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Bordetella pertussis – the returning pathogen

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ABSTRACT

Bordetella pertussis is a non-motile, Gram-negative coccobacillus belonging to the genus *Bordetella*. It is the causative agent of pertussis – a respiratory tract infection also known as whooping cough or 100-days' cough. A similar bacterium, *Bordetella parapertussis*, is responsible for a proportion of pertussis cases, which are distinguished by milder symptoms. Two major virulence factors of *Bordetella pertussis* are adhesins (filamentous hemagglutinin and fimbriae) which facilitate tracheal colonization – the first step leading to infection. In pediatric population *B. pertussis* infection tends to take a highly unpredictable and sometimes fatal course. Antimicrobial drugs of macrolide class are the mainstay of therapy mainly due to their superb activity against *Bordetella pertussis*. However, antibiotic administration is ineffective in alleviating symptoms of whooping cough. Therefore, in the first half of the 20th century there was a collective effort of scientific community to create an efficient vaccine against *B. pertussis*. These endeavors proved fruitful and culminated in the implementation of vaccination programs since 1940s. Despite the widespread availability of vaccines, pertussis remains a significant cause of children morbidity to this day. Epidemiological data gathered in Poland during the last 10 years clearly imply that the disease incidence is steadily increasing. Furthermore, pertussis outbreaks, which happened every few years in the past, nowadays occur almost every other year. The reasons behind the alarming resurgence of pertussis are multifold, but two elements are presumed to play a key role: the waning immunity of adolescents who were vaccinated many years ago; and the parents' reluctance to vaccinate their offspring. On account of the steady growing number of pertussis cases, the definite causes of the current epidemiological state ought to be determined and an adequate approach to combat theses reasons must be undertaken.

***Keywords*:** *Bordetella pertussis*, whooping cough, outbreak, vaccine, epidemiology

1. INTRODUCTION

1. 1. Historical background

The bacterium responsible for pertussis, a disease also known as whooping cough, was discovered in 1900 by two Belgian scientists: Jules Bordet and Octave Gengou. [1] They described a new species of Gram-negative coccobacillus (GNCB), which was later named *Bordetella pertussis* in order to honor one of the discoverers. In 1906 both collaborators invented a medium specifically developed to support *B. pertussis* growth. Developing this medium proved to be challenging since *B. pertussis* is quite a fastidious bacterium. At the very same time, Bordet and Gengou outlined the morphology of *Bordetella pertussis* and characterized its main virulence factors.

The disease itself – whooping cough – was known to doctors for much longer, however; in 1679 an English physician, Thomas Sydenham, named it "pertussis" – which stands for "paroxysmal cough" – due to its outstanding ability to illicit uncontrollable, long-lasting fits of cough. Joseph Lapin a pediatrician working at Bronx Hospital, New York, in 1943 authored a comprehensive treatise on *Bordetella pertussis* and whooping cough. [2]

1. 2. The objective of the study

Whooping cough almost lapsed to the status of forgotten disease with the advent of widespread vaccination programs. However, due to many factors pertussis makes an unexpected comeback nowadays. [3]

The aim of this study is to describe the pathogen, the clinical course of the disease, its treatment and prevention. However, the chief purpose of this analysis is: (1) to depict current epidemiological situation, (2) to indicate the reasons behind the resurgence of whooping cough, and (3) to imply how the epidemic may be countered.

2. RESULTS

2. 1. Pathogen description

Bordetella pertussis is a strictly aerobic bacterium causing whooping cough. Along with *Bordetella parapertussis* and *Bordetella bronchiseptica*, it is a member of *Bordetella* genus. *B. pertussis* has a striking resemblance to *B. parapertussis*; both species are exclusive human pathogens, and *B. parapertussis* – just like *B. pertussis* – also causes whooping cough, though its symptoms are relatively milder compared to the whooping cough caused by *B. pertussis*.

Bordetella genus comprises minute (0.2 – 0.7 μm) Gram-negative cococcobacilli (GNCB) belonging to the phylum Proteobacteria. All *Bordetella* species are catalase-positive and exhibit ability to oxidize amino acids. *Bordetella* thermal optimum lies within 35-37 °C range and corresponds closely to the hosts' body temperature.

While some *Bordetella* species are motile, *B. pertussis* is non-motile; it is infectious only to humans and it has no zoonotic reservoir.

B. pertussis is a remarkably fastidious organism. Consequently, culturing *B. pertussis* is a particularly difficult task. Its growth is restrained by a wide variety of compounds and ingredients commonly used in culture mediums (sulfides, metal ions and fatty acids to name a few). Therefore, a need to create a specific medium supporting *B. pertussis* growth arose. Culture mediums for *B. pertussis* will be discussed later.

2. 2. Pathogenesis

2. 2. 1. Phases of pathogenesis

Four main phases leading to *B. pertussis* infection were identified. These are as follows: (1) adhesion of *B. pertussis* to the host's cells in the respiratory tract (mainly the trachea), (2) dodging the mechanism of host's defense, (3) localized infection and localized tissue destruction, (4) full-blown infection (or symptomatic whooping cough).

2. 2. 2. Virulence factors

B. pertussis has a wide variety of virulence factors at its disposal: filamentous hemagglutinin (FHA), fimbriae (FIM), pertactin (PRN), pertussis toxin (PT), adenylate cyclase toxin (ACT), tracheal cytotoxin (TCT), dermonecrotic toxin (DNT). [4]

2. 2. 3. Description of the virulence factors

Filamentous hemagglutinin and fimbriae belong to adhesins; they play a major role in the colonization of tracheal epithelium. Since they are able to induce immune response, they found a practical application – FHA and FIM are components of acellular pertussis vaccine (DTaP: Diphtheria, Tetanus, acellular Pertussis). Pertactin and pertussis toxin are also thought to act as adhesins.

Adenylate cyclase toxin catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). This in turn inhibits the influx and activation of T-lymphocytes and cells with phagocytic capabilities. [5]

Pertussis toxin is directed against G protein–coupled pathways within the epithelial cells. Pertussis toxin is comprised of two subunits: subunit A and subunit B. Subunit A (active) is responsible for the toxic activity; it does so by adenosine diphosphate (ADP)-ribosylation of G proteins. [6,7] Subunit B (binding), as the name implies, enables binding of the subunit A to the cell surface.

Tracheal cytotoxin damages epithelial cells lining the respiratory tract by increasing the production of NO (nitric oxide) [8], since nitric oxide is lethal for the ciliated tracheal epithelial cells. Dermatonecrotic toxin also induces localized tissue damage. [9]

Contrary to many bacterial infections, the bacterium itself does not enter the blood stream and, therefore, whooping cough is strictly a localized infection. Few systemic symptoms which occur in the course of pertussis are due to the toxins alone, which unlike the bacterium, may cross the blood vessels wall, access the blood circulation and effectively get disseminated throughout the body. It is, thus, chiefly pertussis toxin which gives rise to most systemic manifestations of the disease e.g. lymphocytosis.

2. 3. Epidemiology

2. 3. 1. Global epidemiological situation

Despite widespread vaccination programs, whooping cough poses a major threat to infants' health, and is, hence, considered a major epidemiological problem to this day; this holds true both in developing countries and highly developed countries. According to World Health Organization (WHO) reports there are 16 million cases yearly, 195,000 of which end up in the patient's death. [10,40]

Before vaccines against *B. pertussis* were developed, whooping cough was responsible for more deaths than meningoencephalitis, scarlet fever, measles, diphtheria and poliomyelitis altogether! [11] With the advent of extensive immunization of infant population incidence of pertussis sharply declined.

However, indubitably recent years saw a dramatic resurgence of the disease; [12] nowadays, an outbreak of whooping cough happens somewhere throughout the world almost every other year and presently the number of reported cases is at least twofold higher than it was 10 – 20 years ago. [13]

Before vaccination programs were introduced pertussis predominantly affected children aged 1 to 10. [14] Currently, in pediatric population *B. pertussis* infects mostly newborns. [15] Newborns are at a substantial risk to develop whooping cough owing to the fact that they have not received their first vaccine shot yet. Newborns are also more likely to develop life-threatening complications due to their immature, underdeveloped immune system.

2. 3. 2. Epidemiological situation in selected countries

2. 3. 2. 1. Epidemiological situation in Poland

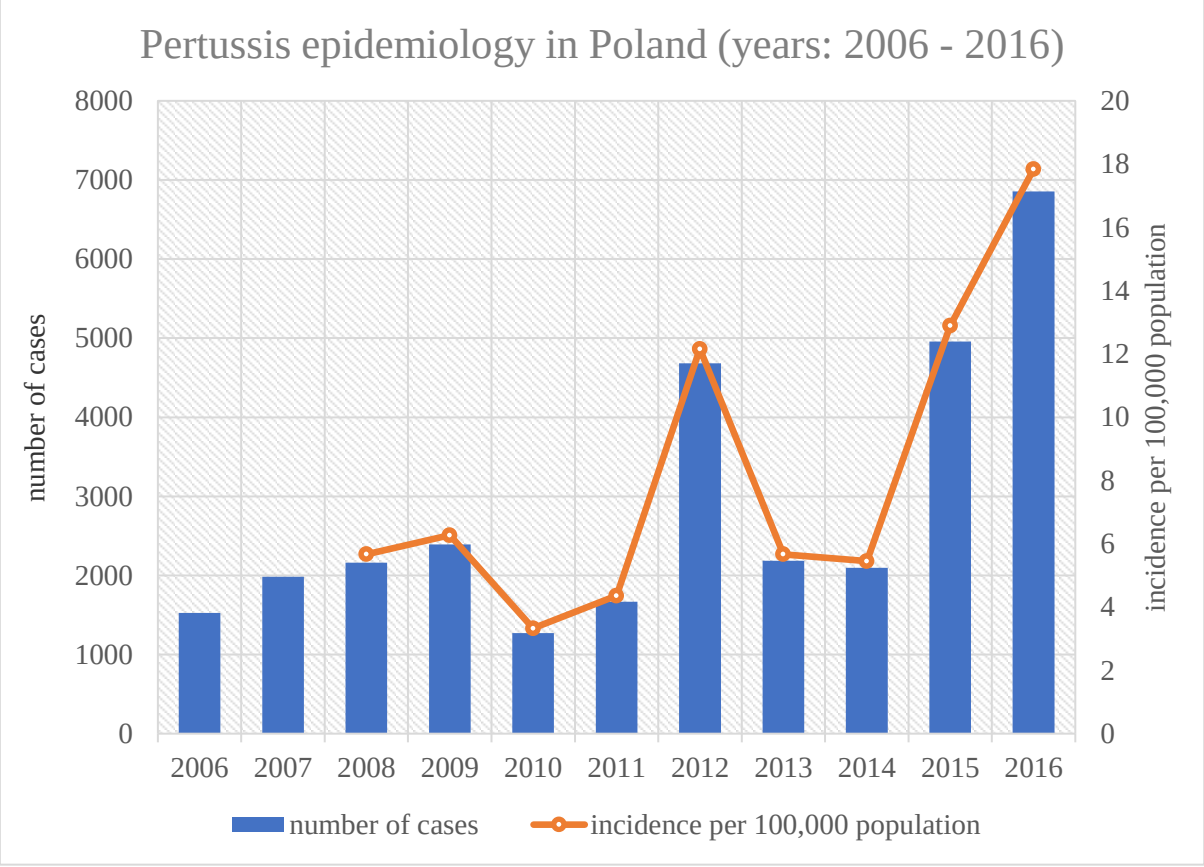

Figure 1. Epidemiology of pertussis in Poland and recent outbreaks (years: 2006 – 2016); based on reports published by National Institute of Public Health – National Institute of Hygiene. [41]

Analysis of the epidemiological data gathered in Poland in the last ten years implies that an undeniable rise in the pertussis incidence takes place. [41] This is in accordance to global trends as pertussis re-emergence is a global phenomenon, not just an isolated incident. While, a few years ago the situation in Poland was quite stable with circa 2000 cases of whooping cough recorded annually, a resurgence of the disease can be clearly noticed currently; this resurgence dates back to around 2012. [42]

This epidemic of 2012 occurred on global scale; it will be described later on in more detail in sections regarding the epidemiological situation in the UK and the USA.

Following this outbreak there was a brief drop in whooping cough incidence. However, it did not last long. Regrettably, a record breaking number of cases was witnessed last year (2016); the number of reported cases rose more than four times compared to data collected 10 years prior (1,526 cases in 2006 [43] versus 6,856 cases in 2016 [44]).

In the Q1 2017 (1st January – 31st March 2017) 1,153 whooping cough cases have already been diagnosed in Poland, [45] which is more than ⅔ of all cases in the entire 2006. Therefore, it is very likely that this year will set another shameful record with the number of recorded pertussis cases.

Table 1. Number of pertussis cases and incidence of pertussis per 100,000 population in Poland (data for years: 2006 – 2016); based on reports published by National Institute of Public Health – National Institute of Hygiene. [41]

year	number of pertussis cases	incidence per 100,000 population
2006	1,526	–[a]
2007	1,984	–[a]
2008	2,163	5.67
2009	2,391	6.27
2010	1,272	3.33
2011	1,667	4.36
2012	4,683	12.16
2013	2,185	5.67
2014	2,098	5.45
2015	4,959	12.89
2016	6,856	17.84

[a] National Institute of Public Health – National Institute of Hygiene did not provide data on incidence per 100,000 population in its reports before 2008.

2. 3. 2. 2. Epidemiological situation in the United Kingdom

The United Kingdom, just like Poland, witnessed an unprecedented rise in the number of diagnosed pertussis cases, particularly in 2012 when 9,367 cases were reported in England alone. [46] Since then the number of confirmed pertussis cases has more than tripled compared to years before this extraordinary outbreak.

Before the epidemic of 2012 the number of evidenced cases fluctuated between 192 (year 2003) and 1051 (year 2013), with a mean of 520 cases per year.

Following this remarkable outbreak, the numbers oscillated in the range of 3,387 – 5,945 for years 2013 – 2016 (mean number of cases per year: 4,536). The number of cases reported in 2013 decreased by half to 4,621 in comparison to 2012. However, that is still 440% the number of cases before the outbreak (1,051 cases in 2011) and 24 times higher than in 2003 when only 192 cases of pertussis were verified.

Since 2014 the number of reported cases is soaring and there seems to be no halt to this continuous trend. In 2016 more cases of whooping cough were evidenced than the total (!) number of observed cases in years 2002 – 2011 (5,199 cases for years 2002 – 2011 as compared to 5,945 in 2016 [47]).

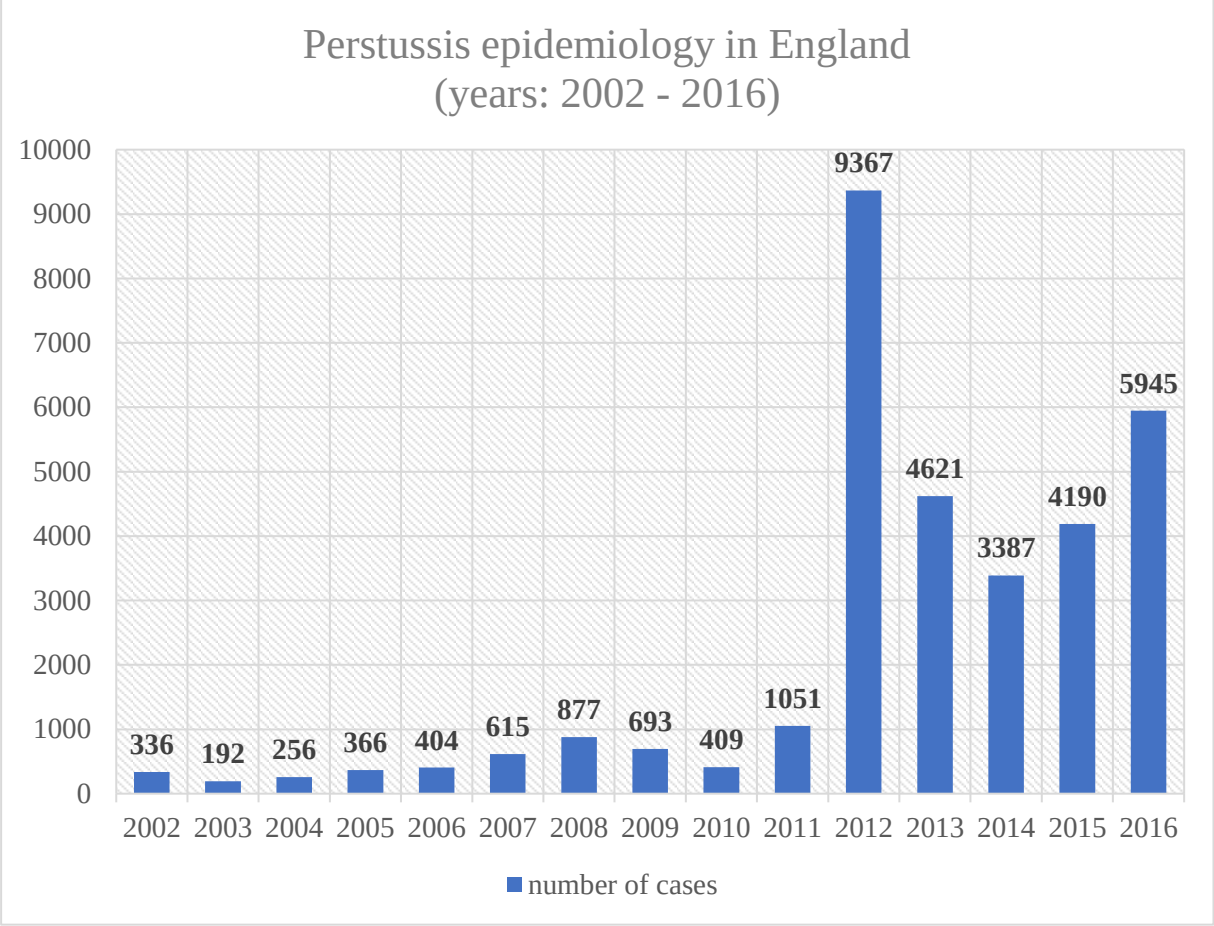

Figure 2. Epidemiology of pertussis in England part of the United Kingdom (years: 2002 – 2016); based on reports published by Public Health England. [46,47]

2. 3. 2. 3. Epidemiological situation in the United States

What was said about the epidemiological situation in the UK mostly holds true for the USA as well. However, unlike in the UK where an unexpected epidemic took place, in the USA there is a slow and steady growth of pertussis incidence with only occasional larger outbreaks every few years [48,49]. These recurrent epidemics were observed on regular basis (2004, 2005 and the most recent one in 2012). [50] However, before 2004 these outbreaks were not as pronounced as they are nowadays. It could be argued that until 2004 these epidemics were of negligible scale, that is the number of cases did not increase considerably compared to "non-epidemic" years.

Presently, the outbreaks are of significantly bigger scale. The epidemic of 2012, [51] which was the greatest whooping cough epidemic in the USA since 1955, understandably strongly correlates with an epidemic which took place in Poland and the UK at the very same year. Because epidemics of whooping cough occur in a cyclical manner, it can be reasoned that another one is just about to be witnessed in the USA.

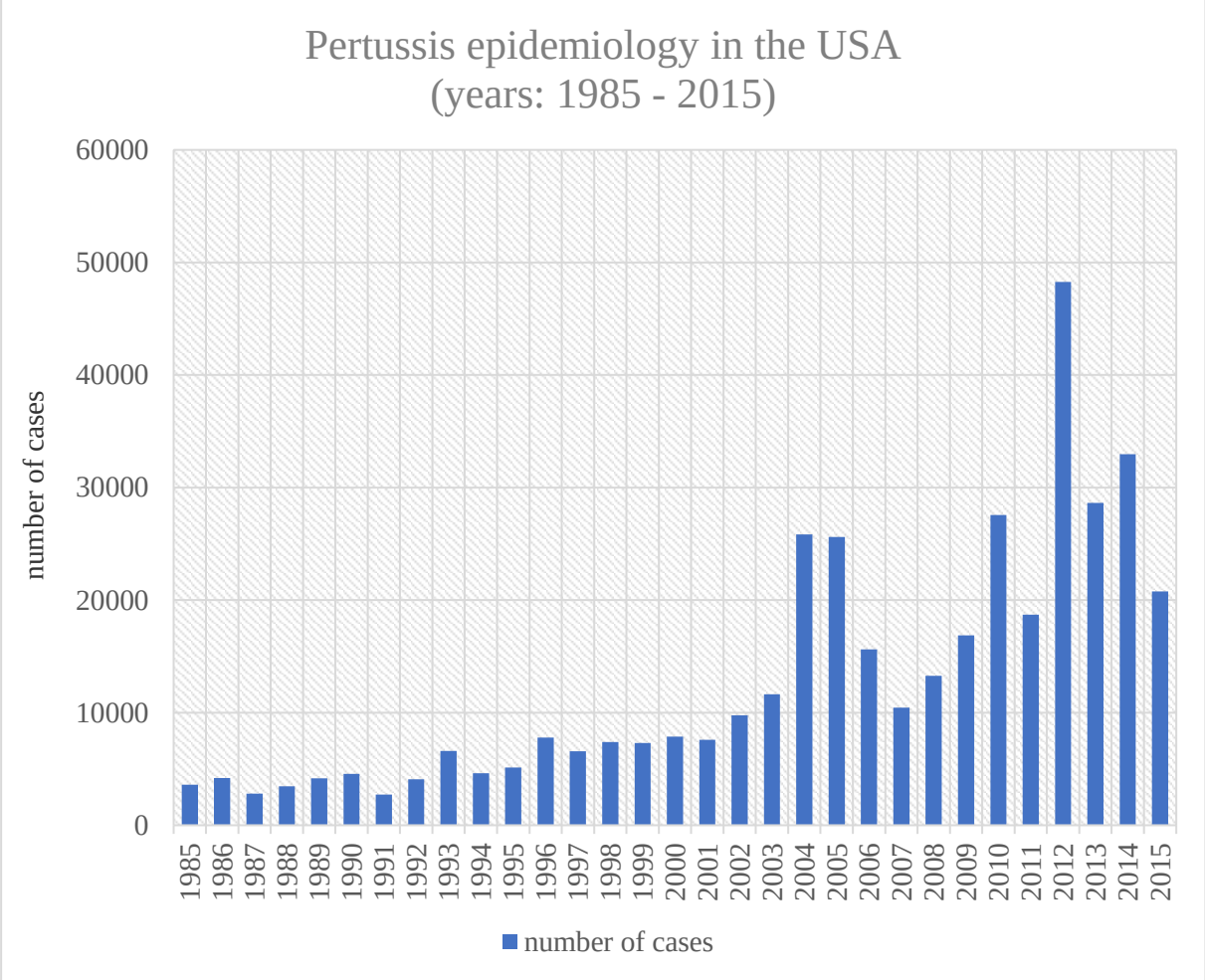

Figure 3. Epidemiology of pertussis in the USA (years: 1985 – 2015); based on reports published by CDC (Centers for Disease Control and Prevention). [49-51]

Table 2. Number of pertussis cases in the USA (data for years: 1985 – 2015); based on reports published by CDC (Centers for Disease Control and Prevention). [49-51]

year	number of pertussis cases	year	number of pertussis cases
1985	3,589	2001	7,580
1986	4,195	2002	9,771
1987	2,823	2003	11,647
1988	3,450	2004	25,827
1989	4,157	2005	25,616
1990	4,570	2006	15,632
1991	2,719	2007	10,454
1992	4,083	2008	13,278
1993	6,586	2009	16,858
1994	4,617	2010	27,550
1995	5,137	2011	18,719
1996	7,796	2012	48,277
1997	6,564	2013	28,639
1998	7,405	2014	32,971
1999	7,298	2015	20,762
2000	7,867		

2. 3. 3. The reasons behind the recent outbreaks in Poland

The reasons behind the alarming resurgence of pertussis are numerous, but two elements are presumed to play a key role: the waning immunity of adolescents who were vaccinated many years ago; and the parents' reluctance to vaccinate their offspring.

The immunity provided by the vaccination does not last entire life; it begins to gradually dwindle by the 3 – 6 year after immunization took place. It is only natural that adults who were vaccinated long time ago develop the disease. Thus, a booster shot is strongly advised in adult people at least every 10 years or so. Periodical booster shots effectively eliminate the threat of infection, though booster shots prove to be unpopular among adults.

In Poland anti-vaccination movements are on the rise. Most prominent of them is known under the name of STOP NOP (STOP Niepożądane Odczyny Poszczepienne), [52] which translates roughly to "STOP Adverse Vaccine Events". Among the movement's numerous demands (such as HBV vaccine discontinuation) one demand directly involves pertussis vaccine. STOP NOP strongly presses for imminent and complete withdrawal

of whole-cell pertussis vaccine, [53] although it is this very type of vaccine which gives the strongest protection against whooping cough; whilst acellular vaccine is safer and its use is associated with fewer complications than whole-cell vaccine, it is nonetheless less efficient in inducing adequate immune response. [16-17]

Regardless of how noble the STOP NOP intentions may be, the replacement of whole-cell vaccine with the acellular one in Poland is simply infeasible from a purely financial standpoint. The insistence of STOP NOP to terminate the use of whole-cell vaccine is all the more disturbing in view of the reports regarding the increase in number of diagnosed pertussis cases. [41] Anti-vaccination movements are seemingly totally oblivious to the fact that the re-emergence of whooping cough stems from the ever lower rates of vaccination among infants. Undoubtedly, acellular vaccine is safer and its use is associated with fewer complications. Even so, the message of the purported vaccination dangers got widely publicized and it will require time-consuming awareness campaigns to denounce all the myths and to reverse all damages.

In the 1970s., there was a suspicion – although never proven – that the whole-cell pertussis vaccine may elicit encephalopathy. [18] This controversy also may have contributed to the decrease in vaccination rates.

Parents who are half-informed and do not further investigate the subject simply decide against vaccinating their children in fear of the potential ramifications, such as the infamous vaccination-induced autism. [19] Although the paper which attributed autism to Measles Mumps Rubella vaccine (MMR vaccine) use was later withdrawn from "*The Lancet*" webpages [54] as it was deemed a pseudoscientific fraud, the belief of the harmful nature of vaccines still lingers on.

Previously, it was widely thought that there is no carrier state of *B. pertussis*. Indeed, there is no evidence of a long-term carrier state. However, recent studies utilizing PCR showed beyond any doubt that a transient carrier state in nasopharynx exists among children who were affected by whooping cough. [20] This *B. pertussis* carriage may also contribute to the spread of whooping cough.

2. 4. Signs and symptoms

The clinical course is utterly different in non-vaccinated children and previously vaccinated adolescents and adults. Clinical manifestation may differ based on many factors e.g. age of the patient, the immunization of the patient, the use of antibiotics and superinfection (a second infection superimposed on an earlier one by a different causative agent).

2. 4. 1. Clinical presentation

2. 4. 1. 1. Clinical presentation in infants and children

Joseph Lapin defined three stages of pertussis: (1) the catarrhal stage or prodromal stage, (2) the paroxysmal stage and (3) the convalescent stage.

The catarrhal stage starts right after the incubation period which usually lasts 7 – 10 days, although it may take even up to 21 days to manifest first symptoms. During the catarrhal stage children complain of typical symptoms of common cold and other upper respiratory tract infections: low-grade fever, rhinorrhea, conjunctivitis, and a mild cough. After 1 – 2 weeks catarrhal stage advances to the next stage.

The most distinctive symptom of the paroxysmal stage is – as the name implies – the paroxysmal cough. A child violently coughs up to 15 times in a row throughout the coughing fits. Since all these coughs happen during one expiration, the child may experience cyanosis due to hypoxia. Each coughing fit ends with a peculiar "whoop" sound, which gave the disease its name. This peculiar sound is attributed to a forceful inspiration through a pharynx, which has not opened yet after a coughing fit. What is bothersome for children and their parents is that the paroxysms tend to occur mainly at night; this leads to insomnia and a chronic fatigue.

Rarely do newborns have violent cough attacks ending with distinctive "whoop" sound. "Whooping" sound is typical for older children.

Sometimes a fit of cough may induce post-tussive vomiting.

The long lasting cough is one of the characteristic findings in pertussis, as it is rarely observed in other respiratory tract infections. After 1 – 6 weeks the paroxysmal stage slowly resolves into the convalescent stage. As the symptoms get milder, the disease enters its last stage – the convalescent stage. Gradually, the coughing fits occur with a decreased frequency and those fits are neither as fierce nor fatiguing for the child as they used to be in the paroxysmal stage. However, coughing fits may recur for up to one year, as evidenced by the other name of pertussis – "100-days' cough".

2. 4. 1. 2. Clinical presentation in adolescents and adults

Whooping cough tends to have an atypical course in adolescents and adults, owing to the fact they were previously vaccinated [21] or have had pertussis a few years prior. Because of that they have a partial immunity against *B. pertussis*, which in turn results in an incomplete clinical presentation. Overall, the course of the illness is gentler.

If the adult was not vaccinated at all or the vaccination took place very long time ago, the patient's immunity against *B. pertussis* is non-existent; it follows that full-blown whooping cough develops in that case.

2. 4. 2. Complications

There is a wide variety of whooping cough complications; most are mild and transient, such as: vomiting, subconjunctival hemorrhage, urinary incontinence, rib fractures or syncope. They are mostly associated with the burst of cough and disappear once the cough subsides.

The most common serious complication of whooping cough is pneumonia. [22] Pneumonia may be caused by *B. pertussis* itself or be a result of superinfection – mainly due to RSV (respiratory syncytial virus). [23]

Another, severe yet rare complication is encephalopathy. [24] It is characterized by seizures, ataxia, aphasia and paralysis, although there were cases of deafness, blindness and even decerebration.

2. 5. Diagnosis

In order to facilitate pertussis diagnosis, WHO defined a confirmed case of whooping cough as a patient suffering from a paroxysmal cough lasting for at least 21 days and one of the following criteria: (1) positive *B. pertussis* culture, (2) a notable rise in IgG or IgA

antibodies against *B. pertussis* antigens or (3) a certain contact with another patient whose disease was diagnosed by a positive culture of *B. pertussis*. [55]

There is also another definition that is less strict in its criteria – definition of a clinical case. Due to its lower requirements it is this very definition that is most important in pertussis surveillance. A clinical case requires a cough of at least 14 days and one of the clinical hallmarks of whooping cough: (1) coughing fits, (2) "whoop" sound during these paroxysms or (3) vomiting which follows cough.

2. 5. 1. *B. pertussis* culture and culture media

Before a culture can be made, appropriate preparations need to take place in order to enhance the sensitivity and specificity of the test. The specimens are collected from nasopharynx: nasopharyngeal aspirates or nasopharyngeal swabs. It is of utmost importance to acquire epithelial cells lining the nasopharynx due to *B. pertussis* predilection towards these cells. When swabbing is performed, it should be carried out with a calcium alginate swab, since cotton – which is commonly used in sample collection – inhibits *B. pertussis* growth.

For the sake of higher specificity, transport media may be enriched with cephalexin which inhibits growth of nasopharyngeal flora; however, too high of an antimicrobial concentration may suppress *B. pertussis* growth as well.

As was previously mentioned, *B. pertussis* is a highly fastidious organism. Its culture is, therefore, a very challenging undertaking. Because of that, discoverers of *B. pertussis* developed a special culture medium for the bacterium growth; it comprises blood, starch and charcoal. It is this traditional medium (Bordet-Gengou medium or BG medium for short) that is most widely used to culture *B. pertussis*. However, as time goes by new media emerge e.g. Regan-Lowe medium (RL medium) enriched with peptones and glycerol which make the medium even more specific than the aging BG medium.

Collected *B. pertussis* strains should be incubated at a temperature of 35-36 °C. Following an incubation period of 3 – 4 days bacterial colonies commence to appear on the medium and, therefore, become viable for further analysis. Colonies of *B. pertussis* are round and shiny with a silvery tint. When incubation is performed on a medium that contains blood (e.g. Bordet-Gengou medium), a hemolysis can be witnessed.

2. 5. 2. Serology

While *B. pertussis* culture is positive only in the early stages of the disease, an undeniable advantage of serology is its usefulness in the later stages, when full-blown clinical symptoms are present.

The most common method of antibodies detection is ELISA (enzyme-linked immunosorbent assay). The array of antibodies identified in laboratories is very wide – these antibodies may be directed against: the whole *B. pertussis* cell, pertussis toxin, filamentous hemagglutinin, fimbriae or against pertactin. Out of all these aforementioned antibodies anti-PT is most commonly used thanks to its high specificity, since pertussis toxin is secreted only by *B. pertussis*. Furthermore, anti-PT antibodies are the most important antibodies in providing immunity against the pathogen. Other antibodies are not as specific as anti-PT e.g. some antibodies are known to cross-react with other respiratory tract pathogens (*Mycoplasma pneumoniae* and *Haemophilus influenzae*).

Most laboratories detect the titer of IgG antibodies; IgA and IgM antibodies are identified less commonly. The increase in IgG levels occurs in the 3rd week of the disease. Since the increase in IgG may be elicited by vaccination it is virtually impossible to differentiate between whooping cough and recent immunization.

The most valuable method of serologic diagnosis is known as "paired sera", which detects the process of seroconversion. A diagnostician observes whether a significant rise in antibodies happen as time goes by. If during the second serology test the titer of antibodies is at least twice as high as before, *B. pertussis* infection can be suspected with a great likelihood. In the event that paired sera cannot be obtained, a single ELISA test must suffice. Consequently, a cutoff level of antibody titer was established. [25]

2. 5. 3. DFA (direct fluorescent antibody) assay

In DFA testing a nasopharyngeal aspirate is collected. Then, the specimen is incubated with an antibody against *B. pertussis* cell antigens. The antibodies in DFA are conjugated with fluorochrome which exhibits fluorescence, and this allows for the detection of *B. pertussis* under a microscope.

The main disadvantage of DFA testing is its low specificity; [26] it is, thus, a method of very limited value and was mostly replaced by other, more reliable methods.

2. 5. 4. PCR (polymerase chain-reaction)

Although culture is considered to be the gold standard in whooping cough diagnosis due to its remarkable specificity, its inconsistent (often inadequate) sensitivity poses somewhat of a problem. For that very reason PCR assay, which detects nucleic acids of *B. pertussis*, superseded culture in many laboratories as the mainstay of whooping cough diagnosis. [27]

Another benefit of PCR test is its speed; results come fairly quickly in comparison to culture of the pathogen. Moreover, positive PCR results may be obtained even in advanced stages of the disease, after a full course of antibiotic therapy. [28] this is a simple consequence of the inherent characteristics of the method – PCR detects both living and dead cells.

Due to its high sensitivity the issue of potential sample contamination arises as the major drawback of PCR [29] e.g. a laboratory staff may transfer DNA of a positive specimen into a specimen collected from a healthy person. This may trigger an outburst of false positive results. False positive results may also originate from a transient carrier state of *B. pertussis*.

2. 6. Treatment

2. 6. 1. Antibiotic therapy

Antimicrobial therapy is of limited benefit to the patient suffering from pertussis. Although antibiotics are effective in eradicating *B. pertussis* from nasopharynx – as evidenced by negative nasopharyngeal swabs – there is little to no benefit in clinical presentation after a course of antibiotics. It is commonly taught that antibiotics mitigate symptoms if given early in the disease – that is during the catarrhal stage; but since the diagnosis in the catarrhal stage is hard to establish, there is no practical use of this theory.

If given during the paroxysmal stage antibiotics do not improve clinical outcome, in spite of complete eradication of *B. pertussis*. [30] This stems from the fact that by the time coughing fits appear the epithelial cells of respiratory tract have already been damaged. This injury to the ciliated epithelium is irreversible, and therefore symptoms do not subside after

antibiotic administration. However, some studies [31] do suggest that even if given during the paroxysmal stage, antibiotics may somewhat alleviate the "whoop"; the effect of antimicrobial therapy is not significant, though.

The treatment of choice are antibiotics of macrolide class: erythromycin, clarithromycin and azithromycin. For many years erythromycin was the gold standard in whooping cough therapy, however, there are some major limitations to its use e.g. erythromycin is strongly contraindicated in infants due to the risk of erythromycin-induced pyloric stenosis. [32] Furthermore, gastrointestinal problems are quite a frequent finding associated with erythromycin administration. Among the most common gastrointestinal symptoms diarrhea, nausea and vomiting can be enumerated. Erythromycin is also known to have numerous interactions with common drugs.

Both clarithromycin and azithromycin have comparable efficacy to erythromycin; [30] they are associated with fewer side effects and the dosage is simpler (erythromycin is given 4 times a day for 14 days versus azithromycin once daily for 5 days). Owing to the high risk of developing pyloric stenosis, infants should be given azithromycin.

Other antimicrobials (for instance trimethoprim-sulfamethoxazole also known as co-trimoxazole) are rarely used and, consequently, are not studied well.

2. 6. 2. Symptomatic and supporting treatment

In case of severe respiratory insufficiency in infants intubation with mechanical ventilation may be required to maintain proper oxygen saturation and to fend off potential apnea and further cyanotic episodes. Children should receive tender care and preventive measures should be taken in order to eliminate the risk of coinfection. However, if a bacterial superinfection does occur, an immediate antibiotic administration is highly advised.

There have been studies which evaluated symptomatic treatment of cough in pertussis; the studied drugs included but were not limited to: short-acting β_2 agonists (salbutamol), antihistamine drugs (diphenhydramine) and glucocorticoids (dexamethasone). [33]

The benefit was dubious if not non-existent: the drugs had no effect on paroxysms frequency nor did they affect the total time children spent in hospital.

2. 7. Prevention

Undoubtedly, the most dependable method and hallmark of whooping cough prevention is vaccination. On account that pertussis may take a highly unpredictable clinical course, efforts to develop an effective pertussis vaccine began early. Initially, attempts were made to create a viable whole-cell vaccine. This kind of vaccine comprises a multitude of antigens, which induce immune response. However, there is one major problem with whole-cell vaccines. Since the vaccine consists of so many varied antigens, it is a challenging issue to create a vaccine which elicits immune response but does not cause adverse effects. Up until late 1930s. the only widely used method to assess the vaccine efficacy was to test the vaccine on human volunteers. In 1940s. Kendrick established a newer, more effective way to assess the vaccine potency [34] – he tested the vaccine on mice and then inoculated them with *B. pertussis*. After 14 days have elapsed, the mice health was evaluated.

Since 1940s. the whole-cell vaccine use was widespread and, thus, public immunization programs began. Because of this the incidence of pertussis plummeted to a level never seen

before. It is to this day that WHO endorses the use of combined vaccine – DTP (diphtheria, tetanus, pertussis) in immunization schedules around the world.

2. 7. 1. Vaccine types

2. 7. 1. 1. Whole-cell vaccine (P)

Whole-cell vaccines, as a rule, cause more local adverse reactions for instance swelling and reddening of the skin. More systemic reactions are observed as well: nausea, vomiting, relentless cry, loss of appetite and feeding problems. Rarely is observed a specific reaction to pertussis vaccine, the so called HHE (hypotonic and hyporesponsive episode) [35].

A subject of ongoing controversy is whether the whole-cell pertussis vaccine may cause neurologic damage, such as encephalopathy and coma. [18] This controversy led to National Childhood Encephalopathy Study which aimed to determine the vaccine-related risk once and for all. [56] The investigation deemed the risk as negligible. Association between whole-cell vaccine and sudden infant death syndrome was suspected but also no definite relation was found.

2. 7. 1. 2. Acellular vaccine (aP)

In response to the high number of local adverse reactions caused by the whole-cell vaccine, acellular vaccine creation was set in motion. It was thought that acellular vaccine, which was to contain fewer *B. pertussis* antigens, would not trigger so many local reactions all the while providing a comparable immunity against whooping cough. Indeed, acellular vaccine proved to be a lot safer and has replaced whole-cell vaccine in a lot of countries, the United Kingdom and the United States included. In other countries whole-cell vaccine is still in use e.g. in Poland.

The first acellular pertussis vaccine was developed in Japan by Yuji Sato and collaborators and it was used first in its home turf in 1981. [36] This very first acellular pertussis vaccine contained two hemagglutinin antigens in order to induce immune response: filamentous hemagglutinin and leukocytosis-promoting factor hemagglutinin. The removal of other antigens, chiefly lipopolysaccharide (endotoxin), resulted in lowered reactogenicity and, hence, much lower rate of adverse reactions. Since then numerous new acellular vaccines were developed. These vaccines include pertussis toxin, fimbriae and pertactin to name a few.

2. 7. 2. Contraindications for pertussis vaccination administration in Poland

Contraindications for whole-cell pertussis vaccine administration are as follows: (1) birth weight of less than 2,500 grams, (2) preterm birth, defined as the birth of a baby at fewer than 37 weeks of gestational age, (3) serious adverse events after the previous administration of whole-cell pertussis vaccine e.g. seizures, persistent crying, fever of > 40 °C, HHE (hypotonic and hyporesponsive episode), (4) age of more than 3 years, (5) progressive central nervous system disease. If contraindications for whole-cell vaccine occur, the acellular pertussis vaccine is administered (DTaP). [57]

Contraindications for acellular pertussis vaccine administration are the same as contraindications for vaccination in general e.g. anaphylaxis or encephalopathy experienced by the child after the previous vaccine administration. [37]

2. 7. 3. Vaccination schedules in selected countries

2. 7. 3. 1. Vaccination schedule in Poland

In Poland three types of pertussis vaccine are given, all of which are combined vaccines: (1) DTP (**D**iphteria, **T**etanus, whole-cell **P**ertussis), (2) DTaP (**D**iphteria, **T**etanus, **a**cellular **P**ertussis) and dTap (**d**iphteria, **T**etanus, **a**cellular **p**ertussis)[bc]. Four doses of DTP are given at the age of: 2 months, 4 months, 5 – 6 months and a complementary shot at the age of 16 months. At the age of 6 years a booster shot of DTaP is given, which is followed by a dTap vaccination at the age of 14 years. [57]

If there are contraindications for the whole-cell pertussis vaccine (DTP), then the child is given the acellular vaccine (DTaP). In the event that the child has contraindications for both whole-cell and acellular vaccine, the child is administered a vaccine without the pertussis component (DT). This vaccine contains only diphtheria and tetanus component.

2. 7. 3. 2. Vaccination schedule in the United Kingdom

Children in the UK are routinely immunized with four doses of vaccine. [58] A combined vaccine DTaP/IPV/Hib (**D**iphtheria, **T**etanus, **a**cellular **P**ertussis, **I**nactivated **P**olio **V**accine, and ***H****aemophilus* ***i****nfluenzae* type **b**)[b], also known as "5-in-one injection", [59] is given at the age of 2 months, 3 months and 4 months. Then, at the age of 3 years and 4 months a preschool booster shot of 4-in-1 vaccine (DTaP/IPV) is administered. [60]

There are no mandatory vaccinations for adults, although immunization in adults is highly recommended. Pregnant women are advised to receive a single dose of DTaP vaccine free of charge; this immunization takes place from the 20th week of gestation onwards in order to ensure adequate protection of the newborn baby against pertussis. [38,61]

2. 7. 3. 3. Vaccination schedule in the United States

In the USA children are given five doses of DTaP vaccine at the age of: 2 months, 4 months, 6 months, 15 – 18 months, and 4 – 6 years. Then, at the age of 11 through 12 years are administered a single dose of Tdap[c] vaccine. [62]

Pregnant women are given a single booster shot of Tdap vaccine during each pregnancy (ideally in the range of 27 through 36 weeks of gestation), regardless of time since last Td or Tdap immunization.

2. 7. 4. Chemoprophylaxis

All household contacts of a confirmed pertussis case should be offered a chemoprophylaxis regimen [63] consisting of antibiotic therapy, that is: erythromycin, clarithromycin or azithromycin. [39] It is of paramount importance to use chemoprophylaxis if the contact has no prior vaccination history (e.g. infants) or the immunization status is questionable. Another strong indication for the chemoprophylaxis is pregnancy, considering how harmful for the unborn child all infections may turn out. For the chemoprophylaxis to have a positive impact on the infection risk the antibiotics ought to be introduced before 21

[b] Bold letters denote letters used in abbreviated name of the vaccine.

[c] Lowercase letters "d" and "ap" denote a reduced dose of diphtheria and acellular pertussis components within the vaccine, respectively.

days have passed since the person had contact with pertussis; otherwise, a full-blown disease is likely to develop.

2. 7. 5. Other methods of prevention

As with most other infectious diseases, pertussis transmission can be effectively thwarted with the traditional measures taken to break the transmission cycle. It is essential to isolate the diseased person. People, who were exposed to a confirmed pertussis case, should be carefully observed whether they develop first symptoms of whooping cough; if so, antibiotic therapy is strongly encouraged.

3. CONCLUSIONS

Under no circumstances may we let ourselves underestimate the risks associated with whooping cough. After all it is a disease that is fatiguing for the organism; it is also characterized by an unpredictable clinical course and is quite tricky to properly diagnose. Infants are faced with the highest risk of developing life-threatening complications and in the case of inappropriate supportive care pertussis may result in the child's death.

Since antimicrobials are of limited use in full-blown pertussis vaccination is paramount. Waning immunity in adults who were vaccinated many years before contributes to the resurgence of the disease. Moreover, parents' reluctance to immunize their offspring plays a key role as well.

It is of utmost importance to educate people that the supposed severe adverse effects of vaccination are very rare and the risk of adverse effects is not well proven if not speculative at all. On the other hand the risk of developing pertussis and complications associated with the disease is very tangible.

Adults whose immunity faded ought to be informed about their vulnerability to whooping cough. If adults acquire the knowledge that immunity provided by the vaccine is only temporary, they may opt to get immunized periodically with non-mandatory paid pertussis vaccines for adults. Thereby, they will put a halt to the spread of the disease to non-immunized people.

Anti-vaccination movements should be reasoned with. Ignoring them is no solution. The only way out is to patiently explain that the adverse effects of vaccination are more of an exception than the rule, that vaccines are generally secure, and that the risks associated with refusal to vaccinate children are far greater.

All in all, educational campaigns and vaccine availability are the principal factors to tackle the issue of whooping cough resurgence. A war against the rise in pertussis incidence needs to be waged. However, given that a collective effort is put in place we may be rewarded with a success in countering whooping cough epidemic.

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