Towards Explaining the Swedish Epidemic of Celiac Disease
— an epidemiological approach

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Abstract

Background: Celiac disease occurs worldwide in approximately 1% of the population, whereof the majority of cases are undiagnosed. Sweden experienced an epidemic (1984-1996) of clinically detected celiac disease in children below 2 years of age, partly attributed to changes in infant feeding. Whether the epidemic constituted a change in disease occurrence and/or a shift in the proportion of diagnosed cases remains unknown. Moreover, the cause of the epidemic is not fully understood.

Objective: To increase the knowledge regarding the occurrence of celiac disease in Sweden, with focus on the epidemic period and thereafter, as well as the etiology of celiac disease in general, by investigating the Swedish epidemic and its potential causes.

Methods: We performed a two-phased cross-sectional multicenter screening study investigating the total prevalence, including both clinically- and screening-detected cases, of celiac disease in 2 birth cohorts of 12-year-olds (n=13 279): 1 of the epidemic period (1993) and 1 of the post-epidemic period (1997). The screening strategy entailed serological markers analyses, with subsequent small intestinal biopsy when values were positive. Diagnosis was ascertained in clinical cases detected prior to screening. Infant feeding practices in the cohorts were ascertained via questionnaires. An ecological approach combined with an incident case-referent study (475 cases, 950 referents) performed during the epidemic were used for investigating environmental- and lifestyle factors other than infant feeding. Exposure information was obtained via register data, a questionnaire, and child health clinic records. All studies utilized the National Swedish Childhood Celiac Disease Register.

Results: The total prevalences of celiac disease were 2.9% and 2.2% for the 1993 and 1997 cohorts, respectively, with 2/3 cases unrecognized prior to screening. Children born in 1997 had a significantly lower celiac disease prevalence compared to those born in 1993 (prevalence ratio, 0.75; 95% confidence interval [CI], 0.60-0.93). The cohorts differed in infant feeding; more specifically in the proportion of infants introduced to dietary gluten in small amounts during ongoing breastfeeding. Of the environmental and lifestyle factors investigated, no additional changes over time coincided with the epidemic. Early vaccinations within the Swedish program were not risk factors for celiac disease. Early infections (≥3 parental-reported episodes) were associated with increased risk for celiac disease (adjusted odds ratio [OR] 1.5; 95% CI, 1.1-2.0), a risk that increased synergistically if, in addition
to having ≥3 infectious episodes, the child was introduced to gluten in large amounts, compared to small or medium amounts, after breastfeeding was discontinued (OR 5.6; 95% CI, 3.1-10). Early infections probably made a minor contribution to the Swedish epidemic through the synergistic effect with gluten, which changed concurrently. In total, approximately 48% of the epidemic could be explained by infant feeding and early infections.

**Conclusion:** Celiac disease is both unexpectedly prevalent and mainly undiagnosed in Swedish children. Although the cause of the epidemic is still not fully understood, the significant difference in prevalence between the 2 cohorts indicates that the epidemic constituted a change in disease occurrence, and importantly, corroborates that celiac disease can be avoided in some children, at least up to 12 years of age. Our findings suggest that infant feeding and early infections, but not early vaccinations, have a causal role in the celiac disease etiology and that the infant feeding practice – gradually introducing gluten-containing foods from 4 months of age, preferably during ongoing breastfeeding – is favorable.

**Keywords:** celiac disease; epidemiology; etiology; infant feeding; infections; prevalence; screening; vaccinations
Original Papers

This thesis is based on the following papers, referred to as Papers I-IV.


*Authors contributed equally to the paper.


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Paper III is reproduced with permission from Pediatrics, Copyright© 2012 by the American Academy of Pediatrics (AAP).
**List of abbreviations**

- **BCG**  
  Bacillus Calmette-Guérin

- **CI**  
  Confidence interval

- **EMA**  
  Endomysial antibodies

- **ESPGHAN**  
  European Society for Pediatric Gastroenterology, Hepatology and Nutrition

- **HLA**  
  Human leukocyte antigen

- **IEL**  
  Intraepithelial lymphocytes

- **OR**  
  Odds ratio

- **tTG**  
  Tissue transglutaminase, refers to both the enzyme and the antibodies
Introduction

*Celiac disease*, defined as a “chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals”,¹ is one of the most common chronic diseases in childhood. In the first century AD, the first description of celiac disease by Aretaeus of Cappadocia referred to a ‘disorder of the abdomen’ (*koiliakos* in Greek).² Almost 2000 years later, in 1888, Samuel Gee published the first description of the ‘celiac affection’ in modern times. Furthermore, he suggested that the treatment would be dietary, even though it was not until the 1950s that Willem Karel Dicke described the factor causing it – the dietary gluten proteins, found in wheat, rye and barley.³–⁴ At that time, and during the following decades, celiac disease was considered a rare European childhood disease, inevitably developing in genetically predisposed children when exposed to gluten.⁵–⁸

In the mid-1980s Swedish pediatricians noticed an increase in the number of severely ill celiac disease cases diagnosed. Via initiation of the National Swedish Childhood Celiac Disease Register⁹ it became evident that the celiac disease incidence rate in children below 2 years of age displayed an epidemic pattern between 1984 and 1996.¹⁰,¹¹ This was an unusual pattern for a genetically dependent disease with autoimmune features, and it was later termed “the Swedish epidemic of celiac disease” (Figure 1).

![Figure 1](image_url)

*Figure 1.* The Swedish epidemic of celiac disease in children below 2 years of age. Reproduced with permission from Pediatrics,¹¹ Copyright © 2008 by the AAP.
The occurrence of the celiac disease epidemic challenged the prevailing understanding of celiac disease as a determined genetic disease and, together with other research, increased awareness about environmental- and lifestyle factors in celiac disease etiology. While the epidemic could have occurred anywhere, this pattern is to date unique to Sweden and, although unfortunate, gives incomparable opportunities for research. Today, almost 2 decades later, the epidemic still contains unanswered questions and subsequently opportunities for increased understanding of the disease.

A multifactorial etiology

Celiac disease is considered to have a multifactorial etiology where a genetic predisposition and exposure to dietary gluten are prerequisites for disease development. However, roughly 25% of the Western general population is considered to have the genetic predisposition and only a minority develop the disease; thus, the genetic predisposition is necessary but generally not sufficient. Consequently, and in contrast to the previous presumption, environmental- and lifestyle factors have a causal role in disease development (Figure 2).

Figure 2. A stipulated model of the multifactorial etiology of celiac disease with all factors (depicted by the circles, not to scale) contributing to immunological responses ultimately leading to celiac disease. Abbreviation: CD=celiac disease. Adapted from Ivarsson et al.14
**Genetic predisposition**

The genetic predisposition for celiac disease encompasses both genes encoding for the human leucocyte antigen (HLA)-DQ2 or DQ8 molecule, and additional non-HLA genes, whereof 39 loci have been associated with the disease through genome-wide association studies. For the associated non-HLA loci, the contribution to disease risk by each individual variant is minor and the underlying molecular mechanisms remain to be elucidated.\(^{15}\)

The primary genetic predisposition is conferred by variants in the genes encoding for HLA-DQ2 seen in 90% of all cases, and in the majority of remaining cases it carries variants encoding for the HLA-DQ8 molecule.\(^{16,17}\) The celiac disease genetic risk is dependent on which allele is carried, and whether the alleles are expressed in a homozygous or heterozygous state; thus a gene-dose effect is seen.\(^{16,18}\)

**Properties of gluten**

The other prerequisite for celiac disease development is exposure to dietary gluten, which constitutes a mix of proteins that give dough its elastic properties.\(^{13}\) Although the term gluten actually refers to the entire protein component of wheat, it is often used as a term encompassing the prolamin (storage proteins) found in wheat (gliadin and glutenin), rye (hordeins), and barley (secalines).\(^1\) These prolamin contain the bulk of components eliciting an immunologic response in individuals with celiac disease, mediated by some of the properties of gluten; high content of the amino acids proline and glutamine, and containment of sequences which are immunogenic and have HLA-DQ2/DQ8 binding specificity.\(^{19,20}\)

When the ingested gluten reaches the gastrointestinal tract it is not fully digested by gastrointestinal proteolysis due to the high content of proline, and subsequently large gluten peptides reach the mucosal surface of the small intestine.\(^{13}\) There the gluten peptides cross the epithelial barrier into the lamina propria where glutamine in the gluten peptide undergoes enzymatic modification (deamidation) by the enzyme tissue transglutaminase (tTG), also called transglutaminase 2, thereby increasing its affinity for HLA-DQ2/DQ8.\(^{17}\) The HLA-DQ2/DQ8 forms a complex with the deamidated gluten peptide and thereby presents it to CD4+ T-cells, thus connecting the genetic risk (HLA-DQ2/DQ8) to the properties of gluten, resulting in an immunological response.\(^{17,20}\)
Celiac disease viewed as an absence of oral tolerance to gluten

The immunological system in the intestine must distinguish between potentially hazardous foreign antigens and food constituents, a process known as oral tolerance. Development of oral tolerance is a complex immunological process involving interactions between genetic factors and environmental- and lifestyle exposures such as infant feeding, gut bacterial colonization, and the microbiota composition.21

Celiac disease can be viewed as a failure to develop, or later loss of oral tolerance to gluten (Figure 3). When tolerance, the default status, is lost, the HLA-DQ2/DQ8–gluten complex bound to the cell surface of antigen presenting cells is recognized by the CD4+ T-cells which become activated and induce an immunological response.12,13 The immune response includes activation of B-cells with ensuing antibody production. Antibodies are produced against the gluten peptide (antibodies against gliadin) as well as against self-antigens (antibodies against tTG, i.e. tTG-antibodies and endomysial antibodies (EMA)).17 Thus, celiac disease has features of an autoimmune disorder.

The inflammatory immune response is characterized by concomitant gluten-specific T-cell responses and responses by intraepithelial lymphocytes (IEL).16,20 The immune responses include production of different pro-inflammatory cytokines, such as interferon-γ (Figure 3).17,22 Involved in the immunological response by the IELs is locally produced interleukin-15, which confers properties (expression of natural killer cell receptors) to some of the IELs giving them the ability to recognize stress- and inflammation-induced ligands on epithelial cells and subsequently to destroy them in an unspecific manner.23

Taken together, the inflammatory responses (Figure 3) ultimately lead to the enteropathy seen in the small intestinal mucosa characterizing fully developed celiac disease: the increased number of IEL, crypt hyperplasia and villous atrophy.
Figure 3. Overview of the celiac disease pathogenesis. Gluten proteins (e.g. gliadin) cross the epithelium into the lamina propria where it becomes deamidated by tissue transglutaminase (tTG). The HLA-DQ2/DQ8 forms a complex with the deamidated gluten which is recognized by CD4+ T-cells. Cytokines are released and antibodies are produced. The inflammatory responses, including the responses by the intraepithelial lymphocytes (IEL), result in the mucosal damage. Reproduced with permission from N Engl J Med, © Massachusetts Medical Society.

Environmental- and lifestyle factors

While the presence of a genetic predisposition and exposure to gluten are necessary for celiac disease development, what triggers the immunological responses to gluten in some individuals remains unknown. Based on twin-studies, structural equation modeling has suggested that the contribution by HLA and non-HLA genes in disease liability is approximately 60-90%, and that the remaining 10-40% (with the exception of 1% attributed to later factors) can be attributed to environmental factors early in life.
Several environmental- and lifestyle factors i.e. any factor in our physical surrounding or aspects of our lifestyle, have been associated with celiac disease in children, but their contribution to the multifactorial etiology is not completely elucidated and the search for additional factors is ongoing. The environmental- and lifestyle factors can be divided into component factors, i.e. potentially causal factors, and associated factors, i.e. factors that do not have a causal effect themselves but could be markers for other causal factors. This division into 2 parts may be a simplification in some respects as it depends on how much is known about the factors and where the focus is in the disease-developing pathway. Nevertheless, the division provides organizational benefits with respect to disease etiology.

A summary of environmental- and lifestyle factors that have been associated with celiac disease is presented in Figure 4. The factors are presented depending on the main direction of the association to celiac disease; decreased or increased risk.

![Figure 4](image-url)

<table>
<thead>
<tr>
<th>Factors associated with decreased risk</th>
<th>Factors associated with increased risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born during winter – weaned during summer</td>
<td>Small for gestational age</td>
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<tr>
<td>Preterm birth</td>
<td>Low birth weight</td>
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<tr>
<td>Breastfeeding</td>
<td>Large amounts of gluten, especially during introduction</td>
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<td>Breastfeeding at gluten introduction</td>
<td>Infections; Gastroenteritis Neonatal</td>
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<td>Age at gluten introduction</td>
<td>Rod-shaped bacteria</td>
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<tr>
<td>&quot;Window of opportunity&quot;</td>
<td>Maternal smoking</td>
</tr>
<tr>
<td>Favorable microbiota composition</td>
<td>Smoking</td>
</tr>
<tr>
<td>Smoking</td>
<td>HLA DQ2/DQ8</td>
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</table>

**Figure 4.** Environmental- and lifestyle factors associated with increased/decreased celiac disease risk, divided into potential component factors (causal factors) and associated factors (markers for other causal factors). Necessary factors are indicated in the middle. Based on reference^14,27-46^
Celiac disease in the clinical setting

In recent decades several changes have occurred in the clinical aspects (clinical presentation, diagnostics and treatment) of celiac disease, with the exception of treatment strategies, which have remained the same since the 1950s. From being considered solely a childhood disease it is now recognized that celiac disease can develop throughout life, more commonly among females than males.\textsuperscript{24,47} The early descriptions of the so-called classical celiac disease case, typically an infant with diarrhea, malnutrition, abdominal distention and failure to thrive, may be encountered less frequently today than other manifestations. Despite an increased awareness of celiac disease, both among health professionals and in the population, as well as the development of more effective diagnostic tools, screening studies have shown that the majority of cases remain undiagnosed.\textsuperscript{24}

A heterogeneous clinical presentation

The classical clinical picture is predominantly seen in young children and includes symptoms and signs of malabsorption, commonly with concomitant irritability.\textsuperscript{48} Other symptoms frequently seen include nausea, vomiting, constipation, abdominal pain, weight loss and short stature.\textsuperscript{7,24,49}

The clinical presentation in older children and adolescents has greater variation, also encompassing symptoms and signs such as anemia, iron deficiency, neurologic findings, thyroid dysfunction, pubertal delay, dermatitis herpiformis, fatigue, tiredness, bloating, and headache.\textsuperscript{7,49} The extraintestinal manifestations can thus derive from any organ or body system and can be the sole presentation or occur with concomitant abdominal symptoms.\textsuperscript{7} In some cases the disease might not result in any perceived symptoms, or the symptoms may only be recognized in retrospect after treatment is initiated.\textsuperscript{50} Hence, the heterogeneous clinical presentation, where the classical symptoms or any perceived symptoms might be absent, complicates diagnosis.

Diagnosis

In the late 1950s the invention of an instrument for performing small intestinal biopsies enabled evaluation of the small intestinal mucosa.\textsuperscript{2} The disease was thereby linked to small intestinal enteropathy, which soon became the hallmark.\textsuperscript{49} Since 1967 small intestinal biopsy has been clinical practice in Sweden,\textsuperscript{51} and demonstration of small intestinal enteropathy remains the gold standard for diagnosis. The enteropathy can be graded based on the Marsh-Oberhauer criteria,\textsuperscript{52} presented in Figure 5.
Figure 5. Schematic presentation of the different grades of inflammation and villous atrophy according to the Marsh-Oberhauer criteria. 0=normal, I=intraepithelial lymphocytosis (>20 IEL/100 enterocytes, II=intraepithelial lymphocytosis + crypt hyperplasia, and IIIa-c=partial- subtotal- and total villous atrophy, respectively.

Villous atrophy and intraepithelial lymphocytosis are, however, not an exclusive sign of celiac disease but can also be present, for example, in an intestinal infection or cow milk protein allergy. The need to determine that gluten was the cause of the demonstrated enteropathy was reflected in the initial diagnostic criteria proposed by the European Society for Pediatric Gastroenterology (today ESPGHAN) recommending 3 consecutive small intestinal biopsies; the first while on a normal diet, the second on a gluten-free diet and the third after gluten challenge (Table 1).

Since the 1980s, serological markers indicative of disease have been available, enabling an improvement in the selection of individuals where a small intestinal biopsy is warranted. Moreover, follow-up (determination of response to a gluten-free diet) can be performed by serological testing. Tests for tTG-IgA or EMA-IgA are recommended, both of which have sensitivity and specificity between 95-100%. In conjunction with serological testing, determining total serum IgA excludes false low values due to IgA-deficiency (seen in 2-2.6% of all celiac disease cases). In case of IgA-deficiency, serological tests for tTG-IgG and EMA-IgG are available. Genotyping for the presence of HLA-DQ2/DQ8 can be of diagnostic value mainly for excluding the disease (high negative predictive value).
Following the introduction of serological tests, the ESPGHAN diagnostic criteria were revised in 1990 and 2011 (Table 1). Comparable diagnostic criteria are present in different parts of the world.56

Table 1. Diagnostic criteria for celiac disease (in summary) proposed by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in the initial and revised versions.

<table>
<thead>
<tr>
<th>Year</th>
<th>Proposed diagnostic procedure</th>
<th>Findings compatible with celiac disease</th>
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</table>
| 197057 | • Initial biopsy on normal gluten-containing diet  
• Second ‘healing’ biopsy during remission (gluten free diet)  
• Third biopsy after gluten challenge | • Small intestinal mucosa with villous atrophy Marsh III  
• Improvement in villous structure  
• Recurrence of villous atrophy |
| 199058 | • Test for serological markers  
• Initial biopsy  
• Clinical and serological follow-upa | • Positive serological markers  
• Marsh III  
• Clear-cut clinical remission in parallel with disappearance of serological markersb |
| 201155 | • Test for serological markers  
• HLA-DQ2/DQ8 genotyping  
• Initial biopsyd  
• Clinical and serological follow-up | • tTG > cut-off x 10 and positive EMAc,d  
• Presence of HLA-DQ2/DQ8  
• Marsh II-IIIe  
• Clinical remission in parallel with disappearance of serological markersf |

Abbreviations: tTG=tissue transglutaminase antibodies, EMA=endomysial antibodies

a For children below 2 years of age, 3 consecutive biopsies were still recommended.
b ‘Healing’ and ‘challenge’ biopsies were recommended if remission was not clear-cut, if there was doubt about the initial diagnosis, or any complicating circumstances.
c The analyses should be performed on blood samples collected at 2 different occasions.
d Initial biopsy can be omitted in case of unambiguous serological markers (see above right column) and presence of HLA-DQ2/DQ8.
e In this situation Marsh I is considered to indicate an uncertain case; recommendation to consider false positive serological markers, false negative biopsy, or early celiac disease. Recommendation to extend evaluation with serology and possibly biopsies.
f Biopsy during remission and/or gluten challenge under special circumstances.
**Treatment**

A strict gluten-free diet constitutes the only treatment for celiac disease, although non-dietary treatments are under investigation. Maintaining a strict gluten-free diet, while relatively safe and effective, can be demanding in everyday life as wheat constitutes a staple food in many countries. In the majority of cases, except individuals with refractory celiac disease, adherence to a gluten-free diet abates the inflammation in the small intestinal mucosa and the normal villous structure recurs.

**Associated conditions and secondary effects**

Celiac disease is associated with a number of other autoimmune diseases, e.g. type 1 diabetes, IgA-deficiency, and thyroid disease. Some of the secondary effects of celiac disease are related to an ongoing small intestinal inflammation and villous atrophy, e.g., low bone mineral density and nutritional deficiencies. While studies of associated diseases in children are limited, several common diseases have been associated with celiac disease in adults, e.g., increased risk for severe infections, asthma, stroke, ischemic heart disease and neuropsychiatric diseases. Whether the increased risk for these diseases is related to persisting inflammation in the small intestine, treatment and/or shared risk factors is unknown.

**Descriptive epidemiology**

Today celiac disease is recognized as occurring worldwide. While the disease generally is said to affect approximately 1% of the population, differences with respect to time, place and person are evident. Moreover, changes in disease definitions, the age group investigated, methods for case finding, and differences in diagnostics renders comparisons problematic.

![Figure 6. Model of the celiac disease iceberg. Adapted from Fasano et al.](image)

In general, the occurrence of celiac disease resembles an iceberg (Figure 6). Here the whole iceberg represents all celiac disease cases with the waterline demarking the difference between the clinically detected cases visible above the waterline and the unrecognized cases below the waterline. To determine the total prevalence, including both the clinically detected cases and the unrecognized cases below the waterline, screening is required.
**Celiac disease occurrence during the 20th century**

One of the earliest studies on disease occurrence in children was performed in the UK during the 1940s with a reported prevalence of 0.0125-0.025%.

During the following decades the reported occurrence was generally somewhat higher and an increasing incidence was seen in several countries up until the 1980s that was attributed to better diagnostic tools.

In the mid-1980s a declining incidence of clinically detected celiac disease was noted in several countries, especially in the UK, although later studies showed that this was due to changes in symptoms and age at diagnosis.

Furthermore, large variations between countries in the incidence of clinically detected celiac disease became evident, although the disease was generally still considered uncommon (prevalence approximately 0.4%).

In the mid-1990s the first population-based screening studies were performed, reporting a large proportion of unrecognized cases even among children.

**Geographical variation in celiac disease occurrence**

While the total prevalence in many areas remains unknown, studies from different parts of the world show a widespread occurrence, with variation between different areas as exemplified in Figure 7. To summarize, the celiac disease total prevalence among children varies between 0.5%-1%, with 1 exception (5.6% reported among Saharawian children).

![Figure 7](image). Map, with Sweden marked in white, exemplifying different prevalences of population-based childhood celiac disease based on screening studies.
Epidemiology of celiac disease in Sweden

In Figure 7 the location of Sweden in northern Europe is marked in white. The first study of celiac disease occurrence among Swedish children was performed in 1964, and until the mid-1980s the occurrence of clinically detected celiac disease displayed similar levels and patterns as in other European countries. 51,89-91

The Swedish epidemic of celiac disease

As previously mentioned, Sweden experienced an epidemic of clinically detected celiac disease among children below 2 years of age (1984-1996). During this period the incidence rate of clinically detected celiac disease was approximately four-fold compared to the time period before and afterwards. Accordingly, the birth cohorts in which the epidemic was seen had a higher cumulative incidence at 2 years of age,10 a difference also seen at 6 years of age (Figure 8).11

Figure 8. Cumulative incidence of clinically detected celiac disease (1973-2003) based on the National Swedish Childhood Celiac Disease Register. The main epidemic cohorts are represented by the darker gray lines. Two examples of statistically significant differences between the epidemic and post-epidemic cohorts at 2 years of age and at 6 years of age are indicated with * and ** respectively. Adapted from Olsson et al.11
The Swedish epidemic has been partly attributed to changes in infant feeding.\textsuperscript{10,31} Subsequent to these changes, which occurred on a population level, the Swedish birth cohorts of the epidemic period and post-epidemic period had different infant feeding practices, more specifically the circumstances related to gluten introduction into the infants’ diet. \textbf{Figure 9} summarizes when different changes in infant feeding occurred. In 1982, approximately 2 years prior to the beginning of the epidemic, Swedish pediatricians recommended postponing gluten introduction in infants from 4 until 6 months of age in an attempt to postpone the development of the disease, in accordance with changed European recommendations.\textsuperscript{92} Later, it was shown that the gluten content of Swedish industrially produced milk-cereal-based follow-on formulas and porridges concurrently had been substantially increased in order to substitute for a reduction in milk, which was decreased to reduce the protein load in the infants.\textsuperscript{10}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{Changes in infant feeding on a population level, to which the Swedish epidemic of celiac disease (1984-1996) partly has been attributed. During the period breastfeeding duration in Sweden increased. The gluten content in industrially produced milk-cereal-based follow-on formulas and porridges was larger during the indicated period. The year of the changes in infant feeding recommendations are marked. Based on data from Ivarsson \textit{et al.}\textsuperscript{10}}
\end{figure}

During the high incidence years a case-referent study was performed indicating that concomitant breastfeeding and gluten introduction, the latter introduced in small-medium amounts compared to large amounts, protects against celiac disease. Age at gluten introduction was not an independent risk factor (\textbf{Figure 10}).\textsuperscript{31}
Breastfeeding continued during the month of gluten introduction and continued beyond was associated with a decreased risk of celiac disease but age at gluten introduction (month 5-6 compared to earlier or later) was not an independent risk factor. Introduction of gluten in large amounts, compared to small-medium amounts, was associated with increased celiac disease risk. Abbreviations: BF=breastfeeding, CI=confidence interval. Based on data from Ivarsson et al.31

In 1996 the Swedish Childhood Celiac Disease Working Group suggested a revised national feeding recommendation, which was introduced by the Swedish Pediatric Society. The revised recommendation advocated that gluten could be introduced from 4 months of age, in small amounts preferably during on-going breast feeding.1 Starting in 1995, the industry decreased the gluten content of the milk-cereal-based follow-on formulas and porridges. During the epidemic period breastfeeding duration in Sweden increased, conceivably affecting the proportion of infants breastfed at gluten introduction. Data from the National Swedish Childhood Celiac Disease Register showed a steep decline in incidence rate to a pre-epidemic level starting in the mid-1990s.10,11

As the Swedish epidemic of celiac disease was seen among clinically detected children it can be considered in 2 fundamentally different ways: either as an increase followed by a decrease in the proportion of clinically detected cases but with a constant prevalence of the disease, or as a true change in celiac disease prevalence.

Moreover, the epidemic has partly (approximately 45%) been attributed to infant feeding, but the cause of more than half of the epidemic remains unexplained.

1 In Swedish the recommendation is: "gluteninnehållande livsmedel, liksom andra livsmedel, kan ges i form av smakportioner från fyra månaders ålder."
Objectives

The main objectives of this thesis were two-fold; to increase the knowledge regarding the occurrence of celiac disease in Sweden, and the etiology of celiac disease in general, by investigating the Swedish epidemic and its potential causes, aiming towards celiac disease primary prevention.

Specific objectives:

- To investigate the total prevalence of celiac disease in Sweden, including both clinically- and screening-detected cases, in 2 birth cohorts of 12-year-olds born during the epidemic period (1993) and the post-epidemic period (1997), respectively.

- Regarding the Swedish epidemic, to discern whether:
  - the differences in cumulative incidence among clinically detected cases seen at 2 years of age remained at 12 years of age
  - it constituted a change in disease occurrence and/or a shift in the proportion of clinically detected cases.

- To relate the findings in celiac disease occurrence in Sweden to changes over time in infant feeding.

- To explore whether there were other changes regarding early exposures (environmental- and/or lifestyle factors) on a population-level that coincided with the Swedish epidemic.

- To assess the possible association between celiac disease and environmental- and lifestyle factors, with focus on early vaccinations and infections.
## Overview of objectives and methods

<table>
<thead>
<tr>
<th>Objective</th>
<th>Study design</th>
<th>Data sources and participants</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>To investigate the total prevalence of celiac disease in Sweden in 2 birth cohorts of 12-year-olds born during the epidemic period (1993) and the post-epidemic period (1997), respectively.</td>
<td>Cross-sectional screenings</td>
<td>Blood samples</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clinical data</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(incl. medical records)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-year-olds:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=7 567 (1993)</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=5 712 (1997)</td>
<td></td>
</tr>
<tr>
<td>Regarding the Swedish epidemic, to discern whether:</td>
<td>Longitudinal surveillance</td>
<td>National Swedish Childhood Celiac Disease Register</td>
<td>II</td>
</tr>
<tr>
<td>-the differences in cumulative incidence among clinically detected cases remained at 12 years of age</td>
<td>Cross-sectional screenings</td>
<td>Cases 0-12 years</td>
<td></td>
</tr>
<tr>
<td>-it constituted a change in disease occurrence and/or a shift in the proportion of clinically detected cases.</td>
<td></td>
<td>(as above)</td>
<td></td>
</tr>
<tr>
<td>To relate the findings in celiac disease occurrence in Sweden to changes over time in infant feeding.</td>
<td>Surveillance</td>
<td>National exposure data (population statistics)</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional / prospective</td>
<td>Questionnaires completed by parents (67%) of 12-year-olds</td>
<td></td>
</tr>
<tr>
<td>To explore whether there were other changes regarding early exposures on a population-level that coincided with the Swedish epidemic.</td>
<td>Ecological approach</td>
<td>National exposure data (population statistics)</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>for the time period 1980-2000</td>
<td>IV</td>
</tr>
<tr>
<td>To assess the possible association between celiac disease and environmental- and lifestyle factors, with focus on early vaccinations and infections.</td>
<td>Incident case-referent</td>
<td>Questionnaires</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Child health clinic records</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children &lt;2 years:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=450 cases</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=950 referents</td>
<td></td>
</tr>
</tbody>
</table>
Methods

An epidemiological approach to the Swedish celiac disease epidemic was used in this thesis. *Epidemiology* has been defined as “the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems”.93

In the 1850s John Snow (1813-58) and William Farr (1807-83) brought medicine and public health surveillance together in their pioneering work on the cholera epidemic in London.94 Snow is often considered the founder of epidemiology, although the concept of population thinking applied to health can be traced back to John Graunt’s (1620-74) work triggered by the plague epidemic 2 centuries earlier. In the early 20th century epidemiology, with its name derived from Greek (*epi* [upon] *demos* [population] *logos* [science]), became an academic field.94 Despite the fact that the epidemiological field of science has evolved, especially during later 20th century, many early concepts still remain.

Conceptual framework for discussing causality

The finding of an association does not automatically entail the existence of a causal relationship.95-97 The assessment of causality cannot be tested by statistical tests alone, as is the case for an association, but requires a broader perspective including logic, methodological considerations, weighing the available evidence, and knowledge about biological processes.98 There are several mathematical and graphical models for evaluating causation99 but for the purpose of discussing causality and integrating the findings from this thesis with current evidence, the ‘9 considerations’ of Sir Austin Bradford Hill,96 presented in Figure 11, have been utilized. In addition, the “pie-chart model”, i.e. the sufficient-component cause model by Rothman, has been used for summarizing the findings (Figure 11).

![Figure 11. A conceptual framework for discussing causality; the 9 considerations of Hill and a schematic illustration of the “pie-chart” model by Rothman (A-C represents 3 component causes for developing a disease). The latter has been used for summarizing the findings.](image-url)
Ever since Sir Austin Bradford Hill published his paper “The Environment and Disease: Association or Causation?” in 1965 his ‘9 criteria’ have been widely used for guidance regarding causal inference. These criteria have been questioned concerning their utility, partly as they cannot be used to rule out causality, although Hill himself never meant them to be ‘causal criteria’ or a ‘checklist’ for affirming or refuting causality, but considered them more as viewpoints and pondered, “In what circumstances can we pass from this observed association to verdict of causation?” The sufficient-component cause model by Rothman portrays the presence or absence of multiple causal factors (necessary and component) in a pie-chart, in which each slice represents a factor, which together results in disease.

**In summary,** the considerations by Hill will serve as a conceptual framework discussing causal inference with respect to findings in this thesis, which will be summarized in suggested “pie-chart” models.

**Overview of the study designs**

Different epidemiological designs were used (Figure 12). All studies utilized the National Swedish Childhood Celiac Disease Register. We performed a two-phased screening study investigating the total prevalence of celiac disease in birth cohorts of the epidemic and the post-epidemic period, respectively. To further explore whether there were changes in environmental- and lifestyle factors coinciding with the epidemic an ecological approach was used. A population-based incident case-referent study, with participants enrolled during the epidemic, was used to assess potential associations between celiac disease and environmental- and lifestyle factors, including potential findings from the ecological approach.

![Figure 12](image_url)

**Figure 12.** Overview of the study designs. All studies were based on the National Swedish Celiac Disease Register (Celiac Disease Register). Solid and dashed lines represent prospectively and retrospectively recorded data, respectively. Coverage of the Swedish child population is given in percentages. The time periods when the case-referent study and the screening study were performed are indicated with black lines and grey lines represents period of case ascertainment/follow-up. The time period of interest in the ecological approach is indicated.
**Swedish setting**

The total population in Sweden is 9.5 million, with an annual birth rate of approximately 100,000 (Statistics Sweden; www.scb.se). Several factors in Swedish society are of assistance in epidemiological studies including: i) the personal identity number, ii) the structure of the child health care system and iii) nationwide registers handled by the Swedish public authorities.

Since 1947 all citizens have been assigned a personal identity number comprising date of birth followed by 4 digits whereof the first 3 identify the individual and the last is a control number, rendering a 10 digit unique combination. The personal identity number is used in all aspects of health care and in registers.

The Swedish child health care system is free of charge and entails primary health care with referral to hospital pediatric departments when appropriate. Well-baby clinics are included in primary health care and 99% of all children are seen there routinely during the first year of life (The National Board of Health and Welfare; www.socialstyrelsen.se)

Statistics Sweden (www.scb.se) handles national demographic population statistics, and there are several nationwide registers with longitudinal surveillance that are handled by different public authorities.

**The National Swedish Childhood Celiac Disease Register**

The incidence register, in short called the *Celiac Disease Register*, was initiated in 1991 and included 14 pediatric departments, covering 40% of the pediatric population, that prospectively reported all new cases. For the period 1973-1991 5 departments, covering 15% of the pediatric population, reported retrospectively. In 1998 the register became nationwide and comprised all 47 pediatric departments (Appendix 1).

Reporting is based on a standardized form including personal identity number, gender, date at first small intestinal biopsy, place of residence and basis for diagnosis (symptoms, serological markers and mucosal evaluation). Included cases were detected within routine clinical care, and had biopsy-verified celiac disease. The register has undergone quality control until 2003. Quality control for the period 2004-2010 is underway.
Screening for celiac disease - the ETICS study

The two-phased cross-sectional screening study was entitled ETICS – Exploring the Iceberg of Celiacs in Sweden (Papers I-II). The first phase was performed in 2005-2006 and the second in 2009-2010. The multicenter study covered the same geographical areas, representing Sweden from north to south, in both phases (Appendix 2). Each of the 5 sites, indicated in Figure 13, included a major city with municipalities in the surrounding suburbs and countryside. The study is part of the PreventCD European project.103

Participants

The screenings included 2 birth cohorts of 12-year-olds, 1 representing the epidemic birth cohorts (born in 1993) and the other representing the post-epidemic cohorts (born in 1997). The birth cohorts differed on a population level in infant feeding, summarized in Table 2.

Table 2. Infant feeding characteristics in the included birth cohorts.

<table>
<thead>
<tr>
<th>Infant feeding recommendation regarding introduction of gluten-containing foods</th>
<th>Birth cohort of 1993</th>
<th>Birth cohort of 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 6 months of age</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>In small amounts, from 4 months of age</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Average daily flour consumption from milk- and cereal-based follow-on formulas&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 g/child/day</td>
<td>24 g/child/day</td>
</tr>
<tr>
<td>Proportion of children breastfed&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 months of age (%)</td>
<td>77</td>
<td>83</td>
</tr>
<tr>
<td>6 months of age (%)</td>
<td>63</td>
<td>74</td>
</tr>
</tbody>
</table>

<sup>a</sup> In children below 2 years of age.<sup>10</sup>

<sup>b</sup> Exclusively or partially breastfed. Statistically significant difference between cohorts at both ages (P<0.001) The National Board of Health and Welfare (www.socialstyrelsen.se)
A total of 10,041 children from the 1993 cohort were invited, with 7,567 (75%) participating and 7,208 (72%) blood sampled. Corresponding numbers for the 1997 cohort were: 8,284 invited, with 5,712 (69%) participating and 5,424 (65%) blood sampled. In the 1993 cohort 8.8% of the population birth cohort was invited (cohort size: n=117,997). The corresponding proportion for the 1997 cohort was 9.2% (cohort size: n=90,502) (Statistics Sweden; www.scb.se).

The screenings were school-based and blood sampling was carried out by research nurses at a visit to the school health nurse. Therefore all children in 6th grade were invited, whereof 95% were born the intended year (1993 and 1997, respectively) and the others during the preceding year or the year thereafter. The proportion of participating girls was similar in both cohorts (48% girls in the 1993 cohort vs. 49% girls in the 1997 cohort, P=0.99).

**Screening strategy and definitions**

*Clinically detected celiac disease*, i.e. celiac disease diagnosed within routine clinical care prior to the study, was reported by the parents at enrollment and ascertained through the Celiac Disease Register and/or the child’s medical record. The other children were screened through analysis of serological markers and all children with positive values were referred to the pediatric department at their study site for case ascertainment with small intestinal biopsy.

Criteria for diagnosis were Marsh III a-c enteropathy, or the combination of Marsh I-II enteropathy, HLA-DQ2/DQ8, symptoms or signs compatible with celiac disease, and clinical response to a gluten-free diet.

*Age at celiac disease diagnosis* in clinically detected cases was defined as the age at the first small intestinal biopsy. The same definition was used in all studies included in this thesis.

A summary of the screening strategy and case ascertainment, on which prevalence comparisons were based, resulting in a diagnosis of *screening-detected celiac disease*, i.e. celiac disease undiagnosed prior to the study and detected through the screening, is presented in **Figure 14**.
**Figure 14.** Screening strategy including case ascertainment through small intestinal biopsy including evaluation by a second pathologist blinded for the previous finding, resulting in a diagnosis of celiac disease. **Bold** indicates that further diagnostics were performed. Abbreviations: tTG=tissue transglutaminase antibodies, EMA=endomysial antibodies

**Laboratory analyses**

All serum samples were analyzed for tTG-IgA and values above 4 U/mL were considered positive. Intermediate values of tTG-IgA (2-4 U/mL) were additionally analyzed for EMA-IgA with values equal to or more than a 1:5 dilution considered as positive. Children with tTG-IgA below 2 U/mL were classified as non-cases.

tTG-IgA was determined by conventional enzyme-linked immunosorbent assay using a commercial kit (Celikey®, Phadia GmbH, Freiburg, Germany). Results were expressed as arbitrary units per milliliter (U/ml). Serum analyses were performed in duplicate within the measuring range 0.1-100 U/ml and the mean value was subsequently used. The manufacturer’s recommended cut-off for positive tTG-IgA was 5 U/ml; however, to increase sensitivity of the test, all values above 4 U/ml were considered elevated. EMA-IgA was analyzed with indirect immunofluorescence technique using tissue sections from marmoset monkey esophagus mounted on glass slides (The Binding Site, Birmingham, UK). Sera yielding fluorescent binding to the endomysial structure were diluted to determine the lowest titer detectable. Analyses were performed according to the manufacturer’s instructions at the same laboratory.
While all samples in the first phase were analyzed for total serum-IgA, this was not performed in the second phase due to low yield (2 additional cases found). IgA-deficiency was defined as serum levels below 0.06 g/l. When total serum-IgA was low (<0.5 g/l), analysis of tTG-IgG was performed. The cut-off for positive tTG-IgG was set at 6 U/ml, and serum samples with intermediate values (3-6 U/ml) were further analyzed for EMA-IgG with 1:5 dilution as the cut-off for positivity.

Total serum-IgA levels were analyzed using a routine nephelometric method (BN Pro Spec® System, Dade Behring, Marburg GmbH, Germany). tTG-IgG and EMA-IgG were determined by the same methods as for the IgA analyses.

Prevalence comparisons were based on the same screening protocol, i.e. excluding the 2 cases found after analyses of IgG antibodies.

**Case ascertainment for screening-detected cases**

All children with positive serological markers were recommended to undergo a small intestinal biopsy (Figure 14). Biopsies were taken using a suction capsule or gastroscopy (4-6 biopsies recommended, from the distal duodenum and the bulb), the former mainly in the first phase of the study in accordance with clinical routine. In case of normal small intestinal mucosa in a biopsy by suction capsule, re-biopsy was mainly performed using gastroscopy. Mucosal specimens were classified according to the revised Marsh-Oberhuber classification (Figure 5). All biopsies were subjected to a second histopathological evaluation by 1 pathologist blinded to the previous result. In case of disagreement, a third pathologist evaluated the biopsy.

All children with screening-detected celiac disease were genotyped for HLA-DQ2/DQ8. DNA was extracted from whole blood using the Blood & Cell Culture DNA Kit (QIAGEN®, Hilden, Germany), mainly the Maxi-kit but for some children the Midi- or Micro-kit was used. Genotyping for HLA alleles encoding for DQ2/DQ8 was performed by multiplex-PCR reactions with oligonucleotide probe hybridization and detection on agarose gel (Eu-DQ® test, Eurhospital SpA, Trieste, Italy).

**Ascertainment of infant feeding**

Differences between the birth cohorts in infant feeding on population level (Table 2) were ascertained via questionnaires, including information regarding breastfeeding duration and age at gluten introduction. Questionnaires were sent to all participating families and completed prior to receiving the result from analyses of serological markers by totally 67%.
**Power of the study and estimation of sample size**

Our prespecified null hypothesis was that there was the same total celiac disease prevalence in the 2 birth cohorts. The acceptable limit for a Type I error was set at 5% (α=0.05), and for a Type II error it was set at 10% (β=0.10), corresponding to a statistical power (1-β) of 90%. Considering an assumed difference in prevalence between the cohorts of 0.5%, it was necessary to have a sample size of approximately 7,000 children, with approximately 5,000 consenting to participate, from each cohort.

**Ecological approach**

To further explore whether there were other changes in early exposures (environmental- and/or lifestyle factors) in Swedish society that coincided and possibly contributed to the celiac disease epidemic, an ecological approach was deemed appropriate (Papers III-IV).

The ecological approach focused on early exposures affecting both the immune system, and possibly celiac disease risk, and a large proportion of Swedish children. In the graph displaying the incidence rate ([Figure 1](#)), we plotted changes over time in environmental- and lifestyle factors focusing on a time period framing the epidemic by 4 years on either side (1980–2000).

Surveillance by Swedish public authorities (register holders) constituted the data sources ([Table 3](#)). Data on changes in the national vaccination program were obtained from The Swedish Council on Technology Assessment in Health Care.105

<table>
<thead>
<tr>
<th>Register holder</th>
<th>Data included in the register</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistics Sweden</td>
<td>Demographic population statistics</td>
</tr>
<tr>
<td><a href="http://www.scb.se">www.scb.se</a></td>
<td>Socioeconomic measuresa</td>
</tr>
<tr>
<td>The National Board of Health and Welfare</td>
<td>Breastfeeding at age 4 and 6 monthsb</td>
</tr>
<tr>
<td><a href="http://www.socialstyrelsen.se">www.socialstyrelsen.se</a></td>
<td>Vaccination program</td>
</tr>
<tr>
<td>Swedish Institute for Infectious Disease Control</td>
<td>Caesarean sectionc</td>
</tr>
<tr>
<td><a href="http://www.smittskyddsinstitutet.se">www.smittskyddsinstitutet.se</a></td>
<td>Vaccination coverage</td>
</tr>
</tbody>
</table>

- a Research on living conditions (ULF/SILC) including data on individual socioeconomic status measured by Socioeconomic Index, see Definitions – environmental- and lifestyle factors.
- b Proportion of infants breastfed at 4 and 6 months of age.
- c Emergency and elective caesarean section jointly.
**Incident case-referent study**

Between 1992 and 1995 all new clinically detected cases of celiac disease reported to the Celiac Disease Register were invited to participate in the study (Papers III-IV). Concurrently, 2 referents were randomly selected from the national population register after fulfilling matching criteria (date of birth, sex and the family’s area of residence). Requirements for participation were informed consent with full personal identity number.

The included cases had biopsy-verified celiac disease according to the 1970 ESPGHAN recommendations. Biopsy procedures (3 consecutive biopsies) were finalized in 1999 and age at diagnosis was set to age at the first biopsy.\(^{31}\) In each respective analysis (Papers III-IV) the inclusion criteria were defined as a matched set of 1 case with 1 or 2 referents below 2 years of age with complete information on main variables of interest.

All families received a questionnaire entitled “Child Health in the 1990s” where parents were asked about a multitude of events and exposures during the first years of their child’s life, including family characteristics, infant feeding, and the child’s general health, without revealing a special interest in celiac disease.

For prospectively recorded data on early vaccinations (Paper III) we requested the child health clinic records from well-baby clinics, with data on type of vaccinations given and date of administration.

**Participants**

Of the included children below 2 years of age (475 cases and their 950 referents), the questionnaire was answered by 455 (96%) cases and 856 (90%) referents, respectively (Figure 15). In Paper III the final analyses included 1015 children; 392 cases (83%) and 623 referents (66%), and in Paper IV the corresponding numbers were 954 children; 373 (79%) cases and 581 (61%) referents (Figure 15).

In the children with ascertained celiac disease the median age at diagnosis was 14-15 months (inter-quartile range 12-18 months). Two thirds of the study population were girls.
Figure 15. Participation in the case-referent study, shown according to inclusion in Paper III and in Paper IV. Abbreviations: CD=celiac disease. Adapted from Papers III-IV.
Definitions – environmental- and lifestyle factors

The duration of breastfeeding was defined as the period of time when the infant was exclusively or partially breastfed. Breastfeeding status at gluten introduction was categorized into discontinued the month before introduction, continued during the month of introduction and continued beyond that time.

Age was used as ‘4 months of age’ meaning after the 4th month of life was completed (120 days) and ‘from 4 months of age’ as after the age of 120 days.

Age at introduction of gluten was set to the first month of life during which flour from wheat, rye or barley was given. The amount of gluten during the introduction corresponded to the amount of gluten-containing flour per day, 2 weeks after the first portion. As previously described in detail elsewhere, the gluten amount was calculated from the food frequency and amount section of the questionnaire and standard recipes, and categorized into small-medium and large amounts with a cut-off at 16 grams/day based on the distribution of flour intake among the referents.

Socioeconomic status of the family was defined according to the Socio-Economic Index of Statistics Sweden (www.scb.se), which is based on the individual’s work position, taking into account the standard education level for each work position in the classification. The Socio-Economic Index was categorized into high-medium and low.

Vaccinations within the national Swedish program (Figure 16) were explored; thus the study was restricted to vaccines in routine use. The program, at the time of interest, included vaccinations against diphtheria/tetanus, pertussis, polio, Haemophilus influenzae type b, measles/mumps/rubella, and for children in risk groups also vaccination against tuberculosis with the live attenuated variant of Mycobacterium bovis, i.e. Bacillus Calmette-Guérin vaccine (BCG).

The child’s vaccination status was defined as vaccinated for a given vaccine after the date the initial dose was given, and unvaccinated until then. Children with celiac disease were considered unvaccinated, with respect to their disease, if vaccinated after diagnosis, and the same cut-off age was used for the respective referent(s).
<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of vaccine</th>
<th>Route</th>
<th>Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphteria (D)</td>
<td>Inactivated toxin</td>
<td>i.m</td>
<td>DT</td>
</tr>
<tr>
<td>Tetanus (T)</td>
<td>Inactivated toxin</td>
<td>i.m</td>
<td>DTPa during trial and</td>
</tr>
<tr>
<td>Pertussis (Pa)</td>
<td>Acellular component</td>
<td>i.m</td>
<td>after Pa introduction</td>
</tr>
<tr>
<td>Polio (IPV)</td>
<td>Inactivated virus</td>
<td>i.m</td>
<td>IPV+Hib after</td>
</tr>
<tr>
<td>Haemophilus influenzae type b (Hib)</td>
<td>Conjugated component</td>
<td>i.m</td>
<td>introduction</td>
</tr>
<tr>
<td>Measles (M)</td>
<td>Attenuated virus</td>
<td>s.c</td>
<td>MMR</td>
</tr>
<tr>
<td>Mumps (M)</td>
<td>Attenuated virus</td>
<td>s.c</td>
<td></td>
</tr>
<tr>
<td>Rubella (R)</td>
<td>Attenuated virus</td>
<td>s.c</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis (BCG)</td>
<td>Live attenuated</td>
<td>i.d</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 16.** Vaccinations within the national Swedish vaccination program, including the main combinations given, and age at the initial dose (upper time line). Abbreviations: route=administration route, i.m=intramuscular, s.c=subcutaneous, i.d=intradermal, BCG=Bacillus Calmette-Guérin. Adapted from Paper III.

**Antibiotic treatment** included any kind of antibiotics received during the first 6 months of life.

An *early* infectious episode was defined as occurring during the first 6 months of life, thereby maintaining a temporal relationship between infectious episodes (prior to) and celiac disease diagnosis.

An *infectious episode* was defined as an infection from the panorama of childhood infections in Sweden – common cold, otitis media, pneumonia, urinary tract infection, gastroenteritis, whooping cough, scarlet fever, exanthema subitum (roseola infantum), or chicken-pox – or an episode of fever, as this could be the only sign of viral infection in children. The parents reported each infection as occurring once, twice, or three or more times. All infectious episodes were summarized and categorized into 2 categories; 0-2 episodes or ≥3 episodes. The cut-off (≥3) was set to the highest level where number of infectious episodes could be discriminated with certainty. In addition, the occurrence of gastroenteritis was analyzed separately with a cut-off set at ≥1 episode, since few children had experienced this during the first 6 months of life.
Statistical analyses

Both the Celiac Disease Register and the ETICS database were stored and handled in Access 2010 (Microsoft, Redmond, WA). PASW Statistics versions 16-20 (SPSS Inc, Chicago, IL), Excel 2003-2010 (Microsoft, Redmond, WA), and Open Access programs WinPepi\(^{107}\) and EpiInfo (both programs available at e.g. www.brixtonhealth.com) were used for basic and statistical analyses. Throughout the thesis, statistical significance was defined as an odds ratio (OR) with confidence interval (CI) not including 1, or a two-tailed \(P<0.05\).

Measurements of disease occurrence

The celiac disease prevalence was calculated as the number of cases divided by the number of children in the cohort. The incidence rate was calculated as the number of new cases divided by the total person-time at risk, approximated as the mid-year population each year. The cumulative incidence per birth cohort at a certain age was calculated as the total number of cases up to this age divided by the number of children in the population birth cohort.

Analytical procedures

Differences in cumulative incidence of celiac disease between birth cohorts were evaluated using the \(\chi^2\)-test. Prevalence comparisons were performed by the standard traditional log-transformation method and expressed as prevalence ratios with 95% CI. Associations were evaluated by conditional logistic regression in both bivariate and multivariate analyses. Variables were presented using proportions and, when appropriate, median values with inter-quartile range. In the case-referent study, complete data on main variables were required, and missing answers for other variables were coded as separate categories. When appropriate, medians were compared by the Mann-Whitney U-test and associations between categorical variables using \(\chi^2\)-tests or Fisher’s Exact test. Stratified analyses were performed, including stratifications for sex. Interaction was analyzed on the additive scale based on the definition by Rothman; interaction equals deviation from additivity.\(^{26}\)

The proportion of cases attributable to exposure was estimated by:

\[
\text{attributable fraction} = \frac{(\text{OR}-1)}{\text{OR}}.
\]

Based on Rothman’s definition of interaction, the etiologic cases were decomposed into the different effects of 3 separate exposures as well as their interactions.\(^{26,108}\)
Ethical Considerations

All studies complied with Swedish legislation (e.g., the Personal Data Act; SFS 1998:204 and the Biobanks in Medical Care Act; SFS 2002:297) and the Helsinki declaration.

The ETICS study was approved by the Regional Ethical Review Board of Umeå University, Umeå, Sweden [dnr 04-156M], and the Swedish Data Inspection Board. The established personal register within the study was listed at Umeå University [dnr UmU 101-2496-04]. The National Swedish Childhood Celiac Disease Register and the case-referent study were approved by all the Research Ethics Committees of all Swedish Medical Faculties [dnr 4741-92 and dnr 97-370], and the Swedish Data Inspection Board.

Informed consent was obtained from all participating families (legal guardians of the children). In the ETICS study, the invitation included a separate page with age-appropriate information for the 12-year-old. The autonomy of the 12-year-old was considered regarding the withdrawal of previously received consent from the legal guardian, i.e. a decision not to participate at the time when the study was conducted, although the reverse was not approved. If in any of the studies the child, when growing up, does not wish to be included in the personal registers within the studies, the child will be removed upon request.
Results

Main findings in this thesis are presented based on each of the 2 parts of the main objective and according to the epidemiological design used.

Two designs for investigating the celiac disease occurrence in Sweden, focusing on the 1993 and 1997 birth cohorts, were used; the National Swedish Childhood Celiac Disease Register and the ETICS screening study.

The etiology of celiac disease was investigated, on one hand by relating the occurrence of celiac disease to changes in infant feeding using population data and the ETICS study, and on the other hand by an ecological approach for exploring the potential causes of the epidemic further, as well as an incident case-referent study for assessing potential associations.

Cumulative incidence of clinically detected celiac disease

Based on the Celiac Disease Register, the cumulative incidence of clinically detected celiac disease at 12 years of age (years 2005/2006 and 2009/2010 for the 1993 and 1997 birth cohorts, respectively) was significantly lower in the 1997 cohort compared to the 1993 cohort (Table 4). The corresponding incidence ratio was 0.80 (95% CI, 0.67-0.93).

Table 4. Clinically detected celiac disease in Sweden based on the National Swedish Celiac Disease Register.

<table>
<thead>
<tr>
<th>Cumulative incidence (cases per 1000 births)</th>
<th>Birth cohort of 1993</th>
<th>Birth cohort of 1997&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 years of age&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 years of age</td>
<td>5.6</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12 years of age</td>
<td>8.1</td>
<td>6.4</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<sup>a</sup>Preliminary data from the time period 2004-2010.

<sup>b</sup>Previously published data; Ivarsson et al. included for comparative purposes.

Although a significant difference was seen at 12 years of age, the 1997 birth cohort had a, to some degree, steeper increase after 2 years of age and subsequently the differences between the cohorts have abated over time (incidence ratio: 0.31, 0.61, and 0.80 for <2, 6 and 12 years of age, respectively)
Screening of the 1993 and 1997 cohorts

The 2 phases in the ETICS screening study were performed in the same geographical areas and included a total of 13 279 12-year-olds (Papers I-II). A summary of the screening procedure according to the same screening protocol is presented below (Figure 17).

**Figure 17.** Flowchart summarizing the results from screening for celiac disease, according to the same screening protocol, in 2 birth cohorts. A total celiac disease prevalence of 2.9% was found in the 1993 cohort, and in the 1997 cohort the figure was 2.2% (Papers I-II). Abbreviations: tTG-IgA=tissue transglutaminase antibodies of IgA-type. Adapted from Paper II.
Clinically detected celiac disease

Of the participating children reporting clinically detected celiac disease prior to the screening, the diagnosis was ascertained in 66 and 34 children from the 1993 and 1997 cohorts, respectively, by small intestinal biopsy in all but 1 of the children from the 1993 cohort and in all from the 1997 cohort (Table 5). Of the clinically detected cases from the respective cohorts 46 (70%) and 22 (65%) were girls.

Table 5. Clinically detected celiac disease in 2 cohorts.

<table>
<thead>
<tr>
<th>Clinically detected celiac disease</th>
<th>Cohort of 1993 number</th>
<th>Cohort of 1997 number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy-verified</td>
<td>65</td>
<td>34</td>
</tr>
<tr>
<td>Without small intestinal biopsy</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>66&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td><strong>34</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> Diagnosis based on EMA-IgA 1/320, family history of celiac disease, and clinical response to a gluten-free diet.

<sup>b</sup> One child initially included (Paper I) was later shown to be misclassified and was subsequently reclassified into the group of children with blood samples without celiac disease (n=7208).

Serological markers

Analyses of the serum samples yielded a total of 192 (2.7%) and 104 (1.9%) children with positive markers from the 1993 and 1997 cohorts, respectively. The distribution between the different levels is summarized in Table 6.

Table 6. Serological markers and distribution in the 2 cohorts.

<table>
<thead>
<tr>
<th>Positive serological markers</th>
<th>Cohort of 1993 n (%)</th>
<th>Cohort of 1997 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tTG-IgA &gt;4 U/mL</td>
<td>167 (87)</td>
<td>85 (82)</td>
</tr>
<tr>
<td>tTG-IgA 2-4 U/mL and EMA-IgA &gt;1:5</td>
<td>20 (10)</td>
<td>19 (18)</td>
</tr>
<tr>
<td>tTG-IgG &gt;6 U/mL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 (3)</td>
<td>-</td>
</tr>
<tr>
<td>tTG-IgG 3-6 U/mL and EMA-IgG &gt;1:5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>192&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td><strong>104</strong></td>
</tr>
</tbody>
</table>

Abbreviations: tTG=anti-tissue transglutaminase antibodies, EMA=anti-endomysial antibodies, IgA=immunoglobulin of IgA type, IgG=immunoglobulin of IgG type

<sup>a</sup> Percentages of positive serological markers.

<sup>b</sup> Analyses performed in all children with serum-IgA <0.5 g/mL.

<sup>c</sup> The 5 children with positive tTG-IgG (Paper I) were excluded in the prevalence comparison (Figure 17).
Serum-IgA levels were analyzed in 7161 (99%) children from the 1993 cohort resulting in 172 children with values <0.5 g/l, of whom 28 had IgA-deficiency (0.4% of all children). Among these 172 children 5 had elevated tTG-IgG, and none had intermediate values in combination with positive EMA-IgG (Paper I).

**Case ascertainment in cases with positive serological markers**

Of the children with positive serological markers a small intestinal biopsy was performed in 184 (96%) and 99 (95%) children from the 1993 and 1997 cohorts, respectively. Results from the evaluation of the small intestinal mucosa, including the blinded re-evaluation,\(^{104}\) are summarized in Table 7.

**Table 7.** Results from the evaluation of the small intestinal mucosa and the distribution according to the Marsh-Oberhauer classification.

<table>
<thead>
<tr>
<th>Small intestinal biopsy evaluation</th>
<th>Cohort of 1993 n (%)</th>
<th>Cohort of 1997 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marsh III a-c</td>
<td>141 (77)</td>
<td>88 (89)</td>
</tr>
<tr>
<td>Marsh II</td>
<td>2 (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>Marsh I and symptoms/signs(^b)</td>
<td>9 (4.9)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Marsh I and no symptoms/signs</td>
<td>3 (1.6)</td>
<td>0</td>
</tr>
<tr>
<td>Marsh 0</td>
<td>26 (14)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Non-interpretable</td>
<td>3 (1.6)(^c)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total number of celiac disease cases</strong></td>
<td><strong>153 (83)</strong>(^d)</td>
<td><strong>89 (90)</strong></td>
</tr>
</tbody>
</table>

\(^a\) Number and percentages of all biopsies

\(^b\) Symptoms/signs: gastrointestinal complaints, irritability, tiredness, associated conditions, family history of celiac disease, deviation in weight and/or height, or deviation in laboratory analyses. See Methods section, Screening strategy and definitions (Figure 14).

\(^c\) One of these children diagnosed with celiac disease and included as a case based on tTG-IgA >100 U/ml as the biopsy was non-interpretable but parents declined re-biopsy. After gluten-challenge: oral rhagades and once more tTG-IgA >100 U/ml.

\(^d\) Differences between the above results (Paper II) and the results presented in Paper I are due to exclusion of 2 cases found through tTG-IgG (Figure 17) and adjustments after the re-evaluation of mucosal specimens by 1 blinded pathologist.\(^{104}\)

In total, 153 and 89 children from the 1993 and 1997 cohorts, respectively, fulfilled the criteria for screening-detected celiac disease, whereof 82 (54%) and 57 (64%) were girls (Paper II). The 2 children with celiac disease diagnosis after findings of positive tTG-IgG were girls (Paper I).
The results of the HLA-DQ2/DQ8 genotyping in the screening-detected cases are summarized in **Table 8**. All successfully genotyped screening-detected cases possessed DQ2, DQ8, or both.

**Table 8.** HLA-DQ2/DQ8 in the screening-detected celiac disease cases.

<table>
<thead>
<tr>
<th>HLA genotyping</th>
<th>Cohort of 1993 n (%)</th>
<th>Cohort of 1997 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQ2</td>
<td>115 (76)</td>
<td>71 (80)</td>
</tr>
<tr>
<td>DQ2/DQ8</td>
<td>23 (15)</td>
<td>8 (9.0)</td>
</tr>
<tr>
<td>DQ8</td>
<td>15 (9.9)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Non-DQ2/DQ8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Genotyping not available</td>
<td>0</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>153</strong></td>
<td><strong>89</strong></td>
</tr>
</tbody>
</table>

*a Number and percentages of all HLA-genotyped screening-detected cases.

*b Diagnosis based on tTG-IgA >100 U/ml and Marsh III enteropathy.

*c The 2 cases found through analyses of tTG-IgG were excluded in the prevalence comparisons.

**Potential celiac disease**

Children with positive serological markers where diagnosis was not ascertained by mucosal evaluation (*potential celiac disease*) were not included in the prevalence estimates. In the 1993 cohort there were a total of 29 (15%) children with potential celiac disease, whereof 26 had normal small intestinal mucosa, and 3 had Marsh I but did not fulfill celiac disease diagnostic criteria. In the 1997 cohort there were 10 (10%) children with positive serological markers but normal mucosa.

Furthermore, in total 12 families, 8 (4%) from the 1993 cohort and 5 (4.8%) from the 1997 cohort, declined investigation with small intestinal biopsy. None of them were included in the prevalence estimates. Whether or not these children had celiac disease remains unknown but at least they had potential celiac disease.
Prevalence comparisons between the 1993 and 1997 cohorts

In children born after the celiac disease epidemic (1997) as compared to during the epidemic (1993) a significantly lower total prevalence of celiac disease was seen (prevalence ratio, 0.75; 95% CI, 0.60-0.93) (Figure 18).

![Graph showing prevalence comparisons between the 1993 and 1997 cohorts. The former birth cohort represents the period of the Swedish epidemic of celiac disease (1984-1996), and the latter the post-epidemic period. A statistically significant difference in the total celiac disease prevalence was observed (Paper II).]

When comparing the prevalence of clinically detected celiac disease between the cohorts, a lower risk in the 1997 cohort as compared to the 1993 cohort was seen, although it was not statistically significant (prevalence ratio, 0.68; 95% CI, 0.45-1.0). We found a difference in the prevalence of positive serological markers (prevalence ratio, 0.74; 95% CI, 0.58-0.94), which remained at the same level for biopsy-verified cases (prevalence ratio, 0.78; 95% CI, 0.6-1.0), although the latter did not reach statistical significance (Figure 18).

In both birth cohorts the proportion of clinically- vs. screening-detected cases was the same (30% vs. 28% clinically detected cases; P=0.59). Considering both cohorts together, celiac disease was more common among girls than boys (prevalence ratio, 1.6; 95% CI, 1.3-2.0), although the difference was more pronounced in the 1997 cohort compared to the 1993 cohort (prevalence ratio 1.8 vs. 1.4) (Paper II).
Changes in environmental- and lifestyle factors in Sweden

As previously described, infant feeding practices changed concurrent with the beginning and end of the Swedish epidemic rendering different infant feeding practices, on a population level, for the 1993 and 1997 cohorts (Table 2). In Figure 19 the changes in Sweden are shown in more detail.

**Figure 19.** The Swedish celiac disease epidemic (black line) and concurrent changes in infant feeding. The proportion of infants breastfed at 6 months of age (light grey line) increased during the period, and the gluten exposure from industrially produced infant foods changed concurrently with the epidemic (dark grey line). Arrows indicate changed infant feeding recommendations. Reproduced with permission from Acta Paediatr© 2000 by John Wiley and Sons.

**Ascertainment infant feeding**

Ascertainment of the infant feeding practices seen on a population level (Figure 19 and Table 2) in the ETICS study (Paper II) showed that the median duration of breastfeeding was 7 months in the 1993 cohort and 9 months in the 1997 cohort (P<0.001), which is in agreement with the proportion of infants breastfed at 6 months of age in the Swedish population. Median age at gluten introduction was 5 months in both cohorts; nevertheless, the proportion of infants with breastfeeding continued beyond gluten introduction was significantly larger in the 1997 cohort (70% vs. 78% in the 1993 and 1997 cohort, respectively, P<0.001). Comparable infant feeding practices were observed for the celiac disease cases and the respective study population in each cohort (data not shown).
Figure 20 A-C shows the results from the ecological approach exploring whether there were any other changes over time in environmental- and lifestyle factors in the Swedish society around the time of the Swedish epidemic of celiac disease.

**Figure 20.** In all parts of the figure the Swedish celiac disease epidemic (incidence rate) is indicated by the thick black line. Ecological findings: A Introduction of acellular pertussis vaccine (thin whole line) coincided with the decrease in incidence rate; none of the other changes in the Swedish vaccination program or coverage were concurrent. Reproduced with permission (Paper III). B The socioeconomic status, indicated by the proportion individuals belonging to the high-medium strata, increased in Sweden during the period, without correlation to the epidemic. C Proportion caesarean sections of all births varied to some extent during the period but without any sharp changes coinciding with the epidemic. Data sources: Statistics Sweden, the National Board of Health and Welfare and the Swedish Institute for Infectious Disease Control, see Methods section.
The Swedish vaccination program underwent various changes during the period of interest (Figure 20A). One of these changes coincided in time with the decrease in incidence rate; the years of large clinical trials for evaluating the effect of an acellular pertussis vaccine. In 1996, the vaccine was introduced into the vaccination program.

Regarding socioeconomic status (Figure 20B), no concurrent changes were observed in Swedish society. The proportion of individuals belonging to high-medium socioeconomic strata increased from 44% to 50% during the epidemic period. Correspondingly, the proportion of individuals belonging to the low socioeconomic stratum decreased (data not shown).

During the time period of interest the proportion of caesarean delivery of all deliveries varied from 10.7% to 14.8%. There was a slight decrease in the end of the 1980s followed by an increase in the end of the 1990s (Figure 20C). Thus, the pattern seems almost inversely correlated with the celiac disease incidence rate, albeit without any sharp changes coinciding with the increase and decrease in incidence rate, respectively. However, Mårild et al. found an increased risk for celiac disease following elective, but not emergency, caesarean section, and there has been an increase in the number of elective cesarean sections performed in the 1990s (National Board of Health and Welfare; www.socialstyrelsen.se). Hence, the changes in proportion of cesarean delivery were probably not associated with the epidemic.

Potential contributing factors with no available data

Antibiotic treatment affects the gut microbiota and might therefore be a risk factor for celiac disease, although there are no studies, to our knowledge, that have assessed this association. Unfortunately, there are no available data on antibiotic treatment in children below 2 years of age for the period 1980-2000 (The National Board of Health and Welfare, personal communication).

It could be hypothesized that the infectious panorama in Sweden changed during the epidemic period. Unfortunately, there are no available ecological data in Sweden to explore such a hypothesis. Interestingly, there was an increased frequency of rod-shaped bacteria seen in biopsies from both untreated and treated celiac disease cases born during the epidemic, as compared to celiac disease cases born afterwards and healthy controls. Whether this constituted an independent phenomenon (an infection that was spreading during the epidemic period), or a secondary effect of the changes in infant feeding affecting the gut microbiota, remains unknown.
Early vaccinations were not risk factors for celiac disease

Based on the ecological approach (Paper III), introduction of acellular vaccine against pertussis could be connected in time to the decrease in celiac disease incidence rate. The case-referent study was performed during the years preceding its introduction into the program (1996). During this period of clinical trials, 26% of the cases and 27% of the referents were vaccinated. No significant association between vaccination against pertussis and celiac disease was seen (OR 0.91, 95% CI, 0.60-1.4).

The findings regarding early vaccinations and association with celiac disease are summarized in Figure 21. Neither vaccination against *Haemophilus influenzae* type b nor measles/mumps/rubella was associated with celiac disease. Risk assessment in relation to vaccination against diphtheria/tetanus or polio was not feasible due to 99% vaccination coverage. We saw no difference in vaccination coverage between boys and girls, and separate risk assessments for boys and girls regarding the above mentioned vaccinations showed similar results (data not shown).

![Figure 21](image)

**Figure 21.** Graph displaying the association between celiac disease and vaccinations. Only BCG was significantly associated. Abbreviations: Pa=acellular pertussis, Hib=*Haemophilus influenzae* type b, MMR=measles/mumps/rubella, BCG=Bacillus Calmette-Guérin, CI=confidence interval. Based on data from Paper III.

**Bacillus Calmette-Guérin and association with celiac disease**

In the case-referent study (Paper III), BCG was less often given to the cases compared to the referents (4.8% vs. 8.5%), corresponding to OR 0.54 (95% CI, 0.32-0.93) (Figure 21) Median age for BCG vaccination was 5.3 months among cases and 0.2 months for referents (P=0.02). The association between decreased risk for celiac disease and BCG vaccination remained in the multivariate analysis when adjusting for infant feeding (adjusted OR 0.54; 95% CI, 0.31-0.94). Further adjustments for socioeconomic status...
(Paper IV) only had minor effects on the risk estimate (adjusted OR 0.53; 95% CI, 0.30-0.93). Girls received BCG more often than boys (9.3% vs. 4.1% P=0.003). Separate assessments showed adjusted OR 0.54 (95% CI, 0.29-0.99) for girls, and adjusted OR 0.48 (95% CI, 0.11-2.07) for boys.

General vaccination against tuberculosis with BCG was replaced with a selective strategy in 1975. The selective strategy involved vaccination of risk groups: i) children potentially exposed to persons with tuberculosis, ii) children whose parents came from countries with a high occurrence of tuberculosis, and iii) children in families planning to stay for longer periods in an area where tuberculosis is common.\textsuperscript{111} Due to the characteristics of the selective vaccination strategy, most children receiving BCG had 1 or 2 parents from a country other than Sweden, both for cases and referents.

The change in vaccination strategy caused a drop in vaccination coverage from about 95% before 1975 to about 2% in 1976/77.\textsuperscript{111} The change in strategy in 1975 made it possible to compare celiac disease occurrence between birth cohorts with a substantial difference in vaccination coverage. Children born in 1973/74 had the same cumulative incidence of celiac disease at 15 years of age as children born in 1976/77 (P=0.47) (Figure 22).

![Figure 22](image)

**Figure 22.** Cumulative incidence of celiac disease in cohorts preceding and after discontinuation of general BCG vaccination (1975). No significant difference between the cohorts was seen. Reproduced with permission (Paper III).

BCG was associated with a protective effect, however, in a subset of the study population. Therefore, and since no change in the cumulative incidence in the general population was seen (Figure 22), residual confounding could not be excluded. Consequently, it was not considered appropriate to translate the effect seen in this group to the whole population, and estimation of the attributable fraction was not performed.
Antibiotic treatment and association with celiac disease

While no data on changes in antibiotic treatment in Sweden were available, the association was investigated using the case-referent study (Paper IV). Considering all kinds of antibiotics, 26% (n=97) of the cases and 23% (n=134) of the referents had been treated during the first 6 months of life. No statistically significantly increased risk for celiac disease was seen regarding antibiotic treatment (OR 1.2; 95% CI, 0.87-1.6). Separate analyses for girls and boys showed similar results.

Socioeconomic status and association with celiac disease

Although the changes in socioeconomic status did not coincide with the epidemic we assessed the association using the case-referent study (Paper IV). Of the cases, 48% (n=180) belonged to a family in the lower socioeconomic stratum, and the corresponding proportion for the referents was 38% (n=223). Compared to the high-medium strata, this was associated with increased celiac disease risk (OR 1.5; 95% CI, 1.2-2.0). Adjusting for infant feeding and early infections reduced the risk estimate but it remained statistically significant (adjusted OR 1.3; 95% CI, 1.0-1.8).

Early infectious episodes were associated with increased celiac disease risk

In children having 3 or more parental-reported infectious episodes during the first 6 months of life, as compared to those with fewer or no infectious episodes, a significant association with increased risk for later celiac disease was found (OR 1.5; 95% CI, 1.1-2.0) (Paper IV). Adjusting for infant feeding and socioeconomic status did not affect the risk estimate (Table 9). Moreover, the results remained when excluding episodes of gastroenteritis, as reported by the parents.

In total, 56 children (29 cases and 27 referents) had experienced gastroenteritis during the first 6 months of life. Gastroenteritis (1 or more episodes) was associated with increased risk for celiac disease (OR 1.8; 95% CI, 1.0-3.2), although it did not reach statistical significance when adjusting for infant feeding and socioeconomic status (OR 1.8; 95% CI, 0.99-3.3) (Table 9). Separate analyses for girls and boys showed similar results (data not shown).
Table 9. Early infectious episodes and association with celiac disease (Paper IV)

<table>
<thead>
<tr>
<th>Exposures(^{a})</th>
<th>Cases [n=373] n (%)</th>
<th>Referents [n=581] n (%)</th>
<th>Bivariate analyses OR (95% CI)(^{b})</th>
<th>Multivariate analyses OR (95% CI)(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infectious episode(s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>241 (65)</td>
<td>422 (73)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>≥3</td>
<td>132 (35)</td>
<td>159 (27)</td>
<td>1.5 (1.1-2.0)</td>
<td>1.5 (1.1-2.0)</td>
</tr>
<tr>
<td>Infectious episode(s) excl. gastroenteritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>250 (67)</td>
<td>429 (74)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>≥3</td>
<td>123 (33)</td>
<td>152 (26)</td>
<td>1.5 (1.1-2.0)</td>
<td>1.4 (1.0-1.9)</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>344 (92)</td>
<td>554 (95)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>≥1</td>
<td>29 (8)</td>
<td>27 (5)</td>
<td>1.8 (1.0-3.2)</td>
<td>1.8 (0.99-3.3)</td>
</tr>
</tbody>
</table>

Abbreviation: OR=odds ratio; CI=confidence interval.
\(^{a}\) Exposure during first 6 months of life.
\(^{b}\) Conditional logistic regression using 373 cases and their matched referents(s).
\(^{c}\) Adjusted for infant feeding and socioeconomic status (Figure 10).

**Interaction between early infections and gluten amount**

We found a statistical interaction, in this case a synergistic effect, between early infections and amount of gluten as depicted by the non-parallelism between the lower and upper 2 lines in Figure 23 (Paper IV).

The interaction was more pronounced among children when breastfeeding had been discontinued prior to gluten introduction. The highest celiac disease risk (OR 5.6; 95% CI, 3.1-10) was seen in children who, in addition to ≥3 infectious episodes, received gluten in large amounts, compared to small-medium amounts, after breastfeeding was discontinued.

Moreover, a synergistic effect between early infections and gluten amount implies that early infections probably contributed to the Swedish celiac disease epidemic. The gluten amounts from industrially produced infant foods changed concurrently with the epidemic (Figure 19). Through the synergistic effect the effects of the changes in gluten amounts consumed were reinforced by early infections and thereby early infections probably contributed to the epidemic, irrespective of changes in the infectious panorama.
Figure 23. Statistical interaction between number of infectious episodes and gluten amount. Large amount of gluten is represented by solid lines, and dotted lines represent small-medium amounts. A synergistic effect between infectious episodes and gluten is depicted by the non-parallelism between the 2 lower and 2 upper lines. The interaction was more pronounced in children when breastfeeding was discontinued prior to gluten introduction (Paper IV). Odds ratios with 95% confidence intervals are included in Table 10.
Celiac disease attributed to infant feeding and early infections

Based on the different combinations between infant feeding and early infections we estimated the number of cases which could be attributed to the exposure (etiologic cases) and the corresponding attributable fraction (Table 10). The number of cases attributed to any of the 8 different combinations of early exposure was in total 178 out of the included 373 cases (Paper IV).

Table 10. Celiac disease risk and the proportion of cases attributable to the different combined effects of early infections and infant feeding (etiologic cases).

<table>
<thead>
<tr>
<th>Breastfeeding status</th>
<th>No. of infections</th>
<th>Gluten amounta</th>
<th>Cases n= 373</th>
<th>Ref n=581</th>
<th>Odds ratio (95% CI)b</th>
<th>Etiologic casesc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding continued</td>
<td>0-2</td>
<td>Small</td>
<td>70</td>
<td>209</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0-2</td>
<td>Large</td>
<td>45</td>
<td>77</td>
<td>1.8 (1.1-2.9)</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>Small</td>
<td>33</td>
<td>76</td>
<td>1.2 (0.8-2.1)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>Large</td>
<td>23</td>
<td>27</td>
<td>2.6 (1.4-5.0)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0-2</td>
<td>Small</td>
<td>54</td>
<td>56</td>
<td>2.7 (1.7-4.3)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>0-2</td>
<td>Large</td>
<td>72</td>
<td>80</td>
<td>2.9 (1.8-4.5)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>Small</td>
<td>32</td>
<td>32</td>
<td>3.2 (1.8-5.9)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>Large</td>
<td>44</td>
<td>24</td>
<td>5.6 (3.1-10)</td>
<td>36</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>178</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: No.=number, ref=referents, CI=confidence interval.

a Gluten amount consumed 2 weeks after introduction. Small amounts include small-medium amounts.
b Odds ratio and 95% CI calculated using conditional logistic regression.
c Number of etiologic cases calculated from the attributable fraction = (OR-1)/OR resulting in the proportion of the cases attributed to the risk, e.g. for the highest risk: ((5.6-1)/5.6)*44=36
To estimate the etiologic cases due to the separate effects of the different factors, including the interaction between them, the etiologic cases in each of the 8 groups were decomposed further into the separate effects of each factor and the interactions between them. Findings are summarized in Figure 24. Breastfeeding (on-going or discontinued) was the factor to which the largest proportion of cases could be attributed (32% of all cases corresponding to 119 of the 178 etiologic cases). Moreover, the effect also superseded the increased risk by gluten and infections as shown by the negative values (the fractional increase in disease burden that would have been the result from separating the factors, i.e. a protective effect by the combination). The attributable fraction for all aspects of infant feeding together was 37% (sum of all values, Figure 24 light grey shaded area). The effect related to early infections alone was 4% (15/178 cases, Figure 24 white area) and the effect of the interactions between early infections and infant feeding was 6% (Figure 24 sum of all values in the dark grey shaded area).

![Figure 24](image)

**Figure 24.** Proportion of etiological cases in percentages attributed to the separate effects of early infections, infant feeding and the interaction between these exposures. (Circles not to scale) Negative values suggest a protective effect by breastfeeding. Total attributable fraction was 48% (sum of all values without round figures) or 178/373 cases.

The overall attributable fraction of celiac disease cases to infant feeding and early infections was 48%. Thus, almost half of all the cases could have been avoided if all children had had the most favorable combination.
Discussion

The main findings in this thesis encompass an unexpectedly high total prevalence of celiac disease in Swedish 12-year-olds (2.2 and 2.9%), with the majority of cases unrecognized prior to screening (2/3 in both cohorts). The Swedish celiac disease epidemic was observed among clinically detected cases below 2 years of age. We have now shown that this difference in clinically detected disease remained at 12 years of age. Furthermore, we showed a statistically significant decrease in total prevalence among children born after the epidemic (1997) compared to during the epidemic (1993). Our finding of a difference in total prevalence (ratio 0.75; 95% CI, 0.60-0.93) in birth cohorts 4 years apart from the same geographical areas corroborates that celiac disease can be avoided in some genetically predisposed children by changing environmental- or lifestyle factors.

The 1993 and 1997 cohorts differed in infant feeding, as shown by the population data ascertained through questionnaires, and our findings suggest that introduction of gluten-containing foods during ongoing breastfeeding is favorable with respect to celiac disease risk. With the exception of infant feeding, no additional changes in environmental- or lifestyle factors in Swedish society could be attributed to the epidemic.

Investigation of other environmental- and lifestyle factors showed that early vaccinations within the Swedish vaccination program were not risk factors for celiac disease. BCG was associated with a protective effect (adjusted OR 0.53; 95% CI, 0.30-0.93), although this has to be interpreted with caution. To our knowledge, the association between vaccinations and celiac disease has not been assessed previously. Among other early exposures we found that having ≥3 parental-reported infectious episodes was associated with an increased celiac disease risk (adjusted OR 1.5; 95% CI, 1.1-2.0), a risk which increased synergistically if, in addition to having ≥3 infectious episodes, the child was introduced to large amounts of gluten after breastfeeding had been discontinued (OR 5.6; 95% CI, 3.1-10). Such an effect has not, to our knowledge, been previously reported and the importance of introducing gluten in small amounts into the infant’s diet during ongoing breastfeeding is substantiated, especially in settings where the infectious load is heavy.

Whether there was a change in the infectious panorama in Sweden could not be determined. Irrespectively, early infections probably made a minor contribution to the Swedish epidemic through the synergistic effect with gluten, which changed concurrently. In total, approximately 48% of the epidemic could be explained by infant feeding and early infections.
Methodological considerations

All the designs used in this thesis are observational, which are more susceptible to error compared to randomized controlled studies or other experimental designs in which this is controlled for more effectively.\textsuperscript{113} However, in medical research randomization might not be feasible, or it might be unethical. As observational studies do not have the intention of intervening, but rather to study what has come to pass, or to follow the individuals over time,\textsuperscript{95,113} these designs are often preferable. Both the design aspect and the main methodological considerations are addressed below.

Design and evidential strength

Of the observational studies, the ecological approach has the lowest degree of evidential strength as it correlates occurrences on a population level (aggregated data).\textsuperscript{113} In this thesis the ecological approach was used to explore whether possible changes in early exposures in Swedish society coincided with the increase and the decrease in the celiac disease incidence rate. Hence ecological data were warranted, albeit not for concluding the cause without additional evidence with higher strength (Papers III-IV).

Surveillance of clinically detected celiac disease in Swedish children is performed through the Celiac Disease Register, which since 1991 is prospective, favoring high quality of data. The population-based incident case-reference study was based on the Celiac Disease Register and utilized data on an individual level (Papers III-IV). This design was chosen instead of a prospective cohort due to requirements for a large cohort encompassing both large costs and a heavy work load. In case of conflicting results between ecological findings and findings from the case-referent study the latter provides a higher strength of evidence.

Identification of unrecognized celiac disease requires a screening (cross-sectional study design). As we aimed to discern whether the proportion of unrecognized celiac disease had changed over time, repeated screenings were necessary (Papers I-II). Comparison of prevalences could be performed with relatively high accuracy; however, relating the findings to infant feeding poses some difficulties. The screenings included 12-year-olds emanating from 2 birth cohorts differing in infant feeding on the population level. Although there is no reason to believe that the relatively large proportion of included children (almost 10\%) should differ from the birth cohort at large, this comparison is of ecological nature. In order to verify the population birth cohort-specific characteristics in the included children on an individual level, and thereby increase the evidential strength, questionnaires were used.
The randomized controlled trial constitutes the gold standard for epidemiological studies, although this design also has its limitations. Together with experimental and intervention studies they provide a higher evidential strength than observational designs.\textsuperscript{113} In some respects the Swedish epidemic could be viewed as a ‘population experiment’ with different exposure patterns to different birth cohorts over time. The revision of infant feeding recommendations in 1996 was based on the emerging knowledge regarding the proposed role of breastfeeding in celiac disease etiology, and aimed at reducing the disease incidence. Therefore, in some respects this could be perceived as an intervention.

\textbf{Representativeness and misclassification}

We believe that the included 12-year-olds in the ETICS study (Papers I-II) are largely representative for Swedish 12-year-old children in the respective cohorts in general, and for the 1993 cohort also for the other birth cohorts from the decade of the epidemic period. The 5 study sites were in different parts of Sweden, participation was high at all study sites, and the female/male ratio corresponded to the national ratio (Statistics Sweden; www.scb.se). Furthermore, the prevalence of clinically detected celiac disease did not differ significantly from the findings in the Celiac Disease Register (\textbf{Table 11}).

As the ETICS study was school-based we did not discriminate by ethnic origin, which could have affected the proportion of invited children with a genetic predisposition, currently defined as carrying genes encoding for HLA-DQ2/DQ8. Preliminary results of genotyping for HLA-DQ2/DQ8 in a random sample in the 1993 and 1997 cohorts, respectively, suggest that the proportion of children predisposed to celiac disease was somewhat larger in the 1997 cohort, thus not explaining the lower prevalence seen in this cohort.

Our screening strategy (serological markers) prioritized high sensitivity in order to identify as many potential cases as possible, but in order to avoid non-cases misclassified as cases, a mucosal evaluation (small intestinal biopsy) and the presence of HLA-DQ2/DQ8 were required for celiac disease diagnosis. To reduce the number of children undergoing an unnecessary small intestinal biopsy (false positive markers) we used 2 serological markers in sequence for some of the children; first tTG with a lowered cut-off to increase sensitivity, and then EMA for all children with intermediate values.

In \textbf{Table 11}, the different methodological considerations regarding representativeness and possible misclassification, including their subsequent possible effect on the prevalence estimate, are summarized (Papers I-II).
<table>
<thead>
<tr>
<th>Methodological consideration</th>
<th>Possible over (↑) or under (↓) estimation of the prevalence estimate and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Representativeness</strong></td>
<td></td>
</tr>
<tr>
<td>Largest study site in south of Sweden</td>
<td>↑ Higher occurrence of celiac disease in south of Sweden.</td>
</tr>
<tr>
<td>Inclination to participate if health problems</td>
<td>↑ Prevalence assuming no cases among non-participants (minimum estimate): 2.1% (1993) and 1.5% (1997)</td>
</tr>
<tr>
<td>Gender of participants</td>
<td>- Celiac disease more common in girls. Similar proportion participating girls and boys.</td>
</tr>
<tr>
<td>Clinically detected cases 1993 cohort</td>
<td>↑ Slightly higher than in the Celiac Disease Register, but not statistically significant (0.87% vs. 0.81%, P=0.58)</td>
</tr>
<tr>
<td>Clinically detected cases 1997 cohort</td>
<td>↓ Slightly lower than in the Celiac Disease Register, but not statistically significant (0.60% vs. 0.64%, P=0.69)</td>
</tr>
<tr>
<td><strong>Classification bias</strong></td>
<td></td>
</tr>
<tr>
<td>False negative tTG</td>
<td>↓ Probably negligible effect; high sensitivity further increased by lowering of the cut-off.</td>
</tr>
<tr>
<td>Absence of serum-IgA analyses in the 1997 cohort</td>
<td>↓ Probably 1-2 cases erroneously classified as non-cases. Prevalence comparisons performed according to same study protocol, excluding 2 cases in the 1993 cohort.</td>
</tr>
<tr>
<td>Exclusion when no biopsy was performed</td>
<td>↓ Declined biopsy (positive serology); 8 (4%) and 5 (4.8%) for the 1993 and 1997 cohorts, respectively.</td>
</tr>
<tr>
<td>Exclusion when positive markers but normal biopsy</td>
<td>↓ Prevalence including all potential cases and assuming the same prevalence among non-participants (maximum estimate): 3.5% (1993) and 2.5% (1997)</td>
</tr>
<tr>
<td>Non-case misclassified as case</td>
<td>↑ Probably minor effect: cases had positive serology, HLA-DQ2/DQ8 and enteropathy (Marsh I-III). Specificity of villous atrophy in Sweden is ~95%$^{114}$</td>
</tr>
<tr>
<td>Inclusion of Marsh I-II if symptoms/signs and HLA-DQ2/DQ8</td>
<td>↑ Restriction to Marsh III; prevalence 2.7% (1993) and 2.1% (1997) with slight effect on the prevalence ratio 0.79 (95% CI, 0.63-0.98).</td>
</tr>
</tbody>
</table>

Symbols: ↑ overestimation, ↓ underestimation, - no effect. Abbreviations: tTG=tissue transglutaminase antibodies, Celiac Disease Register=National Swedish Childhood Celiac Disease Register.
In summary, the prevalence estimate could be somewhat under- or over-estimated. However, the compiled effect does not weigh in any specific direction, indicating that the estimate is reliable (Papers I-II). Importantly, the potential effect on the prevalence estimate was similar in both cohorts, with the exception of the clinically detected cases which probably was slightly overestimated in the 1993 cohort and underestimated in the 1997 cohort (Table 11). Thus, it is not likely to affect the prevalence comparison (Paper II). With respect to the prevalence of clinically detected celiac disease, using the findings from the Celiac Disease Register in the total prevalence estimates affected the estimates by ~0.05% and did not alter the conclusion regarding the comparison (data not shown).

In the case-referent study (Papers III-IV) the cases were diagnosed after 3 consecutive biopsies, rendering the specificity for diagnosis >95%, and referents were randomly selected from the population generating the cases after matching criteria were fulfilled, and they are therefore considered to be representative of the population they originated from. However, we cannot rule out that some of the referents had not yet recognized celiac disease. Considering the size of the Swedish birth cohort from which the referents originated (~100 000 children/year), and a celiac disease prevalence of 1% in children below 2 years,115 we consider the risk for a significant influence on results negligible. Selection bias in the case-referent study could have arisen if the responding individuals differed from those who did not. The response rate was more than 90%, limiting the impact of such a selection bias.

Information bias

In the screening study (Paper II), questionnaires were used for individual infant feeding data (breastfeeding duration and age at gluten introduction). Although this was a cross-sectional study design, the questionnaires were completed before knowledge of the screening results (prospective), thus minimizing recall bias. However, the recall time was 12 years. As parents in both cohorts are likely to remember equally well or poorly, non-differential misclassification pulls the result towards null and underestimates or even conceals a potential difference.26,113 Data from our previous case-referent study performed during the epidemic showed that approximately 70% of the parents followed the infant feeding recommendations at that time. Correspondingly, based on a Swedish prospective cohort of children born after the epidemic, approximately 60% of the parents followed the revised recommendation.43 Difficulties with remembering age at gluten introduction were also reflected by >1300 (13%) missing answers in otherwise completed questionnaires. Taken together, there might have been a difference in age at gluten introduction albeit non-detectable after 12 years.
The cases in the case-referent study were recruited at the time of diagnosis (reported to the Celiac Disease Register), i.e. incident cases and referents were invited simultaneously, and thereby the recall time was limited. Despite a limited recall time, there is an inherent risk for recall bias in the retrospective reports, as the included individuals might remember early exposures differently dependent on the disease status (case or referent). The cases, who know that they have celiac disease, might be more prone to recall exposures than the referents. In an attempt to limit the recall bias with respect to celiac disease, the questionnaire did not reveal this special interest (Paper IV). Prospectively recorded data are not affected by recall bias as they are recorded prior to knowledge of disease status. A limitation concerns available data, but in Sweden the dates of all vaccinations given are recorded in the child health records that were used (Paper III).

**Confounding**

In the case-referent study (Papers III-IV) the issue of potential confounding, i.e. mixing of effects due to another factor unevenly distributed in the population and related to both the disease and the factor under study, was handled on the design stage by matching (age, sex and area of residence) and by collection of data on potential confounders. At the analysis level both stratified analyses and multivariate modeling were used. The data on confounders could be accounted for by inclusion in the conditional logistic regression model. However, we cannot exclude the existence of residual confounding, especially in some analyses (Paper III).

**Null hypothesis and power**

In the screening study (Papers I-II) the null hypothesis was prespecified, and the necessary sample size was estimated in advance, prioritizing a high probability of finding a difference between the 2 cohorts if there was one (β=10%). We used the higher level for β in our a priori sample size estimations to achieve sufficient sample size and power. Albeit 2 large cohorts, the estimated size for comparisons of the total prevalence was not sufficient for stratified analyses.

In the case-referent study (Papers III-IV), most null hypotheses were prespecified, but some analyses were performed following a post hoc approach as one finding lead to the idea of another interesting aspect to consider. As there were no significant findings, adjustments of significance levels were not applicable, although increasing the number of performed analyses increases the risk for chance findings (type I error).
Celiac disease as a public health problem

A *national disease* is defined as a disease occurring in more than 1% of the population. Consequently, as we found a prevalence of at least 2.2%, celiac disease could be defined accordingly (Paper II). In comparison with other childhood diseases, celiac disease is the second most common chronic disease, with asthma/allergy being more prevalent.

*Unexpectedly high prevalence in Sweden*

In the period under study, we found a prevalence of 2.2% among 12-year-olds, which is unexpectedly high (Paper II). Although the prevalence from the epidemic period (2.9%) is even higher (Paper I), 2.2% is the highest prevalence reported among children in Europe and the U.S. The sole exception worldwide is the prevalence of 5.6% (based on EMA positive cases) among the Saharawi children in Algeria. This prevalence was thought to be the result of a high frequency of HLA-DQ2/DQ8, consumption of large amounts of gluten-containing foods in a population where wheat has only been a staple food during the last century, and possibly also a concomitant high infectious burden. In comparison, the frequency of HLA-DQ2/DQ8 in the Swedish population largely unknown. During the epidemic, children were abruptly introduced to large amounts of gluten (Paper II), but although we also found infectious episodes to be associated with increased celiac disease risk in the Swedish child population (Paper IV), Sweden cannot be considered to have a high infectious burden, e.g. rotavirus (gastroenteritis) is relatively uncommon compared to other European countries.

*The majority of cases remain undiagnosed*

The clinical presentation of celiac disease has changed over time towards less and more atypical symptoms. Correspondingly, the age at diagnosis has increased, which could be due to the milder clinical presentation with delayed diagnosis, and/or a separate phenomenon with delayed disease development. In contrast, awareness of celiac disease and its heterogeneous clinical presentation has increased over time, involving increased serological testing at lower clinical suspicion. Irrespective of these counteracting trends, we found the same proportion of screening-detected cases (2/3 cases) among 12-year-olds in both cohorts (Papers I-II). Knowledge regarding which long-term consequences this entails remains limited, although it has rapidly increased during recent years. In short, celiac disease has been associated with several other diseases, increased morbidity, and moderately increased mortality, and diagnosis and treatment are regarded as beneficial.
Celiac disease is increasing in Sweden

The prevalence of celiac disease in the Swedish population has increased over time (Papers I-II). A population-based adult screening performed in the 1990s reported a prevalence of 0.53% (95% CI, 0.25-0.97), as compared to our finding among 12-year-olds (1997) of 2.2% (95% CI, 1.8-2.6). The prevalence is expected to be higher in adults than in children since celiac disease is a chronic disease with only a moderate increase in mortality. Our finding represents an increase over time in celiac disease prevalence, which is in accordance with findings from other countries.

In summary, celiac disease is common, mostly undiagnosed with potentially negative health consequences, and is increasing over time; thus, celiac disease could be regarded not only as a national disease but also as a public health problem, i.e. a threat to public health including the level of health and disease as well as the distribution.

The Swedish epidemic of celiac disease; a change in disease occurrence

The term epidemic has been defined as “a rapid change in the frequency of a health event over time in the same population. Originally used for infectious diseases, now encompassing non-communicable diseases as well as health related behaviour.” As we have now shown a difference in celiac disease occurrence, we can truly refer to “the Swedish epidemic of celiac disease.”

The Swedish epidemic (1984-1996) among children below 2 years of age with clinically detected disease was striking, and a difference between the epidemic cohorts (represented by the 1993 birth cohort) and the post-epidemic birth cohorts (represented by the 1997 cohort) remained at 12 years of age. By investigating the total prevalence at 12 years of age, including both clinically- and screening-detected cases, we showed a significantly reduced total prevalence in the 1997 cohort compared to the 1993 cohort (2.2% vs. 2.9%), indicating that the epidemic constitutes a change in disease occurrence and not solely a change in the proportion of clinically detected cases (Papers I-II). This had been suggested in a previous pilot screening study performed among children at 2.5 years of age who were born in 1993 and 1997. Thus, there seems to have been an increase in both the occurrence of clinically detected celiac disease, as shown through the Celiac Disease Register, and in screening-detected celiac disease, as indicated through the screening-studies, in both cohorts. We hypothesize that the occurrence of celiac disease (total prevalence) has increased gradually in both cohorts (Figure 25), although probably somewhat more promptly in...
the older ages, among the 1997 cohort compared to the 1993 cohort, since part of the difference between the epidemic and post-epidemic cohorts has abated over time. Repeated screenings throughout childhood are needed to determine whether the increase in celiac disease occurrence is gradual or if there is a certain age when children are more likely to lose tolerance to gluten, and only a lifelong follow-up including repeated screening can ultimately determine if the epidemic pattern remains.

**Figure 25.** Hypothetical model for celiac disease development during childhood. A gradual increase in both clinically- and screening-detected cases is proposed (dotted line). Thick black lines indicate the cumulative incidence based on the Celiac Disease Register. Dotted areas indicate screening at 2 an 12 years of age. A statistically significant difference between the 1993 (left white bars) and 1997 (right grey bars) cohorts remains throughout the time period (Paper II). Based on data from Olsson et al.\textsuperscript{11} Carlsson et al.\textsuperscript{115,131} and Papers I-II.

Our findings of a significantly reduced prevalence of celiac disease in the 1997 cohort indicate that primary prevention of celiac disease is feasible. However, before preventive strategies can be considered, the association between celiac disease and different early environmental- and lifestyle factors needs to be evaluated regarding causality.
Environmental- and lifestyle factors in celiac disease etiology

For an early environmental- or lifestyle factor to be a part of the disease etiology the relationship with the disease has to be causal. The discussion regarding causal inference for the associations (potential component causes) found in this thesis, as well as the factors to which the Swedish celiac disease epidemic is attributed, utilizes Hill’s considerations\(^6\) as a conceptual framework. In **Table 12** the 9 considerations, including short clarification of their meaning, are summarized together with a suggested interpretation of the current compiled evidence. Although gluten is a necessary factor, aspects of gluten (age at gluten introduction and amount during the introduction) are included.

**Table 12.** Hill’s considerations for causality, and current evidence (shaded grey when considered relatively satisfactory) regarding environmental- and lifestyle factors (of interest in this thesis) in celiac disease etiology.

<table>
<thead>
<tr>
<th>Hill’s 9 considerations</th>
<th>Aspects of gluten</th>
<th>Breastfeeding</th>
<th>BCG</th>
<th>Infections(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength</strong>-of the association</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Consistency- repeated observations</td>
<td>No</td>
<td>X</td>
<td>Partly</td>
<td>X</td>
</tr>
<tr>
<td>Specificity- one-to-one cause-effect</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Temporality- cause prior to effect</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Biological gradient- dose-response relationship</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Plausibility- biological plausibility</td>
<td>Possibly</td>
<td>Yes</td>
<td>Yes</td>
<td>Possibly</td>
</tr>
<tr>
<td>Coherence- agreement with knowledge</td>
<td>Possibly</td>
<td>Yes</td>
<td>Yes</td>
<td>Possibly</td>
</tr>
<tr>
<td>Experiment- Laboratory or interventions</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Analogy- findings in similar areas</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Possibly</td>
</tr>
</tbody>
</table>

**Abbreviations:** BCG=Bacillus Calmette-Guérin, NA=not applicable, X=Symbol indicating that consistency cannot be evaluated as no other studies have been performed.

\(^a\) Age at gluten introduction.

\(^b\) Early (during first 6 months of life) infectious episodes.
Addressing the considerations assessed equally for all the included factors first. Regarding *temporality*, this is satisfied for all factors under study within this thesis. Gluten is a necessary factor and the aspects of gluten (age and amount) precede disease development. Breastfeeding, when done, is initiated at birth. Early infectious episodes were defined as during the first 6 months of life. Children vaccinated after celiac disease diagnosis were considered unvaccinated with respect to this disease. Specificity of an association is, as Hill concluded, not frequent. A disease has, in most cases, more than 1 cause (multifactorial etiology), and likewise, causes have more than 1 effect, which is seen for all of the factors. The associations that were found had what could be regarded as medium strength.

**Aspects of gluten introduction – age and amount**

There might be a certain age interval that provides a “window of opportunity” for gluten introduction, observed in 1 prospective study but not in other prospective or retrospective studies, i.e. there is a lack of consistency. Immunological studies (experiment) have shown an immature mucosal immunity and increased gut permeability early in life, which could convey an increased celiac disease risk. Indeed, introduction of gluten during the first 3 months of life was associated with increased celiac disease risk. On the other hand, avoidance of gluten until 12 months of age (when the immune system is more mature) did not affect celiac disease risk according to a prospective intervention study; results from the 36-month follow-up suggested that delaying gluten introduction delays celiac disease onset but does not prevent disease development (the significantly different prevalence at the 24-month follow-up did not remain 1 year later). Similar findings were seen in another intervention study, although its main focus was diabetes risk. Thus, the biological plausibility and coherence for age per se (age at gluten introduction and celiac disease risk) beyond the first 4 months of life can be denoted as relatively weak. However, the optimal age for gluten introduction remains to be elucidated. As in the case concerning age at gluten introduction, age at introduction of other complementary foods (e.g. cow’s milk formula) and later risk for type 1 diabetes and/or asthma/allergy has been discussed. Although no definite conclusions with respect to these diseases are available, there are no indications of increased risk for introduction of complementary foods during the period 4-6 months (analogy).

Only our previous study has assessed the relationship between the amount of gluten during introduction and celiac disease risk, and it was shown that larger amounts increased the risk. Our current screening study suggests that the gluten amount affects celiac disease development up to 12 years of
age, as the cohorts were exposed to different amounts from the industry-produced infant foods (Paper II). Whether gluten amount exhibits a dose-response or threshold relationship with celiac disease is not known, although celiac disease cases seem to react in a dose-response fashion to gluten ingestion,\textsuperscript{140} suggesting that the former provides a better model, at least above a minimum point. Based on immunological studies (experiment), an increased amount of gluten in the diet correlates with an increasing number of gluten peptides reaching the lamina propria, rendering more HLA-DQ-gluten complexes, and subsequently an immunological response is more likely to occur (biological plausibility).\textsuperscript{20} Furthermore, the HLA-DQ2/DQ8 dose-effect relationship is thought to be due to the stability of the HLA-DQ molecule, and subsequently the quantity of gluten peptides presented to T-cells.\textsuperscript{17,20} In analogy, changing the amount of gluten available could attain a similar dose-response effect as seen with the different HLA-DQ molecules. Thus, several pieces of evidence point towards a causal relationship not only between gluten and celiac disease, but also with respect to the amount given. This hypothesis is undergoing further investigation in an ongoing randomized controlled trial.\textsuperscript{103}

\textbf{Breastfeeding}

Breastfeeding and celiac disease risk has been discussed at least since the 1940s,\textsuperscript{141,142} although the studies during the 1980s focused on the effect of breastfeeding on the clinical presentation,\textsuperscript{133,143,144} and not as a component in the disease etiology. Case studies have shown a milder clinical presentation and a later median age at diagnosis coinciding with longer breastfeeding.\textsuperscript{145,146} Several studies have shown a reduced celiac disease risk associated with increased duration of breastfeeding, i.e. a dose-response relationship,\textsuperscript{27,33} although no association has been reported from prospective cohort studies with the strength of prospectively collected data but the weakness of relatively few cases (n=27-51).\textsuperscript{43,132,134} In a record linkage study no association between breastfeeding (yes/no) and celiac disease risk was shown,\textsuperscript{37} and the opposite association was seen in a case-referent study.\textsuperscript{28} Despite some controversies, medium consistency is suggested.

As previously described, we have shown a protective effect with concomitant breastfeeding and introduction of gluten-containing complementary foods,\textsuperscript{31} an effect investigated in 3 other studies; it was seen in 2 case-referent studies\textsuperscript{147,148} but not in 1 of the prospective cohorts previously mentioned.\textsuperscript{132} Thus, an alternative hypothesis regarding the impact of age at gluten introduction is the concomitance with breastfeeding, i.e. a larger proportion of infants are more likely to be breastfed when introduced to gluten between 4 and 6 months of age, as compared to later. As our current study also
included screening-detected cases, our findings suggest that breastfeeding during gluten introduction and beyond has a protective effect against celiac disease development, and not only an effect on the clinical presentation and/or delayed diagnosis, at least up to 12 years of age (Paper II). Hence, as the celiac disease etiology is presently viewed as multifactorial, there is coherence, and in analogy, breastfeeding has been associated with reduced risk for several autoimmune and allergic diseases.\textsuperscript{138,149}

Introducing gluten during on-going breastfeeding may increase the chance for developing oral tolerance through several potential molecular mechanisms suggested from experimental studies: \textit{i}) immune-modulating factors in breast milk, \textit{ii}) presence of gliadin in human milk, \textit{iii}) influence on intestinal permeability, \textit{iv}) influence on gut microbiota, and \textit{v}) reduced risk for gastroenteritis.

Breast milk, which is the most essential nutritional source for infants, contains several immune-modulating factors, e.g. transforming growth factor-β, which will favor a tolerogenic response to antigens.\textsuperscript{21} Furthermore, small amounts of gliadin have been detected in human milk,\textsuperscript{150} which could possibly support induction of tolerance to gluten in infants breastfed by mothers on a gluten-containing diet. Breastfeeding might also influence the gut maturation and colonization through growth factors and microbiota-modulating factors found in breast milk.\textsuperscript{21,109,151} Indeed, differences in the microbiota composition between formula-fed and breastfed infants have been shown, which might affect celiac disease risk, as differences in gut microbiota between individuals with and without celiac disease have been reported.\textsuperscript{39,42} Furthermore, breastfeeding has been associated with a reduced risk of gastrointestinal infections, an additional potential risk factor for celiac disease,\textsuperscript{40,151} also suggested in our current findings (Paper IV). The compiled evidence suggests that there are both biological plausibility and supporting experimental evidence for a favorable effect by breastfeeding in promoting tolerance to gluten.

\textbf{Bacillus Calmette-Guérin}

Vaccinations have been discussed as potential protective- or risk factors for autoimmune diseases, but to our knowledge there are no previous studies assessing the relationship between BCG and celiac disease, and thus consistency with our finding of an associated decreased risk (adjusted OR 0.53; 95\% CI, 0.30-0.93) cannot be evaluated (Paper III). The potential mechanisms for how vaccinations can affect the risk for autoimmune diseases are partly the same as proposed for infections and microbiota, including: \textit{i}) molecular mimicry; \textit{ii}) impact on the developing immune
system, e.g. regulatory T-cells; iii) non-specific immunological effects, e.g. heterologous immunity.\textsuperscript{152-157} Thus, there is probably both \textit{plausibility} and \textit{coherence}, although no \textit{experiments} with respect to celiac disease have been performed. The majority of studies on BCG that have shown non-specific effects that cannot be attributed to protection against tuberculosis were performed in low-income countries or they investigated the relationship with asthma (\textit{analogy}).\textsuperscript{158-161} Findings from type 1 diabetes research show no association, but the evidence remains inconclusive.\textsuperscript{162-164} In accordance with our findings from the comparisons between birth cohorts (Paper III), no significant change in cumulative incidence of type 1 diabetes after discontinuation of general BCG vaccination in 1975 was reported.\textsuperscript{165} Our findings of a reduced risk for celiac disease concerned children with increased risk for tuberculosis, and consequently the assumption of exchangeability is compromised; thus the results have to be interpreted with caution until confirmed in other studies.

\textbf{Early infections}

While what triggers loss of oral tolerance to gluten and subsequent celiac disease development remains unknown, it has been hypothesized that it could be due to a gastrointestinal infection,\textsuperscript{20} and \textit{analogical} hypotheses have been proposed for several autoimmune diseases. A number of studies have investigated this hypothesis regarding celiac disease using different methods, and they report different degrees of association, significant or non-significant, between early infectious episodes, or repeated infections indicating a \textit{dose-response} relationship, and celiac disease.\textsuperscript{28,34,38,40,43} We found an increased risk (adjusted OR 1.5; 95% CI, 1.1-2.0) in children with 3 or more early parental-reported infectious episodes (Paper IV), which is in the same range as findings in the 2 studies that had a stronger design (prospective cohort), although the findings were non-significant in 1, possibly due to insufficient power. Thus the findings are relatively consistent.

There are several different ways an infection, in addition to gastroenteritis, could affect the risk for celiac disease, since the gut immune system comprises more than 70% of the total immune system and represents the major site for ‘immune education’.\textsuperscript{109,139} Similar to breastfeeding, infections early in life when the gut microbiota is developing could possibly also result in a different microbiota, affecting both intestinal immune responses and mucosal barrier function and thereby also the risk for celiac disease.\textsuperscript{39,109} From an immunological perspective (\textit{experiments}) it appears biologically \textit{plausible} that when the epithelial permeability is compromised (\textbf{Figure 3}) e.g. in cases of an episode of gastroenteritis, more gluten peptides are likely
to reach the lamina propria.\textsuperscript{5,166} An infection creates a pro-inflammatory environment, e.g. release of interferon-\(\gamma\), which increases HLA expression on antigen presenting cells, thereby increasing the level of HLA-DQ-gluten complexes, as well as release of active tTG, resulting in more deamidated gluten peptides.\textsuperscript{17,20} Taken together, the different effects of an infection make loss of oral tolerance to gluten more likely to occur. Interestingly, several of the effects of an infection would be enhanced by larger amounts of gluten, resulting in an increased risk for celiac disease. It is plausible that the statistical interaction found between early infections and gluten amount constitutes a biologic interaction (Paper IV). Furthermore, the synergistic effect was more pronounced among children where breastfeeding had been discontinued (OR 5.6; 95\% CI, 3.1-10). The effect could be considered \textit{coherent} with disease knowledge, i.e. the combination of increased epithelial permeability and pro-inflammatory environment due to an infection, and large amounts of gluten without the immune-modulating effect of breastfeeding increasing the celiac disease risk.

\textbf{Models for celiac disease etiology}

In the pathway(s) from a genetic predisposition and gluten exposure to the recognition of gluten as hazardous, with subsequent inflammatory reactions, development of villous atrophy and possibly celiac disease symptoms, there are numerous junctures where environmental- or lifestyle factors could affect whether such a pathway continues to be followed or comes to a halt; thus, whether and/or when celiac disease develops.

Following the above reasoning (Table \textbf{12}) and adding the methodological considerations, there are 3 environmental- and lifestyle factors that could play such a role in the celiac disease pathway. The evidence for causal inference regarding aspects of gluten could be considered insufficient with respect to age at gluten introduction (age per se was not supported by current evidence) but stronger with respect to amount at introduction. While the effects of breastfeeding from a life-long perspective remain unsettled, the existing evidence for inferring causality for breastfeeding in celiac disease etiology is relatively strong. Likewise, early infections seem to be part of a causal model, but evidence for BCG is not strong enough for suggesting causal inference (Papers II-IV).

Utilizing the sufficient-component cause “pie-chart” model by Rothman for depicting and summarizing the cause of celiac disease emphasizes that many different sets of component causes could result in the same outcome (celiac disease). Below are 4 different models with suggested sufficient cause for celiac disease development (Figure \textbf{26}).
Figure 26. Four suggested examples of causal models for celiac disease development based on Rothman’s "pie-charts" acting within an eco-social perspective. Each “pie-chart”, although including different sets of causal factors (component factors), results in sufficient cause for disease development. Factor A and B/C are necessary factors depicted by inclusion in all “pie-charts”.

In this setting, it needs to be emphasized that none of the component causes acts alone, and from a broader viewpoint the “pie-chart” model could be seen as part of an eco-social perspective. In our study (Paper IV) we found an association between low socioeconomic status and increased celiac disease risk (adjusted OR 1.3; 95% CI, 1.0-1.8), which was regarded as a potential associated factor for other causal (component) factors. This finding was in accordance with some previous studies. In contrast, a recent large Swedish register-based study found a reverse association; a lower celiac disease risk in the lowest stratum, although this could be due to differences in the proportion of diagnosed cases. How a proposed eco-social perspective affects underlying causal factors and interactions between them needs to be assessed.
Towards explaining the Swedish celiac disease epidemic

A key aspect of the Swedish epidemic of celiac disease elucidated in this thesis is the statistically significant difference in total celiac disease prevalence between the epidemic (1993) and post-epidemic (1997) cohorts. While the epidemic was observed among children below 2 years of age with clinically detected disease, we have now shown that the epidemic, at least up to 12 years of age, did not solely constitute a shift in the proportion of clinically detected cases (Paper II).

To summarize our findings, including the aspects of causal inference, regarding potential causes of the Swedish epidemic of celiac disease, they corroborate that part of the change in celiac disease occurrence can be explained by changes in infant feeding during the period.

It seems plausible that breastfeeding and amount of gluten are causal factors for celiac disease but current evidence does not support age at gluten introduction (preferable age 4-6 months) as a causal factor. On the other hand, according to the alternative hypothesis about age, the recommended age at gluten introduction is imperative for concomitance with breastfeeding, especially in settings where shorter breastfeeding duration is more prevalent than in Sweden, e.g. the majority of countries in Europe where the proportion of breastfed infants at 6 months of age is less than 50%, with comparable reports from Australia (~50%), Canada (~40%) and the US (~45%).

Figure 27 shows a model of how the different infant feeding recommendations for the 1993 and 1997 cohorts, together with increased duration of breastfeeding, affected the proportion of infants breastfed at the time of gluten introduction into the diet, differences ascertained in the cohorts through the questionnaire (Paper II). Thus, this combined effect, as well as the amount of gluten in industrially produced infant foods, changed during the epidemic period. Consequently, however, we cannot distinguish if one aspect was more likely than the other to result in the current difference in celiac disease prevalence, although the attributable proportion indicates that breastfeeding is most important regarding celiac disease risk (Paper IV).
Our efforts to further explore potential changes in environmental- and lifestyle factors in Swedish society coinciding with the epidemic period have not resulted in any major additional explanations for the epidemic pattern. Early infectious episodes probably had a minor contribution through the synergistic effect with gluten amount, as the latter changed concurrently with the beginning and end of the epidemic (Paper IV). Additionally, part of the epidemic could possibly be attributed to the contemporaneous occurrence of rod-shaped bacteria, although there is to date no data to support such a theory. As we found no other concurrent changes, investigating and refuting the possible contribution by vaccinations (Paper III), socioeconomic status, or caesarean section, our findings support that the epidemic is explained by changes in infant feeding, although the whole epidemic is still not fully understood.

**Towards prevention of celiac disease**

Preventive strategies for celiac disease are warranted. Although in theory this would be easily achieved by removing the necessary factor, gluten, from the diet, in reality this is neither warranted nor readily implemented. Preventive strategies can be divided into different levels depending on where in the disease development action is taken; the higher levels concern individuals in whom the disease has developed and the lower levels concern prevention against the disease. For the latter, preventive strategies could be directed towards the entire population or towards high-risk individuals. When considering possible preventive strategies aiming at the whole population, the importance of sufficient evidence that harm is not done is crucial. On the other hand, population-based strategies are most likely to have a larger impact since more individuals, also those with intermediate risks, are influenced.
Levels of prevention with respect to celiac disease

The levels of prevention and possible actions with respect to celiac disease are summarized in Table 13 in an inverted order, illustrating the direction in which we are aiming, although actions need to be taken at all levels in order to improve the situation for individuals with the disease.

Table 13. Levels of prevention regarding celiac disease. The inverted order illustrates an aim towards primary prevention, partly through primordial prevention.

<table>
<thead>
<tr>
<th>In general</th>
<th>Celiac disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiary</td>
<td>Encourage adhering to a gluten-free diet</td>
</tr>
<tr>
<td>Reduce number and/or impact of complications</td>
<td>Increasing knowledge regarding associated conditions and complications</td>
</tr>
<tr>
<td>Secondary</td>
<td>Active case finding</td>
</tr>
<tr>
<td>Early detection</td>
<td>Population based screening?</td>
</tr>
<tr>
<td>Primary</td>
<td>Introduction of gluten-containing foods</td>
</tr>
<tr>
<td>Reduce the incidence of the disease by controlling specific causal factors</td>
<td>Breastfeeding</td>
</tr>
<tr>
<td>Primordial</td>
<td>Probiotica?</td>
</tr>
<tr>
<td>Establish and maintain underlying economic, social and environmental conditions leading to causation that minimize hazards to health</td>
<td>Infant feeding recommendations</td>
</tr>
</tbody>
</table>

At the tertiary level, encouragement to adhere to a gluten-free diet ought to be given. By improving adherence to clinical guidelines, clinical follow-up, including consultations with dieticians and possibly nurses, management of celiac disease in everyday life could increase.\textsuperscript{50,60,173-176} Furthermore, awareness about associated diseases and complications ought to be increased so that they can be avoided, and/or recognized early.

For the secondary level ‘active case finding’ is often recommended today, i.e. testing for celiac disease in relatives of cases, at low clinical suspicion, and in risk groups.\textsuperscript{177} Although ‘active case finding’ has been promoted in Sweden in recent years, the majority of cases remain undiagnosed (2/3) (Papers I-II). Population based screening might be an appropriate intervention for identifying cases. However, before screening can be put into practice, increased knowledge is needed regarding e.g. the natural history of the disease, including the health benefits for screening-detected cases and health economic aspects.
Primary prevention, i.e. intervening before the disease processes have been initiated, requires, in most cases, that causal exposures have been identified and are considered suitable for an intervention. In this thesis breastfeeding and concomitant introduction of gluten in small amounts were suggested as causal factors with potential for celiac disease prevention.

The differences in gut microbiota, the development of which is suggested to be affected by infant feeding and early infectious episodes, between children with and without celiac disease, could, in addition to strategies involving infant feeding, render a possibility for probiotics in celiac disease prevention.

Preventive strategies aimed at reducing the infectious load in children include an immense number of different considerations from the immunological level to the societal level. While possibly desirable in settings where the infectious load is heavy, the immediate requirement does not concern celiac disease, although mathematical predictions have estimated the deaths from celiac disease from a global perspective to be ~4% of all diarrhea-related deaths in children <5 years of age annually (~42,000 of 1.04 million diarrhea-related deaths per year), thus small but not insignificant.

On the other hand, as we found a synergistic effect between early infections and gluten amount, to which approximately 20% of the celiac disease risk could be attributed (Paper IV), whether gluten-containing foods should be avoided during an episode of gastroenteritis, for example, could be a matter for discussion. Furthermore, the synergistic effect was more pronounced, rendering the highest risk for later celiac disease (OR 5.6; 95% CI, 3.1-10), in children where breastfeeding had been discontinued prior to gluten introduction. In settings where the infectious load is heavy, a substantially decreased celiac disease risk could be achieved by avoiding the worst combination, or the worst when the infectious load cannot be affected (OR 1.2; 95% CI, 0.8-2.1 for the combination of small amounts of gluten during on-going breastfeeding and ≥3 infectious episodes). Thus, the importance of breastfeeding is substantiated.

Primordial prevention, i.e. the establishment and maintenance of social and environmental conditions leading to preferable patterns in causal factors concerns changes in associated or structural (societal level) factors. At this level recommendations for infant feeding would be a way to establish/maintain a preferable infant feeding practice in the general population.
**Strategies for celiac disease primary prevention**

Through infant feeding recommendations primary preventive strategies could be put into practice, although different effects must be considered when giving general recommendations.

From the estimation of attributable fraction, breastfeeding was indicated as the factor that had the largest impact on the disease risk, superseding the increased risk inferred by both gluten in large amounts and early infections (Paper IV). Thus promotion of breastfeeding would have the greatest public health effect with respect to celiac disease prevention. In addition, based on the experience of the Swedish celiac disease epidemic, longer duration of breastfeeding would increase the proportion of infants who are introduced to gluten during on-going breastfeeding, especially if recommended from 4 months of age.

Today there are different recommendations regarding duration of exclusive breastfeeding. Since 2001 the World Health Organisation has recommended 6 months, providing a uniform global recommendation. However, the advantage of exclusive breastfeeding for 6 months in areas of Europe, Australia and the US has later been questioned by different governmental and non-governmental agencies, and there is currently no scientific evidence that introduction of complementary foods during the age of 4-6 months is harmful. Therefore, the infant feeding recommendations in many of the above mentioned areas, as well as the most recent (2011) Swedish recommendation from the National Food Agency (www.slv.se), involve introduction of complementary foods, including gluten-containing foods, in small amounts from 4 months of age (exclusive breastfeeding for 4 months). Nevertheless, the scientific evidence-base for the current infant feeding recommendations is based on a heterogeneous collection of findings from observational studies, and thus it is susceptible to differences in interpretation.

In general, breastfeeding is promoted in most parts of the world, and also beyond 4 months of age, although complementary foods may have been introduced. However, promotion of breastfeeding poses some difficulties both with respect to circumstances when breastfeeding might not be optimal and by possibly conveying guilt to non-breastfeeding mothers. Furthermore, infant feeding recommendations should be accompanied by a social structure (practical and economical) providing the mothers and families with the best opportunities to follow the advice they are given; thus there is room for improvement.
Regarding celiac disease, the amount of gluten given to the infant could be affected both by recommendations, e.g. to introduce gluten gradually in small amounts, or by the content of industrially produced infant foods. The latter does not involve individuals following recommendations, nor does it involve the possible necessity to change behavior, and might therefore be more easily put into practice. On the other hand, great caution must be taken as it influences the whole population, without the active choice of the individual, as is evident from our experience with the celiac disease epidemic.

Due to the intrinsic difficulties with randomized trials regarding breastfeeding, the evidence-base in this respect will most likely continue to rely on observational studies. However, in the future the currently ongoing randomized controlled trials regarding amount of gluten and age at gluten introduction\textsuperscript{33,103} will provide a more solid evidence-base for infant feeding recommendations with respect to celiac disease.

While the optimal infant feeding practice with respect to celiac disease remains to be elucidated, our findings suggest that the current infant feeding recommendation in Sweden, as well as in other parts of the world, to introduce gluten-containing foods gradually in small amounts from 4 months of age, preferably during breastfeeding, seems favorable. Through investigations of the Swedish epidemic of celiac disease we have obtained increased knowledge regarding the disease that can hopefully be used to stem the increase in celiac disease prevalence and prevent another celiac disease epidemic elsewhere.
Concluding remarks and future research

In this thesis different epidemiological designs have been used to obtain increased knowledge regarding the occurrence of celiac disease in Sweden, as well as the disease etiology, by investigating the Swedish epidemic of celiac disease in an attempt to also move towards explaining the epidemic. While a piece has been added to the puzzle, further research is warranted.

Through screening, we found the highest celiac disease prevalence (2.9%) reported in Europe or the US in a birth cohort of the epidemic period (1993) (Paper I). Moreover, we found an unexpectedly high prevalence (2.2%) in a birth cohort of the post-epidemic period (1997) (Paper II). In comparison with findings in previous studies, this prevalence indicates that celiac disease has increased in Sweden. Future surveillance will determine whether celiac disease continues to increase in Sweden. The cause of the high celiac disease prevalence in Sweden as well as causes for the increase call for further research. Moreover, in the “global village of celiac disease” there are still gaps to fill regarding total prevalences of the disease in different parts of the world e.g. China and sub-Saharan Africa.

As we found that 2/3 children with biopsy-verified celiac disease were undiagnosed prior to screening (Papers I-II), I would also suggest further research on the topic of population based screening. When in life do most children develop celiac disease? At what age should a screening be performed? How often? What is the benefit for the detected cases? And what are the prerequisites and consequences on a societal and an individual level?

When comparing the prevalences of these 2 cohorts we found a statistically significant difference in total celiac disease prevalence, including both clinically- and screening-detected cases (prevalence ratio 0.75, 95%CI 0.60-0.93), which suggests that celiac disease can be prevented in some cases, at least up to 12 years of age (Paper II). To elucidate if the difference between the epidemic and post-epidemic cohorts is maintained requires longer follow-up, preferably with repeated screening. Moreover, follow-up of the children with potential celiac disease is warranted.

We believe that the differences in celiac disease prevalence can be explained by changes in infant feeding during the period (Paper II). Our findings suggest that gradual introduction of gluten-containing foods from 4 months of age, preferably during ongoing breastfeeding, is favorable. Further studies that distinguish between duration of breastfeeding, gluten amount and the overlap between breastfeeding and gluten introduction are warranted.
Why some individuals develop celiac disease later in life remains largely unknown. Which environmental- and lifestyle factors trigger the loss of previous oral tolerance to gluten? There are no known major changes in Swedish society affecting preschool or school children that could explain the now demonstrated difference in celiac disease prevalence. It could be that infant feeding in some individuals influences if celiac disease develops, but in others it affects when it develops. Thus, I would like to see further research on the effect of early environmental- and lifestyle factors on celiac disease development (including both if and when), as well as on possible environmental- and lifestyle factors triggering celiac disease later in life. In addition, further studies on the role of non-HLA genes in celiac disease etiology are warranted.

In this thesis we found that early vaccinations within the Swedish vaccination program were not risk factors for celiac disease (Paper III). Studies investigating this association in other settings would be advantageous, especially as we could not assess the association between some of the vaccinations due to an almost complete coverage. Additionally, new vaccinations have later been incorporated in the vaccination program. The finding of a protective effect by BCG needs further investigation. We also found an association between early infectious episodes and increased celiac disease risk, and a synergistic effect with gluten amount which was more pronounced in children where breastfeeding had been discontinued (Paper IV). In relation to this finding, the questions I would like to see answered, preferably by prospective studies also using biomarkers, include: What is the molecular mechanism for how infections trigger celiac disease development? What would the results be if this study was replicated in another setting with a higher infectious load? What is the role of gastroenteritis in celiac disease development? What is the relation to the microbiota? Does the usage of different kinds of antibiotics affect celiac disease risk? And does introduction of vaccination against rotavirus affect the occurrence of celiac disease?

Regarding the possibility of celiac disease primary prevention, our findings suggest that promotion of breastfeeding and introduction of gluten in small amounts could be plausible preventive strategies, conceivably put into practice through infant feeding recommendations and regulations regarding the gluten content in industrially produced infant foods. This needs to be further evaluated in intervention studies, preferably (when possible) through randomized controlled trials, both among high risk individuals and the general population. I would also like to see studies further investigating other potential strategies, e.g. probiotica, all aiming towards celiac disease primary prevention.
The Researcher

I was introduced to the field of research in 2004 when writing a research paper on celiac disease at the department of Epidemiology and Global Health, Umeå University. The research paper was an obligatory assignment related to medical school, which I began in 2001 at Umeå University. Prior to medical school I had worked at different places, had taken Psychology and Chemistry courses at Umeå University as well as traveled. While working with my medical school research paper, although the subject was more a coincidence than an active choice, I discovered that this kind of research combined many of my interests, such as moving between molecular level and health on a population level, and my fondness for organizing papers. When I got the opportunity to continue as a project assistant within the ETICS study, for which the planning phase then was about to begin, I decided to take an intermission from medical school to start up my PhD-studies. In the end of 2006 I was accepted as a PhD-student. Over the years thereafter, I combined research with medical school and later medical internship. While this combination, prolonging both processes, has had both pros and cons, I believe that the combination has made me appreciate both processes more. Working with research has made me a better medical doctor and attending medical school and internship contemporaneously with my PhD-studies has improved my scientific skills and provided time to think, reflect and develop my thoughts.

During the PhD-process the content of my thesis has come to change several times. While starting off of as a mixed methodological thesis, including both qualitative and quantitative research, it ended up as “number crunching” only. Still, I value the experience of having worked also with qualitative methodology. Recognizing the different and sometimes contradicting theoretical backgrounds and traditions for qualitative and quantitative research, I, together with many (most?) researcher within the medical field today, believe that there is a valuable scientific contribution from both. I think the combination, as it provides different explanatory power, results in a better overall understanding. Thus, I believe in a physical/biological world which is interpreted and the interpretation depends on who you are. While striving for objectivity when using quantitative methods, I do not believe this can be fully achieved. On the other hand, I think that recognizing this has improved my ability to treat the data in the best possible way increasing both validity and reliability of the results.

Lastly, I would like to share the most frustrating but in the end most interesting question during my PhD-process: What is knowledge?
Acknowledgements

Looking back at the years of PhD-studies there are many people who have made contributions to my process. On the whole, I feel like I have received an entire new family, a “research family”, to whom I wish to express my greatest gratitude. Or as the classical saying; “A picture is worth a thousand words”.

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Lastly, I wish to thank all the participating children and their families for their contributions which enabled the studies.

Celiaki, även kallat glutenintolerans, är en kronisk sjukdom som finns hos ungefär 1% av befolkningen. Sjukdomen innebär att kroppen inte kan hantera mat innehållande proteinet gluten som finns naturligt i framförallt vete, råg och korn. För att man skall utveckla sjukdomen krävs både att man har en genetisk förutsättning (den genetiska varianten HLA-DQ2 eller DQ8 som återfinns hos ungefär 25% av befolkningen) och att man äter mat innehållande gluten. Eftersom sjukdomen triggs av gluten, behandlas sjukdomen genom en strikt glutenfri kost.

Symtomen på celiaki hos små barn är vanligen diarré, näringsbrist, gnällighet och dålig viktuppgång. Man kan dock få sjukdomen när som helst under livet och symtomen kan variera kraftigt med allt från inga symtom alls till tydliga magbesvär. Eftersom symtomen kan vara så varierande har majoriteten av alla personer med celiaki inte blivit diagnosticerande. Man brukar likna förekomsten av sjukdomen vid ett isberg: ovanför vattenytan finns de individer där man hittat sjukdomen (så kallade kliniska fall diagnosticerade inom hälso- och sjukvården) men under vattenyten finns ett stort mörkertal. För att bedöma hur stort mörkertalet är måste man testa för celiaki bland befolkningen (screening).

Bakgrund till och syfte med avhandlingen


Senare studier har visat att den svenska celiakiepidemin till viss del förklaras av förändringar i spädbarnskosten. Men, eftersom det är så många med celiaki som är odiagnostiserade så kvarstår frågan huruvida förändringar i spädbarnskosten kom att påverka andelen barn som fick symtom på celiaki och därmed korrekt diagnos eller om det blev en faktisk ändring i antalet barn med celiaki. Det vill säga; blev hela isberget större under epidemin eller var det bara delen ovanför vattenytan som växte?
På grund av den Svenska celiakiepidemin lyftes även frågan kring varför vissa individer med den genetiska förutsättningen, men inte alla, får celiaki (sjukdomens etiologi). Att det är en så stor skillnad däremellan, samt att förekomsten av diagnosticerad celiaki förändrades kraftigt under kort tid i mönstret av en epidemi, tyder på att även andra bakomliggande faktorer (såsom andra gener och miljöfaktorer) påverkar risken. Idag saknas ännu mycket kunskap kring olika miljöfaktorers roll i sjukdomens etiologi.

Huvudsyftet med denna avhandling är därför att öka kunskapen om celiakins förekomst och etiologi genom att undersöka den Svenska celiakiepidemin närmare. Vi avsåg dels att undersöka huruvida den totala förekomsten av celiaki förändrats i och med epidemin och dels avsåg vi att relatera fynden i förekomst till förändringarna i spädbarnskost under perioden. Utöver detta så undersökte vi även om det fanns andra miljöfaktorer som bidragit till celiakiepidemin och/eller påverkade risken för celiaki, med fokus på barnvaccinationer och tidiga infektioner.

**Epidemiologiska metoder och resultat**

För att försa öka uppnå syftet med avhandlingen användes fyra olika epidemiologiska studier. Dessa var 1) registerstudier baserat på Celiakiregistret som även utgjorde bas för de andra studierna, 2) en screeningstudie i två faser, 3) en ekologisk ansats, samt 4) en populationsbaserad fall-referent studie.


Strategin för screeningen inkluderade dels att alla barn med tidigare diagnosticerad celiaki rapporterades och dels blodprovstagnign för analys av antikroppar tydande på celiaki för resterande. Alla barn med positiva antikroppar kallades till närmaste barnklinik för vidare undersökning med tunntarmsbiopsi samt genetisk kontroll innan diagnos ställdes.

Genom screeningen fann vi att totala celiakiförekomsten bland 12-åringar födda under epidemin (1993) var 2.9%. Detta är den högsta förekomsten som rapporterats i Europa eller USA. Vidare fann vi att den totala förekomsten i födelsekohorten efter epidemin (1997) var 2.2% vilket var statistiskt signifikant lägre i jämförelse. I båda födelsekohorterna var celiaki vanligare bland flickor än pojkar och två av tre fall var tidigare odiagnostiserade och hittades i samband med screeningen.


- Välling och gröt för barn innehöll en större mjölmängd (större mängd gluten) under epidemin jämfört med innan och efter.
- Rekommendationerna angående ålder för introduktion av mat innehållande gluten till spädbarn ändrades 1982 från ”från 4 månaders ålder” till ”från 6 månaders ålder”. 1996 ändrades detta igen till ”kan ges i form av smakportioner från 4 månaders ålder.”
- Amningslängden under perioden ökade: mitt under epidemin ammade ungefär 63% fortfarande vid 6 månaders ålder och efter epidemin var motsvarande siffran 77%.

De två sistnämnda förändringarna kom gemensamt att påverka proportionen spädbarn som introducerades till gluten under pågående amning. Sammanfattningsvis tyder våra resultat på att den spädbarnskost som barn födda 1997 fått är mer gynnsam (jämfört med barn födda 1993) vad gäller risken att utveckla celiaki eftersom vi såg en lägre total förekomst av celiaki (2.2%) bland dem jämfört med barn födda under epidemin (2.9% representerat av kohorten 1993).
För att undersöka om det fanns några ytterligare, med epidemin samtida, förändringar i miljöfaktorer användes en så kallad ekologisk ansats. Vi tog hjälp av data från olika svenska register och jämförde förändringar över tid med förändringen i förekomsten av celiaki. Dock kunde vi inte identifiera någon ytterligare förklaring till epidemin.

Undersökningen huruvida andra miljöfaktorer kan vara riskfaktorer för celiaki gjordes vidare via en populationsbaserad fall-referent studie. Under dryga 2 år bjöds alla nya fall av celiaki som rapporterades till Celiakiregistret in till studien. Samtidigt bjöds två andra slumpvist utvalda barn in som kontroller och de under 2 år inkluderas i denna del av studien. Vid jämförelse av miljöfaktorer mellan fallen och kontrollerna kunde vi se att vaccinationer inom det svenska barnvaccinationsprogrammet inte var riskfaktorer för celiaki. Däremot så rapporterade föräldrarna till barn med celiaki oftare att barnet haft flera infektioner (≥3) under de första 6 månaderna i livet. Denna skillnad var statistiskt signifikant och därmed skulle tidiga infektioner kunna vara en riskfaktor för celiaki. Vidare fann vi att risken ökar ytterligare om barnet dessutom introducerats till gluten i stora mängder. Ökningen var än mer uttalad om barnet inte längre ammade när gluten introducerades. Detta förstärker att amning verkar skyddande mot celiaki.

**Slutsatser**

Genom denna avhandling har vi visat att celiaki är betydligt vanligare bland barn i Sverige än man tidigare trott och majoriteten av dessa fall är odiagnostiserade. Vi fann även en signifikant skillnad i den totala förekomsten av celiaki mellan barnen födda under, jämfört med efter, epidemin vilket betyder att epidemin bestod i en förändring i den totala förekomsten av celiaki (hela isberget) och inte enbart en förändring av andelen med symtom och diagnos (delen ovan vattenytan). Följaktligen betyder detta att celiaki kan förhindras hos vissa individer med den genetiska förutsättningen, åtminstone upp till 12-års ålder. Vi har även visat att upprepade tidigare infektioner, men inte barnvaccinationer, kan vara en riskfaktor för celiaki. Den enda förändringen i miljöfaktorer i det svenska samhället som vi hittills kunnat identifiera är förändringar i spädbarnskosten. Våra fynd tyder på att där det gäller risken för att utveckla celiaki är det gynnsamt att introducera gluten-innehållande mat gradvis i små mängder till spädbarn med start från 4 månaders ålder och helst under pågående amning.
References


153. Shann F. Non-specific effects of vaccination: vaccines have non-specific (heterologous) effects. BMJ 2005;330:844; author reply


## Appendices

### Appendix 1 - National Swedish Childhood Celiac Disease Register

Rapporteurs from different parts of Sweden.

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### Appendix 2 - The ETICS study

Personnel and collaborators in the ETICS study. The responsible researcher at each study site and member of the ETICS study steering group is indicated with *, principal investigator with (PI) and PhD-students within the study with #.

#### Umeå

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<tr>
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<td>Anna Myléus*</td>
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#### Norrköping

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</table>
**Växjö**

*Researchers/clinicians*  
Eva Karlsson*  
Anna Eriksson  
Piroska Lindskog  
Monica Roos

*Research nurses*  

**Lund**

*Researchers/clinicians*  
Annelie Carlsson*  
Cathrine Astermark  
Charlotta Webb*  
Group of pediatric nurses  
Maria van der Pals*

*Research nurses*  

**Collaborators**

*In the ETICS study several collaborators contributed with knowledge within their respective field of knowledge to the collection of data.*

Maria Emmelin  
Qualitative methodology

Britta Halvarsson  
Pathology

Olle Hernell  
Pediatric gastroenterology/nutrition

Agneta Hörnell  
Food and nutrition

Lars Lindholm  
Health economics

Ester Lörinc  
Pathology

Curt Löfgren  
Health economics

Ethel Kautto*  
Food and nutrition

Hans Stenlund  
Epidemiology and statistics

Stig Wall  
Epidemiology and statistics

Erik Winbo  
Illustrations  
etrikwinbo.artworkfolio.com

Several *additional collaborators* were involved in and contributed to papers based on material from the ETICS study.

Part of the ETICS study was incorporated into the European multicenter-study PreventCD (www.preventcd.com), led by Luisa Mearin (PI).