Trials in Vaccinology 5 (2016) 8–14

Contents lists available at ScienceDirect

Trials in Vaccinology

journal homepage: www.elsevier.com/locate/trivac

Original Article

An assessment, in mice, of the safety of the childhood immunization vaccines sourced from three south-eastern states of Nigeria

Oli Angus Nnamdi [a,*], Oli Ugochukwu Chinedum [b], Ejiofor Obiora Shedrack [c], Nwoye Charles Ugochukwu [d], Esimone Charles Okechukwu [a]

[a] Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria
[b] Department of Chemical Pathology, Nnamdi Azikiwe University, Teaching Hospital, Nnewi, Anambra State, Nigeria
[c] Department of Pediatrics, Anambra State University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria
[d] Department of Hematology, Nnamdi Azikiwe University, Teaching Hospital, Nnewi, Anambra State, Nigeria

ARTICLE INFO

Article history:
Received 25 January 2015
Revised 4 September 2015
Accepted 23 October 2015
Available online 26 November 2015

Keywords:
Safety studies
Vaccines
Childhood immunization
Anambra
Ebonyi and Enugu
Nigeria

ABSTRACT

Background: The current and general safety control tests for vaccines have been the use several animal tests. Vaccine is considered safe if it does not cause weight loss or death in laboratory animals at human dose, does not promote leukocytosis by a factor ⩾10 and showed a Leukopenic toxicity value ⩾80% of the Leukopenic toxicity of the control. The study sets to determine the safety of routine immunization vaccines from Anambra, Ebonyi and Enugu states of Nigeria and indirectly evaluate the efficiency of cold-chain facilities.
Method: The study was designed to check the safety of the routine immunization vaccines to the hematopoietic system of mice and mice body weight changes after immunization.
Results: Animal body weight changes test showed that the mice immunized with the vaccines increased in weight at days 3 and 7 post-immunization and exceeded 60% weight gain at day 7 post-immunization. None of the mice died during the observation period. Hematopoietic system toxicity tests showed that the vaccines are non- toxic.
Conclusion: The vaccines were generally safe and non-toxic. The cold-chain systems in the States studied were efficient and had not compromised the safety of the vaccines.

1. Introduction

Vaccines play a vital role in preventive medicine [1]. Vaccine safety is a prime concern to everybody yet no biological or pharmaceutical product ever developed and produced is completely safe and one hundred per cent effective [2]. As a vaccine becomes safer, it becomes less effective [3]. Vaccine development and licensing is usually expensive as well as time consuming. The discovery of effective and reliable method of identifying the safest and most effective vaccine candidate at the early stage would be highly useful [4]. The European Union recommends that both live and killed vaccines be considered safe only after testing according to laid down procedures. They recommend that the assessment of safety in the target species should be based on clinical reaction and laboratory animals' weight gain compared with unvaccinated controls [5]. Animal body weight change test is the most commonly used test to evaluate the toxicity of vaccines. The issue of vaccine safety has discouraged some parents and care-givers, both in developed [6–8] and developing countries [9,10], from immunizing their children and wards. Reassurance of vaccine safety is therefore, vital for population health [6]. Vaccines are biological products and the slightest breech in the cold-chain may make them unsafe for use in immunization programme. Nigeria, like other developing countries of the world, is besieged with the problem of unreliable power supply. This heightens the safety question. In view of the questionable power supply in the country, it is necessary to carry out a sporadic evaluation of the cold-chain system where these thermo labile and live-saving products are stored.

2. Method

2.1. Study area

Anambra, Ebonyi and Enugu states are located in the South-east geo-political zone of Nigeria. The states are predominantly Ibo by

* Corresponding author at: P.O. Box 2130. Tel.: +234 8056306927.
E-mail addresses: a.n.oli@live.com (A.N. Oli), ugoonedu@yahoo.com (U.C. Oli), smejiofor@yahoo.com (O.S. Ejiofor), nwoyecharles@yahoo.com (C.U. Nwoye), co.esimone@unizik.edu.ng (C.O. Esimone).

http://dx.doi.org/10.1016/j.trivac.2015.10.001
1879-4378/

tribe, Christians by religion and procure their immunization vaccines from Abuja via the national allocation. Each state has only one central cold-chain store for vaccine storage and distribution. From there, the vaccines are distributed to the Local Government areas which then feed the immunization centers. Our study concerns the central cold-chain stores in the three of the five states that make the south-east states of Nigeria.

2.2. Vaccines collection and storage

The Expanded Programme on Immunization (EPI) vaccines were donated by the Ministries of Health of Ebonyi, Enugu and Anambra States. Vaccines were transported in an insulated vaccines carrier and stored in the storage facility of Nnamdi Azikiwe University Teaching Hospital, Nnewi within 4 h of collection. Temperature at collection was 4 ± 1 °C and before storage 5 ± 1 °C. Study was conducted within 1 month of vaccine collection and the temperature of the storage facility was charted daily while vaccine storage lasted.

3. Animals used

Albino mice, gender balanced, of weight 12–28 g 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 hematology 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 (Approval #: NAUTH/CS/66/Vol.4/220).

3.1. Mouse identification

Two basic methods exist for the labeling of the animals used in biomedical research. They are temporary identification methods and permanent identification methods [11,12]. For convenience and skill, the temporary identification method was used in this study and the marks were renewed every other day. The animals (age = 20 ± 5 days) used in the study were treated in accordance with the Guidelines stated in the Animal Welfare Act [13] and Australian Government [14].

4. Determination of the toxicity and safety levels of the vaccines

This was done, with some modifications; using the method described by Haruka et al [15].

4.1. Animal body weight changes test

A total of 70 mice grouped into 7 with each group containing 10 mice of equal sex were used. A human dose of OPV, DPT (or pentavalent), BCG, Measles, YFV and HBV was injected into the peritoneum (except for OPV which was by oral route) of mice in group 1–6 respectively. Group 7 animals were given 0.5 ml of 0.9% sodium chloride. All the mice were observed daily for 7 days. A good vaccine should not [16,17] (i) cause a decrease in the weight of the mice 3 days post-vaccination, (ii) produce a less than 60% mean weight gain per mice compared with the control at 7 days post-vaccination (iii) produce greater than 5% animal dead during the 7 days observation period nor any sign of illness on the animals [16].

4.2. Leukocytosis-promoting toxicity test

The toxicity of the vaccines to the hematopoietic system was tested using Haruka et al. [15] method and modified by Oli et al. [20]. Briefly, heparinized capillary tube was inserted just below the eyeball of the mice prior to immunization and 3 days after to allow 1–2 drops of blood flow onto a sterile microscope slide. With a 20 μl micropipette, 20 μl blood samples was picked from the slide and dropped into a sterile eppendoff tube containing 380 μl Turk's solutions. The leukocytes (White Blood Cells) count was done using Heamocytometer. The vaccines were considered safe if the mean peripheral blood leucocyte count 3 days post-immunization is not more than 10 fold pre-immunization [16].

4.3. Leukopenic toxicity test in vaccinated animals

This also tests the toxicity of the vaccines to the hematopoietic system using the general safety test and the leukopenic toxicity test (LTT). The animals used for the body weight changes test were also used for the Leukocytosis-promoting toxicity test. The 8th group was given cyclophosphamide (400 mg/kg) [18] by gavage to serve as positive control. Cyclophosphamide for injection was reconstituted with the 0.9% Sodium Chloride Injection to make a stock solution of 20 mg/ml (Make: Biochem Pharmaceutical Industries LTD, India – Lot #: KB116010). Eighteen hours later, 20 μl blood samples of the mice were collected in sterile eppendoff tube using heparinized capillary tube inserted just below the eye ball, diluted with Turk's solution (380 μl) and leukocytes (White Blood Cells) count done using Heamocytometer. The vaccine is considered safe if it shows a Leukopenic toxicity value greater than or equal to 80% of the Leukopenic toxicity of the control [15,16,19].

$$\text{Leukopenic toxicity [20]} = \frac{\text{WBC count (Test or Control)}}{\text{WBC count (Toxic Substance)}}$$

4.4. Statistical analysis

The results were analyzed using GraphPad Prism version 5.00 for Windows, GraphPad Software, Inc. San Diego California USA, www.graphpad.com". Continuous variables (weights) were compared with baseline using unpaired t test. One-way ANOVA or the non-parametric equivalent was used to compare continuous variables in groups. Two-way ANOVA was used to compare how the continuous variables respond to the effect of two factors (length of time post-vaccination and the different vaccines). Dunnett's Tests of Multiple Comparison and Bartlett's test for equal variances were also carried out. All p values reported are for a one-tailed test. The significance level was chosen at $\alpha = 0.05$.

4.5. Results presentation

None of the mice used in the study showed any sign of abnormality or ill health throughout the 7 days post-immunization observation. There was no weight loss and none died.

As shown in Table 1, the animals immunized with the vaccines all showed on the average some increase in weight gain and the percentage weight gain compared with the mice immunized with 0.9% sodium chloride (the control) was greater than 60%. However, the values given by the measles, yellow fever and hepatitis B vaccines were marginally above the cut-off. Dunnett's Multiple Comparison Test to compare the mean body weights of the immunized mice with that of the control showed that at day 3 post-vaccination, all the mice except those immunized with DPT and BCG, had non-significant weight gain at $p < 0.005$.

At day 7 post-vaccination, the mice immunized with OPV, DPT and BCG had significant weight gain while those immunized with

Table 1
Animal body weight changes test for vaccines from Ebonyi and Enugu states.

Vaccines	Day after vaccination	Mice body weight (g)			% Wt. gain compared to mean Wt. gain of control	*p* Value* (<0.05 = significant)
		Total weight	Mean weight (n = 10)	Mean weight gain		
OPV	0	177.3	17.73	0.00		
	3	183.3	18.33	0.60		0.338
	7	241.3	24.13	6.40	118.430034	<0.0001
DPT	0	216.6	21.66	0.00		
	3	220.8	22.08	0.42		0.4134
	7	275.3	27.53	5.87	100.341297	<0.0001
BCG	0	212.8	21.28	0.00		
	3	214.9	21.49	0.21		0.4443
	7	280.4	28.04	6.76	130.716724	0.0002
Measles	0	153.6	15.36	0.00		
	3	170.5	17.05	1.69		0.0387
	7	201.1	20.11	4.75	62.116041	0.0004
YFV	0	156.4	15.64	0.00		
	3	166.9	16.69	1.05		0.0014
	7	204.8	20.48	4.84	65.1877133	<0.0001
HBV	0	159.4	15.94	0.00		
	3	168.9	16.89	0.95		0.2573
	7	208.5	20.85	4.91	67.5767918	0.0011
Control NS	0	145.2	14.52	0.00		
	3	155.1	15.51	0.99		0.1100
	7	174.5	17.45	2.93		0.0022

Note: day 0 means before vaccination, x = % mean weight gain compared to control (7 days post-vaccination),
* p value is for student t-test.

Measles, Yellow fever and Hepatitis B vaccines had non-significant weight gain at $p < 0.05$. A two-way analysis of variance of the result of the body weight changes test showed that vaccine type and post-vaccination day significantly affected the weight of the animals (p value <0.0001) but the interactive effect of both do not (p value = 0.5308).

Table 2 shows the result of leukocytosis-promoting toxicity test for the vaccines collected from Enugu/Ebonyi States. The vaccines are considered non-toxic if it does not promote leukocytosis by a factor $\geqslant 10$ [16].

The leukocytosis-promoting toxicity study performed on the vaccines (Table 2) revealed that all the vaccines were able to increase the leukocyte count in vaccinated animals by at least two fold. The DPT increased the count by three fold. None of the vaccines studied produced up to ten fold increase in the leukocyte count. Also, the vaccines produced about the same effect (increase in the leukocyte count) compared with the control showing that they are safe. This is confirmed by the Dunnett's Multiple Comparison Test which compared the effect of each vaccine with the control at $p < 0.05$. It may be deduced from the study that the vaccines might not have likely affected the hematopoietic system adversely. A t-test analysis of the data showed significant difference in the mean WBC count at pre- and post-vaccination of the mice with the vaccines studied.

Table 2
Leukocytosis-promoting toxicity test for vaccines from Enugu/Ebonyi.

Vaccines	Day after vaccination	Leukocyte count of the mice ($\times 10^9$/L)		WBC Increased by X folds	*p* Value*
		Total WBC	Mean WBC (n = 10)		
OPV	a	42.90	4.290	2.30	<0.0001
	b	98.55	9.855		
DPT	a	63.05	6.305	3.23	<0.0001
	b	203.95	20.395		
BCG	a	48.00	4.800	2.01	<0.0001
	b	96.35	9.635		
Measles	a	49.80	4.980	2.12	0.002
	b	105.42	10.542		
YFV	a	53.95	5.395	2.13	0.004
	b	114.65	11.465		
HBV	a	48.5	4.85	2.16	0.001
	b	104.65	10.465		
Control NS	a	49.80	4.980	2.21	
	b	110.25	11.025		

Note: a = before vaccination, b = 3 days after vaccination and control NS = control using 0.9% normal saline.
* p value (for Student t-test) < 0.05 = significant.

The two-way ANOVA of the leukocytosis-promoting toxicity test showed that the vaccine type, the post-vaccination day and their interaction all affected the WBC count of the animals (p value <0.0001). The interaction accounts for 9.13% of the total variations in the leukocyte count. The vaccine type accounts for 16.76% while the post-vaccination day accounts for 47.59% variations.

The Fig. 1 shows the leukopenic toxicity test in mice. The vaccines are considered non-toxic if they show Leukopenic toxicity values greater than or equal to 80% of the Leukopenic toxicity of the control. The leukopenic toxic reference used was cyclophosphamide (Control Cp) while the leukopenic non-toxic reference was 0.9% sodium chloride. The leukopenic Toxicity values of the test vaccines and the 0.9% sodium chloride were OPV – 2.713, DPT – 2.655, BCG – 2.825, Measles – 2.879, Yellow fever – 2.654, Hepatitis B – 2.629 and sodium chloride – 3.143. The leukopenic Toxicity value of each of the vaccines was higher than the 80% leukopenic Toxicity value of control NS (Fig. 1). Analysis of the mean leukocyte counts using one way ANOVA shows significant difference ($p < 0.0001$) in the leukocyte counts due to cyclophosphamide compared with the vaccines. Bartlett's test for equal variances also showed that their variances also differ significantly (p value = 0.0018).

The Dunnett's multiple comparison tests for the vaccines showed that the mean leukocyte count in the mice produced by the vaccines and the 0.9% sodium chloride were each significantly

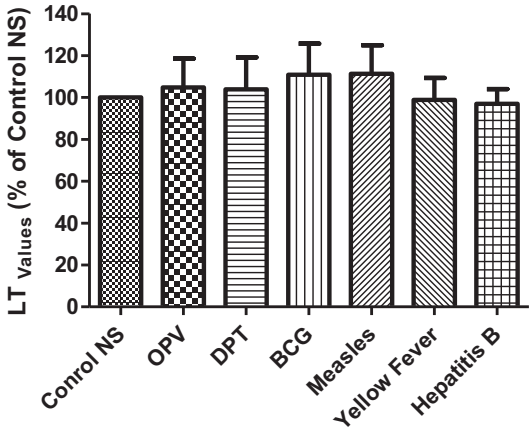

Fig. 1. Leukopenic test for vaccines from Ebonyi/Enugu states. Leukopenic toxicity = WBC count (Test or Control)/WBC count (Toxic Substance). Values represent the leukopenic toxicity values of the vaccines as a percentage of the leukopenic toxicity value of the control (0.9% NaCl).

higher than that produced by the cyclophosphamide at $p < 0.05$. A comparison of the leukocyte count due to the 0.9% sodium chloride and that due to the vaccines using one way ANOVA showed no significant difference (p value = 0.9090). The vaccines were as safe as the normal saline. This is confirmed by the Dunnett's Multiple Comparison. The One-way analysis of the leukopenic toxicity value showed that the leukocyte count is significantly affected by all the treatments (p value <0.0001) – the vaccines, the normal saline and the cyclophosphamide while the Bartlett's test for equal variances showed that the variances are also significantly different.

All the animals used in this study survived with no sign of ill health. A comparison of the mean body weight at pre- and post-vaccination days (Table 3) showed that there was generally non-significant weight gain except in the group immunized with hepatitis B vaccine on day 3 post-vaccination but significant weight gain in all on day 7 post-vaccination at $p < 0.05$. All the vaccines produced beyond 60 per cent weight gain in the immunized mice the least being HBV (60.91%) followed by Pentavalent vaccine (66.45%). The significance of this is that both the hepatitis B and pentavalent vaccines should be used in the shortest possible time.

Dunnett's Multiple Comparison Test showed that at day 3 post-vaccination, all the mice immunized had non-significant weight gain at $p < 0.05$ except those immunized with OPV, compared with the control. At day 7 post-vaccination, all the mice have significant weight gain at $p < 0.05$ compared with the control.

Two-way ANOVA of the result showed that both the vaccine type and the post-vaccination day significantly affected the animals' body weight (p values <0.0001) by 23.69% and 26.12% but the interactions between the two do not (p value = 0.8196). The vaccine type does not have the same effect at all values affected by post-vaccination days. The interactions accounted for only 1.91% of the total variance.

Leukocytosis-promoting toxicity test (Table 4) showed that the vaccines produced less than 2 fold increase in the WBC count between the pre- and post-vaccination period. The increase was well comparable with that of the control. Neither the vaccines nor the control produced as much as ten folds increase in the WBC. A t test analysis showed significant difference, at $p < 0.05$, in the effects produced the vaccines when compared with pre-vaccination period with the exception of BCG. The Dunnett's multiple comparison tests showed that the effects produced by the vaccines were not significantly different from that produced by the control.

Two-way ANOVA of the leukocytosis-promoting test showed also that vaccine type as well as post-vaccination day affected the leukocyte count (p value = 0.003 and <0.0001 respectively) by 11.84% and 16.96% respectively. The vaccine type did not have the same effect at all values of the leukocyte count affected by post-vaccination day. The interactions of the two produced only 0.46% of the total variations.

The vaccines (procured from Anambra State) and the sodium chloride had LT_{values} of 3.362, 3.603, 3.611, 3.356, 3.295, 3.624 and 3.998. The LT_{value} of each of the vaccines was higher than the

Table 3
Animal body weight changes test for vaccines from Anambra state.

Vaccines	Day after vaccination	Mice body weight (g)			% Wt. gain compared to mean Wt. gain of control	p value* (<0.05 = significant)
		Total weight	Mean weight (n = 10)	Mean weight gain		
OPV	0	186.3	18.63	0		
	3	214.2	21.42	2.79		0.114
	7	243.5	24.35	5.72	86.3192182	0.0021
Pentavalent	0	198.3	19.83	0		
	3	203.9	20.39	0.56		0.3227
	7	249.4	24.94	5.11	66.4495114	0.0005
BCG	0	183.2	18.32	0		
	3	192.7	19.27	0.95		0.3316
	7	235.4	23.54	5.22	70.0325733	0.0066
Measles	0	167.7	16.77	0		
	3	173.9	17.39	0.62		0.2475
	7	229.3	22.93	6.16	100.651466	0.0003
YFV	0	199.8	19.98	0		
	3	200.8	20.08	0.1		0.4789
	7	268.7	26.87	6.89	124.429967	0.001
HBV	0	147.0	14.70	0		
	3	159.2	15.92	1.22		0.1042
	7	196.4	19.64	4.94	60.9120521	<0.0001
Control	0	165.8	16.58	0		
	3	172.2	17.22	0.64		0.1419
	7	196.5	19.65	3.07		<0.0001

Note: day 0 means before vaccination.
* p value is for student t test.

Table 4
Leukocytosis-promoting toxicity test for vaccines from Anambra state.

Vaccines	Day after vaccination	Leukocyte count of the mice ($\times 10^9$/L)		WBC increased by X folds	p value* (<0.05 = significant)
		Total WBC	Mean WBC (n = 10)		
OPV	a	43.00	4.300	1.5639535	0.0015
	b	67.25	6.725		
Pentavalent	a	71.35	7.135	1.3398739	0.0235
	b	95.60	9.560		
BCG	a	52.20	5.220	1.5354406	0.086
	b	80.15	8.015		
Measles	a	45.25	4.525	1.6099448	0.0038
	b	72.85	7.285		
YFV	a	66.60	6.660	1.3528529	0.0209
	b	90.10	9.010		
HBV	a	50.60	5.060	1.3488142	0.0069
	b	68.25	6.825		
Control NS	a	59.20	5.920	1.3032095	0.0275
	b	77.15	7.715		

Note: a = before Vaccination, b = 3 days after vaccination.
* p value is for student t-test.

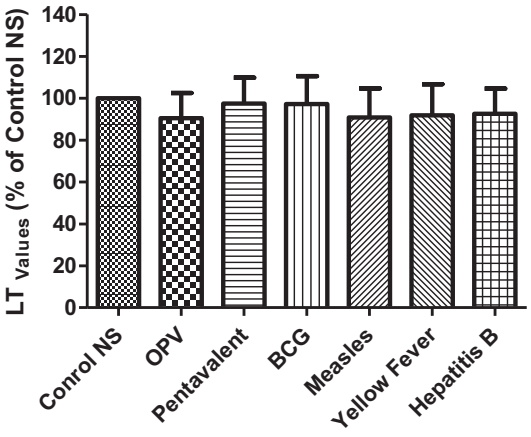

Fig. 2. Leukopenic test for vaccines from Anambra state. Leukopenic toxicity = WBC count (Test or Control)/WBC count (Toxic Substance). Values represent the leukopenic toxicity values of the vaccines as a percentage of the leukopenic toxicity value of the control (0.9% NaCl).

80% LT_{value} of sodium chloride (Fig. 2). One way ANOVA of the mean leukocyte counts shows significant difference ($p < 0.0001$) between the cyclophosphamide and the vaccines. Also, Bartlett's test for equal variances shows that their variances differ significantly.

The Dunnett's multiple comparison tests confirmed the significant differences in the mean leukocyte counts of the vaccines compared with cyclophosphamide but no significant difference when the vaccines and the sodium chloride were compared at $p < 0.05$.

One-way ANOVA of the leukopenic toxicity test shows that the leukocyte count is significantly affected by all the treatments (p value < 0.0001). Bartlett's test for equal variances also show that the variances are also significantly different (p value = 0.0016). Dunnett's Multiple Comparison Test showed that the mean leukocyte count produced by the leukopenic agent – cyclophosphamide is significantly different from that produced by the vaccines. The vaccines have the same effects as the physiological saline (p value = 0.0346).

5. Discussions of results

Immunization with potent and safe vaccines has been reported to be responsible for the reduction in cases infectious diseases outbreak in all age groups [6,21]. Tables 1 and 3 showed the results from animal body weight changes tests. The results showed that the vaccines were safe. However, some vaccines were marginally above the cut-off. The significance of this is that those vaccines, although still safe at the time of collection, should be used up quickly or they risk being unsafe.

That the vaccines were safe could be attributed to the absence of residual toxin and/or chemicals in the vaccines and non-formation of degradation products in the vaccines which results from poor vaccine storage. Two independent groups of researchers [22,23] were able to prove that the Pertussis-containing vaccines that lower animal body weight could cause adverse reactions if administered to children. The safety of vaccines utilized in routine immunization of US children was reported [6]. Another group of researchers [24] showed that the results of abnormal toxicity test correlates well with vaccine toxicity. Generally, the vaccines that showed $\geqslant$100% weight gain (in the immunized mice 7-days post-vaccination) compared to mean weight gain of control mice) have better safety profile.

Tables 2 and 4 showed the results of the leukocytosis promoting test conducted on the vaccines. The leukocytosis-promoting toxicity test showed that none of the vaccines from the states were able to produce up to ten fold increase of the white blood cells in the animals used for the study. This compares well with the control showing that the vaccines did not cause any hematopoietic damage. There effect to the hematopoietic system was as good as the physiological saline used as the control. A study [25] demonstrated that leukocytosis promoting test was better than the mouse body weight changes test in testing for the safety of Whole Cell Pertussis Vaccine. Another study [26] suggested that gene expression analysis of vaccine-treated animals is as accurate as leukocytosis-promoting toxicity test in assessing pertussis vaccine safety.

Figs. 1 and 2 showed the results of the leukopenic toxicity test. This test demonstrated whether or not the vaccines are capable of inducing leucopenia (decrease in the number of white blood cells (leukocytes) found in the blood) thus placing vaccinated individuals at increased risk of infection. From the test of statistics (Dunnett's multiple comparison tests) used in the analysis, the

vaccines were found to be similar to the non-toxic reference (0.9% sodium chloride) used and significantly different (p value < 0.05) from the toxic reference (the cyclophosphamide). It is therefore concluded that the vaccines are not capable of decreasing the number of WBC and so are safe. As a comparison with certain drugs, two groups of researchers [16,27] demonstrated that chloramphenicol at the dose of 3500 mg/kg and cyclophosphamide at the dose of 250 mg/kg respectively was leukopenic to mice.

5.1. Study limitations

This study was a one-point study in that the vaccines were collected at one particular time. It could not give a true picture of the cold-chain facilities over time. None of the vaccines could be evaluated on the day of collection because of poor access road and other logistic problems. However, efforts were made to monitor vaccine temperature throughout the study.

5.2. Conclusion

The EPI vaccines in this study were generally safe and have toxicity profiles comparable to Sodium Chloride 0.9%. The cold-chain systems in Anambra, Ebonyi and Enugu states were efficient as at the time of vaccine collection. The safety of the vaccines were not yet compromised.

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 A.N. wrote the first draft of the manuscript, designed and implemented the study as well as did data analysis and interpretations, Ejiofor O.S. revised the draft critically and cross-checked for important intellectual content, Oli U.C. and Nwoye C.U. helped in data acquisition while Esimone C.O. conceptualized the study. All authors approved the final manuscript.

This study is part of the doctorial research work undertaken by Oli A.N.

Conflict of Interest

None exist except those mentioned in the acknowledgment.

Acknowledgements

African Doctoral Dissertation Research Fellowship award offered by African Population and Health Research Center (APHRC) in partnership with the International Development Research Centre (IDRC): Grant #: ADDRF Award 2012-2014 ADF 020 and The Canadian Commonwealth Scholarship Program (CCSP) administered by the Canadian Bureau for International Education (CBIE) with funding from the Government of Canada's Department of Foreign Affairs and International Trade (DFAIT) funded the research. The States' ministries of Health donated the vaccines.

References

[1] A.N. Oli, R.U. Agu, O.J. Nnadozie, C.O. Esimone, Potency/immunogenicity profile of DPT vaccines used in the expanded programme on immunization in South-East Nigeria, J. Vaccines Vaccin. 5 (2014) 216, http://dx.doi.org/10.4172/2157-7560.1000216.

[2] Centers for Disease Control and Prevention. Vaccine safety. DeKalb county: centers for disease control and prevention. (Online) available from: <http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/safety.pdf>, (accessed on 17.11.2013).

[3] World Health Organization, Supplementary information on vaccine safety – Part 2: background rates of adverse events following immunization, Department of Vaccines and Biologicals, CH-1211 Geneva 27, Switzerland <www.who.int/vaccines-documents/> (accessed on 17.11.2013), 2000.

[4] Rappuoli Rino, Black Steven, Lambert Paul Henri, Vaccine discovery and translation of new vaccine technology, Lancet 378 (2011) 360–368, http://dx.doi.org/10.1016/S0140-6736(11)60440-6).

[5] H.D. Chapman, B. Roberts, M.W. Shirley, R.B. Williams, Guidelines for evaluating the efficacy and safety of live anticoccidial vaccines, and obtaining approval for their use in chickens and turkeys, Avian Pathol. 34 (4) (2005) 279–290, http://dx.doi.org/10.1080/03079450500178378.

[6] A. Maglione, Das Lopamudra, Raaen Laura, Smith Alexandria, Ramya Chari, Newberry Sydne, Shanman Roberta, Perry Tanja, Goetz Matthew Bidwell, Gidengil Courtney, Safety of vaccines used for routine immunization of us children: a systematic review, Pediatrics 134 (2) (2014) 1–15, http://dx.doi.org/10.1542/peds.2014-1079.

[7] McCauley Mary Mason, Kennedy Allison, Basket Michelle, Sheedy Kristine, Exploring the choice to refuse or delay vaccines: a national survey of parents of 6-through 23-month-olds, Acad. Pediatr. 12 (5) (2012) 375–383. http://dx.doi.org/10.1016/j.acap.2012.06.007. Epub 2012 Aug 22.

[8] Favin Michael, Robert Steinglass, Fields Rebecca, Banerjee Kaushik, Sawhney Monika, Why children are not vaccinated: a review of the grey literature, Int. Health 4 (4) (2012) 229–238, http://dx.doi.org/10.1016/j.inhe.2012.07.004.

[9] I. Ojakaa David, Ofware Peter, W. Machira Yvonne, Yamo Emmanuel, Collymore Yvette, Ba-Nguz Antoinette, Vansadia Preeti and Bingham Allison. Community perceptions of malaria and vaccines in the South Coast and Busia regions of Kenya; Malaria J. 10 (2011) 147. http://dx.doi.org/10.1186/1475-2875-10-147.

[10] Ruhul Amin, Telma Joana Corte Real de Oliveira, Mateus Da Cunha, Tanya Wells Brown, Michael Favin, Kelli Cappelier, Factors limiting immunization coverage in urban Dili, Timor-Leste, Glob Health Sci. Pract. 1 (3) (2013) 417–427. <http://dx.doi.org/10.9745/GHSP-D-13-00115>.

[11] Hoogstraten-Miller Shelley, Burns Wendy, and Leja Darryl, Basic Biomethodology for Laboratory Mice. A publication of the National Numan Genome Research Institute, revised 4/6/2004, 2004.

[12] I. Marcel, Perret Gentil, Mouse Biomethodology Handout: Laboratory Animal Resources Center, The University of Texas at San Antonio, 2013.

[13] Animal Welfare Act and Regulations <http://awic.nal.usda.gov/government-and-professional-resources/federal-laws/animal-welfare-act> (accessed on 31st Oct 2011).

[14] Australian Government, Guidelines to Promote the wellbeing of animals used for scientific purposes, The assessment and alleviation of pain and stress in research animals/National Health and Medical Research Council, 2008, pp. 1–189. <http://www.nhmrc.gov.au/index.htm>.

[15] Haruka Momose, TakuoMizukami, Masaki Ochiai, Isao Hamaguchi, and Kazunari Yamaguchi, A new method for the evaluation of vaccine safety based on comprehensive gene expression analysis. J. Biomed. Biotechnol. (2010) 7, Article ID 361841. http://dx.doi.org/10.1155/2010/361841, (Review Article).

[16] T. Kurata, Minimum Requirements for Biological Products, Tokyo, Japan, National Institute of Infectious Diseases, 2006.

[17] G.N. Singh, Diphtheria and tetanus and whole cell pertussis vaccine (Adsorbed), Indian Pharmacopoeia 3 (3) (2007) 744–57).

[18] Masood A Khan, Owais Mohd, Toxicity, stability and pharmacokinetics of amphotericin B in immunomodulators tuftsin-bearing liposomes in a murine model, J. Antimicrob. Chemother. 58 (2006) 125–132, http://dx.doi.org/10.1093/jac/dkl177.

[19] F. Chino, The views and policy of the Japanese control authorities on the three Rs, Develop. Biol. Standard. 86 (1996) 53–62.

[20] Angus Nnamdi Oli, Agu Remigus Uchenna, Ugochukwu Chinedum Oli, Charles Ugochukwu Nwoye, Obiora Shedrack Ejiofor, Charles Okechukwu Esimone, Safety evaluation in mice of the childhood immunization Vaccines from two South-eastern states of Nigeria, Asian Pac. J. Trop. Biomed. 5 (2) (Feb. 2015) 132–137.

[21] U. Ogbuanu Ikechukwu, K. Kutty Preeta, M. Hudson Jean, Blog Debra, R. Abedi Glen, Goodell Stephen, Lawler Jacqueline, Q. McLean Huong, Pollock Lynn, Rausch-Phung Elizabeth, Schulte Cynthia, Valure Barbara, Armstrong Gregory L. and Gallagher Kathleen, Impact of a third dose of measles-mumps-rubella vaccine on a mumps outbreak, Pediatrics 130 (6) (2012). http://dx.doi.org/10.1542/peds.2012-0177.

[22] Isao Hamaguchi, Jun.-ichi Imai, Haruka Momose, Mika Kawamura, Takuo Mizukami, Hiroshi Kato, Seishiro Naito, Jun.-ichi Maeyama, Atsuko Masumi, Madoka Kuramitsu, Kazuya Takizawa, Masayo Mochizuki, Masaki Ochiai, Akihiko Yamamoto, Yoshinobu Horiuchi, Nobuo Nomura, Shinya Watanabe, Kazunari Yamaguchi, Two vaccine toxicity-related genes Agp and Hpx could prove useful for pertussis vaccine safety control, Vaccine 25 (17) (2007) 3355–3364, http://dx.doi.org/10.1016/j.vaccine.2006.12.059.

[23] M.L. Hilton, W.L. Burland, Pertussis-containing vaccines: the relationship between laboratory toxicity tests and reactions in children, Symp. Ser. Immunobiol. Stand. 13 (1970) 150–156.

[24] F.T. Perkins, F. Sheffield, C.L. Miller, J.L. Skegg, The comparison of toxicity of pertussis vaccines in children and mice, Symp. Ser. Immunobiol. Standard. 13 (1970) 150–156.

[25] T. Mizukami, A. Masumi, H. Momose, M. Kuramitsu, K. Takizawa, S. Naito, J. Maeyama, K. Furuhata, M. Tsuruhara, I. Hamaguchi, K. Yamaguchi, An improved abnormal toxicity test by using reference vaccine-specific body weight curves and histopathological data for monitoring vaccine quality and safety in Japan, Biologicals 37 (1) (2009) 8–17.

[26] K.I. van Straatan-van, J.W. van de Gun, F.R. Marsman, C.F. Hendriken, J.M. van de Donk Huib, Collaborative study on test systems to assess toxicity of whole cell pertussis vaccine, Biologicals 25 (1997) 41–57.

[27] J.A. Turton, R. Fagg, W.R. Sones, T.C. Williams, M.C. Andrew, Characterization of the myelotoxicity of chloramphenicol succinate in the B6C3F1 mouse, Int. J. Exp. Pathol. 87 (2006) 101–112.