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

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ABSTRACT

Acute respiratory infections (ARI) are the main cause of morbidity and mortality in children under 5 years worldwide. *Bordetella pertussis* is a highly contagious bacterium that can cause serious illness, and approximately half of infected infants less than 1 year old are hospitalized. Also, pertussis immunization series is not completed until six months of age, leaving young infants vulnerable to pertussis. In Peru, pertussis is an increasing health problem despite immunization efforts, and the role of B. pertussis in ARI is unknown.

We determined the prevalence of B. pertussis among children under 5 years old admitted to Hospital Nacional Cayetano Heredia in Lima with diagnosis of ARI between Jan-2009 and Dec 2010. Epidemiological and clinical features were collected, and presence of B. pertussis was determined by PCR (pertussis toxin and IS481 gene).

A total of 596 nasopharyngeal samples among children under 5 years were analyzed. In 114 (19.1%) samples were positive for B. pertussis. 32.5% of sample positive to *B. pertussis* were diagnosed as viral pneumonia at diagnosis. Importantly, 71.9% of cases were under 12 months of age and 58.8% have been contact with other ARI infected people. Significant differences in clinical symptoms between the total ARI cases and *B. pertussis* cases were not found. The most frequent symptoms in *B. pertussis* cases were fever (100%), rhinorrhea 78%, cough 71.9% and respiratory distress 60.5%. One child died due to the infection. *B. pertussis* cases showed a seasonal distribution with peaks during the months March June and November.

This study shows the high prevalence of *B. pertussis* in infants who were hospitalized due to severe acute respiratory infections in Lima, Peru. Epidemiologic surveillance programs for B. pertussis are essential in the future in Peru

MATERIAL and METHODS

Samples

Two nasopharyngeal samples were obtained per patient. The first, by inserting a swab into both nostrils parallel to the palate (calcium alginate swab, USA) and the second swab was of 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 NaHCO3 and supplemented with 2% fetal bovine serum, penicillin and streptomycin 100 U/ml, amphotericin B 20 □g/ml, neomycin 40 □g/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 the 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 sample 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 procedures described were slightly modified as follows: Fifty µl of reaction mixture containing 25 ul ready mix enzyme (Taq polymerase, 2.5 mM Mg Cl2; 15 mM Tris/HCl PH 8.3, 50 mM KCl, 200 uM each deoxynucleotide) (Kappa Biosyste), 20 pmol of each primer (Macrogen, Seoul, 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 analyzed by electrophoresis on 2.5% gel agarose, containing 3 □g/mL of ethidium bromide, and photographed under ultraviolet illumination. The amplified products were sequenced (Macrogen, Seoul, Korea).

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

RESULTS

•A total of 596 children under 5 years of age 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, being identified in 43% (49/114) and 35.9% (37/103), respectively. A similar sex distribution was observed in both groups. Moreover, around 59% of the children enrolled had had a previous contact with another patient with acute respiratory infection. An equivalent proportion of household contacts was observed for *B. pertussis* and RSV positive samples. (Table 1)

•The frequency of clinical symptoms was similar between patients with *B. pertussis* and RSV. The most common symptoms in both groups were fever, cough, rhinorrhea and respiratory distress, all being present in more than 60% of the cases. However, the patients with a positive RSV sample showed a higher rate of rhinorrhea (88.35%), respiratory distress (76.70%) and pharyngeal congestion (33.98%) 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 infection. The diagnosis of bronchiolitis was more common in children with a positive sample for RSV in 20.39% (20/103). To the contrary, the clinical diagnosis of influenza A H1N1 was clinically diagnosed in 8.77% (10/114) and rhinopharyingitis in 6.14% (7/114) being more frequently observed in patients positive for *B. pertussis*. (Table 3)

•A higher prevalence of *B. pertussis* cases was registered between October and November 2009 and February to April 2010.(Figure 1). The seasonal indexes for *B. pertussis* and RSV positive samples were calculated separately. An increase of pertussis cases was observed from February to March and from October to November with a seasonal index of 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 also showed to be frequent from April to June with a seasonal index between 1.09-2.00. (Figure 2)

Table 1:**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)

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 (%)	Odds	Frequency (n= 103)	Prevalence (%)	Odds
Pneumonia	193	32.38	30	26.32	0,357*	44	42.72	0,746*
Pharyngitis	6	1.01	0	0	0,000	1	0.97	0,010
Rhinopharyngitis	33	5.54	7	6.14	0,065	3	2.91	0,030
Bronchiolitis	57	9.56	9	7.9	0,086*	21	20.39	0,256*
Influenza A Infection	51	8.56	10	8.77	0,096	6	5.83	0,062
Whooping cough-like syndrome	10	1.68	3	2.63	0,027	2	1.94	0,020
Obstruction syndrome to bronchiolar	41	6.88	9	7.89	0,086	11	10.68	0,120
No data	199	33.39	46	40.35	0,676*	15	14.56	0,170*

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

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