Epidemiology of *Bordetella pertussis* in a children's hospital

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ABSTRACT

Introduction. Pertussis or whooping cough continues to be a major cause of morbidity and mortality in infants younger than 1 year old.

Objectives. To describe the clinical and epidemiological profile of *Bordetella pertussis* and to analyze the factors associated with confirmation by PCR and case fatality rate.

Material and Methods. Prospective, cohort study conducted between December 2003 and December 2011. The study included children seen at the Hospital de Niños Ricardo Gutiérrez suspected of pertussis. The factors associated with confirmation by PCR and the case fatality rate by relative risk (RR) with a 95% confidence interval were studied.

Results. Six hundred and twenty patients with a 38% of positive cases (236/620) were included, 3 cases were confirmed by epidemiological link. Confirmed cases (239) showed a seasonal pattern from September through February, a median age of 3 months old, and 89% had received less than three vaccine doses. Eighty six percent of patients were hospitalized: their median length of stay was 7 days. A total of 99% of patients were eutrophic, 98% were immunocompetent and 17.5% required intensive care. The clinical presentation was analyzed in 480 patients. Of them, 38% (184) had a positive PCR result and their symptoms were: 96.2%, cough; 76.5%, paroxysmal cough; 57.9% cyanosis; 55.7%, respiratory distress; 29%, fever; 22.4%, apnea; 21.9%, vomiting after coughing. A multivariate analysis identified the following as independent predictors associated with confirmation of pertussis by PCR: paroxysmal cough (OR 2.52: 1.50-4.22; p= 0.000) and leukocytosis upon admission >20 000 white blood cells/mm^3 (OR 7.96: 4.82-13.17; p= 0.000); having developed fever reduced the chance of having a positive PCR result (OR 0.47: 0.29-0.77; p= 0.003). The case fatality rate for hospitalized patients was 6.8%. Leukocytosis >30 000 white blood cells/mm$_3$ was a predictor of fatality (RR 6.7: 1.88-23.9; p= 0.001).

Conclusions. Confirmed cases were mostly infants younger than 1 year old who were healthy before and who had not completed their primary immunization schedule. Paroxysmal cough and leukocytosis were associated with PCR diagnosis, while leukocytosis was a predictor of mortality.

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INTRODUCTION

Bordetella pertussis infection continues to be a major public health problem, even in countries with a high vaccination coverage. As per the World Health Organization's estimates, in 2008 pertussis accounted for 16 million cases and approximately 195 000 child deaths around the world.[1,2]

Although the use of vaccines with a *pertussis* component in regular immunization schedules has reduced the global burden of disease in 90% from the pre-vaccination stage, a re-emergence of this disease with outbreaks around the world has been observed, both in developed and developing countries.[3-7]

There are several causes for the re-emergence of *pertussis*, including suboptimal levels of vaccine coverage, the natural loss of antibodies following vaccination, and the lack of a long-lasting immunity after a natural infection. In addition, *Bordetella pertussis* is contagious during the catarrhal stage, when it has not yet been diagnosed. Many patients are school-aged children or adolescents with atypical symptoms who are diagnosed late, thus favoring the transmission of the disease due to the lack of adequate treatment and also because no measures are taken in relation to contact with other individuals. Other authors have highlighted the importance of having new laboratory techniques available which are more sensitive and specific, such as the implementation of the PCR diagnosis, which would favor case reporting. Besides, as certain mutations in circulating strains have been described, the correlation with the vaccine strain could be lower,

although the clinical implication of such finding has not yet been determined.[8-11]

In Latin America, many countries have recognized more and more the re-emergence of pertussis and the importance of having a better knowledge on its incidence, hospitalization and mortality rates. In July 2011, the PAHO Technical Advisory Group gathered in Buenos Aires, where they emphasized the problem and issued recommendations on the need to have a better quality epidemiological surveillance system, promote laboratory diagnosis and improve vaccination coverage across the continent.[12]

In Argentina, in 2011, the National Ministry of Health provided data that confirmed whooping cough cases and the death of 70 children; 91% of them younger than 5 months old. Most infants had not been vaccinated or were partially immunized, which is expected at such age (the first dose in Argentina is scheduled to be administered at 2 months old).[13-15]

The National Advisory Committee on Immunization and the National Program for the Control of Vaccine-Preventable Diseases (*Program Nacional de Control de Enfermedades Inmunopreve–nibles*, ProNaCEI) updated the Argentine immunization policy with the aim to control this disease. Based on the international recommendations, now also 11 year old children, members of health teams in contact with children, pregnant women as of 20 weeks of gestation and those living with preterm infants with a weight lower than 1500 grams should also be vaccinated. The basic immunization schedule is still in place with no modifications on the administration of three primary doses and two boosters, one at 18 months old and one at the time of starting primary school.[16]

Infants younger than 6 months old account for the population at highest risk and a major concern should be to reduce morbidity and mortality in this age group, in accordance with international recommendations.[17-19] It is imperative to determine the burden of disease, the clinical characteristics, the complications and the mortality rate of pertussis in this age group so as to contribute to design better public health policies and establish priorities.

OBJECTIVES

To describe the clinical and epidemiological profile of patients seen at Hospital de Niños Ricardo Gutiérrez with a diagnosis of pertussis between December 2003 and December 2011.

To analyze factors associated with the laboratory confirmation of pertussis.

To analyze risk factors associated with mortality from pertussis.

POPULATION

All patients seen at Hospital de Niños Ricardo Gutiérrez with a diagnosis of suspected pertussis between December 1st, 2003 and December 31st, 2011 were included.

MATERIAL AND METHODS

This was a prospective cohort study conducted between December 1st, 2003 and December 31st, 2011.

All patients were selected if they complied with the criteria for a suspected case or laboratory confirmed case, according to the following definitions:[20]

Suspected case of pertussis: a person of any age (especially young children) who has paroxysmal cough, inspiratory stridor, coughs up spinnable mucus, and vomits after coughing. He/she can also present leukocytosis with lymphocytosis. Infants younger than 6 months old can have atypical symptoms, with apnea as the main manifestation. Older children and adults could have persistent cough with no stridor.

Confirmed case: any suspected case with a positive finding of *Bordetella pertussis* in nasopharyngeal secretions obtained by culture isolation or molecular detection by polymerase chain reaction (PCR), or epidemiological link with a laboratory confirmed case.

The nasopharyngeal secretions of suspected cases were analyzed by PCR. In order to confirm the presence of *Bordetella pertussis*, the molecular detection of 2 genes was done by conventional techniques: the insertion sequence IS481 and the *pertussis* toxin promoter sequence. As of 2011, a new diagnostic method was implemented, which allows to distinguish between *Bordetella pertussis*, *B. holmessi* and *B. parapertussis* using a real-time PCR with multiple targets. For interpreting results, an algorithm is applied, which includes the results of the insertion sequence amplification of each insertion sequence (IS481, pIS481, hIS481) and the *pertussis* toxin amplification (ptxS1).

An epidemiological card for each child included in the study was completed with his/her personal data, history of prematurity, malnutrition or pre-existing chronic disease, immunization history, clinical manifestations upon admission, length of stay in days, diagnostic procedures, clinical course and contact with

household members who have symptoms compatible with *Bordetella pertussis* infection.

In 2006, the Ministry of Health of the City of Buenos Aires modified the pertussis surveillance card, and as of that year, clinical data and factors associated with laboratory confirmation have been included.

Data were analyzed using the Epi Info2000 (CDC, Atlanta) software. Categorical outcome measures (comparison of proportions in the studied groups) were assessed using the χ^2 test with Yates's correction or Fisher's exact test, as applicable. A value of $p< 0.05$ was considered significant. Risk factors were analyzed using the relative risk (RR) as a measure of association, with a 95% confidence interval.

RESULTS

Between December 1st, 2003 and December 31st, 2011, 620 patients who complied with the criteria for suspected case were included and samples collected from them were analyzed by PCR; of them, 38% (236/620) were positive for *Bordetella pertussis*. Three other patients were diagnosed by epidemiological link, therefore totaling 239 confirmed cases to be studied. The epidemic curve showed a seasonal pattern from September through February, corresponding to the summer and spring months in the southern hemisphere, more evident in 2006-2007 and 2010-2011 (*Figure 1*).

Characteristics of confirmed cases: the characteristics of all 239 confirmed cases were analyzed. The median age was 3 months old (range: 15 days old to 8 years old); 80.3% were younger than 6 months old at the time of consultation, and 92.1% were younger than 1 year old. Female children accounted for 53.6% of the participants, and 80.3% lived in the province of Buenos Aires.

Immunization history was obtained from 98.7% (236) of confirmed cases. Eighty nine percent of participants had not completed their primary vaccination schedule (3 doses), 48.3% had not started their vaccination schedule (0 doses), and 11% (26 children) had received 3 doses (*Figure 2*).

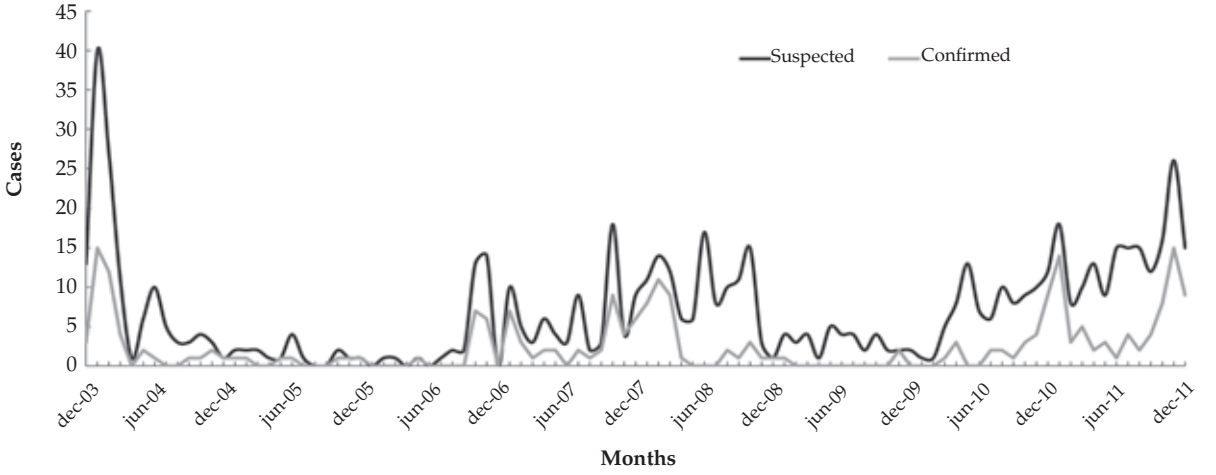
Of the confirmed cases, 86.2% (206) required hospitalization. *Table 1* shows some of the clinical characteristics of these patients.

The mean length of stay was 11.7 days (median: 7 days; range: 1-82 days).

Hospitalization in the intensive care unit was required in 17.5% (36/206) of the cases, with a mean of 18.5 days (median: 12 days; range: 1-75 days). A hospital-acquired infection was found in 9.3% of patients after 6 days hospitalized (median: 9 days; range: 6-99 days).

Blood count was performed in 91.3% of patients; the median white blood cell count upon admission was 22 000/mm^3 (range: 7600-132 000/mm^3). In relation to the patients' epidemiological history, 38.3% had been in contact with a person who had cough in the 7 days prior to symptom onset.

FIGURE 1. *Pertussis epidemic curve. Hospital de Niños R. Gutiérrez. From December 1st, 2003 through December 31st, 2011 (suspected cases: 672; confirmed cased: 239)*



Clinical presentation and comparison among positive and negative PCR patients

The clinical characteristics of cases were analyzed as of October 2006. Since that date, 480 patients were studied by PCR; 38% (184/480) were positive and 62% (296/480) were negative.

Cases confirmed by PCR were characterized by cough (96.2%), paroxysmal cough (76.5%), cyanosis (57.9%), respiratory distress (55.7%), fever (29%), apnea (22.4%), vomiting after coughing (21.9%), pneumonia (6%) and seizures (2.2%) (*Table 2*).

The univariate analysis showed that the following were the factors associated with laboratory confirmation (positive PCR or negative PCR): paroxysmal cough (RR 1.64: 1.23-2.18; p = 0.00049), contact with a person who had cough in the 7 days prior to symptom onset (RR 1.34: 1.05-1.70; p = 0.029) and leukocytosis upon admission >20 000/mm^3 (RR 2.72: 2.13-3.47; p= 0.000); while having fever (RR 0.71: 0.54-0.92; p= 0.008) or a history of perinatal respiratory condition (RR 0.44: 0.19-0.99; p= 0.032) reduced the chance of having a positive PCR result.

FIGURE 2. *Pertussis vaccination in confirmed cases. Hospital R. Gutiérrez (2003-2011)*

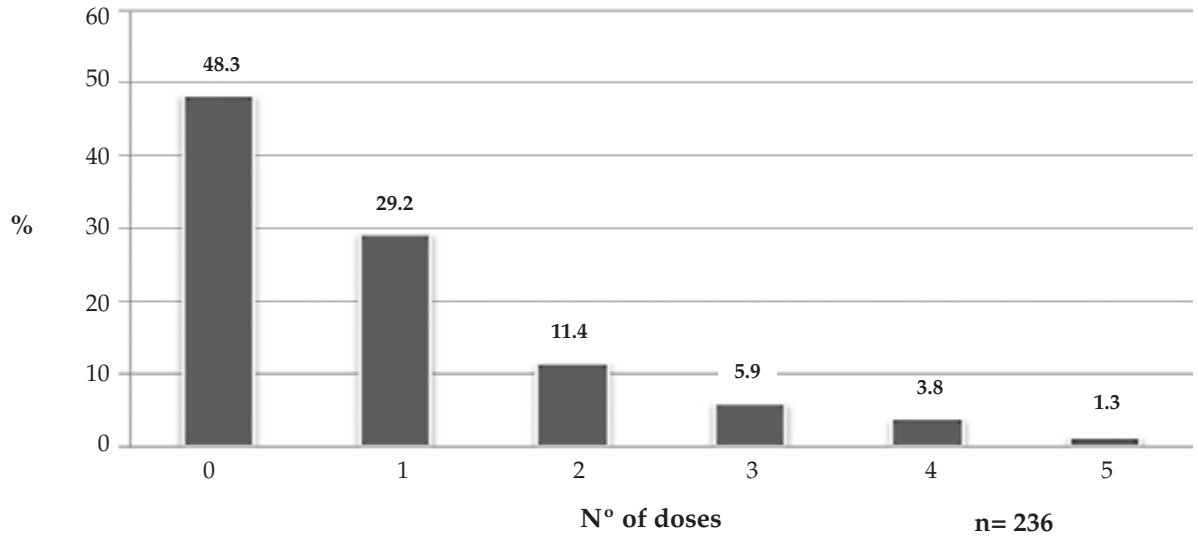


TABLE 1. *Characteristics of patients hospitalized due to pertussis (n= 206)*

Characteristics of hospitalized patients*	n	Percentage
Eutrophic (n= 204)	202	99
Immunocompetent (n= 204)	201	98.5
Infants born at term (n= 204)	183	89.7
Respiratory disease at birth (n= 204)	6	2.9
History of hospitalization due to a respiratory condition (n= 205)	31	15.1
Comorbidity (n= 205)**	33	16.1
Oxygen requirement (n= 197)	158	80.2
Bronchodilators (n= 198)	170	85.9
Mechanical ventilation (n= 206)	25	12.1
Complications (n= 202)	37	18.3
Hospital-acquired infection (n= 204)	19	9.3

* The number between brackets indicates the number of patients available for data collection.
** Of them, 70% had a recurrent bronchial obstructive syndrome.

A multivariate analysis identified the following as independent predictors associated to the confirmation of pertussis by PCR: paroxysmal cough (OR 2.52: 1.50-4.22; $p = 0.000$) and leukocytosis upon admission >20 000/mm^3 (OR 7.96: 4.82-13.17; $p = 0.000$), while having developed fever reduced the chance of having a positive PCR result (OR 0.47: 0.29-0.77; $p = 0.003$).

Fatality risk factors

Of the 239 confirmed cases included in this study between December 2003 and December 2011, 14 children died with a diagnosis of pertussis. The mortality rate in the study group was 6.8%. These children were 2 to 10 months old (median: 2 months old). All patients with information available about their vaccination schedule ($n = 13$) had received at least three doses of the vaccine, 9 had received no dose, 3 had received 1 dose, and 1 had received 2 doses. Upon admission, patients had a white blood cell count higher than 20 000/mm^3 (median: 43 150; range: 20 200-104 400).

A leukocyte count higher than 30 000/mm^3 was considered an independent predictor of mortality (RR 6.7: 1.88-23.9; $p = 0.001$).

DISCUSSION

Whooping cough continues to be a major public health problem. The behavior of pertussis in the studied patients is typical of the re-emergence of this disease in the past years worldwide.[18-22]

In our series, *Bordetella pertussis* was confirmed in 38% of suspected cases, which is higher than the percentage indicated by the ProNaCEI, with a confirmation of 18% of cases in Argentina.[23] In developed countries, more than 50% of cases are confirmed, and for many of them in addition to a routine PCR, a culture is used, which has a high specificity.[24]

It is important that samples sent to the laboratory come from patients who meet the criteria for a suspected case, i.e., clinical observation is essential to obtain better results with a laboratory method such as the PCR. Very low confirmation percentages indicate that cases included do not meet the definition of suspected case, while very high confirmation rates suggest that few cases are suspected of pertussis.

Of the studied cases, 25.6% were recorded in 2011. This is consistent with an outbreak in other regions of Argentina and also with the findings worldwide.[5,7,22]

The series showed a seasonal pattern in spring and summer, similar to what has been reported in other countries.[18,25-27]

The increase in reports might reflect an improvement in the suspicion of pertussis among health team members, especially in the years with a higher pertussis incidence. Of confirmed cases, 80% occurred in infants younger than 6 months old, which means that most individuals in the affected population were not old enough to have a complete primary vaccination schedule. This argument speaks in favor of the importance that is of household members having an adequate vaccination coverage as a prevention strategy for infants younger than 6 months old.[28,29]

In Argentina, vaccination coverage should be improved, especially in relation to the booster dose at 18 months old, which has an 82% coverage, with marked differences observed

TABLE 2. *Clinical signs and symptoms as per the PCR result. Hospital de Niños R. Gutiérrez 2006-2011 (positive PCR: 183; negative PCR: 295)*

Signs and symptoms	Positive PCR % (n)	Negative PCR % (n)	RR (95% IC)	*p*
Cough	96.2 (176)	91.2 (269)	1.86 (0.95-3.63)	0.036
Paroxysmal cough	76.5 (140)	60.3 (178)	1.64 (1.23-2.18)	0.0004
Cyanosis	57.9 (106)	50.8 (150)	1.19 (0.94-1.50)	0.131
Respiratory distress	55.7 (102)	64.0 (189)	0.80 (0.64-1.01)	0.069
Fever	28.9 (53)	41.3 (122)	0.71 (0.54-0.92)	0.008
Apnea	22.4 (41)	17.6 (52)	1.19 (0.91-1.55)	0.199
Vomiting	21.8 (40)	15.2 (45)	1.29 (0.99-1.67)	0.066
Pneumonia	6.0 (11)	9.8 (29)	0.70 (0.41-1.17)	0.14
Seizures	2.2 (4)	2.3 (7)	0.94 (0.43-2.09)	0.894

when comparing provinces and districts within each province. Another aspect to be considered, as mentioned by Gentile, et al., is the impact of the delayed administration of the 18 month booster. Authors found that 29.2% of the doses were administered late. How long were those infants at risk? Such risk is absolutely avoidable.[30]

It draws our attention that our data show that 11% of children had received three or more vaccine doses, which calls to consider the need to conduct studies on the effectiveness of this type of vaccines in the future.

The disease affected mostly previously healthy children, and most of them required hospitalization for one week, with the resulting burden of disease and costs on the health system.

Paroxysmal cough at symptom onset was found to be a risk factor for pertussis. This is relevant information for the clinical practice because it establishes a typical symptom hierarchy which can be observed in young children, but not in adolescents or young adults, who usually have atypical symptoms, especially cough. On the other hand, fever is not associated with this disease and has been an infrequent finding, as mentioned in the bibliography.[8,31]

Another relevant aspect to be analyzed is complete blood count upon admission, specifically to determine the presence of leukocytosis. A leukocyte count equal to or higher than 20 000/mm^3 is a factor associated with pertussis, and a count above 30 000/mm^3 is an independent predictor of mortality.[32,33]

The case fatality rate in our series of patients was 6.8%, with a median age of 2 months old; these data are consistent with those provided by other tertiary care facilities from Argentina.[34] In all cases, deceased patients had a leukocyte count higher than 20 000/mm^3 at pertussis onset, with a median count of 43 150/mm^3. Such finding supports the validity of using a hyperleukocytosis parameter as a predictor of mortality, as described by other authors.[32,33] This is a relevant aspect given that hyperleukocytosis upon admission should be regarded as a predictor of an unfavorable course to warn healthcare professionals and promote active measures aimed at reducing its impact.

The young age of hospitalized patients and deceased infants has forced health authorities to review vaccination strategies. For this reason, in 2012, and facing this new epidemiological reality, a recommendation was issued to vaccinate pregnant women with a single triple acellular dose as of 20 weeks of gestation as a valid strategy to reduce the number of cases in the first three months of life, when infants have not yet received any protection through immunization. Such single dose favors the transmission of antibodies from mother to child, and some pieces of evidence have led to believe that this way infants would be protected during their highest risk period. The current coverage achieved in Argentina for this age group in 2012 is 61.9%. To actively pursue the improvement of coverage levels and of the administration rate of doses at different ages is a task that concerns us all.[13,15]

CONCLUSIONS

Most of the children seen at this children's hospital due to pertussis were otherwise previously healthy, younger than 1 year old and had an incomplete primary vaccination schedule.

Paroxysmal cough and leukocytosis were positively associated with the diagnosis of pertussis by PCR, while a leukocyte count above 30 000/mm^3 was found to be an independent predictor of mortality. ■

REFERENCES

1. World Health Organization. Immunization surveillance, assessment and monitoring *Pertussis*. World Health Organization; 2012. [Accessed on: March 10, 2013]. Available at en http://www.who.int/immunization_ monitoring/diseases/pertussis/en/index.html.
2. World Health Organization. Immunization, Vaccines and Biologicals *Pertussis*. World Health Organization; 2012. [Accessed on: March 10, 2013]. Available at http:// www.who.int/immunization/topics/pertussis/en/index.html.
3. Tan T, Trindade E, Skowronski D. Epidemiology of *pertussis*. *Pediat Infect Dis J* 2005 ;24(5 Suppl):S10-8.
4. WHO (OMS). *Pertussis* vaccines – WHO position paper. Weekly Epidemiological Record. *World Health Organization* 2005;4(80):29-40.
5. California Department of Public Health-Immunization Branch. *Pertussis* Report. 2011. [Accessed on: February 1, 2013]. Available at en http: / / www.cdph.ca.gov / programs / immunize/Pages/Default.aspx.
6. Centers for Disease Control and Prevention. *Pertussis* epidemic--Washington, 2012. *MMWR. Morb Mort Wkly Rep.* 2012;61(28):517-22.
7. Ministerio de Salud - Gobierno de Chile. Informe de coqueluche Año 2011 p. 1-7. [Accessed on: December 22, 2012]. Available at http://www.minsal.gob.cl/portal/ url / page / minsalcl / g_proteccion / g_pni / presentacion_ pni.html.
8. Comité Nacional de Infectología SAP. *Bordetella pertussis*. Libro Azul de Infectología Pediátrica. 4ª ed. Buenos Aires: FUNDASAP; 2012. Págs. 484-93.
9. Long S, Pickering L, Prober C. Principles and Practice of Pediatric Infectious Diseases. 4th ed. Edimburg: Elsevier; 2012.
10. Tatti K, Sparks K, Boney K, Tondella M. Novel multitarget real-time PCR assay for rapid detection of *Bordetella* species in clinical specimens. *J Clin Microbiol* 2011;49(12):4059-66.
11. Fingermann M, Fernánde J, Sisti F, Llanos C, et al. *Bordete-*

lla pertussis y *Bordetella bronchiseptica* aisladas de pacientes pediátricos en Argentina, caracterización molecular e importancia epidemiológica. *Ludovica Pediátr* 2003;4:163-6.
12. Grupo Técnico Asesor sobre enfermedades prevenibles por vacunación. Informe final de la XVIII Reunión del Grupo Técnico Asesor(GTA) sobre Enfermedades Prevenibles por Vacunación de la Organización Panamericana de la Salud, julio de 2011. Buenos Aires, Argentina. 2011.
13. Ministerio de Salud. Programa Nacional de Control de Enfermedades Inmunoprevenibles. Alerta epidemiológico 25/1/2012. Tos convulsa: Aumento de casos y muertes. 2012. [Accessed on: February 10, 2013]. Available at http://www.msal.gov.ar/images/stories/alertas_epidemiologia/2012/alerta-1-tos-convulsa-2012.pdf.
14. Ministerio de Salud de la Nación. Vigilancia de Coqueluche. Boletín Integrado de Vigilancia. 2012;SE 16(116):15-7. [Accessed on: February 17, 2013]. Available at en http://www.msal.gov.ar/images/stories/boletines/ BoletinIntegradoDeVigilanciaVersion_N116-SE16.pdf.
15. Ministerio de Salud de la Nación. Situación coqueluche: Aumento de casos y muertes. Boletín integrado de vigilancia. 2012;SE 4(106):7-20. [Accessed on: February 17, 2013]. Available at http://www.msal.gov.ar/images/ stories / boletines / BoletinIntegradoDeVigilanciaVersion_N106-SE04.pdf.
16. Programa Nacional de Control de Enfermedades Inmunoprevenibles. Fundamentos de la Vacunación de Mujeres Embarazadas con Vacuna Triple Bacteriana Acelular (Dtpa) Argentina 2012. 2012, p. 1-23. [Accessed on: February 17, 2013] Available at http://www.msal. gov.ar/images/ stories/epidemiologia/inmunizaciones/ lineamientos_vacuna_dTpa_%20en_embarazadas.pdf.
17. CDC. Updated Recommendations for Use of Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular *Pertussis* Vaccine (Tdap) in Pregnant Women and Persons Who Have or Anticipate Having Close Contact with an Infant Aged <12 Months-Advisory Committee on Immunization Practices (ACIP) 2011. MMWR Morb *Mortal Wkly Rep* 2011;60(41):1424-6.
18. Gentile A. Infección por *Bordetella pertussis. Arch Argent Pediatr* 2010;108(1):78-81.
19. Gentile A, Romanin V. ¿Podemos controlar la infección por *Bordetella pertussis* en Argentina? Nuevas estrategias. *Rev Hosp Niños B Aires* 2012; 52(236):297-303.
20. GCBA EMS. Coqueluche. Actualización de Normas de Vigilancia y Control Epidemiológico. 2006,1-2.
21. Ulloa-Gutiérrez R, Hernández de Mezerville M, Ávila- Agüero ML. *Bordetella pertussis* en Latinoamérica: ¿estamos reconociendo el problema? *Anales de Pediatría* 2008; 69(3):197-9.
22. Das P. Whooping cough makes global comeback. *Lancet In!ect Dis* 2002;2(6):322.
23. Ministerio de Salud de la Nación-Programa Nacional de Control de Enfermedades Inmunoprevenibles. *Coqueluche Argentina* 2011-2012:1-17.
24. Lind-Brandberg L, Welinder-Olsson C, Lagergárd T, Taranger J, et al. Evaluation of PCR for diagnosis of *Bordetella pertussis* and *Bordetella parapertussis* infections. *J Clin Microbiol* 1998;36(3):679-83.
25. Vickers D, Mainar-Jaime RC, Pahwa P. *Pertussis* in rural populations of Saskatchewan (1995 to 2003): incidence, seasonality, and differences among cases. *Can J Public Health* 2006;97(6):459-64.
26. Canals LM, Labra SF. Análisis no-lineal de la dinámica de enfermedades infecciosas en Chile. *Rev Méd Chile* 1999;127(9):1086-92.
27. Duncan CJ, Duncan SR, Scott S. Whooping cough epidemics in London, 1701-1812: infection dynamics, seasonal forcing and the effects of malnutrition. *Proc Biol Sci.* 1996; 263(1369):445-50.
28. Posse CAR, Miceli INP. Evolución de la coqueluche en la Argentina a finales del Siglo XX. *Medicina* 2005;65:7-16.
29. Romanin V, Salvay MC, Man C, Mistchenko A, et al. Brote de *Bordetella pertussis* en un hospital pediátrico. *Rev Hosp Niños B Aires* 2005;47(214):211-6.
30. Gentile A, Rearte A, Regatky N, Cortez R, et al Esquemas atrasados y oportunidades perdidas de vacunación en niños hasta 2 años atendidos en centros de salud. *Rev Argent Salud Pública.* 2012;3(11):30-6.
31. Guris D, Strebel P, Al. E. Changing epidemiology of *pertussis* in the United States: increasing reported incidence among adolescents and adults, 1990-1996. *Clin In!ect Dis* 1999;28:1230-7.
32. Pierce C, Klein N, Peters M. Is leukocytosis a predictor of mortality in severe pertussis infection? *Intensive Care Med* 2000;26(10):1512-4.
33. Mikelova LK, Halperin SA, Scheifele D, Smith B, et al. Predictors of death in infants hospitalized with *pertussis*: a case-control study of 16 pertussis deaths in Canada. *J Pediatr.* 2003;143(5):576-81.
34. Taffarel P, Bonetto G, Haimovich A. Coqueluche grave, evolución y exanguinotransfusión como tratamiento alternativo. Serie de casos. *Arch Argen Pediatr* 2012;110(4):327-30.