Iranian J Publ Health, Vol.38, No.1, 2009, pp.100-111

Original Article

Short Term Reactogenicity of a Triple Diphtheria-Tetanus-Whole Cell Pertussis Vaccine in Iranian Infants

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(Received 13 Jul 2008; accepted 28 Feb 2009)

Abstract

Background: Immunization against diphtheria, tetanus and pertussis (DTP) has long been applied in Iran using whole cell vaccine. Despite the role of whole cell DTP (DTwP) vaccine in reduction of mortality as a result of disastrous diseases such as diphtheria, tetanus, and pertussis, serious local and systemic complications have been attributed to these vaccines. This study was performed to determine the complications of DTwP vaccine in infants attending some of the health centers of Tehran in 2006-2007.

Methods: In this prospective study, 330 infants were injected with DTwP vaccine manufactured by Razi Institute of Iran. All subjects received DTwP vaccine at 2, 4, and 6 months of age following the national vaccination schedule of Iran. Reactogenicity was assessed by the parents for 7 days post-vaccination using diary cards.

Results: Of the 279 infants who completed the vaccination study, pain was the most frequent local reaction after the primary vaccination (68.1-75.3%). The mean diameters of the redness and swelling at first day post-vaccination were 2.81±6.91 and 2.60±7.93 mm in the first dose, 2.40±6.25 and 1.94±5.74 mm in the second dose and 2.24±5.66 and 2.16±6.03 in the third dose, respectively. Fever (axillary temperature >37.5° C) was the most frequently reported systemic reaction during the primary vaccination (53.8-58.8%). All systemic reactions observed after each dose were either reduced or completely disappeared during a week.

Conclusion: The high incident of complications observed following vaccination with this cellular triple vaccine may be related to the formulation or the bacterial cell fragments used in vaccine production.

Keywords: *Diphtheria-Tetanus-Pertussis Vaccine, Vaccination, Reactogenicity, Infant, Local reaction, Systemic reaction, Iran*

Introduction

Today, immunization of infants against diphtheria, tetanus and pertussis (DTP) is a common practice in most regions of the world. As early as 1925, the vaccine was shown to be effective against DTP. The advent of combination vaccines against diphtheria, tetanus and whole cell pertussis (DTwP) in the 1940s marked the beginning of routine pediatric immunization against serious and often life-threatening childhood diseases which has been part of the WHO program since its launch in 1974 (1, 2). WHO has prepared the World Health Report to help set national and international priorities in health. Such a report creates a challenge with respect to DTP (3). In 2004, WHO reported that the incidence of DTP in Iran was 6, 11 and 98 cases and in 2005 as 15, 8 and 125 cases, respectively (4). In 2002, among diseases for which vaccines are universally recommended, WHO estimated that fewer than 4,000 children aged less than 5 yr died from diphtheria; 198,000 from tetanus and 294,000 from pertussis, worldwide (5). It was the predecessor of the current DTwP vaccines that was in general use for nearly 50 yr in many

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countries, resulting in a drop in the incidence of DTP to very low levels (6-8).

In Iran, immunization against DTP has been applied since 1950s using a local vaccine manufactured by Razi Institute (Razi-DTwP) and the efficacy of the vaccine has been confirmed by previous studies (9-13). Whole-cell pertussis vaccine is a suspension of killed *Bordetella pertussis* organisms. Safety of whole-cell vaccines has been reviewed in detail, and of a range of adverse events considered, evidence suggests a causal relation only for anaphylaxis, prolonged or inconsolable crying, and febrile seizures (1). Concerns about safety have led to the development of acellular pertussis vaccines in the 1970s. Acellular vaccines (DTaP), consisting of up to five specific *B. pertussis* antigens, have been reported to induce lower incidence of both local and systemic complications (14-16).

The present article reports on the short term reactogenicity of Razi-DTwP vaccine in a group of Iranian infants receiving primary triple doses vaccination.

Material and Methods

Population

The study population comprised of 330 healthy male (n=162) and female (n=168) infants aged 2 months at the time of entry into the trial who have not been previously vaccinated against DTP. Infants were excluded if they had hypersensitivity, encephalopathy, fever > 38º C, history of seizures or other neurological disorders, a birth weight of <2500 g, known or suspected immunodeficiencies, treatment with immunosuppressive therapies, or current or planned receipt of immunoglobulins and/or any blood products.

Study design

The prospective study was conducted at 4 health centers affiliated to Shahid Beheshti University of Medical Science in Tehran City from April 2006 to June 2007. After receiving written informed consent from the parents, eligible infants were vaccinated with the Razi-DTwP vaccine which has been approved by the National Vaccination Committee of Iran for universal vaccination. To check for immediate adverse reactions, the infants were under observation for 30 min at the health centers and then were monitored by their parents for vaccine-associated reactions using diary cards. Parents recorded local and systemic reactions on diary cards daily for 7 d after each vaccination. Parents were asked to measure axillary temperature and maximal daily redness as well as swelling using uniform thermometers and ruler provided by the health centers.

Vaccine

Each dose of 0.5 ml of Razi-DTwP vaccine (DTP, Razi Vaccine & Serum Research Institute, Tehran, Iran) contained 15 Lf diphtheria toxoid, 10 Lf tetanus toxoid, 16 IU inactivated *B. pertussis* bacterial cells, 0.3 to 0.6 mg aluminum phosphate (metal ion) and 0.01% merthiolate (instruction sheet provided by the manufacturer). Each dose of vaccine was administered by deep intramuscular injection in the antero-lateral side of the thigh by AD syringes (Soloshot IX, 23 G, 0.6× 25 mm, Becton Dickinson, Fraga, Spain).

Assessment of Safety and Reactogenicity

Members of the study team observed subjects for 30 min after each injection for any immediate local or systemic reactions. Parents recorded local (injection site redness, swelling and pain/tenderness) and systemic reactions [fever (axillary temperature >37.5º C), loss of appetite, gastrointestinal symptoms (diarrhea or constipation), vomiting and eczema] on diary cards daily for seven days following each vaccination. The reactogenicity was graded on a 3-point scale (grade 1= easily tolerated, normal activity, grade 2= discomfort, interferes with normal activity, and grade 3= prevents normal activity). Pain was scored as: minor reaction to touch (grade 1); cries/ protests to touch or limb movement (grade 2) or spontaneous pain (grade 3). Tenderness/swelling diameter was graded: <5 mm (grade 1); 5-20 mm (grade 2); >20 mm (grade 3). Fever was graded: 37.5–38 ºC (grade 1); >38 and <39 ºC (grade 2); ≥39 ºC (grade 3). Other systemic reactions were recorded as yes or no. Parents were also asked to record any additional symptoms occurring within 7 d of vaccination. Parents observ-

ing a large swelling reaction of the injected limb, noticeable diffuse swelling, high temperature or noticeable increase of limb circumference were asked to contact study personnel and bring the child to the health center for evaluation as soon as possible.

Ethics

The study protocol was approved by Avicenna Institute Ethics Committee and the Food and Drug Administration and Health Administration of the Ministry of Health, Treatment and Medical Education of Iran. The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from the parents of all infants before enrollment into the study.

Statistical Analysis

Two-tailed statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Illinois). For evaluation of reactogenicity, the percentage of subjects with a given symptom was calculated for each local and systemic reaction. The data of local and systemic reactions was evaluated by a non-parametric Friedman or Cochran test as appropriate to determine the significance of trend of the decrease of reactogenicity. Binary logistic regression analysis was used to analyze the variables (sex, weight and birth weight) independently associated with local and systemic reactions in the first day of the first dose of vaccination. *P*-values less than 0.05 were considered significant.

Results

Study population

As planned, a total of 330 subjects entered the study of whom 279 (84.5%) completed the study. Twenty subjects in the second dose and 25 subjects in the third dose vaccination withdrew during the study, but none due to an adverse event. The mean age of the subjects at the time of first vaccination was 9.06±1.33 weeks, with a male: female ratio of 1:1.08. Mean birth weights, weights in the first dose, the second dose and the third dose of vaccination were 3.24±0.40, 5.12±0.72, 6.57±0.90 and 7.76±0.90 kg, respectively.

Safety and reactogenicity

All infants who had received triple doses of vaccine were included in the safety analysis. The incidence of local and systemic reactions reported during the 7 d after the first, the second and the third dose of vaccination (primary vaccination) are presented in Table 1, 2 and 3. No serious life-threatening adverse events related to vaccination were reported. The majority of local symptoms were of mild to moderate intensity (grade 1 or 2). Pain was the most frequent local reaction in the first (75.3%), the second (71.7%) and the third (68.1%) dose of vaccination and was observed to have significantly reduced during a week ($P< 0.0001$, Friedman's test). During a week post-vaccination, severity of redness and tenderness of each dose of vaccination was reduced ($P< 0.0001$, Friedman's test) (Tables 1-3). Fever (axillary temperature >37.5° C) was the most frequently reported systemic reaction during the primary vaccination, but only 4 infants in the first dose, 2 in the second dose and 3 in the third dose of vaccination displayed high fever level (grade 3, ≥39° C). During 7 d after each dose of vaccine, fever was declined ($P< 0.0001$, Friedman's test). Fig. 1 shows reducing trend of redness, swelling and body temperature during the primary vaccination course. Other systemic reactions (loss of appetite, gastrointestinal problems and vomiting), with the exception of eczema, were recorded in a small number of vaccinated infants after administration of each dose and were observed to have reduced or completely disappeared during a week. The trend of reduction of these complications over a week after vaccination was found to be significant following administration of the first, second and third vaccine doses ($P< 0.0001$, Friedman's test) (Tables 1-3). Eczema, however, was either not observed or recorded exceptionally in 1-4 subjects, during the follow up period.

The binary logistic regression model of data in the present study revealed no effect of sex, weight and birthday weight of the infants on the

local and systemic reactions in the first day of the first dose of vaccination except effect of gender (female, odds ratio= 1.892, 95%, confidence interval= 1.038-3.450, *P*= 0.037, relative male) on pain and gender (female, odds ratio= 4.895, 95%, confidence interval= 1.359-17.638, *P*= 0.015, relative male) on gastrointestinal problems.

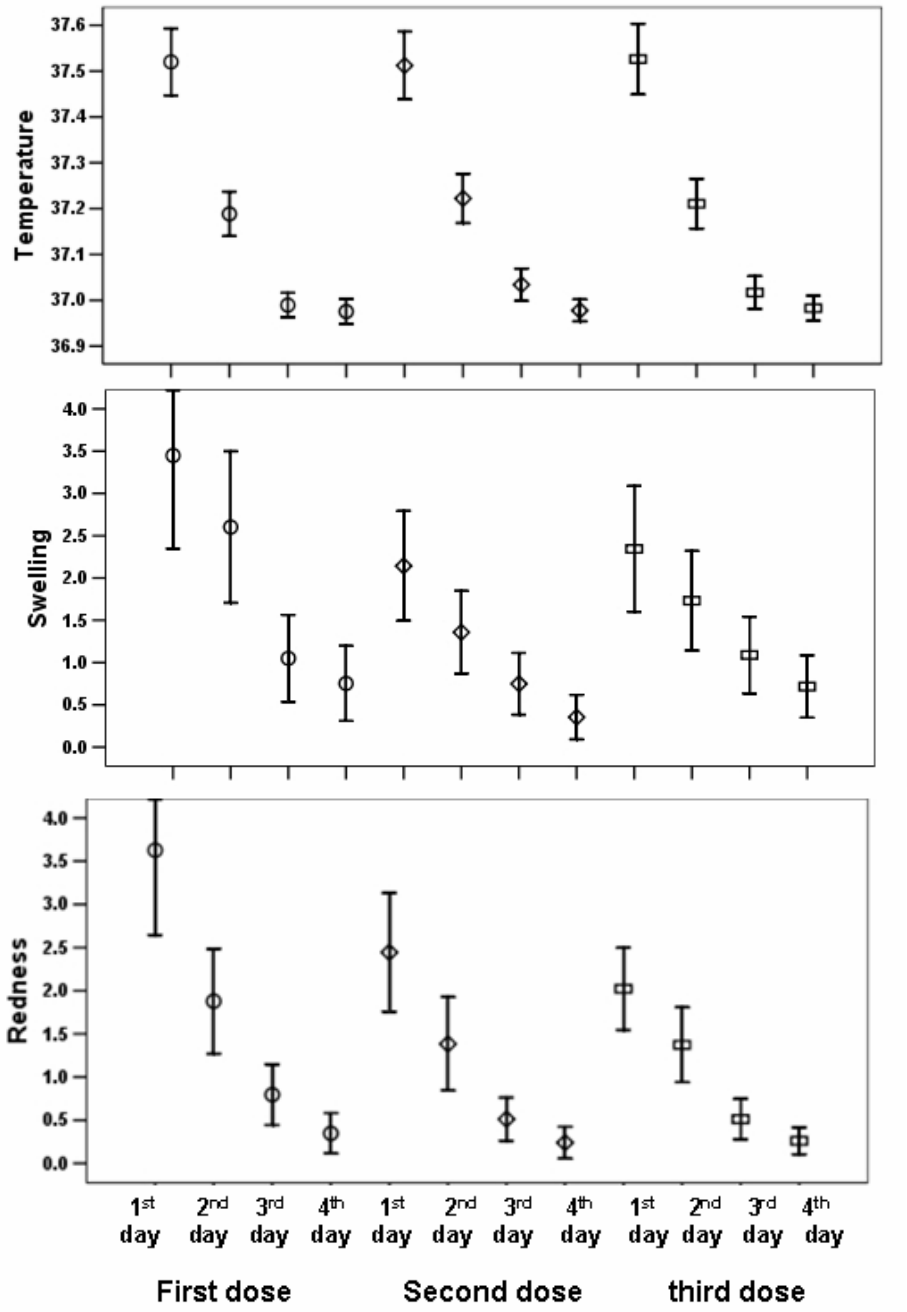

Fig. 1: Comparison of reactogenicity of DTwP vaccine in Iranian infants during the first four days of vaccination following administration of the first, the second or the third vaccine dose

(A) Body temperature, (B) Redness and (C) Swelling

Vertical bars show the 95% confidence intervals of range. (○) first dose; (◇) second dose; (▭) third dose.

Table 1: Frequency of local and systemic reactions reported during the first week follow up after administration of the first vaccine dose of DTwP vaccine in Iranian infants

Symptoms	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	*P*-Value
Local reactions								
Pain								$P < 0.0001$ [a]
No pain	69(24.7)	121(43.4)	213(76.3)	252(90.3)	265(95)	267(95.7)	269(96.4)	
Grade 1	85(30.5)	102(36.6)	51(18.3)	24(8.6)	13(4.7)	12(4.3)	10(3.6)	
Grade 2	98(35.1)	48(17.2)	13(4.7)	3(1.1)	1(0.4)	0	0	
Grade 3	27(9.7)	8(2.9)	2(0.7)	0	0	0	0	
Redness								$P < 0.0001$ [a]
No redness	167(59.9)	210(75.3)	251(90)	266(95.3)	267(95.7)	269(96.4)	270(96.8)	
Grade 1	66(23.7)	42(15.1)	12(4.3)	6(2.2)	5(1.8)	6(2.2)	5(1.8)	
Grade 2	34(12.2)	23(8.2)	16(5.7)	7(2.5)	7(2.5)	4(1.4)	4(1.4)	
Grade 3	12(4.3)	4(1.4)	0	0	0	0	0	
Swelling								$P < 0.0001$ [a]
No swelling	199(71.3)	218(78.1)	250(89.6)	261(93.5)	268(96.1)	269(96.4)	269(96.4)	
Grade 1	45(16.1)	32(11.5)	13(4.7)	7(2.5)	3(1.1)	2(0.7)	3(1.1)	
Grade 2	18(6.5)	17(6.1)	13(4.7)	8(2.9)	6(2.2)	8(2.9)	7(2.5)	
Grade 3	17(6.1)	12(4.3)	3(1.1)	3(1.1)	2(0.7)	0	0	
Systemic reactions								
Auxiliary temperature								$P < 0.0001$ [a]
No fever	115(41.2)	201(72)	268(96.1)	274(98.2)	278(99.6)	278(99.6)	278(99.6)	
Grade 1	140(50.2)	72(25.8)	11(3.9)	5(1.8)	1(0.4)	1(0.4)	1(0.4)	
Grade 2	20(7.2)	6(2.2)	0	0	0	0	0	
Grade 3	4(1.4)	0	0	0	0	0	0	
Loss of Appetite								$P < 0.0001$ [b]
No	226(81)	252(90.3)	261(93.5)	270(96.8)	272(97.5)	275(98.6)	275(98.6)	
Yes	53(19)	27(9.7)	18(6.5)	9(3.2)	7(2.5)	4(1.4)	4(1.4)	
Gastrointestinal problem								
No	261(93.5)	262(93.9)	269(96.4)	273(97.8)	276(98.9)	277(99.3)	278(99.4)	$P < 0.0001$ [b]
Yes	18(6.5)	17(6.1)	10(3.6)	6(2.2)	3(1.1)	2(0.7)	1(0.4)	
Vomiting								$P < 0.0001$ [b]
No	261(93.5)	272(97.5)	276(98.9)	276(98.9)	279(100)	279(100)	279(100)	
Yes	18(6.5)	7(2.5)	3(1.1)	3(1.1)	0	0	0	
Eczema								$P= 0.277$ [b]
No	279(100)	279(100)	278(99.6)	277(99.3)	277(99.3)	278(99.6)	278(99.6)	
Yes	0	0	1(0.4)	2(0.7)	2(0.7)	1(0.4)	1(0.4)	

The results represent number (percent) of cases with or without the specified complications.
Pain: Grade 1, minor reaction to touch; Grade 2, crying/protesting on touch; Grade 3, crying when limb was moved/spontaneously painful Tenderness/swelling: Grade 1,>5 mm; Grade 2, 5-20 mm; Grade 3,>20 mm
Fever: Grade 1, 37.5-38 ^{o} C; Grade 2, >38 and <39 ^{o} C; Grade 3, ≥39 ^{o} C
[a] Friedman test
[b] Cochran test

Table 2: Frequency of local and systemic reactions reported during the first week follow up after administration of the second vaccine dose of DTwP vaccine in Iranian infants

Symptoms	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	*P*-Value
Local reactions								
Pain								$P < 0.0001$ [a]
No pain	79(28.3)	138(49.5)	221(79.2)	258(92.5)	265(95)	268(96.1)	268(96.1)	
Grade 1	106(38)	80(28.7)	40(14.3)	17(6.1)	11(3.9)	8(2.8)	9(3.2)	
Grade 2	70(25.1)	56(20.1)	15(5.4)	3(1.1)	3(1.1)	3(1.1)	2(0.7)	
Grade 3	24(8.6)	5(1.8)	3(1.1)	1(0.4)	0	0	0	
Redness								$P < 0.0001$ [a]
No redness	169(60.6)	214(76.7)	251(90)	267(95.7)	271(97.1)	275(98.6)	276(98.9)	
Grade 1	76(27.2)	48(17.2)	20(7.2)	9(3.2)	6(2.2)	3(1.1)	2(0.7)	
Grade 2	30(10.8)	15(5.4)	8(2.9)	3(1.1)	2(0.7)	1(0.4)	1(0.4)	
Grade 3	4(1.4)	2(0.7)	0	0	0	0	0	
Swelling								$P < 0.0001$ [a]
No swelling	201(72)	228(81.7)	254(91)	266(95.3)	270(96.7)	270(96.7)	271(97.1)	
Grade 1	47(16.8)	28(10)	12(4.3)	8(2.9)	5(1.8)	6(2.2)	6(2.2)	
Grade 2	29(10.4)	22(7.9)	12(4.3)	4(1.4)	3(1.1)	3(1.1)	2(0.7)	
Grade 3	2(0.7)	1(0.4)	1(0.4)	1(0.4)	1(0.4)	0	0	
Systemic reactions								
Auxiliary temperature								$P < 0.0001$ [a]
No fever	122(43.7)	190(68.1)	258(92.5)	276(98.9)	275(98.6)	276(98.9)	278(99.6)	
Grade 1	128(45.9)	84(30.1)	18(6.5)	3(1.1)	4(1.1)	3(1.1)	1(0.4)	
Grade 2	27(9.7)	5(1.8)	3(1.1)	0	0	0	0	
Grade 3	2(0.7)	0	0	0	0	0	0	
Loss of Appetite								$P < 0.0001$ [b]
No	240(86)	257(92.1)	269(96.4)	274(98.2)	274(98.2)	275(98.6)	277(99.3)	
Yes	39(14)	22(7.9)	10(3.6)	5(1.8)	5(1.8)	4(1.4)	2(0.7)	
Gastrointestinal problem								$P < 0.0001$ [b]
No	256(91.8)	262(93.9)	268(96.1)	274(98.2)	273(97.8)	275(98.6)	276(98.9)	
Yes	23(8.2)	17(6.1)	11(3.9)	5(1.8)	6(2.2)	4(1.4)	3(1.1)	
Vomiting								$P < 0.0001$ [b]
No	264(94.6)	274(98.2)	274(98.2)	279(100)	279(100)	279(100)	279(100)	
Yes	15(5.4)	5(1.8)	5(1.8)	0	0	0	0	
Eczema								$P= 0.609$ [b]
No	278(99.6)	278(99.6)	279(100)	279(100)	279(100)	278(99.6)	279(100)	
Yes	1(0.4)	1(0.4)	0	0	0	1(0.4)	0	

See footnote to Table 1

Table 3: Frequency of local and systemic reactions reported during the first week follow up after administration of the third vaccine dose of DTwP vaccine in Iranian infants

Symptoms	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	P-Value
Local reactions								
Pain								$P < 0.0001$ [a]
No pain	89(31.9)	157(56.3)	223(79.9)	248(88.9)	260(93.2)	263(94.3)	267(95.7)	
Grade 1	112(40.1)	83(29.7)	40(14.3)	26(9.3)	17(6.1)	16(5.7)	12(4.3)	
Grade 2	58(20.8)	29(10.4)	15(5.4)	5(1.8)	2(0.7)	0	0	
Grade 3	20(7.2)	10(8.6)	1(0.4)	0	0	0	0	
Redness								$P < 0.0001$ [a]
No redness	169(60.6)	210(75.3)	252(90.3)	265(95)	271(97.1)	272(97.5)	274(98.2)	
Grade 1	80(28.7)	48(17.2)	17(16.1)	10(3.6)	4(1.4)	4(1.4)	2(0.7)	
Grade 2	29(10.4)	20(7.2)	10(3.6)	4(1.4)	4(1.4)	3(1.1)	3(1.1)	
Grade 3	1(0.4)	1(0.4)	0	0	0	0	0	
Swelling								$P < 0.0001$ [a]
No swelling	192(68.8)	218(78.1)	250(89.6)	257(92.1)	261(93.5)	265(95)	267(95.7)	
Grade 1	59(21.1)	34(12.2)	8(2.9)	10(3.6)	9(3.2)	7(2.5)	7(2.5)	
Grade 2	22(7.9)	24(8.6)	20(7.2)	11(3.9)	8(2.9)	6(2.2)	4(1.4)	
Grade 3	6(2.2)	3(1.1)	1(0.4)	1(0.4)	1(0.4)	1(0.4)	1(0.4)	
Systemic reactions								
Auxiliary temperature								$P < 0.0001$ [a]
No fever	129(46.2)	201(72)	263(94.3)	272(97.5)	273(97.8)	275(98.6)	274(98.2)	
Grade 1	113(40.5)	68(24.4)	13(4.7)	6(2.2)	6(2.2)	4(1.4)	4(1.4)	
Grade 2	34(12.2)	10(3.6)	3(1.1)	1(0.4)	0	0	1(0.4)	
Grade 3	3(1.1)	0	0	0	0	0	0	
Loss of Appetite								$P < 0.0001$ [b]
No	227(81.4)	255(91.4)	273(97.8)	276(98.9)	277(99.3)	277(99.3)	277(99.3)	
Yes	52(18.6)	24(8.6)	6(2.2)	3(1.1)	2(0.7)	2(0.7)	2(0.7)	
Gastrointestinal problem								P < 0.0001 [b]
No	257(92.1)	265(95)	271(97.1)	276(98.9)	276(98.9)	276(98.9)	276(98.9)	
Yes	22(7.9)	14(5)	8(2.9)	3(1.1)	3(1.1)	3(1.1)	3(1.1)	
Vomiting								$P < 0.0001$ [b]
No	266(95.3)	271(97.1)	278(99.6)	279(100)	279(100)	279(100)	279(100)	
Yes	13(4.7)	8(2.9)	1(0.4)	0	0	0	0	
Eczema								P =0.137 [b]
No	275(98.6)	276(98.5)	275(98.6)	276(98.9)	277(99.3)	278(99.6)	278(99.6)	
Yes	4(1.4)	3(1.1)	4(1.4)	3(1.1)	2(0.7)	1(0.4)	1(0.4)	

See footnote to Table 1

Table 4: Representation studies reporting reactogenicity of DTwP vaccines in infants

References	Study (year)	Country	Vaccination schedule	Vaccine (manufacture)	Incidence of reactions (%)			
					Pain	Redness	Swelling	Fever
22	Akhavizadegan (1997)	Iran	Primary	Razi	NI [a]	NI [a]	44.8	70.7
23	Ardakani (2000)	Iran	Primary/booster	Razi	44.7	27.7	31.4	54.5
24	Daneshjoo (2000)	Iran	Primary/booster	Razi	63	21	48.7	73
25	Ayatollahi (2005)	Iran	Primary/booster	Razi	55	10.5	26.8	56.8
26	Karami (2006)	Iran	Primary/booster	Razi	67.3	43.1	40.7	54.1
31	Clement (2003)	Brazil	Primary	GlaxoSmithKline	45	37	35	45
31	Clement (2003)	Brazil	Primary	Butantan	36	34	28	39
32	Simondon (1997)	Senegal	Primary	Sanofi Pasteur	14	3.5	24.9	2.2
Present study	Zarei (2009)	Iran	Primary	Razi	75.3	28.7	40.1	58.8

[a] *not identified*

Discussion

Immunization has an essential impact on public health, worldwide (17). Numerous studies have shown the efficacy of different vaccines to protect infants leading to either eradication or significantly reduction of the related diseases in many countries thanks to universal immunization. Nevertheless, a number of individuals (including parents deciding for their infants) do not take advantage of this preventive measure for different reasons such as doubts on their usefulness or concerns over safety (18-21).

This study was undertaken to evaluate the safety and reactogenicity of Razi-DTwP vaccine in healthy Iranian infants. Previous investigations in Iranian infants using the same vaccine indicated a different reactogenicity pattern of Razi-DTwP vaccine (22-26). Karimi et al. reported that of 1295 infants born in Kermanshah City of Iran receiving the primary vaccination of Razi-DTwP vaccine, 67.3%, 65.9% and 64.2% had pain, 41.9%, 42% and 43.4% had redness and 42.6%, 42% and 39.5% had swelling following administration of the first, the second and the third doses of vaccine, respectively (26).

In previous studies, reactogenicity was recorded at only one or two time intervals within the first week of vaccination and frequency of reactogenicity was recorded following in primary and booster vaccinations (Table 4). However, in the present study the symptoms were recorded every day during the first week after vaccination enabling evaluation of the trend of complications over a short term course and it was recorder only in primary vaccination. Contrary to previous local studies, we established a grading system to measure parameters like pain, redness, swelling and fever. According to this measurement system each complication was classified into three grades (see Materials and Methods). Our grading system was

based on guidelines obtained from previous studies (27-29). In the current study, prevalence of grade 3 fever (≥39 ºC) was observed in only 1.4%, 0.7% and 1.1% of subjects after administration of the first, the second and the third doses of vaccine, respectively, with no clinically significant consequences like febrile seizure. Both local and systemic reactions usually resolved within seven days. Comparing the reactogenicity profile of our study with that, reported in other countries reveals some differences.

Previous studies using DTwP vaccine indicated various reactogenicity patterns in other countries (30-32). Kitchin et al. reported that of the children receiving the DTwP/Hib (Act-Hib DTP) vaccine, 54.2%, 42.9% and 36.4% had pain, 51.7%, 60.5% and 58.5% had redness, 42.5%, 44.5% and 44.1% had swelling, 25%, 15% and 19.5% had axillary temperature >37.5 ºC, 50.8%, 34.5% and 28% had decreased feeding, 38.3%, 31.9% and 22.9% had vomiting and 35%, 26.1% and 18.6% had diarrhea following administration of the first, the second and the third doses of vaccine, respectively (30). In most previous studies in other countries, combination vaccines were used. Table 4 summarizes reactogenicity in some previous studies in other countries with respect to pain, redness, swelling and fever and compares them with the present study.

The binary logistic regression model of data in the present study showed increased frequency of pain in the first day of first dose of vaccination in female by 1.9 folds and increased frequency of gastrointestinal problems in the first day of first dose of vaccination in female by 4.9 folds compared, a finding already reported by many other investigators (33).

In some studies, reactogenicity was reported at a higher frequency following administration of the booster dose compared to the primary course (34-38). We have previously observes similar finding after booster vaccination with DTwP in preschool children (39).

Comparison of our results with those reported by other investigators indicates a relatively higher incidence of complications following vaccination with the DTwP vaccine manufactured by Razi Institute compared to the standard WHO approved commercial vaccines. These differences might be related to bacterial strain, bacterial cell preparation and/or formulation process of the vaccine. To resolve these differences, we are planning to perform a prospective vaccination study using Razi-DTwP vaccine in parallel to a WHO-approved standard whole cell DTP vaccine in a group of Iranian infants.

Acknowledgements

The authors gratefully acknowledge the assistance of all parents who agreed to allow their infants to enter the study. We are indebted to the personnel of Mohammadian, Dawazdah Bahman, Safdari and Salavati Health Centers of Shahid Beheshti University of Medical Science for their assistance in vaccination and collection of diary cards, especially Drs. Shirin Bonakddar, Dr. Jalaledin Nasernia, Dr. Anvar Vali, Mrs. Narjes Jamshidi, Narjes Madadian and Hakime Farvardin. The authors wish to thank Dr. Mohammad Ali Akhavizadegan and Mohammad Ali Mansori for consultation. This work was supported by a grant from Food and Drug Administration of the Ministry of Health, Treatment and Medical Education of Iran.

The authors declare that they have no conflict of interests.

References

1. Crowcroft NS, Pebody RG (2006). Recent developments in pertussis. *Lancet*, 367(9526): 1926-36.
2. Anonymous (1999). Combination vaccines for childhood immunization. *Morb Mortal Wkly Rep*, 48(RR-5):1-14. Available from: *www.who.int*
3. Crowcroft NS, Stein C, Duclos P, Birmingham M (2003). How best to estimate the global burden of pertussis? *Lancet Infect Dis*, 3(7): 413-18.
4. Anonymous (2006). Immunization summery 2006. Available from: *www.who.int*

5. Anonymous (2006). Vaccine preventable deaths and the global immunization vision and strategy, 2006-2015. *Morb Mortal Wkly Rep*, 55(18): 511-15. Available from: *www.who.int*
6. Tetanus vaccine-WHO position paper (2006). *Wkly Epidemiol Rec*, 81: 198-208. Available from: *www.who.int*
7. Anonymous (2006). Pertussis vaccines--WHO position paper. *Wkly Epidemiol Rec*, 80 (4): 31-9. Available from: *www.who.int*
8. Anonymous (2006). Diphtheria vaccine-WHO position paper. *Wkly Epidemiol Rec*, 81:21-32. Available from: *www.who.int*
9. Mirchamcy H (1960). Study on diphtheria, tetanus combined immunization in children in some elementary school of Tehran. *Arch Inst Razi*, 12: 9-18.
10. Mirchamcy H (1969). The use of dried whole blood absorbed on filter paper for the evaluation of diphtheria and tetanus antitoxin in mass survey. *Arch Inst Razi*, 21: 7-15.
11. Mirchamcy H (1982). Resultats de immunisation collective des infants en Iran avecles immunogenes de production locale. *Arch Inst Razi*, 33: 73-8.
12. Nazari F (1973). Mass immunity against diphtheria and tetanus in some urban and rural areas in Iran. *Arch Inst Razi*, 25:49-55.
13. Nazari F (1977). A model for developing countries of mass serological survey of children vaccinated against diphtheria and tetanus. *Arch Inst Razi*, 29:3-10.
14. Pichichero ME, Green JL, Francis AB, Marsocci SM, Lynd AM, Litteer T (1994). Comparison of a three-component acellular pertussis vaccine with whole cell pertussis vaccine in two-month-old children. *Pediatr Infect Dis J*, 13(3):193-6.
15. Blennow M, Granstrom M, Jaatmaa E, Olin P (1988). Primary immunization of infants with an acellular pertussis vaccine in a double-blind randomized clinical trial. *Pediatrics*, 82(3): 293-99.
16. Blumberg DA, Mink CM, Cherry JD, Johnson C, Garber R, Plotkin SA et al. (1991). Comparison of acellular and whole-cell pertussis-component diphtheria-tetanus-pertussis vaccines in infants. The APDT Vaccine Study Group. *J Pediatr*, 119(2):194-204.
17. Anonymous (1999). Ten great public health achievements--United States, 1900-1999. *Morb Mortal Wkly Rep*, 48(12): 241-3. Available from: *www.cdc.gov*
18. Fredrickson DD, Davis TC, Arnould CL, Kennen EM, Hurniston SG, Cross JT et al. (2004). Childhood immunization refusal: provider and parent perceptions. *Family Med*, 36(6): 431-39.
19. Freed GL, Clark SJ, Hibbs BF, Santoli JM (2004). Parental vaccine safety concerns. The experiences of pediatricians and family physicians. *Am J of Preven Med*, 26(1):11-4.
20. Poland GA, Jacobson RM (2001). Understanding those who do not understand: a brief review of the anti-vaccine movement. *Vaccine*, 19(17-19): 2440-5.
21. Taylor JA, Darden PM, Brooks DA, Hendricks JW, Wasserman RC, Bocian AB (2002). Association between parents' preferences and perceptions of barriers to vaccination and the immunization status of their children: a study from Pediatric Research in Office Settings and the National Medical Association. *Pediatrics*, 110(6): 1110-6.
22. Akhavizadegan M (1996). Evaluation and comparison of immunogenicity and reactogenicity of DTP vaccines produced in Iran and other countries in Iranian infants. [PhD thesis]. School of Public Health, Tehran University of Medical Sciences, Iran.
23. Ardakani A, Talebian A (2000). Reactogenicity of DTP vaccine in Kashan. *Feiz*, 5(17): 33-7.
24. Daneshjoo K, Hadjizadeh N (2000). Complications of DTP vaccination in preschool children. *Irn J Pediatr*, 3:13-7.

25. Ayatollahi J, Zare A (2005). Evaluation of the side effects of triple vaccine in Yazd in 2005. *Irn J Pediatr*, 16(3): 332-36.
26. Karimi M, Holakouie Naieni K, Rahimi A, Fotouhi A, Eftekhar Ardebili H (2006). Adverse events following immunization with DTP vaccine in infants and children in Kermanshah city: A cohort study. *Irn J Epidemiol*, 1(2):33-9.
27. Kosuwon P, Warachit B, Hutagalung Y, Borkird T, Kosalaraksa P, Bock HL et al. (2003). Reactogenicity and immunogenicity of reduced antigen content diphtheria-tetanus-acellular pertussis vaccine (dTpa) administered as a booster to 4-6 year-old children primed with four doses of whole-cell pertussis vaccine. *Vaccine*, 21(27-30): 4194-200.
28. Tejedor JC, Moro M, Ruiz-Contreras J, Castro J, Gomez-Campdera JA, Navarro ML et al. (2007). Immunogenicity and reactogenicity of primary immunization with a novel combined Haemophilus influenzae Type b and Neisseria meningitidis serogroup C-tetanus toxoid conjugate vaccine coadministered with a diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus vaccine at 2, 4 and 6 months. *Pediatr Infect Dis J*, 26(1):1-7.
29. Lin TY, Wang YH, Chang LY, Huang YC, Kao HT, Lin PY et al. (2007). A fully liquid diphtheria-tetanus-five component acellular pertussis-inactivated poliomyelitis-Haemophilus influenzae type b conjugate vaccine: immunogenicity and safety of primary vaccination in Taiwanese infants. *Int J Infect Dis*, 11(2): 129-36.
30. Kitchin N, Southern J, Morris R, Hemme F, Cartwright K, Watson M et al. (2006). A randomised controlled study of the reactogenicity of an acellular pertussis-containing pentavalent infant vaccine compared to a quadrivalent whole cell pertussis-containing vaccine and oral poliomyelitis vaccine, when given concurrently with meningococcal group C conjugate vaccine to healthy UK infants at 2, 3 and 4 months of age. *Vaccine*, 24(18): 3964-70.
31. Clemens SC, Azevedo T, Homma A (2003). Feasibility study of the immunogenicity and safety of a novel DTPw/Hib (PRP-T) Brazilian combination compared to a licensed vaccine in healthy children at 2, 4, and 6 months of age. *Rev Soc Bras Med Trop,* 36(3): 321-30.
32. Simondon F, Preziosi MP, Yam A, Kane CT, Chabirand L, Iteman I et al. (1997). A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal. *Vaccine*, 15(15):1606-12.
33. Christy C, Pichichero ME, Reed GF, Decker MD, Anderson EL, Rennels MB et al. (1995). Effect of gender, race, and parental education on immunogenicity and reported reactogenicity of acellular and whole-cell pertussis vaccines. *Pediatrics,* 96(3 Pt 2): 584-7.
34. Tregnaghi M, Lopez P, Rocha C, Rivera L, David MP, Ruttimann R et al. (2006). A new DTPw-HB/Hib combination vaccine for primary and booster vaccination of infants in Latin America. *Rev Panam Salud Publica*, 19(3):179-88.
35. Pichichero ME, Deloria MA, Rennels MB, Anderson EL, Edwards KM, Decker MD et al. (1997). A safety and immunogenicity comparison of 12 acellular pertussis vaccines and one whole-cell pertussis vaccine given as a fourth dose in 15- to 20-month-old children. *Pediatrics*, 100(5):772-88.
36. Schmitt HJ, Beutel K, Schuind A, Knuf M, Wagner S, et al. (1997). Reactogenicity and immunogenicity of a booster dose of a combined diphtheria, tetanus, and tricomponent acellular pertussis vaccine at fourteen to twenty-eight months of age. *J Pediatr*, 130(4): 616-23.

37. Tozzi AE, Anemona A, Stefanelli P, Salmaso S, Atti ML, Mastrantonio P et al. (2001). Reactogenicity and immunogenicity at preschool age of a booster dose of two three-component diphtheria-tetanus-acellular pertussis vaccines in children primed in infancy with acellular vaccines. *Pediatrics*, 107(2): E25.
38. Halperin SA, Eastwood BJ, Barreto L, Friesen B, Medd L, Meekison W et al. (1996). Adverse reactions and antibody response to four doses of acellular or whole cell pertussis vaccine combined with diphtheria and tetanus toxoids in the first 19 months of life. *Vaccine*, 14(8): 767-72.
39. Zarei S, Jeddi-Tehrani M, Akhondi M, Zarnani AH, Zeraati H, Ghazanfari M et al. (2007). Short term reactogenicity of cellular DTP vaccine in 4-6 year old children in Tehran, Iran. *Payesh*, 6(3):273-83.