Sensitivity and specificity of the World Health Organization pertussis clinical case definition

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SUMMARY

Objectives: *Bordetella pertussis* continues to circulate even in countries with good childhood vaccination coverage. This study was undertaken to define the relationship between documented disease and the clinical criteria proposed by the World Health Organization (WHO).

Methods: Nasopharyngeal swab samples were collected from previously healthy 6–14-year-old school children in Tehran, presenting with persistent cough of at least 2-week duration. Specimens were examined for *Bordetella pertussis* and *Bordetella parapertussis* by culture and polymerase chain reaction (PCR).

Results: Out of 6601 students, 328 (5.0%) had been coughing for at least 2 weeks. Of these children with cough, 182 (55.5%) experienced whooping, 194 (59.1%) suffered a paroxysmal cough, and 73 (22.3%) had post-tussive vomiting. Twenty-one (6.4%) samples tested positive for *B. pertussis* and six (1.8%) for *B. parapertussis* by PCR. Culture of four (1.2%) specimens was positive for *B. pertussis*. In comparison to PCR, the sensitivity and the specificity of the WHO clinical criteria (year 2000) were 95.2% and 15.0%, respectively.

Conclusions: Pertussis remains one of the etiologies of prolonged cough, even in communities with high immunization in children. The specificity of the WHO criteria is low in diagnosing pertussis compared with PCR.

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1. Introduction

Whooping cough is a highly contagious disease; despite the implementation of large-scale immunization programs, reported cases of pertussis have increased in many populations with high immunization in children during the last decade.1,2 This has been accompanied by a change in the epidemiology of the disease, characterized by a significant increase in pertussis among adolescents and adults, who constitute the main source of infection for infants.3

The World Health Organization (WHO) reports that there are currently around 48.5 million annual cases of pertussis worldwide, with as many as 295 000 fatalities each year.4 Although pertussis may occur at any age, most cases of serious disease and the majority of fatalities are observed in early infancy, particularly in preterm and non-immunized infants.3

In spite of a more than 95% coverage rate for the third dose of pertussis vaccine, a resurgence of pertussis is suspected in Iran; however this has not been formally demonstrated to date, because of a lack of precise surveillance data. The pertussis vaccination program in Iran includes three doses of a whole-cell pertussis vaccine together with diphtheria and tetanus toxoid (DTwP) at months 2, 4, and 6 of life and then two additional booster doses at 1.5 and 4–6 years. The Iranian Centers for Disease Control reported that the incidence of *Bordetella pertussis* in Iran was 0.5 cases per 100 000 population in 2008 – higher than the previous year, 0.19 cases per 100 000 population.4 It has to be considered that this variation may be partly due to the cyclic pattern of disease distribution, with a peak incidence every 3–5 years. The low reported incidence in Iran may be due to inefficient surveillance and also because of the common misconception of Iranian health workers that pertussis has been eradicated by the high vaccination coverage in our country.

The clinical diagnosis of pertussis is complicated by the heterogeneity in disease expression, modification of disease by immunization, mixed infections, and a low index of suspicion among many physicians.1 It is recognized that a key part of achieving disease control is the revision of case definitions and the utilization of the latest laboratory techniques for accurate diagnosis. Definite diagnosis depends on isolation of *B. pertussis*...
or detection of genomic sequences by means of the polymerase chain reaction (PCR) or through positive paired serology. It has been demonstrated that PCR has greater sensitivity than culture and is helpful especially in diagnosing mild cases and in patients who have received antibiotics or are studied late in the course of illness.5

In 1997, the US Centers for Disease Control and Prevention (CDC) proposed definitions for pertussis. Accordingly a ‘clinical case’ was a patient with cough of ≥14 days and at least one of the symptoms of paroxysmal cough, whoop, or post-tussive vomiting and a ‘confirmed case’ was one with positive laboratory findings either by culture or PCR or a patient with a clinical case definition and history of contact with a person with laboratory-confirmed pertussis.3

In 2003 the WHO specified the definition of ‘clinical case’ according to definite criteria, i.e., a case diagnosed as pertussis by a physician or who meets the clinical case definition proposed by the CDC without other apparent cause.6 According to the WHO criteria, a ‘clinically confirmed case’ is a case that meets the clinical case definition but is not laboratory-confirmed and a ‘laboratory confirmed case’ is a case that meets the clinical case definition and is laboratory-confirmed according to culture, PCR, or positive paired serology.6

This study examined the sensitivity and the specificity of the WHO clinical criteria in laboratory confirmed cases of pertussis in whom a definite diagnosis was made by means of positive PCR or positive culture for B. pertussis.

There has been no study to determine the incidence of pertussis in Iranian children and adolescents. This investigation was a population-based study to define the incidence of the disease in 6–14-year-old Iranian school children and to determine the frequency of symptoms in confirmed cases. The selection of this age group has two advantages: first, nearly all the population in this age range is accessible in schools and second, middle school-aged children encompass the group in which waning vaccine efficacy is predicted and elementary school-aged children are presumed to be protected against pertussis because of vaccine-induced immunity.

2. Materials and methods

The study was carried out from September 2007 to November 2008. After obtaining permission from the health and education ministries, the study team distributed questionnaires to school principals to be distributed to parents. These forms included queries about relevant demographic variables and also questions about clinical manifestations of pertussis viz. duration of cough, presence of coughing paroxysms, whooping and vomiting after cough, and cyanosis, and history of vaccination, taking medication, or the presence of any related diseases. After obtaining parental consent, all children aged between 6 and 14 years with a cough of 2-week duration or more, but without any documented underlying diseases, were selected for further investigation. Posterior nasopharyngeal samples were obtained and symptoms rechecked once more by telephone interview with the parents. A team consisting of a subspecialist infectious diseases pediatrician and three trained laboratory technicians were responsible for obtaining the specimens. Children with an immune deficiency or congenital cardiac or chronic lung disease (asthma, cystic fibrosis, gastroesophageal reflux), and also those with an audible heart murmur or respiratory wheezing were excluded from the study.

The population under investigation consisted of the school students in district 9 of the Tehran Board of Education; 20 out of 136 schools in that district were chosen randomly – nine were primary schools (grades 1–5) and 11 intermediate (grades 6–8). A total of 6601 children were studied in these schools. Samples were taken with two flexible Dacron swabs from the posterior nasopharynx (PNP) of the selected children. The swabs were inoculated onto the slant surface of Regan–Lowe medium and the lids of the medium tubes were closed tightly. The specimens were kept at temperatures of 0–4 °C and sent to the laboratory within 6 h. One swab from every specimen was subcultured on fresh Bordet–Gengou medium containing sheep blood and cephalaxin 40%, and incubated at 37 °C with 80% humidity for 3–10 days until the colonies emerged. If no growth was seen on Bordet–Gengou medium after 10 days, the sample was considered negative for culture. For the PCR, the slant surfaces of Regan–Lowe medium of the other series of swabs was washed with buffer solution. The washed off solution was transmitted to micro-tubes for PCR preservation. Extraction of DNA from PNP specimens was performed using the Sinnenagen (Tehran, Iran) extraction kit. The primers for B. pertussis and Bordetella parapertussis were supplied by MWG Company (Germany). Detection of B. pertussis was based on the amplification of a section of the IS481. Amplification of the sequence IS1001 was used for the detection of B. parapertussis (8.1 μl of primer for preparation of 50 μl work stock). The sequences of the oligonucleotide primers were identical to those listed in the report by Kösters et al.7 Positive controls were B. pertussis ATCC 9797 and B. parapertussis ATCC 9305 from the Pasteur Institute of Iran.

3. Statistical analysis

According to the study sampling (random cluster analysis sampling), we defined the schools as primary sampling units and ran survey data analysis in Stata 9.1 (Stata Corp., College Station, TX, USA). Standard errors were computed by linear model and a 95% confidence interval (95% CI) reported for each proportion. To evaluate the effect of certain variables on laboratory results, logistic regression (standard errors were adjusted for cluster of schools) was used. Since the duration of clinical symptoms is not usually more than 6–8 weeks and specimens were obtained from children only at the first visit (i.e., we did not request information on children who developed a cough after the first visit to obtain a specimen), the calculated frequency could be considered as incidence.

4. Results

The total number of students in the selected schools was 6601. Three-hundred twenty-eight children (5.0%) had experienced a persistent cough for at least 2 weeks. Study subjects were aged between 6 and 14 years (mean 11 years). By age group, 93 (28.3%) were aged 6–8 years, 81 (24.7%) were aged 9–11 years, and 154 (47.0%) were aged 12–14 years; 180 (54.9%) were male.

Three hundred and twenty-seven children were fully vaccinated against pertussis; only one had received fewer than three doses of the DTwP vaccine. Antibiotics had been prescribed for 190 (47.0%) children. Eighteen (37.6%) of the DTwP vaccine had been administered against pertussis; only one had received fewer than three doses of the DTwP vaccine. Antibiotics had been prescribed for 190 children (58.3%); only 1.8% of these 190 children had received macrolides or trimethoprim–sulfamethoxazole.

The mean duration of cough was 20 days. Of the 328 children with ≥2 weeks of coughing, 194 (59.1%; 95% CI 48.6–69.7) had a paroxysmal cough, 182 (55.5%; 95% CI 46.1–64.9) had whooping, and 73 (22.3%; 95% CI 16.2–27.9) had post-tussive vomiting (Table 1). Twenty-one of 328 (6.4%; 95% CI 3.1–9.6) children with a persistent cough had a positive PCR test for B. pertussis and six of 328 (1.8%; 95% CI 0–3.62) for B. parapertussis. B. pertussis was detected in culture in four of 328 specimens (1.2%; 95% CI 0–2.74), i.e., all culture-positive cases were PCR-positive. Not a single colony of B. parapertussis was found on culture.

The estimated incidence of B. pertussis in this age group, i.e., the frequency of cases with a positive PCR for B. pertussis, was 318
cases per 100,000 population; the estimated incidence was 2 cases per 100,000 population for *B. parapertussis*. The results of PCR and culture for *B. pertussis* were in agreement in 94.9% of cases (either both positive or both negative); they were not in agreement in 5.1% of cases. PCR was positive and culture negative. The Kappa agreement was 0.31 (p < 0.001). Of those with a persistent cough, 3.2% (3/93) aged 6–8 years, 1.2% (1/81) aged 9–11 years, and 11.0% (17/154) aged 12–14 years had a PCR positive for *B. pertussis*. The frequency of cases, as diagnosed by a positive PCR, increased significantly with age (β = 0.26, p = 0.02), but this relationship did not persist for parapertussis PCR (β = 0.02, p = 0.92). Two hundred and eighty-five children had a cough for 2–6 weeks; 5.3% of these had a positive PCR. For those who had been coughing for ≥6 weeks this figure was 14% (p = 0.04).

Laboratory findings in children presenting with the WHO criteria of pertussis are given in Table 1.

According to these data, if PCR is considered the gold standard for the diagnosis of *B. pertussis*, the sensitivity of the WHO criteria (as given in the year 2000) is 95.2% (20/21). The specificity in this population with ≥2 weeks of cough is 15.0% (Table 2).

The significance of proposed symptoms for predicting confirmed pertussis is shown in Table 3.

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Evidence of pertussis infection</th>
<th>No evidence of pertussis infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of cough, days</strong></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.6 ± 7.44</td>
<td>29.9 ± 4.24</td>
</tr>
<tr>
<td><strong>Median, range</strong></td>
<td>30 (14–360)</td>
<td>20 (14–360)</td>
</tr>
<tr>
<td><strong>Whoop</strong></td>
<td>15/21 (71.4%)</td>
<td>167/307 (54.4%)</td>
</tr>
<tr>
<td><strong>Paroxysms</strong></td>
<td>17/21 (81.0%)</td>
<td>177/307 (57.7%)</td>
</tr>
<tr>
<td><strong>Post-tussive vomiting</strong></td>
<td>13/21 (61.9%)</td>
<td>60/307 (19.5%)</td>
</tr>
<tr>
<td><strong>WHO clinical criteria</strong></td>
<td>20/21 (95.2%)</td>
<td>261/307 (85.0%)</td>
</tr>
</tbody>
</table>

SD, standard deviation; WHO, World Health Organization.

*Defined as cough ≥14 days with either paroxysm of cough, inspiratory whoop, or post-tussive vomiting without other apparent causes.*

### Table 2

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough ≥ 2 weeks + clinical; at least one WHO criterion</td>
<td>95.2%</td>
<td>15.0%</td>
</tr>
<tr>
<td>Cough ≥ 2 weeks + whoop</td>
<td>71.4%</td>
<td>45.6%</td>
</tr>
<tr>
<td>Cough ≥ 2 weeks + post-tussive emesis</td>
<td>61.9%</td>
<td>80.5%</td>
</tr>
<tr>
<td>Cough ≥ 2 weeks + paroxysms</td>
<td>81.0%</td>
<td>42.3%</td>
</tr>
<tr>
<td>Cough ≥ 2 weeks + clinical; at least two WHO criteria</td>
<td>81.0%</td>
<td>66.3%</td>
</tr>
<tr>
<td>Cough ≥ 2 weeks + clinical; at least three WHO criteria</td>
<td>9.5%</td>
<td>91.2%</td>
</tr>
</tbody>
</table>

WHO, World Health Organization; PCR, polymerase chain reaction.

### Table 3

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency of PCR positives (n = 21) OR</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough Positive</td>
<td>17 (8.8%)</td>
<td>3.12</td>
</tr>
<tr>
<td>Cough Negative</td>
<td>4 (3.0%)</td>
<td></td>
</tr>
<tr>
<td>Whoop Positive</td>
<td>15 (8.2%)</td>
<td>2.10</td>
</tr>
<tr>
<td>Whoop Negative</td>
<td>6 (4.1%)</td>
<td></td>
</tr>
<tr>
<td>Vomiting Positive</td>
<td>13 (17.8%)</td>
<td>6.69</td>
</tr>
<tr>
<td>Vomiting Negative</td>
<td>8 (3.1%)</td>
<td></td>
</tr>
</tbody>
</table>

5. Discussion

In our subjects with persistent cough, 59.1% had paroxysms, 55.5% had whooping, and 22.3% had post-tussive vomiting, while the figures from a study in adolescents in Massachusetts were 83%, 30%, and 45%, respectively; both studies show paroxysm as the most common symptom. In our study emesis was the least common finding.

In a hospital-based study in France, Baron et al. defined index and contact cases of pertussis confirmed by culture and/or serology in children or their contacts; paroxysms were seen in 84% of cases, 60% had vomiting, and 31% had whoop.

In our study 95% of culture- or PCR-confirmed cases met the WHO clinical criteria. Moreover, the frequency of positive PCR among those who had vomiting was higher than among students with paroxysmal cough or whoop, i.e., 17.8%, 8.8%, and 8.2%, respectively. The duration of cough was longer in children with a positive PCR than in those with a negative PCR (49 days vs. 30 days). Senzilet et al. found similar results in adolescents and adults ≥12 years old. The patients with laboratory evidence of pertussis had a significantly longer duration of cough and post-tussive vomiting than patients without such laboratory confirmation (56 days vs. 46 days and 45.5% vs. 28.5%, respectively).

According to our data, those who were not PCR-positive for *B. pertussis* in 281 children with cough for ≥2 weeks and at least one symptom of paroxysmal cough, post-tussive emesis, or whooping. If we take a positive PCR as the gold standard for the diagnosis of *B. pertussis*, the sensitivity and specificity of the WHO criteria are 95.2% (20/21) and 15.0% (46/307), respectively.

Cherry et al. claimed that although previous surveys had shown a definition of at least 14 days of cough to be both sensitive (84–92%) and specific (63–90%) for monitoring outbreaks and detecting contacts of culture-positive cases, it was not useful in non-outbreak illness because of the low specificity; only 51% of children who had cough ≥14 days with at least one of the WHO criteria showed laboratory evidence of pertussis or parapertussis infection (based on concurrent WHO criteria). These children were evaluated stringently with repeated PCRs, culture, and paired serology in order to reduce false-negative cases.

Bamberger et al. showed that 76% of unvaccinated, 39% of recently vaccinated, and 40% of post-vaccinated children with a positive PCR did not meet the CDC diagnostic criteria for *B. pertussis*; the conclusion was that clinical criteria had no significant association with infection in recently vaccinated children.

After examining anti-pertussis toxin immunoglobulin levels in both symptomatic and asymptomatic participants, Cagney et al. reported that only a third of coughing illnesses due to pertussis in Australian students met the CDC clinical case definition. They estimated the incidence of the disease according to the CDC clinical case definition to be 1587 cases per 100,000 in 12–14-year-old children, much higher than the figures reported by the CDC.

Gilberg et al. measured the frequency of *B. pertussis* in adults with a duration of cough of 7–31 days. They concluded that 79% of the culture-, PCR-, or serology-confirmed cases met the WHO criteria and that 89% met the CDC criteria. However, 83% of patients with no evidence of pertussis also fulfilled these criteria, indicating that the specificity of these definitions is low for diagnosis in adults and that laboratory confirmation is essential.

In our study, although 95.2% of the confirmed cases met the WHO criteria, 93% of those without laboratory evidence of pertussis also had these manifestations. If we diagnosed all children who fulfilled the WHO clinical criteria for pertussis as actually having the disease, then the estimated incidence of pertussis in the school-aged children would jump to 4257 cases per 100,000 population, an unacceptably high figure. Therefore it is appropriate to indicate that the WHO criteria have a low...
specificity. It has to be mentioned that there is a lack of consistency from parents over case definitions based on subjective symptoms such as whooping and duration of cough. This may explain the high frequency of whooping found in our research.

In order to increase the specificity, stricter criteria should be used for diagnosis, such as cough of more than 3-week duration and the inclusion of at least two or three clinical symptoms as a prerequisite. The sensitivity and specificity for each of these methods, except for the 3-week cough, were measured in our study (Table 2). Improvement in specificity was achieved at the cost of reduced sensitivity.

In 1988, Patriarca et al. showed that the presence of any single acute respiratory symptom such as cough, coryza, nasal congestion, or sore throat, was 100% sensitive but totally non-specific in identifying patients with pertussis. If cough were considered a prerequisite, it would increase the specificity of diagnosis to 28%, with only a negligible change in sensitivity. When duration of cough for 14 days or more was also included in the definition, specificity rose significantly to 63%, with only a modest decline in sensitivity (to 84%). Definitions requiring paroxysmal cough increased the specificity of diagnosis to 83%, but were accompanied by a large reduction in sensitivity (52%).

6. Conclusions

Even in countries where pertussis vaccination coverage for children has been higher than 95% for several years, like Iran, pertussis should be considered as one of the etiologies of prolonged cough. The epidemiology of the disease in Iran is similar to that in other countries which have already started to introduce booster doses in the older age groups. It appears that it is time to consider adolescent and adult immunization programs for pertussis.

Paroxysm was the most common symptom and emesis the least common symptom in our study. The frequency of positive PCR among those who had vomiting was higher than among those with paroxysmal cough or whoop. The duration of coughing was longer in children with a positive PCR than in those with a negative PCR.

The WHO clinical criteria are subjective and have a low specificity. So in addition to clinical definitions presented by the CDC and WHO, rapid, easy-to-use, inexpensive, and standardized laboratory tests need to be available and widely implemented.

Besides improvements in national surveillance, comprehensive population-based studies in different age groups are required. It is necessary to increase awareness in the medical community about adolescent and adult disease. An increased clinical suspicion of infection and more sensitive laboratory diagnostic methods are needed to assess the true incidence of pertussis in very young infants, older adolescents, and those who have been vaccinated.

The efficacy of our vaccines needs to be evaluated, since according to the age at which our booster dose is given and the high vaccine coverage rate we would expect to have immunity to pertussis at least in elementary school-aged children.

Ethical approval

This research was approved by the ethics committee of the Pediatric Infectious Diseases Research Center under the Helsinki rules.

Conflict of interest

No conflict of interest to declare.

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