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Pertussis in the Taiwan Area, 1995

Summary

There was a total of 26 pertussis positive cases in the Taiwan Area in 1995; of them, 12 were culture-positive, and 14 were with clinical symptoms and were epidemiologically related to the culture-positive cases. Their distributions by age, sex, month of onset, residence and immunization status were as follows: of the confirmed cases, 10 (38.5%) were males and 16 (61.5%) females; 8 cases each were either one year and under or in the 5-9 age group, 2 in the 1-4 age group, 1 in the 25-29 age group, 5 in the 30-34 age group, and 2 in the 35-39 age group; with the exception of March, April and September, cases occurred in every month, with more cases (17, 65.4%) in May through August; 9 cases occurred in Taipei County, 7 in Taipei City and 6 in Changhua County. In the 26 confirmed cases, 20 had family clusterings. 16 of them were interviewed: 3 adults could not recall immunization; 7 infants (6 under 2 months, one 3 months old) had not been immunized; 3 had completed 4 doses of DPT (diphtheria-pertussis-tetanus); one had two doses; and two (4 and 6 months old) had only one dose of DPT.

Introduction

Pertussis was first reported by Sydenham in 1679. It is an acute respiratory infection of severe coughing. The Chinese term of "hundred day coughing" for pertussis implies that patient will cough for hundred days, though in effect, the entire disease process takes around six to eight weeks, or about 50 days. The special features of the disease, therefore, are irritating and persistent coughings.

The infectious agents of pertussis are mainly *Bordetella pertussis,* and occasionally *Bordetella parapertussis, Bordetella bronchiseptica* and adenovirus. The latter three agents can produce similar clinical symptoms called the pertussis syndromes. However, *Bordetella pertussis* infection is more common. The typical process of the infection comes in three stages: catarrhal, paroxysmal coughing and convalescent. Clinical symptoms include paroxysmal coughing, whooping or post-tussis[1,2]. *Bordetella pertussis* is vulnerable to environment, and is easily destroyed by chemical disinfectants, dryness

and high temperature. Colonies in culture agars produce variability after several generations and even lose their toxigenicity[3,4].

Serological testings for pertussis are conducted routinely by several teaching hospital in Taiwan. The isolation and identification of strains are not easy and are performed at present only by the National Institute of Preventive Medicine of the Department of Health. There were more pertussis cases in 1995, even some hospital staff were infected. This report analyzes the confirmed cases of pertussis in 1995 by their age, sex, month of onset, place of onset and family clustering. To understand the efficacy of vaccine, 16 cases were interviewed as to their immunization status and clinical symptoms.

Materials and Method

1. Collection of Specimens

Some of the infections are inapparent. Some clinics, for inadequate facilities or poor referral channels for laboratory testings, may fail to diagnose the infections. The National Institute of Preventive Medicine, therefore, deposited some Regon-Lowe agars in some major hospitals such as the National Taiwan University Hospital, Taipei Veterans' General Hospital and Taipei Changgung Memorial Hospital, and some district pediatrics and internal medicine clinics for them to collect soon nasopharyngeal swab and serum specimens of out-patients coughing for more than six days. The specimens were either sent directly or through mail to the laboratory for testings for *Bordetella pertussis* and serological testings.

2. Laboratory Identifications for *Bordetella pertussis*[5]

Isolation of strain from nasopharyngeal or cough specimens:

1) Fresh or mailed specimens are inoculated on the Bordet-Gengou agars (adding 15% of horse blood and Cephalexin), then incubated at 37°C for 3-5 days. Suspected colonies are collected each day for Gram's stain, catalase test, oxidase test and direct fluorescent test.

2) Small Gram-negative colonies with positive results on the catalase test and oxidase test are inoculated on MacConkey agar and Mueller-Hinton agar, and then incubated at 37°C overnight. *Bordetella pertussis* will not grow on these three agars.

3) Colonies that meet the biochemical testings and growth criteria are tested with specific phase 1 serum (Difco Laboratories, Detroit, Michigan, USA) for agglutination test and Congo red absorption test to confirm that they are in phase 1.

3. Serological Testings

Serological testing is an important supporting test for the clinical diagnosis of pertussis. Serum specimens of patients are tested for IgA and IgM of *Bordettella pertussis* (MELJA Diagnostick GmbH, Kassel, Germany) as follows:

1) Sera of patients are mixed at 1:100 with sample diluent. The sample diluent is used as blank test.

2) Take 100 μL each from the undiluted IgA and IgM positive control sera, negative control sera and diluted sera of patients, place them into the wells of the microtiter and incubate at 37°C for one hour.

3) Rinse them three times with 300 μL of washing solution, add diluted anti-IgA-AP or anti-IgM-AP (1:50) into each well, incubate at 37°C for one hour, rinse them with 300 μL washing solution for three times, add 100 μL of the freshly prepared substrate (pnpp), incubater at 37°C for 30 minutes.

4) Stop reaction with 1 M NaOH solution. After thorough mixing, measure absorbance at 405 nm using an ELISA reader.

4. Polymerase Chain Reaction (PCR) Testings[6]

1) 100 μL of reaction mixture (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.01% (v/v) gelatin, 200 μM deoxyribonucleotides, 20 pmol primers, 2.5 U Tag polymerase, 5 μL chromosomal DNA)

2) Denaturation: 94°C, 30 seconds; annealing: 52°C, 10 seconds; extension: 72°C, 10 seconds; total, 30 cycles.

3) Detect products with electrophoresis after they return to room temperature.

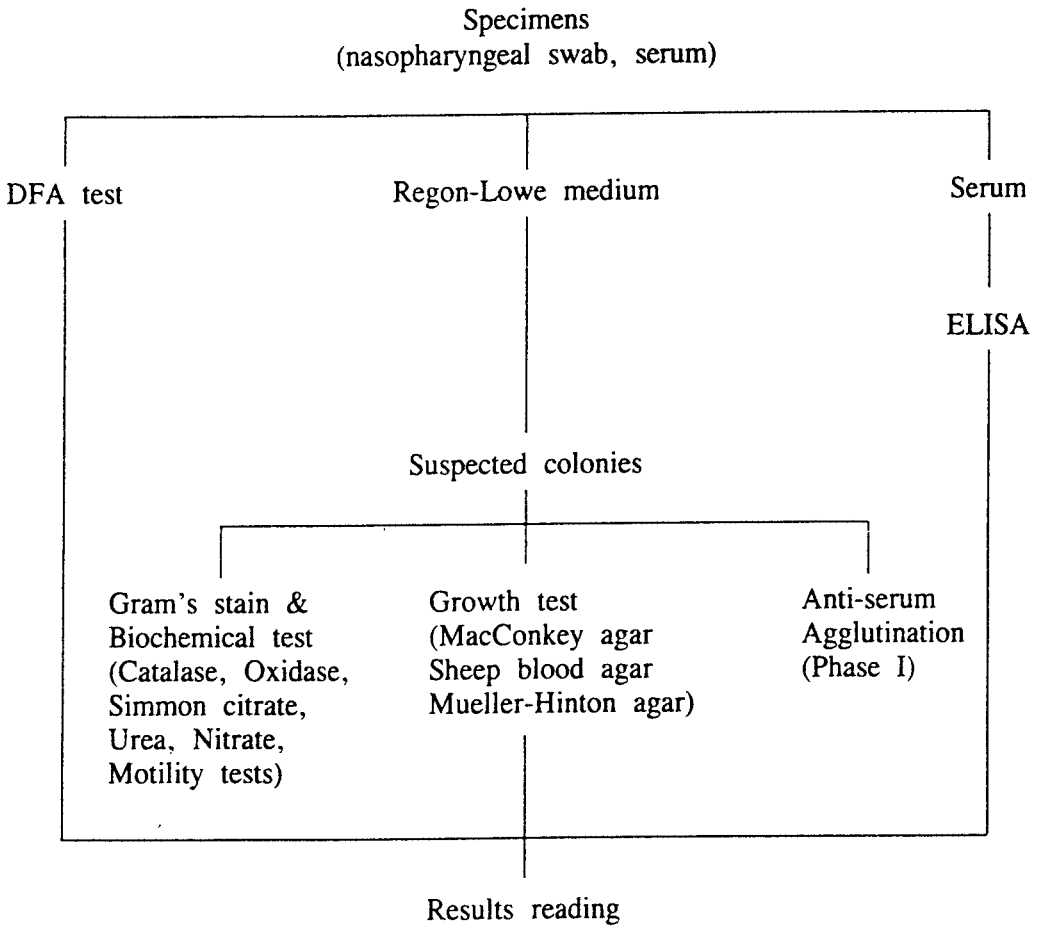
Results

1. Results of Bacteriological Identification

Of the 182 nasopharyngeal swab specimens collected from 168 suspected patients during the year (including 85 reported cases and confirmed cases and their contacts), 14 (12 cases) were culture-positive.

All isolated strains, after agglutination test and Congo red absorption test, were confirmed to be phase I.

Figure 1. Isolation and Identification of *Bordetella pertussis*



2. Results of Serological Testings

Of the 209 serum specimens collected from 168 suspected patients during the year, 84 (61 cases) were antibody-positive; of them, 78 (58 cases) were IgA positive, and 18 (13 cases) IgM positive.

3. Analysis of Positive Cases

1) Age and Sex Distributions of Positive Cases

Following the regulations of US CDC (Centers for Disease Control and Prevention,

USA) and the Department of Health, a pertussis positive case is defined as one whose culture shows positive to *Bordetella pertussis,* or with clinical symptoms and epidemiologically related to *Bordetella pertussis* culture-positive patient.

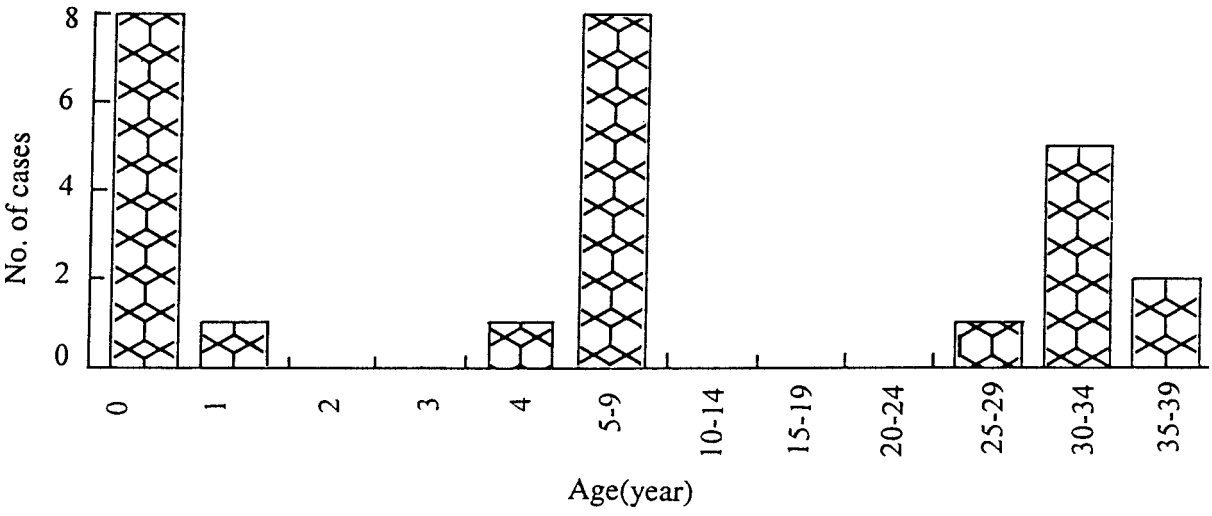
In 1995, a total of 85 pertussis cases was reported (0.41 reported cases per 100,000 population). This was 51 cases (68.8%) more than that of 1994. Specimens were collected from all cases for laboratory testings. Of them, 26 cases were confirmed after either laboratory testings or epidemiological investigations (0.12 confirmed cases per 100,000 population). This was 4.3 times more than the confirmed cases of 1994. Of the 26 confirmed cases, 12 were culture-positive, and 14 were with clinical symptoms and were epidemiologically related to the culture-positive cases. Of them, 10 (38.5%) were males and 16 (61.5%) females.

By age, there were 8 cases each in age groups one year and under and 5-9 years; 2 in the 1-4 age group; 1 in the 25-29 age group; 5 in the 30-34 age group; and 2 in the 35-39 age group (see Figure 2).

2) Month of Onset and Geographical Distribution

The monthly distribution of pertussis cases is shown in Figure 3. With the exception of March, April and September, cases occurred in every month and more so in May through August (17 cases, 65.4%). Geographically, more cases were found in Taipei County (9 cases), Taipei City (7 cases) and Changhua County (6 cases).

Figure 2. Age Distribution of Confirmed Pertussis Cases, 1995



3) Clustering of Cases

Of the 26 confirmed cases, 20 showed family clusterings (from 8 families). Between May and July 1995, a suspected outbreak occurred in a hospital in Taipei City with 5 confirmed cases (2 infants of younger than one year, one adult and two medical care personnel).

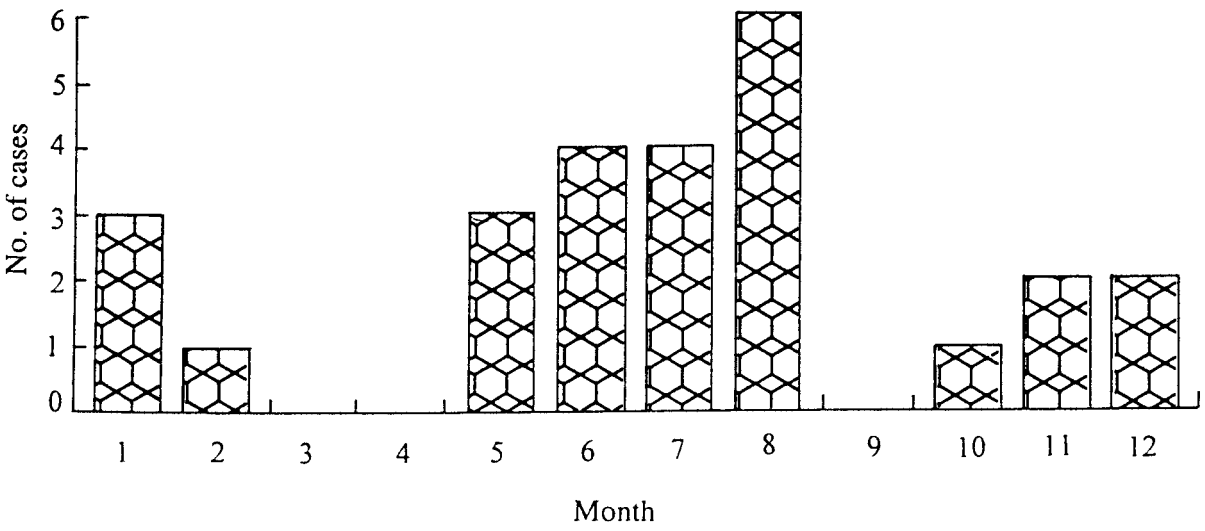
4) Immunization Status

Of the 16 confirmed cases interviewed, 3 adults could not recall any immunization record; 7 infants (6 under 2 months and one 3 months old) had not had any immunization; 3 had four doses of DPT (diphtheria-pertussis-tetanus); one had two doses; and two (four-month and six-month old) had only one dose of DPT.

5) Symptoms

Of the 16 cases interviewed, 10 (62.5%) had paroxysmal coughing, 3 (18.8%) had whooping, 3 (18.8%) showed reddish dark color; and 7 (43.8%) had vomiting, particularly among infants.

Figure 3. Monthly Distribution of Pertussis Cases in Taiwan Area, 1995



Discussion

1. Special Features of the Isolated Strains

Findings of the biochemical testings of the *Bordetella pertussis* strains isolated in the present study were: catalase test (+), oxidase test (+), urea test (−), motility test (−), Simmon citrate (−), nitrate reductase (−). For growth test, they did not grow on blood, MacConkey or Mueller-Hinton agars. By PCR method to magnify specificity of the porin gene, all strains demonstrated the specific 159 bp products, indicating that they were *Bordetella pertussis.*

On culture agars, *Bordetella pertussis* developed gradually from high pathogenic phase I to non-pathogenic phase IV. The agglutination test by specific phase I serum showed that all strains were in phase I. Studies have shown that colonies of phase I *Bordetella pertussis* growing up on solid agar will absorb Congo red stains. All strains showed the same result in the Congo red absorption test. This demonstrated again that all strains isolated in the present study were in phase I.

2. Techniques in Specimen Collection and Laboratory Testings

US CDC specifies that a confirmed case of pertussis should demonstrate clinical symptoms such as coughing for more than two weeks with paroxysmal coughing, whooping or vomiting after coughing for no other apparent reasons, and that *Bordetella pertussis* should be isolated from clinical specimens in laboratories. In 1988, Steketee, et al., reported that the isolation positive rate of *Bordetella pertussis* was related to the duration of onset, 25% positive rate in the third week, 14% in the 4th week and 0% in the 5th week[7]. However, Farizo, et al., reported that pertussis patients under antibiotics treatment could show culture-negative, and if not treated completely, they could later become culture-positive. *Bordetella pertussis* is difficult to collect specimen and culture, in some cases, therefore, no agents were isolated from patients diagnosed by clinicians as pertussis. Therefore, more improvement in the techniques of laboratory testings should be made to upgrade the positive rate of testings.

To upgrade the detection rate of testings, the National Institute of Preventive Medicine of the Department of Health has conducted various studies on the selection of culture agars, the conditions of culture, and the use of PCR for the detection of *Bordetella pertussis*[8]. Adequate method of specimens collection and proper agars for the transportation of specimens are essential to detection rate. The National Institute of Preventive Medicine, with the support of the Bureau of Communicable Disease Control of the Department of Health, has prepared each year the Regon-Lowe transportation agars, and together with instructions (see Figure 4), for the use of hospitals and clinics. A half of the specimens (86 nasopharyngeal swabs) collected for last year came from these hospitals and clinics. The detection rate for *Bordetella pertussis* has made significant improvement over previous years. Since the isolation positive rate of *Bordetella pertussis* is related to the duration of onset and detection is almost impossible after three weeks, to improve detection rate, physicians are alerted to collect specimens in due time.

3. International Comparison of Pertussis Cases

By Farizo, et al., of the 27,826 pertussis cases of the US in 1980-1989, 49.9% occurred in infants under one year, and 21.2% in young children in the 1-4 year group. In contrast in Taiwan, only 30.8% of the cases occurred in infants under one year, and 7.7% in young children in the 1-4 year group. This probably is due to the infections of adults and medical care personnel. With more adults infected, the proportion of children infected becomes smaller.

Figure 4. Instructions for the Collection of Specimens

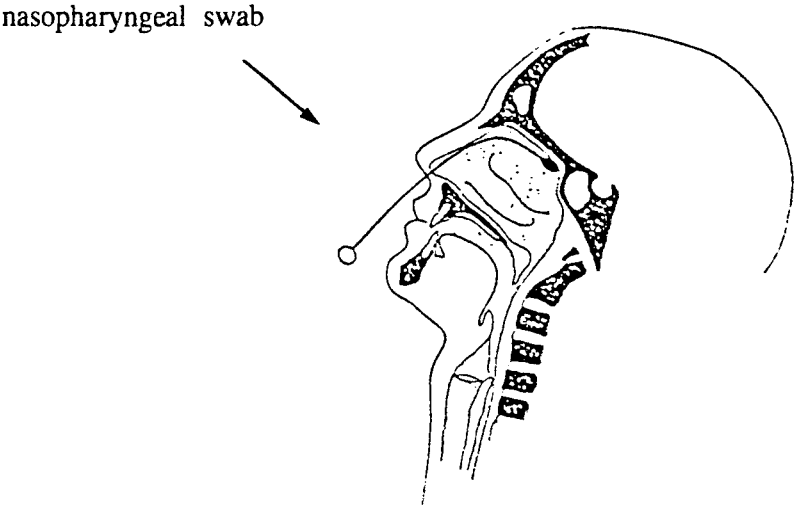
Laboratory testings for *Bordetella pertussis* are provided by the National Institute of Preventive Medicine of the Department of Health.

1. Pertussis is a common respiratory infection of young children. For its high infectivity, it is made a reportable disease. Attending physicians are required to fill out communicable disease report and report to health authorities.

2. Free laboratory testings for pertussis are offered by the National Institute of Preventive Medicine of the Department of Health. Please dial 02-785-7556 for nasopharyngeal swabs before sending specimens for testings.

3. A complete process of test for pertussis includes deep nasopharyngeal swab and serum (1-2 mL). Nasopharyngeal swab is for the isolation and identification of *Bordetella pertussis;* serum is for the analysis of serum antibody. Both must be sent together.

4. Nasopharyngeal specimens should be collected in the following way (see Figure below). After collection, specimens should be placed in the transportation agar and, kept in room temperature if the specimens can reach the National Institute of Preventive Medicine within 12 hours, and in ice otherwise. Serum specimens should be transported in ice to avoid change in potency and thus increase error.



In terms of seasonal variations, 57% of US patients occurred between June and October. Gan and Murphy reported in 1990 that, between 1967 and 1986, of the 182 infant pertussis patients in Dellas, Texas, 48.4% occurred in May through August, corresponding to the 65.4% of the Taiwan patients in this period.

By sex, Farizo, et al., also reported that of patients above five years of age, the incidence of females was higher than that of males; whereas, there was no sex difference in children under five years[4]. Of the 26 positive cases in Taiwan, 10 (38.5%) were males. This finding was close to that of the US.

References:

1. Baron S, Jennings PM. Medical Microbiology, 13th ed., Churchill Livingstone Inc., New York, 1991: 432-440.
2. Mandell GL, Douglas RG, Bennett JE. Principle and Practice of Infectious Diseases, 3rd ed., Churchill Livingstone Co., New York, 1990: 1756-1760.
3. Murray PR, Drew WL, Kobayashi GS, et al. Medical Microbiology, CV Mosby Co., London, 1990: 149-152.
4. Fraizo KM, Cochi ST, Zell ER. Epidemiological features of pertussis in the United States, 1980-1989. Clin Infect Dis 1992; 14: 708-719.
5. Marcon MJ. *Bordetella.* In: Murray PR, Baron EJ, Pfaller MA, et al., eds. Manual of Clinical Microbiology. 6th ed., Am Soc Microbiol, Washington DC, 1995: 566-573.
6. Houard SC, Hackel C, Herzog A, et al. Specific identification of *Bordetella pertussis* by the polymerase chain reaction. Res Microbiol 1989; 140: 477-487.
7. Steketee RW, Burstyn DG, Wassilak SGF. A comparison of laboratory and clinical methods for diagnosis pertussis in an outbreak in a facility for the developmentally disabled. J Infec Dis 1988; 157: 441-449.
8. Pan TM, Lee YS, Chiou SI, et al. Characterization of *Bordetella pertussis* isolated in Taiwan. NSC Report (NSC 83-0412-B-043B-001).

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