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DOCADEA 241478

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2.2 Participants

Children having their 4th birthday in 1998, living in the area where preventive child health care is provided by the Stichting Thuiszorg Oost-Veluwe, were invited to participate in the study by direct mailing from the PEA. Additional information was given to the parents, both in writing (in Dutch and in Arabic & Turkish) as well as orally and parent's questions were addressed. The catchment population was 2068 infants in the cities of Apeldoorn (5 CBs), Epe (1 CB) , Vaassen (1 CB) and Twello (1 CB).
After evaluation of inclusion and exclusion criteria and signing of the informed consent form by the parents, the child was enrolled: the assignment of the child using an in advance generated list, by Unique Trial Number to one of the 8 study groups, was at random. UTN's were assigned in order of enrolment. 32-unit blocks were used to ensure balanced assignment of the children to the 8 groups. This procedure and the block size were not revealed to the study personnel in the clinics.

2.3 Study design

The study was an open randomised controlled study in four year old children with blinded serological evaluation. The vaccines from the different manufacturers could not be blinded because they were supplied in their original packages, with the lotnumbers as a further identifier. Thus both parents and study personnel knew which specific vaccine the child received. Parents were informed about possible adverse events. The first blood sample was taken just prior to the vaccination. The second sampling was scheduled after 4-6 weeks. After vaccination the child remained under direct observation for about fifteen minutes. Any observed adverse events were recorded in a special diary for the seven days following the vaccination by the parents. The observed adverse events were (to be) put on the CRF by the study personnel during the telephone interview 18-30 hours after the vaccination and at the time of the second blood sampling.
Children of the groups not receiving one of the pertussis vaccines were offered either ACV-SB or ACV-WL vaccine, since a single WCV is not available. This was not part of the study and was recommended by the medical ethics committee.

2.4 Vaccination and blood sampling

A physician or a study nurse administered intramuscular injections according to standard procedures (12N-GCP-04 and 12N-GCP-05). DTP-IPV or DT-IPV were to be injected in the left upper arm and the acellular vaccines in the right upper arm. No instruction was given about skin desinfection. The lotnumber and injection site was registered in the CRF. Blood (5-10 ml) was sampled by venapuncture after application of a local anaesthetic (EMLA™). In a subset of the population a heparinized blood sample was also taken. The routing of the blood samples is described in detail in SOP 12N-ALG-04. After transportation to the RIVM at the end of the day serum was separated (SOP 12C-ALG-15) and stored at -20 °C until testing. The heparinized blood samples were handed over to LIO for blood cell isolation. These samples were taken to study the influence of the vaccinations on cellular immunity, in particular T-cell proliferation. The results of this part of the study will be reported later.

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2.5 Immunogenicity

After all serum samples were collected they were thawed and randomized for blinded antibody measurement. Aliquots were divided into marked tubes with a Multiprobe (Canberra Packard, US) (SOP 12N-APP-34). To perform the 11 assays 6 portions were distributed to the participating labs in the following priority order (in case a limited amount of sample would be available):

1. Pertussis	PT-, FHA-, PRN- ELISA	LVO
2. Pertussis	Agglutination assay	LVO
3. Poliomyelitis	Neutralisation assay (3 strains)	LCB
4. Pertussis	FIM2-ELISA	Erlangen, Germany
5. Pertussis	IgA-whole cell ELISA	LIS
6. Diphtheria	Toxin Inhibition Binding assay (ToBI)	LVO
6. Tetanus	Toxin Inhibition Binding assay (ToBI)	LVO

2.5.1 Pertussis anti-PT, anti-FHA, anti-PRN and anti-FIM2 antibodies

ELISA's were performed according to SOP 12C-ALG-36 which is essentially the same procedure as described by Edwards en Meade et al (18, 46). Purified PT (S1-B variant) and FHA-proteins were provided by LPO (RIVM, The Netherlands) and PRN (variant 1) was purchased from CHIRON (Siena, Italy). Their purity was confirmed by SDS-gel electrophoresis. The coat concentration for PT was 1 μg/ml, for FHA 2 μg/ml and 3 μg/ml for PRN. For each serum, 8 twofold dilutions were tested starting with 1: 60 dilution. Alkaline phosphatase conjugated goat antihuman IgG (Sigma, St. Louis, US) was used at a dilution of 1: 30,000. The incubation time in all steps during the assay was 2 hours at 28°C. ELISA unitage was calculated relative to the US Reference Pertussis Antiserum (human), lot 3 for PT and FHA and lot 4 for PRN using the four parameter fit method in KC4 (Kineticalc for Windows) with a BioTek plate reader (EL312e, BioTek Instruments, US) (SOP 12N-APP-50). The results from each plate were accepted if the reference revealed the original amount EU/ml in the linear part of the dose-response curve ± 10%, the slope of the curve had a $r \geq 0.995$ and the control serum was within its predetermined 95% confidence interval (mean ± 2 SD range). The coefficient of variation of the control sera within the whole study (inter-assay variation) was less than 20% for PT, FHA and PRN. The minimum level of detection (MLD) was defined as the minimum amount of antibody present for a serum to have at least one point in the linear part of the reference curve, and was estimated 2 EU/ml for PT and FHA and 4 EU/ml for PRN. For statistical calculations, sera with an unitage below the MLD were assigned a value equal to the half of the MLD, except for PRN 3 EU/ml.

The FIM2 antibodies were determined by Dr. Bartels in Erlangen according to the method described by Meade et al (46). Results were standardized by use of the US Pertussis Antiserum (human) lot 3 and the MLD was 1 EU/ml. Samples below the MLD were assigned the value 0.5 EU/ml.

2.5.2 Pertussis agglutinating antibodies

Agglutinating antibodies were measured according to SOP 12C-ALG-38 which is essentially the method as described by Manclark (44) using *B. pertussis* strain 3838 as antigen. US Pertussis antiserum (rabbit conc.) lot 2 was used as reference and a control serum was included on each plate. For each serum, a twofold dilution series in 8 wells was tested starting with 1:4 dilution. The results were considered valid if the titer of the control serum was

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within one twofold dilution of its previously established titer. The agglutination titer is expressed as the reciprocal of the highest final dilution of serum resulting in agglutination. Sera negative at the starting dilution were scored as 2 for calculation purposes.

2.5.3 Poliovirus neutralising antibodies

Neutralising antibody titers against poliovirus strain 1(Mahoney), 2 (MEF) and 3 (Saukett) were determined according to SOP 17C-IPV11 in essence describes the micro-neutralisation assay by Albrecht et al (2). Each serum was titrated in a twofold dilution series in 12 wells with 100 $CCID_{50}$ of the virus starting at 1 : 2 dilution. The results were considered valid if the titer of the control serum on each plate was within the 95% confidence interval. Titers are expressed as the ^{2}log reciprocal of the highest final dilution of serum resulting in neutralisation. Sera negative at the starting dilution were assigned the value of 1.

2.5.4 IgA-whole cell ELISA

The measurement of IgA antibodies was performed as described by Nagel et al (54, 55) and SOP LBA/M-205 with a crude cell-membrane preparation from 3 different *B. pertussis* isolates. The sera were tested at a 1: 100 and 1: 400 dilution with an internal RIVM standard serum as reference. Titers are expressed as arbitrary units/ml with the negative sera assigned the value of 1. The antigens were prepared following the procedure SOP LBV/13C-PER-01.

2.5.5 Diphtheria and Tetanus antitoxin antibodies

Anti Diphtheria and Tetanus antibodies were measured with the ToBI assay according to SOP 12C-ALG-35 as described earlier by Hendriksen et al (35). Twofold dilutions of sera in 10 wells were preincubated with a fixed amount of toxin and the remaining free toxin is determined in plates coated with a polyclonal antitoxin antibodies. Results were expressed in IU/ml with the use of an internal RIVM standard serum as reference, calibrated on the WHO reference serum. On each plate a control serum was also included. Calculation and acceptance of the results was performed as described in paragraph 2.7.1. The minimum level of detection was 0.01 IU/ml and sera with titers below this level were assigned the value of 0.005 IU/ml.

2.6 Adverse events

Because of the open design of the study possible bias in observation and appreciation of adverse events could not be avoided. Therefore the study has on this aspect a more observational than a controlled character.
All general practitioners in the study area were informed about the study and the possible adverse events. Parents were supplied with a diary in which to record all observed adverse events in three time periods, the first 24 hours, the second to third day and the fourth to seventh day following vaccination. This included systemic symptoms as feverishness with recorded rectal temperature, listlessness, crying, anorexia, nausea, headache and general skin symptoms. Also local reactions as redness and swelling, pain, itching and diminished use of the arm. A digital thermometer and a measuring scale were supplied.
The observed adverse events were put down on the CRF after the telephone interview by a physician (18-30 hours after the vaccination) and at the time of the second blood sampling, when any other adverse events were also reviewed.

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2.7 Data analyses and statistics

2.7.1 Antibody response

Because the data displayed outliers and seemed very skewed, the serological analyses were performed on logarithmically (ln) transformed data except for polio (reciprocal ^{2}log). Pre- and postvaccination geometric mean antibody titers (GMTs) in the eight study groups were compared using One-way Anova.
An immune response to vaccination was defined as at least a fourfold increase of the prevaccination antibody value. Moreover, the post vaccination titer should be at least fourfold greater than the minimum detectable level. For the pertussis antigens PT, FHA, PRN, Aggl. and FIM the percentages of participants showing an immune response were calculated as well as for D, T and polio. As protective titers the cut-off value of 0.1 IU/ml was used for diphtheria and tetanus and 3 (reciprocal ^{2}log) for polio (22, 23, 41, 68, 70).
To analyse the development of antibody levels, the differences of the ln-transformed post- and prevaccination antibody titers were calculated. Multiple linear regression models were built with these differences as dependent variables. Indicator variables were used as independent variables for:

1. whole cell Pertussis; study groups DTP-IPV & DTP-IPVv
2. acellular Pertussis; study groups ACV-SB, ACV-SBv, ACV-WL & ACV-PM
3. Smithkline Biologicals; study groups ACV-SB & ACV-SBv
4. Wyeth Lederle/Takeda; study group ACV-WL
5. Pasteur Merieux MSD; study group ACV-PM
6. Polio-vero; study groups DT-IPVv, DTP-IPVv & ACV-SBv

The forward variable selection method was used (for more detailed information see appendix).
No indicator variable for different diphtheria antigen content was used because it was completely confounded by the WCV-indicator. Thus, any effect apparently attributable to the whole cell pertussis vaccine component may also have been caused by the extra diphtheria dose. For analysis the software programme SPSS version 7.0 was used.

2.7.2 Adverse events

Local and systemic adverse events were recorded for the seven days following vaccination. The occurrence of adverse events in all eight groups are compared, with DT-IPV as control group, by calculating Odds Ratio's with 95% confidence intervals (OR +95%CI). To assess association of specific adverse events with vaccines/vaccine components a logistic regression model was used. Indicator variables were included as independent variables:

1. whole-cell pertussis containing vaccines, groups DTP-IPV and DTP-IPVv
2. acellular pertussis containing combinations, groups ACV-SB, ACV-SBv, ACV-WL or ACV-PM
3. polio-vero containing combinations, groups DT-IPVv, DTP-IPVv and ACV-SBv

For the different diphtheria contents of the vaccines no indicator variable could be used, since this was fully associated with the whole-cell pertussis component.
The occurrence of a specific adverse event at any time in the seven days following vaccination was the dependent variable. All dependent variables were dichotomised.
For the groups with an acellular pertussis vaccine for local reactions the combined local reactions of the left and or the right arm were used.

2.8 Study monitoring

The study was monitored by a full-time monitor from Parexel-MIRAI ®, a contract clinical research organisation in Amsterdam. During the clinical stages of the study, monitoring visits were made to all study sites (CB's) to guard protocol adherence. In addition, all informed consent papers and CRF's were checked for correct and complete data.

3. Results

3.1 Study population

A total of 244 parents responded to the invitation to participate in the study. After additional information was given and after evaluation of the inclusion and exclusion criteria, 180 children were enrolled in the study and an informed consent was obtained from all parents. These participants were randomized to one of the 8 study groups (Table 2). The children have been recruited from a total population of approximately 2000 children living in the area where preventive child health care was provided by the 'Stichting Thuiszorg Oost-Veluwe'.

For one of the participants earlier pertussis disease was reported. After vaccination, one participant was lost to follow-up. Pre- and postvaccination blood samples were not obtained from one participant, and postvaccination samples from two participants. However, data about adverse events were not missing for these children. In two children, the post vaccination blood samples obtained were too small to determine all antibody parameters, so anti-diphtheria and anti-tetanus antibodies testing had to be omitted (see paragraph 2.7). One participant was excluded for the serological analyses, because the interval between vaccination and bloodsampling was too small (3 weeks).

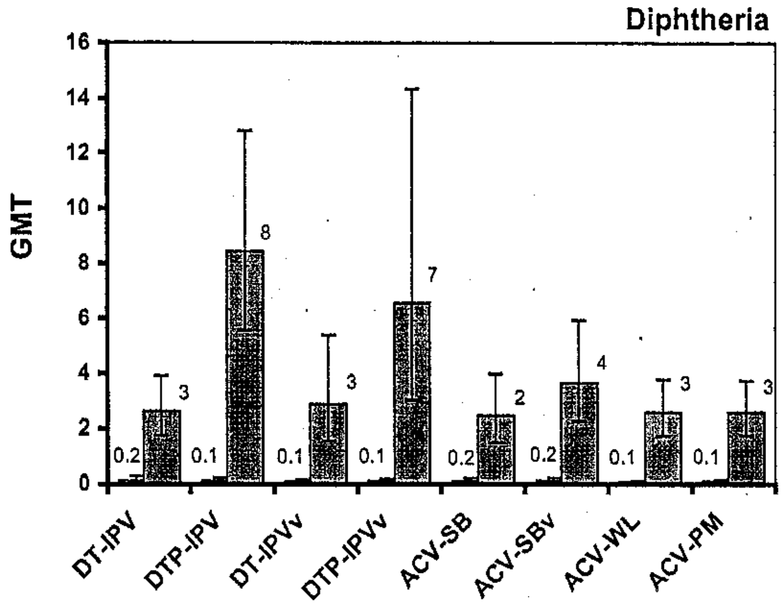
Table 2. Number and sex of participants by study group

Study group	Male	Female	Total
DT-IPV	10	13	23
DTP-IPV	10	11	21
DT-IPVv	12	10	22
DTP-IPVv	9	14	23
ACV-SB + DT-IPV	15	8	23
ACV-SB + DT-IPVv (=ACV-SBv)	11	10	21
ACV-WL + DT-IPV	12	11	23
ACV-PM + DT-IPV	13	11	24
Total	92	88	180

3.2 Antibody response

All data from the different antibody assays are listed in the appendix. Geometric mean titers (GMT) with 95% confidence intervals were calculated for antibodies directed against the six pertussis antigens (PT, FHA, PRN, Aggl., FIM and IgA-WC), diphtheria toxin, tetanus toxin and against poliovirus type 1, 2 and 3 before and after vaccination in the eight different vaccine groups (Table 7 in appendix). The impact of the vaccination with the different vaccines is illustrated in Figure 1.

Prevaccination titers were similar in the eight groups for PT, FHA, PRN, IgA-WC, diphtheria, tetanus and polio type 3. For Aggl. and FIM a statistically significant difference in the prevaccination titers between the groups was observed (One-way Anova; $p<0.05$). The pretiters for Aggl. and FIM were higher in the ACV-SBv group and also somewhat higher in the DT-IPV group compared to the other groups.



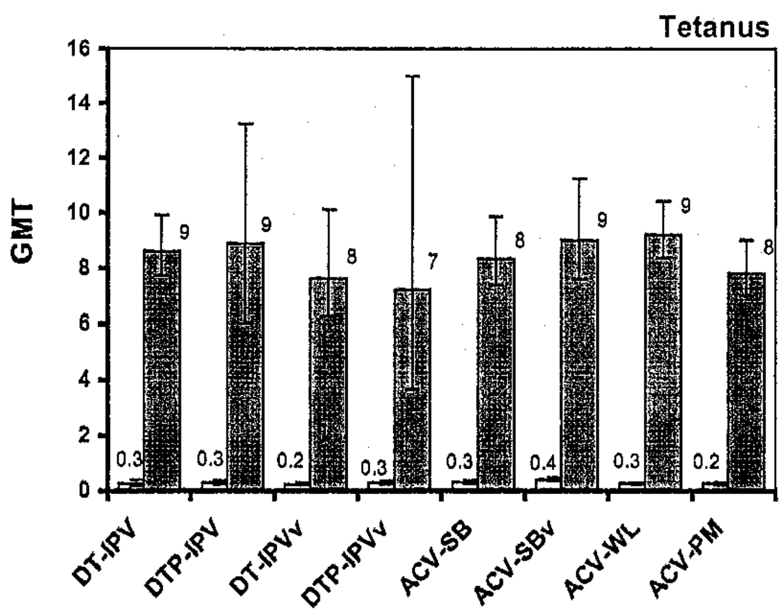
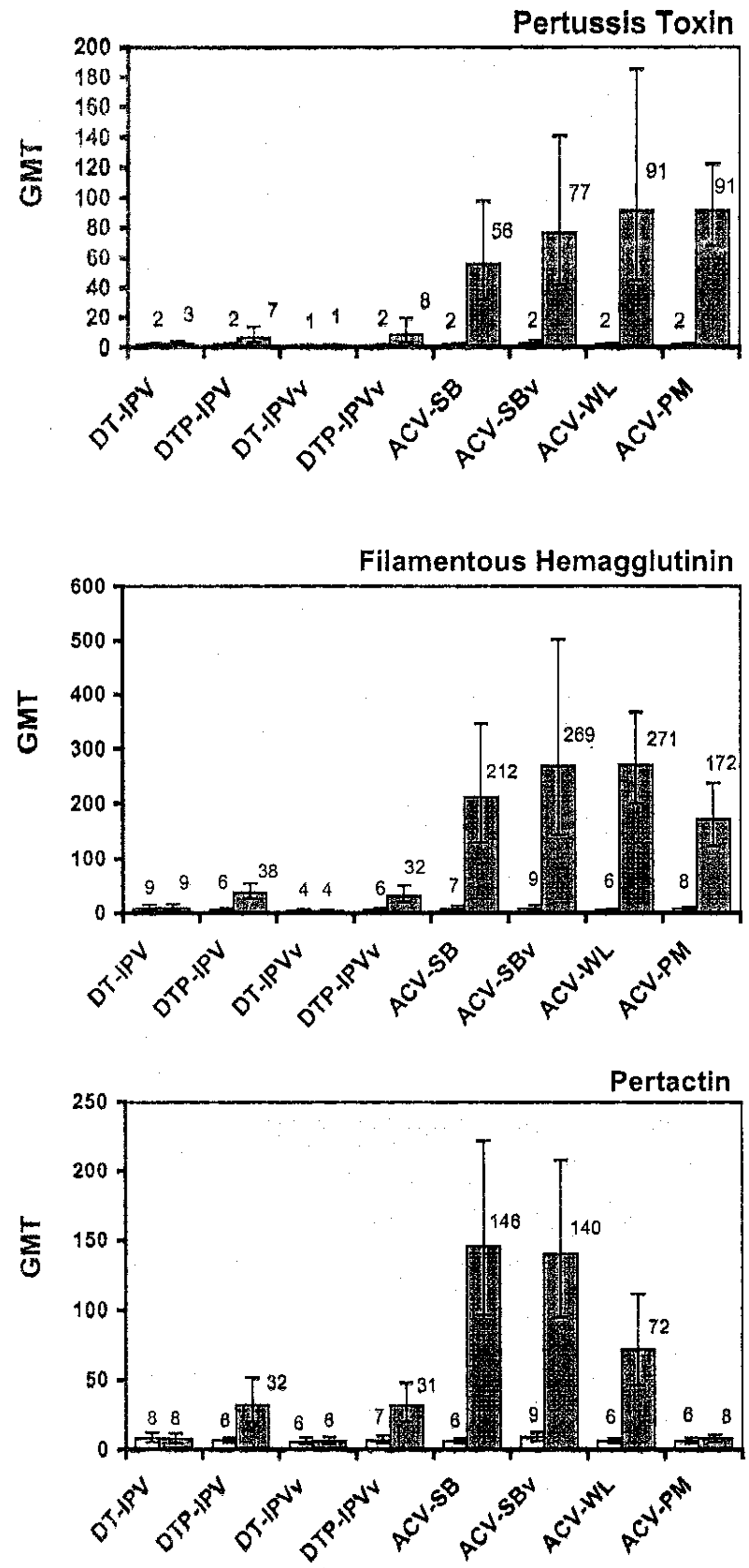
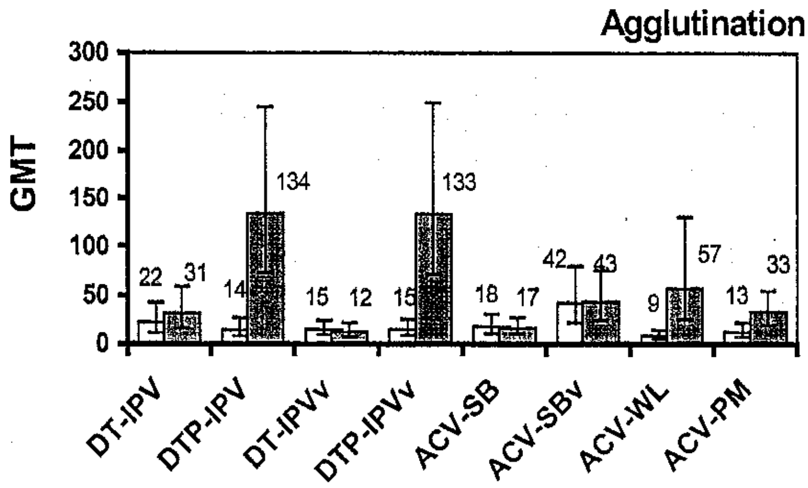
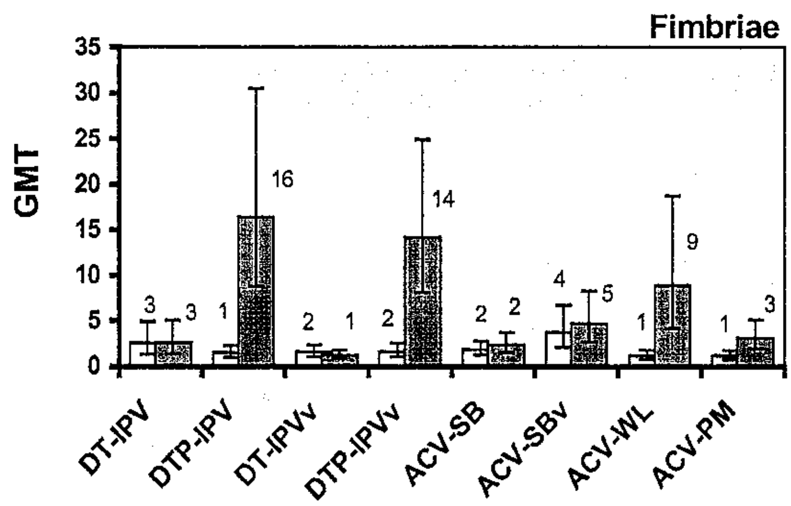
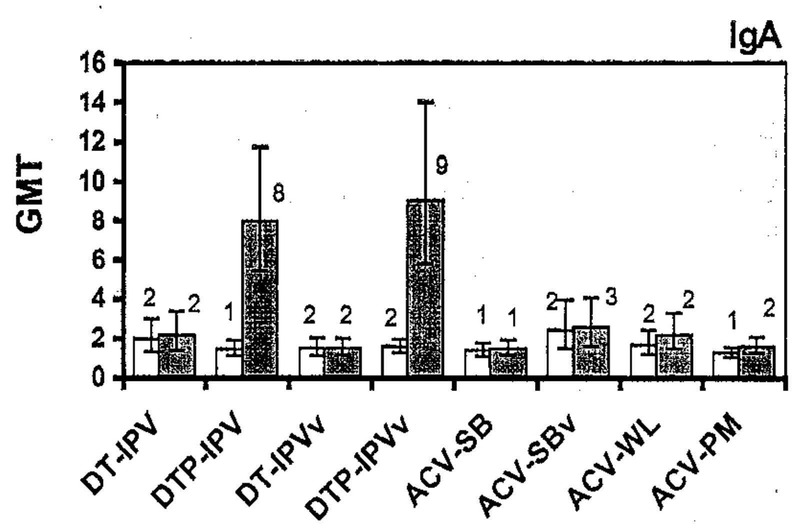


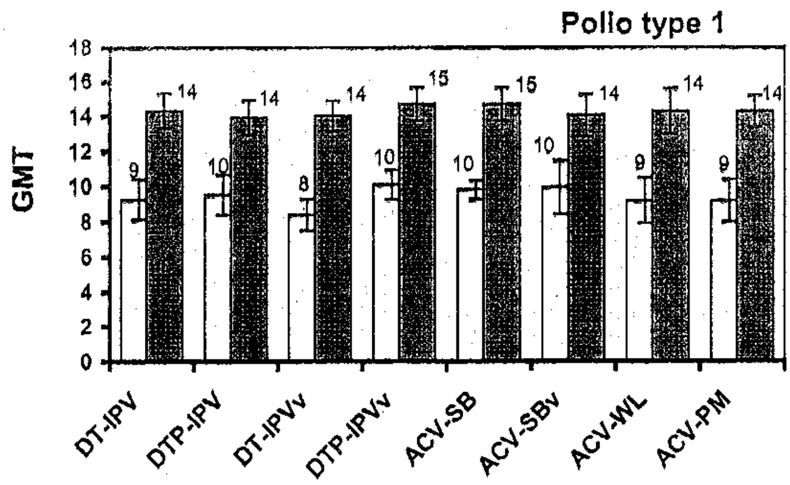
Figure 1. GMT's before and after vaccination (light and dark blue) directed against PT, FHA, PRN, Aggl. FIM and IgA-WC from pertussis, poliovirus type 1, 2 and 3 and diphtheria toxin and tetanus toxin. GMT's are expressed as EU/ml for PT, FHA, PRN and FIM, as reciprocal titres for Aggl., as U/ml for IgA, as IU/ml for D and T and as ^{2}log reciprocal titres for polio. GMT values are indicated in the figure and 95% confidence intervals are indicated. Children in the ACV groups were given also DT-IPV vaccine. Note that GMT's are rounded up/off in the graphs.

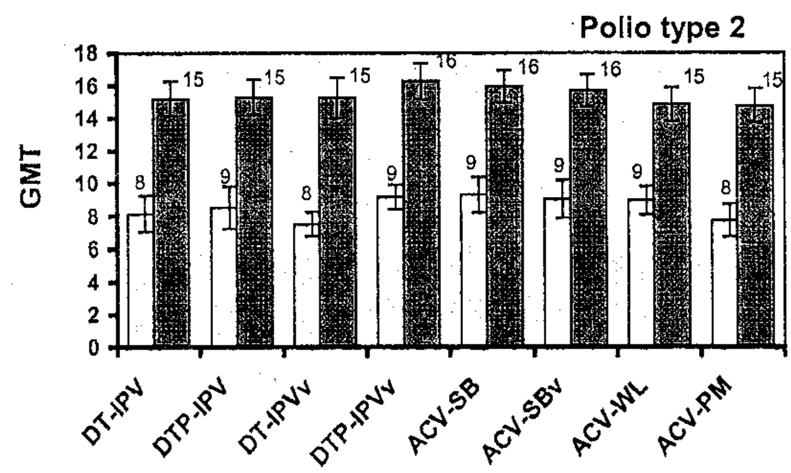

Pertussis Toxin
GMT
200
180
160
140
120
100
80
60
40
20
0
2 3 2 7 1 1 2 8 2 56 2 77 2 91 2 91
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM
Filamentous Hemagglutinin
GMT
600
500
400
300
200
100
0
9 9 6 38 4 4 6 32 7 212 9 269 6 271 8 172
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM
Pertactin
GMT
250
200
150
100
50
0
8 8 6 32 6 6 7 31 6 146 9 140 6 72 6 8
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM

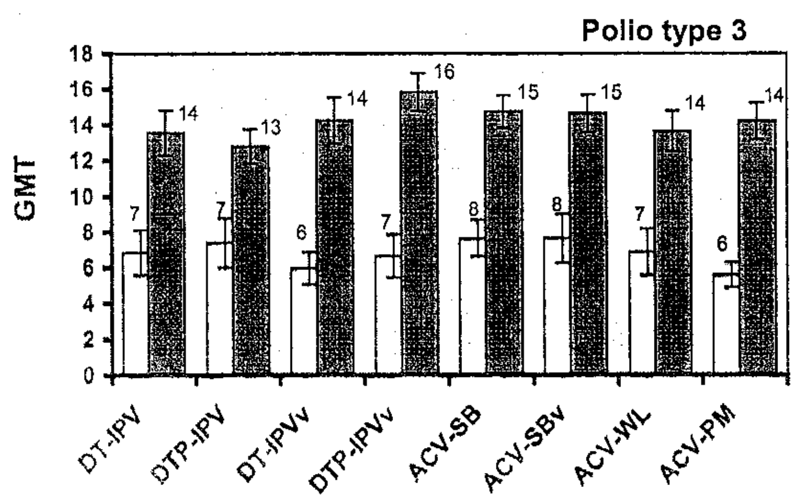

Agglutination
GMT
300
250
200
150
100
50
0
22 31
14 134
15 12
15 133
18 17
42 43
9 57
13 33
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM


Fimbriae
GMT
35
30
25
20
15
10
5
0
3 3
1 16
2 1
2 14
2 2
4 5
1 9
1 3
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM


IgA
GMT
16
14
12
10
8
6
4
2
0
2 2
1 8
2 2
2 9
1 1
2 3
2 2
1 2
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM


Polio type 1
GMT
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM


Polio type 2
GMT
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM


Polio type 3
GMT
DT-IPV
DTP-IPV
DT-IPVv
DTP-IPVv
ACV-SB
ACV-SBv
ACV-WL
ACV-PM

Antibody titers for polio virus type 2 were marginally different before vaccination (One-way Anova; $p<0.05$) probably due to fluctuations in titers and the small number of children in the groups. After vaccination all titers for the six pertussis antigens, diphtheria and for polio type 3 were significantly different between the 8 groups (One-way Anova; $p<0.05$). The titers for tetanus, polio type 1 and 2 did not show this difference.

3.2.1 Pertussis antigens

The number of participants who responded with a fourfold rise in the immune response (IgG antibody titer) to the pertussis antigens PT, FHA, PRN, FIM and Aggl. is shown in Table 3. The percentages with the fourfold rise is illustrated in Fig. 2, where no distinction was made between the IPV-MK and IPV-vero groups. The immune response to PT after vaccination was good for all 3 ACV's (=90% with a fourfold rise), while the WCV only scored 45% (see Fig. 2).
A similar high result was obtained with the ACV's for the response to FHA (=95%) and PRN (=90%) except for ACV-PM in the latter case because the vaccine does not contain PRN. The WCV gave a better response for these two antigens (FHA 70% and PRN 60%) than for PT. The fourfold rise in response to Aggl. and FIM after vaccination with ACV-WL amounts to =60%. The ACV-PM gives a fourfold rise to these antigens in 17% of the children, which is remarkable because this vaccine does not contain FIM. The WCV performed good for the Aggl. and FIM titers resulting in a percentage =85% of the children with a fourfold rise.

In the DT-IPV group 2 children showed a fourfold rise in the PT-titer (see Fig. 2). One child was probably infected with *B. pertussis* during the month between the first (prevaccination) and the second (postvaccination) blood sample because the other pertussis antigen titers were also elevated except PRN (see appendix 4). The other child already had high titers in the prevaccination sample except for PT suggesting a recent *B. parapertussis* infection. In the same vaccine group 4 other children showed an isolated fourfold rise in the agglutination titer, while the other pertussis antigens showed no rise. No explanation could be given for the rise of the agglutination titer in these cases but the sometimes rather difficult, subjective reading procedure of this assay might be the reason.

Table 3. Number of participants showing a fourfold rise in immune response against the different pertussis antigens

antigen → study group	N	PT n	FHA n	PRN n	Aggl. n	FIM n
DT-IPV	23	2	1	0	3	1
DTP-IPV	21	8	15	13	18	18
DT-IPVv	22	0	0	0	2	0
DTP-IPVv	23	10	16	12	20	16
ACV-SB + DT-IPV	23	21	21	21	1	1
ACV-SB + DT-IPVv	21	20	21	19	1	0
ACV-WL + DT-IPV	23	19	22	20	15	14
ACV-PM + DT-IPV	24	23	23	0	8	4

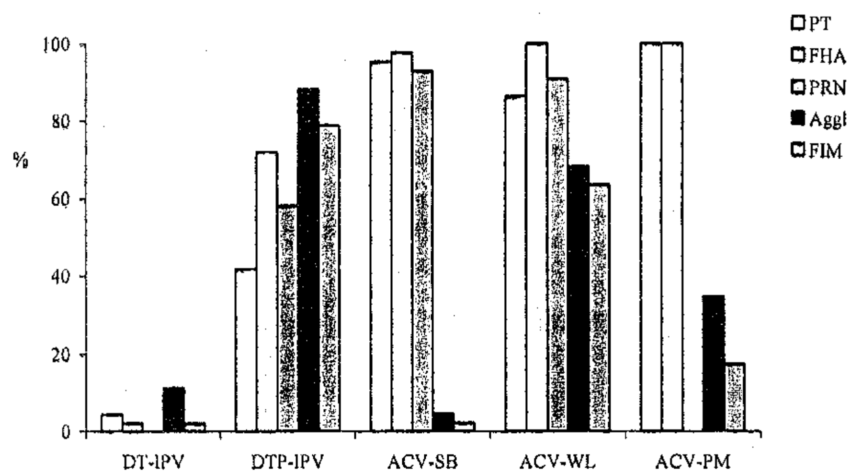


Figure 2. Percentage of participants showing an fourfold immune response against the different pertussis antigens (due to missing samples valid percentages are indicated).

To assess the effect of the different vaccines/vaccine components (WCV, ACV-SB, ACV-WL and ACV-PM) on the development of antibody titers, multiple linear regression was used. Table 4 shows the statistically significant regression coefficients for all measured antigens. After vaccination with DTP-IPV, the regression coefficient for Aggl. is 2,19, i.e. vaccination with WCV leads to a $e^{2,19}$ = 9-times increase in agglutination antibody titer when compared to vaccination without WCV. This method provides a clear picture of the vaccination effect evoked by the different vaccines/vaccine components on the different antibody titers, separately. Vaccination with WCV resulted in a large increase of the

Table 4. Association between antibody levels and vaccine components, as measured by regression coefficients (only statistically significant regression coefficients (r.c.) and p-values are shown)

Antibody	Aggl	FIM	IgA	PRN	FHA	PT	dipht	tet	polio 1	polio 2	polio3
DTP-IPV											
r.c.	2.19	2.24	1.63	1.72	1.82	1.38	0.88	-	-	-	-
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				
ACV-SB											
r.c.	-*	-	-	3.06	3.30	3.52	-	-	-	-	-
p				<0.001	<0.001	<0.001					
ACV-WL											
r.c.	1.77	1.93	-	2.61	+0,56**	3.52	-	-	-	-	-
p	<0.001	<0.001		<0.001	0.02	<0.001					
ACV-PM											
r.c.	0.90	0.91	-	-	3.30	3.52	-	-	-	-	2.13
p	<0.001	<0.001			<0.001	<0.001					0.01
P_{vero}											
r.c.	-	-	-	-	-	-	-	-	-	-	1.65
p											<0.01

* no statistically significant regression coefficient (r.c.),

** regression coefficient is similar for all acellular vaccines (3.30), however for ACV-WL an additional regression coefficient of 0.56 was found

agglutination antibody titer compared to DT-IPV. The vaccination with ACV-WL and ACV-PM also showed an increase in Aggl. antibody titer but smaller, while vaccination with ACV-SB yielded no statistically significant increase as this vaccine does not contain this component. A similar vaccination effect was observed for FIM antibody titers (see table 4). Vaccination with ACV's caused large increases in PT-, FHA- and PRN antibody titers compared to DT-IPV, except for ACV-PM in case of PRN, as this vaccine does not contain PRN. ACV-WL caused even a significantly higher response to FHA than ACV-SB and ACV-PM. Vaccination with WCV caused smaller increases in the antibody titer against these 3 Pertussis antigens.
It is in this study not possible to distinguish between the WCV component and the effect of the other components of combined vaccines or of interaction/potentiation between the different vaccine components. The linear regression model does not allow this distinction either.
A similar picture was obtained when the increase in GMT between postvaccination and prevaccination for the 6 pertussis antigens was calculated as GMTpost/GMTpre. This increase is clearly visualised in Fig 3. It is important to keep in mind that the increase in ratio post/pre GMT only gives a broad outline of the effect of the different vaccines while the linear regression method was performed weighing all the participants individually.

The correlation between the different pertussis antigen assays was also analysed. A good correlation was only found between the Agglutination assay and the Fimbriae ELISA resulting in R=0.894 for all samples in the study. For the other antigens no good correlation could be observed even between PT, FHA and PRN (data not shown).

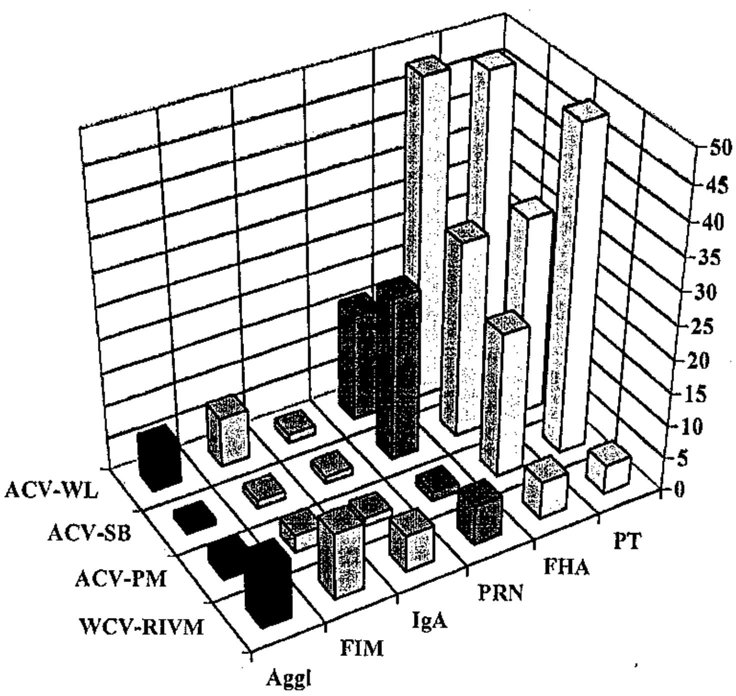


Figure 3. Rise in GMT of postvaccination titers compared to prevaccination titers for the pertussis antigens (x-axis) evoked by the 4 vaccines (y-axis). On the z-axis the ratio post/pre is indicated.

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3.2.2 IgA-WC

IgA-antibody titers only rose after vaccination with DTP-IPV (5-fold increase, see table 3). From the 43 children in the DTP-IPV and DTP-IPV$_v$ groups 16 showed an increase between pre- and postvaccinaton sample in their titer to a level =10 U/ml, while 17 others also had a rise in their titers but smaller (=5 U/ml). In all the other vaccine groups only one child showed a significant increase in its IgA titer to a level of 10 U/ml and three children a smaller increase (level between 3 and 6 U/ml). The IgG titers to the other Pertussis components in the DTP-IPV groups showed generally a good increase with the exception of PT where an increase to a level =10 U/ml was observed only in 18 out of 43 children. From these 18 children only 7 had significant elevated postvaccination titers for both IgA to *B. pertussis* and IgG to PT.
From the total of 180 participants, 15 children spread over the different groups had elevated IgA titers in their pre- as well as postsamples which may indicate on a recent natural infection. In 6 children a significant elevated titer to a level =10 U/ml was observed, while the other 9 showed a smaller increased level (=4 U/ml).

3.2.3 Diphtheria and Tetanus

The immune respons to diphtheria was very good and similar for the DT-IPV and the ACV groups (see Fig 1). The DTP-IPV group however showed a 2-fold increase compared to the other vaccine groups (table 4). The immune response to tetanus was also very strong and similar for all vaccine groups (Fig.1). None of the vaccine groups showed a significant difference in the rise in tetanus titers after vaccination (see table 4).
Before immunisation, 70 children (39.3%) had antibody levels <0.1 IU/ml to diphtheria and 32 children (18.0%) to tetanus. After vaccination, 8 children did not show a fourfold titer rise of diphtheria toxin antibodies and 9 children for tetanus. Thus the percentage with a fourfold rise amounted to =95%. From this group, 4 children had high pre-titers for diphtheria and 6 children for tetanus.
From the same group, 4 children did not show a titer rise both for diphtheria and tetanus and one child of these 4 had high pre-titers. From these 4 children, one child was still not protected against diphtheria, and one child was not protected against both diphtheria and tetanus (antibody levels <0.1 IU/ml). These 2 children were offered an additional DT-IPV vaccination.

3.2.4 Polio

The postvaccination titers to all three poliomyelitis strains were very high (note that the GMT's are expressed as a reciprocal ^{2}log in figure 1). There was only a small difference in immune response to the vaccine groups DTP-IPVvero and IPV-MK + ACV-PM for Polio type 3 (see Fig. 1 and table 4). For all other groups no difference was found between the IPVvero and IPV-MK for the three polio strains. In the DTP-IPVvero group the difference between the pre and post titer to polio type 3 was 1.65 (no ln, see paragraph 2.7.1) and after vaccination with DT-IPV+ACV-PM this difference was 2.13. When the results from the IPV-MK and IPVvero from the different groups were combined (5 and 3 groups, respectively) the GMT's were similar for pre and post samples for the three polio types with the exception of the post polio type 3 titers (table 5). A small but significant difference was found in favour of IPVvero for polio type 3. Remarkably, the GMT's for polio type 2 were slightly higher than for polio type 1. The percentages of the children with a fourfold rise after vaccination

Table 5. Geometric mean titers with 95% confidence interval in IPV-MK and IPVvero groups expressed as reciprocal ^{2}log titers.

		polio type 1		polio type 2		polio type 3	
		GMT	95%CI	GMT	95%CI	GMT	95%CI
IPV-MK	pre	9.39	[8.92-9.85]	8.53	[8.06-8.99]	6.84	[6.34-7.34]
	post	14.29	[13.85-14.73]	15.23	[14.76-15.67]	13.80	[13.34-14.26]*
IPVvero	pre	9.48	[8.84-10.12]	8.59	[8.07-9.12]	6.74	[6.07-7.40]
	post	14.25	[13.68-14.82]	15.77	[15.16-16.37]	14.91	[14.26-15.56]*

* statistically significant difference between IPV-MK and IPVvero (t-test $p<0.01$)

amounted up to 88, 93 and 95% for type 1, 2 and 3, respectively. The children that did not show a fourfold rise in titer had already very high pretiters. Before vaccination only 6 children were not protected against type 3 and after vaccination all children were protected.

3.3 Adverse events

For each study group both systemic adverse events and injection site reactions were recorded for the seven days following the vaccination. No serious adverse events occurred and no children were hospitalised and none were seen by a physician because of an adverse event. Sixteen children received paracetamol at any time in the first three days, mainly for fever and pain, thirteen in the whole-cell pertussis vaccine groups, two in the DT-IPV groups and one of the recipients of an acellular pertussis vaccine.
Frequency and severity was highest in the first 24 hours after vaccination and declined thereafter. In most children the symptoms lasted for one day or less. The most frequent complaint lasting into the second observation period was listlessness, in the majority without other systemic discomfort (25 cases). None of the children had continuation of systemic symptoms in the 4-7 day period. Therefore only symptoms in the first three days are presented.
The incidence rates of the systemic adverse events in the first day and the second-third day period for the different study groups are shown in Fig. 4A. The local reactions are displayed in Fig. 4B. Valid percentages are given (see for detailed recordings of adverse events Table 8A and Table 8B of the appendix). For comparison between the different groups the local reactions of the left arm in the WCV groups were compared with the accumulated reactions of the left and right arm in the ACV groups. The Odds Ratio's are given in Table 6.

Systemic and local symptoms were prominent in the whole-cell combination vaccine recipients, both in frequency as in severity or duration.
Fever, with recorded temperature ≥38.5°C occurred in 18/44 (40%) children at any time during the three days following the vaccination, in 5 children for more than one day. In the acellular pertussis vaccine recipients fever occurred in 3/91 (3.3%) children and in the DT-IPV groups in 2/45 (4%) cases, once for more than one day. The highest temperature recorded was 40.0°C in the WCV groups and 39.8 °C in the ACV or DT-IPV groups. With inclusion of low grade elevated body temperature (≥37.5°C) the respective numbers are 28/44, 7/91 and 5/45 (62%, 8%, 11%).

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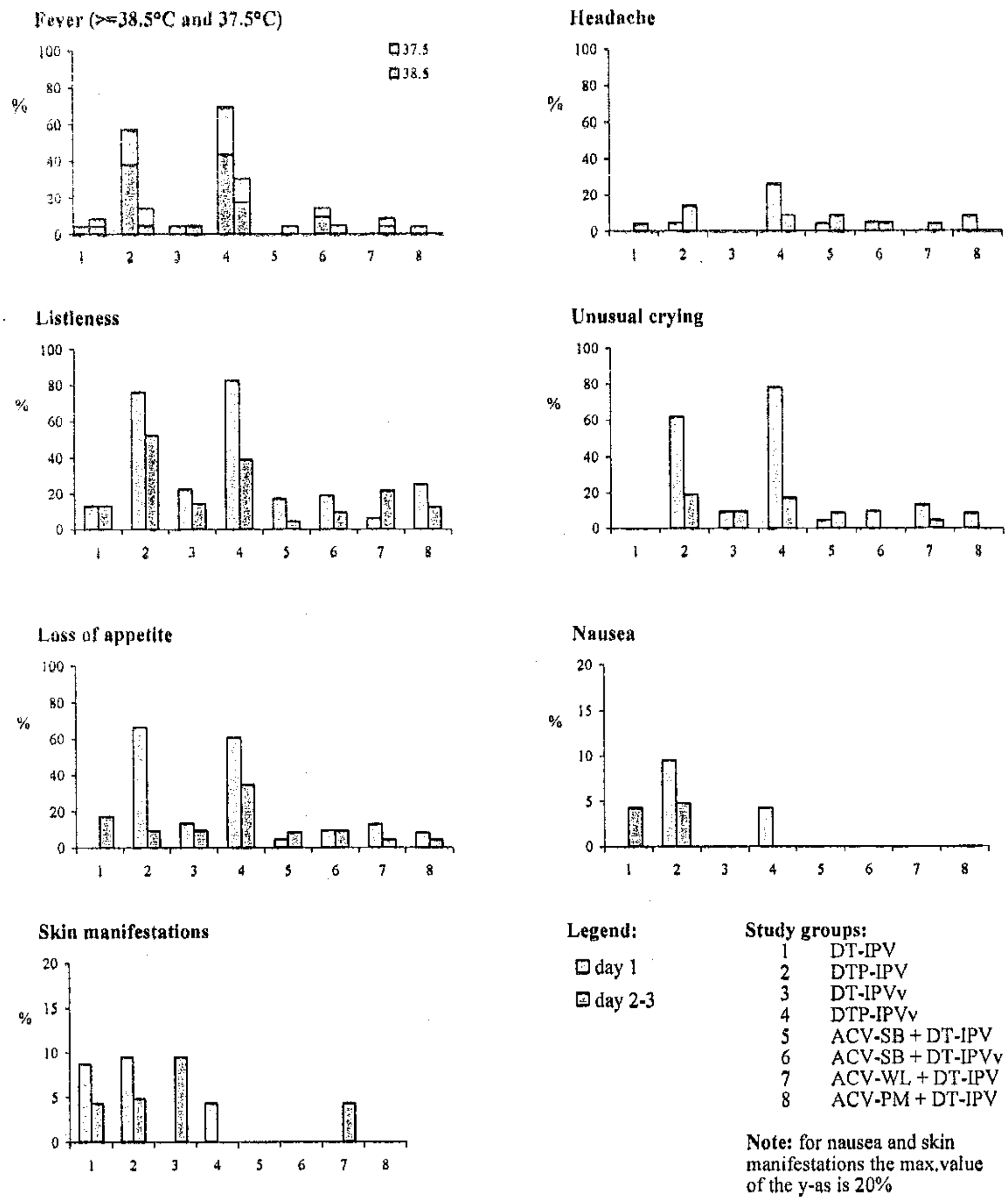
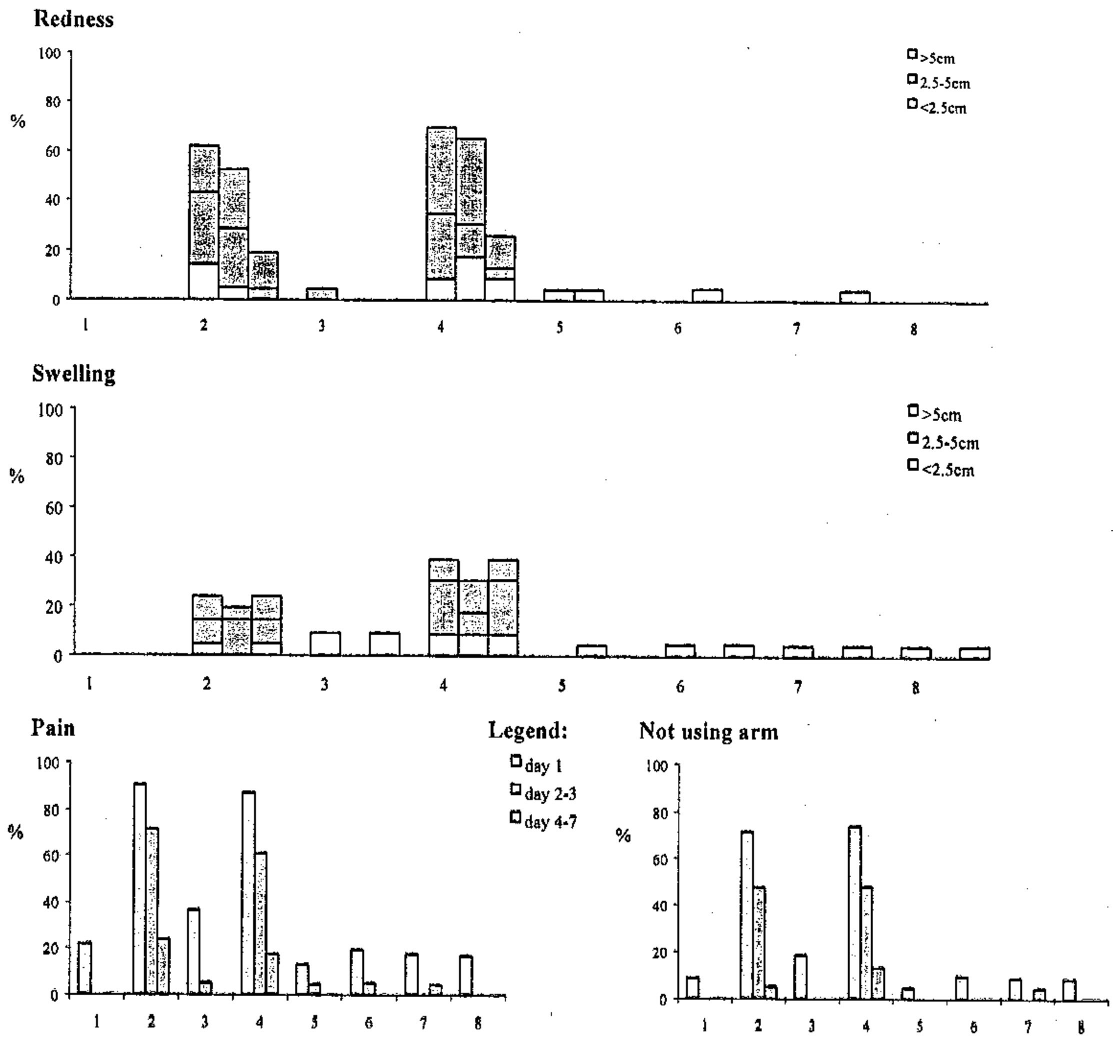


Figure 4A. Systemic adverse events occurring in percentages of children in the different vaccine groups.



Study groups:

1 DT-IPV
2 DTP-IPV
3 DT-IPVv
4 DTP-IPVv
5 ACV-SB + DT-IPV
6 ACV-SB + DT-IPVv
7 ACV-WL + DT-IPV
8 ACV-PM + DT-IPV

Figure 4B. Local adverse events occurring in percentages of children in the different vaccine groups for the three observation periods (day 1; 2-3; 4-7).

Listlessness and crying occurred in 69-80 % of whole-cell pertussis combination vaccine recipients often accompanied by loss of appetite, with much lower frequencies in the other group with 15-30% and 9-27% respectively in the ACV and DT-IPV groups (see table 6 for frequencies and Odds Ratio's). Nausea and skin manifestations were rare events in all study groups.
Local reactions were also much more common in the WCV groups as was the severity and duration of the symptoms. Of the children in the WCV groups 23 experienced local redness for more than one day of which 10 extended into the third observation period. Swelling was less frequent and of shorter duration. Tenderness was very frequent and lasted in 28/44 cases for more than one day with in 9 cases into the third observation period. This was in the majority of cases accompanied with diminished use of the affected arm 35/44 which lasted in 18/44 for more than one day and in 4 cases more than 3 days. Injection site reactions for acellular vaccines and DT-IPV were much less frequent.
For the DT-IPV-only group redness and swelling occurred in up to 4% and keeping the arm still in 13 % with noted pain in 42 %. Only once pain lasted for more than one day.
The DT-IPV injection site reactions in the ACV groups were for redness and swelling 3.3-6.7% with pain in 25% and diminished use of the arm in 11%.
For the ACV injection site the aggregated local reactions were slightly lower with redness and swelling in up to 5.5% and pain and still arm in 18.7% and 7.7% respectively.

Logistic regression analyses was used to assess the association of the specific vaccines or components and the occurrence of specific adverse events. Fever, listlessness, crying, anorexia and headache occurring at any time during the seven day follow-up were used as dependent variables. As were the local reactions redness, swelling, pain or diminished use of the affected arm. As dummy variables were used WVC component containing vaccines, ACV component vaccines and IPV-vero component containing vaccines. Fever, headache, listlessness, crying and loss of appetite were significantly more prevalent in the WCV containing vaccine groups than in the groups with ACV and /or DT-IPV. This difference was most prominent on the first day and for listlessness still statistically significant in the second observation period. The effect of WCV containing vaccines on the four injection site symptoms was also statistically significant.

Table 6. Proportion and Odds Ratio's of adverse events in DTP-IPV and ACV groups vs. DT-IPV-groups

Symptom	DT-IPV	DTP-IPV	OR	[95%CI]	ACV	OR	[95%CI]
General adverse events							
fever (=38.5°C)	2/44	18/44	14.54	[3.12-67.86]	3/91	0.72	[0.12-4.45]
headache	1/44	10/44	12.65	[1.54-103.72]	7/90	3.63	[0.43-30.44]
listlessness	12/45	36/44	12.38	[4.50-34.03]	27/91	1.16	[0.52-2.58]
crying	4/45	31/44	24.44	[7.26-82.29]	11/91	1.41	[0.42-4.70]
loss of appetite	8/44	30/44	9.64	[3.57-26.07]	10/91	0.56	[0.20-1.52]
Local symptoms							
redness	1/45	33/44	132.00	[16.22-1073.93]	6/91	3.11	[0.36-26.61]
swelling	2/45	15/44	11.12	[2.36-52.33]	6/91	1.52	[0.29-7.84]
not using arm	6/45	35/44	25.28	[8.17-78.20]	13/91	1.08	[0.38-3.07]
pain	13/45	41/44	33.64	[8.83-128.20]	29/91	1.15	[0.53-2.51]

It is in this study not possible to distinguish between the WCV component and the effect of the other components of combined vaccines or of interaction/potentiation between the different vaccine components. The logistic regression model does not allow this distinction either.
There was no statistically significant difference between the groups receiving ACV and DT-IPV-only recipients, although there may be some attribution of ACV on systemic symptoms that could not reach statistically significance level because of the small numbers. For local symptoms the used model cannot distinguish between ACV and DT-IPV because the aggregated results of left and right arm together were used.
There was no statistically significant influence of IPVvero on systemic events.

4. Discussion

In the past few years several vaccine trials with a large number of ACV's in combination with DT have been performed (6, 7, 18, 20, 26, 28, 30, 39, 56, 61, 63, 76). Most of those studies involved the primary vaccinations in the first half year of life of the children and a booster at one year or in the second year of life. Only in a few trials ACV- and WCV-administration as a booster vaccination in children 4 to 6 years of age has been studied (3, 7, 8, 39, 59, 82). In this trial we have studied the immunogenicity and reactogenicity of 3 different ACV's given simultaneously with the regular DT-IPV and the RIVM-WCV in the combination vaccine DTP-IPV administered as a booster dose in children 4 years of age. Furthermore, the immune response of a new IPV (produced on Vero cells) was evaluated in comparison with the regular IPV (produced on MK cells) in the same group of children. The children in this study were previously immunized with 4 doses of the RIVM DTP-IPV (containing WCV and IPV-MK) at the age of 3, 4, 5 and 11 months.

4.1 Antibody responses

4.1.1 ACV

The GMT's of the pertussis antigens PT, FHA and PRN determined after vaccination with the 3 different ACV's in this study are in agreement with the titers observed in other trials with these vaccines (18, 28, 30, 56). All three ACV's induce an excellent response to the pertussis components that are included in the vaccines (fourfold rise in antibody titer = 90%). The near equal GMT-PT in the different ACV groups, despite the 8-fold lower PT content in ACV-WL may reflect the different detoxification procedures of PT. For FHA the antibody levels of the ACV-PM were slightly lower, although antigen content was equal for the different ACV's. The antibody responses to PRN do reflect the antigen contents of the different vaccines. The immune response to Aggl. and FIM in 30% and 15% of children vaccinated with ACV-PM is surprising since the vaccine is not supposed to contain FIM in sufficient amounts. But the response indicates that a small amount of fimbriae is likely to be present in this vaccine. In the ACV-WL group 4 out of 22 children had no response against FIM, probably reflecting the low content of this antigen in the vaccine. ACV-SB does not contain FIM and did not evoke an immune response.

4.1.2 WCV

The WCV groups showed a very broad immune response to all tested antigens. Especially the response to FIM and Aggl. with a fourfold titer rise was very good (up to 90%). The absolute titers against PT, FHA and PRN were considerably lower than observed for the ACV's, which reflects the smaller amounts of PT, FHA and PRN present in the RIVM-WCV. The lower GMT's may not be clinically relevant since conferred immunity is not reflected by the level of antibodies, especially in WCV recipients but also in ACV.
About 50% of the children in the WCV group did not respond at all to PT, but did respond to the other pertussis antigens. If the non-responders to PT are omitted from the WCV groups the GMT to PT doubles (21-48 EU/ml). Apparently, the booster vaccination with WCV induces a good or a non-detectable (all or nothing?) response against PT. The antigen content of PT present in the RIVM-WCV is low indeed due to the production procedures, purposefully introduced some decades ago (15). The production process for the RIVM-WCV was modified in 1996. This modification resulted in a better standardisation of the potency.

In the DT-IPV and the ACV-SB group a high GMT to Aggl. and FIM in the prevaccination samples was observed. This elevated level was maintained after vaccination but no rise was observed. Because of their composition these two vaccines are not likely to induce a rise in the Aggl. and FIM antibody titers. Therefore, the high GMT in the pre- and postvaccination samples must be due to the high titers of several children in these groups most likely induced by natural infection.

4.1.3 IgA

The presence of IgA antibodies reflects contact with *B. pertussis* (54). Elevated IgA titers were observed in the paired pre- and postvaccination samples of 15 children from the total study group (8%). In 5 of these 15 children the pre-titers were very suggestive for a recent *B. pertussis* infection (high titers for all the pertussis antigens as well as for IgA), in 4 cases for a *B. parapertussis* infection (high titers to all the pertussis antigens except PT) and in the other 6 the data were not so conclusive. These 15 children were evenly distributed over the different vaccine groups with the exception of the DTP-IPV and ACV-PM groups in which no significant elevated IgA titers in paired samples could be found.
On the contrary, in the DTP-IPV groups 32 out of 43 children showed a rise in the IgA titer after vaccination (=5 U/ml). From these 32 children only in 4 cases the prevaccination samples were also slightly elevated in IgA titer (4 to 7 U/ml). In one of these 4 the overall titers were suggestive for a recent *B. pertussis* infection. In a few cases from the 31 children the rise in IgA titer as well as in the other pertussis antigen titers was so dramatic that this might be caused by an infection intermittent with the study. However, parents did not report clinical symptoms of *B. pertussis* at the second blood sampling. None of the children of the ACV groups showed a substantial IgA response.
All the data taken together seem to indicate that vaccination with DTP-IPV causes a small but significant rise in the IgA titer in the serum of most children. The relevance of this titer increase remains to be investigated. It is interesting to determine the specificity of these IgA antibodies. According to the literature they should be directed largely against FHA (25) but preliminary results show that these IgA antibodies are directed not only against FHA but also against PRN and to a lesser extent against PT. Another very important question is whether there might be a link/correlation to an increase of the titer of secretory IgA on the mucosa of the respiratory tract.

4.1.4 D and T

The immune response to Diphtheria toxoid is significantly larger in the DTP-IPV groups than in the other vaccine groups. The greater amount of toxoid present in this vaccine (15 Lf versus 2.5 Lf in DT-IPV) and/or the adjuvant effect of the pertussis WCV-component on the diphtheria response in this combination vaccine can explain the larger titer increase. It should be realized that this adjuvant effect is lost of course should WCV be replaced by an ACV. The immune response to Tetanus toxoid was also very good and no difference was observed between the groups.

4.1.5 IPV

The response to the IPV-MK and IPVvero was excellent against all three virus types. The small difference observed in the ACV-PM group for polio type 3 is probably due to a few low pretiters of some children in this group resulting in a low preGMT. When the data from all IPV-MK and all IPVvero vaccinations are compiled this difference was no longer observed due to the larger number of children in the groups. The difference in the response to polio

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type 3 found in the DTP-IPVvero group was still significant after compiling the results from the different groups. This might reflect the difference in the amount of D-Ag of type 3 between the IPV-MK and IPVvero composition. The IPV-MK as a component of DT(P)-IPV was composed according to current Dutch guidelines (with a minimum of 20-2-3.5 D-Ag for type 1, 2 and 3, respectively) and the IPVvero to the international requirements (at least 40-8-32 D-Ag for the 3 strains). Both vaccines used in this study comply with these requirements (see table 1). While the twofold difference in the amount of polio type 2 present in the two vaccines seems to have no influence on the response, the fourfold difference in the amount of type 3 may result in a significant difference in response. However, the antibody titers after vaccination with both vaccines are so high that it is doubtful if such a difference will have any influence on protection.
Both vaccines perform very well in this study in which the children proved to be excellently primed. If both vaccines will contain a similar composition of the three strains, most probably no difference in response will be observed between them in future field trials.

4.2 Adverse events

In this study the rate of adverse events in the whole cell pertussis vaccine recipients was much higher than in the acellular pertussis vaccine recipients. This was to be expected since WCV is much more reactogenic than ACV. This difference is most pronounced in the frequent mild adverse reactions.
It was shown in the Sweden trial that the rarer more serious adverse events occurred in infants in near equal frequency following WCV and ACV (56). This study with four year old children is far to small to detect any adverse event that is relatively rare.
It should be stressed that even for the common adverse events this study does not provide for direct comparison of the whole cell pertussis component with the acellular component. The whole cell component is administered in a combination vaccine with known interaction whereas the acellular component vaccines are single vaccines.
So the results should be interpreted with some reservation. Also because possible attention bias may play a role since the study was not blinded and parents were advised on what to expect and how to deal with occurring adverse events. Although the WCV recipients had frequent systemic and local symptoms, generally there was only mild to moderate discomfort, mainly limited to the first day after vaccination. Compared to studies in infants, the rate of adverse events in this group of four year olds is favourable (64, 81). The results do not suggest higher reactogenicity of WCV in older children as is commonly believed (53). Considering the higher potency of the WCV component of the vaccine used in the current study this seems to be even less the case.
Comparing safety data between different studies is nearly always hampered by different designs, level of ascertainment and cut-off values. Differences in vaccine constituents other than the WCV may attribute substantially to observed differences in reactogenicity. In this study there does not seem to be significant differences in the rate of reactions between the IPVvero and IPV-MK containing vaccines.
In the light of the satisfactory serologic profile, with a very broad response to all tested antigens and the proven long-term effectivity of whole cell pertussis vaccines, it certainly seems worthwhile and feasible to invest in lowering the reactogenicity of the current vaccine (4, 5, 16, 45), which is necessary for maintaining the high acceptance of the RVP in the population. Additionally, programmatic adjustments like changing the injection site from M. Deltoideus to M. Triceps may alleviate local reactions to some extent. The use of profylactic paracetamol as is advised in some other countries may deserve attention also.

4.3 Considerations and recommendations

4.3.1 Booster response and memory

For the pertussis antigens, the titers after administration with acellular vaccines are high and comparable with other field trials where a 3 or 4 dose schedule with the last vaccination as booster is used. Titers of non-vaccinated children at 4 years of age are not known in the Netherlands due to the massive participation in the national immunisation programme. However, titers found after vaccinations with WCV at 3, 4 and 5 months (priming) and even at 11 months (booster) are much lower compared to the titers observed in this study (41, 80). It is obvious from the immune responses to the different vaccines/vaccine components measured in this study that the children are indeed primed by the vaccinations with the RIVM-WCV administered in the first year of life. This observation indicates that the 4 year old children have sufficient immunological memory to produce a good booster response. Moreover, it should be noted that in the serum of about 10% of children before and during the trial there were signs of natural *B.pertussis* or *B. parapertussis* infection. They showed high titers to all or almost all measured pertussis antigens. However, only one child reported clinical symptoms. So there is evidence that the RVP schedule and vaccine do protect against infection or colonization and prevent clinical symptoms or complications, again suggesting sufficient immunological memory for protection against natural infection. But it also shows that the risk of pertussis is high, and it stresses the importance of timely vaccination. For the RVP, it carries a warning against unnecessary postponement and omitting the pertussis component in the first four vaccinations.

4.3.2 Importance of antibodies

The persistence of high antibody levels after vaccination with ACV or WCV and natural infection is generally limited (26, 47, 79). The pretiters of antibodies directed against the different pertussis antigens were low in this study about 3 years after the previous pertussis immunisation, but also in other field trials it has been observed that the titers almost disappeared in not more than 2-3 years. It is not to be expected that there will be a difference in persistence of antibodies induced by the ACV and WCV vaccines used in this trial. It seems that in contrast with Diphtheria, Tetanus and in particular Polio vaccines, the pertussis antibody titers as a result of administration of ACV or WCV and even after natural infection have a much shorter half life. This is illustrated by the high rate of reinfections in the whole population and multiple subclinical or asymptomatic infections individually during life (84). In this respect, it should be noted that high titers as induced by ACV's do not necessarily mean better protection because these titers also rapidly decrease. Therefore, the importance of the antibodies is still under discussion: do they contribute to protection or not? Recently, in pertussis field trials in Sweden and Germany a correlation was found between the presence of antibodies in individuals and the degree of protection in 2 year follow-up studies. Both research groups came to the conclusion that besides PT also PRN and FIM antibodies seem to be relevant for protection (11, 79). The antibodies against FHA were found not to contribute to protection. These findings support historical data indicating that agglutinating antibodies are associated with protection (44) and suggest that anti-PT, anti-PRN and anti-FIM antibodies may be used as surrogate markers of protection. As a consequence antibodies really contribute to protection although, as it seems, on a short term basis because the titers against pertussis antigens rapidly decrease within 2-3 years (17, 27, 37, 65, 66). The vaccine induced protection against *B. pertussis* seems to last longer (5-10 years) than the measured antibody levels and upon subsequent natural infection the disease is much milder. Although many biases can affect efficacy studies based on attack rates, in this manner calculated vaccine efficacy seems to amount to around 50% after 5 years (21, 38).

4.3.3 Polymorphism of *B. pertussis*

Another important point to take into consideration is the polymorphism in the virulence factors PT and PRN of *B. pertussis* found not only in the Netherlands but also in strains in other countries. There is accumulating evidence that the selection pressure caused by the vaccination programme during the past 45 years may have selected for strains carrying PRN and PT variants distinct from those found in the vaccine strains. This has already been proven for PT and PRN but is possibly also the case for other pertussis components (52). If this vaccine-driven evolution of *B. pertussis* should prove to be a major cause of the Dutch epidemic in 1996-97 (49), then the consequences for both WCV's and ACV's will be enormous, the route to further improve the pertussis vaccines will be longer and the shrew of pertussis even more difficult to be tamed (14).
In this respect, it should be noted that the ACV-WL contains FIM2 as vaccine component which is at the moment only present in the 10-20% of the circulating strains of *B. pertussis* in the Netherlands (80-90% of circulating strains contain FIM3). The RIVM-WCV is composed of two strains (509 and 134) which ensures the presence of both FIM2 and FIM3 in the vaccine. Both the WCV and the ACV's contain the variant PRN1 while the majority of the circulating strains of *B. pertussis* in the Netherlands since 1990 were found to contain variant PRN2 or PRN3 (43). Immunological effects of the antigenic differences found in PRN has been suggested in the results of proliferative T-cell responses (33, 51). A similar situation applies for the S1 subunit of PT with variants B and D present in all vaccines and mainly variant A in the circulating strains, but it should be noted that the polymorphism of PT is restricted to a conservative substitution of 3 amino acids (52).

4.3.4 Other parameters

It will be worthwhile to measure in these serum samples the biologically active, functional antibodies in more detail, like with the PT-assay in Chinese Hamster Ovary cells, inhibition of adhesion to epithelial cells, the subclass of the antibodies etc. Moreover, the samples can be used to localize immunologically important epitopes of PT, FHA and PRN with the use of monoclonal antibodies, synthetic peptides and affinity measurements.
In this field trial, heparinized blood samples were taken also in study groups DT-IPV, DTP-IPV and ACV-SB to study the influence of vaccination on the cellular immunity in more detail (29). In particular, the ability of T-cells to proliferate after stimulation with different peptides from the various pertussis antigens and the cytokine profile of these stimulated T-cells will be studied. All these studies can contribute to a better understanding of the correlates of protection in case of *B. pertussis* infection.

4.3.5 Possible effects of extra vaccination

Since 1953, when pertussis vaccination was introduced in the Netherlands, the whole cell vaccine has lowered the incidence of clinical pertussis tremendously. Protection has been most pronounced in young children in whom the rate of complications, sequelae and death is highest. There is probably also cross-protection against *B. parapertussis* (34).
Due to the recent epidemic (1996-1997) doubts have been raised about the efficacy of the WCV, but it was shown that there still is protection in the majority of the population and that vaccination reduced severity of symptoms. Most reported cases were seen in the 4-9 year old age group. It is debated that the inclusion of pertussis vaccine in the RVP at four years of age

will either or not diminish the substantial burden of disease in the (very) young children and may provide a substantial contribution to the prevention of epidemics, which is the long term purpose of such a booster vaccination (10).
In the USA there seems to be a shift in the important source of *B. pertussis* infection to older age groups in the child bearing age, who may transmit the disease to susceptible infants. It is hypothesized that this shift of infection is due to revaccination at 4-6 years of age, postponing natural reinfection at that age which might have caused a more solid long lasting immunity. The importance of this hypothesis needs to be further established (1).
Whether including pertussis vaccine in the regular schedule in the four year olds will effectively lower clinical pertussis incidence in young children in the Netherlands remains to be seen. An extra pertussis vaccination at this age will probably decrease *B. pertussis* circulation in siblings of young infants. It is not very likely, however, that it will decrease the circulation among parents of babies in the Netherlands-with an average age of mothers of first-borns near thirty- contrary to suppositions made in other countries. There is accumulating evidence that persisting immunity in vaccinated populations depends on subclinical or mild infections to booster waning immunity in later years (21). These infections seem to occur more frequently in elderly children and adults than has been commonly recognized. These individuals may function as a major reservoir for transmission of pertussis to infants too young to be vaccinated (13, 17, 21).

4.3.6 Choice of pertussis vaccine for 4 year old children

On the basis of this study and the serological results it is not possible to make a choice between the three different ACV's, because they all show very good responses reflecting their variable composition, but a high titer level is not related directly to protection. Moreover the data available about long term efficacy of different ACV's are limited yet (62, 76). It seems reasonable based on former trials to prefer an acellular vaccine with the most pertussis components (18, 28, 56). The necessary incorporation of any acellular vaccine into a combined DT-IPV vaccine will take time and further field trials. As discussed by Gangarosa et al., the choice between WCV and ACV involves trade-offs between safety, efficacy, practicality, and cost (24). In addition to fewer mild adverse events, acellular vaccines could interrupt disease transmission by means of their potential use in adolescents and adults. However, the best acellular vaccine may not provide protection equal to that of the best whole-cell vaccines (62). Replacement of WCV with ACV might conceivably lead to less effective control at substantially higher costs (24).

If it is considered necessary in the control of B. pertussis and for protection of the most vulnerable age group, to include pertussis vaccination of the four year olds immediately, then the easiest, fastest and cheapest way is to use the regular DTP-IPV vaccine. Despite the relatively high rate of common adverse events, the balance of the safety profile and the broad immunogenicity profile and long term proven effectivity of the whole cell pertussis vaccine components seem to warrant such a choice. Moreover it will save the children of this age group the emotional stress of a second injection and some additional injection site complaints and it will save money and time because it will be just one injection. If a multicomponent acellular vaccine is chosen for the booster dose the ACV with the most components should be preferred and a combination with the regular DT-IPV seems a requirement to guarantee a single injection for the 4 year old children.

4.4 Conclusion

The main goal of this study was to investigate whether a booster response could be induced after vaccination with acellular and whole cell pertussis vaccines in children primed with WCV and to demonstrate whether the two polio vaccines induced similar booster responses. In general, the answer to those two questions can be positive for all vaccines.

With respect to pertussis, after vaccination with the ACV's almost all the titers are high against the different pertussis components and generally reflect the composition of these components present in the vaccines. The titers are comparable with those observed in other trials with these vaccines. After vaccination with the WCV the titers against Aggl. and FIM are good, against FHA and PRN still reasonable but against PT they are low. The reason for this is perfectly clear. The amount of PT was kept as low as possible in the vaccine for historical reasons as this was believed to prevent serious adverse events. Therefore, half of the children vaccinated with WCV showed no PT-booster reaction and the GMT of PT for the other half was reasonable. On the other hand, the response to the WCV was broad as expected but also showed an unexpected IgA-response in the majority of the children. A drawback of the WCV is the rate of adverse events, although mostly mild and of limited duration. This may hamper the acceptance of the vaccine in the population. But this will almost certainly be the case if there is a need for two injections instead of one. The emotional upset and pain and additional injection site reactions will have to be taken into account in the trade-off between different pertussis vaccines, as will be the costs of purchase, production and of the (extra) time needed by the vaccine administrators.

With respect to polio, the answer is far less complex. Both the IPV-MK and the IPVvero have proven to induce excellent booster responses in this trial. Although the composition of the vaccines was not identical, the protection after the booster vaccinations with the IPVvero produced under the international requirements will be as good or even better as achieved with the present vaccine.