THE DEVELOPMENT AND PRODUCTION OF TYPHOID FEVER VACCINES TOXICITY AND POTENCY TESTS IN MICE

GARBA, S.A., AGBA, M.I.* NGBEDE, J.*, ODUGBO, M.*, FASANYA, O.O.A., OKE, S.A., MOHAMMED, K., ABDULLAHI, H. AND MOHOMMED, I.N.+

Federal University of Technology,
P.M.B. 65, Minna, Nigeria.

*National Veterinary Research Institute,
Vom-Jos, Nigeria.

+ Ministry of Health, Minna, Nigeria.

ABSTRACT

Sixty-six different types of typhoid 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 were used at the rate of 60 mice per vaccine. The mice were vaccinated intraperitonially with 0.5ml of sterile batch of vaccines. Three hundred mice died of toxicity. For the patency test the vaccinated mice were challenged with 0.5ml intraperitionially of a mixed 24 hour culture (2 x 10^{10} cfu/ml) of *Salmonella typhi, Salmonella paratyphi* A, B, C. after 3 weeks of vaccination. 1,388 mice died after the challenge. A total of 26 vaccines passed the tests, the twelve vaccines that produced 80% survival of mice and 14 vaccines that produced 100% survival. The results of the potency testing also reveal that Adjuvant A is the most effective followed by Adjuvant B. Fourteen vaccines that attained 100% survival will be used in the next round of potency testing in laboratory animals.

INTRODUCTION

Typhoid fever has been a problem of the Third 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 (Lennette, E.H. *et al.,* 1985; Davis, B.D. *et al.,* 1973; Wistreich, G.A. *et al.*, 1988). 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 the faeces which will further contaminate water, food or reingested directly. The small scale farmers farming along river or stream banks apply human dungs as manure on their farms. The microorganism loaded faeces manure will then contaminate the vegetables, carrots, garden eggs and so on. the innocent buyers may eventually come down with typhoid fever. Our Water Works all over Nigeria now find it difficult to get sufficient quantities of allum and chlorine needed to sediment and sterilize water before being suitable for human consumption.

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 (Lennette, *et al.,* 1985, Ketchum, 1988) makes it necessary to take appropriate steps to protect the populace against the disease.

If a potent vaccine can be produced vaccination is the most reliable method of protection against infection (Robbins and Robbins, 1984). This work is aimed at developing, producing and testing typhoid fever vaccines with the hope of finding a highly immunogenic vaccine that will give protection against typhoid fever.

MATERIALS AND METHODS

Isolation and Characterization of Local Strains

Specimen were collected from Typhoid Fever patients at the Minna General Hospital and Kowa Clinic. *Salmonella typhi, Salmonella paratyphi* A, B & C stains 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 Cultures, London) were used in these investigations. the imported strains were to be used in parallel with the local strains for comparison.

Culturing of Organisms

The 4 organisms locally isolated as well as the 4 duplicate imported stains were cultured in beef infusion broth for 48 hours. Viable counts were carried out on the cultures. The cultures were killed by heat.

Preparation of Vaccines

Each vaccine combination was treated with adjuvants A, B and C. A total of 66 vaccines were prepared and labelled.

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 well 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 assay.

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 intraperitionially (i/p) to each mice. The vaccinated mice and the controls were challenged after 3 weeks of vaccination.

Potency Test

The vaccines were each potency-tested by challenging the vaccinated mice 14 days post vaccination. A mixture of four strains of typhoid-causing *Salmonella* (*S. typhi* and *S.*

paratyphi A, B & C) were prepared and used as combined challenge culture. The challenge dose (mixture) 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 for each strain. Each proportion of the 4 diluted strains were pooled and used as the combined challenge (infection) dose of 0.5ml of the mixture being administered intraperitionially per mouse.

The challenged mice were observed for 3 days post challenge for survival or death. The controls were challenged along side the vaccinates.

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 or any reactions caused by the vaccines. No observable damaging reactions at the site of inoculation.

RESULTS

Toxicity Test

Three hundred mice died of toxicity (Table 1).

Potency Test

On challenge, 1,388 mice died (Table 1). Six vaccines had zero per cent survival, 8 vaccines produced 20% survival, and 17 vaccines had 40% survival. Seven, twelve and fourteen vaccines had 60%, 80% and 100% survival respectively (Table 2). All vaccines that produced 80% survival or above can be said to have produced adequate protection (Tables 1 & 2).

Performance of Adjuvants

Considering the vaccines that passed the test, the performance of adjuvants are indicated in Table 3. Adjuvant A has produced the best enhanced protection.

CONCLUSION

Some of the vaccines have produced very high immunity in mice. These vaccines will now be tested in other laboratory animals.

ACKNOWLEDGEMENT

These investigations have been sponsored by the Unipetrol Plc. We than the Federal University of Technology, Minna and National Veterinary Research Institute, Vom for allowing us to use some of their facilities.

Table 1: Toxicity and Potency Tests in Mice

Vaccine No.	No. of Mice 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	24/48	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	48/48	80
b	60	13 deaths	47/47	80
c	60	No deaths	60/60	100
5 a	60	No deaths	60/60	100
b	60	35 deaths	24/25	40
c	60	24 deaths	36/36	60
6 a	60	23 deaths	36/37	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	48/49	80
c	60	36 deaths	24/24	40
8 a	60	No deaths	60/60	100
b	60	No deaths	60/60	100
c	60	25 deaths	35/35	60
9 a	60	No deaths	23/60	40
b	60	12 deaths	13/48	20
c	60	No deaths	11/60	20
10 a	60	No deaths	49/60	80
b	60	No deaths	36/60	60
c	60	No deaths	25/60	40
11 a	60	No deaths	47/60	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	-	-
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
15 a	60	13 deaths	23/47	40
b	60	No deaths	24/60	40
c	60	No deaths	12/60	20

Vaccine No.	No. of Mice Vaccinated	Toxicity Tests (No. of Deaths	Potency Tests Survival/No. challenged*	Remarks % Survived
16 a	60	No deaths	60/60	100
b	60	No deaths	35/60	60
c	60	No deaths	11/60	20
17 a	60	No deaths	48/60	80
b	60	No deaths	25/60	40
c	60	12 deaths	48/48	80
18 a	60	No deaths	60/60	100
b	60	No deaths	49/60	80
c	60	No deaths	24/60	40
19 a	60	11 deaths	48/49	80
b	60	No deaths	48/60	80
c	60	12 deaths	48/48	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	25/37	40
22 a	60	No deaths	60/60	100
b	60	13 deaths	0/47	0
c	60	No deaths	60/60	100
		CONTROL =	0/60	0

* Number of Survival challenged after toxicity test.

Table 2: Percentage Survival After Challenge

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

Table 3: Performance of Adjuvants

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

REFERENCES

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

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

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

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

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