# ACKNOWLEDGEMENTS

*I would like to express my thanks to Allah the Most Great and Mmost Merciful.*

It is a pleasure to express my deep appreciation to my supervisors **Dr. Mohammed Abboud Al- Qaraguli & Dr. Yahya A. Al-Tufayli** for their valuable guidance during the progress of the present work.

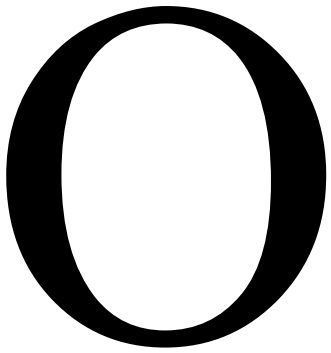
I am grateful to *Mrs.* Sandra Williams *(MedQuich Test Co. Los Anglos-California) for her help* in accomplishing *this study.*

*I extend my thanks to Mrs. Bushra Ibraheem (Head of Laboratories Section) , Mr. Hamza Muhsin, Mrs. Kareema Abdolhamza, Mrs. Khadeeja Obead, Abear Abed Al-Ameer, Miss. Fatima Ali, Mr. Ayad Jawad and Mrs. Lamyaa Baqeer in Bacteriology Department – Babylon Maternity and Children Hospital.*

*I would like to thank each of Mr. Hussein Kadhum, Mr. Dergham Ali, and Mr. Sachit Abbas in Isolation Unit in Babylon Maternity and Children Hospital.*

*I am grateful to Dr. Amal Mirza (Head of Public Health Laboratory in Hilla).*

*I am also indebted to Mr. Ali Hussein and Miss. Shaimaa Obead (M Sc. Students in college of Medicine Babylon University).*



(سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَى كُلِّ شَيْءٍ شَهِيدٌ)

صدق الله العظيم

(سورة فصلت: الآية 53)

# Declaration

*We certify this thesis was prepared under our supervision at the University of Babylon as partial requirements for degree of M.Sc. in Medical Microbiology….*

***Signature**:*

*Name:* ***Dr. Mohammad A.Muhsin***
***Ass. Prof.***
*Address:* ***Department of Microbiology***
***College of Medicine***
***University of Babylon***

*Date: / / 2005*

***Signature**:*

*Name:* ***Dr. Yahya Abd AL***
***Ass. Prof.***
*Address:* ***Department of Pediatrics***
***College of Medicine***
***University of Babylon***

*Date: / / 2005*

# Recommendation of Head of Microbiology Department

*According to the available recommendations, I introduce this thesis to discussion.*

*Signature:*

*Name:* ***Ass. Prof. Dr. Mohammad Sabri***
*Address:*
***Head of Microbiology Department***
***College of Medicine***
***University of Babylon***

# Decision of discussion committee

***We, the examining committee , after reading this thesis and examining the student in its contents , find it adequate as a thesis for the degree of Master of Science in Medical Microbiology.***

*Signature:*

***Prof.***
***Dr. Ibrahim M. S. Shnaw***
***Chairman***

*Signature:*

***Ass. Prof.***
***Dr. Salman Aziz***

***Member***

*Signature:*

***Ass. Prof.***
***Dr. Shaker Kareem Katea***

***Member***

*Approved by the College Committee on Graduate Studies*
*Signature:*

***The Dean of Medical College***

# List of contents

# List of Tables

# List of Figures

# List of Abbreviations

| *Symbol* | *Description* |
|---|---|
| **Abs** | Antibodies |
| **ACT** | Adenylate cyclase |
| **ADP** | Adinosin diphosphate |
| **AGGs** | Agglutinogens |
| ***B. pertussis*** | *Bordetella pertussis* |
| **BrkA** | *Bordetella* resistance to killing genetic locus, frame A |
| **CD** | Cluster of differentiation |
| **CDC** | Center for disease control and prevention |
| **CFA** | Complement fixation test |
| **CIEPT** | Counterimmunoelectrophoresis test |
| **DFA** | Direct fluorescent |
| **DNA** | Deoxyribonuclic acid |
| **DTP vaccine** | Diphtheria – tetanus – pertussis vaccine |
| **EDTA** | Ethylin diamine terta aciticacid |
| **ELISA** | Enzyme linked immunosorbent assay |
| **EPI** | Expanded program on immunization |
| **Fc** | Fragment crystilizable |
| **FHA** | Filamentous hemagglutinin |
| **FIMs** | Fimbrial agglutinogens |
| **HLT** | Heat labile toxin |
| **IFN-γ** | Interferon gamma |
| **IgA** | Immunoglubulin A |
| **IgG** | Immunoglubulin G |
| **IgM** | Immunoglubulin M |
| **IHAT** | Indirect hemagglutination test |
| **IL** | Interlukin |
| **LOS** | Lipooligosaccharide |
| **LPS (HST)** | Lipopolysaccharide (heat stable toxin) |
| **O. D.** | Optical density |
| **OMP(PRN)** | Outer membrane protein (pertactin) |
| **PBS** | Phosphate buffer solution |
| **PCR** | Polymerase chain reaction |
| **PT (LPF)** | Pertussis toxin (Lymphocytosis promoting factor) |
| **PT-IgA** | Pertussis toxin-Immunoglobulin-A |

| *Symbol* | ***Description*** |
|---|---|
| **PT-IgG** | Pertussis toxin-Immunoglobulin-G |
| **SAT** | Slide agglutination test |
| **TCT** | Tracheal cytotoxin |
| **TMB** | Tetramethylbenzidine |
| **U/ml** | Unit per milliliter |
| **WBC** | White blood cells |
| **WHO** | World Health Organization |

# Abstract

The aims of this study is to describe some of the epidemiological features of pertussis in Babylon Province in Iraq, and to evaluate the specific anti *Bordetella pertussis* IgG and IgA ELISA technique in the laboratory diagnosis of pertussis, and to determine the humoral immune response to DTP vaccine.

Blood samples were obtained from the following groups: 68 (36 unvaccinated and 32 vaccinated)clinically diagnosed hospitalized pertussis patients, 103 normal healthy subject of similar age groups (49 vaccinated and 54 unvaccinated) and 20 healthcare workers in Babylon Province.

The anti *Bordetella pertussis* IgG and IgA antibodies were tested by ELIZA technique in all study groups.

The study shows that the highest incidence of pertussis was noticed in patients less than 1 year (49% of the total pertussis patients). The male to female ratio was 1.27:1. There were 71% of pertussis cases from rural area compared with 29% from urban area. 36 (53%) of the patients were unvaccinated and 26 (38%) of the patients were partially vaccinated, while only 6 (9%) are fully

vaccinated (3 doses of DTP vaccine). Complications were noticed in (88%) of the patients; they are mainly apnea (88%), seizure (26%), pneumonia (29%), and death (4%). Most cases were noticed between March and July.

The anti *Bordetella pertussis* IgG diagnostic sensitivity and specificity in unvaccinated subjects were 77%, 100% respectively; while in vaccinated subjects they were 67%, 91% respectively. The anti *Bordetella pertussis* IgA diagnostic sensitivity and specificity in unvaccinated subjects were 33%, 100% respectively, while in vaccinated subjects they were 72%, 86% respectively. The best diagnostic sensitivity and specificity was observed in samples collected after > 21 days of the onset of pertussis symptoms. The higher diagnostic sensitivity and specificity were obtained by using anti *Bordetella pertussis* IgG or IgA positive levels.

The study shows that the anti *Bordetella pertussis* IgG mean level in DPT vaccinated normal subjects was 56.41 U/ml (range 19.77-143.89 U/ml), while it was 29.33 U/ml (range 1.01-56.9 U/ml) in unvaccinated subjects. The anti *Bordetella pertussis* IgA mean level in vaccinated normal subjects was 9.05 U/ml (range 3.6-29.74 U/ml), while it was 4.79 U/ml (range 3.05- 7 U/ml) in unvaccinated subjects.

There is 10.7% (11/103) of the normal subjects which have a positive anti *Bordetella pertussis* IgG or IgA levels. In

addition, 70% (14/20) of the healthcare workers in this study have a positive anti *Bordetella pertussis* IgG or IgA levels.

# Introduction

Pertussis **(**Whooping cough) is an acute respiratory disease affecting over 60 million infants and is responsible for approximately half million dying each year (Holder, and Mortimer.1992 and Pizza *et al*. 1989). It remains an important public health problem in the developing world and represents a reemerging public health problem in developed countries (Holder, and Mortimer.1992). At the turn of the twentieth century in United States approximately 5 of every 1,000 infants born alive die of the disease before their fifth birthdays. Today fewer than 10 deaths are reported annually in the United States (Mortimer. 1998).

The disease was characterized by a paroxysmal cough which often starts after an incubation period of 7 to 10 days, with a range of 4 to 21 days (Hoppe *et al*. 1998.). The classical pertussis illness has three stages: the catarrhal, paroxysmal and convalescent. Complications are mainly due to the effect of the paroxysms, and include anoxia, cyanosis, atelectasis and bronchiectasis. Pneumonia is due to secondary bacterial infection such as *Hemophilus influenzae, Streptococcus pneumonia*, *Streptococcus pyogenes or Staphylococcus aureus*. The central nervous system complication includes encephalitis and seizure (Hoppe *et al*. 1998 and Zackrisson *et al*. 1990).

*Bordetella pertussis* (*B. pertussis*), the causative agent of whooping cough (Beier, *et al*. 1996) is a small gram negative coccobacilli. *B. pertussis* produces a number of virulence factors such as Pertussis toxin (PT), Filamentous hemagglutinin (FHA), pertactin, tracheal cytotoxin, demonecroting toxin and adenylate cyclase (Manklark and Hill. 1984).

The majority of pertussis causes occur in children under the age of 5; most deaths occur during the first year of life (Brooks, *et al*. 2004).

Attempts to develop effective vaccine were unsuccessful until the late 1930s. In the United States, whole killed bacterial vaccines appeared to be of sufficient efficacy to warrant licensure and generally in young infants in 1940s (Mortimer. 1998). During the 1960s the number of notified cases and of hospitalized cases of pertussis decreased substantially. However, from the beginning of the 1970s the incidence of pertussis increased despite a continued high vaccination rate (Nelson *et al*. 1996). The whole cell vaccine have been associated with mild reactions, such as redness, swelling and fever that occur in 20-70% of the vaccinates. In addition to these mild reactions severe side effects such as anaphylaxis, brain damage, and death have been associated in rare cases 3 to 9 cases per million doses, with pertussis vaccination (Peppolni *et al*. 1995). In 1981 Japan introduced acellular pertussis vaccines, major components of this vaccines were PT and FHA. The acellular pertussis vaccines were more immunogenic with less local and systemic reactions than the whole cell pertussis vaccine (Afari *et al*. 1996).

The diagnosis of pertussis primarily depends on clinical signs and symptoms, but these symptoms do not occur until week 3 of infection, during the paroxysmal phase of illness (Pittman. 1984). Techniques routinely available in clinical laboratories include culture and direct immunoflorecent on nasopharyngeal secretions, and Polymerase Chain Reaction (PCR). These techniques have either low sensitivity and/or low specificity (de Melker *et al*. 1999). Some investigators have suggested that serological methods may increase the diagnostic sensitivity. The ELISA technique which detects anti

PT and/or FHA antibodies in serum samples was considered to be the most sensitive laboratory method to diagnose pertussis (Halperin *et al.* 1989). However, the ELISA technique needs further evaluation and standardization (Kosters *et al.* 2000).

# Aims of the study

1. To describe the epidemiological features (age and sex distributions, vaccination status, residential and seasonal influence) of pertussis infection in Babylon Province.
2. To evaluate the role of anti *Bordetella pertussis* IgG and/or IgA antibodies by ELISA technique in diagnosis of whooping cough, and demonstrate the influence of age, vaccination status and timing of serum collection on sensitivity and specificity of this technique.
3. To determine the levels of anti *Bordetella pertussis* IgG and IgA as a response to DTP vaccination.
4. To determine the carrier state of *Bordetella pertussis* infection in the general population.
5. To determine the role of healthcare workers in nosocomial transmission of *Bordetella pertussis* infection.

# Literature Review

## 2-1 Historical background

The first recorded description of the whooping cough dated back to the 16th century (Holder and Mortimer. 1992; Mortimer. 1998). No definitive description of whooping cough syndrome was found earlier than that of Gillian De Daillou who described an outbreak in Paris during the year 1578 (Holder, and Mortimer.1992; Altemeier-III. 1981) in which infants and young children were primarily affected. It is curious and unclear why *B. pertussis* infection eluded earlier description. Epidemics of pertussis followed settlers to the New World (Holder, and Mortimer.1992). An explanatory hypothesis from this curiosity includes the possibility that pertussis was a disease new to human beings at that time perhaps as a consequence of mutation of some similar organism that affected lower animals (Mortimer. 1998). Another hypothesis is that physicians of the past paid less attention to the disease in the presence of many other more devastating diseases (Mortimer. 1998). Yet whooping cough has enough unique features that it should have been recognized far earlier. Its prevalence as a common communicable disease of childhood, occurrence in epidemics, and prolonged clinical course with paroxysmal cough and whoops should have made pertussis at least as recognizable as was mumps to Hippocrates or measles to the ancient Arabian physician Al-Rhazes (Altemeier-III. 1981). Sydenham first used the term pertussis in 1670, but the illness has also been known as kink, which pertained to a Scottish word for “fit” or paroxysm and chin cough derived from the German kindhoest of child’s cough. In 1897 Frohlich

was apparently the first to note the lymphocytosis which characterized pertussis, a useful diagnostic tool (Altemeier-III. 1981).

The organism was recognized in respiratory secretion and pathological specimens in the late 19$^{th}$ century but was not recovered on culture until 1906 (Mortimer. 1990), when Bordet and Gengou isolated the organism on potato starch infusion, glycerol and defibrinated blood (Holder, and Mortimer.1992; A combined Scottish studies. 1970) and was named after these two scientists *Heamophilus pertrussis* and finally *Bordetella pertussis* became accepted nomenclature. The complex medium designed by these two workers is, with slight modification, used today to isolate this organism and is appropriately known as Bordet-Gengou medium.

Following isolation of pertussis bacillus, many workers tried unsuccessfully to prepare effective vaccines. Sauer, a pioneer of vaccination in the United States recognized the importance of using freshly isolated strain in adequate number and produced a vaccine that conferred good protection during a period when others were having many problems (Altemeier-III. 1981). Because of poor understanding of the organism of biological anatomy as well as difficulties in propagation, attempts to develop effective vaccines were unsuccessful until the late 1930s (Mortimer. 1998).

## 2-2 The causative agent (*Bordetella pertussis*)

### 2-2-1 Definition and Classification

*Bordetella* spp. are minute, gram-negative, aerobic, non-acid-fast, non-sporing coccobacilli. They do not ferment carbohydrates. Their optimum temperature is 35-37°C (Bemi. 1992). The genus *Bordetella* consists of seven

species, *B. pertussis, B. parapertussis, B. bronchiseptica, B. avum* and the recently added *B. hinzii, B. holmesii* and *B. termatum* (Altemeier-III. 1981). *B. pertussis* and *B. parapertussis* are responsible for the majority of *Bordetella* infections in human. Most of the other species have been recognized to cause human infection under special circumstances (Parton. 1996). For example, *B. hinzii* has been isolated from symptomatic patients with cyctic fibrosis (Vandamme *et al*. 1995; Funk *et al.* 1996). *B. holmesii* has been associated with septicemia (Tang *et al.* 1998; Weyant *et al.* 1995) and *B. trematum* has been recovered from wounds and ear infection in human (Vandamme *et al.* 1996). *B. bronchiseptica* causes respiratory diseases such as kennel cough in dogs, snuffles in rabbits, pneumonia in koalas, and atrophic rhinitis in swine, but it is also infrequently responsible for chronic respiratory infection in humans, especially immunocompromised hosts and persons who have contact with animals (Bauwens *et al.* 1992; Cosh and Tranter *et al.* 1979).

## 2-2-2 Culture

The preferred medium is charcoal blood agar to which cephalixin has been added; the antibiotic inhibits other organism that commonly found in specimens and only minimally restricts the growth of *Bordetella* species (Ruijs. 1991). This medium is superior to Bordet-Gengou medium in that it supports a heavier growth of the organism on primary isolation, and the colonies are larger (Ruijs. 1991). Recent work suggests that this medium may be stored at 4 °C in airtight, sealed plastic bags for up to 14 weeks without significant deterioration in the efficiency of culture (Ruijs. 1991). Primary

isolation of *B. pertussis* on charcoal blood agar is slow and incubation should be continuing for at least 5 days. Plates or slops should be incubated at 35 °C with enhanced humidity. Usually a growth will be recognizable after 72h as greish white colonies with a shiny surface and high convex shape: the typical 'bisected pearl' or 'mercury drop' appearance (Onorato and Wassilak. 1987).

## 2-2-3 Biochemical activity

Studies on the metabolic activities of the *Bordetella* have been concerned mainly with defining nutritional requirements or with biochemical tests for identification and for taxonomic and phylogenitic purposes. The biochemical testing has a limited value for differentiation between *B. pertussis, B. parapertussis and B. bronchiseptica,* which can be distinguished by some biochemical tests (Table-1) (Vandamme, *et al.*1995).

**Table (1): The biochemical activity of medically important *Bordetella* Spp (Vandamme, *et al.*1995):**

| Characteristic | *B. pertussis* | *B. parapertussis* | *B. bronchiseptica* |
|---|---|---|---|
| Growth on blood agar | - | + | + |
| Oxidase | + | - | + |
| Motility | - | - | + |
| Urease | - | + | + |

## 2-2-4 Susceptibility to antimicrobial agents

*B. pertussis* was mentioned to be susceptible to several antibiotics, such as tetracycline, erythromycin, and chloramphenicol; the efficacy of these drugs in patients during the paroxysmal phase is not convincing. Treatment

with erythromycin, which is usually considered the antibiotic of choice, will eliminate viable *B. pertussis* organisms from the respiratory tract within a few days. The treatment, however, has no influence on the course of the disease. Antipertussis hyperimmuneglobulin is still used occasionally (Balagtas *et al.* 1971). Further treatment is symptomatic. (Blackall *et al.* 1995).

## 2-2-5 Antigenic characters

*B. pertussis* has three major antigens, designated 1,2 and 3, which can be detected by slide agglutination tests. In recent years the commonest serotype in Britain has been types 1 and 3 in well immunized communities and types 1 and 2 in poorly immunized communities (Preston,. 1985). However, it is now clear that thorough epidemiological investigation requires more sophisticated procedures such as restriction fragment analysis of DNA (Khattak, *et al.*1992).By using this technique it has become clear that multiple strains of *B. pertussis* circulate in the population at one time and that considerable variant in type of the organism exists in different geographical areas. (Preston. 1985)

## 2-2-6 Virulence factors

*B. pertussis* produces an array of toxins, aggressins and adhesions that are presumed to be important in colonization of their respective hosts and ensuring the prolongation of their survival and propagation. (Weiss *et al.* 1984).

**2-2-6-1 Pertussis toxin (PT)**

Pertussis toxin (PT) has a complex structure. It is composed of five dissimilar subunits, named S1-S5 (fig-1). PT is a member of the A-B toxins, in which the A moiety, composed of the S1 subunit, is an enzyme, and the B moiety, composed of subunits S2-S5, constitutes the target-cell receptor-binding portion. The B oligomer of PT can in turn be divided into two dimers, named D1 and D2, composed of subunits S2-S4 and subunit S3-S4, respectively (Tamura *et al.* 1982).

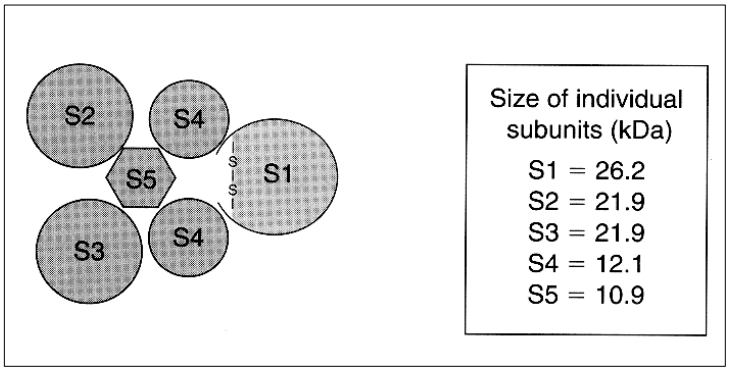



**Fig (1): Structure of pertussis Toxin (Locht and Antoine. 1997).**

The steps of PT biological activity include (Locht and Antoine. 1997)(fig-2):

1. Binding of toxin to the target – cell receptors via the B oligomer, with specific involvement of D1 or D2.
2. Membrane translocation of the enzymatically active S1 subunit.
3. Expression of the ADP-ribosyltransferase activity catalyzed by the internalized S1 subunit.

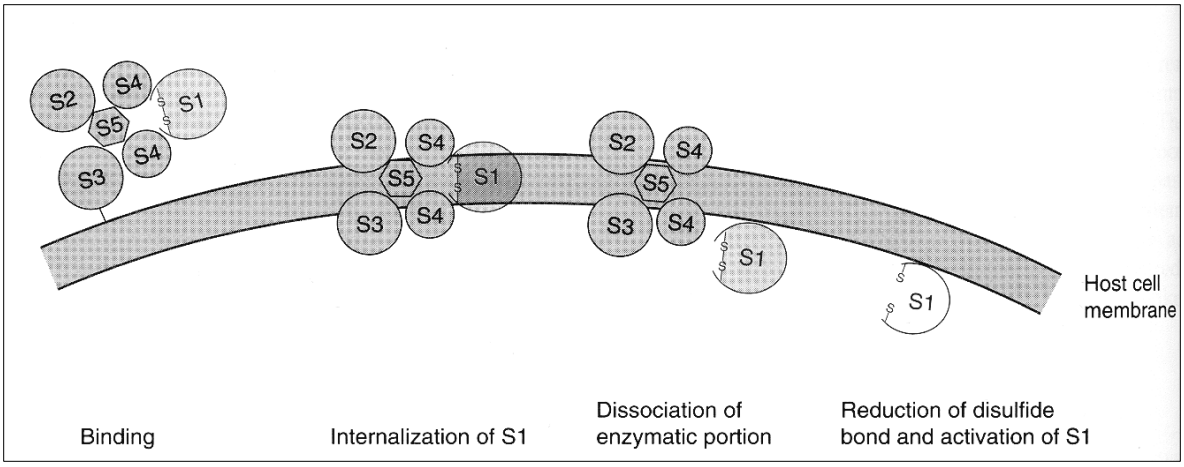



**Fig (2): Hypothetical steps involved in the binding, internalization, and activation of pertussis toxin (Locht and Antoine. 1997).**

All the three steps are required for the full expression of most – but not all – biological activities of PT. Depending on the target cell; the physiological effects of PT may vary tremendously. These effects are responsible for most of the systemic features of whooping cough (Pittman. 1984). Accordingly, the existence of PT was first predicted by the expression of its numerous biological activities detected after infection with *B. pertussis* or administration of whole cell pertussis vaccine. These activities include the biological effects that have been related to the action of PT in tissue cultures including suppression of cell proliferation, morphological changes, exocrine secretion, inhibition of histamine secretion and stimulation of lipolysis (Munoz. 1985). *In vivo*, the biological effects are recognized as Langerhan's islet activation, lymphocyte promoting activity, histamine sensitization and increase of vascular permeability (Munoz. 1985), some of which are hallmarks of systemic pertussis in patients (Pittman. 1984). PT is considered

as a potent protective antigen in vaccines against whooping cough (Ad Hoc group. 1988).

### 2-2-6-2 Filamentous Hemagglutinin (FHA)

Filamentous protein with an estimated molecular weight of 130,000 is derived from fimbirae on the surface of the organism (Manklark and Hill. 1984; Rodrignez *et al.* 1996). Its major and perhaps sole, function in the pathogenesis of whooping cough is facilitating adherence of the organism to cilia. It is likely that both PT and FHA are important in this process (Manklark and Hill. 1984; Mortimer. 1990) (Fig-3).

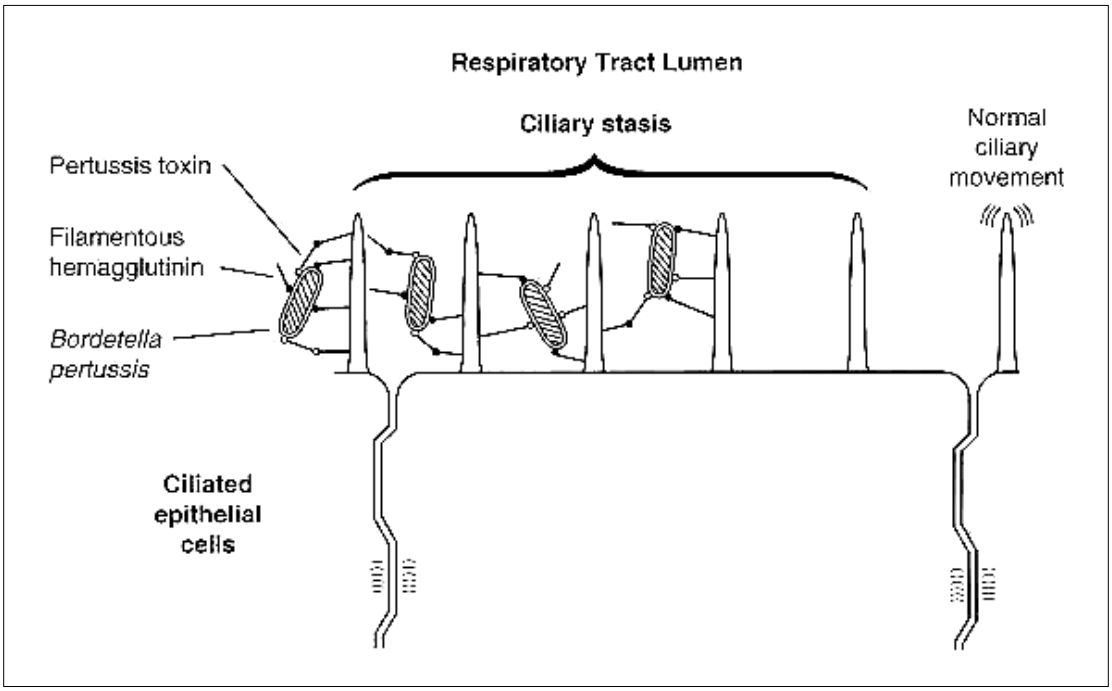



**Fig (3): Synergy between pertussis toxin and the filamentous hemagglutinin in binding to ciliated respiratory epithelial cells (Pittman. 1979).**

Although FHA is difficult to study in vivo in humans, in vitro studies employing human and other mammalian cells are strongly suggestive of such a role, not in the least due to the fact that Ab to FHA impedes adherence in such models, FHA appears to be nontoxic and does not appear to adhere (Mortimer. 1990). FHA is produced by three *Bordetella* species *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* (Muller *et al*. 1997). Antibodies to FHA develop in human following whooping cough or immunization with whole cell pertussis vaccine and acellular vaccine (Mortimer. 1990). There is also evidence that these Abs are necessary for optimum protection. This evidence from the Swedish field trial of two acellular pertussis vaccines one containing only PT and the other containing PT and FHA, point estimate of efficacy indicated greater efficacy for latter preparation (Mortimer. 1990). In all age groups, FHA Abs was more commonly found than PT Abs (Wirsing van Konig and Schmitt. 1996). Abs to PT and FHA increase with age (Zackrisson *et al*. 1990), and there is evidence that these Abs contribute to clinical protection against pertussis (Mortimer. 1998) by preventing the adherence of these bacteria to respiratory epithelial cells. FHA is immunogenic, therefore, like PT, is strong candidate for inclusion in an acellular pertussis vaccine (Mortimer. 1991).

**2-2-6-3 Lipopolysaccharide LPS (Heat Stable toxin HST)**

LPS or endotoxin of the cell wall is heat stable and basically similar to the endotoxins of enterobacteraceae except for differences in macromolecular structure. It consists of two different polysaccharides each terminated by a molecule of 3-deoxy-2-octulosonic acid. Two distinct lipid fragments-lipid A and Lipid X, are present, and contain glucosamine, fatty acids and esterified

phosphate in similar proportions. Lipid X which is the minor lipid has 2-methyl, 3-hydroxydecanoic and tetradecanoic acids that are absent from lipid A. Lipid X appears to be responsible for the acute toxicity of this endotoxin (Mortimer. 1998; Manklark and Hill. 1984).

**2-2-6-4 Fimbrial Agglutinogens (FIMs)**

More than a dozen agglutinogens are present on the cell envelope of the three species of the genus *Bordetella*; most, if all of these agglutinogens are fimbrial (Stanbridge and Preston. 1974). Only agglutinogens 1,2 and 3 are considered to be of importance in disease pathogenesis and immunity. Antibodies to these agglutinogens have been useful in seroepidemiological studies (Preston and Stanbridge. 1972). The FIMs appear to participate in the attachment of *B. pertussis* to respiratory cilia, although their role probably is less than that of FHA or PT. The FIMs are also immunogenic (Public Health Laboratory Service. 1973). Serum Abs to the FIMs is found almost universally after natural disease or after immunization with vaccines containing these proteins (Preston. 1976).

**2-2-6-5 Pertactin PRN or (69 kD protein) or (OMP)**

The pertactin is an outer membrane protein (OMP), originally called 69 kD protein because of its molecular weight of 69,000 Dalton (Muller *et al*. 1997). Pertactin, which is a membrane-associated Ag of *B. pertussis* and which is present in many acellular vaccines against whooping cough, has been reported to be similar to the homologous protein in *B. bronchiseptica* (Pagliaccia *et al*. 1997). Pertactin has been identified as being associated either with adenylate cyclase or one of the agglutinogens. The activity of the

pertactin in animal preparations suggests that it play a role in clinical immunity and antigenisity in human (Mortimer. 1990). Abs to PRN appear following natural disease. Pathogenesis in human as well as in the development of immunity is unknown at present (Trollfors *et al.* 1992; Mortimer. 1991). Abs to pertactin were determined in subset of serum samples by ELISA technique (Kimura *et al*. 1991).

**2-2-6-6 Adenylate Cyclase (ACT)**

Adenylate Cyclase is an enzyme that is released into culture medium and it is present in all virulent strain of *B. pertussis* , and is non-cytoplasmic apparently being located on the cell membrane. In pertussis, ACT compromises phygocytic cell function (including chemotaxis, phygocytosis, and bacterial killing **(**Hewlett & Grondon. 1988**)** by augmenting production within the phygocyte of cyclic adenosine monophosphate from adenosine triphosphate, resulting in an excessive accumulation of cyclic adenosine monophosphate and paralysis of the various phygocytic functions. ACT may contribute to the excessive production of bronchial secretion during pertussis. In the mouse model of aerosol infection, PT and ACT appear to be the two most important virulence factors.**(**Weiss, *et al* .1990**)** ACT is immunogenic **(**Arciniega, *et al. 1991***)**; in the mouse models of intracerebral aerosol challenge, prior active immunization with Adenylate Cyclase was shown to be similar in protective efficacy to whole- cell vaccine **(**Guiso, *et al* .1990**).** In addition, it has been shown that ACT antibodies interfere with the multiplication of organisms in these models **(**Guiso, *et al* .1990**)**

**2-2-6-7 Tracheal Cytotoxin (TCT)**

Tracheal cytotoxin appears to be a fragment released from the peptidoglycan of the *B. pertussis* cell wall. It can be recoeverd from culture supernatant; and it is a very a small molecule, and is nonimmunogenic **(**Cookson, *et a.* 1989**)**. *In vitro* models suggest that it has a single major function: the paralysis and destruction of respiratory ciliated cells. Tracheal cytotoxin appears to be the only component of *B. pertussis* to exhibit this function. **(**Cookson, *et al.* 1989**)**

**2-2-6-8 Heat-labile toxin (HLT)**

Heat labile toxin, so called because it is inactive at 56°C, is also known as the dermonecrotic or mouse-lethal toxin because it's of effect in experimental animals. It is produced by all virulent *Bordetella* species **(**Weiss, *et al* .1990**)**. Located inracellularly, it can be recovered by disruption of *B. pertussis* cells. The mechanism of production of cutaneous lesions after injection of the toxin in animals appears to be vasoconstriction. The role, if any, of heat-labile toxin in the pathogenesis of pertussis is unknown. No consistent effect on cells has been recognized in vitro. It is a weak immunogen, antibodies to it are non-protective in animal challenge tests, and its absence does not diminish the lethality of experimental pertussis infection in mice. **(**Weiss, *et al* .1990**)**

**2-2-6-9 *Bordetella* resistance to killing genetic locus, frame A (BrkA)**

BrkA Is another outer membrane protein of *B. pertussis* similar in structure to PRN mediates adherence to respiratory cells and protects the bacterium against complement. **(**Fernandez & Weiss. 1994**)**

## 2-2-7 Pathogenesis

*B. pertussis* is strict human pathogens. It has not been isolated from animals or as member of normal flora of healthy persons (Nicosia *et al*. 1987). Knowledge of the action of the various components of *B. pertussis* permits the development of the hypothesis about the series of pathogenic events that occur in the course of whooping cough (fig-4). Because the likelihood of infection varies directly with the intimacy and duration of contact, it is probable that large numbers of organisms are required to infect the respiratory tract (Mortimer. 1998). Following inhalation of infected droplets, the organism colonizes the respiratory tract (Manklark and Hill. 1984). PT and FHA facilitate attachment of the organism to the respiratory cilia. After attachment; PT and ACT presumably play major roles in this process; it is necessary for the organism to evade host defenses. It is logical that tracheal toxin and HLT also participate. Cell damage is a consequence of the action of PT and adenylate cyclase. It is probable that tracheal and HLT also contribute (Mortimer. 1998). The role of pertactin is uncertain; it may relate to cell adherence. There is no evidence that agglutinogens play a role in pathogenesis. Similarly, there is no evidence that the rather weak endotoxin of *B. pertussis* contributes to disease manifestation (Mortimer. 1998). Tissue invasion does not occur (Mortimer. 1998), because the non ciliated cells are not involved and maintain the integrity of the epithelial lining is maintained (Nicosia *et al*. 1987) and because invasion of other tissue by *B. pertussis* is related to the lack of receptors for the organism on the other cell types (Manklark and Hill. 1984). It’s presumed that the incubation period and initial mild symptoms of rhinitis, cough, sneezing and sometimes

conjunctivitis are caused by local multiplication of the organism in the respiratory tract (Manklark and Hill. 1984). In the second stage the bacterium grows in the dense masses in the trachea and large bronchi cause the production of thick mucus that impedes cilia (Case *et al*. 1986). The cough appears to be due to interference with normal mechanism of bronchial toilet, mucoid secretions accumulate which are responsible for ineffective, repetitive, paroxysmal coughing and ultimately, for varying degree of bronchial obstruction, atelectasis and bronchopneumonia (Mortimer. 1998). The thick ropy secretions that accumulate are very hard to expel, resulting in episodes of repetitious, paroxysmal coughing often followed by vomiting. The mechanism of vomiting is probably the accumulation of this viscid material in the pharynx. The characteristic whoop follows a protracted spasm that has nearly emptied the brochopulmonory tree of air and represents an attempt to inspire though vocal cords may be particully narrowed of secretions and consequent spasm. An obvious contribution of PT is the characteristic lymphocytosis of pertussis (Mortimer. 1998). The persistence of cough and lymphocytosis is a result of the fixation of toxin to cells. Such adherence is essential for the production of disease, as specific Abs can prevent damage to ciliated cells in vitro, or prevent disease in an animal model (Manklark and Hill.1984).

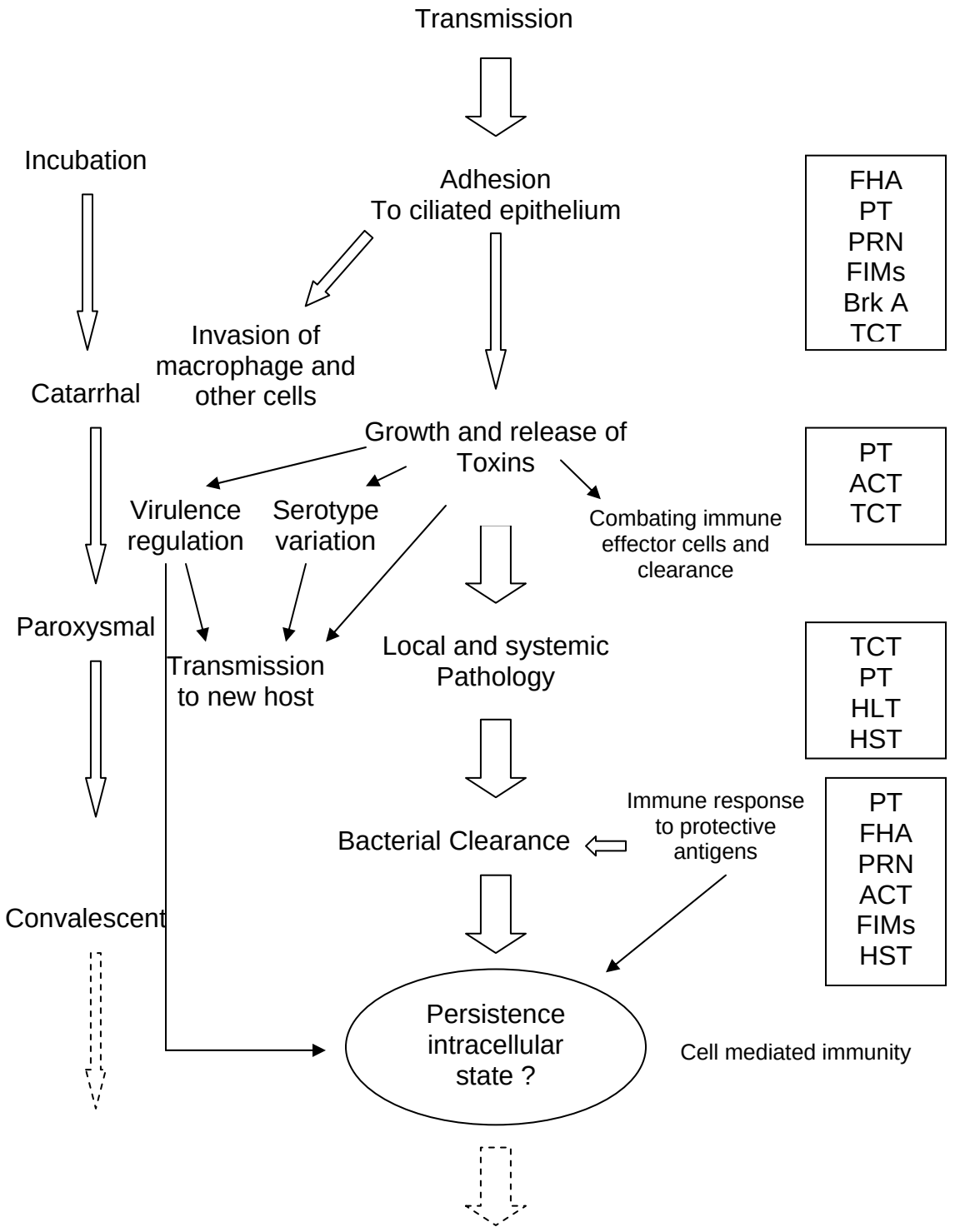



**Fig (4): The sequence of events in pertussis ((Monack, *et al*:, 1989).**

# 2-3 The immune response to *B. pertussis*

## 2-3-1 Humeral immune response:

### 2-3-1-1 IgG response:

There is compelling evidence nowadays that anti pertussis toxin IgG (PT IgG) antibodies alone provide passive protection against pertussis. Although protective levels of PT IgG antibodies have not been determined, vaccine trials and specifically those using single component acellular PT vaccines have shown that antibodies against PT alone can protect against pertussis. The addition of other antigens such as filamentous hemagglutinin (FHA), agglutinins, and 69-kDa protein only add minimal protection to that achieved by PT alone. It is known that patients convalescing from pertussis mount a high antibody response to PT, which approximates the onset of gradual recovery from the disease. (Ad Hoc Group for the Study of Pertussis Vaccines. 1988)

After vaccination with DTP vaccine, IgG and IgM directed against both FHA and PT were detected. Generally, antibody titers increased with subsequent injections and the age of the children. Maternal antibodies against FHA and PT were detected in cord blood, consistent with reports that there is placental transfer of antibodies to *B. pertussis*. All cord blood antibodies detected were of the IgG class. This was expected and was used as an internal control since IgM and IgA antibodies do not cross the placenta (Olin, and Storsaeter. 1989).

The IgG activity directed against the specific adherence of *B. pertussis* to human ciliated cells clearly exists. It appears that this activity involves specific antibody. Activity appears after infection and, to a lesser extent, after

immunization. In both cases, activity can be abolished by absorption of immunoglobulin classes from serum. Both the IgG and IgA fractions isolated from convalescent serum have antiadherence activity. This evidence suggests that for *B. pertussis*, antibody that prevents bacterial adherence may one of the factors that contribute to lower respiratory tract immunocompetence. (Olson, 1975).

In a study by Mills *et al*., it was demonstrated that cell-mediated immunity and PT-IgG antibody response play complementary roles in conferring immunity in the aerosol challenge model. A comparison between three whole-cell and five acellular pertussis vaccines have demonstrated a high correlation between clinical vaccine efficacy in children and *B. pertussis* clearance from the lungs of immunized mice with the aerosol challenge model (Mills *et al*. 1998). In another study there is evidence for the important role of anti-PT IgG antibodies in the immune response but do not exclude the complementary role of cell-mediated immunity. (Bruss. and George. 1999). There is evidence that PT antibodies not only play a major role in passive protection but also can reverse symptoms of established disease for at least 7 days in mice in the aerosol challenge model. (Bruss. and George. 1999)

**2-3-1-2 IgA response**

*B. pertussis* is noninvasive and is found exclusively on mucosa of the respiratory tract. Since IgA represents the predominant antibody isotype at mucosal surfaces, a role for IgA in anti-*B. pertussis* mechanisms is possible. (Nagel, and Poot Scholtens. 1983). IgA is generally believed to function by neutralizing and agglutinating pathogens or by preventing their attachment to mucosal surfaces (Childers, *et al*. 1989). The role of IgA, however, may be

much broader because of the effectors functions induced by binding to IgA receptors. The prototypic IgA receptor (FcaRI [CD89]) is found exclusively on cells of the myeloid lineage: monocytes, macrophages, neutrophils, and eosinophils (Kerr, Mazengera, and Stewart. 1990). Increasing evidence shows that FcaRI exhibits potent proinflammatory capacities. FcaRI binding with IgA molecule that attached to bacteria readily induces phagocytosis, degranulation, respiratory burst, antibody-dependent cellular cytotoxicity, and the release of proinflammatory cytokines (Van Spriel, and van de Winkel. 1999). IgA-mediated anti-*B. pertussis* activity was also observed in a murine pertussis infection model. High IgA titers in sera of human pertussis patients younger than 1 year of age were found to correlate with reduced duration of positive pertussis culture and PCR in throat samples. These findings pointed to bactericidal effects of anti-*B. pertussis* IgA in humans.( Van der Zee. *et al.* 1996).

A pertussis vaccines trial in The Netherlands showed that, boosting of 4-year-old children with the Dutch whole-cell pertussis vaccine induced anti-*B. pertussis*-specific serum IgA, in contrast to boosting with acellular vaccines (Berbers, *et al*. 1999). IgA capable of inducing anti- *B. pertussis* activity may be important in the evaluation of vaccines. For years IgA has been considered to play a passive, “noninflammatory” role in immunity; by blocking microbial interaction with host tissue, it may prevent cell damage and inflammation. However, IgA proved to be very effective in inducing cellular immune functions via FcaRI expressed on myeloid cells. A number of studies have already reported IgA-mediated phagocytosis of different microorganisms and tumor cells (Deo, *et al*. 1998).

**2-3-1-3 Role of complement**

Complement is present in serum, but it is also exuded from the blood to mucosal surface (Persson *et al.* 1991). Mucosal pathogens have mechanism to resist complement (Fearon and Locksley. 1996). *B. pertussis* is a mucosal pathogen that produces potent toxins that contribute to the disease. In general the complement cascade can be activated by either of two pathways: carbohydrate on bacterial surfaces (LPS) which can activate the alternate pathway (Moffitt and Frank. 1994), while the deposition of Abs on bacterial surface can activate the classical or primarily antibody-dependent pathway of complement. The alternative pathway does not appear to mediate activity against *B. pertussis* (Fernandez and Weiss. 1994). Abs to *B. pertussis* can activate the classical pathway, but the BrkA protein confers resistance to killing by complement (Fernandez and Weiss. 1994).

## 2-3-2 Cell mediated immunity

The mice genetically deficient in mature B cells given aerosol *B. pertussis* infection develop a persistent infection that never be resolved but does not disseminate (Mahon, *et al*. 1997). In addition, recent studies have demonstrated that pertussis-specific human (Ryan, *et al*. 1997) and murine (Mills, *et al*. 1993) T cells, particularly CD4+ T cells, secrete IL-2 and IFN-γ in response to specific stimulation. In a mouse model of respiratory infection, transfer of these Th1 cells resulted in bacterial clearance in the apparent absence of antibodies (Mills, *et al*. 1993). Furthermore, after aerosol infection, mice lacking IFN-γ did not control bacterial growth well (Barbic, *et al*. 1997), and mice lacking IFN-γ receptors had disease with aberrant organ

pathology, atypical dissemination of bacteria outside of lungs, and occasional deaths (Mahon, *et al*. 1997). Consistent with controversial reports suggesting that *B. pertussis* survives, if not replicates, within murine (Cheers, & Gray. 1969), rabbit, or human (Saukkonen, *et al.* 1991) macrophages;, the latter results suggest that cell-mediated immunity may play a significant role in controlling pertussis infection.

T cell mediated response may be induced as a result of infection or immunization with the whole cell vaccine and, when induced by infection, it is accompanied by only minimal antibody responses. This result agrees with a report indicating the critical role of cellular immunity in the clearance of a *B. pertussis* primary infection from the murine respiratory tract (Mills, et al. 1993) and also demonstrates the importance of T cells in protective immunity acquired by immunization. Until recently, pertussis was considered to be a noninvasive mucosal infection giving rise to a toxin-mediated disease (Pittman,. 1979). Consistent with this view, most investigations of immunity to the disease have centered on humoral responses, particularly the levels of serum IgG, to selected antigens, mainly PT and putative adhesins (Charles, *et al.*. 1991.). However, more findings demonstrating the invasive potential of *B. pertussis* (Ewanowich, *et al*. 1989) and the presence of antigen-specific T-cell responses following infection (De Magistris, *et al*. 1988) have necessitated a reevaluation of potential protective mechanisms.

# 2-4 Clinical manifestation

## 2-4-1 The incubation period

The incubation period of pertussis is commonly 7 to 10 days, with a range of 4 to 21 days, and may rarely be as long as 42 days. (Gordon. *et al. 1994*).

## 2-4-2 The clinical course of the illness:

### 2-4-2-1 Catarrhal stage:

It is characterized by the insidious onset of coryza (runny nose), sneezing, low-grade fever, and a mild, occasional cough, similar to the common cold. The cough gradually becomes more severe (Lenette *et al*. 1974).

### 2-4-2-2 Paroxysmal stage:

After 1-2 weeks the paroxysmal stage begins. It is during the paroxysmal stage that the diagnosis of pertussis is usually suspected. Characteristically, the patient has bursts, or paroxysms of numerous rapid coughs, apparently due to difficulty expelling thick mucus from the tracheobronchial tree. At the end of the paroxysm, a long inspiratory effort is usually accompanied by a characteristic high-pitched whoop. During such an attack, the patient may become cyanotic (turn blue). Children and young infants, especially, appear very ill and distressed. Vomiting and exhaustion commonly follow the episode (Lenette *et al*. 1974). The patient usually appears normal between attacks. Paroxysmal attacks occur more frequently at night, with an average of 15 attacks per 24 hours. During the first or second week of this stage the attacks increase in frequency, remain at the same level for 2 to 3 weeks, and

then gradually decrease (Mortimer. 1998). The paroxysmal stage usually lasts 1 to 6 weeks, but may persist for up to 10 weeks. Infants younger than 6 months of age may not have the strength to have a whoop, but they do have paroxysms of coughing (Manklark and Hill. 1984).

**2-4-2-3 Convalescent stage**:

Recovery is gradual. The cough becomes less paroxysmal and disappears in 2 to 3 weeks (Guermonprez *et al*. 1999). However, paroxysms often recur with subsequent respiratory infections for many months after the onset of pertussis. Fever is generally minimal throughout the course of pertussis (Altemeier-III. 1981).

## 2-4-3 Complication

Young infants are at highest risk for acquiring clinical pertussis, and for pertussis-associated complications. The most common complication, and the cause of most pertussis-related deaths, is secondary bacterial pneumonia (He *et al*. 1996; Johnston *et al*. 1998). Data from 1997-2000 indicate that pneumonia occurred among 5.2% of all reported pertussis cases, and among 11.8% of infants <6 months of age. Neurologic complications such as seizures and encephalopathy may occur as a result of hypoxia from coughing, or possibly from toxin (Mortimer. 1990). Neurologic complications of pertussis are more common among infants. In 1997-2000, seizures and encephalopathy were reported between 0.8% and 0.1%, respectively, of all cases, and among 1.4% Pertussis and 0.2%, respectively, of infants <6 months of age (Sherris *et al.* 1984). Other less serious complications of pertussis include otitis media, anorexia, and dehydration. Complications

resulting from pressure effects of severe paroxysms include pneumothorax, epistaxis, subdural hematomas, hernias, and rectal prolapse (Mortimer. 1998). In USA 1997-2000, 20% of all reported pertussis cases required hospitalization, including 63% of all infants <6 months of age. In a 4-year period, 62 deaths were due to pertussis (case-fatality rate 0.2%). Fifty-six (90%) of these deaths occurred in children <6 months of age (CDC. 2002).

## 2-5 Laboratory Diagnosis

The diagnosis of pertussis is usually based upon a characteristic history and physical examination. However, laboratory tests may be useful in young infants, atypical cases, and cases modified by vaccine. The standard and preferred laboratory test for diagnosis of pertussis is:

### 2-5-1 Isolation of *B. pertussis* by culture:

A positive culture for *B. pertussis* confirms the diagnosis. Fastidious growth requirements make *B. pertussis* difficult to isolate (Mertsola *et al.* 1983). Isolation of the organism using direct plating is most successful during the catarrhal stage (fig-5). Specimens from the posterior nasopharynx, not the throat, should be obtained using Dacron or calcium alginate (not cotton) swabs and should be plated directly onto selective media (Lenette *et al.* 1974). Success in isolating the organism declines with prior antibiotic therapy effective against pertussis (erythromycin or trimethoprim-sulfamethoxazole) or delay in specimen collection beyond the first 2 weeks of illness, or in vaccinated persons. (Marcon, *et al.* 1987)

## 2-5-2 Polymerase chain reaction (PCR):

Polymerase chain reaction testing of nasopharyngeal swabs or aspirates can be a rapid, sensitive, and specific method for diagnosing pertussis (Mastrantonio *et al*. 1998). Currently, it is only available in certain laboratories; the assays vary among laboratories and are not standardized (Muller *et al*. 1997). PCR should be used in addition to culture, not as a replacement for culture, because bacterial isolates may be required for the evaluation of antimicrobial resistance, or for molecular typing. (Fry, *et al*. 2004).

## 2-5-3 Direct fluorescent antibody (DFA):

DFA testing of nasopharyngeal specimens may be useful as a screening test for pertussis (Holder, and Mortimer.1992). Because direct fluorescent antibody testing of nasopharyngeal secretions has been shown in some studies to have low sensitivity and variable specificity, it should not be relied on as a criterion for laboratory confirmation (Halperin, *et al*.. 1989).

## 2-5-4 Lymphocyte count

An elevated white blood cell count with a lymphocytosis is usually present in classical disease. The absolute lymphocyte count often reaches 20,000 or greater. However, there may be no lymphocytosis in infants and children or in mild or modified cases of pertussis. (Birkebaek, *et al* . 1999)

## 2-5-5 Serological testing:

Laboratory conformation of whooping cough is best achieved by the isolation of *B. pertussis,* as carriers occur rarely if at all. Where isolation has not been possible, serological methods may be useful in individual cases and

for epidemiological studies. Serology is also used to measure the response to vaccination (MacAulay. 1979).

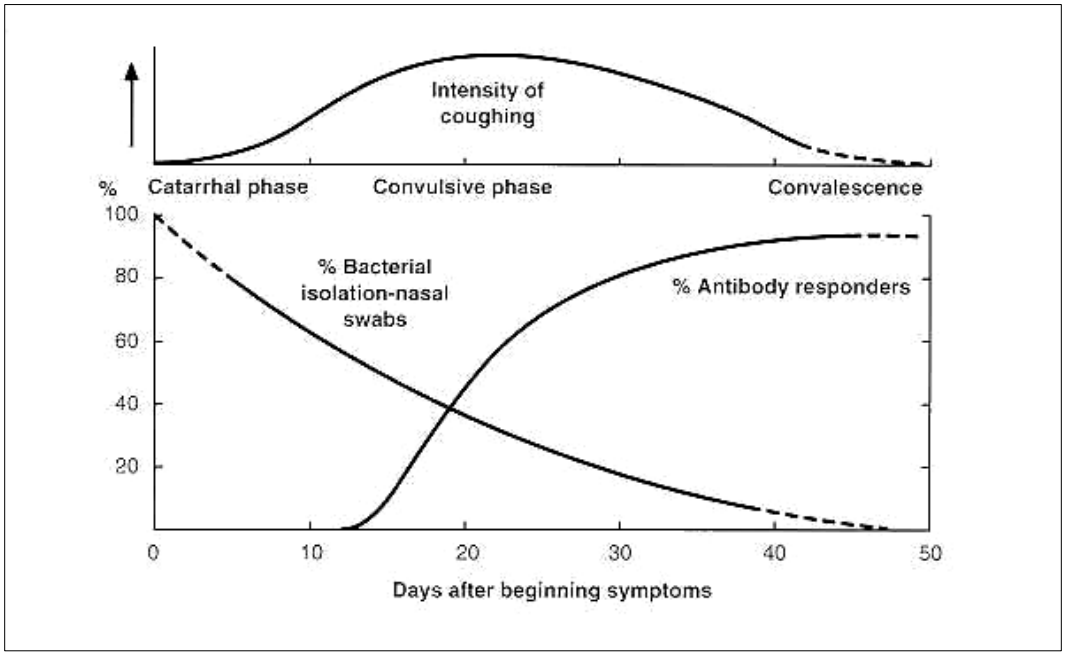



**Fig (5): The sensitivity of culture and serology after the onset of pertussis symptoms. (American Academy of Pediatrics. 2003)**

### 2-5-5-1 Slide agglutination test (SAT)

A slide agglutination test can be performed with a standard inoculum of organism and specific antiserum (Manklark and Hill .1984). Slide agglutination test can be performed with specific antiserum as soon as growth is apparent on Bordet-Gengou plates (Lenette *et al*. 1974). A variety of methods for performing agglutination tests on pertussis antisera have been proposed (Macauly. 1979; Wilkins *et al.* 1971). Immunization with pertussis vaccine or recovery from pertussis disease may not always result in the production of agglutinins, and immunity may exist in the absence of demonstrable agglutinin; however, disease does not occur in the presence of agglutinins of high titer (Kendrick *et al.* 1969).

**2-5-5-2 Counterimmunoelectrophoresis test (CIEPT)**

The technique was used for the identification of Ag-Ab reaction by precipitation and purity (Vancanneyt *et al*. 1995; Mohammed. 1997). CIEPT is a well-established technique for the rapid diagnosis of bacterial meningitis and pneumonia. Its major advantage is that it detects Ag and, therefore, it is positive early in the course of illness (Borel and Gnlesph. 1984, Vancanneyt *et al*. 1995). CIEPT was more sensitive than culture methods early in the course of the illness when the importance of symptoms is more difficult to assess (Borel and Gnlesph. 1984; Thompson. 1977).

**2-5-5-3 Complement fixation test (CFT)**

The simple complement fixation test can be used to test either of Ag-Ab interaction or the presence or absence of complement (Tietz. 1970). The serological response of pertussis disease or immunization has been measured by the CFT (Bradstreet *et al.* 1972).

**2-5-5-4 Indirect haemagglutination test (IHAT)**

Erythrocytes are not only good passive carrier of Ag, but also easily coated with foreign proteins and can easily be obtained and stored (Campell *et al*. 1970). The use of sheep RBCs sensitized with specific Ag provides a method which is more sensitive and specific than standard agglutination test for pertussis diagnosis (Mastrantonio *et al*. 1998).

**2-5-5-5 Enzyme Linked Immunosorbent assay (ELISA)**

Most of the serological assays mentioned above lack sensitivity and/or specificity. The ELISA procedure has been used to measure the serological response to pertussis infection or immunization (Garnstorm G *et al.* 1988;

Baraff *et al.* 1983; Burstyn *et al.* 1983; Wassilak *et al.* 1983; Garnstorm M *et al.* 1982; Viljanen *et al.* 1982; Goodman *et al.* 1981; Ruuskanen *et al.* 1980).

By using whole *B. pertussis* cells as the antigen in an ELISA, Ruuskanen *et al* (1980) reported an immunoglobulin G (IgG) response but low or undetectable IgM and IgA response in sera from vaccines. The same ELISA methods and antigens were used by Viljanene *et al* (1982), who documented IgG, IgM and IgA responses in sera from pertussis patients and proposed that the presence of an IgG or IgA response was evidence of recent disease. Goodman *et al*, (1981) used the ELISA to detect secretory IgA in nasopharyngeal secretions during and after natural infection, but they were not able to detect IgA after vaccination.

The enzyme-linked immunosorbent assay, using purified pertussis factors such as pertussis toxin (PT) or filamentous hemagglutinin (FHA), has been used extensively in epidemiological studies (Meade, *et al*. 1990). This technique has proven to be reasonably sensitive and specific (Granström,. *et al*. 1988). It is generally concluded that a significant increase in specific anti-PT immunoglobulin G (IgG) or IgA and/or anti-FHA IgG or IgA in paired sera correlates with *Bordetella pertussis* infection (Garnstorm *et al.* 1988). The ELISA has been used for diagnosis of pertussis based on single serum sample using age-specific values for deferent populations (Wirsing von Konig *et al.* 1999). Several studies used the ELISA to determine the immune response to pertussis vaccine (Giammanco *et al.* 1997; Olin *et al.* 1997; Wirsing von konig *et al.* 1996; Hallander, *et al*. 1991).

# 2-6 Pertussis epidemiology

## 2-6-1 Mode of transmission

Pertussis is a highly contagious disease (Mortimer. 1998). The source of infection is usually a patient in the early catarrhal stage of disease (Brooks *et. al.* 2004). Transmission of pertussis infection probably occurs via secretion of the mouth and nose if infected individual, which serves as a primary vehicle to infect other individuals either directly (Nester *et al*. 1973; Wyss and Eklund. 1971), through droplets expelled into the air or indirectly, via contaminated handkerchiefs, towels, food, linens and similar objects (Wyss and Eklund. 1971). Humans beings are the only known source of *B. pertussis* (Manklark and Hill. 1984). In adults, 20-47% of those exposed to the disease develop infection. Lack of awareness of adult pertussis in patients with prolonged cough and the high incidence of subclinical disease (40%) results in intra-familial and nosocomial disease (Franso, *et al* , 1992).

## 2-6-2 Age and sex distribution

Pertussis may occur at any age (Hoppe. 1998). Infants are susceptible to pertussis within the first few weeks or months of life, when mortality from whooping coughs are highest. For many years, it was assumed that one attack of pertussis provided lifelong immunity. Before the widespread vaccination, this belief was reflected by the age distribution of pertussis: approximately 20% of all whooping cough cases occurred in infants younger than 1 year, and as much as 60% among children aged 1 to 4 years (Luttinger. 1998).

More recently, in countries such as the United States in which pertussis vaccination is common, the age distribution of pertussis has markedly changed. Between 1992 and 1994, 28% of reported pertussis cases occurred in individuals 10 years and older (CDC. 1995), in contrast to less than 3% of patients being older than 15 years before the advent of widespread vaccination, 41% of cases providing age data occurred in infants younger than 1 year, compared with 45% in prior 3 years (Luttinger. 1998). Serologic surveys demonstrate a high frequency of non-diagnosed infection in young men (Cherry. 1995), and studies of adults with cough lasting more than 1 to 2 weeks demonstrate evidence of recent pertussis infection in more than 20% (Mink, *et al.1992* ; Wright, *et al. 1995*).

Although both sexes are presumably equally affected, pertussis is unusual in that, inexplicably, the disease is more often reported in females, who also exhibit higher mortality rates. It may be that the smaller larynx of female infants places them in greater jeopardy of severe disease (Mortimer. 1998; Mortimer. 1990; Mortimer. 1998).

## 2-6-3 Pertussis incidence in vaccinated and unvaccinated patients

Prior to mass immunization, pertussis incidence was not decreasing. The introduction of mass immunization was associated with a five-to-100 fold reduction in pertussis incidence in Canada, England, Wales and the USA between 1930 and 1980. (Varughese. 1985; Cherry. 1984; Pollard. 1980).

Countries with consistently low pertussis incidence rates have had consistently high immunization coverage rates (e.g. Hungary and the former

East Germany) (Joo. 1991; Finger, *et al* .1991). Higher pertussis incidence rates in some countries now, and in others at intervals in the recent past, have been due to low immunization coverage and use of vaccines with low efficacy, or less than optimal immunization schedules. The higher incidence of pertussis in Canada compared with the USA during the 1980s and 1990s was due to the lower efficacy of the pertussis vaccine used in Canada. (DeSerres *et al* . 1996).

Countries that have experienced large increases in pertussis incidence in association with reductions in vaccine coverage include the UK, Sweden and Japan (Miller, *et al* 1992; White, *et al*. 1996; Romanus, *et al.* 1987; Noble. *et al.* 1987; Kimura & Kuno-Sakai. 1990).

## 2-6-4 Seasonal influence on pertussis incidence

Pertussis is endemic with superimposed epidemics in most densely populated areas all over the world (Black. 1997). Cycles every 3-4 years after accumulation of susceptible cohort (Rota *et al*. 1998). The majority of cases occur in late winter and early spring (. Rota *et al*. 1998), some reports indicate a higher incidence in summer when young infants probably are in more with each other, and some alleged no seasonal variation (Mortimer. 1998).

## 2-6-5 Nosocomial Transmission

Transmission of pertussis in hospital settings has been documented in numerous reports (Shefer, *et al* 1995). The number of patients and staff who have developed clinical pertussis indicates that these outbreaks have been of limited size, and no deaths due to nosocomial transmission have been

reported; however, their impact has been large (Linnemann, *et al* 1975). These outbreaks have resulted from failure to recognize and isolate infected infants and children, failure to recognize and treat disease in staff members, and failure to institute control measures rapidly (Kurt. *et al* 1972). Either a health-care worker or a patient may introduce *B. pertussis* into the hospital or clinic, and subsequent transmission to patients or health-care workers (or both) may occur. The risk of developing pertussis for patients or staff during these outbreaks is often difficult to quantify because exposure is not well defined **(**Kurt, *et al*. 1972).

## 2-7 Pertussis immunization

### 2-7-1 Passive immunization

**2-7-1-1 Transplacental:** Antibodies to PT and FHA readily cross the placenta and are found in infants sera in concentration comparable to those in maternal sera. The half-life of these transplacental antibodies appears to be about 6 weeks, and they disappear by 4 months of age (Van Savage *et al.* 1990). However, these antibodies appear to offer little or no clinical protection, because infants of mothers, who are presumed to be immune to pertussis, whether because of immunization or disease, are susceptible to pertussis on exposure. Indeed, from 1980 to 1989, 31% of the cases of pertussis reported to CDC were in children younger than 6 months, and the highest average annual age-specific incidence rate during those years was among infants 1 to 2 months of age (Farizo *et al.* 1992).

**2-7-1-2 Therapeutic:** Before the widespread use of pertussis vaccine and the availability, passive immunization using whole sera was employed in an

effort to prevent the spread of disease to exposed susceptible people and to modify the course of the illness in those who had already acquired whooping cough (Plotkin and Orenstein. 1999). In most of the studies of preventive efficacy, individuals were not controlled; a few studies that did include control subjects suggested that as many as 40% of recipients were protected (Pittman. 1984). The subsequent development of methods for purification of serum immune globulin led to the commercial production of pertussis hyperimmune globin, composed largely of IgG (Department of Health and Human Services, FDA. 1985). Although this preparation was widely used for prophylaxis and treatment, controlled studies showed that it had no effect, and production was discontinued (Balagtas *et al.* 1971). More recently, new preparation of pertussis immune globulin has been prepared and evaluated in animal models and human. Sato and Sato (1991) demonstrate that both monoclonal and polyclonal antibodies to PT improved survival of sucking mice after aerosol challenge with live pertussis organisms. The ability to generate high-titer immune globulin by immunizing adults with safe acellular pertussis vaccine led to a reassessment of the use of immune globulin as therapy for pertussis. In 1991, Granstorm and colleagues conducted a double-blind, randomized, placebo-controlled trial of a pertussis immune globulin prepared from adults immunized with an acellular vaccine containing PT or PT plus FHA. A significant reduction in the duration of whoops was demonstrated in recipients of the pertussis immune globulin compared with placebo recipients.

## 2-7-2 Active immunization

### Pertussis vaccine

Whole-cell pertussis vaccine is composed of a suspension of formalin-inactivated *B. pertussis* cells. It was developed in the 1930s, and used widely in clinical practice by the mid-1940s. Whole cell vaccine plays an important role in the control of pertussis in the world and the vaccine proved to be very successful during nearly 50 years of its use (Galazka. 1997). Based on controlled efficacy trials conducted in the 1940s and on subsequent observational efficacy studies, a primary series of four doses of whole-cell DTP vaccine was 70% to 90% effective in preventing serious pertussis disease (Tozzi *et al*. 1998; Grnoff and Rappuoli. 1997). Protection from pertussis vaccine decreased with time, resulting in little or no protection 5 to 10 years following the last dose (Beque and Griprel. 1996; Edward and Decker. 1997). Local reactions such as redness, swelling, and pain at the injection site occurred following up to half of the doses of whole-cell DTP vaccines. Fever and other mild systemic events were also common. More severe systemic reactions, such as convulsions and hypotonic hyporesponsive episodes occurred less frequently (one case to 1,750 doses administered) (He *et al*. 1994). In 1981 acellular pertussis vaccines were introduced in Japan. The traditional acellular pertussis vaccines was made of the portion of culture supernatant of *B. pertussis* Tahoma strain phase 1 which contain hemagglutinin activity. The major components of this portion were PT and FHA (Kimura and Kuno-Sakai. 1990; Edwards and Karazon. 1990; Mills *et al*. 1998). The acellular DPT vaccine was either one containing FHA,

pertactin and PT inactivated formalin and glutaraldehyde (Hewiett .1997), or one with FHA pertactin and genetically detoxified pertussis toxin (Mahon *et al*. 1996; Greco *et al*. 1996). Acellular pertussis vaccine has been shown to have a high clinical efficacy and to be less reactogenic than whole cell vaccine (Atti *et al*. 1999; Sato T *et al*. 1990; Stewart *et al*. 1996; Cassone *et al*. 1997).

## 2-8 Pertussis in Iraq

Pertussis (whooping cough) and diphtheria, rarely seen in middle-income countries, continued to occur throughout Iraq (WHO. 2003). The last documented pertussis epidemic occurred from June-December 1996 with 40% of those affected under 5 years of age. Almost 20% of children 1-4 years were not immunized. Pertussis has no seasonal pattern but tends to favor the summer/autumn months (June to Oct) (WHO. 2003).

**Table (2): The epidemiological features of pertussis in Iraq (WHO. 2003).**

| | |
|---|---|
| **Burden** | **Number of cases reported (incidence rate per 100,000):**<br>**2001:** 2,312 cases (8.7)<br>**2000:** 407 cases (1.6)<br>**1999:** 466 cases (1.9)<br>**1998:** 1,271 cases (5.3)<br>**1997:** 535 cases (2.3)<br>**1996:** 1,179 cases (5.4)<br>**1995:** 475 cases (2.3)<br>**1994:** 526 cases (2.6)<br>**1993:** 767 cases (3.8)<br>**1992:** 1,601 cases (8.3)<br>**1991:** 1,537 cases (7.8)<br>**1990:** 489 cases (2.5)<br>**1989:** 368 cases (2.0) |
| **Geographical distribution** | Throughout the country |
| **Seasonality** | Pertussis has no distinct seasonal pattern, but may increase in the summer and fall. |
| **Recent epidemics** | *June-December 1996 – Basrah:* a total of 133 referred cases presented with pertussis; 53 (39.8%) were children under 5 years. Of these 53, 10 were non-immunized infants under 2 months of age; 5 were non-immunized children aged more than 1 year.<br>The male:female ratio for pertussis was 2:8 and 71.4% of the 133 cases had been immunized.<br>Many more unreferred cases occurred. |

The incidence of pertussis is still high in Iraq (2,312 reported cases in 2001) and appears to be increasing (Table-2). The high incidence (fig-6) despite high vaccination coverage can be attributed to the following factors:

- Immunization protection mostly covers children 5-9 years of age who have taken four or five doses of DTP. Infants under 6 months of age who have not yet received the full benefit of immunization, and adolescent and adult populations with diminishing effectiveness of the vaccine, are usually the most affected groups.
- Poor nutrition is common, especially among infants, leading to malnutrition, and severely increasing the risk of infection and disease **(WHO. 2003)**.

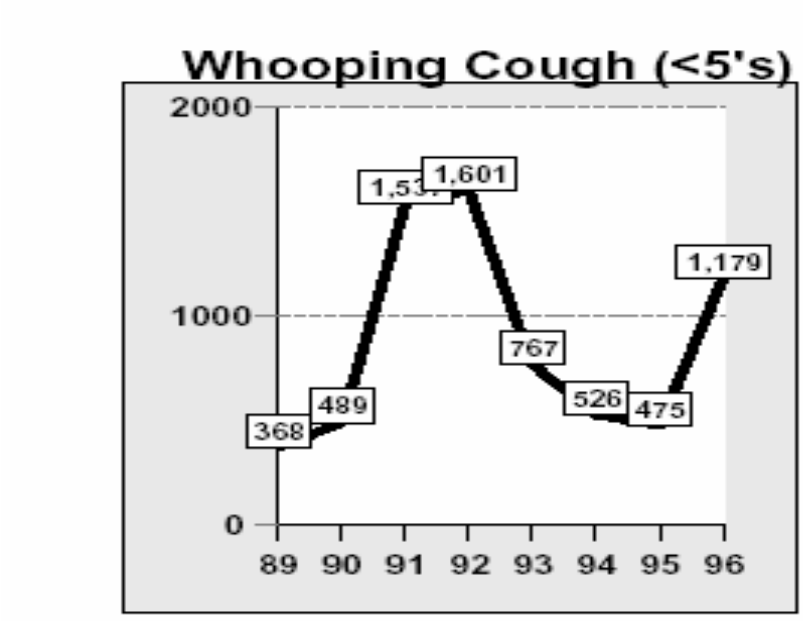



**Figure (6): Trends of Whooping cough in Iraq (South/Centre) 1989 to 1996. (UNICEF/Iraq. 1998)**

Since 1990, Iraq's immunization services were interrupted (Table-3). The Expanded Program on Immunization (EPI) coverage was affected, with disruption of vaccine supply, of the cold chain and in the health service upheaval in general. This compromised protection against preventable childhood diseases and their incidence rose steeply in 1991 and in some - measles and whooping cough continued rising in 1992. The decline in most conditions is a reflection of successful EPI efforts (UNICEF/Iraq. 1998).

Pertussis is a potential problem if introduced into crowded refugee settings with many non-immunized children DTP3 coverage (UNICEF/Iraq. 1998).

**Table (3): DPT vaccine coverage in Iraq (UNICEF/Iraq. 1998).**

| The year | DTP coverage (%) | Source of information |
|---|---|---|
| 1980 | 13 | Official country estimation |
| 1990 | 83 | Official country estimation |
| 1998 | 86 | Official country estimation |
| 1999 | 90 | WHO-UNICEF survey database |
| 2000 | 86 | Official country estimation |
| 2001 | 74 | WHO-UNICEF estimation |
| 2002 | 67 | WHO-UNICEF estimation |

# Materials & Methods

## 3-1 Study Design and Study Groups

### 3-1-1 Patients:

Blood samples were collected from 68 Hospitalized patients with clinically diagnosed pertussis; it was conducted from January 2004 to December 2004 in the Babylon Maternity and Children Hospital in Hilla City. The blood samples were collected from patients of less than 18 years old.

The diagnosis of each case depends on clinical diagnosis and that was based on the following criteria (CDC. 1997): patients with a cough lasting at least 2 weeks with one of the following:

- Paroxysmal cough
- Inspiratory whoop
- Post-tussive vomiting
- Early diagnosis of an epidemiological link to pertussis case.

Serum samples from patients with clinical symptoms of pertussis were stored at – 10 °C in the laboratory of the Babylon Maternity and Children Hospital. The ELISA tests were done in the Public Health Laboratory in Hilla. For each patient, a complete information indicating age, sex, duration of illness, sign and symptoms, immunization status, residency, history of close contact with a pertussis case within or external to the family was collected.

## 3-1-2 Normal population

Blood samples collected from 103 normal subjects in 7 age groups with different immunization status (Table-4):

**Table (4): The age groups and immunization status of the normal subjects.**

| Age groups | Immunization status | | Total |
|---|---|---|---|
| | Immunized | Unimmnized | |
| > 2 months | 0 | 14 | 14 |
| 2-4 months | 7 | 7 | 14 |
| 5-7 months | 7 | 7 | 14 |
| 8 months – 1 year | 7 | 7 | 14 |
| 2 – 6 year | 7 | 7 | 14 |
| 7 – 18 year | 7 | 7 | 14 |
| Adults | 14 | 5 | 19 |
| Total | 49 | 54 | 103 |

The blood samples from normal children were done in 3 different health-care centers in Hilla City in addition to Babylon Maternity and Children Hospital. The adult samples were collected from students of medical college, Babylon University.

## 3-1-3 Healthcare workers

In order to show the role of the healthcare workers in the hospital in nosocomial infection and epidemiology of pertussis 20 blood samples were collected from medical staff, including doctors, nursing staff, laboratory staff and other heath worker in Babylon Maternity and Children Hospital.

# 3-2 Material

## 3-2-1 Equipments and tools

**Table (5): Equipments and tools used in this study.**

| Device | Company | Origin |
|---|---|---|
| Refrigerator | Liebher | Austria |
| Freezer | Liebher | Austria |
| Centrifuge | Heraeus | Germany |
| Automatic Pipette 5, 50, 100, 500 ml | Slamed | Germany |
| Microtiter plate spectrophotometer | Beckman coulter | Austria |
| Microtiter plate washer | Beckman coulter | Austria |

## 3-2-2 Bordetella pertussis IgG and IgA ELISA kits :

The ELISA *Bordetella pertussis* (IgG, IgA) kits were provided by IBL (Immuno-Biological Laboratories) – Hamburg-Germany, in association with MEDQUICK Test Company – Los Anglos – California USA.

### 3-2-2-1 Contents of the test kits:

**A) *Bordetella pertussis* IgG ELISA kit:**

- **Microtiter Strips**: strips with 8 breakable wells each, coated with *Bordetella pertussis* antigens (Pertussis toxin, Filamentous Hemagglutinin, different Liopopolysaccharides.
- **Standard A-D**:

| Standard | A | B | C | D |
|---|---|---|---|---|
| Concentration of IgG in U/ml | 1 | 20 | 45 | 150 |

**Standard A**: 2 ml, protein solution diluted with PBS, contains no IgG antibodies against *Bordetella pertussis*. Addition of 0.01%, with 0.01 potassium tetraiodomercurate

**Standard B**: 2 ml human serum diluted with PBS contains a low concentration of IgG antibodies against *Bordetella pertussis*. , with 0.01 potassium tetraiodomercurate.

**Standard C**: 2 ml, human serum diluted with PBS, contains a medium concentration of IgG antibodies against *Bordetella pertussis*. , with 0.01 potassium tetraiodomercurate

**Standard C**: 2 ml, human serum diluted with PBS, contains a high concentration of IgG antibodies against *Bordetella pertussis*. , with 0.01 potassium tetraiodomercurate

- **Enzyme Conjugate**: 12 ml, anti-human-IgG-HRP (rabbit), conjucated to protein-containing buffer solution.
- **Tetramethylbenzidine (TMB) Substrate Solution:** 12 ml.
- **TMB Stop Solution**: 12 ml, 0.5 M sulfuric acid ($H_2SO_4$).
- **Sample Diluent**: 60 ml, PBS/BSA buffer. Addition of 0.01 % potassium tetraiodomercurate.
- **Washing Buffer concentrates (10x):** 60 ml, PBS + Tween 20, 10x concentrate.
- **Plastic Foils**: 2 pieces to cover the microtiter strips during the incubation.
- **Plastic Bag**: Resealable, for the dry storage of non-used strips.

**B) *Bordetella pertussis* IgA ELISA kit**

- **Microtiter Strips**: strips with 8 breakable wells each coated with a *Bordetella pertussis* antigens (Pertussis toxin, Filamentous Hemagglutinin, different Liopopolysaccharides serotype 1,2 and 3).
- **Standard A**-D:

| Standard | A | B | C | D |
|---|---|---|---|---|
| Concentration of IgA in U/ml | 1 | 10 | 20 | 50 |

**Standard A**: 2 ml, protein solution diluted with PBS, contains no IgA antibodies against *Bordetella pertussis*. Addition of 0.01%, with 0.01 potassium tetraiodomercurate

**Standard B**: 2 ml human serum diluted with PBS, contains a low concentration of IgA antibodies against *Bordetella pertussis*. , with 0.01 potassium tetraiodomercurate.

**Standard C**: 2 ml, human serum diluted with PBS, contains a medium concentration of IgA antibodies against *Bordetella pertussis*. , with 0.01 potassium tetraiodomercurate

**Standard C**: 2 ml, human serum diluted with PBS, contains a high concentration of IgA antibodies against *Bordetella pertussis*. , with 0.01 potassium tetraiodomercurate

- **Enzyme Conjugate**: 12 ml, anti-human-IgA-HRP (rabbit), conjucated to protein-containing buffer solution.
- **Tetramethylbenzidine (TMB) Substrate Solution:** 12 ml.
- **TMB Stop Solution**: 12 ml, 0.5 M sulfuric acid ($H_2SO_4$).
- **Sample Diluent**: 60 ml, PBS/BSA buffer. Addition of 0.01 % potassium tetraiodomercurate.
- **Washing Buffer concentrates (10x):** 60 ml, PBS + Tween 20, 10x concentrate.
- **Plastic Foils**: 2 pieces to cover the microtiter strips during the incubation.

**Plastic Bag**: Resealable for the dry storage of non-used strips.

# 3-3 Methods

## 3-3-1 Specimens Collection and Storage

The blood samples were obtained by venipunture from all study groups after cleaning the skin with 70 % alcohol, then the blood samples were stored in plastic tubes, To separate the blood samples they were allowed to clot undisturbed for about 1 h at room temperature. Then loosed the clot gently from the tube wall by means of a wooden stick, then centrifuge for 10 min at about 1200g. Then transfer the serum for other tubes for storage under -10 to - 20 °C. (Lewis, et al . 2001) .

## 3-3-2 Samples storing

After the collection of serum samples, they were stored at – 10 °C until they were tested by ELISA technique for quantitative determination of anti *Bordetella pertussis* IgG and IgA.

### 3-3-3 ELISA assay procedure

The researcher depended on IBL Co. instructions in ELISA assay (IBL Catalog No. RE 561 31).

#### 3-3-3-1 Preparation of samples and reagents

**1) Samples**

Before use, the patient's serum samples were diluted 1 to 100 with sample diluent (5µl of sample + 500 µl buffer).

**2) Wash Buffer**

The wash buffer was diluted with distilled water 1 to 10 (10 ml concentrate + 90 ml distilled water).

**3-3-3-2 Assay procedure**

1. A sufficient amount of microtiter wells was prepared for the standard controls and samples.
2. 100 µL from each of the diluted (1:100) samples and the standards were pipeted into appropriate wells of the strip.
3. The plates were covered with the enclosed foil and incubated at room temperature for 60 minutes.
4. The incubation solution was discarded, and the wells were rinsed 3x with 300 µl of wash buffer.
5. 100 µl of enzyme conjugate was added to each well in sequence.
6. The plates were covered with enclosed foil and incubated for 30 minute at room temperature.
7. The incubation solution was discarded, and the wells were rinsed 3x with 300 µl of wash buffer.
8. 100 µl of TMB substrate solution was pipeted into the rinsed wells.
9. The plates were covered with enclosed foil and incubated for 20 min at room temperature.
10. The reaction stopped by adding 100 µl of TMB stop solution to each well.
11. The microtitter strips shaken gently and the reading was at 450 nm with in 60 minutes.

## 3-3-4 Quantitative evaluation

For quantitative evaluation the absorption of the standards is graphically drawn against their concentration. From the resulting standard

curve and their linear equation the concentration for each patients sample can be extracted in relation to his absorption.

### 3-3-4-1 The standard curve and the linear equation of *Bordetella pertussis* IgG

The standard curve was drawn depending on the absorption of the standards (Y) and their concentration (X):

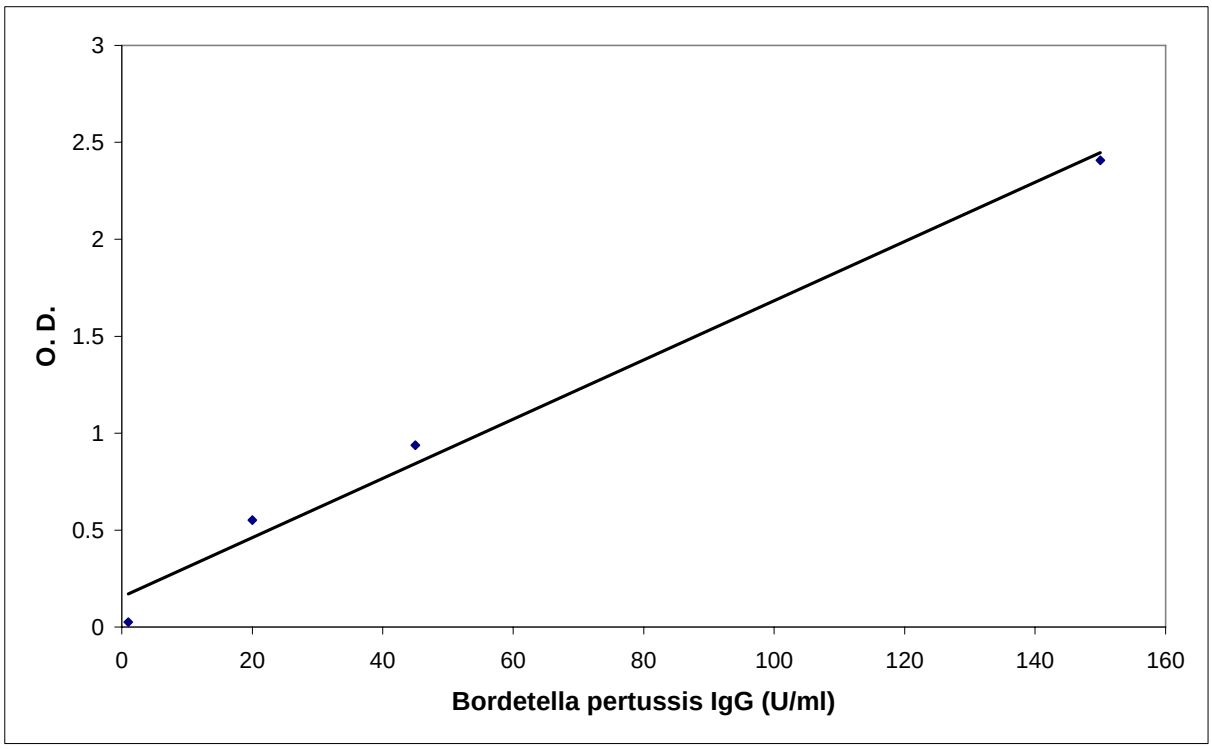



**Figure (7): The standard curve of anti *Bordetella pertussis* IgG.**

## Extraction of the linear equation

**Table (6): Statistical data of the linear equation of IgG.**

| Y* | X** | XY | $Y^2$ | $X^2$ |
|---|---|---|---|---|
| 0.025 | 1 | 0.025 | 0.000625 | 1 |
| 0.551 | 20 | 11.02 | 0.303601 | 400 |
| 0.938 | 45 | 42.21 | 0.879844 | 2025 |
| 2.407 | 150 | 361.05 | 5.793649 | 22500 |
| 3.921 | 216 | 414.305 | 6.977719 | 24926 |

*** O. D. value**
**** IgG concentration (U/ml)**

$$b = \frac{\sum xy - (\sum x \frac{\sum y}{n})}{\sum x^2 - \left[\frac{(\sum x)^2}{n}\right]}$$

$$b = \frac{414.305 - (216 \frac{3.921}{4})}{24926 - \left[\frac{(216)^2}{4}\right]}$$

$$b = 0.0152745$$

$$a = \overline{Y} - b\overline{X} = \frac{3.921}{4} - 0.0152745\frac{216}{4}$$

$$a = 0.1554247$$

$$\boxed{Y = 0.1554 + 0.0153X}$$

### 3-3-4-2 The standard curve and the linear equation of *Bordetella pertussis* IgA

The standard curve was drawn depending on the absorption of the standards (Y) and their concentration (X) :

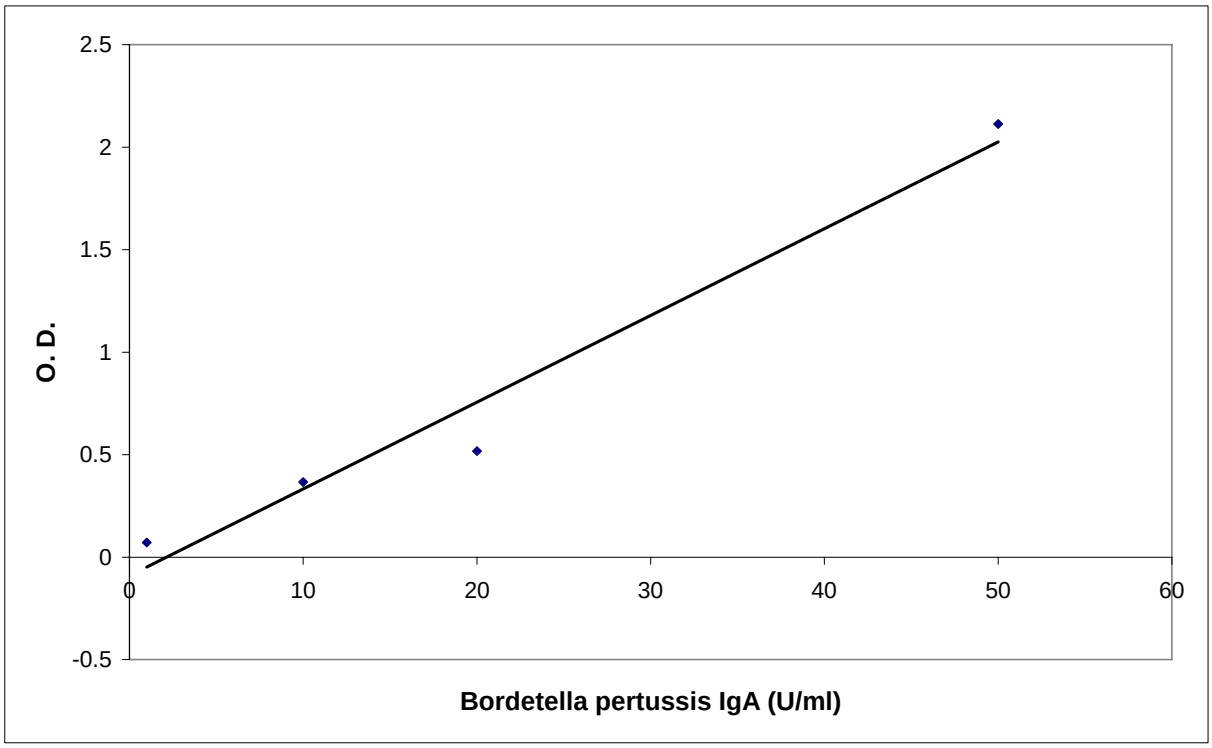



**Figure (8): The standard curve of anti *Bordetella pertussis* IgA.**

## Extraction of the linear equation

**Table (7): Statistical data of the liner equation of IgA.**

| Y* | X** | XY | $Y^2$ | $X^2$ |
|---|---|---|---|---|
| 0.071 | 1 | 0.071 | 1 | 0.005041 |
| 0.366 | 10 | 3.66 | 100 | 0.133956 |
| 0.517 | 20 | 10.34 | 400 | 0.267289 |
| 2.112 | 50 | 105.6 | 2500 | 4.460544 |
| 3.066 | 81 | 119.671 | 3001 | 4.86683 |

*** O. D. value**
**** IgA concentration (U/ml)**

$$b = \frac{\sum xy - (\sum x \frac{\sum y}{n})}{\sum x^2 - \left[\frac{(\sum x)^2}{n}\right]}$$

$$b = \frac{119.671 - (81 \frac{3.066}{4})}{3001 - \left[\frac{(81)^2}{4}\right]}$$

$$b = 0.0423182$$

$$a = \overline{Y} - b\overline{X} = \frac{3.066}{4} - 0.0423182 \ \frac{81}{4}$$

$$a = -0.0904435$$

$$Y = -0.0904 + 0.0423X$$

### 3-3-5 Interpretation of the results

The results of each sample were assessed as the following (Melker, H.E., *et al,* 1999; Redhead K *et al*. 1993; Baughhman AL *et al.*2004):

- IgG level (in U/ml) at least 100 U/ml considered a diagnostic of recent or active infection with *Bordetella pertussis.* Such levels are present in less than 1% of the population and are reached in most pertussis patients
- IgA level (in U/ml) at least 12 U/ml is considered a diagnostic of active infection.

### 3-3-6 Statistical analysis:

Differences between means were compared by student's *t* test under confidence level of 0.95; the P value $\leq 0.05$ was considered a significant deference and P value $> 0.05$ was considered a non significant differences.

### 3-3-7 Sensitivity and Specificity

To calculate diagnostic sensitivity and specificity the following formula is used (Rodgers, 1994):

$$\text{Diagnostic Sensetivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}$$

$$\text{Diagnostic Specificity} = \frac{\text{True Negative}}{\text{True Negative} + \text{False positive}}$$

# Results

## 4-1 Age distribution of the pertussis patients

The age distribution of the 68 pertussis patients presented in this study is shown in Fig (10). The age of patients ranged (1 month-16 years). There were 33 (49%) of the patients under 1 year old, 22 (32%) 1-3 years, 3 (4%) 4-6 years, 7 (10%) 7-9 years, 0 in 10-12 years, 2 (3%) 13-15 years, and 1 (2%) 16-18 years (fig. 9). The data show that the No. of cases is reduced by the increase in age. The higher rate of pertussis is in infants under 1 year old. In this age group 58% of the patients are unvaccinated with DTP vaccine (Table-8).

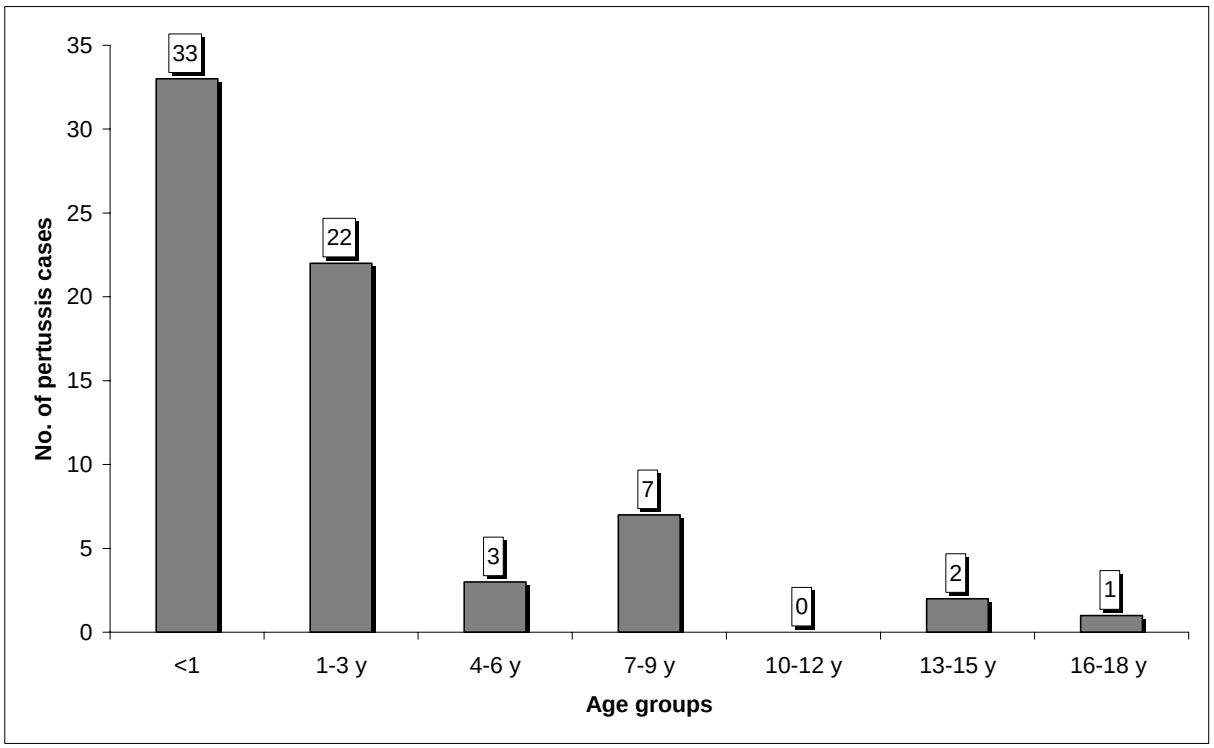



**Figure (9): Age distribution of the pertussis patients**

**Table (8): Age distribution and vaccination status of the patients.**

| Age group (years) | No. of cases (%) | | Total |
|---|---|---|---|
| | Vaccinated | Unvaccinated | |
| less than 1 | 14 (42) | 19 (58) | 33 (49) |
| 1-3 | 8 (36) | 14 (64) | 22 (32) |
| 4-6 | 2 (67) | 1 (33) | 3 (4) |
| 7-9 | 7 (100) | - | 7 (10) |
| 10-12 | - | - | - |
| 13-15 | - | 2 (100) | 2 (3) |
| 16-18 | 1 (100) | - | 1 (2) |
| Total | 32 (47) | 36 (53) | 68 (100) |

## 4-2 Sex distribution of the patients

The sex distribution of the patients was shown in Figure (10). There were 38 (56%) males of the patients and 30 (44%) were females. The male to female ratio was 1.27:1.

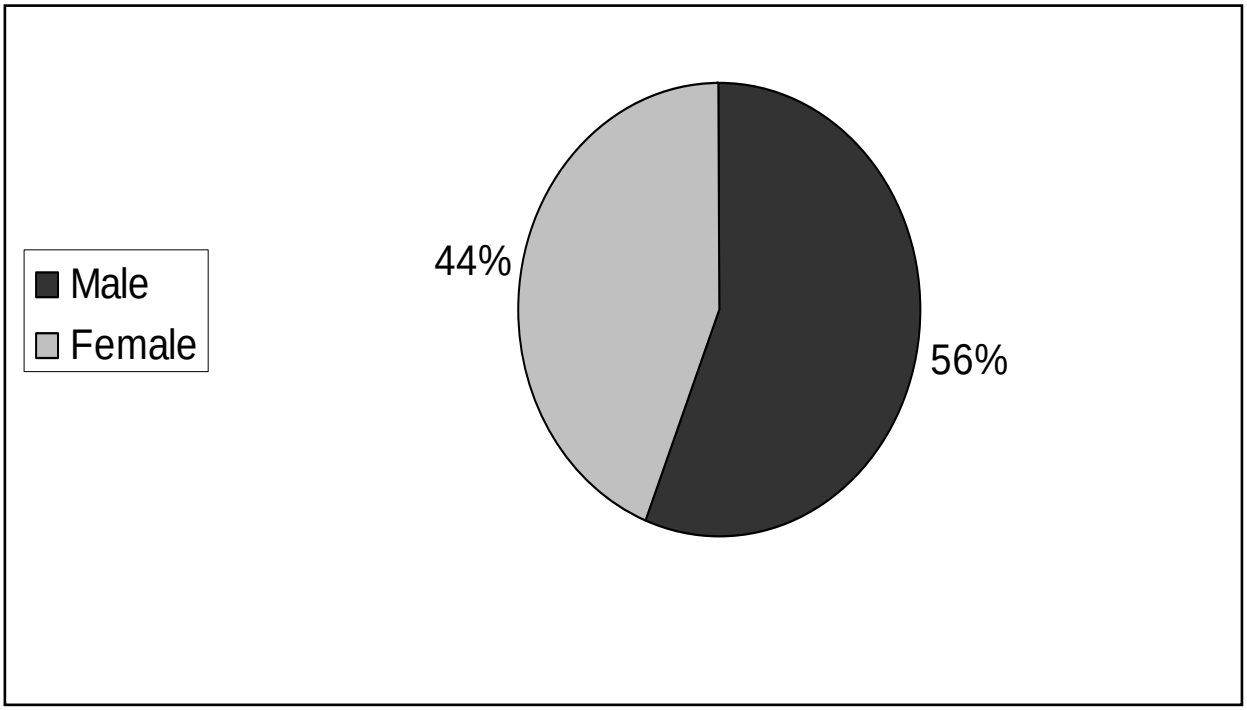



**Figure (10): Sex distribution of the pertussis patients.**

# 4-3 Residential distribution of the patients

The residential distribution of the patients is shown in Figure (11). 48 (71%) of the patients were rural residents, and 20 (29%) of the patients were urban residents.

Table (9) shows that, of the 48 rural area resident patients, there were 34 (71%) (50 % to the total No. of patients) unvaccinated patients. Only 14 (29%) (21% of the total No of patients) was vaccinated patients. While, for urban 26 % were vaccinated and 3 % were unvaccinated.

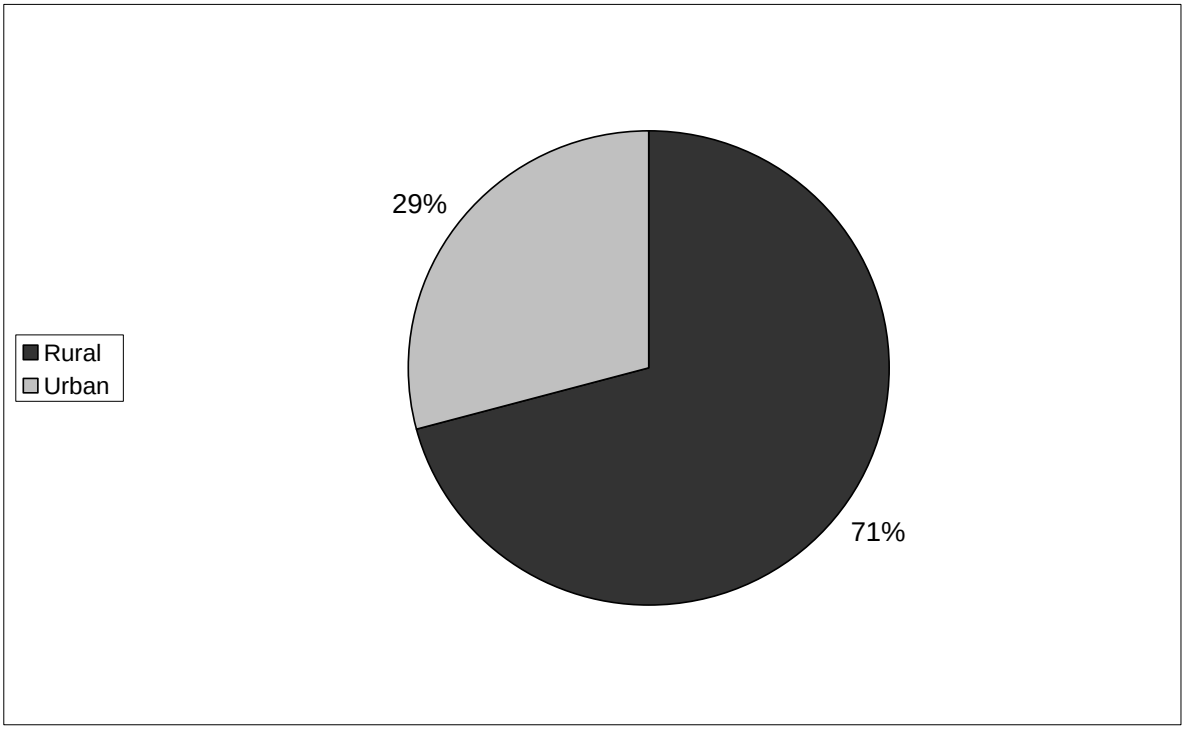



**Figure (11): The residential distribution of the pertussis patients**

**Table (9): The residential distribution of the vaccinated and unvaccinated patients with DTP vaccine.**

| Vaccination status | Rural area resident | Urban area resident | Total |
|---|---|---|---|
| **Vaccinated** | 14 (21%) | 18 (26%) | 32 (47%) |
| **Unvaccinated** | 34 (50%) | 2 (3%) | 36 (53%) |
| **Total** | 48 (71%) | 20 (29%) | 68 (100%) |

# 4-4 Vaccination status of the patients

The vaccination status of the patients in this study with DTP vaccine was shown in Figure (12). There were 36 (53%) of the patients unvaccinated, 26 (38%) of the patients was vaccinated with one (1) dose of DTP vaccine; there is no patient who has 2 doses of DTP vaccine, and 6 (9%) was vaccinated with 3 doses of DTP vaccine. The higher rate of pertussis in this study was noticed in unvaccinated and 1 dose vaccinated patients. There were no children who had received any booster doses after 1 year.

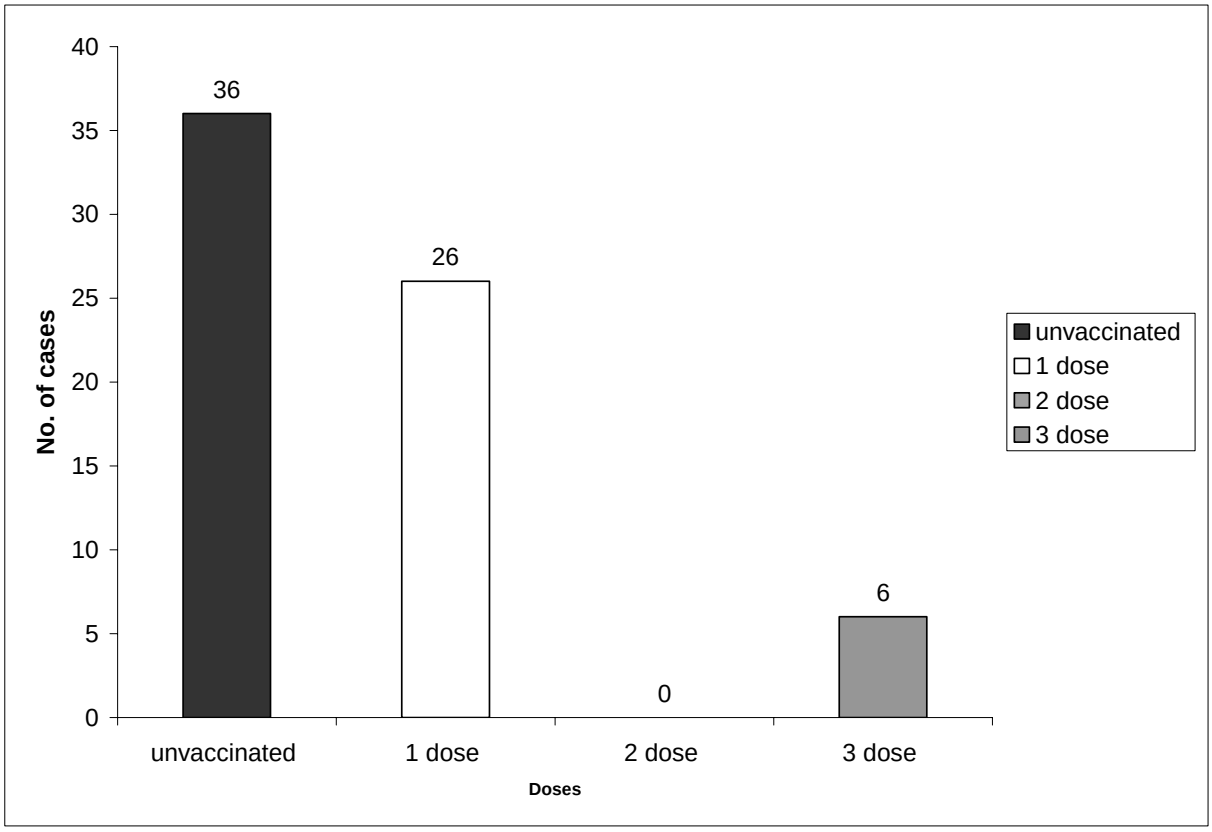



**Figure (12): Vaccination status of the pertussis patients**

# 4-5 The complication of pertussis

Several complications were noticed in pertussis patients. Apnea was the most common pertussis complication which has been noticed. Of the 68 pertussis patients there were 60 (88%) suffering from apnea. The other complications noticed were, seizure 18 (26%), pneumonia 20 (29%) and death 3 (4%).

The age distribution of complicated pertussis cases shows that a high incidence of complications was noticed in patients aged less than 3 years. Fig (13)

The vaccination status of complicated pertussis show high incidence of complication in unvaccinated patients, especially pertussis patients with pneumonia. 75% of the patients with pneumonia were noticed in unvaccinated patients. All three deaths reported in this study were unvaccinated patients. Fig (14)

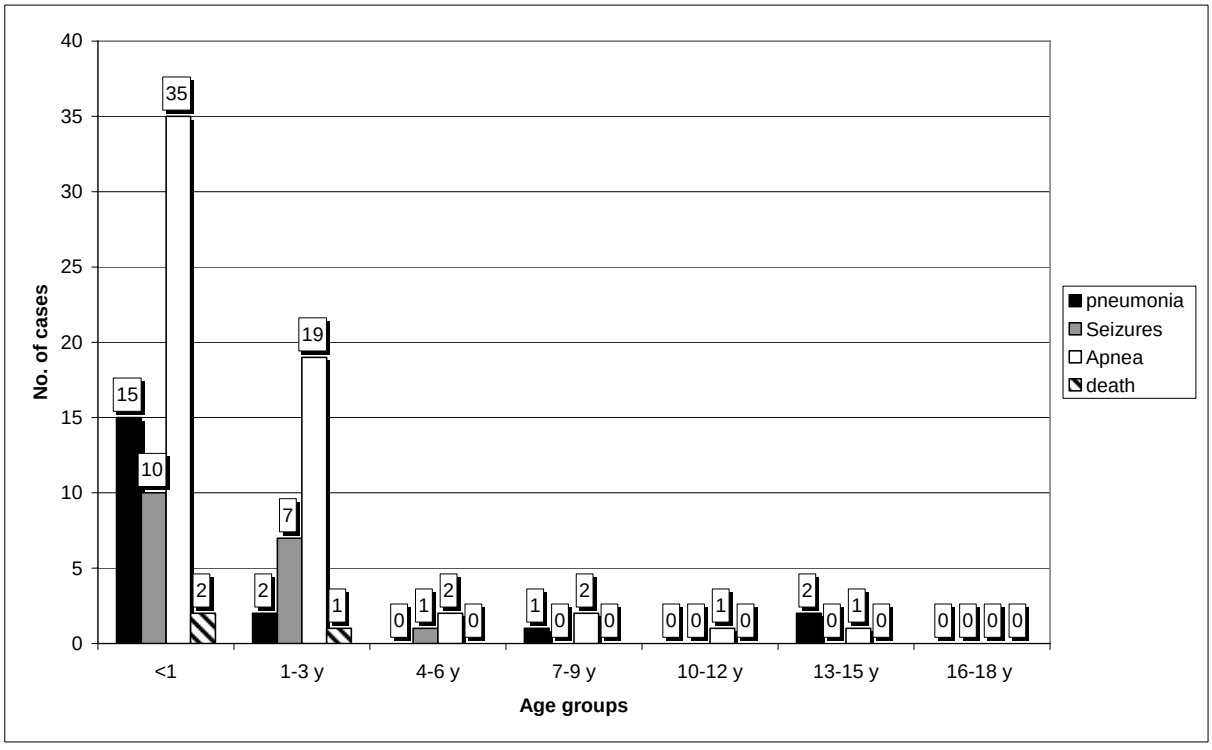



**Figure (13): Age distribution of the pertussis complication.**

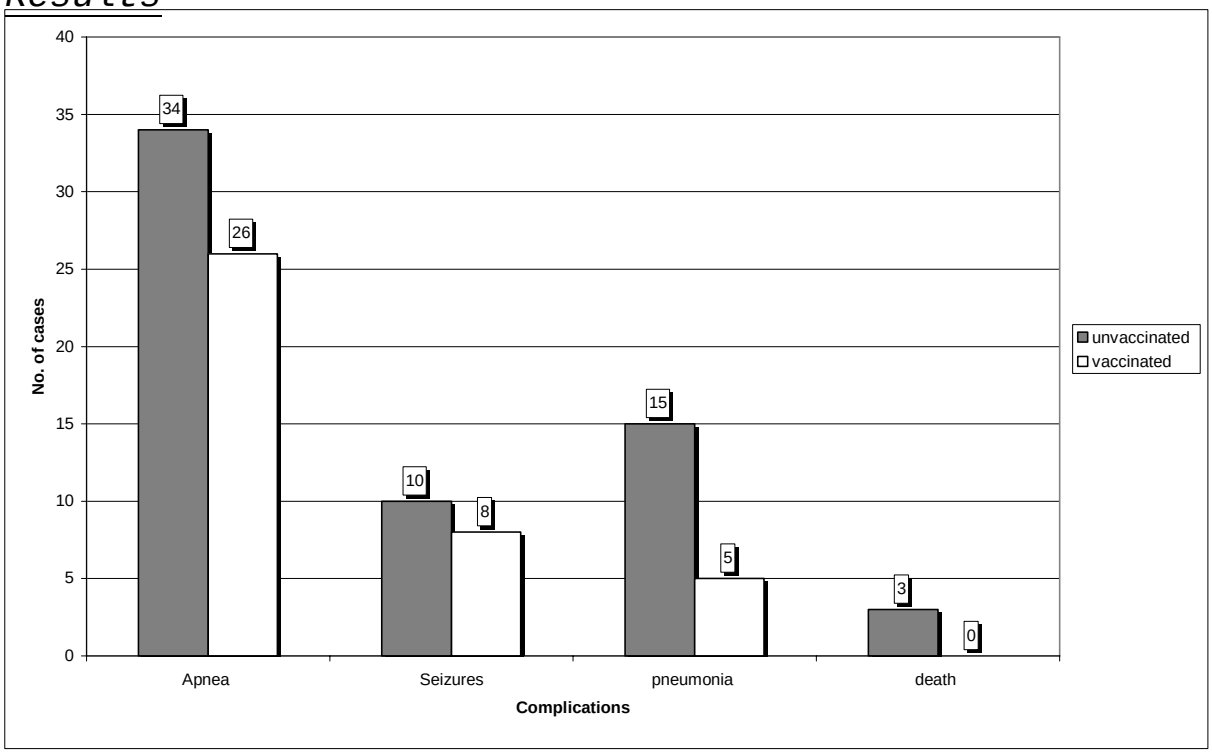



**Figure (14): Vaccination status of complicated pertussis cases.**

# 4-6 The seasonal distribution of pertussis cases

Fig (15) shows the distribution of pertussis cases presented in this study during the study period. The pertussis cases show a high incidence in the period between March and July, (in summer and spring).

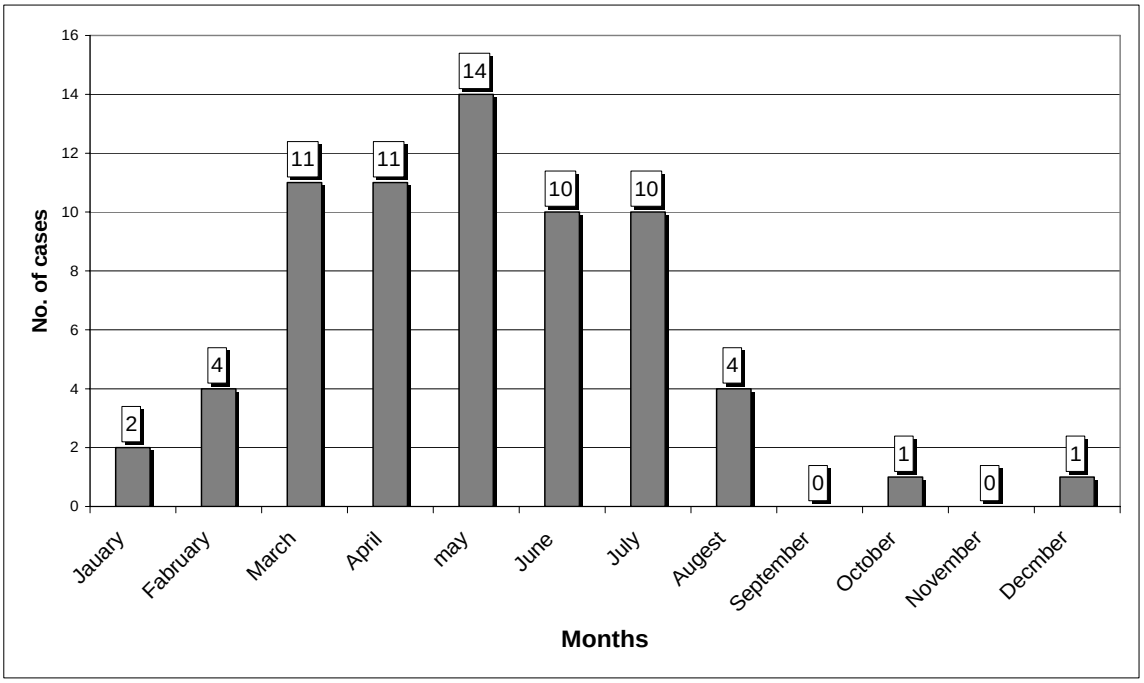



**Fig (15): Distribution of pertussis cases in the study period.**

## 4-7 Anti *Bordetella pertussis* IgG response in unvaccinated patients and normal population:

The specific IgG antibodies against *Bordetella pertussis* surface antigens (pertussis toxin, filamentous heamagglutinin and lipopolyssacharids) were measured by the ELISA technique in 36 unvaccinated patients and the results were compared with 49 unvaccinated normal children in the same age groups. Figure (16) and table (11) shows the means of anti *Bordetella pertussis* IgG levels in unvaccinated patients and normal children. The mean anti *Bordetella pertussis* IgG level in unvaccinated patients was (149.75) U/ml compared with (30.51) U/ml in normal children. The levels of anti *Bordetella pertussis* IgG were significantly higher ($p<0.05$) in unvaccinated patients than in unvaccinated normal children in all age groups.

Table (12) shows the diagnostic sensitivity and specificity and the statistical analysis of differences between the patients and normal children. The age groups (< 2 months), (5-7 months), (8 months-1year) and (7-18 year) shows 100% sensitivity. The age group (2-4 months) shows 75% sensitivity. The lowest sensitivity 50% was in age group (2-6 years). The anti *Bordetella pertussis* IgG ELISA shows 77% sensitivity and 100% specificity in unvaccinated children.

**Table (11): The Anti *B. pertussis* antibody mean level in study groups**

| Group | Anti *B. pertussis* antibody mean level (U/ml) | | | |
|---|---|---|---|---|
| | Vaccinated | | Unvaccinated | |
| | IgG | IgA | IgG | IgA |
| **Patients** | 128.04 | 11.30 | 149.75 | 13.33 |
| **Normal** | 56.41 | 9.05 | 29.33 | 4.79 |

**Table (12): The *P* value and sensitivity & specificity of anti *Bordetella pertussis* IgG in unvaccinated patients and normal children.**

| Age groups | Means IgG levels U/ml | | *P* value | No. of positive cases / total | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| | Patients | Normal | | | | |
| < 2 months | 198.68 | 19.14 | 0.0001 | 5/5 | 100 % | 100 % |
| 2-4 months | 108.95 | 41.81 | $8.9 \times 10^{-5}$ | 6/8 | 75 % | 100 % |
| 5-7 months | 127.37 | 22.12 | $6.03 \times 10^{-7}$ | 4/4 | 100 % | 100 % |
| 8 m-1 year | 128.04 | 46.28 | $2.93 \times 10^{-7}$ | 5/5 | 100 % | 100 % |
| 2-6 year | 103.51 | 30.16 | 0.007 | 6/12 | 50 % | 100 % |
| 7-18 year | 231.99 | 23.6 | $3.64 \times 10^{-8}$ | 2/2 | 100 % | 100 % |
| Total | | | | 28/36 | 77% | 100% |

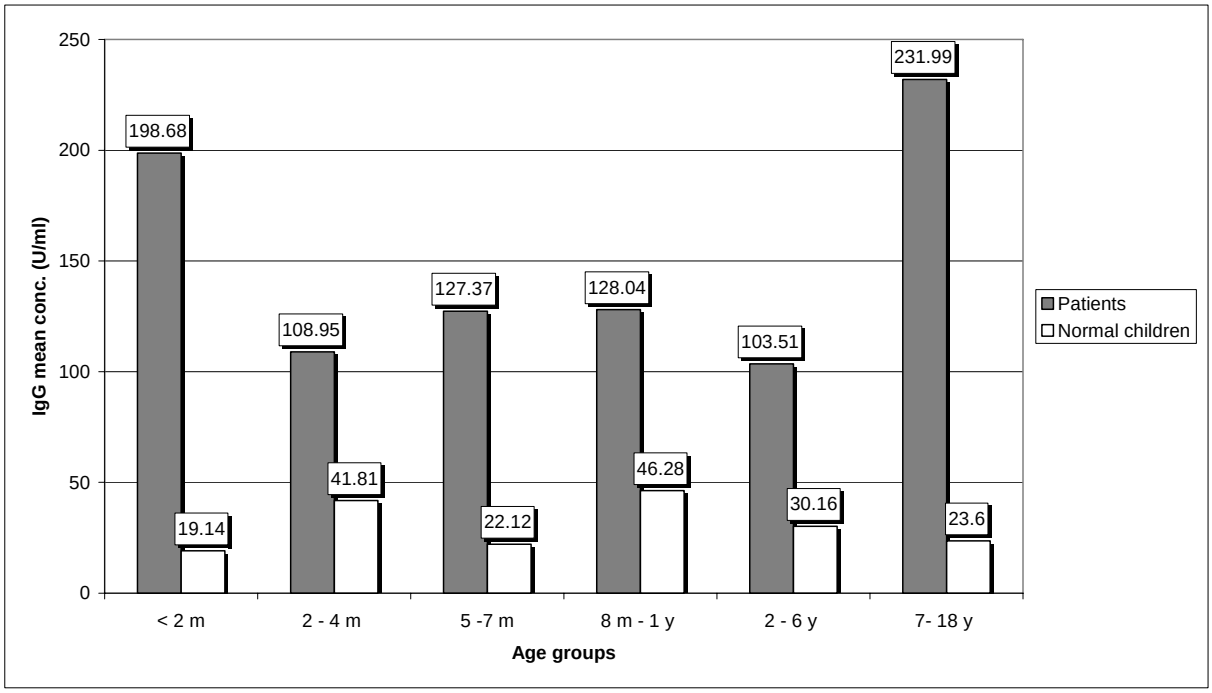



**Figure (16): Anti *Bordetella pertussis* IgG mean levels in unvaccinated patients and normal children.**

## 4-8 Anti *Bordetella pertussis* IgG response in vaccinated patients and normal population:

The specific IgG antibodies against *Bordetella pertussis* surface antigens (pertussis toxin, filamentous heamagglutinin and lipopolyssacharids) were measured by ELISA technique in 32 vaccinated patients and the results were compared with 35 vaccinated normal children in the same age groups. Figure (17) shows the means of anti *Bordetella pertussis* IgG levels in vaccinated patients and normal children. The mean anti *Bordetella pertussis* IgG level in vaccinated patients was (128.04) U/ml compared with (52.43) U/ml in normal children. The levels of anti *Bordetella pertussis* IgG were significantly higher ($p<0.05$) in vaccinated patients than in vaccinated normal children in all age groups.

Table (13) shows the diagnostic sensitivity and specificity and the statistical analysis of differences between the patients and normal children. The highest sensitivity (100%) was in age group (7-18 years), and the lowest sensitivity (50%) was noticed in age group (2-4 months). The specificity of 100% was noticed in age groups; (5-7months) and (2-6 years). The specificity of 86% were noticed in age groups (2-4 months), (8 months - 1 year) and (7-18 years). The anti *Bordetella pertussis* IgG ELISA shows 67 % sensitivity and 91 % specificity in vaccinated children.

**Table (13): *P* value and sensitivity & specificity of anti *Bordetella pertussis* IgG in the vaccinated patients and normal children:**

| Age groups | Means IgG levels U/ml | | *P* value | No. of positive cases / total | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| | Patients | Normal | | | | |
| 2-4 m | 117.74 | 49.41 | 0.04 | 3/6 | 50 % | 86 % |
| 5-7 m | 96.18 | 45.90 | 0.02 | 2/3 | 67 % | 100 % |
| 8-1 y | 121.17 | 56.85 | 0.03 | 4/7 | 71 % | 86 % |
| 2-6 y | 144.16 | 43.75 | 0.0005 | 12/15 | 80 % | 100 % |
| 7-18 y | 160.95 | 66.26 | 0.02 | 1/1 | 100 % | 86 % |
| Total | | | | 22/32 | 67% | 91% |

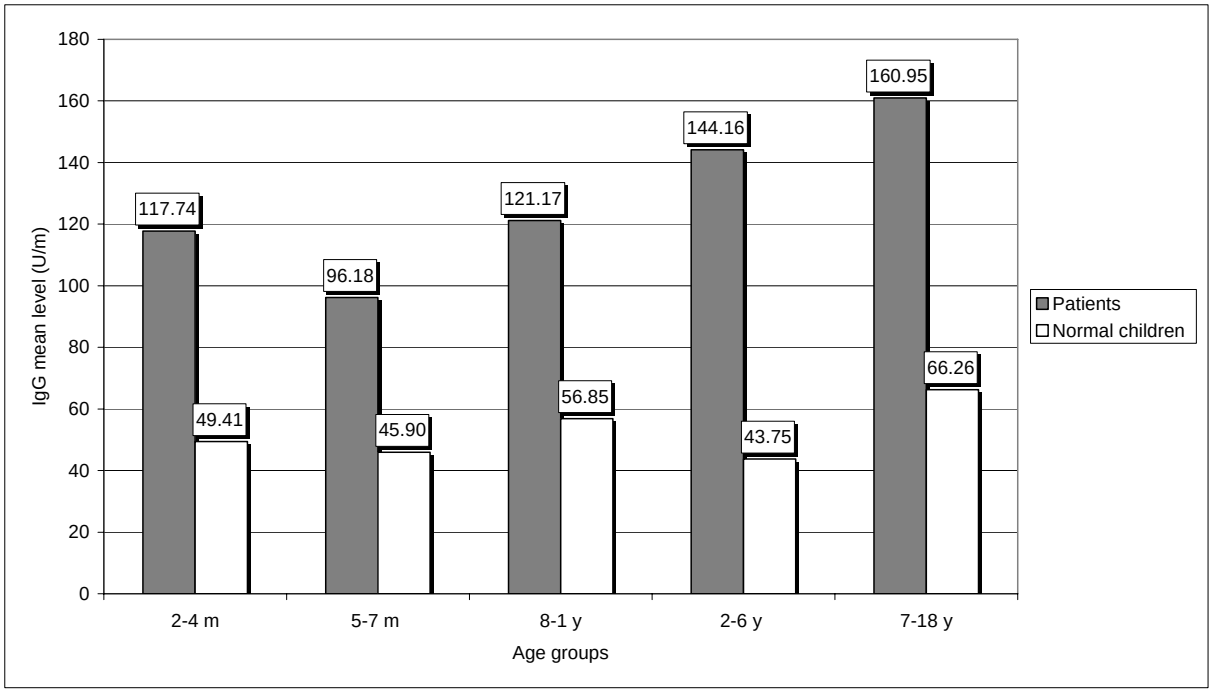



**Figure (17): Anti *Bordetella pertussis* IgG mean levels in vaccinated patients and normal children.**

## 4-9 Anti *Bordetella pertussis* IgA response in unvaccinated patients and normal population:

The specific IgA antibodies against *Bordetella pertussis* surface antigens (pertussis toxin, filamentous heamagglutinin and lipopolyssacharids) were measured by ELISA technique in 36 unvaccinated patients and the results were compared with 49 unvaccinated normal children in similar age groups. Figure (18) shows the means of anti *Bordetella pertussis* IgA levels in unvaccinated patients and normal children. The mean of anti *Bordetella pertussis* IgA level in unvaccinated patients was (11.30) U/ml compared with (4.96) U/ml in normal children.

Table (14) shows the diagnostic sensitivity and specificity and the statistical analysis of differences between the patients and normal children. The levels of anti *Bordetella pertussis* IgA were significantly higher ($p \leq 0.05$) in unvaccinated patients than in unvaccinated normal children in age groups; (<2 months), (8 months – 1 year) and (7-18 years). There are no significant differences ($p > 0.05$) in age groups; (>2-4 months), (5-7 months) and (2-6 years).

The highest diagnostic sensitivity was noticed in age group (7-18 years), and the lowest sensitivity was 12 % noticed in age group (2-4 months). The anti *Bordetella pertussis* IgA ELISA shows 33 % sensitivity and 100 % specificity in unvaccinated children.

**Table (14): *P* value and sensitivity & specificity of anti *Bordetella pertussis* IgA in unvaccinated patients and normal children.**

| Age groups | Means IgA levels U/ml | | *P* value | No. of positive cases/ total | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| | Patients | Normal | | | | |
| < 2 months | 8.28 | 4.27 | 0.004 | 1/5 | 20 % | 100 % |
| 2-4 months | 8.53 | 4.41 | 0.06 | 1/8 | 12 % | 100 % |
| 5-7 months | 9.32 | 4.70 | 0.07 | ¼ | 25 % | 100 % |
| 8 m-1 year | 12.92 | 5.21 | 0.01 | 3/5 | 60 % | 100 % |
| 2-6 year | 8.17 | 5.69 | 0.1 | 4/12 | 33 % | 100 % |
| 7-18 year | 20.61 | 5.52 | $9.93 \times 10^{-5}$ | 2/2 | 100 % | 100 % |
| Total | | | | 12/36 | 33% | 100% |

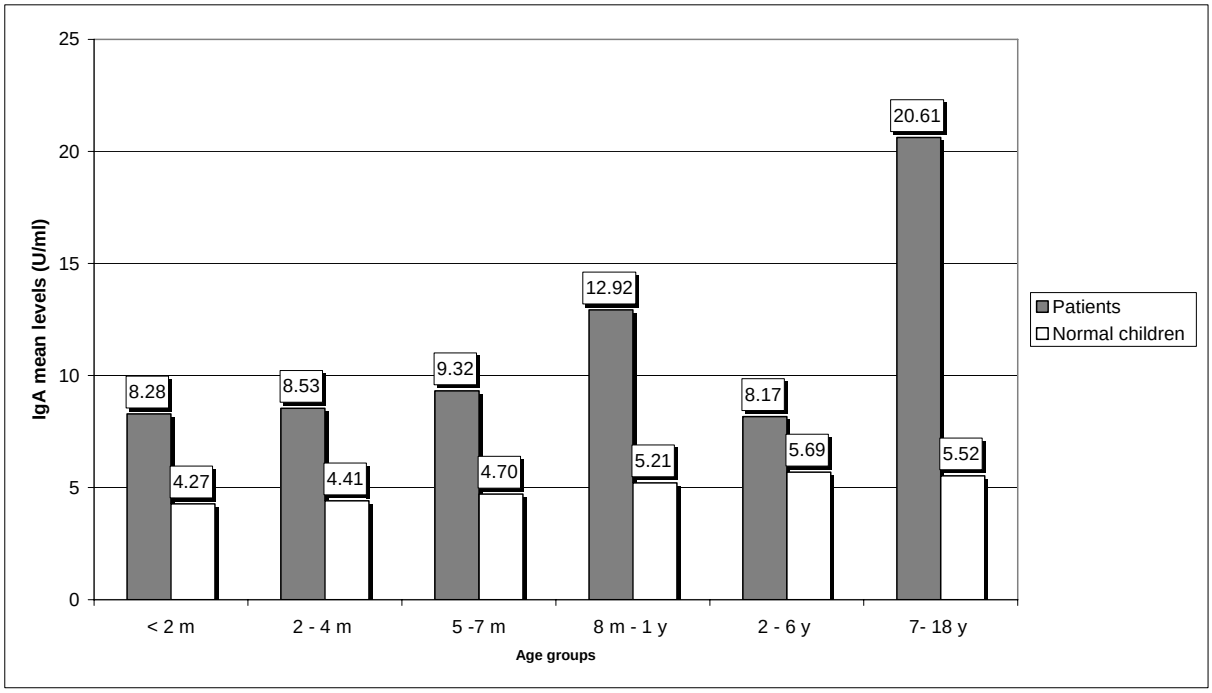



**Figure (18): Anti *Bordetella pertussis* IgA mean levels in unvaccinated patients and normal children.**

## 4-10 Anti *Bordetella pertussis* IgA response in vaccinated patients and normal population:

The specific IgA antibodies against *Bordetella pertussis* surface antigens (pertussis toxin, filamentous heamagglutinin and lipopolyssacharids) were measured by ELISA technique in 32 vaccinated patients and the results were compared with 35 vaccinated normal children in same age groups. Figure (19) shows the means of anti *Bordetella pertussis* IgA levels in vaccinated patients and normal children. The mean anti *Bordetella pertussis* IgA level in vaccinated patients was (13.33) U/ml compared with (8.94) U/ml in normal children.

Table (15) shows the diagnostic sensitivity and specificity and the statistical analysis of differences between the patients and normal children. The levels of anti *Bordetella pertussis* IgA were significantly higher ($p \leq 0.05$) in vaccinated patients than in vaccinated normal children age groups; (2-4 months), (5-7 months) and (8 months – 1 year). There were no significant differences in age groups (2-6 years) and (7-18 years).

The highest sensitivity (100%) was in age group (7-18 years), and the lowest sensitivity (33%) was noticed in age group (2-4 months). The sensitivity was increased with the increase in age group. The highest specificity was noticed in age groups (2-4 months). The lowest specificity was noticed in age group (7-18 years). The anti *Bordetella pertussis* IgA ELISA shows 72 % sensitivity and 86 % specificity in vaccinated children.

**Table (15): *P* value and sensitivity & specificity of anti *Bordetella pertussis* IgA in vaccinated patients and normal children.**

| Age groups | Means IgA levels U/ml | | *P* value | No. of positive cases/ total | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| | Patients | Normal | | | | |
| 2-4 m | 9.38 | 4.90 | 0.01 | 2/6 | 33 % | 100 % |
| 5-7 m | 14.04 | 8.01 | 0.05 | 2/3 | 67 % | 86 % |
| 8-1 y | 12.73 | 6.89 | 0.01 | 6/7 | 86 % | 86 % |
| 2-6 y | 13.71 | 13.59 | 0.48 | 12/15 | 87 % | 71 % |
| 7-18 y | 16.81 | 11.34 | 0.27 | 1/1 | 100 % | 57 % |
| Total | | | | 23/32 | 72% | 86% |

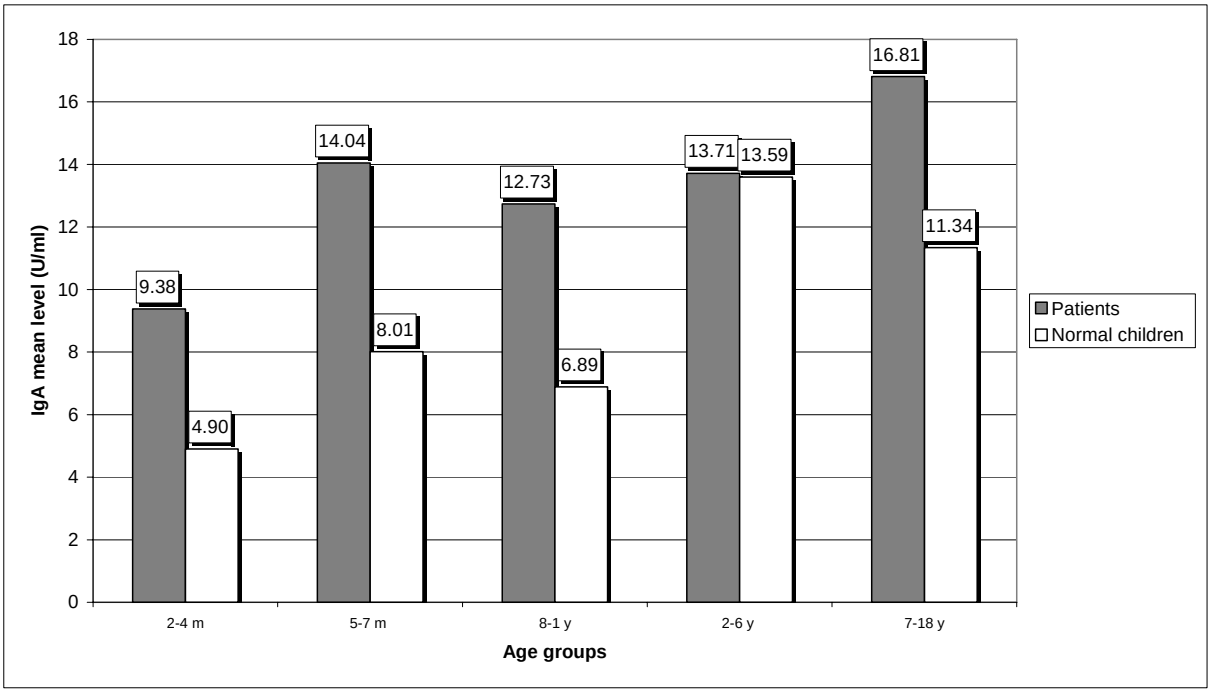



**Figure (19): Anti *Bordetella pertussis* IgA mean levels in vaccinated patients and normal children.**

## 4-11 The anti *Bordetella pertussis* IgG and IgA response in relation to pertussis duration

The 68 pertussis cases were classified to 4 categories (<7, 7-14, 15-21 and >21 days) according to the timing of sample collection after the onset of pertussis symptoms (paroxysmal cough, respiratory whoop… Etc).

Figure (20) shows the sensitivities of anti *Bordetella pertussis* IgG and IgA according to timing of sample collection after the onset of disease symptoms. The samples were collected and tested after 1,2,3 weeks or more; they had a significantly higher ($p<0.05$) anti *Bordetella pertussis* IgG and IgA level as compared with the samples collected after less than 1 week. The anti *Bordetella pertussis* IgG ELISA showed a sensitivity of 40% in samples collected after less than 7 days after the onset of disease symptoms. Higher sensitivities were observed in 7-14 day (69%) and 15-21 days (71%) after the onset of symptoms. The anti *Bordetella pertussis* IgA ELISA shows a 20%, 46%, 50% and 57% for the samples collected after less than 7, 7-14, 15, 21 and more than 21days after the onset of symptoms. The lowest sensitivity was observed in samples collected after less than 7 days after the onset of disease symptoms.

The anti *Bordetella pertussis* IgA shows a lower sensitivity as compared with *Bordetella pertussis* IgG in all the four categories (Table -16 and Table -17). A higher sensitivity was obtained by using anti *Bordetella pertussis* IgG or IgA as compared with the using of anti *Bordetella pertussis* IgG and IgA as a single test (Table -18).

**Table (16): The diagnostic sensitivity of anti *Bordetella pertussis* IgG according to the timing of sample collection.**

| Timing of sample collection | No. of positive cases/ total No. of cases | Diagnostic sensitivity |
|---|---|---|
| < 7 days | 2/5 | 40 % |
| 7 – 14 days | 24/35 | 69 % |
| 15 – 21 days | 10/14 | 71 % |
| > 21 days | 14/14 | 100 % |

**Table (17): The diagnostic sensitivity of anti Bordetella pertussis IgA according to the timing of sample collection.**

| Timing of sample collection | No. of positive cases / total No. of cases | Diagnostic sensitivity |
|---|---|---|
| < 7 days | 1/5 | 20% |
| 7 – 14 days | 16/ 35 | 46% |
| 15 – 21 days | 7/14 | 50% |
| > 21 days | 8/14 | 57% |

**Table (18): The diagnostic sensitivity of anti Bordetella pertussis IgG or IgA according to the timing of sample collection.**

| Timing of sample collection | No. of positive cases/ total No. of cases | Diagnostic sensitivity |
|---|---|---|
| < 7 days | 3/5 | 60% |
| 7 – 14 days | 29/35 | 83% |
| 15 – 21 days | 11/14 | 76% |
| > 21 days | 14/14 | 100% |

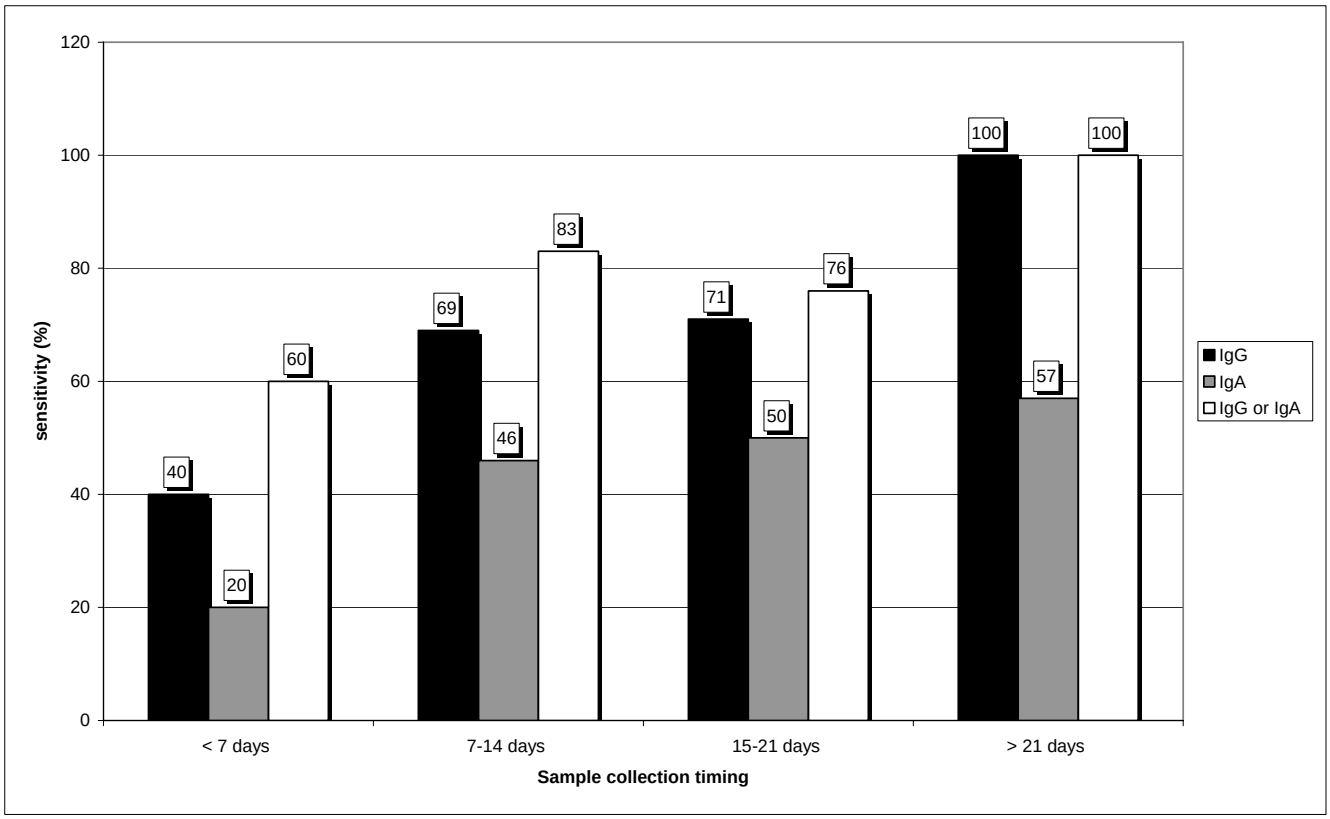



**Figure (20): Anti Bordetella pertussis IgG and IgA sensitivities according to timing of sample collection.**

## 4-12 The diagnostic sensitivity and Specificity of anti *Bordetella pertussis* IgG and IgA measured by ELISA technique in laboratory diagnosis of pertussis:

Table (19) shows the diagnostic sensitivity and Specificity of the anti *Bordetella pertussis* IgG and IgA. The sensitivity and specificity of anti *Bordetella pertussis* IgG were (74%) and (96%) respectively. The sensitivity and specificity of anti *Bordetella pertussis* IgA were (50%) and (94%) respectively. The sensitivity and specificity of anti *Bordetella pertussis* IgG and IgA were (41%) and (99%) respectively. The best sensitivity and specificity were the anti *Bordetella pertussis* IgG or IgA: (82%) and (92%) respectively.

**Table (19): The diagnostic sensitivity and Specificity of the anti *Bordetella pertussis* IgG and IgA**

| The test | No. of True positive | No. of false negative | No. of false positive | No. of true negative | Diagnostic sensitivity | Diagnostic specificity |
|---|---|---|---|---|---|---|
| **IgG** | 50 | 18 | 3 | 81 | 74 % | 96 % |
| **IgA** | 34 | 34 | 5 | 79 | 50 % | 94 % |
| **IgG and IgA** | 28 | 40 | 1 | 83 | 41 % | 99 % |
| **IgG or IgA** | 56 | 2 | 7 | 77 | 82 % | 92 % |

## 4-13 Anti *Bordetella pertussis* IgG response in DTP vaccinated normal population

A total of 103 serum samples were collected from normal population, and anti *Bordetella pertussis* IgG levels were determinate by ELISA technique. 49 samples obtained from DTP vaccinated people were tested and compared with 54 samples obtained from unvaccinated people.

Figure (21) shows the mean levels of anti *Bordetella pertussis* IgG according to DTP doses. The mean of anti *Bordetella pertussis* IgG level in unvaccinated normal people was (28.89) U/ml. The mean level of anti *Bordetella pertussis* IgG in vaccinated people with 1,2 and 3 doses of DTP vaccine was (46.53), (55.33) and (55) U/ml respectively. The anti *Bordetella pertussis* IgG levels in people vaccinated with 1, 2 and 3 doses of DTP vaccine were significantly higher ($p < 0.05$) than anti *Bordetella pertussis* IgG levels in unvaccinated people.

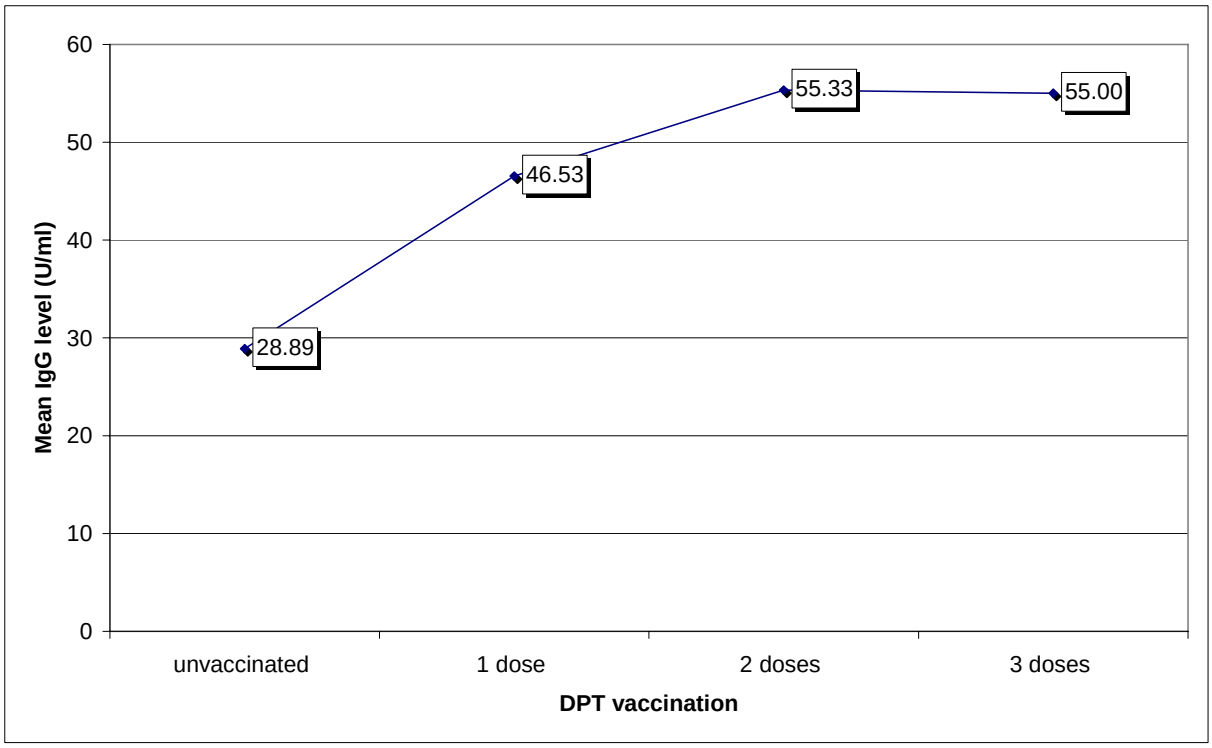



**Figure (21): Anti *Bordetella pertussis* IgG mean levels according to DTP vaccine doses.**

## 4-14 Anti *Bordetella pertussis* IgA response in DTP vaccinated normal population

A total of 103 serum sample collected from normal population, and anti *Bordetella pertussis* IgA levels was determinate by ELISA technique. 49 samples obtained from DTP vaccinated people were tested and compared with 54 samples obtain from unvaccinated people.

Figure (22) shows the mean levels of anti *Bordetella pertussis* IgA according to DTP doses. The mean of anti *Bordetella pertussis* IgA levels in unvaccinated normal people was (4.87) U/ml. The mean levels of anti *Bordetella pertussis* IgA in vaccinated people with 1,2 and 3 doses of DTP vaccine were (6.15), (6.16) and (12.56) U/ml respectively. The anti *Bordetella pertussis* IgA levels in vaccinated children with 1 and 2 doses of DTP vaccine show no significant increase as compared with

unvaccinated children. A significant increase of *Bordetella pertussis* IgA was only observed in people who received 3 doses of DTP.

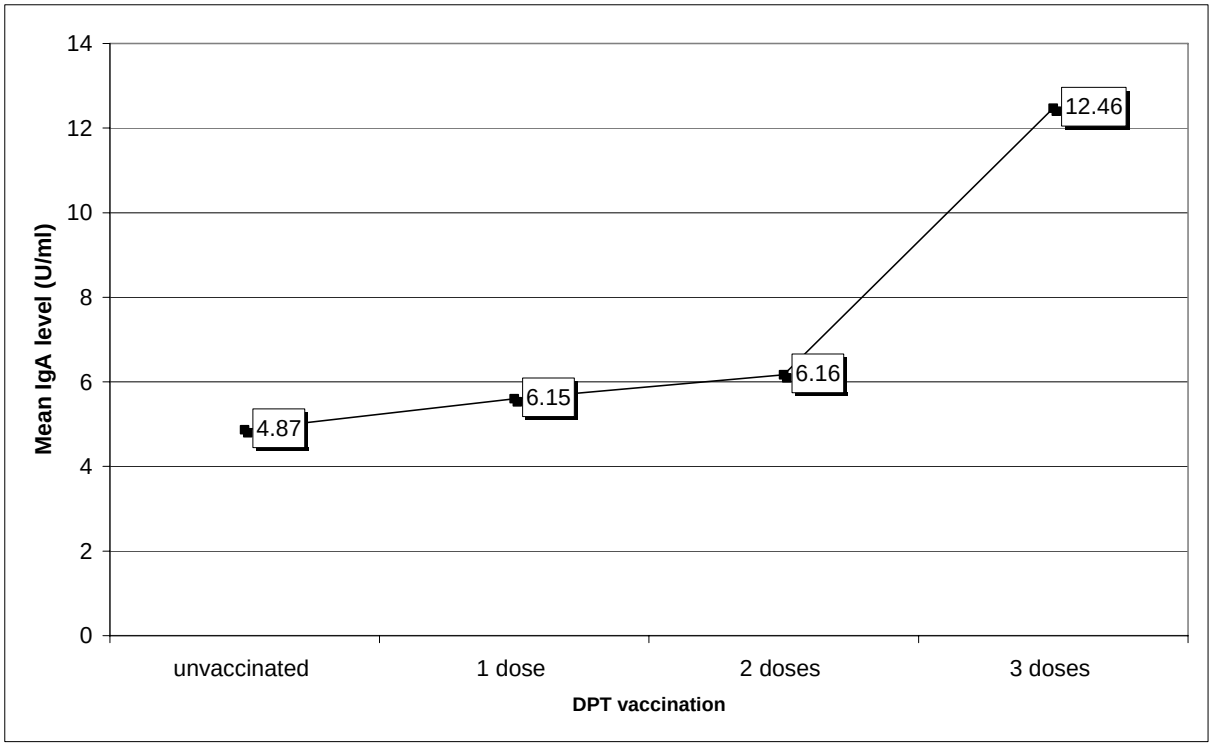



**Figure (22): Anti *Bordetella pertussis* IgA mean levels according to DTP vaccine doses.**

## 4-15 Anti *Bordetella pertussis* IgG response in DTP vaccination & unvaccinated and normal population according to age groups

According to age groups, the results of 103 tested serum samples obtained from normal population were arranged in 7 age groups and their mean anti *Bordetella pertussis* IgG levels were compared. There are 49 samples from vaccinated population compared with 54 samples from DTP unvaccinated people.

In vaccinated people, the mean of anti *Bordetella pertussis* IgG levels was 56.41 U/ml (range 19.77-143.89 U/ml). The highest mean level was in vaccinated adults (76.29 U/ml), while the lowest mean level was observed in age group (2-6) years (43.75 U/ml). Fig (23)

In unvaccinated people, the mean of anti *Bordetella pertussis* IgG levels was 29.33 U/ml (range 1.01-56.9 U/ml). The highest mean level was in age group 8m – 1y (46.28 U/ml), while the lowest mean level was observed in age group < 2 months (19.14 U/ml). Fig (23)

The anti *Bordetella pertussis* IgG levels of DTP vaccinated people were significantly higher ($p \leq 0.05$) than anti *Bordetella pertussis* IgG levels of unvaccinated people in age groups (5-7 m) , (2-6 years), (7-18 years) and adults. There is no significant deference between DTP vaccinated and unvaccinated people in age groups (2-4 months) and (8 month – 1 year). The data show that, there is a positive IgG level (> 100 U/ml) in 6 cases in vaccinated people, 3 of them are among the adult age group.

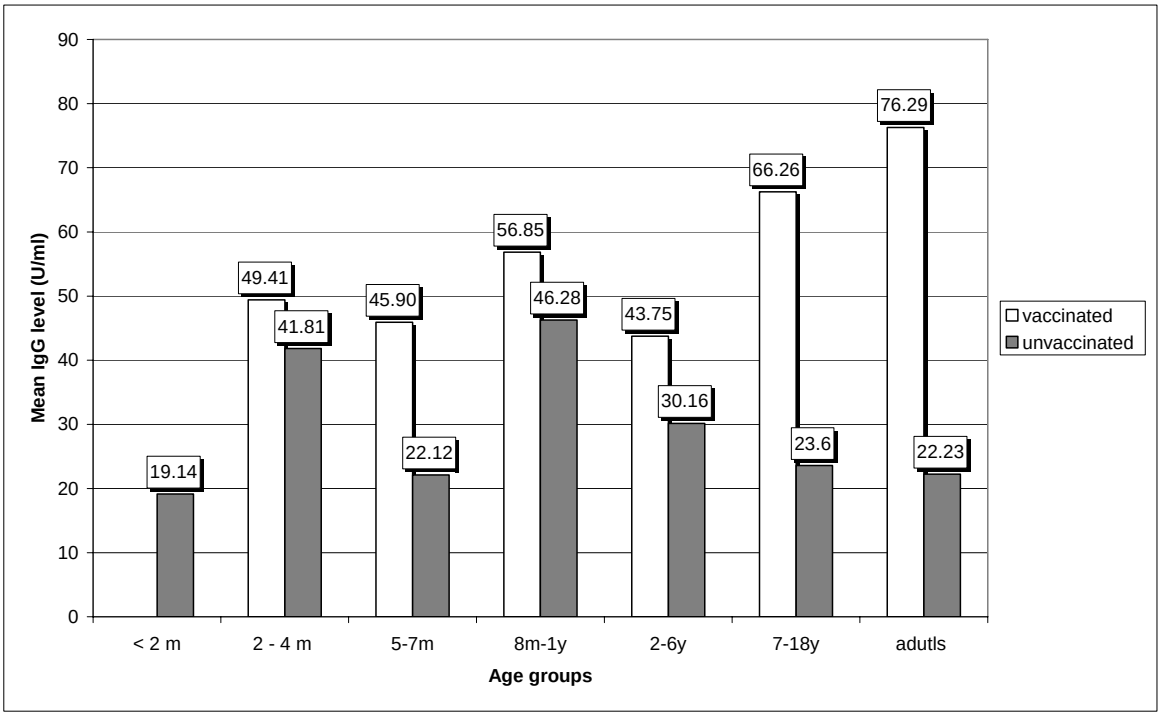



**Figure (23): Anti *Bordetella pertussis* IgG response to DTP vaccine according to age groups in vaccinated and unvaccinated population.**

## 4-16 Anti *Bordetella pertussis* IgA response in DTP vaccination & unvaccinated normal population according to age groups

According to age groups, the results of 103 tested serum samples obtained from normal population were arranged in 7 age groups and their mean anti *Bordetella pertussis* IgA levels compared. There are 49 samples from vaccinated population compared with 54 samples from DTP unvaccinated people.

In vaccinated people, the mean of anti *Bordetella pertussis* IgA levels was 9.05 U/ml (range 3.6-29.74 U/ml). The highest mean level was in 2-6 years (13.59 U/ml), while the lowest mean level was observed in age group 2-4 months (4.90 U/ml). Fig (24)

In unvaccinated people, the mean of anti *Bordetella pertussis* IgA levels was 4.79 U/ml (range 3.05-7 U/ml). The highest mean level was in age group 2-6 year (5.69 U/ml), while the lowest mean level was observed in adult's age group (3.77 U/ml). Fig (24)

The anti *Bordetella pertussis* IgA levels of DTP vaccinated people were significantly higher ($p \leq 0.05$) than anti *Bordetella pertussis* IgG levels of unvaccinated people in all age groups except for the age group (8 months-1year) where there is no significant difference.

Our data show that, there is a positive IgA level (> 100 U/ml) in 7 (7%) cases in vaccinated people, 2 of them are among the adult age group.

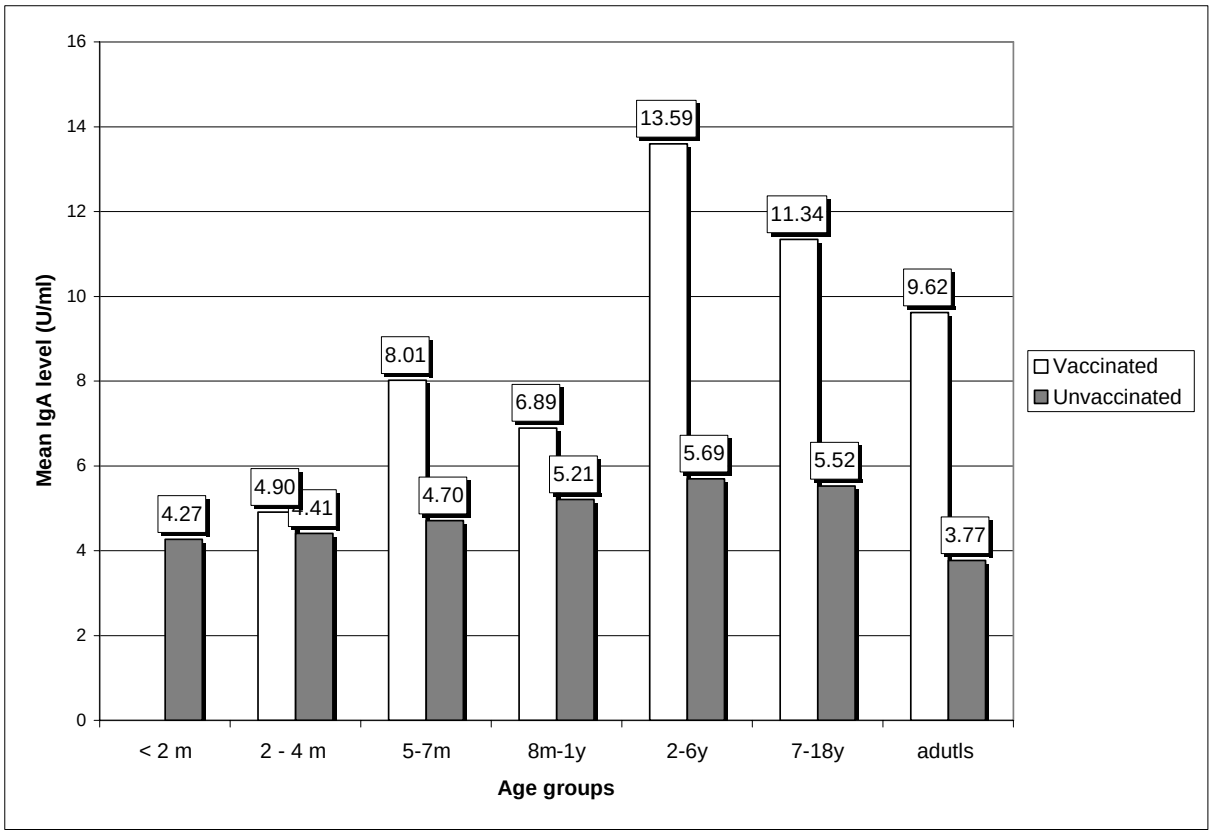



**Figure (24): Anti *Bordetella pertussis* IgA response to DTP vaccine according to age groups in vaccinated and unvaccinated population.**

## 4-17 Anti *Bordetella pertussis* IgG, IgA levels in medical staff:

A total of 20 serum samples from medical staff in Babylon Maternity and Children Hospital in Hilla were tested for anti *Bordetella pertussis* IgG and IgA levels and compared with 19 tested samples from normal adults as a control.

The mean of anti *Bordetella pertussis* IgG, IgA levels in medical staff was significantly higher ($p \leq 0.05$) than the control group. The anti *Bordetella pertussis* IgG mean levels in medical staff was (107.37) U/ml compared with (62.06) U/ml in control group. The anti *Bordetella*

*pertussis* IgA mean level in medical staff was (14.19) U/ml compared with (8.08) U/ml in control group. Figure (25).

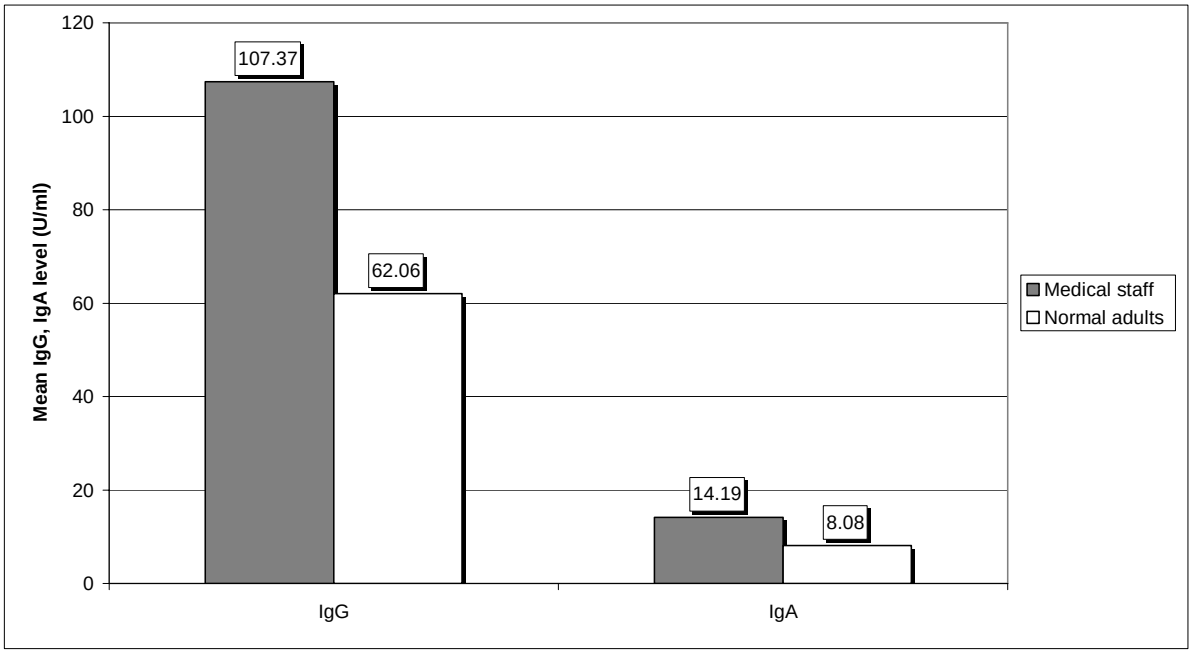



**Figure (25): Anti *Bordetella pertussis* IgG, IgA levels in medical staff**

Table (20) shows the Number of positive results of medical staff compared with control group. There is 14 (70%) of the medical staff showing a positive level of IgG or IgA compared with 4 (21%) for control group.

**Table (20): The No. of positive results noticed in medical staff.**

| Diagnostic test | No. of positive cases | |
|---|---|---|
| | Medical staff | Control |
| IgG positive level | 9 (45%) | 3 (16 %) |
| IgA positive level | 9 (45%) | 2 (11%) |
| IgG and IgA positive level | 4 (20%) | 1 (5%) |
| IgG or IgA positive level | 14 (70%) | 4 (21%) |

# Discussion

## 5-1 Age distribution of the pertussis patients

The age distribution of the pertussis cases presented in this study shows that, the higher incidence of pertussis occur in infants under 1 year old, (Fig-10). They represent 49% of all cases of this study. Our data show that the rate of pertussis is reduced with the increase of age groups. These results explain that most of the patients in these age groups are either unvaccinated which represent 58% of the patients in this age group (table-8), or partially vaccinated with DTP vaccine. Some of the patients are under 2 months before the first scheduled dose of DTP vaccine.

The incidence of pertussis has dramatically changed after presentation of DTP vaccine. In the prevaccine era, pertussis primarily affected children between 1 to 5 years of age, at least in part because of passive protection during first year of life through maternal antibody. For example, in a study from 1937, 60.2% of pertussis patients were 1 to 5 years of age (Luttiger. 1977). In that setting with most adult having had pertussis as children and being repeatedly exposed in the population, there was high level of adult immunity. Whole cell vaccine, pertussis vaccine has clearly been the major cause of overall reduction in the incidence of disease, yet it has probably also affected a shift in the United States occur in children younger than 1 year of age (Anonymous, 1984). In 1980-1989, the children under 1 year old had the highest annual incidence of disease (62.8 per 100000) (Farizo. 1992). The incidence of reported cases of pertussis among infants increased 49% in the

1990s compared with the incidence in the 1980s. Increase in the incidence of cases and the number of the deaths among infants during the 1990s primarily was among those aged 4 months or younger, contrasted with a stable incidence of cases among infants aged 5 months or older (Tanaka. *et al*. 2003).

There are many studies which agree with our findings. In Al-Marzoki JM *et al* (2004) study**,** more than 95% of patients were below 6 years, and more than 60% were below one year. In pertussis outbreak in Basra – Iraq, 39.8% were children under 5 years of age, between them 18.9% infants under 2 months of age who were not immunized and only 19.4% children aged more than 1 year who had no pertussis immunization (Al-Bargish. 1999).

In Australia a study of 140 pertussis patients, 97 (69%) of infants who had not been vaccinated for pertussis, 63 (65%) were less than 2 months (Elliott, *et al*. 2004).

In Taiwan, a study of 46 pertussis cases shows that 52.2% were patients younger than 1 year of age. The mean age was 4.3 years (range, 24 day to 37years) and the median age was 10.5 months (Lin. 2004).

The proportion of susceptible children to pertussis is affected by vaccine coverage. In poor vaccination coverage (< 70%) in 1999 there is about 30 % of cases is under 1 year, and for countries with good coverage (> or = 70%) there is 10% of patients is under 1 years old (Crowcroft. 2003). The DTP coverage in Iraq was expanded from 13% in 1980 to 90% in 1999 and it declined after 2000 to reach 67% in 2002 (WHO. 2003).

**5-2 Sex distribution of the pertussis patients**

The sex distribution of the pertussis patients in this study shows slightly higher incidence of pertussis in males (56%) compared with females (44%), (Fig -11).

This result agrees with some studies. In Lin PY, *et al* study, there is higher rate of pertussis in males (54.3%) (Lin, *et al*. 2004). In pertussis epidemic in Cincinnati, USA, the incidence of pertussis is slightly higher in males (53%) (Bisgard, *et al*. 2001). In England and Wales, between 1975 and 1979, the percentage of female among notificated cases of pertussis was 49%, 52% to 53% and 64% for children < 1 year, 1 to 14 years and ≥ 15 years of age, respectively (Gordon and Hood. 1980).

Some studies indicate that there is no effect of gender on pertussis incidence. Ulrich *et al* found an even distribution of pertussis between both genders in children (male : female 49.3% :50.7%) (Ulrich, *et al* .1997). Data from the Center of Disease Control revealed that up to the age of 15 years, a similar number of boys and girls (50% to 51%) developed pertussis, where as a female predonderance (55% to 69%) was noted in older groups (CDC. 2002). A study of finger H. and Wirsing von Konig CH., shows that there is no sex differences found in children suffering from pertussis. In adult patients, however, women were more often affected (Finger. and Wirsing von Konig. 1992).

Other studies were disagreeing with our findings. In Basra – Iraq the outbreak of pertussis the male/female ratio was 2:8 (Al-Bargish. 1999). In Al-Marzoki JM *et al,* the females outnumbered males (60%) **(**Al-Marzoki *et al.*2004). In Poland, more cases occurred among females (7/100000) than

males (5.4/100000) (Sitarska-Golebiowska. and Zielinski. 2003). In Yih. *et al*, the incidence of pertussis was slightly higher for females than males – 8.8 and 8.1 per 100000 populations, respectively. Incidences (cases per 100000 populations) for females and males, respectively, were 56.1 and 55.2 for infants; 5.7 and 5 for children 1-10 years of age; 49 and 44.2 for adolescents 11-19 years of age and 2.9 and 2 for adults ≥ 20 years. The ratio of females incidence to male incidence was greater in adults (1.45) than in the younger age groups (1.01-1.14) (Yih. *et al* . 2000). Syedabubakar *et al* , indicate that there is statically a significant finding including a higher proportion of isolations from females patients (Syedabubakar. *et al*.1995).

**5-3 Residential distribution**

The residential distribution of the pertussis patients are shown in Fig (12). Our data show a high incidence of pertussis in rural areas as compared with urban areas in Babylon. Of the 68 patients presented in this study, 48 (71%) were rural area residents compared with 20 (29%) of patients in urban area. These findings may be explained by low vaccination coverage in the rural area of Babylon. To examine this explanation, we analyze the vaccination status of the urban and rural area resident patients in this study (Table-9). Our data show that, of 48 rural area resident patients, there are 34 (17%) (47 % to the total No. of patients) unvaccinated patients. Only 14 (29%) (19% of the total No of patients) was vaccinated patients. These data suggest low vaccination coverage in the rural area in Babylon, and that explains the high incidence of pertussis in rural area residents.

There are many studies that agree with our finding. In Iraq, the rural: urban ratio was 1.37:1 **(**Al-Marzoki *et al.* 2004). In Abdalla BA *et al* (1998) in

Sudan, there is 50% of the pertussis patients from periurban area and the majority (83.3%) were living in crowded houses and the primary vaccination was low (2.8%).

In India, Dhadwal. *et al* (1997) show that the immunization coverage of DTP vaccine was 1% in the rural area and 8% in urban area and there is an association between high incidences of pertussis in rural areas.

In Chile, Sanchez. *et al* (1992) show that high lethality of pertussis cases are recorded in rural areas.

In serological survey in Mexico, the children living in rural areas and those classified as belonging to lower socioeconomic level showed lowest seropositivity prevalence (Sapian-Lopez. *et al*. 1992).

In Poland, Zielinski's study disagrees with our results and shows that the incidence of pertussis in urban areas was (507/100000) compared with (3/100000) in rural areas, but he justifies that by insufficient sensitivity of surveillance in rural areas of Poland (Zielinski. 2004).

**5-4 Vaccination status of patients**

The vaccination status of the pertussis patients presented in this study was shown in Fig (13). Our findings indicate that the higher rate of pertussis was among unvaccinated and partially vaccinated patients with only one dose of DTP vaccine. That high rate of pertussis in unvaccinated children is due to several reasons. First, the low vaccination coverage in rural area in Babylon. The rural area resident patients represent 94% (47% of the total patients) of the unvaccinated patients in this study (Table-10). Second, some of the patients are under the age that the first scheduled dose of DTP vaccine received.

There are many studies which reported the high incidence of pertussis in unvaccinated or under vaccination populations. In Al-Marzuki *et al* (2004) study, there was one third of the patients who were not vaccinated, and 16.6% of them were partially vaccinated**.** In Lin. *et al* (2004), 50 % of the pertussis patients were unvaccinated or partially vaccinated children. In Liese *et al* (2003), of the 180 pertussis cases, there were 69% fully vaccinated, 7% partially vaccinated and 24% were not vaccinated against pertussis. Juretzko. *et al* (2002) show that 2.3% of the pertussis patients were vaccinated and 76.8% were not vaccinated. Fruhwirth. *et al*. (2002) indicate in there study that out of the patients with *Bordetella pertussis* infection, 62% were not vaccinated and 11.2 were too young to have received the first recommended dose of pertussis vaccine.

Our findings show that, there were 9 % of pertussis cases which occurred in vaccinated patients. Pertussis occurred among children, who had been appropriately immunized (Saleh. 2000). These results may be explained by following:

1) Lessened potency of the pertussis vaccine (Christie. *et al*.1994).
2) Waning of vaccine-induced immunity (cherry. 2003).
3) A mismatch between the vaccine and the circulating strains of *Bordetella pertussis* (Mooi. *et al*.1998; De Melker. *et al*.1997; vanderZee. *et al*.1996; Mooi. *et al*.1995).

Our results show that, all the patients presented in this study did not received 4 or 5 or 6 booster doses of DTP vaccine given in 1, 2 year of age and preschool age. Several studies suggest that the major cause of the resurgence of pertussis was waning immunity after vaccination due to lack of

regular natural or vaccine boosters (Baron. *et al*. 1998 ; Grimprel. *et al*. 1993). Another study suggests that the booster doses of pertussis vaccine may prevent cough illness related to pertussis among adolescents and adults (Ward. 2002 ; Ward. 2001).

**5-5 The seasonal distribution of pertussis patients**

The distribution of pertussis cases during the study period is shown in Fig (14). These results show a high incidence of pertussis cases between March and July, and the peak rate was in May. These findings agree with the study of Al-Marzoki JM *at al* study that includes hospitalized pertussis patients over a period of 5 years **(**Al-Marzoki *et al*.2004).These findings indicate that the high incidence of pertussis was during spring and autumn.

The seasonal distribution of pertussis varies between other studies. In de Melker *et al*. study there is an increasing of cases in March and April 1996 (de Melker. *et al*. 2000). Bisgard. *et al*. (2001) show that the peak incidence of pertussis cases was during the period between July to September. Yih. *et al*. (2000) indicate that the peak incidence in 7 years was in the fourth quarter of the year. In Taiwan Lin. *et al*. show that the pertussis cases were reported throughout the year, and the peak incidence was in summer and autumn (Lin. *et al*. 2004). Most of these studies studied the influence of seasonal variation on pertussis incidence in countries which have a different climate from that of Iraq. In Iraq, Al-Bargish (1999) reported that the higher number of pertussis cases was noticed in the first months of the year particularly in July, and that is agrees with our findings.

**5-6 Complications of pertussis patients**

The principal complications of pertussis are secondary infections, such as otitis media and pneumonia, and physical sequelae of paroxysmal cough (Plotkin and Orenstein. 1999). Our findings show that the pertussis complications are common among pertussis patients. Complications are noticed in (60/68) 88% of the patients. Apnea was the most common pertussis complications; it is noticed in 88% of the patients, Pneumonia was noticed in 29% of the patients, while seizure is presented in 26% of the patients. Death was noticed in 4% of the cases. In Al-Marzuki *et al* (2004) study the complications were notified in 52.29% of the patients, the most common was pneumonia followed by diarrhea, convulsion, and hemorrhages; the death percentage was 1.06%**.**

Pertussis complications were directly related to age, the disease being most severe in children under 1 year, especially those under 6 months (Miller and Fletcher. 1976; Mandell *et al*.2000; Benneson. 1995). In our results, incidence of pertussis complications was affected by age (Fig-14). The higher incidence of complications was noticed in age group < 1 years. Halperin *et al* (1999) in a study of 1082 pertussis cases show that pneumonia was reported in 9.4%, seizures in 2.3% and death in 0.9, all children < or = 6 months pf age. In a study by Nielsen and Larsen (1991), pneumonia developed in 12%, seizures in 2% under one year and 5 % over year, apnea occurred in 10% under one year and 2% over one year.

Rates of complications were generally higher among unvaccinated children than among who had received three or more doses of DPT vaccine (Farizo *et al*. 1992). In our findings the DTP vaccination has an important

influence on complications incidence in pertussis patients (fig-15). There was increasing evidence that previously vaccination reduces the complication of the disease but the vaccination program does not cover very young children (Miller and Fletcher. 1976). Pertussis deaths increased among infants too young to be fully protected by immunization (Vitek *et al*. 2003).

### 5-7 Anti *Bordetella pertussis* IgG response in pertussis patients

The IgG specific response to *Bordetella pertussis* surface antigens (pertussis toxin, filamentous heamagglutinin and lipopolyssacharids) were determined by the ELISA technique from 68 patients. The results were compared with 84 healthy children from different age groups.

Our findings show a significantly higher anti *Bordetella pertussis* IgG in patients than in normal subjects. The mean of anti *Bordetella pertussis* IgG was 149.75, 30.52 U/ml in unvaccinated patients and normal children, respectively. The mean of anti *Bordetella pertussis* IgG was 130.21, 52.44 U/ml in vaccinated patients and normal children, respectively.

The mean of anti *Bordetella pertussis* IgG level was significantly higher ($p \leq 0.05$) in unvaccinated patients than vaccinated. This finding may be explained by the fact that vaccinated children have an earlier, lower, and briefer IgG response when the disease occurs. If the response to exposure *Bordetella pertussis* among unvaccinated children is considered a primary immune response and the response among vaccinated children is considered a secondary immune response, the lower briefer IgG response observed among vaccinated children is a-typical (Simondon *et al*. 1998). Comparable observations were reported for tetanus and leishmaniasis (Ipsen. 1961). This

response may be related to an increase in the avidity of the antitoxin produced during maturation of the immune response. It may also reflect less-sever disease among vaccinated children, rather than temporary immunodeficiency associated with vaccine failure (Simondn *et al*. 1998).

Of the 36 unvaccinated patients, there were 28 patients who give a positive IgG levels (≥ 100 U/ml), and that gives sensitivity of 77%, while none of the normal children has a positive IgG level (100% specificity). In unvaccinated patients, the assay of PT-IgG and FHA-IgG in the serum samples is highly sensitive for the diagnosis of pertussis (Trollfors *et al*. 1999). The IgG1 and IgG3 were mainly produced in unvaccinated pertussis patients (Giammance *et al*. 2003).

Of the 32 vaccinated patients, there were 22 patients having a positive anti *Bordetella pertussis* IgG and that is gives a sensitivity of 67%. This sensitivity was lower than the sensitivity observed in unvaccinated patients. The IgG response to pertussis toxin was more sensitive in unvaccinated patients than those immunized with DTP vaccine.

In Granstorm G *et al*, (1988) all but one of the unvaccinated children with disease duration of less than 3 weeks at the time of first sample showed titer raises, while in vaccinated patients, rapid booster response after 1 or 2 weeks of disease were noted. These results imply that diagnosis by IgG response may be difficult to obtain in a population vaccinated with DTP. In Trollfors B *et al* (1999), the ELISA assay of PT-IgG and FHA-IgG in paired serum sample is highly sensitive for diagnosis pertussis in unvaccinated individuals, while this assay was less sensitive in vaccinated children.

Our finding shows that, the specificity of anti *Bordetella pertussis* IgG was lower than 100% in vaccinated children in some age groups (Table-13). These results may be explained by temporary elevation of anti *Bordetella pertussis* IgG as a response to vaccination, or indicate asymptomatic infection of *Bordetella pertussis*. Elevated levels of anti *Bordetella pertussis* IgG occurred in individuals with asymptomatic or a mild cough illness and IgG level likely reflected gradual declining in the months following *Bordetella pertussis* infection (Heininger *et al*. 2004 ; Tennis *et al*. 2002).

Our findings show a little influence of age on the sensitivity of anti *Bordetella pertussis* IgG. These findings agree with Granstorm G *et al* study that shows 100% sensitivity of anti FHA-IgG and PT-IgG in all age groups (Granstorm G *et al*. 1988). The low sensitivities noticed in some age groups (Table-11 & Table-12), may be due to other respiratory tract infection that cause "pertussis like" symptoms (Wirsing van Konig *et al*. 1998) or rapid decline of previously high IgG level, or absence of IgG response after *Bordetella pertussis* infection (de Melker *et al.* 1999). Other explanations of these results were the short interval between the onset of symptoms and sample collection. The median time for increasing anti *Bordetella pertussis* IgG was 9 days after the onset of symptoms (Simondin *et al*. 1998).

## 5-8 Anti *Bordetella pertussis* IgA response in pertussis patients

The IgA specific response to *Bordetella pertussis* surface antigens (pertussis toxin, filamentous heamagglutinin and lipopolyssacharids) were determined by the ELISA technique in 68 patients from different age groups

and vaccination status. The results were compared with 84 healthy children from different age groups. (Table 14 & 15)

The results show that the levels of anti *Bordetella pertussis* IgA were significantly higher ($p \leq 0.05$) in patients than normal children. The mean level of anti *Bordetella pertussis* was 11.3 and 4.96 U/ml in unvaccinated patients and normal children, respectively. While the mean level of anti *Bordetella pertussis* IgA was 13.33 and 8.94 U/ml in vaccinated patients and normal children, respectively.

A high titer of immunoglobulin A (IgA) antibodies are detected in nasal secretions of pertussis patients (Long *et al*. 1990; Thomas *et al*. 1990), in saliva (Zackrisson *et al.* 1990) and in serum (Granstorm *et al.* 1988). There are several studies have reported the use of the IgA ELISA to certain *Bordetella pertussis* antigens for the diagnosis of whooping cough (Nagel and Poot-Scholtens. 1983; Granstorm M *et al.* 1982; Granstorm M *et al.* 1982;).

Our findings show that, the anti *Bordetella pertussis* IgA shows a diagnostic sensitivity of 33% and 72% in unvaccinated and vaccinated patients, respectively. These results agree with Granstorm G *et al* who documented a lower IgA response in unvaccinated patients. The anti *Bordetella pertussis* IgA for both pertussis toxin and filamentous heamagglutinin was significantly higher in vaccinated patients than in unvaccinated patients (Granstorm G *et al.* 1988).

Our findings show that there is a higher sensitivity of anti *Bordetella pertussis* IgA in children of more than 1 year than infants of less than 1 year in both vaccinated and unvaccinated patients (Table-13 & Table-14). These

findings agree with the studies of Granstorm G *et al.* 1982 and Granstorm G *et al*. 1988.

Our results show a specific of anti *Bordetella pertussis* IgA of 100% and 86% in unvaccinated and vaccinated children, respectively. The low specificity in vaccinated children may be due to asymptomatic pertussis infection rather than a response due to vaccination.

**5-9 Anti *Bordetella pertussis* IgG and IgA response in relation to disease duration**

The sensitivities of anti *Bordetella pertussis* IgG and IgA according to the timing of sample collection are shown in Fig (21). The anti *Bordetella pertussis* IgG ELISA shows a sensitivity of 40% in samples collected after less than 7 days after the onset of disease symptoms. Higher sensitivities were observed in 7-14 days (69%) and 15-21 days (71%) after the onset of symptoms. The highest sensitivity was observed in samples collected after > 21 days (100%). Granstorm G *et al* show a sensitivity of 85%, 88%, 63 and 80% for samples collected after 1-7, 8-14, 15-21 and more than 21 days after the onset of symptoms, respectively (Garnstorm G *et al.* 1988). Wong and Skelton indicate that the lowest IgG titer for pertussis toxin was collected from patients 1 week after the onset of clinical symptoms, and the highest titer was found in convalescent patients 7 weeks after clinical onset (Wong and Skelton. 1988). While Simondon F *et al* documented that the anti pertussis toxin increase was observed in a median of 42 days, in pertussis patients (Simondon *et al.* 1998).

The anti *Bordetella pertussis* IgA ELISA shows 20%, 46%, 50% and 57% for the samples collected after less than 7, 7-14, 15,21 and more than

21days after the onset of symptoms. These findings agree with the study of Zackrisson *et al*, (1990) that they show that, samples obtained from patients with symptoms of less than 6 days lack IgA antibodies against FHA, while all samples obtained after 50 days or more of symptoms had detectable IgA antibodies. Granstorm G *et al* (1988) study indicates that the sensitivity of ELISA IgA in nasopharyngeal samples increased with disease duration, from 65% during the first week to 92% after more than 3 weeks of disease.

The anti *Bordetella pertussis* IgA shows a lower sensitivity as compared with *Bordetella pertussis* IgG in all the four categories in our study. That may be explained by the anti *Bordetella pertussis* IgG apparent before anti *Bordetella pertussis* IgA antibodies (Nagel *et al.* 1983).

The use of anti *Bordetella pertussis* IgG or IgA ELISA as a diagnostic procedure elevates the sensitivity of the anti *Bordetella pertussis* IgG and IgA as a single test. The anti *Bordetella pertussis* IgG or IgA ELISA shows sensitivity of 60%, 83%, 76% and 100% for <7, 7-14, 15-21 and > 21 days.

In our study, only 5 (7%) of the patients had a disease duration of less than 7 days at the time of diagnosis, while 63(93%) patients had a disease duration of 7 or more at the time of diagnosis.

The increase in the rate of positive results with disease duration in the anti *Bordetella pertussis* IgG, IgA assay shows the opposite kinetics of sensitivity of culture for *Bordetella pertussis* (Al- Bargish. 1999). So the anti *Bordetella pertussis* IgG or IgA ELISA therefore represent a valuable complement to culture.

As a conclusion, anti *Bordetella pertussis* IgG or IgA ELISA gives a sensitivity of 87% in samples obtained after at least 7 days after the onset of was disease symptoms.

**5-10 The sensitivity and specificity of anti *Bordetella pertussis* IgG and IgA ELISA assay in diagnosis of pertussis**

Tables (16) & (17) show the diagnostic sensitivities and specificities of anti *Bordetella pertussis* IgG, IgA ELISA assay. The use of anti *Bordetella pertussis* IgG alone gives a 74% and 96% sensitivity and specificity. The anti *Bordetella pertussis* IgA alone gives a 50%, 94% sensitivity and specificity, respectively. While the use of anti *Bordetella pertussis* IgG or IgA criteria gives the best diagnostic sensitivity (82%) and a high specificity (92%) (Table -17).

Other serological techniques were investigated to diagnosis of whooping cough, but these techniques have a lower sensitivity than what the ELISA has. In Saleh (2000) study, serological techniques (Indirect hemagglutination test, Slide agglutination test, Countrimmunoelecrophoresis test and Complement fixation test) evaluated in the diagnosis of pertussis. The best and most sensitive method which gave higher positive samples was the Slide agglutination test, with 54.16% sensitivity.

Table (21) shows a comparison between our findings and other researchers' findings about the using of ELISA technique in laboratory diagnosis of *Bordetella pertussis* infection.

**Table (21): Comparison between the present study and other researchers' studies.**

| Study | Sensitivity (%) | Specificity (%) | No. of cases | Description |
|---|---|---|---|---|
| **Present study** | **82** | **92** | **68** | **Anti *Bordetella pertussis* IgG or IgA** |
| Aoyam *et al.* 1997 | 67 | - | 51 | |
| Halperin *et al*. 1989 | 87 | - | 77 | PT- antibodies |
| Lawrence and Paton. 1987 | 77 | - | 1240 | Antibodies to PT & FHA |
| Klement *et al*. 2005 | 53.8 | 98 | - | Antibodies to whole bacteria |
| Baughman *et al.*2004 | 80 | 93 | - | Whole sonicate IgA Elisa |
| Hallander *et al.* 1991 | 89 | - | 37 | Anti-PT IgG Elisa |
| Mertsola *et al.*1990 | 61 | 98 | 50 | Whole bacteria |
| | 90 | 92 | | PT |
| | 90 | 72 | | Outer membrane proteins |
| Campbell *etal.*1988 | 24 | - | 395 | Whole *Bordetella pertussis* IgA |
| Conway *et al.* 1988 | 57 | 100 | 113 | IgA – Elisa |
| Granstorm G *et al.* 1988 | 100 | - | 90 | IgA & IgG to FHA & PT |
| Granstorm G *et al.* 1988 | 81 | - | 54 | Anti PT & FHA in nasopharyngeal secretions |

## 5-11 The anti *Bordetella pertussis* IgG, IgA to DTP vaccination

To evaluate the humoral immune response (IgG and IgA) to DTP vaccination process, a total of 103 serum samples are collected from several age groups of 49 vaccinated and 54 unvaccinated people.

The anti *Bordetella pertussis* IgG (anti PT, FHA and LPS) levels show a significant elevation after vaccination with 1, 2 and 3 DTP vaccine doses (Fig-22).

Several studies documented high levels of anti *Bordetella pertussis* IgG antibodies to pertussis toxin and filamentous hemagglutinin and Lipopolysaccharide of *Bordetella pertussis* after vaccination of DTP vaccine (Samz *et al.* 2002; vanden Berg *et al.* 2000; Tomoda *et al.* 1991).

The anti PT-IgG has shown to be protected in several animal models, including the intracerebral inoculation model (Sato Y *et al.* 1985; Sato H and Sato Y. 1984; Oda *et al*. 1984), the inranasal challenge model (Robinson *et al*. 1989) and the aerosol challenge model (Robinson *et al.* 1989; Gupta *et al.* 1988; Halperin *et al*. 1988; SatoY. 1981; Pittman *et al.* 1984; Sato Y *et al.* 1980).

A pertussis vaccine could prevent the disease either by neutralizing toxins activities of *Bordetella pertussis* or by eliminating the microbe. The current whole-cell and the new acellular vaccines seem to protect severe disease but not necessarily infection by the bacterium (Poltkin and Cadoz. 1997; Hewlett. 1997; Mink *et al*. 1994; Ad Hoc Group. 1988; Pittman. 1979).

There is a correlation between high levels of serum IgG to *Bordetella pertussis* antigens and the rapid initial clearance of bacteria, which may be due to the transduction of antibodies into the lungs (Redhead *et al*. 1993).

The bacterial activity against *Bordetella pertussis* Lipopolysaccharides associated with the presence of certain IgG subtype, specifically the presence of $IgG_3$ and the subtype that is the most efficient at fixing complement (Weiss *et al.* 1998). The elicitation of pertussis toxin neutralizing IgG antibodies in the lungs as well as in the systemic circulation is considered one mechanism of protection against respiratory infection with *Bordetella pertussis* (Shahin *et al.* 1990).

Our results show that, the anti *Bordetella pertussis* IgA shows a non-significant elevation in children vaccinated with 1 and two doses of DTP. The significant elevation of *Bordetella pertussis* IgA was noticed in children who received 3 doses of DTP. These findings may explain that the DTP immunization did not induce a high level of anti *Bordetella pertussis* IgA (Mertsola *et al*. 1984; Nagel and Poot-Scholtens. 1983; Burstyn *et al.* 1983; MacAulay. 1981; MacAulay. 1979). *Bordetella pertussis* is not an invasive organism, but it induces the pathogenic process from colonization of the ciliated cells in respiratory tract (Pitman. 1984), so factors inhibit colonization should be protective. Pertussis toxin and FHA are considered to be important for the attachment of the organism to ciliated cells (Tuomanen and Weiss. 1985). The anti PT and FHA IgA have a high antiadherence activity (Tuomanen *et al.*1984). The IgA response to FHA and PT was lower in vaccinated children than in pertussis patients (Thomas *et al.* 1989) and that will reduce the anti adherence activity to *Bordetella pertussis* in serum and recreations and that may contribute to the lack of efficacy of the current pertussis vaccine (Tuomanen *et al.* 1984).

Our findings show an important influence of age on humoral immune response to DTP vaccination. The results show higher levels of IgG and IgA in adolescents than those levels of younger children. (Figures 24 and 25).

Polyzou *et al* indicate that, the anti *Bordetella pertussis* antibody concentrations were significantly elevated with age, and show that the anti *Bordetella pertussis* antibodies were significantly higher in adults than in less than 10 years children (Polyzon *et al.* 2004). Stroffolini *et al* (1989) show that, a high anti *Bordetella pertussis* IgG after vaccination in 67% of 11-12 years old children compared with 24% noticed in 1-3 years old children. Some of vaccinated healthy children showed low serological results because of waned immunity with time (Saleh. 2000).

The susceptibility of newborn infants to infection may be the result of the lack of IgA antiadherence antibody, which is presumably absent by virtue of the exclusion of this antibody class from transplacantal transport (Tuomanen *et al.* 1984).

Our study shows an anti *Bordetella pertussis* IgG mean level of 19.14 U/ml in unvaccinated less than 2 months infants (Fig 24). This result explained by placental transfer of IgG antibodies to *Bordetella pertussis*. The maternal antibodies against FHA and PT were detected in cord blood, consistent with reports that there is placental transfer of IgG antibodies to *Bordetella pertussis* (Burstyn *et al*. 1983). The newborn children do not gain transplacental protection even when mothers are immuned because of natural infection (Gordon and Hood. 1981). The presence of such antibodies adversely affects the ability of an infant to respond to immunization with pertussis vaccine (Burstyn *et al*. 1983).

Our findings show that there is 11% (11/103) of normal population in this study which has a positive anti *Bordetella pertussis* IgG or IgA level. Of those 11 positive cases, there are 4 noticed in adults. These results suggest an asymptomatic *Bordetella pertussis* infection. That incidence of pertussis in adults may be explained by decline in immunity induced by pertussis vaccine (Lambert. 1985; Jenkinson. 1988). The rate of children with negative clinical history of pertussis and serological evidence of *B. pertussis* infection is high in the general population (Saleh. 2000). The asymptomatic carriage and mildly symptomatic adults represent the source of spread of pertussis infection (Nelson. 1978; Herwadt. 1991; CDC. 1995).

## 5- 12 The anti *Bordetella pertussis* IgG & IgA in health-care workers

The outbreak among health-care workers is of special concern because of the risk of transmission to vulnerable patients (Wright *et al*. 1999). These outbreaks can result in substantial public health and economic costs (Calugar *et al*. 2003).

To evaluate the role of the health-care workers in epidemiology of pertussis, 20 serum samples were collected from medical staff in Babylon Maternity and Children Hospital to determine the anti *Bordetella pertussis* IgG and IgA levels. The results show that, 14 (70%) of the health-care staff has a positive IgG or IgA level (Table-19). These findings indicate an exposure to pertussis cases and a circulation of *Bordetella pertussis* in the

hospital environment, or it indicates asymptomatic pertussis cases between health-care workers.

There are several studies which show the role of health-care workers in the transmission of pertussis. Christie *et al* show that a serological evidence of asymptomatic pertussis infection was evident in 44% of the health-care workers (Christie *et al.*2001). Bassinet *et al* describe pertussis outbreak in health-care workers in French general hospital. The index cases transmitted pertussis to other health-care worker, who in turn, contaminated other staff and two immunosuppressed patients (Bassinet *et al.* 2004). Ainatblian N *et al* in a study of 12 healthcare workers 7(58%) of the subjects have a positive *Bordetella pertussis* DNA by using PCR (Aintablian *et al.* 1998). de Juanes JR *et al* show that the prevalence of *Bordetella pertussis* antibodies by using the ELISA technique was 51.7% of 487 healthcare workers (de Juanes *et al.* 2004).

# Conclusions & Recommendations

## Conclusions

1. The highest incidence of pertussis occurs in unvaccinated patients of less than 1 year old infants.
2. A high incidence of pertussis complications occur in unvaccinated and less than one year of old infants.
3. High incidences of pertussis infection occur in the rural area of Babylon Province as a result of low vaccination coverage of DTP in these areas.
4. The anti *Bordetella pertussis* IgG or IgA ELISA technique is a useful diagnostic tool in the laboratory diagnosis of *Bordetella pertussis* infections.
5. The best sensitivity and specificity obtained in anti *Bordetella pertussis* IgG or IgA ELISA were obtained in samples collected after at least 7 days after the onset of pertussis symptoms.
6. A significant increase occurs in anti *Bordetella pertussis* IgG levels after vaccination with DTP, and these levels are affected by the age of the vaccinated subjects.
7. Lower IgA responses occur after DTP vaccinated especially in subjects less than 1 year old.
8. A high rate of asymptomatic *Bordetella pertussis* infection occurs in the population of Babylon province, especially in adults.
9. A high rate of asymptomatic *Bordetella pertussis* infection occurs in healthcare workers.

# Recommendations

1. Use the anti *Bordetella pertussis* IgG and IgA Elisa technique in the diagnosis of whooping cough, and in serological surveys or vaccine efficacy trials.
2. DTP booster doses in 1, 2 and preschool age should be considered.
3. Improve the vaccination coverage in the rural area of Babylon Province.
4. Give attention to the nosocomial transmission of pertussis via healthcare workers by diagnosis, treatment, and prevention of the *Bordetella pertussis* infections of those healthcare workers.
5. Evaluate of the new acellular pertussis vaccines and compare their efficacy with the whole cell pertussis vaccine efficacy.
6. Give attention to asymptomatic or mild *Bordetella pertussis* infections in adolescents and adults.

# Appendix A

The O. D. values and IgG, IgA level in ELISA U/ml of all subjects in this study:

## 1) The O. D. values and IgG, IgA level of the patients:

| No. | O. D | IgG (U/ml) | O.D | IgA (U/ml) |
|---|---|---|---|---|
| 1. | 3.734 | 233.89 | 0.633 | 17.10 |
| .2 | 3.531 | 220.62 | 0.211 | 7.12 |
| .3 | 3.421 | 213.43 | 0.124 | 5.06 |
| .4 | 2.546 | 156.24 | 0.210 | 7.10 |
| .5 | 2.745 | 169.25 | 0.123 | 5.04 |
| .6 | 1.128 | 63.56 | 0.200 | 6.86 |
| .7 | 2.387 | 145.85 | 0.352 | 10.45 |
| .8 | 3.997 | 251.08 | 0.584 | 15.94 |
| .9 | 3.135 | 194.74 | 0.143 | 5.61 |
| .10 | 0.528 | 24.35 | 0.109 | 4.71 |
| .11 | 0.567 | 26.90 | 0.448 | 12.72 |
| .12 | 1.879 | 112.65 | 0.970 | 25.06 |
| .13 | 1.902 | 114.15 | 0.062 | 3.60 |
| .14 | 2.806 | 173.24 | 0.147 | 5.61 |
| .15 | 1.802 | 107.62 | 0.168 | 6.10 |
| .16 | 1.702 | 101.08 | 0.211 | 7.12 |
| .17 | 1.906 | 114.41 | 0.174 | 6.25 |
| .18 | 1.103 | 61.93 | 0.147 | 5.61 |
| .19 | 1.480 | 86.57 | 0.112 | 4.78 |
| .20 | 1.978 | 119.12 | 0.836 | 21.90 |
| .21 | 2.200 | 133.63 | 0.165 | 6.03 |
| .22 | 0.703 | 35.79 | 0.511 | 14.21 |
| .23 | 2.273 | 138.40 | 0.148 | 5.63 |
| .24 | 1.834 | 109.71 | 0.810 | 21.28 |
| .25 | 1.942 | 116.77 | 0.840 | 4.12 |
| .26 | 2.368 | 144.61 | 0.174 | 6.25 |
| .27 | 3.634 | 227.35 | 0.421 | 12.08 |
| .28 | 1.936 | 116.37 | 0.556 | 15.28 |
| .29 | 3.521 | 219.97 | 0.431 | 12.32 |
| .30 | 1.934 | 116.24 | 0.504 | 14.05 |
| .31 | 1.236 | 70.62 | 0.517 | 14.35 |
| .32 | 0.904 | 48.92 | 0.077 | 3.95 |
| .33 | 0.901 | 48.73 | 0.634 | 17.12 |
| .34 | 2.166 | 131.41 | 0.107 | 4.66 |
| .35 | 2.166 | 131.41 | 0.117 | 4.90 |
| .36 | 1.954 | 117.55 | 0.801 | 21.07 |
| .37 | 2.173 | 131.86 | 0.603 | 16.93 |
| .38 | 2.114 | 128.01 | 0.632 | 17.07 |
| .39 | 1.865 | 111.73 | 0.422 | 12.11 |
| .40 | 1.763 | 105.07 | 0.543 | 14.97 |
| .41 | 2.594 | 159.83 | 0.148 | 5.63 |

| | | | | |
|---|---|---|---|---|
| .42 | 1.146 | 64.74 | 0.107 | 4.66 |
| .43 | 0.644 | 31.93 | 0.186 | 6.53 |
| .44 | 0.421 | 17.35 | 0.026 | 2.75 |
| .45 | 2.597 | 159.58 | 0.714 | 19.01 |
| .46 | 2.546 | 156.24 | 0.104 | 4.59 |
| .47 | 0.607 | 29.51 | 0.098 | 4.45 |
| .48 | 0.458 | 19.77 | 0.093 | 4.33 |
| .49 | 3.590 | 224.48 | 0.761 | 20.12 |
| .50 | 3.726 | 233.37 | 0.446 | 12.68 |
| .51 | 0.527 | 24.28 | 0.604 | 16.41 |
| .52 | 3.137 | 194.87 | 0.157 | 5.84 |
| .53 | 0.570 | 27.09 | 0.628 | 16.98 |
| .54 | 1.841 | 110.16 | 0.141 | 5.47 |
| .55 | 1.547 | 90.95 | 0.432 | 12.34 |
| .56 | 3.124 | 194.02 | 0.512 | 14.24 |
| .57 | 1.157 | 65.46 | 0.102 | 4.54 |
| .58 | 2.874 | 177.68 | 0.551 | 15.16 |
| .59 | 3.162 | 196.50 | 0.986 | 25.44 |
| .60 | 3.317 | 206.64 | 0.438 | 12.49 |
| .61 | 2.127 | 128.86 | 0.570 | 15.61 |
| .62 | 3.182 | 197.81 | 0.435 | 12.42 |
| .63 | 3.415 | 213.04 | 0.400 | 11.59 |
| .64 | 2.178 | 132.19 | 0.519 | 14.40 |
| .65 | 2.168 | 131.54 | 0.598 | 16.06 |
| .66 | 3.971 | 249.38 | 0.580 | 15.84 |
| .67 | 3.439 | 214.61 | 0.984 | 25.39 |
| .68 | 2.618 | 160.95 | 0.621 | 16.81 |

## 2) The O. D. values and IgG, IgA level of the Normal subjects:

| No. | O. D | IgG (U/ml) | O.D | IgA (U/ml) |
|---|---|---|---|---|
| 1. | 0.732 | 37.68 | 0.089 | 4.24 |
| .2 | 0.480 | 21.21 | 0.039 | 3.05 |
| .3 | 0.582 | 27.88 | 0.073 | 3.86 |
| .4 | 0.300 | 9.45 | 0.071 | 3.81 |
| .5 | 0.330 | 11.41 | 0.087 | 4.19 |
| .6 | 0.510 | 23.17 | 0.177 | 6.32 |
| .7 | 0.574 | 27.35 | 0.189 | 6.60 |
| .8 | 0.206 | 3.30 | 0.076 | 3.93 |
| .9 | 0.171 | 1.01 | 0.081 | 4.05 |
| .10 | 0.776 | 40.56 | 0.044 | 3.17 |
| .11 | 0.460 | 19.90 | 0.059 | 3.53 |
| .12 | 0.634 | 31.28 | 0.093 | 4.33 |
| .13 | 0.172 | 1.08 | 0.099 | 4.47 |
| .14 | 0.350 | 12.71 | 0.091 | 4.28 |
| .15 | 0.772 | 40.30 | 0.076 | 3.93 |
| .16 | 0.789 | 41.41 | 0.155 | 5.80 |
| .17 | 0.743 | 38.40 | 0.158 | 5.87 |
| .18 | 1.724 | 102.52 | 0.120 | 4.97 |
| .19 | 1.089 | 61.01 | 0.086 | 4.17 |
| .20 | 0.739 | 38.14 | 0.135 | 5.32 |

| | | | | |
|---|---|---|---|---|
| .21 | 0.525 | 24.15 | 0.091 | 4.28 |
| .22 | 0.410 | 16.64 | 0.101 | 4.52 |
| .23 | 0.950 | 51.93 | 0.093 | 4.33 |
| .24 | 1.025 | 56.83 | 0.100 | 4.50 |
| .25 | 0.699 | 35.52 | 0.093 | 4.33 |
| .26 | 0.990 | 54.54 | 0.108 | 4.69 |
| .27 | 0.745 | 38.53 | 0.093 | 4.33 |
| .28 | 0.748 | 38.73 | 0.086 | 4.17 |
| .29 | 0.786 | 41.21 | 0.181 | 6.41 |
| .30 | 0.884 | 47.62 | 0.530 | 14.66 |
| .31 | 0.834 | 44.35 | 0.174 | 6.25 |
| .32 | 1.411 | 82.06 | 0.189 | 6.60 |
| .33 | 0.743 | 38.40 | 0.187 | 6.55 |
| .34 | 0.668 | 33.50 | 0.246 | 7.95 |
| .35 | 0.679 | 34.22 | 0.235 | 7.69 |
| .36 | 0.627 | 30.82 | 0.124 | 5.06 |
| .37 | 0.339 | 12.00 | 0.102 | 4.54 |
| .38 | 0.460 | 19.90 | 0.103 | 4.57 |
| .39 | 0.214 | 3.83 | 0.099 | 4.47 |
| .40 | 0.801 | 42.19 | 0.132 | 5.25 |
| .41 | 0.358 | 13.24 | 0.063 | 3.62 |
| .42 | 0.659 | 32.91 | 0.144 | 5.45 |
| .43 | 0.788 | 41.34 | 0.112 | 4.78 |
| .44 | 0.689 | 34.87 | 0.155 | 5.80 |
| .45 | 0.864 | 46.31 | 0.116 | 4.87 |
| .46 | 0.802 | 42.26 | 0.578 | 15.80 |
| .47 | 0.518 | 23.69 | 0.183 | 6.64 |
| .48 | 1.159 | 65.59 | 0.195 | 6.74 |
| .49 | 2.357 | 143.89 | 0.062 | 3.60 |
| .50 | 1.021 | 56.57 | 0.103 | 4.57 |
| .51 | 1.005 | 55.52 | 0.162 | 5.96 |
| .52 | 1.026 | 56.90 | 0.109 | 4.71 |
| .53 | 1.000 | 55.20 | 0.179 | 6.36 |
| .54 | 0.851 | 45.46 | 0.104 | 4.59 |
| .55 | 0.381 | 14.74 | 0.153 | 5.75 |
| .56 | 0.761 | 39.58 | 0.102 | 4.54 |
| .57 | 0.780 | 40.82 | 0.242 | 7.85 |
| .58 | 1.255 | 71.86 | 0.158 | 5.87 |
| .59 | 0.607 | 29.51 | 0.227 | 7.50 |
| .60 | 0.846 | 45.13 | 0.157 | 5.84 |
| .61 | 0.458 | 19.77 | 1.158 | 29.51 |
| .62 | 0.789 | 41.41 | 1.168 | 29.74 |
| .63 | 1.039 | 57.75 | 0.286 | 8.87 |
| .64 | 0.590 | 28.40 | 0.151 | 5.70 |
| .65 | 0.781 | 40.88 | 0.134 | 5.30 |
| .66 | 0.570 | 27.09 | 0.105 | 4.61 |
| .67 | 0.438 | 18.47 | 0.206 | 7.00 |
| .68 | 0.612 | 29.84 | 0.161 | 5.94 |
| .69 | 0.658 | 32.84 | 0.124 | 5.06 |
| .70 | 0.670 | 33.63 | 0.174 | 6.25 |
| .71 | 0.739 | 38.14 | 0.232 | 7.62 |
| .72 | 1.056 | 58.86 | 0.348 | 10.36 |
| .73 | 0.810 | 42.78 | 0.217 | 7.26 |

| | | | | |
|---|---|---|---|---|
| .74 | 0.980 | 53.89 | 0.347 | 10.34 |
| .75 | 2.311 | 140.88 | 1.148 | 29.27 |
| .76 | 1.151 | 65.07 | 0.158 | 5.87 |
| .77 | 1.138 | 64.22 | 0.276 | 8.66 |
| .78 | 0.490 | 21.86 | 0.141 | 5.47 |
| .79 | 0.680 | 34.28 | 0.124 | 5.06 |
| .80 | 0.470 | 20.56 | 0.105 | 4.61 |
| .81 | 0.337 | 11.86 | 0.206 | 7.00 |
| .82 | 0.511 | 23.24 | 0.151 | 5.70 |
| .83 | 0.557 | 26.24 | 0.114 | 4.83 |
| .84 | 0.571 | 27.16 | 0.164 | 6.01 |

## 3) The O. D. values and IgG, IgA level of the adults

| No. | O. D | IgG (U/ml) | O.D | IgA (U/ml) |
|---|---|---|---|---|
| 1. | 1.900 | 114.02 | 0.222 | 7.38 |
| .2 | 1.315 | 75.79 | 1.058 | 27.14 |
| .3 | 1.054 | 58.73 | 0.112 | 4.78 |
| .4 | 1.057 | 58.92 | 0.260 | 8.28 |
| .5 | 1.019 | 56.44 | 0.201 | 6.88 |
| .6 | 1.017 | 56.31 | 0.154 | 5.77 |
| .7 | 1.091 | 61.15 | 0.269 | 8.94 |
| .8 | 2.134 | 129.32 | 0.236 | 7.71 |
| .9 | 2.001 | 120.62 | 1.001 | 25.80 |
| .10 | 1.031 | 57.22 | 0.202 | 6.91 |
| .11 | 1.156 | 65.39 | 0.134 | 5.30 |
| .12 | 1.357 | 78.53 | 0.205 | 6.98 |
| .13 | 1.240 | 70.88 | 0.128 | 5.16 |
| .14 | 1.146 | 64.74 | 0.234 | 7.66 |
| .15 | 0.480 | 21.21 | 0.051 | 3.34 |
| .16 | 0.670 | 33.63 | 0.068 | 3.74 |
| .17 | 0.460 | 19.90 | 0.071 | 3.81 |
| .18 | 0.347 | 12.52 | 0.087 | 4.19 |
| .19 | 0.521 | 23.89 | 0.070 | 3.79 |
| .20 | 0.480 | 21.21 | 0.051 | 3.34 |

## 4) The O. D. values and IgG, IgA level of the Healthcare workers:

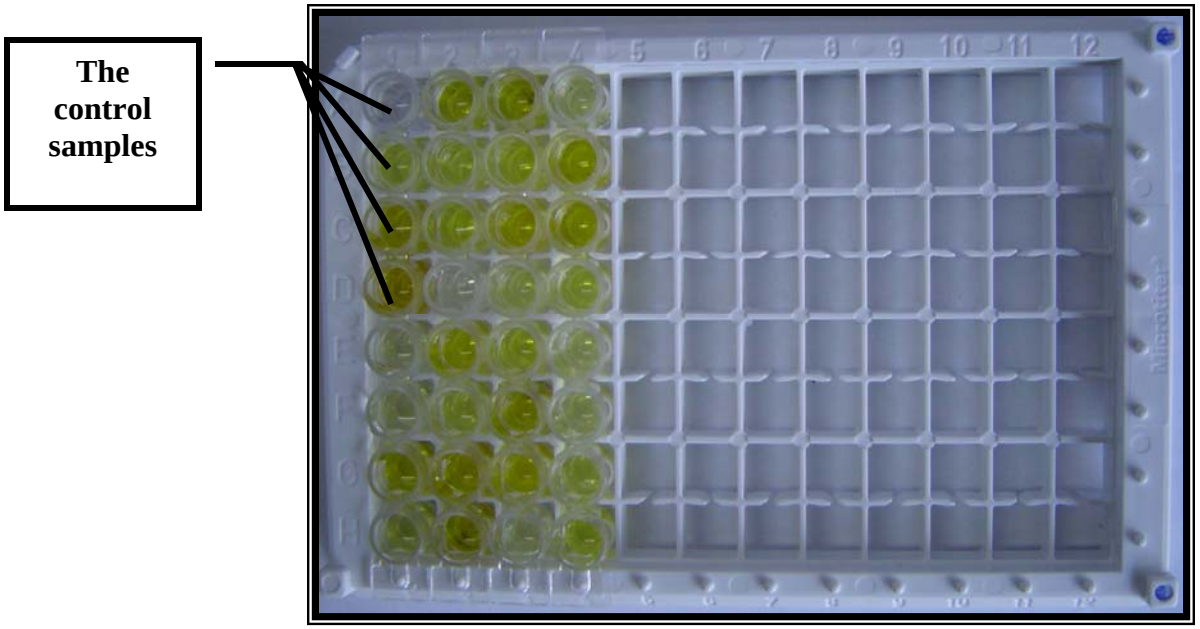
| No. | O. D | IgG (U/ml) | O.D | IgA (U/ml) |
|---|---|---|---|---|
| 1. | 1.019 | 56.44 | 0.780 | 20.57 |
| .2 | 1.486 | 86.96 | 0.798 | 21.00 |
| .3 | 1.585 | 93.43 | 0.357 | 10.57 |
| .4 | 2.522 | 154.67 | 0.148 | 5.63 |
| .5 | 1.570 | 92.45 | 0.317 | 9.63 |
| .6 | 2.546 | 156.24 | 0.255 | 8.16 |
| .7 | 2.695 | 165.98 | 0.754 | 19.96 |
| .8 | 1.054 | 58.37 | 0.890 | 23.17 |
| .9 | 1.342 | 77.55 | 0.647 | 17.43 |
| .10 | 1.841 | 110.16 | 0.150 | 5.68 |
| .11 | 2.114 | 128.01 | 0.347 | 10.34 |
| .12 | 0.770 | 40.16 | 0.848 | 22.18 |
| .13 | 2.634 | 162.00 | 0.741 | 19.65 |
| .14 | 2.407 | 147.09 | 0.562 | 15.42 |
| .15 | 2.556 | 156.90 | 0.233 | 7.64 |
| .16 | 2.346 | 143.17 | 1.121 | 28.63 |
| .17 | 1.215 | 69.25 | 0.354 | 10.50 |
| .18 | 1.132 | 63.83 | 0.344 | 10.03 |
| .19 | 1.342 | 77.55 | 0.365 | 10.76 |
| .20 | 1.175 | 66.64 | 0.204 | 6.95 |

# Appendix B

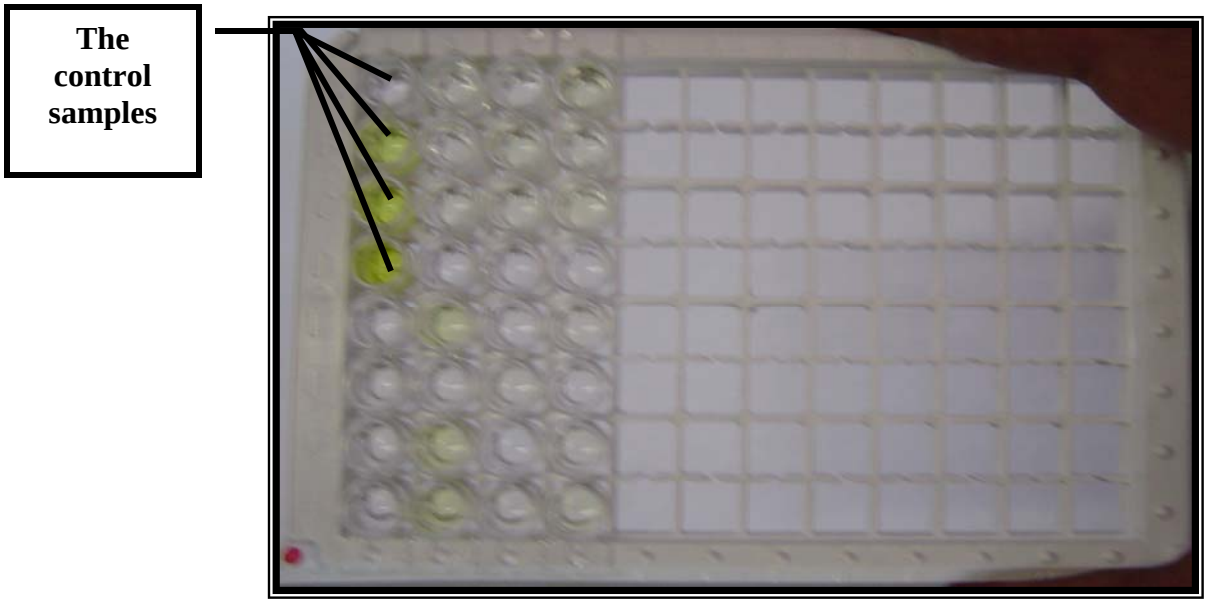
## The ELISA test plates of IgG and IgA:

- The first four wells of the plate contain control samples, all the rest of wells contain the test samples.

### 1) The ELISA test plate of IgG




### 2) The ELISA test plate of IgA



# References

Abdalla BA, Salin MA, Yousif EA and Omer MI. 1998. Whooping cough in Sudanese children. East. Afr. Med. J. Jun;75 (6): 353-7.

Acombined Scottish studies. 1970. Diagnosis of whooping cough, comparison of serology tests with isolation of Bordetella pertussis. Brit. Med. J. 4: 637-639.

Ad Hoc Group for the Study of Pertussis Vaccines. 1988. Placebo-controlled trial of two acellular pertussis vaccines in Sweden—protective efficacy and adverse events. Lancet i:955–960.

Afari EA, Kamiya Y and Nkrumah FK. 1996. Randomized controlled trail of acellular diphtheria tetanus and pertussis vaccines in southern Chana. Ann. Trop. Perdiatr. 16 (1): 39-48.

Aintablian N, Walpita P and Sawyer MH. 1998. Detection of *Bordetella pertussis* and respiratory synctial virus in air samples from hospital rooms. Infect. Control. Hosp. Epidemiol. Dec; 19 (12): 918-23.

Al-Bargish KA. 1999. Outbreak of pertussis in Basra, Iraq. East Mediterr Health J. May; 5 (3) : 540-8.

Al-Marzoki JM, Salih HA and Al-Janabi IA. 2004. A cross sectional study of pertussis cases diagnosed over a period of five years in Babylon Maternity and Children Hospital. J. Babylon University. 10 (4).

Altemeier-III WA. 1981. Pertussis and Parapertussis. Kelley V. C. practice of pediatric, 3: 1-20.

American Academy of Pediatrics. 2003. Pertussis. In: Pickering LK ed. Red Book: 2003 Report of the Committee on Infectious Diseases. 26th ed. Elk Grove Village, IL: American Academy of Pediatrics,:472-86.

Anonymous. Pertussis – United States 1982 and 1983. 1984. MMWR Morb Mortal Wkly Rep. 33: 573-575.

Aoyama T, Kato T, Takenchi Y, Kato K, Morokuma K and HiRai T. 1997. Simple, speedy, sensitive and specific serodiagnosis of pertussis by using particle agglutination test. J. Clin. Mircobiol. 35 (7): 1859-1861.

Arciniega JL, Hewlett EL, Johnson FD. 1991. Human serologic response to envelope-associated proteins and adenylate cyclase toxin of B. pertussis. J Infect Dis 163:135-142.

Atti MCD, Anemona A, Tozzi AE, Stefanelli PG, Giammanco A and Salmaso S. 1999. Reactogenicity of a three-dose pertussis acellular vaccine catch-upon children 21-40 months of age. Vaccine. 17: 2030-2035.

Balagtas RC, Nelson KE and Kevin S. 1971. Treatment of pertussis with pertussis immune globulin. J. Pediatr. 79: 203-208.

## References

Baraff LJ, Leake RD, Burstyn DG, Payne T, Cody CL, Manclarck CR and Geme Jr. JW. 1983. Immunologic response to early and routine DTP immunization in infants. Pediatrics. 73: 37-42.

Barbic, J., Leef, M.F., Burns, D.L., Shahin, R.D. 1997. Role of gamma interferon in natural clearance of *Bordetella pertussis* infection. Infect. Immun. 65:4904-4908.

Baron S, Njamkepo E and Grinprl E. 1998. Epidemiology of pertussis in French hospitals in 1993: thirty years after routine use of vaccination. Pediatr. Infect. Dis. J. 17: 412-8.

Bassinet L, Motrat M, Njamkapo E, Aberrano S, Housset B and Guiso N. 2004. Nosocomial pertussis outbreak among adult patients and health-care workers. Infect. Control. Hosp. Epidemiol. Nov; 25 (11): 995-7.

Baughaman AL, Bisgard KM, Guris D, Decker MD, Holland K, Meade BD and Lyum F. 2004. Establishment of diagnostic cutoff point for levels of serum antibodies to pertussis toxin, filamentous hemagglutinin and fimbriae in adolescents and adults in the United State. Clin. Diagn. Lab. Immunol. Nov; 11 (6): 1045-53.

Bauwens JE, Spach DH and Schacker TW. 1992. *Bordetella bronchiseptica* pneumonia and bactermia following bone marrow transplantation. J. Clin. Microbiol. 30: 2474-2475.

Beier D, Fuchs TM, Graff-Wohllen H and Gress R. 1996. Signal transduction and virulence regulation in *Bordetella pertussis.* Microbiol. 12 (2): 185-96.

Bemis DA. 1992. Bordetella and mycoplasma respiratory-infections in dogs and cats, ver clin North Am Small anim Pract, 22: 1173-86.

Bennesson AS. 1995. Control of communicable disease manual. 16th ed. Washington, DC: American Public Health Association. 403-11.

Beque P and Griprel E. 1996. Futher combined vaccine. J. Infect. Dis. 174 (suppl 3): S295-S297.

Berbers, G., A. B. Lafeber, B. Labadie, P. E. Vermeer-de Bondt, D. J. A. olscher, and A. D. Plantinga. 1999. A randomized controlled study with hole-cell or acellular pertussis vaccines in combination with regular DTiPV accine and a new poliomyelitis (IPV-Vero) component in children 4 years of age in The Netherlands. Report no. 105000001. National Institute of Health and the Environment, Bilthoven, The Netherlands. Dekker, New York, N.Y.

Birkebaek NH, Kristiansen M, Seefeldt T. 1999. Bordatella pertussis and chronic cough in adults. Clin Infect Dis ;29:1239-42.

Bisgard KM. , Christie CD. , Reising SF. , Sanden GN. , Cassiday PK. , Gomersall C. Wattigney WA. , Roberts NE. and Strebel PM. 2001. Molecular epidemiology of *Bordetella pertussis* by pulsed – filed gel

electrophoresis profile: Cincinati, 1989-1996. J Infect Dis ; 183 May. 1360-1367.

Black S. 1997. Epidemiology of pertussis. Pediatr. Infect. Dis. J. 16 (4): S85-S89.

Blackall PJ, Eaves LE, Fegan M. 1995. Antimicrobial sensitivity testing of Australian isolates of B. avum and the B.avum-like organism, Aust Ver J, 72:97-100.

Borel P and Gnlesph S. 1984. Technical methods, conuterimmuesis in the diagnosis of whooping cough. J. Clin. Pathol. 37: 950-955.

Bradstreat CMP, Tammahill AJ, Edwards JMB and Beuson PF. 1972. Detection of *Bordetella pertussis* antibodies in human sera by complement-fixation and immunofluorescence. J Hyg. 70: 75-83.

Brook GF, Butel JS, and Morse SA. 2004. Jawetz, Melnick & Adelberg's, Medical Microbiology. 23rd. MacGraw Hill Co. 282-284.

Bruss JB. and George RS. 1999. Protective Effects of Pertussis Immunoglobulin (P-IGIV)in the Aerosol Challenge Model. Clinical and Diagnostic Laboratory Immunology, Vol. 6, No. 4, p. 464-470.

Burstyn DG, Baraf LJ, Peppler MS, Leake DR, Gene JS and Manclarck CR. 1983. Serological response to filamentous hemagglutinin and lymphocytosis promoting toxin of Bordetella pertussis. Infect. Immun. Sept. 41 (3): 1150-1156.

Calugar A, Orrega-Sanchez I, Oakes I, Jahre J, Tiwari T and Murph TV. Economic impact of noscomial pertussis among heath-care worker – Pennsylvania, 2003. 53rd Annual Epidemic Intelligence Service Conference, April 19-23, 2004. Atlanta, GA.

Campbell PB, Masters PL and Rohwedder E. 1988. Whooping cough diagnosis a clinical evaluation of complementing culture and Immunoflorescence with ELISA of pertussis immunoglobulin A in nasopharyngeal secretions. J. Med. Microbiol. Dec; 27 (4): 247-54.

Campell DH, Garvey JS, Cremer NE and Sussdorf DH. 1970. Methods in immunology. 2nd edition. Canada published simultaneously. 315-323.

Case TF, Tortora GJ, Funke BR and Case CL. 1986. Microbiology (An introduction). 3rd edition. New York. Inc. 173-175.

Cassone A, Ausiello CM and Urbani F. 1997. Cell mediated and antibody response to *Bordetella pertussis* antigens in children vaccinated with acellular or whole cell pertussis vaccine. Arch. Pediatr. M. Adolesc. Med. 151: 382-389.

CDC. 1995. Pertussis United State, January 1992-june 1995. MMWR Morb. Mortal. Wkly. Rep. 44: 525-529.

CDC. 1997. Case definitions of infectious conditions under public health surveillance. MMWR 46 (RR-10):1-57.

## References

CDC. 2002. Pertussis deaths. United State, 2000. MMWR. July/51 (28); 616-618.

Charles, I. G., J. Li, M. Roberts, K. Beesley, M. Romanos, D. J. Pickard, M. Francis, D. Campbell, G. Dougan, M. J. Brennan, C. R. Manclark, M. A. Jensen, I. Heron, A. Chubb, P. Novotny, and N. F. Fairweather. 1991. Identification and characterization of a protective immuno-dominant B cell epitope of pertactin (P.69) from Bordetella pertussis. Eur. J. Immunol. 21:1147- 1153.

Cheers, C., Gray, D.F. 1969. Macrophage behaviour during the complaisant phase of murine pertussis. Immunology. 17:875-887.

Cherry JD. 1984. The epidemiology of pertussis and pertussis immunization in the United Kingdom and United States: a Comparative study. Curr Probl Pediatr 14:1.

Cherry JD. 1995. Comparison of values of antibody to *Bordetella pertussis* antigens in young German and American men. Clin Infect Dis 20: 1271.

Cherry JD. 2003. The science and fiction of the "resurgence" of pertussis. Pediatrics. 112; 405-406.

Childers, N. K., M. G. Bruce, and J. R. McGhee. 1989. Molecular mechanisms of immunoglobulin A defense. Annu. Rev. Microbiol. 43:503–536.

Chosh HK, Tranter J. 1979. *Bordetella bronchiseptica* infection in man. Review and a case report. J. Clin. Pathol. 32: 546-548.

Christie C, Max ML, Matchant CD and Reising F. 1994. The 1993 epidemic of pertussis in Cincinnati. Resurgence of disease in highly immunized population of children. N. Engl. J. Med. 331: 16-21.

Christie CD, Garrison KM, Kielly L, Gupta RK, Fleubi J and Marchant CD. 2001. A trial of acellular pertussis vaccine in hospital workers during the Cincinnati pertussis epidemic of 1993. CID. 33-997-1003.

Conway SP, Balfour AH and Ross H. 1988. Serologic diagnosis of whooping cough by Enzyme-Liked Immunosorbent Assay. Pediatr. Infect. Dis. J. Aug; 7(8) 570-4.

Cookson BT, Cho HL, Herwaldt LA. 1989. Biological activities and chemical composition of purified tracheal cytotoxin of B. pertussis. Infec Immun 57:2223-2229.

Crowcroft NS. , Stein C. , Duclos P. and Brimingham M. 2003. How best to estimate the global burden of pertussis. Lancet infect Dis. Jul; 3(7): 413-8.

de Juanes JR, Gil A, Gonzalez A, Arrazola MP, Sam-Martin M and Esteban J. 2004. Seroprevalence of pertussis antibody among health-care personal in Spain. Eur. J. Epidemiol. 19 (1): 69-72.

## References

De Magistris, M. T., M. Romano, S. Nuti, R. Rappuoli, and A. Tagliabue. 1988. Dissecting human T cell responses against Bordetella species. J. Exp. Med. 168:1351-1362.

de Melker HE, Conyn-van Spaendonck MAE, Rumko HC, Wijngaarden JK, Mooi FR and Schellekens JFP. 1997. Pertussis in Netherlands: an outbreak despite high level of immunization with whole cell vaccine. Emerg. Infect. Dis. 3: 175-8.

de Melker HE, Schellkers JFP and Neppelenboeek SE, Mooi FR, Rumke HC, and Conyu-von Spandonck MAE. 2000. Reemergence of pertussis in highly vaccinated population of the Netherlands: Observations and surveillance data. Emerging Infectious Disease. 6 (4): 348-357.

de Melker HE, Verstcech FGA, Conyn van Spaendonck MAE, Elvers LH, Berbers GAM, Zee A and Schellekens JFP. 1999. Specificity and sensitivity of high level of immunoglobulin G antibodies against pertussis toxin in single serum sample for diagnosis of infection with Bordetella pertussis. J. Clin. Microbiol. 38 (2): 800-806.

De Serres G, Boulianne N and Duva B. 1996. Effectiveness of whole cell pertussis vaccine in child-care centers and schools. Pediatrics Infecet. Dis. J. 15 (6): 519-24.

Deo, Y. M., K. Sundarapandiyan, T. Keler, P. K. Wallace, and R. F. Graziano. 1998. Bispecific molecules directed to the Fc receptor for IgA (Fc alpha RI, CD89) and tumor antigens efficiently promote cell-mediated cytotoxicity of tumor targets in whole blood. J. Immunol. 160:1677–1686.

Department of Health and Human Services, FDA. 1985. Biological products; bacterial vaccines and toxoids; implementation of efficacy review; proposed rule. Fed. Register. 50:51002-51117.

Dhadwal D, Sood R, Gupta AK, Ahluwalia SK, Valsayan A, Sharama R. 1997. Immunization coverage among urban and rural children in the Shimla hills. J. Commun. Dis. June; 29 (2): 127-30.

Edward KM and Decker MD. 1997. Combination vaccines consisting of acellular pertussis vaccines. Pediatr. Infect. Dis. J. 16 (4 Suppl): 97-102.

Edwards KM and Karazon DT. 1990. Pertussis vaccines. Pediatr. Clin. North. Am. 37 (30): 549-566.

Elliott E. , Mclntyre P. ,Ridley G. ,Morris A. ,Massie J. , McEniery J. And Knight G. 2004. National study of infants hospitalized with pertussis in the acellular vaccine era. Pediatr Infect Dis J. Mar :23 (3): 246-52.

Ewanowich, C. A., A. R. Melton, A. A. Weiss, R. K. Sherburne, and M. S. Peppler. 1989. Invasion of HeLa 229 cells by virulent Bordetella pertussis. Infect. Immun. 57:2698-2704. model. Microb. Pathog. 8:37-45.

## References


Farizo KM. , Cochi SL. and Zell ER. 1992. Epidemiological features of pertussis in the United States 1980-1989. Clin Infec Dis. 14: 708-719.

Fearon DT and Locksley RM. 1996. The instructive role of innate immunity in the acquired immune response. Science. 272: 50-54.

Fernandez RC, Weiss A. 1994. Cloning and sequencing of B. pertussis serum resistance locus. Infect Immun , 62: 4727-4738.

Finger H, Wirsing von konig CH, Tacken A and Wassilak SG. 1991. The epidemiological situation of pertussis in the Federal Republic of Germany. Dev. Biol. Stand. 37: 343-55.

Finger H. and Wirsing von Konig CH. 1992. The epidemiology of whooping cough. Gesundheitswesen. Oct; 54 (10): 541-5.

Franso KM, Cochi SL, Zell ER. , 1992. Epidemiologic features of pertussis in the united states, 1980-1989. Clin Infect Dis 14:708.

Fruhwirth M, Neher C, Schmidt-Schlapfer G and Allerberger F. 2002. *Bordetella pertussis* and Bordetella parapertussis infection in Austrian pediatric out patient's clinic. Wien. Klin. Wochenschr. Jun 14; 114 (10-11): 377-82.

Fry NK, Tzivra O, Li YT, McNiff A, Doshi N, Maple PA, Crowcroft NS, Miller E, George RC, Harrison TG. 2004. Laboratory diagnosis of pertussis infections: the role of PCR and serology. J Med Microbiol. 53(Pt 6):519-25.

Funke G, Hess T and Vandamme P. 1996. Characterization of *Bordetella hinzii* sp. nov. isolated from a cyctic fibrosis patients over 3-year period. J. Clin. Microbiol. 34: 966-969.

Galazka A. 1997. Can we control pertussis better? II. Old and new vaccine against pertussis. Epidemiol. 51 (3): 285-295.

Gammanco A, Taormina S, Genovese M, Mangiaracina G, Giammanco G and Chiarini A. 1997. Serological response to infection with *Bordetella pertussis*. Dev. Biol. Stand. 89: 213-220.

Giammanco A, Taormina S, Chiarini A, Dardanoni G, Stesanelli P, Salmaso S and Mastrantonio P. 2003. Analogous IgG subclass response to pertussis toxin in vaccinated children, healthy or affected by whooping cough. Clin. Infect. Dis. Jun; 6 (3): 183-187.

Goodman YE, Wort AJ and Jackson FL. 1981. Enzyme linked Immunosorbent assay for detection of pertussis immunoglobulin A in nasopharyngeal secretions as an indicator or recent infection. J. Clin. Microbiol. 13: 286-292.

Gordon JE, Hood RL. 1981. Whooping cough and its epidemiological anomalies. Am. J. Med. Sci. 222: 333-7.

Gordon JE. and Hood RJ. 1980. whooping cough and its epidemiological abnormalities. Am J Med Sci ; 222:333-61.

## References

Gordon M, David HD, Golden R. 1994. Clinical and microbiological features of children presenting with pertussis to Canadian pediatric hospital during an eleven-year period. Pedatr Infect Dis J 13:617.

Granstrom G, Askelof P and Granstrom M. 1988. Specific immunoglobulin A to *Bordetella pertussis* antigens in mucosal secretion for rapid diagnosis of whooping cough. J. Clin. Microbiol. 26(5): 869-874.

Granstrom G, Wertlind B, Salenstedt CR and Granstrom M. 1988. Evaluation of serologia assays for diagnosis of whooping cough. J. Clin. Microbiol. 26 (9): 1818-1823.

Granstrom M, Lindberg AA, Askelof P and Hederstedt B. 1982. Detection of antibodies in human serum against fimbrial hemagglutinin of *Bordetella pertussis* by Enzyme-Linked Immunosorbent assay. J. Med. Microbiol. 15: 85-96.

Granstrom M, Lindberg AA, Askelof P and Hederstedt B. 1982. Serological diagnosis of whooping cough by an Enzyme-Linked Immunosorbent assay using fimbrial hemagglutinin as antigens. J. Infect. Dis. 146: 741-745.

Granstrom M, Olinder-Nielsen AM and Holmblad P. 1991. Specific immunoglobulin for treatment of whooping cough. Lancet. 338: 1230-1233.

Greco D, Samaso S, Masrtantonio P, Giuliano M, Tozzi AE and Anemona A. 1996. A controlled trail of two acellular vaccine and one whole cell vaccine against pertussis. New Engl. J. Med. 334 (6): 341-348.

Grimprel E, Begue P, Anjak I, Betson F and Guiso N. 1993. Comparison of polymerase chain reaction, culture and Westren Immunoblot serology for diagnosis of *Bordetella pertussis* infection. J. Clin. Biol. 31: 2745-50.

Grnoff DM and Rappuoli R. 1997. Are serological response to acellular pertussis antigens sufficient criteria to ensure that new combination vaccines are effective for prevention of disease. Bev. Biol. Stand. 89: 379-389.

Guermonprez P, Ladant D, Karimova G, Ullmann A and Leclerc C. 1999. Direct delivery of the *Bordetella pertussis* adenylate cyclase toxin to the MHC class I Ag presentation pathway. J. Immuno. 162: 1910-1916.

Guiso N, Szatanik M, Rocancourt M. 1990. *Bordetella pertussis* adenylate cyclase: A protective antigens against lethality and bacterial colonization in murine respiratory and intracerebral models. Bethesda, MD, department of human Services, United state Public health service, pp207-231.

Gupta RK, Saxena SN, Suniti BS and Ahuja S. 1988. The effect of purified pertussis components and lipolysaccharide on the results of the mouse test. J. Biol. Stand. 16: 321-331.

## References

Hallander HO, Storsaeter J and Mollby R. 1991. Evaluation of serology and nasopharyngeal cultures for diagnosis of pertussis in a vaccine efficacy trail. J. Infect. Dis. May; 163 (5): 1046-54.

Halperin SA, Bortolussi R and Wort AJ. 1989. Evaluation of culture, immunofluoresenes and serology for diagnosis of pertussis. J. Clin. Mirobiol. 29 (4): 1653-1659.

Halperin SA, Heifetz SA and Kasina A. 1988. Experimental respiratory infection with *Bordetella pertussis* in mice: comparison of two methods. Clin. Investig. Med. 11: 297-303.

Halperin SA, Wang EE, Lan B, Mills E, Morris R, Dery P, Lebel M, MacDonald N, Jadvji T, Vandry W, Scheifele D, Delage G and Duclose P.1999. Epidemiological features of pertussis in hospitalized patients in Canada, 1991-1997 report of the immunization monitoring program active. Clin. Infect. Dis. Jun. 28 (6): 1238-43.

He Q, Schmidt-Schlapfer G and Just M. 1996. Impact of Polymerase Chain Reaction on clinical pertussis research: Finnish and Swiss experience. J. Infect. Dis. 174 (6): 1288-1295.

He Q, Vilijanen MK and Olander RM. 1994. Antibodies to filamentous hemagglutinin of *Bordetella pertussis* and protection against whooping cough in school children. J. Infect. Dis. 170 (3): 705-708.

Heininger U, Chery JD and Stehr K. 2004. Serologic response and antibody decay in adult with pertussis. Clin. Infect. Dis. 38: 591-594.

Herwaldt L. 1991. Pertussis in adult- what physicians need to know. Arch. Intern. Med. 151: 1510.

Hewlett EL, Gordon VM. 1988. Adenylate cyclase toxin of B. pertussis. Pathogenesis and immunity in pertussis. New York, John Wiley and Sons, pp 193-209.

Hewlett EL. 1997. Pertussis: current concept of pathogenesis and prevention. Pediatr. Infect. Dis. J. 16 (4th Suppl.): S78-S84.

Holder SL, and Mortimer EA Jr.1992. Epidemiology of pertussis and reaction to pertussis vaccine. Hopkins J. Epidemiological reviews. USA, Johns Hopkins University School of Hygiene and public health. 14: 243-267.

Holmes WH. 1940. Bacillary and Rickttsial infections. New York, Macmillan, pp 395-398.

Hoppe JE, Murray PR, Baron E, Pfaller MA Tenover FC and Yolken RH. 1998. Manual of Clinical Microbiology. 17th edition. Washington DC. ASM press. 614-624.

Hoppe JE. 1998. Pertussis current aspects of diagnosis, therapy and prevention. Ther. Umsch. 55 (1): 22-25.

## References

Ipsen J. 1961. Change in immunity and antitoxin level immediately after secondary stimulus with tetanus toxoid in rabbits. J. Immunol. 86: 50-54.

Jenkinson D. 1988. Duration of effectiveness of pertussis vaccine in United State. Br. J. Med. 296: 612-4.

Johnston IDA, Strachan DP and Anderson HR. 1998. Effect of pneumonia and whooping cough in childhood on adult lug function. New Engl. J. Med. 338 (9): 381-387.

Joo I. 1991. Epidemiology of pertussis in Hungary. Developments in Biological Standardization 73: 357–9.

Juretzko P, Kries RV, Herman M, Wirsing von konig CH, Weil J and Giani G. 2002. Effectiveness of acellular pertussis vaccine assessed ay hospital-based active surveillance in Germany. CID ; 35: 162-7.

Katz SL, Gershon AA and Hotez PJ. Krugman's infectious disease of children. 10th edition. Mosby. 335-361.

Kaufman S and Bruyn HB. 1975. Pertussis, a clinical study. Am. J. Dis. Child. 99: 417-22.

Kendrick PL, Gottshall RY, Anderson HD, Volk VK, Bunney WE and Top FH. 1969. Pertussis agglutinins in adults. Public health Rep. 84: 9-15.

Kerr, M. A., R. L. Mazengera, and W. W. Stewart. 1990. Structure and function of immunoglobulin A receptors on phagocytic cells. Biochem. Soc. Trans. 18:215–217.

Khattak MN, Matthews RC, Burnie JP. 1992. Is *Bordetella pertussis* clonal?. British Medical Journal. 304: 813-815.

Kimura M, Kuno-Sakai H, Sato Y, Kimura H, Nill R and Isomura S. 1991. A comparative trial of the reactogenicity and immuogenicity of Takeda acellular pertussis vaccine combined with tetanus and Diphtheria toxoids. J. Dis. Child. 145: 734-741.

Kimura M, Kuno-Sakai H. 1990. Developments in pertussis immunisation in Japan. Lancet 336(8706): 30–2.

Klement E, Kagan N, Hagian L, Kayouf R, Boyman J, Cohen D and Orr. N. 2005. Correlation of IgA, IgM and IgG antibody-detecting assays based on Filamentous hemagglutinin, pertussis toxin and *Bordetella pertussis* sonicate in a strictly adult population. Epidemiol. Infect. Feb; 133(1): 149-58.

Koster K, Rifflelmann M, Dohrn B and Wirsing von Konig CH. 2000. Comparison of five commercial ELISA for detection of antibodies to *Bordetella pertussis*. Clin. Duagn. Lab. Immunol. 7 (3): 422-426.

Kurt TL, Yeager AS, Guenette S. 1972. Spread of pertussis by hospital staff. JAMA 221:264.

## References

Lagergern G. 1973. The white blood cell count and ESR in pertussis. Acta. Pediatr. 52: 405.

Lambert HJ. 1985. Epidemiology of a small pertussis outbreak in Kent County-Michigan. Pub. Health. Rep. 8: 365-9.

Lawrence AJ and Paton JC. 1987. Efficacy of Enzyme-Linked Immunosorbent Assay for rapid diagnosis of *Bordetella pertussis* infection. J. Clin. Microbiol. 25 (11): 2102-2104.

Lenette EH, Spindling E and Hand Truant JP. 1974. Manual of clinical microbiology. 5th edition. Washington society for microbiology. 29-35, 303-314.

Lewis, S. M. , Bain, B. J. and Bates, I. 2001. Dacie and Lewis practical hematology. 19th ed. Churchill livingstone. Pp: 1-5.

Liese LG, Renner C, Stojanor S, Belohradsky BH and the Munich Vaccine Study Group. 2003. Clinical and epidemiological picture of *Bordetella pertussis* and Bordetella parapertussis infection after introduction of acellular pertussis vaccine. Arch. Dis. Child. ; 88: 684-687.

Lin PY. ,Chin PW. , Wang YH. , Su LH. , Chia JH. , Hang YC. , Chung PW. , Wu TL. and Lin TY. 2004. *Bordetella pertussis* infection in northern Taiwan, 1997-2001. J Microbiol Immunol Infect ; 37:388-294.

Linnemann CC, Ramundo N, Perlstin PH. 1975. Use of pertussis vaccines in an epidemic involving hospital staff. Lancet 2:540.

Locht C and Antoine R. 1997. Bacterial toxins – tools in cell biology and pharmacology. 1st ed. Chapman & Hall. (3): 33-60.

Long SS, Welko C and Clark J. 1990. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J. Infect. Dis. 161: 480-486.

Luttinger P. 1977. The epidemiology of pertussis. Am J Dis Child. 290-315.

MacAulay ME. 1979. The serological diagnosis of whooping cough. J. Hyg. 83: 95-102.

MacAuly ME. 1981. The IgM and IgG response to *Bordetella pertussis* vaccination and infection. J. Med. Micronbiol. 14: 1-7.

Mahon BP, Ryan MS, Griffin F and Mills KH. 1996. Interlukin-12 is produced by macrophages in response to live or killed *Bordetella pertussis* and enhance efficacy of an acellular vaccine by promoting induction of Th1 cells. Infect. Immun. 64 (12): 5295-5301.

Mahon, B.P., Sheahan, B.J., Griffin, F., Murphy, G., Mills, K.H. 1997. Atypical disease after *Bordetella pertussis* respiratory infection of mice with targeted disruptions of interferon-γ receptor or immunoglobulin μ chain genes. J. Exp. Med. 186:1843-1851.

## References

Mandell GL, Bennett JE and Dolin R. 2000. Douglas and Bennett's principle and practice of infectious disease. 5th ed. New York: Churchill Livingstone. 78-84.

Manklark CR and Hill JC. 1984. Bordetella. Joklik WK, Willett HP and Amons DB. Zinesser Microbiology. 18th edition. USA appletion-century crofts. Narwalk: Connecticut: 519-526.

Marcon, M. J., A. C. Hamoudi, H. J. Cannon, and M. M. Hribar. 1987. Comparison of throat and nasopharyngeal swab specimens for culture diagnosis f *Bordetella pertussis* infection. J. Clin. Microbiol. 25:1109–1110.

Mason, P. 2004. Why, what's and when's of blood tests. The Pharmaceutical Journal 272: 419-421.

Mastrantonio P, Stefanelli P, Giuliano M, Herrera-Rojas M and Tozzi AE. 1998. Bordetella parapertussis infection in children, Epidemiology and microbiology of isolates. J. Clin. Microbiol. 36 (4): 999-1002.

Meade, BD, G. Mink, C. R. Manclark. 1990. Serodiagnosis of pertussis,. In Proceedings of the Sixth International Symposium on Pertussis. Food and Drug Administration, Bethesda, Md. p. 322-329.

Mertsola J, Kuronen T, Turunen A, Viljanen MK and Ruvskanen O. 1984. Diagnosis of pertussis. J. Infect. Dis. 8: 149-156.

Mertsola J, Runskanen O, Kuronen T and Viljanen MK. 1990. Serologic diagnosis of pertussis comparison of enzyme-linked immunosorbent assay (ELISA) and bacterial agglutination. J. Infect. Dis. 147 (2): 252-257.

Miller CL and Fletcher WB. 1976. Severing of notified whooping cough. Br. Med. J. Jan 17; 1 (6002): 117-9.

Mills KHG, Ryan E, Ryan M and Mahon BP. 1998. A murine model in which production reveals complementary roles for humoral and cell mediated immunity in protection against Bordetella pertussis. Infect. Immun. 66 (2): 594-602.

Mills, K.H., Barnard, A., Watkins, J., Redhead, K. 1993. Cell-mediated immunity to Bordetella pertussis: role of Th1 cells in bacterial clearance in a murine respiratory infection model. Infect. Immun. 61:399-410.

Mink CM, Cherry JD, Christenson P. 1992. A search of *Bordetella pertussis* in university students. Clin infec Dis 14:464.

Mink CM, Sirota NM and Nugent S. 1994. Outbreak of pertussis in a fully immunized adolescent and adult population. Arch. Pediatr. Adesc. Med. 148: 153-157.

Moffitt MC and Frank MM. 1994. Complement resistance in microbes. Spring Semin. Immunopathol. 15: 327-344.

## References

Mohammed SH. 1997. Epidemiology of malaria in Al-Tamim procince, with study of some biochemical changes in blood infected with Plasmodium vivax. M. Sc. Thesis. Tikrit University.

Monack D, Munoz JJ, Peacock MG.1989. Expression of pertussis toxin correlates with pathogenesis in Bordetella species. J Infect Dis 159:205.

Mooi FR, van Orischot H, Heuvelman K, Vander Heide HGJ, Gastra W and willems RJL. 1998. Polymorphism in the *Bordetella pertussis* virulence factors P. 69/pertactin and pertussis toxin in the Netherlands: temporal trend and evidence of vaccine-driven evolution. Infect. Immun. ; 66: 670-5.

Mooi FR, van Orischot H, Pecters J and Willens RJL. 1995. Antigenic variation of the acellular vaccine component pertactin in the Dutch *Bordetella pertussis* population. Bilthoven, the Netherlands: National Institute of Public Health and the environment. Annual Report. 74-5.

Mortimer EA Jr. 1990. Pertussis and its prevention, a family affair. The J. of Infect. Dis. 161: 473-479.

Mortimer EA Jr. 1990. Pertussis vaccine. Lancet. 1-33.

Mortimer EA Jr. 1991. Pertussis and pertussis vaccine, 15 years of change. Pediatr. Infec. Dis. 2 (2): 82-90.

Mortimer EA Jr. 1998. Pertussis (whooping cough), Katz SL, Gershon AA, and Hotez PJ. Infectious disease of children. 10th edcition. USA Company. 335-349.

Mortimer EA Jr. 1998. Pertussis. Evans AS and Brachman PS. Bacterial infections of humans, epidemiology and control. 3rd edition. New York: Pelnum medical book company: 529-543.

Muller FMC, Hoppe JE and Wirsing-von Konig CH. 1997. Laboratory diagnosis of pertussis, state of the Artin 1997. J. Clin. Microbiol. 35 (10): 2435-2443.

Munoz JJ. 1985. Biological activities of pertussigen (pertussis toxin). Infect. Immun. 33: 820-826,

Nagel J and poot-Scholtens EJ. 1983. Serum IgA antibodies to *Bordetella pertussis* as an indicator of infection. J. Med. Micobiol. 16: 417-426.

Nelson JD. 1978. The changing epidemiology of pertussis in young infants. The role of adults as reservoirs of infection. Am. J. Dis. Child. 132: 371-6.

Nelson WE, Behrman RE, Kliegman RM and Arvin AM. 1996. Nelson text book of pediatrics. 15th edition. United State. W. B. Saunders Company. 779-784.

Nester EW, Roberts CE, McCatrhy BJ and Pearsall NN. 1973. Microbiology, Molecules, Microbes and Man, USA. Holt Rinehart and Winston. 460-461.

## References

Nicosia A, Bartoloni A, Perugini M and Rappuoli. 1987. Expression and immunological properties of the five subunits of pertussis toxin. Infect. And Immun. 55 (4): 963-967.

Nielsen AM and Larsen SO. 1991. Whooping cough necessitating hospitalization in Denmark, 1980-1986. Ugeskr Laeger. May. 20. 153 (21) : 1477-82.

Noble GR, Bernier RH, Esber EC, et al. 1987. Acellular and whole-cell pertussis vaccines in Japan. Report of a visit by US scientists. JAMA 257(10): 1351–6.

Oda M, Cowell JL and Burstyn AC. 1984. Protective activities of filamentous hemagglutinin and the lymphocytosis-promoting factor of *Bordetella pertussis* in mice. J. Infect. Dis. 150: 427-469.

Olin P, Rasmussen F, Gustafsson L, Hallander HO and Heijbel H. 1997. Randomized controlled trail of two-component, three-component acellular pertussis vaccines compared with whole cell vaccine. Lancet. 350: 1569-1577.

Olin, P., and J. Storsaeter. 1989. The efficacy of acellular pertussis vaccines. JAMA 261:560.

Olson, L. C. 1975. Pertussis. Medicine 54:427–469.

Onorata IM, Wassilak SGF. 1987. Laboratory diagnosis of pertussis: the state of art. Pediatr. Infect. Dis. 6: 145-151.

Pagliaccia C, Manetti R and Rappuoli R. 1997. Pertactin antigens extract from *Bordetella pertussis* and Bordetella bronchiseptica differ in the isoelectric point. Arch. Microbiol. 168 (5): 437-440.

Parton R. 1996. New perspective on *Bordetella* pathogenicity. J. Med. Misrobiol. 44: 233-235.

Peppolni S, Pizza M, Magistris MTD, Batoloni A and Rappuoli R. 1995. Acellular pertussis vaccine composed of genetically inactivated pertussis toxin. Physiol. Chem. Phys & Med. NMR. 27 : 355-361.

Persson CG, Erjefalt AI, Alkner U, Baumgarten C, Grief L, Gustafsson B, Luts A, Pipkorn U. Sundler F, Svensson C and Wollmer P. 1991. Plasma exudation as a first line respiratory mucosal defence. Clin. Exp. Allergy. 21: 17-24.

Pittman M. 1979. Pertussis toxin: The cause of the harmful effect and prolonged immunity of whooping cough. A hypothesis. Rev. Infect. Dis. 1: 401-412.

Pittman M. 1984. The concept of pertussis as a toxin-mediated disease. Pediatr. Dis. J. 3: 467-486.

Pizza M, Covacci A and Batoloni A. 1989. Mutants of pertussis toxin suitable for vaccine development. Science. J. 246: 497-500.

## References

Plotkin SA ande Cadoz M. 1997. The acellular pertussis vaccine trails: an interpretation. Pediatr. Infect. Dis. J. 16: 508-617.

Pollard R. 1980. Relation between vaccination and notification rates for whooping cough in England and Wales. Lancet 1(8179): 1180–2.

Poltkin SA and Orenstein WA. 1999. Vaccines. W B sanders Co. 3rd edition: 293-294.

Polyzon A, Pouranras S, Dafni V, Sofianou D, Christeli E, Patrion S and Tsakris A. 2004. Seroepidemiology of *Bordetella pertussis* immune response in a healthy population in northern Greece. J. Clin. Lab. 18 (3): 211-4.

Preston NW and Stanbridge TN. 1972. Efficacy of pertussis vaccines: A brighter horizon. BMJ. 3: 448-451.

Preston NW. 1976. Prevalence serotype of *Bordetella pertussis* in non-vaccinated communities. J. Hyg. Camb. 77: 85-91.

Preston NW. 1985. Change in the serotype of pertussis infection in Britain. Lancet i:510.

Public Health Laboratory Service. 1973. Efficacy of whooping cough vaccines used in the United Kingdom before 1968. BMJ. 1: 259-262.

Redhead K, Watkins J, Barnard A and Mills KHG. 1993. Effective immunization against *Bordetella pertussis* respiratory infection in mice is dependent on induction of cell-mediated immunity. Infect. Immun. 16: (8): 3198-3205.

Robinson A, Gorringe A and Fernandez M. 1989. Serospecific protection of mice against intranasal infection with *Bordetella pertussis* vaccine. 7: 321-324.

Rodgers, RPC. 1994. Clinical laboratory methods for detecting of antigens and antibodies. In Stites, Terr DP & Parslow TG. Basic & clinical immunology. 18th ed. Prentice-Hall International Inc., London. pp 151-194.

Rodrignez ME, Hasbro DF and Yantorno OM. 1996. Effect of hydromechanical stress on cellular antigens of Bordetella pertussis. J. Ind. Mricrobiol. 17 (1): 53-55.

Romanus V, Jonsell R, Bergquist SO. 1987. Pertussis in Sweden after the cessation of general immunization in 1979. Pediatric Infectious Disease Journal 6(4): 364–71.

Rota MC, Ausiello CM and Damelio R. 1998. Prevalence of markers of exposure to *Bordetella pertussis* among Italian young adults. Clin. Infect. Dis. 26: 297-302.

Ruijs GJ, groenendijk TW, Biever M. 1991. Shelf life of prepared Bordet-Gengou and Regan-Lowe agar plates forisolation of Bordetella pertussis. European Journal of Clinical Microbiology and infectious diseases 10:974-978.

Ruuskanen O, Viljanen MK, Salmi TT, Lehtonen OP and Peltonen T. 1980. DTP and DTP-inactivated polio vaccines: comparison of adverse reactions and

IgG, IgM and IgA antibody responses to DTP. Acta. Paediatr. Scand. 69: 177-182.

Ryan, M., Murphy, G., Gothefors, L., Nilsson, L., Storsaeter, J., Mills, K.H. 1997. *Bordetella pertussis* respiratory infection in children is associated with preferential activation of type 1 T helper cells. J. Infect. Dis. 175:1246-1250.

Saleh RH. 2000. A comparative serological study of whooping cough in vaccinated children in Salah Al- Deen Province. M. Sc. Thesis. Tikrit University.

Samz JC, Fernandez M, Sagues MJ, Ramirez R, Castaveda R, Barranco D and de Org F. 2002. Comparison of three Elisa techniques for the evaluation of IgG seroprevalence against *Bordetella pertussis* in children vaccinated with three doses of DTP. Emferm. Infect. Microbiol. Clin. Jan; 20 (1): 10-15.

Sanchez I, Repetto G, Saenger A. 1994. Whooping epidemiology in Chile (1950-1990). The adult as new reservoir of infction. Rev. Med. Chil.122 (3) : 334-45.

Sapian-Lopez LA, Valdespino JL, Salvatierra B, Tapia-conyer R, Gutierrez G, Macedo J and Sepu;veda J. 1992. Seroepidemiology of whooping cough in Mexico. Salud. Publica. Mex. Mar-Apr; 34 (2) : 177-85.

Sato H and Sato Y. 1984. *Bordetella pertussis* infection in mice: correlation of specific antibodies against two antigens, pertussis and filamentous hemagglutinin with mouse protectively in an intracerebral or aerosol challenge system. Infect. Immun. 46: 415-421.

Sato H, Sato Y. 1991. Relationship between structure and biological and protective activities of pertussis toxin. Dev. Biol. Stand. 73: 121-132.

Sato T, Akamatsu T and Aoyama T.1990. Efficacy of acellular pertussis vaccine. Kansenshogaku-Zasshi. 64 (5): 564-569.

Sato Y, Kazumi I, Sato H, Cowell JL and Manclarck CR. 1985. Role of antibody to filamentous hemagglutinin and to leukocytosis promoting factor-hemagglutinin in immunity to pertussis. Semin. Infect. Dis. 4: 380-385.

Sato Y, Lzumiya K, Sato H, Cowell JL and Manclarck CR. 1980. Aerosol infection of mice with Bordetella pertussis. Infect. Immun. 29: 261-266.

Sato Y. 1981. Role of antibody to leukocytosis-promoting factor, Hemagglutinin and to filamentous hemagglutinin in immunity to pertussis. Infect. Immun. 1223-1231.

Saukkonen, K., Cabellos, C., Burroughs, M., Prasad, S., Tuomanen, E. 1991. Integrin-mediated localization of *Bordetella pertussis* within macrophages: role in pulmonary colonization. J. Exp. Med. 173:1143-1149.

## References

Shahin RD, Witvliet MH and Manclarck CR. 1990. Mechanism of pertussis toxin β oligomer-mediated protection against *Bordetella pertussis* respiratory infection. Infect. Immun. Dec; 58 (12): 4063-4068.

Shefer A, Dales L, Nelson M. 1995. Use and safety of accelular pertussis vaccine among adult hospital staff during a outbreaj of pertussis. J infect Dis 171: 1053.

Sherris JC, Ryan KJ, Ray CG, Pleorde JJ, Corey L and Spizizen J. 1984. Medical microbiology an introduction to infectious disease. New York: Elsevier science publishing, Co. Inc. 223-225.

Siber GR, Samore MW. Pertussis. In: Fauci AS, 1998. Harrison's Principles of Internal Medicine, 14th ed. McGraw Hill, New York, 933–35.

Simondon F, Lteman I, Preziosi MP, Yan A and Guiso N. 1998. Evaluation of immunoglobulin G Enzyme Linked Immunosorbent Assay for pertussis toxin and hemagglutinin in diagnosis of pertussis in Senegal. Clin. Diagn. Lab. Immunol. 5 (3): 130-134.

Sitarska – Golebiowska J. and Zielinski A. 2003. Pertussis in Poland in 2001. Przegl Epidemaiol; 57 (1): 27-32.

Stanbridge TN and Preston NW. 1974. Variation of serotype in strain of *Bordetella pertussis*. J. Hyg. Camb. 73: 305-310.

Stewart TA, Nolan TM, Hogg G, and Mcewen J. 1996. Antibodies to Diphtheria, Tetanus and pertussis in infants before and after immunization with DTP (triple antigen) vaccine. J. Pediatr. Child. Health. 32 (5): 378-381.

Storffolini T, Giammanco A, De Crescenzo L, Lupo F, Nicosia V, Vanlenza AR, Cascio A, Tauormina S and Nistico L. 1989. prevalence of pertussis IgG antibodies in children in Palermo, Italy. Sep. Oct. 17 (5): 280-3.

Streble PM, Cochi SL, Farizo KM, Payne BJ, Hanauer SD and Baughlan AL. 1993. Pertussis in Missouri: Evaluation of nasopharyngeal culture, direct fluorescent antibody testing and clinical case definitions in the diagnosis of pertussis. Clin. Infect. Dis. Feb. 16 (2): 276-85.

Sydabubakar SN. , Mathewes RC. , Preston NW. , Owen D. and Hillier V. 1995. Application of pulsed field gel electrophoresis the 1993 epidemic of whooping cough in the UK. Epidemiol Infect. Aug; 115 (1): 101-13.

Tamura M, Nogimori K and Murai S. 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry. 21: 5516-5522.

Tanaka M. , Vitek CR. , Pascual FB. , Bisgard KM. ,Tate JE. And Morphy TV. 2003. Tends in pertussis among infants in the United State, 1980-1999. JAMA. Dec 10; 290 (22): 2968-75.

## References

Tang YW, Hopkins MK and Kolbert CP. 1998. *Bordetella holmessi*-like organisms associated with septicemia, endocarditis and respiratory failure. Clin. Infect. Dis. 26: 389-392.

Tennis PFM, van der Heijden OG, de Melker HE, Schellekens JFP, Versteegh FGA and Kretzschmar. 2002. Kinetics of the IgG antibody response to pertussis toxin after infection with Bordetella pertussis. Epidemiol. Infect. 129: 479-489.

Thomas MG, Ashworth LAE, Miller E and Lamert HP. 1990. Nasal immunoglobulin A response to pertussis toxin, filamentous hemagglutinin 2 and 3 after infection with *Bordetella pertussis* and immunization with whole-cell vaccine, p 330-334. In C. R. Manclark (ed), preceding of the Sixth International Symposium on pertussis. Department of health and human Service publication No. 90-1164. Department of Health Services, Bethesda, Md.

Thompson RA. 1977. Techniques in clinical immunology. 1st edition. Black Well scientific publication, 43-64.

Tietz NW. 1970. Fundamental of clinical chemistry. 3rd edition. Convention: Saunders Company. 75-83.

Tomoda T, Ogura H and Kurashige T. 1991. Immune response to *Bordetella pertussis* infection and vaccination. J. Infect. Dis. Mar; 163 (3): 559-63.

Tozzi AE, Atti ML, Wassilak SGW, Salmaso S, Panei P and Anemona A. 1998. Predictors of adverse events after the administration of acellular and whole cell diphtheria-tetanus-pertussis vaccine. Vaccine. 16 (2/3): 320-322.

Trollfors B , Taranger J, Lagergard T, Sundh V, Bryla DA, Schneerson R and Robbins JB. 1999. Serum IgG antibody response to pertussis toxin and filamentous hemagglutinin in unvaccinated and vaccinated children and adults with pertussis. Clin. Infect. Dis. Mar; 28 (3): 552-9.

Trollfors B, Zackrisson G, Taranger J and Lagergard T. 1992. Serum antibodies against a 69-Kilo Dalton outer membrane protein, pertactin, from *Bordetella pertussis* in non-vaccinated children with and without a history o clinical pertussis. J. Pediatr. 120 (6): 924-926.

Tuomanen E and Weiss A. 1985. Characterization of two adhesins of *Bordetella pertussis* for human ciliated respiratory epithelial cells. J. Infect. Dis. 152: 118-125.

Tuomanen EI, Zapianin LA, Galvan P and Hewlett EL, 1984. Characterization of antibody inhibiting adherence of *Bordetella pertussis* in human respiratory epithelial cells. J. Clin. Microbiol. Aug; 20 (2): 167-170.

Ulrich H. , Kerstin K. , Klemen S. and Cherry JD. 1997. Clinical findings in *Bordetella pertussis* Surveillance study. Pediatrics; 100: 1-6.

References

UNICEF/Iraq. 1998. Situation analysis of children and women in Iraq. 30 April 1998. pp: 49.

van den Berg BM, David S, Beekhuizen H, Mooi R and van Furth R. 2000. Protection and humoral immune response against *Bordetella pertussis* infection in mice immunized with acellular or cellular pertussis immunogens. Vaccine. Dec; 19 (9-10): 118-28.

Van der Zee, A., C. Agterberg, M. Peeters, F. Mooi, and J. Schellekens. 1996. A clinical validation of *Bordetella pertussis* and Bordetella parapertussis polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J. Infect. Dis 174:89–96.

Van Savage J, Decker MD and Edwards KM. 1990. Natural history of pertussis antibody in the infant and effect on vaccine response. J. Infect. Dis. 161: 487-492.

Van Spriel, A. B., and J. G. J. van de Winkel. 1999. CD89 (FcaRI) review. Encycloped. Mol. Med., in press.

Vancanneyt M, Vandamme P and Kersters K. 1995. Differentiation of Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica by whole-cell protein electrophoresis and analysis. Int. J. Syst. Bacteriol. 45 (4): 843-847.

Vandamme P, Heyndrickx M, and Vancanneyt M. 1996. *Bordetella trematum* sp. nov. isolated from wounds and ear infections in humans, and reassessment of *Alcaligenes denitrificans* Ruger and Tan 1983. Int. J. Syst. Bacteriol. 46: 849-858.

Vandamme P. Hommez J. 1995. Bordetella Spp isolation from poultry and humans, Int J Syst Bacteriol, 45: 37-45.

Varughese P. 1985. Incidence of pertussis in Canada. CMAJ 132(9): 1041–2.

Viljanen MK, Ruuskanen O, Granberg C and Salmi TT. 1982. Serological diagnosis of pertussis: IgM , IgA and IgG antibodies against *Bordetella pertussis* measured by enzyme linked immunosorbent assay (ELISA). Scand. J. Infect. Dis. 14: 117-122.

Vitek CR, Pascual FB, Baughman AL and Murphy TV. 2003. Increase in deaths from pertussis among young infants in the united State in 1990s. Pediatr. Infect. Dis. J. Jul; 22(7) : 628-34.

Ward J. 2001. Pertussis epidemiology and acellular vaccine efficacy in older children. Can. Commun. Dis. Rep. 26: 1-8.

Ward J. 2002. The APERT study. Canada Communicable Disease report. Vol. 2953.

Wassilak S, Smith G, Garbe P, Burstyn D, Manclarck C and Orenstein W. 1983. Pertussis outbreak in Colorado: use of newly-developed diagnostic test,

P. 37-41. in RF Campolucci (ed.), 18th immunization Conference proceedings. Center fro disease control, Atlanta.

Weiss AA, Goodwin M St. M, Allison N. 1990. Investigation of the role of B. pertussis virulence factors in disease. Bethesda, MD, department of human Services, United state Public health service, pp202-205.

Weiss AA, Hewlett EL. 1984. Pertussis toxins and extracytoplasmic adenylate cyclase as virulence factors of B. pertussis, J Infect Dis, 150: 219-22.

Weiss AA, Mobberley PS, Fernandez RC and Mink CM. 1998. Characterization of human bactericidal antibodies to Bordetella pertussis. Infect. Immun. Mar; 67 (3): 1424-1431.

Weyant RS, Hollis DG and weaver ER. 1995. *Bordetella holmessi* sp. nov. a new gram-negative species associated with septicemia. J. Clin. Microbil. 33: 1-7.

White JM, Fairley CK, Owen D. 1996. The effect of an accelerated immunisation schedule on pertussis in England and Wales. Communicable Disease Report. CDR Review 6(6): R86–91.

WHO. 2003. Communicable disease profile in Iraq. Update 19 March 2003. 6-7.

Wilkins J, Williams FF, Wherle PF and Portnoy B. 1971. Agglutinin response to pertussis vaccine. I. Effect of dosage and interval. J. Pediatr. 79: 197-202.

Wirsing van Konig CH and Schmitt HJ. 1996. Epidemiological aspects and diagnostic criteria for a protective efficacy field trail of a pertussis vaccine. The J. Infect. Dis. 74 (supple 3): S281-S286.

Wirsing van Konig CH, Roh H, Bogaerts H and Schmitt HJ. 1998. A serologic study of organism possibly associated with pertussis-like coughing. Pediatr. Infect. Dis. J. 17: 645-649.

Wirsing von Konig CH, Gounis D, Laukamp S, Bogaerts H and Schmitt HJ. 1999. Evaluation of single serum sample serological technique for diagnosing pertussis in unvaccinated children. Eur. J. Clin. Microbiol. Infect. Dis. 18: 341-345.

Wong KH and Skelton SK. 1988. New practical approach to detecting antibodies to pertussis toxin for public health and clinical laboratories. J. Clin. Microbiol. ; July. 26 (7): 1316-1320.

Wright SW, Decker MD and Edwards KM. 1999. Incidence of pertussis infection in health care workers. Infect. Control. Hosp. Epidemiol. 26: 120-3.

Wright SW, Edward KM, Decker MD. 1995. Pertussis infection an adult with persistent cough. JAMA 273:1044.

Wyss O and Eklund C. 1971. Microorganism and man. New York: Jimntene Onsly. 245-248,254.

## References

Yih WK. , Lett SM. , Vignes FN. , Garrison KM. , Sipe PL. and Marchant CD. 2000. The increasing incidence of pertussis in Massachusetts Adolescents and adults, 1989-1998. JID ; 182 November: 1409-16.

Zackrisson G, Lagergar T, Trollfors B and Krautz I. 1990. Immunoglobulin A antibodies to pertussis toxin and filamentous hemagglutinin in saliva from patients with pertussis. J. Clin. Microbiol. 28: 1502-1505.

Zielinski A. 2004. Pertussis in Poland in 2002. Przegl Epidemiol. ; 58(1): 29-34.

# الخلاصة


**دف هذه الدراسة الى وصف بعض الخصائص الوبائية للسعال الديكي** في محافظة بابل **وتقييم الأضداد المتخصصة للـ *Bordetella pertussis* IgG و IgA** باستخدام تقنية الـ **ELISA للتشخيص المختبري للسعال الديكي، هذا بالاضافة الى قياس الاستجابة المناعية الخلطية للقاح الثلاثي (DPT).**

**جمعت عينات الدم من: 68 طفل مصاب بالسعال ديكي راقد** في **المستشفى (36** غير **ملقح و 32 ملقح) ، 103 من اشخاص اسوياء (49 شخصا ملقحا، 54 شخصا** غير **ملقح) و 20 من العاملين بالخدمة الصحية** في محافظة **بابل.**

**اضداد *Bordetella pertussis* IgG و IgA** تم **قياسها بواسطة تقنية** الـ **ELISA لكل مجموعات الدراسة. التعداد الكلي والتفريقي للخلايا البيضاء اجري** للـ**68 مريض و 20 من مجموعة السيطرة.**

**اظهرت الدراسة ان اعلى مستوى حدوث للسعال الديكي لوحظ** في **المرضى اقل من عمر سنة (49% من مجموع الحالات). كانت نسبة الذكور للاناث 1.27 :1. 71% من المرضى مقيمين** في **المناطق الريفية مقارنة بـ 29 %** في **المناطق الحظرية. 36 (53%) من المرضى كانوا غير ملقحين و 26 (38%) كانوا ملقحين جزئيا، بينما 6 (9%) تلقوا تلقيحا كاملا (3 جرعات من اللقاح الثلاثي). لوحظت مضاعفات السعال الديكي** في **88% من المرضى، والتي كانت بصورة رئيسية الاختناق (88%)، اختلاجات (26%)، ذات الرئة (29%)، والوفاة (4%). كما لوحظ ارتفاع** في **تعداد الخلايا البيضاء** في **64% من المرضى و ارتفاع** في **تعداد الخلايا اللمفاوية** في **69% من المرضى. معظم الحالات سجلت خلال الفترة** بين **شهر نيسان و تموز.**

**كانت الحساسية والتخصص التشخيصي لاضداد الـ *Bordetella pertussis* IgG** في **الاطفال غير الملقحين كانت 77% و 100% على التوالي،** في حين **كانت الحساسية والتخصص التشخيصي لأضداد *Bordetella pertussis***

IgG في الاطفال الملقحين 67%، 91% على التوالي. كانت الحساسية والتخصص التشخيصي لاضداد الـ *Bordetella pertussis* IgA في الاطفال غير الملقحين كانت 33% و 100% على التوالي، في حين كانت الحساسية والتخصص التشخيصي لأضداد *Bordetella pertussis* IgA في الاطفال الملقحين 72%، 86% على التوالي. لوحظت افضل حساسية وتخصص تشخيصي في العينات التي جمعت بعد أكثر من 21 يوما بعد ظهور أعراض السعال الديكي.

تم الحصول على أعلى حساسية وتخصص تشخيصي (92، 82% على التوالي) بأستعمال مستوى موجب لـ أضداد *Bordetella pertussis* IgG او IgA.

أظهرت الدراسة ان متوسط مستوى اضداد *Bordetella pertussis* IgG في الاسوياء الملقحين كانت U/ml 56.41 (المدى 19.77-143.89 U/ml)، بينما بلغ متوسط اضداد *Bordetella pertussis* IgG في الاسوياء غير الملقحين U/ml 29.33 (المدى 1.01-56.9 U/ml).

كان متوسط مستوى اضداد *Bordetella pertussis* IgA في الاسوياء الملقحين كانت U/ml 9.05 (المدى 3.6-29.74 U/ml)، بينما بلغ متوسط اضداد *Bordetella pertussis* IgA في الاسوياء غير الملقحين U/ml 4.79 (المدى 3.05- 7 U/ml).

أظهرت الدراسة ان 10.7 % (11/103) من الاسوياء يظهرون مستوى موجب لاضداد *Bordetella pertussis* IgG أو IgA، في حين اظهر 70% (14/20) من العاملين في الخدمة الصحية في هذه الدراسة مستوى موجب لاضداد *Bordetella pertussis* IgG أو IgA .