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Original Article

Incidence and severity of pertussis among hospitalized infants, Sarawak, Malaysia, 2015-2021

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Abstract

Introduction: A resurgence of pertussis has been reported in numerous countries. This study aimed to determine the incidence, clinical characteristics, and outcome of pertussis among infants in Sarawak, Malaysia.

Methodology: We conducted a descriptive retrospective study of infants aged < 12 months with laboratory-confirmed pertussis admitted to Bintulu Hospital in Sarawak, Malaysian Borneo, from 2015 until 2021. Pertussis was confirmed in all patients using a polymerase chain reaction of nasopharyngeal aspirates.

Results: Of 588 infants who had a nasopharyngeal aspirate, 108 (18%) had laboratory-confirmed pertussis. The average annual incidence was 482 per 100,000 infants aged < 12 months between 2015 and 2019, with a marked decline in 2020 and 2021. Eighty-two (76%) were < 3 months of age. Seventy-eight (72%) were unvaccinated for pertussis, including 75 (96%) who were too young to receive the first dose. A third of the cases had atypical presentations. Severe disease characterized by hypoxemia, pulmonary hypertension, recurrent apnea, encephalopathy, or cardiovascular dysfunction occurred in 32%. Forty-eight percent required humidified high-flow nasal cannula oxygen therapy and 22% required invasive ventilation. Twenty-four percent overall needed intensive care. One (1%) infant had a fatal outcome. Nearly all cases of severe disease or those that required invasive ventilation or intensive care had received ≤ 1 dose of pertussis vaccination.

Conclusions: A high incidence of pertussis with a high rate of severe disease was observed in Sarawak, Malaysia, predominantly among infants too young to be vaccinated. Additional vaccination strategies such as maternal vaccination or cocooning should be considered.

Key words: Pertussis; whooping cough; *Bordetella pertussis*; infants; vaccination; Malaysia.

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Introduction

Pertussis, or whooping cough, is a severe respiratory infection caused by the Gram-negative obligate human pathogen *Bordetella pertussis* [1]. Before the introduction of pertussis vaccines, this highly contagious disease was extremely widespread and mainly affected children [2]. In the United States, for example, the average yearly rate of reported pertussis was 157 per 100,000 population with adult cases accounting for < 3% [3]. In this prevaccine era, only 7-11% of all reported cases were recognized in infants; however, this was likely due to underdiagnosis of pertussis in infants, as infections in this age group were usually attributed to other respiratory pathogens. Meanwhile, pertussis deaths were recorded in children of all ages and even in adults [4].

The introduction of the whole-cell pertussis (wP) vaccine in the 1940s resulted in a rapid and massive decline in pertussis case incidence and fatalities [5,6]. However, due to concerns about its safety, including purported causation of a vaccine encephalopathy and sudden infant death syndrome, less reactogenic acellular pertussis (aP) vaccines were introduced in the late 1990s. In the three decades since, many countries have reported a significant resurgence of pertussis, despite the high uptake of pertussis vaccination among infants [1,7]. This resurgence has been attributed to the failure of aP vaccines to induce an optimal immune response, the rapid waning of aP vaccine-elicited

immunity, genotypic and phenotypic variations between vaccine reference and circulating strains of *B. pertussis*, and improved case detection through better laboratory diagnostics [2,8]. Virtually all pertussis deaths, however, now occur in infants < 4 months of age [4], and this is likely a reflection of the inherent limitations of pertussis vaccination among infants that typically begin after 2 months of life.

The use of polymerase chain reaction (PCR) for confirmation of *B. pertussis* infection has superseded other less sensitive methods such as bacterial culture and serology [9,10]. With reported sensitivities of 73-100%, PCR has enabled the detection of *B. pertussis* in patients and population groups not previously known to be burdened by the infection [11]. In adults, for example, who typically present late and are only tested after weeks of cough, confirmation of pertussis was rarely achieved with bacterial isolation, as sensitivity was only 1-3% in this situation [12]. Indeed, improved detection with PCR has now established adult infection as an important reservoir of *B. pertussis* and that parents are the most common source of infection for infants [13].

Pertussis is a mandatory notifiable disease in Malaysia. The number of reported cases has increased from a low of 3 cases in 1997 to a high of 939 cases in 2015 [14]. The largest increase was recorded from 2011 onwards, likely related to the introduction of PCR diagnosis at the national reference laboratory in 2010. Overall population incidences and mortality rates, however, have remained relatively low at 0.9-3.1 per 100,000 population and 0-0.07 per 100,000 population, respectively, despite the availability of PCR diagnostics [15]. In Malaysia, wP vaccine was introduced in 1958 and replaced by aP vaccines in 2008. Vaccine uptake has been consistently > 95%, and it is given at 2,3, and 5 months of age with a booster at 18 months.

Bintulu Hospital is a 312-bed hospital that provides primary and secondary health services to a total population of 256,000, including 72,000 children aged < 15 years, residing in Bintulu Division and Belaga district of Kapit Division in central Sarawak, Malaysian Borneo. It is the only healthcare facility within this 32,000 km^2 area with pediatric critical care services.

Limited data are available on the age-specific incidence and burden of pertussis in Malaysia. In this study, we aimed to determine the incidence of laboratory-confirmed pertussis among infants in the region. Additionally, the study aimed to provide a detailed description of clinical characteristics, severity, and outcome of these infections.

Methodology

Inclusion and exclusion criteria

We performed a descriptive retrospective study of all infants aged < 12 months with laboratory-confirmed pertussis admitted to Bintulu Hospital over a 7-year period from January 2015 to December 2021. Infants who had clinically diagnosed pertussis without laboratory confirmation were excluded.

Case definitions

A laboratory-confirmed pertussis case was defined as an infant who tested positive for *B. pertussis* by PCR in a nasopharyngeal aspirate, regardless of the clinical presentation. In Bintulu Hospital, a decision to perform a nasopharyngeal aspirate for *B. pertussis* detection is made in infants and children with any pertussis-like symptom, even in the absence of a typical presentation, at the discretion of the treating clinician. To identify cases, we conducted a manual search of the microbiology laboratory logbooks and electronic database. Medical records of identified cases were then retrieved and reviewed. Details on demography, clinical, laboratory and radiological findings, case management, and outcome were collected using a standardized case report form.

Typical pertussis presentation was defined either by presence of apnea or by cough associated with ≥ 1 of paroxysms, whoop, or post-tussive vomiting [16]. Atypical presentation was defined by the detection of *B. pertussis* in a patient who had no typical symptoms. For each pertussis vaccine dose, we defined an infant as vaccinated if he/she had received the dose of interest > 14 days before symptom onset [16]. Unvaccinated infants were those who had not received any dose or who had received the first dose ≤ 14 days before symptom onset. A potential source of infection was defined as a person with a cough who had contact with the case in the 2-3 weeks before the date of symptom onset of the case. Leukocytosis was defined by an elevated white blood cell count > 15×10^9/L in infants aged ≤ 6 months and > 12×10^9/L in those aged > 6 months. Severe pertussis was defined by the presence of hypoxemia (oxygen saturation < 90%), pulmonary hypertension, recurrent apnea, encephalopathy, or cardiovascular dysfunction. Pulmonary hypertension was diagnosed by echocardiography using measurement of tricuspid regurgitation velocity, and it was defined as mean pulmonary arterial pressure > 25 mmHg [17]. Recurrent apnea was defined by the presence of ≥ 2 episodes of apnea with cyanosis or bradycardia. Encephalopathy was diagnosed when altered sensorium or seizures were present in the

absence of other plausible diagnoses. Cardiovascular dysfunction was defined according to Goldstein organ dysfunction criteria or by signs of cardiac failure on echocardiography [18].

Microbiological methods

For *B. pertussis* detection, nasopharyngeal aspirate samples were transported at 2-8 °C to the national reference laboratory, the Institute for Medical Research Malaysia, in Kuala Lumpur, for PCR analysis. DNA extraction was performed using MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Switzerland), according to the manufacturer's instructions. The DNA was kept at -20 °C until testing. Real-time PCR amplification targeting IS481 was conducted using specific primers on LightCycler 480 platform (Roche, Switzerland) [19]. The real-time PCR results were analysed with the LightCycler software (1.5.1.62 SP3) where the melting curves were compared to those produced by a positive control (NCTC 10739 *B. pertussis*) [20].

Statistical analysis

Statistical analysis was performed using SPSS Statistics 21. Fisher's exact test was used to compare categorical variables. The Mann-Whitney *U* test or Kruskal-Wallis *H* test was used for continuous variables. The correlation between monthly incidence and average monthly rainfall and between lymphocyte count and both duration from onset to admission and age was examined using Spearman's rank co-efficient test. Population data were obtained from the Malaysian Census Data 2010. Meteorological data were obtained from the Malaysian Meteorological Department.

Ethics statement

The study was approved by the Malaysian Medical Research Ethics Committee (NMRR ID-23-01294-1PW). As all data analyzed in this retrospective study were anonymized, the need to obtain consent was formally waived by the ethics committee.

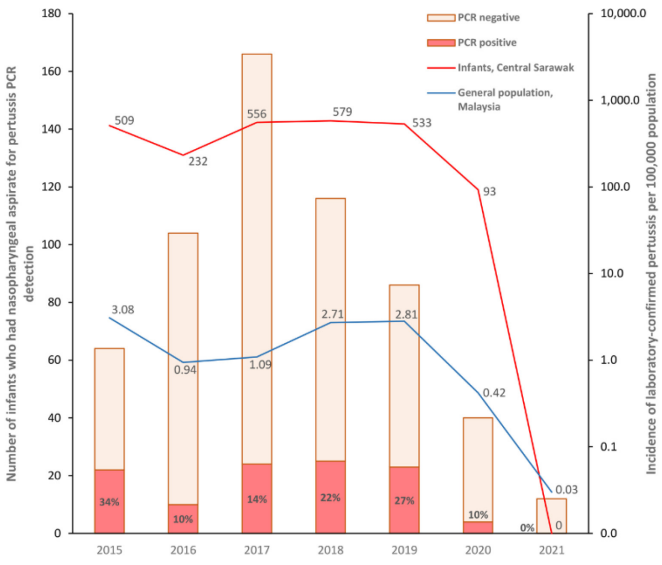
Results

Patient demographics

From January 2015 to December 2021, 588 infants aged < 12 months, including 562 (96%) aged < 6 months, were admitted to Bintulu Hospital and had a nasopharyngeal aspirate for detection of *B. pertussis* by PCR. Of these, 108 (18%) infants had laboratory-confirmed pertussis (22 in 2015, 10 in 2016, 24 in 2017, 25 in 2018, 23 in 2019 and 4 in 2020) (Figure 1). Medical records of all identified cases were available for analysis.

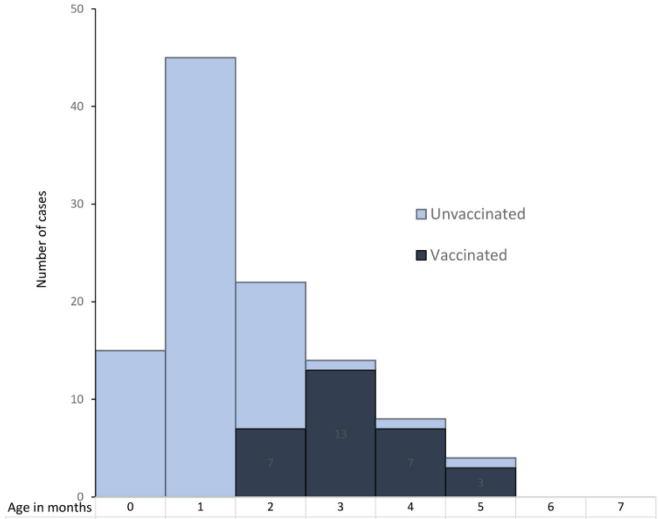
Of the 108 cases, 66 (61%) were male. All were aged < 6 months; 82 (76%) were < 3 months of age

Figure 1. Distribution of infants investigated for pertussis and laboratory-confirmed pertussis incidence, Bintulu Hospital (Sarawak, Malaysia), 2015 - 2021.



The bar chart shows the distribution of infants who had a nasopharyngeal aspirate for pertussis PCR detection and the number and percentage who had a positive PCR according to year. The line graph shows the incidence of laboratory-confirmed pertussis among infants in central Sarawak compared to the incidence of laboratory-confirmed pertussis among the general population in Malaysia.

Figure 2. Distribution of the 108 pertussis cases admitted to Bintulu Hospital (Sarawak, Malaysia) from 2015 to 2021 by age.



Age in months	0	1	2	3	4	5	6	7
Unvaccinated	15	45	15	1	1	1	0	0
Vaccinated	0	0	7	13	7	3	0	0

The histogram shows the distribution of the 108 laboratory-confirmed pertussis cases according to age in completed months. Pertussis vaccination status of cases in each age group is shown.

(Figure 2). Seventy-eight (72%) were unvaccinated for pertussis, including 75 (96%) who were too young to receive the first dose. Seventeen (16%), 12 (11%), and 1 (1%) infants had received 1, 2, and 3 doses of pertussis vaccine, respectively. Sixty-two (57%) were from urban areas, while 46 (43%), including 35 (76%) who lived in traditional longhouses, resided in rural areas.

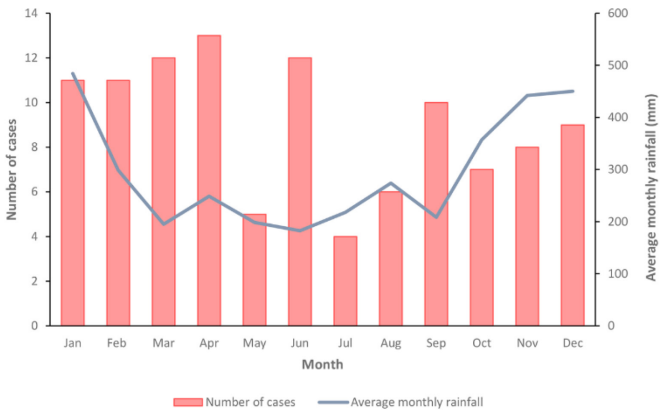
Population incidence

From 2015 to 2019, the average annual incidence of pertussis was 482 per 100,000 infants aged < 12 months, with the highest incidence recorded in 2018 (579 per 100,000 infants) (Figure 1). In 2020, the incidence of pertussis dropped dramatically to 93 per 100,000 infants, and no cases were diagnosed in 2021. Although fewer pertussis cases occurred during the dryer months of the year (Figure 3), no significant correlation was found between the monthly incidence and average monthly rainfall (r_s = -0.15; p = 0.64).

Patient risk factors

Of 98 (91%) infants with birth information recorded, 81 (83%) were delivered term (i.e., ≥ 37 weeks gestation), 12 (12%) late preterm (i.e., ≥ 34 to < 37 weeks), and 5 (5%) preterm (< 34 weeks). The median birthweight was 2.8 kg (interquartile range [IQR] 2.5-3.1 kg, range 0.8-4.1 kg) among 99 (92%) infants with data available. Only 8 (7%) infants had comorbidities (chronic lung disease [n = 3], congenital heart disease [n = 2], developmental delay with vocal cord palsy, cleft lip and palate, and failure to thrive [n = 1, each]). Sixty-six (61%) out of 108 infants had contact with a potential source of infection within the household.

Figure 3. Distribution of the 108 pertussis cases admitted to Bintulu Hospital (Sarawak, Malaysia) from 2015 to 2021 and average rainfall by month.



The bar chart shows the distribution of the 108 pertussis cases according to the month of admission. The average monthly rainfall in central Sarawak is shown in the line graph.

Clinical presentation and physical findings

Pertussis was categorized as typical in 71 (66%) cases; 37 (34%) had atypical pertussis presentations. The median duration of symptoms prior to admission was 4 days (IQR 2-7 days, range 0-15 days). Cough was the predominant presenting manifestation, recorded in 97% (105/108) of infants (Table 1). Paroxysms, post-tussive vomiting, and whoop were documented in 55 (52%), 39 (37%), and 3 (3%) of these infants with cough, respectively. Seven (6%) infants had apneic episodes, including one who had no cough. Fever was present in 40% (43/108) of cases but reported to be low grade (< 38 °C) in 32 (74%). Cyanosis was only reported in those with typical pertussis and was related to paroxysms or apnea.

Nearly all (95%, 103/108) infants were tachypneic at admission, and 94% (101/108) had chest recessions. Chest auscultation revealed crepitations or rhonchi in 44% (48/108) and 11% (12/108) of infants, respectively; the remaining 44% (48/108) had no added respiratory sounds. Overall, demographic characteristics and clinical manifestations were similar in both typical and atypical pertussis presentation groups (Table 1). Additionally, no significant differences in clinical characteristics were found between younger and older infants or between those who had or had not been vaccinated (data not shown).

Laboratory and radiological findings

Among 94 (87%) infants with blood counts performed at admission, the median white blood cell count was 13.4 × 10^9 cells/L (IQR 10.3-17.8 × 10^9 cells/L, range 4.0-66.5 × 10^9 cells/L). Thirty-seven (39%) had leukocytosis. Of 87 (81%) with differential counts, the median lymphocyte count was 6.9 × 10^9 cells/L (IQR 5.1-9.6 × 10^9 cells/L, range 1.9-39.9 × 10^9 cells/L). Twenty (23%) had blood lymphocyte counts > 10.0 × 10^9 cells/L, and 11 (13%) had blood lymphocyte counts > 15.0 × 10^9 cells/L. Using either cut-off value, lymphocytosis was significantly more common in those with typical presentations (Table 1). Additionally, lymphocyte counts correlated with duration between onset and admission (r_s = 0.23; p = 0.03) but did not correlate with the patient's age (r_s = -0.17; p = 0.11). There was no significant difference in the lymphocyte count among those with severe and non-severe disease (11.3 × 10^9 cells/L [IQR 4.6-15.8 × 10^9 cells/L] vs 8.0

$\times 10^9$ cells/L [IQR 5.2-9.5 $\times 10^9$ cells/L]; $p = 0.95$). Of 104 (96%) infants whose admission chest radiograph findings were recorded, alveolar opacities or atelectasis were reported in 43% (45/104). Thirty-one percent (32/104) had peri-hilar reticulonodular haziness and 24% (25/104) had clear lung fields. Ground glass haziness was recorded in two (2%) infants.

Case management

Ninety-four (87%) of the 108 cases were initially treated with nasal oxygen; 52 (48%) subsequently required humidified high-flow nasal cannula oxygen therapy (HHFNC) (Table 2). Over a fifth (24/108) ultimately required invasive ventilation, including 23/95 (24%) infants who had received ≤ 1 dose of pertussis vaccine and a single (1/13, 8%) infant who had received ≥ 2 doses; this difference, however, was not statistically significant ($p = 0.29$). In total, 60 (56%) and 26 (24%) infants needed high-dependency unit or intensive care unit admission, respectively. All infants received a macrolide antibiotic.

Acute complications and outcome

Hypoxemia was observed in 30 (28%) infants overall, including 27/95 (28%) infants who had received ≤ 1 dose of pertussis vaccine and 3/13 (23%) who had received ≥ 2 doses ($p > 0.99$). Pulmonary hypertension, recurrent apnea, encephalopathy, and cardiovascular dysfunction were documented in 9 (8%), 6 (6%), 3 (3%), and 2 (2%) of infants, respectively.

Table 1. Clinical, laboratory, radiological, and outcome characteristics of 108 infants with laboratory-confirmed pertussis admitted to Bintulu Hospital (Sarawak, Malaysia) from 2015 to 2021.

Characteristic	All cases (n = 108)	Typical presentation (n = 71)	Atypical presentation (n = 37)	*p*
Demography				
Male sex	66 (61)	43 (61)	23 (62)	> 0.99
Age, months, median (IQR)	1.7 (1.2-3.0)	1.8 (1.2-3.1)	1.6 (1.1-2.8)	0.22
Pertussis vaccination status				
unvaccinated	78 (72)	49 (69)	29 (78)	-
1 dose	17 (16)	11 (15)	6 (16)	-
≥ 2 doses	13 (12)	11 (15)	2 (5)	0.35
Clinical manifestations				
Time between onset and admission, days, median (IQR)	4 (2-7)	4 (2-7)	3 (2-5)	0.13
Cough	105 (97)	70 (99)	35 (95)	0.27
Paroxysms	55 (52)	55 (77)	-	-
Post-tussive vomiting	39 (37)	39 (55)	-	-
Whoop	3 (3)	3 (4)	-	-
Apnea	7 (6)	7 (10)	-	-
Nasal symptoms	69 (64)	46 (65)	23 (62)	0.84
History of fever or fever on admission	43 (40)	27 (38)	16 (43)	0.68
Poor feeding	22 (20)	14 (20)	8 (22)	0.81
Cyanosis	20 (19)	20 (28)	0 (0)	< 0.001
Tachypnea[#]	103 (95)	66 (93)	37 (100)	0.16
Crepitations	48 (44)	36 (51)	12 (32)	0.10
Rhonchi	12 (11)	8 (11)	4 (11)	> 0.99
No added respiratory sounds	48 (44)	27 (38)	21 (57)	0.07
Laboratory findings[§]				
WBC, $\times 10^9$ cells/L, median (IQR)*	13.4 (10.3-17.8)	13.6 (10.5-19.4)	12.4 (9.3-16.5)	0.09
Leukocytosis*[€]	37 (39)	26 (43)	11 (33)	0.51
Lymph, $\times 10^9$ cells/L, median (IQR)[†]	6.9 (5.1-9.6)	7.9 (5.5-11.9)	5.8 (4.5-8.1)	0.009
Lymph > 10.0×10^9 cells/L[†]	20 (23)	17 (30)	3 (10)	0.03
Lymph > 15.0×10^9 cells/L[†]	11 (13)	10 (18)	1 (3)	0.04
Chest radiograph finding[§¶]				
Alveolar opacities or atelectasis	45 (43)	29 (43)	16 (44)	> 0.99
Peri-hilar reticular-nodular haziness	32 (31)	22 (32)	10 (28)	0.66
Clear lung fields	25 (24)	16 (24)	9 (25)	> 0.99
Treatment and outcome				
HHFNC	52 (48)	33 (46)	19 (51)	0.69
Invasive ventilation	24 (22)	13 (18)	11 (30)	0.22
ICU admission	26 (24)	14 (20)	12 (32)	0.16
Severe disease	35 (32)	22 (31)	13 (35)	0.67
Died	1 (1)	1 (1)	0 (0)	> 0.99

IQR: interquartile range; WBC: white blood cell count; Lymph: lymphocyte count; HHFNC: humidified high flow nasal cannula oxygen therapy; ICU: intensive care unit. Data are No. (%) unless otherwise indicated. [#]Tachypnea was defined as a respiratory rate ≥ 60 breaths/minute in infants < 2 months and ≥ 50 breaths/minute in infants 2-12 months of age. [§]Only laboratory and radiological investigations done on the day of admission were included. *In 94 children with blood counts (typical presentation: n = 61; atypical presentation: n = 33). [€]Leukocytosis was defined by an elevated white blood cell count > 15×10^9/L in infants aged ≤ 6 months and > 12×10^9/L in those aged > 6 months. [†]In 87 children with differential counts (typical presentation: n = 57; atypical presentation: n = 30). [¶]In 104 children who had chest radiography (typical presentation: n = 68; atypical presentation: n = 36).

These complications were only noted in infants who had received ≤ 1 dose of pertussis vaccine; however, this difference was not statistically significant (16/95 [17%] vs 0/13 [0%]; p = 0.21). Additionally, no differences in treatment, severity, or outcome were observed between typical and atypical pertussis presentation groups (Table 1).

One (1%) infant had a fatal outcome. This was a previously healthy 3-month-old who had received a single dose of pertussis vaccine and was admitted with a typical presentation but had a blood lymphocyte count of only 3.7×10^9 cells/L. She required intubation and invasive ventilation at admission for respiratory failure and succumbed a day after to refractory pulmonary hypertension and right heart failure.

Long-term outcome among survivors

Among the 107 (99%) survivors, the median duration of hospitalization was 7 days (IQR 4-14 days, range 1-135 days). Thirty-two (30%) had ≥ 1 further hospitalization for a respiratory illness in the year after the admission for pertussis; 16 (15%) had recurrent respiratory admissions (2 admissions, n = 8; 3 admissions, n = 2; 4, 5, and 7 admissions, n = 1 each; 8 admissions, n = 2; 10 admissions, n = 1). In 66 (62%) with complete follow-up records, 20 (30%) had been initiated on inhaled corticosteroids within 18 months of the pertussis admission for recurrent wheezing episodes requiring either hospitalization or outpatient visits.

Discussion

To our knowledge, this study is the first detailed report of laboratory-confirmed pertussis in Sarawak, Malaysian Borneo, and it shows a large and underappreciated burden of *B. pertussis* infection among infants in the region. The study adds to the growing evidence of the global pertussis resurgence, which began to be reported in the early 2000s, and its associated mortality and morbidity in both high-income as well as low- and middle-income countries.

Over the 7-year study period, we identified 108 laboratory-confirmed pertussis cases among infants admitted to Bintulu Hospital. The average annual incidence of pertussis-associated hospitalization in this central region of Sarawak was estimated to be 482 per 100,000 infants aged < 12 months between 2015 and 2019, with a marked decrease in 2020-2021. Globally, few studies have reported age-specific incidences of pertussis: studies in Zambia, Pakistan, and South Africa reported incidences of 520, 247, and 220 per 100,000 infants aged < 12 months, respectively [21]. In the United States, pertussis is considered the most common vaccine-preventable disease, with an incidence of 75.3/100,000 infants < 12 months [7]. The high pertussis incidence reported in the present study can be attributed to the high index of suspicion and the low threshold to attain laboratory confirmation in infants admitted with respiratory infections, even in the absence of typical pertussis presentations. While the rigorous testing likely enhanced the detection rate, the notable incidence suggests widespread pertussis under detection within the wider community. The findings are consistent with global observations of a resurgence of pertussis, and this resurgence probably extends beyond Malaysian Borneo, to other regions in Malaysia. Rigorous prospective hospital- and population-based

Table 2. Treatment and outcome of 108 infants with laboratory-confirmed pertussis admitted to Bintulu Hospital (Sarawak, Malaysia) from 2015 to 2021 based on pertussis vaccination status.

Characteristic	All cases (n = 108)	Unvaccinated (n = 78)	1 dose (n = 17)	≥ 2 doses (n = 13)	*p*
Complexity/level of care required					
Duration of hospitalization, median (IQR), days	7 (4-14)	7 (5-14)	5 (3-20)	6 (3-10)	0.43
HHFNC	52 (48)	39 (50)	7 (41)	6 (46)	0.86
Duration, median (IQR), days	3 (1-5)	3 (1-5)	6 (1-7)	3 (1-5)	0.71
Invasive ventilation	24 (22)	17 (22)	6 (35)	1 (8)	0.23
Duration, median (IQR), days	5 (4-11)	5 (4-9)	8 (2-12)	-	0.98
HFOV	7 (7)	5 (6)	2 (12)	0 (0)	0.56
Duration, median (IQR), days	10 (6-20)	12 (9-20)	8 (6-10)	-	0.57
ICU admission	26 (24)	19 (24)	6 (35)	1 (8)	0.22
Duration, median (IQR), days	8 (5-14)	7 (4-15)	9 (6-14)	-	0.92
Complications/outcome					
Uncomplicated disease	73 (68)	56 (72)	7 (41)	10 (77)	0.04
Hypoxemia	30 (28)	18 (23)	9 (53)	3 (23)	0.05
Pulmonary hypertension	9 (8)	6 (8)	3 (18)	0 (0)	0.17
Recurrent apnea	6 (6)	5 (6)	1 (6)	0 (0)	> 0.99
Encephalopathy	3 (3)	1 (1)	2 (12)	0 (0)	0.10
Cardiovascular dysfunction	2 (2)	0 (0)	2 (12)	0 (0)	0.04
Died	1 (1)	0 (0)	1 (6)	0 (0)	0.28

IQR: interquartile range; HHFNC: humidified high-flow nasal cannula oxygen therapy; HFOV: high-frequency oscillatory ventilation; ICU: intensive care unit. Data are No. (%) unless otherwise indicated.

studies are needed to determine the true extent of this resurgence. Meanwhile, public health managers and clinicians should be made aware of the possible resurgence, to improve diagnostic suspicion, laboratory testing and confirmation, and notification of pertussis among infants in Malaysia.

Pertussis infections in Sarawak were associated with severe disease in a third of infants. Eighty-seven percent overall required nasal oxygen, nearly half of all patients progressed to require HHFNC, and over a fifth ultimately required invasive ventilation. Overall, 56% required high-dependency unit admission and nearly 25% ultimately required intensive care. In the literature, pertussis pneumonia and death were recorded in 20-23% and 1-1.6% of infants, respectively [1]. However, a recent multi-center prospective study in Malaysia showed that over 70% of infants with laboratory-confirmed pertussis had pneumonia and comparable proportions to our study required nasal oxygen (80%) and invasive ventilation (18%) [22]. Similar to our study, a quarter of hospitalized patients in China and Europe required intensive care [16,23]. While these studies demonstrate a selection bias by focusing exclusively on hospitalized cases, they highlight the substantial health and critical care resources that are consumed in the treatment of this vaccine-preventable disease. These data also inform healthcare workers and clinicians that close monitoring is often required and critical care expertise and facilities should be readily available when caring for infants with suspected or confirmed pertussis.

Most of the pertussis burden was borne by infants aged < 3 months who were too young to be protected by routine infant vaccination. Over 75% of cases in the study were infants aged < 3 months, and 91% of those with severe disease and 96% of those requiring invasive ventilation had received ≤ 1 dose of pertussis vaccine. This aligns with recent findings from Peru, where nearly 75% of infant pertussis cases occurred in those aged < 3 months [24], and Canada, where the hospitalization rate for infants under 4 months of age was 8.2 times higher than those aged 4-11 months [25]. These findings have led to the deployment of additional pertussis prevention strategies, including maternal vaccination, adolescent and adult booster vaccinations, and cocooning, to reduce the burden of pertussis in young infants. For example, in the Canadian study, a two-fold decline in hospitalization among infants < 4 months of age was recorded in the year following the recommendation for maternal vaccination. In the United Kingdom, the vaccine effectiveness of the maternal pertussis immunization programme against infant deaths was estimated to be 95% [26]. In addition, our study revealed that 61% of infants had known contact with a possible household source of infection. The data did not, however, differentiate between adults and children among these potential sources, underscoring an important area for future research. Although less commonly implemented or studied, adolescent and adult booster vaccinations could potentially also be beneficial.

An apparent reduction of the pertussis burden was observed in 2020-2021, likely related to the adoption of COVID-19 mitigation measures–usage of face masks, widespread lockdowns, and school closures–that inadvertently reduced the transmission of other respiratory pathogens, including *B. pertussis*. Similar observations were seen globally. In England, the annual pertussis incidence among infants declined sharply from 24.5/100,000 between July 2014 to June 2019 to 0.5/100,000 between July 2020 to June 2021 [27]. With the easing of COVID-19 restrictions, we anticipate a rebound to pre-pandemic pertussis incidence and burden, highlighting the urgent need for effective control measures.

One-third of infants with pertussis in this study had atypical presentations. The absence of apnea, paroxysms, whoop, or post-tussive vomiting suggests that a significant proportion of infants with pertussis do not manifest typical clinical features. This has been alluded to in several previous reports. For example, the use of either the WHO or the Global Pertussis Initiative clinical case definitions, which rely on the presence of typical clinical features, demonstrated a sensitivity of < 70% for laboratory-confirmed pertussis among infants < 4 months in South Africa [28]. One out of five pertussis cases in the Netherlands would have been missed if pertussis diagnostics were only performed when physicians had clinical suspicion of pertussis [29]. These observations highlight an important consideration for clinicians: reliance on the typical symptoms may lead to significant underdetection of pertussis cases.

Our study identified leukocytosis and lymphocytosis in only 39% and 23% of infants, respectively. Leukocytosis attributable to lymphocytosis is a hallmark of pertussis and this was recognized over 100 years ago [30,31]. In a large prospective multicenter surveillance study, 72% and 76% of children with pertussis had leukocyte and lymphocyte values, respectively, above the mean and 30% and 35% of patients had significant leukocytosis and lymphocytosis (values above the upper limit of the 95% confidence interval of the age-specific mean) [30].

However, our findings should be interpreted cautiously, as only white cell counts obtained on admission were analyzed. Considering the median duration from symptom onset to admission was 4 days, the inclusion of subsequent white cell counts during hospitalization could potentially reveal higher proportions of infants with lymphocytosis. Leukocytosis and lymphocytosis tend to be observed in the later stages of the disease [32]. Moreover, our data showed a significant correlation between lymphocyte count and the duration between symptom onset and admission, suggesting the potential influence of disease progression on these laboratory markers. Interestingly, however, a study conducted in South Africa reported that lymphocytosis had a sensitivity of only 31% when compared to PCR diagnosis in infants < 4 months, and adding lymphocytosis to the analysis only marginally improved the diagnostic sensitivity of clinical case definitions [28]. Despite these findings, the lymphocyte count remains a valuable investigation in young infants with respiratory infection, given the significantly higher occurrence of lymphocytosis in pertussis than in other infections [33,34]. Nevertheless, the absence of leukocytosis and lymphocytosis should not deter clinicians from considering a diagnosis of pertussis.

This study has several limitations. Firstly, the detection of *B. pertussis* in the nasopharyngeal aspirates was performed using single-target PCR for insertion sequence gene IS481 and confirmed using a melt curve analysis. Because IS481 is present in up to 250 copies per *B. pertussis* genome but is also present in fewer quantities in *Bordetella holmesii* and *Bordetella bronchiseptica*, it is especially susceptible to false-positive results due to both contamination and detection of non-pertussis *Bordetella* species. Multiple-target PCR is therefore recommended [10]. The use of multiple-target PCR, however, may reduce the sensitivity for *B. pertussis* diagnosis as alternate target genes are present in much lower copy numbers and may not be detected when IS481 cycle threshold values are high [10]. In a setting where stringent laboratory control measures were available to prevent and detect IS481 contamination and no *B. holmesii* and *B. bronchiseptica* infections were detected, it was shown that all IS481 positive specimens should be considered as *B. pertussis* infection [35]. Moreover, in a large study involving > 11,000 nasopharyngeal swabs of Dutch and Finnish patients, *B. holmesii* DNA was not found in any of the samples analysed, suggesting that it is not among the causative agents of pertussis-like symptoms [36]. *B. bronchiseptica*, meanwhile, has rarely been isolated from humans: very occasionally as a commensal or colonizer and rarely as a pathogen in the severely immune-compromised [37]. These findings, in addition to the use of the melt curve analysis, likely ensured a high specificity of the molecular diagnosis despite the use of single-target IS481 PCR. Second, none of the cases were routinely investigated for additional pathogens such as respiratory viruses or other bacterial infections (except for bacterial blood cultures, which were routinely performed). Co-infections between *B. pertussis* and common respiratory pathogens have been extensively documented, although no marked differences in disease severity between infants with *B. pertussis* infection alone and those with coinfections were recorded [38,39]. Whether similar co-infections are present and the extent these additional pathogens contribute to the clinical presentation and outcome of infants with *B. pertussis* infection in Sarawak remains to be determined. Other than these two potentially important limitations, the inherent biases related to the descriptive retrospective monocentric design of the study may have also affected the findings.

Conclusions

In conclusion, the average annual incidence of laboratory-confirmed pertussis in Sarawak, Malaysia, was extremely high at 482 per 100,000 infants aged < 12 months. Severe disease was present in a third of infants, and nearly one-quarter overall needed intensive care. Infants aged < 3 months accounted for over 75% of cases, and nearly all cases of severe disease or those that required invasive ventilation or intensive care had received ≤ 1 dose of pertussis vaccination. Additionally, about a third of cases overall had atypical presentations. Implementation of additional strategies such as maternal vaccination or cocooning should be urgently considered to protect these youngest and most vulnerable infants.

References

1. Kilgore PE, Salim AM, Zervos MJ, Schmitt HJ (2016) Pertussis: microbiology, disease, treatment, and prevention. Clin Microbiol Rev 29: 449-86. doi: 10.1128/cmr.00083-15.
2. Esposito S, Stefanelli P, Fry NK, Fedele G, He Q, Paterson P, Tan T, Knuf M, Rodrigo C, Weil Olivier C, Flanagan KL, Hung I, Lutsar I, Edwards K, O'Ryan M, Principi N, World Association of Infectious Diseases and Immunological Disorders (WAidid) and the Vaccine Study Group of the European Society of Clinical Microbiology and Infectious Diseases (EVASG) (2019) Pertussis prevention: reasons for resurgence, and differences in the current acellular pertussis vaccines. Front Immunol 10: 1344. doi: 10.3389/fimmu.2019.01344.
3. Cherry JD (1984) The epidemiology of pertussis and pertussis immunization in the United Kingdom and the United States: a

comparative study. Curr Probl Pediatr 14: 1-78. doi: 10.1016/0045-9380(84)90016-1.
4. Cherry JD (2016) Pertussis in young infants throughout the world. Clin Infect Dis 63: S119-S22. doi: 10.1093/cid/ciw550.
5. Cherry JD (2019) The 112-year odyssey of pertussis and pertussis vaccines-mistakes made and implications for the future. J Pediatric Infect Dis Soc 8: 334-41. doi: 10.1093/jpids/piz005.
6. Guiso N, Meade BD, Wirsing von Konig CH (2020) Pertussis vaccines: the first hundred years. Vaccine 38: 1271-6. doi: 10.1016/j.vaccine.2019.11.022.
7. Skoff TH, Hadler S, Hariri S (2019) The epidemiology of nationally reported pertussis in the United States, 2000-2016. Clin Infect Dis 68: 1634-40. doi: 10.1093/cid/ciy757.
8. Fullen AR, Yount KS, Dubey P, Deora R (2020) Whoop! There it is: the surprising resurgence of pertussis. PLoS Pathog 16: e1008625. doi: 10.1371/journal.ppat.1008625.
9. Faulkner AE, Skoff TH, Tondella ML, Cohn A, Clark TA, Martin SW (2016) Trends in pertussis diagnostic testing in the United States, 1990 to 2012. Pediatr Infect Dis J 35: 39-44. doi: 10.1097/inf.0000000000000921.
10. van der Zee A, Schellekens JF, Mooi FR (2015) Laboratory diagnosis of pertussis. Clin Microbiol Rev 28: 1005-26. doi: 10.1128/cmr.00031-15.
11. Wood N, McIntyre P (2008) Pertussis: review of epidemiology, diagnosis, management and prevention. Paediatr Respir Rev 9: 201-11. doi: 10.1016/j.prrv.2008.05.010.
12. Viljanen MK, Ruuskanen O, Granberg C, Salmi TT (1982) Serological diagnosis of pertussis: IgM, IgA and IgG antibodies against *Bordetella pertussis* measured by enzyme-linked immunosorbent assay (ELISA). Scand J Infect Dis 14: 117-22. doi: 10.3109/inf.1982.14.issue-2.08.
13. Wiley KE, Zuo Y, Macartney KK, McIntyre PB (2013) Sources of pertussis infection in young infants: a review of key evidence informing targeting of the cocoon strategy. Vaccine 31: 618-25. doi: 10.1016/j.vaccine.2012.11.052.
14. WHO (2021) Pertussis reported cases by country. Available: https://apps.who.int/gho/data/node.main.WHS3_43?lang=en. Accessed: 4 July 2023.
15. Malaysia MOH (2021) Health facts. Available: https://www.moh.gov.my/index.php/pages/view/58?mid=19. Accessed: 4 July 2023.
16. Merdrignac L, Ait El Belghiti F, Pandolfi E, Jane M, Murphy J, Fabianova K, Garcia Cenoz M, Flem E, Guillot S, Tozzi AE, Carmona G, Habington A, Zavadilova J, Navasues A, Boas H, Levy-Bruhl D, Ferretti B, Lanaspa M, O'Sullivan N, Krizova P, Fernandino L, Bekkevold T, Hanslik T, Munoz-Almagro C, Bacci S, Spiteri G, Valenciano M, Moren A, PERTINENT Group (2021) Incidence and severity of pertussis hospitalisations in infants aged less than 1 year in 37 hospitals of six EU/EEA countries, results of PERTINENT sentinel pilot surveillance system, December 2015 to December 2018. Euro Surveill 26. doi: 10.2807/1560-7917.es.2021.26.4.1900762.
17. Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, Hanna BD, Rosenzweig EB, Raj JU, Cornfield D, Stenmark KR, Steinhorn R, Thebaud B, Fineman JR, Kuehne T, Feinstein JA, Friedberg MK, Earing M, Barst RJ, Keller RL, Kinsella JP, Mullen M, Deterding R, Kulik T, Mallory G, Humpl T, Wessel DL, American Heart Association Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation, Council on Clinical Cardiology, Council on Cardiovascular Disease in the Young, Council on Cardiovascular Radiology and Intervention, Council on Cardiovascular Surgery and Anesthesia, The American Thoracic Society (2015) Pediatric pulmonary hypertension: guidelines from the American Heart Association and American Thoracic Society. Circulation 132: 2037-99. doi: 10.1161/cir.0000000000000329.
18. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric S (2005) International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 6: 2-8. doi: 10.1097/01.pcc.0000149131.72248.e6.
19. WHO (2014) Laboratory manual for the diagnosis of whooping cough caused by *Bordetella pertussis/Bordetella parapertussis*: update 2014. Available: https://apps.who.int/iris/handle/10665/127891. Accessed: 4 July 2023.
20. Koidl C, Bozic M, Burmeister A, Hess M, Marth E, Kessler HH (2007) Detection and differentiation of *Bordetella* spp. by real-time PCR. J Clin Microbiol 45: 347-50. doi: 10.1128/jcm.01303-06.
21. Muloiwa R, Kagina BM, Engel ME, Hussey GD (2020) The burden of laboratory-confirmed pertussis in low- and middle-income countries since the inception of the Expanded Programme on Immunisation (EPI) in 1974: a systematic review and meta-analysis. BMC Med 18: 233. doi: 10.1186/s12916-020-01699-3.
22. Mohamed TJ, Fong SM, Nadarajaw T, Choo CM, Yusoff NKN, Nachiappan JP, Chan KC, Koh MT, Amran F, Hashim R, Jabar KA, Teh CSJ, Macina D, Ibrahim HM (2022) Burden of pertussis among young infants in Malaysia: A hospital-based surveillance study. Vaccine 40: 5241-7. doi: 10.1016/j.vaccine.2022.07.019.
23. Shi T, Wang L, Du S, Fan H, Yu M, Ding T, Xu X, Zhang D, Huang L, Lu G (2021) Mortality risk factors among hospitalized children with severe pertussis. BMC Infectious Diseases 21: 1-10.
24. Castillo ME, Bada C, Del Aguila O, Petrozzi-Helasvuo V, Casabona-Ore V, Reyes I, Del Valle-Mendoza J (2015) Detection of *Bordetella pertussis* using a PCR test in infants younger than one year old hospitalized with whooping cough in five Peruvian hospitals. Int J Infect Dis 41: 36-41. doi: 10.1016/j.ijid.2015.10.020.
25. Bhagat D, Saboui M, Huang G, Domingo FR, Squires SG, Salvadori MI, Li YA (2023) Pertussis epidemiology in Canada, 2005-2019. Can Commun Dis Rep 49: 21-8. doi: 10.14745/ccdr.v49i01a05.
26. Amirthalingam G, Campbell H, Ribeiro S, Fry NK, Ramsay M, Miller E, Andrews N (2016) Sustained effectiveness of the maternal pertussis immunization program in England 3 years following introduction. Clin Infect Dis 63: S236-S43. doi: 10.1093/cid/ciw559.
27. Tessier E, Campbell H, Ribeiro S, Rai Y, Burton S, Roy P, Fry NK, Litt D, Amirthalingam G (2022) Impact of the COVID-19 pandemic on *Bordetella pertussis* infections in England. BMC Public Health 22: 405. doi: 10.1186/s12889-022-12830-9.
28. Muloiwa R, Nicol MP, Hussey GD, Zar HJ (2020) Diagnostic limitations of clinical case definitions of pertussis in infants and children with severe lower respiratory tract infection. PLoS One 15: e0235703. doi: 10.1371/journal.pone.0235703.
29. van den Brink G, Wishaupt JO, Douma JC, Hartwig NG, Versteegh FG (2014) *Bordetella pertussis*: an underreported pathogen in pediatric respiratory infections, a prospective

cohort study. BMC Infect Dis 14: 526. doi: 10.1186/1471-2334-14-526.
30. Heininger U, Klich K, Stehr K, Cherry JD (1997) Clinical findings in *Bordetella pertussis* infections: results of a prospective multicenter surveillance study. Pediatrics 100: E10. doi: 10.1542/peds.100.6.e10.
31. Lagergren J (1963) The white blood cell count and the erythrocyte sedimentation rate in pertussis. Acta Paediatr (Stockh) 52: 405-9. doi: 10.1111/j.1651-2227.1963.tb03798.x.
32. Carbonetti NH (2016) Pertussis leukocytosis: mechanisms, clinical relevance and treatment. Pathog Dis 74. doi: 10.1093/femspd/ftw087.
33. Barger-Kamate B, Deloria Knoll M, Kagucia EW, Prosperi C, Baggett HC, Brooks WA, Feikin DR, Hammitt LL, Howie SR, Levine OS, Madhi SA, Scott JA, Thea DM, Amornintapichet T, Anderson TP, Awori JO, Baillie VL, Chipeta J, DeLuca AN, Driscoll AJ, Goswami D, Higdon MM, Hossain L, Karron RA, Maloney S, Moore DP, Morpeth SC, Mwananyanda L, Ofordile O, Olutunde E, Park DE, Sow SO, Tapia MD, Murdoch DR, O'Brien KL, Kotloff KL, Pneumonia Etiology Research for Child Health (PERCH) Study Group (2016) Pertussis-associated pneumonia in infants and children from low- and middle-income countries participating in the PERCH Study. Clin Infect Dis 63: S187-S196. doi: 10.1093/cid/ciw546.
34. Vittucci AC, Spuri Vennarucci V, Grandin A, Russo C, Lancella L, Tozzi AE, Bartuli A, Villani A (2016) Pertussis in infants: an underestimated disease. BMC Infect Dis 16: 414. doi: 10.1186/s12879-016-1710-0.
35. Moosa F, du Plessis M, Wolter N, Carrim M, Cohen C, von Mollendorf C, Walaza S, Tempia S, Dawood H, Variava E, von Gottberg A (2019) Challenges and clinical relevance of molecular detection of Bordetella pertussis in South Africa. BMC Infect Dis 19: 276. doi: 10.1186/s12879-019-3869-7.
36. Antila M, He Q, de Jong C, Aarts I, Verbakel H, Bruisten S, Keller S, Haanpera M, Makinen J, Eerola E, Viljanen MK, Mertsola J, van der Zee A (2006) Bordetella holmesii DNA is not detected in nasopharyngeal swabs from Finnish and Dutch patients with suspected pertussis. J Med Microbiol 55: 1043-51. doi: 10.1099/jmm.0.46331-0.
37. Woolfrey BF, Moody JA (1991) Human infections associated with *Bordetella bronchiseptica*. Clin Microbiol Rev 4: 243-55. doi: 10.1128/cmr.4.3.243.
38. Frassanito A, Nenna R, Nicolai A, Pierangeli A, Tozzi AE, Stefanelli P, Carsetti R, Concato C, Schiavoni I, Midulla F, Pertussis study group (2017) Infants hospitalized for *Bordetella pertussis* infection commonly have respiratory viral coinfections. BMC Infect Dis 17: 492. doi: 10.1186/s12879-017-2567-6.
39. Jiang W, Mao L, Wang K, Wang Y, Hao C, Shao X, Xu J (2021) Prevalence of *B. pertussis* infection in children with clinically suspected pertussis. J Microbiol Immunol Infect 54: 693-700. doi: 10.1016/j.jmii.2020.03.006.

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