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Circulation of *Bordetella pertussis* in vaccinated Cambodian children: A transversal serological study

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ABSTRACT

Background: The Cambodia pertussis immunization schedule includes three doses given at age 6, 10 and 14 weeks using a whole-pertussis vaccine. No booster doses are included. Pertussis biological diagnosis is unavailable in Cambodia and its burden remains unclear. This study aimed to provide accurate data on pertussis serological status of Cambodian children and adolescents, and to evaluate vaccination timeliness.

Methods: Fully vaccinated children aged 3–15 years were recruited at the Rabies Prevention Center, Institut Pasteur in Cambodia, Phnom Penh. Capillary blood samples and information on pertussis vaccination history were collected. Anti-pertussis toxin (PT) IgG titers were quantified by ELISA.

Results: Compliance with the national immunization schedule was 95.1%. Initiation of vaccination after 8 weeks of age was observed for 29.0% of the children, but was less frequent in the youngest children (13.0%) compared with the oldest ones (46.4%). Rate of children exhibiting anti-PT IgG varied across age groups, and increased from 35.7% to 55.0% in 3–5 and 12–15 years age groups, respectively.

Conclusion: Pertussis circulates among vaccinated Cambodian children and adolescents. These data support the need for public health authorities to strengthen pertussis surveillance and use local epidemiological data to make evidence-based decision for the establishment of an optimal vaccination strategy.

Introduction

Whooping cough, also known as pertussis, is a highly contagious disease (Clark, 2012; Kretzschmar et al., 2010). The etiological agent is the Gram-negative bacterium *Bordetella pertussis* (*B. pertussis*) that typically causes respiratory illness lasting several weeks. The incidence of this disease has greatly decreased with the mass vaccination that began in the 1950s in high-income countries (HIC) and was then introduced into the developing world with the World Health Organization's (WHO) Expanded Programme on Immunization (EPI) from 1974 (Guiso et al., 2020; World Health Organization, 2015b). However, whooping cough is still endemic and its burden was estimated to be around 24 million cases and 160,000 deaths in children aged <5 years in 2014 (Yeung et al., 2017).

The most severe cases of pertussis occur in young infants, particularly in those aged <3 months who have not yet started their primary vaccination. However, due to waning immunity induced by the vaccine or by natural infection, pertussis has been recognized to be an important cause of morbidity in older children, adolescents and adults, which in turn represents a source of contamination for infants (Jenkinson, 1988; Wiley et al., 2013; Zepp et al., 2011). To better control the circulation of the pathogen, the WHO recommends several booster doses in a lifetime, including in

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toddlers and children (World Health Organization, 2015b). However, in many low and middle-income countries (LMIC) these additional vaccinations are not included in the national immunization program and only the primary vaccination is given through the EPI, using whole-cell vaccines (wPVs). Although less reactogenic acellular vaccines (aPVs) have been developed since the 1980s, the WHO continues to recommend vaccination with wPV in national immunization programs for children aged <7 years (World Health Organization, 2015b). wPV production, made of inactivated *B. pertussis*, is complex and despite guidance provided by the WHO (World Health Organization, 2007), the features of the wPVs may vary (Steinhoff et al., 1995) and affect vaccine immunogenicity (Edwards and Berbers, 2014). Many studies performed in the 1980–1990s on wPVs used in HIC before their switch to aPVs have shown that there was a high variability between the brands, with vaccine efficacy ranging 46–92% (World Health Organization, 2015b). Contemporary wPVs used in LMICs are distinct, and their immunogenicity and efficacy have not been evaluated in humans.

Despite global concern on pertussis resurgence, lack of understanding in contemporary wPVs and limited national immunization schedules, little attention on pertussis has been paid in LMIC. Epidemiological information is limited and cases are most likely to be underdiagnosed and underreported in those regions of the world, as surveillance systems and diagnostic laboratory capacity are lacking or scarce. Local data on pertussis burden and circulation are needed (Guiso and Taieb, 2019; von Koenig and Guiso, 2017).

The EPI was launched in Cambodia in 1986. National recommendations include primary immunization, with injections at 6, 10 and 14 weeks of life using a combined vaccine that is either tetravalent – which includes diphtheria and tetanus toxoids, wPV, and *Haemophilus influenzae* type b vaccine – or pentavalent – which also includes hepatitis B vaccine. wPVs are provided by UNICEF and, importantly, may come from different sources from one year to another. Sources of wPVs used in Cambodia in the period 2002–2019 included Staten Serum Institute, GlaxoSmithKline Biologicals, Serum Institute of India, Berna Biotech Korea Corp, Biological E-limited, LG CHEM LTD Korea, and Shantha Biotechnics Private Limited.

Pertussis has been detected in Cambodia (Moriuchi et al., 2017); however, its burden is still unclear. A few cases are reported almost every year through the passive surveillance system (World Health Organization, 2019a), which are mostly diagnosed based on the WHO clinical definition of pertussis that lacks sensitivity (Ghanaie et al., 2010; Gopal Krishnan et al., 2019). Monitoring of pertussis disease by active hospital-based surveillance and accurate molecular testing is critical, but complex to establish. Serological testing in general populations offers an easy tool with which to estimate pertussis circulation, especially in older children, adolescents and adults who may have subclinical or symptomless pertussis (Barkoff et al., 2015). Anti-pertussis toxin (PT) antibodies are lost within months or a few years; therefore, although vaccine and infection-derived antibodies cannot be distinguished, presence of serological anti-PT immunoglobulin G (IgG) in the absence of recent vaccination is a sign of recent contact with the bacteria (Guiso et al., 2011).

This study aimed to evaluate serological status in fully vaccinated Cambodian children aged 3–15 years, and to assess compliance with the national immunization schedule.

Methods

Study population

Children were recruited at the Rabies Prevention Center, Institut Pasteur in Cambodia, Phnom Penh. The inclusion criteria were: being aged 3–15 years and having completed the three injections of pertussis primary vaccination at least a year ago, as documented in the vaccination booklet of the child. A blood sample from the tip of a finger (approximately 200 μL) was collected using a lancet (Becton Dickinson, Sentry 23G) and a serum separating tube microtainer (Becton Dickinson, reference 365968). Information related to pertussis vaccination history, sex, age, and blood sampling were reported on a standardized questionnaire and then collated into a computer database. No information related to past or current respiratory illness was collected.

Serology analysis

Upon collection, blood sample was transferred to the Medical Biology Unit, Institut Pasteur in Cambodia, Phnom Penh, and serum was collected after short spinning and then stored at −80 °C until analysis. Anti-PT Immunoglobulin G (IgG) titers were quantified using a commercial purified PT-containing enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN; reference EI 2050-G) (Riffelmann et al., 2010) and the WHO reference serum available from the National Institute for Biological Standards and Control (NIBSC). All tests had internal negative and positive controls and passed the validity criteria. Results were reported as International Units (IU)/mL. The lower limit of quantitation was defined as 5 IU/mL. A titer of <5 IU/ mL corresponded to being seronegative and children with anti-PT IgG levels ≥5 IU/mL were considered as seropositive. Anti-PT titers were categorized using 40 IU/mL and 100 IU/mL cut-offs to evidence children who had contact with *B. pertussis* sometime during the past 12 months when [40–100[IU/mL, and 6 months when ≥100 IU/mL (Guiso et al., 2011).

Timeliness of pertussis immunization

The Cambodian pertussis immunization schedule includes three doses: at 6, 10 and 14 weeks of age. Compliance with the national recommendation schedule was defined as having received the first injection at ≥38 days of life, and the second and third injections 24–70 days after the previous dose.

Statistical analysis

Continuous variables were described using the median, interquartile range (IQR), minimum and maximum, and comparisons were conducted using two-sided Kruskal–Wallis test. For categorical variables, percentages were estimated, and comparisons were conducted using the two-sided Chi-squared test. Logistic regression models were used to identify factors associated with compliance and with anti-PT IgG ≥5 IU/mL. Statistical analyses were undertaken using Stata software, version 15.1. A 5% alpha risk was considered for statistical tests.

Ethical aspects

After giving the information to the parents/guardians, informed consent of the parents/guardians and the oral assent for children aged >7 years were collected. Only children meeting the inclusion criteria and for whom written and oral consent/assent were obtained participated to the study. The study protocol was reviewed and approved by the Institutional Review Board of Institut Pasteur, France (decision number 2016-04/IRB), and by the National Ethics Committee for Health Research in Cambodia (decision number 311 NECHR). Authorization for data processing was obtained from the French legal authority (*Commission Nationale Informatique et Liberté*) and each participant was assigned a unique code for pseudonymization. The ClinicalTrials.gov identifier of the study is: NCT02983487.

Table 1
Study population description: sex and age at first pertussis immunization shown by age groups.

Age group (years)		3–5	6–8	9–11	12–15	Total	*p* value
	N	154	152	303	151	760	–
Sex							
Boys	n (%)	81 (52.6)	85 (55.9)	151 (49.8)	87 (57.6)	404 (53.2)	*0.386*
Initiation of primary pertussis immunization							
Age (weeks)	Median	6.7	6.9	7	7.6	6.9	<***0.001***
	IQR	6.6–7.0	6.6–7.7	6.6–8.9	6.6–10.3	6.6–8.6	–
	Min–max	4.3–15.0	5.1–23.6	4.7–24	4.4–62.7	4.3–62.7	–
Age >8 weeks	n (%)	20 (13.0)	33 (21.7)	98 (32.3)	70 (46.4)	221 (29.1)	<***0.001***

Results

Description of study population

A total of 761 children participated to the study and were recruited over a period of 14 months from January 2017 to February 2018. There was one withdrawal. Analyses were performed on information collected from the 760 remaining children. Age groups were defined as 3–5, 6–8, 9–11, and 12–15 years; each group included more than 150 children (Table 1). Proportions of girls and boys were similar across the age groups. Overall, 29% (221/760) of the children started their pertussis vaccinations at age >8 weeks, which was significantly associated with age groups (Table 1). Age at initiation increased across the age groups, with children aged 12–15 years having started their primary immunization significantly later than younger children (Table 1). Two children initiated their vaccination at age >6 months and one child at age >12 months; they belonged to the 12–15 age group.

Age at pertussis injections and timeliness

Details regarding age at each injection are presented in Table 2. Overall, median age at injection 1 was in accordance with national recommendations. Median age at injections 2 and 3 was older than recommended. Forty-seven and four children were not yet fully vaccinated at 6 and 12 months old, respectively. Timeliness for the entire primary immunization, based on national recommendations, was observed for 95.1% (723/760) of the children (Figure 1). Eight children only started their vaccination earlier than the minimum age, and 15 and 19 had too long an interval in between doses 1 and 2, or 2 and 3, respectively. The longest delay was 5 and 8 months for the second and third injection, respectively.

Anti-pertussis toxin serology

The shortest timespan since the last pertussis immunization was 2.7 years. The seropositivity rate was 47% (357/760), including 8.6% (65/760) of children exhibiting titers ≥40 IU/mL indicating contact with the bacteria in the past 12 months (Table 3). The proportion of seropositive children increased with age, starting from 35.7% (55/154) in the 3–5 age group; seropositivity reached 55% (83/151) of children in the 12–15 years group (Table 3 and Figure 2). Children aged 6–8 years exhibited the highest anti-PT IgG titers, with 13.2% (20/152) of them having titers ≥40 IU/mL (Figure 2). Sex and compliance with the national immunization schedule were not associated with seropositivity.

Table 2
Age at pertussis vaccine injections.

Pertussis injection	1	2	3
Recommended age	6 weeks	10 weeks	14 weeks
Age at injection (weeks)			
Median	6.9	11.6	16.4
25–75%	6.6–8.6	11–13.7	15.6–19
10–90%	6.4–10.7	10.9–16.6	15.3–22.7
Min–max	4.3–62.7	8.7–67.1	13.3–71.1

Discussion

This study is the first pertussis sero-epidemiological study implemented in Cambodia. It aimed to evaluate the serological status of Cambodian children aged 3–15 years and vaccinated during their infancy with three doses of wPV.

Cambodia has made significant progress in coverage with tetravalent and pentavalent vaccines over the years, as shown in Cambodian Demographic and Health Surveys conducted every 4–5 years since 2000 and investigating approximately 15,000 nationally representative households each time (National Institute of Public Health et al., 2006; National Institute of Statistics et al., 2015; National Institute of Statistics et al., 2011; National Institute of Statistics et al., 2001). Notably, while 63% and 43% of children had received their first and third doses by the age of 12 months in 2000, coverage increased to 94% and 82% in 2014, respectively (National Institute of Statistics et al., 2015). More recently, estimates from WHO and UNICEF reported 94% and 92% of coverage in 2018 for the first and third doses, respectively, reaching 90% coverage with the third dose, as recommended by the WHO (World Health Organization, 2015b, 2019b). Although the WHO/UNICEF estimates tend to be higher than those from surveys, and may suffer from inaccuracy in denominators, coverage trends are similar (World Health Organization, 2015a). The sharp reduction in numbers of pertussis cases in Cambodia since 2011 may be, at least in part, attributed to increasing coverage since 2008 (World Health Organization, 2019a).

In this study, all participating children had received their three doses, as it was an inclusion criterion. Yet, four of them received their third dose when older than 12 months, and may be considered as dropouts (i.e. a child who received at least one dose of tetravalent/pentavalent vaccine but failed to receive the third dose of the vaccine during the first year of life). In addition, 29% of the participating children initiated their vaccinations at ≥9 weeks. However, it is critical to start vaccinating as early as 6 weeks and no later than 8 weeks of age, as the longer the infant remains without vaccination, the longer he/she remains susceptible to severe and sometimes deadly disease (World Health Organization, 2015b). Interestingly this study showed increasing compliance with early vaccination over the years, and thus negatively correlated with age, which could be a direct consequence of the growing performance of national immunization campaigns. Overall, based on national recommendations, timeliness was very good, with 95.1% of children complying with dose intervals. Yet, given the study inclusion criteria, these children may be representing a particularly compliant sub-population.

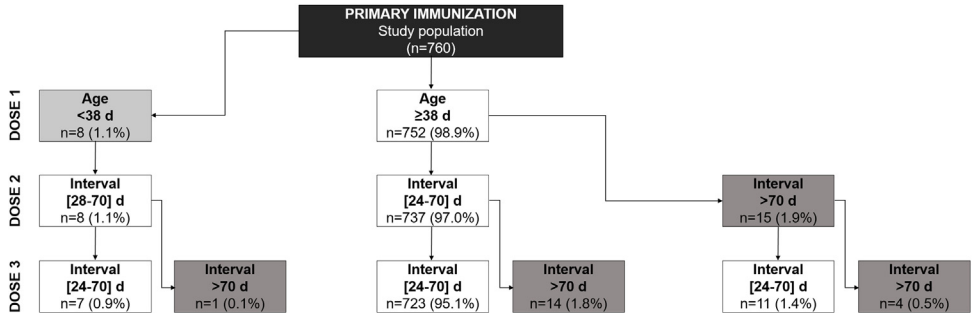

Figure 1. Compliance with the national recommendation schedule for pertussis vaccination.
Numbers (n) and proportions (%) of children who received early (light grey), on time (white) or late (dark grey) pertussis vaccine doses as compared with Cambodian recommendations are shown for each dose. Intervals in between the first and second, and second and third doses are shown with respect to compliance with the first and second dose, respectively.
d, days.

Table 3
Stratified analysis of pertussis seropositivity according to age groups and other factors.

		Children with anti-PT IgG titers ≥5 IU/mL			
	N	n	%	OR (95% CI)	*p* value
Total	760	357	46,97	–	–
Age group (years)					
3–5	154	55	35.71	1	–
6–8	152	72	47.37	**1.6 (1.0–2.6)**	**0.039**
9–11	303	147	48.51	**1.7 (1.1–2.5)**	**0.009**
12–15	151	83	54.97	**2.2 (1.4–3.5)**	**0.001**
Sex					
Boys	404	192	47.52	1	0.746
Girls	356	165	46.35	1.0 (0.7–1.3)	
Compliance with primary immunization schedule					
No	37	20	54.05	1	0.376
Yes	723	337	46.61	0.7 (0.4–1.4)	

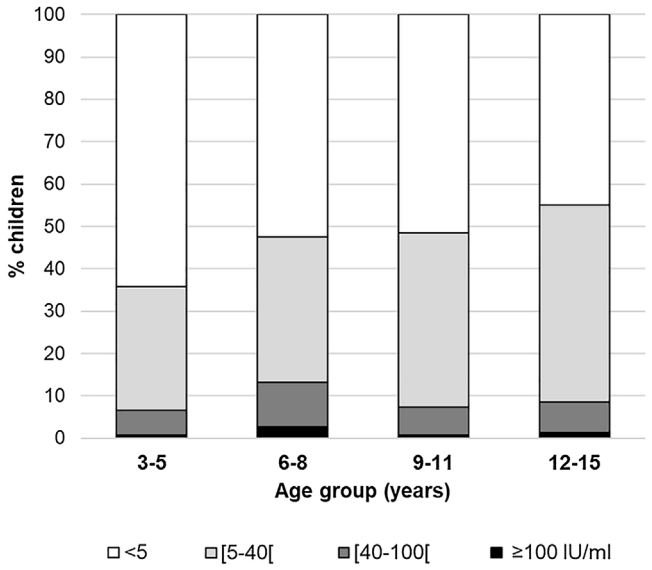

Figure 2. Anti-PT IgG titer distribution by age groups.
Anti-PT IgG titers are represented as proportions of seronegative children (<5 IU/mL) and seropositive children exhibiting titers [5–40[, [40–100[or ≥100 IU/mL

Procurement of vaccines for the National Immunization Program is through UNICEF, and the origin may vary from one year to another as it is governed by manufacturer and local authority requirements (Dellepiane and Pagliusi, 2018). Since 2006, a tetravalent and pentavalent vaccine have replaced the trivalent diphtheria-tetanus-wPV vaccine in Cambodia. Immunogenicity and duration of protection conferred by these wPVs have not been studied (World Health Organization, 2015b) and the pertussis epidemiological situation is obscure in Cambodia. Laboratory capacity for pertussis diagnosis is absent in the country, and there have been few or no cases reported annually since 2011, based on the WHO clinical case definition (World Health Organization, 2019a). Recently, a study on >600 nasopharyngeal swabs collected during 2008–2016 in Cambodia, and sent to Japan for molecular analyses, showed that 82 samples tested positive for *B. pertussis* infection (Moriuchi et al., 2017). Pertussis-positive children were aged 0–13 years but no information was provided regarding the vaccination status of the children. However, these results confirmed the circulation of *B. pertussis* in Cambodia, such as it was shown in other Southeast Asian countries, including in older children and adolescents (Thisyakorn et al., 2019).

This serological study showed that 47% of children, aged 3–15 years and who were fully vaccinated during infancy, had anti-PT IgG in their bloodstream. This indicated a previous contact with *B. pertussis* bacteria, since there was no recent vaccination (shortest timespan since vaccination was 2.7 years). Among those, 8.6% had that contact sometime in the past 12 months. More importantly, it was observed that the proportion of seropositive children significantly increased with age, from 36% of seropositive children in the 3–5 age group to 55% in the 12–15 age group. Similar seropositivity rates were observed in a recent study in Lao People's Democratic Republic, a bordering country where the pertussis vaccination strategy is the same as in Cambodia. Approximately half (52.2%) of a cohort of 6–19-year-old schoolchildren were seropositive for pertussis, including 10% with titers ≥40 IU/mL (Kleine et al., 2020); the most recent vaccination dated back >5.5 years for these children. In Nigeria, which applies the same vaccination strategy, a study also highlighted the rise of anti-PT titers from 3-year-old children (Bassey et al., 2020).

Knowledge on duration of protection conferred by these contemporary vaccines and after only three doses is scant. Nowadays, most studies are performed in countries using one or multiple booster doses and mostly using aPV. Information regarding duration of protection induced by wPVs is mostly derived from older studies, which thus evaluated distinct wPVs and showed a rapid decrease in vaccine effectiveness over time (Broutin et al., 2004; Jenkinson, 1988). Incidence was greatly reduced after introduction of vaccination; however, infection still occurred in vaccinated children but the age at first infection was delayed and was associated with vaccine coverage (Broutin et al., 2004; Préziosi et al., 2002). Herd immunity was observed but was insufficient to stop pertussis circulation and prevent infection and disease in older children (Broutin et al., 2004). The protection conferred by pertussis vaccines wanes overtime, whatever the

initial vaccine efficacy (Jenkinson, 1988; Préziosi et al., 2002; Sheridan et al., 2014). Today all HIC and some LMIC have included a toddler and, sometimes, a 5–7 years booster to their pertussis immunization schedule, as recommended by the WHO (World Health Organization, 2015b). These additional doses are critical to further enhance herd immunity, as wPV vaccination reduces not only the disease but also transmission (Broutin et al., 2010; Jackson and Rohani, 2014). Nevertheless, pertussis is highly contagious and maintaining high overall coverage in infants is necessary to prevent outbreaks such as the one recently described in Southern Ethiopia, which recorded numerous deaths and severe cases in very young infants (Mitiku et al., 2020).

The current data were derived from children who were recruited at Institut Pasteur in Cambodia when seeking the rabies vaccination. In Cambodia, rabies is endemic and mainly transmitted by dogs that represent a remarkably large population, with about 80% of its population living in the countryside (Ly et al., 2009). According to data collected during 1998–2007, 90% of the patients reaching the IPC Rabies Prevention Centre reside in Phnom Penh, or in the six surrounding provinces, within approximately 200 km from the Centre (Ly et al., 2009). Thus, the study population may not be representative of the entire population of the country, but may predominantly represent children living in the countryside of South Cambodia and Phnom Penh provinces. Furthermore, the contribution of seropositive children in disease burden was not established, as no clinical information were collected in this study; nonetheless, they constitute a share in *B. pertussis* circulation.

Conclusion

The timeliness of vaccination in Cambodia was very good, yet efforts should continue to initiate vaccination as early as 6 weeks of age. This transversal study found that *B. pertussis* substantially circulates among Cambodian children vaccinated during infancy, with increasing contact from 3–15 years old. These is local evidence of the need for improving pertussis surveillance to inform policy and reinforce herd immunity among the Cambodian population. These findings provide information to help fill in the gap in pertussis epidemiology in LMIC using contemporary wPV.

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Conflict of interest

None.

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References

Barkoff A-M, Gröndahl-Yli-Hannuksela K, He Q. Seroprevalence studies of pertussis: what have we learned from different immunized populations. Pathog Dis 2015;73(7), doi:http://dx.doi.org/10.1093/femspd/ftv050 ftv050–ftv050.

Bassey GE, Ekanem EE, Okpara HC, Eyong KI. Bordetella pertussis antibody levels in DPT/pentavalent immunized preschool Nigerian children: a cross-sectional study. J Pediatr Infect Dis 2020;15(01):011–5, doi:http://dx.doi.org/10.1055/s-0039-1693484.

Broutin H, Simondon F, Rohani P, Guégan J, Grenfell BT. Loss of immunity to pertussis in a rural community in Senegal. Vaccine 2004;22(5):594–6, doi:http://dx.doi.org/10.1016/j.vaccine.2003.07.018.

Broutin H, Viboud C, Grenfell BT, Miller MA, Rohani P. Impact of vaccination and birth rate on the epidemiology of pertussis: a comparative study in 64 countries. Proc Biol Sci 2010;277(1698):3239–45, doi:http://dx.doi.org/10.1098/rspb.2010.0994.

Clark TA. Responding to Pertussis. J Pediatr 2012;161(6):980–2, doi:http://dx.doi.org/10.1016/j.jpeds.2012.07.014.

Dellepiane N, Pagliusi S. Challenges for the registration of vaccines in emerging countries: differences in dossier requirements, application and evaluation processes. Vaccine 2018;36(24):3389–96, doi:http://dx.doi.org/10.1016/j.vaccine.2018.03.049.

Edwards KM, Berbers GAM. Immune responses to Pertussis vaccines and disease. J Infect Dis 2014;209(suppl_1):S10–5, doi:http://dx.doi.org/10.1093/infdis/jit560.

Ghanaie RM, Karimi A, Sadeghi H, Esteghamti A, Falah F, Armin S, et al. Sensitivity and specificity of the World Health Organization pertussis clinical case definition. Int J Infect Dis 2010;14(12):e1072–5, doi:http://dx.doi.org/10.1016/j.ijid.2010.07.005.

Gopal Krishnan S, Fun WH, Ramadras MD, Yunus R, Lye YF, Sararaks S. Pertussis clinical case definition: time for change in developing countries?. PLoS One 2019;14(7), doi:http://dx.doi.org/10.1371/journal.pone.0219534 e0219534–e0219534.

Guiso N, Berbers G, Fry NK, He Q, Riffelmann M, Wirsing von König CH, et al. What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis 2011;30(3):307–12, doi:http://dx.doi.org/10.1007/s10096-010-1104-y.

Guiso N, Meade BD, Wirsing von König CH. Pertussis vaccines: the first hundred years. Vaccine 2020;38(5):1271–6, doi:http://dx.doi.org/10.1016/j.vaccine.2019.11.022.

Guiso N, Taieb F. Pertussis in low and medium income countries: a pragmatic approach. Adv Exp Med Biol 2019;, doi:http://dx.doi.org/10.1007/5584_2019_409 0065-2598 (Print).

Jackson DW, Rohani P. Perplexities of pertussis: recent global epidemiological trends and their potential causes. Epidemiol Infect 2014;142(4):672–84, doi:http://dx.doi.org/10.1017/s0950268812003093.

Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study. Br Med J (Clin Res ed) 1988;296(6622):612–4, doi:http://dx.doi.org/10.1136/bmj.296.6622.612.

Kleine D, Billamay S, Chanthavilay P, Mongkhoune S, Keokhamphoui C, Souksakhone C, et al. Pertussis in Lao PDR: seroprevalence and disease. Int J Infect Dis 2020;95:282–7, doi:http://dx.doi.org/10.1016/j.ijid.2020.03.074.

Kretzschmar M, Teunis PFM, Pebody RG. Incidence and reproduction numbers of pertussis: estimates from serological and social contact data in five European countries. PLOS Med 2010;7(6), doi:http://dx.doi.org/10.1371/journal.pmed.1000291 e1000291–e1000291.

Ly S, Buchy P, Heng NY, Ong S, Chhor N, Bourhy H, et al. Rabies situation in Cambodia. PLoS Negl Trop Dis 2009;3(9), doi:http://dx.doi.org/10.1371/journal.pntd.0000511 e511–e511.

Mitiku AD, Argaw MD, Desta BF, Tsegaye ZT, Atsa AA, Tefera BB, et al. Pertussis outbreak in Southern Ethiopia: challenges of detection, management and response. Research Square. .

Moriuchi T, Vichit O, Vutthikol Y, Hossain MS, Samnang C, Toda K, et al. Molecular epidemiology of Bordetella pertussis in Cambodia determined by direct genotyping of clinical specimens. Int J Infect Dis 2017;62:56–8, doi:http://dx.doi.org/10.1016/j.ijid.2017.07.015.

National Institute of Public Health, National Institute of Statistics [Cambodia] & ORC Macro, Cambodia Demographic and Health Survey 2005. Phnom Penh, Cambodia and Calverton. Maryland, USA: National Institute of Public Health, National Institute of Statistics and ORC Macro; 2006.

National Institute of Statistics, Directorate General for Health & ICF International, Cambodia Demographic and Health Survey 2014. Phnom Penh, Cambodia, and Rockville. Maryland, USA: National Institute of Statistics, Directorate General for Health, and ICF International; 2015.

National Institute of Statistics, Directorate General for Health & ICF Macro, Cambodia Demographic and Health Survey 2010. Phnom Penh, Cambodia and Calverton. Maryland, USA: National Institute of Statistics, Directorate General for Health, and ICF Macro; 2011.

National Institute of Statistics, Directorate General for Health [Cambodia] & ORC Macro, Cambodia Demographic and Health Survey 2000. Phnom Penh, Cambodia, and Calverton. Maryland USA: National Institute of Statistics, Directorate General for Health, and ORC Macro; 2001.

Préziosi MP, Yam A, Wassilak SG, Chabirand L, Simaga A, Ndiaye M, et al. Epidemiology of pertussis in a West African community before and after introduction of a widespread vaccination program. Am J Epidemiol 2002;155(10):891–6, doi:http://dx.doi.org/10.1093/aje/155.10.891.

Riffelmann M, Thiel K, Schmetz J, Wirsing von Koenig CH. Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to bordetella pertussis. J Clin Microbiol 2010;48(12):4459–63, doi:http://dx.doi.org/10.1128/JCM.01371-10.

Sheridan SL, Frith K, Snelling TL, Grimwood K, McIntyre PB, Lambert SB. Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. Expert Rev Vaccines 2014;13(9):1081–106, doi:http://dx.doi.org/10.1586/14760584.2014.944167.

Steinhoff MC, Reed GF, Decker MD, Edwards KM, Englund JA, Pichichero ME, et al. A randomized comparison of reactogenicity and immunogenicity of two whole-cell pertussis vaccines. Pediatrics 1995;96(3):567.
Thisyakorn U, Tantawichien T, Thisyakorn C, Buchy P. Pertussis in the Association of Southeast Asian Nations: epidemiology and challenges. Int J Infect Dis 2019;87:75–83, doi:http://dx.doi.org/10.1016/j.ijid.2019.07.016.
von Koenig CHW, Guiso N. Global burden of pertussis: signs of hope but need for accurate data. Lancet Infect Dis 2017;17(9):889–90, doi:http://dx.doi.org/10.1016/S1473-3099(17)30357-2.
Wiley KE, Zuo Y, Macartney KK, McIntyre PB. Sources of pertussis infection in young infants: a review of key evidence informing targeting of the cocoon strategy. Vaccine 2013;31(4):618–25, doi:http://dx.doi.org/10.1016/j.vaccine.2012.11.052.
World Health Organization. Annex 6: recommendations for whole-cell pertussis vaccine. Technical report series. 2007 No 941.
World Health Organization. Assessing and improving the accuracy of target population estimates for immunization coverage. 2015.
World Health Organization. Pertussis vaccines: WHO position paper. Wkly Epidemiol Rec 2015b;90:433–58.
World Health Organization. Incidence time series for Cambodia (KHM). WHO vaccine-preventable diseases: monitoring system 2019 global summary. 2019 Available from: https://apps.who.int/immunization_monitoring/globalsummary/incidences?c=KHM. [Accessed 23 June 2020].
World Health Organization. WHO UNICEF estimates time series for Cambodia (KHM). WHO vaccine-preventable diseases: monitoring system. 2019 global summary. 2019 Available from: https://apps.who.int/immunization_monitoring/globalsummary/estimates?c=KHM. [Accessed 3 July 2020].
Yeung KHT, Duclos P, Nelson EAS, Hutubessy RCW. An update of the global burden of pertussis in children younger than 5 years: a modelling study. Lancet Infect Dis 2017;17(9):974–80, doi:http://dx.doi.org/10.1016/S1473-3099(17)30390-0.
Zepp F, Heininger U, Mertsola J, Bernatowska E, Guiso N, Roord J, et al. Rationale for pertussis booster vaccination throughout life in Europe. Lancet Infect Dis 2011;11(7):557–70, doi:http://dx.doi.org/10.1016/S1473-3099(11)70007-X.