

# Epidemiological characteristics of whooping cough in Paraguay (2005-2019)

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## Abstract

**Objective:** To describe the epidemiological characteristics of whooping cough in Paraguay.

**Methods:** 5327 samples of children and adults of all ages with suspected whooping cough and their contacts were included in this study. 745 samples were processed per microbiological culture and 4582 samples per microbiological culture and PCR.

**Results:** The overall frequency of cases and their laboratory-confirmed contacts was 9.1%. A total of 361 cases and 123 close case contacts were detected as positive. Positive cases were more common in the < 2 months (70.6%), followed by the 2-6 month (11.9%).

**Conclusion:** In Paraguay it is a public health problem that mainly affects children under 2 months, who have not yet received the vaccine. Knowing the epidemiology of whooping cough or pertussis helps us to know the burden of the disease as well as to measure the effectiveness and impact of vaccines.

**Keywords:** Whooping cough, *Bordetella pertussis*, epidemiology

## Características epidemiológicas de la tos ferina en Paraguay (2005-2019)

### Resumen

**Objetivo:** Describir las características epidemiológicas de la tos ferina en Paraguay.

**Métodos:** Se incluyeron 5327 muestras de niños y adultos de todas las edades con sospecha de tos ferina y sus contactos. Se procesaron 745 muestras por cultivo microbiológico y 4582 muestras por cultivo microbiológico y PCR.

**Resultados:** La frecuencia global de casos y sus contactos confirmados por laboratorio fue del 9,1%. Se detectaron como positivos un total de 361 casos y 123 contactos cercanos de casos. Los casos positivos fueron más frecuentes en los < 2 meses (70,6%), seguidos de los 2-6 meses (11,9%).

**Conclusión:** En Paraguay es un problema de salud pública que afecta principalmente a niños menores de 2 meses, que aún no han recibido la vacuna. Conocer la epidemiología de la tos ferina o tos ferina nos ayuda a conocer la carga de la enfermedad así como a medir la efectividad y el impacto de las vacunas.

**Palabras clave:** tos ferina, *Bordetella pertussis*, epidemiología

## Introduction

Whooping cough is a disease caused by the bacterium *Bordetella pertussis*<sup>1</sup>. It is a preventable respiratory disease that mainly affects children and is a major cause of hospitalization and death in infants who have not yet been vaccinated (<2 months)<sup>2</sup>. In 2013, an estimated 136,000 cases worldwide were reported<sup>3</sup>. More than 80% of cases and 95% of deaths occurred in developing countries, where vaccination coverage is low<sup>4</sup>.

It is a infectious disease transmitted from person to person through sneezing or coughing. In adults and adolescents, pertussis can occur with few symptoms. Vaccination is the

main prevention strategy<sup>5</sup>. Vaccination does not induce long-term immunity, but five to ten years after the last booster dose. Older contacts are often the source of infection for children under 6 months of age, who are at increased risk of morbidity and mortality<sup>6</sup>.

Lack of booster immunization and improvement in diagnostic methods, low vaccine coverage and genetic changes contribute to the onset of the disease<sup>7</sup>. Since 2002, many Latin American countries have reported increases in the number of cases, including Argentina, Brazil, Chile, Colombia, Panama and Mexico<sup>8,9</sup>. In Paraguay it is a disease of immediate and individual notification. In 1978 the DPT (against diphtheria, whooping cough and tetanus) is incorporated and since

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2002 the pentavalent vaccine (against diphtheria, convulsive cough, tetanus, hepatitis B and *H. influenzae b*) has been included in the vaccination schedule. The whooping cough vaccination scheme, used in our country includes a primary series of three doses (2, 4 and 6 months; with the pentavalent vaccine), and two reinforcements (1 and 4 years; with the DPT vaccine). And in 2013 the triple acellular bacterial vaccine (Tdap) was incorporated for pregnant women from 20 weeks gestation, 10-year teens and health personnel<sup>10</sup>.

Diagnoses recommended by the World Health Organization (WHO) include cultivation and PCR of nasopharyngeal samples and also serological tests<sup>11</sup>. Laboratorial surveillance in Paraguay began in 2005 with conventional microbiological culture and in 2011 molecular techniques (real-time PCR) were incorporated to improve diagnostic sensitivity at the Central Laboratory of Public Health, which is the reference center for surveillance in Paraguay.

Strong epidemiological and laboratory surveillance is crucial to understand epidemiological characteristics and implementing strategies that reduce the impact of this disease.

The objective of this study is to describe the epidemiological characteristics of whooping cough in Paraguay.

## Methods

The type of study has a non-experimental design, with a quantitative and cross-sectional approach. It is a non-probabilistic type, all the samples from patients were considered and they were represented by confirmed cases of *Bordetella pertussis*, by phenotypic or genotypic techniques.

### Samples

This study included 5327 samples of children and adults of all ages with suspected whooping cough and their contacts, who attended the different health services of Paraguay's 18 departmental regions, during the period 2005-2019, within the framework of national whooping cough surveillance.

A total of 3971 nasopharyngeal swabs and 1356 nasopharyngeal aspirates were studied, with 745 samples processed per conventional microbiological culture and 4582 samples per conventional microbiological culture and PCR.

### Case definitions

Suspect case: anyone who coughs for at least 14 days, and one of the following symptoms: paroxysm (cough crisis), stridor, vomiting immediately after coughing without other apparent causes. In children under 3 months of age, it is considered a suspicious case associated with apnea and cyanosis. Laboratory-confirmed case: anyone presenting positive culture for *Bordetella pertussis* or PCR positive.

Epidemiological nexus-confirmed case: any suspected case with epidemiological link to a laboratory-confirmed case.

## Isolation and identification

Nasopharyngeal swab and nasopharyngeal aspirate were cultured in Regan-Lowe agar plates with cephalixin, incubated at 37 °C with high humidity and examined daily for seven to ten days. The colonies were dyed to verify the presence of gram-negative coccobacilli and *Bordetella species* were confirmed by biochemical tests such as oxidase, urease, nitrate reduction, citrate, as well as slide agglutination with specific antisera<sup>11</sup>.

Nucleic acid extraction was performed with automated equipment (Magpurix, Taiwan) using the Bacterial DNA Extraction Kit - V1.1. Molecular diagnosis was performed with a commercial kit (Rida® gene, R-biopharm, Darmstadt, Germany Germany), according to manufacturer's recommendations.

## Data analysis

This study is limited to the description of the data and does not include any inferential statistical analysis. Calculations of absolute frequency and percentage ratios were performed. The variables analyzed were the age and sex of patients and contacts, sample identification code, place of origin (departmental region), sample type (nasopharyngeal aspirate and nasopharyngeal swab), sampling date, microorganism (confirmed by conventional microbiological culture or genotypically by PCR).

Statistical analyses were carried out with the Stata 11.0 Program (StataCorp. 2011. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

## Results

A total of 5327 samples of patients suspected of whooping cough, predominantly female and < 2 months, were studied. Table 1.

Confirmed by conventional microbiological culture 64/484 (13.2%) and by real time PCR 420/484 (86.8%).

According to the sample type, 186/1356 (13.7%) were found in nasopharyngeal aspirates and 297/3971 (7.4%) in nasopharyngeal swabs.

The overall frequency of cases and their laboratory-confirmed contacts was 9.1%, during the period 2005 to 2019, with predominance of the female sex 62.0%. The distribution according to the year of study: 10/104 (9.6%) in 2005, 6/109 (5.5%) in 2006, 4/71 (5.6%) in 2007, 7/240 (2.9%) in 2008, 1/129 (0.8%) in 2009, 2/92 (1.1%) in 2010, 11/171 (6.4%) in 2011, 34/363 (9.4%) in 2012, 73/467 (15.6%) in 2013, 108/663 (16.3%) in 2014, 39/545 (7.2%) in 2015, 34/475 (7.2%) in 2016, 50/629 (7.9%) in 2017, 63/620 (10.2%) in 2018, 42/649 (6.5%) in 2019. Figure 1.

The distribution of suspected and confirmed cases according to the month of notification resulted in the highest number of suspected cases in June (N=724) and the month of September the highest number of confirmed cases (N=145). Figure 2.

**Table 1.** Distribution of suspected cases of whooping coughing in Paraguay (2005-2019) (N=5327)

Year	N	Male	Female	< 2 months	2-6 months	7-11 months	1-4 years	5-9 years	10-14 years	15-19 years	>20 years
2005	104	41	63	20	41	2	15	8	7	0	11
2006	109	48	61	12	41	7	25	6	3	3	12
2007	71	32	39	10	35	1	5	1	12	1	6
2008	240	100	140	50	104	8	14	6	10	9	39
2009	129	54	75	38	43	6	12	3	2	8	17
2010	92	43	49	23	40	11	5	3	1	1	8
2011	171	83	88	35	62	6	14	6	7	6	35
2012	363	157	206	180	0	0	50	10	14	29	80
2013	467	170	297	197	0	0	49	26	23	35	137
2014	663	246	417	289	3	0	62	12	12	56	229
2015	545	201	344	287	2	0	33	13	5	41	164
2016	475	188	287	263	1	0	26	12	11	26	136
2017	629	245	384	257	4	0	54	34	15	44	221
2018	620	246	374	73	134	26	56	25	48	42	216
2019	649	257	392	72	168	18	60	28	20	35	248
<b>Total</b>	<b>5327</b>	<b>2111</b>	<b>3216</b>	<b>1806</b>	<b>678</b>	<b>85</b>	<b>480</b>	<b>193</b>	<b>190</b>	<b>336</b>	<b>1559</b>

A total of 361 positive cases and 123 close case contacts were positive. Positive cases were more common in the < 2 months (70.6%) cohort, followed by the 2-6 month cohort (11.9%) and between contacts in the group of > 20 years (74.0%). Table 2.

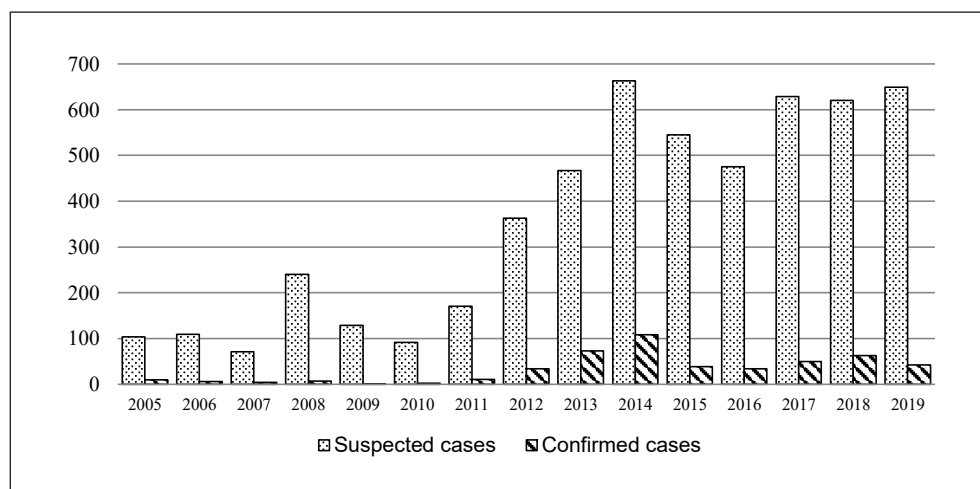
Laboratory-confirmed contacts were 123/361 (34.0%), being mothers 91/123 (74.0%), grandmothers 6/123 (6.5%), fathers 7/123 (5.7%), sisters 6/123 (4.9%), brothers 6/123 (4.9%), aunts 4/123 (3.2%) and grandfather 1/123 (0.8%).

The departmental regions with the most positive cases were Central (Sanitary Region XI) with 243/484, Capital (Sanitary Region XVIII) with 54/484 and Amambay (Sanitary Region XIII) with 34/484.

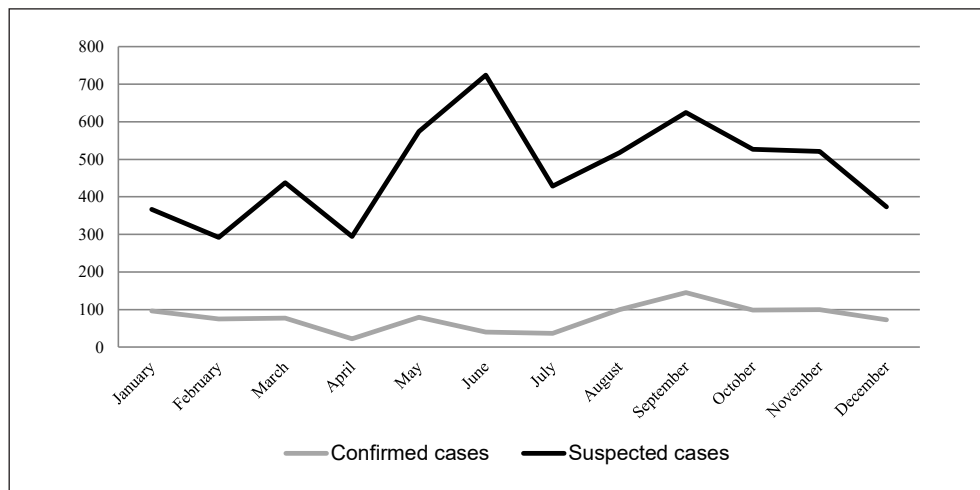
## Discussion

Whooping cough has resurfaced in many countries around the world as a major cause of infant morbidity and mortality. The resurgence of the disease is not fully understood but could be explained by the replacement of the whole cell vaccine with the acellular vaccine which could have led to a selection within the circulating clones of *B. pertussis* and consequently increased the number of cases. Another hypothesis is that the introduction of molecular techniques improved diagnostic sensitivity<sup>12,13</sup>.

During the study period there was an increase in the number of suspected cases of the disease, which may be indicating an expansion and improvement of the whooping cough sur-



**Figure 1.** Distribution of laboratory-confirmed cases and contacts by study year (N=5327)



**Figure 2.** Distribution of suspected and confirmed cases according to month of notification (2005-2019) (N=5327).

veillance system in the country. In Paraguay, PCR was introduced in 2011 as part of national surveillance procedure and since then an increase in the number of confirmed cases can be observed.

In this study the overall frequency turned out to be 9.1%, being 2013 and 2014 the years with the highest frequency of cases. Studies in Brazil (2007-2016) showed a frequency of 43.0% and 19.6% (117/597) respectively during a 5-year study period<sup>14,15</sup>. In Santa Fé, Argentina, a group of researchers recorded 102 (9.49%) confirmed cases of 1074 samples of individuals that were determined by PCR. A decrease in cases was observed over the years of the study (2006-2010), with no positive cases found in the last two years of the study<sup>16</sup>. Conversely, other studies found a frequency of 53% amongst patients and contacts<sup>17</sup>.

These study showed a high prevalence in children under 2 months of age, followed by the 2-6 month group, indicating that this disease occurs in children who did not receive the vaccine yet and children with the incomplete vaccination scheme, as previously stated by other authors<sup>15,18</sup>. Similar to these results in Quito, Ecuador, it was found that the majority of positive cases of *B. pertussis* infections corresponded to infants belonging to the 0-3 months of age group, reaching a prevalence of 65%<sup>19</sup>. According to previous publications, in the age group of children under 3 months the rates of hospitalizations, complications and deaths are significantly higher and > 90% of all deaths associated with whooping cough occurred in infants who have not reached protection, since in most countries it begins at 2 months of age<sup>12,20</sup>.

Contacts could be confirmed in 34.0% of cases, where the main source of transmission was the mothers. Several studies state that the main epidemiological contact is intra-domiciliary. Additionally, they indicate that 53% of contacts were confirmed by laboratory being the most likely source of infection of the baby, a sibling (41%), mother (38%) or father (17%)<sup>21</sup>. In Spain, they concluded that mothers were the main source of transmission (21.0%)<sup>22</sup>. In the United States, brothers were found to be the main source (35.5%) followed by mothers (20.6%) and fathers (10%)<sup>23</sup>. In Chile, they found mainly mothers and fathers (40%) and in Peru, the mother was the likely epidemiological contact (17.86%), followed by siblings (14.29%), uncles (14.29%), cousins (10.71%) and grandparents (3.57%), all intradomiciliary<sup>5</sup>.

A greater number of cases were confirmed in the month of September. Some countries have suggested increases during the spring-summer months<sup>2,25</sup>, however, there are also studies that have suggested that whooping cough may be more evident during the winter months<sup>26</sup>. A study in Germany showed that after the introduction of a vaccine against *B. pertussis* the seasonal pattern with the highest number of infections in the spring to summer months changed<sup>27</sup>.

A total of 13.2% of *Bordetella pertussis* strains could be recovered by bacteriological culture, which is the gold standard in diagnosis, but has only a sensitivity of 12-60%<sup>24</sup>. Several studies suggest low sensitivity compared to the PCR technique (1.4%)<sup>28</sup>.

The highest number of cases was observed in the central department region and in the capital, which are the departments with the highest population density.

**Table 2.** Distribution of confirmed cases of whooping cough and their contacts according to age group (N=484).

	< 2 months	2-6 months	7-11 months	1-4 years	5-9 years	10-14 years	15-19 years	>20 years	Total
Cases	255	43	8	35	3	11	6	0	361
Contacts	0	0	0	3	4	6	19	91	123
Total	255	43	8	38	7	17	25	91	484

Strengthening disease surveillance and knowing the epidemiology of whooping cough or pertussis helps us to know the burden of the disease as well as to measure the effectiveness and impact of vaccines.

### Ethical disclosures

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**Conflict of interest.** The authors declare that they have no conflict of interest.

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