

Prevalence of *Bordetella pertussis* and *Bordetella parapertussis* in Samples Submitted for RSV Screening

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Background: The clinical presentation of *Bordetella pertussis* can overlap with that of respiratory syncytial virus (RSV); however, management differs.

Hypothesis: First, the prevalence of *B. pertussis* is less than 2% among patients screened for RSV, and second the prevalence of *B. parapertussis* is also less than 2% among these patients.

Methods: Nasal washings submitted to a clinical laboratory for RSV screening were tested for *B. pertussis* and *B. parapertussis*, using species-specific real-time polymerase chain reaction (PCR) assays. These were optimized to target conserved regions within a complement gene and the *CarB* gene, respectively. A *Bordetella* spp. genus-specific real-time PCR assay was designed to detect the *Bhur* gene of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*. RSV A and B subtypes were tested by reverse transcription-PCR.

Results: Four hundred and eighty-nine clinical samples were tested. There was insufficient material to complete testing for one *B. pertussis*, 10 RSV subtype A, and four RSV subtype B assays. *Bordetella pertussis* was detected in 3/488 (0.6%) (95% CI 0.1% to 1.8%), while *B. parapertussis* was detected in 5/489 (1.0%) (95% CI 0.3% to 2.4%). Dual infection of *B. pertussis* with RSV and of *B. parapertussis* with RSV occurred in two and in three cases respectively. RSV was detected by PCR in 127 (26.5%).

Conclusion: The prevalence of *B. pertussis* in nasal washings submitted for RSV screening was less than 2%. The prevalence of parapertussis may be higher than 2%. RSV with *B. pertussis* and RSV with *B. parapertussis* coinfection do occur.

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INTRODUCTION

The clinical presentation of *Bordetella pertussis* can

overlap with and be clinically indistinguishable from that of respiratory syncytial virus (RSV).^{1,2} The potential for

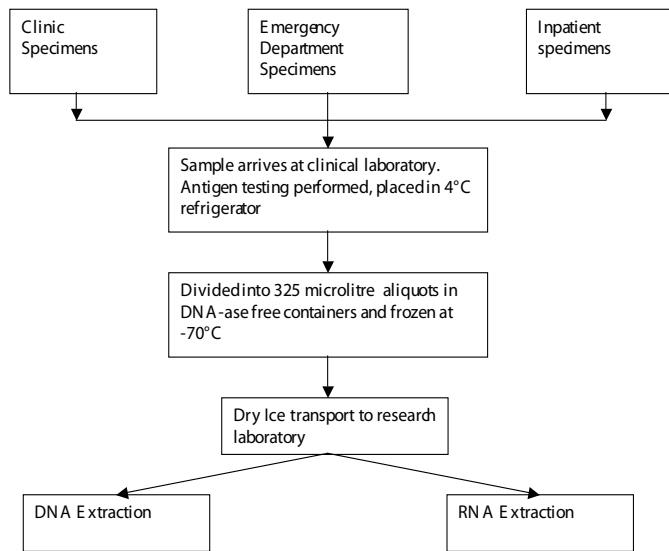


Figure 1. Specimen flow through the study.

morbidity and mortality is higher with *B. pertussis*.^{2,3} Apnea occurs in 15.9% of previously healthy infants less than six months of age who contract pertussis.⁴ Apnea rates for RSV are lower, even in hospitalized infants.⁵ This risk is largely confined to infants less than two months of age and to those with co-morbidities.⁶⁻⁹ Admission criteria reflect these differences with mandatory admission recommended for those with pertussis up to six months of age.¹⁰ Mandatory admission for infants with RSV is controversial, with conflicting published evidence and opinions, but many opt to admit all less than two months of age.^{11,12,13}

RSV epidemiological trends have remained steady¹⁴ whereas pertussis infections have climbed both locally (Kern County Department of Public Health data) and nationally¹⁵ with genomic subtypes not contained in vaccinations being increasingly represented.¹⁶ Previous work among children admitted to intensive care for bronchiolitis in the UK, and among infants less than six months of age hospitalized with bronchiolitis in Finland, suggests that despite the apparently viral nature of their illness (typically RSV), pertussis is under diagnosed.^{1,17} However this experience is not universal with some reporting the opposite.¹⁸ These studies raise the question whether infants and toddlers screened for RSV should also be screened for *B. pertussis*.

While baseline population prevalence data can help inform such a decision, these estimates from public health departments are of limited value. They reflect cases with more typical presentations in which the diagnosis was clinically suspected; even then they are prone to under reporting.^{19,20} Determining the overall baseline-expected prevalence of *B. pertussis* in patients with presentations more typically associated with RSV requires large numbers. It also requires a sample representative of the full spectrum of RSV disease that presents for treatment (primarily bronchiolitis). We tested de-identified specimens previously submitted for RSV screening to determine the prevalence of *B. pertussis* in this population.

We considered that a prevalence of less than 2% would represent an acceptable miss rate for most emergency physicians (EPs) for pertussis and tested the hypothesis that the prevalence of *B. pertussis* is less than 2% in nasal washings screened for RSV. Our secondary hypothesis was that the prevalence of *B. parapertussis* is less than 2% in nasal washings screened for RSV.

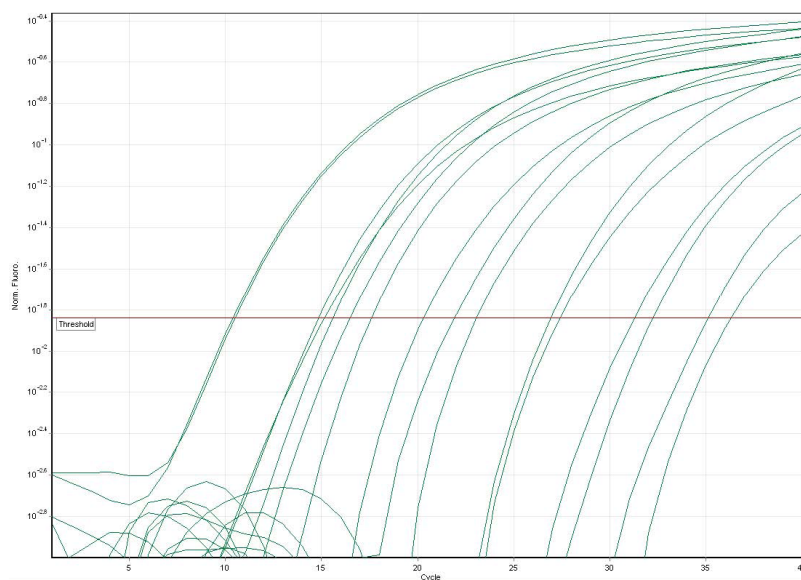


Figure 2. *B. pertussis* range of detection and standard curve ranging from 1×10^{10} copies to 1×10^2 copies.

METHODS

Our institutional review board exempted the study from review.

Setting: A single clinical laboratory serving an emergency department (ED), pediatric clinic, family practice clinic and a county hospital and a diagnostic testing laboratory specializing in the use of molecular amplification assays for the detection of viruses, bacteria, and fungi in biological specimens.

Nasal washings samples collected between October 2005 and May 2006 were first tested for the RSV antigen by the clinical laboratory. They were collected in test tubes without additives using normal saline. The remaining part of each sample was refrigerated to 4°C prior to being divided into 325 µl aliquots into DNA-ase-free transport containers and frozen to -70°C. The flow of specimens is shown in Figure 1.

Upon receipt at the diagnostic testing laboratory the specimens were thawed, and DNA was extracted using an automated Corbett Robotics X-tractor Gene system. Extracted DNA from these specimens were subsequently tested by *Bordetella pertussis* and *Bordetella parapertussis* species-specific real-time polymerase chain reaction (PCR) assays designed and optimized to target conserved regions within a complement gene and the *CarB* gene, respectively. A *Bordetella spp.* genus-specific real-time PCR assay was also designed to detect the *Bhur* gene of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*. The characteristics and detection limits of this PCR assay (detection limit for *B. pertussis* 100 copies per reaction, detection limits for *B. parapertussis* 10 copies per reaction) are shown in Figures 2 and 3. Specificity was assessed by testing the assay against known samples of the viral, bacterial, and fungal pathogens listed in Table 1. No amplification for any of these agents was

Table 1. Pathogens for which the real time probe and primer specificity was assessed.

<i>Gardnerella vaginalis</i>	<i>Candida parapios</i>	<i>Bartonella bacilliformis</i>
<i>Neisseria gonorrhoea</i>	<i>Candida tropicalis</i>	<i>Bartonella quintana</i>
<i>Neisseria meningitidis</i>	<i>Candida lusitanae</i>	Human rhinovirus 6
<i>Trichomonas vaginalis</i>	<i>Candida albicans</i>	Human rhinovirus 11
<i>Chlamydia trachomatis</i>	<i>Candida dublinensis</i>	Adenovirus type 10
<i>Chlamydia pneumoniae</i>	<i>Candida utilis</i>	Adenovirus
<i>Ureaplasma urealyticum</i>	<i>Candida krusei</i>	Coxsackie virus
Herpes simplex virus I	Influenza A	<i>Cryptococcus neoformans</i>
Herpes simplex virus II	Influenza B	<i>Babesia microti</i>
Human papilloma virus	<i>Aspergillus fumigans</i>	<i>Bacteroides fragilis</i>
Epstein Barr virus	Human herpes virus 8	<i>Mobiluncus curtsii</i>
Cytomegalovirus	Human herpes virus 6	<i>Mobiluncus mulieris</i>
<i>Mycoplasma fermentens</i>	Canine herpes virus	<i>Legionella pneumophila</i>
<i>Mycoplasma pneumoniae</i>	HTLV 1	<i>Brucellosis ovis</i>
<i>Mycoplasma genitalium</i>	Parainfluenza 2	<i>Borrelia burgdorferi</i>
<i>Mycoplasma hominis</i>	Parainfluenza 3	<i>Streptococcus pneumoniae</i>
		<i>Moraxella catarrhalis</i>

detectable for the *B. pertussis*, *B. parapertussis* or *Bordetella spp.* assays. These assays are available for clinical use from a commercial CLIA approved laboratory.

For the RSV assays, RNA was extracted from the washings using either a modified protocol with the Corbett Robotics X-tractor Gene System or with the QIAamp Viral RNA purification kit (Qiagen, Valencia, CA). Extracted RNA was subsequently tested for RSV-A and RSV-B using two separate one-step reverse transcription PCR assays targeting

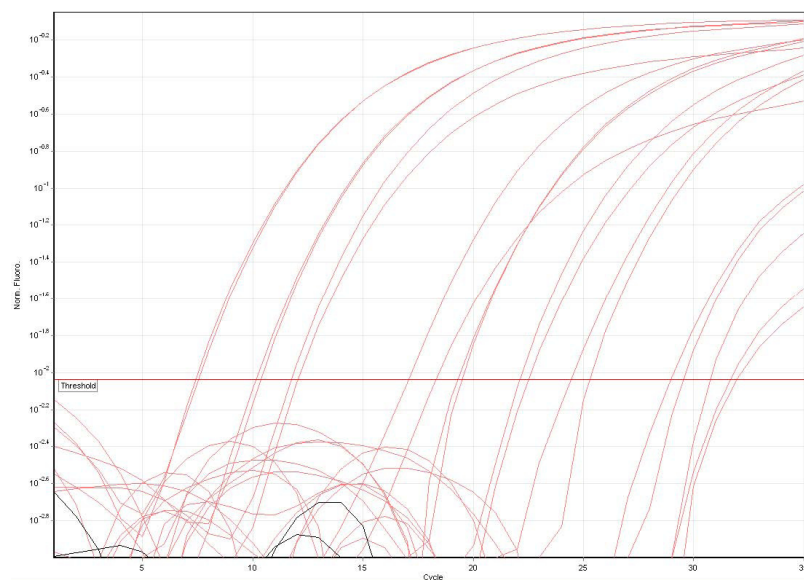


Figure 3. *B. parapertussis* range of detection and standard curve ranging from 1×10^{10} copies to 1×10^2 copies.

Table 2. Results by subtype

	RSV	<i>B. pertussis</i> +	<i>B. parapertussis</i> +
RSV A +	75 (16%)	1 (0.2%)	2 (0.4%)
RSV B +	52 (11%)	1 (0.2%)	1 (0.2%)
RSV A -	404 (84%)	1 (0.2%)	2 (0.4%)
RSV B -	434 (89%)		

genes, which encodes a phosphoprotein for each strain. The detection threshold for RSV-A and RSV-B was 100 copies per reaction.

Because we were not allowed to use patient identifiers, we used total population characteristics for all RSV samples submitted to the clinical laboratory during the study period to estimate population characteristics.

Statistical Analysis: Prevalence and exact confidence intervals were calculated for *B. pertussis*, *B. parapertussis* and RSV and co-infection. We tested the hypotheses that the proportion of cases screening positive for *B. pertussis* and *B. parapertussis* would exceed 2% with exact methods using STATA 9.2 (Stata Corp LP, College Station, TX).

RESULTS

The median age of the population sampled was 4.8 months (IQR 8.2); 356/648 (54.9%) were male. Six hundred and forty-eight specimens were received at the clinical lab. Most of these, 571 (88.4%) came from the ED, 49 (7.6%) from the pediatric and family practice clinics, and 26 (4.0%) from inpatients. Four hundred and eighty-nine (75.5%) of these submitted clinical samples were captured for additional testing. There was insufficient material to complete testing for one *B. pertussis*, 10 RSV subtype A, and three RSV subtype B assays. *Bordetella pertussis* was detected in 3/488 (0.61%) (95% CI 0.13% to 1.79%) while *B. parapertussis* was detected in 5/489 (1.02%) (95% CI 0.33% to 2.37%). Dual infection with *B. pertussis* and RSV was found in two cases. Dual infection with *B. parapertussis* and RSV occurred in three cases. The results are detailed in Table 2. One case of *B. bronchiseptica* ('kennel cough') was also found. RSV A was found in 75/479 (16%) samples and RSV B in 52/486 (11%). Local prevalence trends for the preceding 10 years are shown in Figure 4 to place the results in context.

DISCUSSION

Despite the rising local and national trend of reported cases of *B. pertussis* at the time of the study, we found very low rates of co-infection of *B. pertussis* and RSV. Our study does provide some evidence for clinicians who discount concerns about missing pertussis during RSV season, even during the current apparent resurgence of pertussis.

Our results are consistent with that of a retrospective

study of 166 children less than six-years-old hospitalized with lower respiratory tract symptoms in which a 0.6% (95% CI: 0.0% to 3.3%) prevalence was noted.¹ However, Siberry et al.¹⁸ was performed at a low point in local disease prevalence (five cases in all age groups in Baltimore) and drawn from an older population than ours; the studies are therefore not directly comparable. Our results contrast with those of Korppi and Hiltunin,¹⁷ which found an 8% prevalence in infants hospitalized with bronchiolitis who were less than six months old.

Our methodology allowed us to obtain large numbers of clinical specimens but not to obtain individual level clinical information on the patients from whom they were obtained, much less the clinicians' reasons for ordering the test. This is a feature of studies using so called "discarded clinical specimens." Such studies' results are most useful inferentially when they find a high or a low prevalence of an infectious agent. They are useful in triangulation, i.e. supporting a particular hypothesis by three different research methodologies. Triangulation is warranted when investigating conditions with high morbidity (such as causes of apnea) or when existing research provides conflicting answers.

This study suggests that when the community prevalence of pertussis is low, (our reported prevalence was 7.9/100,000 during the study period), and a 2% miss rate is acceptable to clinicians, routine screening for pertussis in patients suspected of having RSV is unwarranted. This is in contrast to studies that have looked at selected subgroups and found *B. pertussis* to be common. The definitiveness of this statement will depend on the results being replicated over a number of seasons.

LIMITATIONS

Local test-ordering patterns may help explain our low RSV rate. The sampling would have included patients

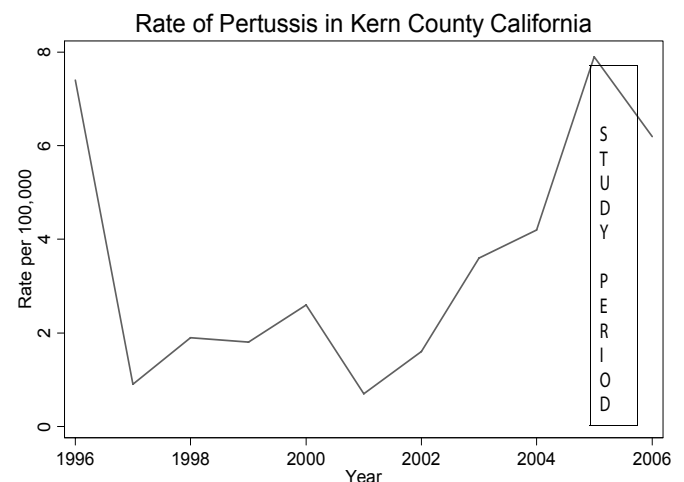


Figure 4. The context in which our sampling was performed. The box represents the sampling period.

screened for RSV early and late in the bronchiolitis season when other viruses are generally responsible. In-patient screening samples required for cohorting would also have been included in this analysis. RSV testing may have been obtained in cases of fever without source, or as part of apparent-life threatening event (ALTE) evaluations. False-negative PCR results for RSV are also a likely potential reason. This may in part be due to dilution of virus inherent in the collection of nasal aspirates. Initial specimen collection used unpreserved saline in clinical test tubes. RNA is less stable than DNA. During the uncontrolled phase (specimen going from patient to clinical laboratory) and despite refrigeration prior to aliquoting into DNA-ase-free containers, some RNA degradation could have occurred. Nasal aspirates have been shown to be markedly inferior to universal transport medium for room temperature (UTM-RT) based collection methods for PCR-based testing for respiratory viruses in general and RSV in particular.^{21,22} While this may artificially lower our detection of RSV it is less likely to have such an effect on *Bordetella spp.* Detection of bacterial DNA is much less susceptible to the collection method used (unpublished data). All of these factors may account for the difference between RSV detection in this and another contemporaneous study in our institution where a clinical diagnosis of bronchiolitis was the entry criterion and in which RSV prevalence was 55%.²³

The use of nasal washings rather than nasopharyngeal swabs (NPS) could potentially have decreased the yield of pertussis. While only NPS are considered acceptable for culture, the sensitivity of PCR mitigates against the potential loss of sensitivity by our methods.

Mistaken attribution of respiratory illness to *B. pertussis* has been described due to poor PCR assay specificity but high sensitivity.²⁴ We feel this is unlikely to be the case in our study based on the specificity testing results for our assay and the actual study results obtained.

We have not addressed here the broader epidemiology of those clinical presentations that typically prompt RSV testing. The clinical role for testing for these other agents is currently evolving. We could not control the age of patients whose samples were included; however, based on the age profile of the patients whose samples were submitted, our results should be considered applicable only to infants and toddlers.

We included only samples from a single clinical laboratory serving a single county system of clinics and hospital sites. Although this system serves a mixed urban, suburban and rural population, our patients tend to be drawn from lower socio-economic groups. This would tend to increase the prevalence of infectious diseases in general and may overestimate the prevalence that would be found in a more affluent setting. On the other hand vaccine refusal, which increases vulnerability to pertussis, tends to occur in higher socio-economic groups. Local DPT vaccination and

pertussis patterns mirror those of the rest of the state and country as a whole.

Our results must be viewed in the context of the overall prevalence of pertussis within the community. A higher prevalence could have changed our results, although the degree of change necessary to change the outcome is speculative. Nonetheless, although low by historical standards, prevalence was at a 10-year high during our study period. This is based on Department of Public Health reported case, data that inevitably carry their own limitations. Although the number of cases reported is low, it is likely an underestimate. It is estimated that between one-third and one in 20 of actual pertussis cases are reported.^{19,20} Nonetheless, the increasing trend of reported cases matches that for state and national reporting.

Our geographic locale is large. It is also a relatively sparsely populated, poor, and a medically underserved county with very low per person medical care expenditure,²⁵ again suggesting that the actual number of pertussis cases is probably higher than that reported.

Future reports will address these limitations by enrolling only ED patients and addressing clinical variables and outcomes over a number of seasons.

CONCLUSION

In a combined outpatient clinic, ED, and inpatient population, the prevalence of *B. pertussis* in nasal washings submitted for RSV antigen screening was less than 2%. The prevalence of parapertussis may have been higher than 2%. RSV with *B. pertussis* and RSV with *B. parapertussis* coinfection did occur.

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