

NEUTRALIZATION OF EXCESS FORMALIN BY SODIUM META-BISULFITE IN COMBINED ANTHRAX & CLOSTRIDIAL VACCINE

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Summary

Excess formalin in combined anthrax & Clostridial vaccine was neutralized by different quantities of a suspension (50 gr in 100 ml D.W.) of sodium meta-bisulfite. A final concentration of 3 per cent could almost completely neutralize the excess formalin without harming the live anthrax spores, remaining in the combined vaccine.

The same method was employed for two experimental combined vaccines and viability of anthrax spores were checked by colony count method. Results showed that the minimum requirements of anthrax live spores were well met in a vaccinal dose when tested after two years.

Introduction

During the past two decades, bacterial and viral combined vaccines prepared with various natures have been used and developed for animals mostly with satisfactory results. Usage of formalin in definite quantities has been known as a suitable chemical compounded for inactivating the live organisms in the vaccines but when a monovalent or polyvalent formalized vaccine such as clostridial vaccine is mixed with a live spore vaccine like anthrax, the bactericidal effect of excess formalin should be eliminated or neutralized by some chemical substances so that no harmful effects appear either in vivo or in vitro.

Sodium meta-bisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) is a good substance which is able to neutralize the excess formalin without detrimental effects on immunological properties of the vaccine. Jansen (1) has reported the good effect of sodium meta-bisulfite in neutralizing the excess formalin after evaluating such formalin treated antigens by flocculation tests.

Materials and Methods

In this survey, four samples of Blackleg vaccine inactivated by

0.5 per cent commercial formalin were prepared, using local strain. 50% W/V Sodium meta-bisulfite solution in distilled water was then added to each sample to final concentrations of 0.5, 1, 2, and 3 gm%. Flasks containing the foregoing samples were shaken well, and then kept at 4° C for 4 days, after which, each sample was mixed with anthrax living spores of vaccine strain 34 F2, so that, the quantity of anthrax spores added, be approximately 7×10^8 per ml of final preparation.

Similar vaccine mixture, but without Sodium meta-bisulfite was also treated likewise, to be used as control.

Determination of the number of living spores present in experimental samples was carried out by colony counting method, 4 hours, 5 months, 9 months, and 19 months post vaccine combination.

Results and Discussion

Results indicated that, neutralization of excess formalin in combined vaccines, is best achieved, when 50% W/V sodium meta-bisulfite solution is added to a final concentration of 3 gr% W/V, (table 1).

As demonstrated in table 2, the results of anthrax spores colony count indicated that both vaccines had still enough live spores in vaccinal doses of three or five ml for the protection of animals over a period of two years.

In the present time, mass vaccination is made against anthrax and clostridial diseases in Iran. Large scale production and wide administration of the above vaccines were the main causes which necessiated this study towards the obtainment of an effective combined vaccine.

Acknowledgments

The authors are indebted to Dr. H. Mirchamsy and also to Dr. M. Ardehali for their valuable advices in this paper.

References

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Table 1

The results of spore count per ml of combined anthrax and blackleg vaccines, neutralized by various concentrations of sodium meta-bisulfite.

Adjuvant	7 days	45 days	1 year	2 years
Saponine	3×10^6	2.1×10^6	1.5×10^6	400×10^3
Aluminum hydroxide	3×10^6	2.9×10^6	1.7×10^6	525×10^3

Table 2

The comparison of spore count per ml of combined anthrax and clostridial vaccines at different times post preparation.

Na2S2O5	4 hours	5 months	9 months	19 months
0.5%	7×10^6	No growth	No growth	No growth
1%	7×10^6	4.7×10^6	4×10^6	1.2×10^6
2%	7.1×10^6	4.6×10^6	4×10^6	1.3×10^6
3%	7.1×10^6	4.8×10^6	4.2×10^6	1.5×10^6
Control	7×10^6	No growth	No growth	No growth