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The Production and Testing of Typhoid Fever Vaccine in Nigeria

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ABSTRACT: Sixty-six different types of vaccines were prepared using three adjuvants and using both local and imported strains of *Salmonella typhi* and *Salmonella paratyphi* A, B, & C. About 3,960 mice, 90 rabbits, 5 monkeys and hundreds of humans were vaccinated with 0.5 ml of vaccine and after 3 weeks of vaccination challenged (except humans) with 0.5ml of live culture of causative organisms. Twenty six vaccines sufficiently protected the vaccinates. The best eight of the 26 vaccines were used to vaccinate rabbits and after challenge 3 vaccines that had antibody tires of $^{1}/160$ using Widal test were used for monkey testing. All the 5 monkeys used were solidly protected against typhoid fever. The vaccine used for the test in monkeys was also used for human testing. Human vaccinates reported some reactions at the site of inoculation. The level of reaction varied from person to person. A few people complained of feverish condition on the first day of vaccination. Reports from the vaccinates and our own tests in the laboratory indicate that people are protected against the disease for up to 3 years.

Key Words: Typhoid fever; Vaccine development; Vaccine production and Testing; *Salmonella typhi*; *Salmonella paratyphi*.

Introduction

Typhoid fever has been a problem of the Developing World especially those countries where keeping to high standard of hygiene is a problem. The disease is caused by Salmonella typhi and related microorganisms which are acquired by the ingestion of contaminated food and water (Lennete, et al, 1985; Davis, et al, 1973; Nistreich and Lectman, 1983). Nigeria is presently faced with the problem of controlling the disease. The disease is now endemic in most communities in Nigeria.

Many individuals are chronic carriers of the organism causing the disease. By this the organisms will continue to be excreted in faeces which will further contaminate water, food or reingested directly. In Nigeria the small scale farmers farming along the river or stream banks apply human dungs as manure on their farms. The microorganism loaded faeces manure then contaminate the vegetables, carrots, garden eggs and so on. The innocent buyers may eventually come down with typhoid fever.

Furthermore, the causative organism may not readily respond to drugs (Pasteur, Merieux, 2nd Edition). Many people have to repeat the dosages more than twice before been cured. The fact that the disease can cause intestinal haemorrhages or perforation of the bowel and deaths (Lennete et al, 1985; Ketchum, 1988; van Basten and Stocken, 1994; Adesunkanmi & Ahao, 1997; Akgun et al. 1995; Meier & Tarpley, 1998)

makes it necessary to take appropriate steps to protect individuals against the disease. Vaccination has been the most reliable method of protection against infection[9] (Robbins & Robbins, 1984). This work is aimed at developing, producing and testing typhoid fever vaccines with the hope of finding a highly potent vaccine that will give protection against typhoid fever.

Materials and Methods

Isolation and Characterization of Local Strains

Specimens were collected from Typhoid Fever patients at the Minna General Hospital and Kowa Clinic. *Salmonella typhi, Salmonella paratyphi* A, B and C strains were isolated and were characterized by Colindale National Center for Type Cultures, London.

Strains Used for Vaccine Production

The four locally isolated strains and four typed strains obtained from Colindale (National Collection of Type Culture, London) were used in these investigations. The imported strains were used in parallel with the local strains for comparison.

Culturing of Organisms

The four organisms locally isolated as well as the four duplicate imported strains were cultured in beef infusion broth for 48 hours. Viable counts were carried out on the cultures. Roux flats (flasks) containing nutrient agar were inoculated with the cultures. After 48 hours, the growth on the agar was washed with sterile beads and sterile saline. Viable counts were then conducted on each type of organism. The partially purified whole cell cultures were killed by heat.

Preparation of Vaccines

Each partially purified whole cell killed vaccine combination was treated with sterile adjuvants A, B and C which have been tested for their toxicity. A total of 66 vaccines were prepared and labeled. Each vaccine type differed from the other depending on the type(s) of Salmonella strain(s), and the type of adjuvant it contains.

Sterility Test

The vaccines were checked for sterility before killing the cultures and after the vaccines were bottled. All contaminated cultures or vaccines were destroyed.

Breed of Mice Used

Albino mice were bred and maintained on a balanced pelleted ration. The mice were fed ad-lib throughout the duration of the experiment. Mice of 6 – 8 weeks old were used for the work. All mice experiments were carried out at the National Veterinary Research Institute, Vom, Nigeria.

Inoculation of Mice

Three thousand nine hundred and sixty (3,960) mice were used for these investigations at the rate of 60 mice per vaccine type. 0.5ml dose of vaccine was given intraperitonially (i/p) to each mice. The vaccinated mice and the controls were challenged after 3 weeks of vaccination.

Potency Test in Mice

The vaccines were potency-tested by challenging the vaccinated mice 21 days post vaccination. A mixture of four strains of typhoid-causing Salmonella (*S. typhi* and *S. paratyphi* A, B and C) were prepared and used as combined challenge culture. The challenge dose was obtained by growing each strain separately in Beef Infusion Broth for 24 hours. The culture was then diluted to obtain approximate concentration of 10^{10} colony forming units (cfu) per challenge dose of 0.5ml. Each proportion of the 4 diluted strains were pooled and used as the combined challenge dose of 0.5ml being administered intraperiotoneally per mouse. The challenged mice were observed for 3 days post challenge for survival or death. The controls were challenged alongside the vaccinates. Eight of the 14 vaccines or combinations that gave 100% protection were used in the rabbit potency testing. The experiments were repeated with 3,960 mice with similar results.

Toxicity Test

After the vaccination of the mice with 0.5ml dose of the vaccine intraperitonially, the mice were observed for 14 days post vaccination for any toxicity which may result in deaths or any reactions caused by the vaccines. No observable damaging reactions at the site of inoculation.

Potency Test in Rabbits

Ninety stable rabbits were vaccinated with 8 vaccines from mice potency testing at 10 rabbits per vaccine type (A – H). Each rabbit received 0.5ml vaccine intraperitoneally. The vaccines were not toxic to rabbits and no abnormal skin reactions were observed. After 2 weeks of vaccination, 5 of the rabbits in each vaccine type received booster dose of 0.5ml vaccine intraperitoneally. The rabbits were then observed for another 2 weeks, bleeding every other day for antibody titres using Widal test (Chow, et al, 1987). The observations and results were noted. The repeat experiments with fresh stable 90 rabbits gave similar results.

Challenge Test for Best 3 Vaccines

Vaccines C, D and E that gave Widal antibody titre of $^1/160$ were used in this test. Thirty healthy rabbits were vaccinated with 1.0ml of vaccines C, D and E subcutaneously at 10 rabbits per vaccine. Five out of 10 immunized rabbits received a booster dose of 1.0ml subcutaneously after two weeks of primary vaccination.

All the rabbits vaccinated were challenged with 1.0ml of LD_{50} of pooled virulent strains of Salmonella (typhi, paratyphi A, B and C) two weeks after booster dose.

Test of Vaccine in Monkeys

Vaccines produced for humans are normally tested in monkeys before they are used in man. This aspect of the work was carried out at the National Institute for Pharmaceutical Research, and Development, Idu, Abuja, Nigeria. Five monkeys were purchased and maintained in large wired rooms where they could move around freely. The monkeys were allowed to acclimatize to the new environment for one month before they were vaccinated with vaccine E (from rabbit testing results). Before vaccination, the monkeys were bled and Widal test was carried out on the blood serum. The results showed that four monkeys were Widal test negative and one monkey had a titre of $^1/80$. This means that the one positive monkey was likely to have some typhoid fever organisms. The five monkeys were vaccinated with 0.5ml of vaccine E which contained Sal. typhi; Sal. A, B & C. The monkeys were observed for 3 weeks before they were challenged.

Challenge of Vaccinated Monkeys

After 3 weeks of vaccination, the monkeys were challenged using 1.0ml of a mixed culture of causative organisms per monkey. Bloood was collected from the monkeys after 72 hours of challenge. The blood was cultured for possible isolation of typhoid microorganisms.

Preliminary human testing

A large batch of the vaccine was prepared. Special attention was paid to the sterility of all the materials used in the production of the batch for in the production of the batch for examples: equipment, glasswares, vials, chemicals and adjutants. The special laboratory where the vaccine was prepared was sterilized by fumigation. The personnel took special sterile precautions to prevent their contaminating the vaccine.

The vaccine was tested for toxicity in mice and it was found not to be toxic. The vaccine was applied subcutaneously in humans using a dose of 0.5ml. Immediately following the launching of the vaccine in 1999, about 512 persons were vaccinated at the Federal University of Technology, Minna; and 1,348 persons vaccinated at the General Hospital, Minna. From that time the vaccine had been made available at the University Clinic for people that want vaccination.

Duration of Immunity

Two hundred and twenty female mice were used for this aspect of the work. Ninety mice were vaccinated with a fresh batch of vaccine using 0.5ml intraperianlly per mice. Thirty mice were kept as controls. Five vaccinated mice were removed every two months to receive challenge dose. This was done for a period of 2 years six months.

Adjutants Testing

Adjutants A, B, C were tested for purity and toxicity before and after they were sterilized. They were separately tested for their reactions in mice and rabbits before they were used in the vaccine production. They were found to be safe for use in humans.

Results

Potency Test in Mice

The results obtained showed that mice vaccinated with 7 types of vaccines did not survive the challenge, 8 types of vaccine could protect only 20% of vaccinates, 17 types of vaccine protected 40% of vaccinates, 7 types of vaccine protected 60% of mice, 12 types of vaccine protected 80% of vaccinates and 14 types of vaccine protected 100% of vaccinates (Tables 1 and 2). Vaccine 12c was not tested. The control mice had zero survival. One thousand six hundred and forty-one mice died after challenge.

Potency Test in Rabbits

Five of the eight vaccines tested had average antibody titre of $^{1}/80$ while 3 vaccines C, D and E had average antibody titre of $^{1}/160$ (Table 4). It was observed that the vaccines C, D and E were non-toxic, protective and they were recommended to be tried in primates for safety and potency.

Test of Vaccine in Monkeys

There were no side reactions in 4 of the 5 monkeys. The monkey that had a titre of $^{1}/80$ Widal test was sick on the first day of vaccination but recovered by the second day (Table 5). This may mean that the vaccine should not be given to persons that have the disease.

Challenge of Vaccinated Monkeys

There was no growth of Salmonella organisms on solid and broth media. Similarly, there were no side reactions (Table 6). The results of the challenge revealed that the monkeys were solidly immunized and protected. It also shows that the vaccine can be applied in man without fear of toxicity or adverse reactions.

Preliminary Human Testing

There were some reported reactions and feverish conditions which rapidly wanes away within 24 hours. Severity vary from person to person (Table 7).

Duration of Immunity

Both human and mice vaccinates were protected against Typhoid fever for upward of 2 years. It will be safe to recommend 2 years as the duration of immunity (Table 8).

Performance of Adjuvants

Table 3 indicates the performance of the three adjuvants. Adjuvant A seems to give the best performance. It was recommended for other batch productions.

Table 1: Toxicity and Potency Tests in Mice

Vaccine No. **	Vaccine No. Vaccinated	Toxicity Tests No. of Deaths	Potency Tests Survival/No. Challenged*	Remarks % Survived
1 a	60	No deaths	23/60	40
b	60	No deaths	25/60	40
c	60	12 deaths	19/48	40
2 a	60	No deaths	25/60	40
b	60	No deaths	24/60	40
c	60	No deaths	12/60	20
3 a	60	No deaths	0/60	0
b	60	No deaths	0/60	0
c	60	No deaths	0/60	0
4 a	60	12 deaths	38/48	80
b	60	13 deaths	37/47	80
c	60	No deaths	60/60	100
5 a	60	No deaths	60/60	100
b	60	35 deaths	10/25	40
c	60	24 deaths	22/36	60
6 a	60	23 deaths	22/37	60
b	60	No deaths	60/60	100
c	60	No deaths	60/60	100
7 a	60	No deaths	60/60	100
b	60	11 deaths	39/49	80
c	60	36 deaths	10/24	40
8 a	60	No deaths	60/60	100
b	60	No deaths	60/60	100
c	60	25 deaths	21/35	60
9 a	60	No deaths	24/60	40
b	60	12 deaths	10/48	20
c	60	No deaths	12/60	20
10 a	60	No deaths	48/60	80
b	60	No deaths	36/60	60
c	60	No deaths	24/60	40
11 a	60	No deaths	48/60	80
b	60	No deaths	60/60	100
c	60	No deaths	60/60	100
12 a	60	12 deaths	0/48	0
b	60	No deaths	24/60	40
c	60	Not done	-	-

Vaccine No. **	Vaccine No. Vaccinated	Toxicity Tests No. of Deaths	Potency Tests Survival/No. Challenged*	Remarks % Survived
13 a	60	No deaths	13/60	20
b	60	No deaths	36/60	60
c	60	No deaths	12/60	20
14 a	60	No deaths	23/60	40
b	60	No deaths	49/60	80
c	60	No deaths	12/60	20
15a	60	13 deaths	19/47	40
b	60	No deaths	24/60	40
c	60	No deaths	12/60	20
16 a	60	No deaths	60/60	100
b	60	No deaths	35/60	60
c	60	12 deaths	11/60	20
17 a	60	No deaths	48/60	80
b	60	No deaths	25/60	40
c	60	12 deaths	38/48	80
18 a	60	No deaths	60/60	100
b	60	No deaths	49/60	80
c	60	11 deaths	24/60	40
19 a	60	No deaths	38/49	80
b	60	12 deaths	48/60	80
c	60	No deaths	38/48	80
20 a	60	No deaths	0/60	0
b	60	No deaths	24/60	40
c	60	No deaths	0/60	0
21 a	60	No deaths	60/60	100
b	60	No deaths	35/60	60
c	60	23 deaths	14/37	40
22 a	60	No deaths	60/60	100
b	60	13 deaths	0/47	0
c	60	No deaths	60/60	100
		CONTROLS =	0/60	0

*Number of Survival/Number challenged after toxicity test.
**a, b & c represent Adjuvant A, B & C respectively.

Discussion

Several parameters have been used in determining the quality of the vaccines. These include sterility tests of (1) the raw materials, (2) equipment used, and (3) at various stages of production: toxicity and potency tests in mice, rabbits and monkeys; challenge test on the vaccinated animals and (4) the repeat of experiments to confirm results. After all these precautions, the results obtained at the end of the experiments may be considered reliable.

Handling such large number of mice and rabbits may involve stress on the animals. Since the animals, especially the mice, survived the vaccination stage and a large number were healthy for the challenge the number of deaths before challenge was likely to be due to toxicity of some of the vaccines (Tables 1 and 2). It can also be assumed that all the 26 vaccines that produced 80% to 100% survival of vaccinated mice after challenge should be regarded as good vaccines especially the 14 vaccines that gave 100% protection (Table 2).

Table 2: Percentage survival after challenge

Vaccine					
0%	20%	40%	60%	80%	100%
3a, 3b, 3c	2c, 9b	1a, 1b, 1c	5c, 6a	4a, 4b	4c, 5a
20a, 20b	9c, 13a	2a, 2b	8c, 10b	7b, 10a	6b, 6c, 7a
	13c, 14c	5b, 7c, 9a	13b, 16b	11a, 14b	8a, 8b, 11b, 11c
15c, 16c	9a, 10c	10c	21b		16a, 18a
		12b, 14a		18b, 19a	21a, 22a
		15a, 15b		19b, 19c	22c
		17b, 18c		17a, 17c	
		12c			
		21c			
7	8	17	7	12	14

Table 3: Performance of Adjuvants from 80% to 100% survival.

Adjuvant A	Adjuvant B	Adjuvant C
4a, 5a, 7a	4b, 6b, 7b	4c, 6c
8a, 10, 11a	8b, 11b, 14b	11c, 17c
16a, 18	18b, 19b	19c, 22c
19a, 21a		
22a		
12	8	6

Table 4: Serological Response by Rabbits Vaccinated with Vaccines A – H.

Vaccine	No. of Rabbits	Toxicity	Potency/Average Highest Antibody titre	Remarks
A	10	Nil	1/80	
B	10	Nil	1/80	
C	10	Nil	1/160	Selected for challenge test in monkeys.
D	10	Nil	1/160	
E	10	Nil	1/160	
F	10	Nil	1/80	
G	10	Nil	1/80	
H	10	Nil	1/80	
I (Control)	10	Nil	Nil	

Table 5: Toxicity and Potency Test in Monkeys

S/No of Monkey	Widal test on blood serum before vaccination	Dose of vaccine used	Remarks
1.	Negative	0.5 ml	No side reactions
2.	1/80	0.5 ml	Sick on the first day but recovered by the second day
3.	Negative	0.5 ml	No side reactions
4.	Negative	0.5 ml	No side reactions
5.	Negative	0.5 ml	No side reactions

Table 6: Challenge of vaccinated monkeys.

S/No	Challenge dose*	Culture of blood after 72 hours of challenge	Remarks
1.	1 ml	No growth of Salmonella	No side reactions
2.	l ml	No growth of Salmonella	No side reactions
3.	1 ml	No growth of Salmonella	No side reactions
4.	1 ml	No growth of Salmonella	No side reactions
5.	1 ml	No growth of Salmonella	No side reactions

*3×10^{-10} colony forming units per milliliter

Table 7: Preliminary Human Testing

Place of vaccination	Dosage*	No. vaccinated	Reported reactions	Remarks
1. Federal University of Technology, Minna.	0.5 ml	512	Some reactions at site of inoculation and 10 people complained of feverish condition on the first day of vaccination. Severity varies from person to person.	The result of the human test is encouraging.
2. General Hospital	0.5 ml	1,348	Some reactions at site of vaccination.	

*Contains 10^9 colony forming units per milliliter.

Table 8: Duration of Immunity.

Vaccinates	Length of Immunity	Comments
Human vaccinates	The people we were able to contact indicated that they had not contacted typhoid fever since they received the vaccination two years earlier. Some people received immunity for about 3½ years. For the 2½ years that the experiment was conducted, the mice were still highly protected.	It is safe to recommend 2 years as the duration of immunity. A repeat dose can be given every 2 years.

Only eight vaccines were chosen from the 14 vaccines that gave 100% protection to be tested on rabbits to reduce the large number of rabbits that would be involved. The test of the vaccines in rabbits revealed three outstanding vaccines C, D and E. (Table 4) that are good candidates for the test in Monkeys.
The toxicity, potency and challenge tests in monkeys (Tables 5 and 6) revealed that the vaccine was not toxic and that the monkeys were solidly protected against the disease. The vaccinated people who reported some reactions at the site of inoculation also reported that the reactions rapidly wane away in one or two days. The level of reaction varied from person to person depending on whether the individual was rubbing or scratching the site with hand. Reactions or itching at the site of inoculation are common with the vaccinations of many vaccines. A few people complained of feverish conditions on receiving the vaccine but the severity of fever also varied from person to person.

The experiments carried out on the duration of immunity of the vaccine and the report obtained from human vaccinates after two years of vaccination indicated that the vaccine could provide immunity for more than two years. There were reports that the vaccine gave protection for more than 3 years.
Two years is recommended as the period that the immunity is adequate. A repeat vaccination can be every two years.

It is concluded therefore that the results of this work are good enough to give us the confidence that this vaccine can supply the immunity needed against Typhoid fever. A large scale production of the vaccine is recommended. Nigeria, Africa and indeed the developing World will eventually be protected against Typhoid fever and the total eradication is assured.

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References

Adesunkanmi, A.R., Ajao, O.G. (1997). The prognostic factors in typhoid ileal perforation: a prospective study of 50 patients. J. R. Coll. Surg. Edinb. 42:395-399.

Akgun, Y., Bac, B., Boylu, S., Aban, N., Tacyildz, I. (1995). Typhoid enteric perforation. Br. J. Surg. 82:1512-1515.

Chow, C. B., Wang, P. S., Chung, M. W., Yan, W. W., Leung, N. K., (1987). Diagnostic value of the Widal test in childhood typhoid fever. Pediatr Infect Dis. J. 6:914-917.

Davis, B. D., Dulbecco, R., Elsen, H. N., Ginsberg. H. S., Wood, W.B. and McCarthy, M. (1973. Microbiology 2nd Edition, Harper and Row Publishers Hagerstown, Maryland.

Lennete, E.H., Belows, A., Hausler, Jr. W. J. and Sahdomy, H.J. (1985. Manual of Clinical Microbiology, 4th Edition. America Society for Microbiology. Washington D. C.

Ketchum, P. A. (1988). Microbiology, Concepts and Applications. Published by John Wiley and Sons Inc., U.S.A.

Meier, D. E., and Tarpley, J. L. (1998). Typhoid intestinal perforations in Nigerian children. World J. of Surg. 22:319-323.

Robbins, J.D. and Robbins, J.B. (1984). Re-examination of the protective rate of the consular polysaccharide (V-antigen) of *Salmonella typhi.* Inf. Des. Vd. 150:436 – 449.

Van Basten, J.P., Stocken brugger, R. (1994). Typhoid perforation. A review of the literature Since 1960. Trop. Geogr. Med. 46(6): 336 – 339.

Wistreich, G.A. and Lectman, M.D. (1983). *Microbiology* 5th Edition Macmillan PublishingCompany, New York.