the incidence of T1D has increased in Finland over the last decades, the enterovirus (EV) infections have declined (Oikarinen et al., 2014). Nevertheless, EV infections, especially coxsackievirus B (CVB) infections, have been proposed to be one of the main environmental triggers of T1D (Hyöty et al., 2018). To address this relationship, the “polio hypothesis” was proposed in 2000 (Viskari et al., 2000).

The “polio hypothesis” is based on the observation that higher paralytic poliomyelitis frequency was associated with lower rate of polio infections in the population (Viskari et al., 2000). With an analogy, the hypothesis proposes that as mothers have encountered less EV infections in Finland, they cannot provide maternal antibodies against these infections for the offspring. Furthermore, children have infections later in life and are not protected by maternal antibodies anymore at the time of infection. Thus, this could lead to increased T1D incidence in the population through impaired protection against CVB infections.

Many antenatal complications have been associated with CVB infections including spontaneous abortions, fetal myocarditis, and neurodevelopmental delays and the infections can be life-threatening for newborns and infants (Sin et al., 2015). Also, in addition to T1D (Sioofy-Khojine et al., 2018), CVBs have been associated with celiac disease (Oikarinen et al., 2021), another chronic disease that often manifests early in life.

During pregnancy, placenta permits transportation of immune mediating factors from mother to the fetus (Albrecht et al., 2022). Maternal antibodies provide protection against infections during the first months of life and gradually diminish while the child’s adaptive immune system develops (Viskari et al., 2005). The immune system in early life is often described to be immature, and there are many unresolved mechanisms behind the questions of why in early life the immune system fails to produce a long-lasting response to some recurring infections and antigens introduced through vaccination, while during the first year of life a sufficient antibody response is induced through vaccination e.g. against poliovirus (Labeur-lurman and Harker, 2024).

This thesis work aimed to study the frequency of CVB infections and maternal neutralizing antibodies against five CVB serotypes (CVB1-CVB5) in Finland. Also, the aim was to investigate if, and for how long, the maternal antibodies protect the child from CVB infections. In that regard, it was important to determine the age of first CVB infections. Serum samples from 528 children at 4 different timepoints (cord blood, 3, 6, 12, months) were tested for neutralizing antibodies. The blood samples and clinical health records utilized in this thesis have been collected as part of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study.

The longitudinal dataset from DIPP study provided a unique opportunity to study the kinetics of eliminating maternal antibodies and determine how long maternal antibodies are present in child's blood circulation. Thus, the waning of maternal antibodies and the antibody responses in natural infections, that occurred during the first year of life, were assessed.

2. LITERATURE REVIEW

2.1 Type 1 diabetes

In type 1 diabetes (T1D), insulin-producing beta cells in the islets of Langerhans are destroyed due to autoimmune reactions. This leads to severe imbalance in blood glucose levels and a need for a lifelong insulin treatment. T1D is a heterogenetic disease with multiple disease subtypes recognized (Redondo and Morgan, 2023; Sioofy-Khojine et al., 2018).

The appearing of autoantibodies against beta cell antigens marks the beginning of the autoimmunity process. Recent studies have shown that both the number of different islet autoantibodies (IAbs) and the order of their appearance, together with the individual's genetic background, have an impact on the disease development (Ziegler et al., 2013).

T1D is a complex disease. Genetic susceptibility to T1D is associated most importantly with the Human Leukocyte Antigen (HLA) region of chromosome 6p21 (Redondo et al., 2018). The highest risk comes with DR4-DQ8 and DR3-DRQ2 haplotypes. The second most important genetic risk is associated with insulin gene *INS* polymorphism. Different T1D risk genotypes have been associated with different autoimmune processes before the clinical onset of T1D (Sioofy-Khojine et al., 2018). For example, haplotype HLA-DR4-DQ8 and *INS* polymorphism have been associated with the autoimmune process in which the insulin autoantibody (IAA) is the first IAb to appear, whereas HLA-DR3-DQ2 haplotype is associated with glutamic acid decarboxylase 65kDa isoform (GAD65) autoantibody (GADA) as the first appearing antibody.

In addition to genetic susceptibility, environmental factors affect the development of T1D. Enterovirus (EV) infections are proposed to be one of the main environmental triggers (Hyöty et al., 2018). Especially, Coxsackievirus B1 (CVB1) serotype has been identified as a major risk associated virus (Laitinen et al., 2014; Sioofy-Khojine et al., 2018).

2.2 Enteroviruses

EVs are the most common human viruses including over 200 genotypes (Hyöty et al., 2018). Polioviruses, echoviruses, rhinoviruses, enterovirus 71, and coxsackievirus groups A and B are all members of the *Enterovirus* genus that is part of *Picornaviridae* family. EVs are positive sense single stranded RNA (+ssRNA) viruses with an icosahedral protein capsid and are transmitted mainly through the fecal-oral route.

The lymphatic tissues of the intestine and oropharynx are the primary EV replication sites (Hyöty et al., 2018). Primary infection can spread to blood and cause viremia, which can lead to secondary infections in other organs. Virus specific tropism to different tissues determines which organs are affected. Tissue tropism can be mediated through expression of viral receptors on the cell surface, intracellular regulation factors of viral replication, and the tissue specific innate immune response. However, these mechanisms are not fully understood with EVs.

2.2.1 Coxsackievirus B

CVB infections can be subclinical or cause mild common cold-like illness, but they are also associated with severe conditions, such as, myocarditis, pancreatitis, meningitis, encephalitis, and hepatitis (Tapparel et al., 2013). For newborns and infants CVB infections can be even life-threatening (Bissel et al., 2014; Foulis et al., 1990), and CVBs have been associated with antenatal complications (Sin et al., 2015). Furthermore, CVBs have been associated with certain chronic diseases that often manifest early in life, including T1D (Sioofy-Khojine et al., 2018) and celiac disease (Oikarinen et al., 2021). There are six known CVB serotypes (CVB1-CVB6) which have distinct genetic and antigenic properties.

Icosahedral CVB capsid, represented in Figure 1., is approximately 30nm in diameter (Sin et al., 2015). Like other EVs, CVBs have +ssRNA genome with a size ranging between 7 to 8 kilobase and encoding four structural capsid proteins (VP1-VP4) and seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C and 3D). The structural proteins VP1-VP3 are located on the surface of the viral capsid and the VP4 lies inside it.

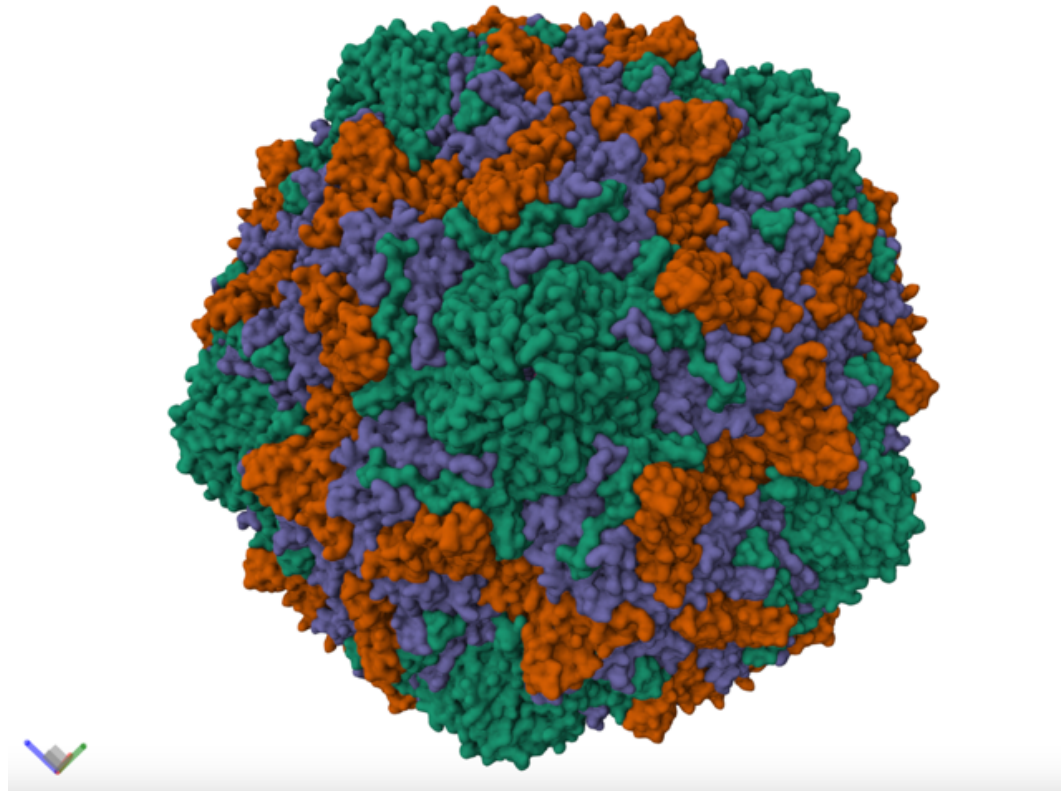


Figure 1. Cryo-Electron Microscopic structure of CVB1 particle. VP1 in green, VP2 in orange, VP3 in purple and VP4 inside the capsid (not showing). Image from PDB ID: 7DPF, (Xu et al., 2021).

Decay Accelerating Factor DAF/CD55 serves as a cell surface anchoring receptor for CVBs (Bergelson et al., 1995). The virus gains access into the host cell through Coxsackie and Adenovirus Receptor (CAR) induced endocytosis (Bergelson et al., 1997). While the capsid is inside the endosome the viral genome is uncoated and is released into the cytoplasm (Xu et al., 2021). The schematic overview of CVB lifecycle is represented in Figure 2.

A single polyprotein is translated at host ribosomes which is then cleaved by viral proteases to generate non-structural proteins, including the viral RNA-dependent polymerase (Wells and Coyne, 2019). The polymerase replicates the viral genome through intermediate double-stranded RNA state in which the +ssRNA is used as a template for negative strand and then, the negative strand acts as a template for new +ssRNA genomes. The replication takes place in replication organelles which are derived from host membranes and protect the process from innate immune pattern recognition receptors (Wells and Coyne, 2019) which detect single- and double-stranded viral RNA (Kato et al., 2006).

The viral capsids are assembled from structural proteins VP0, VP1, and VP3 by first forming protomers and then pentamers (Baggen et al., 2018). The emerging viral genome is packed inside the provirion at the vicinity of the replication machinery. Finally, genome induced maturation of virions includes processing VP0 into VP2 and VP4 capsid proteins (Baggen et al., 2018). Viral particles can be released from the host cell through lysis but non-cytolytic release in vesicles is also possible (Wells and Coyne, 2019).

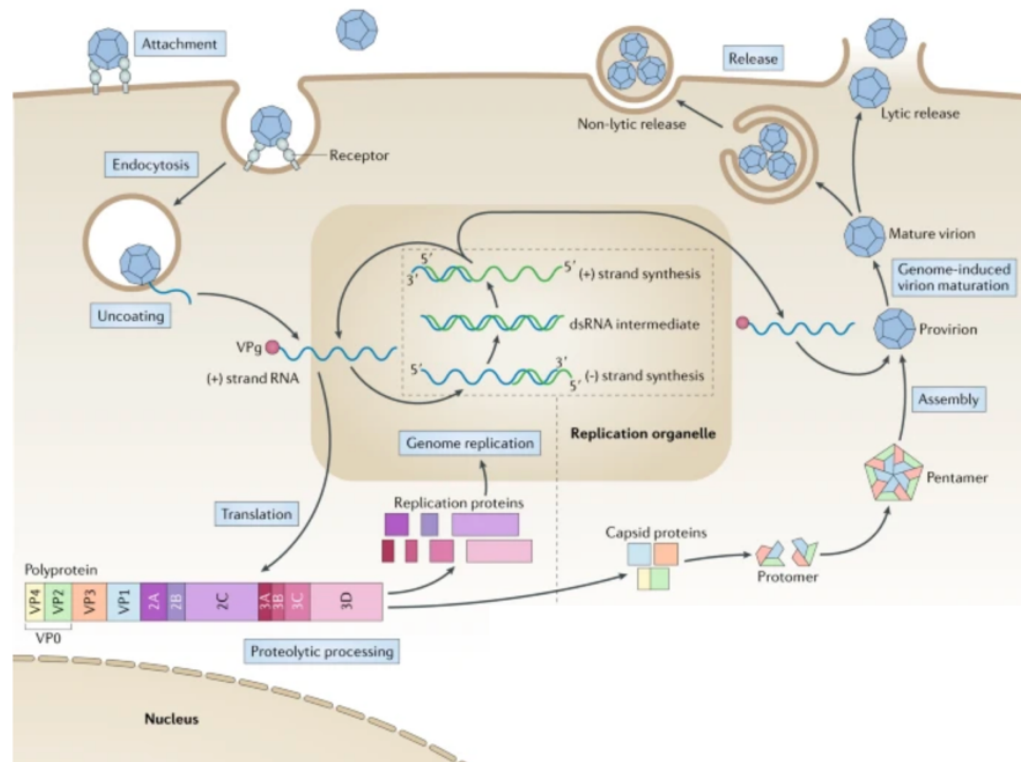


Figure 2. Schematic representation of CVB infection cycle. The virus gains access into the host cell through membrane bound receptors which induces endocytosis. The +ssRNA viral genome is uncoated and released into cytoplasm. A single polyprotein is translated at host ribosomes and then cleaved by viral proteases to generate non-structural proteins, including the viral RNA-dependent polymerase. The replication occurs through intermediate double-stranded RNA state in which the +ssRNA is used as a template for negative strand synthesis. Then the negative strand acts as a template for new +ssRNA genomes. The replication takes place in replication organelles which are derived from host membranes. The viral capsids are assembled from structural proteins VP0, VP1, and VP3 to form first protomers and then pentamers. The emerging viral genome is packed inside the provirion at the vicinity of the replication machinery. Finally, genome induced maturation of virions includes processing VP0 into VP2 and VP4 capsid proteins. Viral particles can be released from the host cell through lysis or inside vesicles. Image modified from (Baggen et al., 2018)

Along with its role as a viral receptor, CAR is a multifunctional protein in human body. CAR is encoded by human CXADR gene, and it serves as a cell-cell adhesion protein in

tight junctions (Pazirandeh et al., 2011). CAR is developmentally and functionally important for many tissues such as heart and pancreas. In addition to transmembrane CARs, there are soluble isoforms due to splicing variants. The soluble CARs can inhibit the infectivity of CVBs (Dörner et al., 2004). The interaction with CAR induces the uncoating of CVB capsid (Xu et al., 2021). When invading to the host cell, this occurs after endocytosis. In contrast, when the soluble CAR isoform binds the virus, the viral genome can be released outside any host cell, and the virus loses infectivity. On the other hand, soluble CARs might occupy CAR binding sites to prevent the CVB binding on the host cell surface.

CAR is a member of immunoglobulin superfamily (Dörner et al., 2004), thus, its protein structure resembles the one of antibodies. There is evidence that the CAR binding sites on CVB capsid can also be epitopes for neutralizing antibodies (Xu et al., 2021). Correspondingly to soluble CARs, the neutralizing antibodies can neutralize the viral infectivity by e.g. occupying the receptor binding sites or disrupting the structure of protein capsid.

2.3 Immune system and immunological memory

Human body interacts constantly with the environment and encounters innumerable antigens throughout life. Some of the antigens may act as immunogens inducing a host immune response against them. This immune response will eliminate the non-self-structures or organisms through various mechanisms which can be divided into innate and adaptive immune responses.

Innate immunity is complex and more ancient than the adaptive immune system. It has evolved to prevent infections and attack pathogens with non-specific and fast mechanisms, from minutes to hours post exposure. The innate immune system consists of different components on multiple levels. For example, on tissue level, skin and mucosa prevent microbial entrance as a physical barrier but also by secreting proteins and enzymes that block pathogen entry. On cellular level, granulocytes, monocytes, macrophages and dendritic cells participate in phagocytosis and secrete cytokines and enzymes. Natural killer cells lyse infected cells and active macrophages. Finally, there are multiple soluble mediators that mediate and regulate immune responses (e.g. chemokines) and can sustain resistance to viral and other pathogen infections (e.g. interferons). As part of the adaptive immune system, T and B cells recognize non-self-structures, that are not produced by the individual, via their cell surface receptors. The recognition leads to proliferation and differentiation of immune cells and finally to immunological memory.

The adaptive immune system is highly specific and reacts with cellular and humoral immunity. However, the response is slower than the one of innate immune system.

Antigen presenting cells (e.g. macrophages, B cells, and dendritic cells) process antigens into short polypeptides and present them on the cell surface in complex with human leukocyte antigen class II (HLA-II). CD4⁺ helper T cells can interact with the HLA-II-antigen complex and induce a downstream immune response. On the other hand, virus infected cells can present virus-derived peptides in complex with the HLA class I (HLA-I) which is detected by CD8⁺ cytotoxic T cells.

B cells have many roles including cytokine secretion and antigen presenting to activate T cells. Moreover, they are essential in acquiring the immunological memory. B cell receptors interact with large and complex immunogenic molecules which leads to proliferation and differentiation of antibody-producing B cells (Virella, 2020). Memory B cells and plasma cells are the main cell types to compose the humoral immunity (Amanna and Slifka, 2010).

Memory B cells express membrane bound immunoglobulins, i.e. antigen specific B cell receptors, and can elicit a rapid immune response when reencountering antigens (Amanna and Slifka, 2010; Andraud et al., 2012). Plasma cells, on the other hand, are fully differentiated and produce circulating antibodies even without antigen's presence. After an infection, the long-term antibody production is maintained by plasma cells in the bone marrow. It is not fully understood which mechanisms lie beneath the long-term antibody production, and why some antigen-specific antibody responses last for a lifetime while others decline with the time (Amanna and Slifka, 2010; Medzhitov and Iwasaki, 2024).

2.4 Maternal antibody transfer

During pregnancy, placenta acts as an interface between mother and fetus and allows transporting oxygen, nutrients, and hormones together with immune mediating factors (Albrecht et al., 2022). The latter can include antibodies, cytokines, and even cells. The transfer of antibodies through placenta requires active receptor mediated transport (Jennewein et al., 2017). Maternal antibodies provide protection against infections during the first months of life and gradually diminish before the child's adaptive immune system develops (Viskari et al., 2005). Thus, maternal antibodies may serve as a vaccination against early infections.

The most prevalent antibody isotype in newborn's serum is IgG (Jennewein et al., 2017). The molecular and cellular mechanism of maternal antibody transfer is represented in Figure 3. Maternal IgG antibodies bind to neonatal Fc-receptors (FcRn) in placental syncytiotrophoblast's endosomes (Jennewein et al., 2019). The binding is triggered by the acidic conditions in the endosomes. The antibodies are transported and released into the fetal circulation upon returning to the physiological pH (Albrecht et al., 2022). The transfer of antibodies is selective (Jennewein et al., 2019), and the efficiency is antigen specific (Fu et al., 2016). Furthermore, it has been found that the maternal antibody transfer is dependent on individual placental function (Albrecht et al., 2022). After birth, infants receive maternal IgA antibodies through breast milk. Maternal antibody transfer through placenta during pregnancy and through breast milk after birth are also referred to as naturally acquired passive immunity.

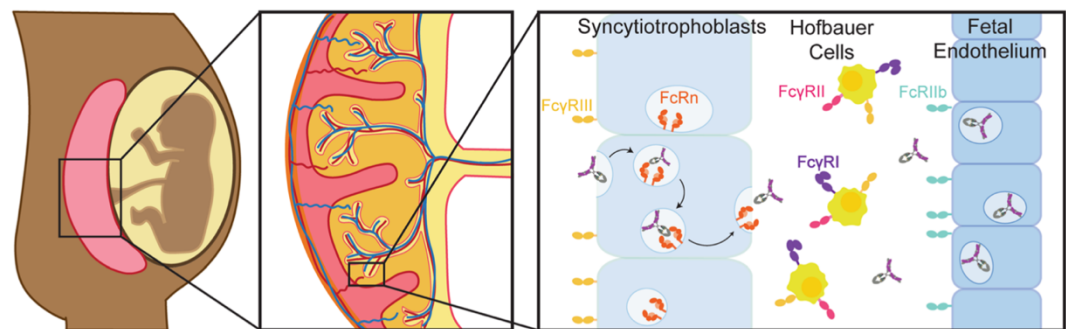


Figure 3. During pregnancy, placenta permits transplacental transfer of maternal (IgG) antibodies, through binding neonatal Fc-receptors (FcRn) to placental syncytiotrophoblast's endosomes. The antibodies are transferred and released to fetal circulation due to pH-gradient between endosome and fetal blood. Image modified from (Jennewein et al., 2017)

Maternal antibodies protect the newborn from pathogens that are present in the community (Jennewein et al., 2017). Thus, to be able to provide maternal antibodies against a specific antigen, the mother must come across the antigen before or during pregnancy which can happen through vaccination or a natural infection.

2.5 Immune system in early life

The immune system of a newborn faces a substantial transition from rather sterile environment *in utero* to an environment with numerous antigens at birth (Jain, 2020). During this shift, the immune system is simultaneously developing tolerance to self, microbiota,

and environmental antigens while balancing between exaggerated inflammation and accurate response to pathogens (Labeur-lurman and Harker, 2024). While many measures are taking place in early life for developing the immune system, it is also often seen as an immature system. Many pathogens, such as CVBs that usually cause only mild disease in healthy adults can be life-threatening to newborns and infants

In the fetal liver, B cell precursors are found as early as seven weeks post-conception, and the B cell production begins at mid-gestation in fetal bone marrow (Jain, 2020). Immediately, dendritic cells can start presenting antigens to B cells. In the work of Blanco et. al (Blanco et al., 2018) plasma cells were not found in cord blood samples, and in samples from newborns they were detected only in low numbers. However, in the samples of 1-to-5-month-old children the number of plasma cells were shown to increase. Plasma cell number was the highest in children from 1-to-2-years-old after which the counts started to decrease gradually throughout life (Blanco et al., 2018).

The capability of producing antigen specific high affinity antibodies relies on CD4⁺ T cell help which is not fully available in early life (Labeur-lurman and Harker, 2024). It also seems that in early life, antigens that induce T cell – independent antibody responses give more effective protection compared to antigens that require T cell-dependent response. However, later in life the T cell-independent responses are known to produce only short-term antibody responses and T cell-dependent responses are known to last from years to decades (Amanna and Slifka, 2010).

While B cells of newborns have distinct characteristics from the ones of adults, including the limited antibody production and somatic hypermutations together with enhanced IgM production (Labeur-lurman and Harker, 2024), during the first 3 months, B cells develop adult-like phenotypes (Jain, 2020). Human IgG half-life is known to be approximately 17.5-26 days and IgG accounts for 75% of the antibody isotypes in adults (Andraud et al., 2012). Also in newborn's serum, IgG is the main antibody isotype as it is the most efficiently transferred isotype of maternal antibodies (Blanco et al., 2018; Jennewein et al., 2017).

IgM and IgA are the other main antibody isotypes in humans. These types have low concentrations in newborn's serum (Blanco et al., 2018). In early life, the maternal IgGs diminish and the IgM produced by the child becomes the predominant isotype. Adult-level IgM concentration in newborns is eventually reached by the age of two years while

the IgA and IgG concentrations are only at 60-70% of the adult concentrations of the same types by the age of three years (Labeur-lurman and Harker, 2024).

2.6 CVB and immune reactions

CVBs cause acute cytolytic infections, which are eradicated by the host's immune system (Chapman, 2022). However, they can also establish chronic infections, which persist in the tissue, regardless of the adaptive immune system's reaction. CVBs are known to persist at least in pancreatic (Sane et al., 2013) and heart tissues (Chapman and Kim, 2008). Together with innate immune system, especially B cells have an essential role in immune responses against CVBs (Wells and Coyne, 2019).

B cells produce antibodies, which can neutralize the infectivity of the virus by e.g. occupying receptor binding sites on the viral capsid or disrupting the capsid structure (Xu et al., 2021). Thus, neutralizing antibodies are essential in blocking CVB infections (Sin et al., 2015). Additionally, they provide a long-lasting protection against a given pathogen and may persist in the body for years or even decades (Oikarinen et al., 2014).

2.7 Vaccination and maternal immunization

Vaccines are one of the most important achievements of biomedicine as they have improved the public health status by reducing global morbidity and mortality of the infectious diseases (Centers for Disease Control and Prevention (CDC), 1999). Many of the vaccines in national vaccination programmes are given at a young age to provide optimal protection at the right time (Finnish Institute for Health and Welfare, 2025a). For example, in Finland the national vaccination programme provides vaccinations against 13 different diseases, related secondary diseases, and long-term adverse effects for all children and young people. Many vaccinations, including the vaccine against poliovirus, are given already during the first six months of life (Finnish Institute for Health and Welfare, 2025a).

It is not fully understood why certain antigens provide robust antibody response in early life while others do not (Labeur-lurman and Harker, 2024). For example, respiratory syncytial virus (RSV) is an example of a viral pathogen that does not seem to elicit long-lasting or fully functional immunity during infancy (Openshaw et al., 2017), thus RSV vaccines have not been as successful in producing antibody responses in early life as e.g. achieved by poliovirus and tetanus vaccines (Labeur-lurman and Harker, 2024). However, it is known that live and live-attenuated vaccines induce robust and long-lasting antibody response and vaccines that aim to protect from pathogens that consist mainly

of one serotype, and are not mutating or transmitted rapidly, seem to be the effective ones (Medzhitov and Iwasaki, 2024).

In addition to vaccinating the youngest age groups to obtain optimal protection, it has been shown that maternal immunization through vaccination during prenatal period provides an efficient and safe tool to protect both infants and mothers from adverse outcomes of infectious diseases (Quincer et al., 2024). There are already several vaccines available that can prevent early life infections through maternal immunization, including influenza, SARS-CoV-2, RSV, tetanus, diphtheria, and pertussis (Tdap) (Albrecht et al., 2022; Finnish Institute for Health and Welfare, 2025b; Quincer et al., 2024).

There are commercial formalin-inactivated EV vaccines against poliovirus and enterovirus 71, but currently no commercial CVB vaccine is available (Hyöty et al., 2018; Stone et al., 2020). It has been estimated that multivalent vaccine targeting all CVB serotypes could prevent up to 60% of new T1D cases (Hyöty et al., 2018). Also, it has been shown that EV vaccine-induced antibody responses do not differ between the general population and the population with the genetic predisposition to T1D (Sioofy-Khojine et al., 2023) furthermore supporting broad applicability for preventing both T1D and severe CVB infections in early life.

A multivalent vaccine including five formalin-inactivated CVB serotypes CVB1-5 has recently passed the phase I clinical trial in Finland which provided preliminary evidence that the vaccine is safe and well tolerated in healthy adults (Hyöty et al., 2024). Also, the vaccine induced robust neutralizing antibody responses against the five CVB serotypes provided in the vaccine formula. Poliovirus vaccine-induced neutralizing antibodies are shown to mediate the protection against poliovirus infections which suggests that similar mechanism should exist against CVBs using the CVB vaccines.

2.8 Bayesian statistics

Bayesian statistical approaches provide a comprehensive framework for analyzing multilevel and longitudinal data (Puga et al., 2015; Verissimo, 2025). The underlying principle of Bayesian statistics is to incorporate prior knowledge in the model with the evidence from new data to yield an updated current understanding. Bayesian models express the resulting parameter estimates as full probability distributions which allows intuitive quantification of uncertainty. Bayesian framework also facilitates hierarchical and mixed effects models which allow estimating both population level parameters and individual level

variation. This is especially valuable when studying complicated systems such as early-life immune development where both biological variation and measurement uncertainty are present.

In traditional frequentist methods, the parameters yield point estimates; however, in Bayesian statistics, the parameter estimates are considered as quantifiable random variables and the certainty around them is described through a full probability distribution (Puga et al., 2015; Veríssimo, 2025). While obtaining full probability distributions is computationally more demanding than the point estimates, Bayesian statistics involve many advantages over the traditional methods such as more intuitive interpretation and incorporating prior knowledge. The prior knowledge is incorporated to the model as a distribution that represents the current belief of the matter. This belief is updated after considering the data which then yields the posterior distribution. This is formally described in Bayes theorem:

$$P(\theta|y) \propto P(y|\theta) \times P(\theta) \tag{1}$$

Bayes theorem produces a conditional probability from its inverse (Veríssimo, 2025). Here $P(\theta|y)$ is the posterior parameter distribution which gives the probability of different population parameter values given the data y . The posterior distribution is constructed from large number of samples collected during the model fitting process. For example, in brms R package, that was used in this thesis project, the default is 4000 posterior samples for each parameter (Bürkner, 2017).

To obtain the posterior parameter distribution, two components are needed: $P(y|\theta)$ gives the probability of the data given each parameter value which is the “likelihood” of frequentist methods (Veríssimo, 2025). Furthermore, $P(\theta)$ gives the distribution of parameter values independently of the data which is the distribution of the prior knowledge. The prior distribution can be assigned to be wide and weakly informative if no robust prior knowledge is available and, in this case, the prior only outlines the most extreme values. With a narrower and more informative prior distribution it is, however, possible to improve the model.

The results from a Bayesian statistical model are the estimated posterior parameter distribution and the 95%-Credible Interval (95%-CrI) (Veríssimo, 2025). The 95%-CrI of the posterior distribution can be interpreted as the bounds within the parameter value lies with 95% probability. If the 95%-CrI is very wide there is more uncertainty around the

parameter estimate and in case of a narrower interval, the parameter estimate is more confident.

2.9 Bayesian mixed effects modelling

The function of immune system is complex, and the knowledge of underlying mechanisms of humoral immunity and antibody kinetics are still limited (Garcia-Fogeda et al., 2023). While the complexity of immune system is a shared feature across individuals, there is also substantial biological variation between individuals. To study such phenomena, mechanistic mathematical approach utilizes longitudinal antibody measurements and mixed effects modelling to include both population level parameters and individual level variation.

Mixed effects modelling is a statistical approach used to analyze data with multiple levels of variability e.g. measurements nested within individuals or individuals nested within groups (Bürkner, 2017). Mixed effect models include both fixed effects i.e. parameters that describe the population, and random effects i.e. parameters that describe the variation across individuals or groups of the given population.

There are several different approaches available to perform mixed effects modelling. The modelling can be based on frequentist methods, such as Maximum Likelihood Estimation (MLE), or Bayesian statistics (Bürkner, 2017), and in some cases the method can be a mixture of both (Garcia-Fogeda et al., 2023). The relationship between predictors and response can be assumed to be linear and residuals normally distributed (Veríssimo, 2025), or in a generalized mixed effect model, there can be a non-linear relationship between the response and predictor variables, and the residuals can be assumed to have e.g. binomial or Poisson distribution (Bürkner, 2018). In case of a complicated non-linear mixed-effects model, the informative prior distributions become crucial for the model to function as intended.

As the computational capacity has increased and sophisticated algorithms, such as Markov Chain Monte Carlo (MCMC) algorithms, have been developed during the last few decades, it has become possible to utilize Bayesian statistics across sciences and yield posterior probability distributions for even complicated multilevel models (Bürkner, 2017). As an example, Stan is a software developed to utilize such sophisticated algorithms as Hamiltonian Monte Carlo and its extension, the No-U-Turn Sampler (NUTS) (Stan Development Team, 2025). These algorithms are designed to converge quickly for

high-dimensional models without the requirement for the priors to be conjugated which is a demand in other similar algorithms (Bürkner, 2017).

Stan allows modelling flexibility by using its own programming language and all models can be written and optimized by the user. Nevertheless, to provide simple and accessible way to perform Bayesian mixed effects modelling, the R package “brms: Bayesian Multilevel Model using Stan” was developed to allow “the user to benefit from the merits of Stan only by using simple ... formula syntax” (Bürkner, 2017).

The general model framework in brms adapts both linear and non-linear multilevel models. In linear multilevel models the response variable y is predicted through the linear combination η of predictor terms transformed by the inverse link function f assuming certain distribution D (Bürkner, 2017). This is formally described by:

$$y_i \sim D(f(\eta_i), \theta), \quad (2)$$

which emphasizes the dependency on the i^{th} observation. The parameter θ describes distribution specific parameters that do not vary across observations, such as standard deviation for normal distribution. The linear predictor term η can be described as:

$$\eta = X\beta + Zu, \quad (3)$$

where β and u denote the population-level and group-level coefficients, respectively, while X and Z are the design matrices (Bürkner, 2017). In mixed effects modelling, response variable y and the design matrices X and Z correspond to the data and the estimated parameters are β , u and θ .

In non-linear mixed effects models the predictor terms can alternatively have a non-linear form (Bürkner, 2018), as presented in:

$$\eta = f(c_1, c_2, \dots, \phi_1, \phi_2, \dots). \quad (4)$$

Here the function f is defined by the user, which can be, for example, the exponential decay function (5) as in this thesis work. The terms c and ϕ denote the known covariates and non-linear parameters, respectively (Bürkner, 2018). Each of the non-linear parameters have their own linear predictor term η_ϕ and, in this approach, the non-linear parameters are rather placeholders for linear predictor terms than actual parameters themselves. This means that the model estimates the linear predictor terms instead of the non-linear parameters.

3. OBJECTIVES

This master's thesis is conducted in the Virology research group at Tampere University. The work aimed to study the frequency of CVB infections and maternal neutralizing antibodies against five CVB serotypes (CVB1-CVB5) in Finland. Also, the aim was to investigate if, and for how long, maternal antibodies protect the child from CVB infections. In that regard, it was important to determine the age of first CVB infections.

The longitudinal dataset from DIPP study provided a unique opportunity to study the kinetics of elimination of the maternal antibodies, and to determine how long the maternal antibodies are present in child's blood circulation. Thus, the kinetics of elimination of the maternal antibodies and the antibody responses in natural infections that occurred during the first year of life, were determined.

The objective of this thesis work was to assemble a complete data matrix of maternal neutralizing antibody titers against five CVB serotypes (CVB1-CVB5) from 528 children at 4 different timepoints during the first year of life. The neutralizing antibody titers were studied with plaque neutralization assays. The frequency of CVB infections was computed by using an algorithm for which the infection is determined as "persistent seroconversion to CVB neutralizing antibody positivity in consecutive samples". Antibody elimination kinetics was visualized and calculated. Finally, the kinetics of elimination of the maternal antibodies were modelled using a Bayesian mixed effects approach.

4. MATERIALS AND METHODS

4.1 Study cohort

The blood samples and clinical health records utilized in this thesis have been collected as part of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study. DIPP is a prospective birth-cohort study, initiated in 1994, and is still recruiting children with genetic predisposition to T1D in three different clinical centers in Finland (www.dipp.fi).

Prospective birth cohort study is especially useful for studying the disease etiology of T1D where both genetical predisposition and environmental factors can contribute to the process of the T1D. Longitudinal data provides an opportunity to record the initiation of autoimmune processes before the clinical onset of T1D and to trace any possible preceding environmental triggers.

The DIPP study has been approved by the ethical committees of the hospital districts of Southwest Finland (Turku University Hospital), Pirkanmaa (Tampere University Hospital), and Northern Ostrobothnia (Oulu University Hospital). The parents of the participating children have given their informed written consent to the participation in the study.

4.2 Study design

The complete data matrix assembled in this thesis included samples from 528 children born 1995 - 2009, with the following time points: cord blood (CB), 3, 6, and 12 months of age. From these 4 timepoints antibody titers against five CVB serotypes, CVB1-CVB5, were measured. CVB6 was not included in this study because the aim was to assemble a full data matrix from the same serotypes that are used in the multivalent vaccine including five formalin-inactivated CVB serotypes (CVB1-5) that has recently passed the phase I clinical trial in Finland (Hyöty et al., 2024).

The study was designed to have case-control matched-groups with a 1:2 ratio. The case children developed multiple (≥ 2) T1D related biochemical islet autoantibodies. The groups were matched for HLA-DQ mediated T1D risk, sex, and the year and month of birth (± 2 months). Descriptive statistics of the participants are reported in Table 1. In this study, the case-control status of the child was not included in the analysis, as the aim was to assess the overall protective effect of maternal antibodies.

Table 1. Descriptive statistics of the participants. IAbs = islet autoantibodies, CB = cord blood.

Total number of children	528
Controls	360 (68%), girls 136 (37.8%)
Cases (developed at least 2 T1D related IAbs)	168 (32%), girls 62 (36.9%)
Measured timepoints	CB, 3, 6, 12 months
Virus serotypes used	CVB1-CVB5
Boys	330 (62.5%)
Girls	198 (37.5%)

4.3 Plaque neutralization assay

Neutralizing antibody titers can be measured from blood serum samples and are used as a sign of past infections (Sioofy-Khojine et al., 2018). The assays are highly specific for the virus serotype. In the context of this thesis project, neutralizing antibody titers were measured for five CVB serotypes (CVB1-CVB5) using a plaque neutralization assay method blinded to the case/control status. The work was conducted at the Department of Virology at Tampere University, Finland.

Four-fold dilutions of antisera (serum including antibodies) were incubated with equal volumes of 100 plaque-forming unit (PFU) of one type of the infectious virus for 1h at 37°C followed by an overnight incubation at room temperature (RT). The antisera-virus reactions were made to 100µl with Hanks' Balanced Salt Solution (HBSS)-HEPES and the solutions were incubated with confluent Green Monkey Kidney (GMK) cells in 12-well plates for 0.5h at 37°C. Cells were then covered with semi-solid media (minimum essential medium supplemented with 0.67% carboxymethyl cellulose) and were incubated for 2 days at 37°C in humid chamber with 5% CO₂ to develop virus induced plaques. Cells were fixed and stained with a crystal violet-formaldehyde solution after which the virus plaques were quantified. An example of stained plaque neutralization assay plate is represented in Figure 4.

A positive result was achieved if the reduction in plaque number was $\geq 75\%$ compared to mock serum-treated virus suspension. The resulting titer corresponds to the highest dilution that still yield a positive result i.e. inhibited the infectivity of the virus. In the four-

fold dilution series the possible titer values are 0, 4, 16, 64, 256, 1024, and 4096 (as the reciprocal of the dilution factors).

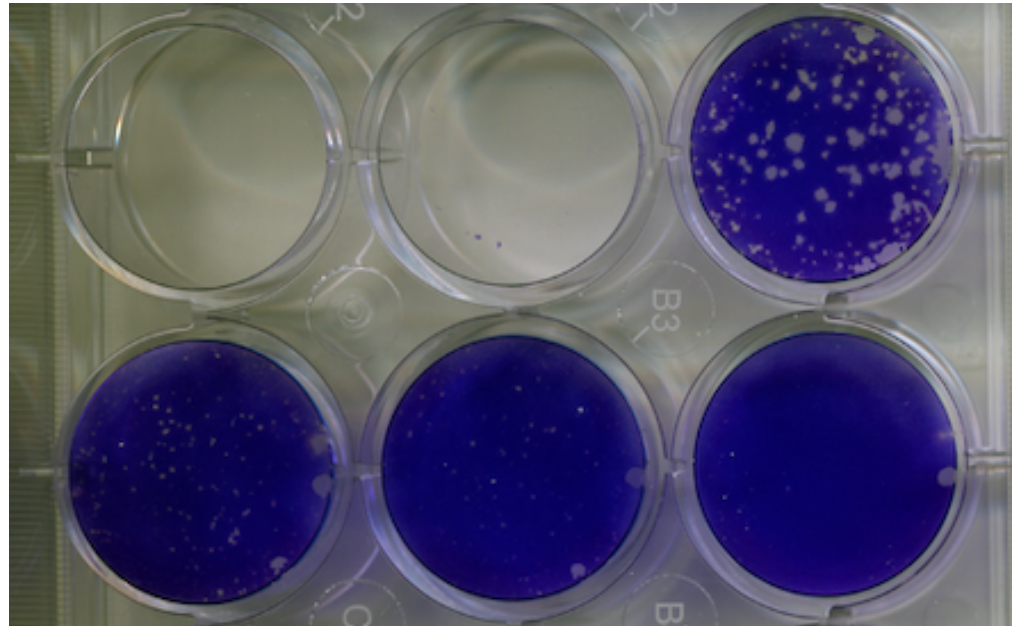


Figure 4. Example image of the plaque neutralization assay plate after crystal violet-formaldehyde fixation-staining. The cell monolayer in the right well of the bottom row is confluent and not infected by the virus (the small round wholes are caused by scratching the monolayer by suction tip). Furthermore, in the left and middle wells of the bottom row, there are small virus plaques in the middle of the wells. In the left and middle wells of the top row, the cell monolayer is fully destroyed by the virus. In the right well, the cell monolayer has large virus plaques.

4.4 Data analysis

The resulting antibody titers from plaque neutralization assay were processed for the analysis that was conducted by using R version 4.3.1 in RStudio version 2025.05.0+496. R packages and the versions used in the analysis were brms (2.22.0), car (3.1-3), dplyr (1.1.4), epiDisplay (3.5.0.2), ggplot2 (3.5.2), reshape2 (1.4.4), tidybayes (3.0.7), and tidyr (1.3.1).

The frequency of maternal antibodies was calculated by using titer 16 as a threshold for antibody positivity. When calculating the frequency of child's CVB infections, an algorithm was used first to remove maternal antibody levels from the measured ones. Then the infections were recorded by using an algorithm for which the infection was deter-

mined as persistent seroconversion to CVB neutralizing antibody positivity in consecutive samples for antibody negative samples and 4-fold increase in titer for antibody positive samples (Sioofy-Khojine et al., 2018). The algorithms used in the analysis were previously designed for other studies in the research group.

Kinetics of the maternal antibody elimination were visualized by plotting the antibody levels from all available timepoints for each child that was assessed to have maternal antibody titer ≥ 16 and not to have any persistent seroconversion against the respective serotype. This yielded in a varying number of children for each of the serotypes (Table 2).

Table 2. *The number of children who had CB titer ≥ 16 and no persistent seroconversion during the first 12 months by each serotype. The kinetics of elimination of the maternal antibodies from the child's blood circulation were determined based on this dataset.*

Serotype	Number of children
CVB1	86
CVB2	199
CVB2	248
CVB4	295
CVB5	161

To understand the average trend in the kinetics of maternal antibody elimination, the geometric mean titer (GMT) was calculated separately for each time point of the respective serotype. For this purpose, the antibody titers were \log_2 -transformed before calculations. Additionally, the geometric standard deviation factor (GSD) was calculated. The maternal antibody decay was assumed to be exponential (White et al., 2014) by the definition:

$$mAb_t = mAb_0 e^{-\mu t} \quad (5)$$

Here, maternal antibody titer was labelled as mAb , decay rate constant as μ , and time as t . The GMTs were used to calculate the decay rate constant μ according to the following:

$$\mu = \frac{\ln(mAb_0) - \ln(mAb_t)}{t} \quad (6)$$

Finally, maternal antibody half-life $T_{1/2}$ and estimated lifespan E were calculated respectively:

$$T_{1/2} = \ln(2)/\mu \quad (7)$$

$$E = 1/\mu \quad (8)$$

4.4.1 Bayesian mixed effects modeling

Calculating the characteristic values of maternal antibody kinetics through GMTs provides only approximate information as it does not consider the individual variation in the underlying biological process that occurs in eliminating the antibodies from blood circulation. To address this issue, the kinetics of eliminating maternal CVB1 antibodies were modelled through Bayesian non-linear mixed effects model using brms R package (Bürkner, 2017). The analysis was conducted for a subset of the dataset with the following inclusion criteria: the CVB1 cord blood titer was 16 or higher and there was no persistent seroconversion during the first year of life (N=86), see Table 2. for CVB1.

In the model, the observed maternal antibody titers made the response variable y . The equation of exponential decay (5) was used as the non-linear function to describe the exponential decay of maternal antibodies over time. The decay rate constant μ and the initial level of maternal antibodies mAb_0 were modelled to have individual-specific variation i.e. random effects. These non-linear parameters were used as placeholders for linear parameter terms η_ϕ in the model, as described (4).

The response distribution was assumed to have hurdle-lognormal distribution. This assumption was justified based on the visualization of the data distribution in Figure 5. Based on this, considerable proportion of the data values were zeros. A hurdle model uses binomial probability to divide the distribution into zero and non-zero values (Min and Agresti, 2005). In the hurdle-lognormal distribution, non-zero values yield lognormal distribution. Thus, the model distribution separates the two phenomena that are present in the dataset: either the child is positive (titer > 0) or negative (titer = 0) for maternal antibodies.

The parameters μ and mAb_0 were assumed to be lognormally distributed and used to predict the maternal antibody titer in an individual i at timepoint j ($mAb_{pred,ij}$) (Andraud et al., 2012). Measured antibody-titers mAb_{obs} were \log_{10} -transformed with an additive residual error:

$$\log_{10}(mAb_{obs,ij}) = \log_{10}(mAb_{pred,ij}) + \varepsilon_{ij}. \quad (9)$$

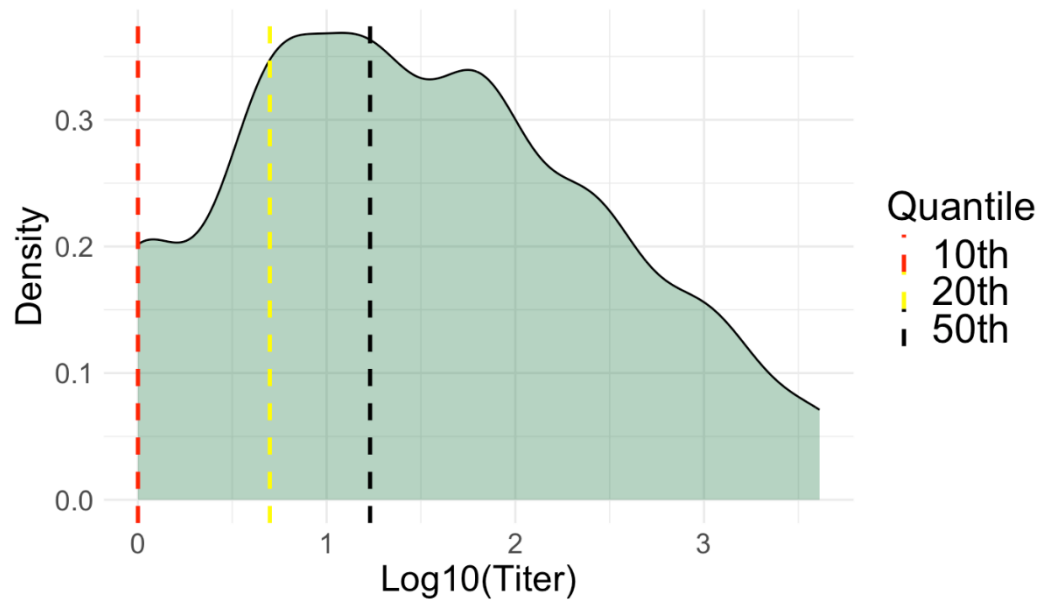


Figure 5. Density plot for visualizing the data distribution of maternal CVB1 antibody levels. The maternal antibody titers have been \log_{10} -transformed. The red, yellow, and black vertical lines represent the 10th, 20th, and 50th quantiles of the data distribution, respectively.

The prior distribution for μ was assigned to have mean 0 with standard deviation 1 to permit wide and weakly informative prior distribution in attempt to learn from the data. For the initial antibody titer, the prior distribution was set to have a mean 6.2 with standard deviation 0.3 which allowed the prior distribution to assign the mean initial titer to approximately 500 and vary approximately between 278 and 900 (95% range of the distribution).

The posterior distributions were constructed from samples collected during the fitting process. Altogether 12,000 posterior samples were collected for each parameter. In the analysis, 95% Credible Intervals (95% CrI) were assigned for the population parameters, multilevel hyperparameters, and distributional parameters. The multilevel hyperparameters describe the average level of individual variation for each estimated population parameter.

5. RESULTS

5.1 Maternal antibody frequency

Approximately half of the children had protective neutralizing antibody titer ≥ 16 against CVB2-CVB5 serotypes. However, only around 25% of the children had titer ≥ 16 against CVB1. When including titer 4, the proportions were more similar across different serotypes. The proportion of children with maternal antibody titer from level 0 to 4096 against CVB1-CVB5 is represented in Table 3.

Table 3. *Maternal neutralizing antibody titers, measured from the cord blood samples, for each of the CVB1-CVB5 serotypes at the time of birth. The proportion of children with protective neutralizing antibody titer (≥ 16) against CVB1 differed from the other four serotypes. The proportions across serotypes were more similar if titer 4 was also included. High antibody titers against CVB2-CVB5 were more common than against CVB1.*

Maternal antibody titer	CVB1	CVB2	CVB3	CVB4	CVB5
0	295 (55.9%)	265 (50.2%)	271 (51.3%)	216 (40.9%)	281 (53.2%)
4	99 (18.8%)	18 (3.4%)	6 (1.1%)	5 (0.9%)	7 (1.3%)
16	28 (5.3%)	20 (3.8%)	12 (2.3%)	13 (2.5%)	13 (2.5%)
64	24 (4.5%)	26 (4.9%)	13 (2.5%)	14 (2.7%)	26 (4.9%)
256	37 (7.0%)	49 (9.3%)	43 (8.1%)	29 (5.5%)	83 (15.7%)
1024	28 (5.3%)	88 (16.7%)	93 (17.6%)	96 (18.2%)	74 (14.0%)
4096	17 (3.2%)	62 (11.7%)	90 (17.0%)	155 (29.4%)	44 (8.3%)
≥ 16	134 (25.4%)	245 (46.4%)	251 (47.5%)	307 (58.1%)	240 (45.5%)
≥ 4	233 (44.1%)	263 (49.8%)	257 (48.7%)	312 (59.1%)	247 (46.8%)

The effect of considering titer 4 as protective was investigated more closely by visualizing the density plots of the maternal antibody data by serotype (Figure 6.). The titer values are \log_2 -transformed for the visualization. In each figure, the titer 4 and 16 have been

labelled with blue and red vertical lines correspondingly. For CVB2-CVB5 the data distribution is bimodal, suggesting two subpopulations that could be interpreted as antibody negatives on the left and antibody positives on the right. For these serotypes, it is notable that the titer 4 is located at the antibody negative side of the bimodal distribution as the titer 16 is in between the two. For CVB1 the distribution is irregular and different from the other serotypes.

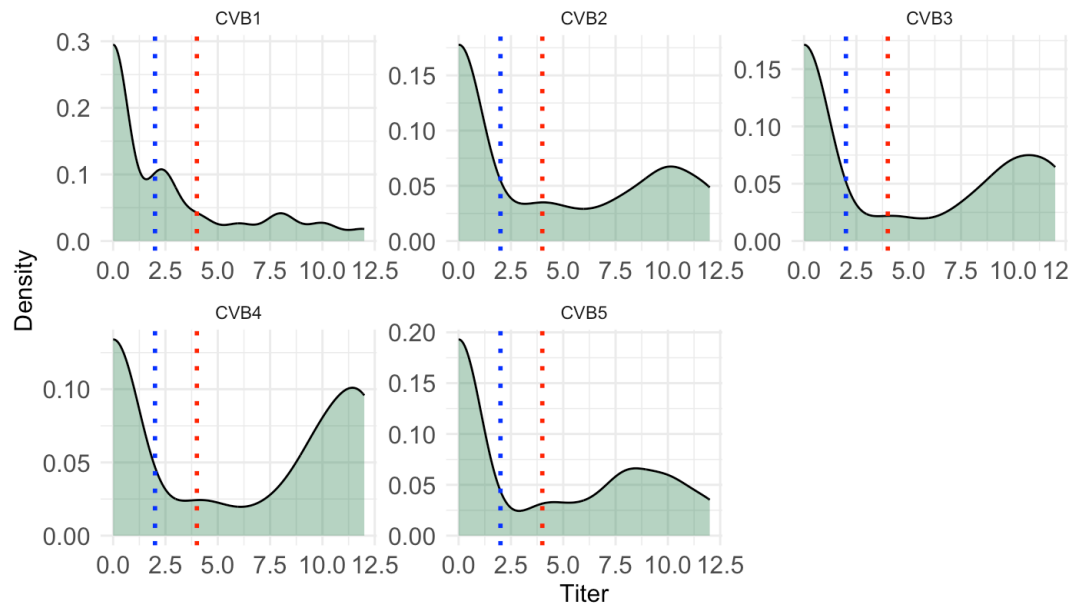


Figure 6. Density plots by serotype to visualize the data distributions of maternal antibody titers. The titer values are \log_2 -transformed. In each figure the titer 4 and 16 have been labelled with blue and red vertical lines correspondingly. For CVB2-CVB5 the density plot is bimodal, suggesting two subpopulations that could be interpreted as antibody negatives on the left and antibody positives on the right. For these serotypes it is notable that the titer 4 is located at the antibody negative side of the bimodal distribution and titer 16 is in between the two. For CVB1 the distribution is different from the other serotypes.

For all serotypes, the most common maternal antibody titer was 0. Only for CVB4, the frequency of positive antibody titers (≥ 4) together exceeded the frequency of negative antibody titer 0. However, there were some differences between serotypes in regard of the second most common titer. For CVB1 it was 4, but for other serotypes it was higher: 1024 for CVB2, 1024 and 4096 almost equally for CVB3, 4096 for CVB4, and 256 for CVB5. Thus, higher antibody titers against CVB2-CVB5 were more frequent than against CVB1. These findings are highlighted in Table 3.

5.2 CVB infections and maternal antibody-mediated protection

CVB1 and CVB2 infections were more common than CVB3, CVB4, and CVB5 infections. During the first year of life, 53.2% of the children acquired CVB1 and 40.3% of the children acquired CVB2 infection (Table 4.A. and Table 4.B.). The same percentages for CVB3, CVB4, and CVB5 were 1.9%, 3.6%, and 2.3% respectively (data not shown).

Table 4. *A. Maternal antibody titers against CVB1 reported with the proportions of children that had no CVB1 infection during the first year of life and the proportions of children that had infections around each of the sample timepoints. B. Provides the same statistics for CVB2. The “Total” column summarizes the number of children with given cord blood titer and their proportion of that group. The “Total” row summarizes the children that had no infection or had an infection at a given timepoint and their proportion of the total number of children (N=528).*

A.

Maternal antibody titer against CVB1	No Infection in 12 months	Infection at 3 m	Infection at 6 m	Infection at 12 m	Total
0	122 (41.4%)	78 (26.4%)	36 (12.2%)	59 (20%)	295 (100%)
4	39 (39.4%)	33 (33.3%)	8 (8.1%)	19 (19.2%)	99 (100%)
16	11 (39.3%)	0 (0%)	5 (17.9%)	12 (42.9%)	28 (100%)
64	9 (37.5%)	0 (0%)	2 (8.3%)	13 (54.2%)	24 (100%)
256	30 (81.1%)	0 (0%)	0 (0%)	7 (18.9%)	37 (100%)
1024	24 (85.7%)	0 (0%)	0 (0%)	4 (14.3%)	28 (100%)
4096	12 (70.6%)	0 (0%)	0 (0%)	5 (29.4%)	17 (100%)
Total	247 (46.8%)	111 (21%)	51 (9.7%)	119 (22.5%)	528 (100%)

B.

Maternal antibody titer against CVB2	No Infection in 12 months	Infection at 3 m	Infection at 6 m	Infection at 12 m	Total
0	113 (42.6%)	74 (27.9%)	21 (7.9%)	57 (21.5%)	265 (100%)
4	3 (16.7%)	7 (38.9%)	1 (5.6%)	7 (38.9%)	18 (100%)
16	9 (45%)	0 (0%)	5 (25%)	6 (30%)	20 (100%)
64	15 (57.7%)	0 (0%)	3 (11.5%)	8 (30.8%)	26 (100%)
256	35 (71.4%)	0 (0%)	0 (0%)	14 (28.6%)	49 (100%)
1024	81 (92%)	0 (0%)	0 (0%)	7 (8%)	88 (100%)
4096	59 (95.2%)	0 (0%)	0 (0%)	3 (4.8%)	62 (100%)
Total	315 (59.7%)	81 (15.3%)	30 (5.7%)	102 (19.3%)	528 (100%)

From the children that had maternal antibody titer ≤ 4 , 28.2% and 28.6%, acquired CVB1 and CVB2 infection, respectively, by the age of 3 months. CVB3-CVB5 infections were acquired later, closer to the age of 12 months (data not shown). At the age of 3 months, none of the children with maternal antibody titer ≥ 16 had CVB1 or CVB2 infections. At the age of 6 months, children that had maternal antibody titer 256 or higher had no CVB1 or CVB2 infections. These findings are highlighted in Table 4.A. and Table 4.B.

At the age of 12 months, both CVB1 and CVB2 infections were assessed in all maternal antibody levels, regardless of the initial titer. Larger proportion of children who had CB titer ≥ 1024 did not have CVB2 infections compared to CVB1 infections at the age of 12 months. When the maternal antibody titer against CVB2 was 1024 or 4096 the proportion of children that had no infections was 92% and 95.2%, respectively. However, these antibody titer levels against CVB1 resulted with 85.7% and 70.6% proportions of children not having infections. The effect of maternal antibody titer levels on the acquisition of CVB1 infections is visualized in Figure 7.

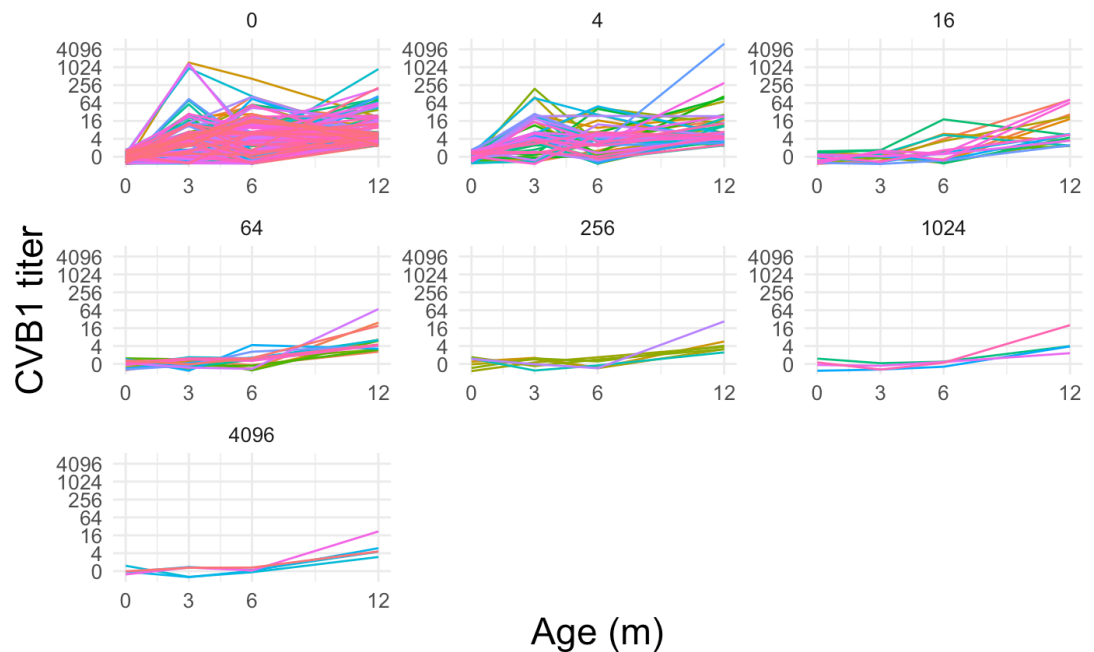


Figure 7. Each of the figures show CVB1 neutralizing antibody responses (titers) in CVB1 infections that occurred by the age of 12 months. Label above each figure shows the initial maternal antibody titer. At very low maternal antibody titers (≤ 4) the infection rate is higher, and the infections occur at a very young age. As the maternal antibody titer increases (≥ 16), the infection rate is reduced, and the infections are acquired later.

5.3 The kinetics of elimination of the maternal antibodies

The kinetics of maternal antibody levels over time were visualized for each of the children that had no infections in 12 first months. This was performed for all serotypes separately to gain an overview of the patterns in maternal antibody waning. The visualization of maternal CVB1 neutralizing antibodies over time is shown in Figure 8. as an example.

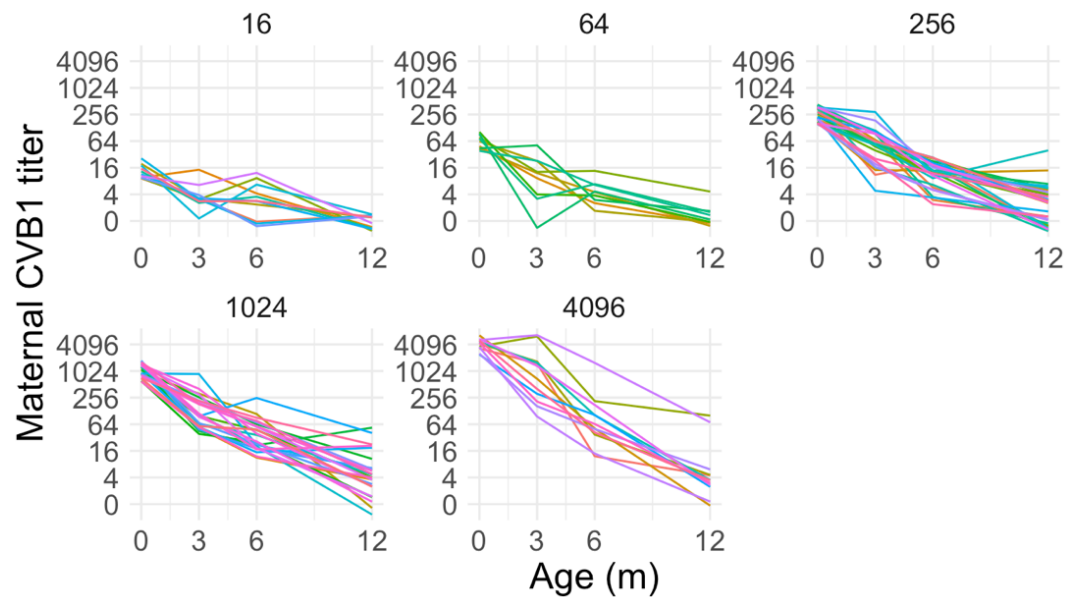


Figure 8. Visualization of maternal antibody titers against CVB1 over time. Each of the lines show maternal antibody levels of an individual child. Label above each figure shows the initial maternal antibody titer.

The geometric mean titer (GMT) was calculated separately for each time point of the respective serotype. For this purpose, the antibody titers were \log_2 -transformed before calculations. Additionally, the geometric standard deviation factor (GSD) was calculated. The GMTs and GSDs are visualized over time in Figure 9.

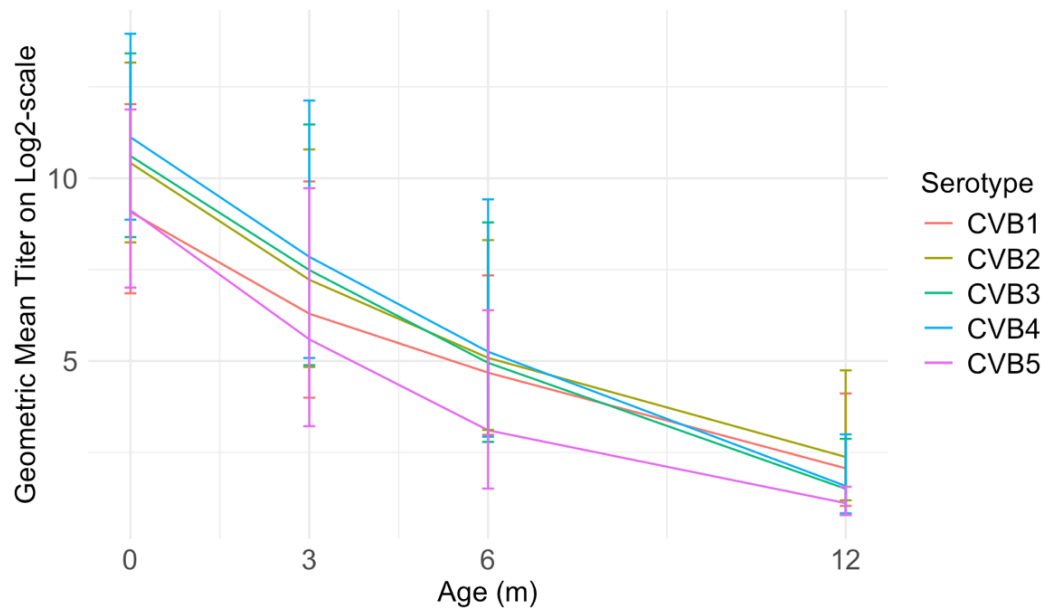


Figure 9. The kinetics of elimination of the maternal antibodies from the child's blood stream during the first year of life by serotype. Geometric mean titer and geometric standard deviation factor was calculated for each timepoint. Note that on a log-scale an exponential decay in antibody titers appear as linear decay.

To obtain general understanding of maternal antibody diminishing from child's blood circulation, the half-life (7) and estimated lifespan (8) of maternal antibodies were calculated based on the definition of exponential decay (5). The results of these calculations are represented for each serotype in Table 5. The half-life of maternal CVB neutralizing antibodies was approximately 4-6 months and estimated lifespan was 6-8 months. The shortest half-life of maternal antibodies was against CVB5 (4.0 months) while the half-life of antibodies against CVB1 and CVB2 was the longest (5.6 months).

Table 5. The calculated maternal antibody half-lives and estimated lifespans by serotype. The shortest half-life of maternal antibodies was against CVB5 (4.0 months) while the half-life of antibodies against CVB1 and CVB2 was the longest (5.6 months).

Serotype	CVB1	CVB2	CVB3	CVB4	CVB5
<i>mAb</i> $T_{1/2}$ (months)	5.6	5.6	4.3	4.3	4.0
<i>mAb</i> E (months)	8.1	8.1	6.1	6.2	5.7

When back transforming the GMT values to linear scale and visualizing them over time in Figure 10., it was possible to see more clearly than from the \log_2 -transformed scale, that for all serotypes there is a rapid initial waning phase during the first three months followed by slower decay phase during the following three months.

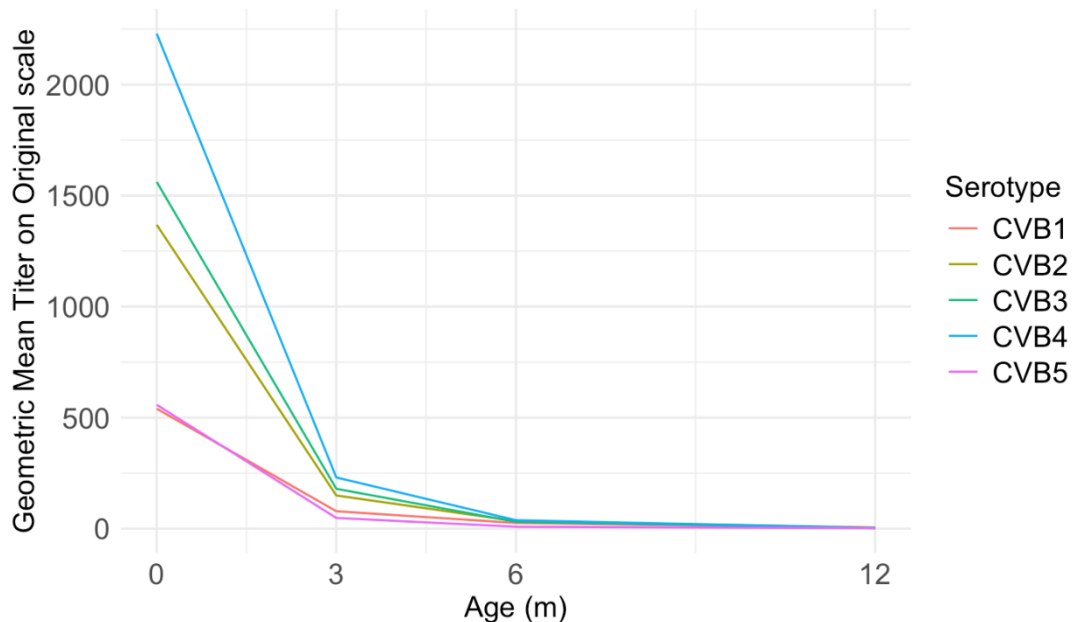


Figure 10. The geometric mean titers of maternal antibodies visualized over time on linear scale to highlight the possible bi-phasic exponential decay.

5.4 Bayesian mixed effects model for maternal antibody kinetics

Calculating the characteristic values of maternal antibody kinetics through GMTs provided only approximate information as the approach does not consider the individual level variation of the biological processes underlying the kinetics of eliminating the antibodies from the child's blood circulation. To address this issue, the kinetics of elimination of the maternal antibodies against CVB1 were modelled through Bayesian non-linear mixed effects model as described in the methods section of this thesis.

The results of the population level parameter estimates, i.e. fixed effects, and individual level variation, i.e. random effects, are compiled in Table 6. and Table 7., respectively. The posterior mean of the population level decay rate constant indicates that, on average at the population level, the half-life of maternal antibodies against CVB1 was 2.65 months

[2.44, 2.87; 95%-CrI]. Correspondingly the estimated lifespan was 3.82 months [3.53, 4.14; 95%-CrI]. Due to individual level variation, the half-life could typically range between 2.01 and 3.37 months across individuals. Similarly, the results imply individual level variation for the estimated lifespan of maternal antibodies against CVB1 between 3.0 and 4.85 months.

Table 6. *The posterior mean of the population level parameter estimates (fixed effects), standard deviation (SD) of the posterior distribution, and the quantile-based bounds of 95%-Credible Interval on logarithmic scale.*

Parameter	Estimate	SD	Q2.5	Q97.5
μ	-1.34	0.04	-1.42	-1.26
mAb_0	2.42	0.12	2.21	2.69

Table 7. *The posterior mean of the standard deviation of the individual level variation (random effects) with standard error, and the quantile-based bounds of 95%-Credible Interval on logarithmic scale.*

Parameter	Estimate	Est. Error	Q2.5	Q97.5
$SD(\mu)$	0.24	0.05	0.15	0.33
$SD(mAb_0)$	0.87	0.10	0.70	1.10

The average expected value for maternal antibody titer over time, based on the posterior distributions of the decay rate constant and the initial maternal antibody titer, is represented by green line in Figure 11. The purple lines show the bounds of 95%-CrI i.e. the bounds within the predicted maternal antibody titer lies with 95% probability. Including the initial maternal antibody titer as a non-linear parameter to the model allowed the model to capture the data distribution as shown in Figure 12. By setting an informative prior to this parameter, it was possible to optimize the magnitude of the expected antibody level over time. For both non-linear parameters estimated in this model, the shape of the posterior distribution is visualized as violin plot in Figure 13.

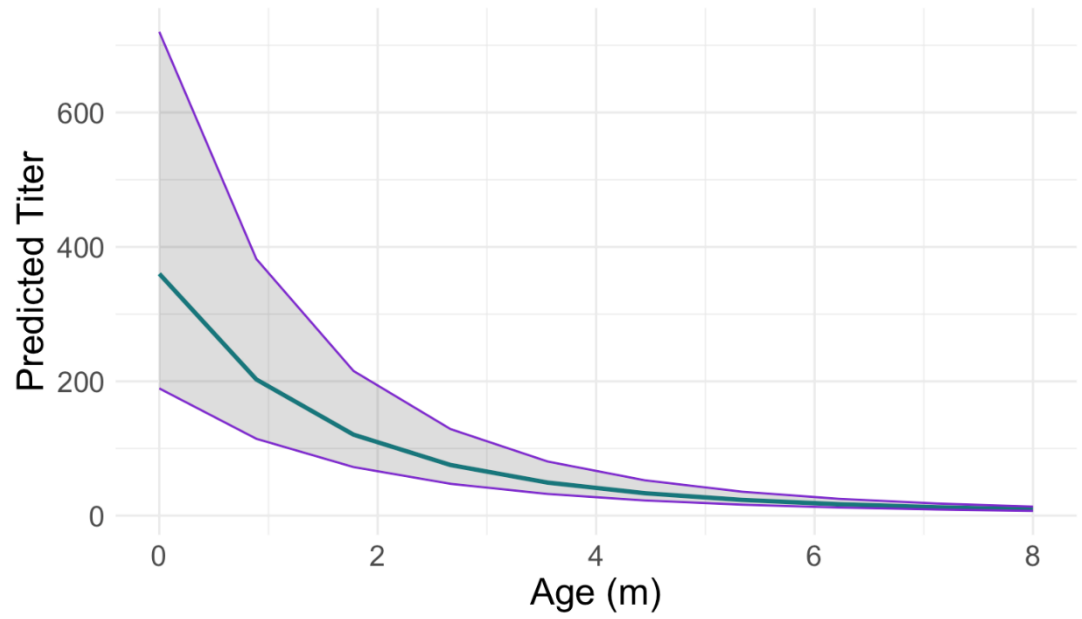


Figure 11. The average predicted maternal antibody titer over time (months) is colored with green and the bounds of 95%-CrI are colored with purple.

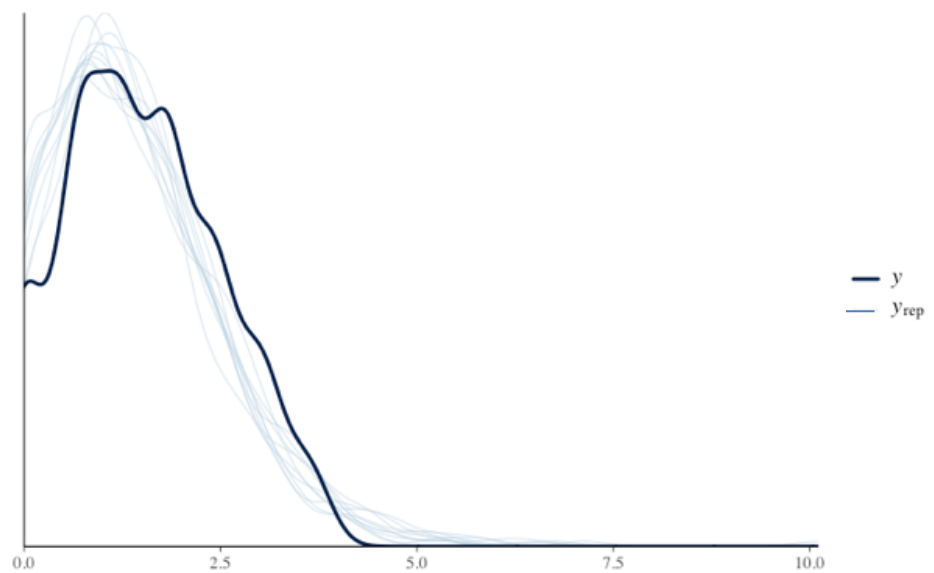


Figure 12. The hurdle-lognormal model distribution was able to capture the data distribution. Black line (y) represents the actual data distribution, and the blue lines (y_{rep}) represent the posterior predictive simulations.

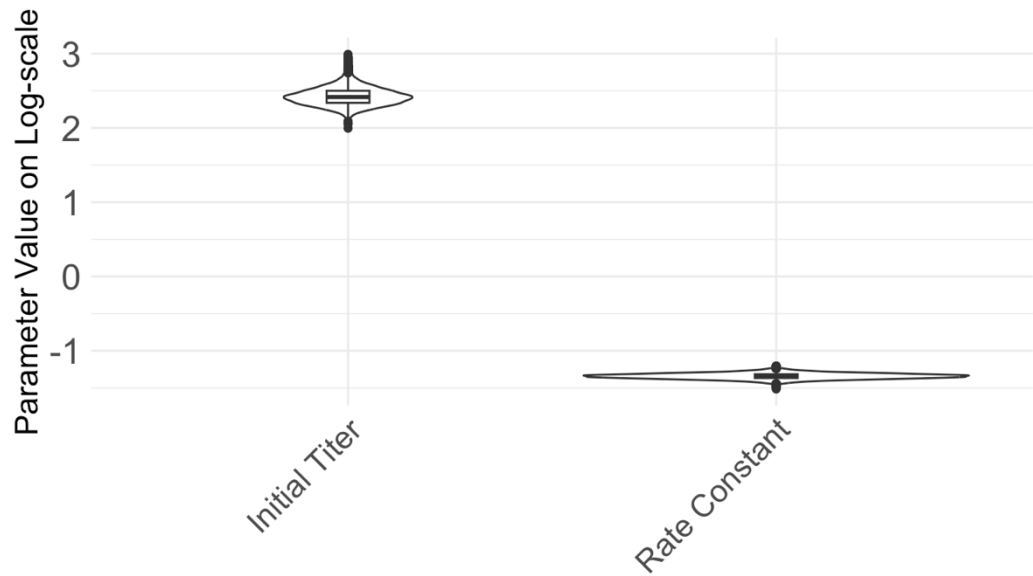


Figure 13. Posterior parameter distributions as violin plots on logarithmic scale.

For Bayesian mixed effects models, there are several ways to evaluate the model fit. Rhat value is provided as part of the model summary in brms R package, and it describes the convergence of the algorithm (Bürkner, 2017). The present model returned Rhat value 1.00 for all parameter estimates which indicated good convergence of the chains.

Bayes R^2 is another measurement of model fit, and it provides a Bayesian version of the classical R^2 that is used for frequentist statistical models (Gelman et al., 2019). It measures the proportion of the variance in the observed data that is explained by the model. The Bayesian R^2 is computed by the definition:

$$R^2 = \frac{Var(\hat{y})}{Var(\hat{y}) + Var(\varepsilon)} \quad (10)$$

where $Var(\hat{y})$ is the variance of the predicted values and $Var(\varepsilon)$ is the variance of the residuals.

The Bayes R^2 provides a full posterior distribution of R^2 values. In the case of the present model, the average R^2 value was 0.821 for which the standard error was 0.0194 and the quantile-based bounds of 95%-CrI were [0.779, 0.855]. The closer the R^2 value is to 1, the more the model explains the variance in the observed data. In the case of Bayesian approach, and especially in the case of non-linear mixed effects model, the interpretation of Bayes R^2 is non-trivial, however, it can be used as part of the overall evaluation of the model behavior.

Finally, by visualizing the observed values against the predicted values from the model it is possible to evaluate the accuracy of the model predictions. As visualized in Figure 14, the observed antibody titer levels form seven distinct levels across the dashed identity line. Due to the ordinal nature of observed values and the continuous scale of predicted values, the data points do not align perfectly along the identity line. The model overestimates zeros to be positive values while underestimating the largest titers. The best accuracy is found for titers 4 and 16. Similar effect is seen in Figure 15, when visualizing the predicted values from the model against the residuals which allows to evaluate the distance between the model prediction and the given observation.

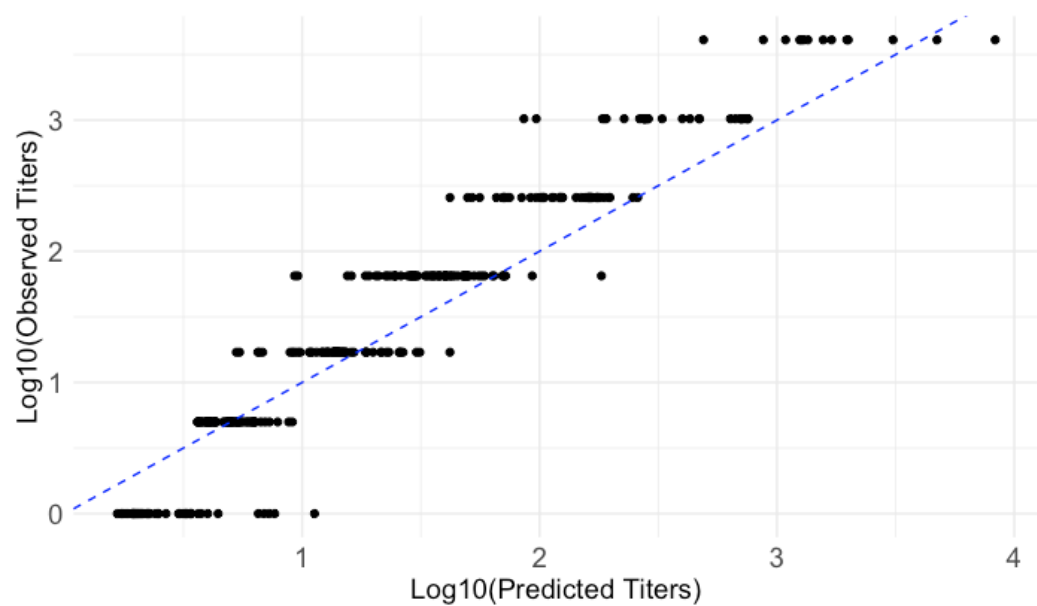


Figure 14. \log_{10} -transformed observed titers plotted against \log_{10} -transformed model predicted titers. Observed values form seven distinct levels across the dashed identity line which represents the data structure. However, the predicted values deviate from this.

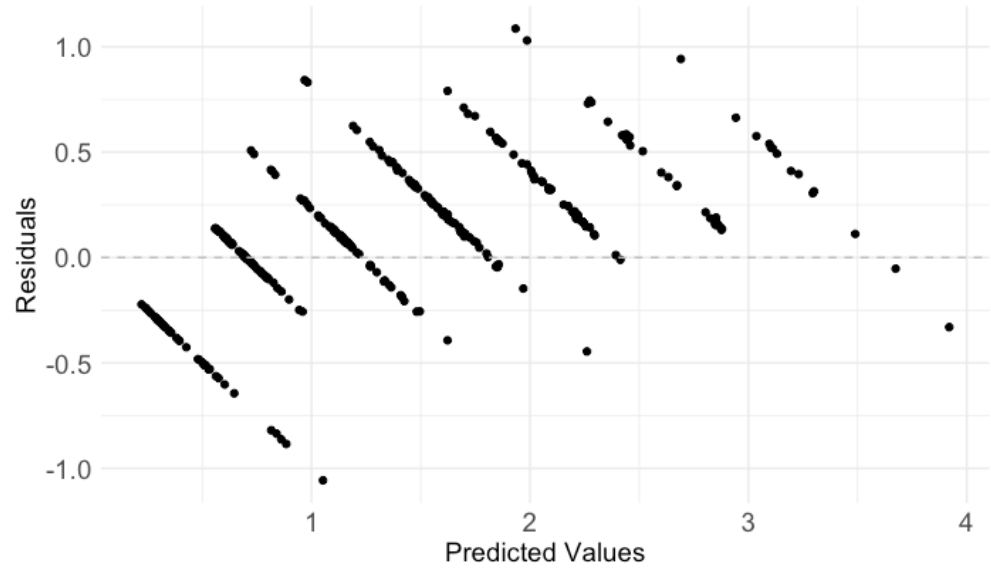


Figure 15. Residuals visualized against the predicted values from the model. This figure describes the distance between the model prediction and the given observation.

6. DISCUSSION

In this thesis, the aim was to study the frequency of CVB infections and maternal neutralizing antibodies against five CVB serotypes (CVB1-CVB5) in Finland. Also, the aim was to investigate if, and for how long, maternal antibodies protect the child from CVB infections. In that regard, it was important to determine the age of first CVB infections. Furthermore, the kinetics of elimination of the maternal antibodies and the antibody responses to natural infections occurring during the first year of life, were assessed.

The key findings of this thesis implied that in Finland, among the children that have genetic risk for T1D, around 50-75% did not have maternal antibody protection against CVB at the time of birth. Maternal antibodies against CVB1 were less common than against CVB2-CVB5. CVB1 and CVB2 infections were frequent during the first year of life. In contrast, CVB3, CVB4, and CVB5 infections were less frequent and were acquired later, closer to the age of 12 months. Maternal CVB antibodies provided effective protection against early life CVB infections. The results of the maternal antibody waning provided further evidence on how transplacental passive immunity against CVBs wanes during the first year of life.

6.1 Frequency and variability of maternal antibody protection against CVBs

The overall frequency of maternal CVB antibodies was relatively low as 50-75% of the children lacked maternal antibody protection against the five CVB serotypes. As many factors contribute to the frequency and variability of maternal antibodies, no comprehensive study reporting the level of maternal antibodies for EVs in general exists. According to the report by Zhou and colleagues, 42% of neonates in Chinese cohort had a positive (≥ 8) maternal antibody titer against Coxsackievirus A16 (CVA16) (Zhou et al., 2022). However, comparing the results is not straightforward as the neutralization method and the threshold for antibody positivity were different from the ones used in the present study. Additionally, the subjects in that study represented more general population whereas in this study the children were recruited based on the genetic risk for type 1 diabetes. However, there is no evidence to suspect that such specific group with a known genetic risk might differ from the general population.

The most frequent cord blood titer for all serotypes was 0, meaning that in most cases, the child did not have maternal antibodies against the given serotype. Only for CVB4, the frequency of positive antibody titers (≥ 4) together exceeded the frequency of negative antibody titer 0. Maternal antibodies against CVB1 were less common than antibodies against CVB2–CVB5 as 25% of the children had effective maternal antibody protection (titer ≥ 16) against CVB1 while around 50% of the children had the same level of protection against CVB2–CVB5 serotypes. There are a number of factors that can affect the frequency and variability of maternal antibodies, such as the efficacy of the antibody transfer which seems to be antigen specific (Fu et al., 2016). Also, maternal antibody transfer depends on the functionality of individual's placenta (Albrecht et al., 2022). The transplacental passive immunity is conferred only against pathogens that are either circulating within the community or have been introduced to the mother through vaccination (Jennewein et al., 2017). Thus, reduced maternal antibody level against a specific antigen may reflect epidemiological as well as biological differences.

In this work, the threshold for protective titer was 16. If titer 4 was also considered protective, the frequency of maternal antibody protection against CVB1 resembled the other CVB serotypes. As the pioneering work of David Bodian with poliovirus in 1950s showed, antibody titer 3 provided only little protection against paralytic poliomyelitis, while for titers 6 and 11 the corresponding levels of protection were about 50% and 90%, respectively (Nathanson, 2005). Thus, the threshold 16 as protective antibody titer is more robust and justified than threshold 4. When investigating more closely the potential of the titer 4 to be protective, the results showed that for CVB2–CVB5 the data distribution is bimodal, suggesting two subpopulations that could be interpreted as antibody negatives on the left and antibody positives on the right (Figure 6.). For CVB1 the distribution was irregular and different from the other serotypes.

The most frequent positive antibody level for CVB1 was the lowest positive level 4 for which the level of protection is not clear. For other serotypes, the most frequent positive antibody level was much higher ≥ 256 . Thus, higher antibody titers against CVB2–CVB5 were more frequent than against CVB1. Interestingly, a larger proportion of children having a high maternal antibody titer (≥ 1024) did not have CVB2 infections compared to CVB1 infections at the age of 12 months. When the maternal antibody titer against CVB2 was 1024 or 4096 the proportion of children having no infections was 92% and 95.2%, respectively. In contrast, the same antibody titer levels against CVB1 resulted with 85.7% and 70.6% proportions of children not having infections. Whether this is indicative of better protection against CVB2 is not clear as in this study the data is not able to separate

the 12-months-old children that did not have the infection due to maternal antibody protection from the ones that were not exposed to the virus.

The different data distribution and the lower frequency of maternal antibody protections against CVB1 might indicate serotype-specific biological or epidemiological features. For example, mothers may have encountered fewer CVB1 infections than infections for the other serotypes. On the other hand, it might be possible that CVB1 induces lower or more rapidly waning antibody response than the other serotypes. However, CVB serotype-specific differences in immunogenic properties are not fully elucidated.

6.2 Early-life susceptibility and timing of primary CVB infections

CVB1 and CVB2 infections were frequent already during the first year of life as 53.2% and 40.5% of the children acquired CVB1 and CVB2 infections during the follow-up, respectively. The high infection frequencies imply that CVB1 and CVB2 are common pathogens among the Finnish population and children are exposed to them in early infancy.

The first infections were acquired already at very young age when maternal antibody protection was low or did not exist. 28.2% and 28.6% of the children who had maternal antibody titer ≤ 4 , acquired CVB1 and CVB2 infection, respectively by the age of 3 months. Contrasted to the relatively low level of maternal antibody protection against CVB1 and CVB2, these findings suggest that many infants may be susceptible to the systemic spread of the virus and for more severe outcomes of the infection. While CVB3-CVB5 infections were less frequent, they were also acquired later, closer to the age of 12 months. However, the results of this thesis indicate that by that time, also the maternal antibody protection had waned.

6.3 Protective role and waning of maternal antibodies

During the first months of life, maternal antibodies provided effective protection against CVB infections. At the age of 3 months, none of the children with maternal antibody titer ≥ 16 had CVB1 or CVB2 infections. At the age of 6 months, the maternal antibody protection against CVB1 or CVB2 infections was still evident for the children that had maternal antibody titer 256 or higher. These results are consistent with previous observations that higher levels of maternal antibodies are protective against early life infections (Albrecht et al., 2022).

The concept of temporary passive immunity involves the perception that maternal antibodies wane during the first year of life. In this study, maternal antibody protection waned by the age of 12 months as both CVB1 and CVB2 infections were detected at that age regardless of the initial CB antibody titers. Also, the results of maternal antibody elimination kinetics supported this idea as the half-life and estimated lifespan of maternal CVB neutralizing antibodies were around 4-6 and 6-8 months, respectively, when performing approximate calculations using geometric mean titers (GMT). The shortest half-life of maternal antibodies was against CVB5 (4.0 months) while the half-life of antibodies against CVB1 and CVB2 was the longest (5.6 months). In contrast, the results yielded from Bayesian mixed effects modelling implied that on average the half-life of CVB1 maternal antibodies was around 2.65 months and correspondingly, the estimated lifespan was 3.82 months on average. The model showed that there was individual-specific variation in these estimates.

In general, the half-life of human IgG is estimated to be approximately 17.5-26 days (Andraud et al., 2012), however, individual variation has been reported up to 42 and 80 days (Amanna and Slifka, 2010). In their work, Zhou and colleagues (Zhou et al., 2022) reported that the half-life of maternal antibodies against CVA16 (which also belongs to *Enterovirus genus*) was 2.0 months, whereas the maternal RSV antibodies are reported to wane with a half-life of approximately 38 days (Openshaw et al., 2017). When comparing the estimations in this thesis to the reported half-lives in other studies, maternal antibody half-lives seem to yield or even exceed the general upper limits of human IgG half-lives.

The half-life estimate from Bayesian mixed effects model was closer to the reported maternal antibody half-lives against other pathogens (Openshaw et al., 2017; Zhou et al., 2022) than the approximate calculations using GMTs. This is expected, since the GMT-based method does not consider individual level variation in the underlying biological processes of maternal antibody waning.

6.4 Limitations of the study

The data was collected as part of the DIPP study that recruits children with genetic predisposition to T1D in Finland. While being highly valuable study cohort, the data is biased in respect to the HLA-genotypes which must be considered when applying the results to general population. Also, the results represent the situation in Finland during the years

1995-2009 which might differ from other parts of the world or at different time. Furthermore, the study in this thesis did not utilize the full potential of the present dataset as it did not compare the findings between cases and controls in terms of end point autoimmunity or T1D. In addition, in this dataset, the four-fold dilution series was terminated at 1/4096 dilution due to the limitations to the time and workload in laboratory. This means that the highest measured titer might not be the actual end-point-titer in all cases.

In the case of the present study, the ordinal nature of the data challenged the Bayesian mixed effects modelling as the predicted titer values from the model were continuous and thus, did not align perfectly with the observed titer values. Another point of consideration in the Bayesian mixed effects model is the selected model function. In this thesis, the maternal antibody waning was assumed to follow the kinetics of exponential decay based on previous literature (Saha et al., 2025; White et al., 2014). However, there is currently no consensus on how antibody titers wane over time. As an alternative to exponential decay, also bi-phasic exponential waning and power-law decay models have been proposed (Saha et al., 2025).

A bi-phasic exponential decay includes two phases, first a prompt decay phase followed by gradual decay. When visualizing the GMTs over time in Figure 9. and Figure 10., it was possible to see that there is a rapid initial waning phase during the first three months followed by slower decay phase during the next three months. This observation was not studied further in this thesis but could serve as a motivation for future modelling. However, even with limitations, the exponential decay model used in this thesis could arguably provide more nuanced information about the maternal antibody waning than the characteristic values for antibody kinetics calculated from the GMTs.

In future studies, the Bayesian mixed effect modelling could be also used to investigate the maternal antibody waning of other CVB serotypes, and the model could be extended to include other immunological mechanisms, such as, antibody responses after natural infection or vaccination (Garcia-Fogeda et al., 2023). Furthermore, in addition to antibody decay, different mechanistic compartments, such as, antibody production by short- and long-lived antibody secreting cells could be modelled to quantify the waning of immunity (Amanna and Slifka, 2010; Andraud et al., 2012; White et al., 2014). Finally, it could be justified to explore different data types for such models, for example, antibody levels from ELISA test (Andraud et al., 2012).

7. CONCLUSIONS

Enteroviruses (EVs), and especially CVB infections, have been proposed to be one of the main environmental triggers of T1D (Hyöty et al., 2018). To address the relationship between the increasing T1D incidence and the declined EV infection rates in Finland, the “polio hypothesis” was proposed based on the observation that higher paralytic poliomyelitis frequency was associated with lower rate of polio infections in the population due to increased hygiene (Viskari et al., 2000).

With an analogy, the hypothesis proposes that as mothers have encountered less EV infections in Finland, and there is no EV vaccine available, they cannot provide maternal antibodies against EVs for the offspring (Viskari et al., 2000). Furthermore, children have infections later in life and are not protected by maternal antibodies anymore at the time of infection as the maternal antibody protection wanes during the first year of life. Thus, this could lead to increased T1D incidence in the population through impaired protection against CVB infections.

Even though this study did not evaluate differences between the case and control subjects analyzed and thus address the relationship between CVB infections and T1D per se, it is still connected to T1D through the genetic susceptibility that the children in this study carry. The results provide evidence for the “polio hypothesis” because they imply that maternal neutralizing antibody titers might be relatively low in Finland as around 50-75% of the children in this dataset were not protected against CVB1-CVB5 serotypes. The results also indicate that CVB, especially CVB1 and CVB2, infections are frequent already during the first year of life.

Most of the early infections occurred in children who lacked maternal antibodies leaving them vulnerable and susceptible to the systemic spread of the virus. In the present dataset, maternal neutralizing antibodies provided protection from early CVB infections at the age of 6 months, however, due to the waning of passive immunity by the age of 12 months, the maternal antibodies alone are not sufficient for sustainable protection against virus infections. These findings support the rationale for developing a vaccine that can induce neutralizing antibodies against CBVs and for administering the vaccine early in infancy to prevent the early CVB infections.

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