

An outbreak of pertussis among young Israeli soldiers

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SUMMARY

In winter 2001, an outbreak of pertussis involving an estimated 75 people occurred among soldiers serving in an infantry regiment of the Israeli Defense Forces (IDF). Nasopharyngeal swabs were obtained from patients and contacts for culture and PCR. Serum samples were obtained and assayed by ELISA for the presence of IgA, IgM and IgG antibodies to a lysate antigen of *Bordetella pertussis*. The calculated attack rate was 21% based on clinical signs alone (cough lasting 30 days or longer) and 9.5% based on clinical signs with laboratory confirmation (by PCR, IgA or IgM). A high carriage rate was observed; 20% of the asymptomatic and previously symptomatic subjects were PCR-positive for *B. pertussis*. These findings emphasize the importance of *B. pertussis* as a causative agent of epidemic respiratory infections in young adults and reveal the occurrence of a significant proportion of pertussis transient carriers during an outbreak of the disease.

INTRODUCTION

Pertussis is caused by the fastidious Gram-negative coccobacillus *Bordetella pertussis*. During the early part of the twentieth century, the incidence of the disease was very high and claimed the lives of many young children. Introduction of whole-cell pertussis vaccine in the 1940s led to a sharp decline in the incidence of the disease in the developed world. However, during the past two decades, there has been a rise in the incidence of pertussis in the United States, with peaks occurring every 3 years [1]. A similar trend has been documented in Israel [2]. In parallel, there has been a rise in the proportion of cases of adolescents and adults among all pertussis cases [3, 4].

Studies in various clinical settings have shown the proportion of sporadic pertussis cases among adults with persistent cough to be between 12 and 32% [5–10]. Localized, as well as widespread outbreaks of pertussis involving adults, have also been described [11–15]. Transient carriage confirmed by PCR or culture has been reported in children [16, 17] and in adults [12, 18]. However, its role in the epidemiology and the transmission of the disease is unclear.

These data are important because adults, adolescents and children older than 1 year can serve as the source of infection to young, incompletely vaccinated infants amongst whom the disease can be fatal [19–23].

Here, we report the occurrence of an outbreak of pertussis in a previously vaccinated adult population (soldiers) in Israel with a high carriage rate of *B. pertussis* among close contacts.

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MATERIALS AND METHODS

Study population and sample collection

In April 2001, it was reported to the Army Health Branch of the IDF (Israeli Defense Forces) Medical Corps that an outbreak of a cough illness suspected to be caused by *B. pertussis*, had occurred in an infantry regiment.

Epidemiological investigation revealed that between March and May 2001, 18 soldiers distributed in four companies of an infantry regiment in the IDF visited the regimental clinic with complaints of persistent cough. A representative sample of the regimental personnel, comprising 96 soldiers from the same regiment, distributed across the four companies (21–28 soldiers from each company) was randomly selected. These subjects were exposed equally to the members of the initial group of 18 coughing subjects. These two groups (a total of 114 soldiers) were asked to fill in a questionnaire under the guidance of a physician. The questionnaire included questions regarding the duration, description of the illness and the date of cough onset. The subjects were also interviewed about smoking habits, possible history of asthma and possible contact with others suffering from persistent cough. A total of 107 of these questionnaires were valid for statistical analysis.

Specimens for PCR and culture were collected by trained physicians, using dacron nasopharyngeal swabs (Pernasal swabs, MW&E, Corsham, Wiltshire, UK). All the samples were obtained on 23 April 2001 except two, which were obtained during June 2001. Ninety-six nasopharyngeal samples were obtained for PCR; 89 serum samples were tested for *B. pertussis* serology; 17 nasopharyngeal samples were obtained for culture from the first group of 18 patients.

The study subjects (111 males and 3 females) were aged from 19 to 44 years (90% were aged between 19 and 22 years). Of 107 subjects for whom eligible data on the birth country existed, 90 subjects were born in Israel, and hence were eligible for vaccination four times during infancy (at ages 2, 4, 6 and 12 months). The remaining 17 soldiers were born in other countries and their exact vaccination status was not clear. All the subjects were healthy otherwise, except for four reporting a history of mild asthma.

Twelve subjects were treated with antibiotics for other reasons, 3 weeks to 3 months before the blood and nasopharyngeal samples were obtained; five were treated with erythromycin and azithromycin was prescribed to two subjects for the treatment of the

coughing illness 1–6 days before the samples were obtained.

Laboratory procedures

Culture

Culture was performed on selective charcoal Bordetella agar (Hy-labs[®], Rehovot, Israel) and incubated at 36 °C for 14 days as described previously [24].

PCR

PCR was performed as described previously [17]. Nasopharyngeal swabs for PCR were placed in sterile physiological saline solution and stored at –70 °C. Upon thawing swabs were removed after vigorous mixing and cell suspensions were transferred to Eppendorf tubes. Bacteria were pelleted by centrifugation at 1300 *g*, 4 °C for 10 min. DNA was extracted from the pellet using QIAmpR DNA kit (Qiagen, Hilden, Germany).

Primers previously published, specific for the repeated insertion sequences were used in semi-nested PCR assay [25]. Upstream primer sequence gAT-TCAATAggTTgTATgCATggTT and downstream primer sequence AATTgCTggACCATTcAgTC-gACG were used in the first PCR which included 15 μ l sample DNA, reaction buffer (10 mM Tris–HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100), 1 μ M each primer, 200 μ M dNTPs and 1 U *Taq* polymerase (Boehringer–Mannheim, Germany) in a total 25 μ l volume. Thirty-five PCR cycles were performed (94 °C for 45 s denaturation, 59 °C for 45 s annealing, and 75 °C for 45 s extension) in a thermal cycler (MJ Research, Waltham, MA, USA).

A second round of amplification using a 2 μ l aliquot of first-round PCR product and upstream internal primer, gCTTCaggCACAAACTTgATGG and previous downstream primer was performed for 25 cycles. The second PCR products were visualized by UV following electrophoresis in ethidium bromide-stained 3% agarose gels (Sigma, St Louis, MO, USA). Molecular-weight markers, negative controls (PCR mix without sample DNA or irrelevant DNA) and positive controls (obtained from *B. pertussis* cultures) were run with each assay. Potential inhibitory activity was monitored by amplifying sample DNA together with minimal quantities of bacterial DNA obtained from cultures. A positive result of this assay ruled out a potential false negative result.

Serology

The presence of specific IgG, IgM and IgA to *Bordetella pertussis* was examined using an enzyme immunoassay (ELISA) based on whole-cell lysate antigens (PanBio, East Brisbane, Australia).

IgM, IgA and IgG results are given as ELISA units obtained according to the manufacturer's instructions by calculating the ratio of the sample absorbance to a cut-off calibrator serum absorbance and multiplying by 10. For IgA and IgM, a result of 11 U or higher was considered positive. A result of between 9 and 11 U was considered equivocal and was arbitrarily assigned as negative. A result of less than 9 was considered negative.

An IgG value of 19.97 U or higher was considered indicative of recent infection. This value was calculated by adding 1.96 standard deviations to the mean value of the anti-*B. pertussis* IgG levels of 86 soldiers from another infantry regiment, unrelated to the outbreak. As these soldiers did not have clinical evidence of recent infection with *B. pertussis*, values above this cut-off represent 2.5% of the normal (not recently infected) population and thus, type I error of the IgG ELISA is 2.5% and specificity is estimated to be 97.5%.

Subjects who started coughing less than 10 days before the blood sample was obtained were excluded from serological analysis.

Data analysis

The rates of seropositives in the study groups were compared, using the Fisher exact test or the χ^2 test. The data were analysed using SPSS 8.0 for Windows. A *P* value of 0.05 or less was considered significant.

RESULTS

The 107 subjects eligible for analysis were divided into three subgroups according to presence and duration of cough (Fig. 1a-c):

- (1) Subjects coughing for at least 30 days (persistent cough) (*n* = 34, median = 60 days).
- (2) Subjects coughing less than 30 days (short duration cough) (*n* = 18, median = 5 days).
- (3) Subjects without cough (*n* = 55).

Table 1 shows the frequency of clinical signs among patients who complained of coughing. All 17 nasopharyngeal swabs that were obtained from

Table 1. Prevalence of additional clinical signs among subjects suffering from cough

	Cough less than 30 days (<i>n</i> = 18)	Cough for 30 days or longer (<i>n</i> = 34)
Paroxysmal cough	33 %	91 %
Whooping	6 %	55 %
Posttussive emesis	0 %	29 %
Rhinorrhoea	61 %	77 %
Sputum production	67 %	91 %

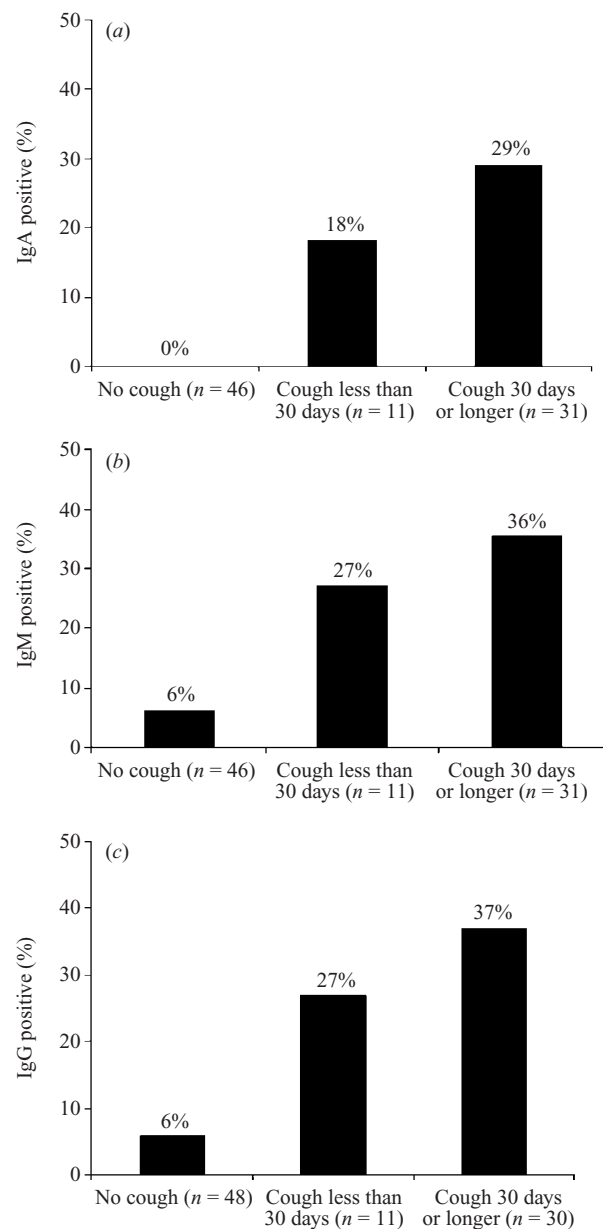


Fig. 1. Proportions of positive results: (a) IgA, (b) IgM, (c) IgG within the study groups.

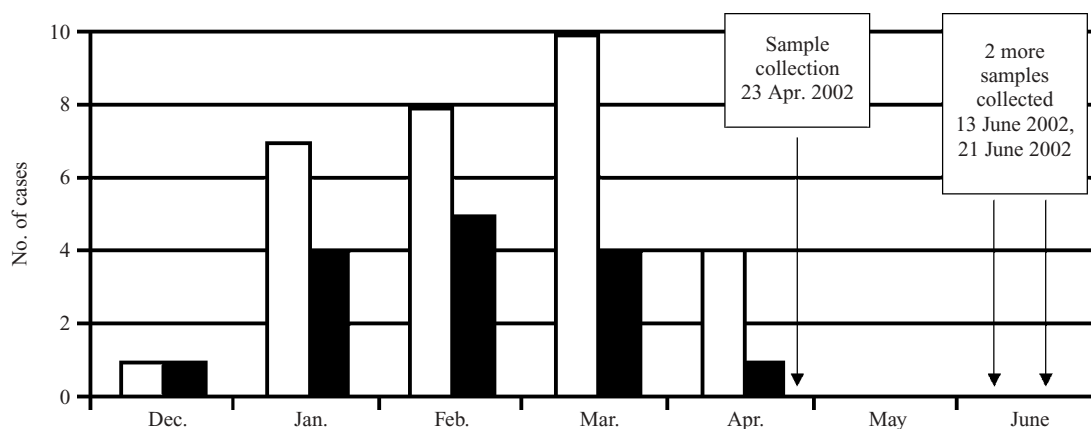


Fig. 2. Cases of pertussis between December 2000 and April 2001. □, Coughing for at least 30 days; ■, coughing for at least 30 days with laboratory confirmation (PCR, IgM or IgA). Analysis was performed for 30 of the 34 cases with persistent cough, due to lack of available data regarding onset of symptoms on the remaining cases.

participants with persistent cough were culture negative for pertussis.

Seropositivity for IgA, IgM and IgG differed significantly between the three subgroups; percentage positivities are shown in Figure 1 [IgA ($P < 0.001$), IgM ($P = 0.005$), IgG ($P = 0.003$)]. Nineteen subjects (20%) were positive for PCR. Nine of these were asymptomatic during all of the outbreak period. Two epidemiological curves were constructed, based on the data obtained from the questionnaires about the onset of cough in each patient (Fig. 2).

The overall attack rate of pertussis in the regiment was then estimated. The attack rate in the random sample of 96 soldiers was calculated first (17 of 96). It was then extrapolated for the entire four involved companies of the regiment. The 18 patients who came to the clinic with persistent coughing were added to the numerator. This led to a calculated attack rate of 21% and a total estimated number of 75 patients in the entire regiment. Using the same method, we calculated an attack rate of 9.5% for patients suffering from cough which lasted longer than 30 days and confirmed positive by laboratory results (PCR, IgA or IgM). No association was found between smoking or a history of asthma and incidence of pertussis.

DISCUSSION

Outbreaks of pertussis among adults have been described previously [12–15], but to the best of our knowledge, this is the first description of an outbreak of pertussis involving a closed population of otherwise healthy young adults (combat soldiers who return home for the weekend, approximately once every

3 weeks). The disease symptoms in this outbreak caused significant complaints amongst young soldiers and in some cases interfered with their daily activities.

During an outbreak, the CDC defines a clinical case of pertussis as a patient with at least 14 days of persistent cough [26]. Since in our study the outbreak occurred during wintertime, when other agents causing upper respiratory tract infections are prevalent, we preferred to adopt a more conservative definition of a pertussis case as any participant who was coughing for at least 30 days. Based on this clinical definition we calculated an attack rate of 21% in the regiment. When PCR or serology (IgA or IgM) was used for laboratory confirmation, an attack rate of 9.5% was calculated.

The isolation rate of *B. pertussis* is lower in the later stages of the disease and is negatively correlated with age and with the number of pertussis vaccinations previously received by the patient [24]. Since in our study most of the subjects were encountered at the end of the paroxysmal stage or in the convalescent stage, the failure to isolate *B. pertussis* from the 17 nasopharyngeal swabs obtained for culture was not unexpected. Although the bacteria were not isolated from any of the subjects, the aetiology of *B. pertussis* as the causative agent of the outbreak was confirmed by the clinical manifestations, backed up by positive PCR and serology results. It was further supported by the high correlation between serology results and the duration of cough.

PCR samples were obtained at the later stages of the disease. The sensitivity of PCR in these stages has been reported to be low [24]. In a study performed by He et al. [18], none of the patients with symptoms

lasting more than 7 weeks was diagnosed based on PCR. For this reason, the importance of PCR in this study is for the detection of asymptomatic carriers.

The existence of a carrier state of pertussis has been a subject for debate for several decades. Early studies on asymptomatic contacts of pertussis patients in which cultures were used for detection, gave a low rate of isolation of *B. pertussis* [27–29]. However, more recent studies using PCR as a detection tool, have given much higher rates of positives for this pathogen among asymptomatic contacts of pertussis patients [17, 18]. Our results are similar to those reported by He et al. [18], who found the rates of positives by PCR in asymptomatic and symptomatic adults and adolescents involved in an outbreak to be 29 and 30% respectively [18]. In another outbreak occurring among children, cultures from positive asymptomatic contacts grew a significantly lower number of colony-forming units compared to cultures from symptomatic subjects [16]. Thus it is probable that a transient asymptomatic carriage state of pertussis exists, in which the bacteria are present in the nasopharynx in very small numbers that can be detected by PCR but not by culture, as most probably was the case in our study. The high percentage of asymptomatic carriers of *B. pertussis* based on PCR as opposed to serology may result from a high prevalence of contamination with the bacteria that does not elicit a detectable immune response.

Health-care policy in Israel provides infants with four consecutive doses of DTP vaccine starting at the age of 2 months. Children and adolescents in Israel are not vaccinated against pertussis after the age of 1 year, nor does the IDF provide pertussis vaccines to new recruits. Ninety of the 107 subjects who participated in this study were born in Israel, hence at least 90 subjects were probably vaccinated four times during their infancy. Data generated in previous studies of outbreaks of pertussis suggest that immunity acquired by vaccination wanes gradually and that after 7 years the efficacy of the vaccine reaches only 46% [30]. A previous report showed that only 58.6% of Israeli military recruits were positive for pertussis IgG antibodies [31]. We assume that the onset of the outbreak described in this paper and the risk for pertussis in the IDF are the result of the waning immunity and lack of protection against the disease in a significant proportion of the IDF recruits.

The possible role of adults in the transmission of the disease to infants should bring into consideration the potential benefits of re-vaccination of young

adults. Acellular pertussis vaccines have favourable safety profiles, are highly immunogenic when administered to adults [32] and might be effective in preventing outbreaks of the disease.

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