The pertussis paradox: why are cases increasing despite vaccination efforts?

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ABSTRACT

Introduction
Pertussis, a highly transmissible respiratory infection mainly caused by *Bordetella pertussis*, remains one of the most widespread public health concerns despite the establishment of global vaccination initiatives. The disease manifests as paroxysmal coughing followed by a characteristic high-pitched “whooping” sound, and post-tussive vomiting. Severe complications can include pneumonia, encephalopathy, and even mortality, particularly in infants.

Aim of the study
This study aims to provide a comprehensive analysis of pertussis, encompassing its clinical presentation, complications, diagnostic methods, epidemiological aspects, and vaccination strategies, with a special focus on the development and potential impact of a novel nasal pertussis vaccine.

Brief description of the state of knowledge
Diagnosis of pertussis often relies on recognizing typical symptoms supported by laboratory confirmation through culture methods, polymerase chain reaction testing, or enzyme–linked immunosorbent assays. This disease leads to considerable morbidity and mortality worldwide, accompanied by profound medical, social and economic challenges.

Summary
To address the limitations of existing vaccines, a novel nasal pertussis vaccine has been proposed. This vaccine aims to elicit robust mucosal immune responses, thereby providing enhanced protection against infection and preventing the asymptomatic transmission of *Bordetella pertussis*. The study offers a detailed examination of pertussis and comprehensive statistics to support the analysis, providing valuable insights into the epidemiology, highlighting the need for improved vaccination strategies to mitigate the disease's impact.

Key words: pertussis; whooping cough; paroxysms; infection
INTRODUCTION
Pertussis, also known as whooping cough, is a highly contagious respiratory infection primarily caused by the gram-negative bacterium *Bordetella pertussis*. However, other species, such as *Bordetella parapertussis* and *Bordetella bronchiseptica*, have also been identified as causative agents. [1, 2] Pertussis presents a global public health challenge, contributing significantly to morbidity and mortality rates worldwide. Although it is commonly regarded as a childhood disease, the age distribution of clinically notable pertussis cases has been evolving in countries with high primary vaccination rates. [3] The bacterium is transmitted via airborne droplets and is characterized by paroxysmal coughing episodes of significant severity. [3, 4] The clinical syndrome itself is distinctive enough to be identified in the majority of cases. [5] However, an atypical presentation during the early, highly contagious stages of pertussis can lead to misdiagnosis. If adolescents and adults who frequently exhibit mild symptoms remain untreated, they may unwittingly transmit the disease to vulnerable infants. [4] Recent data show an increasing number of reported cases among older children and adults, particularly in areas where acellular pertussis (aP) vaccines have replaced whole-cell pertussis (wP) vaccines for primary immunization. [3]

Pertussis emerged as a prominent source of childhood illness and death in the early decades of the 20th century, but with global childhood pertussis vaccination programs, incidences have markedly decreased. [1] However, despite this significant decrease in the incidence of pertussis, there has been a recent global resurgence in its occurrence. This resurgence is attributed to factors such as waning immunity, the transition from whole-cell to acellular vaccines, enhanced active surveillance, improved diagnostic methodologies, and heightened awareness. [6]

This paper reviews the current literature on pertussis, encompassing its clinical manifestations, associated complications, epidemiological aspects, different methods of diagnosis, vaccination, and current research on new vaccine. Additionally, the review explores various factors contributing to the observed trend of pertussis resurgence while presenting up-to-date statistical data on its prevalence.

THE CLINICAL PRESENTATION
Pertussis is an illness that can manifest in individuals of any age, although it is commonly viewed as a childhood ailment. [6] Various factors influencing the clinical presentation of
*Bordetella pertussis* include patient age, prior immunization or infection, presence of passively acquired antibodies, and antibiotic therapy. Moreover, other factors, such as the initial bacterial load, host genetic and acquired characteristics, and the genotype of the organism, may also contribute to the clinical manifestations. [7]

The incubation period of pertussis extends from 7 to 10 days, surpassing the typical 1- to 3-day duration seen in most viral upper respiratory tract infections. [2] The typical duration of illness is 6 to 12 weeks, although in some cases, it may extend beyond this timeframe. [1] Subsequently, patients may exhibit a spectrum of presentations ranging from asymptomatic to mild respiratory tract infections, culminating in persistent, severe coughing episodes. [2]

Pertussis symptoms are categorized into three stages: catarrhal, paroxysmal, and convalescent. [4] The symptoms of each stage are presented in the table [Tab. 1].

<table>
<thead>
<tr>
<th>Stage</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catarrhal</td>
<td>Low fever, rhinorrhea, malaise, dry, nonproductive cough, lacrimation with conjunctival injection</td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>Paroxysmal coughing (the paroxysms are repetitive burts of coughing during a single exhalation, followed by an inspiratory effort resulting in the characteristic high-pitched “whooping” sound when the patient inhales forcefully following a severe coughing attack), post-tussive vomiting, cyanosis, and emesis, salivation, bulging eyes, leukocytosis, lymphocytosis, weight loss In infants &lt; 6months old – bradycardia, apnea, poor feeding, absence of wheezing</td>
</tr>
<tr>
<td>Convalescent</td>
<td>Gradual reduction in both the frequency and severity of cough attacks, white blood cells count normalizes</td>
</tr>
</tbody>
</table>

Tab. 1 Stages of pertussis infection. [1, 2, 4, 7, 8, 9]
After the incubation period, pertussis begins with the catarrhal phase, which lasts from 1 to 2 weeks and has an insidious onset and progressively worsening symptoms. It is highly contagious and characterized by nonspecific cold-like symptoms that can be misdiagnosed as they resemble the viral infection of upper respiratory tract. The paroxysmal stage typically peaks 2 weeks after the onset of the illness and lasts 1 to 6 weeks. [2, 4] Between paroxysms attacks, the patient appears relatively healthy, without any respiratory distress. [1] The convalescent stage lasts approximately 2 to 12 weeks, during which there is a gradual alleviation of paroxysms, although coughing might persist for several months. [4] This illness leads to significant morbidity among adults and older children, although fatal outcomes are infrequent. Infants under four months old who have not yet received complete vaccination face the highest risk of mortality. Therefore, promptly identifying and treating pertussis in adults and adolescents could be helpful in minimizing transmission to vulnerable infants. [10] It is noteworthy that children who have experienced classical pertussis frequently exhibit recurrent bouts of characteristic coughing paroxysms when they contract respiratory viral infections. [7]

In countries adhering to vaccination protocols, manifestations of pertussis in adolescents and adults often deviate from the classical presentation observed in unvaccinated children. Symptoms frequently resemble those of upper respiratory tract infections and bronchitis. [11] Subtle clinical manifestations frequently occur in partially immunized children, adolescents, and adults with fading immunity. Such individuals may only exhibit a prolonged dry cough lasting more than 21 days, often leading to diagnostic challenges as it resembles atypical pneumonia, bronchitis, sinusitis, or allergic conditions. [8] Pertussis may also manifest asymptomatically, which can lead to misdiagnosis during the initial, highly contagious phases. [4]

Adolescents and adults with pre-existing conditions, such as asthma, chronic obstructive pulmonary disease (COPD), or obesity, may face a heightened susceptibility to pertussis infection. Immunodeficiency and smoking have likewise been linked to exacerbated pertussis symptoms and an elevated rate of hospitalization due to pertussis-related complications. [12] The reported cases of pertussis represent merely the surface of the issue. Officially reported pertussis cases are 40 to 160 times lower than the actual rates of illness. Asymptomatic infections occur at a rate 4 to 22 times higher than symptomatic infections. [9]

**COMPLICATIONS**
In adolescents and adults, persistent cough linked with pertussis may endure for up to six weeks. Throughout this period, patients may endure weight loss, urinary incontinence, cough-induced syncope, pneumothorax, subdural hematoma and rib fractures due to elevated intrathoracic pressure during severe coughing episodes. Other systemic problems resulting from prolonged coughing encompass sleep disturbances, subconjunctival hemorrhage, abdominal hernias, back pain, or even intracranial hemorrhage and stroke resulting from vertebral artery dissection. [1, 2, 11]

Furthermore, among the common complications of classic pertussis are pneumonia, otitis media, sinusitis, seizures, and encephalopathy. [7, 11, 13] The disease can also result in subarachnoid and intraventricular hemorrhage, spinal epidural hematoma, ulcer or laceration of the frenulum of the tongue, epistaxis, melena, rupture of the diaphragm, umbilical and inguinal hernia, rectal prolapse, apnea, severe alkalosis with associated tetanic seizures, and dehydration. [7] Furthermore, cases of herniated intervertebral discs, hearing loss, angina attacks, and carotid artery dissection have also been reported. [1]

Respiratory complications encompass atelectasis, pneumothorax, apnea, respiratory arrest, cyanosis, *B. pertussis* pneumonia, and viral or bacterial superinfections. Among the less common neurological complications are seizures, blindness, and deafness. [8, 14] Cardiac arrhythmias and episodes of intractable hypoglycemia have also been reported [15].

In infants, complications arise from the respiratory and systemic impact of pertussis toxin, compounded by hypoxemia resulting from paroxysmal coughing. [2] Therefore, they are highly susceptible to severe complications, such as seizures due to brain ischemia, encephalopathy, secondary infections like bacterial pneumonia and otitis media, as well as episodes of apnea and pulmonary hypertension. Furthermore, pertussis stands as a contributing factor to sudden infant death syndrome. Death is mostly associated with hypotension and organ failure. [9, 11] Elevated levels of leukocytosis and lymphocytosis could serve as predictors of fatal outcomes among pediatric hospital admissions. [15] In the most lethal instances of pertussis, predominantly in pediatric patients, the illness is complicated by pneumonia. [9, 11]

**DIAGNOSIS**

Diagnosis can be made when the typical clinical symptoms of the patient occur, which are characterized by a persistent cough lasting over 14 days, accompanied by episodes of paroxysmal coughing, inspiratory "whoop", or post-tussive vomiting without other apparent
cause, and lack of significant fever. Moreover, laboratory diagnosis has to be performed to confirm the disease. [1, 11, 16]

Common laboratory diagnostic methods include bacterial culture, polymerase chain reaction (PCR) and serologic diagnosis - ELISA. [7] Culture and PCR are employed to identify *B. pertussis* in respiratory secretions, which are collected through nasopharyngeal washes or swabs. [11]

Growing *B. pertussis* in culture can be challenging. Direct agar inoculation or careful transport in specialized media is typically required before inoculation. [4] A positive culture of nasopharyngeal secretions is cultivated on selective Regan-Lowe medium and is regarded as the primary diagnostic standard for pertussis. The bacterium can be isolated from patients only during the initial 3 to 4 weeks of the illness. The sensitivity of the culture is constrained by the fastidious characteristics of *B. pertussis*. For instance, the yield is diminished in patients with prolonged illness durations, in children who have received prior immunization, or when specimens are collected following antibiotic treatment. [7, 11]

PCR offers a notable advantage due to its results provided in one to two days and significantly higher sensitivity compared to conventional culture methods. However, PCR sensitivity declines with prolonged symptom duration because it relies on microorganism detection. Currently, the primers that are most commonly utilized for pertussis diagnosis include IS481 and IS1001. False-positive outcomes represent a potential challenge linked with PCR in the diagnosis of pertussis and various other respiratory conditions. [1, 2, 7, 15]

Another method to detect the presence of *B. pertussis* is the ELISA test, which is more beneficial than methods such as culture or PCR, particularly in cases with a duration of illness exceeding 3 weeks or with prior antibiotic treatment. [1] Serological testing relies on detecting notable changes in IgG or IgA antibody titers against the most relevant virulence factors of *B. pertussis* between the acute and convalescent stages of the illness. The primary antigens targeted in such assessments typically include pertussis toxin (PT), filamentous hemagglutinin, and pertactin. However, the only consistent immune responses observed in both vaccinated and unvaccinated patients are those directed against pertussis toxin. Therefore, the greatest sensitivity and specificity for serological diagnosis of *B. pertussis* infection is attained through ELISA, along with the measurement of IgG and IgA antibodies to PT. Following natural infection with *B. pertussis*, the serum concentrations of IgA, IgG, and IgM antibodies that target specific antigens increase. [7, 15] Serological analysis is not utilized for diagnosing pertussis in infants younger than six months due to potential interference from maternal antibodies. [1]
VACCINATION AGAINST PERTUSSIS

Prior to the implementation of pertussis vaccinations in the 1940s, the average annual incidence of reported pertussis cases in the United States was 157 per 100,000 individuals, with cyclic peaks recurring every two to five years. However, underreporting may suggest that the true rates were considerably higher. In the 1980s, the number of reported cases reached its nadir, decreasing to more than 1000 reported cases. [4, 17]

Currently, combination vaccines containing tetanus toxoid, diphtheria toxoid, and acellular pertussis (Tdap) with reduced antigen content are readily accessible for vaccination across various age groups, including children, adolescents, and adults. Moreover, in several countries, booster vaccination programs are advised to be implemented every ten years. [18]

Various vaccine strategies are currently being implemented across nations. Typically, the primary immunization regimen involves three successive doses administered during the initial year of life, followed by a fourth dose in the second year and a fifth dose during preschool age. [19] While these various immunization schedules are implemented worldwide, the World Health Organization recommends initiating pertussis vaccination at 6 weeks of age and completing the three-dose regimen within 6 months. [13] Furthermore, pregnant women are advised to receive a singular dose of Tdap, which is ideally administered early in the third trimester of each pregnancy, to provide protection for both the mother and the infant. [20]

Halting the onward spread of the pathogen could be accomplished through vaccines that either prevent or mitigate disease manifestations, such as coughing, which facilitate pathogen transmission. With regard to acellular pertussis (aP) vaccines, any decrease in transmission among recently vaccinated individuals may be offset by heightened transmission rates due to the quicker waning of immunity compared to immunity conferred by natural infection or whole-cell pertussis (wP) vaccines. [21]

The acellular pertussis vaccine, which is less reactogenic and has been utilized across numerous jurisdictions since the 1990s, exhibits lower efficacy compared to the previously employed whole-cell vaccine, with immunity diminishing over time. [21] While wP vaccines are not utilized in the majority of developed nations, they persist in use across many low- and middle-income countries. [22]

The global rise in vaccination coverage against pertussis has significantly diminished the morbidity and mortality linked to this illness. [15] The coverage of a third dose of the vaccine providing protection against diphtheria, tetanus, and pertussis increased worldwide from 81% in 2021 to 84% in 2022. [23] Vaccination coverage for pertussis in the United States for 2020-
2021 is presented below. Figure 1 [Fig. 1] displays percent of children vaccinated by age 24 months, with birth years 2010-2016, while figure 2 [Fig. 2] presents coverage among adolescents aged 13-17 years.

**Fig. 1.** Vaccination coverage for pertussis in the United States by age 24 months with birth years 2010–2016. [24]

**Fig. 2.** Vaccination coverage for pertussis in the United States among adolescents aged 13-17 years. [24]
Stopping the spread of the infection requires achieving a high level of immunization coverage within the population, ideally exceeding 92% (>92%). Moreover, it is believed that immune protection diminishes after 4 to 12 years. [16] In addition to the fact that natural immunity resulting from past infection or vaccination does not confer lifelong protection, the absence or inconsistency of recommendations for booster vaccinations in adults, leading to low vaccine coverage in certain populations or regions, may also significantly contribute to the recent resurgence of pertussis. Therefore, administering pertussis booster vaccinations throughout life is crucial for preventing pertussis infection and its transmission among those at risk. [12, 18] Currently, research on a new vaccination method against *Bordetella pertussis* is underway. This investigation focuses on a nasal vaccine for pertussis that has the potential to revolutionize the treatment of the disease. The most favorable candidates for intranasal acellular pertussis vaccines are those that stimulate both local humoral and cellular immune responses, effectively preventing *Bordetella pertussis* infection of the upper and lower respiratory tracts, including the nasal cavity. [25] Conducted studies in humans, baboons, and mice have demonstrated that prior infection triggers opsonizing and *Bordetella pertussis*-specific IgG as well as systemic Th1 and Th17 responses. Conversely, parenterally delivered aP vaccines primarily elicit Th2 responses and IgG capable of transuding into the lungs and nasal mucosa, where they counteract bacterial toxins, thus averting disease. Nevertheless, aP vaccines do not elicit immunoglobulin A (IgA) or tissue-resident memory T cells (T_{RM}) in the respiratory tract, failing to prevent nasal *B. pertussis* infection. Furthermore, preclinical mouse studies have revealed that nasal administration of live-attenuated pertussis vaccines, pertussis OMV vaccines, or aP vaccines supplemented with mucosal adjuvants triggers the production of IgA and T_{RM} cells in the lungs and nasal passages. CD4 T_{RM} cells in nasal tissue secrete IL-17, fostering antimicrobial peptide (AMP) production and recruiting neutrophils to eliminate *B. pertussis*. Conversely, lung CD4 T_{RM} cells secrete IFN-γ, activating macrophages that, along with opsonizing antibodies, phagocytose and kill *B. pertussis*. [25, 26] Additionally, a recent study showed that while intranasal, oral and intramuscular immunization of rats induced systemic IgG, only intranasal vaccination induced mucosal IgA. [27]
In summary, there is increasing agreement on the necessity of replacing current vaccines and developing ones that stimulate mucosal immune responses in the respiratory tract, providing protective immunity against infections in both the nasal cavity and the lungs.

STATISTICS AND EPIDEMIOLOGY
Although the incidence of pertussis has decreased dramatically since the introduction and widespread use of the pertussis vaccine, recent epidemiological data have revealed a resurgence in the pertussis incidence, especially among adolescents and adults. It is believed that every three to five years, larger outbreaks are expected, despite achieving high vaccination coverage rates.

The number of reported pertussis cases in EU/EEA countries increased in 2023 and 2024, following a period of notably low pertussis activity during the COVID-19 pandemic, spanning from mid-2020 to the end of 2022. Specifically, there were 25,130 reported cases between January 1, 2023, and December 31, 2023, followed by an additional 32,037 cases reported between January 1 and March 31, 2024. Similar trends were observed in 2018 (35,627) and 2019 (34,468). [28, 29] Reported cases of pertussis from 2016 to 2024 are presented in the figure 3 [Fig. 3], and the notification rate per 100,000 population (n/100000) associated with these cases is shown in another figure [Fig. 4].

![Reported Pertussis Cases (EU/EEA)](image)

**Fig. 3.** Reported cases of pertussis from 2016 to 2024 in the EU/EEA. [28, 29]
Fig. 4. The notification rate per 100,000 population (n/100000) of reported pertussis cases in the EU/EEA. [29]

According to publicly available data, as of March 20, 2024, an escalation in pertussis incidence has been documented in numerous countries globally, including Australia, Brazil, Bolivia, Canada, China, Israel, Montenegro, Serbia, the United States, and the United Kingdom. [28]

The current epidemiological updates regarding Poland are concerning as well: from January 1st to April 15th, 2024, the National Institute of Public Health – National Institute of Hygiene received reports of 1053 pertussis cases, compared to 277 cases during the same period in 2023. [30] Figure 5 [Fig. 5] presents the reported cases of pertussis in Poland since 2017. An increasing trend in the incidence of whooping cough has been observed in recent years. For comparison of incidence rates, figure 6 [Fig. 6] presents the cases of pertussis in selected European countries in 2023.
In 2023 and 2024, infants (those under one year old) accounted for the highest reported incidence in 17 EU/EEA countries, while in six countries, adolescents aged 10-19 years had the highest incidence. The majority of fatalities were recorded among infants. However, these surveillance findings should be interpreted cautiously due to known differences in member state surveillance systems, laboratory method availability, testing practices, and vaccination schedules. Additionally, case identification may differ across age groups. [28]
From 2011 to 2022, there were 103 reported fatalities from pertussis, comprising 69 (67%) infants and 25 (24%) individuals aged 60 years and older. Between January 2023 and April 2024, 19 deaths were reported: 11 (58%) infants and eight (42%) older adults (aged 60+). [28]

FACTORS CONTRIBUTING TO THE ESCALATION OF PERTUSSIS OUTBREAKS

The increased number of outbreaks of pertussis could appear due to a combination of different factors.

One of the reasons is the waning immunity of the vaccine. This phenomenon leads to a rise in low immunization rates among adults. The duration of vaccine-induced immunity typically spans 12–15 years, with the wP vaccine providing immunity for 6–8 years after the final dose (with a booster given at 16–18 months), while efficacy of the aP vaccine starts to diminish 4–6 years after the last immunization. Furthermore, the absence of cellular immunity to pertussis in vaccinated individuals who have not been naturally infected contributes to this phenomenon. [11]

Incorrect diagnosis of an atypical presentation in adults may be another reason for this resurgence. The correct diagnosis of whooping cough may not be made correctly due to the presence of mild or atypical manifestations of pertussis, as well as clinicians’ potential oversight of pertussis as a possible cause of cough, particularly in older children and adults. [4a, 34a] The infected adult serves as a reservoir of infection for unvaccinated infants and children with suboptimal immunization coverage. [10, 11]

Transmitting low antibody levels from mothers to neonates can also contribute. We depend on herd immunity and maternal antibodies to protect young infants until they are eligible for direct protection through vaccination. [11, 34]. Waning maternal immunity increases the vulnerability to infection among the youngest individuals, who have yet to receive a minimum of two vaccine doses. [11]

Another factor contributing to the increase in the incidence of pertussis outbreaks is the change in the type of vaccine – from whole-cell pertussis (wP) to acellular pertussis (aP). In nations that transitioned to the acellular pertussis vaccine during the 1990s, we currently observe plenty of children who not only posses diminished protection against pertussis but also potentially display reduced responsiveness to booster doses. This is because the initial vaccine administered to a child may influence their immune reaction to subsequent booster vaccinations. Furthermore, in contrast to natural infection and whole-cell pertussis vaccination, acellular pertussis vaccines have limited ability to stimulate cellular immunity and Th1 responses. These immune responses, along with Th17 responses, have been
demonstrated to be crucial for eliminating *Bordetella pertussis* and could represent the cornerstone for maintaining long-term protection. [21] Another reason is insufficient vaccination coverage. This occurrence can lead to the continued presence of pertussis in the population and can sporadically cause significant outbreaks. [35] Contributing factors may include barriers to access to immunization and other healthcare services, as well as the presence of individuals who are only partially vaccinated due to the lack of booster vaccination. [18] The resurgence of pertussis may also be attributed to the reduced involvement of natural immunity reinforcement across the entire population during the COVID-19 pandemic. [28] Another factor contributing to the persistence and resurgence of pertussis is pathogen adaptation. These modifications involve antigenic drift from vaccine strains and heightened pertussis toxin production. Antigenic drift is expected to impact both the recall of immune memory and antibody effectiveness, while increased pertussis toxin levels may escalate the suppression of both innate and acquired immunity. [36]

**THE SUMMARY**

Pertussis, also known as whooping cough, is experiencing a resurgence, posing a significant epidemiological challenge despite high vaccination coverage. This paper provides a comprehensive review of pertussis, investigating and highlighting various aspects of pertussis, including clinical symptoms, associated complications, diagnostic approaches, vaccinations, with emphasis on a newly proposed vaccine, epidemiological aspects and statistics, and factors contributing to pertussis resurgence.

Recent epidemiological data indicate a notable increase in the pertussis incidence, particularly among adolescents and adults. Several factors contribute to this resurgence, including waning immunity of the vaccine, incorrect diagnosis of an atypical presentation leading to misdiagnosis, the switch from whole-cell to acellular vaccines, insufficient vaccination coverage, and pathogen adaptation.

The global rise in vaccination coverage against pertussis has notably decreased the morbidity and mortality linked to the illness. However, the resurgence of pertussis every few years underscores the necessity for enhanced vaccination strategies and continuous monitoring of pertussis epidemiology. Essential interventions include developing a new nasal vaccine that induces longer-lasting immunity and better mucosal protection against nasal and pulmonary infections, increasing the frequency of booster doses for adolescents and adults to maintain
immunity, and educating healthcare providers and the public about the signs and symptoms of pertussis across different age groups to improve early diagnosis and treatment. In conclusion, the increasing number of pertussis cases in Europe signifies a growing epidemiological crisis that demands urgent attention. This paper contributes to the understanding of the current pertussis landscape by analyzing recent data and trends, highlighting the complexity and multifaceted nature of this growing public health issue. Increasing awareness about the pertussis and improving preventing strategies is critical to protect vulnerable populations and to achieve sustained control of pertussis in Europe.

DISCLOSURE

Author’s contribution:
Conceptualization: KB; methodology: MB, KM, NH, KD; check: KT, KB, MB; formal analysis: KM, NH; investigation: KD, KT; resources: NH, KB; data curation: MB, KT, NH; writing - rough preparation: KB, KD, KM; writing - review and editing: KB, MB, NH; visualization: KT, KM, KD; supervision: KM, NH, MB; project administration: KD, KT, KB
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