FACULTY OF HEALTH AND MEDICAL SCIENCES
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Master's thesis

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Epidemiology and surveillance of three diarrhoeagenic *Escherichia coli* in Denmark between 2000 - 2012

- Can surveillance be improved?

Epidemiologi og overvågning af tre diaréfremkaldende *E. coli* bakterier i Danmark, 2000 – 2012

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Preface

The present work was carried out at the Statens Serum Institut, Copenhagen, during my time at the Department of Infectious Disease Epidemiology from February 2013 to July 2013 as part of my studies in Public Health Science at University of Copenhagen.

I am greatly indebted to the Director of the department, *Kåre Mølbak*, M.D, who was also my main supervisor, for the excellent working conditions offered me. I have really appreciated the counselling, feedback, valuable suggestions and inputs. Also it has been a privilege to be part of the everyday routines in the department.

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I also want to thank the rest of my colleagues at the department, for welcoming me, for their support and encouragement throughout the past six months.

Laura Espenhain, July 2013.

Abstract

***Introduction**:* Diarrhoeal illness is one of the major causes of morbidity and mortality in paediatric populations throughout the world. While infectious diarrhoea is associated with modest mortality rates and is generally less severe in the industrialized world, it still represents a considerable disease burden. *Escherichia coli* strains are important aetiological agents in the overall burden of illness. Verocytotoxin-producing *E. coli* (VTEC) is often seen in food-borne outbreaks and with increased international trade of food products so is Enterotoxigenic *E. coli* (ETEC). Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhoea in infancy and early childhood in Denmark, but little is known about risk factors and prevention. The *objectives* of the study were to describe the epidemiology of VTEC, ETEC and EPEC in Denmark between 2000 and 2012; the surveillance systems in place for detecting the three pathogens in terms of diagnostic methodology and indication for testing; and to assess the possibilities of using *epiMiBa* in the surveillance and monitoring of DEC in the future.

***Methods and material**:* Data from two laboratory-based and a physician-based database was used for the descriptive epidemiology. The observations were linked to the Danish Civil Registration System in order to get geographical information. *KMAs* were given a questionnaire on diagnostic methods and principles for testing. An epiMiBa extraction from September 2012 was compared with data from the same period in existing databases.

***Results**:* An increase in the overall number of reported cases was seen for all three pathogens over the period from 2000 to 2012. VTEC and EPEC infections were primarily seen in children below 5 years of age whereas ETEC infections were common in all age groups. The majority of ETEC infections were acquired abroad. Most *KMAs* are following the recommendations of the DSKM with regards to principles for testing for DEC however, diagnostic methodology varies between *KMAs.*

***Conclusions**:* EPEC continues to be an important pathogen in small children and is probably underdiagnosed. More knowledge of the burden of illness is needed. Outbreaks of ETEC might go undetected. The surveillance of VTEC has long been prioritised and is reflected in the elaborateness and completeness of the *E. coli* database. However, varying principles and methods for testing are reflected in the regional incidences. Enhanced diagnostics and surveillance would inform priorities as regards to improved detection, typing, outbreak response and understanding of risk factors in order to possibly improve prevention.

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0.0 Introduction

It is July 26th 2011, the epidemiologists from Robert Koch Institute (RKI) in Berlin, Germany, together with regional and local public health authorities, clinics and the investigations of the federal food safety authorities, have worked hard over the past months; today they can declare the VTEC O104:H4 outbreak over. 4011 persons have been reported as infected, many more affected, more companies have been boycotted and/or lost money, and 53 people are dead – 35 of kidney failure as sequelae from the *Escherichia coli* O104:H4 infection (1). The outbreak was primarily limited to Northern Germany but also travel-associated cases were seen in 13 other European countries, including Denmark.

At the beginning of May 2011 an increase in the reported number of VTEC cases was noted by RKI. On May 20th they initiated an outbreak investigation and by this time three women had died and 276 cases had developed HUS. Epidemiological, microbiological, food-safety and trace-back investigations were set in place immediately in order to describe the outbreak and identify the source in order to stop it. 10 days later the accumulated number of HUS cases had reached 400 and an additional 843 people had been reported to be infected with VTEC. Many people had been hospitalized, several requiring intensive care.

The initial epidemiological investigation suggested that cucumber, tomatoes or lettuce could be the vehicle of infection and by June 2nd 2842 samples of cucumber, tomatoes and leafy salads had been examined. Two cucumbers were found positive for VTEC (later shown to be *E. coli* O8:H19 (1)); this became a confounding and politically challenging lead. However on June 5th, 3 days later, sprouts became the suspected vehicle as sprouts were suspected in a concurrent outbreak in France. Re-interviews showed that most people had eaten sprouts, however they had not remembered them in the first interview. On June 10th a warning concerning sprouts was released, and tomatoes, salad and cucumber were withdrawn from the list of suspected food items.

Once sprouts had been identified as the source of the infection, and their distribution stopped at the beginning of June, there were no further clusters associated with the consumption of sprouts. In the late stages of the outbreak however, cases of secondary transmission by infected persons via close contact within households occurred, as well as distinct localized outbreaks that could be attributed to secondary contamination of food products by employees in the food industry that shedded the bacteria (1:26). Also a few laboratory infections were recorded (1:31).

Within a relatively short period of time, epidemiological studies and systematic tracing of food products led to the discovery of fenugreek sprouts from Lower Saxony imported as seeds from Egypt as the vehicle of infection. A well-functioning surveillance system, with adequate or appropriate detection methodology in the primary diagnostic clinics and hospitals, outbreak investigation as well as response system, are crucial for determining the source of the outbreak, eliminate future spread, morbidity, and mortality.

Diarrheal disease

Diarrhoeal illness is one of the major causes of morbidity and mortality in paediatric populations throughout the world (2). Although infectious diarrhoea is associated with modest mortality rates and generally is of low severity in the industrialised world, it affects a high number of people and represents a considerable disease burden. A cross sectional study carried out in Denmark in 2009 (3), found that almost 10% of the respondents had experienced diarrhoea within a four week period. This corresponds to a standardized incidence rate of 1.4 episodes of illness per person *per year* – highest amongst children. Far from all of these episodes is an iatrotropic stimulus which is not seen by a doctor. Episodes are in general self-limiting and will resolve within days. However infections may, in rare instances, lead to serious sequelae or even death as seen in the German outbreak depicted above. Despite the somewhat low seriousness of the infections, the high incidence of diarrhoeal diseases can have a significant socioeconomic impact as a result of both lost working days for parents and medical costs in more severe cases (4;5).

Escherichia coli

Escherichia coli is a Gram-negative, typically rod-shaped bacterium that normally lives in the intestines of people and warm-blooded animals. *E. coli* is a heterogeneous group of bacteria; most are harmless, and rarely cause disease - many *E. coli's* are in fact an important part of a healthy human intestinal tract (6), however certain subtypes or clones possess combinations of gene expressions that enable them to cause serious illness in humans; such pathotypes are more frequently observed in certain serotypes than others. Pathogenic *E. coli* that are associated with diarrhoea are referred to as diarrhoeagenic *E. coli* (DEC). In this project the focus will be on the three pathotypes which are recognised as diarrhoeagenic:

Verocytotoxin-producing E. coli (VTEC) also referred to as Shiga toxin-producing *E. coli* (STEC) or enterohemorrhagic *E. coli* (EHEC). These types can cause the most severe sequelae as kidney failure (HUS) and death. The most common O group for this pathotype is the O157, but also other O groups are seen in outbreaks – e.g. the O104:H4 in Germany.

Enterotoxigenic E. coli (ETEC) refers to a group of *E. coli* that produce at least one of two enterotoxins: a heat-stable toxin (known as ST) and a heat-labile toxin (LT). Although different strains of ETEC can secrete either one or both of these toxins, the illness caused by each toxin is similar.

Enteropathogenic E. coli (EPEC) are in Denmark defined as *E. coli* that are diarrhoeagenic, produce the A/E lesion on intestinal cells, belongs to one of a number of serogroups which will be discussed later, and that do not produce Verocytotoxin.

Diarrhoeagenic *Escherichia coli* strains are important aetiological agents in the overall burden of diarrhoeal illnesses throughout the world. ETEC strains predominate in terms of global impact because they are important causes of acute watery diarrhoea among children in developing countries and also a leading cause of travellers' diarrhoea (2;5) VTEC is of particular concern as a food-borne infection, and in particular in the US, much of the improvements in food safety has been prompted by large outbreaks of *E. coli* O157 from ground beef. Infections with diarrhoeagenic *Escherichia coli's* are also common in Denmark. The reported episodes of all diarrhoeagenic *Escherichia coli* (VTEC, EPEC, ETEC, Enteroinvasive *E. coli* (EIEC) and attaching and effacing *E. coli* (A/EEC) – the latter two will not be discussed in this thesis) accounts for around one third of the total reported episodes of gastrointestinal illness in the registry for enteric bacteria.

VTEC has often been seen in food-borne outbreaks in Denmark and abroad (7-11). As the infection can have detrimental effects, the severe cases will eventually be seen in the health sector. However, in outbreak situations it is crucial to identify cases as early as possible in order to find the source of the infection and limit further spread. The ETEC infections are often in Denmark perceived as *travellers' diarrhoea* as many infections are acquired during travel in developing countries (12), nevertheless ETEC have been seen in multiple food-borne outbreaks in Denmark and abroad (13-16).

EPEC is an important cause of diarrhoea in infancy and early childhood in Denmark (17) and abroad, but has not been associated with food-borne outbreaks in Denmark. It was a main cause – together with *Shigella* - of outbreaks of diarrhoea amongst children in institutions, also in Denmark. It is highly infectious amongst children and can lead to long-term or chronic diarrhoea that can affect the growth and the wellbeing of the infected child (18:72).

Diagnostics of DEC is often challenging compared with diagnosis of other bacteria that can cause diarrhoea. However, public health surveillance is based on diagnostics, and surveillance is essential to determine trends, detect and control outbreaks, understand disease dynamics, to measure burden, and make priorities in preventive strategies.

1.0 Objectives

The objectives of this project are to provide a detailed description of the epidemiology of the reported DEC[1] in Denmark from 2000 – 2012 in terms of time, place, person; describe and discuss the current surveillance of DEC in Denmark in terms of diagnostic methods and indication for test for DEC, and to assess the possibilities of using a new surveillance database epiMiBa in the surveillance and monitoring of DEC in the future.

[1] VTEC, ETEC, EPEC

2.0 Background

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 the control of health problems* (19:9). In 1854 Snow used epidemiological reasoning as the foundation for preventing deaths from cholera in London by defining its water-borne transmission. Already in the 17th century Graunt assembled 100 years' worth of vital statistics into tables that defined the basic facts of human mortality (19). Last century Doll and Hill established the causal link between cigarette smoking and lung cancer in a series of epidemiological studies performed in England during the 1950s (20;21). The domain of epidemiology has expanded over time though, epidemiologists are not only studying health issues, diseases and conditions for which biological inferences can be drawn, but also topics like behaviour e.g. homicide and suicide, or social problems, such as unplanned pregnancy among teenagers (19:10).

In Gregg's book 'Field Epidemiology', Goodman and Buehler define *Field epidemiology* as the application of epidemiology carried out under four general conditions:

- The problem being studied is unexpected
- An immediate response may be demanded
- Public Health epidemiologists must travel to and work in the fields to solve the problem
- The extent of the investigation is likely to be limited because of the imperative for timely intervention (22:4).

In the context of field and intervention epidemiology, epidemiological methods are most often used to identify the agent(s) causing disease, modes of transmission, factors of susceptibility, risk, or exposure, and environmental determinants. Often, investigations are accompanied with a need for urgent action such as evidence-based recommendations to manage a new challenge for public health or to control an ongoing outbreak. In the early 1980s epidemiology enabled public health epidemiologists to determine transmission mechanisms and groups at risk for acquired immunodeficiency syndrome (AIDS) and to develop recommendations for its prevention three years before the causative virus was identified (19:10).

Nowadays we do rely more on data from the diagnostic laboratories concerning the pathogens' characteristics as it can tell us, for instance, whether the *Salmonella* found in two patients are identical and thus likely have been acquired from the same source or if

they are different and thus might not have the same origin. This knowledge is important to guide the epidemiological investigations as the picture can become blurred otherwise.

All epidemiologic studies – be it field or 'academic' – obtain data on a study population and capture facts to analyse (23:16). The main purpose for the field epidemiologist though is to use the data for action. Getting timely health-related data, either in a hurry or on an ongoing basis and using this information in public health is referred to as surveillance (23:16). Thacker refers to CDC's definition of surveillance from 1986 in his chapter on Surveillance in Gregg's book 'Field Epidemiology' and defines it as *the ongoing and systematic collection, analysis, and interpretation of outcome-specific data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know. The final link of the surveillance chain is the application of these data to the control and prevention of human disease and injury* (23:16).

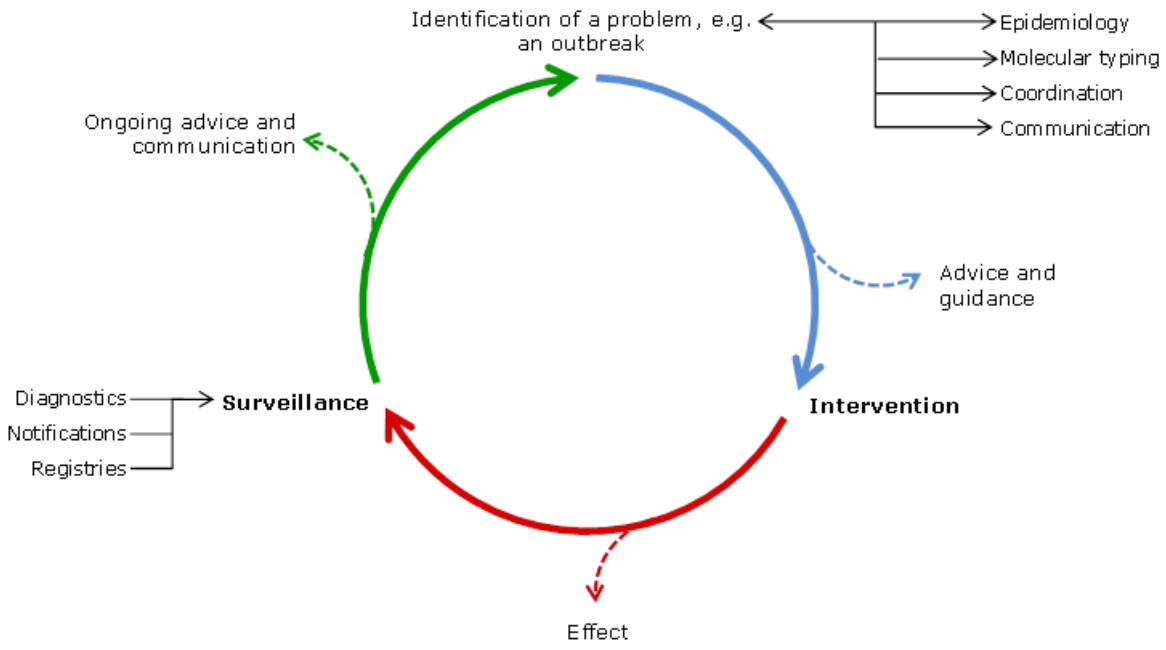

Figure 1 - Cycle in which data generated during surveillance enter into, to be analysed, communicated and acted upon (24)

Surveillance can be categorised as either active or passive. *Passive* surveillance ascertains cases by clinical notifications from physicians, laboratories and other health care professionals required to submit such reports as defined by public health legislation. The people that do not seek medical assistance for e.g. their gastrointestinal illness are not captured by this method.

Active disease surveillance is also based on public health legislation and refers to daily, weekly or monthly contacting of physicians, hospitals, laboratories, schools or others to "actively" search for cases. This type of surveillance is usually set up periodically to coincide with periods of high disease frequency and generally yields a much higher percentage of actual cases as compared to passive surveillance.

Surveillance is the base on which field epidemiology lies as it provides an ongoing means for detecting important health problems (19:14). Figure 1 (24) illustrates the cycle data that are generated through surveillance enter into; here it is analysed, communicated, outbreaks are detected and acted upon, and interventions or preventive measures are taken.

Whether you are investigating an epidemic in the field or implementing a national programme of prevention, surveillance is the cornerstone, the management tool, for public practice. A good surveillance provides the data needed to give:

- An accurate assessment of the status of health in a given population
- A quantitative base to define objectives for action
- Measures to define specific priorities
- Data to define strategies
- Measures to evaluate interventions, programs, and outcomes
- Information to plan and conduct research.

In short, surveillance data should provide a scientific, factual basis for appropriate policy decisions in public health practice and allocation of resources (23:18).

One of the most basic and important tasks an epidemiologist faces is organising and describing data in terms of *how much* (e.g. how much disease is occurring), *when*, *where*, and *to whom*. The three latter are referred to as *time*, *place* and *person*. Characterising epidemiologic data along these three dimensions serves several purposes. First, this approach provides a systematic method for dissecting a health event or problem and it ensures that one is familiar with the basic dimensions of that health event or problem. Second, the approach provides a detailed characterisation of the problem in basic terms that can easily be communicated and understood. Third, it identifies populations at increased risk of the health problem under investigation, enabling the epidemiologist then to generate testable hypothesis relevant to aetiology, mode of spread, and other aspects of the problem (25:60).

Field and intervention epidemiology is in the crossfire between 'academic epidemiology' and surveillance where the detail of each observation is weighted against timeliness and practicalities. The Danish surveillance registries, some of which will be described later, are of high quality and are used for research; nevertheless, data is collected by clinicians for whom the immediate goal is the individual patient – and the other patients in line – and not the surveillance as such.

2.1 Surveillance in Denmark in general

The surveillance of infectious diseases is the responsibility of Statens Serum Institut (SSI). Along the lines with Thacker's definition it is stated on the website of SSI that '*A modern surveillance system does not only entail the collection and register of disease information, but also a timely and continuously dissemination of knowledge to the authorities responsible for infection control, treatment and prevention*.' (26). Surveillance of infectious diseases, microorganisms and vaccination coverage is a central part of the national and international disease preparedness in Denmark. The national surveillance system comprises mainly diseases of serious character, diseases that are particularly infectious, and most of the vaccine-preventable diseases.

SSI is a public enterprise under the Danish Ministry of Health and for more than 100 years SSI's main task has been to secure the preparedness towards infectious diseases and congenital disorders. The tasks have been expanded since, and today, SSI is an international research, production and service enterprise (26). SSI is responsible for research-based health surveillance, rational use of IT in the Danish healthcare system and prevention and control of infectious diseases, biological threats and congenital disorders. SSI aims to ensure advanced control of infectious diseases, including new infections and biological threats (26).

Additional to the general themes of a surveillance pointed out above, the Danish surveillance system serves several purposes:

- Detection of disease outbreaks
- Estimation of tendencies and development over time
- Identification of population groups with special risks of certain diseases, i.e. incidence according to age, gender, geography, and personal characteristics in the form of for instance ethnicity

- Detection of changes in bacteria and virus, e.g. occurrence of resistance towards antibiotics or certain pathogenic germs and - via this - prioritization of prevention and control (26).

2.1.1 Surveillance of food-borne illnesses

Food is a source of transmission of infectious diseases to humans, and food-borne infections are often seen as the cause of acute gastrointestinal infection with diarrhoea. The micro-organisms which are often transmitted through food can be divided into two groups: Zoonoses and non-zoonoses. *Zoonoses are* transmitted naturally from animals to humans through the food chain. *Salmonella* and *Campylobacter* are the most common food-borne bacterial zoonoses in Denmark (27). Non-zoonotic infections are caused by direct or indirect contamination of food by human faeces. Viral gastroenteritis caused by e.g. norovirus is transmitted this way, but also food-borne outbreaks with virus that do not cause diarrhoea have been seen. During the spring 2013 a food-borne outbreak of Hepatitis A has been ongoing in Denmark (28).

Food can become contaminated with an infectious agent during the production and processing as well as at preparation, cooking and the serving of the food. Individuals who shed bacteria or virus can contribute to the contamination of food. Most often gastrointestinal symptoms will usually resolve without treatment. For some bacteria though, the risk of severe sequelae is high and if the illness is caused by a source that can infect others, it is important to try to eliminate it.

The Danish surveillance system for food-borne illness is passive i.e. it relies on reports supplied by physicians and laboratories. During outbreaks though, active surveillance is used to identify additional cases.

2.1.1.1 Mandatory notification systems

During the period of interest (2000-2012), the surveillance of DEC has been carried out on the basis of two regulatory community frameworks. In the course of 2013, SSI has, amongst others, worked towards improving the regulatory framework in order to make it more up to date and flexible.

The current regulatory community framework for the national surveillance in Denmark is The National Board of Health Statutory Order on Physicians' Notification of Infectious Diseases etc., including later amendments:

- Statutory Order on Physicians' Notification of Infectious Diseases No 277 of 14 April 2000. (29)
- Statutory Order on Physicians' Notification of Severe Acute Respiratory Syndrome (SARS) No 616 of 27 June 2003. (30)
- Statutory Order on Physicians' Notification of Methicillin-resistant Staphylococcus aureus (MRSA) No 1002 of 6 October 2006. (31).

Individually notifiable diseases

In the Statutory Order a number of diseases and infections that are individually notifiable for physicians and general practitioners (GPs) are stated. The notifications are also called "clinical notifications"; they include relevant information about the patient, and are notified on a paper form (*1515 form*) to the regional public health officer and to Statens Serum Institut. VTEC is on the list of individually notifiable diseases in § 4 of Act no 277 of 14 April 2000 as a separate item, but also 'food-borne infections' and 'water-borne infections' are listed and could include e.g. ETEC (29).

Laboratory notification system

There also exists a parallel laboratory-based surveillance system for a large number of micro-organisms. The clinical-microbiological laboratories (abbreviated '*KMA*') are obliged to notify findings of certain micro-organisms as well as information on the patient on a weekly basis. In the period 2000-2012, the surveillance of DEC has been carried out through the *Tarmbakteriologisk Register* – Register of enteric bacteria (abbreviated '*TBR*').

In chapter 4 of Act no 277 of 14 April 2000, § 11, concerning the laboratory based surveillance, it is stated that *infections with enteric bacteria are notifiable, and that the reporting should be done on a weekly basis* (§ 12). In § 13 *enteric bacteria* are defined as *both known pathogenic bacteria as Salmonella species, Campylobacter jejuni/coli , Yersinia enterocolitica , Shigella species, Vibrio cholerae, diarrhoeagenic E. coli and other bacteria which the KMA believe have caused intestinal symptoms in the patient*.

The *KMA* thus report their findings of enteric bacteria on a weekly basis, though more often if an outbreak is suspected or on going. When a laboratory-report of VTEC has been received at the Infectious Disease Epidemiology department at SSI, the personnel in charge of VTEC-surveillance will check whether a 1515 form has been received from the treating doctor. If not, the doctor is contacted and a 1515 form is requested (Cf. Figure 4 page 25).

2.1.1.2 The organization of the outbreak response in Denmark

Food- or water-borne outbreaks can be identified in several ways. e.g. from notifications from GPs or hospitals seeing more patients with gastrointestinal symptoms than usual, through notifications from the public to the local food or health authorities, through notifications from the laboratories receiving more samples, finding more of the same pathogen or by finding clusters of the exact same subtype of a pathogen. Findings of identical subtypes of a certain pathogen point towards a common source of infections, however contamination with multiple agents is not rare.

The Danish outbreak response system is divided in local/regional and national outbreak response. Local or regional food-borne outbreaks will usually be dealt with by the local public health inspector, the regional offices of The Danish Veterinary and Food Administration and the *KMAs* in the affected area. National outbreaks are handled by the national authorities, often under the auspices of The Central Outbreak Coordination Group (Den Centrale Udbrudsgruppe (DCUG)) which is a collaboration between SSI, DTU National Food Institute and the Danish Veterinary and Food administration. Each of the institutions in the DCUG are independent units that work with food-borne outbreaks from different perspectives.

2.1.1.3 KMA's (Clinical microbiological department)

There are twelve clinical microbiological departments covering the whole of Denmark (with KMA Midt-Vest Herning and Viborg counting as one). The location of these are shown on Figure 2 where also the five regions and the 11 landsdele are illustrated. The *KMAs* are the backbone of the laboratory-based surveillance system; they test for a wide range of bacteria, viruses, and other pathogens such as parasites. Most KMAs are located at a hospital and serve this as well as the surrounding GPs – except for the *KMA* at the SSI and the Rigshospitalet. SSI is, evidently not connected to a hospital, and Rigshospitalet do not receive samples from GPs.

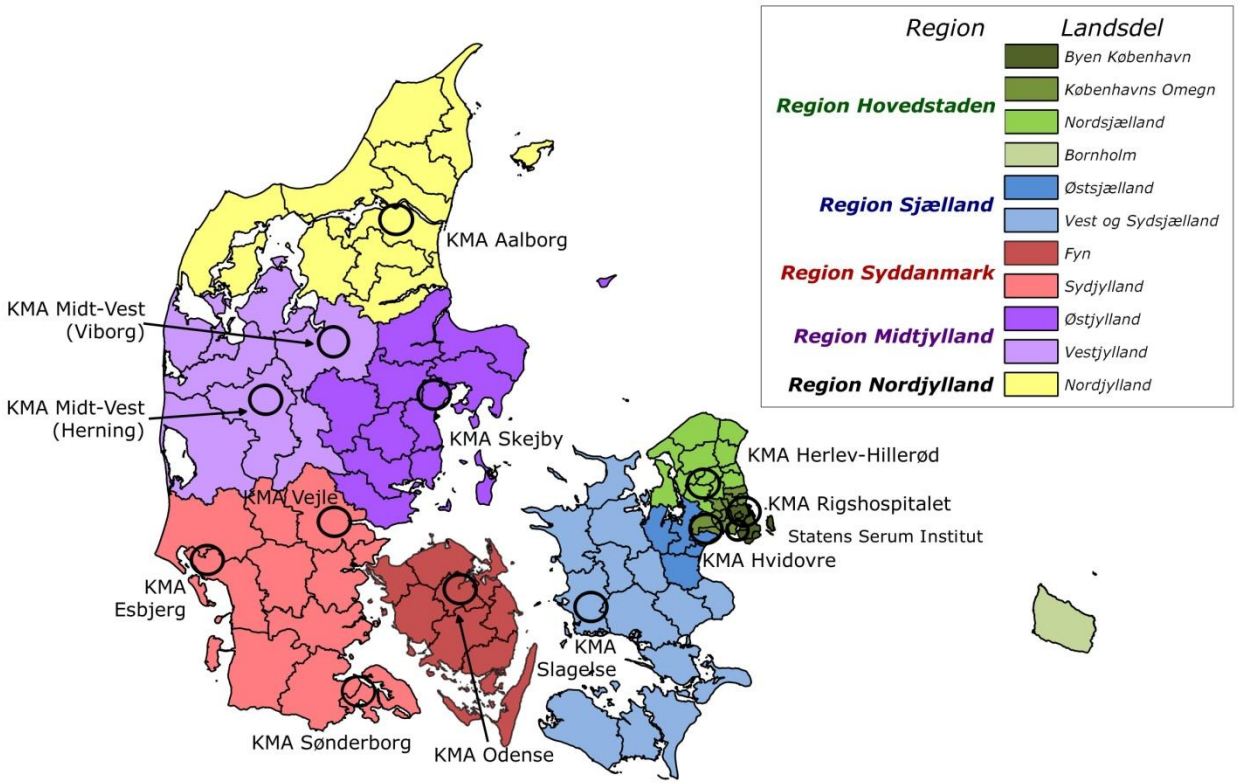

Figure 2 – Overview of the five *regioner*, 11 *landsdele* and 12 *KMAs* in Denmark

In the beginning of the 2000s, the diagnostic laboratory at SSI was responsible for most of the DEC diagnostics. Throughout the 2000s the *KMAs* began to take over the responsibility of the diagnostics' concurrently with them acquiring the proper equipment. The SSI also serves as the National reference laboratory.

2.1.1.4 Outbreaks

VTEC

There have been four larger general outbreaks with VTEC in Denmark since 2000. The first known general outbreak of VTEC O157 occurred over a six month period from September 2003 until March 2004. During this period 18 children and seven adults with indistinguishable pulsed-field gel electrophoresis (PFGE) DNA profiles were identified through routine surveillance. None developed haemolytic uraemic syndrome (HUS). The outbreak was restricted to Copenhagen and the surrounding areas. A case-control study indicated that shopping in a specific supermarket chain in Copenhagen and surrounding area was associated VTEC O157:H- infection with a matched odds ratio (OR) of 8.7 (95%CI 1.1-71). After excluding three assumed secondary cases, only consumption of a particular kind of organic milk from a small dairy was associated with disease (OR 8.7 95%CI 1.6-48). Environmental and microbiological investigations at the suspected dairy did not

confirm the presence of the outbreak strain, but the outbreak stopped once the dairy was closed and thoroughly cleaned (8).

In 2004, a local outbreak of VTEC O157 amongst children a one adult who had been visiting a 'petting zoo' (*besøgsgård*) in the former *Frederiksborg amt* 10 were reported from four different childcare institutions (32).

In 2007 a signal of an outbreak with VTEC O26:H11 was detected through routine surveillance by the means of PFGE typing of isolates. The signal was recognised on March 9. Samples from 20 people were found positive for the outbreak strain from February 1st until May 1st 2007 - 18 from children and two from adults. The mean age was 2 years (range, 0-51 years). In general the symptoms were mild, one with bloody diarrhoea and there were no cases of HUS. The hypothesis generating questionnaires of cases (or parents to cases) did not give a lead and credit card information was used to trace back what the patients had bought three weeks prior to the date of onset. Also a case-control study was conducted during March 28-30ieth 2007. A multivariate matched analysis found beef sausage to be associated with disease (matched OR 2.8 95%CI 1.4-170). Microbiological investigations of sausages and frozen beef used to make the sausages were sampled and subsequently tested positive for the outbreak strain (7;33).

During the German VTEC O104:H4 outbreak in May and June 2011 26 Danes were reported infected. 20 of these were submitted to hospital and ten developed HUS – none of the Danish cases died (34).

Latest, an outbreak with VTEC O157 with a rare and severe toxin gene subtype profile was detected through notification from a paediatric department of four cases of HUS. The annual number of HUS cases in Denmark ranges from two to six (35). In total 13 cases were identified (from nine families): 11 with VTEC O157:H7 with eae, vtx1a and vtx2a genes or HUS + a serology-confirmed VTEC O157 infection, and two who were diagnosed with HUS but had no laboratory confirmed VTEC infection. Eight developed HUS. Date of onset ranged from September 18 to October 2012. The cases were distributed throughout the country, eight were female and the median age was 14 years (range 3-68 years). Hypothesis generating questionnaires suggested minced beef steaks and a case by case comparison from a recent salmonella outbreak supported this hypothesis. The short shelf life of ground beef (approximately 7 days) may have limited the size of the outbreak. If the source of infection had been a food item with longer shelf life, the public health impact

could have been much larger (9). Later the strain was isolated from imported minced meat and a case from Sweden was also seen.

ETEC

In 2006, there was an outbreak of ETEC (and salmonella) at a high school with 217 people affected. On November 14th 2006 the regional public health authorities were contacted by the director of a high school who informed them about an outbreak of diarrhoea and vomiting among participants at a school dinner party held on November 11th 2006. Almost all of the students and teachers of the school (750 people) had attended the party and a retrospective cohort survey was performed in order to identify the source of infection. The cohort was defined as students and teachers, who had attended the party at the high school on November 11th and a case was a person from the cohort, who presented with diarrhoea (looser stools than normal ≥3 times in 24 hours) or vomiting within 48hours after the meal. Stool samples from 48 persons were examined, 18 tested positive for ETEC O92:H- - out of which four also tested positive for *Salmonella* Anatum - and one tested positive for ETEC O153:H2.

A multivariate analysis found the consumption of pasta salad with pesto to be associated with disease and the environmental investigation found the leftovers of the pasta salad to be heavily contaminated with generic *E. coli* (up to 10^5 bacteria/g) and *S.* Anatum was also detected in the pasta salad leftovers. The fresh basil leaves used in the pesto were believed to be the source of contamination (14).

In 2010, an equally big outbreak was detected. From January 18 -20 2010, a series of outbreaks of gastroenteritis were reported to the Danish authorities. At least 11 outbreaks were included in the cluster comprising approximately 480 potentially exposed persons and approximately 260 cases with symptoms of gastroenterititis. The outbreaks took place in the eastern part of Denmark (*Fyn* and *Sjælland*) and all occurred in groups of people (companies, courses etc.) that had had lunch – sandwiches or 'smørrebrød' – delivered from catering companies. Results from several questionnaire studies and a retrospective cohort study indicated that sandwiches containing lettuce (imported Lollo Bionda lettuce) were associated with illness. Norovirus and ETEC O6:K15:H16 was found in 23 and 11 patients respectively from the outbreak, and norovirus was found in heads of the lettuce collected from two of the implicated catering companies. An additional 15 persons infected with ETEC O6:K15:H16 were found through routine diagnostics of stool samples performed at SSI in January and connected to undetected outbreaks also from sandwiches from catering companies (13).

ETEC O27:H7 outbreak in 2011. Cases of illness possibly connected to a canteen delivering lunch to 6 companies, approximately 250 persons total. Preliminary investigation showed that 87 of 241 persons became ill. ETEC O27:H7 was isolated from 5 of 6 faecal samples. Investigative interviews were performed and the illness seemed correlated with eating lunch on June 9 or 10. A full scale cohort study was performed including all dishes served in the canteen where the patients ate in the period 9-10 June. Specifically eating a salad composed of asparagus, broccoli and mange tout peas with a dressing of vinegar and oil seemed significant. Both asparagus and broccoli were steamed prior to serving. The peas were served raw in both salads. The two mentioned salads both contained peas from the same possible lot. Unfortunately no stock from that consignment was left on the market. Samples have been taken from later shipments from the same exporter and none of these samples showed presence of *Escherichia coli*.

On January 2nd 2012, a signal of a fourth outbreak of ETEC appeared. The Food Authorities East received notifications from various companies whose employees had developed persistent diarrhoea after having eaten sandwiches from a sandwich shop in the centre of Copenhagen at the end of December 2011. 49 people from five different companies were sick. A cohort study carried out pointed towards Lucerne sprouts. In this outbreak Salmonella was also found, both in patients and in samples. Seven samples from patients tested positive for ETEC O169:H41 with identical PFGE patterns.

Two of four of the general VTEC outbreaks were detected through routine laboratory surveillance and one was detected through notification to the authorities. None of the ETEC outbreaks were detected through the routine diagnostics – except for the additional clusters of O6:K15:H16 that were found after the diagnostic activity intensified due to the Lollo Bionda outbreak. As later discussed, ETEC diagnosis is not implemented to the same extent as diagnosis for other gastrointestinal bacteria, and they are not serotyped (or subtyped by other methods). Hence, outbreaks are not usually detected through the laboratory surveillance.

2.2 *Escherichia coli*

Three general clinical syndromes can result from an infection with one of the pathogenic *E. coli*: Enteric or diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis (6: 123). In the early 1940s, 'summer diarrhoea' was a significant clinical problem in infants in Europe and North America.

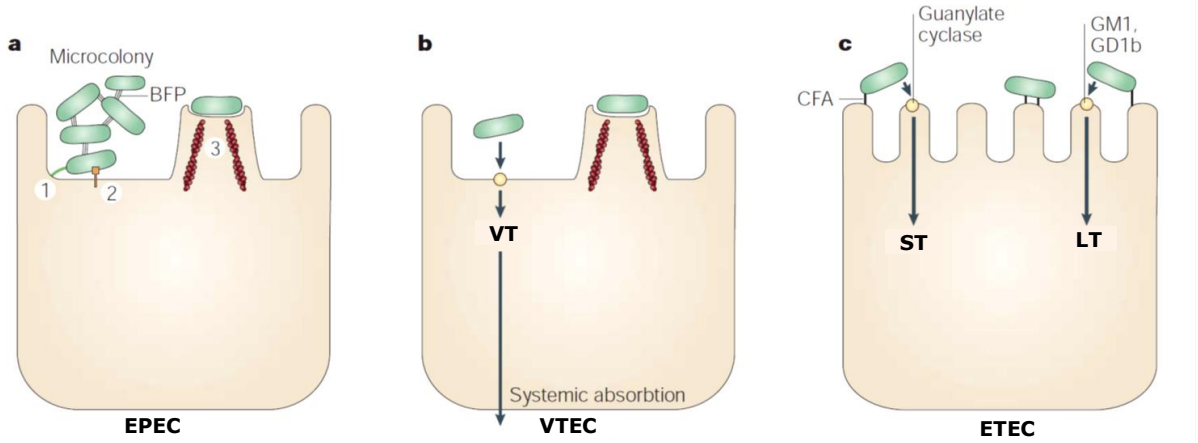

Figure 3 - Pathogenic schema of diarrhoeagenic E. coli **a** | EPEC adhere to small bowel enterocytes, but destroy the normal microvillar architecture, inducing the characteristic attaching and effacing lesion. Cytoskeletal derangements are accompanied by an inflammatory response and diarrhoea. 1. Initial adhesion, 2. Protein translocation by type III secretion, 3. Pedestal formation. **b** | VTEC also induce the attaching and effacing lesion, but in the colon. The distinguishing feature of VTEC is the elaboration of verocytotoxin (VT), systemic absorption of which leads to potentially life-threatening complications. **c** | Similarly, ETEC adhere to small bowel enterocytes and induce watery diarrhoea by the secretion of heat-labile (LT) and/or heat-stable (ST) enterotoxins (Kaper, 2004:124).

The microbiologist – John Bray – and the paediatrician – John Beavan – studied the phenomenon (36:378). They were aware that the routine stool cultures in use at that time detected *Shigella* and *Salmonella,* but failed to identify pathogens in most infants. Bray and Beavan hypothesized that some strains of *E. coli* that appeared to be strains of normal flora by colonial morphology on agar, might actually be pathogenic. To pursue this hypothesis, they used an immunizing strain of *E. coli* isolated from an infant who had summer diarrhoea and who had no other pathogens, to prepare an antiserum that might identify the homologous and related strains of *E. coli*. They examined cultures of stools from infants with summer diarrhoea and from healthy control infants from strains of *E. coli* that could be agglutinated by the antiserum that they had developed from a rabbit (36:378). Nowadays the diarrhoeagenic *E. coli* are divided into various pathotypes according to distinct virulence properties, different interactions with the intestinal mucosa, distinct clinical syndromes, difference in epidemiology, and distinct O:H serotypes (36:377). Diarrhoeagenic *E. coli* are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles (2:144;36:377). The three pathotypes that are the focus of this report are characterized by their interaction with intestinal mucosa (Cf. Figure 3).

Enteropathogenic *E. coli* adhere to small bowel enterocytes via "bundle-forming pilus" (BFP) (cf. Figure 3– 1 (6:128)) and destroy the microvillar architecture by inducing the characteristic attaching and effacing lesion (cf. Figure 3 a,3). The cytoskeleton derangements are accompanied by an inflammatory response, active ion secretion, increased intestinal permeability, loss of absorptive surface area resulting from microvillus effacement and, as a result of this, diarrhoea (6:128).

Clinical EPEC illness is characterized by fever, malaise, vomiting, and diarrhoea with a prominent amount of mucus but usually without gross blood. The *reservoir* of EPEC is unknown, but is believed to be symptomatic or asymptomatic children and asymptomatic adult carriers, (2:161). *Spread* is mostly by the faecal-oral route (2:161). The *infective dose* of EPEC in infants is very low and EPEC is highly infectious for infants (2:161). EPEC illness in infants tends to be clinically more severe than many other diarrhoeal infections in this group. Some infants will develop prolonged diarrhoea that persists for >14 days. In the few documented cases of adult diseases, the infective dose is thought to be relatively high at 10^6 - 10^11 organisms. The *incubation time* is 18-72 hours (average 36 hours). *Treatment*: Antibiotics can shorten the duration of the illness and reduce the risk of complications and person-to-person spread.

Some Verocytotoxin-producing *E. coli* also induce the attaching and effacing lesion, however, whereas EPEC induce the lesion in the small intestines, VTEC induce it in the colon (6:124). The distinguishing feature of VTEC is the elaboration of verocytotoxin that is absorbed systemically (cf. Figure 3b) and can lead to life-threatening complications (6:124). Verocytotoxin-producing *E. coli* (VTEC) are defined as: *E. coli* with the presence of vtx gene(s) and/or production of verocytotoxin (VT).

Clinical VTEC illness is characterized by acute gastrointestinal infection with diarrhoea stomach cramps and vomiting and no or light fever. 1/3 experiences bloody diarrhoea (37) and this has been shown to be associated with an increased risk of developing severe sequelae such as acute renal failure (HUS) and neurological impairment such as paralysis (38). HUS is a serious and often life-threatening condition and up to 50% of patients with HUS may develop long term renal damage or blood pressure related complications (39;40). Children in the age group 2-6 are at significantly increased risk of developing HUS. The risk for a VTEC infection to progress to HUS depends upon the characteristics of the infecting strains, in particular the subtype of the vtx gene; some are designated as HUS-inducing or high-risk strains (35). *Reservoirs*: VTEC occur naturally in the intestines of ruminants e.g. cattle, sheep, and goats (41:26), primarily cattle (42-46) and can be transmitted during

slaughtering if the meat gets in contact with the animals' manure (2:169). *Sources of infection* are beef, contaminated fruit and vegetables, raw milk, or milk that has not been pasteurised properly. VTEC can be transmitted by food and water and as the *infectious dose* is very small (fewer than 100 VTEC bacteria) person-to-person transmission is a risk. Carriers preparing food or working with vulnerable groups (children in day-care, hospitalized patients, the elderly etc.) are quarantined if they have a VTEC infection and cannot go to work or institution before they have had two negative samples taken (38). The *incubation time* is 1-8 days and the duration of the illness is usually 5-10 days. *Treatment* of a VTEC infection is limited to supportive care. It is recommended that antibiotic or anti-diarrhoeal treatment in the acute phase is avoided as these has been shown to increase the risk of HUS among children in some studies (47;48)

Enterotoxigenic *E. coli* is defined as containing the *E. coli* strains that elaborate at least one member of two defined groups of enterotoxins: ST and LT. ETEC strains were first recognized as causes of diarrhoeal disease in piglets, where the disease continues to cause lethal infection in newborn animals. Studies of ETEC in piglets first elucidated the mechanisms of disease, including the existence of two plasmid encoded enterotoxins (2).

The ETEC bacteria colonize the proximal small intestine – the critical site of host-parasite interactions – where they elaborate LT or ST (36:379). These toxins stimulate the lining of the intestines causing them to secrete excessive fluid (cf. Figure 3) that result in diarrhoea. The *clinical features* of ETEC infections are watery diarrhoea, nausea, abdominal cramps and low-grade fever (6:129;36:379). The major O serogroups associated with ETEC are O6, O8, O15, O20, O27, O63, O78, O80, O85, O115, O128ac, O139, O148, O153, O159 and O167 (36:379). *Sources* of ETEC infections are drinking- or bathing water or fruit or vegetables that have been contaminated with human faeces. The *incubation time* is 8 - 44 hours with the average being 26 hours. The illness lasts from 3 to 19 days. *Treatment*: Antibiotics have been shown to decrease both the duration of diarrhoea and the intensity of ETEC excretion; however antibiotic resistance in ETEC strains is an emerging problem and is why rehydration is often the preferred treatment.

2.2.1 Testing methods

2.2.1.1 Slide agglutination for determination of O group

Agglutination refers to the reaction that occurs when antibodies are mixed with their corresponding antigens on the surface of large, easily sedimented particles such as animal cells, erythrocytes, or bacteria, the antibodies cross-link the particles, forming visible clumps. For DEC this can be done on various media such 'SSI Enteric Medium' made at SSI,

XLD-plate or a CIN plate (49). The Danish Society for Clinical Microbiology (abbreviated *DSKM*) in Denmark recommends that agglutination is done in three serum pools: O26, O103, O111, O145, O157; O55, O119, O125ac, O127, O128ab and O86, O114, O121, O126 and O142.

2.2.1.2 Molecular Detection Methods

Diarrhoeagenic *E. coli* strains were among the first pathogens for which molecular diagnostic methods were developed. Molecular methods are the most reliable techniques for differentiating diarrhoeagenic strains from non-pathogenic members of the stool flora and distinguishing one category from another. Substantial progress has been made both in the development of nucleic acid-based probe technologies (hybridisation) as well as *polymerase chain reaction* (PCR) methods (2:145). PCR is a major advance in molecular diagnostics of pathogenic microorganisms, including *E. coli*. PCR primers have been developed successfully for several of the categories of diarrhoeagenic *E. coli*. Advantages of PCR include great sensitivity in *in situ* detection of target templates. However, substances within stools have been shown to interfere with the PCR, thus decreasing its sensitivity; several methods have been used successfully to remove such inhibitors (2:147).

2.2.2 Diagnostics

VTEC is diagnosed at the *KMA* by detection of the verocytotoxin-producing genes vtx1 and/or vtx2 through PCR or hybridisation followed by slide agglutination for determination of the most common O and/or K groups (O26, O103, O111, O145 or O157 (49:22)). Some *KMAs* only perform slide agglutination for common O groups and forward the isolate to the reference laboratory at SSI for further testing.

EPEC is diagnosed at the *KMA* by detection of the intimin gene (eae for *E. coli* attaching and effacing) through PCR or hybridisation followed by slide agglutination for determination of the most common O groups (O26, O55, O86, O111, O114, O119, O125ac, O126, O127, O128ab, O142, O158, O103, O145 or O157 (49:22)). As for VTEC, some KMAs only perform slide agglutination for common EPEC O groups and forward the isolate to the reference laboratory for further testing. VTEC and EPEC have some O groups in common and this is why many EPEC isolates are forwarded to the reference laboratory to confirm the diagnosis.

At the reference laboratory *classical* EPEC is defined by *E. coli* isolates (vtx-negative) with the eae gene belonging to one of the following serotypes: O26:H–, O26:H11, O26:H34, O55:H–, O55:H6, O55:H7, O86:H–, O86:H34, O111:H–, O111:H2, O111:H25, O114:H–,

O114:H2, O119:H–, O119:H2, O119:H6, O125:H–, O125:H6, O125:H21, O126:H–, O126:H2, O126:H21, O126:H27, O127:H–, O127:H6, O126:H21, O128:H–, O128:H2, O128:H7, O128:H12, O142:H–, O142:H6, O158:H– and O158:H23. Additionally the serotypes O39:NM, O88:H25, O111:H8, O111:H9, O126:H12, O127:H4; O145:H45; O157:H8 and O157:H45 are classified as *new* EPEC (5).

ETEC is diagnosed at the *KMA* by detection of genes that codes for LT, STh, and/or STp through PCR or hybridisation. The *KMAs* will normally not try to determine the O group (49:22).

DSKM recommendations

The *DSKM* recommends that all VTEC and EPEC isolates are sent to the national reference laboratory at SSI for confirmation and virulence characterisation (49:22).

In 2003 DSKM recommended that all faeces samples containing blood (visible or in the anamneses) and all samples sent in for gastroenteritis from children below 7 years should be tested for VTEC. For EPEC their recommendation was to test all samples sent in for gastroenteritis from children below 2 years (50). In 2012 they revised the recommendations to that all samples sent in for gastroenteritis from children below 7 years of age, from people who have been travelling abroad, or from people who is believed to have HUS should be tested for DEC (VTEC, ETEC, EPEC, EIEC and A/EEC) (49).

2.3 MiBa – Modernisation of the laboratory-based surveillance

The laboratory-based surveillance has in the past been based on submitted lists or forms from the diagnostic labs. During the previous couple of years a new overarching database has been developed: the Danish microbiological database (MiBa). The MiBa database receives copies of reports from all Danish KMAs (Cf. Figure 4). From MiBa the result will automatically be mapped and transformed into a somewhat conform "language" and appear in EpiMiBa. EpiMiBa is intended to be the focal point in the national surveillance of infectious diseases and microorganisms.

The goal of MiBa is a) to provide health care personnel with nationwide access to microbiology reports and b) to enable real time surveillance of communicable diseases and microorganisms. This will facilitate identifying and reacting to outbreaks and make it easier to convey relevant information to the health inspectors and national and international authorities.

3.0 Methods

3.1 Databases

Use of databases:

For the description of the epidemiology of VTEC, EPEC and ETEC in Denmark between 2000 and 2012, information from three databases was used.

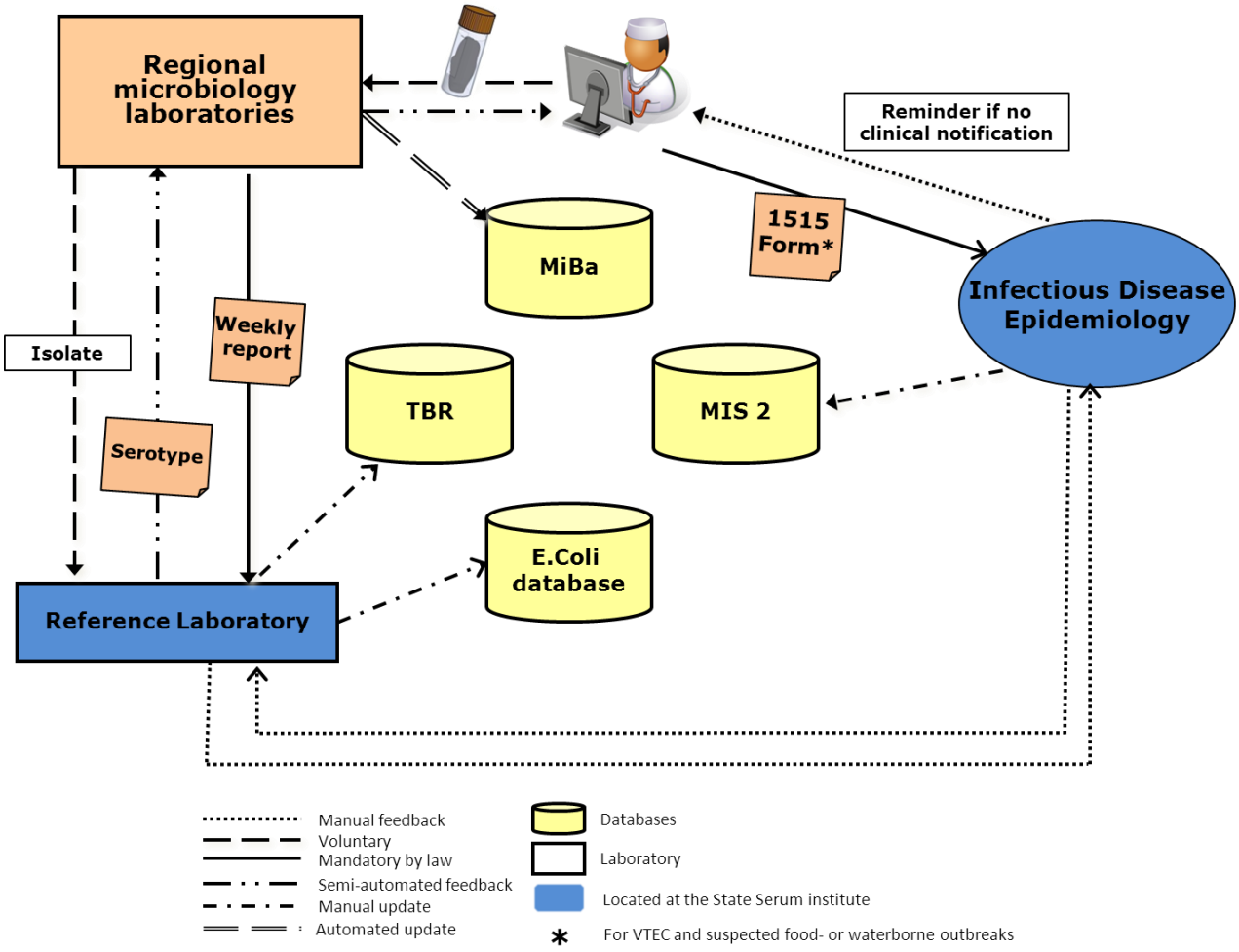

Figure 4 - Overview of the DEC surveillance reporting in Denmark

3.1.1 Database for Enteric bacteria (TBR)

Information found through the laboratory-based surveillance is kept in *TBR*; this database is supposed to contain all positive test results made in the *KMA*s. The database is updated on a weekly basis via reports from each *KMA* (cf. Figure 4). The database contains a record of each identified serotype in an isolate and these are given a unique identifier *provnr*. The *TBR* also contains information about the CPR number, age, sex, name of the patient, date of reception of the sample, whether the patient has been travelling (if the GP has provided

this information on the 'order form'). There is a code for the person ordering the test (GP or hospital section), but no code for which *KMA* has done the test. It is, however, possible to distinguish between a) samples that have only been tested at the *KMA* and reported to the *TBR*, b) samples that have both been tested locally at the *KMA* and forwarded to the reference laboratory for further testing, and c) samples that have not been tested locally but forwarded to the reference laboratory for testing.

3.1.2 MIS-2

Information gathered from the 1515 form is entered into the *MIS-2 database*. The 1515 form often has fields for date of onset, date of sampling, symptoms, travel history and occupation. MIS-2 is an epidemiological case-based database i.e. multiple infections in one individual at the same time will count once. Each case is given a unique identifier, the *epilbnr* – as opposed to the *TBR* that is episode based.

3.1.3 *E. coli* database

This is the database of the results of the test done on the isolates that are sent to the reference laboratory. This database contains results from the typing done in the laboratory with extensive information about resistance patterns, O, H and K groups, and much more. The reference laboratory primarily receives potential VTEC isolates, but both EPEC and VTEC which have intimin gene (eae) and share some of the same serotypes are also forwarded to the reference laboratory for further analysis. The isolates received are given a unique identifier in the lab, but keeps the identifier given in the *TBR* (the provnr) for merging purposes. Also the reference laboratory will enter the *epilbnr* and add any additional information on the case from the 1515 form on VTEC cases when it is received at Infectious Disease Epidemiology, SSI. The database contains one record for each subtype detected as *TBR*.

3.1.4 CPR

In order to get geographical information about all cases, the database was linked to the Danish Civil Registration System by the means of the CPR number. After the municipality reform in 2007 the administrative division *Region* was introduced as well 270 municipalities were merged and reduced into 98 municipalities. *Region (NUTS2), landsdel (NUTS3)*, and municipality information for all observations had been converted to the current divisions before the linking. The CPR registry contains information – address, former addresses, parents, children, etc. about everyone with a CPR number.

3.1.5 Dataflow

The extraction from the *E. coli* database of episodes from January 1st 2000 until December 31st 2012 was merged with *TBR* by Flemming Scheutz on April 10th 2013 by means of *provnr*. In order to get geographical information on these records, this database was merged – using *provnr* - with an extraction of the *TBR* made by Steen Ethelberg on the 13th of March 2013 with all ETEC, EPEC and VTEC registrations made from January 1st 2000 and onwards in *TBR*. Afterwards the MIS-2 database was merged with the VTEC episodes from the combined dataset by the means of the *epilbnr*. In order to get as much information as possible on each case, some manual corrections were done. As an example case reports received through the 1515 form as VTEC, might be found to be ETEC in the laboratory and will therefore not get an *epilbnr* and did thus not merge with MIS2. 60 observations out of 8601 (0.7%) were corrected manually.

3.2 Case definition

VTEC: A person for whom a test result has been categorised as *VTEC* and reported to TBR within a six months period between January 1st 2000 and December 31st 2012. If samples have not been received at the KMA, a report via the 1515 form will – until (dis)confirmed at the *KMA* or at the national reference laboratory, count as a case.

EPEC: A person for whom a test result has been categorised as *EPEC* at a *KMA* and reported to the TBR within a six months period between January 1st 2000 and December 31st 2012.

ETEC: A person for whom a test result has been categorised as *ETEC* at a *KMA* and reported to the TBR within a six month period between January 1st 2000 and December 31st 2012.

3.3 Statistics Denmark

In order to be able to calculate the incidences, information about the number of people in each age group, *region*, *landsdel* and municipality was obtained from the '*STATBANK'* at Statistics Denmark's website (51). The geographic information on each observation had been converted into the new administrative entities – *regioner* and *kommuner* from 2007. The population from 2008 available from STATBANK were used for calculating the incidences in the *regions* and *landsdele* prior to 2008. The population from 2006 available from STATBANK were used for calculating the incidences in the municipalities prior to 2006. For calculating the incidence in various age groups, nominators for all years were available.

3.4 Software

3.4.1 SAS

SAS version 9.3 was used to do the merging, data cleaning and the descriptive epidemiology.

3.4.2 Quantum GIS

The geographical software *Quantum GIS* version 1.8.0 was used to draw maps of Denmark with the incidences. In the CPR extraction, all municipality-codes had been converted into the existing municipalities from 2007. A geographical database with municipality borders in vector-format (SHP file) was obtained from 'Kortforsyningen' under the Danish Geodata Agency.

3.5 Questionnaire development

A web-based questionnaire was developed in order to unveil the diagnostic methods used by each *KMA*, the principles for testing, and the periods of time these methods and practices had be used. This information should shed light on the second objective of this project – to describe and discuss the current surveillance of DEC in Denmark in terms of diagnostic methods and indication for test for DEC – as well as supporting the interpretation of the results.

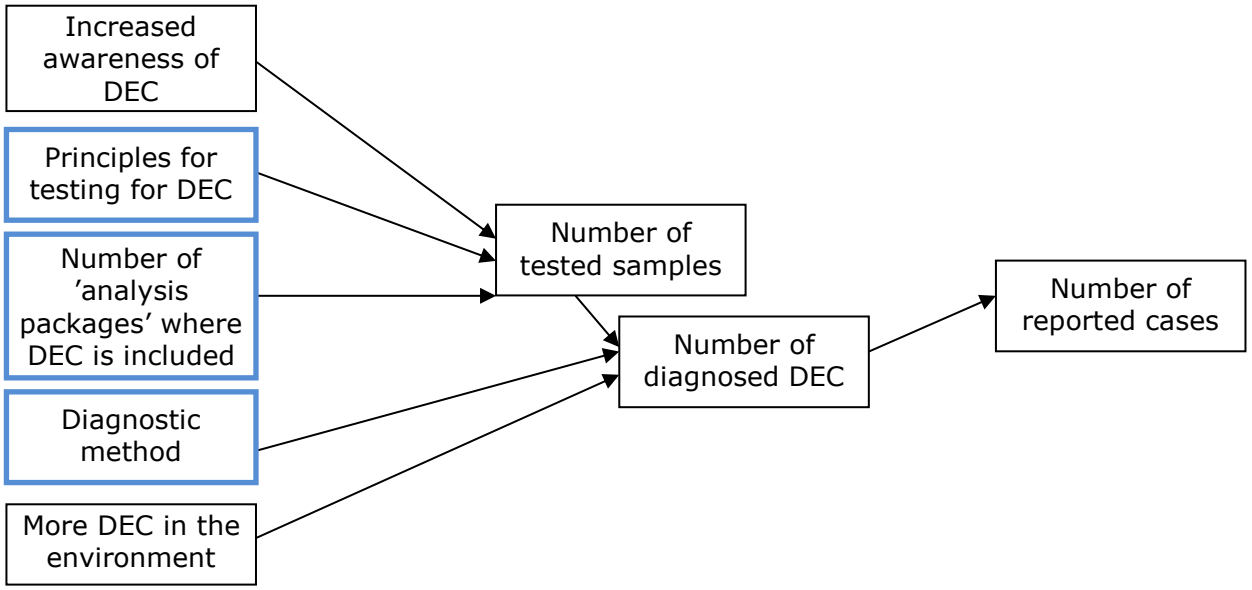

Figure 5 - Model of what affects the number of reported cases used in the creation process of the questionnaire to the KMAs

The background for the questions is the hypothesis that at least five elements affect the number of reported cases either through affecting the number of samples that are tested or the number of samples that are found positive for DEC:

1) *Increased awareness of DEC*, in the sense that if the public becomes more aware of the fact that their gastrointestinal symptoms can be caused by *E. coli*, they might ask for this test specifically. Or if doctors, for instance, become more attentive towards DEC in samples that come out negative in the first test for *Salmonella* and *Campylobacter*, they may send in more samples for testing of DEC.
2) *Principles for testing for DEC,* the hypothesis that a *KMA* that test all samples for DEC will find more DEC than a *KMA* only testing a fraction of samples for DEC.
3) *Number of 'analysis packages'*, i.e. the more analysis packages that include DEC, the more likely it is that an analysis including DEC will be requested.
4) *Diagnostic methods*, i.e. the better the diagnostics method, the more DEC detected.
5) *Increased DEC in the environment*, in the sense that the more DEC there is in the environment, the more people will be exposed and the higher risk they have of getting infected and ultimately getting tested positive.

3.5.1 Target group

The target group of the questionnaire was the *KMAs* which is why the items in the questionnaire are only covering the three elements in the hypothesis that the KMA is responsible for, namely the diagnostic methods, the principles for testing and the constellation of 'test or analysis packages' that the *KMA* offers.

3.5.1 Conceptualisation and operationalisation

Before the three elements from the model of what affects the reported number of DEC cases could be transformed into items, the dimensions were conceptionalised (52;53) i.e. all relevant themes needed to be covered in each element were explored. Once the survey concepts had been established, they were translated into observable variables, operationalised into items (53).

Diagnostic methods: Initially '*Molecular detection methods'* was one of three options in the item covering diagnostic methods. This, however, was changed after feedback from staff at infectious disease epidemiology, SSI and a more nuanced version that differentiates between PCR and Hybridisation was used. This was done in order to heighten face validity (52;53) by trying to accommodate the respondents need for providing specific answers and cover as much as possible. 'Agglutination for determination of O group' and 'Other' were the two other options.

Principles for testing: This item should cover whether the *KMAs* are, or have been, testing for DEC in addition to what the doctor orders. It was believed that a *KMA* testing more samples for DEC than requested will find more DEC.

The implication of the answer to this item unfortunately is not straight forward as the 'DEC test ordering activity' of the GPs and physicians is not known. If all doctors in the *KMA's* area as standard request tests for DEC or if a 'test package' for enteric bacteria exists and this as standard includes DEC, i.e. analysis for DEC is requested in almost all instances, it is of little help that the *KMA* is testing additional to the doctors request. On the other hand, if few doctors send in samples for testing and thus only few samples are actually received at the *KMA*, it is of little help that all samples are tested for DEC. It is only possible to reveal what the *KMA's* principles for testing are.

In a questionnaire to the *KMAs* it is not possible to determine a) whether there are differences in how often the treating doctors send in samples or b) how often the treating doctor orders a 'test package' that includes DEC. This limitation will be accommodated in MiBa where all tests and results are registered, negative and positive.

Constellation of 'test or analysis packages': In order to try to accommodate some of the limitations to the items concerning principles for testing, two items about the constellation of 'test or analysis packages' were added: One item about whether the pathotype in question (VTEC, ETEC, or EPEC) was a part of the standard test for 'enteric bacteria' if such a standard test existed, and an item where the *KMA* were asked to list any additional 'analysis/test packages' that included VTEC, ETEC and EPEC respectively.

Year of testing
As most diagnostics were done at the diagnostic lab at SSI in 2000, questions about when the *KMA* started to offer testing for VTEC, ETEC and EPEC respectively locally were posed.

In order to ensure (content) validity of the questionnaire as well as a reasonable operationalisation of the conceptionalisation, an iterative process of commenting and changing the type of questions took place. Personnel from the lab and from Infectious Disease Epidemiology at SSI in turn commented. The final questionnaire was tested by the Chief science officer and clinical laboratory manager in Microbiology and Infection Control – i.e. the diagnostic laboratory at SSI.

The questionnaire was web-based and constructed in Google Drive as a 'Form'. It was constructed as a fold-out questionnaire i.e. the answers determined what questions the respondent was asked. The questionnaire should cover practices from 2000 – 2012 and the first items therefore dealt with practices in 2000 – or the year the *KMA* started to offer DEC diagnostics – and whether these had been changed since then. The fold-out questions were capable of handling up to three diagnostic methods – i.e. two changes in practice since 2000. For the principles for testing for DEC exceeding the doctor's request one question was asked: Whether the *KMA* is or has been testing additional to the doctor's request. If the *KMA* answered positively to this question, they were asked to specify in which period and in which instances they had been doing this.

The answers were collected in an Excel spread sheet, interpreted in collaboration with Flemming Scheutz from the reference laboratory. The participating *KMAs* were contacted if there were doubts about the answers.

An overview of when the diagnostic laboratory at SSI stopped being responsible for testing samples for enteric bacteria in each *amt* from 1995 – 2004 was obtained from the infectious disease epidemiology department and this was used, in combination with the questionnaires. For *KMAs* that did not participate, information available at their websites were used in combination with knowledge from personnel at SSI.

3.6 *E. coli* in MiBa

An extraction from EpiMiBa of all test results that had found a sample positive for *'Escherichia coli'* (Cdmcode 7702) in faeces (code: 10030433) from September 1st 2012 until September 30th 2012 were compared (by means of the CPR number) with the observations recorded in the same period from the dataset used to describe the epidemiology of DEC in Denmark. The observations that did not match on CPR were manually looked up in MiBa and in the whole data material, with no restriction on dates.

4.0 Results

The results of the questionnaires, the epidemiology of the three diarrhoeagenic *E. coli's* and the comparison between MiBa/EpiMiBa and the data generated through the current surveillance are presented in the following section. First, the answers from the questionnaires will be presented in order to formulate a basis on which the epidemiology can be understood.

4.1 Diagnostics at the KMAs

Of the current 12 KMAs in Denmark, eight participated: *Slagelse* (also responded for the former *KMA Næstved), Aalborg, SSI, Odense, Herlev* and *Hillerød* (are now merged but responses for both were provided), *Esbjerg, Skejby* and *Hvidovre*.

In the 1990s, the diagnostic laboratory at SSI was responsible for most of the enteric bacteria diagnostics in Denmark. In the late 1990ies the, at that time administrative entities, *'Amter',* began to undertake the diagnostics of enteric bacteria themselves and thus the uptake area of the diagnostic laboratory at SSI shrunk. By 2000 the diagnostic laboratory at SSI covered approximately 50% of the Danish population. Figure 6 illustrates which *KMA* that has covered what *landsdel* or areas within *landsdele* from 2000 to 2013 in terms of population in the *KMAs* uptake area. SSI covered the *old Roskilde Amt (now landsdel Østsjælland*) until 2010. Information about when the responding KMA started

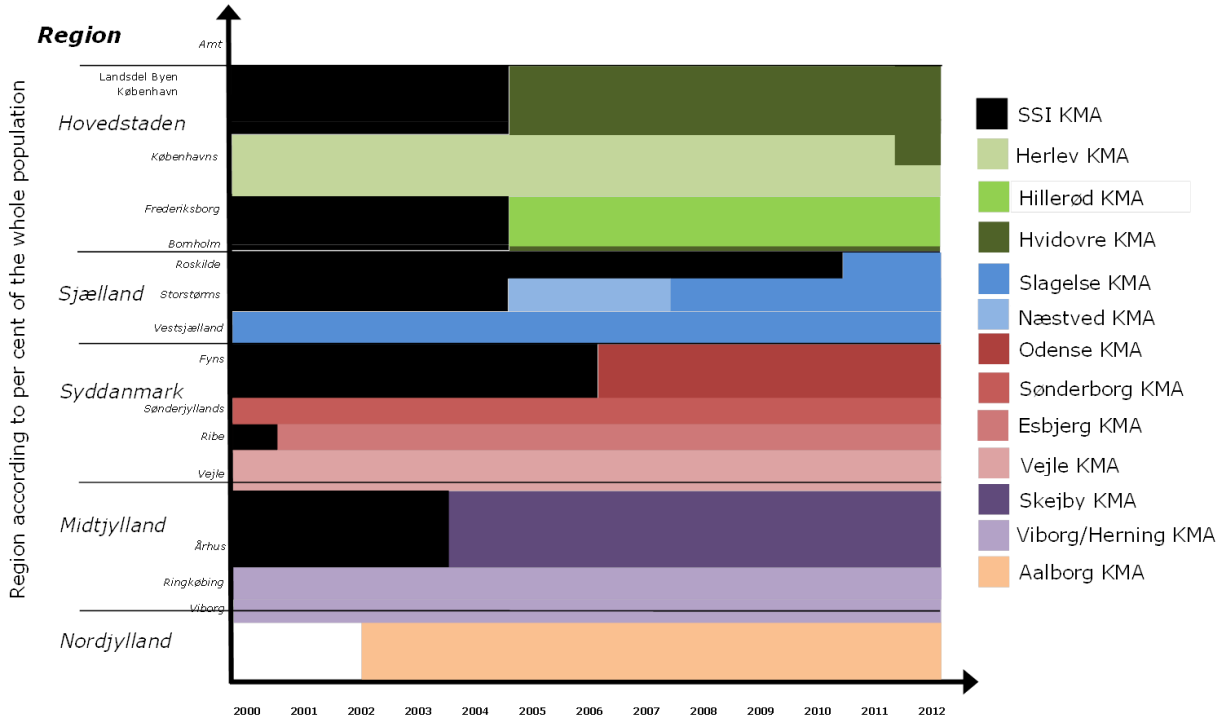

Figure 6 –DEC-diagnostics uptake area for each *KMA* by year and proportion of the population in the coverage in relation to the total Danish population

testing for each of the pathogroups was obtained through the questionnaires. Already before 2000 seven *amter* had undertaken the responsibility of the diagnostics for enteric bacteria: *Københavns, Vestsjællands, Sønderjyllands, Vejle, Ringkøbing, Viborg*, and *Nordjyllands amter*. The KMA in *Nordjyllands amt* – now *Region Nordjylland* – did not start testing for VTEC until 2002, and have not offered tests for EPEC and ETEC but referred them to SSI if necessary. *KMA Viborg/Herning* (light purple Figure 1) do not offer tests for DEC locally (54) and through the questionnaire it was learned that *KMA Skejby* have been testing samples for VTEC and EPEC from *KMA Viborg/Herning*. *KMA Viborg* did not participate in the questionnaire, however from their website it appears that they are doing PCR and testing for all three pathotypes. Since which year they have been using PCR is not known. *KMA Esbjerg*, covering the old *Ribe Amt* undertook the diagnostics for enteric bacteria in 2000/2001 and has done object glass agglutination since then.

Molecular methods for testing (hybridization) for DEC were introduced by the SSI in the mid-90s and replaced by PCR in 2004. As time passed, more and more *amter* undertook the diagnostics, now *KMA* SSI is only responsible for a small fraction of the primary diagnostics Denmark (Cf. Figure 6).

Table 1 - Use of diagnostic methods and indication for test for DEC in the period from 2000 – 2012 in nine *KMAs*

		Prior to 2000	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Diagnostic method	Objectglas agglutination	Slagelse Aalborg Herlev		Esbjerg											
	Hybridisation	SSI				Skejby	Næstved								
	PCR or RT-PCR						SSI Hillerød		Odense	Hvidovre		Slagelse			Skejby*
	Other				Aalborg¥										

¥ : ELISA, not including EPEC and ETEC
* Not including ETEC

Table 1 illustrates when a diagnostic method has been introduced or changed at the participating *KMAs*. *KMA Odense*, *Hvidovre* and *Hillerød* started out with PCR when they undertook the diagnostics from the diagnostic laboratory at SSI in 2004, 2006, and 2007 respectively. *KMA Slagelse* changed to PCR in the beginning of 2009. Skejby changed from hybridisation to PCR in January 2012, but does not yet offer ETEC diagnostics. They announced that they are planning on implementing ETEC diagnostics. Also *Aalborg* and *Esbjerg KMAs* declared that they are looking more into introducing PCR and thus diagnostics for all three: VTEC, EPEC and ETEC.

4.1.1 Principles for testing

Of the eight participating *KMAs*, seven indicated that they offered an 'analysis package' for enteric bacteria (*Slagelse, SSI, Odense, Skejby, Hvidovre, Esbjerg, Herlev-Hillerød*) and that this included DEC this if certain indications are met. The majority tested according to the *DSKM* 2003 recommendations, for the pathogens they were able to detect locally.

In May 2010 *KMA Odense* started testing all samples from GPs for DEC. In May 2011 they extended this to also include hospital-samples. Prior to May 2010 – since they started testing in 2006 – *KMA Odense* tested for VTEC, EPEC, and ETEC as recommended by DSKM in 2012, additionally they also tested samples from people with persistent diarrhoea for DEC.

Since May 2009 *KMA Slagelse* have tested as recommended by DSKM in 2012; however travel to Norway, Sweden or Finland does not prompt a test. *KMA Hillerød* have also been testing on the same principles, however their DEC test is only prompted by travel outside Europe.

KMA Hvidovre has since they started been testing after the DSKM principles from 2012. For physicians ordering a test online (via WebReq) there is a mandatory field for travel history. On the old paper form there was also a field specifically for travel anamneses.

KMA Skejby and *KMA Esbjerg* have been testing for VTEC and EPEC after the DSKMs recommendations for VTEC from 2003, i.e. blood or samples from patients below 7 years. They also test for the two pathogens in patients suspected to have HUS or thrombotic thrombocytopenic purpura (TTP). In *Skejby* there is a mandatory field for travel history in the WebReq. This, however, was not reported to promt at DEC test.

KMA Aalborg tests on the DSKM principles from 2003 for VTEC plus samples from patients with HUS.

KMA Vejle did not participate, but lists the DSKM recommendation on their website. It is not known for how long they have been testing after these principles.

At the diagnostic laboratory at SSI they are testing for DEC according to the DSKM recommendations from 2012. They offer four 'analysis packages' in which DEC are included: Acute gastroenteritis, persistent diarrhoea, travel diarrhoea and 'standard enteric bacteria incl. DEC'.

4.2 Epidemiology of DEC

The data material on which this thesis builds comprises a total of 8272 individuals, experiencing a total of 8473 episodes of diarrhoea with 8601 types of the three pathotypes. In isolates from 250 episodes of diarrhoea, multiple VTEC, ETEC or EPEC were identified. 227 of these had a double infection, 21 patients were diagnosed with three *E. coli's*, and two people had a total of four different types of *E. coli* (two VTEC serotypes and two EPEC serotypes) in one course of illness. The combination of bacteria in episodes with two and three types of *E. coli* is listed in Table 3 and Table 2.

Table 3 – Combination of infections people for who two types of DEC was isolated (n= 227)

		Second infection		
		VTEC	EPEC	ETEC
First infection	VTEC	**31**	**51**	**28**
	EPEC		**59**	**49**
	ETEC			**9**

Table 2 – Combination of infections people for who three types of DEC was isolated (n= 21)

First infection	Second infection	Third infection	
		EPEC	ETEC
VTEC	VTEC	**2**	
VTEC	EPEC	**3**	**3**
EPEC	EPEC	**7**	**6**

The epidemiological results are based on *two* datasets – one containing one observation per serotype per six month i.e. potentially several observations per case (n=8601), and one containing only one observation per case (n=8473).

Eighty-eight percent of the isolates found positive for EPEC were forwarded to the reference laboratory for further analysis in the period from 2000 to 2012. This is the case for 7% of the ETEC isolates and 97% of the isolates found positive for VTEC. 81% of the EPEC isolates sent to the reference laboratory were confirmed. From Table 4 it is seen that most of the dis-concordance for EPEC happened prior to 2007, where the forwarded isolates did not meet the EPEC definition in the reference laboratory concerning serotype. This was primarily isolated O group 145 that were found to be O145:H28 or H:34 in the reference laboratory and thus did not meet the EPEC serotype criteria: O145:H45.

Table 4 - Difference between the findings at the reference lab and the KMA for EPEC isolates that are forwarded to SSI (n= 2425) and that are *not* confirmed to be EPEC (n=453)

Categorisation at reference laboratory	Before 2007 n (%)	2007 or later n (%)
-	31 (2.4)	34 (2.9)
A/EEC (Attaching and effacing *E. coli*)	314 (24.8)	34 (2.9)
EAggEC (enteroaggregative *E. coli*)	4 (0.3)	2 (0.2)
EAST1 (Enteroaggregative *E. coli* heat-stable enterotoxin 1)	9 (0.7)	13 (1.1)
EIEC (Enteroinvasive *E. coli*)	1 (0.1)	3 (0.3)
ETEC	3 (0.2)	3 (0.2)
VTEC	3 (0.2)	1 (0.1)
Total isolates received	**1267**	**1158**

Disregarding these isolates, which were found to be attaching and effacing (A/EEC), but did not meet the EPEC serotype-criteria, the concordance between the *KMA* and the reference laboratory findings increases to 96%. Fifty-eight percent of the ETEC isolates sent to the reference laboratory for subtyping are confirmed as ETEC. For 30% of the isolates, it is not possible to confirm the finding (-) – most of these prior to 2007 – and for the rest of the isolates, other *E. coli* were identified (cf. Table 6). 97% of the VTEC found at the *KMA* was confirmed at the reference laboratory.

Table 6 - Difference (%) between the findings at the reference lab and the *KMA* for ETEC isolates that are forwarded to SSI (262) and that are *not* confirmed to be

Categorisation at reference laboratory	Before 2007 n (%)	2007 or later n (%)
-	79 (39.5)	1 (1.6)
A/EEC	11 (5.5)	
EAggEC	5 (2.5)	4 (6.5)
EAST1	1 (0.5)	
EIEC	4 (2.0)	1 (1.6)
EPEC	3 (1.5)	
Total isolates received	**200**	**62**

Table 5 - Difference (%) between the findings at the reference lab and the *KMA* for VTEC isolates that are forwarded to SSI (n = 1937) and that are *not* confirmed

Categorisation at reference laboratory	Before 2007 n (%)	2007 or later n (%)
-	26 (2.9)	25 (2.4)
A/EEC	1 (0.1)	2 (0.2)
E. herbicola	1 (0.1)	
EAST1	3 (0.3)	4 (0.4)
EPEC	2 (0.2)	2 (0.2)
ETEC	1 (0.1)	
New EPEC	1 (0.1)	1 (0.1)
Total isolates received	**887**	**1050**

4.2.1 O groups

O groups O26, O55, O128, O145 and O157 are the most prevalent amongst the tested EPEC isolates from 2000 – 2012 (Cf. Figure 7). They are found in 13%, 22%, 10%, 11%, and 12% respectively of the isolates that are sent to the reference laboratory at SSI. The number of O145 sent in to the reference laboratory almost halved from 2000-2006 to

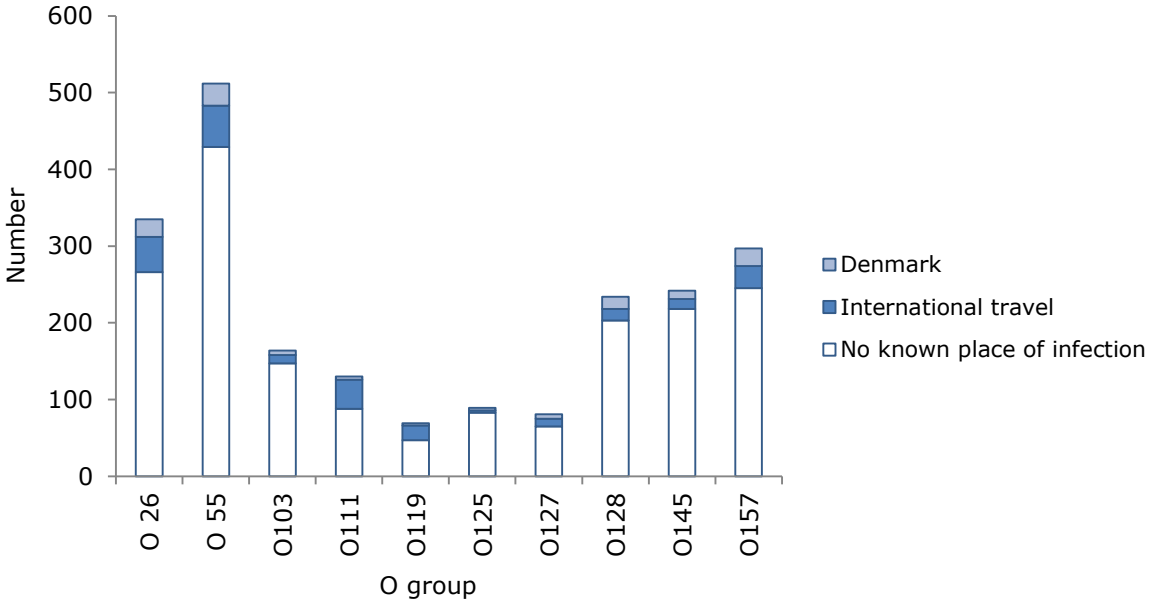

Figure 7 – The 10 most common EPEC O groups identified in the reference laboratory from 2000 – 2012 according to place of infection

2007-2012. Often no place of infection is registered for EPEC.

As mentioned, not many ETEC isolates are sent in for subtyping (7%) and no or only very few *KMA*s are can subtype ETEC locally. Primarily isolates from suspected outbreaks are forwarded. The most prevalent O group amongst the tested ETEC isolates is O6 – 24 of these were part of the lettuce outbreak.

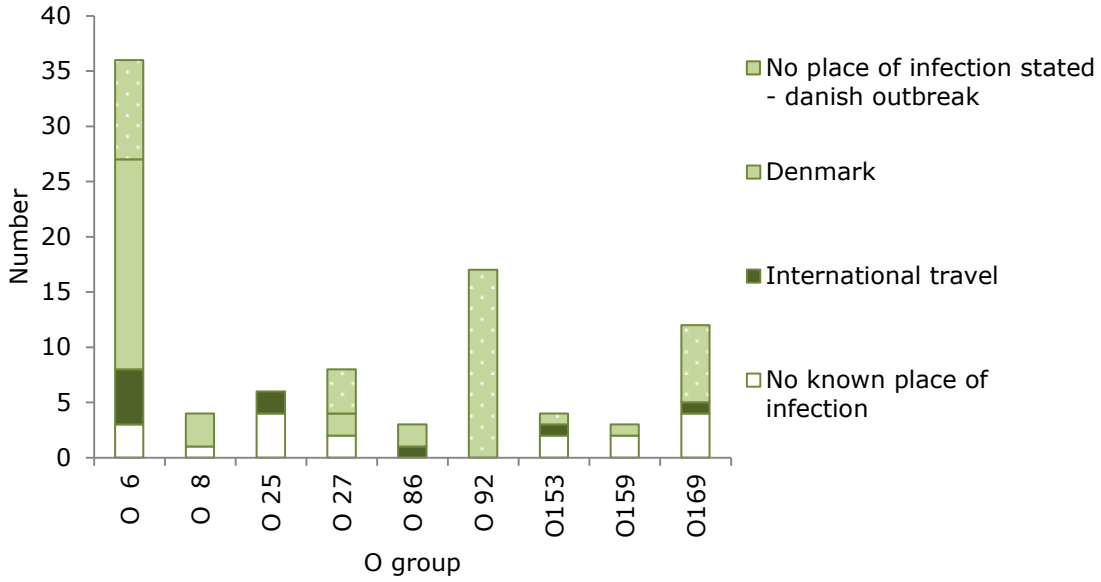

Figure 8 – The 9 most common ETEC O groups identified in the reference 2000 – 2012 according to place of infection

O157 is the most commonly detected VTEC O group; it is detected in 13% of the isolates that are sent to the reference laboratory. Also O26 and O103 are common (cf. Figure 9) they are found in 10% and 11% respectively of the isolates sent to the reference laboratory. The majority of these are reported as acquired in Denmark (>70%) – O26 and

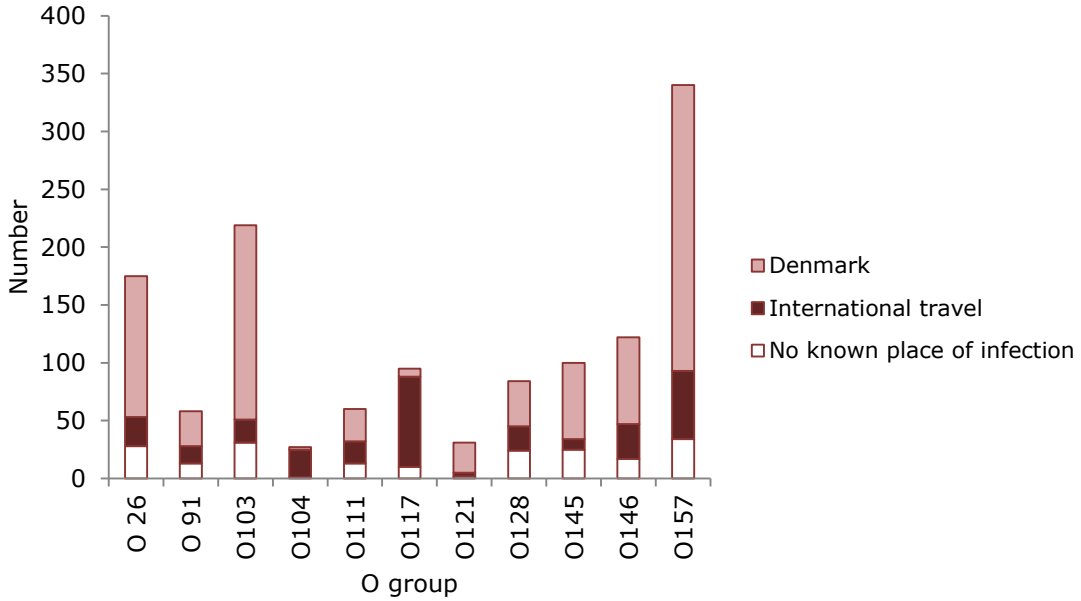

Figure 9 – The 11 most common VTEC O groups identified in the reference laboratory from 2000 – 2012 according to place of infection

O157 were seen in the previously described Danish outbreaks. O117 has been isolated from 95 samples and 82% are reported as acquired abroad; primarily (38%) in Africa. All the O104 with travel history are from the German outbreak in 2011, except for one case from 2008 who was infected in Afghanistan.

4.2.2 Time and seasonal variation

The total number of reported cases of VTEC, EPEC, and ETEC has increased twofold during the period from 2000 – 2012, from a total of 332 reported cases in 2000 to 705 reported cases of the three pathogens in 2012. For EPEC the average increase is 7.5 more reported cases or a 5.2% increase each year. Figure 11 illustrates the seasonal variations in the EPEC infections by travel status. For the EPEC infections an oscillation pattern with peaks in July and valleys in the winter months is constant over the years.

Figure 10 - Number of KMA reported and reference laboratory-confirmed cases of EPEC, 2000-2012

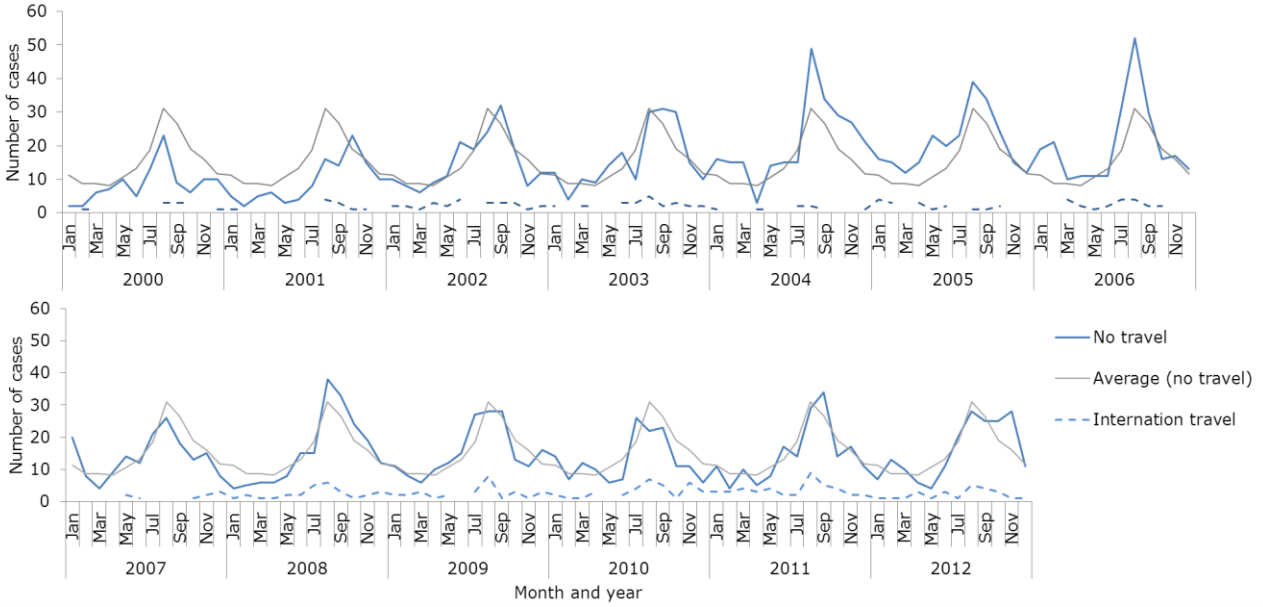

Figure 11 – Annual oscillation of EPEC cases. Number of cases by travel status (known international travel vs Denmark or unknown place of infection), month and year in Denmark 2000-2012 and average (2000-2012) monthly number of EPEC infections with no travel history.

There has not been much variation over the years when comparing with the average (grey line, Figure 11). In 2000-2001, where the diagnostics were still in the revival phase, the observed numbers of cases were below the average over the period, in 2004 to 2006 the observed number of cases in the summer peaks is exceeding the average over the period.

When focusing on the two most common O groups, a seasonal pattern is seen for both, with a marked peak in August for both O groups, and with a tail into October for O55 (cf. Figure 12). When stratifying on *region*, it is seen that the peak of O26 infections in *Region Hovedstaden* is smaller than in *Region Sjælland* and *Region Syddanmark*. Instead, a marked peak of O55 infections is seen for *Region Hovedstaden* (cf. Figure 13).

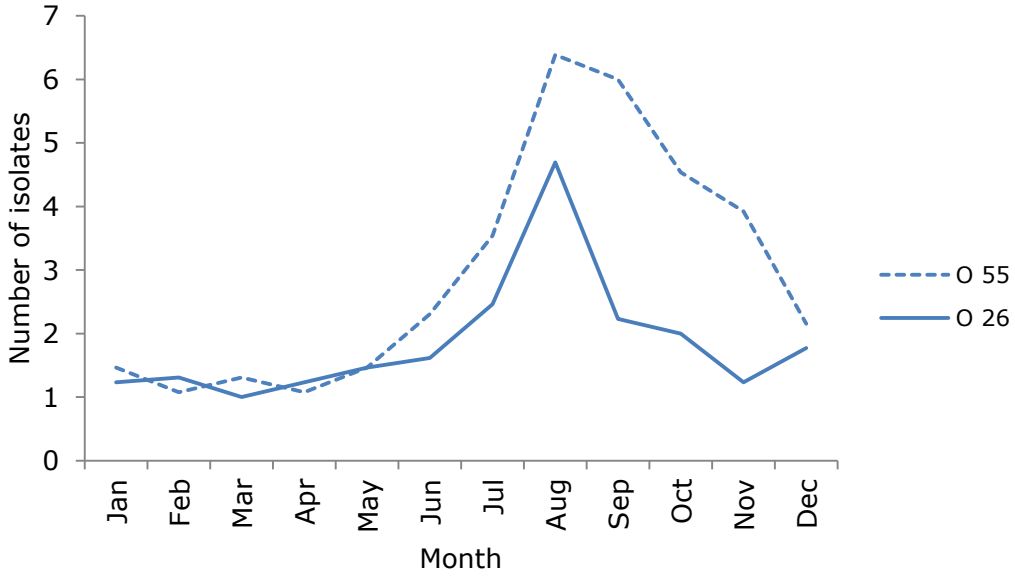

Figure 12 – Seasonal distribution of EPEC O 26 and O 55. Average number of O 26 and O 55 isolate at the reference laboratory from patients without travel anamneses, in Denmark from 2000- 2012, by month.

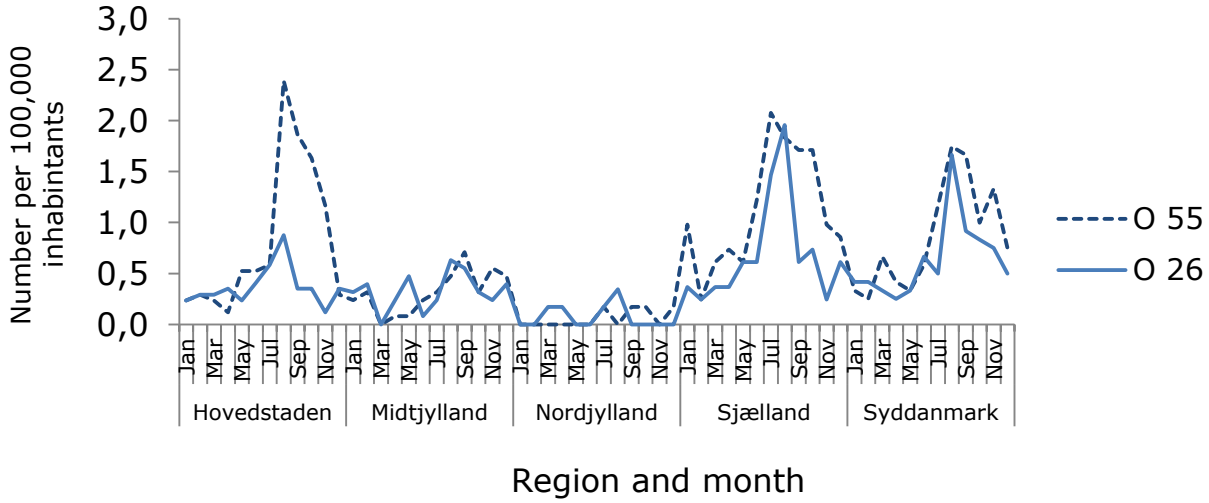

Figure 13 – Seasonal and regional distribution of EPEC O 26 and O 55. Number of O 26 and O 55 isolated at the reference laboratory in Denmark from 2000- 2012 (excl. travelers), by month.

The yearly number of ETEC cases has risen from 124 to 289 from 2000 to 2012, this is close to 10 cases, or 5.5%, per year.

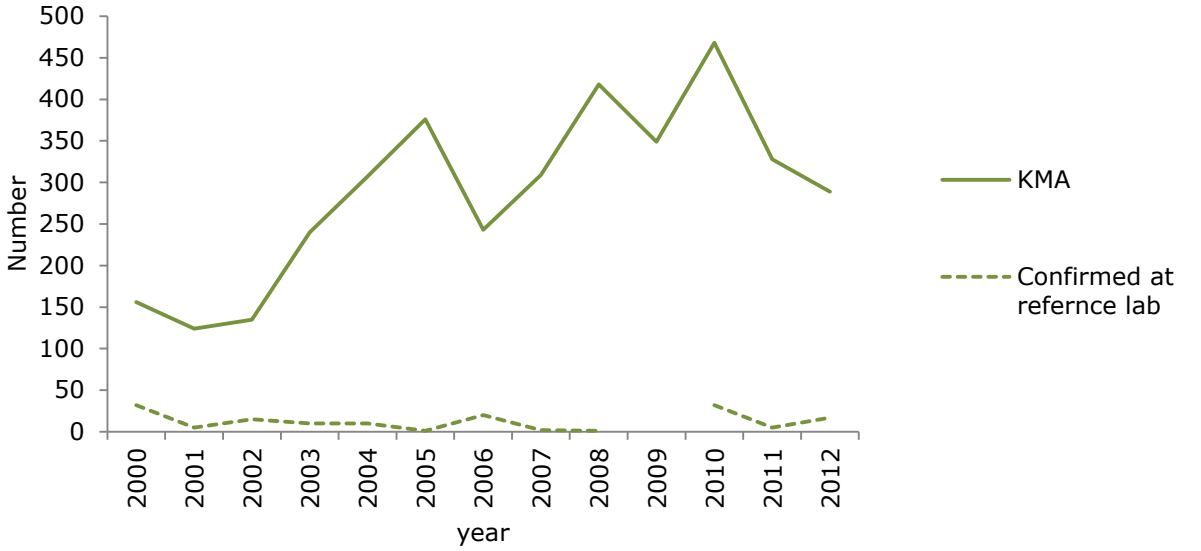

Figure 14 - Number of KMA reported and reference laboratory-confirmed cases of ETEC, 2000-2012

A seasonal pattern with peaks in August and valleys from December to June is seen for both travel-related and ETEC infections for which there is no information on place of infection (Cf. Figure 15).

When looking at the infections by year and month (cf. Figure 16), the ETEC outbreaks in November 2006 and in January 2010 and 2012 appear on the graph. In the beginning of the period, not many were tested for ETEC at all, but from 2003/2004 the number began to rise. From 2007, an increasing proportion of the cases of ETEC have a registered place of infection, and a travel-seasonal pattern, with peaks in August and in the winter- and/or Easter holiday months. In 2010 a marked peak of infections acquired during international travel in people from all over Denmark was seen. The majority of these were acquired in Turkey (44%) and Egypt (27%).

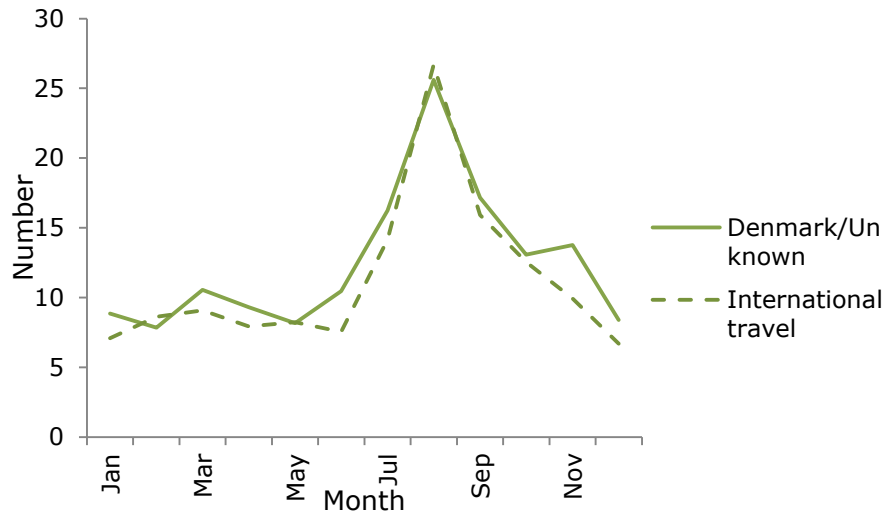

Figure 15 - Seasonal distribution of ETEC. Average number of cases (excl. outbreak cases) in Denmark from 2000- 2012, by month.

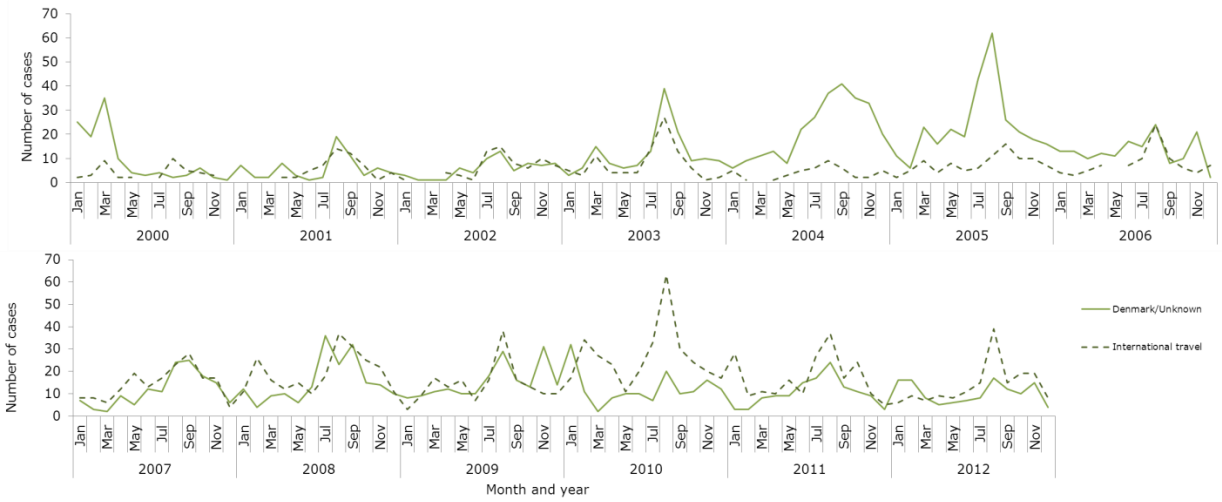

Figure 16 – Annual oscillation of ETEC cases. Number of cases by travel status (known international travel vs Denmark or unknown place of infection), month and year in Denmark 2000-2012.

The number of reported VTEC cases increased on average by 10 cases (~10%) each year from 2000 – 2012; from 61 in 2000 to 203 in 2012.

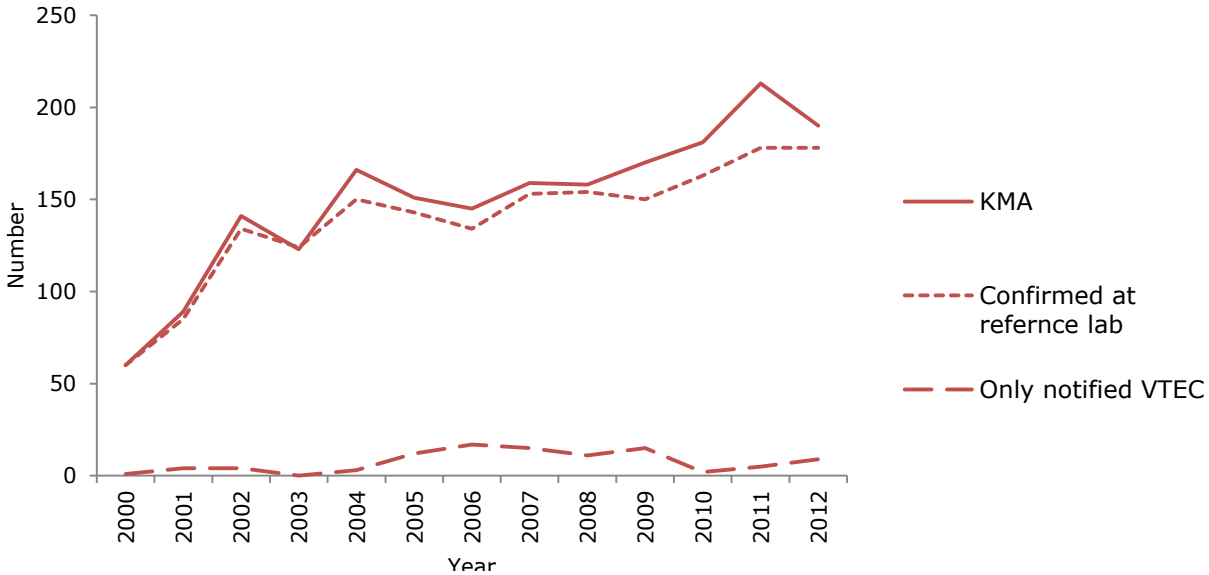

Figure 17 - Number of notified, KMA reported and reference laboratory-confirmed cases of VTEC, 2000-2012

The VTEC outbreaks in the beginning of 2004, January 2007, May 2011 and October 2012 are seen on Figure 18. As for EPEC and ETEC and an oscillation pattern in the number of infections is also seen, however the numbers are lower and fluctuations from the average are more common.

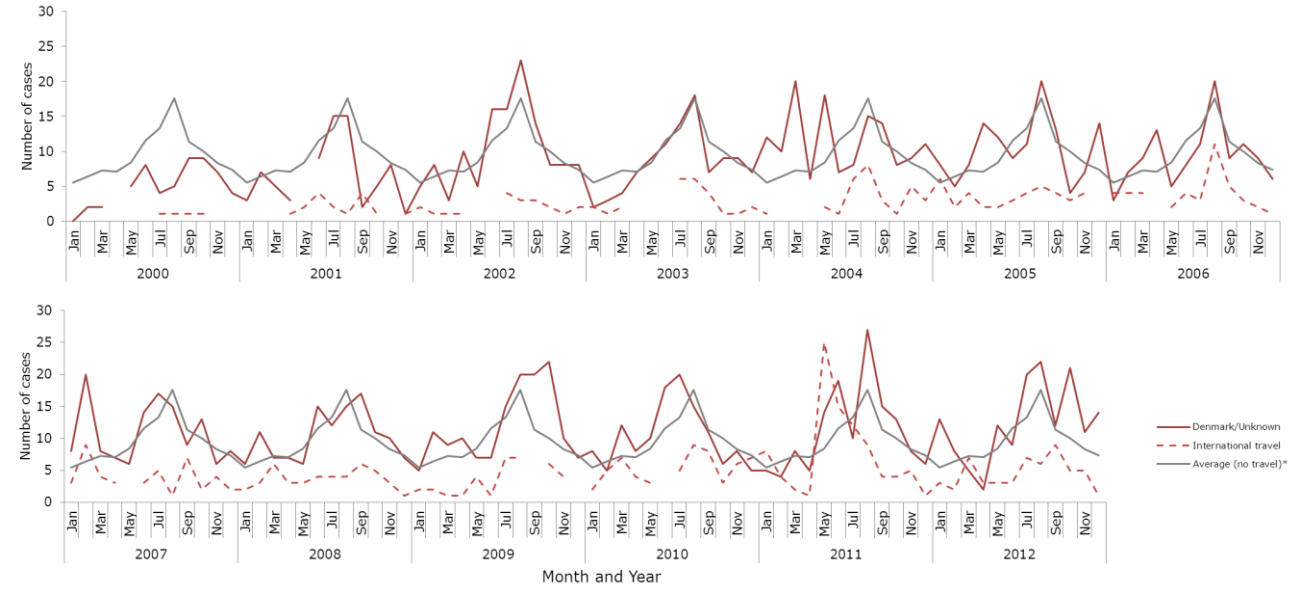

Figure 18 – Annual oscillation of VTEC cases. Number of cases by travel status (known international travel vs. Denmark or unknown place of infection), by month and year in Denmark 2000-2012 and average (2000-2012) monthly number of VTEC infections (excl. travel history and outbreaks).

A seasonal pattern for the two most common VTEC O groups (excluding isolates from outbreaks and from travellers) is seen, with a marked summer-peak of O103 infections and valley from November to May; and a more constant number of O157 infections throughout the year, though with a peak in August and valley in November-January.

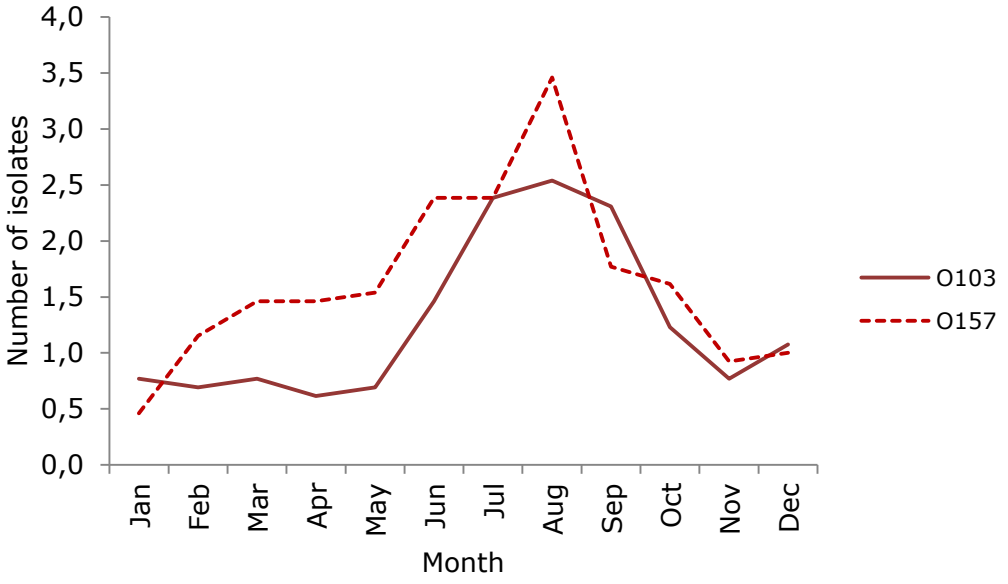

Figure 19 - Seasonal distribution of VTEC O groups O103 and O157. Total number of VTEC O103 and VTEC O157 isolated at the reference laboratory from patients that have not been travelling and are not a part of an outbreak in Denmark from 2000- 2012, by month.

4.2.3 Place

4.2.3.1 Travel

In 2012, 12% of the reported EPEC cases had a place of infection registered. Fifty-seven percent of the ETEC infections had been acquired outside of Denmark. This was the case for 27% of the reported VTEC cases and (cf. Figure 21). The proportion of the reported cases with known travel history who had acquired their infection in Europe was twice as high for VTEC as for EPEC, and more than three times as high as for ETEC – also after exclusion of cases from the German outbreak. 30% of the ETEC cases with known travel history had travelled to Egypt (cf. Figure 20), this was the case for 17% of the EPECs and only 4% of the VTEC cases with a known travel history.

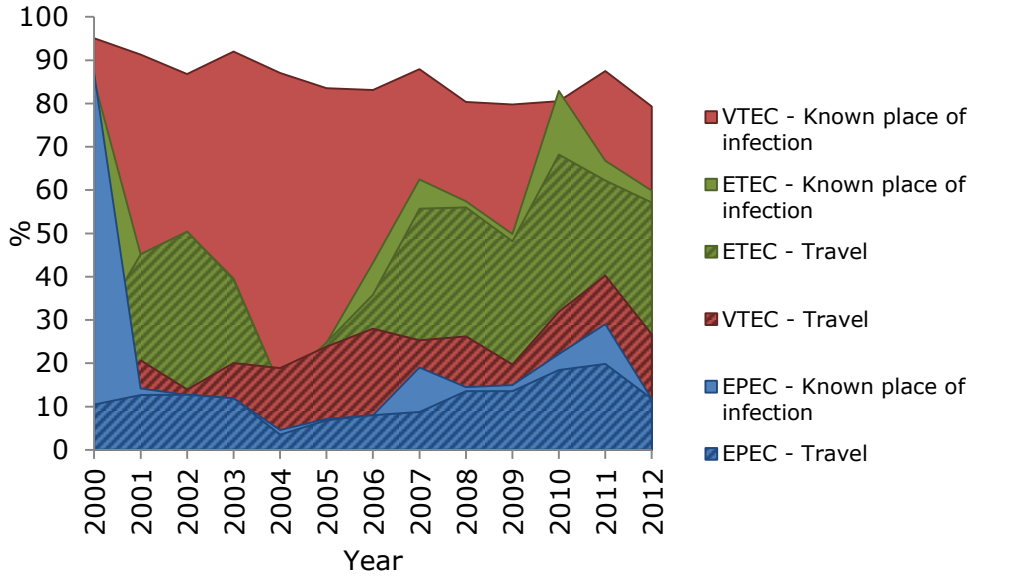

Figure 21 – Place of infection. Percentages of cases with known travel history and a registered place of infection (Denmark + international travel)

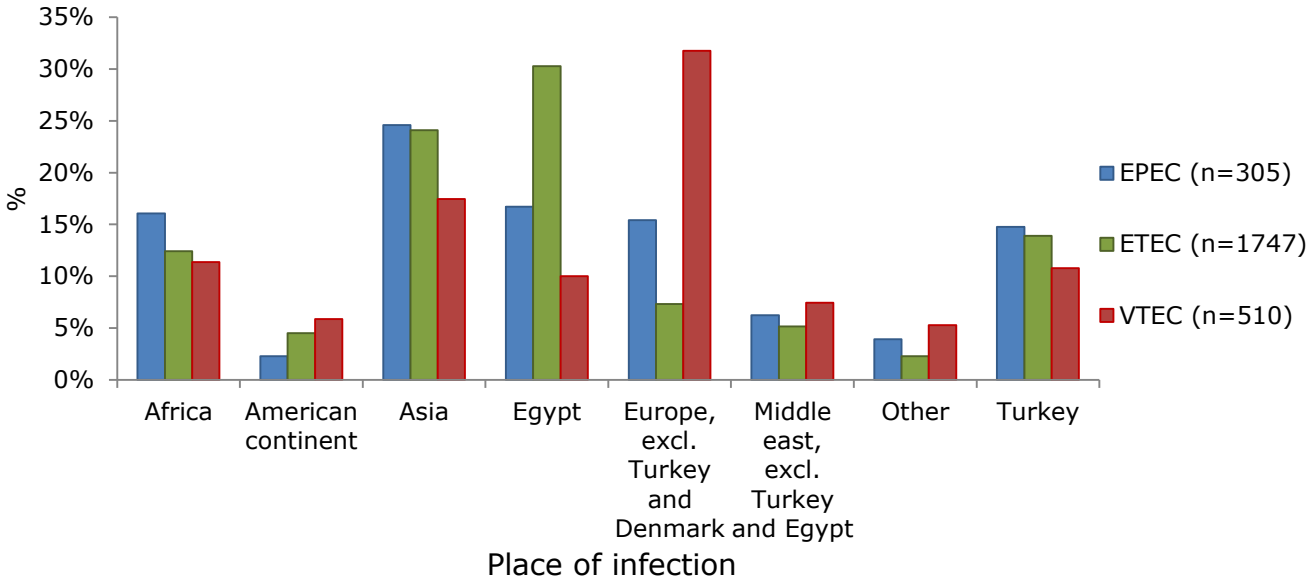

Figure 20 – Places of infection during international travel (% of cases with known travel anamneses) by pathogen

4.2.3.2 Regioner and landsdele

In the following section, the reported incidence of the three pathogens will be showed in *regions* and *landsdele*. *Region Nordjylland* is the same as *landsdel Nordjylland* and for that reason *Nordjylland* will only be portrayed on the graph illustrating the incidences by *region*.

The reported incidence of EPEC has been increasing in *Region Syddanmark*. In *Region Nordjylland* the incidence has been stable at below 1 case per year per 100,000 inhabitants and no cases since 2009.

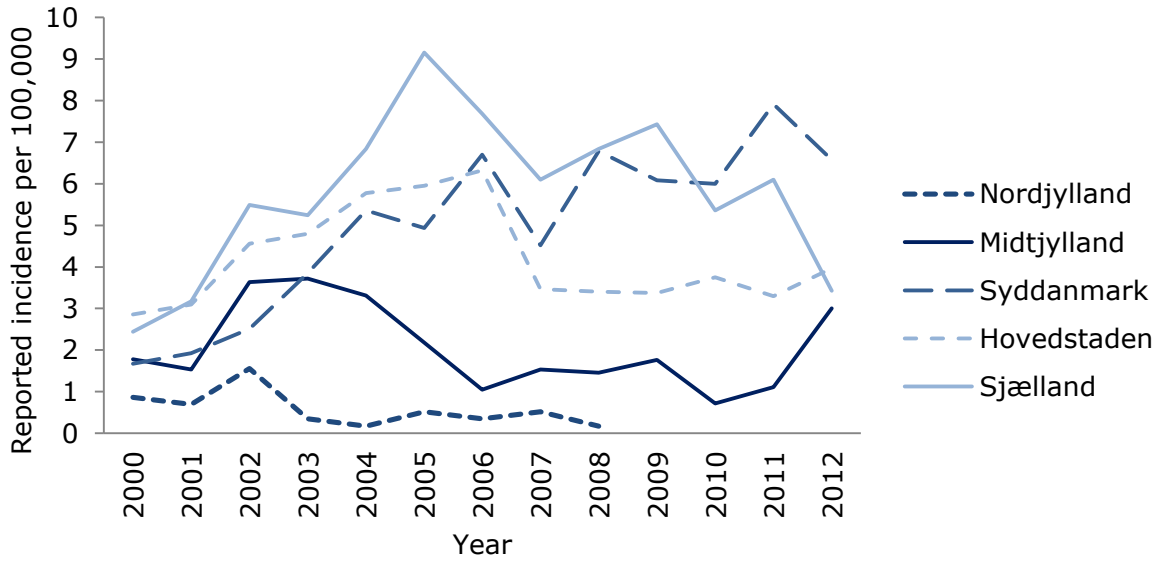

Figure 22 - Reported incidence of EPEC in Denmark 2000 – 2012 by *region*

The incidence of EPEC on *Region Sjælland* increased until 2005, but dropped in several sittings after that. Part of the decline can maybe be explained by the fact that SSI used to serve as a diagnostic lab for the Roskilde area. This was transferred to *Slagelse KMA* in 2010. In the beginning of 2009 Octaplex PCR was introduced in *Slagelse KMA* as well as a principle of testing of samples from certain patients despite the doctor's request. This should manifest itself by an increased reported incidence when this method is being more widespread.

When disregarding 2011 - as there may have been tested more for DEC in general in this period due to the German VTEC outbreak, the decrease seen for *Region Sjælland* seems to be caused by a decrease in both *landsdele*. What also appears when splitting up the regions into *landsdele* for *Region Sjælland*, is that the peak in 2009 for *Region Sjælland* was caused by an increased incidence in *Østsjælland*. The reported incidence of EPEC in *Region Syddanmark* has been increasing throughout the period with the highest incidence in 2011, the year of German outbreak and the year they started to test all samples for DEC; in 2011 the incidence reached almost 8/100,000.

When splitting up *Region Syddanmark* into *landsdele*, it appears that Fyn was accountable for the majority of the reported cases of EPEC.

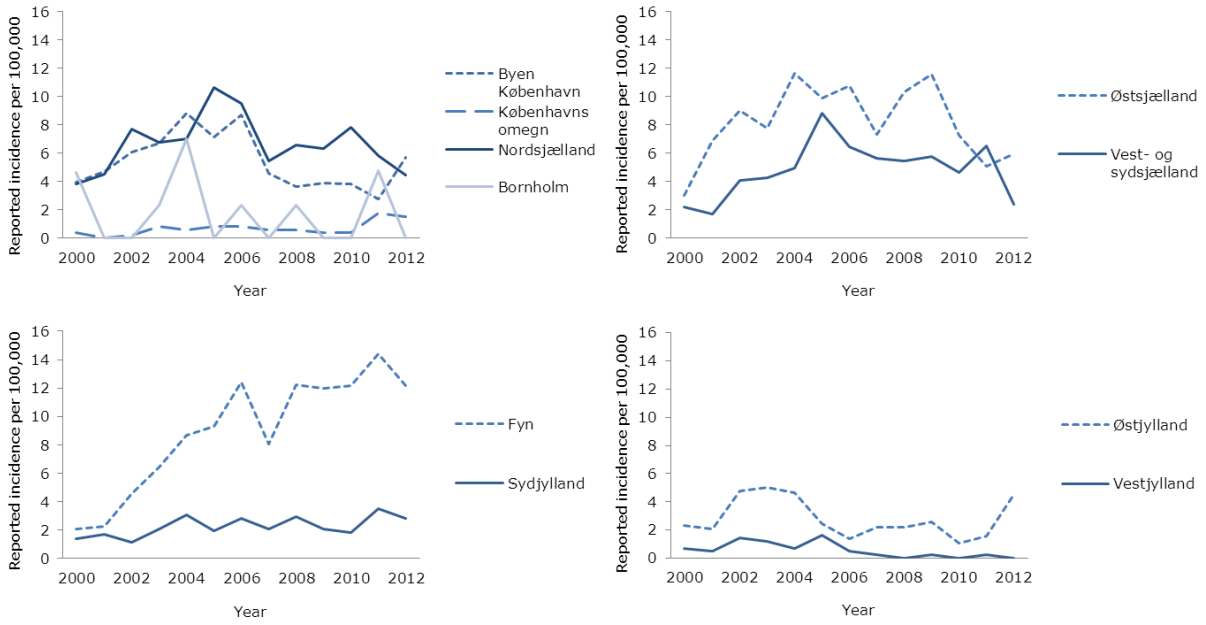

Figure 23 - Reported incidence of EPEC in Denmark 2000 – 2012 by *region* and *landsdel*

When splitting up *Region Hovedstaden*, it appears that the incidence of EPEC in *landsdel København og Omegn* has been very low until 2010 where it increases from 0.2 to 1.5. The reported incidence of *Bornholm* is very low; the population on the island is so small that one case is equivalent to an incidence of 2.3/100,000.

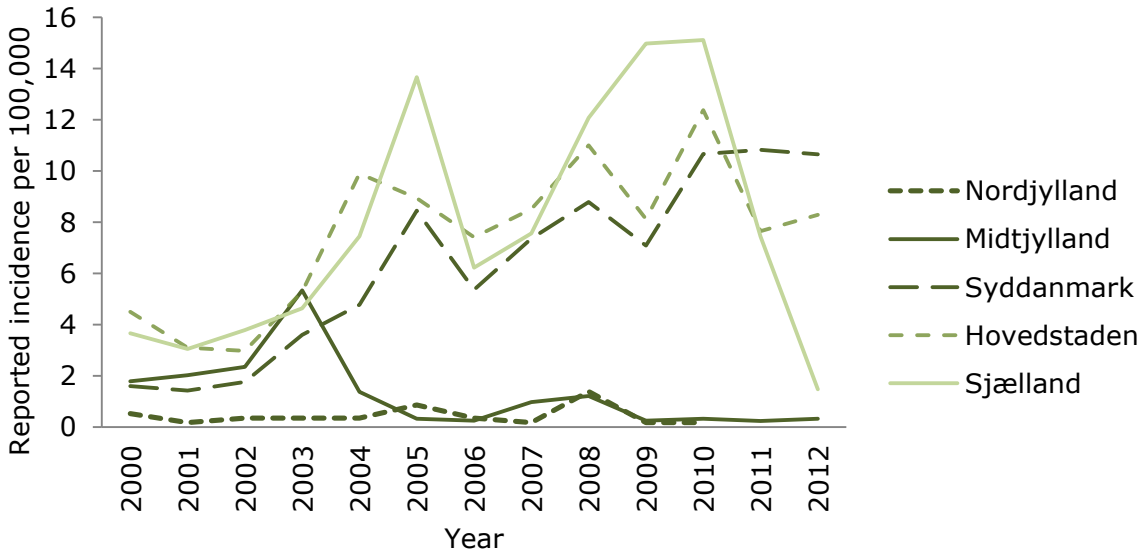

Figure 24 - Reported incidence of ETEC in Denmark 2000 – 2012 by *region*

When looking at the reported incidence of ETEC according to *region*, it appears that the incidence in *Region Syddanmark* and *Region Hovedstaden* has increased. What leap out at

us is *Region Sjælland* and the peaks in 2005 and in 2008-2010 – followed by a huge drop from 2010 to 2012 from 15/100,000 to 1.3/100,000. When splitting up on *landsdele*, it seems as if *Østsjælland* was accountable for the first peak in 2005 and for the increase in 2008, solely. Reported cases from *Vest- og Sydsjælland* then accounted for the 'continued increase' in 2009 and 2010. A small peak of reported ETEC cases was also seen in *Vest- and Sydsjælland* in 2005, but not as dramatic as the peak for *Østsjælland*.

The incidence in *Region Nordjylland* has been stable with an incidence well below 1/100,000 – and no cases in 2011 and 2012. The *KMA* in *Nordjylland* (Aalborg) do not offer diagnostics of ETEC or EPEC why samples has to be sent to SSI if it is suspected to be infected with one of these. Even if the tests come out negative for *Salmonella*, *Campylobacter* etc. which are in the standard pathogenic enteric bacteria 'package' in most *KMAs* the sample is unlikely to be forwarded to SSI for further testing as the patient may already have recovered.

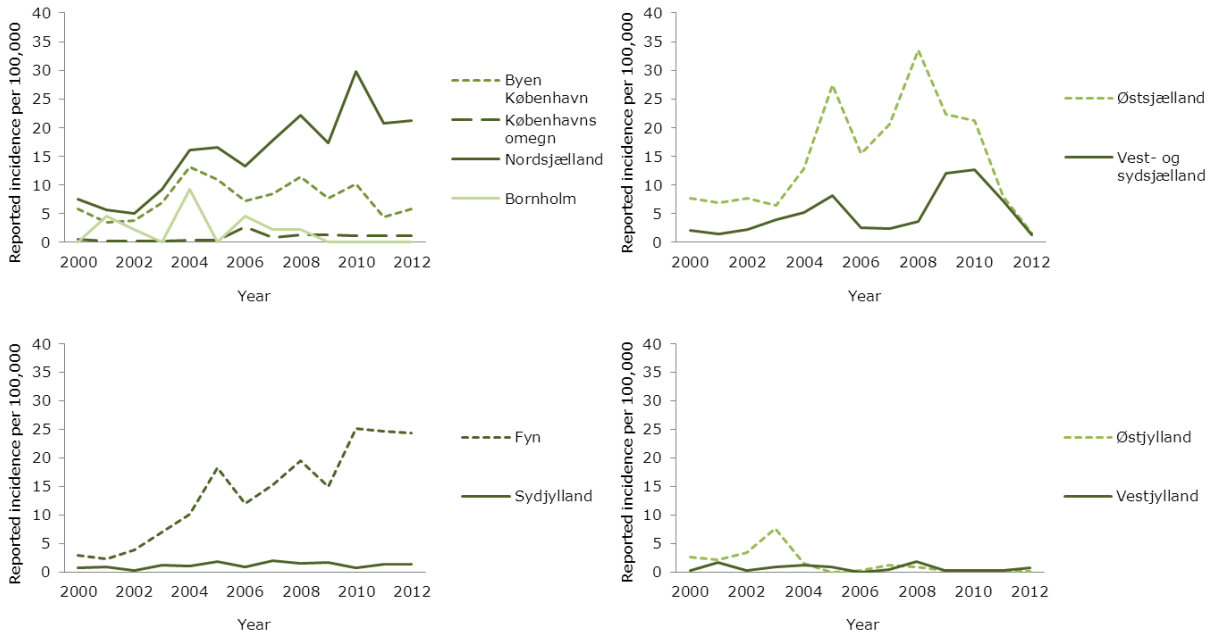

Figure 25 - Reported incidence of ETEC in Denmark 2000 – 2012 by *region* and *landsdel*

The reported incidence of ETEC in *Region Midtjylland* is around 2/100,000 all years except for 2003 where the incidence was 5.3. As was the case for EPEC, the reported incidence in *Region Syddanmark* has increased steadily throughout the period, due to an increase on *Fyn*. Contrary to the picture for EPEC, the reported incidence of ETEC in *Region Hovedstaden* is increasing steadily from 2000 to 2012.

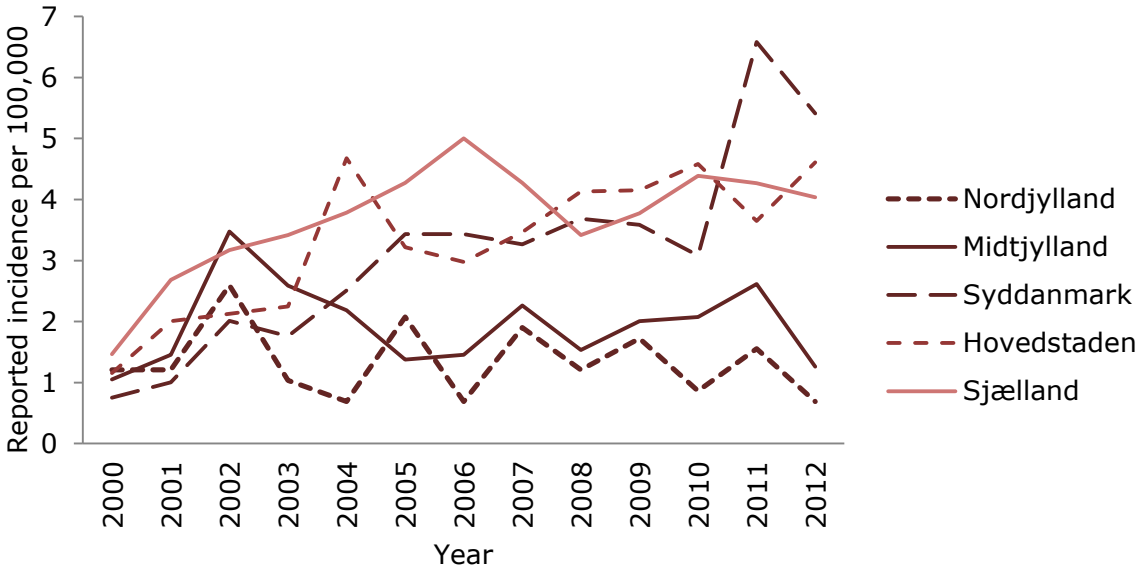

Figure 26 - Reported incidence of VTEC in Denmark 2000 – 2012 by *region*

The incidence of VTEC has increased threefold from 2000- 2012 in three *regions*, namely *Region Hovedstaden*, *Syddanmark*, and *Sjælland* from around 1/100,000 in 2000 to more than 4/100,000 in 2012. In *Region Syddanmark*, a great peak is seen in 2011 with 6.7 reported cases per 100,000 inhabitants due to the German outbreak and increased testing. In *Region Hovedstaden* a peak is seen in 2004, possibly due to the VTEC O157 outbreak at the visiting farm in *Nordsjælland*.

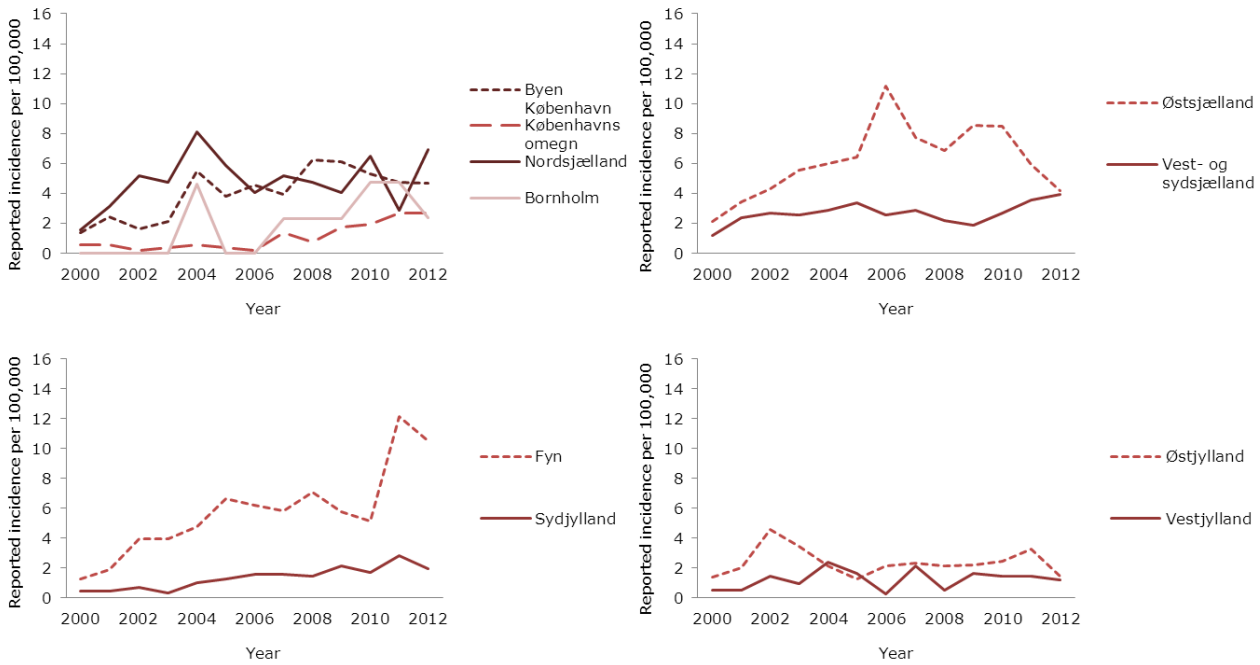

Figure 27 - Reported incidence of VTEC in Denmark 2000 – 2012 by *region* and *landsdel*

When splitting each *region* up on *landsdele*, it is found that the peak in *Region Sjælland* in 2006 was caused by an increased number of cases in *Østsjælland*. As was the case for EPEC and ETEC, the increase in the reported incidence of *Region Syddanmark* seems to be driven by an increased number of cases from *Fyn*. However, the incidence of *Syddanmark* also seems to have been increasing over the period, from below 1/100,000 to 2/100,000.The peak seen in *Region Midtjylland* in 2002 seems to have been caused by an increase in the reported incidence of *Østjylland.*

4.2.3.3 Municipality

The islands Christiansø and Læsø had no reported cases of any of the pathogens between 2000 and 2012 – these are the two least populated municipalities (population of 103 and 1897 persons in 2012), so this is not unexpected.

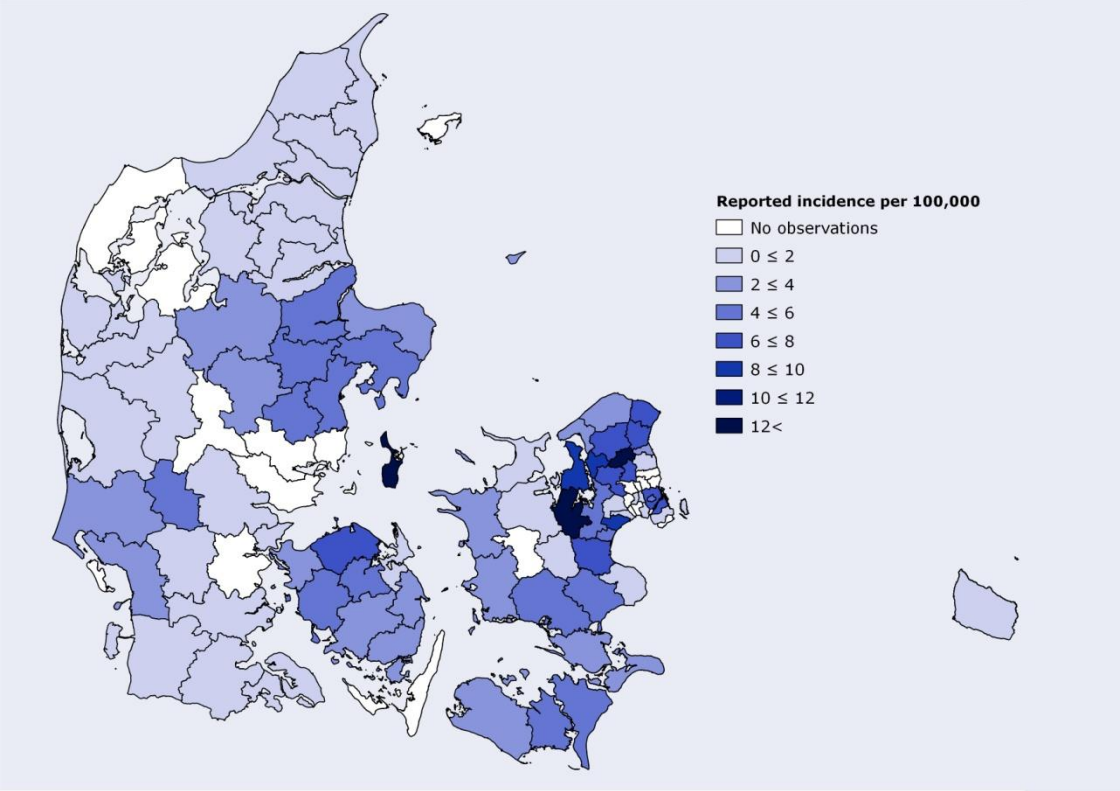

Figure 28 - Average yearly reported incidence of EPEC in Denmark from 2000-2003, by municipality

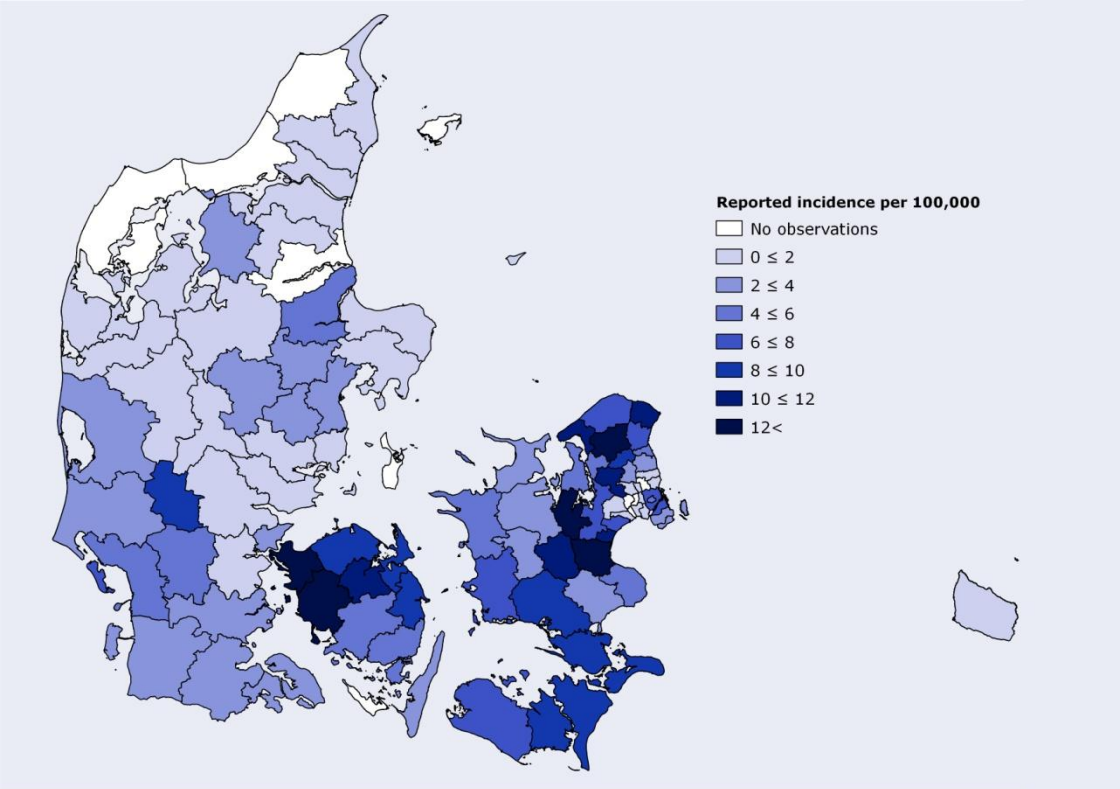

Figure 29 - Average yearly reported incidence of EPEC in Denmark from 2004-2008, by municipality

There were no reported EPEC cases from Albertslund, Mors, or Thisted additional to Christiansø and Læsø from 2000 to 2012. Figure 28 shows the average yearly reported incidence of EPEC by municipality in 2000-2003. The average reported incidence ranged from 0 in 23 municipalities to 12.6/100,000 on Samsø. On Figure 29 the average reported incidence in 2004-2008 is mapped. Here the blue colour of *Fyn* and most parts of *Sjælland* has darkened, symbolising a rise in the reported incidences. From 2004-2008 13 municipalities had no reported cases of EPEC. The incidence of EPEC in Lejre municipality was higher compared to the surrounding municipalities in both periods; this area (*Roskilde Amt/Landsdel Østsjælland*) has been served by the SSI laboratory until approximately 2010.

In the period from 2009-2012 almost no cases of EPEC was reported in the western and northern part of Jutland (cf. Figure 30). Only *Struer* municipality had reports of EPEC cases – a sample from a least one of these were sent directly by a GP to SSI. Most of *Fyn* reached the darkest blue colour with a reported incidence of above 12 with *Kerteminde* reaching 22 cases per 100,000 inhabitants. Ringsted appears as a dark centre on *Sjælland* with a reported incidence of 11.4 from 2009-2012.

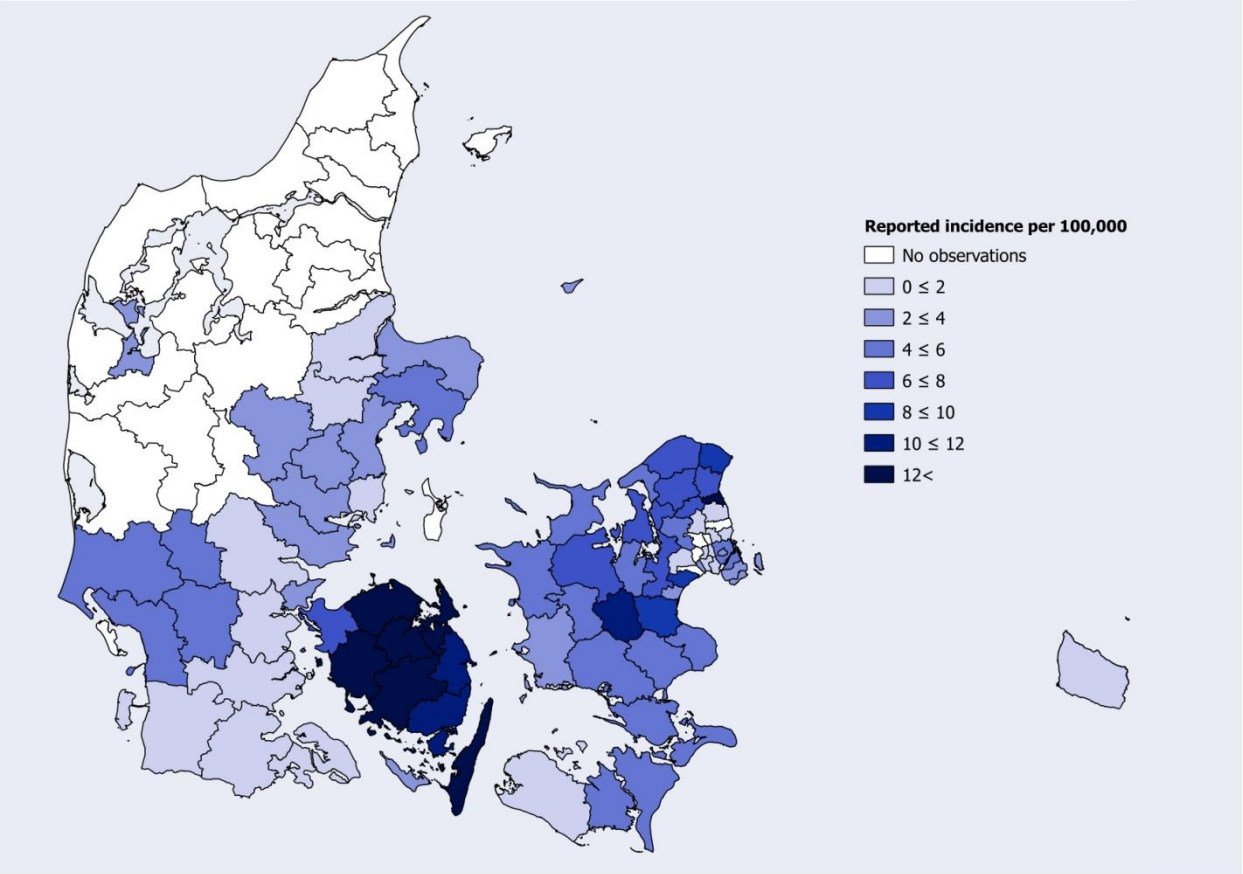

Figure 30 - Average yearly reported incidence of EPEC in Denmark from 2009-2012, by municipality

Six municipalities had no reported cases of ETEC between 2000 and 2012: Vallensbæk, Christiansø, Lemvig, Samsø, Morsø and Læsø. From 2000-2003 27 municipalities had no reported cases of ETEC - Mainly in the north and western part of Jutland, south Jutland and around Copenhagen. The incidence in the rest of the municipalities ranged from 0.3 to above 10 cases per 100,000 in Fredensborg, Allerød and Hørsholm (11.5, 12.8, and 15.4 per 100,000 inhabitants respectively).

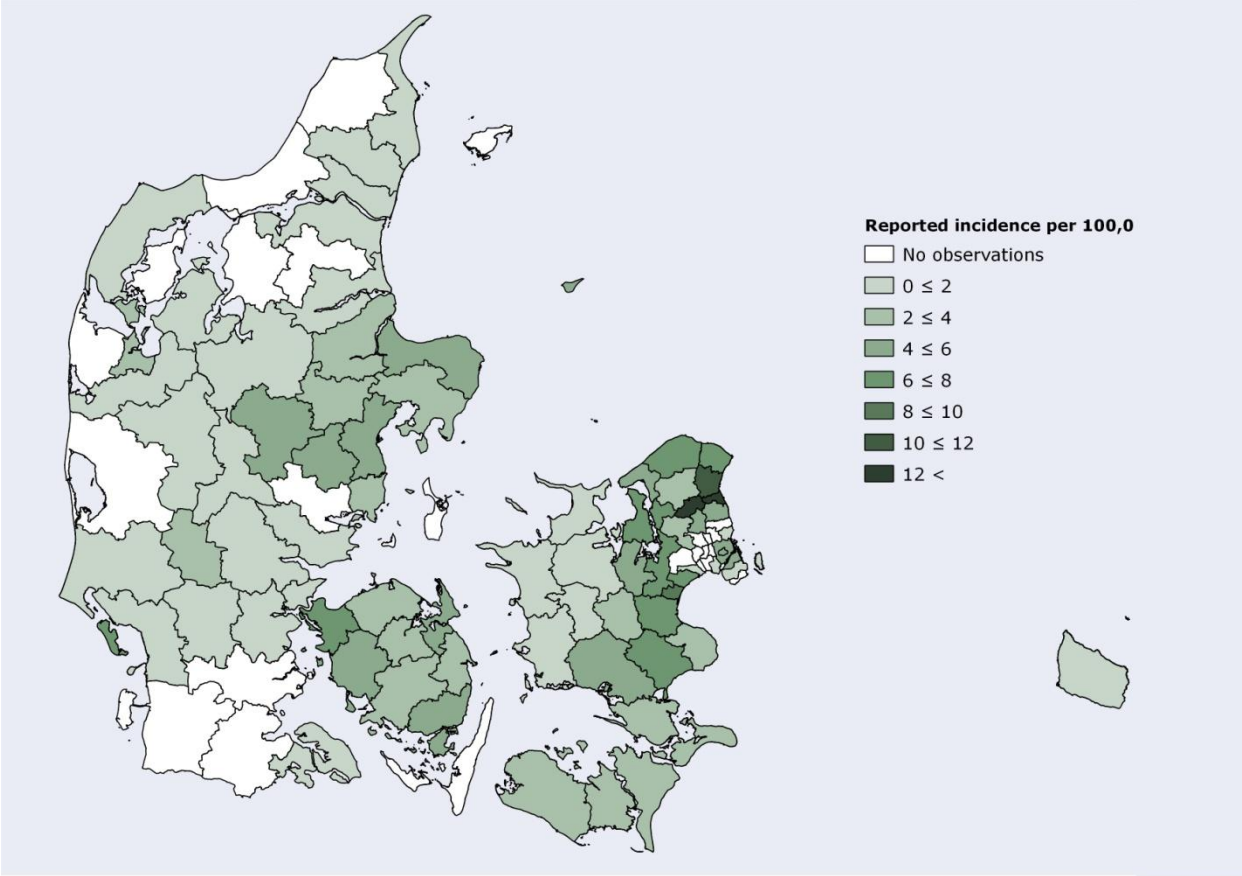

Figure 31 - Average yearly reported incidence of ETEC in Denmark from 2000-2003, by municipality

From 2004-2008, the average yearly incidence ranged from 0 in 16 municipalities to around 28 in Allerød and Solrød. The reported incidence in the municipalities in *Nordsjælland* was higher during this period than from 2000-2003. Hillerød took over the responsibility of DEC testing in *Nordsjælland* and introduced PCR in 2004. Also the incidences on *Fyn* increased. *København og Omegn* i.e. the municipalities around Copenhagen remained light green. On Figure 33 illustrating the average yearly reported incidence in 2009-2012, the colour of the western part of *Sjælland* has darkened compared to 2004-2008, however the *Østsjælland* is lighter green in this period compared to 2004-2008. In 2009 *Slagelse KMA* introduced PCR and in 2010 they took over the diagnostics of *landsdel Østsjælland*. Almost all municipalities on *Fyn* had a reported incidence of above 12/100,000. 12 cases in *region Midt-* and *Nordjylland* were seen, in MiBa it appear that eight of these were sent directly from GPs to SSI, and one was diagnosed in Odense.

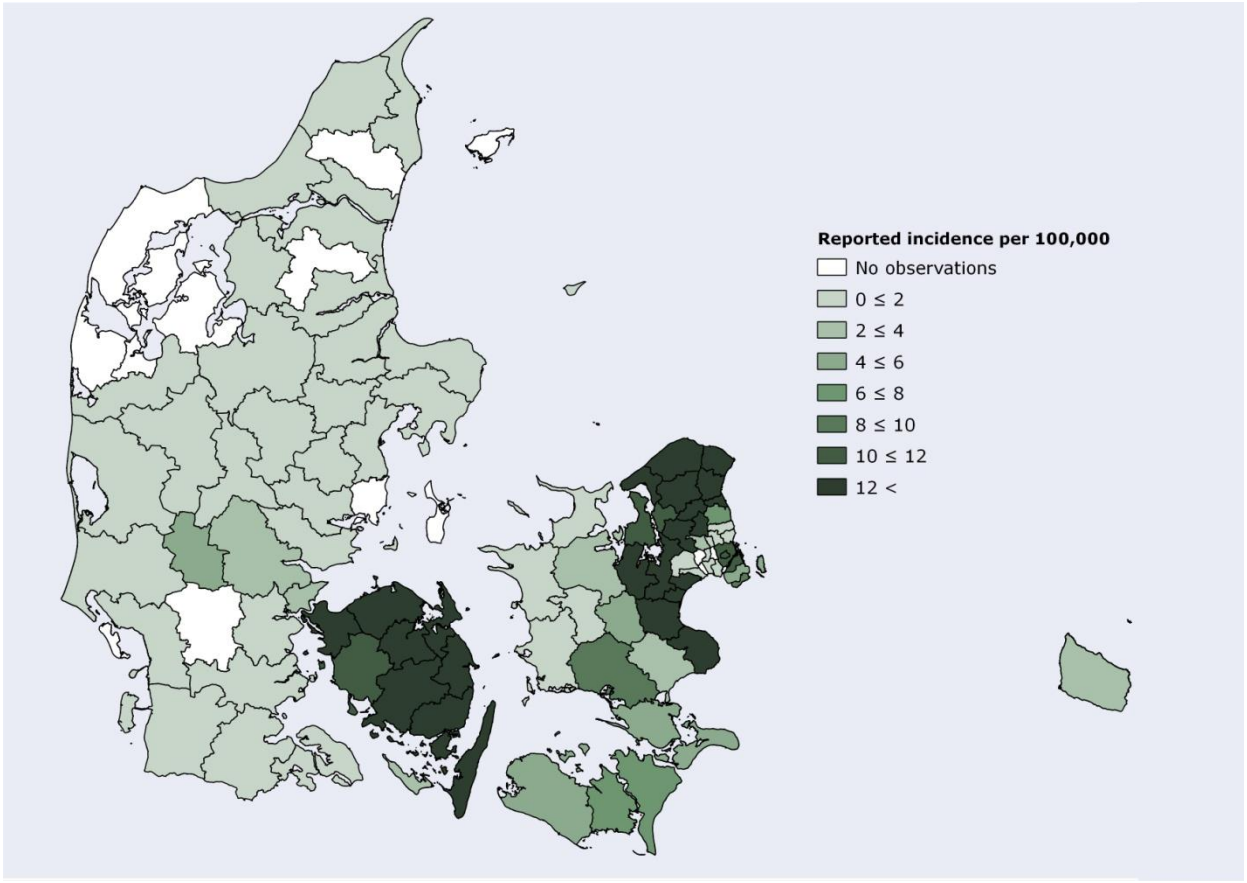

Figure 33 - Average yearly reported incidence of ETEC in Denmark from 2004-2008, by municipality

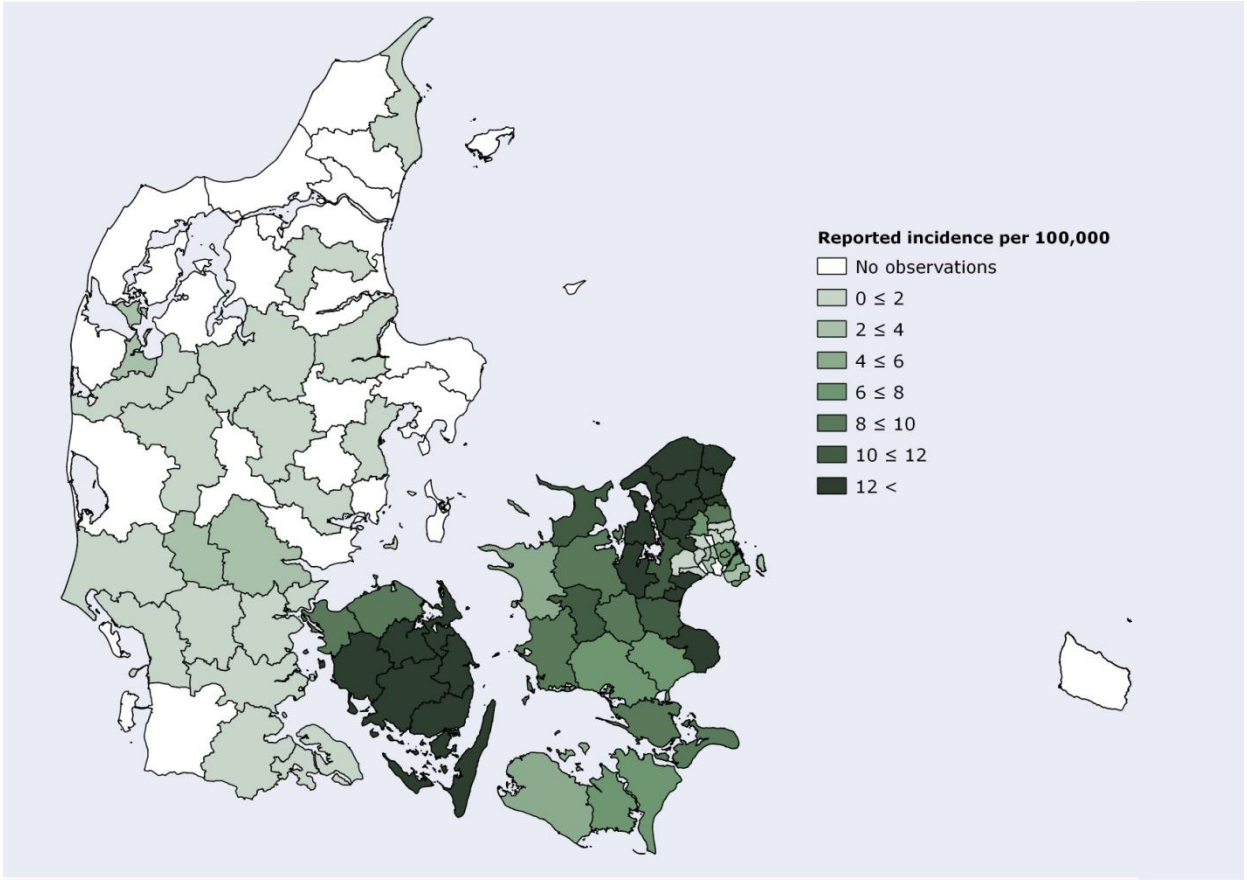

Figure 32 - Average yearly reported incidence of ETEC in Denmark from 2009-2012, by municipality

From 2000-2012 three municipalities had no cases of VTEC – namely Christiansø, Fanø and Læsø. 27 municipalities had no reported cases of VTEC in the period from 2000-2003. The incidence in the rest of the municipalities was between 0.4 and 7.6 reported cases per 100,000 person years. Egedal, Ærø and Fredensborg had incidences of above 7/100,000. In the period 2004-2008 the incidence in Lejre and Køge municipality was higher than the surrounding municipalities as was also the case for EPEC and ETEC. Only few cases in the western part of *Sjælland* are reported. The incidence in *København og Omegn* remains low.

Figure 35 shows the average reported incidence of VTEC in the period 2009-2012 by municipality. As was the case for EPEC and VTEC, Fyn has a higher incidence than the rest of the country. In 2010 the *KMA Odense* started to test all samples from GPs for DEC and since 2011, in connection to the Germany VTEC outbreak, all samples have been tested for DEC.

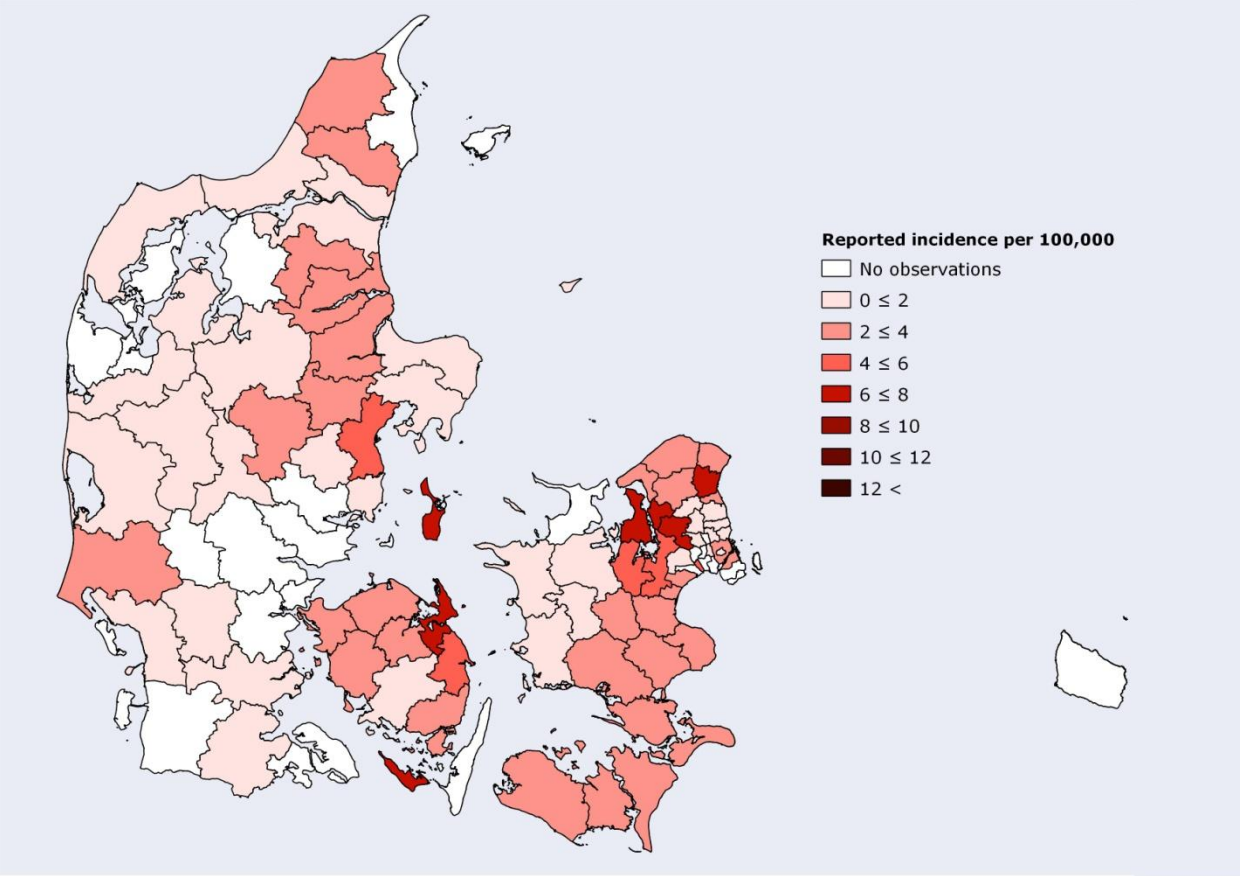

Figure 34 - Average yearly reported incidence of VTEC in Denmark from 2000-2003, by municipality

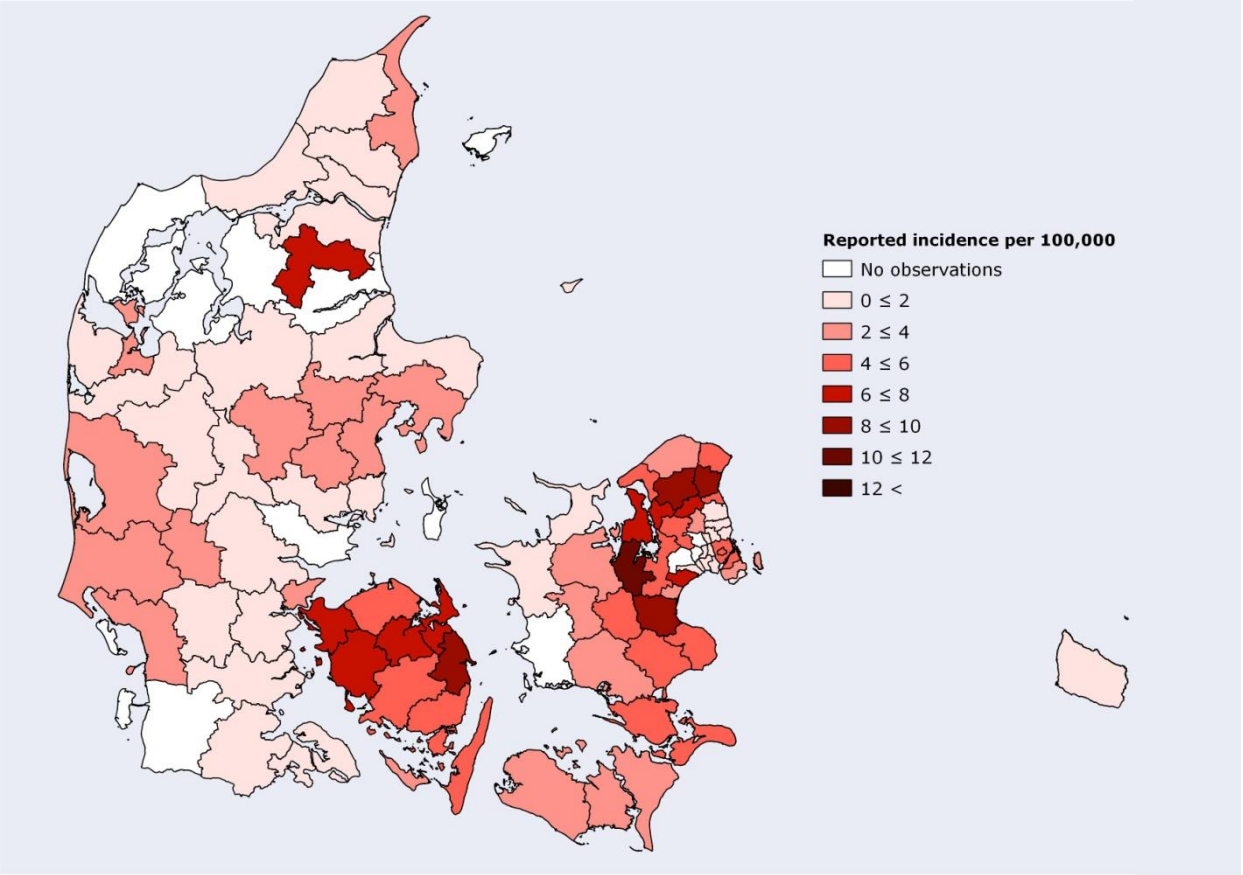

Figure 36 - Average yearly reported incidence of VTEC in Denmark from 2004-2008, by municipality

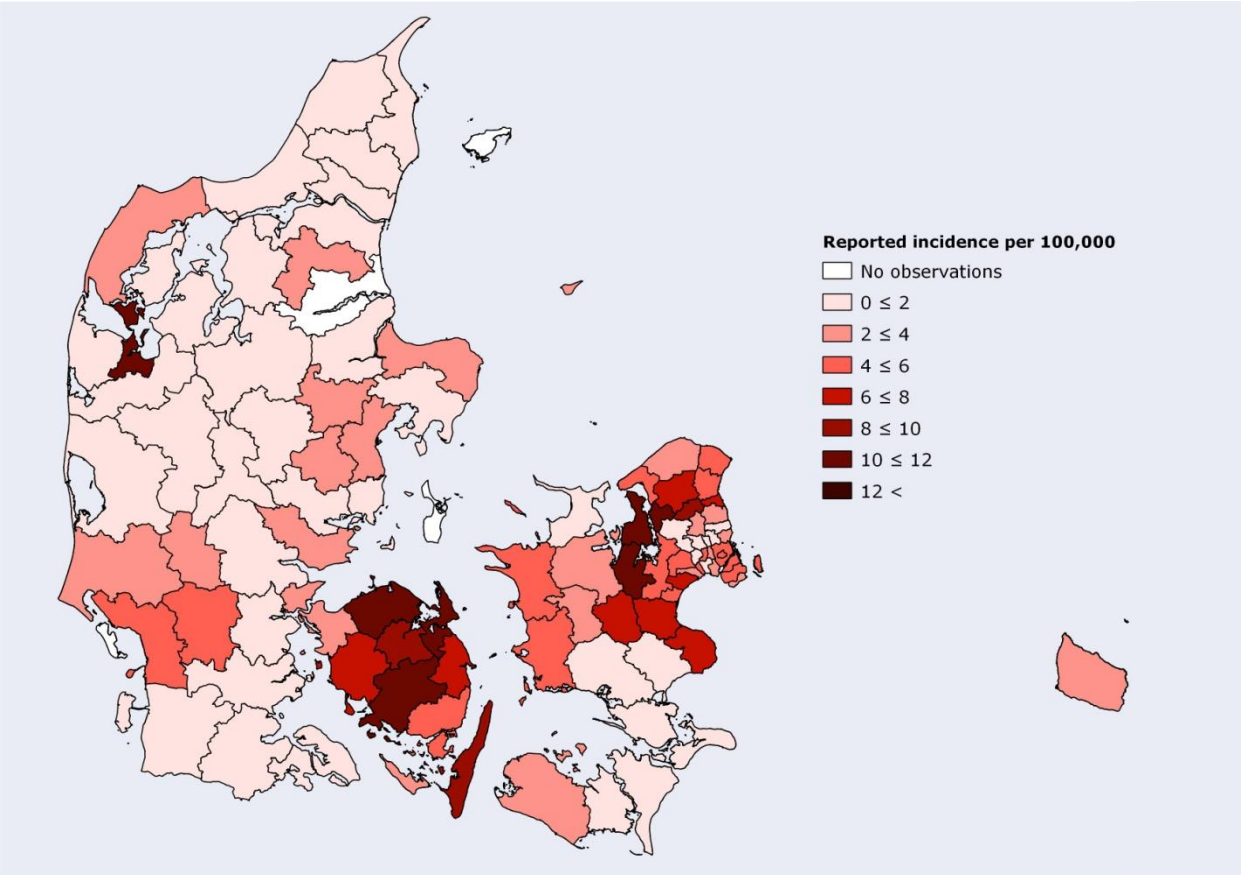

Figure 35 - Average yearly reported incidence of VTEC in Denmark from 2009-2012, by

4.2.4 Person

4.2.4.1 Sex

From Figure 37a it appears that there are more male than female reported cases of EPEC. Approximately equally many men and women are reported as having an ETEC infection. The peak in 2008 consisted of more male cases (cf. Figure 37b).

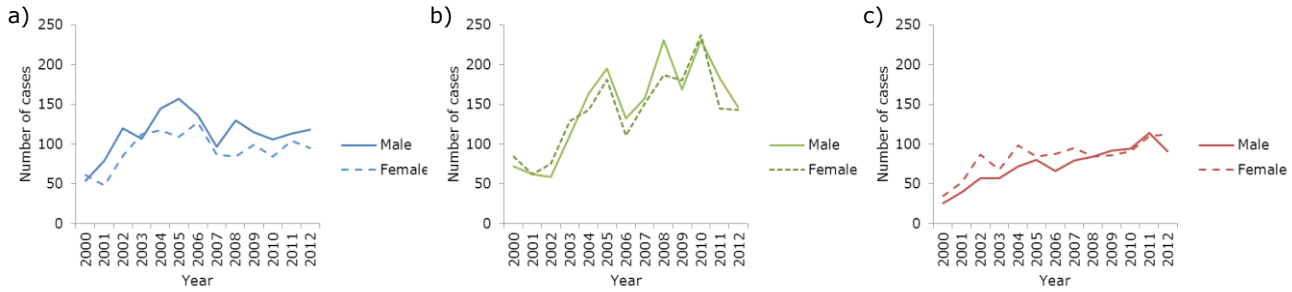

Figure 37 - No. of reported **a|** EPEC, **b|** ETEC and **c|** VTEC cases in Denmark 2000-2012, by sex

The two peaks seen for VTEC seen on Figure 17, are made up of reports on female cases (cf. Figure 37c) whereas the reported number of male cases, increase with a constant slope. In 2012, 115 female and 90 male cases of VTEC were reported.

4.2.4.2 Age

Figure 38a shows the incidence of EPEC by age group. In the year 2000 the reported incidence for the age groups 1-4 years and below 1 year was around 20 cases per 100.000 person years. The incidence for all other age groups was below 1 per 100,000. The incidence of the below 5 years old's increased from 2000 until 2004/2005 where the incidence amongst the 1-4 year olds levelled at around 40-45/100,000.

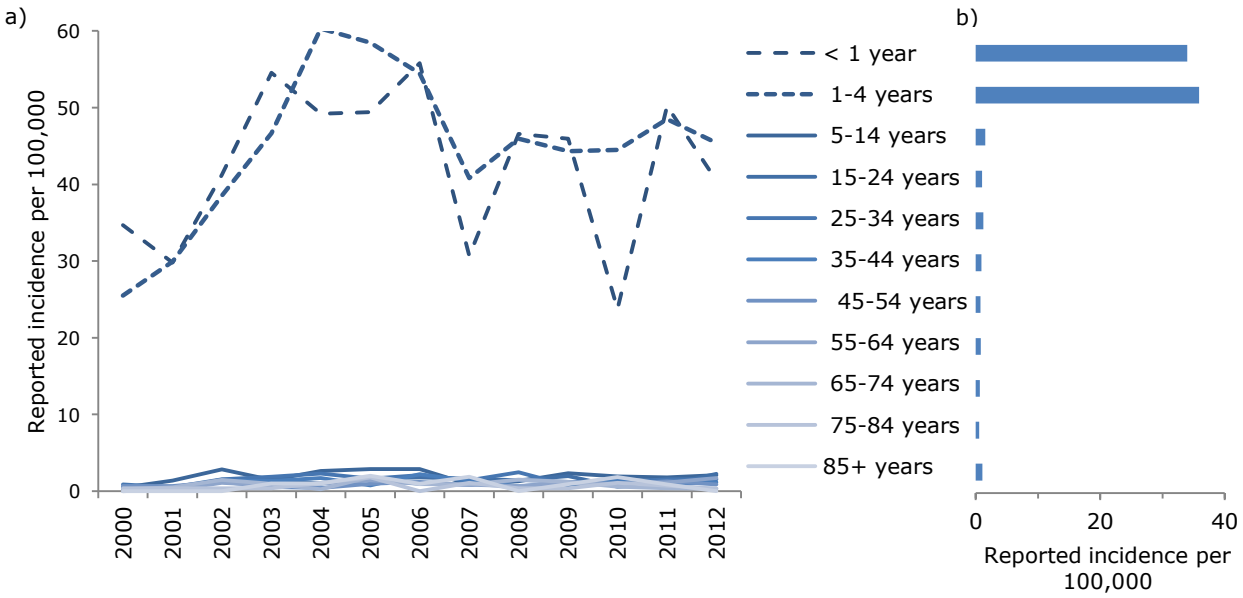

Figure 38 – Incidence of EPEC in Denmark according to age **a|** Reported incidence of EPEC in Denmark, 2000 - 2012 by age group **b|**Average incidence of EPEC 2000 – 2012 by age group

Figure 38b illustrates the average incidence of EPEC from 2000 to 2012 by age group. Here it is evident that the majority of the reported cases are in the below 5 years old's and almost no cases are seen in the older age groups.

Unlike EPEC, ETEC affects not only children. The highest incidence of ETEC is, nevertheless, seen amongst the 1-4 year olds (>10/100,000), as these are more often tested, but also in the 15-64 years age groups an incidence of above 5/100,000 is seen. The lowest reported incidences (<3/100,000) are seen in the age groups 5-14 and in the 65 and older (Cf. Figure 39b). Figure 39a shows the development in the incidence of ETEC from 2000 to 2012 by age groups. No obvious patterns in the incidence leap into the eye, the incidence of most age groups are varying throughout the period.

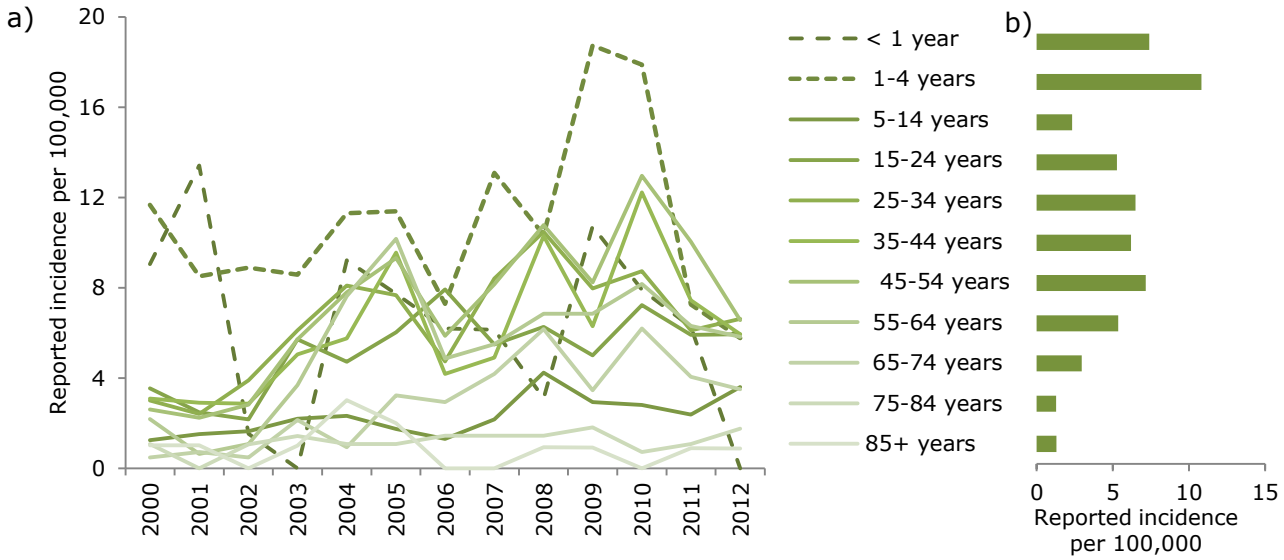

Figure 39 - Incidence of ETEC in Denmark according to age **a|** Reported incidence of ETEC in Denmark, 2000 - 2012 by age group **b|**Average incidence of ETEC 2000 – 2012 by age group

Figure 40a portraits the development in the reported incidence of VTEC in Denmark from 2000 to 2012 by age groups. It seems as if there has been a slight increase in the reported incidence in all age groups. The reported incidence of the <1 year olds was around 9 in 2000 and 13 in 2012. As was the case for EPEC, VTEC is primarily reported amongst children below 5 years of age (cf. Figure 40b), but the pattern is less skewed towards small children. It is likely that a large part of the age-specific pattern can be ascribed to diagnostic practices with a focus on diagnosis in children <7 years of age cf. the recommendations of DSKM.

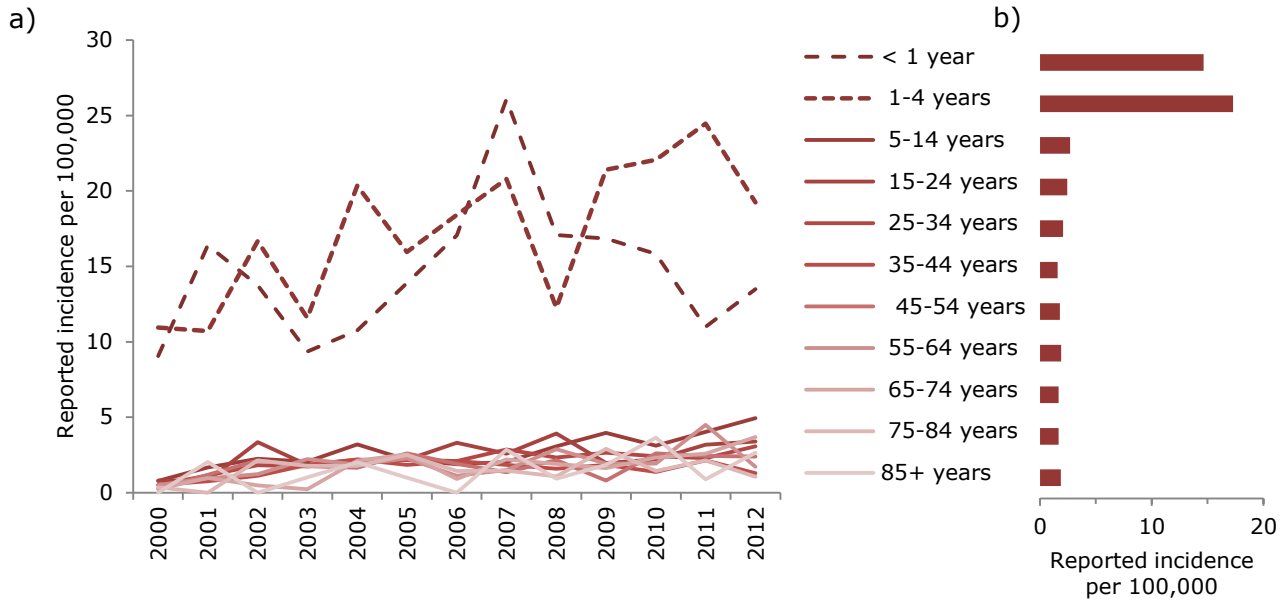

Figure 40 - Incidence of VTEC in Denmark according to age **a|** Reported incidence of VTEC in Denmark, 2000 - 2012 by age group **b|**Average incidence of VTEC 2000 – 2012 by age group

4.2.5 Potential outbreaks

ETEC isolates are not serotyped in routine surveillance, for this reason outbreaks are not necessarily detected. None of the ETEC outbreaks there have been in Denmark were found through routine surveillance – additional clusters, though, have been found through increased serotyping in outbreak situations.

Through working with data; presenting it along various dimensions of time, place and person at least three ETEC peaks called for an even closer look. They are presented chronological in the following section.

Already on Figure 14, describing the annual development in the number of ETEC cases in Denmark overall, a peak in 2005 stands out. On Figure 16 showing the number of cases per year and month, a peak not seen before - or since - appeared in August 2005. On Figure 25 illustrating the development of ETEC over time by *landsdel* it became clear that the increase was only seen in *Østsjælland*, on *Fyn* and to a lesser extend in *Vest- og Sydsjælland*. When focusing on the monthly number of cases with no travel history per 100.000 inhabitants in five *landsdele* with more than 10 cases in the whole 2005 it appears that the peak is most pronounced for *Østsjælland (*cf. Figure 41). The monthly number of cases in the years 2004 and 2006 (excluding outbreak cases) are also plotted on Figure 41 as a reference. Sixteen cases with no travel history from *Østsjælland* were reported. Within this *landsdel* primarily Lejre, Greve and Køge were experiencing relatively high numbers per inhabitants. Both male and females were infected.

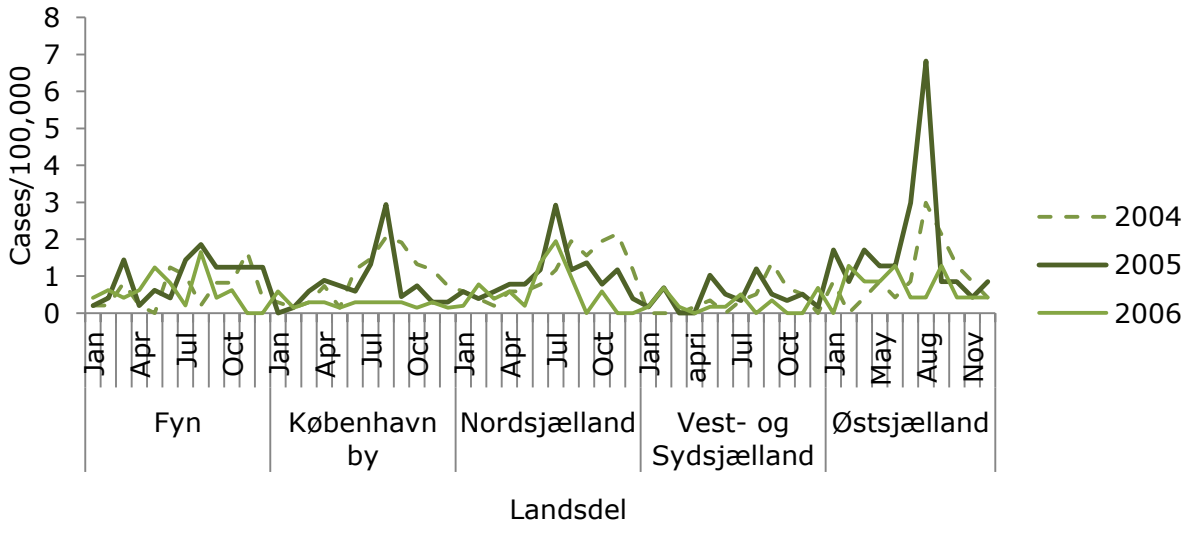

Figure 41 - Peaks of ETEC infections (excl. travel and outbreak cases) per 100,000 inhabitants in five *landsdele*. Year 2004, 2005 and 2006

Another peak was seen in July 2008 in *Østsjælland*. This peak consisted of 11 cases between 15 and 65 year old. Three were from Solrød and four from Køge. Another peak was seen in 2008. In September/October 13 cases from all over the *landsdel* were reported. More males were infected in the September-October peak. The numbers in 2008 are lower than in 2005.

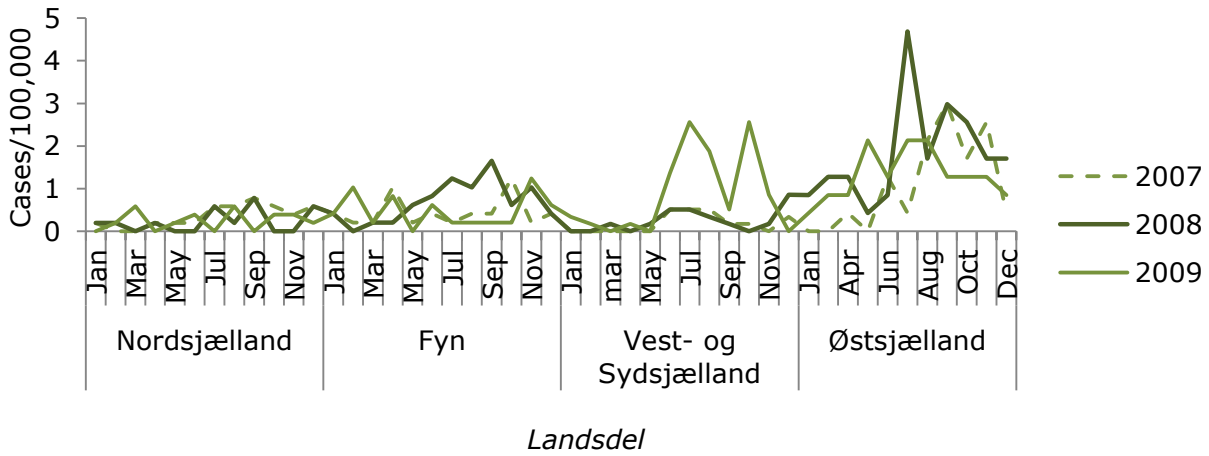

Figure 42 - Peaks of ETEC infections (excl. travel and outbreak cases) per 100,000 inhabitants in four *landsdele*. Year 2007, 2005 and 2009

What also appear on Figure 42 is peaks in 2009 in *landsdel Vest og Sydsjælland*. This year the incidence of ETEC in all other *landsdele* fell compared to 2008 (cf. Figure 14). From 2000-2012 the infections in the below 5 years old children accounted for 15% of the total number of ETEC infections that were not part of an outbreak of acquired abroad. In 2009 40% (25 cases) of the ETEC infections were in children below five years. The infections were seen from August to November. They were spread out of the landsdel, from Odsherred and Holbæk in August and to Faxe and Stevns in October and November.

4.2.6 Extrapolating from a *landsdel* with high incidence

From the questionnaire it was seen that the *KMA* on Fyn has tested all faeces samples that are sent in for diagnostics for gastroenteritis since 2011. In Table 7- Table 9 the incidence of the *landsdel Fyn* is extrapolated to the 10 other *landsdele* in order to estimate the added value of enhanced diagnostics.

When extrapolating the incidence of *Fyn* – as a proxy for the 'real' incidence (i.e. the incidence of the episodes of VTEC, ETEC or EPEC that exceeds the iatrotropic threshold) the 1:1 extrapolation for EPEC shows a diagnostic benefit of 3 times more reported cases of EPEC each year.

Table 7 - Number of reported cases of EPEC in 2012, extrapolated number of cases, and diagnostic benefit, by *landsdel*

EPEC	Inhabitants	Number of reported cases	Reported incidence per 100,000 inhabitants	Extrapolated number of cases with an incidence of 12.2/100,000	Extra cases
Byen København	704,108	40	5.7	86	46
Københavns omegn	520,784	8	1.5	63	55
Nordsjælland	448,291	20	4.5	55	35
Bornholm	41,406	0	0	5	5
Østsjælland	236,429	14	5.9	29	15
Vest- og sydsjælland	581,478	14	2.4	71	57
Fyn	485,190	59	**12.2**	59	0
Sydjylland	716,152	20	2.8	87	67
Østjylland	839,710	38	4.5	102	64
Vestjylland	426,972	0	0	52	52
Nordjylland	579,996	0	0	71	71
Total cases		**213**		**679**	**466**

The biggest potential lays in the diagnostics of ETEC, where the number of reported cases is estimated to increase by 474% - more than 1050 additional cases – when applying the *Fyns* incidence to the whole country.

Table 8 - Number of reported cases of ETEC in 2012, extrapolated number of cases, and diagnostic benefit, by *landsdel*

ETEC	Inhabitants	Number of reported cases	Reported incidence per 100,000 inhabitants	Extrapolated number of cases with an incidence of 24.3/100,000	Extra cases
Byen København	704,108	41	5.8	171	130
Københavns omegn	520,784	6	1.2	127	121
Nordsjælland	448,291	95	21.2	109	14
Bornholm	41,406	0	0	10	10
Østsjælland	236,429	4	1.7	58	54
Vest- og sydsjælland	581,478	8	1.4	141	133
Fyn	485,190	118	**24.3**	118	0
Sydjylland	716,152	10	1.4	174	164
Østjylland	839,710	1	0.1	204	203
Vestjylland	426,972	3	0.7	104	101
Nordjylland	579,996	0	0	141	141
Total		**286**		**1357**	**1071**

For VTEC there is also a potential, but here the current practices are more similar. It is estimated that we could expect 390 more reported cases each year if all *landsdele* had the same incidence of VTEC as *Fyn*.

Table 9 - Number of reported cases of VTEC in 2012, extrapolated number of cases, and diagnostic benefit, by *landsdel*

VTEC	Inhabitants	Number of reported cases	Reported incidence per 100,000 inhabitants	Extrapolated number of cases with an incidence of 10.5/100,000	Extra cases
Byen København	704,108	33	4.7	74	41
Københavns omegn	520,784	14	2.7	55	41
Nordsjælland	448,291	31	6.9	47	16
Bornholm	41,406	1	2.4	4	3
Østsjælland	236,429	10	4.2	25	15
Vest- og sydsjælland	581,478	23	4.0	61	38
Fyn	485,190	51	**10.5**	51	0
Sydjylland	716,152	14	2.0	75	61
Østjylland	839,710	12	1.4	88	76
Vestjylland	426,972	5	1.2	45	40
Nordjylland	579,996	3	0.5	61	58
Total		**197**		**587**	**390**

4.3 DEC in MiBa

The 555 observations in the EpiMiBa extraction from September 2012 contained 245 duplicates on CPR number and pathogen. Of the 310 remaining observations, 59% (182 isolates) had information about the pathotype: 21 were EPEC, 10 were ETEC and 3 were VTEC and 148 were found positive for other *E. colis* such as A/EEC and EAEC. 128 observations did not have any information about the pathotype or O group of the *E. coli* isolated.

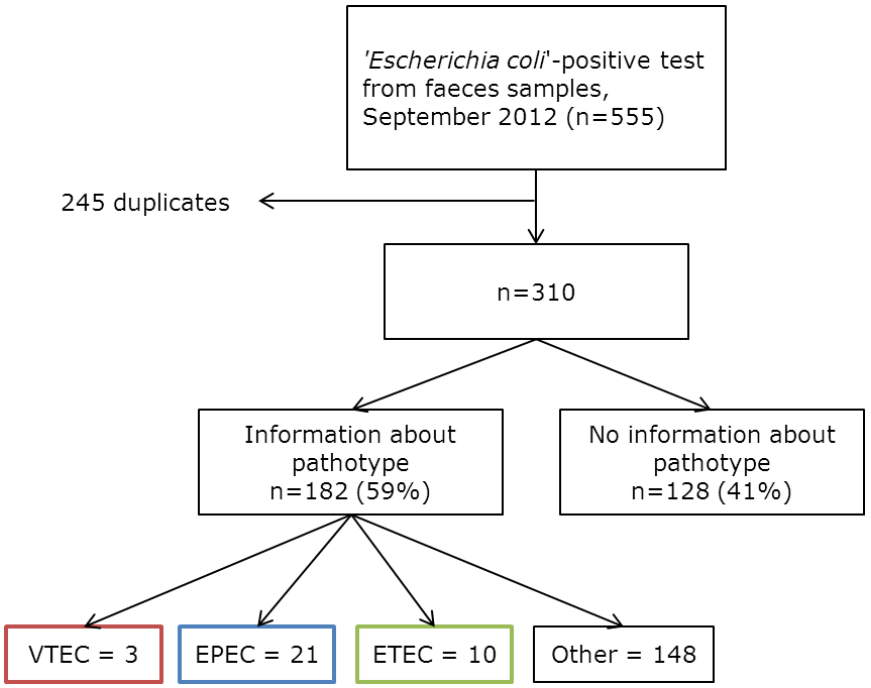

Figure 43 - Overview of data in the EpiMiBa extration from September 2012

For EPEC, 24 observations matched on CPR number – for 14 of these, information about pathotype was available from the EpiMiBa extraction i.e. for ten observations this information was not available, and manual look up in MiBa had to be done. Seven were tested in *KMA Odense, one in KMA Esbjerg* and two in *KMA Hillerød*. Six observations of EPEC from Skejby and one from *KMA Herlev/Hillerød* was found in the EpiMiBa extraction but not in *TBR* equivalent to 23% additional, unknown, cases. For all of these, information of pathotype (EPEC) was available from the EpiMiba extraction. For none of the 31 EPEC observations in epiMiBa there was information about O groups. The four observations that did not appear in the EpiMiBa extraction were from the diagnostic laboratory at SSI and thus were not in the extraction at all. All four could be manually looked up in MiBa.

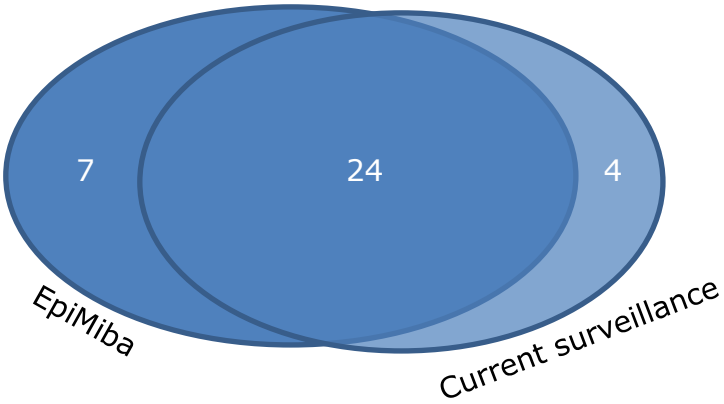

Figure 44 – Illustration of the EPEC observations from September 2012 caught by the different surveillance systems. EpiMiBa (left circle) and the current surveillance (right circle)

Sixteen ETEC cases were found in both the EpiMiBa extraction and through the current surveillance system. One of these cases, however, did match on CPR number, but were only noted positive for intimin producing *E. coli* in the EpiMiBa extraction – in MiBa this person was found positive for both at the same time.

Ten cases were not found in EpiMiBa – six were diagnosed at SSI, three were from *KMA Hillerød* and one was registered in October in MiBa and thus did meet the date criterion. All could be looked up manually in MiBa. Ten ETEC observations were only found in EpiMiBa equivalent to 40% additional cases than captured by the existing system. All observations were from *KMA Slagelse*.

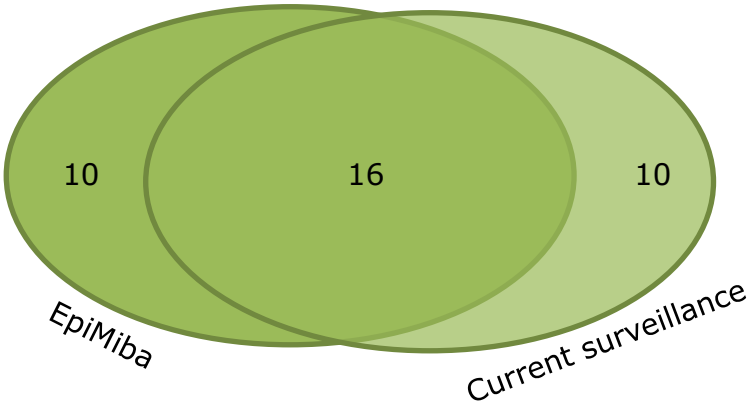

Figure 45 - Illustration of the ETEC observations from September 2012 caught by the different surveillance systems. EpiMiBa (left circle) and the current surveillance (right circle)

Sixteen VTEC cases were found in both EpiMiBa and the existing surveillance – 13 of the VTEC observations from EpiMiBa did not have information about patho- or serotype available in EpiMiBa. Four VTEC cases were not found in the EpiMiBa extraction, three were diagnosed at SSI and one case from *Nordsjælland* did not appear in MiBa at manual look up. One case of VTEC was found only in EpiMiBa, this observation was known to the reference laboratory but had not been confirmed.

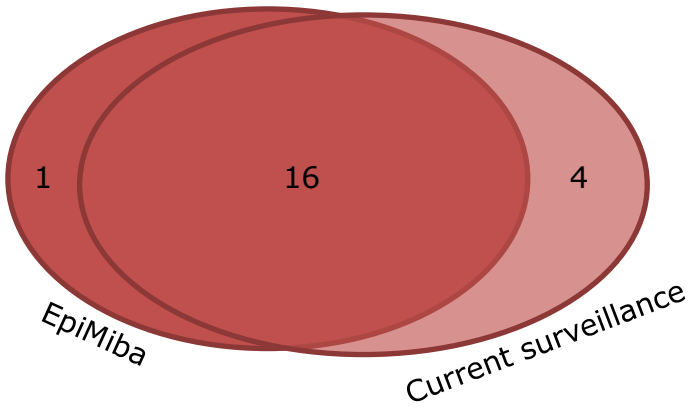

Figure 46 - Illustration of the VTEC observations from September 2012 caught by the different surveillance systems. EpiMiBa (left circle) and the current surveillance (right circle)

5.0 Discussion

The quality and the completeness of the data collected through the routine surveillance of food-borne pathogens in Denmark is very high compared to other European countries where many public health institutions do not keep records of ETEC or EPEC infections at all (55;56). Furthermore, many diagnostic laboratories in other countries test only for VTEC O157 whereas Denmark has always kept a focus on the non-O157 VTEC as well. For these reasons only a few studies have looked at the epidemiology of EPEC and ETEC, also it was pertinent to address the VTEC surveillance to see whether there is room for additional improvements. The general understanding is that ETEC is travel related and that EPEC is only seen in young children. The objectives of the project were to provide a detailed description of the epidemiology of EPEC, ETEC and VTEC in Denmark from 2000 – 2012 in terms of time, place and person; to describe and discuss the current surveillance of DEC in Denmark in terms of diagnostic methods and indication for test for DEC, and to assess the possibilities of using epiMiBa in the surveillance and monitoring of DEC in the future.

5.1 Epidemiology

5.1.1 EPEC

The incidence of EPEC amongst the under five years olds – especially the under 2 years olds was increasing until 2004 in six of 11 *landsdele*. Since 2004, the incidence has been stable throughout Denmark, except on *Fyn* where the incidence continued to rise.

The main point for discussion is that EPEC continues to be an important pathogen in small children. EPEC is probably underdiagnosed by at least a factor of three – if all of Denmark had the same diagnostic practices as currently carried out at *KMA Odense*, the case count would be around 679. The seasonal pattern is very marked; this has been described by others (57-59), but is not well explained. Other gastrointestinal infections associated with faecal oral transmission among children tend to be clustered in wintertime when in-house crowding facilitates transmission (e.g. rotavirus and norovirus). We need more knowledge of the burden of illness etc. to learn if episodes of EPEC are associated with failure to thrive and long standing gastrointestinal problems and if some of these could be food-related. On this basis, it would be easier to make priorities as regards improved detection, typing, outbreak response and understanding of risk factors in order to possibly improve prevention. There are lots of known unknowns.

5.1.2 ETEC

As expected, many of the ETEC infections in Denmark were acquired while travelling abroad. Many authors find ETEC to be the most common pathogen in patients with travel associated diarrhoea (60-64). However, only a few studies have focused on the proportion of ETEC infections that are acquired during travel, as this information is rarely available. Konishi et al found that of 142 ETEC strains isolated from sporadic cases of diarrhoea at hospitals in Tokyo, Japan between 2006 and 2009, 80% (114) were acquired during overseas travel (12). When discarding the cases that were part of the three outbreaks in Denmark 64% of the Danish cases were reported to have been acquired abroad between 2010 and 2012. That a place of infection is registered for the majority of ETEC cases is an important quality of the Danish surveillance system and is rarely seen in most other European countries; nevertheless an under-reporting of travel anamneses is still not unlikely. After the first ETEC outbreak in 2006, the proportion of ETEC cases with a registered travel history has been increasing, maybe indicating that increased awareness of the problem of locally acquired infections makes doctors note the anamneses down. In 2010 the Infectious disease epidemiology department at SSI called all ETEC cases with no place of infection registered and interviewed them in order to determine the extent of the problem with locally acquired infections. That year the proportion of ETEC cases with an infection acquired while travelling reached 72%, excluding the outbreak cases.

$^{1}/_{3}$ of the Danish cases with a known international travel history had travelled to Egypt, ¼ to Asia, primarily India (30% travelling to Asia had been in India, next was Thailand and Pakistan). Hill and Beeching (65) found that ETEC caused travellers' diarrhoea about 30% of the time in Latin America and the Caribbean, Africa, and South Asia, but only 7% in Southeast Asia. Not many Danes are travelling to Latin America compared to Egypt and Asia which could explain only a small share of the infections is reported as acquired in that part of the world.

The current study shows that there is a huge under-diagnosis of ETEC in Denmark, and it is likely that major food-borne outbreaks, caused by, for example, contaminated imported produce, are overlooked. Furthermore, ETEC are rarely serotyped in routine surveillance, which is why little is known about which serotypes prevail in Denmark. However, this is true for most countries and ETEC surveillance and control have currently relative low priority by both public health and food safety agencies. However, this may change in the future. Nishikawa reported in 1995 that O169:H41 was the most prevalent ETEC serotype in Japan (66). Wolf (67) reported that serogroups O6, O78, O8 and O128 were the most

frequently isolated ETEC serotypes from samples collected from various regions throughout the world.

In Denmark there have been at least four outbreaks with ETEC, with O92:H-, O6:K15:H16, O27:H7, and O169:H41. O169:H41 is according to Beatty et al the most common O group identified in food-borne outbreaks of ETEC in the States (68) and Konishi et al (12) looked at ETEC isolated in Tokyo, Japan between 1966 and 2009 and found that ETEC O169 have been seen in both food and water-borne outbreaks in Tokyo since the early 90s. O6 is also commonly seen in ETEC outbreaks in the States; in fact O6:H16 used to be the most commonly detected O group in ETEC outbreaks up until 1995 (69;70). Konishi et al found ETEC O6 to have caused food-borne outbreaks since the 70s in Tokyo. From 2001-2009 there were reports of 35 outbreaks with six main types of ETEC (O6, O27, O148, O159, O25, and O169) in Tokyo. Neither Konishi et al nor Beatty et al report any outbreaks or sporadic cases of ETEC O92.

With increased international trade of food, focus on ETEC is warranted both from a diagnostic and a public health perspective.

5.1.3 VTEC

The surveillance of VTEC in Denmark has been a priority since the end of the 1990s and the database kept at the reference laboratory at SSI is of high quality: it is very complete and elaborate in terms of symptoms, genes, O, K, and H groups, resistance patterns and place of infection. Almost all VTEC isolated at the *KMA*s are sent to the reference lab for further testing.

The incidence of VTEC in Denmark in 2012 was 3.6/100,000 inhabitants, this is lower than in Sweden where they had a reported incidence of 4.9/100,00 (71) in 2012, but higher than in the rest of the Nordic countries. Norway had an incidence of VTEC of 1.5/100,000 (72) and Finland an incidence of 0.5/100,000 equivalent to 30 cases in 2012 (73). In 2007, the Nordic Meeting on *detection and surveillance of VTEC infections in humans* was held in Copenhagen. At this time the incidence of the countries also differed in the same way; with Norway and Finland having lower incidences and Denmark and Sweden on about the same level (38). Sakuma et al found the average annual incidence of VTEC to be 2.74 per 100,000 (11) in Japan from 1999-2004.

The most common O group isolated in Denmark in the period from 2000 – 2012 was O157, also when disregarding the outbreaks; O157 was detected in 16% of the isolates sent to

the reference laboratory. This is lower than in the Nordic countries where they found O157 in 39-50% of the samples tested from 2002 – 2006 (38:13). Other common O groups in Denmark are O103 and O26, this is also the case in the other Nordic countries (38:13).

The seasonal variation of VTEC infections was less apparent than for EPEC and ETEC. The numbers are lower though, and the seasonal variation did show on the monthly average from 2000-2012. A sole peak in June until October is seen for O103, whereas O157 is seen all year, but with a summer peak.

A seasonal variation in VTEC has been reported in past studies from other countries. Albihn et al stated that most O157:H7 infections in Sweden were reported during the summer (42) and Sakuma et al found a marked seasonal oscillation pattern for VTEC infections with peaks centred in July and August in Japan (11). Sakuma et al found the fluctuation in the incidence over time to be associated with climate (average weekly air temperature), socioeconomic, and population factors, for example the beef cattle/population index. Albihn et al found that VTEC O157:H7 was more often present in bovine faeces in Sweden in July and August than the rest of the year (42) and various other studies from e.g. Finland (74), Holland (46) (75) and England (44) show similar patterns. A Danish study made by Nielsen et al also indicated that there is a seasonal variation in the prevalence of VTEC O157 in bovines in Denmark (45) and in a study by Roldgaard et al they found that there was a significant overlap of the vtx/phage type combinations of Danish bovine and human clinical isolates that indicated that cattle are an important reservoir of VTEC O157 (76). None of these studies looked at the seasonal variation in the serotypes O103 specifically.

As also seen in other countries (11) VTEC is isolated the most amongst children under 5 in Denmark. Most *KMAs* can test for VTEC, half via PCR or hybridisation followed by agglutination, half only by agglutination for the most common VTEC O groups. All *KMAs* are testing samples sent in for infectious gastroenteritis from children under 5 years for VTEC, which is why it is to be expected that there is more VTEC found in this group.

The proportion of VTEC infections that are acquired during international travel has been increasing during the period; however the majority of VTEC infections are acquired in Denmark.

5.2 Current surveillance system – diagnostics and indication

The current work underscores that public health surveillance depends on the primary diagnostic activities, and I have documented large differences in these practices. One aim was to understand the *KMAs'* ability to test for and detect *E. coli* as well as their principles for when they test a sample for DEC, and how these practices have developed over time. This was not fully uncovered; nonetheless some conclusions can be drawn.

EPEC was for many a forgotten pathogen in the 1970s and 1980s, but a gradual revival of the awareness of EPEC and changes in diagnostic methodology and/or indication for testing from 2000 – 2004 seem to have affected the number of reported cases. Since 2004 the incidence has been stable. At least two *KMAs* are not able to tests for EPEC locally, and samples have to be referred elsewhere for diagnostics. In some periods *KMA Midt-Vest* (Viborg/Herning) has sent to *KMA Skejby* and *KMA Aalborg* has sent to SSI. From 2009 and onwards however, only two cases of EPEC were reported in these *KMAs'* uptake area.

Few *KMAs* offer tests for ETEC and at least one of the big *KMAs* indicated that only a few samples were referred to SSI for ETEC testing. Some doctors are forwarding samples directly to SSI and it has also been seen that some have sent them to *Odense KMA*.

Most VTEC isolates are forwarded to the national reference laboratory as the DSKM recommends, however differences in the diagnostic methodology and principles for testing for VTEC affect the number of VTEC isolated. This is reflected in the lower incidence in some parts of Denmark.

Most KMAs are following the DSKM recommendations, at least for the tests they offer locally.

5.2.1 Improvements

Many authors have pointed out that ETEC is seen more often in food-borne outbreaks than in the past, and combined with the fact that some *KMAs* in Denmark are not able to test for this particular pathogen, it is of interest whether national ETEC outbreaks could be overlooked. Many authors have put this point forward. Already in 1999 Dalton et al (70) suggested that ETEC outbreaks may go unrecognized, and opportunities for treatment and prevention may be missed. Six years later Beatty et al. repeated this point and stated that the number of outbreaks caused by ETEC is likely to be underestimated in the States as not all diagnostic laboratories are able to test for ETEC (68). Also Devasia et al pointed out the danger that ETEC is traditionally recognized as a common cause of travellers' diarrhoea,

but in fact is becoming a more frequent cause of food-borne disease outbreaks in the United States (77). They suggest that it is important for public health practitioners and clinicians to be aware of ETEC as a domestic cause of gastroenteritis.

ETEC outbreaks have often been associated with non-durable food items such as parsley, basil, lettuce, sprouts etc. which one could argue would only affect people in a limited time span so that preventive measures or product recall would not be feasible by the time the source had been pointed out through epidemiological studies. However, Naimi et al found two concurrent *Shigella* and ETEC outbreaks reported to the Minnesota Department of Health to be related to the same vehicle of infection (parsley). One month later another outbreak occurred, this time the source of infection was cilantro. The cilantro was traced back to the same producer as the parsley (78). The authors suggest that the latter outbreak could have been prevented with improved surveillance (78).

Through working with data three potential outbreaks appeared. The search for outbreaks was unsystematic and not all peaks and changes were elaborated on. At least one major peak and two smaller but conspicuous trajectories were seen in the period from 2000 to 2012. In the light of a combination of no serotyped isolates and a not 100% complete recording of place of infection , it is hard to determine whether these peaks symbolises domestic or a travel related outbreak – or an unusually high number of sporadic ETEC infections.

The extrapolation from the incidence on *Fyn* showed a huge gap between the different parts of Denmark and indicates a potential for improved diagnostics of ETEC. Even if 80% of the detected cases are acquired abroad, it is estimated that 200 additional ETEC infections acquired in Denmark will be diagnosed each year. If the characteristics of these were to be revealed in terms of serotypes or the like, it is likely that more outbreaks would be detected and potentially solved. The knowledge generated through an increased focus could be directed toward preventive measures. At the moment the surveillance is too geographically biased so that there is a fear that signals will be overlooked.

That the DEC surveillance, or diagnostics, is geographically uneven, is also a concern when it comes to proper treatment of these infections and to limiting person-to-person spread. The three pathogens may belong to the same species, but they are treated differently and doctors treating 'in blind' – or not treating at all – may cause unnecessary harm or delay.

KMA Aalborg and *KMA Esbjerg* are looking into introducing PCR and *KMA Skejby* is looking into incorporating ETEC in their PCR diagnostics. Better diagnostics will first of all ensure proper treatment and precautions towards further spread of the infection and secondly, may strengthen the outbreak signals and through that the ability to pick up the signal and act – especially if isolates are sent to the reference laboratory for subtyping. It will be interesting to follow the development in the number of detected cases of ETEC and EPEC after the enhanced diagnostic in the three *KMAs*.

For VTEC and EPEC the practices are less skewed, and most of the isolates are forwarded to the national reference laboratory so there potentially can be acted upon findings of similar subtypes, PFGE patterns etc. However, more knowledge on the un-diagnosed cases that is estimated to be lost in unequal diagnostic practices etc., would help shed light on patterns, risk factors, and could potentially guide and inform preventive measures.

5.3 MiBa

The possibilities in MiBa are many: real time surveillance data and with that a possibility of reacting faster to signals. Better mapping of results, including epidemiological markers, such as serotypes is a prerequisite and should have high priority. Forty per cent of the observations were not mapped in EpiMiba and no information on serotypes was transferred from MiBa to the EpiMiBa extraction. This loss of valuable information in the transition from MiBa to EpiMiBa will need to be resolved before EpiMiBa will be of relevance in the daya-to-day surveillance.

Seven EPEC cases and 10 ETEC cases that had not been registered in TBR were discovered in EpiMiBa. Out of 54 cases registered in TBR, this is an under-reporting of 30% and underlines the need for a system that does not rely on manual registration. Some of the EPEC cases that were found in MiBa can potentially be A/EEC's that the *KMA* in the first place though were EPEC but that did not fulfil the serogroup criteria when sent to the reference laboratory. They should, nevertheless, still be in TBR. The SSI findings were not in the EpiMiba extraction and this limited the possibility of comparing findings if these EPEC isolates were forwarded to the reference laboratory.

5.4 Limitations

The data on which this project builds is collected through routine surveillance by clinicians as a part of their everyday duties. Information change hands many times (patient, doctor, person testing at *KMA*, person registering at *KMA*, reference laboratory, etc.) before reaching TBR, MIS-2 or the *E. coli* database and information is sometimes lost in the transfer. Especially for EPEC infections, a place of infection is rarely registered in TBR. It is also not unlikely that travel history is under-reported for ETEC infections. VTEC has been prioritised at SSI and the information on the requisition has been combined with information on the 1515 forms for a long period. The quality of the Danish surveillance databases, in terms of elaborateness and completeness, are high compared to elsewhere and by the means of CPR numbers geographical information can be tied to each observation.

The surveillance of food-borne bacteria is passive and the *TBR* therefore only contain information on people for whom a sample has been tested positive for e.g. DEC. To be registered in TBR the person experiencing acute gastrointestinal symptoms first has to a) seek medical help b) meet a doctor who think of sending in a sample for testing c) provide a faeces sample d) send it to a *KMA* that either look for DEC or refer the sample to a laboratory that is capable of detecting DEC. An underestimation of the true number of people infected with VTEC, ETEC and EPEC cases is for this reason expected.

Whether the regional differences in the incidences of the three groups of DEC is due to varying risk factors in some parts of the country, to varying sampling-activity amongst GPs or to varying diagnostics methodology and/or principles for testing cannot be completely revealed in the present study. The number of samples received, or tested for that matter, at each *KMA* is currently not known and it is therefore not possible, with the data from the surveillance databases, to determine how much of the differences in the reported incidence can be ascribed to differences in submitted samples, diagnostic methods or principles for testing. A light has been shed on the diagnostic practices and the principles for testing however. The regional differences in the incidences are so marked, that it would be unlikely that all could be ascribed to varying sample-activity, risk factors and/or iatrotropic thresholds in different parts of the country. In the future become possible to get the denominator through Miba; the number of samples received at each *KMA* and also which *KMA* that has tested and/or referred which sample. With this figure, it would be possible to calculate the proportion of samples e.g. from patients below 7 years of age that are found positive for DEC and compare this figure amongst the *KMAs.*

The case definition used in this report has been based on the *KMAs* categorisation of their findings. Prior to 2007 more than 20% of the EPEC was not confirmed at the national reference laboratory; the vast majority of these did not meet the H group criteria in the EPEC definition and were overturned to 'A/EEC'. From 2007 and onwards only a small proportion of the isolates have not been confirmed. The incidence of EPEC can, as a result of this, be overestimated in the period from 2000-2006. Twelve percent of the isolated EPEC is not forwarded to the reference laboratory and it is unknown whether these in fact live up to the serotype criteria in the EPEC definition or if they potentially could be A/EEC. After 2007 the discrepancy for the forwarded isolates has been negligible and that this should be much different for the not-forwarded isolates is unknown but not likely.

For the number of ETEC cases the sole option was to rely on the findings at the KMAs as only very few samples are sent in for verification and subtyping at the reference laboratory. ETEC can only be detected with molecular methodology and the sensitivity of this is high which is why there is no reason not to trust the *KMA's* diagnosis.

For VTEC this issue is almost non-existing, as almost all VTEC isolated at the *KMAs* are forwarded to the reference laboratory and a high proportion is confirmed. Each year few 1515 forms are received at the Infectious Disease Epidemiology department at SSI for which no sample is found positive or even submitted. Fifty-five 1515 notifications were counted as VTEC cases in the period from 2000 – 2012 i.e. 2.6% of the cases was not clinically confirmed. These reports were from all over the country (however to a lesser extend *Region Nordjylland*) so it is not believed that they distort the overall picture.

The incidence on *landsdel Fyn* was used in the extrapolation for estimating the diagnostic benefit. There is no reason to believe that *Fyn* is different in terms of risk factors for acquiring DEC or determinants within the population. The age and sex distribution are not noticably different than the rest of the country and on Fyn there is both bigger cities and country side.

6.0 Conclusion

The objectives with this report were to describe the epidemiology in Denmark of VTEC, EPEC and ETEC in terms of time, place and person from 2000 – 2012; to describe and discuss the current surveillance of DEC in Denmark in terms of diagnostic methods and indication for test for DEC, and to assess the possibilities of using EpiMiBa in the surveillance and monitoring of DEC in the future.

Denmark is one of the leading countries when it comes to awareness of DEC, and the data generated through the routine surveillance is of high quality and the databases are elaborate. Since 2000, where the incidence of the three DEC groups was about the same throughout the country, the development in the incidences have had very different trajectories. The experience from *KMA Odense*, where they tests all faeces samples sent in for gastroenteritis diagnostics for DEC is an example of how common these infections in fact are. Not all *KMAs* are testing, or referring samples, according to the DSKM recommendations. Implementing the DSKMs recommendations in terms of when to test for DEC would enhance public health and clinical practices. As we have learnt, not all *KMAs* can test for ETEC and EPEC. This is not a problem as such, but the results indicate that the EPEC and ETEC infections - that we expect *do* occur – are not captured by any other diagnostic laboratory and thus remain undiagnosed and un- ,or not properly, treated. Extrapolations from the incidences on Fyn indicate an incredible under-diagnosing, of all three pathogens, but especially of ETEC. This suggests a potential in improving diagnostic methods in some parts of the country.

Enhanced diagnostics, based on the DSKM recommendations, will first of all limit the number of infections that otherwise go un-diagnosed. This could ensure timely and proper treatment of these infections, with the potential of reducing person-to-person spread. Second, a better picture of the burden of illness and monitoring of the developments in all parts of Denmark can facilitate and direct preventive measures as data can be used to explore major risk factors and outcomes for the three pathogens as well as detecting and eventually follow up upon outbreaks.

The result shows that MiBa catches observations that do not appear in TBR and the potentials with MiBa therefore seem promising. MiBa will ensure real time surveillance and thus the opportunity to react faster. Better mapping of results, including epidemiological markers such as serotypes, PFGE, travel information, etc., is a prerequisite and should have high priority.

7.0 Public Health Recommendations

1. Denmark is in the forefront as regards awareness of DEC, however, the implementation of the recommendations prepared by DSKM would enhance public health and clinical practices
2. VTEC surveillance works well and diagnostics has improved. There is still room for improvement.
3. ETEC is underdiagnosed, and with the globalisation of food supply, much can be done to detect patients, and conduct subtyping in particular as regards domestic cases.
4. EPEC is also underdiagnosed, but less than ETEC. There are many unknowns as regards the epidemiology of EPEC. A good start would be to define the burden of illness and to clarify major risk factors and outcomes of EPEC. This basis would enable to put the findings in a public health perspective
5. An increased focus on DEC should be followed by exploring the potentials of improved/increased testing for detecting outbreak signals in order to enforce public health response
6. MiBa will ensure real time surveillance and thus the option to react faster. Better mapping of results, including epidemiological markers, is a prerequisite and should have high priority

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