Epidemiology, reemergence of pertussis and vaccine development in Latin America: an overview

Celso Pérez-Bolaños, Lourdes Lisbet Proenza-Alfonzo and Rafael Fando-Calzada.

Departament of Molecular Biology, Biotechnology Division, National Center for Scientific Research, P. O. Box 6414 Havana, Cuba.

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RESUMEN. Pertussis o tosferina es una enfermedad bacteriana aguda del tracto respiratorio, causada principalmente por *Bordetella pertussis* y en menor medida por *Bordetella parapertussis*. *Bordetella pertussis* ocupa el quinto lugar en la lista de muertes atribuidas a enfermedades prevenibles por vacunas en niños menores de cinco años en todo el mundo. Se ha reportado que provoca morbilidad y mortalidad significativa, tanto en países desarrollados como en países en desarrollo. La enfermedad es más severa en niños pequeños, pero su prevalencia ha sido observada en todo el mundo en todos los grupos de edad, aún después del desarrollo de vacunas a partir de células completas contra pertussis en los años cuarenta del pasado siglo. Desde la última década ha sido reportada una re-emergencia de pertussis en muchos países desarrollados, incluidos aquellos con una elevada cobertura de vacunación por años. Varios factores pudieran provocar la re-emergencia de pertussis, por ejemplo, una mayor conciencia del problema, un mejor diagnóstico mediante la implementación de técnicas de PCR, disminución de la cobertura de vacunación, la utilización de vacunas de baja protección, baja inmunidad inducida por vacunas y adaptación del patógeno. Este trabajo revisa el estado actual de la epidemiología y la re-emergencia de la enfermedad en América Latina y enfoca la situación actual en Cuba, para dar un panorama de la aplicación de estrategias novedosas en el desarrollo de vacunas futuras, así como medidas generales, recomendadas para el tratamiento de la enfermedad.

ABSTRACT. Pertussis or whooping cough is a highly contagious acute bacterial disease involving the respiratory tract, caused mainly by *Bordetella pertussis* and to a lesser extent by *Bordetella parapertussis. Bordetella pertussis* remains in fifth place in the list of deaths attributed to vaccine-preventable diseases in children under five years old around the world. It has been reported to cause significant morbidity and mortality in both developed and developing countries. The disease is most severe in young infants, but its prevalence has been observed worldwide occurring in all age groups, even after the development of whole-cells vaccines against pertussis in the 1940s. Since the last decade it has been reported a re-emergence of pertussis in many developed countries, including those that have shown high vaccination coverage for many years. Various factors might cause pertussis re-emergence, for instance, increased awareness, improved diagnostics through the PCR-technique implementation, decreased vaccination coverage, sub-optimal vaccines, waning vaccine-induced immunity and pathogen adaptation. This paper reviews the nowaday state of the epidemiology and re-emergence of the disease in Latin America, focusing the current Cuban situation, just for giving a panorama of the application of novel strategies for future vaccine development as well as general recommended means of treating the disease.

INTRODUCTION

The Gram-negative bacterium *Bordetella pertussis* is the causal agent of Whooping cough or Cocheluque-its present name in French being a strict human pathogen with no known animal or environmental reservoir.[1] Transmission of the disease seems to occur through respiratory droplets and although it was first described by Guillaume de Baillou (1538-1616) as an epidemics in France, 1578, mentioning of the disease dated from 1540 in Moulton's *The Mirror of Health* and even a paper by Nils Rosen von Rosenstein suggested that the illness began in France in 1414.[2,3] *B. bronchiseptica*, *B. parapertussis*, and *B.holmesii* belonging to the Genus *Bordetella* have been associated with respiratory infections in humans and other mammals.[1,4] At present, pertussis is submitted to worldwide vaccination programs. Nevertheless,

Correspondencia:
Lic. Celso Pérez Bolaños
Departamento de Biología Molecular, Biotecnología, Centro Nacional de Investigaciones Científicas, Ave. 25 y 158, Cubanacán, Playa, Apartado Postal 6414, La Habana, Cuba. Correo electrónico: celso.perez@cnic.edu.cu

an estimation of 48.5 million yearly worldwide cases and 295 000 deaths have been reported recently, so that the circulation of *B. pertussis* pathogenic strains remains, as judged by its re-emergence everywhere, mostly in areas of where vaccine use is low.[5,6] Currently, pertussis is still one of the top 10 causes of death in children under 1 year old worldwide, reaching as many as 400 000 pertussis-related deaths annually, 90 % in developing countries.[6-8] According to reports from the World Health Organization (WHO), developing countries have the highest disease burden.

In the case of pertussis, estimating rates is rather difficult, mostly due to lack of access to diagnostic methods, misdiagnosis, under-reporting, and different reporting criteria among countries.[6,9,10]

The re-emergence of pertussis has been recorded and well-documented in developed countries having immunization programs,[10-15] i.e., the United States, Australia, Canada or Israel. Two broad age groups may be defined: those over the age of 10 years and those under the age of 5 months. The former group was often born in an era of low immunization coverage, with waning immunity in those who were vaccinated while the latter cannot be fully protected by current vaccine schedules.[15-18] It has been observed a significant increasing incidence of the disease in adolescents and adults, which otherwise do not affect changes the antimicrobial resistance pattern of clinical isolates, susceptible to macrolides.[19] Both groups have been identified as an important source of infection for unvaccinated or incompletely vaccinated infants.[20-22] As a whole, various factors might cause pertussis re-emergence, for instance, increased awareness, improved diagnostics, decreased vaccination coverage, suboptimal vaccines, waning vaccine-induced immunity, and pathogen adaptation. This behavior has been also associated to toxin increased production of current circulating *Bordetella pertussis* strains in The Netherlands.[23] Besides, the lowering of protective immunity related to age may condition adolescents and adults as the primary source for pertussis infection of infants and new-born, on-milky infants,[20,24,25] since those groups show non-specific clinical manifestations, even the persistent cough is the only symptom of the disease.[26]

New vaccination strategies have been recommended mainly to protect infants before 2 months of age, who are the most vulnerable ones having a greater risk of hospitalizations, complications and death. These include vaccination with the tetanus, diphtheria and pertussis vaccine in adolescents, adults and postpartum women. However, vaccination of pregnant women is still controversial. Potential benefits of vaccinating newborns with monovalent or combined acellular pertussis vaccines have been demonstrated. Nevertheless, no vaccine is yet approved for this age group.[27] Based on previous studies from other authors, this paper reviews the current state of the epidemiology and reemergence of the disease in Latin America, focusing Cuban situation and giving a panorama of the application of novel strategies for vaccine development.

The situation of pertussis in Latin America

Various serious efforts have been carried out in order to know the situation of pertussis in Latin America.[19,28,29] It is worldwide recognized that usually outbreaks of this disease occurs every 2-5 years but under-reporting is a limiting factor, including Latin America.[9,19] The main reasons may be related to disease unawareness, inadequate epidemiological surveillance or poor sensitivity and specificity laboratory identification techniques (sometimes unaffordable) as well as resemblance of pertussis to other upper or lower respiratory tract diseases.[19] The problem is reinforced by the non-universal application of the standard WHO clinical case definition. Therefore, despite of the paucity of information, estimates from the WHO reveal that America as a whole was responsible for approximately 7 % of all pertussis by 2005.[19,30] Moreover, after a WHO/UNICEF joint reporting, the number of cases by 2006, 2007 and 2008 in Latin America (Table 1).[19,31] From the analysis of this table, it is apparently observed a rising of reports in most countries. However, any conclusion may result from the table and probably misinformation is affecting the whole data.

Table 1. Distribution of number of cases of pertussis in 2006, 2007 and 2008 in Latin American countries, in alphabetical order.[1]

	Number of cases for Year		
Country	2006	2007	2008
Argentina	1 607	2 587	3 085
Brazil	797	596	1 275
Colombia	233	125	408
Dominican Rep.	17	10	11
Ecuador	23	84	125
Guatemala	48	97	60
Honduras	138	71	224
Mexico	171	164	99
Nicaragua	148	51	25
Panama	132	78	108
Peru	84	47	59
Uruguay	15	26	128
Venezuela	1 183	–	0
TOTAL	4 596	3 936	5 607

[1] WHO/UNICEF joint reporting forms.[31] Data from the poorest countries were not considered. (–) Means non-reported cases.

According to a report from the Caribbean Epidemiology Center (CAREC), the rationale for pertussis surveillance is monitoring the impact of the immunization system, identify high risk areas and detecting outbreaks, which must then be investigated.[29] Data from Figure 1 corresponds to the period 1980-2005. It was observed as a whole an improved surveillance among countries belonging to CAREC (Fig. 1). The feature was particularly remarkable in number of cases rising from 1980 to 1981 when routine vaccination against diphtheria, pertussis, tetanus and poliomyelitis were conducted in all 19 Caribbean countries.

Although confirmation of cases by laboratory methods were already available, most cases were clinically confirmed by physicians. Between 1999 and 2005, 22 % of the 85 cases reported were laboratory confirmed.

The decrease in cases was reflected in the history of the EPI program (Expanded Program on Immunization). In 1980, 40 % of children less than 1 year of age had received the diphtheria, pertussis and tetanus (DPT) vaccine. The annual vaccine coverage figures increased steadily so that by 1985, it had exceeded 80 %. Thereafter, with the exception of 1988, the combined coverage figure for member countries continued to exceed 80 %, with some countries achieving 100 % vaccine coverage (CAREC, Historical Review of EPI, 1994).[29]

Widespread DTP vaccination has not eliminated pertussis in Latin America and pertussis has re-emerged as

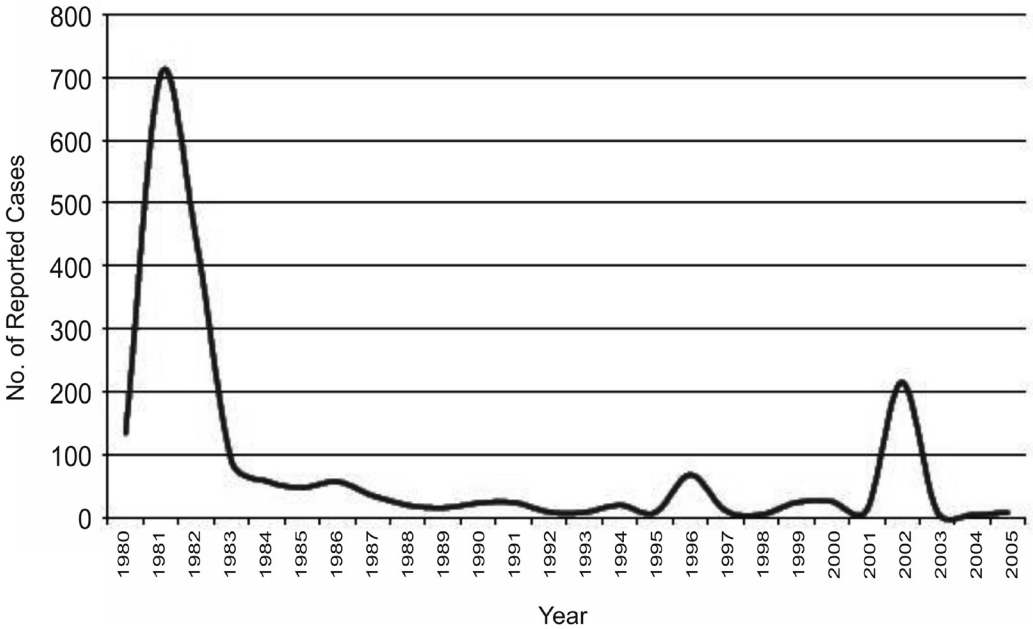

Fig. 1. *Reported (clinically and laboratory confirmed) Cases of Pertussis in all CAREC Member Countries 1980 – 2005.*

a health problem, just like in the rest of the world. Due to scarcity of epidemiology information for many countries, data are obtained mostly from Argentina, Brazil, Colombia, Costa Rica and Mexico.[19,32,33] For instance, it was observed that pertussis was identified most frequently in pediatric patients under 1 year of age, being most likely to be hospitalized.[33] Hospitalization rates from three community-based studies showed a dependency on age with rates of 9.4 % (median age, 9 years), 62 % (median age, 3 months) and 72 % (median age, unreported). In hospital-based studies, laboratory case confirmation was reported at rates of 10.2-78.4 %, whilst an overall rate of 28.4 % was seen in all patients with suspected pertussis.

Mortality predominates among children under 1 year with the rising prevalence of pertussis attributed to waning immunity in adolescents and adults. Laboratory confirmation was made only in approximately 25 % of suspected cases, making accurate estimates of disease burden problematic, thus contributing to under-reporting in all age groups.

An overview of the epidemiology reported in various Latin American countries

As pointed out before, information regarding pertussis epidemiology in Latin America is scarce mainly due to non-uniformity of the methods involved in diagnosis as well as the non existence of regular reports in the number of cases. Nevertheless, data from various countries have been reviewed and may reflex a rough panorama of the matter.

According to a review by Ulloa-Gutiérrez, an epidemiological study from 25 666 Mexican serum samples of 1-14 years of age revealed an increasing prevalence of antibody levels to *B. pertussis* but decreasing titers with increasing age, which suggests the waning immunity associated with the disease and consequent risks.[19] Furthermore, the highest rates of pertussis morbidity and mortality were associated to the lowest seroprevalence. Another study in Jalisco demonstrated that the most affected group ranged from 3 to 11 months of age and 85 % of cases being household contact infection. A most recent report in Mexico City by 2002-2003 revealed from a group of 61 High School students, 12-15 years old, a seroprevalence of *B. pertussis* isolates in 32.8 % of cases with a cough persisting for more than 2 weeks.[19]

In Costa Rica,[19] this review reported an outbreak of pertussis from 2000 to 2001 both hospitalized and ambulatory children, most of them before 1 year and too young to be vaccinated. It was known that in most of the cases the mother was the source of infection. Moreover, a more severe outbreak occurred from the end of 2005 to 2007, with decreasing numbers by 2008. The outbreak was considered as the most severe one over the last 40 years so that the government launched a TdaP nationwide vaccination program to mothers within 48 hours postpartum at the public maternities of the country.

On the other hand, it had been observed in Panama a national increase of pertussis in number of cases and deaths by 2006 corresponding to a rate of 132/100 000. Previous outbreaks occurred in 1998 and 2002 showing rates of 226/100 000 and 88/100 000 respectively, which suggests the cyclic nature of the disease. This behavior seems to be the result of accumulation of susceptible individuals, mainly pre-scholars and young people.[34] An interesting study concerning hospitalizations at the Hospital del Niño from 2001 to 2008 was carried out.[35] In order to lowering the impact of *B. pertussis* in infant population, the authors have recommended two main strategies: reinforcing immunity by vaccination in High School or University adolescents and vaccination of mothers *post-partum*, which enhances antibody levels after 2–3 weeks of immunization, thus reducing the risk of newborn infection. Both strategies was recently introduced since the high incidence and mortality in newborns before 3 months age.[34]

A relatively poor information about epidemiology of pertussis have been reported from Colombia.[36] Since 1978, when a regular DPT vaccination program was implemented in infants before 5 years old, there was a considerable reduction of morbidity and mortality. Following the review of Ulloa-Gutiérrez and Avila-Abreu,[19] in 2005, a total of 139 cases were confirmed, being 80.6 %

younger than 1 year, 14.4 % of 1-4 years old, then 2.9 % of 5-14 years old and 1.4 %, 15-44 years old. It was also observed a mortality rate increase from 1.7 % in 2004 to 6.5 % in 2005. In addition, the roll of healthy carrier's adolescents of 12-19 years old in transmission of pertussis was searched in the Department of Tolima, but the authors have not reached to conclusive results.[37]

Concerning Argentina, the review of Hozbor reports a steady increase of pertussis cases from 2002.[32] In fact, the authors have detected that most of them were younger than 6 months of age with lesser than 3 vaccine doses, although adolescents and adults have been detected as well. These findings seem to be associated to waning immunity due to antigenic divergence between local and clinical isolates and vaccine strains or lack of current vaccines to induce long-lasting immunity. The rate of cases x 100 000 inhabitants from 1997 to 2006, according to the Argentinean National Epidemiological Surveillance System (SINAVE) firstly showed a decline from 1997 to 2002, which was followed by an increment till 2006, even before the introduction of PCR techniques in diagnosis. Further data from the Argentinean Pertussis National Reference Center covers from 2004 to 2007, where 8 823 samples from various provinces were registered and analyzed. The increment was as follows: 188 in 2004, 206 in 2005, 523 in 2006 and 617 in 2007. Data of patient age were available in 1 460 cases. Pertussis confirmed cases by age were most of them younger than 6 months old (865 of the 1 460 cases, 59.2 %); the rest was distributed as follows: 16.06 % (235) between 6 and 17 months of age, 12.8 % (188) between 18 months and 6 years old, and 11.7 % (172 cases) older than 6 years. The authors also reported an outbreak from the province Neuquén, which was analyzed separately.[32] The outbreak occurred in 2005, where from a total of 2 806 suspected pertussis cases, 568 were laboratory confirmed but out of 352 of them had data of age and vaccination status of patients. The outbreak showed a behavior rather different in percentage of cases per age from the previously ones in the rest of the country, being most of cases in the range from 18 months to 6 years old, that is, it was observed a shift to older ages cases having more vaccine doses. These observations were assumed to be the result of a change in the pathogen population so that the vaccine used conferred less protection.[32,38] A similar behavior was also reported in The Netherlands, during the 1996-1997 outbreak.[39] The effect of this waning immunity may be aggravated by the antigenic divergence between clinical isolates and pertussis vaccine strains. In fact, the authors have shown molecular evidences of virulence factors changes in circulating *B. pertussis* strains respect to those for current vaccine strains.

Various reports from Brazil have followed well-documented epidemiological data. As a whole, results have been pointed out that adults and other household members as the potential source for pertussis transmission, although mass-vaccination with whole-cell pertussis DTwP was established in the 1970s.[19,40] Re-emergence of pertussis was not firstly recognized until 2006, when detected in some areas of Rio de Janeiro. The authors of this paper were looking for a mathematical model to analyze the dynamics of the disease and they found mathematically a higher incidence than that reported, specially in older children, adolescents and adults.[41] Some other reports from other regions of the country revealed increasing numbers in positive cases, mostly in infants younger than 6 months as well as adolescents and adults. For instance, a rising from 202 samples in 2001 to 821 in 2005 was reported in Sao Paulo.[19] Furthermore, a paper from Baptista[40] revealed loss of childhood protective DwPT vaccine effect in infected individuals older than 19 years. Vaccine reduces the transmissibility of breakthrough vaccinated cases, which suggests that revaccination of adolescents and adults may decrease pertussis transmission.

The epidemic outbreak of pertussis in California: its relation to Latin America

By the time of writing this manuscript, an epidemic outbreak of pertussis in California, USA was taking place.[42,43] Howsoever California is next to Latin America for historical reasons, the current development, approach and treatment of the disease must be important and helpful for our purposes of this review. The outbreak was detected on March, this year and between Jan. 1st and July 16, there were 1 496 reported cases of pertussis and 700 possible cases under investigation. Six infants have died from the bacterial respiratory disease. California has reported that the number of reported deaths and illnesses this year due to whooping cough (pertussis) is the highest in 52 years.[44]

Since pertussis vaccine immunity is strongest for about 3 years and then gradually decreases over the next 2 to 7 years, it is important for teens and adults renewal vaccination. It has been recognized that in this case, teens and adults probably have failed to renew protection against pertussis through Tdap vaccination, so that if they get sick, can infect infants who have not completed their three dose primary series of pertussis vaccines when they are about six months old. The fact that some parents were refusing vaccines or using alternative immunization schedules likely was not helping prevent these types of outbreaks of vaccine preventable infections either. Therefore, the Center for Disease Control and Prevention has established recommendations for vaccinating children, adolescents and adults against the bacterial respiratory disease. The following are the Pertussis vaccination guidelines:[45]

- Infants and children need five doses of the DTaP (diphtheria, tetanus, acellular pertussis) vaccine. One dose is recommended at each of the following ages: 2 months, 4 months, 6 months, 15 to 18 months, and 4 to 6 years.
- Adolescents age 11 or 12 should receive one dose of the Tdap booster vaccine.
- Adults age 19 through 64 should receive one dose of Tdap if they never had the booster, and if their most recent tetanus toxoid-containing vaccine was received at least 10 years earlier.
- Adults in close contact with an infant younger than 12 months who previously have not received Tdap should receive one dose.
- Health professionals with direct patient contact who previously have not received Tdap should receive one dose.

Epidemiology, re-emergence of pertussis and vaccination in Cuba

Cuba has informed the elimination of pertussis in 1994 as a consequence of the application of a National Program of Immunization of preventable diseases.[46] Nevertheless, re-emergence of pertussis may occur, being this phenomenon worldwide observed. As far as the authors know, there is no report from Cuba dealing with pertussis re-emergence in the recent decade.[47]

Pertussis vaccine in Cuba is carried out as a DTP (diphteria, tetanus, pertussis) whole-cell inactivated

pertussis component of the trivalent[46,47] preparation, even as a part of tetravalent or pentavalent vaccines have been developed and applied.[48] Scheme of vaccination begins at early stages of childhood, which ranges in doses from 2 months of age, 4, 6 and 15 months, the final dose. Hence, this scheme of vaccination might have contributed to eradication of pertussis in this case as suggested before.[47]

Despite of all above, non-reporting of pertussis may occur due to cumbersome procedures for isolation, cultivation and identification of the bacterium[9] so that long-lasting cough might mask actual cases of the disease. This is particularly important for adolescents and adults, who are recognized and identified elsewhere as risk groups for household infection of infants. Moreover, it has been reported that adolescents and adults may become infected with *B. pertussis* showing milder or asymptomatic disease[49] or indistinguishable from other respiratory infections. Therefore, a practical approach would be developing and applying such procedures as a part of public medical assistance.

Concerning vaccines and vaccination, current antipertussis Cuban formulae contain whole cell inactivated preparations as an active ingredient.[50] Protective efficacy of whole cell vaccines ranges from 70 to 85 %. Since this kind of vaccine is described to cause adverse reactions such as fever, pain and even neurological disorders, acellular vaccines have become a much more practical option for pertussis vaccination in the whole World, both in developed and developing countries.[51] Such vaccines contain structural immunogenic components of the bacteria in various amounts, i.e., detoxified pertussis toxin filamentous hemagglutinin, pertactin, and fimbrial antigens and show similar levels of protection as the whole-cell vaccines.[49]

In order to produce a Cuban acellular pertussis vaccine, various serious efforts have been carried out.[52,53] Vaccine strains that are currently used were submitted to genetic manipulation. The different strategies comprise toxin genetic inactivation for enhanced expression as well as pectartin and fimbrial expression in these strains.[52] The resulting strains must be evaluated in animal models, i,e., sensitization to histamines in mice[54] before growing in bench fermentors and further scaling-up for production.

Another approach in this matter is the development of an acellular vaccine candidate based on a proteoliposome derived from *B. pertussis*.[53] Protective ability and reactogenicity evaluation of the proteolisosome indicates a high protective immune response on clinical strains. Moreover, proteomic analyses showed the presence of outer membrane proteins including pertactin and fimbriae, which are normal components in vaccines as well as lipopolysaccharide (LPS), conferring to proteolisosome adjuvant activity as immunomodulator. Therefore, the proteoliposome may be considered as a potential vaccine and adjuvant.

CONCLUSIONS

Despite of being a disease well-know and treated, even vaccination programs applied, whooping cough or pertussis, named the coqueluche, is still one of the most important maladies affecting Mankind, mainly due to its Worldwide re-emergence. Besides other preventable diseases, it is fatal for newborn infants and may affect adolescents and adults as well, both in developed and developing countries. These age groups have been demonstrated that constitute the source of infection for infants as surrounding household.

This behavior has also been observed in Latin America, from Mexico to Argentina and the Caribbean, and according to Ulloa-Gutiérrez,[19] the actual burden of the disease in the Region is unknown so that epidemiological and reporting surveillance must be improved, which involves pertussis molecular diagnosis. Respect to vaccination coverage, acellular vaccines must substitute whole-cell vaccines and universal adolescent-adult vaccination should be recommended.

In addition, other strategies must be considered: selective immunization of mothers and close family contacts of newborns; selective immunization of health care workers; selective immunization of child care workers; preschool booster at 4-6 years of age; and reinforcement and improvement of current infant and toddler immunization strategies.[55,56]

All the above are general considerations, which may be adapted to every country and immunization program, provided that further data are necessary to support every particular situation.

BIBLIOGRAPHIC REFERENCES

1. Cotter PA, Miller JF. *Bordetella*. In: E. A. Groisman (ed). Principles of bacterial pathogenesis. London, United Kingdom: Academic Press, Ltd: 2001:pp.619-674.
2. Lapin JH. Whooping cough. Charles C Thomas, Springfield, IL:1943.p.155-174.
3. Baillou G. Constitutio aestiva. In: Classic Description of Disease, 3rd edn, 6th print. Edited by Major RH. Charles C Thomas, Springfield, IL: 1965:pp.210-212.
4. Mattoo S, Foreman-Wykert AK, Cotter PA, Miller JF. Mechanisms of *Bordetella* pathogenesis. Front. Biosci. 2001;6:E168-E186.
5. Cherry JD, Heininger U. Pertussis and other *Bordetella* infections, In: RD Feigin, JD Cherry, GJ Demmler and S Kaplan (ed.), Textbook of pediatric infectious diseases, 5th ed. Philadelphia, Pa: The W. B. Saunders Co: 2004.p.1588-1608.
6. Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? Lancet Infect. Dis. 2003;3:413-418.
7. Bamberger ES, Srugo I. What is new in pertussis? Eur J Pediatr. 2008;167:133-139.
8. Tan T, Trindale E, Skowronski D. Epidemiology of pertussis. Pediatr Infect Dis J. 2005;24 Suppl:S10-S18.
9. Wood N, McIntyre P. Pertussis: review of epidemiology, diagnosis, management and prevention. Paediatric Resp. Rev. 2008;9:201-212.
10. World Health Organization. Global Burden of Disease Estimates 2002. [Date accessed: 14 July 2010].Available at: http://www.who.int/healthinfo/bodgbd2002revised/en/index.html.
11. Baron S, Mjamkepo E, Gimprel E *et al.* Epidemiology of pertussis in French hospital in 1993-1994: thirty years after a routine use of vaccination. Pediatr Infect Dis J.1998;17:412-418.
12. Tanaka M, Vitek C, Pascual F *et al.* Trends in pertussis among infants in the United States, 1980–1999. JAMA. 2003;23:2968-2975.
13. Godfroid F, Denoel P, Poolman J. Are vaccination programs and isolate polymorphism linked to pertussis re-emergence. Expert Rev Vaccines. 2005;4:757-778.
14. De Melker H, Versteegh F, Schellekens J *et al.* The incidence of *Bordetella pertussis* infections estimated in the population from a combination of serological surveys. J Infect. 2006;53:106-113.
15. Hochwald O, Bamberger E, Srugo I. The Return of Pertussis: Who is Responsible? What Can Be Done? Israel Med Ass J. 2006;8:301-307.
16. National Centre for Immunisation Research, Surveillance of Vaccine Preventable Diseases. Vaccine preventable diseases and vaccination coverage in Australia, 1999-2000. Commun Dis Intell. 2002;26 Suppl 1:S1-S111.
17. Galanis E, King A, Varughese P *et al.* Changing epidemiology and emerging risk groups for pertussis. CMAJ. 2006;174:451-452.

18. Bettinger J, Halperin S, De Serres G *et al.* The effect of changing from whole p-cell to acellular pertussis vaccine on the epidemiology of hospitalized children with pertussis in Canada. Pediatr Infect Dis J.2007;26:31-35.
19. Ulloa-Gutiérrez R, Avila-Agüero ML. Pertussis in Latin America: current situation and future vaccination challenges. Expert Rev Vaccines. 2008;7(10):1569-1580.
20. Bisgard KM, Pascual FB, Ehresmann KR *et al.* Infant pertussis: who was the source? Pediatr Infect Dis J. 2004;23:985-989
21. Wendelboe AM, Njamkepo E, Bourillon A *et al.* Transmission of *Bordetella pertussis* to young infants. Pediatr Infect Dis J. 2007;26:293-299.
22. Schellekens J, Von König CH, Gardner P. Pertussis source of infection and routes of transmission in the vaccination era. Pediatr Infect Dis J. 2005;24:819-824.
23. Mooi F R, Van Loo I H M, Van Gent M, He Q, Bart M J, Heuvelman K J *et al. Bordetella pertussis* strains with increased toxin production associated with Pertussis resurgence. Emerging Infectious Diseases. 2009;15(8):1206-1213.[Date accessed: 20 July 2010]. Available at: www.cdc.gov/eid
24. Wendelboe AM, Van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J. 2005;24:S58-61.
25. He Q, Mertsola J. Factors contributing to pertussis resurgence. Future Microbiol. 2008;3:329-339.
26. Senzilet LD, Halperin SA, Spika JS, *et al.* Pertussis is a frequent cause of prolonged cough illness in adults and adolescents. Clin Infect Dis. 2001;32:1691-1697.
27. Knuf M, Schmitt HJ, Wolter J *et al.* Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. J. Pediatr. 2008;152(5):655-660.
28. Luz PM, Codeço CT, Werneck GL. A reemergência da coqueluche em países desenvolvidos: um problema também para o Brasil? (The resurgence of pertussis in developed countries: a problem for Brazil as well?) Cad. Saúde Pública, Rio de Janeiro. 2003;19(4):1209-1213.
29. Pertussis. Data Source: CAREC Surveillance Database as at October 2008. [Date accessed: 19 July 2010]. Available at: www.carec.net/pdf/mobidityreview/20.%20Pertussis.pdf
30. WHO vaccine-preventable diseases: monitoring system. 2006 global summary. [Date accessed: 14 July 2010]. Available at: www.who.int/vaccines-documents/GlobalSummary/GlobalSummary.pdf
31. Number of Reported Pertussis Cases in the Americas, 1990-2008. *Source:* Country communications, EPI Tables 1999-2003 and Country reports using PAHO-WHO/UNICEF Joint Reporting Forms (JRF) since 2004. Updated: 1st June 2010. [Date accessed: 14 July 2010]. Available at: http://amro.who.int/english/ad/fch/im/Pertussis_NumberCases.pdf
32. Hozbor D, Mooi F, Flores D, Weltman G, Bottero D, Fossati S *et al.* Pertussis epidemiology in Argentina: trends over 2004-2007. Journal of Infection. 2009;59:225-231.
33. Whitford D, Lima T, Sauboin C, Cohen C. Prevalence of pertussis in Latin America. [Date accessed: 14 July 2010]. Available at: www.kenes.com/espid2010/abstracts/pdf/583.pdf.
34. Ministerio de Salud de Panamá. Boletín Estadísticas de Salud. Años1999-2007. [Date accessed: 14 August 2010]. Available at: http://www.minsa.gob.pa
35. Nieto Guevara J, Luciani K, Montes de Oca A, Mateos M, Estripeaut D. Hospitalizaciones por *Bordetella pertussis*: experiencia del Hospital del Niño de Panamá, periodo 2001-2008. An Pediatr (Barc). 2010. [Date accessed: 14 July 2010]. Available at: doi:10.1016/j.anpedi.2009.11.012
36. Instituto Nacional de Salud de Colombia, Subdirección de Vigilancia y Control en Salud Pública. Colombia, Bogotá. Informe Quincenal Epidemiológico Nacional. 2006;11(5):73-76.
37. Morón Duarte LS, Moreno J, Gracia M, Realpe ME, Peña Daza GL. Estado de portadores de *Bordetella pertussis* en adolescentes de 12 a 19 años en el Departamento del Tolima, Colombia, 2007. Investigaciones Andina. 2008;10(17):7-26.
38. Fingermann M, Fernández J, Sisti F, Rodríguez ME, Gatti B, Bottero D *et al.* Differences of circulating *Bordetella pertussis* population in Argentina from the strain used in vaccine production. Vaccine. 2006;24(17):3513-3521.
39. Van Boven M, De Melker HE, Schellekens JFP, Kretzschmar M. A model based evaluation of the 1996-7 pertussis epidemic in the Netherlands. Epidemiology Infect. 200;127:73-85
40. Baptista PN, Magalhães VS, Rodrigues LC. The role of adults in household outbreaks of pertussis. Int J Infect Dis. 2010;14(2):111-4.
41. Luz PM, Codeço CT, Werneck GL, Struchiner CJ. A modelling analysis of pertussis transmission and vaccination in Rio de Janeiro, Brazil. Epidemiol Infect. 2006;134:850-862.
42. Billingsley K. State of California-Health and Human Services Agency, California Department of Public Health. 2010. [Date accessed: 20 August 2010]. Available at: www.mbc.ca.gov/lnc-af1-10-24.pdf
43. Moyer CS. Pertussis epidemic in California linked to vaccination gaps. Posted July 26, 2010. [Date accessed: 14 July 2010]. Available at: www.ama-assn.org/amednews/2010/07/26/prl10726.htm
44. Barrett S. Pertussis Epidemic in California. 2010); [Date accessed: 14 August 2010]. Available at: www.quackwatch.org/.../immu/pertussis_california.html
45. Centers for Disease Control and Prevention. [Date accessed: 24 August 2010]. Available at: www.cdc.gov/vaccines/vpd-vac/pertussis/in-short-both.htm
46. Collazo MM, Pérez-Cristiá R. Programa Nacional de Inmunización en Cuba. Implicaciones económicas y beneficios obtenidos. [Date accessed: 14 July 2010]. Available at: www.economiadelasalud.com/ediciones/56/08_pdf/cuba.pdf
47. Arredondo A., Amores J. Enfermedades reemergentes: factores causales y vigilancia. AMC. 2009;13(2):pp.1-15. [online]. Available at: http://scielo.sld.cu/scielo.php?script=sci_pdf&pid=S1025-02552009000200016&lng=es&nrm=iso&tlng=es
48. López E, Silva R, Acevedo B, Buxadó JA, Aguilera A, Herrera L. Biotechnology in Cuba: 20 years of scientific, social and economic progress. J Comm Biotechnology. 2006;13:1-11.
49. Versteegha FGA, Schellekensb JFP, Fleerc A, Roordd JJ. Pertussis: a concise historical review including diagnosis, incidence, clinical manifestations and the role of treatment and vaccination in management. Rev Med Microbiology. 2005;16:79-89.
50. Edwards KM, Decker MD. Pertussis vaccine. In: S. Potkin and W.A. Orestein (ed.), Vaccines, 4th ed. Philadelphia, Elsevier: 2004:p.471-528.
51. Robbins JB, Schneerson R, Keith JM, Shiloach J, Miller M, Trollors B. The rise in pertussis cases urges replacement of chemically-inactivated with genetically-inactivated toxoid for DTP. Vaccine. 2007;25(15):2811-2816.
52. Proenza LL. Construcciones genéticas para la expresión episomal y cromosomal de toxoide pertúsico en *Bordetella pertussis*. [Tesis en opción al grado de Máster en Bioquímica mención Biología Molecular]. La Habana, Centro Nacional de Investigaciones Científicas, 2010.
53. Pérez JL, Gaillard ME, Fingermann M, Bottero D, Reyes G, Fernández S *et al. Bordetella pertussis* derived proteoliposome as acellular vaccine candidate. preliminary antigenic and biological characterization. International Workshop on Vaccine Adjuvants and Parasitic Vaccines, 13-18 April 2008. Club Amigo Varadero Hotel, Matanzas, Cuba; 2008.
54. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other Bordetella subspecies. Clin Microbiol Rev. 2005;18(2):326-382.
55. Forsyth K, Tan T, Von König CH, Caro JJ, Plotkin S. Potential strategies to reduce the burden of pertussis. Pediatr Infect Dis J. 2005;24(5 Suppl):S69-74.
56. Ulloa-Gutiérrez R. Estrategias actuales de vacunación contra tos ferina en niños y adultos (Current vaccination strategies against pertussis in children and adults). Acta Pediátr Costarric. 2008;20(2):81-87.