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Potency/Immunogenicity Profile of DPT Vaccines Used in the Expanded Programme on Immunization in South-East, Nigeria

Oli AN[1]*, Agu RU[2], Nnadozie OJ[3] and Esimone CO[1]

[1]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria*
[2]*Biopharmaceutics and Drug Delivery Laboratory, College of Pharmacy, 5968 College Street, PO Box 15000, Dalhousie University, B3H 4R2, Halifax, NS, Canada*
[3]*Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria*

Abstract

Objectives: This study tries to validate the DPT vaccines used in South-east Nigeria for the routine childhood immunization.

Study design: The Antibody Induction Method in mice was used. The neutralizing IgG antibody titers in a control group (7 mice given DPT stored at 37°C for 1 year) and test group (7 mice given DPT vaccines from the South-Eastern States of Nigeria) were compared after 30 days post-immunization using One-Way ANOVA, Bartlett's test for equal variances and Dunnett's Multiple Comparison Test.

Results: The vaccines from the States produced similar Pertussis and Tetanus antibody titers which were significantly higher than the control (P<0.0001). The Diphtheria antibody titer produced by the vaccine from Enugu/Ebonyi States was higher than the vaccines from other States. The control produced much less Diphtheria IgG antibody titer (P<0.0001). The vaccines do not differ significantly from each other on the level of antibody titer (protection) and their individual protection can last for the maximum number of days needed for a repeat vaccination except for the control.

Conclusions: There is statistical difference in the antibody titer evoked by the control compared to that evoked by the vaccines from the states. The vaccines from the States show 100% potency and immunogenicity. This indicates adequate cold-chain maintenance and monitoring of South-eastern Nigeria. Continuous monitoring of efficiency of cold- chain maintenance and vaccine potency testing would translate to good vaccine strategy.

Keywords: Routine immunization; DPT vaccine; Potency/Immunogenicity; South-east Nigeria

Introduction

Preventive medicine has been recognized to be a cheaper way of avoiding treatment cost, averting disability and preventing death and vaccines are well known for their role in such [1-3]. Because they are bio-products, they are affected by storage temperature/conditions and their potency tends to reduce with time. Childhood immunisation is one of the most important preventive health actions undertaken to promote children's health [3,4]. It protects them against the most dangerous childhood diseases. Potency and immunogenicity assays are essential in the quality control of vaccines for human and animal use [5,6] and are integral parts in biopharmaceutical products' life cycle and development [7]. Immunogenicity studies generally provide important information with respect to optimization of adjuvant formulations (where necessary) and the evaluation of immunological characteristics of the antigen including the ability to induce functional antibodies and/or protection from challenge [8]. While immunogenicity refers to a vaccine's ability to induce a specific immune response, potency refers to ability to induce a protective immune response [9].

DPT is a class of combination vaccines developed to provide protection against three infectious human diseases: diphtheria, pertussis (whooping cough) and tetanus. The vaccine components include diphtheria and tetanus toxoids, and killed whole *Bordetella pertussis* cells - the organism that causes pertussis - all adsorbed on insoluble aluminium salts that serve as adjuvants. Some other combination vaccines include the Pentavalent vaccine (a combination of five vaccines in one: diphtheria, tetanus, whooping cough, hepatitis B and *Haemophilus influenza* type b (the bacteria that causes meningitis, pneumonia and otitis)), Hexaxim [10,11] - "a new, thiomersal-free, fully liquid, hexavalent combination pediatric vaccine containing diphtheria and tetanus toxoids, acellular pertussis, inactivated poliovirus, recombinant hepatitis B virus surface antigen produced in the yeast *Hansenula polymorpha,* and *Haemophilus influenzae* type b polysaccharide (polyribosylribitol phosphate) conjugated to tetanus toxoid" and MMR vaccine which give protection against measles, mumps and rubella. The pentavalent vaccine is expected to replace the current Hepatitis B and DPT vaccinations in the immunisation programme during the 6,10 and 10 week after birth and reduces the incidence of pneumonia and meningitis caused by *Haemophilus influenzae* type b (Hib) bacteria [4,12,13].

This study evaluates, indirectly, if the DPT and Pentavalent vaccines used in routine Immunization Programme in South-east Nigeria contains sufficient quantity of antigens of suitable characteristics (quality) to induce a protective immune response.

Methods

Vaccine samples

The DTP vaccines were donated by the Ministries of Health of

***Corresponding author:** Oli AN, Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria, E-mail: oli_an@yahoo.com

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Ebonyi, Enugu and Imo States while Pentavalent (DTP/IPV/Hib) were donated by the Ministries of Health of Abia and Anambra States. Vaccines were transported in vaccines carrier and stored in the storage of facility Nnamdi Azikiwe University Teaching Hospital, Nnewi within 4 hours of collection. Study was conducted within 1 month after vaccine collection. The temperature of the storage facility was charted daily while vaccine storage lasted.

Animals

Swiss albino mice (15-26 g) were used. They were accommodated under standard conditions (temperature: 26 ± 2°C, relative humidity: 45 ± 2%) and provided with standard pellet diet and water. The study was carried out in Pharmacology and Toxicology Laboratory of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus and in Chemical Pathology laboratory of Nnamdi Azikiwe University Teaching Hospital, Nnewi. The research protocols were approved by the Research and Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi.

Antibody development by animal immunization

Modified Antibody Induction Method in mice [14] was used. Briefly, fourteen mice of similar weight and sex were distributed in to 2 groups with each group containing 7 mice. The first group was immunized with one human dose of the test DPT or Pentavalent vaccine by intra-peritoneal injection. The second group was immunized with DPT vaccine previously stored at 37°C for 12 months. The animals were well fed while the study lasted (30 days). During the feeding period, the animals were checked daily for any abnormality before they were bled.

Bleeding and serum extraction

The animals were bled using heparinized capillary tube inserted via the eye (just below the eye ball) and their blood collected in sterile eppendoff tube. The blood was allowed to clot before centrifuging at 4000 rpm for 30 minutes. The sera were carefully pipetted out, transferred into another sterile eppendoff tube and preserved by freezing until ready to use.

Antibody measurement

Antibody quantifications were carried out using the protocols stated by the manufacturer (Bioactiva Diagnostica GmbH, Homburg, Germany) of the ELISA kits used. All specimens and reagents were allowed to reach room temperature (25 ± 2°C). Serum samples/specimens were diluted carefully mixing 10 µl serum + 1 ml of sample diluents. A volume of 100 µL of *Clostridium tetani* 5S IgG or *Corynebacterium diphtheriae* IgG toxin IgG standards (A-E) and diluted serum samples/specimens containing diphtheria or tetanus antibodies were pipetted into microtitre strip wells pre-coated with inactivated specific *Corynebacterium diphtheriae* or *Clostridium tetani* toxin (toxoid) antigens (as the case may be) to bind the antibodies. The samples/specimens were put in duplicate wells and the substrate blank was dispensed into the well A1. The wells were covered with foil supplied in the kits and the plate incubated at 37 ± 1°C for 1 hr ± 5 minutes. The wells were aspirated and washed 5 times for 30 seconds with washing solution (350µl/well) using automatic microplate washer (Stat Fax – 2600, model #: H009775). After washing the wells to remove all the unbound sample material, 100 µl of *C. diphtheriae* or *Clostridium tetani* toxin horseradish Peroxidase (HRP) labeled anti-mouse IgG conjugate was added into all the wells (to bind the captured *Corynebacterium diphtheriae* or *C. tetani* toxin-specific antibodies) except the blank well and covered. The plates were incubated for 30 minutes at room temperature (23 ± 2°C) away from direct light. Well aspirating and washing was repeated as done previously. The immune complex formed by the bound conjugate was visualized by adding 100 µl Tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the quantity of *Corynebacterium diphtheriae* or *C. tetani* toxin-specific IgG antibodies in the serum/specimen. The colour development is stopped by pipetting 100 µl 0.2M Sulphuric acid into the wells of the plates. The absorbance at 450 nm was read using Stat Fax - 2100 microplate reader (manufacturer – Awareness Technology, USA) and the result recorded as mean absorbance. A graph of the absorbance – concentration was developed from the Standards A- E and concentration of the IgG antibody was calculated from the graph.

For the pertussis, 100 µL diluted serum samples/specimen and five standards (A – E) of volume 100µl each, were pipetted into microtitre strip wells pre-coated with *Bordetella pertussis*/toxin (antigen) and incubated at 37 ± 1°C for 1 hr ± 5 minutes. The substrate blank was dispensed into the well A1, the negative controls into the wells B1 and C1, the cut-off controls into wells D1, E1, F1 and G1 while the positive controls were in wells H1 and A2. The rest of the wells contained the samples in duplicates. The wells were aspirated and washed 5 times for 30 seconds with washing solution 350 µl/well using automatic microplate washer (Stat Fax – 2600, model #: H009775). A volume of 100 µl of *Bordetella pertussis* anti-mouse IgG horseradish peroxidase conjugate was dispensed into all the wells except for the blank well and covered. The plate was incubated for 30 minutes at room temperature (23 ± 2°C) away from direct light. Well aspirating and washing was repeated as done previously. Tetramethylbenzidine (TMB) substrate, 100 µl volume, was pipetted into all the wells (including the blank well) and the plate was incubated for 15 minutes in the dark at room temperature. Stop solution (0.2M Sulphuric acid) of volume 100 µl was pipetted into the wells which changed the colour from blue to yellow. The absorbance was read at 450 nm using Stat Fax-2100 microplate reader (manufacturer-Awareness Technology, USA) and the result recorded as a ratio of mean absorbance of specimen and the cut-off.

Results

Table1 shows diphtheria IgG antibody titer in laboratory animals used (mice). It is the recommendation of the manufacturer of the ELISA kit used that titer/results <0.01 IU/mL means absence of protective

Vaccine from	ANTIBODY TITER IN THE MICE (IU/mL)								
	1	2	3	4	5	6	7	Mean	±SEM
Control	0.057	0.076	0.103	0.058	0.058	0.061	0.071	0.0691	±0.0063
Enugu/Ebonyi	0.719	1.429	1.449	2.651	1.427	1.364	1.474	1.502	±0.2164
Imo State	1.179	1.109	0.954	1.294	0.872	0.700	0.904	1.002	±0.0769
Anambra	0.943	1.189	1.163	0.664	0.986	1.185	0.956	1.012	±0.0713
Abia	0.637	1.109	0.969	0.664	0.897	0.823	1.452	0.9359	±0.1063

Note: Control was the DPT stored at 37°C for 12 months

Table 1: Diphtheria IgG Antibody quantification in immunized Mice.

antibody level and so immediate full course of basic immunization is recommended. Titers of 0.01-0.09 IU/mL means absence of reliable protection (i.e. antibody level is too low). Booster vaccination is recommended within 4-6 weeks. Titers 0.1-1.5 IU/mL means presence of reliable protection but booster vaccination is needed after 5 years. Titres >1.5-2.0 IU/mL means presence of reliable protection but booster vaccination is needed after 7 years while titers >2.0 IU/mL mean presence of long term protection but vaccination may be needed after 10 years. All the vaccines from the states have titres between 0.9 and 1.5 IU/mL and so offer protection.

Table 2 shows pertussis IgG antibody titer in laboratory animals used (mice). The manufacturer of the ELISA kit used recommended a Cut-off of 10 NTU to mean no reliable protection. The antibody level is insufficient to confer protection and so, booster vaccination is needed. A Grey Zone=9 -11 NTU means that protection is in doubt. Booster vaccination is needed A negative protection =<9 NTU meaning no protective antibody and so immediate vaccination is needed. A positive protection =>11 NTU means there is reliable protection. All the vaccines except the control have antibody titer well above 11 NTU.

Table 3 shows tetanus IgG antibody titer in laboratory animals used (mice). By the recommendations of the manufacturer of the ELISA kit used antibody titer/results <0.01 IU/mL means absence of protective antibody level. Immediate full course of basic immunization is recommended. Titers 0.01-0.10 IU/mL means absence of reliable protection (antibody level is too low). Booster vaccination recommended within 4 weeks. Titers 0.11-0.5 IU/mL means presence of reliable protection but booster vaccination needed after 4-6 weeks. Titers 0.51-1.0 IU/mL means presence of reliable protection but booster vaccination needed after 2 years. Titers 1.1-5.0 IU/mL means presence of long term protection but control after 5-10 years. Titers >5.0 IU/mL means presence of long term protection but control after 10 years. All the vaccines except the control produced titers>0.10 IU/mL.

In Figure 1 all values are expressed as Mean ± SEM and $p<0.05$. The control vaccines produced sub-optimal antibody titer (Figure 1).

Discussion

Storage of the DPT or Pentavalent vaccine outside the recommended temperature for too long is strongly discouraged [15,16]. Several reports show that adsorbed vaccines such as DPT stored at freezing temperatures irreversibly loose both their potency and immunogenicity [17-20]. The vaccine from the States' cold stores produced sufficient DPT IgG antibody titers to believe that they have not been stored outside the recommended temperature (2-8°C). The Tetanus antibody titers (Table 3) produced by the vaccines from Enugu/Ebonyi, Imo, Anambra and Abia States were 0.1770, 0.1726, 0.1686 and 0.1759 IU/mL respectively and significantly higher than the control (0.0761 IU/ml). The pertussis IgG antibody evoked by the vaccines from the States was 20.453, 21.463, 19.633 and 19.896 NTU/mL (Table 2) which is also higher than that of the control vaccine (8.455 NTU/mL). The Diphtheria antibody titer produced by the vaccine from Enugu/Ebonyi States was higher (1.502 IU/mL) than the antibodies from the rest of the States – 1.002, 1.012 and 0.9359 IU/mL respectively for Imo, Anambra and Abia States (Table 1). The control produced much less antibodies. Figure 1 above shows a graphic presentation of the antibody titers evoked by the DPT/Pentavalent vaccines. It was observed that the control vaccines did not produce enough DPT antibodies, their potency and immunogenicity being compromised by the storage temperature. One-way analysis of variance shows that the mean antibodies titers evoked by the vaccines differ significantly at $\alpha<0.05$ (P value<0.0001 for all the DPT components) while Bartlett's test for equal variances show that the variances differ except for Pertussis component which has a P value of 0.6184. The Bartlett's test P values for Tetanus and Diphtheria antibody titers were each <0.0001. Dunnett's Multiple Comparison Test shows that the mean antibodies titers for the components of the vaccines differ significantly from the titers evoked by the control vaccines.

Conclusion

There is statistical difference in the antibody titer evoked by the control compared to that evoked by the vaccines from the states. The vaccines from the States' central cold-chain stores show 100% potency and immunogenicity. This indicates adequate cold-chain maintenance and monitoring of the storage facilities at these Central Stores in South-east Nigeria. Continuous monitoring of efficiency of cold- chain maintenance and vaccine potency testing would translate to good vaccine strategy [21-23]. The vaccines from the States do not differ significantly from each other on the level of antibody titer (protection) and their individual protection can last for the maximum number of days needed for a repeat vaccination except for the control.

The scientific insight provided in this study is the systematic way of monitoring the efficiency of cold-chain system for vaccine storage and vaccine potency testing which will translate to good vaccine strategy.

Study Limitation

The cold stores for the States are usually in the cities and are fitted with stand-by generators (alternative power supply). The result of this study therefore may not have reflected the state of these vaccines at the Local Government and end-use stores. It is therefore recommended that this study be replicated with samples from these two sources.

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Vaccine from	MEAN ANTIBODY TITRE IN NTU VALUES								
	1	2	3	4	5	6	7	Mean	±SEM
Control	5.006	11.704	7.067	12.440	7.582	3.828	11.557	8.455	± 1.309
Enugu/Ebonyi	17.740	18.476	20.169	24.807	23.629	17.740	20.611	20.453	± 1.072
Imo State	20.979	24.660	20.022	19.065	24.660	23.114	17.740	21.463	±1.036
Anambra	17.814	18.255	19.654	21.494	17.740	21.273	21.200	19.633	±0.644
Abia	20.169	25.985	18.550	17.667	18.108	18.623	20.169	19.896	±1.078

Note: Control was the DPT stored at 37 °C for 12 months
Calculated Antibody Titer (in "Nephelometric Turbidity Unit" (NTU)) = Sample (mean) absorbance Value x 10/Cut-off

Table 2: Pertussis IgG Antibody quantification in immunized Mice.

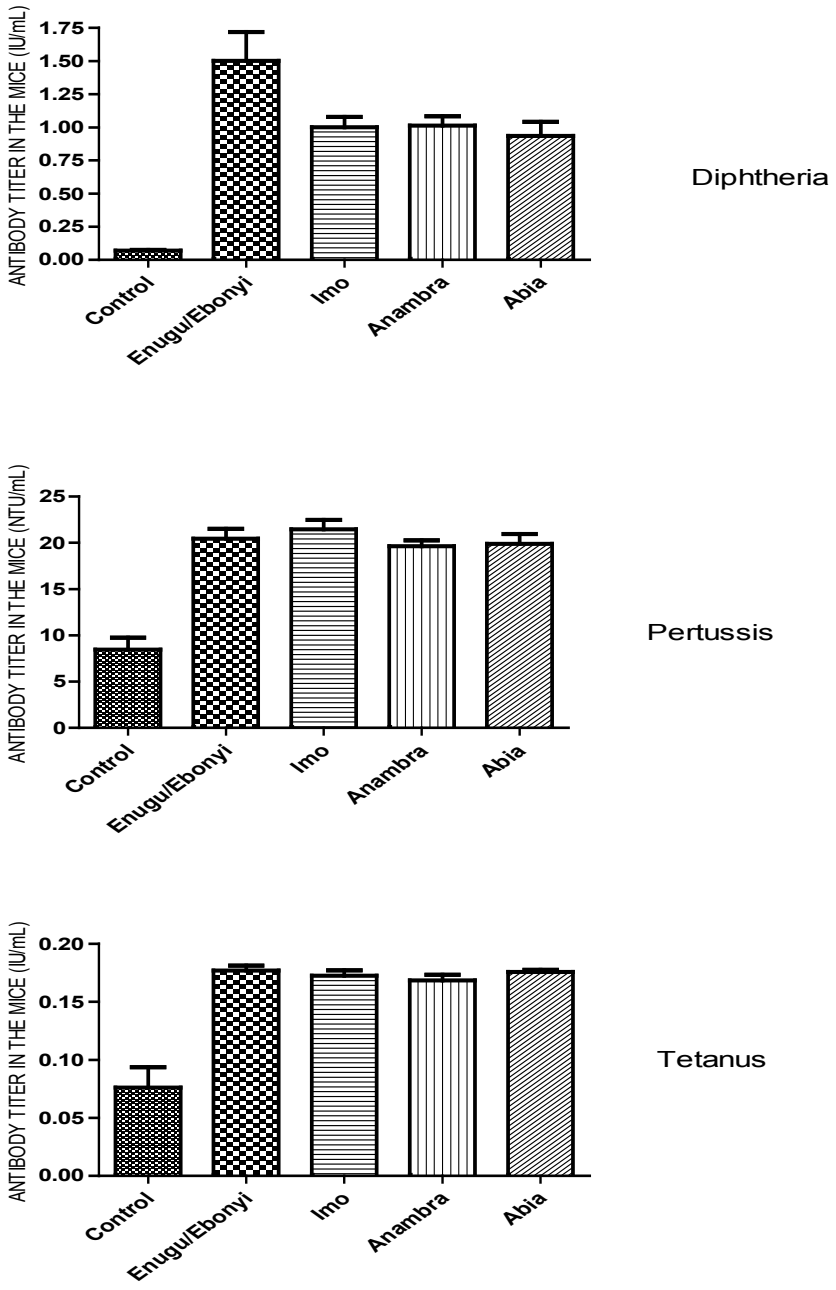

Figure 1: Diphtheria/Pertussis/Tetanus (DPT) Vaccines IgG Antibodies Concentration in immunized mice.

Vaccine from	ANTIBODY TITER IN THE MICE (IU/mL)								
	1	2	3	4	5	6	7	Mean	±SEM
Control	0.103	0.103	0.104	0.103	0.104	0.009	0.007	0.0761	± 0.0176
Enugu/Ebonyi	0.165	0.177	0.174	0.193	0.165	0.174	0.191	0.1770	± 0.0042
Imo State	0.180	0.196	0.163	0.164	0.161	0.175	0.169	0.1726	± 0.0047
Anambra	0.165	0.160	0.172	0.195	0.168	0.155	0.165	0.1686	± 0.0049
Abia	0.173	0.181	0.172	0.170	0.175	0.184	0.176	0.1759	± 0.0019

Note: Control was the DPT stored at 37°C for 12 months.

Table 3: Tetanus IgG Antibody Quantification in immunized Mice.

Ethical Issues

The work described in this article was approved by the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi (Approval #: NAUTH/CS/66/Vol.4/220)

Authors' Contribution

Oli AN wrote the first draft of the manuscript, designed and implemented the study as well as did data analysis and interpretations, Agu RU revised the draft critically and cross-checked for important intellectual content, Nnadozie OJ helped in data acquisition while Esimone CO conceptualized the study and also revised the manuscript critically.

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