UNIVERSIDAD PERUANA DE CIENCIAS APLICADAS

FACULTAD DE CIENCIAS DE LA SALUD
CARRERA DE MEDICINA

"PREVALENCIA Y FACTORES ASOCIADOS A INFECCIÓN POR BORDETELLA PERTUSSIS EN NIÑOS MENORES DE 5 AÑOS CON INFECCIÓN RESPIRATORIA AGUDA (IRA) EN UN HOSPITAL DE LIMA."

Para optar el título profesional de:

MEDICO CIRUJANO

Autores:

Ivana Pavic-Espinoza

Sandy Bendezú-Medina

Angella Herrera-Alzamora

Asesora:

Juana del Valle Mendoza

Monterrico, 25 de enero de 2016

DEDICATORIA

A nuestra amiga y profesora Juanita por todo el apoyo brindado en este largo proceso y a nuestros padres por el amor y paciencia en esta larga carrera.

AGRADECIMIENTOS

Al Centro de investigación de la Universidad Peruana de Ciencias Aplicadas, nuestra alma mater, y al Instituto de Investigación Nutricional, por permitirnos utilizar sus instalaciones para la realización práctica de este estudio de investigación. Asimismo, a nuestra asesora por su orientación y motivación en este camino.

TABLA DE CONTENIDO

2. ARTÍCULO CIENTÍFICO

High prevalence of *Bordetella pertussis* in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru.

Nombre de los autores:

Ivana Pavic-Espinoza
Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.
+51997012381
Correo electrónico ivanita.89@gmail.com

Sandy Bendezú-Medina
Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.
+51978003120
Correo electrónico sandyta291@hotmail.com

Angella Herrera-Alzamora
Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.
+51989269381
Correo electrónico angella_h@hotmail.com

Juana del Valle Mendoza
Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.
+51988040532

Correo electrónico **jdelvall@upc.edu.pe**

Corresponding autor

Juana del Valle Mendoza

Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.

+51988040532

Correo electrónico **jdelvall@upc.edu.pe**

High prevalence of *Bordetella pertussis* in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru.

Abstract

Background: Pertussis diagnosis may go unrecognized when other pathogens, such as respiratory syncytial virus (RSV) circulate.

Methods: A prospective cross-sectional study was conducted in Lima, Peru from January 2009 to September 2010. A total of 596 children under 5 years old admitted with clinical diagnoses of acute respiratory infections were test for *B. pertussis* and RSV detection by polymerase chain reaction (PCR).

Results: The pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the cases and the respiratory syncytial viruses (RSV-A and RSV-B) were identified in 17.28% (103/596) of patients. Infants under 3 months old were the most frequently affected by this pathogens in 43% (49/114) and 35.9% (37/103) respectively. An increase of *B. pertussis* was observed from February to March and from October to November with a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively.

Conclusions: Epidemiologic surveillance for *B. pertussis* is essential in Peru, especially in children that could most benefit from the vaccine. *B. pertussis* should be suspected in infants hospitalized for acute respiratory symptoms for early treatment and prevent complications.

Keywords: Bronchiolitis, *Bordetella pertussis*, Infants, Vaccine

INTRODUCTION

Pertussis is an endemic vaccine-preventable disease with the highest morbidity and mortality in the youngest infants.[1] Worldwide, there are an estimated of 16 million cases of pertussis, 95% of which occur in developing countries, resulting in about 195 000 children deaths per year.[1,2] In the last years, an increase in reported cases of pertussis has been noted, even in countries with high vaccination coverage.[3-6]

Bordetella pertussis is a fastidious gram-negative coccobacillus which causes Pertussis disease, a highly contagious infection of the human respiratory tract also known as "whooping cough".[3,5] Pertussis is characterized by three phases: catarrhal, paroxysmal, and convalescent; being the most infectious periods the catarrhal and early paroxysmal phases.[7]. This classic presentation is well-known, but is observed less often since the start of immunization.[4]

The standard diagnostic criteria for *B. pertussis* identification and epidemiological surveillance are culture and molecular techniques such as polymerase chain reaction (PCR). The DNA amplification techniques (e.g., PCR) for *B. pertussis* detection are faster, and have increase the sensitivity by approximately 19% the overall percentage of laboratory-confirmed cases, being the preferred method.[8,9] However, in clinical practice the diagnosis is generally reached without microbiological confirmation leading to a possible lack of clinical awareness to start early treatment and prevent complications.[10]

Pertussis can be especially difficult to diagnose in children under 1 year of age during winter season, when other pathogens, such as respiratory syncytial virus (RSV) or Influenza, are prevalent. In these difficult cases, pertussis acute respiratory symptoms can overlap with those of bronchiolitis or other unspecific acute respiratory infections.[10,11] This is especially worrisome in infants too young to be immunized in whom atypical and more severe presentations have been reported, often requiring hospitalization for respiratory or other complications.[7,12,13]

In Peru, pertussis is a major health problem that has been raising in the last 5 years.[14] Furthermore, the most affected are infants under 1 year old representing 38% of cases, despite a national immunization coverage of 92% in this age group.[15, 16] Currently, the "whole cell" *B. pertussis* vaccine (DTwP) is the only available presentation in Peru; and the national coverage level for this vaccination is 88.3% for the 3 doses of the pentavalent vaccine (DTwP-Hib-HepB) according to the 2014 epidemiology reports.[17]

To study the Pertussis epidemiology in Peru is essential in order to understand the real impact of the disease, especially in the most vulnerable population. The aim of this study is to determine the prevalence, epidemiological and clinical characteristics of *B. pertussis* and Respiratory syncytial virus cases in infants under 5 years old hospitalized with acute respiratory infections in a Peruvian hospital between 2009 and 2010.

MATERIALS AND METHODS

Patients

A prospective cross-sectional study was conducted in children under 5 years old admitted to *"Hospital Nacional Cayetano Heredia. Lima - Peru"* with diagnosis of acute respiratory infection (ARI). A total of 596 patients were studied from January 2009 to September 2010. The study region had a representative population, since Lima is recognized as *B. Pertussis* endemic area and have a vaccine coverage similar to the national reports.

Epidemiological and clinical features were registered, including: age, gender and clinical symptoms (fever, rhinorrhea, cough, respiratory distress, malaise, wheezing, pharyngeal congestion, expectoration, vomiting, diarrhea, among others).

This study was approved by the Research Ethics Board of the *"Hospital Nacional Cayetano Heredia and Universidad Peruana de Ciencias Aplicadas"*. An informed consent was sign by parents or children's caregivers before enrollment.

Samples

Two nasopharyngeal samples were obtained per patient. The first one, by inserting a swab into both nostrils parallel to the palate (calcium alginate swab, USA) and a second

swab for the posterior pharyngeal and tonsillar areas (Viral Culture, Becton-Dickinson Microbiology Systems, MD, USA). Both nasal and pharyngeal swabs were placed into the same tube containing viral transport medium (a minimal essential medium buffered with $NaHCO_3$ and supplemented with 2% fetal bovine serum, penicillin and streptomycin 100 U/ml, amphotericin B 20 μg/ml, neomycin 40 μg/ml). The samples were then stored at 4°C until being sent to the Laboratory of molecular biology at "*Universidad Peruana de Ciencias Aplicadas (UPC)*". On receipt of the samples the swabs were discarded and tubes were centrifuged to pellet the cells, which were re-suspended in 0.8 ml of PBS 1X. Two aliquots of 200μl of each fresh specimen were used for the extraction of nucleic acids and 200μl for bacterial culture.

DNA extraction

DNA was extracted from a volume of 200μl of each samples using a commercial kit (High Pure Template Preparation Kit, Roche Applied Science, Germany) according to the manufacturer´s instructions. DNA extraction was assayed immediately or stored at -80°C until use.

PCR amplification.

The presence of *B. pertussis* was determined using two PCR assays, each specific for an independent region of the *B. pertussis* genome. A fragment of 191-bp of the pertussis toxin S1 gene (PTxA) was amplified using the primers PTp1: 5´-CCAACGCGCATGCGTGCAGATTCGTC-3´ and PTp2:5´-CCCTCTGCGTTTTGATGGTGCCTATTTTA- 3´.[18] Meanwhile a 145 bp fragment of the insertion sequence IS481 was amplified using the primers IS481F: 5'-GATTCAATAGGTTGTATGCATGGTT-3' and IS48R: 5'-

TTCAGGCAGACAAACTTGATGGGCG-3´.[19] The described procedures were slightly modified as follows: Fifty µl of reaction mixture containing 25 ul ready mix enzyme (Taq polimerase, 2.5 mM Mg Cl2; 15 mM Tris/HCl PH 8.3, 50 mM KCl, 200 uM each deoxynucleotide) (Kappa Biosyste), 20 pmol each primer (Macrogen-Korea), water and 5 ul DNA were amplified using a pre-denaturation of 5 min at 95°C, followed by 55 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C and elongation for 45 sec at 72°C, with a final elongation of 10 min at 72°C. The presence and size of amplification products were analysed by electrophoresis on 2.5% gel agarose, containing 3 µg/mL of ethidium bromide, and photographed under ultraviolet illumination. The amplified products were sequenced (Macrogen, Seoul, Korea).

Respiratory syncytial virus (RSV-A and RSV-B) were identified by multiplex RT-PCR as previously described by Coiras et. al., 2004.[20]

Statistical analysis

Qualitative variables were reported as frequencies and percentages. All analyses were processed with the IBM Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Chicago, IL, USA). The chi-square test (χ^2-) was used to assess associations between categorical variables while z-Test was used to 30 is significant. A p-value <0.05 was considered statistically significant. A seasonal index was calculated for *Bordetella* and Respiratory syncytial virus PCR-confirmed cases from January 2009 to September 2010. Seasonal indexes were calculated dividing the monthly frequency of confirmed cases by the average of cases per year.

RESULTS

A total of 596 children under 5 years diagnosed with an acute respiratory infection were admitted to the *"Hospital Nacional Cayetano Heredia. Lima - Peru"* from January 2009 to September 2010. The pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the cases. Respiratory syncytial viruses (RSV-A and RSV-B) were identified in 17.28% (103/596) of patients. Co-infections between *B. pertussis* and RSV-A were observed in 14 patients and only one sample was positive for *B. pertussis* and RSV-B. (Table 1)

Positive samples for *B. pertussis* and RSV were analyzed according to age distribution, and infants under 3 months old were the most frequently affected in 43% (49/114) and 35.9% (37/103) respectively. A similar sex distribution was observed in both groups. Moreover, around 59% of enrolled children had a previous contact with another patient with acute respiratory infections. An equivalent proportion of household contacts was observed for *Bordetella pertussis* and RSV positive samples. (Table 1)

A similar clinical symptoms frequency was observed between patients with *B. pertussis* and RSV. The most common symptoms in both groups were fever, cough, rhinorrhea and respiratory distress, all of them present in more than 60% of cases. However, among the patients with a positive RSV sample a higher rate of Rhinorrhea 88.35%,

Respiratory distress 76.70% and pharyngeal congestion 33.98% was observed, in comparison with the Pertussis-positive group. (Table 2)

Pneumonia was the most frequent clinical diagnosis in 32.38% (193/596) of the total of patients hospitalized with acute respiratory infections. The diagnosis of Bronchiolitis was more common in children with a positive sample for RSV in 20.39% (20/103). On the contrary, the diagnosis of rhinopharyingitis 6.14% (7/114) was more common in patients with positive *B. pertussis.* (Table 3)

A higher prevalence of *B. pertussis* cases were registered between October and November 2009 and February to April 2010.(Figure 1) Seasonal indexes were calculated for *B. pertussis* and RSV positive samples separately. An increase of pcases was observed from February to March and from October to November with a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively. A similar predominance was observed in RSV cases from November to December. However, RSV showed to be also frequent from April to June with a seasonal index between 1.09-2.00. (Figure 2)

DISCUSSION

Bordetella pertussis is a strict human pathogen which causes whooping cough, an endemic illness responsible of significant morbidity and mortality, especially in infants under 6 months old.[1,2,5] Although regional differences exist, Pertussis represents a considerable global disease burden that has been increasing, even in countries with high vaccination coverage.[2,5,13] In Peru, an alarming increase of cases has been observed in the last 5 years, and 56% of cases are reported in infants under 1 year old.[12,14,15,16] This have raise especial concern since infants under 6 months old are more vulnerable to disease related complications and carry a higher mortality.[7, 21, 22]

The most common clinical manifestations of *B. pertussis* infections are prolonged and paroxysmal coughing, accompanied by inspiratory stridor.[1, 3] However, several factors are known to affect the disease presentation and Pertussis diagnosis may go unrecognized when other pathogens, such as respiratory syncytial virus (RSV) or Influenza virus circulate.[10, 23, 24] A retrospective study in Italy, from a group of infants hospitalized from October 2008 to April 2010 for acute respiratory symptoms reported that most of Pertussis cases were infants under 6 months with median of 71.5 days old and a male: female ratio of 6:13.[10] In our study pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the patients admitted with an acute respiratory

infection and infants under 3 months old were the most frequently affected in 43% (49/114) with a similar sex distribution.

Co-infection between *Bordetella pertussis* and RSV has been previously described to cause severe infections.[10, 11] A study conducted in a group of infants hospitalized for RSV bronchiolitis showed that almost 2% of patients were co-infected with *B. pertussis.*[25, 26] In our series, co-infections were observed in 14 patients between *B. pertussis* and RSV-A and 1 sample was positive for *B. pertussis* and RSV-B. Moreover, 6 out of 9 cases of co-infections were clinically diagnosed as Bronquiolitis and *B. pertussis* was not suspected at the time of admission. Influenza virus and *B. pertussis* co-infections have been also identified as a possible pathogen present in children with community-acquired pneumonia; and the pertussis toxin-mediated suppression have been postulate to be responsible to produce more sever presentations.[27, 28]

Multiple studies have reported Paroxysmal cough (76.5-91.1%), cyanosis (46.7-81.7%) and respiratory distress (47.8-55.7%) as the most common symptoms in children.[13, 29, 30] However, several clinical features might help to suspect the diagnosis of pertussis in infants hospitalized for acute respiratory symptoms.[10]

One study in 2013, compared infants with Pertussis and confirmed RSV bronchiolitis; and the clinical characteristics showed that the percentage of infants with paroxysmal cough was significantly higher in infants with *B. pertussis*. Additionally, cough at admission lasted longer in infants with pertussis than in control infants. Also, fever was significantly lower in infants with pertussis, and more common in patients with bronchiolitis. In our study population, a similar clinical symptoms frequency was

observed between patients with B. pertussis and RSV. The most frequently reported symptoms were fever, cough, rhinorrhea and respiratory distress, in more than 60% of cases. However, the presence of rhinorrhea 88.35%, respiratory distress 76.70% and pharyngeal congestion 33.98% was more common among patients with RSV. This higher frequency of symptoms in our study may be related to fact that more than 52% of our patients were hospitalized infants under 6 months old.

The clinical diagnosis of Pertussis in infants can be challenging, especially in children with incomplete immunizations, and some patients may be catalogued as acute viral respiratory infections, before laboratory confirmation. Thus delaying the appropriate antibiotic treatment and isolation measures.[11, 24] In our series, pneumonia was clearly the most frequent diagnosis in 26.32% (30/114) of the patients with positive *B. Pertussis*. However, other diagnosis were considered in this group, such as rhinopharingitis, bronchiolitis and influenza infections. In contrast, the diagnosis of Bronchiolitis was more common in 20.37% (21/103) of children with a positive sample for RSV.

For *Bordetella pertussis* seasonality, a pattern corresponding to the summer and spring months have been reported in the southern hemisphere.[13] Comparably, a previous study in infants under 6 month of age from 2003 to 2008 in Lima, registered more hospitalizations due to whooping cough during the months of February and September. In our study, a similar distribution was observed with an increase of *B. pertussis* cases from February to March and from October to November and a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively.

Pertussis represents a considerable disease burden in Peru and the diagnosis is complicated by the limitations of currently available diagnostic tests. Therefore, the only diagnostic tests that are recommended for case confirmation in national reporting are culture and polymerase chain reaction (PCR).[7, 31] However, in Peru the use of PCR for surveillance was started recently in 2012 and there is still evidence of a deficient report and registration of cases that limit the analysis of the real disease burden.[32]

CONCLUSION

As in other Latin American countries, epidemiologic surveillance for *B. pertussis* is essential in Peru, especially in children that could most benefit from the vaccine. This study demonstrates a considerable incidence of *B. pertussis* in children previously diagnosed as acute respiratory infections and highlights the importance of possible co-infections that may difficult the diagnosis and prognosis of patients. There is an increasing need for further investigations to better establish the impact of the disease and improve vaccination programs especially in hospitalized children were more severe presentations have been reported.

REFERENCES

1. Centers for Disease Control and Prevention (CDC) Childhood Whooping Cough Vaccine Protects Most Children For At Least 5 years. Atlanta, Georgia. [Accessed June 18, 2015; Cited on July 13, 2015]. Available at: http://www.cdc.gov/pertussis/surv-reporting.html.
2. World Health Organization (WHO). Immunizations, vaccines and biological: Pertussis. Geneva, Switzerland. [Accessed July 08, 2015; Cited on July 15, 2015]. Available at: http://www.who.int/immunization/topics/pertussis/en/
3. Torres R, Santos T, Torres R, et al. Resurgence of pertussis at the age of vaccination: clinical, epidemiological, and molecular aspects. J Pediatr (Rio J). 2015 Jan 23. pii: S0021-7557(15)00006-6
4. Van den Brink G, Wishaupt J, Douma J, et al. (2014) Bordetella pertussis: an underreported pathogen in pediatric respiratory infections, a prospective cohort study. BMC Infect Dis 30;14:526.
5. Gao F, Mahoney J, Daly E, et al. (2014) Evaluation of a Multitarget Real-Time PCR Assay for Detection of Bordetella Species during a Pertussis Outbreak in New Hampshire in 2011. J Clin Microbiol52(1):302-306.

6. Nikbin V, Shahcheraghi, Lotfi M, et al. (2013) Comparison of culture and real-time PCR for detection of *Bordetella pertussis* isolated from patients in Iran. Iran J Microbiol. 5(3):209-214.
7. Murphy T, Slade B, Broder K, et al. Prevention of pertussis, tetanus and diphtheria among pregnant and postpartum women and their infants recommendations of the Advisory Committee on Immunization Practices(ACIP). MMWRRecommRep.2008;57(RR-4): 1–51.
8. Hajia M, Rahbar M, Fallah F. Detection of *Bordetella pertussis* in Infants Suspected to have Whooping Cough. Open Respir Med J. 2012;6:34-6.
9. Riffelmann M, Wirsing von König C, Caro V, et al. Nucleic Acid amplification tests for diagnosis of Bordetella infections J Clin Microbiol. 2005 Oct;43(10):4925-9.
10. Nicolai A, Nenna R, Stefanelli P, et al. *Bordetella pertussis* in infants hospitalized for acute respiratory symptoms remains a concern. BMC Infect Dis. 2013 Nov 8;13:526.
11. Crowcroft N, Booy R, Harrison T, et al. Severe and unrecognized: pertussis in UK infants. Arch Dis Child. 2003 Sep;88(9):802-6.
12. Perret C, Viviani T, Peña A, et al. Source of infection in young infants hospitalized with *Bordetella pertussis*. Rev Med Chile 2011; 139: 448-454.
13. Gentile A, Romanin V, Juarez Mdel V, et al. Epidemiology of *Bordetella pertussis* in a children's hospital. Arch Argent Pediatr. 2014 Feb;112(1):26-32.
14. Red Nacional de Epidemiologia (RENACE). Distribución de Tos Ferina por distritos Peru 2015. [Internet]. Lima, Perú. Dirección General de Epidemiología (DGE). [Accessed on June 12, 2015; Cited on July 09, 2015] Available at: http://www.dge.gob.pe/portal/docs/vigilancia/sala/2015/SE23/tosf.pdf

15. Ministerio de Salud del Peru (MINSA).Alerta Epidemiológica: Brote de Tos Ferina en el País. [Internet]. Lima, Peru. Dirección General de Epidemiología (DGE). [Accessed June 12, 2015; Cited on July 13, 2015] Available at: http://www.inr.gob.pe/transparencia/Epidemiolog%C3%ADa/alertas%20epidemiologicas/ALERTA%20N%C2%BA%2003%20BROTE%20DE%20TOS%20FERINA.pdf
16. Ministerio de Salud del Peru (MINSA). Boletín Epidemiológico 06. [Internet]. Lima, Peru. Dirección General de Epidemiología (DGE). [Accessed June 22, 2015; Cited on July 10, 2015] Available at: http://www.dge.gob.pe/portal/docs/vigilancia/boletines/2013/06.pdf
17. Oficina General de Epidemiologia e Informática (OGEI) – Ministerio de Salud (MINSA): Boletín: Bordetella Pertussis; Lima, Peru; 2015. (Unpublished report)
18. Houard S, Hackel C, Herzog A, Bollen A. Specific identification of *Bordetella pertussis* by the polymerase chain reaction. Res Microbiol. 1989 Sep;140(7):477-87.
19. Glare EM, Paton JC, Premier RR, Lawrence AJ, Nisbet IT. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J Clin Microbiol. 1990 Sep;28(9):1982-7.
20. Coiras M, Pérez-Breña P, García M, et al. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J Med Virol. 2003 Jan; 69(1):132-44.
21. Tan T, Trindade E, Skowronski D. Epidemiology of Pertussis. Pediatr Infect Dis J. 2005 May;24(5 Suppl):S10-8.

22. Quian J, Cerisola A, Russomano F, et al. Infecciones por *Bordetella pertussis* en niños menores de un año hospitalizados y sus contactos en el hogar. Arch Pediatr Urug. 2006; 77(3):229-236.
23. Matto S, Cherry J. Molecular Pathogenesis, Epidemiology, and Clinical Manifestations of Respiratory Infections Due to Bordetella pertussis and Other Bordetella Subspecies. Clin Microbiol Rev. 2005 Apr;18(2):326-82.
24. Siberry G, Paquette N, Ross T, et al. Low prevalence of pertussis among children admitted with respiratory symptoms during respiratory syncytial virus season. Infect Control Hosp Epidemiol. 2006 Jan;27(1):95-7.
25. Walsh P, Overmeyer C, Kimmel L, et al. Prevalence of B*ordetella pertussis* and Bordetella parapertussis in samples submitted for RSV screening. West J Emerg Med 2008, 9:135–140.
26. Walsh PF, Kimmel L, Feola M, et al. Prevalence of *Bordetella pertussis* and Bordetella parapertussis in infants presenting to the emergency department with bronchiolitis. J Emerg Med 2011, 40:256–261.
27. Holter JC, Müller F, Bjoran O, et al. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. BMC Infect Dis. 2015 Feb 15;15:64
28. Ayala VI, Teijaro JR, Farber DL, et al. *Bordetella pertussis* infection exacerbates influenza virus infection through pertussis toxin-mediated suppression of innate immunity. PLoS One. 2011 Apr 20;6(4):e19016.
29. Nieto-Guevara J, Luciani K, Montesdeoca-Melian, et al. Hospital admissions due to whooping cough: experience of the del niño hospital in Panama. Period 2001-2008. An Pediatr (Barc). 2010 Mar;72(3):172-8.

30. Castillo M, Petrozzi V, Vera M, et al. Study Clinical and Epidemiological of Whooping Cough in Breast-Fed Babies under 6 months admitted in three Hospitals of Lima - Peru over the years 2003 – 2008. Rev Peru Pediatr.2012; 65 (1): 21-32
31. Pan American Health Organization (PAHO). Alerta Epidemiológica: Tos Ferina (Coqueluche). [Internet]. Washington, D.C. [Accessed May 22, 2015; Cited on July 11, 2015] Available at: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=19325&Itemid
32. Ministerio de Salud del Peru (MINSA). Boletín Epidemiológico 06. [Internet]. Lima, Peru. Dirección General de Epidemiología (DGE). [Accessed June 22, 2015; Cited on July 10, 2015] Available at: http://www.dge.gob.pe/portal/docs/vigilancia/boletines/2013/06.pdf

Table 1: General characteristics of *Bordetella pertussis* and RSV cases.

CHARACTERISTIC	**Total ARI patients**	***Bordetella pertussis***	**RSV**
	Frequency (n= 596) N (%)	**Frequency (n=114) N (%)**	**Frequency (n=103) N (%)**
Gender			103
Female	243 (40.8)	52 (45.6)	46 (44.7)
Male	353 (59.2)	62 (54.4)	57 (55.3)
Age			
Newborn (≤ 28 days)	112 (18.8)	17 (14.9)	11 (10.7)
29 days – ≤ 3 months	121(20.3)	32 (28.1)	26 (25.2)
3 – 5 months	82 (13.8)	13 (11.4)	11(10.7)
6 – 11 months	115(19.3)	20 (17.6)	26 (25.2)
1 – 5 years	166 (27.9)	32 (28.1)	29 (28.2)
Contact with another people with ARI			
Yes	353 (59.2)	67 (58.8)	59 (57.3)
Not	243 (40.8)	47 (41.2)	44 (42.7)

Table 2: Clinical symptoms observed in patients with positive *B. pertussis* and RSV by PCR.

CLINICAL SYMPTOMS	Total of patients	Patients positive for *Bordetella pertussis*	Patients positive for RSV
	Frequency (n=596) N (%)	Frequency (n= 114) N (%)	Frecuency (n=103) N (%)
Fever	596 (100)	114 (100)	103 (100)
Cough	448 (75.2)	82 (71.9)*	92 (89.32)*
Rinorrhea	448 (75.2)	90 (78.9)	91(88.35)
Respiratory distress	366 (61.4)	69 (60.5)*	79 (76.70)*
Wheezing respiratory	230 (38.6)	40 (35.1)*	59 (57.28)*
Malaise	150 (25.2)	28 (24.6)	24 (23.30)
Pharyngeal congestion	150 (25.2)	25 (21.9)*	35 (33.98)*
Expectoration	142 (23.8)	28 (24.6)	30 (29.13)
Vomits	79 (13.3)	16 (14)	16(15.53)
Diarrhea	71(11.9)	13 (11.4)	15 (14.56)
Asthenia	52 (8.7)	13 (11.4)	9 (8.74)
Conjunctival congestion	23(3.9)	5 (4.4)	5 (4.85)
Abdominal pain	21(3.5)	2 (1.7)	2 (1.94)
Headache	16 (2.7)	3(2.63)	4 (3.88)
Otalgia	6 (1.0)	2 (1.75)	1 (0.97)
Myalgia	6 (1.0)	1(1.75)	1 (0.97)

* z-Test: Patients positive for *Bordetella pertussis* vs Patients positive for RSV, $p<0.05$

Others (< 10% of cases): Ear pain, photophobia, conjunctival congestion, abdominal pain, lymphadenopathy, fatigue, headache, myalgia, skin rash

* 3 children died, one of them in the *B.pertussis* infection group

Table 3: Clinical diagnosis observed in patients with positive *B. pertussis* and RSV by PCR.

CLINICAL DIAGNOSIS	Total of patients		Patients positive for *Bordetella pertussis*			Patients positive for *RSV*		
	Frequency (n=596)	Prevalence (%)	Frequency (n= 114)	Prevalence (%)	p-value**	Frequency (n= 103)	Prevalence (%)	p-value**
Pneumonia	193	32.38	30	26.32*	0.124	44	42.72*	0.014
Pharyngitis	6	1.01	0	0	0.231	1	0.97	0.968
Rhinopharyngitis	33	5.54	7	6.14	0.754	3	2.91	0.200
Bronchiolitis	57	9.56	9	7.9*	0.327	21	20.39*	<0.05
Influenza A Infection	51	8.56	10	8.77	0.927	6	5.83	0.276
Whooping cough-like syndrome	10	1.68	3	2.63	0.378	2	1.94	0.819
Obstruction syndrome to bronchiolar	41	6.88	9	7.89	0.634	11	10.68	0.094

* z-Test: Patients positive for *Bordetella pertussis* vs Patients positive for RSV, $p<0.05$

** χ^2-Test

Others (1% of cases): Sinusitis, respiratory distress syndrome, sepsis late atypical febrile seizure status epilepticus, atypical febrile seizure, gastroenteritis.

Figure 1. ***Bordetella pertussis*** **confirmed cases (2009-2010).**

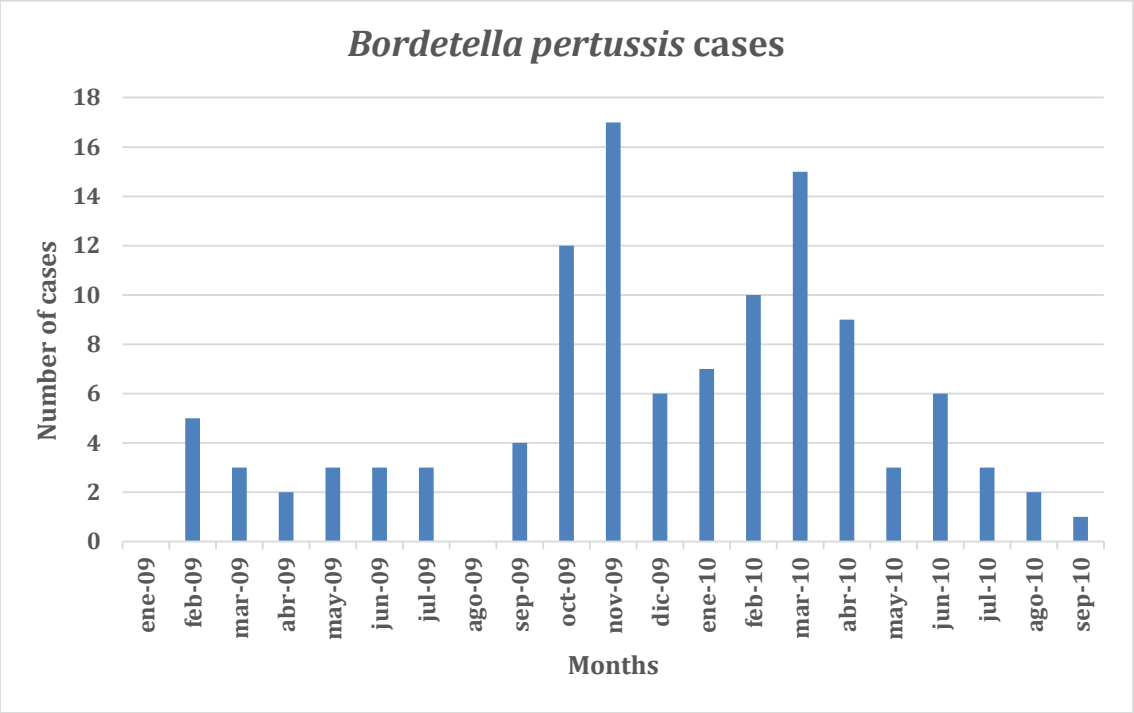
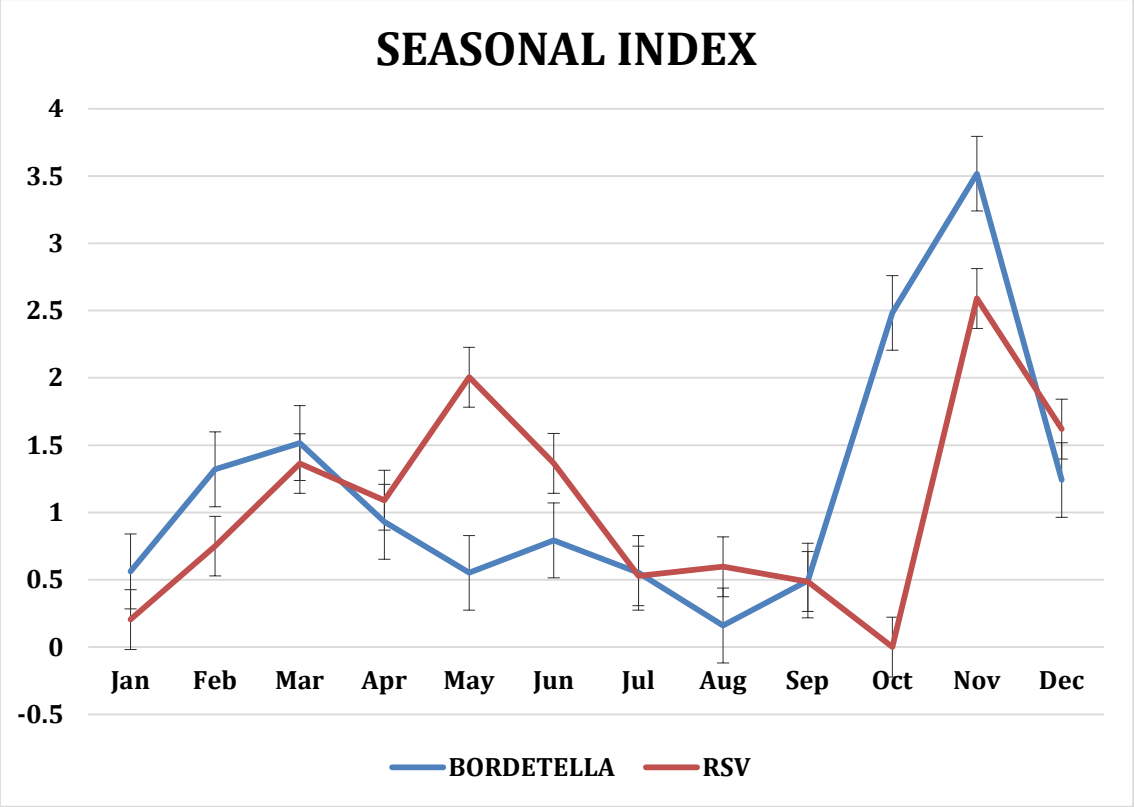


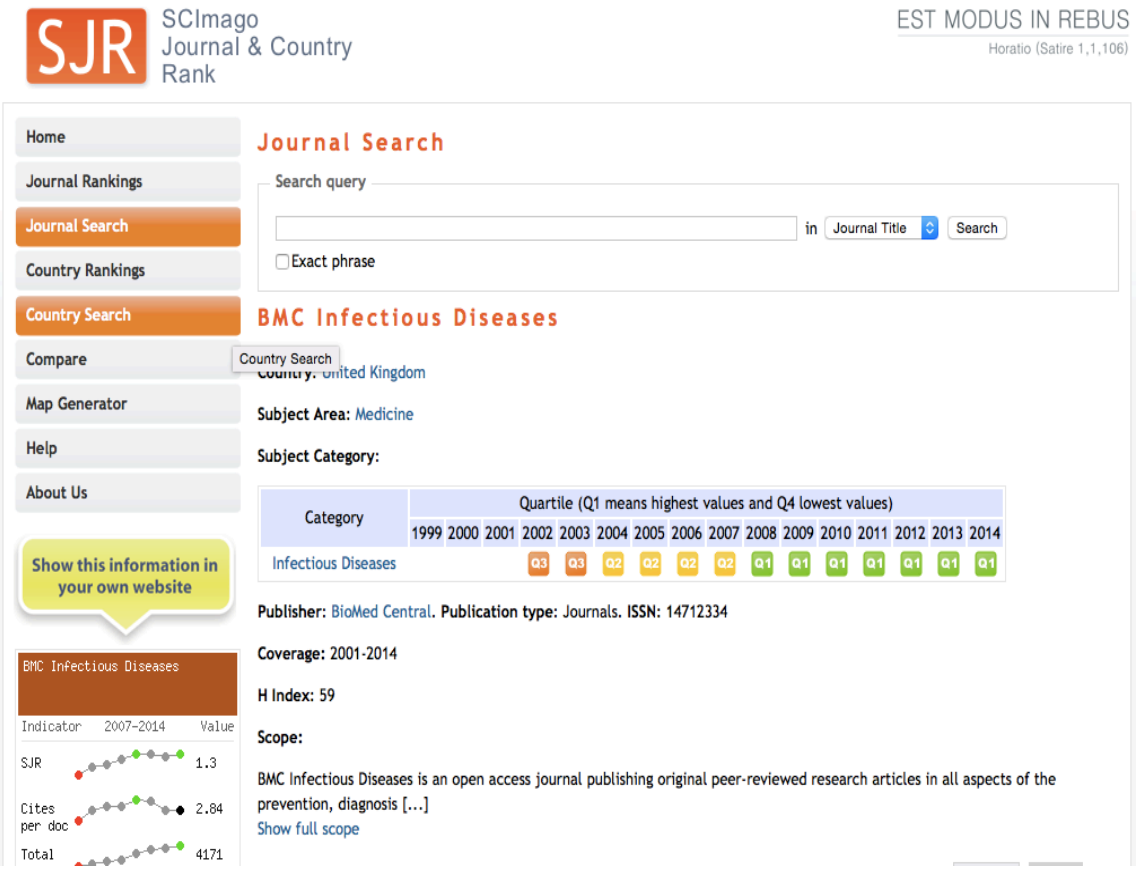
Figure 2. ***Bordetella pertussis*** **and RSV seasonal index (2009-2010).**



3. REVISTA A PUBLICAR

BMC Infectious Diseases

Se optó por esta revista en particular, por ser idónea respecto a nuestro tema y además, se encuentra en el primer cuartil según el ranking de SCImago para revistas científicas.



4. PROCESO DE REVISIÓN

4.1. RECEPCIÓN DEL ARTÍCULO A LA REVISTA

---------- Mensaje reenviado ----------
De: **BMC Infectious Diseases Editorial Office** <em@editorialmanager.com>
Fecha: jueves, 27 de agosto de 2015
Asunto: INFD-D-15-00389 - your submission is being processed
Para: Juana Del Valle <joana.del.valle@gmail.com>

Dear Dr Del Valle,

We are pleased to inform you that your submission entitled: "High prevalence of Bordetella pertussis in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru." has been assigned to the Editor(s).

The manuscript id is: INFD-D-15-00389
Please refer to this number in any future correspondence.

You may track the status of your submission via: http://infd.edmgr.com/

If you have forgotten your username or password please use the "Send Username/Password" link to get your login information. For security reasons, your password will be reset.

Thank you for your submission to BMC Infectious Diseases.

Best wishes,

Editorial Office
BMC Infectious Diseases
http://www.biomedcentral.com/bmcinfectdis

4.1.1 PRIMERA VERSIÓN DEL ARTÍCULO

- *Fecha: 7 de Octubre del 2015*

High prevalence of *Bordetella pertussis* in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru.

Ivana Pavic-Espinoza[1]; Sandy Bendezú-Medina[1]; Angella Herrera-Alzamora[1]; Pablo Weilg[1]; María J. Pons[1]; Miguel Angel Aguilar-Luis[1]; Verónica Petrozzi-Helasvuo[1]; Juana del Valle Mendoza[1*].

[1]Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.

*Corresponding author

Juana del Valle
Universidad Peruana de Ciencias Aplicadas
Av San Marcos cuadra 2, Chorrillos, Lima-Peru

E-mail: jdelvall@upc.edu.pe
Phone +51 1988040532
Fax. +51 1 3496023

E-mail addresses for all authors

Ivana Pavic-Espinoza: ivanita.89@gmail.com

Sandy Bendezú-Medina: sandyta291@hotmail.com

Angella Herrera-Alzamora: angella_h@hotmail.com

Pablo Weilg: pablo.weilg@gmail.com

María J. Pons: mariajesus.pons@isglobal.org

Verónica Petrozzi-Helasvuo: pcnumpet@upc.edu.pe

Miguel Aguilar-Luis: pcmemiag@upc.edu.pe
Juana del Valle Mendoza: jdelvall@upc.edu.pe

Abstract

Background: Pertussis diagnosis may go unrecognized when other pathogens, such as respiratory syncytial virus (RSV) circulate.

Methods: A prospective cross-sectional study was conducted in Lima, Peru from January 2009 to September 2010. A total of 596 children under 5 years old admitted with clinical diagnoses of acute respiratory infections were test for *B. Pertussis* and RSV detection by polymerase chain reaction (PCR).

Results: The pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the cases and the respiratory syncytial viruses (RSV-A and RSV-B) were identified in 17.28% (103/596) of patients. Infants under 3 months old were the most frequently affected by this pathogens in 43% (49/114) and 35.9% (37/103) respectively. An increase of *B. Pertussis* was observed from February to March and from October to November with a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively.

Conclusions: Epidemiologic surveillance for *B. pertussis* is essential in Peru, especially in children that could most benefit from the vaccine. *B. pertussis* should be suspected in infants hospitalized for acute respiratory symptoms for early treatment and prevent complications.

Keywords: Bronchiolitis, *Bordetella pertussis*, Infants, Vaccine

INTRODUCTION

Pertussis is an endemic vaccine-preventable disease with the highest morbidity and mortality in the youngest infants.[1] Worldwide, there are an estimated of 16 million cases of pertussis, 95% of which occur in developing countries, resulting in about 195 000 children deaths per year.[1,2] In the last years, an increase in reported cases of pertussis has been noted, even in countries with high vaccination coverage.[3-6]

Bordetella pertussis is a fastidious gram-negative coccobacillus which causes Pertussis disease, a highly contagious infection of the human respiratory tract also known as "whooping cough".[3,5] Pertussis is characterized by three phases: catarrhal, paroxysmal, and convalescent; being the most infectious periods the catarrhal and early paroxysmal phases.[7]. This classic presentation is well-known, but is observed less often since the start of immunization.[4]

The standard diagnostic criteria for *B. Pertussis* identification and epidemiological surveillance are culture and molecular techniques such as polymerase chain reaction (PCR). The DNA amplification techniques (e.g., PCR) for *B. pertussis* detection are faster, and have increase the sensitivity by approximately 19% the overall percentage of laboratory-confirmed cases, being the preferred method.[8,9] However, in clinical practice the diagnosis is generally reached without microbiological confirmation leading to a possible lack of clinical awareness to start early treatment and prevent complications.[10]

Pertussis can be especially difficult to diagnose in children under 1 year of age during winter season, when other pathogens, such as respiratory syncytial virus (RSV) or Influenza, are prevalent. In these difficult cases, pertussis acute respiratory symptoms

can overlap with those of bronchiolitis or other unspecific acute respiratory infections.[10,11] This is especially worrisome in infants too young to be immunized in whom atypical and more severe presentations have been reported, often requiring hospitalization for respiratory or other complications.[7,12,13]

In Peru, pertussis is a major health problem that has been raising in the last 5 years.[14] Furthermore, the most affected are infants under 1 year old representing 38% of cases, despite a national immunization coverage of 92% in this age group.[15, 16]

To study the Pertussis epidemiology in Peru is essential in order to understand the real impact of the disease, especially in the most vulnerable population. The aim of this study is to determine the prevalence, epidemiological and clinical characteristics of *B. pertussis* and Respiratory syncytial virus cases in infants under 5 years old hospitalized with acute respiratory infections in a Peruvian hospital between 2009 and 2010.

MATERIALS AND METHODS

Patients

A prospective cross-sectional study was conducted in children under 5 years old admitted to *"Hospital Nacional Cayetano Heredia. Lima - Peru"* with diagnosis of acute respiratory infection (ARI). A total of 596 patients were studied from January 2009 to September 2010. Epidemiological and clinical features were registered, including: age, gender and clinical symptoms (fever, rhinorrhea, cough, respiratory distress, malaise, wheezing, pharyngeal congestion, expectoration, vomiting, diarrhea, among others).

This study was approved by the Research Ethics Board of the "*Hospital Nacional Cayetano Heredia and Universidad Peruana de Ciencias Aplicadas"*. An informed consent was sign by parents or children's caregivers before enrollment.

Samples

Two nasopharyngeal samples were obtained per patient. The first one, by inserting a swab into both nostrils parallel to the palate (calcium alginate swab, USA) and a second swab for the posterior pharyngeal and tonsillar areas (Viral Culture, Becton-Dickinson Microbiology Systems, MD, USA). Both nasal and pharyngeal swabs were placed into the same tube containing viral transport medium (a minimal essential medium buffered with $NaHCO_3$ and supplemented with 2% fetal bovine serum, penicillin and streptomycin 100 U/ml, amphotericin B 20 μg/ml, neomycin 40 μg/ml). The samples were then stored at 4°C until being sent to the Laboratory of molecular biology at "*Universidad Peruana de Ciencias Aplicadas (UPC)*". On receipt of the samples the

swabs were discarded and tubes were centrifuged to pellet the cells, which were re-suspended in 0.8 ml of PBS 1X. Two aliquots of 200µl of each fresh specimen were used for the extraction of nucleic acids and 200µl for bacterial culture.

DNA extraction

DNA was extracted from a volume of 200µl of each samples using a commercial kit (High Pure Template Preparation Kit, Roche Applied Science, Germany) according to the manufacturer´s instructions. DNA extraction was assayed immediately or stored at -80°C until use.

PCR amplification.

The presence of *B. pertussis* was determined using two PCR assays, each specific for an independent region of the *B. pertussis* genome. A fragment of 191-bp of the pertussis toxin S1 gene (PTxA) was amplified using the primers PTp1: 5´-CCAACGCGCATGCGTGCAGATTCGTC-3´ and PTp2:5´-CCCTCTGCGTTTTGATGGTGCCTATTTTA- 3´.[17] Meanwhile a 145 bp fragment of the insertion sequence IS481 was amplified using the primers IS481F: 5'-GATTCAATAGGTTGTATGCATGGTT-3' and IS48R: 5'-TTCAGGCAGACAAACTTGATGGGCG-3´.[18] The described procedures were slightly modified as follows: Fifty µl of reaction mixture containing 25 ul ready mix enzyme (Taq polimerase, 2.5 mM Mg Cl2; 15 mM Tris/HCl PH 8.3, 50 mM KCl, 200 uM each deoxynucleotide) (Kappa Biosyste), 20 pmol each primer (Macrogen-Korea), water and 5 ul DNA were amplified using a pre-denaturation of 5 min at 95°C, followed by 55 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C and elongation for 45 sec at 72°C, with a final elongation of 10 min at 72°C. The presence and size of

amplification products were analysed by electrophoresis on 2.5% gel agarose, containing 3 μg/mL of ethidium bromide, and photographed under ultraviolet illumination. The amplified products were sequenced (Macrogen, Seoul, Korea).

Respiratory syncytial virus (RSV-A and RSV-B) were identified by multiplex RT-PCR as previously described by Coiras et. al., 2004.[19]

Statistical analysis

Qualitative variables were reported as frequencies and percentages. A seasonal index was calculated for *Bordetella* and Respiratory syncytial virus PCR-confirmed cases from January 2009 to September 2010. Seasonal indexes were calculated dividing the monthly frequency of confirmed cases by the average of cases per year.

RESULTS

A total of 596 children under 5 years diagnosed with an acute respiratory infection were admitted to the *"Hospital Nacional Cayetano Heredia. Lima - Peru"* from January 2009 to September 2010. The pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the cases. Respiratory syncytial viruses (RSV-A and RSV-B) were identified in 17.28% (103/596) of patients. Co-infections between *B. pertussis* and RSV-A were observed in 14 patients and only one sample was positive for *B. pertussis* and RSV-B. (Table 1)

Positive samples for *B. pertussis* and RSV were analyzed according to age distribution, and infants under 3 months old were the most frequently affected in 43% (49/114) and 35.9% (37/103) respectively. A similar sex distribution was observed in both groups. Moreover, around 59% of enrolled children had a previous contact with another patient with acute respiratory infections. An equivalent proportion of household contacts was observed for *Bordetella pertussis* and RSV positive samples. (Table 1)

A similar clinical symptoms frequency was observed between patients with *B. pertussis* and RSV. The most common symptoms in both groups were fever, cough, rhinorrhea and respiratory distress, all of them present in more than 60% of cases. However, among the patients with a positive RSV sample a higher rate of Rhinorrhea 88.35%, Respiratory distress 76.70% and pharyngeal congestion 33.98% was observed, in comparison with the Pertussis-positive group. (Table 2).

Pneumonia was the most frequent clinical diagnosis in 32.38% (193/596) of the total of patients hospitalized with acute respiratory infections. The diagnosis of Bronchiolitis was more common in children with a positive sample for RSV in 20.39% (20/103). On the contrary, the clinical diagnosis of Influenza A H1N1 8.77% (10/114) and the diagnosis rhinopharyingitis 6.14% (7/114) were more frequently made in patients positive for *B. pertussis*. (Table 3)

A higher prevalence of *B. Pertussis* cases were registered between October and November 2009 and February to April 2010 (Figure 1). Seasonal indexes were calculated for *B. Pertussis* and RSV positive samples separately. An increase of *B. Pertussis* cases was observed from February to March and from October to November with a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively. A similar predominance was observed in RSV cases from November to December. However, RSV showed to be also frequent from April to June with a seasonal index between 1.09-2.00. (Figure 2)

DISCUSSION

Bordetella pertussis is a strict human pathogen which causes whooping cough, an endemic illness responsible of significant morbidity and mortality, especially in infants under 6 months old.[1,2,5] Although regional differences exist, Pertussis represents a considerable global disease burden that has been increasing, even in countries with high vaccination coverage.[2,5,13] In Peru, an alarming increase of cases has been observed in the last 5 years, and 56% of cases are reported in infants under 1 year old.[12,14,15,16] This have raise especial concern since infants under 6 months old are more vulnerable to disease related complications and carry a higher mortality.[7, 20, 21]

The most common clinical manifestations of *B. pertussis* infections are prolonged and paroxysmal coughing, accompanied by inspiratory stridor.[1, 3] However, several factors are known to affect the disease presentation and Pertussis diagnosis may go unrecognized when other pathogens, such as respiratory syncytial virus (RSV) or Influenza virus circulate.[10, 22, 23] A retrospective study in Italy, from a group of infants hospitalized from October 2008 to April 2010 for acute respiratory symptoms reported that most of Pertussis cases were infants under 6 months with median of 71.5 days old and a male: female ratio of 6:13.[10] In our study pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the patients admitted with an acute respiratory infection and infants under 3 months old were the most frequently affected in 43% (49/114) with a similar sex distribution.

Co-infection between *Bordetella pertussis* and RSV has been previously described to cause severe infections.[10, 11] A study conducted in a group of infants hospitalized for RSV bronchiolitis showed that almost 2% of patients were co-infected with *B. pertussis*.[24, 25] In our series, co-infections were observed in 14 patients between *B. Pertussis* and RSV-A and 1 sample was positive for *B. Pertussis* and RSV-B. Moreover, 6 out of 9 cases of co-infections were clinically diagnosed as Bronquiolitis and *B. Pertussis* was not suspected at the time of admission. Influenza virus and *B. Pertussis* co-infections have been also identified as a possible pathogen present in children with community-acquired pneumonia; and the pertussis toxin-mediated suppression have been postulate to be responsible to produce more sever presentations.[26, 27]

Multiple studies have reported Paroxysmal cough (76.5-91.1%), cyanosis (46.7-81.7%) and respiratory distress (47.8-55.7%) as the most common symptoms in children.[13, 28, 29] However, several clinical features might help to suspect the diagnosis of pertussis in infants hospitalized for acute respiratory symptoms.[10]

One study in 2013, compared infants with Pertussis and confirmed RSV bronchiolitis; and the clinical characteristics showed that the percentage of infants with paroxysmal cough was significantly higher in infants with *B. pertussis*. Additionally, cough at admission lasted longer in infants with pertussis than in control infants. Also, fever was significantly lower in infants with pertussis, and more common in patients with bronchiolitis. In our study population, a similar clinical symptoms frequency was observed between patients with *B. pertussis* and RSV. The most frequently reported symptoms were fever, cough, rhinorrhea and respiratory distress, in more than 60% of cases. However, the presence of rhinorrhea 88.35%, respiratory distress 76.70% and

pharyngeal congestion 33.98% was more common among patients with RSV. This higher frequency of symptoms in our study may be related to fact that more than 52% of our patients were hospitalized infants under 6 months old.

The clinical diagnosis of Pertussis in infants can be challenging, especially in children with incomplete immunizations, and some patients may be catalogued as acute viral respiratory infections, before laboratory confirmation. Thus delaying the appropriate antibiotic treatment and isolation measures.[11, 23] In our series, pneumonia was clearly the most frequent diagnosis in 26.32% (30/114) of the patients with positive *B. pertussis*. However, other diagnosis were considered in this group, such as rhinopharingitis, bronchiolitis and influenza infections. The diagnosis of Bronchiolitis was more common in 20.37% (21/103) of children with a positive sample for RSV. In contrast, the clinical diagnosis of Influenza A H1N1 8.77% (10/114) was more frequent in patients with positive for *B. pertussis*, probably because Influenza A H1N1 is associated with severe presentations and was easily suspected during the 2009 outbreak in Peru.

For *Bordetella pertussis* seasonality, a pattern corresponding to the summer and spring months have been reported in the southern hemisphere.[13] Comparably, a previous study in infants under 6 month of age from 2003 to 2008 in Lima, registered more hospitalizations due to whooping cough during the months of February and September. In our study, a similar distribution was observed with an increase of *B. pertussis* cases from February to March and from October to November and a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively.

Pertussis represents a considerable disease burden in Peru and the diagnosis is complicated by the limitations of currently available diagnostic tests. Therefore, the only diagnostic tests that are recommended for case confirmation in national reporting are culture and polymerase chain reaction (PCR).[7, 30] However, in Peru the use of PCR for surveillance was started recently in 2012 and there is still evidence of a deficient report and registration of cases that limit the analysis of the real disease burden.[31]

As in other Latin American countries, epidemiologic surveillance for *B. pertussis* is essential in Peru, especially in children that could most benefit from the vaccine. This study demonstrates a considerable incidence of *B. pertussis* in children previously diagnosed as acute respiratory infections and highlights the importance of possible co-infections that may difficult the diagnosis and prognosis of patients. There is an increasing need for further investigations to better establish the impact of the disease and improve vaccination programs especially in hospitalized children were more severe presentations have been reported.

ACKNOWLEDGEMENTS

This work has been supported partiality the “Concurso Incentivo a la Investigación de la Universidad Peruana de Ciencias Aplicadas” (UPC). Lima-Perú

AUTHOR’S CONTRIBUTIONS

IV-E, SB-M and AH-A performed the PCR for *Bordetella pertussis* and RSV. VP and JV designed the study protocol; JV was responsible for obtaining funding and

laboratory work supervision. PW, MA-L and MJP was responsible for the clinical assessment, samples collection and database completion. PW, MJP, VP and JV drafted the manuscript. All authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Conflicts of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest or funding related to this study

4.2 CARTA DE RESPUESTA CORRECION

- *Fecha: 14 de Octubre del 2015*

Lima, 14 de octubre 2015

Dear Editor,

Enclosed please find the revised version of the manuscript: INFD-D-15-00389 "High prevalence of Bordetella pertussis in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru". Juana Del Valle, PhD; Ivana Pavic-Espinoza; Sandy Bendezú-Medina; Angella Herrera-Alzamora; Pablo Weilg; María J. Pons; Miguel Angel Aguilar-Luis; Verónica Petrozzi-Helasvuo. The reviewer's considerations have been taken into account as is stated below:

Comments to the Author

Reviewer #1:

- **Throughout the manuscript *B. pertussis* or *Bordetella pertussis* should be written in italics and pertussis without capital P.**
  The English should be polished.

  Grammatical and spelling errors were corrected

- **INTRODUCTION: without microbiological confirmation a lot of cases are missed, and in today's modern medicine I think it is clear that on clinical grounds aRSV infection cannot be distinguished from any other respiratory**

tract infection. (Versteegh et al, Clin Microbiol Infect 2005;11:801-807)(Wishaupt et al, Pediatrics 2011;128:e1113-1120)

Regarding the following paragraph:

"The standard diagnostic criteria for *B. pertussis* identification and epidemiological surveillance are culture and molecular techniques such as polymerase chain reaction (PCR). The DNA amplification techniques (e.g., PCR) for *B. pertussis* detection are faster, and have increase the sensitivity by approximately 19% the overall percentage of laboratory-confirmed cases, being the preferred method.[8,9] However, in clinical practice the diagnosis is generally reached without microbiological confirmation leading to a possible lack of clinical awareness to start early treatment and prevent complications.[10]"

The authors are aware that it is impossible to differentiate aRSV from other respiratory tract infections. However, we consider important to mention that in Peru most of the cases of Pertussis are suspected and treated only by clinical criteria and are not reported to national surveillance. Thus, molecular techniques, such as PCR, are necessary to ensure an accurate early detection and treatment of Pertussis.

The authors considered that aRSV is one of the most important causes of acute respiratory infections, as well as a common cause of *B. Pertussis* coinfections that should be study in our media.

Within the group of respiratory virus RSV is the most common virus in children, for the project we consider these studies

Cosnes-Lambe C, Raymond J, Chalumeau M, Pons-Catalano C, Moulin F, de Suremain N, Reglier-Poupet H, Lebon P, Poyart C, Gendrel D. Pertussis and respiratory syncytial virus infections. Eur J Pediatr. 2008 Sep;167(9):1017-9. Epub 2007 Nov 23.

Korppi M, Hiltunen Pertussis is common in nonvaccinated infants hospitalized for respiratory syncytial virus infection. J. Pediatr Infect Dis J. 2007 Apr;26(4):316-8.

Moshal KL, Hodinka RL, McGowan KL. Concomitant viral and Bordetella pertussis infections in infants. Pediatr Infect Dis J. 1998 Apr;17(4):353-4.

- **MATERIALS AND METHODS: the authors should explain how they calculate the seasonal index.**

  Seasonal indexes were calculated dividing the monthly frequency of confirmed cases by the average of cases per year.

- **RESULTS: the authors should explain how they make the clinical diagnosis of Infl A H1N1. In the methods it is not clear**.

  The phrase has been removed "In contrast, the clinical diagnosis of Influenza A H1N1 8.77% (10/114) was more frequent in patients with positive for *B. pertussis*, probably because Influenza A H1N1 is associated with severe presentations and was easily suspected during the 2009 outbreak in Peru"

  The authors considered that the diagnosis of Influenza A H1N1 cannot be done without microbiological confirmation. All of these 10 cases were reported as "highly suspected Influenza A H1N1" during the 2009 outbreak. However, we believe that this "clinical diagnosis" only adds confusion to the manuscript and should not be considered as valid diagnosis.

- **TABLE 2 AND 3: Statistics should be added to the data shown in order to show whether there are significant differences between the total group and the subgroups with Bp and RSV.**

  In the table 2 and 3, the analyses were processed with the IBM Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Chicago, IL, USA). The chi-square test (χ^2-) was used to assess associations between categorical variables while z-Test was used to 30 is significant. A p-value <0.05 was considered statistically significant.

- **TABLE 2 AND 3: Also the data of the group with no pathogen found should be described separately.**

Corrections have been made in table 2 and 3.

- **The authors should explain why they only tested for Bp and RSV and not for other virus like Rhinovirus, Influenza, Adeno, Entero etc. and why they did not test for other Bordetella like parapertussis, holmesii or bronchiseptica.**

  One of the main objectives of this study was to assess the number of B. pertussis and aRSV coinfections. Since aRSV is one of the most common etiologies of acute respiratory infections in Peruvian children.

  The clinical presentation of other Bordetella spp. e.g. parapertussis, holmesii or bronchiseptica is usually milder than B. pertussis. And, even though there is evidence that aRSV and parapertussis coinfections might be more common that with B. Pertussis. The authors considered that since B. Pertussis is associated with a worse prognosis and represents a national health problem, this study should first address the number of coinfections only with B. pertussis. We also encourage future studies to address the prevalence of coinfections with other Bordetella spp.

- **DISCUSSION: The authors state that co-infection causes more severe infections. But on the other hand there is sufficient literature that co-infection with Bp might protect against severe disease. This should be discussed more extensively. Co-infection RSV and Bp is in other studies higher than 2 %.**

  The following references mention that B. Pertussis and aRSV coinfections can be more severe and are important to study:

  1) Nicolai A, Nenna R, Stefanelli P, et al. Bordetella pertussis in infants hospitalized for acute respiratory symptoms remains a concern. BMC Infect Dis. 2013 Nov 8;13:526.
  2) Walsh P, Overmeyer C, Kimmel L, et al. Prevalence of Bordetella pertussis and Bordetella parapertussis in samples submitted for RSV screening. West J Emerg Med 2008, 9:135–140.

3) Gimenes-Sanchez F, Cobos-Carrascosa E, Sanchez-Forte M, et al. Clinical and epidemiological differences between Bordetella pertussis and respiratory syncytial virus infections in infants: a matched case control study. Enferm Infecc Microbiol Clin. 2014 Jun-Jul;32(6):359-62.
4) Ayala VI, Teijaro J, Farber D, et al. Bordetella pertussis infection exacerbates influenza virus infection through pertussis toxin-mediated suppression of innate immunity. PLoS One. 2011 Apr 20;6(4):e19016.
5) Moreno Samos M, Amores Torres M, Pradillo Martin M, et al. [Incidence and severity of pertussis in infants with a respiratory syncytial virus infection. Enferm Infecc Microbiol Clin. 2015 Aug-Sep;33(7):476-9.

INTRODUCTION: The authors should explain something about the vaccination program in Peru, the vaccination coverage in Peru and especially in the region were the study was performed.

was added "Currently, the "whole cell" B. pertussis vaccine (DTwP) is the only available presentation in Peru; and the national coverage level for this vaccination is 88.3% for the 3 doses of the pentavalent vaccine (DTwP-Hib-HepB)" (Add Reference 17)

- **I don't think the seasonality adds much to the manuscript.**

  There is no evidence of B. pertussis seasonality in Peru and the authors consider that studying the frequency of cases might help us to better understand the B. pertussis epidemiology.

Reviewer #2:

- The findings in the manuscript that the rate of detection of pertussis was the same as the rate of detection of RSV in children admitted to the hospital for acute respiratory infections in Peru is quite remarkable. This has not been reported in other settings when these two pathogens are compared. The detection of pertussis by PCR is highly sensitive but is also fraught with issues of contamination and false positive results. The authors are to be commended for using two different pertussis probes to evaluate the presence of pertussis but they do not indicate in the paper how often the two PCR results are concordant. This needs to be added to the manuscript. In addition, where any of

the PCR results confirmed by culture? **This would greatly add to the confidence of the reviewer that the large number of pertussis cases detected are real.**

The microbiological culture has many limitations due to slow growth and identification of the bacteria. There are studies that samples the sensitivity and specificity of both techniques, microbiological culture being displaced by biochemical or molecular techniques
The limitations of the laboratories of the health centers do not facilitate the development of microbiological techniques and that is why we have standardized PCR to have a faster and more reliable diagnosis. They were considered positives when both PCR amplified PtxA and IS48R.

- **The authors do not make it clear why they have selected the very restricted time period of the study from January 2009 to September 2010. Was this during a confirmed pertussis outbreak in the city? Was this a convenience sample? What has happened since that time and what was happening in the community during that time? The authors need to put this period in a greater context.**

  The study period was directly subject to our financial budget. We initiated in January, since we tried to include at least 2 consecutive summers.

- **The clinical manifestations of the patients with pertussis do not appear to be significantly different than those with RSV, although no statistical comparisons were made between the two. This would have enhanced Table 3.**

  Corrections have been made in table 3.The analyses were processed with the IBM Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Chicago, IL, USA). The chi-square test (χ^2-) was used to assess associations between categorical variables while z-Test was used to 30 is significant. A p-value <0.05 was considered statistically significant.

- **In addition the very high rate of fever in patients with pertussis is not generally seen. What was the definition of fever?**

  We are aware that all of our cases had fever. Fever was determinate by the attending physician as temperature >37.5 °C. We believe that this high amount of cases with fever might be related to the fact that all of our patients were hospitalized due to a severe presentation.

- **Lymphocytosis is also very frequently seen in young children with pertussis and no mention is made of that laboratory parameter. Was it measured in the patients with pertussis and did it differ from those with RSV? No tenemos este dato supongo xq no era parte del diseño de studio. Also infants with pertussis are often prone to severe disease and even death. Were any of these children severely ill? Did any of them develop pulmonary hypertension? Were any of them on the ventilator? Were there any deaths?**
  We believe that all that information would have add valuable data to the study. However, due to the limitations in the hospital and the poor register in the clinical records, we were not able to obtain it. Mortality data could not be provided by the hospital because they handle this internally

- **The rates of immunization in the children should be added to the paper. Did the pertussis detection rates in the immunized children differ from those in the unimmunized group? What vaccines were used in the population, whole cell? These data should be added to Table 1.**

  We believe that the rates of immunization in the children would have add valuable data to the study. However, due to the limitations in the hospital and the poor register in the clinical records, we were not able to obtain it.

4.2.1 VERSION MODIFICADA

- *Fecha: 14 de Octubre del 2015*

High prevalence of *Bordetella pertussis* in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru.

Ivana Pavic-Espinoza[1]; Sandy Bendezú-Medina[1]; Angella Herrera-Alzamora[1]; Pablo Weilg[1]; María J. Pons[1]; Miguel Angel Aguilar-Luis[1]; Verónica Petrozzi-Helasvuo[1]; Juana del Valle Mendoza[1*].

[1]Centro de Investigación de la Facultad de Ciencias de la Salud. Universidad Peruana de Ciencias Aplicadas - UPC. Av. San Marcos cdra. 2. Cedros de Villa. Lima, Perú.

*Corresponding author

Juana del Valle
Universidad Peruana de Ciencias Aplicadas
Av San Marcos cuadra 2, Chorrillos, Lima-Peru

E-mail: jdelvall@upc.edu.pe
Phone +51 1988040532
Fax. +51 1 3496023

E-mail addresses for all authors

Ivana Pavic-Espinoza: ivanita.89@gmail.com
Sandy Bendezú-Medina: sandyta291@hotmail.com
Angella Herrera-Alzamora: angella_h@hotmail.com
Pablo Weilg: pablo.weilg@gmail.com
María J. Pons: mariajesus.pons@isglobal.org
Verónica Petrozzi-Helasvuo: pcnumpet@upc.edu.pe
Miguel Aguilar-Luis: pcmemiag@upc.edu.pe
Juana del Valle Mendoza: jdelvall@upc.edu.pe

Abstract

Background: Pertussis diagnosis may go unrecognized when other pathogens, such as respiratory syncytial virus (RSV) circulate.

Methods: A prospective cross-sectional study was conducted in Lima, Peru from January 2009 to September 2010. A total of 596 children under 5 years old admitted with clinical diagnoses of acute respiratory infections were test for *B. pertussis* and RSV detection by polymerase chain reaction (PCR).

Results: The pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the cases and the respiratory syncytial viruses (RSV-A and RSV-B) were identified in 17.28% (103/596) of patients. Infants under 3 months old were the most frequently affected by this pathogens in 43% (49/114) and 35.9% (37/103) respectively. An increase of *B. pertussis* was observed from February to March and from October to November with a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively.

Conclusions: Epidemiologic surveillance for *B. pertussis* is essential in Peru, especially in children that could most benefit from the vaccine. *B. pertussis* should be suspected in infants hospitalized for acute respiratory symptoms for early treatment and prevent complications.

Keywords: Bronchiolitis, Bordetella pertussis, Infants, Vaccine

INTRODUCTION

Pertussis is an endemic vaccine-preventable disease with the highest morbidity and mortality in the youngest infants.[1] Worldwide, there are an estimated of 16 million cases of pertussis, 95% of which occur in developing countries, resulting in about 195 000 children deaths per year.[1,2] In the last years, an increase in reported cases of pertussis has been noted, even in countries with high vaccination coverage.[3-6]

Bordetella pertussis is a fastidious gram-negative coccobacillus which causes Pertussis disease, a highly contagious infection of the human respiratory tract also known as "whooping cough".[3,5] Pertussis is characterized by three phases: catarrhal, paroxysmal, and convalescent; being the most infectious periods the catarrhal and early paroxysmal phases.[7]. This classic presentation is well-known, but is observed less often since the start of immunization.[4]

The standard diagnostic criteria for *B. pertussis* identification and epidemiological surveillance are culture and molecular techniques such as polymerase chain reaction (PCR). The DNA amplification techniques (e.g., PCR) for *B. pertussis* detection are faster, and have increase the sensitivity by approximately 19% the overall percentage of laboratory-confirmed cases, being the preferred method.[8,9] However, in clinical practice

the diagnosis is generally reached without microbiological confirmation leading to a possible lack of clinical awareness to start early treatment and prevent complications.[10]

Pertussis can be especially difficult to diagnose in children under 1 year of age during winter season, when other pathogens, such as respiratory syncytial virus (RSV) or Influenza, are prevalent. In these difficult cases, pertussis acute respiratory symptoms can overlap with those of bronchiolitis or other unspecific acute respiratory infections.[10,11] This is especially worrisome in infants too young to be immunized in whom atypical and more severe presentations have been reported, often requiring hospitalization for respiratory or other complications.[7,12,13]

In Peru, pertussis is a major health problem that has been raising in the last 5 years.[14] Furthermore, the most affected are infants under 1 year old representing 38% of cases, despite a national immunization coverage of 92% in this age group.[15, 16] Currently, the "whole cell" *B. pertussis* vaccine (DTwP) is the only available presentation in Peru; and the national coverage level for this vaccination is 88.3% for the 3 doses of the pentavalent vaccine (DTwP-Hib-HepB) according to the 2014 epidemiology reports.[17]

To study the Pertussis epidemiology in Peru is essential in order to understand the real impact of the disease, especially in the most vulnerable population. The aim of this study is to determine the prevalence, epidemiological and clinical characteristics of *B. pertussis* and Respiratory syncytial virus cases in infants under 5 years old hospitalized with acute respiratory infections in a Peruvian hospital between 2009 and 2010.

MATERIALS AND METHODS

Patients

A prospective cross-sectional study was conducted in children under 5 years old admitted to *"Hospital Nacional Cayetano Heredia. Lima - Peru"* with diagnosis of acute respiratory infection (ARI). A total of 596 patients were studied from January 2009 to September 2010. The study region had a representative population, since Lima is recognized as *B. Pertussis* endemic area and have a vaccine coverage similar to the national reports.

Epidemiological and clinical features were registered, including: age, gender and clinical symptoms (fever, rhinorrhea, cough, respiratory distress, malaise, wheezing, pharyngeal congestion, expectoration, vomiting, diarrhea, among others).

This study was approved by the Research Ethics Board of the *"Hospital Nacional Cayetano Heredia and Universidad Peruana de Ciencias Aplicadas"*. An informed consent was sign by parents or children's caregivers before enrollment.

Samples

Two nasopharyngeal samples were obtained per patient. The first one, by inserting a swab into both nostrils parallel to the palate (calcium alginate swab, USA) and a second swab for the posterior pharyngeal and tonsillar areas (Viral Culture, Becton-Dickinson Microbiology Systems, MD, USA). Both nasal and pharyngeal swabs were placed into the same tube containing viral transport medium (a minimal essential medium buffered with $NaHCO_3$ and supplemented with 2% fetal bovine serum, penicillin and streptomycin 100 U/ml, amphotericin B 20 μg/ml, neomycin 40 μg/ml). The samples were then stored at 4°C until being sent to the Laboratory of molecular biology at "*Universidad Peruana de Ciencias Aplicadas (UPC)*". On receipt of the samples the swabs were discarded and tubes were centrifuged to pellet the cells, which were re-suspended in 0.8 ml of PBS 1X. Two aliquots of 200μl of each fresh specimen were used for the extraction of nucleic acids and 200μl for bacterial culture.

DNA extraction

DNA was extracted from a volume of 200μl of each samples using a commercial kit (High Pure Template Preparation Kit, Roche Applied Science, Germany) according to the manufacturer´s instructions. DNA extraction was assayed immediately or stored at -80°C until use.

PCR amplification.

The presence of *B. pertussis* was determined using two PCR assays, each specific for an independent region of the *B. pertussis* genome. A fragment of 191-bp of the pertussis toxin S1 gene (PTxA) was amplified using the primers PTp1: 5´-CCAACGCGCATGCGTGCAGATTCGTC-3´ and PTp2:5´-CCCTCTGCGTTTTGATGGTGCCTATTTTA- 3´.[18] Meanwhile a 145 bp fragment of

the insertion sequence IS481 was amplified using the primers IS481F: 5'-GATTCAATAGGTTGTATGCATGGTT-3' and IS48R: 5'-TTCAGGCAGACAAACTTGATGGGCG-3´.[19] The described procedures were slightly modified as follows: Fifty µl of reaction mixture containing 25 ul ready mix enzyme (Taq polimerase, 2.5 mM Mg Cl2; 15 mM Tris/HCl PH 8.3, 50 mM KCl, 200 uM each deoxynucleotide) (Kappa Biosyste), 20 pmol each primer (Macrogen-Korea), water and 5 ul DNA were amplified using a pre-denaturation of 5 min at 95°C, followed by 55 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C and elongation for 45 sec at 72°C, with a final elongation of 10 min at 72°C. The presence and size of amplification products were analysed by electrophoresis on 2.5% gel agarose, containing 3 µg/mL of ethidium bromide, and photographed under ultraviolet illumination. The amplified products were sequenced (Macrogen, Seoul, Korea).

Respiratory syncytial virus (RSV-A and RSV-B) were identified by multiplex RT-PCR as previously described by Coiras et. al., 2004.[20]

Statistical analysis

Qualitative variables were reported as frequencies and percentages. All analyses were processed with the IBM Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Chicago, IL, USA). The chi-square test (χ^2-) was used to assess associations between categorical variables while z-Test was used to 30 is significant. A p-value <0.05 was considered statistically significant. A seasonal index was calculated for *Bordetella* and Respiratory syncytial virus PCR-confirmed cases from January 2009 to September 2010. Seasonal indexes were calculated dividing the monthly frequency of confirmed cases by the average of cases per year.

RESULTS

A total of 596 children under 5 years diagnosed with an acute respiratory infection were admitted to the *"Hospital Nacional Cayetano Heredia. Lima - Peru"* from January 2009 to September 2010. The pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the cases. Respiratory syncytial viruses (RSV-A and RSV-B) were identified in 17.28% (103/596) of patients. Co-infections between *B. pertussis* and RSV-A were observed in 14 patients and only one sample was positive for *B. pertussis* and RSV-B. (Table 1)

Positive samples for *B. pertussis* and RSV were analyzed according to age distribution, and infants under 3 months old were the most frequently affected in 43% (49/114) and 35.9% (37/103) respectively. A similar sex distribution was observed in both groups. Moreover, around 59% of enrolled children had a previous contact with another patient with acute respiratory infections. An equivalent proportion of household contacts was observed for *Bordetella pertussis* and RSV positive samples. (Table 1)

A similar clinical symptoms frequency was observed between patients with *B. pertussis* and RSV. The most common symptoms in both groups were fever, cough, rhinorrhea and respiratory distress, all of them present in more than 60% of cases. However,

among the patients with a positive RSV sample a higher rate of Rhinorrhea 88.35%, Respiratory distress 76.70% and pharyngeal congestion 33.98% was observed, in comparison with the Pertussis-positive group. (Table 2)

Pneumonia was the most frequent clinical diagnosis in 32.38% (193/596) of the total of patients hospitalized with acute respiratory infections. The diagnosis of Bronchiolitis was more common in children with a positive sample for RSV in 20.39% (20/103). On the contrary, the diagnosis of rhinopharyingitis 6.14% (7/114) was more common in patients with positive *B. pertussis.* (Table 3)

A higher prevalence of *B. pertussis* cases were registered between October and November 2009 and February to April 2010.(Figure 1) Seasonal indexes were calculated for *B. pertussis* and RSV positive samples separately. An increase of pcases was observed from February to March and from October to November with a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively. A similar predominance was observed in RSV cases from November to December. However, RSV showed to be also frequent from April to June with a seasonal index between 1.09-2.00. (Figure 2)

DISCUSSION

Bordetella pertussis is a strict human pathogen which causes whooping cough, an endemic illness responsible of significant morbidity and mortality, especially in infants under 6 months old.[1,2,5] Although regional differences exist, Pertussis represents a considerable global disease burden that has been increasing, even in countries with high vaccination coverage.[2,5,13] In Peru, an alarming increase of cases has been observed in the last 5 years, and 56% of cases are reported in infants under 1 year old.[12,14,15,16] This have raise especial concern since infants under 6 months old are more vulnerable to disease related complications and carry a higher mortality.[7, 21, 22]

The most common clinical manifestations of *B. pertussis* infections are prolonged and paroxysmal coughing, accompanied by inspiratory stridor.[1, 3] However, several factors are known to affect the disease presentation and Pertussis diagnosis may go unrecognized when other pathogens, such as respiratory syncytial virus (RSV) or Influenza virus circulate.[10, 23, 24] A retrospective study in Italy, from a group of infants hospitalized from October 2008 to April 2010 for acute respiratory symptoms reported that most of Pertussis cases were infants under 6 months with median of 71.5 days old and a male: female ratio of 6:13.[10] In our study pertussis toxin and IS481 genes were detected in 19.12% (114/596) of the patients admitted with an acute respiratory

infection and infants under 3 months old were the most frequently affected in 43% (49/114) with a similar sex distribution.

Co-infection between *Bordetella pertussis* and RSV has been previously described to cause severe infections.[10, 11] A study conducted in a group of infants hospitalized for RSV bronchiolitis showed that almost 2% of patients were co-infected with *B. pertussis.*[25, 26] In our series, co-infections were observed in 14 patients between *B. pertussis* and RSV-A and 1 sample was positive for *B. pertussis* and RSV-B. Moreover, 6 out of 9 cases of co-infections were clinically diagnosed as Bronquiolitis and *B. pertussis* was not suspected at the time of admission. Influenza virus and *B. pertussis* co-infections have been also identified as a possible pathogen present in children with community-acquired pneumonia; and the pertussis toxin-mediated suppression have been postulate to be responsible to produce more sever presentations.[27, 28]

Multiple studies have reported Paroxysmal cough (76.5-91.1%), cyanosis (46.7-81.7%) and respiratory distress (47.8-55.7%) as the most common symptoms in children.[13, 29, 30] However, several clinical features might help to suspect the diagnosis of pertussis in infants hospitalized for acute respiratory symptoms.[10]

One study in 2013, compared infants with Pertussis and confirmed RSV bronchiolitis; and the clinical characteristics showed that the percentage of infants with paroxysmal cough was significantly higher in infants with *B. pertussis*. Additionally, cough at admission lasted longer in infants with pertussis than in control infants. Also, fever was significantly lower in infants with pertussis, and more common in patients with bronchiolitis. In our study population, a similar clinical symptoms frequency was

observed between patients with B. pertussis and RSV. The most frequently reported symptoms were fever, cough, rhinorrhea and respiratory distress, in more than 60% of cases. However, the presence of rhinorrhea 88.35%, respiratory distress 76.70% and pharyngeal congestion 33.98% was more common among patients with RSV. This higher frequency of symptoms in our study may be related to fact that more than 52% of our patients were hospitalized infants under 6 months old.

The clinical diagnosis of Pertussis in infants can be challenging, especially in children with incomplete immunizations, and some patients may be catalogued as acute viral respiratory infections, before laboratory confirmation. Thus delaying the appropriate antibiotic treatment and isolation measures.[11, 24] In our series, pneumonia was clearly the most frequent diagnosis in 26.32% (30/114) of the patients with positive *B. Pertussis*. However, other diagnosis were considered in this group, such as rhinopharingitis, bronchiolitis and influenza infections. In contrast, the diagnosis of Bronchiolitis was more common in 20.37% (21/103) of children with a positive sample for RSV.

For *Bordetella pertussis* seasonality, a pattern corresponding to the summer and spring months have been reported in the southern hemisphere.[13] Comparably, a previous study in infants under 6 month of age from 2003 to 2008 in Lima, registered more hospitalizations due to whooping cough during the months of February and September. In our study, a similar distribution was observed with an increase of *B. pertussis* cases from February to March and from October to November and a Seasonal index between 1.32-1.51 and 1.24-3.5 respectively.

Pertussis represents a considerable disease burden in Peru and the diagnosis is complicated by the limitations of currently available diagnostic tests. Therefore, the only diagnostic tests that are recommended for case confirmation in national reporting are culture and polymerase chain reaction (PCR).[7, 31] However, in Peru the use of PCR for surveillance was started recently in 2012 and there is still evidence of a deficient report and registration of cases that limit the analysis of the real disease burden.[32]

CONCLUSION

As in other Latin American countries, epidemiologic surveillance for *B. pertussis* is essential in Peru, especially in children that could most benefit from the vaccine. This study demonstrates a considerable incidence of *B. pertussis* in children previously diagnosed as acute respiratory infections and highlights the importance of possible co-infections that may difficult the diagnosis and prognosis of patients. There is an increasing need for further investigations to better establish the impact of the disease and improve vaccination programs especially in hospitalized children were more severe presentations have been reported.

ACKKNOWLEDGEMENTS

This work has been supported partiality the “Concurso Incentivo a la Investigación de la Universidad Peruana de Ciencias Aplicadas” (UPC). Lima-Perú

AUTHOR’S CONTRIBUTIONS

IV-E, SB-M and AH-A performed the PCR for *Bordetella pertussis* and RSV. VP and JV designed the study protocol; JV was responsible for obtaining funding and laboratory work supervision. PW, MA-L and MJP was responsible for the clinical assessment, samples collection and database completion. PW, MJP, VP and JV drafted the manuscript. All authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Conflicts of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest or funding related to this study

REFERENCES

1. Centers for Disease Control and Prevention (CDC) Childhood Whooping Cough Vaccine Protects Most Children For At Least 5 years. Atlanta, Georgia. [Accessed June 18, 2015; Cited on July 13, 2015]. Available at: http://www.cdc.gov/pertussis/surv-reporting.html.
2. World Health Organization (WHO). Immunizations, vaccines and biological: Pertussis. Geneva, Switzerland. [Accessed July 08, 2015; Cited on July 15, 2015]. Available at: http://www.who.int/immunization/topics/pertussis/en/
3. Torres R, Santos T, Torres R, et al. Resurgence of pertussis at the age of vaccination: clinical, epidemiological, and molecular aspects. J Pediatr (Rio J). 2015 Jan 23. pii: S0021-7557(15)00006-6
4. Van den Brink G, Wishaupt J, Douma J, et al. (2014) Bordetella pertussis: an underreported pathogen in pediatric respiratory infections, a prospective cohort study. BMC Infect Dis 30;14:526.
5. Gao F, Mahoney J, Daly E, et al. (2014) Evaluation of a Multitarget Real-Time PCR Assay for Detection of Bordetella Species during a Pertussis Outbreak in New Hampshire in 2011. J Clin Microbiol52(1):302-306.

6. Nikbin V, Shahcheraghi, Lotfi M, et al. (2013) Comparison of culture and real-time PCR for detection of *Bordetella pertussis* isolated from patients in Iran. Iran J Microbiol. 5(3):209-214.
7. Murphy T, Slade B, Broder K, et al. Prevention of pertussis, tetanus and diphtheria among pregnant and postpartum women and their infants recommendations of the Advisory Committee on Immunization Practices(ACIP). MMWRRecommRep.2008;57(RR-4): 1–51.
8. Hajia M, Rahbar M, Fallah F. Detection of *Bordetella pertussis* in Infants Suspected to have Whooping Cough. Open Respir Med J. 2012;6:34-6.
9. Riffelmann M, Wirsing von König C, Caro V, et al. Nucleic Acid amplification tests for diagnosis of Bordetella infections J Clin Microbiol. 2005 Oct;43(10):4925-9.
10. Nicolai A, Nenna R, Stefanelli P, et al. *Bordetella pertussis* in infants hospitalized for acute respiratory symptoms remains a concern. BMC Infect Dis. 2013 Nov 8;13:526.
11. Crowcroft N, Booy R, Harrison T, et al. Severe and unrecognized: pertussis in UK infants. Arch Dis Child. 2003 Sep;88(9):802-6.
12. Perret C, Viviani T, Peña A, et al. Source of infection in young infants hospitalized with *Bordetella pertussis*. Rev Med Chile 2011; 139: 448-454.
13. Gentile A, Romanin V, Juarez Mdel V, et al. Epidemiology of *Bordetella pertussis* in a children's hospital. Arch Argent Pediatr. 2014 Feb;112(1):26-32.
14. Red Nacional de Epidemiologia (RENACE). Distribución de Tos Ferina por distritos Peru 2015. [Internet]. Lima, Perú. Dirección General de Epidemiología (DGE). [Accessed on June 12, 2015; Cited on July 09, 2015] Available at: http://www.dge.gob.pe/portal/docs/vigilancia/sala/2015/SE23/tosf.pdf
15. Ministerio de Salud del Peru (MINSA).Alerta Epidemiológica: Brote de Tos Ferina en el País. [Internet]. Lima, Peru. Dirección General de Epidemiología (DGE). [Accessed June 12, 2015; Cited on July 13, 2015] Available at:

http://www.inr.gob.pe/transparencia/Epidemiolog%C3%ADa/alertas%20epidemiologicas/ALERTA%20N%C2%BA%2003%20BROTE%20DE%20TOS%20FERINA.pdf

16. Ministerio de Salud del Peru (MINSA). Boletín Epidemiológico 06. [Internet]. Lima, Peru. Dirección General de Epidemiología (DGE). [Accessed June 22, 2015; Cited on July 10, 2015] Available at: http://www.dge.gob.pe/portal/docs/vigilancia/boletines/2013/06.pdf
17. Oficina General de Epidemiologia e Informática (OGEI) – Ministerio de Salud (MINSA): Boletín: Bordetella Pertussis; Lima, Peru; 2015. (Unpublished report)
18. Houard S, Hackel C, Herzog A, Bollen A. Specific identification of *Bordetella pertussis* by the polymerase chain reaction. Res Microbiol. 1989 Sep;140(7):477-87.
19. Glare EM, Paton JC, Premier RR, Lawrence AJ, Nisbet IT. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J Clin Microbiol. 1990 Sep;28(9):1982-7.
20. Coiras M, Pérez-Breña P, García M, et al. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J Med Virol. 2003 Jan; 69(1):132-44.
21. Tan T, Trindade E, Skowronski D. Epidemiology of Pertussis. Pediatr Infect Dis J. 2005 May;24(5 Suppl):S10-8.
22. Quian J, Cerisola A, Russomano F, et al. Infecciones por *Bordetella pertussis* en niños menores de un año hospitalizados y sus contactos en el hogar. Arch Pediatr Urug. 2006; 77(3):229-236.

23. Matto S, Cherry J. Molecular Pathogenesis, Epidemiology, and Clinical Manifestations of Respiratory Infections Due to Bordetella pertussis and Other Bordetella Subspecies. Clin Microbiol Rev. 2005 Apr;18(2):326-82.
24. Siberry G, Paquette N, Ross T, et al. Low prevalence of pertussis among children admitted with respiratory symptoms during respiratory syncytial virus season. Infect Control Hosp Epidemiol. 2006 Jan;27(1):95-7.
25. Walsh P, Overmeyer C, Kimmel L, et al. Prevalence of B*ordetella pertussis* and Bordetella parapertussis in samples submitted for RSV screening. West J Emerg Med 2008, 9:135–140.
26. Walsh PF, Kimmel L, Feola M, et al. Prevalence of *Bordetella pertussis* and Bordetella parapertussis in infants presenting to the emergency department with bronchiolitis. J Emerg Med 2011, 40:256–261.
27. Holter JC, Müller F, Bjoran O, et al. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. BMC Infect Dis. 2015 Feb 15;15:64
28. Ayala VI, Teijaro JR, Farber DL, et al. *Bordetella pertussis* infection exacerbates influenza virus infection through pertussis toxin-mediated suppression of innate immunity. PLoS One. 2011 Apr 20;6(4):e19016.
29. Nieto-Guevara J, Luciani K, Montesdeoca-Melian, et al. Hospital admissions due to whooping cough: experience of the del niño hospital in Panama. Period 2001-2008. An Pediatr (Barc). 2010 Mar;72(3):172-8.
30. Castillo M, Petrozzi V, Vera M, et al. Study Clinical and Epidemiological of Whooping Cough in Breast-Fed Babies under 6 months admitted in three Hospitals of Lima - Peru over the years 2003 – 2008. Rev Peru Pediatr.2012; 65 (1): 21-32
31. Pan American Health Organization (PAHO). Alerta Epidemiológica: Tos Ferina (Coqueluche). [Internet]. Washington, D.C. [Accessed May 22, 2015; Cited on July 11, 2015] Available

at: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=19325&Itemid

32. Ministerio de Salud del Peru (MINSA). Boletín Epidemiológico 06. [Internet]. Lima, Peru. Dirección General de Epidemiología (DGE). [Accessed June 22, 2015; Cited on July 10, 2015] Available at: http://www.dge.gob.pe/portal/docs/vigilancia/boletines/2013/06.pdf

Table 1: General characteristics of *Bordetella pertussis* and RSV cases.

CHARACTERISTIC	**Total ARI patients**	***Bordetella pertussis***	**RSV**
	Frequency (n= 596) N (%)	**Frequency (n=114) N (%)**	**Frequency (n=103) N (%)**
Gender			103
Female	243 (40.8)	52 (45.6)	46 (44.7)
Male	353 (59.2)	62 (54.4)	57 (55.3)
Age			
Newborn (≤ 28 days)	112 (18.8)	17 (14.9)	11 (10.7)
29 days – ≤ 3 months	121(20.3)	32 (28.1)	26 (25.2)
3 – 5 months	82 (13.8)	13 (11.4)	11(10.7)
6 – 11 months	115(19.3)	20 (17.6)	26 (25.2)
1 – 5 years	166 (27.9)	32 (28.1)	29 (28.2)
Contact with another people with ARI			
Yes	353 (59.2)	67 (58.8)	59 (57.3)
Not	243 (40.8)	47 (41.2)	44 (42.7)

Table 2: Clinical symptoms observed in patients with positive *B. pertussis* and RSV by PCR.

CLINICAL SYMPTOMS	Total of patients	Patients positive for *Bordetella pertussis*	Patients positive for RSV
	Frequency (n=596) N (%)	Frequency (n= 114) N (%)	Frecuency (n=103) N (%)
Fever	596 (100)	114 (100)	103 (100)
Cough	448 (75.2)	82 (71.9)*	92 (89.32)*
Rinorrhea	448 (75.2)	90 (78.9)	91(88.35)
Respiratory distress	366 (61.4)	69 (60.5)*	79 (76.70)*
Wheezing respiratory	230 (38.6)	40 (35.1)*	59 (57.28)*
Malaise	150 (25.2)	28 (24.6)	24 (23.30)
Pharyngeal congestion	150 (25.2)	25 (21.9)*	35 (33.98)*
Expectoration	142 (23.8)	28 (24.6)	30 (29.13)
Vomits	79 (13.3)	16 (14)	16(15.53)
Diarrhea	71(11.9)	13 (11.4)	15 (14.56)
Asthenia	52 (8.7)	13 (11.4)	9 (8.74)
Conjunctival congestion	23(3.9)	5 (4.4)	5 (4.85)
Abdominal pain	21(3.5)	2 (1.7)	2 (1.94)
Headache	16 (2.7)	3(2.63)	4 (3.88)
Otalgia	6 (1.0)	2 (1.75)	1 (0.97)
Myalgia	6 (1.0)	1(1.75)	1 (0.97)

* z-Test: Patients positive for *Bordetella pertussis* vs Patients positive for RSV, $p<0.05$

Others (< 10% of cases): Ear pain, photophobia, conjunctival congestion, abdominal pain, lymphadenopathy, fatigue, headache, myalgia, skin rash

* 3 children died, one of them in the *B.pertussis* infection group

Table 3: Clinical diagnosis observed in patients with positive *B. pertussis* and RSV by PCR.

CLINICAL DIAGNOSIS	Total of patients		Patients positive for *Bordetella pertussis*			Patients positive for *RSV*		
	Frequency (n=596)	Prevalence (%)	Frequency (n= 114)	Prevalence (%)	p-value**	Frequency (n= 103)	Prevalence (%)	p-value**
Pneumonia	193	32.38	30	26.32*	0.124	44	42.72*	0.014
Pharyngitis	6	1.01	0	0	0.231	1	0.97	0.968
Rhinopharyngitis	33	5.54	7	6.14	0.754	3	2.91	0.200
Bronchiolitis	57	9.56	9	7.9*	0.327	21	20.39*	<0.05
Influenza A Infection	51	8.56	10	8.77	0.927	6	5.83	0.276
Whooping cough-like syndrome	10	1.68	3	2.63	0.378	2	1.94	0.819
Obstruction syndrome to bronchiolar	41	6.88	9	7.89	0.634	11	10.68	0.094

* z-Test: Patients positive for *Bordetella pertussis* vs Patients positive for RSV, $p<0.05$

** χ^2-Test

Others (1% of cases): Sinusitis, respiratory distress syndrome, sepsis late atypical febrile seizure status epilepticus, atypical febrile seizure, gastroenteritis.

Figure 1. *Bordetella pertussis* confirmed cases (2009-2010).

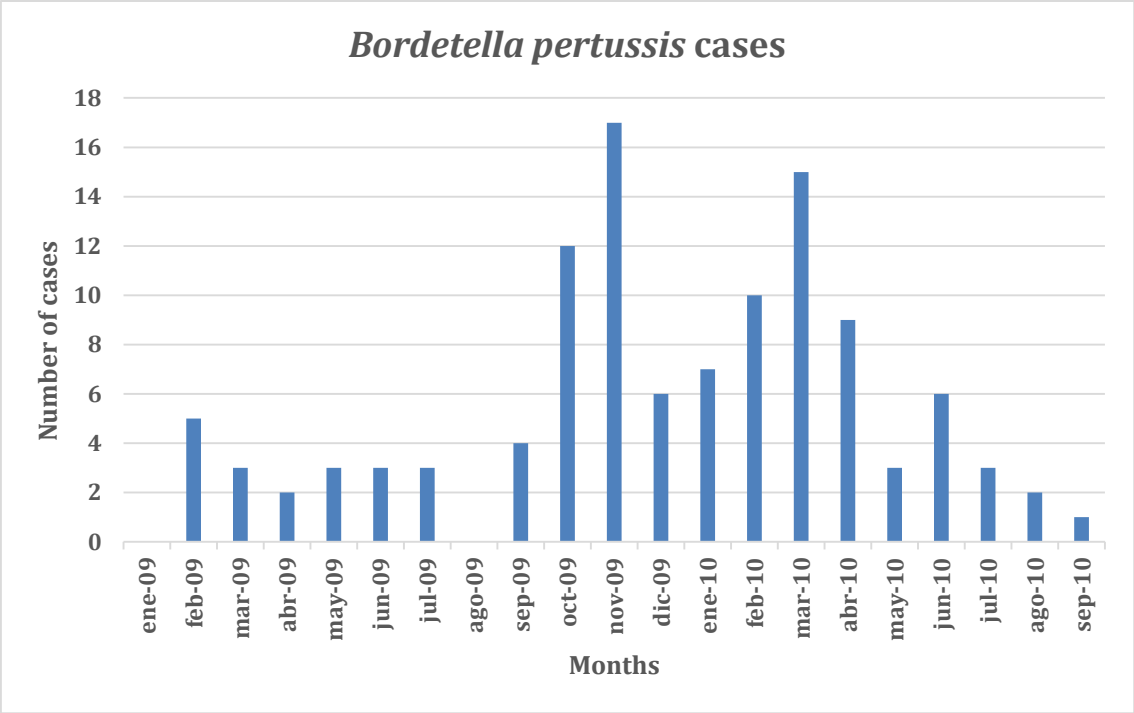
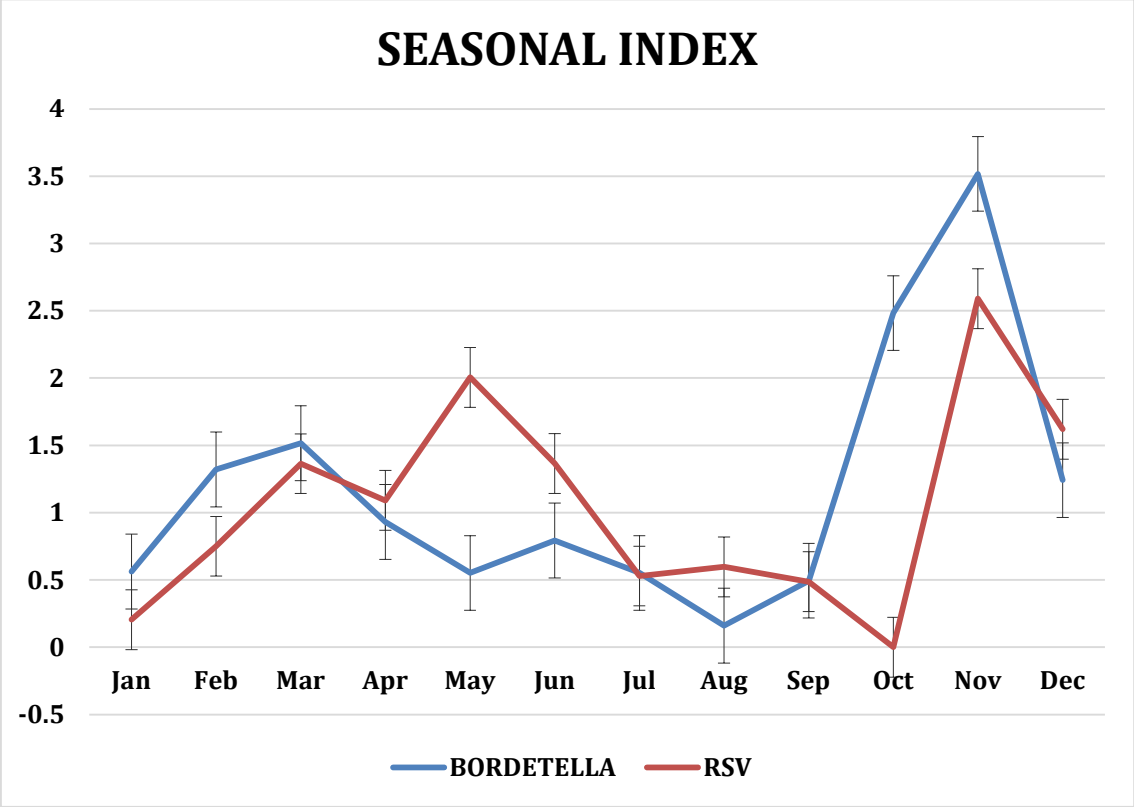


Figure 2. *Bordetella pertussis* and RSV seasonal index (2009-2010).



5. PUBLICACIÓN

5.1 COMPROMISO DE PUBLICACIÓN

Monterrico, 20 de Setiembre del 2015

COMPROMISO DE PUBLICACIÓN

Sr. Decano de la Facultad de Ciencias de la Salud

Por medio de la presente nos dirigimos a Ud. a fin de comunicarle nuestro interés por publicar en la revista " BMC Infectious Diseases" el artículo titulado:

High prevalence of *Bordetella pertussis* in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru.

El cual es tema de tesis de los autores: "Ivana Pavic Espinoza", "Sandy Bendezú Medina", "Angella Herrera Alzamora " y fue supervisado por la asesora: Juana del Valle Mendoza.

En este sentido, el autor se compromete a realizar el seguimiento respectivo con la

finalidad de publicar el artículo en la revista mencionada.

Atentamente,

Ivana Pavic Espinoza

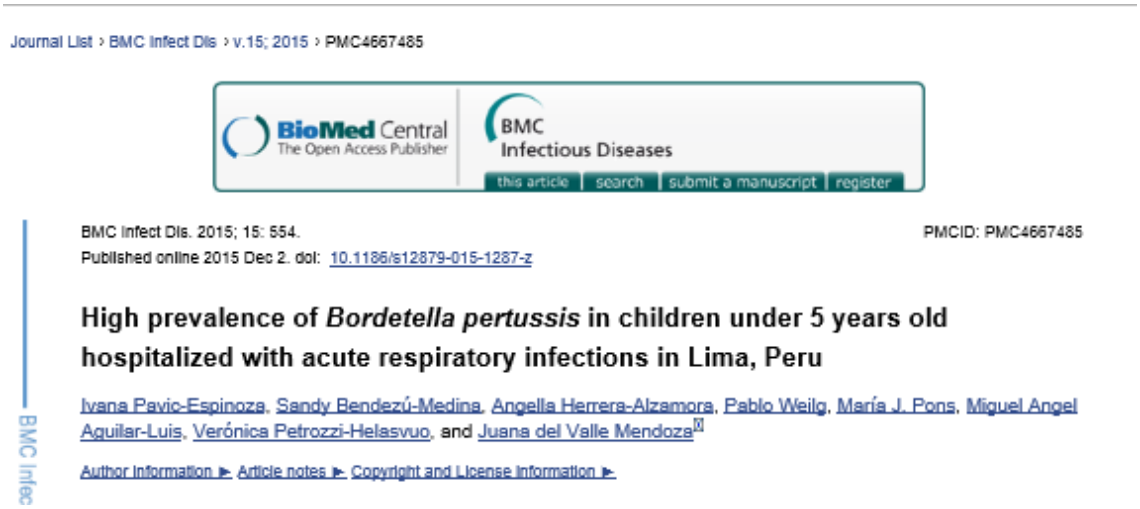
Sandy Bendezú Medina

Angella Herrera Alzamora

Juana del Valle Mendoza

5.2 ESTADO DE PUBLICACIÓN

El artículo ha sido aceptado y publicado el 2 de Diciembre del 2015por la Revista BMC Infectious Diseases, publicación on line open Access: http://www.ncbi.nlm.nih.gov/pubmed/26626910.



Journal List › BMC Infect Dis › v.15; 2015 › PMC4667485

BioMed Central The Open Access Publisher

BMC Infectious Diseases

this article | search | submit a manuscript | register

BMC Infec

BMC Infect Dis. 2015; 15: 554. PMCID: PMC4667485

Published online 2015 Dec 2. doi: 10.1186/s12879-015-1287-z

High prevalence of *Bordetella pertussis* in children under 5 years old hospitalized with acute respiratory infections in Lima, Peru

Ivana Pavic-Espinoza, Sandy Bendezú-Medina, Angella Herrera-Alzamora, Pablo Weilg, María J. Pons, Miguel Angel Aguilar-Luis, Verónica Petrozzi-Helasvuo, and Juana del Valle Mendoza

Author information ▶ Article notes ▶ Copyright and License information ▶