

PIIA JÕGI

Epidemiological and  
clinical characteristics  
of pertussis in Estonia



DISSERTATIONES MEDICINAE UNIVERSITAS TARTUENSIS

**267**

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UNIVERSITY OF TARTU  
Press

Department of Microbiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Estonia

This dissertation has been accepted for the commencement of the degree of Doctor of Philosophy in Medicine on February 21, 2018 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

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Commencement: April 16, 2018

Publication of this dissertation is granted by University of Tartu.

This study was supported by a grant from the Estonian Science Foundation (9259), Estonian Research Council (IUT 34-24), and European Union through the European Regional Development Fund.

ISSN 1024-395X

ISBN 978-9949-77-701-3 (print)

ISBN 978-9949-77-702-0 (pdf)

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University of Tartu Press  
[www.tyk.ee](http://www.tyk.ee)

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## LIST OF ORIGINAL PUBLICATIONS

1. Jõgi P, Oona M, Toompere K, Leedo S, Epstein J, Lutsar I. 2014. Seroprevalence of IgG antibodies to pertussis toxin in children and adolescents in Estonia. *Vaccine* 32(41):5311–5.
2. Jõgi P, Oona M, Toompere K, Lutsar I. 2015. Estimated and reported incidence of pertussis in Estonian adults: A seroepidemiological study. *Vaccine* 11;33(38):4756–61.
3. Jõgi P, Oona M, Kaart T, Toompere K, Maskina T, Koort I, Rätsep A, Lutsar I. 2018. Pertussis and parapertussis in children and adults with a persistent cough: An observational study. *Infection* 2018;46(1):83–91.

### **Author's personal contribution:**

In all papers, Piia Jõgi participated in the study design, was in charge of sample collection, conducted data analyses, and wrote the draft of papers.

## LIST OF PRESENTATIONS AT INTERNATIONAL CONFERENCES

- Jõgi P, Oona M, Kaart T, Maskina T, Koort, I, Rätsep A, Lutsar I. Pertussis study group of Estonia. Pertussis antibodies in entire population, among patients with pertussis and the time course up to three years after the disease. 35th Annual Meeting of the European Society for Paediatric Infectious Diseases. Madrid, Spain, May 23–27, 2017
- Jõgi P, Oona M, Kaart T, Maskina T, Koort, I, Rätsep A, Lutsar I. Patients with persistent cough need a laboratory evaluation to exclude the presence of pertussis. 15th Baltic States Conference “Vaccination Day”. Riga, Latvia, September 2–3, 2016
- Jõgi P, Oona M, Kaart T, Maskina T, Koort, I, Rätsep A, Lutsar I. Pertussis study group of Estonia. Children with persistent cough should undergo laboratory evaluation to exclude presence of pertussis. 34th Annual Meeting of the European Society for Paediatric Infectious Diseases. Brighton, England, May 10–14, 2016
- Jõgi P, Oona M, Lutsar I. Pertussis epidemiology and clinical characteristics in Estonia. 3rd Baltic Paediatric Congress. Riga, Latvia, August 19–21, 2015
- Jõgi P, Oona M, Toompere K, Lutsar I. Estimated versus reported incidence of pertussis in Estonian adults. 25th European Congress of Clinical Microbiology and Infectious Diseases. Copenhagen, Denmark, April 25–28, 2015
- Jõgi P, Oona M, Epstein J, Lutsar I. Epidemiology of pertussis in Estonian infants over the last 25 years. 33rd Annual Meeting of the European Society for Paediatric Infectious Diseases. Leipzig, Germany, May 12–16, 2015
- Jõgi P, Oona M, Toompere K, Epstein J, Lutsar I. Does the number of boosters and the diagnostic method influence the notification of pertussis? 32nd Annual Meeting of the European Society for Paediatric Infectious Diseases. Dublin, Ireland, May 6–10, 2014
- Jõgi P, Oona M, Toompere K, Maskina T, Koort, I, Rätsep A, Lutsar I. Prevalence of pertussis in patients with progressive cough. 32nd Annual Meeting of the European Society for Paediatric Infectious Diseases. Dublin, Ireland, May 6–10, 2014
- Jõgi P, Oona M, Toompere K, Leedo S, Lutsar I. Seroprevalence of IgG antibodies to pertussis toxin in children and adolescents in Estonia. 31st Annual Meeting of the European Society for Paediatric Infectious Diseases. Milan, Italy, 28–June 1, 2013

## ABBREVIATIONS

AU	arbitrary units
aP	paediatric acellular pertussis vaccine
ap	preteens, teens, and adults acellular pertussis vaccine
CDC	Centers for Disease Control and Prevention
CI	confidence interval
DTaP	paediatric diphtheria, tetanus, and acellular pertussis vaccine
dTap	preteens, teens, and adults diphtheria, tetanus, and acellular pertussis vaccine
DTwP	diphtheria, tetanus, and whole cellular pertussis vaccine
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
ESEN	European Sero-Epidemiology Network
FHA	filamentous hemagglutinin
FIM	fimbriae
GMC	geometric mean concentration
GP	general practitioner
Hib	<i>Haemophilus influenzae</i> type b vaccine
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IPV	inactivated poliovirus vaccine
IQR	interquartile range
IS	insertion element
IU	international unit
LR+	positive likelihood
LR-	negative likelihood
MCA	multiple correspondence analysis
mL	millilitre
NPs	nasopharyngeal swab
OR	odds ratio
PCR	polymerase chain reaction
PRN	pertactin
PT	pertussis toxin
$R_0$	reproduction number
ROC	receiver operating characteristic
Th	T-helper cell
UK	United Kingdom
WHO	World Health Organization
wP	whole cellular pertussis vaccine

# 1. INTRODUCTION

Pertussis, or whooping cough, is a traditional childhood disease which has assumed to be vaccine-preventable. Pertussis can be a life-threatening disease, particularly for unimmunised newborns and partly immunised infants (1, 2). For adolescents and adults, pertussis might entail persistent or prolonged cough (3, 4). Routine immunisation with killed pertussis vaccine started in the 1950s and was effective, reducing incidence by more than 90% (5). However, after almost 70 years of vaccination, pertussis is the most common vaccine-preventable disease, even in countries with high immunisation coverages (6). Furthermore, during the last two or three decades, the resurgence of pertussis has been seen across most of the world (7). The reasons of the spread of pertussis are not entirely clear (8-10).

In Estonia, trends in the incidence of pertussis have been similar to in other countries with high immunisation rates (11). After the low incidence in the 1970s and 1980s, the reported incidence rates started to increase and were highest in 2010 at 97/100,000 (data from Health Board of Estonia), the highest in Europe (11).

However, in Estonia, the real incidence of pertussis before our studies was unknown, mainly for three reasons:

- 1) Only qualitative serology tests with mixed antigens were available until 2012, and this test has low specificity; therefore, false positives may have occurred.
- 2) Despite the high immunisation coverage of pertussis vaccines in infants, the highest incidence has been always reported among children.
- 3) As adolescents and adults often have a mild or even asymptomatic course of the disease, their pertussis might be underdiagnosed and underreported.

Pertussis is mandatory to report to the surveillance organisations in most countries, including Estonia (11), and therefore epidemiological studies based on reported data are easy to conduct. However, as pertussis might be mild or even asymptomatic, it is often unrecognised, undiagnosed, and thus unreported (12). Prospective studies in patients with persistent cough enable us to determine the prevalence of pertussis and clinical characteristics of the disease. However, only symptomatic cases are enrolled. To capture both symptomatic and asymptomatic cases, seroprevalence studies should be conducted (13).

To date, four epidemiological studies about Estonia, all based on national surveillance data, have been published between 2000 and 2016 (6, 14-16). As stated previously, the main disadvantage of studies based on the nationally reported data is low sensitivity, as not all cases are diagnosed and reported. In addition, we suspected overdiagnosis of pertussis among children. Therefore, to determine the real prevalence of pertussis in Estonia, we conducted a prospective study in patients with persistent cough and population-based seroprevalence studies.

## 2. REVIEW OF THE LITERATURE

### 2.1. Definition of pertussis

Pertussis is a highly contagious disease of the human respiratory tract caused by the Gram-negative coccobacillus *Bordetella pertussis*, which belongs to the genus *Bordetella* in the family *Alcaligenaceae* (17). Less frequently, other species of genus *Bordetella*, such as *B. parapertussis*, *B. bronchiseptica* or *B. holmesii* have been identified as the cause of pertussis-like disease (18).

*B. pertussis* is a strict human pathogen with no known animal or environmental reservoir (19). *B. pertussis* is a fragile bacterium and is therefore very sensitive to external conditions, requiring special agar and conditions to grow in medium (20). *B. pertussis* is transmitted via aerosolised respiratory droplets and is highly contagious (21). In susceptible populations, the *B. pertussis* transmission rate is close to 90% (22). The basic reproduction number ( $R_0$ ) indicates the number of other people that one patient infects during the period of infectivity (23). In the case of pertussis,  $R_0$  is estimated to be about 10 in pre-vaccine (24) and about 5.5 in highly immunised European countries (25). For example, in the cases of influenza and Ebola,  $R_0$  is evaluated to be about 1.5 and 2.0, respectively (26, 27), but in the case of measles about 12 (28).

### 2.2. First description of the disease and detection of the causative organism of pertussis

The first description of pertussis was published after an epidemic in Paris in 1578 by Guillaume de Baillou, who described it as a serious paroxysmal cough with posttussive emesis which mostly attacked children of four to ten months (29). In 1900, the causal agent of pertussis was described as ovoid bacillus in sputum of a 6-month-old infant with pertussis by Jules Bordet, but he was unable to isolate the microbe because of its instability (30, 31). Bordet and Octave Gengou first cultivated the pathogen at the Pasteur Institute in 1906 in a medium containing potato extract and blood, which is now known as Bordet Gengou agar (30, 32). In tribute to Bordet, this Gram-negative bacterium was called *B. pertussis* (30).

### 2.3. Pathogenesis of pertussis

Transmission of *B. pertussis* is only possible through inhalation of aerosol droplets containing the bacteria (21). Patients with either cough or asymptomatic carriage might transmit *B. pertussis* to naive contacts (21, 33, 34). The bacteria adhere to ciliated airway epithelium in the upper portion of the respiratory system to multiply and produce toxins. The toxins paralyse the cilia to

obstruct the clearing of the airways, damage epithelium, and cause local inflammation of the respiratory tract and systemic effects (35, 36).

*B. pertussis* has many virulence factors: some of them are important for adhesion and others for producing toxins (35, 36) (Table 1).

**Table 1: Main known virulence factors of *B. pertussis***

<b>Adhesins</b>	<b>Toxins</b>
Fimbriae	A-subunit of pertussis toxin
B-subunit of pertussis toxin	Adenylate cyclase toxin
Filamentous hemagglutinin	Tracheal cytotoxin
Autotransporters*	Lipopolysaccharide
Polysaccharide capsule	Dermonecrotic toxin
Pertactin	Autotransporters*
	Polysaccharide capsule
	“Cough toxin”

\* Vag8, BekA, BapC, SphB1, and TcFA

The adhesins responsible for binding bacteria to host cells are B-subunit of pertussis toxin (PT), fimbriae (FIM), filamentous hemagglutinin (FHA), auto-transporters (including pertactin [PRN]), and polysaccharide capsule (37).

A-subunit of PT, adenylate cyclase toxin (ACT), lipopolysaccharide (LPS), tracheal cytotoxin (TCT), and autotransporters have roles in blocking the innate immune response, which allows the infection to progress (37, 38).

The only unique virulence factor expressed by *B. pertussis* is PT, which is an A-B exotoxin. A-subunit possesses enzyme activity, and B-subunit binds PT to the host cell and allows the A-subunit to enter the cells (39). Once in the cell cytosol, A-subunit hydrolyses cellular NAD and transfers the released ADP-ribose to G-proteins. This increases intracellular levels of cAMP, leading to interference with cellular functions and suppression of the phagocytic function of phagocytes. The systemic effects of the toxin regulated by cAMP include lymphocytosis, alteration of hormonal activities, and increased insulin and histamine production (37, 40, 41).

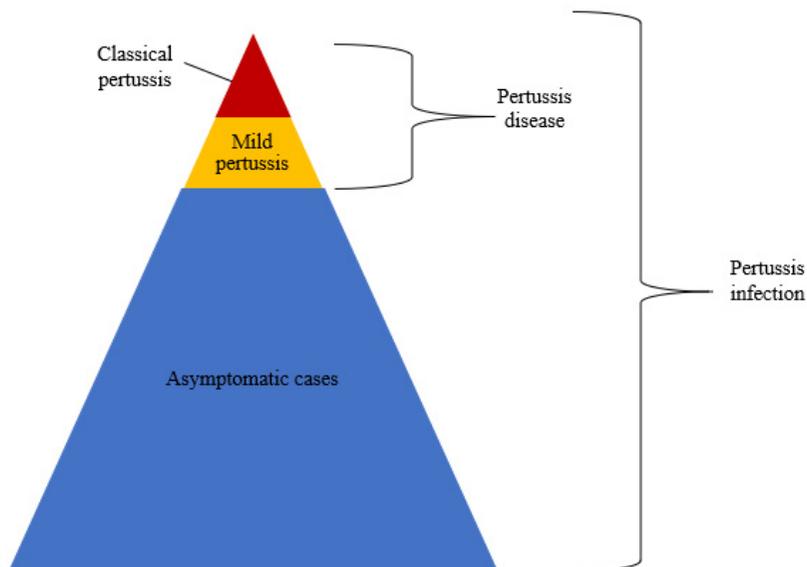
In infants, the presence of hyperleukocytosis may be associated with fatal outcomes (42). The hyperleukocytosis is not caused by the increased leucocyte production; instead, there is an increase in the release of B and T lymphocytes into the blood from extravascular sites, and these cells recirculate rather than emigrate from the blood as they normally would (43, 44). Increased leucocyte mass in small pulmonary vessels can diminish blood flow by increasing pulmonary vascular resistance, which may result in pulmonary hypertension, cardiac failure, and shock (45).

To date, it remains unclear how the bacterium is cleared from immune or nonimmune hosts and why the symptoms may last for many months even after the causative organism has been eliminated by host immune response or antibiotics (46). Pittman et al. (47) and Robbins et al. (48) have suggested that paroxysmal cough, the main manifestation of pertussis, is PT-mediated. However, infections caused by *B. parapertussis*, which does not express PT, also cause paroxysmal cough (49). The long duration of paroxysmal cough has also been associated with local tissue damage, but even in the case of the classical course of the disease, the paroxysms persist even longer than the expected recovery of the local tissue damage; therefore, Mattoo and Cherry have suggested that another, unidentified toxin might contribute to the continued paroxysmal cough (43).

## 2.4. Clinical signs and symptoms of pertussis

The incubation period is usually 7–10 days but may range from 7 to 21 days (50).

The severity of clinical courses of pertussis may vary from asymptomatic carriage, typically in adolescents and adults, to a life threatening disease in newborns and infants (18, 50, 51) (Figure 1).



**Figure 1. Overview of possible clinical presentation of pertussis**

Pertussis disease covers all symptomatic cases (both classical and mild pertussis). Asymptomatic cases have no symptoms, but *B. pertussis* colonises their nasopharynx, and transmission to naive subjects is possible. Pertussis infection comprises both symptomatic and asymptomatic cases.

The classical course of pertussis is mostly seen in unimmunised children and consists of three periods (52). During the catarrhal period, which lasts one to two weeks, pertussis is highly contagious, and the clinical characteristics are like those of typical upper respiratory tract infections: malaise, rhinorrhoea, sneezing, lacrimation, and mild cough with no or low-grade fever (18). During the catarrhal period, the severity of cough progresses (52). In the paroxysmal period, which usually lasts from three to six weeks, the classical symptoms of pertussis occur. During the paroxysms, the patient cannot breathe until the coughing has passed, and paroxysms might be followed by a sudden inspiration, the “whoop” (52). Frequency of paroxysms may vary from only once a day to as often as every 15 minutes (52). Sometimes after the paroxysms, posttussive emesis appears (52). The cough is particularly severe at night (38, 53). The patient generally feels fine and has no coughing episodes between paroxysms (52). The last period is called the convalescence stage, which may last for several months, during which the cough slowly improves (54).

Adolescents and adults who have had previous contact with pertussis antigens usually have an atypical course of the disease (3, 13). Sometimes mild pertussis, characterised by nonparoxysmal cough for 1 to 2 weeks without classical pertussis symptoms, is presented. Though the infection remains mostly asymptomatic in these age groups, *B. pertussis* colonises their nasopharynx, and they can transmit the pathogen to others (12, 55, 56). McGuinness et al. have published that among those over 50-years of age, the most common conditions diagnosed prior to pertussis were cough and bronchitis in cases of symptomatic pertussis (57).

The other age groups with an atypical course of the disease are newborns and young infants, who may have no paroxysms or cough at all, but poor feeding, dyspnoea, cyanosis, bradycardia, and apnoea can occur (41, 58, 59). Infants are at the highest risk of serious disease and death due to pertussis (60, 61).

## 2.5. Main complications

Type and frequency of pertussis complications are associated with the age and immune status of the patient. The rate of complications is four times higher in infants under six months old than in others because this age group is un- or partly immunised (53).

Major complications of pertussis are as follows (41, 54):

- pulmonary (pneumonia, pulmonary hypertension, atelectasis, pneumothorax, pneumomediastinum, subcutaneous emphysema, and rib fracture)
- neurologic (seizures, encephalopathy, and intracranial haemorrhage)
- nutritional (emesis, dehydration, hypoglycaemia, and weight loss)

Hospitalisation rates are highest among infants at 63% and 28% in those under 6 months and those from 6 to 11 months, respectively (62). Among adolescents and adults, only 1–2% of patients are hospitalised, but in those at least 50 years of age, the hospitalisation rate is 6% (4). Between 1997 and 2000 in the USA,

20% of all reported pertussis cases were hospitalised, 5.2% had pneumonia, 0.8% had seizures, 0.1% had encephalopathy, and 0.2% died (62). Bacterial and viral superinfections like influenza and respiratory syncytial virus (RSV) may also occur, which leads to a more severe clinical course (63, 64).

The pertussis mortality rate is highest among infants (1). The risk factors associated with infant death from pertussis are low birth weight, young gestational age, young age at the time of cough onset, and high peak white blood cell and lymphocyte counts (65). Chow et al. (1) have systematically reviewed studies about pertussis mortality rates and concluded that overall pertussis mortality rate in the pre-vaccine era varied from 2 cases per million in Italy to 1500 cases per million in Denmark (66, 67), pertussis mortality rate in infants in the pre-vaccine era varied from 307 per million in United Kingdom (UK) (68) to 6,200 per million in Senegal (69). The overall pertussis mortality rate ranged from 3.4–5.3 per million in the early vaccine era and decreased by 90% in later periods (1). Furthermore, the infant mortality rate decreased from 16.5 per million births in the early vaccine era to a later 7.2 in the UK and from 12.3 to 2.4 in the USA (1, 64, 68, 70, 71).

## 2.6. Laboratory testing of pertussis

Direct methods such as nasopharyngeal culture and polymerase chain reaction (PCR) and the indirect method enzyme-linked-immunosorbent serologic assay (ELISA) are reliable and mostly used in diagnosing pertussis (22, 72). The sensitivity, specificity, timing, advantages, and disadvantages of the different tests for diagnosing pertussis are presented in Table 2.

The gold standard for the diagnosis of pertussis, **culture**, is highly specific but has poor sensitivity which decreases with the duration of the disease, treatment of antibiotics, and increasing age of the patient (75, 80, 83). *B. pertussis* is very susceptible to environmental conditions, and therefore improper sampling and culture techniques may give false negative results. Because the immediate culture of specimen is usually unavailable in clinical practise, the transport medium of Regan-Lowe agar to prevent the loss of *B. pertussis* and inhibit the growth of other bacteria should be used (43). Bordet-Gengou and Regan-Lowe agars are the media most used to isolate *B. pertussis* (20), but the slow growth of bacteria may delay diagnosis up to 10 days (20, 84).

Many assays to detect *B. pertussis* by **PCR** are available. However, none of them have been standardised across clinical laboratories until now (85). The most frequently used target genes of *B. pertussis* are insertion element (IS) 481 and promotor of PT gene (ptxA-Pr) (72, 86). In clinical practise, PCR is more used than culture because of its higher sensitivity, faster diagnosis, and ability to detect inviable bacteria (84, 87). However, PCR is also not ideal because of its inability to distinguish between the colonisation and the disease (88), potential cross-reaction with other *Bordetella* species (19), and potential contamination during management (89).

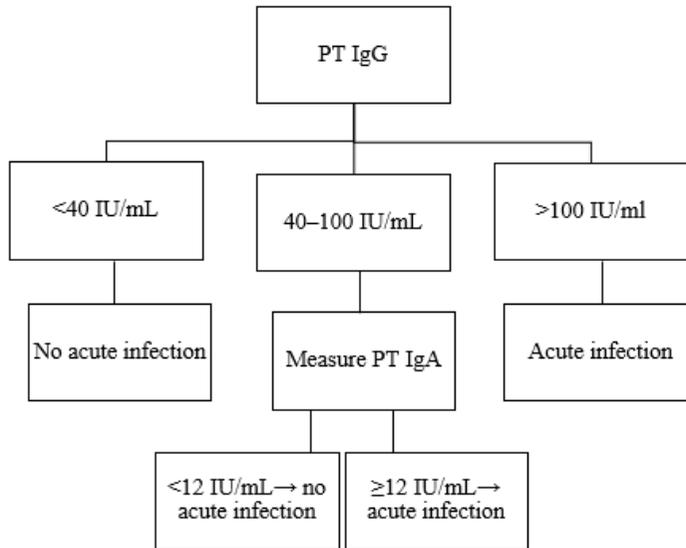
**Table 2.** Summary of the diagnostic tests for pertussis (19, 20, 73-81)

<b>Test</b>	<b>Sensitivity*</b>	<b>Specificity*</b>	<b>Timing</b>	<b>Advantages</b>	<b>Disadvantages</b>
<b>Culture</b>	12–60%	~100%	< 2–3 weeks post cough onset	- high specificity - antibiotic sensitivity testing - typing of <i>B. pertussis</i>	- low sensitivity - special transport media - slow growth
<b>PCR</b>	70–99%	86–100%	< 3–4 weeks post cough onset	- rapid result - sensitive - unviable microbe - usable during 5-day antibiotic treatment	- specificity varies - sensitivity decreases during time - cross-reaction with other <i>Bordetellas</i> - contamination during management - unable to distinguish between carriage and disease - no standardisation
<b>Paired sera (PT IgG)</b>	90–92%	72–100%	At symptom onset and 4–6 weeks later	- usable in late stage of the disease (culture and PCR negative) - antibiotic treatments do not influence diagnosis	- late diagnosis (pathogen is already transmitted to others) - unusable if last pertussis vaccine was < 12mo ago
<b>Single sera (PT-IgG and PT IgA if PT IgG is between 40–100 IU/mL)</b>	36–76%	99%	At least 2 weeks after cough onset, ideally 4–8 weeks post-cough	- usable in late stage of the disease (culture and PCR negative) - antibiotic treatments do not influence diagnosis	- diagnostic cutoffs not validated
<b>Oral fluid antibodies</b>	80%	97%	At least 2 weeks of cough	- non-invasive - late stage of the disease (culture and PCR negative) - antibiotic treatments do not influence diagnosis	- available only for 5–16 years olds in UK - not validated or standardised - unusable if last pertussis vaccine was < 12mo ago

\* sensitivity and specificity values obtained from Wendelboe and Van Rie, 2006 (73) except antibodies in oral fluid (82)

In terms of serologic tests, many different ELISA tests are in use. Serum Reference Standards are essential for comparison of both intra- and inter-laboratory estimates. Therefore, the World Health Organization (WHO) established the First International Standard for Pertussis Antiserum (coded 06/140) for the measurement of pertussis antibody concentrations in human serum in 2008 (90). The long-used qualitative ELISA tests with mixtures of antigens such as PT and FHA or with whole *B. pertussis* cells have low specificity and are not recommended anymore (81, 91). Only PT antibodies are specific to *B. pertussis*; therefore, concentration of PT antibodies should be measured, and the result should be expressed in international units per millilitre (IU/ml) (20, 81, 91, 92). Previously, serological data has been published in many different units, but the numerical values of IU/ml are equivalent to the previously used ELISA units/ml (EU/ml) (81, 91), and the European Sero-Epidemiology Network (ESEN) units/ml (93) and 100 (Dutch) u/ml are equivalent to 125 IU/ml (94, 95).

Serologic confirmation of pertussis can be performed using either paired sera or single-sample serology. **Paired serology** looks for on  $\geq 100\%$  increase or decrease in PT immunoglobulin G (IgG) concentration for at least two weeks (92). If there is no  $\geq 100\%$  change, but one of the samples shows antibody levels above the cutoff for single-sample serology (see below), the result can be interpreted as evidence of recent infection of *B. pertussis* (92). **Single-sample serology** is often used in clinical practise, but the cutoff values for PT IgG are not conclusive (20) and vary between 40 and 200 IU/mL (96-101). This might be influenced by the ELISA kit used, age group of study subjects, or immunisation status and schedule of the country. The EU Pertstrain group have suggested that a single sample cutoff of PT IgG with optimal sensitivity and specificity may be in the range of 60–75 IU/mL based on receiver operating characteristic (ROC) curve analysis of data from Denmark, the Netherlands, and the UK (81). However, de Melker et al. suggested two cutoffs of PT IgG: 125 IU/ml (sensitivity 81.0% and specificity 99.2%) and 62.5 IU/mL (sensitivity 92.7% and specificity 96.4%) (96). The latter values have been used in a large seroepidemiological survey conducted in Western Europe, where the higher value indicates infection in the last 6 months and the lower value in the last 7–12 months (93). The lower cutoff of PT IgG is also suggested for outbreak situations (100). If diagnosis cannot be confirmed with single-sample serology but pertussis is suspected based on clinical symptoms, then PT IgG should be measured in a second (convalescent) serum sample two to four weeks later (92). If a second serum sample cannot be obtained, measurement of the concentration of PT immunoglobulin A (IgA) in addition to PT IgG is an alternative (92). The cutoff value of PT IgA is not broadly accepted, but in view of the relatively high specificity and low sensitivity, it may be between 10 and 20 IU/mL (56, 81). In addition, in children under four years old, the IgA response to *B. pertussis* infection may be low or even absent (74, 102). A serologic diagnostic algorithm suggested by Riffelmann et al. (91) is presented in Figure 2.



**Figure 2. Serologic diagnostic algorithm suggested by Riffelmann et al. (91)**

Currently, measurement of immunoglobulin M (IgM) against *B. pertussis* antigen is not recommended because its reaction is very slow and often absent in immunised or previously infected populations (20, 103). However, Otsuka et al. have recently developed a novel IgM capture ELISA that measures serum anti-*Bordetella pertussis* Vag8 (autotransporter protein) IgM levels with higher diagnostic accuracy than commercial PT IgG ELISA tests; in addition, it was shown that anti-Vag8 IgM antibodies were induced earlier than PT IgG antibodies in consecutive patients' sera (104). However, this method is not available on the commercial market yet.

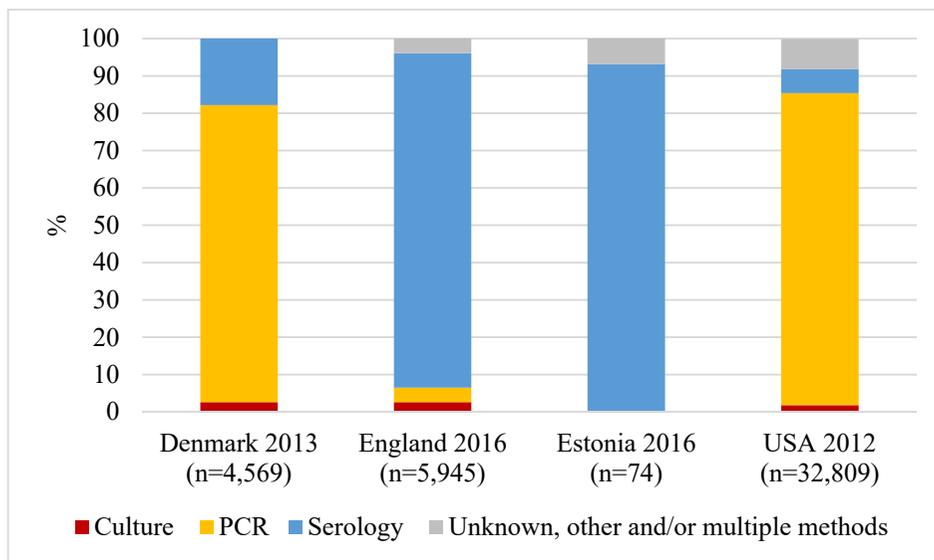
Available serological tests cannot be used for one year after pertussis immunisation to confirm the diagnosis, as these tests are unable to differentiate antibodies from natural infection and vaccination (80, 81). However, Otsuka et al. have shown that paediatric diphtheria, tetanus, and acellular pertussis (DTaP) vaccines did not induce anti-Vag8 IgG antibodies in mice and therefore might be applicable as a diagnosis conformation method in recently immunised individuals (104).

Litt et al. have found a correlation between the level of PT IgG in serum and PT IgG in **oral fluid**. Using cutoff points for PT IgG of 100 IU/mL for serum and 70 arbitrary units (AU) for the oral fluid, it was reported that 80% of seropositive subjects were also positive according to the oral fluid assay, and 97% of seronegative subjects were also negative according to the oral fluid assay (82).

He et al. conducted a study to describe the methods of laboratory confirmation for pertussis in the EU and European Economic Area/European Free

Trade Association (EEA/EFTA) countries (n=27) in 2010 and concluded that culture, PCR, and ELISA were available in 17, 18, and 20 countries, respectively (72). All three methods were simultaneously available in only ten countries (72). However, the distribution of confirmed cases according to laboratory methods was not studied.

The distribution of laboratory-confirmed cases according to the laboratory methods in Denmark, England, Estonia, and the USA is presented in Figure 3.



**Figure 3. Distribution of laboratory confirmation methods used by country (105-107) (data from Health Board of Estonia)**

In Estonia more than 90% of all pertussis cases are confirmed by serology. This result is similar to the data reported from England. In Denmark and the USA, direct methods like PCR and culture are mostly used to confirm pertussis diagnosis.

## 2.7. Clinical case definitions of pertussis

Many **clinical case definitions** of pertussis are used worldwide (108). The WHO (109), European Centre for Disease Control and Prevention (**ECDC**) (110) and US Centers for Disease Control and Prevention (**CDC**) (111) define clinical cases of pertussis similarly as any person with a cough lasting at least two weeks and combined **with at least one of the following symptoms: paroxysms of coughing, inspiratory whooping**, and posttussive vomiting (i.e. vomiting immediately after coughing) without other apparent cause (Table 3). In addition, the ECDC and CDC accept apnoeic episodes as a clinically defining symptom in infants (110, 111), while the WHO and ECDC accept a case diagnosed as pertussis by physician as a clinical case (109, 110).

**Table 3. Overview and comparison of the WHO's, ECDC's and CDC's pertussis clinical and laboratory case definitions and case classifications of pertussis**

Organisation/ Year	Clinical definition	Criteria for laboratory confirmation	Case classification	Epidemiological linkage
WHO, 2000 (109)	<p>a case diagnosed as pertussis by a physician</p> <p><b>or</b></p> <p>a person with a cough lasting <math>\geq</math> 2 week with at least 1 of the following symptoms:</p> <ul style="list-style-type: none"> <li>- paroxysms of coughing</li> <li>- inspiratory whooping</li> <li>- posttussive vomiting without other apparent cause</li> </ul>	<ul style="list-style-type: none"> <li>- isolation of <i>B. pertussis</i></li> <li><b>or</b></li> <li>- positive PCR</li> <li><b>or</b></li> <li>- positive paired serology</li> </ul>	<p><b>Clinically confirmed:</b> clinical criteria are met</p> <p><b>Laboratory confirmed:</b> clinical and laboratory criteria are met</p>	no
ECDC, 2012 (110)	<p>Similar to the WHO's clinical definition plus</p> <p>apnoeic episodes in infants</p>	<ul style="list-style-type: none"> <li>- isolation of <i>B. pertussis</i></li> <li><b>or</b></li> <li>- positive PCR</li> <li><b>or</b></li> <li>- <i>B. pertussis</i>-specific antibody response (serology results need to be interpreted according to the vaccination status)</li> </ul>	<p><b>Possible case:</b> clinical criteria are met</p> <p><b>Probable case:</b> clinical criteria are met plus epidemiological link</p> <p><b>Confirmed case:</b> clinical and laboratory criteria are met</p>	<p>human-to-human transmission (incubation period 6–20 days, most often 10 days)</p>

Organisation/ Year	Clinical definition	Criteria for laboratory confirmation	Case classification	Epidemio-logical linkage
CDC, 2014 (111)	<p>in the absence of a more likely diagnosis, a cough illness lasting <math>\geq 2</math> week, with at least 1 of the following symptoms:</p> <ul style="list-style-type: none"> <li>- paroxysms of coughing</li> <li>- inspiratory whooping</li> <li>- posttussive vomiting</li> </ul> <p><b>or</b></p> <ul style="list-style-type: none"> <li>- apnoea (with or without cyanosis) (for &lt; 1 year only)</li> </ul>	<ul style="list-style-type: none"> <li>- isolation of <i>B. pertussis</i></li> </ul> <p><b>or</b></p> <ul style="list-style-type: none"> <li>- positive PCR</li> </ul>	<p><b>Probable case:</b> clinical criteria are met for &lt; 1 year:</p> <p>acute cough illness of <u>any duration</u> plus at least 1 of the symptoms of the clinical case definition plus positive PCR</p> <p><b>or</b></p> <p>for &lt; 1 year:</p> <p>acute cough illness of <u>any duration</u> plus at least 1 of the symptoms of the clinical case definition plus a contact with a laboratory-confirmed case</p> <p><b>Confirmed case:</b></p> <p>acute cough illness of <u>any duration</u> plus isolation of <i>B. pertussis</i></p> <p><b>or</b></p> <p>clinical criteria are met plus positive PCR</p> <p><b>or</b></p> <p>clinical criteria are met plus contact with a laboratory-confirmed case*</p>	<p>contact with a laboratory-confirmed case*</p>

\* An illness meeting the clinical case definition should be classified as “probable” rather than “confirmed” if it occurs in a patient who has contact with an infant aged < 1 year who is PCR positive and has  $\geq 1$  symptom and cough duration < 14 days.

### 2.7.1. Sensitivity and specificity of clinical case definitions

Studies assessing the sensitivity and specificity of the WHO's clinical case definition of pertussis have showed that these criteria perform poorly in distinguishing pertussis from other causes of cough lasting at least seven days. Although the sensitivity of the definition in studies conducted in the USA, Iran, and France was relatively high and varied between 79% and 100%, the specificity was low in all studies, ranging between 15% and 17% (112-114). In the French study, pertussis was defined as at least 21 days of paroxysmal cough according to the WHO's recommendations in 1991 (113, 115).

We were not able to find any studies assessing the sensitivity and specificity of the ECDC clinical case definition. However, Gilberg et al. have estimated of the CDC's 1990 case definition (a cough illness lasting at least two weeks with paroxysms of coughing, inspiratory "whoop", or posttussive vomiting and without other apparent cause (as reported by a health professional) and found high sensitivity (89%) and low specificity (11%) similar to those in the above-described studies (113, 116).

In 2011, Cherry et al. proposed more age-specific case definitions separately for the ages 0–3 months, 4 months–9 years, and 10 years and older. The key indicators of pertussis for younger infants are afebrile nature of the illness, cough that increases in frequency and severity, and a coryza that remains watery (108). The addition of apnoea, seizures, cyanosis, emesis, or pneumonia increases both sensitivity and specificity (108). For children 4 months–9 years old, the presence of a worsening paroxysmal, non-productive cough of  $\geq 7$  days in an afebrile child with coryza that has not become purulent might also be sensitive and specific to pertussis (108). The addition of classical pertussis symptoms like inspiratory whooping, posttussive emesis, and apnoea will increase specificity (108). For patients at least 10 years old, the triad listed for 4 months to 9 years would result in high sensitivity and good specificity (108). The presence of sweating episodes in older children and adults will significantly increase specificity (108). To the best of our knowledge, the prediction accuracy of these criteria has not been studied in any of these age groups until now.

## 2.8. Laboratory criteria of pertussis

The WHO, ECDC, and CDC have all defined their criteria for laboratory confirmation of pertussis (Table 3). All accept isolation of *B. pertussis* from a clinical specimen and/or detection of *B. pertussis* nucleic acid in a clinical specimen as confirmation (109-111). The differences between these three agencies are found in their use of serology for confirmation of diagnosis. The WHO recommends only positive paired serology, whereas the ECDC criteria require specific antibody response to *B. pertussis* and emphasise the necessity of interpretation according to immunisation status (109, 110). However, neither organisation defines accurate cutoff values. The CDC does not accept serology

as a laboratory confirmation method (111). However, US National Notifiable Diseases Surveillance System also collects the results of serology tests, and the percentage of reported pertussis cases with serology is increasing; therefore, the CDC analyses serologic assays currently available in the USA to help guide their routine use (107).

The EU Pertstrain group has different recommendations for neonates and young infants than for older children and adults (Table 4) (81).

**Table 4. EU Pertstrain group recommendations for testing suspected pertussis (81)**

	Duration of cough	Culture	PCR	PT-IgG*
Neonates and young infants	As soon as possible post-onset of symptoms	yes	yes	no
Vaccinated children, adolescents, and adults	< 2 weeks	yes	yes	no
Adolescents and adults	2–3 weeks	no	yes	yes
Adolescents and adults	> 3 weeks	no	no	yes

\* Serology cannot be interpreted if the last pertussis immunisation was less than 1 year ago.

## 2.9. Pertussis vaccines and their effectiveness

There are two types of pertussis vaccines available: whole-cell pertussis vaccine (wP), which contains the inactivated *B. pertussis* (~3,000 antigens), and acellular pertussis vaccines (aP) consisting of detoxified PT and up to four other *B. pertussis* antigens (FHA, PRN, FIM 2, and FIM 3) (38, 117).

Pertussis vaccines are routinely administered in combination with diphtheria and tetanus toxoid (DTwP or DTaP) (38). For adolescents and adults, dTap, with reduced concentration of diphtheria toxoid and pertussis antigens compared to DTaP, has been introduced (118).

The wP vaccine has been available since 1950s, and different manufactures have produced it using different strains of *B. pertussis*, technology to kill the bacteria by heating or with formalin, and adjuvants; therefore the available wP vaccines are relatively heterogeneous (38). The wP vaccines are not licenced for routine use in children, adolescents, or adults because of higher reactogenicity in these age groups (38).

Since the 1980s, wP vaccines have been replaced with aP vaccines in many industrialised countries because of concerns about adverse reactions associated with them. The safety of wP vaccines has been reviewed, and there is no evidence that they cause brain damage or severe neurological disease (119, 120). Alleged cases of wP vaccine encephalopathy appear to have been inborn genetic disorders (121, 122). Therefore, wP vaccines are still used, mainly in developing countries because of their lower cost (7, 30).

In developed countries, aP vaccines are widely used. Vaccines differ from each other in the number of antigen components, used bacterial clones, methods of purification and detoxification, and added adjuvants (38). Generally aP vaccines are less reactogenic than wP vaccines (123). The main reported side-effects have been local redness and swelling and fever (58). The rate of these local side-effects after primary vaccination is very low, but the frequency and severity tend to increase with each subsequent dose of the aP vaccine (19, 58). However, among children who receive a booster dose of DTaP, the reported prevalence of local swelling is 2–6% (58).

Measuring the effectiveness of pertussis vaccines depends on the case definition, age of vaccination, settings of exposure, time since vaccination, and vaccine characteristics, but similar efficacy levels have been suggested for both wP vaccines (46–92%) and aP vaccines (67–89%) (19, 38). In more recent studies, Fulton et al. estimated the effects of aP vaccines and wP vaccines on WHO-defined pertussis in children under 5 years old in meta-analysis and concluded that the effectiveness of wP was 94% (95% confidence interval [CI] 88–97) and aP vaccine (three component and five component) 84% (95% CI: 81–87) (124). As the herd immunity threshold for pertussis is estimated at 90–94% (125), even a vaccine coverage rate of 100% would be insufficient to achieve complete elimination of pertussis (124). In the case of aP vaccines, one-component and two-component vaccines have had lower efficacies than vaccines with  $\geq 3$  components (67–70% vs 80–84%) (38, 126, 127). Generally, in the first year after primary vaccination, the effectiveness against fatal and severe disease has been estimated at 100%, against typical disease at 90%, and against mild disease at 70%, and a single dose of vaccine is probably sufficient to prevent death from pertussis (19). In addition, in several studies, a milder course of the disease and shorter duration of cough in immunised patients with pertussis than in nonimmunised has been reported (128, 129).

Initial data from the comparative clinical trials suggested that the duration of immunity from wP vaccines and aP vaccines is similar (130, 131), but epidemiological data suggested that immunity from aP vaccines wanes more rapidly, and a clear increase in reported incidence has been observed in adolescents who have been immunised only with aP vaccines (132–134). Wendelboe et al. have reviewed the published data on duration of immunity and concluded that the estimated durations of immunity after natural infection, after immunisation with the wP vaccines, and after immunisation with the aP vaccines are 7–20 years, 4–12 years, and approximately 6 years, respectively (135). McGirr et al. conducted a meta-analysis of the duration of pertussis immunity and estimated that every additional year after the last dose of DTaP, the odds of pertussis increased by 1.33 times (95% CI: 1.23–1.43), that 10% of children vaccinated with DTaP would be immune to pertussis 8.5 years after the last dose, and that there was no difference in the duration of immunity from 3- vs 5-dose DTaP regimens (136). In addition, in adolescents immunised with dTap, the vaccine effectiveness during the first year after vaccination was 68.8% (95%

CI: 59.7–75.9), decreasing to 8.9% (95% CI: 30.6–36.4) by  $\geq 4$  years after immunisation (137).

Although after immunisation the level of antibodies produced by antigens included in aP vaccines is higher than the level of the same antibodies after administration of wP vaccines, (138) the half-life of antibodies to pertussis antigens following natural infection after immunisation with aP vaccines and wP vaccines are similarly short at 6–12 months (139–141). It is known that the relationship between the level of antibodies and protection is not straightforward, and protective immunity against pertussis need both antibodies to a number of pertussis antigens and cell-mediated immune memory (132, 142). A recent study from Finland demonstrated that cell-mediated immunity persists even when antibodies have decayed (143). Data suggests that natural infection and wP vaccines induce T-helper cell 1 (Th1) responses, while the aP vaccine induces Th 2 or mixed Th2/Th1 responses (132, 144–147). After infection and vaccination with DTwP strong Th17 response in addition to Th1 memory has been reported in nonhuman primate models (33). Th17 and Th2 are specialised to control the extracellular bacterial and fungal infection at mucosal surfaces, and Th1 immune response provides protection from intracellular pathogens (132). As *B. pertussis* is an extracellular pathogen, then the role of Th1 is unclear (132).

### 2.9.1. Immunisation schedule

According to the WHO, there are more than 80 different pertussis immunisation schedules used worldwide (54). However, most of them consist of three doses in the first year of life followed by the first booster dose in the second year (148). In some countries, a second booster for children entering school and third for adolescents are added to the immunisation schedule (148).

The WHO recommends a three-dose primary series to induce an immunological memory, with the first dose administered between 6 and 8 weeks of age; subsequent doses should be given 4–8 weeks apart (58). According to the ECDC, 22 countries start primary immunisation of pertussis at the age of 2 months, and 9 countries start one month later (148). In addition, two types of schedules are used for immunisation under 24 months of age: the 2p+1 schedule (two doses of primary immunisation during the first 6 months of life and a booster dose at 11–12 months) and the 3p+1 schedule (three doses of primary immunisation during the first year of life and a booster dose in the second) (149). All EU/EEA countries have shifted to aP vaccines except Poland, which still uses wP vaccines for primary immunisation and the first booster dose (148).

As unmeasurable or low antibody response will develop in some children after the primary doses and the immunity post-immunisation wanes, the booster doses are scheduled (150). The first booster dose is recommended at the age of 1–6 years (at least 6 months after the last primary dose). In developed countries,

the first booster dose is usually administered at the age of 18–24 months, and the second booster dose to increase herd immunity at preschool age (148). In addition, some countries have already added a third booster dose to adolescents at the age of 14–16 years, as the prevalence of pertussis in this age group is highest in developed countries (93, 148).

Adult boosters with dTap have also been recommended in some countries, but immunisation rates are generally low (148, 151).

Recently, in many countries (the USA, the UK, Australia, Belgium, Ireland, Israel and some regions in Spain), maternal pertussis immunisation has been implemented to prevent pertussis in newborns (152-154). After immunisation of pregnant women, a high level of pertussis antibodies has been achieved, and some of them pass placenta and protect newborns during the first months of life, before the infant's pertussis immunisation program starts (153). The WHO recommends immunising pregnant woman with dTap in the second or third trimester of pregnancy (at least 15 days before the expected delivery) (58). However, in a study conducted in the UK, blunt responses to some vaccines in the infant immunisation schedule after maternal immunisation were shown (155), but this should be monitored and according to Cherry, may be prevented by administering a booster dose in the second year of life (156).

### **2.9.2. Pertussis immunisation in Estonia**

In Estonia, routine childhood immunisation using wP vaccines combined with diphtheria and tetanus toxoids was introduced in 1957 (Table 5) (14, 15). In 2008, all doses of DTwP vaccines were replaced with DTaP (data from Health Board of Estonia).

In general, the immunisation rate has been high ( $\geq 90\%$ ), except between 1991 and 1998, when 76% of 1 year olds had the primary vaccination because of vaccine delivery problems (data from Health Board of Estonia) (15).

**Table 5. Significant events in pertussis immunisation schedule in Estonia (14-16)**

<b>Year</b>	<b>Vaccine type</b>	<b>Event</b>
1957	DTwP, produced by Research Institutes of Moscow	DTwP was introduced; schedule: 5–6 months, 6–7 months, 7–8 months, 1–1.5 years, 3–4 years
1967	DTwP, produced by Odessa I. I. Mechnikov National University	Schedule changed: 5–6 months, 6–7 months, 7–8 months, 2–2.5 years, 6 years
1980	DTwP, produced by Odessa I. I. Mechnikov National University	Schedule changed: 5–6 months, 6.5–7.5 months, 8–9 months, ~2.5 years (the second revaccination was removed)
1993	DTwP (TetrACT-HIB®)	Schedule changed: 3 months, 4.5 months, 6 months, 2 years
2008	DTaP (Tetraxim®, Infanrix Polio®, Infanrix-IPV+Hib®, Pentaxim®)	All doses of DTwP replaced with DTaP; schedule changed: 3 months, 4.5 months, 6 months, 2 years, 6–7 years
2012	DTaP (Tetraxim®, Infanrix Polio®, Infanrix-IPV+Hib®, Pentaxim®) and for 15–16 years dTap (Boostrix®)	Schedule changed: 3 months, 4.5 months, 6 months, 2 years, 6–7 years, 15–16 years

## 2.10. Global epidemiology of pertussis between 1940 and 2017

Pertussis is an endemic disease with sporadic outbreaks and unique epidemic peaks every 3–5 years in both the pre-vaccine and vaccination eras (50, 58).

Before vaccines became widely available in the 1950s, pertussis was one of the most common childhood diseases worldwide, affecting mostly children younger than five years, with the highest mortality rate occurring in children under one year of age (14, 50). In the USA, *B. pertussis* caused more than 200,000 cases and up to 7,500 deaths yearly in the pre-vaccine era (5). The introduction of infant vaccination programmes was associated with a deep decline in the number of pertussis cases and deaths in children by the 1970s (58, 62, 157). For example, the declines between the pre-vaccine and vaccine eras in cases and deaths of pertussis were 92.2% and 99.3%, respectively, in the USA (5). In Canada the reported pertussis incidence of 160/100,000 in the pre-vaccine era was reduced to 11/100,000 by 1974–1983 (158). While *B. pertussis* caused about 73,000 deaths in the USA (mostly among infants) between 1922 and 1931, the number of deaths decreased to 56 between 1983 and 1992 (159).

The WHO has estimated that in 2008, about 16 million cases of pertussis occurred worldwide and 195,000 children died from the disease (160, 161). In 2010, the number of reported cases worldwide was about 161,000 (162). The gap between estimated and reported numbers is huge, mainly because of under-reporting due to unrecognition and underdiagnosing of pertussis (152).

Domenech de Celles et al. reviewed the reported pertussis incidence data from 63 countries between 1980 and 2012 and showed that 32 countries had at least one period of increase in pertussis incidence (zero in Africa, eight in the Americas, four in the Eastern Mediterranean, eleven in Europe, five in southeast Asia, and four in the Western Pacific), but of those, 28 also had at least one period of decrease in pertussis incidence, whereas Australia, Israel, the Netherlands, and the USA experienced no decrease during the period studied (8). The switch to aP vaccines for primary vaccination has been associated with pertussis resurgence (33), but in Australia, the USA, Israel, the Netherlands, Finland, and Bulgaria, for example, the resurgence occurred prior to switching to aP vaccines (8, 163, 164). In addition, the decrease in reported pertussis incidence occurred after the switch to aP vaccines in different pertussis epidemic cycles: for example, in Finland after the period of high incidence, in Italy after the period of low incidence, and in Sweden after the period of no vaccination (8). Similar trends have been noted in countries where wP vaccines are used as a primary vaccination; the reported pertussis incidence increased alongside immunisation coverage in Brazil and Columbia, but decreased in Bolivia, Thailand, and Vietnam (8). However, the differences in the sensitivity of surveillance programmes might have influenced the analysis by Domenech de Celles et al. (8).

### 2.10.1. Pertussis surveillance and epidemiology in Europe

In Europe, the ECDC routinely collects surveillance data on pertussis. In 2015, 29 EU/EEA countries, excluding Liechtenstein and France, reported national data according to the ECDC's pertussis case definition (including clinical, epidemiological, and laboratory criteria) (154).

The reported number of pertussis instances was about 40,000 in 2015, and incidence varies widely between countries (11). The highest incidence rate was in Norway, with 37/100,000, and no cases were reported from Malta or Luxembourg (11). The mean notification rate between 2006 and 2015 in Europe has been 4–12/100,000 (11). During 2006–2015, a total of 101 pertussis-related deaths were registered in Europe (11). The distribution of the average notification rate of pertussis by age group in Europe is presented in Figure 4.

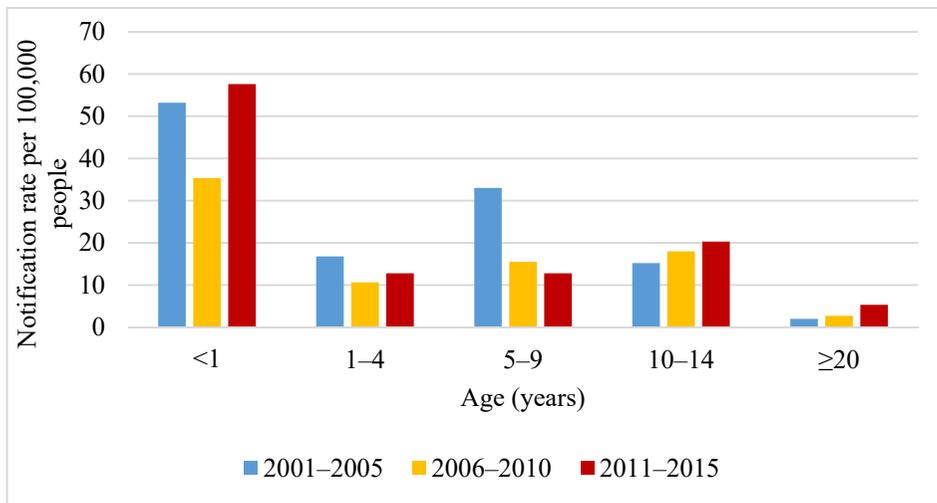
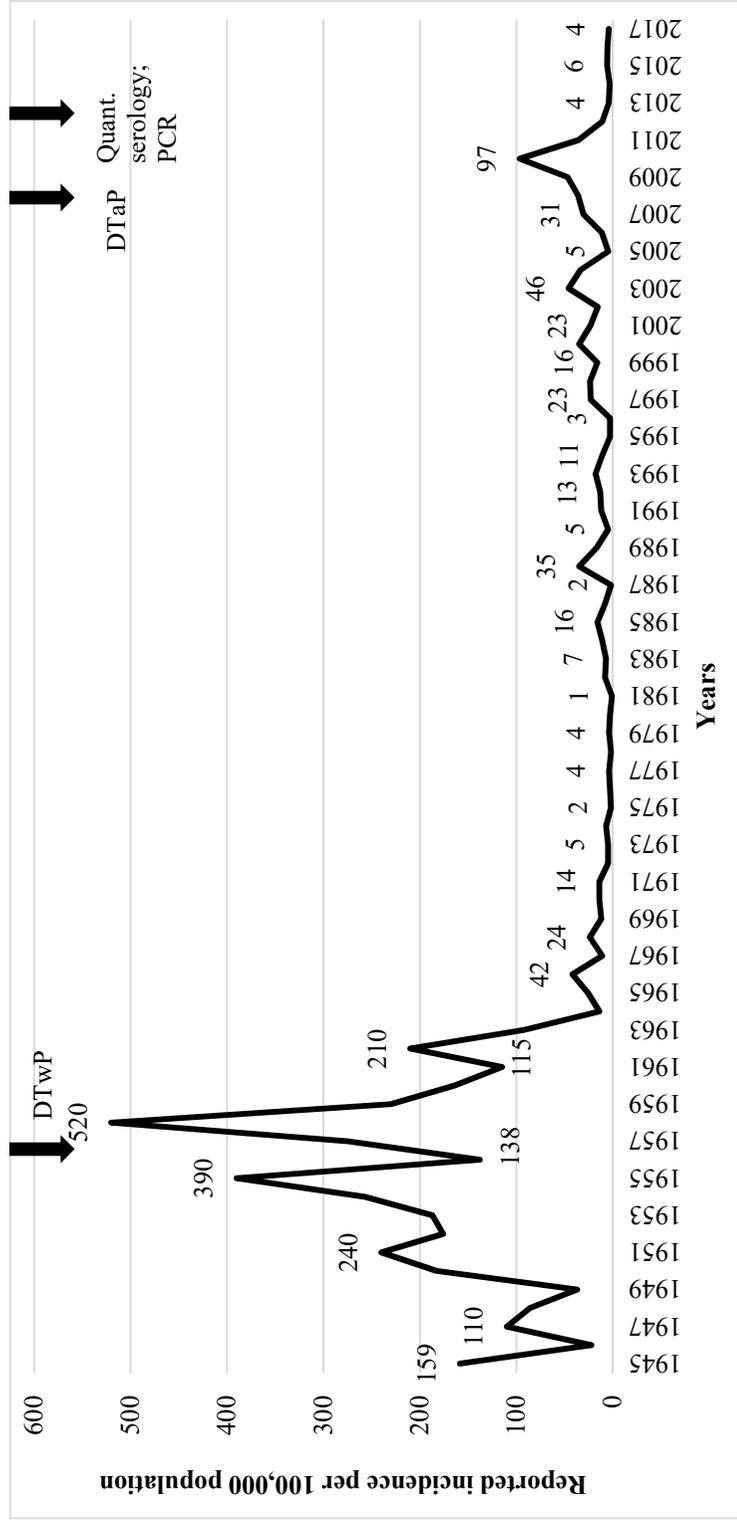


Figure 4. Distribution of the average notification rate of pertussis in different age groups in 2001–2005, 2006–2010, and 2011–2015 in Europe (11)

### 2.10.2. Pertussis epidemiology in Estonia from 1945 to 2017

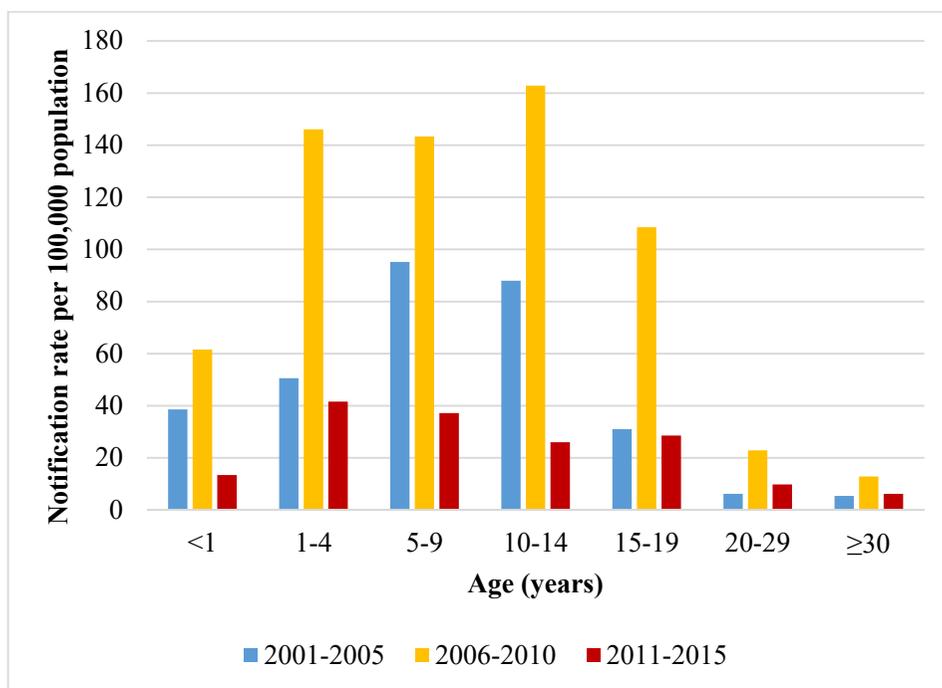
The data on pertussis epidemiology has been collected by the Health Board of Estonia since 1945. The reporting system is based on mandatory reporting by physicians (passive surveillance). The epidemic curve of reported pertussis in the last 70 years is very similar to that in other developed countries (Figure 5) (16, 165). Prior to the adoption of national immunisation in 1957, the reported incidence rate had increased to 400/100,000. The immunisation programme was successful, and the reported incidence rate decreased to 2–4/100,000 in the 1970s (15). The slight increase in the reported incidence rate started in the 1980s, and the highest peak after the adoption of immunisation was achieved in 2010 (incidence 97/100,000) (Figure 5) (165).



**Figure 5. Reported incidence of pertussis (black line) in Estonia between 1945 and 2017 (16, 165)**

Immunisation with the DTwP began in Estonia in 1957 (arrow with DTwP), before which the notification rate was 400/100,000. Immunisation was effective, and the reported incidence rate decreased to 2–4/100,000 in the 1970s. Since the 1980s, the notification rate has slightly increased, and the last epidemic peak was in 2010, with 97/100,000. In 2008, the DTwP was replaced with the DTaP (arrow with DTaP), and in 2012 qualitative serology was replaced with PT-based quantitative serology, and the PCR method became available (arrow with Quant. serology; PCR).

The distribution of the average notification rate of pertussis in different age groups in Estonia is presented in Figure 6. Mortality from pertussis has been very low in Estonia. The last reported death was in 2007 in a one-month-old child (6).



**Figure 6. Distribution of the average notification rate of pertussis among different age groups in 2001–2005, 2006–2010, and 2011–2015 in Estonia (11)**

## 2.11. Factors that may contribute to resurgence of pertussis

Several factors have been observed to contribute to increase in pertussis, but the real role of each of them is still unknown:

- a) Waning natural and vaccine-induced immunities (76, 166): Immunity after both pertussis disease and immunisation (both wP and aP) wanes. It is estimated that infection-acquired immunity lasts a maximum of 7–20 years, whereas protective immunity after immunisation with wP and aP wanes faster, after 4–12 years and approximately 6 years, respectively (135, 167, 168).
- b) Lack of natural booster (76): Lavine et al. have suggested that because a primed immune system can respond to a lower dose of antigen than a naive one, during the pre-vaccine era, adolescents' and adults' primed immunities

- were frequently boosted by re-exposure, leading to individual long-lasting immunity. In the vaccine era, low pathogen circulation allows immunity to be lost before re-exposure occurs, which might explain the increased incidence among adolescents and adults (169).
- c) Use of less immunogenic vaccines (170): Cherry has proposed that efficacy of a vaccine increases directly with the number of antigens in it (171). Therefore, aP vaccines with higher quantities of *B. pertussis* antigens are preferable. However, wP vaccines contain ~3,000 *B. pertussis* proteins, many of which might contribute to protection (43).
  - d) Improved diagnostic methods (166, 172): Development of more sensitive (though not as specific as culture) diagnostic methods such as PCR and serology, in addition to culture, afforded a better opportunity to diagnose pertussis over a longer period of time and in adolescents and adults (9, 84, 173).
  - e) Increased awareness: Cherry has pointed out that initial observations of increased pertussis in the 1980s and 1990s were due to a general increased awareness of pertussis because of the hundreds of publications at the time related to the development and study of acellular pertussis vaccines (174). In addition, increased awareness of pertussis in adolescents and adults was fostered by the use of single-sample serology (174).
  - f) Changes in the genomic content of *B. pertussis* and differences between *B. pertussis* vaccine strains and circulating strains (166, 175-180): Data suggest that *B. pertussis* strains have changed over time and between the pre-vaccine and vaccine eras. Changes have been found in PT, FIM, and PRN. So far there is no evidence of a decrease in effectiveness of wP vaccines because of antigenic drift (181). However, in the case of aP vaccines, circulation of antigen-deficient isolates, especially non-expressed PRN, has been detected (182-185).
  - g) Low vaccine coverage: A correlation between the decrease of vaccine coverage and increase of the disease has been reported in several studies (68, 186). The WHO expects at least 90% of pertussis vaccine coverage in infants with three doses to ensure a high level of protection in children under five years of age (58). However, as the effectiveness of wP and efficacy of aP vaccines have been evaluated at 94% and 84%, respectively (124), and the herd immunity threshold for pertussis is estimated at 90–94% (125), even a vaccine coverage rate of 100% would be insufficient to achieve complete elimination of pertussis (124).
  - h) Unvaccination or undervaccination (individuals who have received fewer doses of pertussis-containing vaccine than recommended according to their ages): Phadke et al. have reviewed 32 reports of pertussis outbreaks in the USA and concluded that unvaccinated and undervaccinated individuals have an increased risk of pertussis compared to fully vaccinated individuals (187). Glanz et al. have demonstrated that both unvaccinated and undervaccinated children higher risk of laboratory-confirmed pertussis than fully vaccinated children (188-189).
  - i) Real increase of pertussis.

## 2.12. Methods of studying pertussis epidemiology

Pertussis epidemiology can be studied in the following three ways: (1) studies based on national surveillance systems, (2) prospective studies in patients with persistent cough, and (3) population-based seroprevalence studies (13). However, it is still difficult to compare the results of the same type of studies conducted in different countries at different times. In addition to real differences, the results might be affected by differences in the stage of pertussis epidemic cycle (the highest rates occur during outbreak as compared to non-outbreak), age of population, vaccination coverage, type of vaccine used and immunisation schedules, and differences in laboratory methods used, and it is difficult to assess the impact of each factor (6, 190-192).

Studies based on a **national surveillance system** analyse the cases collected by mandatory reporting and are divided into passive and active surveillance. Passive surveillance is the easiest method for getting the overview of the epidemiology of the disease, as its data relies on routine reporting. However, it has the main disadvantage of low sensitivity because of unrecognised and thus unreported pertussis cases (193). In active or enhanced surveillance, additional data of routinely reported cases is collected, and possible cases are actively pursued (166, 194, 195). Studies conducted by Plans et al. and Dominguez et al. have shown that under passive surveillance, a higher percentage of severe cases, cases in infants under one year of age, primary cases, hospitalisations, presence of apnoea, and laboratory-confirmed cases have been reported than under active surveillance (166, 194). If the household contacts of confirmed cases are not investigated, then 40% of cases will be missed, mainly in the adult population (166). The huge differences in pertussis prevalence in studies based on national reporting systems conducted in different countries and at different times (paragraph 2.12.1) may be explained by the sensitivity and specificity of the surveillance system and reporting only confirmed and/or confirmed and/or suspected cases, in addition to previously mentioned factors (6, 190-192).

Prospective studies in patients with **persistent or prolonged cough** enable determination of the prevalence of pertussis. Because acute persistent or prolonged cough (of duration between three and eight weeks) is the most frequent symptomatic reason for visiting the general practitioner (GP), pertussis is not always investigated (196-200). During prospective studies, description of pertussis-specific symptoms, immunisation status, data of possible contact with coughing subjects, etc. can be studied in addition to prevalence. However, usually only symptomatic cases are enrolled, and a large number of asymptomatic cases remain unknown. The overall high variability in prevalence of pertussis in patients with persistent cough (paragraph 2.12.2) may be partly explained by the recruitment criteria, methods of pertussis confirmation, and differences in doctor-seeking behaviour (201, 202).

During population-based **seroprevalence studies**, levels of pertussis antibodies are measured, and the estimated pertussis incidence can be calculated. Theoretically, this is an easy way to estimate the incidence rate of pertussis

infection, as it captures both pertussis disease and asymptomatic infection and enables comparison between times and countries (93, 152, 203-208). However, in addition to previously mentioned factors, the main difference between studies might be caused by the sensitivity and specificity of the ELISA tests used and differences in calculating estimated pertussis incidence (different cutoff values, use of simple percentage above the cutoff or of a specific formula such as de Melker's [(203)]). In previous studies, levels of PT IgG of  $\geq 125$  IU/mL or  $\geq 100$  IU/mL have indicated pertussis infection during the last 6 months, with  $\geq 62.5$  IU/mL or 50 IU/mL suggesting pertussis infection in the previous year (93, 95, 96, 204). Various PT IgG levels, ranging from  $< 1$  IU/mL (209) to  $< 30$  IU/mL (206), have been used to describe negative sera in different studies, but most authors define sera with  $< 5.0$  IU/mL as negative (81, 93, 96, 204, 205, 207). However, sometimes this level may instead be the lower limit of detection of the test. Protective antibody levels are still not defined, probably because in addition to humoral immunity, the protection against pertussis is insured with cellular one (33, 96, 102, 152).

### **2.12.1. Previous studies based on surveillance systems**

The overview of previously published epidemiological studies based on surveillance data in different countries is presented in Table 6, and the reported incidence varies widely from 0.6 to 3,697.6/100,000.

There is a great difference in incidence rates between studies based on passive and active surveillance, with higher incidence rates from the active ones during outbreaks (210-212) (Table 6). However, in two long-term studies based on passive surveillance conducted in Australia, similarly high yearly incidence rates have been reported (157, 213). The highest incidence is usually reported among infants (Table 6).

One of the first comparative assessments of pertussis epidemiology, based on passive surveillance in 16 Western European countries in 1998–2002, was published by Celentano et al. in 2005 (Table 6) and observed high variability between countries. The highest incidence was reported in Northern and Central European countries: 124/100,000 in Switzerland, 57/100,000 in Norway, 33/100,000 in the Netherlands, 22/100,000 in Sweden, and 10/100,000 in Germany. These five countries reported 89% of all cases. In all other countries, the incidence was less than 10/100,000 (214). Seven of 13 countries reported highest incidence among children under one year of age, while in Switzerland, it exceeded 1,000/100,000 and in five other countries 100/100,000 (214). The incidence was highest in 1–4-year-olds in the Netherlands, in 5–9-year-olds in Sweden and in Malta, and in 10–14-year-olds in Norway and in Germany (214). The highest incidence in children other than infants might be explained by recent achievement of high immunisation coverage among infants and the lack of booster doses after primary immunisation (214, 215). However, the incidence among people older than 14 years doubled, and the median age of reported

cases increased from 7 years to 11 years between 1998 and 2002, which may be an effect of increased immunisation coverage and decreased frequency of natural boosters causing more cases in adolescence and adults (214).

Recently, Heininger et al. undertook an epidemiological survey of annual incidences of pertussis based on national surveillance systems from 2000–2013 in 10 Central and Eastern European countries and similarly highlighted large differences of reported pertussis incidence ranging from 0.01 in Hungary and Serbia to 96/100,000 in Estonia. In five countries, the highest burden was found among infants under one year old, but in others the highest incidence shifted to older children. This study identified the common problems with surveillance systems and a necessity to standardise pertussis detection and confirmation in surveillance programs across Europe (6).

As presented in Table 6, the highest yearly incidence (3,698/100,000) is reported from an active surveillance study conducted during an outbreak in small town in Ireland in 2010 (210). Despite high immunisation coverage in this region, 70% of all cases were diagnosed among  $\leq 18$ -year-olds, with the highest attack rates among  $< 3$ -year-olds (149/1,000) (210).

A relatively long-term active surveillance study was conducted in Austria between 2000 and 2005, and the yearly incidence rate (9/100,000) was lower than in studies conducted during short outbreaks, as expected (216). However, the highest incidence was reported among  $< 1$ -year-olds (39/100,000) (216).

**Table 6. Overview of epidemiological studies worldwide**

Location	Study period	Case definition	Cul- ture	PCR	Serology	Popu- lation in millions	Number of pertussis cases	Yearly incidence per 100,000	Highest incidence per 100,000/ age group	Hospitalisation (%)/proportion (%) of hospitalised cases in infants
<b>Studies of passive surveillance</b>										
16 EU countries (214)	1998–2002	National case definition	yes	yes	yes	323	79,217	9.0	~80/< 1y	NS/70 < 1y
Australia (157)	1995–2005	Clinical, epidemiological, or laboratory-confirmed	yes	yes	yes	19.2	75,458	39.6	NS/ < 6mo	NS
New South Wales, Australia (213)	1993–2005	National case definition (217)	yes	yes	yes	7.6	35,695	42.8	NS	NS
Estonia, Lithuania, Romania, the Czech Republic, Poland, Turkey (14)	1945–2005	WHO criteria* plus laboratory confirmation or epidemiological link, except Romania only WHO criteria*	yes	yes	yes	NS	NS	0.6–16.2	NS	NS
Argentina (218)	2004–2007	Acute cough illness of any duration plus positive culture or WHO criteria* plus positive PCR or WHO criteria* plus positive serology (if the last vaccine dose was received at least 4 years ago) or WHO criteria* plus epidemiological link	yes	yes	yes	38	2,102	1.4	NS	NS

Location	Study period	Case definition	Culture	PCR	Serology	Population in millions	Number of pertussis cases	Yearly incidence per 100,000	Highest incidence per 100,000/age group	Hospitalisation (%)/proportion (%) of hospitalised cases in infants
Catalonia, Spain (190)	2004–2008	A confirmed case: WHO criteria* plus laboratory confirmation or WHO criteria* plus epidemiological link A suspected case: WHO criteria* without laboratory confirmation and without epidemiological link	yes	yes	no	7.5	963: 555 (58%) confirmed, 408 (42%) suspected	2.7	123/< 1y	31.5/56 < 1y
Washington, USA (219)	Jan–Jun 2012	WHO criteria* plus laboratory confirmation or epidemiological link	yes	yes	no	0.6	2,069	37.5	210/10 y	NS/21.9 < 1y
<b>Studies of active surveillance</b>										
Austria (216)	2001–2005	WHO criteria* plus laboratory confirmation	yes	yes	yes	8.1	4,395	9.0	39/< 1y	NS/79 < 1y
Ireland (210)	Jan–Jun 2010	A possible case: WHO criteria* A probable case: WHO criteria* plus epidemiological link A confirmed case: WHO criteria* plus laboratory confirmation	yes	yes	Yes (PT IgG 100 IU/ml)	0.003624	67: 4 probable 3 confirmed 60 possible	3,697.6	14,900/< 3y	NS

Location	Study period	Case definition	Culture	PCR	Serology	Population in millions	Number of pertussis cases	Yearly incidence per 100,000	Highest incidence per 100,000/age group	Hospitalisation (%)/proportion (%) of hospitalised cases in infants
California, USA (211)	2010/ outbreak	A confirmed case: a cough illness and positive culture or a cough illness of at least 2 weeks with positive PCR or epidemiological link A probable case: a cough illness of at least 2 weeks but not laboratory-confirmed and not linked epidemiologically A suspected case: a cough illness of any duration plus positive PCR or epidemiologic link and at least 1 of the following: paroxysms, inspiratory whoop or posttussive vomiting	yes	yes	no	37.3	9,154: 5,482 (60%) confirmed, 1,706 (19%) probable, 1,966 (22%) suspected	23.4	446/< 6mo	8.8/ 46 < 6mo
Valles, Spain (212)	2011	1)WHO criteria* plus laboratory confirmation 2) WHO criteria* plus epidemiological link	yes	yes	no	1.3	421	33.0	448/< 1y	8/ 30 < 1y

\* WHO clinical case definition: A person with a cough lasting at least 2 weeks with at least one of the following symptoms: paroxysms (i.e. fits) of coughing, inspiratory whooping, posttussive vomiting (i.e. vomiting immediately after coughing) without other apparent cause (109).  
NS – not studied

In all studies, all age groups were included.

### 2.12.2. Previous clinical studies in patients with acute onset persistent cough

The prevalence of pertussis among patients with persistent cough also varied greatly from 2.3% in Canada (201) to 37% in England (220) (Table 7).

In 2016, Marchello et al. published a meta-analysis of prevalence of atypical pathogens in patients with cough and community-acquired pneumonia and reported an overall prevalence of pertussis of 12.4% among mainly outpatients (95% CI: 4.9–19.8) (221). The prevalence was higher in studies conducted only in children (17.6%; 95% CI: 3.4–31.8) than in adults and children (8.9%; 95% CI: 6.7–11.2) (221).

Recently, Teepe et al. conducted a prospective study in GP practices in 12 European countries by enrolling 3,104 adults with cough lasting  $\leq 28$  days between 2007 and 2010, reporting a 3.0% prevalence of pertussis (95% CI: 2.5–3.7). The prevalence varied from 0.0% in Italy to 6.2% in Sweden. Teepe et al. suggested that the differences among European countries might be caused by diversity in immunisation schedules and vaccines used, genetic variations of *B. pertussis*, and differences in doctor-seeking behaviour (202).

A much higher prevalence of pertussis was reported in a prospective study conducted in England between 2001 and 2005 among immunised 5–16-year-old (at least one year previously) school children with cough lasting at least 14 days; 64 of the 172 participants (37%; 95% CI: 30–44) had PT IgG more than 100 IU/mL or an increase of at least four times in paired sample method (220). In another study based on an oral fluid PT IgG titre conducted in England among a similar age group approximately 10 years later, the prevalence was almost half that of the previous study (20%; 95% CI: 16–25) (222). Both studies were conducted in the moderate-low stage of the pertussis epidemic cycle and with immunisation coverage of at least 90%. This difference might be explained by the vaccines used, as England was switching from DTwP to DTaP during the first study, and the differences of the diagnosis confirmation methods. The high prevalence of pertussis in both English studies could be explained by the selected age group, as the national reported incidence of pertussis has almost always been lower in the UK than in other EU/EEA countries (11). However, another study on a moderate stage of a pertussis epidemic cycle using oral fluid serology with similar inclusion and laboratory criteria and approximately 10 years after the DTaP was introduced, conducted in New Zealand, found a similar 17% prevalence of pertussis among school-age children (223).

There were two studies conducted in countries still using DTwP. In a study conducted in Iran in 2007–2008 among children between ages 6 and 14 years with a cough lasting at least two weeks, the prevalence of pertussis based on culture and PCR was 6.4% (95% CI: 3.1–9.7) (114). In a study conducted in Brazil in 2010 and 2011, among subjects aged 10 years and older with a cough lasting between 14 and 30 days, the prevalence was a similar 5.2% (95% CI: 2.0–8.4) based on culture, PCR, and epidemiological linkage (224).

**Table 7. Studies of patients with persistent cough to determine the prevalence of pertussis**

Location	Age group (years)	Study time	Change from wP to aP <sup>a</sup>	Immuni- sation coverage (DTP3) <sup>b</sup> (%)	Stage of pertussis epidemic cycle <sup>b</sup>	Duration of cough days	Cul- ture	PCR	Case confirmation laboratory methods			Pre- valence (%)
									Serology (quantitative)	Number of parti- cipants	Number of pertussis cases	
England (220)	5–16	Oct 2001–March 2005	2001 booster, 2004 all	91	Moderate-Low	14–28 days	no	No	single: > 100 pertussis IU/ml; paired: 4x change	172	64	37.2
France (113)	≥ 18	Apr–Dec 1999	2002	97	N/A	7–31 days	yes	Yes	paired: 2x change	217	70	32.3
Korea (225)	≥ 11	Dec 2009–Dec 2011	1982 <sup>c</sup>	94 <sup>d</sup>	N/A	N/A	yes	Yes	single: PT > 24 EU/mL	310	76	24.5
France (226)	> 13	May 2008–March 2009	2002	98	N/A	> 7 days	no	Yes	single: IgG > 100 IU/mL	204	46	23
New Zealand (223)	5–49	May 2011–Oct 2011	2000 <sup>d</sup>	95	Moderate	≥ 2 weeks	no	No	oral fluid, IgG ≥ 70aU	225	23	20.1
England (222)	5–15	Nov 2010–Dec 2012	2001 booster, 2004 all	95	Moderate-Low	2–8 weeks	no	No	oral fluid, IgG ≥ 70aU	279	56	20.1
Netherlands (227)	≤ 18	Sept 2001–Sept 2003	2001 booster, 2005 all	97	Moderate	1–3 weeks	yes	Yes	single: IgG > 100 IU/mL; paired: 4x increase	135	23	17.0
Denmark (228)	≥ 16	Nov 1996–May 1997	1997	77–92	Moderate-low	2–12 weeks	yes	Yes	single: IgG ≥ 2 SD above the GMC in the control group; paired: 4x increase	201	34	16.9
Turkey (229)	6–14	Nov 2004	2007	85	High	> 14 days	no	No	single > 100 IU/ml; paired: 4x increase	307	51	16.6
Japan (230)	Mean 39 (16–77)	Apr 2005–Mar 2012	1981	99	Moderate-High	N/A	no	yes	single: ≥ 100 IU/ml (in acute or follow up sera); paired: 4x increase	1,325	183	13.8
USA (112)	10–49	Jan 1995–Dec 1996	1997	95	Moderate	7–34 days	yes	yes	single: IgG ≥ 3 SD above the mean of age-matched control values; paired: 2x increase IgG or IgA (≥ 20 U/mL);	212	27	12.7

Case confirmation laboratory methods

Location	Age group (years)	Study time	Change from wP to aP <sup>a</sup>	Immunisation coverage (DTP3) <sup>b</sup> (%)	Stage of pertussis epidemic cycle <sup>b</sup>	Duration of cough	Culture	PCR	Serology (quantitative)	Number of participants	Number of pertussis cases	Prevalence (%)
China (200)	All	Jan 2010–Dec 2012	2007	99	High	N/A	yes	yes	single IgG ≥ 80 IU/mL; paired: 4x increase	1,089	113	10.4
Turkey (231)	10–39	Oct 2010–May 2011	2007	97	Moderate	≥ 2 weeks	yes	yes	No	214	15	7.0
Korea (232)	≥ 11	July 2011–June 2012	1982 <sup>c</sup>	94 <sup>d</sup>	N/A	≤ 30 days	yes	yes	No	490	34	6.9
Iran (114)	6–14	Sept 2007–Nov 2008	wP is in use	99	Moderate	≥ 2 weeks	yes	yes	No	328	21	6.4
Denmark (101)	≥ 8	Oct 2006–Jun 2008	1997	87–93	Low	NA	no	no	single: IgG > 100 IU/mL	265	14	5.3
Brazil (224)	≥ 10	Aug 2010–July 2011	wP is in use <sup>e</sup>	99	Moderate–Low	14–30 days	yes	yes	No	192	10	5.2
12 EU countries (202)	≥ 18	Oct 2007–April 2011	Only Poland used wP, others switched to aP during study <sup>f</sup>			≤ 28 days	no	yes	single: IgG ≥ 125 (in day 28)	3,074	93	3.0
Canada (201)	≥ 12	Oct 1996–Dec 1997	1997–1998g	86–90	Moderate–High	7–56 days	yes	yes	paired: 4x increase	442	10	2.3

<sup>a</sup> Data of change from DTwP to DTaP is recovered from Barkoff et al. 2015 (152)

<sup>b</sup> Data from file of the Ministry of Health for New Zealand (233)

<sup>c</sup> Data of immunisation coverage and stage of epidemic cycle is recovered from the WHO web page (234).

<sup>d</sup> (235)

<sup>e</sup> (236)

<sup>f</sup> (148)

<sup>g</sup> (237)

NA – not available

GMC – geometric mean concentration

### 2.12.2.1. Clinical characteristic of patients with pertussis and with cough of another or unknown aetiology

Most of the studies conducted in patients with persistent cough assess only the prevalence of pertussis and do not compare the clinical characteristics in patients with pertussis and those with cough of another or unknown aetiology. If the symptoms are described and compared, then often no symptoms distinguishing patients with pertussis from those with a cough of another origin are reported (223, 226).

However, in some studies the prevalence of paroxysms, posttussive emesis and inspiratory whooping are significantly higher in patients with confirmed pertussis than in others (12, 112, 201, 220, 230, 232, 238) (Table 8).

**Table 8. Overview of studies which estimate the significant differences in prevalence of symptoms between patients with confirmed pertussis and with cough of another or unknown aetiology**

Location	Age group (years)	Number of participants/ Number of confirmed cases	Clinical characteristics (%)				Other significant symptoms
			Laboratory methods <sup>a</sup>	Paroxysms	Inspiratory whoop	Post-tussive emesis	
USA (112)	10–49	212/27	Culture, PCR, serology	-	-	+	Posttussive gagging
England (220)	5–16	172/64	Serology	-	+	+	Sputum production, wheezing
Iran (114)	6–14	328/21	Culture, PCR	-	-	+	N/A
Spain (190) <sup>b</sup>	All ages	963/555	Culture, PCR	+	-	+	Cough ≥ 2 weeks, apnoea, fever
France (226)	> 13	204/46	PCR, serology	-	-	-	N/A
Japan (230)	16–79	1,325/183	PCR, serology	+	+	-	Posttussive gagging
Korea (232)	≥ 11	490/34	Culture, PCR	-	-	+	Sputum production, rhonchi
Turkey (231)	10–39	214/15	Culture, PCR	+	+	+	N/A

<sup>a</sup> At least one of the laboratory methods used should be positive to confirm pertussis.

<sup>b</sup> Spain's (190) laboratory-confirmed cases + cases epidemiologically linked to the laboratory-confirmed cases

+ presence of significant differences in prevalence of symptoms between patients with pertussis and with cough of another or unknown aetiology

- - no significant differences in prevalence of symptom between patients with pertussis and with cough of another/unknown aetiology was reported

N/A – not available

Harnden et al. found that among 5–16-year-olds, the prevalence of whooping (odds ratio [OR] 2.9; 95% CI: 1.4–5.8), vomiting (OR 4.4; 95% CI: 2.0–9.3), and sputum production (OR 2.4; 95% CI: 1.2–5.0) were significantly higher, but prevalence of wheezing (OR 0.5; 95% CI: 0.2–1.0) was significantly lower among patients with confirmed pertussis than in others (220).

In a lengthy study conducted in Japan from 2005 to 2011, the prevalence of paroxysms, posttussive gagging, whooping, and exposure to cough by others at home, work, or school were significantly more associated with laboratory-confirmed pertussis cases than with other causes of coughing (230).

In 2017, two meta-analyses to determine the diagnostic accuracy of clinical characteristics of pertussis were published (239, 240). Ebell et al., based on 22 studies with a total of 15,909 patients, concluded that overall clinical impression was the most accurate predictor of pertussis (positive likelihood ratio [LR+] 3.3; negative likelihood ratio [LR–] 0.63) (239). Although the overall clinical impression was quite specific (0.85), it lacked sensitivity (0.47); therefore, more than half of patients with pertussis might have been missed (239). The presence of whooping (LR+, 2.1) and posttussive vomiting (LR+, 1.7) increased the likelihood of pertussis, whereas the absence of paroxysms (LR–, 0.58) and sputum (LR–, 0.63) decreased it (239). Moore et al., based on 53 studies with a total of 23,790 patients, concluded that if an adult patient does not have paroxysmal cough or has a fever, they probably do not have pertussis, but the presence of posttussive vomiting or whooping raises suspicion of pertussis (240). In children, only posttussive vomiting was moderately sensitive (60.0%; 95% CI: 40.3–77.0) and specific (66.0%; 95% CI: 52.5–77.3) (240).

The duration of cough in patients with confirmed pertussis is usually significantly longer than in patients with cough of another or unknown aetiology (Table 9). However, the overall duration of cough is a hindsight marker and does not aid in diagnosis.

**Table 9. Median duration of cough in days (range) in patients with confirmed pertussis and with cough of another or unknown aetiology**

Location	Pertussis	Cough of another or unknown aetiology	<i>p</i> -value
UK (220)	112 (38–191)	58 (24–192)	N/A
12 European countries (202)	17 <sup>a</sup> (N/A)	12 <sup>a</sup> (N/A)	0.008
USA (112)	42 (27–66)	35 (4–77)	0.01
Canada (201)	56 (N/A)	46 (N/A)	0.0006
Iran (114)	30 (14–360)	20 (14–360)	N/A

N/A – not available

<sup>a</sup> duration of cough after the index consultation

### 2.12.3. Previous seroprevalence studies

The first large seroepidemiological study conducted by ESEN covered four countries with high (> 90%) immunisation coverage (Finland [DTwP], France [DTwP + DTaP], the Netherlands [DTwP] and East Germany [DTaP]) and three countries with moderate to low (< 90%) immunisation coverage (Italy [DTaP], West-Germany [DTaP] and the UK [DTwP]) and concluded that high concentration (> 125 IU/mL) sera were found across all countries and all age groups, but recent infection was more likely in adolescents (10–19 years old) and adults in countries with high immunisation coverage and more likely in children (3–9 years old) in countries with low immunisation coverage (93).

Barkoff et al. have reviewed 44 seroprevalence studies from 23 countries all over the world and found that the seroprevalence of pertussis (PT IgG > 62.5 IU/mL) ranged from 16 to 22,000 per 100,000 (152). These great differences depended mostly on the age of the study groups, the sensitivity and specificity of the ELISA test used, the cutoff value, the timing of the study during the natural cycle of pertussis, immunisation coverage, vaccine type, immunisation schedule, and diagnostic methods available in clinical practice (152). However, all studies demonstrated a significant difference between nationally reported incidence and seroepidemiological data, and this indicates the spread of asymptomatic and/or mild disease, which causes the unrecognition and underreporting of pertussis (152). According to the seroepidemiological studies, it is difficult to conclude whether the change from wP vaccines to aP vaccines has affected *B. pertussis* circulation in the population, but booster doses of the aP vaccines have postponed the incidence peaks from children to adults (152). A similar shift has been not noted in countries still using wP vaccines (152).

Both, the reported incidence and seroprevalence rate of pertussis were relatively high in two Australian studies conducted in 1997–1998 (DTwP primary, DTaP boosters) and 2007 (DTaP), and the seroprevalence was about 300 times greater than the reported incidence rates (94, 204). A reduction in the percentage of population with antibody levels  $\geq 62.5$  IU/ml from 19% (95% CI: 16–22) to 5% (95% CI: 4–6) in the ten years between the two studies was revealed (204). However, at the same time, the overall percentage of the population with PT IgG < 5 IU/mL increased significantly from 17% (95% CI: 14–20) in 1997–1998 to 38% (95% CI: 36–40) in 2007 (204). The same trend occurred in all age groups. The collection of sera in 1997–1998 occurred during an epidemic period, which is consistent with the large percentage of population with high concentration of antibodies (204). After the 2007 study (with a high number of participants with PT IgG at undetectable levels), a large pertussis epidemic was reported in Australia (234). Despite high (89%) and moderate (55–76%) immunisation coverage with booster doses at ages of 4–5 years and 15–17 years, respectively, the percentage of high level antibodies was about 10% in both age groups in 2007 (241, 242). Campbell et al. concluded that vaccine-produced immunity wanes quickly in communities with low *B. per-*

*tussis* circulation, in which natural boosting opportunities are reduced, and the low level of PT IgG antibodies may be an indicator of susceptibility at population level (204).

Sweden is the only European country where no universal pertussis immunisation program existed between 1979 and 1996 (209). The prevalence of PT IgG was studied in two seroprevalence studies, the first performed one year after the resumption of the immunisation programme in 1997 (n=3,420) and the second 10 years later in 2007 (n=2,379) among subjects at least 2 years old (children vaccinated within the two years preceding the survey were excluded) (209). The proportion of sera with PT IgG < 1 IU/mL without vaccination was low (3.8%) in 1997 but had increased to 16.3% in 2007 (209). The proportion of sera with high PT IgG (> 100 IU/mL) was about 3% in both studies (209). In 1997, the highest proportion of sera, with PT IgG > 100 IU/mL, occurred among 4–5-year-olds (approximately 11%), but 10 years later, it was only about 1% ( $p=0.014$ ), suggesting rapid decline of PT IgG antibodies after immunisation (209). In two non-primary vaccinated age groups (14–15-year-olds and 17–18-year-olds) the proportion of children with PT IgG > 100 IU/mL was higher in 2007 (~6%) than in 1997 (~4%) ( $p=0.11$ ) (209). The difference was significant if a PT IgG cutoff of 50 IU/mL was used ( $p=0.046$ ) (209). Among adults, the seroprevalences for a PT IgG cutoff of 50 IU/mL were 7–10% and 4–8% in 1997 and 2007, respectively ( $p=0.052$ ) (209). Hallander et al. concluded that children's pertussis vaccination has slowed down the transmission of pertussis, with a possible negative effect on population immunity (209).

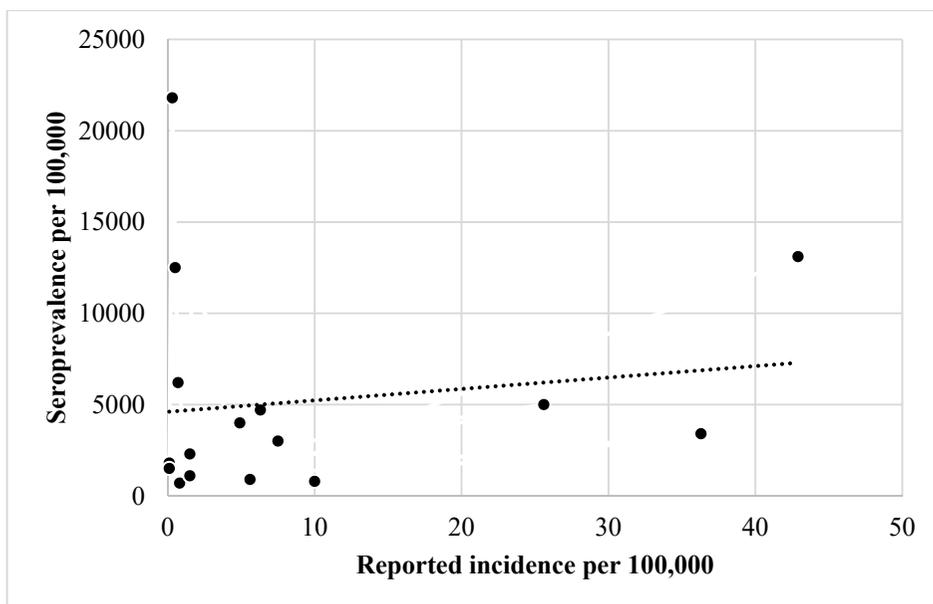
Seroprevalence studies are superior to epidemiological studies based on reported data and studies conducted in coughing patients because they are able to determine all pertussis cases (from asymptomatic to classical ones). In Europe, the gap between the rate of seroprevalence and reported incidence varies from about 80 times in The Netherlands in 1995–1996 to about 9,000 times in Italy in 2004–2005 (Table 10). As shown in Figure 7, no correlation was found between reported incidence and seroprevalence based on the data from Table 10.

**Table 10. Reported incidence and seroprevalence of pertussis in different countries**

Country	Study period (years)	Age of study group (years)	Number of participants	Reported incidence per 100,000 <sup>a</sup>	Sero-prevalence per 100,000	Gap between seroprevalence and reported incidence	PT IgG cutoff
<b>Studies in the DTwP era</b>							
Australia (94)	1997–1998	≥ 1	1,054	42.9	18,700	436	> 62.5 IU/mL
Israel (208)	2000–2001	≥ 3	1,982	5.6	13,100	305	> 125 IU/mL
Turkey (243)	2000–2001	All	2,085	0.5	2,300	411	≥ 62.5 IU/mL
The Netherlands (96)	1995–1996	3–79	7,756	10	900	161	≥ 125 IU/mL
Gambia (244)	2008	2–90	1,067	N/A <sup>b</sup>	27,800	55,600	≥ 50 IU/mL
					12,500	25,000	≥ 100 IU/mL
					3,600	360	≥ 62.5 IU/mL
					800	80	≥ 125 IU/mL
					6,200	N/A	≥ 62.5 IU/mL
					1,800		≥ 125 IU/mL
<b>Studies in the DTaP era</b>							
The Netherlands (245)	2006–2007	≥ 9	7,903	36.3	3,400	94	≥ 125 IU/mL
Czech Republic (246)	2011–2012	≥ 18	2,000	0.8	699	350	≥ 62.5 IU/mL
Australia (204)	2007	≥ 1	3,224	25.6	5,000	195	> 62.5 IU/mL
China (206)	2010	0–95	1,313	0.1	1,800	18,000	≥ 100 IU/mL
Sweden (209)	2007	≥ 3	2,132	7.5	3,000	400	≥ 100 IU/mL
Italy (247)	1996–1997	all	3,565	6.3	4,700	746	> 125 IU/mL
Belgium (248)	2012	20–49	1,500	4.9	8,000	1,633	≥ 50 IU/mL
Slovenia (207)	2000	0–60	3,418	1.5	4,000	816	≥ 100 IU/mL
Denmark (249)	2006–2008	18–72	3,440	1.5	2,300	1,533	≥ 125 IU/mL
Italy (250)	2004–2005	≥ 12	1,304	0.7	3,000	2,000	> 75 IU/mL
Mexico (251)	2010	1–95	3,334	0.3	1,100	1,650	> 125 IU/mL
China (252)	2008–2009	2–20	1,616	0.1	6,200	8,857	≥ 100 IU/mL
					21,800	72,667	> 125 IU/mL
					1,500	15,000	> 125 IU/mL

<sup>a</sup> Reported incidence is recovered from the WHO database of vaccine preventable diseases: monitoring system or referred publications (234).

<sup>b</sup> N/A – not available



**Figure 7. Correlation between reported incidence and seroprevalence per 100,000 people**

Each point indicates the results of reported incidence and seroprevalence of a study from Table 10. Cutoff values of at least 100 IU/mL were used. The line indicates the trendline. No correlation between reported incidence and seroprevalence among different studies was found.

The main problem of seroprevalence studies is recent immunisation, as both immunisation and contact with *B. pertussis* increase antibody levels (81, 93, 203, 253). Therefore, it is not possible to estimate pertussis seroprevalence by the increase in PT IgG antibodies in recently immunised populations, but in these age groups, the level of these antibodies could be compared after immunisation with different vaccines in cases of different immunisation schedules.

In studies conducted in the Netherlands in 1995–1996 (the DTwP era) and 2006–2007 (the DTaP era), the levels of PT IgG in recently immunised children were studied (245). While in the first study, only 0.9% (95% CI: 0–2) of one-year-olds had PT IgG  $\geq$  62.5 IU/mL, in the second study already 32% (95% CI: 21–43) had PT IgG  $\geq$  62.5 IU/mL (245). However, a sharp decrease occurred, and only 2% had PT IgG  $\geq$  62.5 IU/mL a year later. The next peak of a high percentage of participants with PT IgG  $\geq$  62.5 IU/mL was revealed at the age of booster dose, but the following peak started only four years later and might have been associated with waning immunity and new natural infection (245).

Pebody et al. have also studied the proportion of PT IgG  $>$  125 IU/mL in infants primary-vaccinated with DTwP or DTaP and found prevalences of 0–4.3% and 4–13.3%, respectively (93).

## 2.13. Summary of literature

Despite high childhood pertussis immunisation rates, reported incidence has been increasing in many countries recently, including Estonia (10). As pertussis is mandatory to report to national surveillance organisations in most countries, most epidemiological studies have been based on passive surveillance because they are easiest to perform. However, such studies have low sensitivity due to unrecognised and underreported cases (193). Another way to characterise pertussis epidemiology is to conduct seroepidemiological studies, which capture both symptomatic and asymptomatic cases. To date, no studies of this type have been conducted in Estonia. Even those conducted in the other countries have not captured entire lifespan, having instead been focused on specific populations, usually children.

Cough is a common reason to seek medical care; however, the cause of cough often remains unknown (199). In the pre-vaccine era, pertussis was mostly a childhood disease and mostly diagnosed based on classical pertussis symptoms such as paroxysmal cough, posttussive emesis, and inspiratory whoop (109). In the vaccine era, the sensitivity and specificity of the clinical characteristics and course of pertussis are unknown.

### 3. AIMS OF THIS RESEARCH

The general aim of this thesis is to describe the epidemiology and clinical characteristics of *B. pertussis* infection in Estonia in a population with a high rate of childhood pertussis immunisation and to evaluate whether the current immunisation schedule is sufficient in preventing pertussis.

The study had the following objectives:

1. To determine the concentration of the PT IgG type antibodies in the Estonian population across an entire lifespan;
2. To estimate the incidence of *B. pertussis* infection among adults and children not recently immunised;
3. To compare estimated incidence of pertussis based on PT IgG with the officially reported national figures on pertussis disease;
4. To define the prevalence of pertussis and parapertussis in patients who have sought medical care for a cough of unknown aetiology that had lasted for  $\geq 7$  days;
5. To determine and compare the clinical characteristics of pertussis to those of parapertussis and cough of another or unknown aetiology and to determine how well the WHO's clinical case definition of pertussis predicts the disease;
6. To determine the course of pertussis and parapertussis.

## 4. MATERIALS AND METHODS

This thesis is based on the results of three studies as detailed in Table 11.

**Table 11. Description of studies of the thesis**

<b>Study name</b>	<b>Study populations</b>	<b>Timing</b>	<b>Primary aims</b>	<b>Publication</b>
<b>Study 1:</b> A seroprevalence study in children	Subjects 0–17 years old (n=1,053) with available laboratory leftover sera	09.04.2012– 23.08.2012	To estimate the incidence of <i>B. pertussis</i> infection among 9–14-year-olds; To compare the estimated and reported incidence of pertussis.	I
<b>Study 2:</b> A seroprevalence study in adults	Subjects 18–99 years old (n=3,425) with available laboratory leftover sera	07.01.2013– 27.02.2013	To estimate the incidence of <i>B. pertussis</i> infection; To compare the estimated and reported incidence of pertussis.	II
<b>Study 3:</b> A study in coughing patients	Outpatient and hospitalised patients with persistent cough of all ages (n=549)	23.04.2012– 31.12.2014	To determine the prevalence of pertussis and parapertussis; To compare the symptoms of pertussis, parapertussis, and coughs of another or unknown aetiology.	III

### 4.1. Design and population of the studies

**A seroprevalence study in children** consisted of cross-sectional serosurveys and included consecutive laboratory leftover sera of hospitalised and outpatient subjects younger than 18 years old with any diagnosis. Sera was collected at the Children’s Clinic of Tartu University Hospital, one of the two paediatric hospitals in Estonia. Sera from patients with suspected or confirmed pertussis were excluded. The only information available was the subject’s age.

**A seroprevalence study in adults** also consisted of cross-sectional serosurveys and included consecutive laboratory leftover sera of outpatient subjects aged 18–99 years with any diagnosis. Sera was collected at Synlab Eesti OÜ laboratories (the largest private medical laboratory in Estonia). Sera from patients with suspected or confirmed pertussis were excluded. The only information available was the subject’s age and gender.

**A study of coughing patients** was a prospective observational study conducted among 25 GP practices, two referral hospitals (Tartu University Hospital and Tallinn Children’s Hospital), and one district hospital (Järvamaa

District Hospital). It included patients of all ages with cough of unknown aetiology that had lasted for  $\geq 7$  days with no signs of recovery. We used time-dependent inclusion criteria to identify cough lasting  $\geq 7$  days instead of the  $\geq 14$  days recommended by the WHO, ECDC, and CDC to also detect clinically mild, culture, and PCR-positive cases.

## 4.2. Study procedures

In **seroprevalence studies**, 200  $\mu$ l to 2 mL of randomly selected leftover laboratory sera were collected to the studies according to the subjects' age. In a seroprevalence study in children, samples were first stored at +2 to +8°C for a maximum period of one week and then at -80°C for a maximum period of eight months until analysed. In a seroprevalence study in adults, all sera were stored at +2 to +8°C and analysed within 48 hours of collection. Persons between 20 and 99 years old with PT IgG  $\geq 62.5$  IU/mL were identified. These subjects' GPs were contacted and asked whether their patient(s) had complained of coughing during the six months preceding serum sampling.

**Data collection and monitoring in a study of coughing patients.** During patients' first visits, demographic data, onset time, and presence of basic symptoms (paroxysm, inspiratory whooping, posttussive emesis, apnoea, and fever) and additional symptoms (other complaints patients submitted to doctors, e.g. sleeping disturbances, rhinitis, sore throat, sweating, etc.), severity of cough on a Likert scale from 0–10 (0 = no cough; 5 = discomfort, cough interferes with sleeping; 10 = cough is unbearable, very painful, with constant fear of suffocation) and number of paroxysms according to the patients and/or their parents or guardians were collected in an electronic case report form created specifically for this project. Details of patients' pertussis immunisations based on their immunisation passports, GP's database, or self-reporting were also collected in an electronic case report form. Only patients with confirmed pertussis and parapertussis were further monitored by recording presence of symptoms via weekly telephone or e-mail contact until they had recovered or 12 weeks had passed, whichever occurred first. The presence of cough was monitored until complete recovery.

**Sample collection and storage in a study of coughing patients.** Two nasopharyngeal swabs (NPS) and one sample of whole blood were collected. One NPS was placed into a Regan-Lowe transport medium (Copan Italia®, Brescia, Italy) and stored on site at +4°C for a maximum of 24 hours before being transported to the laboratory. The second NPS was placed into a sterile tube and stored at room temperature for a maximum of 12 hours or at +4°C for a maximum of five days before being analysed in the laboratory. Approximately 1 ml of whole blood was collected in a tube with a clot activator and stored at +4°C for a maximum of five days.

### 4.3. Laboratory testing

The swabs of Regan-Lowe transport medium were analysed in Health Board of Estonia or Tartu University Hospital laboratories. After defreezing, the swabs were placed directly into charcoal agar (OXOID CM0119®, Basingstoke, UK) and incubated at 35°C and 60–70% humidity for 10 days. Plates were visually inspected daily and microorganisms identified using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany).

Swabs of sterile tubes were analysed using an in-house PCR test at Synlab Eesti OÜ or a commercial PCR kit (Diagenode Bordetella [pertussis/parapertussis] Real-Time PCR kit; Liège, Belgium) at Tartu University Hospital's laboratory. For the in-house PCR test, two primers derived from unique deoxyribonucleic acid (DNA) sequences, upstream of the porin genes of *B. pertussis* (5'-ATGCTTATGGGTGTTTCATC CGGCC-3') and *B. parapertussis*/*B. bronchiseptica* (5'-CGTCCAC CAGGGGTGGTAGGAGAT-3'), and a third primer (5'-TGCAACATCCTGTCCCTTAATCC-3') shared by both species were used (254).

Antibodies against PT were analysed at the Tartu University Hospital or Synlab Eesti OÜ laboratories using a commercial ELISA kit (Euroimmun®, Lübeck, Germany) according to the manufacturer's protocol. Concentration of the PT IgG was measured in all studies. In addition, PT IgA was measured in a study of coughing patients if PT IgG was 40–100 IU/mL. The sensitivity and specificity of the PT IgG assay were 100% and 95.5%, respectively, and of the PT IgA assay 100% in both categories.

### 4.4. Definitions

**Adults** – people aged  $\geq 18$  years

**Children** – people aged 0–17 years

**Cough of another or unknown aetiology** – a persistent cough without pertussis or parapertussis confirmation by culture, PCR, and/or serology

**High concentration of PT IgG antibodies** – PT IgG 62.5 to  $< 125$  IU/mL, suggesting pertussis infection or immunisation in the preceding 7–12 months (93, 95, 96, 204)

**Infants** – people aged 0–12 months

**Mid-range concentration of PT IgG antibodies** – PT IgG 5 to  $< 62.5$  IU/mL, suggesting pertussis infection or immunisation more than 12 months previously (93, 95, 96, 204)

**Parapertussis** – *B. parapertussis* isolated by culture and/or positive PCR

**People not recently immunised** – cohorts not immunised with pertussis vaccine during the previous year

**Persistent or prolonged cough** – cough of duration between three and eight weeks (196, 197)

**Pertussis** – *B. pertussis* isolated by bacterial culture and/or positive PCR and/or a single PT IgG > 100 IU/ml or PT IgG 40–100 IU/ml and PT IgA > 12 IU/ml in the absence of immunisation for pertussis within the previous 12 months (91)

**Undetectable concentration of PT IgG antibodies** – < 5 IU/mL (93, 95, 96, 204)

**Very high concentration of PT IgG antibodies** – PT IgG  $\geq$  125 IU/mL, suggesting pertussis infection or immunisation in the last 6 months (93, 95, 96, 204)

## 4.5. Statistics and data analysis

Data on the annual notified incidence of pertussis and immunisation coverage were derived from reports of the Health Board of Estonia (<http://www.terviseamet.ee/nakkushaigused/nakkushaigustesse-haigestumine.html> and <http://www.terviseamet.ee/nakkushaigused/vaktsineerimine/riiklik-immuniseerimiskava-ja-selle-taitmine.html>). The size of the general population was obtained from the databases of Statistics Estonia ([http://pub.stat.ee/px-web.2001/Database/Rahvastik/01Rahvastikunaitajad\\_ja\\_koosseis/04Rahvaarv\\_ja\\_rahvastiku\\_koosseis/04Rahvaarv\\_ja\\_rahvastiku\\_koosseis.asp](http://pub.stat.ee/px-web.2001/Database/Rahvastik/01Rahvastikunaitajad_ja_koosseis/04Rahvaarv_ja_rahvastiku_koosseis/04Rahvaarv_ja_rahvastiku_koosseis.asp)).

All statistical analysis was performed using Microsoft Excel 2013 and Stata 12.1 for seroprevalence studies and R 3.1.1 ([www.r-project.org](http://www.r-project.org)), SAS 9.4 (SAS Institute Inc., Cary, NC, USA), MS Excel 2013, and Medcalc® (<https://www.medcalc.org/index.php>) for study of coughing patients.

### 4.5.1. Sample size calculations

For the seroprevalence studies, the required sample sizes were calculated based on the estimated incidence of pertussis infection of 7–9% in the Netherlands (203, 245). Accordingly,  $55 \pm 6$  subjects were required in each yearly cohort for ages 0–19 and at least 369 subjects for each eight 10-year age cohort (20–29, 30–39, ... 90–99) for other ages.

According to previously conducted studies, the prevalence of pertussis among patients with persistent cough varies widely (Table 7). We hypothesised that the estimated prevalence of pertussis in patients with persistent cough is about 10% in Estonia. Accordingly, 900 patients, or at least 100 laboratory-confirmed pertussis cases, would allow for an estimation of the prevalence of pertussis with a 95% CI:  $\pm 2\%$ .

#### **4.5.2. Analysis of PT IgG concentration in the entire population**

PT IgG antibodies were presented as geometric mean concentrations (GPs) with 95% CIs. As the PT IgG ELISA calibration curve was linear between 5 and 174 IU/mL, the arbitrary PT IgG cutoff values were given as 2.5 IU/mL and 175 IU/mL, respectively, to calculate the GMC.

The antibody levels were further divided into four categories as follows:  $\geq 125$  IU/mL: very high, suggesting pertussis infection or immunisation in the preceding 6 months; 62.5 to  $< 125$  IU/mL: high, suggesting pertussis infection/immunisation in the preceding 7–12 months; 5 to  $< 62.5$  IU/mL: mid-range, suggesting exposure to pertussis infection or immunisation  $> 12$  months previously;  $< 5$  IU/mL: undetectable (93, 95, 96, 204).

Associations between age groups and GMC were assessed using the Kruskal-Wallis' test; pairwise comparisons between age groups were made using Mann-Whitney's U test. A chi-squared test or Fisher's exact test was used to test whether the prevalence of the specified PT IgG values was associated with age group. The prevalence of different PT IgG values among age groups was further compared by a two-proportions z-test if the chi-squared test or Fisher's exact test showed a statistically significant association.

#### **4.5.3. Calculation of estimated incidence of pertussis**

Estimated incidence of pertussis infection was calculated only for people not recently immunised aged 9–14 years and  $\geq 20$  years in seroprevalence studies. A cutoff level of 62.5 IU/mL was chosen (93), and the average time for the very high PT IgG after infection to decrease to 62.5 IU/mL was expected to be 208.9 days (95% CI 195.4–223.3) (203). The estimated yearly incidence was calculated as described by de Melker et al. (203) as  $365.25/208.9 \times$  the percentage of the population sera containing PT IgG of at least 62.5 IU/mL.

Estimated incidence rates of infection were compared using a chi-squared test. Adjustments for multiple testing were made using the Holm-Bonferroni method.

#### **4.5.4. Analysis of data in a study of coughing patients**

Patients with persistent cough were analysed in three groups: pertussis, parapertussis, and cough of another or unknown aetiology. All analyses were performed for the whole cohort and separately for children and adults. In addition, the prevalences of pertussis and parapertussis were calculated separately for five age groups ( $< 1$  y – infants, 1–9y – younger children, 10–17y – older children, 18–64y – adults, and  $\geq 65$  y – elderly).

Patients' symptoms were categorised as basic (paroxysms, inspiratory whoop, posttussive emesis, apnoea, and fever) and additional (other complaints submitted to doctors).

To describe patients with pertussis, parapertussis, and cough of another or unknown aetiology, the arithmetic means ( $\pm$  standard deviation [SD]) were evaluated for numerical variables such as age and number and duration of symptoms. For categorical binary demographic and personal characteristics such as age group, gender, hospitalisation, immunisation, etc. and for presence of symptoms, prevalence (with 95% CI) was evaluated. For pairwise comparison of study groups, the Wilcoxon and Fisher's exact tests were used in cases of numerical and categorical variables, respectively. Odds ratio (OR) with 95% CI: was calculated for all categorical variables.

To evaluate common patterns among the five basic symptoms and three diagnoses (pertussis, parapertussis, and cough of another or unknown aetiology), multiple correspondence analysis [MCA] was performed. The sensitivity, specificity, area under the receiver operating characteristic (ROC)-curve, positive likelihood ratio, and negative likelihood ratio were used to estimate the accuracy of the WHO's clinical case definition of pertussis (109) and the diagnostic accuracy of basic symptoms. Multiple logistic regression analyses were performed to look for alternative predictive models of pertussis.

## 4.6. Ethics

All study protocols have been approved by the Research Ethics Committee of the University of Tartu. For seroprevalence studies, informed consent was not considered necessary because there were no interventions and all sera were collected and analysed anonymously. After complementary approval by the Research Ethics Committee of the University of Tartu for seroprevalence study in adults, the GPs informed us about coughing episodes of patients at least 20 years old with PT IgG  $\geq$  62.5 IU/mL. For the study of coughing patients, all patients and/or their parents or guardians, as appropriate, gave their signed informed consent.

## 5. RESULTS AND DISCUSSION

### 5.1. Participants

A total of 5,027 subjects were enrolled across all studies, 4,478 to the seroprevalence studies (1,053 and 3,425 to the studies in children and adults, respectively) and 549 to the study of coughing patients (Table 12).

**Table 12. Demographic data of the studies**

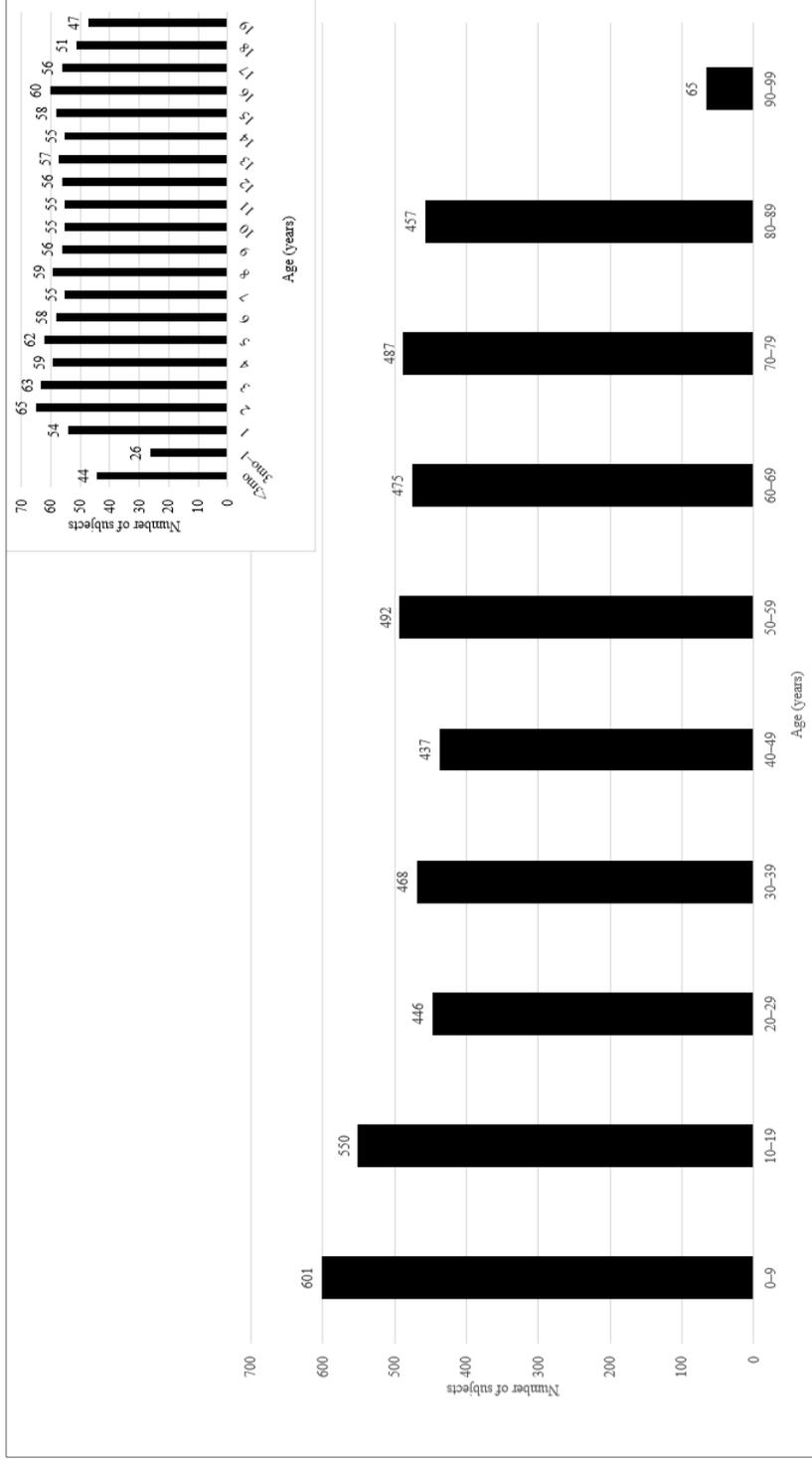
<b>Characteristics</b>	<b>Seroprevalence studies (n=4,478)</b>	<b>Study of coughing patient (n=549)</b>
Infants – n (%)	70 (1.6)	7 (1.3)
Children – n (%)*	1053 (23.5)	145 (26.4)
Adults – n (%)	3425 (76.5)	404 (73.6)
Age in years – mean ( $\pm$ SD)	43.6 (27.0)	35.0 (21.6)
Male-female ratio	N/A	1:2
Male-female ratio in children	N/A	1:1
Male-female ratio in adults	1:2	1:4
Duration in days between start of illness and enrolment in the study		
mean ( $\pm$ SD)	N/A	25.7 (21.0)
median ( $\pm$ SD)	N/A	18.0 (21.0)
Hospitalised – n (%)	N/A	9 (1.6)

N/A – not available

\* Infants are also included

#### 5.1.1. Participants in the seroprevalence studies

The samples were evenly distributed between age cohorts, with a mean number of samples per 10-year age groups of 490 (SD $\pm$ 53). The 90–99-year-old group was an exception, with only 65 samples available (Figure 8). The male to female ratio for  $\geq 20$  years was 1:2 in all age groups.

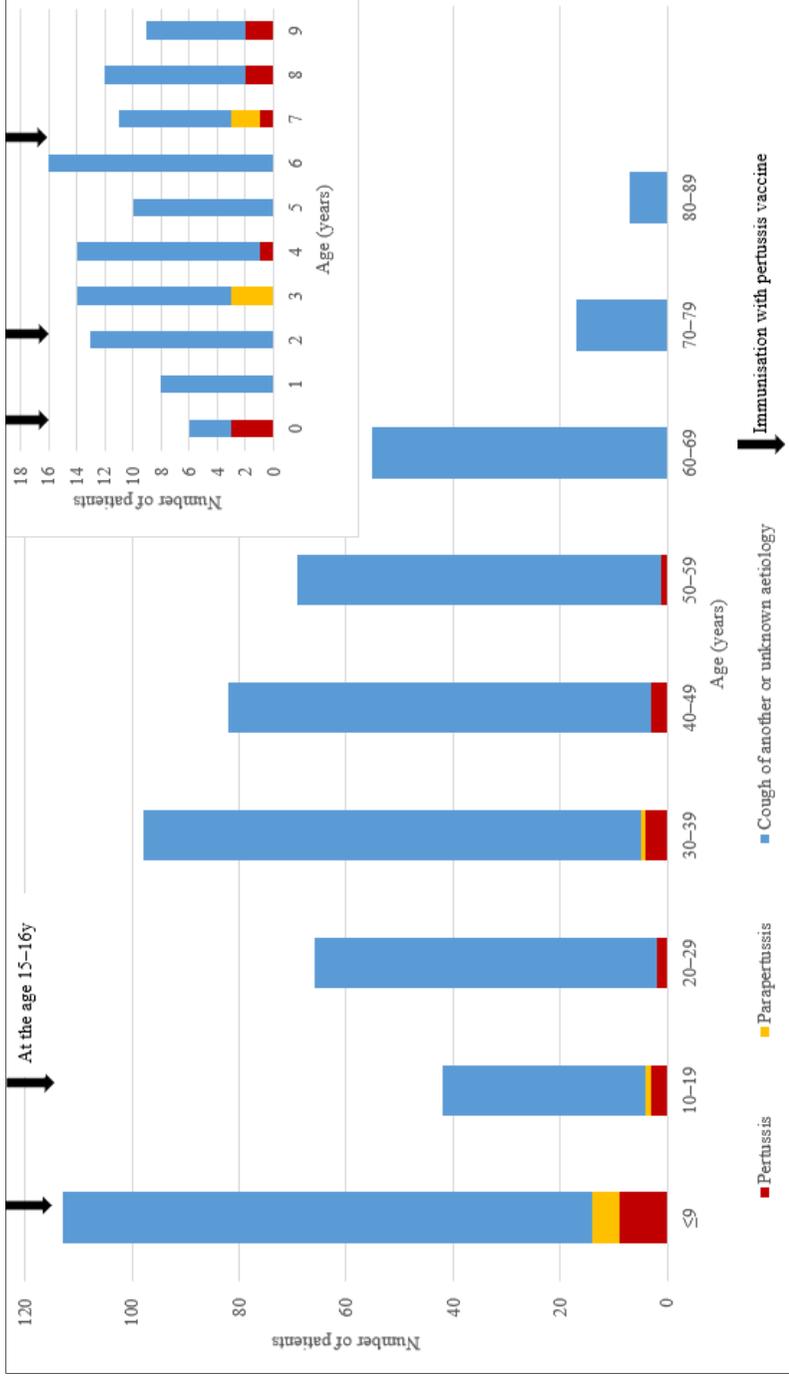


**Figure 8. Age distribution of the study subjects in 10-year age groups in seroprevalence studies**  
 In the subfigure, the age distribution of 0–19 years is presented in yearly cohorts, except that an additional distribution for infants is added, as children under three months have been never immunised.

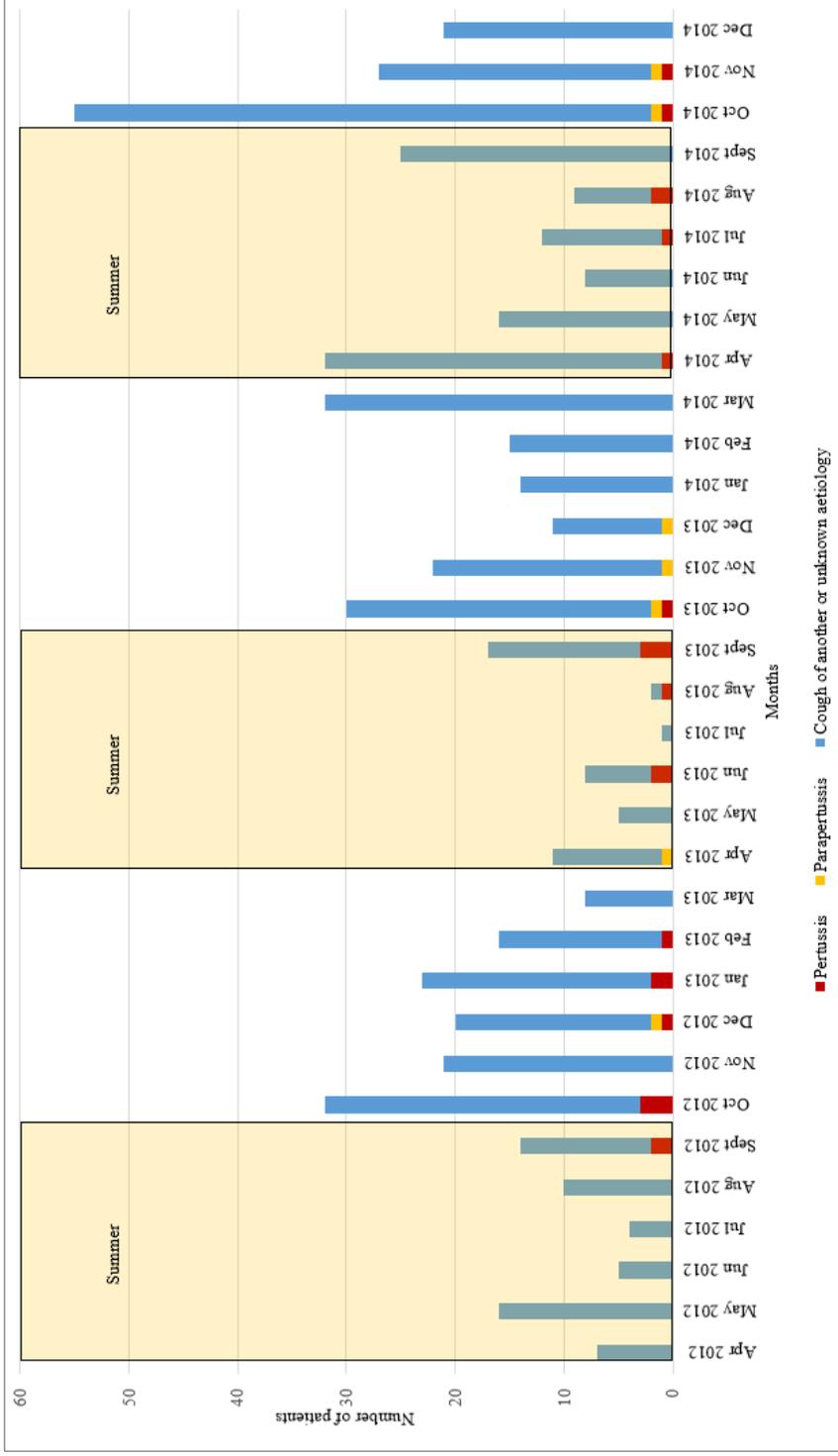
### 5.1.2. Participants in the study of coughing patients

The age distribution of the participants in the study of coughing patients is presented in 10-year age groups in Figure 9. The youngest patient was 24 days old, and the oldest 84 years. The highest number of patients was < 10-year-olds, which might be due to the fact that children more often have respiratory (viral) infections and receive medical care more often than adults (255, 256). However, among adults, the highest number of patients enrolled were of the group 30–39 years old, which is consistent with data published from Korea (232). To the best of our knowledge, only one study covering the entire lifespan of patients with persistent cough to determine the prevalence of pertussis has been published previously (200) (Table 7).

Consistently with other studies' findings, more patients with persistent cough seek medical care in winter than in summer; therefore, 63.2% of the study population were enrolled during winter, from October to March (altogether 15 months), and 36.7% in summer, from April to September (altogether 18 months) (Figure 10). No differences occurred in enrolment between summers or winters of different years; however, the study period was short. The ECDC has reported that pertussis activity typically has no seasonal pattern, which is consistent with our study, in which about half of all pertussis cases were enrolled during summer and another half in winter (154).



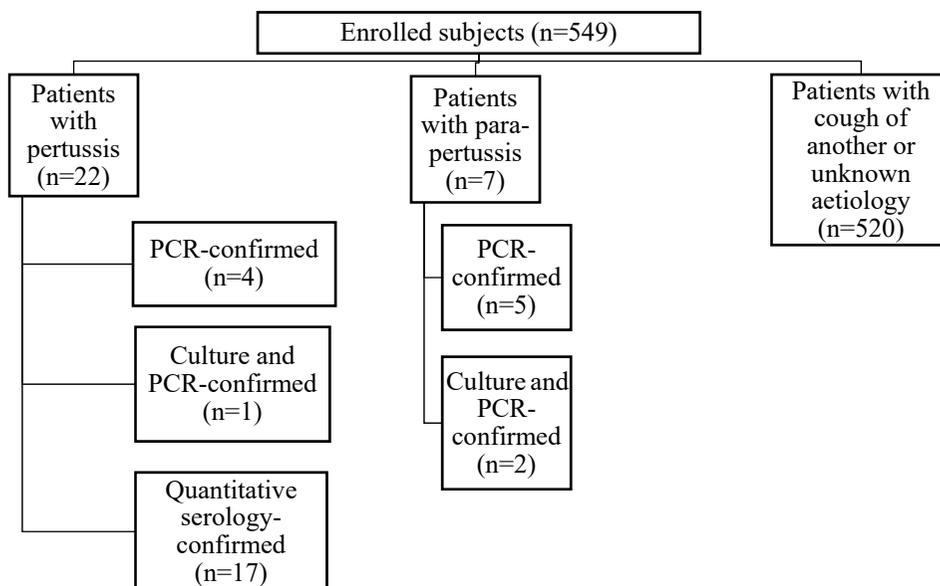
**Figure 9. Age distribution of patients with pertussis, parapertussis, and cough of another or unknown aetiology in study of coughing patients**  
 Red indicates pertussis, yellow parapertussis, and blue cough of another or unknown aetiology. Black arrows indicate ages children in Estonia are immunised with pertussis vaccine. In the subfigure, the distribution of children < 10 years old is presented in yearly cohorts.



**Figure 10. Seasonal distribution of enrolled patients**  
 Red indicates pertussis, yellow parapertussis, and blue cough of another or unknown aetiology.

Of all participants, nine (1.6%) were hospitalised in the study of coughing patients. The highest hospitalisation rate was among infants, at 4/6 (66.6%). Among 1–9-year-olds, 5/107 (4.7%) were hospitalised. In other age groups, no one was hospitalised.

Patient flow according to diagnosis and diagnosis confirmation method are presented in Figure 11.



**Figure 11. Study outline and patient flow in a study of coughing patients**

All patients (n=549) with acute persistent cough of unknown aetiology that had persisted for at least 7 days with no tendency of recovery were included as participants.

The mean and median duration of cough before enrolment were 25.7 and 18.0 days, respectively (Table 12). Yaari et al. have reported that the mean coughing duration before the enrolment in their study was similar at 23±15 days (257). In other previously conducted studies, median duration of cough before enrolment has varied from 14 to 24 days (113, 201, 226, 232).

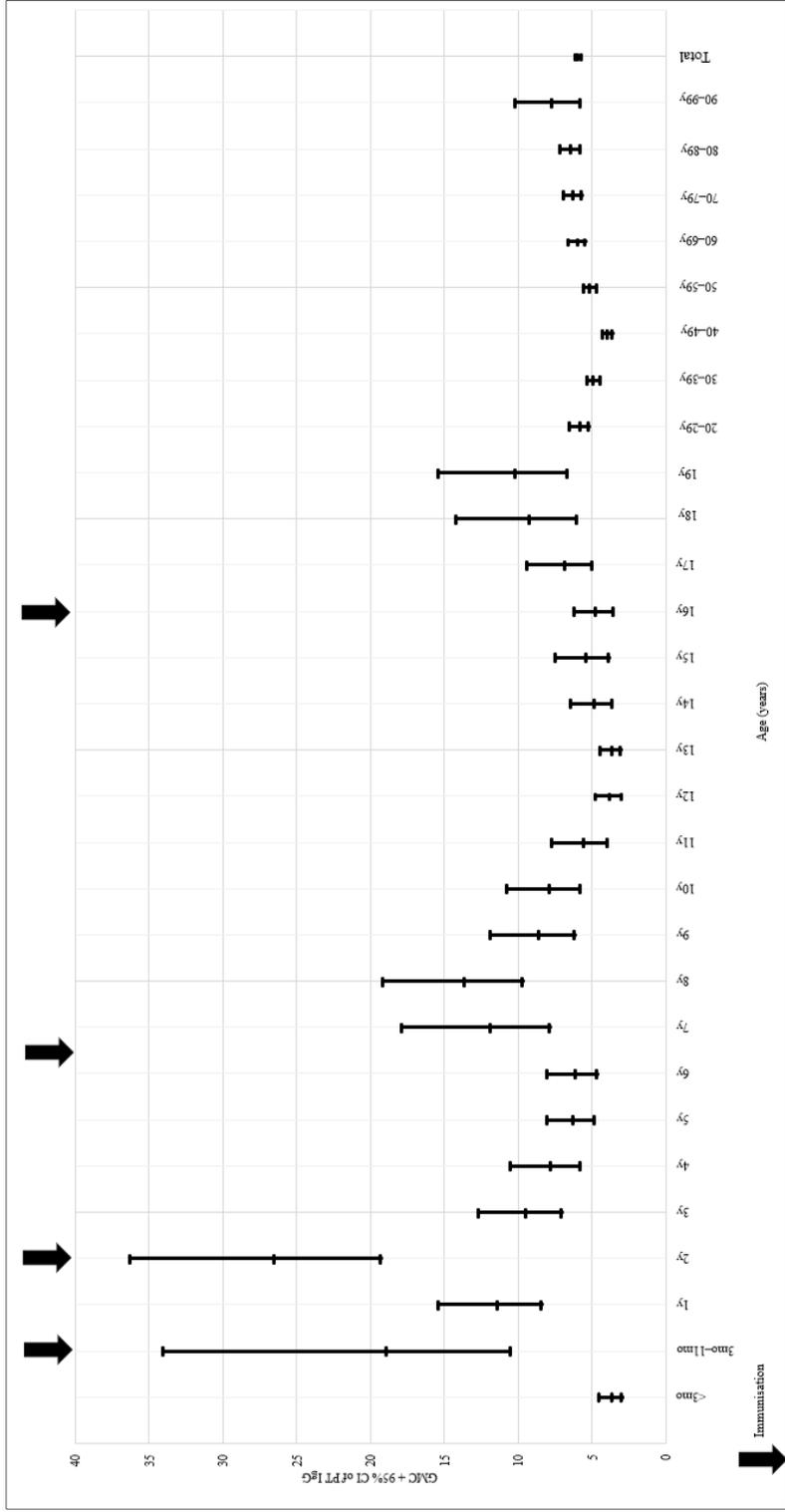
## 5.2. GMC of PT IgG in entire population

The GMC of PT IgG of the entire cohort was low at 5.9 IU/mL (95% CI: 5.7–6.1) (Figure 12). This is comparable to the findings of studies conducted in Gambia and Thailand (both in the wP era), which found 4.9 IU/mL (95% CI: 4.1–5.8 IU/mL) and 5.8 IU/mL (95% CI: 5.3–6.4) (244, 258). Interestingly, the GMC in two Italian studies, a study conducted in Australia in 1997–1998, and a study in China in 2010 showed numerically higher GMC of PT IgG at 19.2 IU/mL, 16.3 IU/mL, 18.8 IU/mL, and 14.2 IU/mL, respectively (94, 206, 247, 250). These differences may be real, but could also have been caused by use of ELISA tests with different sensitivities and specificities (91). In addition, studies conducted in different regions at different time periods might be influenced by the cutoff value used, timing of the study during the natural cycle of pertussis, immunisation coverage, vaccine type, and immunisation schedule (152).

In our study, the GMC of PT IgG was higher in younger age groups at the time of the primary immunisation and first booster dose, after which a sharp decline was observed from age 3 onwards (Figure 12). Similar GMC changes were reported by Rota et al. in Italy and Socan et al. in Slovenia, both of which countries were using aP vaccines similar to those in our study (207, 247). However, in a study conducted in China (where both aP and wP are in use), the GMC tends to be higher in subjects at least 13 years old than among younger children (206). Because in China, the immunisation schedule consists of primary immunisation and a single booster dose at 18–24 months old, this higher prevalence in older children might be an indication of waned vaccine-induced immunity leading to increased prevalence of natural pertussis infection (206).

The high peaks of GMC of PT IgG among infants (primary immunisation) and at the time of the first booster dose were seen exactly at these ages (3–11 months and 2 years). Interestingly, the expected increased levels of GMC of PT IgG linked to the second and third booster doses (at the ages of 6–7 years and 15–17 years) actually occurred one to two years later at the ages of 7–8 years and 18–19 years. Furthermore, there was a trend of antibody response to immunisations and booster doses, with GMC of PT IgG being significantly greater in younger age groups (3 months–1 year and 2 years) than in older age groups (7–8 years and 18–19 years), with 24.0 IU/mL (95% CI: 18.2–31.5) and 11.2 IU/mL (95% CI: 9.2–13.6), respectively ( $p < 0.001$ ). The higher rate of GMC of PT IgG in the younger age group might be due to repeated doses or the specifics of the immune system in different age groups.

Among subjects aged at least 20 years, the GMC of PT IgG of 3.9 IU/mL (95% CI: 3.6–4.2) among 40–49-year-olds was significantly lower than the GMC of PT IgG of other 10-year age groups, except 30–39-year-olds (Figure 12).



**Figure 12. Age-specific distribution of PT IgG GMC with 95% CI**

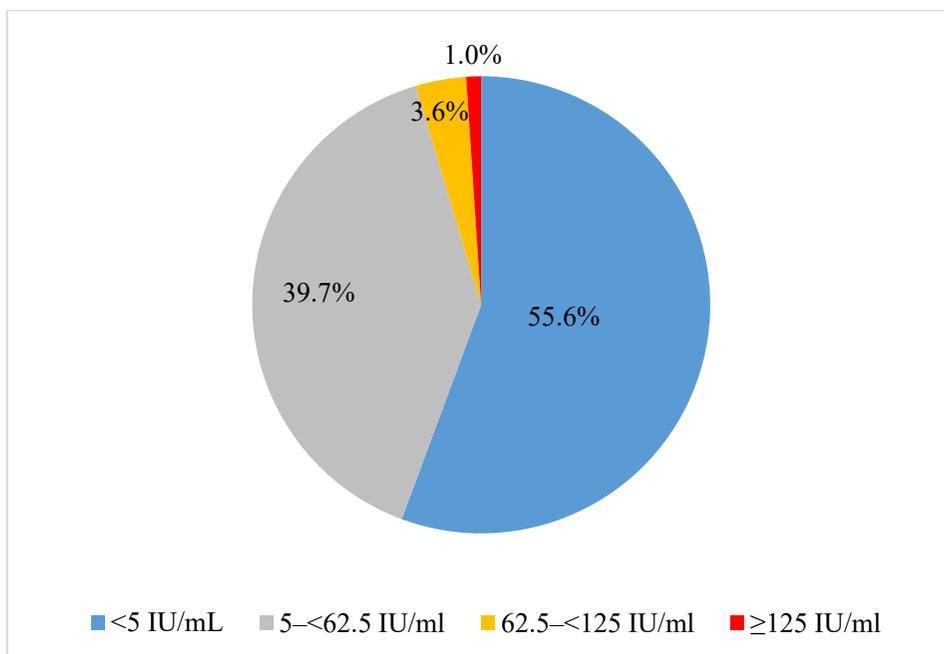
Black arrows indicate ages when children are immunised with pertussis vaccine in Estonia. The GMC was higher at these age groups. Among subjects aged at least 20 years, the GMC was significantly lower among 40–49-year-olds than among other 10-year age groups (except 30–39-year olds).

### 5.3. Seroprevalence of PT IgG antibodies in entire population

Of all studied subjects, 25 (0.6%; 95% CI: 0.4–0.8%) had PT IgG over the highest detectable level (174 IU/mL), with more than half (55.6%; 95% CI: 54.2–57.1%) under the detectable level (Figure 13). The prevalence of an undetectable level of PT IgG in our study is slightly higher than in studies conducted in other countries, in which it has varied from 11.5% (95% CI: 8.4–14.7%) in Slovenia in 2000 to 38% (95% CI: 36–40%) in Australia in 2007 (204, 207). As discussed above, the use of different ELISA kits in measuring PT antibodies may explain differences between studies, and one cannot conclude that PT IgG antibody level is different in different countries. In our study, the highest rates of undetectable levels of PT IgG were among 40–49-year-olds (74.4%; 95% CI: 70.0–78.4%) and 12- to 14-year-olds (72.0%; 95% CI: 64.6–78.7%). However, very few subjects in these age groups had antibody levels indicative of recent infection, suggesting that despite low antibodies, these age groups are rather well protected against infection. This confirms that the protective value of PT IgG is not clearly established. Storsaeter et al. have shown that 47% of those making household contact with *B. pertussis* with PT IgG levels < 2 IU/mL developed cough, compared to 15% of those with PT IgG levels from 2–35 IU/ml ( $p < 0.00001$ ) (259). If to the low PT level the criteria of “no history of previous pertussis or pertussis immunisation” were added then the risk of symptomatic pertussis raised to 71% ( $p = 0.0007$ ) (259). Campbell et al. have suggested that undetectable antibodies may be an indicator of increased susceptibility to pertussis at the population level (204).

A total of 1.0% (95% CI: 0.8–1.4%) of subjects studied had PT IgG concentration at a very high level (Figure 13). A similar prevalence of PT IgG  $\geq 125$  IU/mL has previously been reported in the Netherlands (203, 245), Israel (208), Denmark (249), and Italy (247), while a very high percentage (21.8%) of subjects with PT IgG > 125 IU/mL was reported in Mexico (251).

When comparing the 10-year age groups, the highest percentage of subjects with very high PT IgG concentration were children aged 0–9 years (likely because of their recent immunisations), followed by young adults (20–29 years old) (Table 13). The highest percentage of subjects with high antibody concentration was similarly among 0–9-year-old children, followed by 90–99-year olds (Table 13). While high rates among 0–9-year olds could be explained by recent immunisations, the high rate of PT IgG among very old people is not well understood.



**Figure 13. Distribution of subjects with different PT IgG levels**

**Table 13. Distribution of subjects with different PT IgG values in percentage by 10-year age group**

Age group (years)	% < 5 IU/mL (undetectable)	% 5 to < 62.5 IU/mL (mid-range)	% 62.5 to < 125 IU/mL (high)	% ≥ 125 IU/mL (very high)
0-9	37.8	51.7	7.8	2.7
10-19	60.5	33.5	4.0	2.0
20-29	59.9	33.9	4.7	1.6
30-39	62.8	34.6	2.1	0.4
40-49	74.4	23.3	2.1	0.2
50-59	59.6	38.6	1.4	0.4
60-69	52.4	45.1	2.1	0.4
70-79	50.5	45.6	3.1	0.8
80-89	50.5	45.7	3.3	0.4
90-99	41.5	52.3	6.2	0.0

Children had significantly lower prevalence of PT IgG in undetectable levels (48.7%; 95% CI: 45.7–51.6%) than adults (58.1%; 95% CI: 56.4–59.8%),  $p < 0.001$ . At the same time, children had significantly higher prevalence of high (6.0%; 95% CI: 4.7–7.5%) and very high (2.3%; 95% CI: 1.6–3.4%) rates of PT IgG than adults (2.7%; 95% CI: 2.2–3.4% and 0.6%; 95% CI: 0.4–0.9%, respectively) ( $p < 0.001$  in both categories). However, if we compare  $\geq 20$ -year-olds to the children not recently immunised (ages 9–14), the prevalence for all categories is similar (Table 14). This suggests that pertussis immunisation influences the concentration of PT IgG antibodies.

**Table 14. Distribution of PT IgG values in percentage in not recently immunised children and adults**

	9–14y	20–99y	<i>p</i>
% < 5 IU/mL	60.5	58.1	0.4
% 5 to < 62.5 IU/ml	35.9	38.6	0.3
% 62.5 to < 125 IU/ml	2.7	2.7	1.0
% $\geq 125$ IU/ml	0.9	0.6	0.5

Among children, the highest percentage of subjects with significantly increased PT IgG antibody concentrations was seen in the age cohorts following immunisation, at the ages of 3 months to < 1 year, 2 years, 7–8 years, and 18–19 years (Figure 14). Accordingly, the lowest number of subjects with undetectable concentrations was observed at the age of 2 years (12.3%; 95% CI: 5.5–22.8%). Grouping children into frequently and less frequently immunised (e.g. below and above age 10) groups, it appeared that a significantly higher percentage of those aged 10 to 19 years had undetectable antibody levels than those from 3 months–9 years (60.5%; 95% CI: 56.3–64.7% and 38.1%; 95% CI: 34.1–42.2%, respectively [ $p < 0.001$ ]), suggesting that after immunisation, the concentration of PT IgG antibodies increases, but sharp decline occurs soon thereafter.

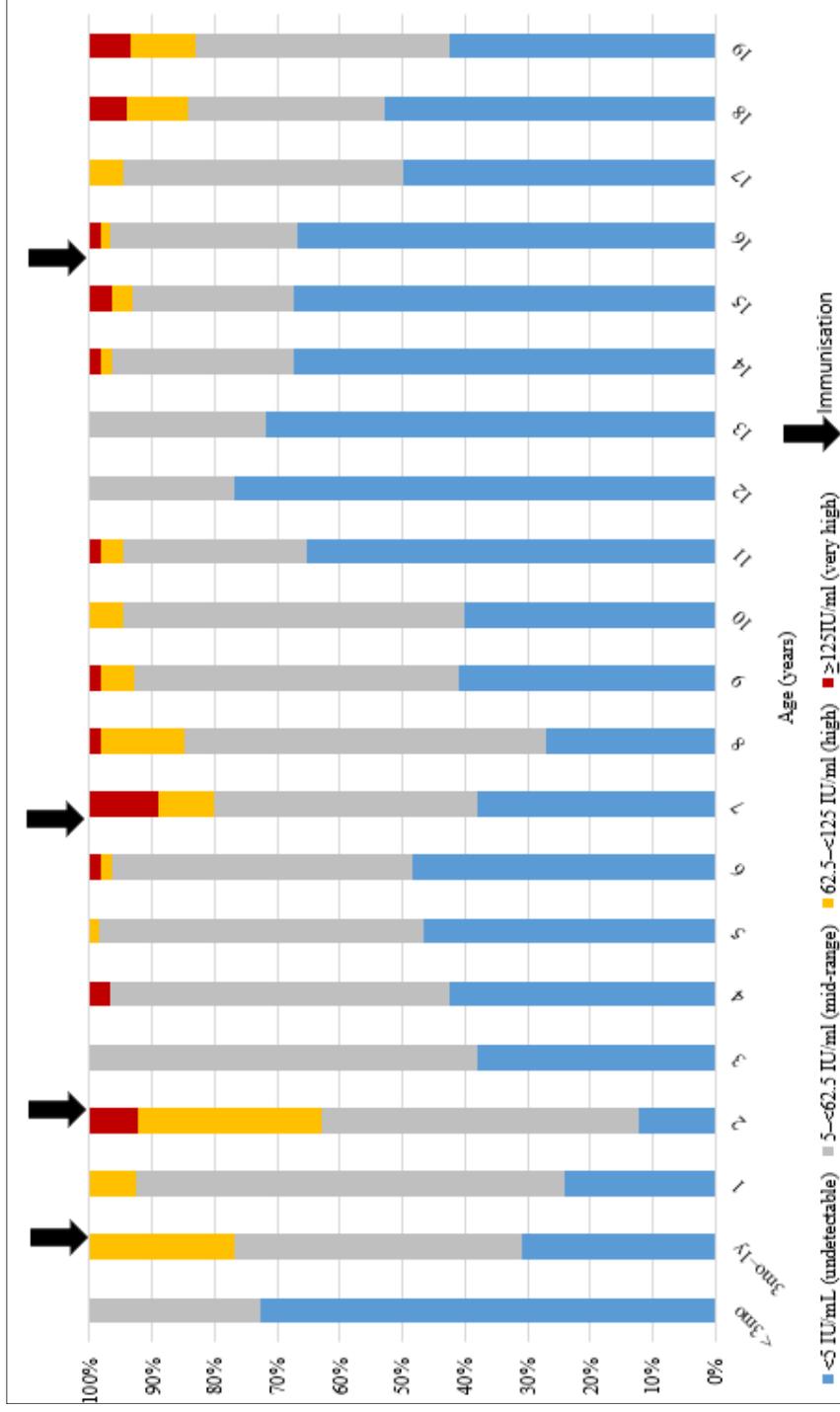


Figure 14. Distribution of the percentage of the subjects aged 0–19 years, with different PT IgG levels in yearly cohorts. Blue indicates < 5 IU/mL (undetectable), grey 5 to < 62.6 IU/mL (mid-range), yellow 62.5 to < 125 IU/mL (high), and red ≥ 125 IU/mL (very high)

Among  $\geq 20$ -year-olds, the highest prevalence of very high antibodies was among people of childbearing age (20–39 years old), with similar distribution among females 55.6% and males 33.3% ( $p=0.9$ ). De Melker et al. have reported the highest prevalence of high and very high levels of PT IgG among 20–24-year-olds in the Netherlands (203).

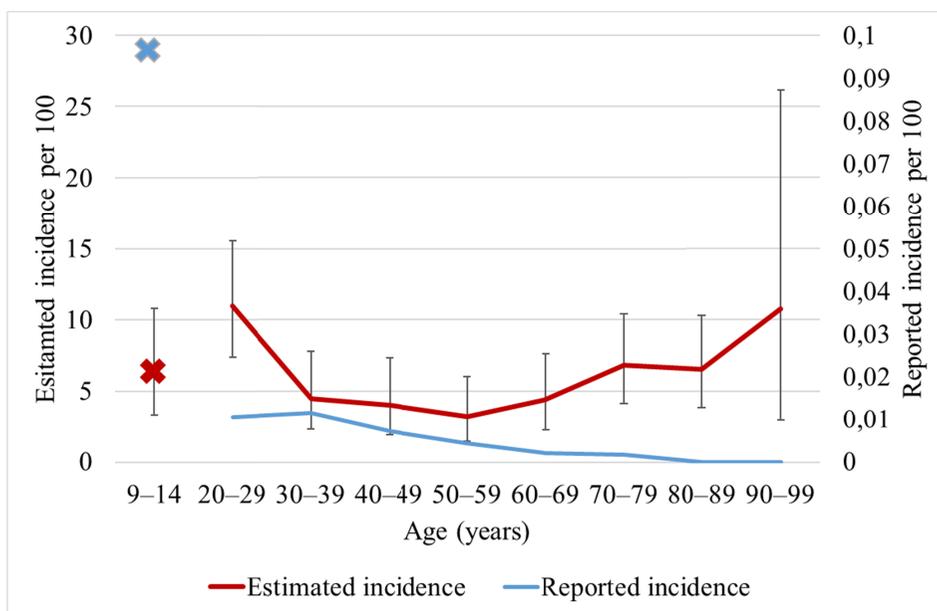
#### **5.4. Estimated incidence of pertussis infection based on seroprevalence studies and gap between estimated and reported pertussis**

Based on serology, there were 123 subjects (3.4%; 95% CI: 2.8–4.0%) in not recently immunised cohorts (children 9–14 years and adults from 20 years onwards) with a PT IgG concentration of at least 62.5 IU/mL. Taking this as an evidence of recent infection, we estimated the incidence of *B. pertussis* infection in the year before serum sampling at 5.9% (95% CI: 4.9–7.0%). This is consistent with similar studies: 6.6% in the Netherlands (203) and 2.4% in Israel (208). Kretzschmar et al. have estimated pertussis infection incidence rates based on seroepidemiology in five Western European countries using two different methods and found similar figures to ours (25).

As the prevalence of high and very high levels of PT IgG was high among young adults 20–29 years old, the estimated incidence rate was also higher in this age group (11.0%; 95% CI: 7.4–15.6%) than in other age groups, being significantly higher when compared to that of 50–59-year-olds (3.2%; 95% CI: 1.5–6.0%) ( $p=0.036$ ) (Figure 15). Furthermore, in a Dutch study, young adults aged 20–24 years had numerically higher estimated incidence of pertussis infection than other age groups, whereas in Israel, the highest peaks were observed among 15–19-year olds and those over 60 years (203, 208).

Another increase in estimated pertussis infection incidence, similar to that in Israel (208), was observed from age 60 years onwards (Figure 15). In the Netherlands, however, the estimated incidence of pertussis infection was lower among subjects over 60 years than among those between the ages of 25 and 59 years old (203).

While in all age groups older than 20 years, the estimated incidence of pertussis was higher than that reported among 9–14-year-olds it was an opposite (Figure 15). In Estonia the reported incidence of pertussis in children has almost always been higher than in adults (data from Health board of Estonia).



**Figure 15. Annual estimated (red line and cross, primary y-axis) (+95% CI) and reported (blue line and cross, secondary y-axis) incidence of pertussis per 100 people in people not recently immunised age groups**

Reported incidence of pertussis disease among 9–14-year-old subjects (in 2011) and  $\geq 20$ -year-old subjects (in 2012) in Estonia was 0.013% (12.5/100,000), while the calculated incidence of pertussis infection based on seroepidemiology is 470 times greater than that. This is consistent with the data from Israel (with a gap of 400 times) and the Netherlands (with a gap of 685 times) (203, 208). In our study, large differences between reported and estimated incidences of 66 times in 9–14-year-olds to  $> 8,000$  times in those over 80 years old were observed (Figure 15), suggesting that asymptomatic pertussis is much more common than symptomatic disease. Based on officially reported figures (data from Health Board of Estonia), pertussis among those over 60 years of age is rare. In the seroprevalence study, however, the estimated incidence of pertussis is similar in all age groups. This may indicate that the course or symptoms of pertussis in the elderly are much different from in other age groups, or that the elderly have a mainly asymptomatic course of the disease.

The gap between estimated and reported incidence of pertussis infection suggests that while current vaccines are effective in preventing symptomatic disease, they do not provide protection against pertussis infection. In addition, one must remember that none of the surveillance systems are good enough to register all cases, and national notification rates almost always underestimate the real incidence of the disease (193).

## 5.5. Prevalence of pertussis in patients with persistent cough

Of 549 recruited patients, 22 had pertussis (prevalence of 4.0%; 95% CI: 2.5–6.0%). This prevalence is very similar to that found in a recent study of GP practices throughout Europe, with a reported prevalence of 3.0% (95% CI: 3.5–3.7%) (202). However, our study included both hospitalised and ambulatory patients. Nevertheless, when comparing the prevalence of pertussis in different studies of coughing patients, we noticed wide variations (from 2–37%) (112, 113, 201, 202, 220, 222, 223, 227) and that the prevalence in our study is at the lower end in the context of previous studies (Table 7). This could partly be explained by methodology: enrolling only laboratory-confirmed cases, using single-sample serology, and conducting the study between epidemic peaks but in a country with a high child immunisation rate and in which adolescent immunisation was introduced in 2012.

The prevalence of pertussis was higher among children than adults (7.6%; 95% CI: 3.9–13.2% and 2.7%; 95% CI: 1.4–4.8% respectively) ( $p=0.027$ ). The highest prevalence of pertussis was 50.0% among those aged < 1 year. However, it is important that four of six of those patients were recruited by hospitals rather than in ambulatory settings. In many epidemiological studies, which are based on nationally reported figures, the highest incidence has been reported among children less than one year old (190, 191, 210, 212). This could be expected in the vaccine era, without maternal pertussis immunisation; because of waned immunity in adults, there are no antibodies transmitted through the placenta, and infants' immunisation schedules have not yet started or are incomplete.

The vast majority of pertussis cases were diagnosed by clinical findings and positive serology (17/22 cases) in our study (Table 15). This is consistent with clinical practise in Estonia (Figure 3) but differs from the study conducted in 12 EU countries, in which the majority of cases were confirmed by PCR (202).

**Table 15. Overview of laboratory methods confirming pertussis and parapertussis**

Diagnosis method	Pertussis (n=22)	Parapertussis (n=7)
Culture	0	0
PCR	4	5
Culture + PCR	1	2
Serology	17	0

As shown in Table 16, patients with pertussis were younger and more often male than those with cough of another or unknown aetiology. Patients with pertussis were more likely to have been hospitalised than those with a cough of another or unknown aetiology or caused by to parapertussis (Table 16).

The median time between patients' last immunisations with pertussis vaccine and the onset of pertussis was 2.2 years (interquartile range [IQR] 1.8–7.1) among children with known immunisation status. In a study conducted during the pertussis outbreak in a preschool in the USA, the reported average time between the last immunisation and start of illness was in the similar range of 22 months (260).

**Table 16. Characteristics of patients with pertussis, parapertussis, and cough of another or unknown aetiology**

	Pertussis (n=22)	Para- pertussis (n=7)	Cough of unknown aetiology (n=520)	Pertussis compared to unknown aetiology	Parapertussis compared to unknown aetiology	Pertussis compared to parapertussis
	Mean ( $\pm$ SD)			<i>p</i> -value		
Age (years)	21.6 (17.2)	12.0 (12.5)	35.9 (21.5)	<b>0.003</b>	<b>0.006</b>	0.161
	Number of patients (prevalence %)			Odds ratio (95% CI)		
Children (< 18 years old)	11 (50.0)	6 (85.7)	128 (24.6)	<b>3.1</b> <b>(1.2–7.8)</b>	<b>18.3</b> <b>(2.5–417.4)</b>	0.2 (0.0–1.6)
Male	12 (54.5)	5 (71.4)	146 (28.1)	<b>3.1</b> <b>(1.3–7.3)</b>	<b>6.7</b> <b>(1.3–45.7)</b>	0.5 (0.1–3.5)
Hospitalised	3 (13.7)	1 (14.3)	5 (1.0)	<b>16.0</b> <b>(3.1–70.6)</b>	16.9 (0.6–162.1)	1.0 (0.1–28.5)
Immunised 1–5 years ago	4 (18.2)	4 (57.1)	98 (18.8)	1.0 (0.3–2.8)	<b>5.7</b> <b>(1.2–29.4)</b>	0.2 (0.0–29.4)

Statistically significant differences are presented in boldface.

## **5.6. Prevalence of parapertussis in patients with persistent cough**

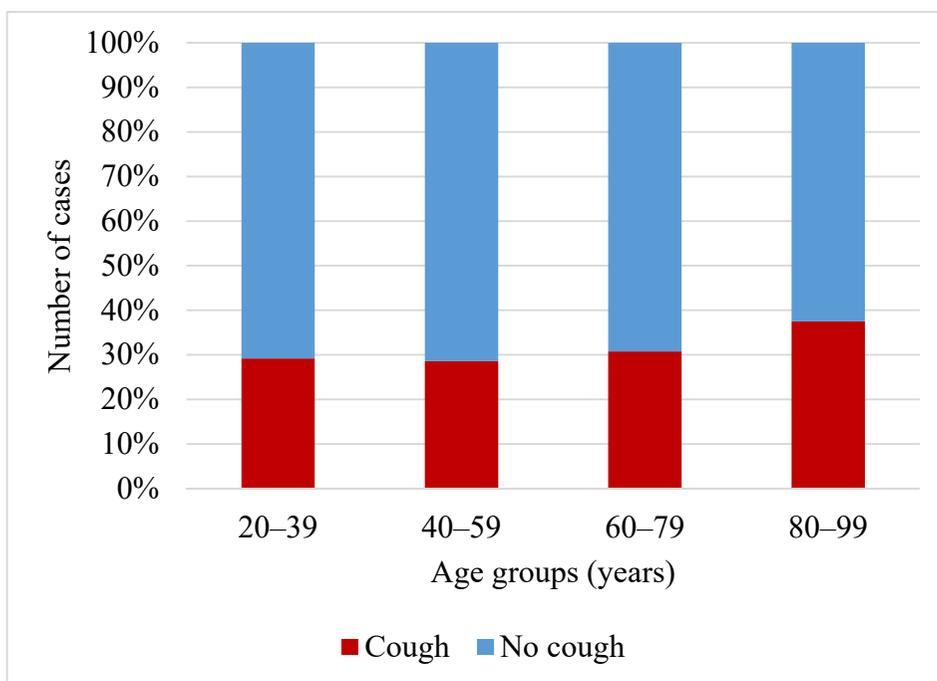
Only seven cases of parapertussis were confirmed in patients with persistent cough, and six of them were among children. Therefore, the overall prevalence of parapertussis is lower (1.3%; 95% CI: 0.5–2.6%) than the prevalence of pertussis. Similar to that of pertussis, the prevalence of parapertussis was higher among children (4.1%; 95% CI: 1.5–8.8%) than adults (0.3%; 95% CI: 0.0–1.4%) ( $p=0.003$ ). All parapertussis cases were diagnosed using PCR.

Patients with parapertussis were younger and more often male than those with cough of another or unknown aetiology (Table 16).

## **5.7. Clinical characteristics and course of pertussis and parapertussis in the era of immunisations**

### **5.7.1. Cough in subjects with very high and high concentrations of PT IgG**

Based on the seroprevalence study in adults, 111 subjects at least 20 years old had PT IgG  $\geq 62.5$  IU/mL, indicating pertussis infection during the last 12 months. Data about visits to GPs within the previous six months were available for 80 subjects. Of these, 25 (31.3%) had complained of a cough to their GP. The percentage of subjects with cough was similar in all 20-year age groups (Figure 16). No pertussis was suspected or diagnosed in these subjects. In a study conducted in the Netherlands, the prevalence of patients with cough over the past year of serum sampling among subjects with PT IgG  $\geq 62.5$  IU/mL was slightly lower at 17% in 1995–1996 and 25% in 2006–2007 (245).



**Figure 16. Distribution of subjects with PT IgG  $\geq$  62.5 IU/ml and their coughing complaints to their GPs**

31.3% of all subjects had complained of a cough to their GPs. Red indicates subjects who had complained of a cough to their GP, and blue sections indicate those who did not.

### 5.7.2. Clinical characteristics and course of pertussis and parapertussis in patients with persistent cough

As presented in Table 17, patients with pertussis, parapertussis, and cough of another or unknown aetiology had all basic symptoms, except for apnoea in patients with parapertussis at time of enrolment. However, patients with pertussis had significantly more inspiratory whooping and posttussive emesis than those with coughs of another or unknown aetiology (Table 17). In a meta-analysis on clinical characteristics of pertussis-associated cough based on 53 papers, the presence of inspiratory whooping and posttussive emesis was highly specific to pertussis, although with low sensitivity (240).

All patients with pertussis had at least one basic symptom in addition to cough, and the overall number of basic symptoms was significantly higher among patients with pertussis than among those with a cough of another or unknown aetiology (Table 18). The highest mean number of paroxysms in 24 hours was reported from patients with parapertussis. However, patients with parapertussis reported a milder cough on a numeric scale than other patients.

To determine the complex of symptoms to distinguish pertussis from other causes of cough, we conducted the MCA (Paper III, Figure 1), which revealed that in children and adults, the most remarkable result is the relatively small proportion of overall variability described by the first two dimensions (36.4% and 48.0% for children and adults, respectively). This suggests a lack of common patterns among different symptoms and diagnoses. However, as the patterns discovered are the most common, we can still conclude that adults with pertussis most often had inspiratory whooping, posttussive emesis, and apnoea, while children had the above plus fever. Children with parapertussis formed a separate group, but the analysis did not reveal any common pattern of symptoms related to parapertussis, which is also the reason why omitting patients with parapertussis changed the results very little.

The mean ( $\pm$ SD) durations of a cough among patients with pertussis and with parapertussis were similar at 104.1 ( $\pm$ 52.5) days and 78.3 ( $\pm$ 62.9) days, respectively ( $p=0.194$ ). In the case of pertussis, there were no differences in the mean ( $\pm$ SD) duration of cough between children and adults at 103.2 ( $\pm$ 64.4) and 105.0 ( $\pm$ 40.3) days, respectively.

Two significant differences between the courses of pertussis and parapertussis were revealed. The mean duration of paroxysms was longer in pertussis patients than parapertussis patients at 5.0 ( $\pm$ 3.9) and 1.9 ( $\pm$ 4.5) weeks, respectively ( $p=0.012$ ), and the mean duration of inspiratory whooping was longer among pertussis patients than parapertussis patients at 3.7 ( $\pm$ 4.5) and 0.0 ( $\pm$ 0.0) weeks, respectively ( $p=0.012$ ). All patients recovered.

**Table 17. Presence of basic clinical symptoms in patients with pertussis, parapertussis, and cough of another or unknown aetiology**

	Pertussis (n=22)	Para- pertussis (n=7)	Cough of unknown aetiology (n=520)	Pertussis compared to cough of unknown aetiology	Parapertussis compared to cough of unknown aetiology	Pertussis compared to parapertussis
	<b>Number of patients (prevalence %)</b>			<b>Odds ratio (95% CI)</b>		
Cough without any other symptoms	0 (0.0)	0 (0)	35 (6.7)	0.0 (0.0–2.7)	0.0 (0.0–8.27)	N/A
Paroxysms	21 (95.5)	6 (85.7)	438 (84.2)	3.9 (0.6–81.2)	1.1 (0.2–25.8)	3.3 (0.1–140.8)
Inspiratory whooping	13 (59.1)	3 (42.9)	173 (33.3)	<b>2.9 (1.2–7.3)</b>	1.5 (0.3–7.0)	1.9 (0.3–11.8)
Posttussive emesis	14 (63.6)	2 (28.6)	118 (22.7)	<b>9.9 (2.5–14.9)</b>	1.4 (0.2–6.7)	4.2 (0.6–35.6)
Apnoea	7 (31.8)	0 (0.0)	95 (18.3)	2.1 (0.8–5.2)	0.0 (0.0–2.7)	N/A
Fever, axillary (≥ 37.3 °C)	6 (27.3)	2 (28.6)	203 (39.0)	0.6 (0.2–1.5)	0.6 (0.1–3.1)	0.9 (0.1–8.3)

N/A – not available

CI – confidence interval

Statistically significant differences (odds ratio) are presented in boldface.

**Table 18. Mean ( $\pm$ SD) of characteristics and pairwise comparison of patients with pertussis, parapertussis, and cough of another or unknown aetiology (Wilcoxon test)**

	Pertussis (n=22)	Parapertussis (n=7)	Cough of unknown aetiology (n=520)	Pertussis compared to cough of unknown aetiology	Parapertussis compared to cough of unknown aetiology	Pertussis compared to parapertussis
	<b>Mean (<math>\pm</math>SD)</b>			<b>p-value</b>		
Duration between onset of illness and enrolment in study (days)	28.9 (20.5)	28.6 (28.5)	25.6 (21.0)	0.362	0.921	0.557
Number of paroxysms in 24 hours	9.0 (8.5)	12.9 (8.1)	11.4 (9.8)	0.775	0.156	0.122
Severity of cough on scale of 0–10 <sup>a</sup>	6.2 (2.1)	5.3 (1.0)	6.3 (1.8)	0.788	0.104	0.296
Number of basic symptoms <sup>b</sup>	2.8 (1.1)	1.9 (1.3)	2.0 (1.2)	<b>0.001</b>	0.802	0.102
Number of additional symptoms <sup>c</sup>	0.7 (0.9)	1.1 (0.9)	0.8 (1.0)	0.278	0.230	0.089
Duration of cough (days)	104.1 (52.5)	78.3 (62.9)	N/A	N/A	N/A	0.194

Data were compared using Wilcoxon's test.

N/A – not available

SD – standard deviation

Statistically significant differences (p-values) are presented in boldface.

<sup>a</sup> 0 = no cough; 5 = discomfort, cough interferes with sleeping; 10 = cough is unbearable, very painful, constant fear of suffocation

<sup>b</sup> Basic symptoms include paroxysm, inspiratory whooping, posttussive emesis, apnoea and fever.

<sup>c</sup> Additional symptoms are other complaints patients told their doctors.

### 5.7.2.1. Clinical characteristics of pertussis and parapertussis in children

At enrolment, 90.9% and 45.5% of children with pertussis had paroxysms and posttussive emesis, respectively. Respective numbers in parapertussis were 100% and 33%. As shown in Table 19, there were no differences in the prevalence of basic symptoms between children with different diagnoses. However, children with parapertussis tended to have more paroxysms in 24 hours than children with pertussis or cough of another or unknown aetiology (Table 19; Table 20). In some studies, conducted in children, the prevalence of inspiratory whooping and/or posttussive emesis has been higher among children with pertussis than those with cough of another or unknown aetiology (114, 220).

**Table 19. Prevalence and odds ratio of basic clinical characteristics in children with pertussis, parapertussis, and cough of another or unknown aetiology**

	Children with pertussis (n=11)	Children with parapertussis (n=6)	Children with cough of unknown aetiology (n=128)	Pertussis compared to unknown aetiology	Parapertussis compared to unknown aetiology	Pertussis compared to parapertussis
	Number of patients (prevalence %)			Odds ratio (95% CI)		
Paroxysms	10 (90.9)	6 (100.0)	110 (85.9)	1.6 (0.2–75.6)	N/A	N/A
Inspiratory whooping	4 (36.4)	3 (50.0)	31 (24.2)	1.8 (0.4–7.7)	3.1 (0.4–25.0)	0.6 (0.0–7.5)
Posttussive emesis	5 (45.5)	2 (33.3)	36 (28.1)	2.1 (0.5–9.1)	1.3 (0.1–9.5)	1.7 (0.1–29.7)
Apnoea	4 (36.4)	0 (0.0)	18 (14.1)	3.5 (0.7–15.8)	N/A	N/A
Fever, axillary (> 37.3°C)	2 (18.2)	2 (33.3)	44 (34.4)	0.4 (0.0–2.2)	1.0 (0.1–7.1)	0.4 (0.0–9.5)

N/A – not available

CI – confidence interval

Statistically significant differences (odds ratio) are presented in boldface.

**Table 20. Mean ( $\pm$ SD) of characteristics and pairwise comparison of children with pertussis, parapertussis, and cough of another or unknown aetiology (Wilcoxon test)**

	Children with pertussis (n=11)	Children with parapertussis (n=6)	Children with a cough of unknown aetiology (n=128)	Pertussis compared to unknown aetiology	Para-pertussis compared to unknown aetiology	Pertussis compared to parapertussis
	Mean ( $\pm$ SD)			<i>p</i> -value		
Duration between onset of illness and enrolment in study (days)	26.1 (19.8)	29.8 (31.0)	28.7 (24.2)	0.806	0.787	0.882
Number of paroxysms in 24 hours	6.8 (4.4)	15.0 (6.3)	9.8 (8.8)	0.941	<b>0.015</b>	<b>0.023</b>
Severity of cough on a scale of 0–10 <sup>a</sup>	6 (2.3)	5.3 (1.0)	5.8 (1.9)	0.904	0.449	0.615
Number of basic symptoms <sup>b</sup>	2.3 (1.0)	2.2 (1.2)	1.8 (1.1)	0.207	0.549	0.837
Number of additional symptoms <sup>c</sup>	0.5 (0.9)	0.8 (0.4)	0.8 (0.9)	0.135	0.495	0.098
Period of coughing (days)	103.2 (64.4)	82.2 (68.0)	N/A	N/A	N/A	0.555

N/A – not available

SD – standard deviation

Statistically significant differences (*p*-values) are presented in boldface.

<sup>a</sup> 0 = no cough; 5 = discomfort, cough interferes with sleeping; 10 = cough is unbearable, very painful, constant fear of suffocation

<sup>b</sup> Basic symptoms include paroxysm, inspiratory whooping, posttussive emesis, apnoea, and fever.

<sup>c</sup> Additional symptoms are other complaints patients told their doctors.

### 5.7.2.2. Clinical characteristics of pertussis in adults

In contrast to children, among adults, inspiratory whooping and posttussive emesis were significantly more characteristic of pertussis than cough of another or unknown aetiology (Table 21). Moor et al., in their meta-analysis on clinical characteristics of pertussis-associated cough, also reported that presence of inspiratory whooping and posttussive emesis have high specificity in adults (240).

**Table 21. Prevalence and odds ratio of clinical characteristics in adults with pertussis and cough of another or unknown aetiology**

	Adults with pertussis (n=11)	Adults with cough of unknown aetiology (n=392)	Pertussis compared to cough of unknown aetiology
	<b>Number of patients (prevalence %)</b>		<b>Odds ratio (95% CI)</b>
Paroxysms	11 (100.0)	328 (83.7)	N/A
Inspiratory whooping	9 (81.8)	142 (36.2)	<b>7.9 (1.6–76.5)</b>
Posttussive emesis	9 (81.8)	82 (20.9)	<b>17.0 (3.4–165.3)</b>
Apnoea	5 (45.5)	77 (19.6)	3.4 (0.8–13.9)
Fever, axillary (> 37.3°C)	3 (27.3)	159 (40.6)	0.5 (0.1–2.3)

N/A – not available

CI – confidence interval

Statistically significant differences (odds ratio) are presented in boldface.

One adult patient with paraptussis was not included in statistical analysis.

Among adults with pertussis, the number of basic symptoms was significantly higher than among patients with cough of another or unknown aetiology (Table 22).

**Table 22. Mean ( $\pm$ SD) of characteristics and pairwise comparison of adults with pertussis and cough of another or unknown aetiology (Wilcoxon test)**

	Adults with pertussis (n=11)	Adults with a cough of unknown aetiology (n=392)	Pertussis compared to cough of unknown aetiology
	Mean ( $\pm$ SD)		<i>p</i> -value
Duration between onset of illness and enrolment in study (days)	31.6 (21.7)	24.5 (19.7)	0.137
Number of paroxysm in 24 hours	11.2 (11.0)	11.9 (10.0)	0.548
Severity of cough on a scale of 0–10 <sup>a</sup>	6.4 (2.0)	6.4 (1.7)	0.913
Number of basic symptoms <sup>b</sup>	3.3 (0.9)	2.0 (1.2)	<b>&lt; 0.001</b>
Number of additional symptoms <sup>c</sup>	0.9 (0.8)	0.9 (1.1)	0.934
Period of coughing (days)	105.0 (40.3)	N/A	N/A

N/A – not available

SD – standard deviation

Statistically significant differences (*p*-values) are presented in boldface.

<sup>a</sup> 0 = no cough; 5 = discomfort, cough interferes with sleeping; 10 = cough is unbearable, very painful, constant fear of suffocation

<sup>b</sup> Basic symptoms include paroxysm, inspiratory whooping, posttussive emesis, apnoea, and fever.

<sup>c</sup> Additional symptoms are other complaints patients told their doctors.

### 5.7.2.3. Pertussis in the elderly

A total of 54 patients aged at least 65 years were enrolled in the study of coughing patients (composing 9.8% of all enrolled patients), but none of them had pertussis. However, in the seroprevalence study, we noticed that in concentration of PT IgG, the group of elderly people did not differ from other groups, and 3% of all the elderly had PT IgG of at least 62.5 IU/mL, indicating a recent contact with *B. pertussis*, and 32% of them had reported cough within the previous 6 months to their GPs (none of which reports were diagnosed as pertussis). Both findings raise questions about the clinical course of pertussis among the elderly.

Ridda et al. have reviewed recent literature on pertussis in the elderly and have described the increase in notification rates in those aged 60 year and older (261). The number of epidemiological studies in this age group is limited, and

diagnosis is difficult because of the lack of specific symptoms (261). In a retrospective study conducted in the USA among those aged 50 and older, the most frequent initial diagnoses before pertussis diagnosis were cough and upper respiratory tract infection (57). However, pertussis can cause significant morbidity, hospitalisation, and death among the elderly (261, 262).

### **5.8. Diagnostic value of symptoms corresponding to the WHO's clinical case definition and basic symptoms of pertussis**

Of the 22 patients with confirmed pertussis, 17 met the WHO's clinical case definition of pertussis (109), and of the 527 patients with cough of another or unknown aetiology or parapertussis, 201 did not meet the definition. The sensitivity and specificity of this definition were 0.77 (95% CI: 0.55–0.92) and 0.38 (95% CI: 0.34–0.42), respectively (Paper 3, Table 4). Due to low sensitivity and specificity, the overall prediction accuracy of the WHO's clinical case definition was poor at 0.58 (95% CI: 0.49–0.67) (Paper 3, Table 4). Furthermore, the high sensitivity of the WHO's clinical case definition, varying from 0.79–1.00, has been reported previously; however, the specificity has been lower, varying from 0.15–0.17 (112-114).

The step-wise multiple logistic regression analysis revealed that the optimal combination of symptoms for the entire cohort and for adults consists of inspiratory whooping, posttussive emesis, and fever (prediction accuracy 0.77 [95% CI: 0.67–0.87] and 0.89 [95% CI: 0.82–0.97], respectively). For children, no multiple logistic regression model fit the data better than single symptoms. The sensitivity of multivariate logistic regression models did not differ from the sensitivity of the WHO's clinical case definition, while the specificity and overall prediction accuracy of logistic regression models were higher (Paper 3, Table 4).

A summary of the three statistical analyses to determine the pertussis-specific symptoms for the entire cohort, for children, and for adults is presented in Table 23. Inspiratory whooping and posttussive emesis in addition to cough (as inclusion criteria) were the symptoms which most frequently indicated pertussis. However, the sensitivity and specificity of these symptoms are not reliable enough for decision making in clinical practise. Furthermore, Ebell et al., in their meta-analysis, also did not find a reliable clinical decision rule (239).

**Table 23. Summary of statistical analysis to determine pertussis-specific symptoms**

	All patients with pertussis (n=22)	Children with pertussis (n=11)	Adults with pertussis (n=11)
Prevalence of symptoms	Inspiratory whooping, Posttussive emesis	Not found	Inspiratory whooping, posttussive emesis
MCA	N/A	Inspiratory whooping, posttussive emesis, apnoea, fever	Inspiratory whooping, posttussive emesis, apnoea
Step-wise multiple regression analysis	Inspiratory whooping, posttussive emesis, fever	Not found	Inspiratory whooping, posttussive emesis, fever

N/A – not available

## 6. GENERAL DISCUSSION

### 6.1. Is pertussis still an important disease in Estonia?

Over the last ten years, the reported incidence of pertussis has varied from 3–97/100,000 (0.003–0.097%) in Estonia. According to our study of coughing patients, the prevalence of pertussis is about 4%. Based on seroprevalence studies, about 6% of subjects not recently immunised have had pertussis infection yearly. This suggests that *B. pertussis* is still circulating in highly immunised populations with long immunisation history and in all age groups.

It is mandatory to report pertussis to the surveillance system in Estonia. National surveillance systems are effective at collecting severe and classical cases, but mild and asymptomatic cases are not included. Seroprevalence studies are unique because they capture all types of pertussis infections: classical, atypical, mild, and even asymptomatic. The huge gap between reported and estimated incidence (based on seroprevalence studies) of pertussis indicates that the majority of pertussis cases are either atypical, mild, or asymptomatic and thus neither recognised nor reported to the official reporting systems. Mild and asymptomatic cases are unimportant from a clinical perspective, but they are important epidemiologically. Seroepidemiological studies yielding a real overview of the epidemiology of the disease should be conducted occasionally.

As expected, more severe cases leading to hospitalisations occurred mostly among infants. The last reported death from pertussis in Estonia was in an infant in 2007 (data from Health Board of Estonia). Furthermore, in the other EU/EEA countries, mortality from pertussis is low; only 101 pertussis-related deaths were reported between 2006 and 2015 in these countries (11). In 2015, all pertussis-related deaths occurred in infants, about 90% of them in children under three months (263). Usually pertussis immunisation starts at the age of 2–3 months (148). Therefore, we suggest that the vaccines currently used are sufficiently effective to serve their main purpose, to prevent death due to pertussis, at least in countries with high immunisation coverage and high levels of medical assistance.

After the start of universal immunisation with pertussis vaccine, the importance of pertussis has diminished as the notification rate of pertussis (classical and severe cases) has significantly decreased. However, vaccines currently used are unable to prevent pertussis infection and mild disease. To avoid all cases of pertussis, a new vaccine which also prevents colonisation by *B. pertussis* is needed.

### 6.2. Do we need pertussis immunisation in adults?

Pertussis immunisation in adults is recommended to prevent the transmission of *B. pertussis* to unprotected younger infants, to protect adults from the disease, and to reduce the circulation of pertussis (264). However, as shown by Warfel

et al., aP vaccines currently used do not prevent colonisation and transmission by *B. pertussis* from immunised subjects to pertussis-naive subjects, at least in a baboon model (33). In addition, studies of postpartum immunisation and cocooning have shown no reduction in the prevalence of pertussis disease in infants of immunised parents when compared to non-immunised parents (265, 266). Furthermore, dTap immunisation was only moderately effective to prevent PCR-confirmed pertussis among adolescents and adults in the USA (267).

As in other studies, pertussis among adults in our studies was mostly mild to moderate: there were no hospitalisations among adults because of severe pertussis in a study of coughing patients. Only 30% of adults with a high or very high level of PT IgG, which indicates recent pertussis, had complained of coughing to their GP in our seroprevalence study. In our studies, the prevalence of pertussis among coughing adults and the estimated incidence of pertussis infection among adults based on serology were relatively low at 2.7% and 5.8%, respectively. We believe that this rate is too low to recommend universal national pertussis immunisation in adults. In addition, in a study conducted in adolescents immunised with one dose of dTap, the effectiveness was only about 9% by the fourth year after immunisation (137). This suggests that an effective immunisation schedule should include multiple doses with a high immunisation rate, but this is expensive and complicated to achieve. However, we should remember that sometimes pertussis in adults can be an uncomfortable disease with prolonged cough (224), and pertussis immunisation may help to prevent it at the individual level (267).

Pertussis immunisation for health care worker safety and patient safety has been recommended (268). However, the same problems as those of pertussis immunisation in adults (duration of effective immunity and transmission of *B. pertussis*) persist. We are not aware of any consensus recommendation on pertussis immunisation of health care professionals by the ECDC; however, the CDC recommends a one-time dose of dTap as early as possible if not received previously (269). Based on the data above, the real benefit of this suggestion is unclear. In Estonia, no official recommendations about pertussis immunisation of health care workers have been published. As long as the duration of immunity after immunisation is unknown, and more importantly, as long as pertussis vaccines are unable to prevent colonisation and transmission, we cannot recommend universal pertussis immunisation for health care workers. However, everybody can immunise themselves to prevent pertussis at the individual level.

Pertussis is a life-threatening disease mainly for unimmunised infants. In EU/EEA countries, primary pertussis immunisation starts at the age of 2–3 months (148). Maternal pertussis immunisation to protect infants under this age has been implemented in the UK, Belgium, the Czech Republic, Greece, Ireland, Italy, and some regions in Spain (270). According to a recent systematic review, maternal pertussis immunisation during pregnancy is an effective and safe method to prevent disease in infants < 3 months (270). However, after examining epidemiological data from Estonia, we have observed a similar decline in pertussis cases among infants without maternal immunisation, likely

due to epidemiological trends (271). According to the Health Board of Estonia, to date fewer than three cases of pertussis have been diagnosed among children < 6 months old annually from 2011–2017. In addition, based on our study of coughing patients, the severe cases of pertussis among infants in Estonia are rare; therefore, at present we recommend the careful monitoring of pertussis in infants. Immunisation of pregnant women may be considered if the epidemiological situation changes.

Thus, we believe that implementation of universal adult pertussis immunisation is not currently needed in Estonia, as the main purpose of pertussis immunisation (preventing death by pertussis) is achieved without it. To stop the circulation of *B. pertussis*, a vaccine which also prevents colonisation and grants life-long immunity is needed.

### **6.3. Is the current pertussis immunisation schedule optimal in Estonia?**

The Estonian pertussis immunisation schedule consists of three doses of DTaP vaccine for primary immunisation at the ages of 3, 4.5, and 6 months and three booster doses at the ages of 2 years, 6–7 years (since 2008), and dTap for 15–16 years (since 2012). This immunisation schedule offers one of the highest numbers of pertussis vaccine doses for children in Europe (148). However, there are no booster doses for adults and no recommendations for pertussis immunisation for specific groups, for example health care workers or pregnant women, in Estonia's immunisation schedule.

After the implementation of the second and the third booster doses, the notification rate of pertussis decreased to 4–5 cases per 100,000 people. As this low level has stood since 2012 without any observed increase, this is the longest low-incidence period in Estonia following re-independence (Figure 5). Therefore, we believe that changes in the immunisation schedule in 2008 and 2012 through implementation of additional booster doses at school entry and leaving has been effective in preventing pertussis. However, the decreased rate of pertussis may not be result only of the changing immunisation schedule. In the same era, improved diagnostic methods (quantitative serology and PCR in 2012) and additional education of health care workers (for example not to perform serology in recently immunised children) were implemented, and it is difficult to assess the roles of these. In any case, reported incidence of pertussis in infants (in most vulnerable group) is low. The low incidence should be regarded as real since infants with serious pertussis cases are hospitalised and the disease recognised. In addition, the overall mortality rate in infants is minimal in Estonia, and we know the causes of infant death.

The reasons not to implement adult and/or maternal immunisation in Estonia are presented above (paragraph 6.2).

We believe that the current pertussis immunisation schedule is effective enough to prevent serious cases at least in inter-epidemic years and that eradication of pertussis is not possible with the vaccines currently available.

#### **6.4. Management of patients with persistent cough**

Persistent cough is a very common reason for visiting a doctor (198, 199). However, the aetiology of cough is difficult to define based solely on its characteristics (199). About half of all visits for cough are by children less than 15 years of age (198). Similarly, in our study, the age group with the highest number of patients with persistent cough was < 10-year-olds. This might be due to the more frequent upper respiratory tract infection among children and the fact that parents might worry about the health of their children more than their own and therefore visit doctor more readily over concerns related to them.

The most important reason to detect the aetiology of a cough is for treatment and management of the patient. In addition, determination of the aetiology of a cough is relevant for surveillance purposes to assess the situation, interfere quickly if necessary, and plan future actions at the level of public health. With data on the aetiology of coughs and incidences of diseases, there is greater hope for better medications and vaccines.

Although in our study the inclusion criteria included at least seven days of cough, the mean duration between start of illness and enrolment in the study was still 26 days, suggesting that patients seek medical care if cough persists and/or doctors suspect respiratory viral infection for cough of short duration. The most frequently described symptoms at enrolment were presence of paroxysms and posttussive emesis for pertussis, presence of paroxysms and inspiratory whooping for parapertussis, and presence of paroxysms and fever for cough of another or unknown aetiology. However, after conducting several statistical analyses, we were unable to identify pertussis-specific symptoms in this highly immunised population, which suggests that no symptoms are pathognomonic for pertussis. However, in our study we only examined classical symptoms of pertussis rather than all symptoms patients may have had, and other symptoms such as sleep disturbance and sweating should be examined more carefully.

If the reason for cough is not revealed through careful medical-history and physical examination, then diagnostic tests (laboratory and imaging tests) should be performed. Infectious agents are easy to diagnose using PCR cough panels, where a majority of the important pathogens (both bacterial and viral) are included in one test. However, in addition to several disadvantages that PCR has, in Estonia the price of these panels is too high for GPs to use them routinely.

## 6.5. Laboratory criteria of pertussis

As mentioned above, pertussis cannot be diagnosed based on clinical signs alone. Although in most countries, culture, PCR, and serology of pertussis are available (6), methods used vary (Figure 3). In addition, laboratory criteria specifically about pertussis serology vary (through quantitative vs qualitative tests, single- vs paired-sample serology, and different cutoff values) (6, 81).

In our study of patients with cough, the number of patients with pertussis and parapertussis confirmed by PCR was unexpectedly low despite the fact that PCR was done in almost all patients (n=546). For example, about 80% of all pertussis cases are confirmed by PCR in Denmark and the USA (Figure 3) (105, 107). We speculate that the main reason for the low number of PCR-confirmed cases in our study was the long duration of cough (a mean of 26 days) between onset of the illness and seeking medical care. The late enrolment is consistent with the high rate of serology-confirmed cases. If we had used the laboratory criteria of the CDC, which only accepts culture and PCR (111), we would have missed more than 75% of pertussis cases. Furthermore, in clinical practise, about 95% of all pertussis cases are confirmed with single-sample serology in Estonia (data from Health Board of Estonia). Therefore, in our opinion, PT-based serology tests with high specificity and sensitivity should be used to detect late-stage pertussis cases in Estonia. Results from PT IgG ELISA can be standardised using the WHO International Standard Pertussis Antiserum (90). The controls of the ELISA test used in our studies were calibrated using this antiserum (272). However, the exact cutoff values for both paired and single-sample serology may vary among studies and should be determined in the future.

In clinical practice, the importance of *B. pertussis* culture has decreased since the introduction of PCR, mainly because of logistical reasons and the long duration between collection of the sample and the result. However, culture is still the gold standard for diagnosing pertussis and the only method for determining the antibiotic sensitivity of pathogen. Therefore, we recommend performing cultures.

To the best of our knowledge, pertussis PCR has not yet been approved by the Food and Drug Administration or European Medicines Agency, which is a serious problem. Nevertheless, Burd has published a guideline for validation of laboratory-developed molecular assays for infectious diseases (273). Another problem is detection of *B. pertussis* in asymptomatic patients. For example, 4.8% of asymptomatic Chinese schoolchildren had positive PCR to *B. pertussis* (88). Whether transmission from asymptomatic carriers to others is possible is still debated (274, 275). A study conducted by Warfel et al. (33) indicated that asymptomatic transmission is possible, and studies of postpartum immunisation and cocooning have shown no reduction in the prevalence of pertussis disease in infants of immunised parents compared to non-immunised parents (265, 266). It is widely known that pertussis is most infectious during the catarrhal stage, when people do not even have severe cough and paroxysms, but *B. pertussis* is still spread via aerosolised respiratory droplets (21). However, the

CDC does not recommend testing asymptomatic subjects, even in cases of close contact with laboratory-confirmed cases, as it increases the likelihood of obtaining false positive results, and the decision of the post-exposure prophylaxis should not be based on PCR (85).

In 2012, most medical laboratories in Estonia switched from qualitative to quantitative serology tests, as recommended by the EU Pertstrain group (81), as these contains only PT antigen specific to *B. pertussis* and the result is expressed in IU/mL. Previously used qualitative tests included several *B. pertussis* antigens or even whole bacteria, and cross-reactivity with other microbial antigens such as other *Bordetella* species, *Haemophilus* species, *Mycoplasma pneumoniae*, and *Escherichia coli* might occur (81). The low specificity of qualitative serology tests might cause false positive cases; therefore, only ELISAs with PT as the only antigen should be used.

## 6.6. Limitations of the study

Some limitations of our studies should be noted.

First, the weakness of all seroprevalence studies is the indirect method of estimating incidence; however, this method also has the strength of being the only way to estimate the spread of pertussis infection at a population level.

Second, we did not have many data about the subjects in the seroprevalence studies. The only information available was a subject's age in the study in children and a subject's age and gender in the study in adults. However, we know that none of the subjects had been diagnosed with clinical or laboratory-confirmed pertussis. As the sera collected were medical laboratory leftovers of hospitalised and ambulatory patients with any diagnosis, we cannot exclude the possibility of selection bias towards a disproportionately sick population. However, Kelly et al. found no significant differences when measuring immunity to five vaccine-preventable diseases in children between laboratory leftovers and population-based cluster sampling methods (276).

Third, all studies were conducted in the interepidemic period. Pertussis incidence follows the cyclical pattern of the disease, and one cycle usually lasts 3–5 years, indicating that our studies were conducted in low incidence years.

Fourth, in the study of coughing patients, the planned sample size was not achieved. We calculated the sample size based on the cases in an extraordinary epidemic year (97/100,000 in 2010) in Estonia and planned to enrol 900 subjects. During the study period, the officially reported incidence of pertussis dramatically declined to 4–5/100,000 per population. Therefore, enrolment was much slower and took much longer than expected. We suspect the notification rate of pertussis in the epidemic year may also have included false positive cases, because qualitative serology was used and almost all cases were confirmed using serology and occurred among children in the age groups in which children in Estonia are immunised with pertussis vaccine (data from Health Board of Estonia). The statistical requirement for sample size (pre-

valence of pertussis with a 95% CI:  $\pm 2\%$ ) was met by 549 enrolled patients due to the lower prevalence of pertussis than expected.

Fifth, we suspect that not all patients who met inclusion criteria were enrolled in the study of coughing patients. Doctors probably did not follow the study protocol. While we did not specifically study the reasons for that, we suspect that less severe cases were not included because of busy schedules. Due to a lack of resources, we were not able to use screening logs or a special trial nurse. In addition, we mostly investigated classical pertussis symptoms, but other symptoms should also be examined carefully.

Sixth, due to resource restrictions, we were unable to evaluate other causative agents of coughs or monitor duration of symptoms in patients with cough of another or unknown aetiology in the study of coughing patients. However, the mean duration of acute cough has been evaluated as 18 days (277), and in our study the mean duration of cough before enrolment was already 26 days; therefore, we suspect that most viral upper respiratory tract infections were excluded.

We believe that none of these limitations prevented us from drawing adequate conclusions.

## 6.7. Future considerations

Despite performing several analyses, we were unable to propose clinical criteria clearly superior to the WHO's for distinguishing pertussis from other causes of persistent cough. Although patients with pertussis were more likely to have inspiratory whooping and posttussive emesis than others, the presence or absence of these symptoms did not confirm or exclude pertussis. Therefore, a new, more age-specific, clinical case definition is needed. The Global Pertussis Initiative group have published a new clinical case definition algorithm (108). They state that high sensitivity and good specificity should be expected with the presence of the triad: afebrile/low-grade fever, a paroxysmal cough that does not improve, and watery coryza. The addition of apnoea, seizures, cyanosis, emesis, or pneumonia should further increase both sensitivity and specificity among 0–3-month-olds (108). In older children (4 months to 9 years old), the addition of whooping, apnoea, and posttussive emesis should increase specificity, and in those  $\geq 10$  years old, the addition of sweating episodes between paroxysms should significantly increase specificity (108). However, these criteria are not validated, and this should be done in the future.

Pertussis symptoms among the elderly are largely unknown. As we had no pertussis cases among coughing patients aged 55 and older, they probably do not have persistent cough. Although based on the seroprevalence study, we can clearly state that pertussis infection occurs in the elderly with similar frequency to that in any other age group.

Pertussis vaccines, which are currently used quite effectively to prevent severe disease and death, are not as effective as other vaccines that have stopped the circulations of pathogens such as smallpox, *Haemophilus influenzae* type B,

poliomyelitis, etc. Therefore, a new pertussis vaccine is needed. Immunity from the vaccine should last the recipient's entire life (278) after few immunisations (ideally one), preferably administered by a method other than injection (279). A high level of effective immunity without side effects should be rapidly achieved, and the vaccine should prevent the disease in all age groups (including infants and the elderly) and transmit maternal protection to the foetus (279). Broad protective immunity against all variants of *B. pertussis* at a low cost and easy storage requirements are also characteristics of the ideal vaccine (279). Most importantly, to prevent the disease, the new vaccine should also prevent colonisation to avoid shedding (279).

Despite the similar pertussis vaccines and immunisation schedules in most European countries, the reported incidence of pertussis and its prevalence among coughing patients varies widely (11) (Table 7). The most important reason is probably the heterogeneity in methods used for laboratory confirmation of pertussis. Therefore, standardisation of pertussis detection and confirmation methods are also needed.

In primary care, point-of-care testing of pertussis would be most useful to encourage GPs to check for pertussis. The test should be usable in all patients with the cold-like symptoms of the early stage of the disease, which would allow early treatment and prevent the spread of pertussis. In addition, the test should be usable without special equipment or user training and at a low cost and should yield quick results, preferably within 10 minutes, within the appointment. We are not aware of a test fulfilling this description on the healthcare market but recently Salminen et al. have developed a rapid lateral flow immunoassay for serological diagnosis of pertussis which fulfils some of the criteria noted above (280).

It is still unclear how to define protective immunity against *B. pertussis*. Storsaeter et al. have shown that symptomatic pertussis is more frequently found among people with a low initial level of PT IgG, especially those without a previous history of pertussis vaccination or disease (259). Campbell et al. have suggested that the high prevalence of people with undetectable PT IgG may be an indicator of increased susceptibility to pertussis at the population level (204). However, the level of the protective value of PT IgG has not been established. Therefore, different authors have used different levels of PT IgG to assess the number of subjects with seroprotection. Most authors have used < 5.0 IU/mL as negative sera (93, 96, 204, 205). Some authors have used the cutoff value of > 10 IU/ml as seroprotection based on the lowest measured antibody titre among children recovering from pertussis (208, 281). Zhang et al. have used the Virion/Serion anti-PT IgG Elisa kit and, following the instruction of the kit's manual, considered subjects bearing more than 30 IU/mL of IgG against PT seropositive, with below 20 IU/mL considered seronegative (206). However, recent studies have demonstrated that in addition to humoral immunity, cellular immunity has an important role in ensuring immunity against *B. pertussis* (33). As far as we now, there is still no single reliable marker for evaluating immunity against *B. pertussis* at both the population and individual levels.

## 7. CONCLUSIONS

The reported pertussis incidence has dramatically decreased (from 97/100,000 in 2010 to 4.3/100,000 in 2017) during our studies in Estonia. Over the last five years, the reported incidence has been stable at 4–5/100,000. This is the longest low reported incidence period since the re-independence of Estonia. Furthermore, infants are well protected; the last death due to pertussis was in 2007, and in the last five years, fewer than 3 cases have been diagnosed in this age group each year. Similarly, in our study of coughing patients, the prevalence of pertussis was low, which suggests that the current pertussis immunisation schedule is sufficiently effective in controlling pertussis disease, at least between epidemics. However, the large gap between reported and estimated incidence of pertussis indicates that *B. pertussis* is still circulating and is mostly asymptomatic or mild in the vaccine era. The eradication of pertussis requires a new vaccine which also prevents colonisation of *B. pertussis*.

The following specific conclusion can be drawn from this research:

1. The population GMC of PT IgG is low in Estonia. Higher levels of GMC of PT IgG were detected at the ages of pertussis immunisation according to the Estonian immunisation schedule. However, sharp decline of GMC of PT IgG already occurs in the first year following immunisation. This suggests that serologic methods are not reliable for diagnostic purposes when the last pertussis immunisation occurred during the last year.  
Although the majority of study subjects had PT IgG at undetectable levels, a very few had antibodies at high or very high levels, indicating recent *B. pertussis* infection. This suggests that despite the low level of antibodies, people are well protected against pertussis, and factors other than antibodies play an important role in ensuring protection against the disease. Thus, antibody measurement cannot be used to characterise population protection levels against pertussis.
2. The estimated incidence of pertussis based on serology occurred in similar frequencies in all adult age groups and children not recently immunised. This indicates permanent circulation of *B. pertussis* in the Estonian population and that current vaccines are ineffective to stop that circulation.
3. The estimated incidence of pertussis infection based on PT IgG was about 500 times greater than the officially reported incidence of pertussis disease. This suggests that pertussis is mostly mild or asymptomatic in immunised subjects or pertussis symptoms are unknown and thus poorly recognised.
4. The prevalence of pertussis among coughing patients is low in Estonia. The highest prevalence of pertussis, among infants, is associated with absent or incomplete immunisation in this age group. The absence of confirmed cases of pertussis among those at least 65 years old with cough indicates that an asymptomatic course or symptoms other than cough are specific to pertussis in this age group. The overall low prevalence indicates that the pertussis

vaccine and immunisation schedule currently used are sufficient to reduce symptomatic pertussis to a minimal level in inter-epidemic periods at present. The prevalence of parapertussis among coughing patients was even lower than the prevalence of pertussis.

5. No parapertussis-specific symptoms were revealed in the study of coughing patients. Although patients with pertussis had inspiratory whooping and posttussive emesis more often than patients with cough of another or unknown aetiology, the sensitivity and specificity of classical symptoms of pertussis is low in the highly immunised population. New, age specific clinical criteria could improve the overall prediction accuracy of the WHO's clinical case definition of pertussis.
6. Symptomatic pertussis and parapertussis result in long-lasting cough, even in the vaccine era. However, the mortality of pertussis in developed countries with high immunisation coverage and a high level of medical assistance is extremely low.

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## 9. SUMMARY IN ESTONIAN

### Läkakõha epidemioloogia ja sümptomid Eestis

Läkakõha on klassikaline lastehaigus, mida kirjeldas esmaskordselt Guillaume de Baillou 1578. aastal kui raske kõhaga kulgevat ja kõrge suremusega haigust 4- kuni 10-kuuste laste hulgas (29). Haigus on põhjustatud Gramm-negatiivse bakteri *Bordetella pertussise* poolt, mis isoleeriti 1906. aastal kahe prantsuse teadlase Jules Bordet and Octave Gengou poolt Pasteur instituudis Pariisis (32).

Vastavalt Maailma Tervishoiuorganisatsiooni (MTO) läkakõha kliinilisele definitsioonile on läkakõha vähemalt 14 päeva kestev kõhaga haigestumine, millele lisandub vähemalt üks järgmistest sümptomitest: paroksüsmaalsed kõhahood, inspiratoorseid repriisid või kõhahoojärgne oksendamine (109). Tänapäeval esineb MTO definitsioonile vastavat klassikalist läkakõha suhteliselt harva, peamiselt vaid mittevaktsineeritud laste hulgas (52). Teismelised ja täiskasvanud, kes on läkakõha antigeenidega varem kokku puutunud, kas siis eelneva vaktsineerimise või haiguse läbipõdemise teel, kuid kelle immuunsus on vananenud, põevad haigust kergemini. Tavaliselt kulgeb neil läkakõha 1–2 nädalat kestva mitteparoksüsmaalse kõhana või põevad nad täiesti asümptoomselt. Viimasel juhul esineb *B. pertussise* kandlus ninaneelus ning inimene on nakkusohtlik (3) kuid ise täiesti vaevusteta. Imikutel on haiguse kulg kõige raskem koos apnoede, tsüanoosihoogude ja bradükardiaepisoodidega (41).

Kuna tänapäeval võivad nii läkakõha kulg kui ka sümptomid olla väga erinevad, siis tuleb läkakõha diagnoosi kinnitamiseks või välistamiseks kasutada laboratoorseid meetodeid. Mikroobi väljakülvamine on kuldne standard ja ainuke meetod, mis võimaldab määrata bakteri antibiootikumtundlikkust, kuid madala tundlikkuse ning kuni 10-päeva pikkuse diagnoosi kinnitumise aja tõttu on see meetod harva kliinilises praktikas kasutusel (20). PCR meetod on suurema tundlikkusega ja oluliselt kiirema diagnoosi kinnitumise ajaga kui mikrobioloogiline väljakülv ja seetõttu enam kasutatav kliinilises praktikas, kuid *B. pertussise* tuvastamiseks kasutatavad praimerid ei ole veel standardiseeritud (20). Eestis on siiani läkakõha diagnoosimiseks enamasti kasutatud seroloogilisi meetodeid. MTO soovitab kasutada paarisseejumite meetodit, mille kohaselt on läkakõha diagnoosi kinnituseks vajalik oluline antikehade kontsentratsiooni tõus kahes vähemalt kahe nädalase vahega võetud vereanalüüsis (109). Selle meetodi puuduseks on samuti diagnoosi hilinemine. Seetõttu kasutatakse kliinilises praktikas enam ühekordse seroloogia meetodit, mis annab võimaluse haiguse diagnoosi kinnitada ka haiguse hilises faasis, kui PCR ja külv ei ole enam informatiivsed (20). Samas, seroloogiat ei saa kasutada inimestel, kes on läkakõha vastu vaktsineeritud viimase aasta jooksul ning teiseks meetodi puuduseks on valideerimata diagnostilised piirväärtused (20).

Arenenud riikides hakati läkakõha vastu vaktsineerima 1950. aastatel. Vaktsineerimine oli efektiivne ning haiguse avaldumus (*incidence*) langes mõõdunud sajandi 70–80. aastatel enam kui 90% võrreldes vaktsineerimiseelse ajaga (5). Viimastel aastakümnetel on läkakõha avaldumus oluliselt tõusma

hakanud ning läkaköha on tänapäeval kõige enam levinud vaktsiinivõiditav haigus ka nendes riikides, kus läkaköha vaktsiiniga hõlmatus on kõrge (5). Ka Eestis on riiklikult registreeritud läkaköha avaldumuse trendid sarnased teiste kõrge immuniseerimistasemega riikidega (11). Kui 1970. ja 1980. aastatel oli läkaköha avaldumus ~4–5/100 000 elaniku kohta siis alates taasiseseisvumisajast on avaldumus oluliselt tõusnud ning 2010. aastal registreeriti Eestis läkaköha 97 juhtu 100 000 elaniku kohta (11). See oli kõrgeim aastane läkaköha avaldumus Euroopas (11).

Eestis ei olnud enne käesolevate uuringute alustamist teada tegelikku läkaköhasse haigestumist järgmistel põhjustel:

- 1) Kuni 2012. aastani olid Eestis kasutusel ainult kvalitatiivsed seroloogilised testid. Nende testide puuduseks oli madal spetsiifilisus, st. need andsid ristreaktsioone teiste tekitajatega ja seega ka valepositiivseid vastuseid. Alates 2012. aastast on enamikus Eesti laborites kasutusel kõrge tundlikkuse ja kõrge spetsiifilisusega kvantitatiivsed seroloogilised testid, kus määratakse antikehi vaid pertussise toksini ehk läkaköha toksini (PT) suhtes. Läkaköha seroloogilise testi kvaliteedi paranemine on väga oluline, kuna Eestis enam kui 90% juhtudest kinnitatakse läkaköha diagnoos ühekordse seroloogilise testiga.
- 2) Terviseameti andmetel on läkaköha Eestis siiani diagnoositud peamiselt laste hulgas. Kuid ~95% 1-aastastest lastest on vaktsineeritud kolme doosi läkaköha vaktsiiniga (165). Tõenäoliselt on haigus laste hulgas ülediagnoositud.
- 3) Kuna teismelised ja täiskasvanud on läkaköha antigeenidega varem kokku puutunud, kas haiguse eelneva läbipõdemise teel või vaktsineerimise tõttu, siis neil on läkaköha kulg ja sümptomid muutunud. Enamasti põevad nad läkaköha kergelt või täiesti asümptoomselt ning nad ei pruugi arsti poole pöörduda või siis arst ei mõtle läkaköha peale ja ei uuri neid läkaköha suhtes.

Läkaköha haigestumist saab uurida nii riiklikul statistikal põhinevate epidemioloogiliste uuringutega, prospektiivsete uuringutega kõhivatel inimestel kui ka seroepidemioloogiliste uuringutega, kus määratakse inimeste vereseerumist PT IgG tüüpi antikehade kontsentratsioon ja selle põhjal hinnatakse läkaköhasse haigestumist.

Läkaköhasse haigestumise kohta Eestis on varem avaldatud neli artiklit, mis kõik põhinevad riiklikul statistikal (6, 14-16). Kuna arstid peavad Eestis diagnoositud läkaköha juhtudest teavitama Terviseametit, siis on riiklikul statistikal põhinevaid uurimistöid suhteliselt lihtne teha. Samas, ükski järelevalve süsteem pole ideaalne ning sel viisil kogutud andmed on suhteliselt madala tundlikkusega (193). Seega, et selgitada välja tegelik läkaköha avaldumus (*incidence*) ja levimus (*prevalence*) Eestis, viisime me läbi seroepidemioloogilise uuringu hindamiseks *B. pertussise* avaldumust ja prospektiivse uuringu kõhivatel patsientidel.

## Uurimistöö eesmärgid

Uurimistöö peamiseks eesmärgiks oli selgitada välja *B. pertussise* poolt põhjustatud infektsioonide epidemioloogia ja sümptomid Eestis ning hinnata, kes hetkel kasutusel olev immuniseerimisskeem on läkaköha suhtes optimaalne.

Konkreetsed eesmärgid:

1. määrata PT IgG antikehade kontsentratsiooni erinevates vanuserühmades;
2. hinnata läkaköha infektsiooni avaldumust hiljuti mittevaktsineeritud laste ja täiskasvanute hulgas;
3. võrrelda omavahel antikehadel põhinevat hinnangulist läkaköha avaldumust ja riiklikult registreeritud avaldumust;
4. kindlaks määrata läkaköha ja paraläkaköha esinemine vähemalt seitse päeva kõhinud teadmata etioloogiaga kõhaga arsti vastuvõtule pöördunud patsientide hulgas;
5. kirjeldada ja omavahel võrrelda läkaköhaga, paraläkaköhaga ja muu kõhaga patsientidel esinevaid sümptomeid ning kindlaks määrata, kui hästi MTO läkaköha kliiniline definitsioon ennustab haiguse olemasolu;
6. kirjeldada läkaköha ja paraläkaköha kulgu.

## Patsiendid ja meetodid

**Laste läbilõikelisse seroepidemioloogilisse uuringusse** kogusime ajavahemikul 09.04.2012–23.08.2012 Tartu Ülikooli Kliinikumi Ühendlabori lastekliiniku osakonda saadetud alla 18-aastaste laste jääkvereseerumid koos vanuse andmetega.

**Täiskasvanute läbilõikelisse seroepidemioloogilisse uuringusse** kogusime ajavahemikul 07.01.2013–27.02.2013 Synlab Eesti OÜ laboritesse saadetud 18–99-aastaste täiskasvanute jääkvereseerumid koos vanuse ja soo andmetega.

Mõlemasse uuringusse kaasati vaid vereseerumid, mis koguti inimestelt, kellel ei olnud kahtlust läkaköhale. PT IgG kontsentratsiooni mõõtsime kvantitatiivselt kommertsiaalse ELISA testiga (Euroimmun®, Lübeck, Saksamaa). Läkaköha infektsiooni levikut hindasime ainult 9–14-aastaste laste ja  $\geq 20$ -aastaste täiskasvanute hulgas, et vältida läkaköhavastase vaktsineerimise mõju antikehade kontsentratsioonile. Eeldasime, et PT IgG  $\geq 62,5$  IU/mL näitab kokkupuudet *B. pertussis*’ega vereanalüüsi andmisele eelneval aastal (203). Hinnangulise haigestumuse arvutamiseks kasutasime de Melkeri ja kaasautorite valemit:  $365.25/208.9 \times \text{populatsiooni protsent}$ , kellel on PT IgG  $\geq 62,5$  IU/mL (203). Üle 20-aastaste hulgas identifitseerisime inimesed, kellel PT IgG kontsentratsioon oli  $\geq 62,5$  IU/mL ja selgitasime välja, kas nad olid vereanalüüsile eelnenud kuue kuu jooksul kaevanud oma perearstile kõha.

**Prospektiivse kõhivate patsientide uuringu** viisime läbi 25 perearstikeskuses (Harjumaal, Tartumaal, Järvamaal, Jõgevamaal ja Võrumaal) ja kolmes haiglas (SA Tartu Ülikooli Kliinikum, SA Tallinna Lastehaigla ja AS Järvamaa haigla) ajavahemikul 23.04.12–31.12.14. Uuringusse kaasasime vähemalt seitse

päeva kestnud ägeda köhaga lapsed ja täiskasvanud, kelle köha ei hakanud selle aja jooksul taanduma ja kelle köhale ei olnud teist teadaolevat põhjust. Lisaks pidi uuritav ja/või tema lapsevanem/hooldaja andma kirjaliku nõusoleku uurin-gus osalemiseks.

Esimesel visiidil registreeriti viie põhilise sümptomi (paroksüsmaalsed köha-hood, inspiratoorsed repriisid, köhahoojärgne oksendamine, apnoed ja palavik) olemasolu, hinnati köha raskust Likert'i skaalal (0 – köha ei ole, 5 – köha on ebamugav, segab magamist, 10 – köha on väljakannatamatu, väga valus, pidev hirm lämbumise ees) ja võeti patsiendilt kaks analüüsi tampooniga ninaneelust ja veenivere analüüs. Ühest ninaneelust kogutud proovist tehti PCR-analüüs *B. pertussis*'ele ja *B. parapertussis*'ele/*B. bronchiseptica*'le ja teisest mikrobioloogiline väljakülv. Vereseerumist määrati ELISA meetodi abil kvantitatiivselt PT IgG kontsentratsioon ja kui see jäi vahemikku 40–100 IU/mL, siis määrati lisaks ka PT IgA kontsentratsioon.

Läkaköha diagnoosi kinnitas *B. pertussis* isoleerimine mikrobioloogilisel väljakülvil ja/või *B. pertussis*'e DNA tuvastamine PCR meetodil ja/või kui PT IgG > 100 IU/mL või kui PT IgG oli 40–100 IU/mL siis PT IgA pidi olema  $\geq 12$  IU/mL. Paralaköha diagnoosi kinnitas *B. parapertussis*'e isoleerimine mikrobioloogilisel väljakülvil ja/või *B. parapertussis*'e DNA tuvastamine PCR meetodil.

Läkaköha/paralaköha põdevatelt patsientidelt (või tema vanemalt) küsiti üks kord nädalas kuni tervistumiseni infot patsiendi haiguskulu kohta (maksimaalselt 12 nädala jooksul). Köha kestvust hinnati kuni köha tegeliku taandumiseni.

Läkaköhaga, paralaköhaga ja muu köhaga patsientidel esinevate sümptomite analüüsimiseks kasutasime järgimisi statistilisi meetodeid: sümptomite levimuse analüüs, mitmemõõtmeline korrespondentsanalüüs ja mitmene regressioonanalüüs.

## Peamised tulemused

Kokku osales uuringutes 5027 uuritavat, kellest 4478 osales seroepidemioloogilises uuringus (1053 laste ning 3425 täiskasvanute uuringus) ja 549 prospektiivses köhivate patsientide uuringus.

**Läbilõikelises seroepidemioloogilises uuringus** oli kogu populatsiooni üldine PT IgG geomeetriline keskmine (GMC) madal (5,9 IU/mL; 95% usaldusvahemik (UV): 5,7–6,1). GMC oli numbriliselt kõrgem nendes vanusrühmades, kus lapsi oli hiljuti vaktsineeritud läkaköha vastu, kuid sellest juba järgmisel aastal langes GMC taas järsult madalale tasemele. Nooremates vanusrühmades, kus Eestis lapsi läkaköha vastu vaktsineeritakse (3-kuused–1-aastased ja 2-aastased) tõusis GMC oluliselt kõrgemale tasemele kui vanemates vaktsineerimise vanusrühmades (7–8-aastased ja 18–19-aastased) – vastavalt 24,0 IU/mL (95% UV: 18,2–31,5) ja 11,2 IU/mL (95% UV: 9,2–13,6);  $p < 0,001$ . Täiskasvanute

hulgas oli GMC 40–49-aastaste hulgas (3,9 IU/mL; 95% UV 3,6–4,2) oluliselt madalam kui teistes 10-aastastes vanusrühmades.

Kõikidest seroepidemioloogilises uuringus osalenud uuritavatest 1,0% (95% UV: 0,8–1,4) olid PT IgG kontsentratsioon väga kõrges väärtuses ( $\geq 125$  IU/mL), mis näitas kokkupuudet *B. pertussis*'ega või vaksineerimist läkaköha vaktsiiniga viimase 6 kuu jooksul, 3,6% (95% UV: 3,1–4,2) väärtuses  $62,5 < 125$  IU/mL, mis näitas kokkupuudet *B. pertussis*'ega või vaksineerimist läkaköha vaktsiiniga viimase 7–12 kuu jooksul ja 39,7% (95% UV 38,3–41,2)  $5,0 < 62,5$  IU/mL, mis näitas kokkupuudet *B. pertussis*'ega või vaksineerimist läkaköha vaktsiiniga enam kui 12 kuud tagasi ning 55,6% (95% UV 54,2–57,1) alla 5,0 IU/mL ehk mittemääratavas tasemes, mis samas ei tähenda, et inimesel ei oleks kaitset läkaköha eest. Seda kinnitab ka uuringu tulemus, kus kõige enam mittemääratavas tasemes antikehadega uuritavaid oli 40–49-aastaste ja 12–14-aastaste hulgas, kuid samas oli neis vanusrühmades ka väga vähe inimesi, kelle antikehade tase viitaks hiljutisele läkaköha infektsioonile. Laste hulgas oli kõige enam kõrges või väga kõrges tasemes antikehadega uuritavaid vanusgruppides, kus meil Eestis lapsi läkaköha vastu vaksineeritakse ning täiskasvanute hulgas 20–29-aastaste seas. Need noored täiskasvanud on olnud lapsed ajal kui Eestis oli läkaköhaga vaktsiiniga hõlmatus madal ning lastele manustati vaid üks tõhususdoos ning võib oletada, et eelnev on tinginud suurema infektsiooni leviku selles vanusgrupis. Samas, just selles vanuses saadakse lapsevanemateks ja need 20–29-aastased ohustavad oma veel vaksineerimata või osaliselt vaksineeritud imikuid.

PT IgG põhinev hinnanguline läkaköha avaldumus vereanalüüsile eelnenud aasta jooksul oli 5,9%; 95% UV 4,9–7,0. Hinnanguline avaldumus oli kõrgem noorte täiskasvanute ja üle 60-aastaste inimeste hulgas. Samas, ainult 30% neist olid vereanalüüsile eelnenud 6 kuu jooksul kaevanud köha oma perearstile, kuid kedagi läkaköha suhtes ei uuritud ja seega ka mitte kellelgi läkaköha ei diagnoositud.

Riiklikult registreeritud läkaköha avaldumus 9–14-aastaste laste ja  $\geq 20$ -aastaste täiskasvanute hulgas 2011. ja 2012. aastal oli 0,013% (12,5/100,000). See-ga oli hinnanguline seroepidemioloogial põhinev avaldumus 470 korda kõrgem kui riiklikult registreeritud haiguse avaldumus.

**Prospektiivses kõhivate patsientide uuringus** diagnoositi läkaköha 22 patsiendil (levimus 4,0%; 95% UV: 2,5–6,0). Läkaköha levimus oli oluliselt kõrgem laste ( $< 18$ -aastased) 7,6%; 95% UV: 3,9–13,2 kui täiskasvanute hulgas 2,7%; 95% UV: 1,4–4,8;  $p=0.027$ . Kõrgeim läkaköha levimus oli  $< 1$ -aastaste imikute hulgas 50,0, kuid üle 65-aastaste seas ühtegi läkaköha juhtu ei kinnitunud. Läkaköha diagnoos kinnitus suuremal osal juhtudest (17/22) ühekordse seroloogilise testiga, mida võib seostada suhteliselt pika ajaperioodiga haiguse algusest kuni uuringusse kaasamiseni – keskmiselt 26 (standardhälve (SD) $\pm$  21,0) päeva.

Paraläkaköha diagnoositi seitsmel patsiendil (levimus 1,3%; 95% UV: 0,5–2,6) ja kõikidel diagnoos kinnitus PCR meetodiga. Ka paraläkaköha levimus oli

laste hulgas kõrgem 4,1%; 95% UV: 1,5–8,8 kui täiskasvanute hulgas 0,3%, 95% UV: 0,0–1,4);  $p=0.003$ .

Läkaköhaga ja paraläkaköhaga patsiendid olid keskmiselt oluliselt nooremad kui muu köhaga patsiendid, keskmine vanus vastavalt 21,6 (SD±17,2) aastat, 12,0 (SD±12,5) aastat ja 35,9 (SD±21,5) aastat. Hospitaliseerimise määr oli läkaköhaga patsientide hulgas oluliselt kõrgem (13,7%) kui muu köhaga (1,0%) patsientide hulgas; suhteline risk (SR) 16,0; 95% UV: 3,1–70,6. Alla 18-aastaste laste hulgas oli keskmine aeg viimase läkaköha vaktsiini doosi ja läkaköhasse haigestumise vahel 2,2 aastat (kvartiilide vahe (IQR) 1,8–7,1). Läkaköhaga patsientidest ligi 80% olid läkaköha vastu vaktsineerimata või vaktsineeritud rohkem kui viis aastat tagasi.

Läkaköhaga patsientidel esines uuringusse haaramisel rohkem põhisümptomiteid (2,8 ±1,1) kui muu köhaga patsientidel (2,0±1,2),  $p=0,001$ .

Sümptomite levimuseanalüüsist selgus, et läkaköhaga patsientidel esines oluliselt rohkem nii köhahoojärgset oksendamist kui ka inspiratoorseid repriise kui muu köhaga patsientidel; SR vastavalt 9,9; 95% UV: 2,5–14,9 ja 2,9, UV: 1,2–7,3.

Vastavalt mitmemõõtmelisele korrespondents analüüsile ühtegi kindlat seost registreeritud põhisümptomite ja diagnooside vahel ei ilmnenud, kuid läkaköhaga täiskasvanutel esines enam inspiratoorseid repriise, köhahoojärgset oksendamist ja apnoesid ning lastel ka palavikku kui paraläkaköhaga ja muu köhaga patsientidel. Paraläkaköhaga lapsed olid sümptomite poolest sarnased ja moodustasid eraldi grupi, kuid ühtegi kindlat paraläkaköhale iseloomulikku sümptomit ei leitud.

Kõigist läkaköhaga patsientidest 17 vastas ja 527-st paraläkaköhaga ja muu köhaga patsiendist 201 ei vastanud MTO-i läkaköha kliinilisele definitsioonile (109). Seega on MTO-i läkaköha kliinilise definitsiooni tundlikkus, spetsiifilisus ja ennustustäpsus vastavalt 0,77 (95% UV: 0,55–0,92), 0,38 (95% UV: 0,34–0,42) ja 0,58 (95% UV: 0,49–0,67) antud populatsioonis.

Vastavalt mitmesele regressioonianalüüsile on läkaköhale iseloomulik nii üldpopulatsioonis kui ka täiskasvanute hulgas inspiratoorsete repriiside, köhahoojärgse oksendamise ja palaviku esinemine. Laste hulgas polnud ükski sümptomite kompleks suurema diagnostilise väärtusega kui üksiksümptomid.

Keskmine köha kestvus nii läkaköha kui ka paraläkaköha korral oli sarnane, vastavalt 104.1 (±52.5) ja 78.3 (±62.9) päeva. Pärast uuringusse haaramist püsisid läkaköhaga patsientidel paroksüsmaalsed köhahood ja inspiratoorseid repriisid oluliselt kauem kui paraläkaköhaga patsientidel, vastavalt (5.0 (±3.9) vs 1.9 (±4.5) nädalat ja 3.7 (±4.5) vs 0.0 (±0.0) nädalat; mõlemal juhul  $p=0.012$ . Kõik uuringus osalenud läkaköhaga ja paraläkaköhaga patsiendid paranesid.

## Järeldused

Alates meie uuringute algusest on Eestis riiklikult registreeritud läkaköha avaldumus oluliselt langenud (97/100 000 2010.a.ja 4,3/100 000 2017.a.). Riiklikult

registreeritud läkaköha avaldumus on püsinud stabiilselt madalana (4-5/100 000) alates 2012. aastast. Nii pikka ni madala riiklikult registreeritud läkaköha avaldumusega perioodi pole Eesti taasiseseisvumise ajal varem olnud. Ka riiklikult registreeritud läkaköha avaldumus imikute hulgas on madal (alla kolme juhu aastas viimase viie aasta jooksul). Viimane läkaköhaast tingitud surmajuht oli 2007 aastal. Läkaköha levimus oli madal ka köhivate patsientide uuringus. Seega, hetkel Eestis kasutuses olev läkaköhaavastane immuniseerimiskava on piisavalt efektiivne, et hoida läkaköha avaldumus madal tasemel. Suur vahe seroepidemioloogilistel uuringutel põhineva hinnangulise läkaköha avaldumuse ja riiklikult registreeritud avaldumuse vahel näitab, et *B. pertussis* ringleb ja, et läkaköha on enamasti asümptomaatiline või kerge kuluga haigus kõrge vaktsineerimistasemega riikides. Hetkel kasutuses olevate vaktsiinidega on võimalik vähendada läkaköha avaldumust, kuid haiguse täielik likvideerimine pole tõenäoliselt võimalik.

Uurimistöö konkreetsete järeldused:

1. Eestis on PT IgG GMC madal. PT IgG GMC on kõrgeim hiljuti vaktsineeritud hulgas, kuid langeb kiiresti madalale tasemele pärast vaktsineerimist. Igapäevapraktikas peab meeles pidama, et seroloogiline meetod ei sobi läkaköha diagnoosimiseks, kui viimasest läkaköhaavastasest immuniseerimisest on möödunud vähem kui üks aasta.

Enamusel seroepidemioloogilises uuringus osalejatest oli PT IgG mittemäärataval tasemel. Samas, väga väiksel hulgal uuritavatest olid PT IgG kõrgel või väga kõrgel tasemel, mis näitab hiljutist kokkupuudet *B. pertussis*'ega. Seega, vaatamata üldisele väga madalale antikehade tasemele uuritavate hulgas on nad suhteliselt hästi kaitstud läkaköha eest. Sellest järeldub, et kaitse läkaköha eest tagavad lisaks PT vastastele antikehadele veel muud faktorid ning populatsiooni immuunsust ei saa hinnata pelgalt antikehade taseme põhjal.

2. Seroepidemioloogial põhinev hinnanguline läkaköha avaldumus on sarnane kõikides täiskasvanute vanusrühmades ja hiljuti mittevaktsineeritud laste hulgas. Seega *B. pertussis* ringleb keskkonnas ja hetkel kasutuses olevate vaktsiinidega pole võimalik seda peatada.
3. Hinnanguline läkaköha avaldumus on ligi 500 korda kõrgem kui riiklikult registreeritud avaldumus hiljuti mittevaktsineeritud laste ja täiskasvanute hulgas, mis näitab asümptoomse ja/või kergekujulise *B. pertussise* infektsiooni levikut Eestis või siis pole läkaköha sümptomid teada ja seega ei tunda läkaköha ära.
4. Läkaköha levimus köhivate patsientide hulgas Eestis on madal. Kõrgeim levimus imikute hulgas on seotud vanusest tingitud puuduva ja/või mitetäieliku vaktsineerimisega selles vanusrühmas. Kuna üle 65-aastaste hulgas ühtegi läkaköha juhtu ei kinnitunud, siis on läkaköha selles vanusrühmas asümptoomne või pole köha esinemine läkaköha korral tavaline. Üldine madal läkaköha levimus näitab, et hetkel kasutuses olevad läkaköhaavastased vaktsiinid ja Eestis kasutusel olev immuniseerimiskava suudavad hoida

- sümptomaatilise läkaköha levimuse madalal tasemel. Paraläkaköha levimus oli veel madalam kui läkaköha levimus.
5. Läkaköhaga patsientidel esines enam inspiratoorseid repriise ja köhahoojärgset oksendamist kui muu köhaga patsientidel. Siiski on klassikaliste läkaköha sümptomite tundlikkus ja spetsiifilisus kõrge immuniseerimistasemega riikides madal. Seega on ka hetkel kasutuses oleva MTO-i läkaköha kliinilise definitsiooni ennustustäpsus ebapiisav. Lisaks ei sobi antud definitsioon läkaköha diagnoosimiseks üle 65-aastaste patsientide hulgas. Läkaköha paremaks diagnoosimiseks oleks vajalik välja töötada uued easpetsiifilised läkaköha kliinilised kriteeriumid. Paraläkaköhale iseloomulikke sümptomeid ei leitud.
  6. Isegi kõrge vaktseerimistasemega riikides võivad läkaköha ja paraläkaköha kulgeda pikaajalise ja piinava köhaga, kuid kõrge meditsiinilise tasemega riikides on surmajuhtumid harvad.

## 10. ACKNOWLEDGEMENTS

This work was carried out in the Department of Microbiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, in 25 general practitioner practices, in three hospitals (Tartu University Hospital, Tallinn Children's hospital, and Järvamaa District hospital), in Synlab Eesti OÜ (former Quattromed HTI Laborid OÜ), and in the Health Board of Estonia.

Studies were supported by the Estonian Science Foundation (grant 9259 and IUT34-24) and Basic Financing and Target Financing of the Estonian Ministry of Education and Research (SF0180004s12), and European Union through the European Regional Development Fund.

The studies conducted in this thesis have been based on teamwork, with the contribution of many great people to whom I wish to express my deepest gratitude.

In particular, I would like to thank my supervisors:

- Professor Irja Lutsar for setting my focus and giving me the opportunity to research into this topic. For constructive guidance, asking insightful questions, and for sharing her immense knowledge and insights to medical science.
- Associate Professor Marje Oona for her everlasting enthusiasm to keep me going when times were tough, intelligent and useful discussions and for giving invaluable advice.

I can truly not imagine having better supervisors for my doctoral studies.

I am very grateful to Professor Ruth Kalda and Associate Professor Matti Maimets for their critical reading of the manuscript of dissertation and valuable comments. I appreciate the time they took with my work.

This study would never have been possible without the help and contribution of my co-authors and co-workers at the general practitioner practises and laboratories.

- I am very thankful to the following family doctor centres' GPs and their nurses for helping me conduct the study of coughing patients: Tereza Maskina FIE, University Family Doctors' Centre OÜ, Merekivi Family Doctors' Centre OÜ, Lembi Põlder Family Doctor's Centre OÜ, Ilme Last FIE, Ülle Trumm FIE, Tabasalu Family Doctors' Centre OÜ, Family doctor Tiiu Tootsi OÜ, Türi Health Centre OÜ, Vee Family Doctors' Centre OÜ, Pirtia-Kose Family Doctors' Centre OÜ, Medicum AS, Family Doctor Hiie Karelson OÜ, Linnamõisa Family Doctors' Centre OÜ, Saku Health Centre OÜ, Ädala Family Doctors' Centre OÜ, Family doctor Tarvo Kiudma OÜ, Eve Mõistuse Family Doctors' Centre OÜ, Family doctor Mairi Kotsar OÜ, Helvi Kansi OÜ, Plaks ja Pilv Family Doctors' Centre OÜ, Dr Aune OÜ,

Family doctor Ljudmila Jakobson OÜ, Family doctor Sirje Saar OÜ, and Family Doctor Marje Koha OÜ.

- I thank the doctors and nurses of the Children’s Clinic of Tartu University Hospital and the paediatric intensive care units of Tartu University Hospital, Tallinn Children’s Hospital, and Järvamaa District Hospital, who helped conduct the study of coughing patients.
- I thank the staff of Synlab Eesti OÜ (former Quattromed HTI OÜ Laborid) for taking and testing the samples. I am especially thankful to Kai Lauri for helping organise the studies and Ulla Märtsen for helping to solve all unexpected situations.
- I thank the staff of United Laboratories of Tartu University Hospital, especially Dr Sirje Leedo for helping conduct the study of seroprevalence in children and Dr Krista Lõivukene for helping organise the study of coughing patients.
- I thank the staff of the Health Board, especially Kaie Otsmaa for helping to organise the study of coughing patients in Tallinn and Jevgenia Epštein for valuable data on nationally reported figures.
- I am very thankful to all my colleagues from the Department of Microbiology, especially Merit Pauskar and Kristi Huik for managing and storing the samples.
- Thank you, Karolin Toompere, Tanel Kaart, and Aivi Themas for advice on statistical analyses.

I am very grateful to all people who participated in the studies for their cooperation.

I would like to thank all my colleagues from the Children’s Clinic of Tartu University Hospital, especially Dr Eda Tamm, Dr Inga Vainumäe, Dr Marika Kirss, Dr Margit Närska, Dr Aime Pütsepp, Dr Siiri Torm, and Dr Marje Kleemann who made it possible for me to have time to write my papers and finish the thesis and their help and support.

I cherish the people who have been beside me through the years I have been focusing on my research: my close friends Aivi, Klari, Mari-Liis, Mari-Anne, Lenne-Triin, and Jane for interesting discussions, sincere support, and friendship and my children’s nanny Aili for taking care of Oskar and Karl while I was away.

My greatest gratitude goes to my family: my father Andres and Maire, Kristi, and Marit for all their support through the difficulties and their genuine happiness for my success.

And last, but not least, I could not overrate the importance of the love and backing from my beloved husband Klaus and our dear children, Karl and Oskar. I love you very much.



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### Professional employment

2011– Children's Clinic of Tartu University Hospital Paediatrician,  
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### Scientific work

5 articles in international peer reviewed medical journals, 3 articles in Estonian Doctor, and 9 oral or poster presentations at international scientific conferences. The topics of my research activities have been epidemiology and surveillance of vaccine-preventable diseases.

### Professional organisations

- Estonian Paediatric Association (since 2005)
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- European Society for Paediatric Infectious Diseases (since 2007)
- Estonian Society of Infectious Diseases (since 2011)
- Central and Eastern Europe Pertussis Awareness Group (since 2017)

### Publications

1. **Jõgi P**, Oona M, Kaart T, Toompere K, Maskina T, Koort I, Rätsep A, Lutsar I. Pertussis and parapertussis in children and adults with a persistent cough: an observational study. *Infection* 2018; 46(1), 83–91;
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Olen avaldanud koos kaasautoritega 5 teadusartiklit rahvusvahelistes eelretsenseeritavates ajakirjades, 3 artiklit ajakirjas „Eest Arst“ ja olen esinenud 9 suulise ja posterettekandega rahvusvahelistel teaduskonverentsidel.

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- Eesti Lastearstide Selts (alates 2005)
- Eesti Arstide Liit (alates 2005)
- Euroopa Laste Infektsioonhaiguste Selts (alates 2007)
- Eesti Infektsioonhaiguste Seltsi (alates 2011)
- Kesk ja Ida-Euroopa läkaköha uurimisgrupp (alates 2017)

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## DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

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