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The immunity conferred by anthrax avirulent uncapsulated live vaccine following different methods (intradermal and subcutaneous) of vaccination.

By:
Aarabi, I.and Sotoodehnia, A.

INTRODUCTION

Sterne in 1939 used the anthrax vaccine prepared from avirulent uncapsulated **Bacillus anthracis** strain 34F2. The vaccine was inoculated subcutaneously and the immunity lasted, at least, one year for sheep and goats (11).Delpy and Mirchamsy (1,2,3,4,8) used Pasteur strain No II for preparation of anthrax vaccine in Iran. Later on, virulent field strain (C2) was attenuated by the Sterne method (10) and was designated C5 avirulent strain . In 1978, according to WHO recommendations and minimum requirements for anthrax spore vaccine (12), Streme strain 34F2 was used by the authors of this paper.

Until 1971, in spite of some difficulties, intradermal inoculation method was commonly used for anthrax vaccination in Iran. In this year intradermal (I/D) and subcutaneous (S/C) vaccination methods were compared with anthrax spore vaccine under a research project by Aarabi, I. and Ardalan, A. (13). Intradermal route had been recommended by Besredka in I 921 (9) and has been used in some of the infected countries with success. However, few years later Pasteur Institute substituted the S/C method for I/D method of vaccination. In this study, we have compared the results obtained from the I/D method with thos of S/C method in sheep and goats in Iran.

MATERIALS AND METHODS

I – Vaccine:

Anthrax sporulated live vaccine was prepared with uncapsulated avirulent strain C5 of **B.anthracis** Roux flasks which contained pepton free agar medium were cultured with the strain. Dense cultures were harvested in physiological saline plus one in 20000 Merthiolate. Glass beads were used to remove the organisms from the surface of the agar. O.1% MT Saponine was added to the final product. No adjuvants were used for intradermal vaccine*.

2 – Susceptible animals and vaccination:

Fifty seven sheep and goats with equal number of males and females were selected from an area which was free of anthrax disease. No vaccine had

* This research work was carried out in 1971 under a research project for determination of duration of immunity of anthrax spore vaccine.

previously been administered to these animals and they were about one year old. Half of the animals were vaccinated by I/D route and the other half by S/C route. One vaccinal dose contained 4–5 million spores.

3 – Sterility and safety tests of the vaccine:

Sterility and purity tests were made in nutrient broth, nutrient agar and thioglycollate media. For safety tests, the vaccine was inoculated subcutaneously into guinea – pigs which were checked for a week.

4 – Challenge tests:

The vaccinated animals were divided into 10 groups. Groups 1– 2 – 3 – 4 – 5 comprised of those animals that had received the vaccine intradermally and groups 6-7-8-9-10 of those which had been subcutaneously vaccinated. Each group contained 10 animals, 5 sheep and 5 goats, except groups 2 and 9 which contained 12 animals, 6 sheep and 6 goats. A control animal was included in each challenge test. Groups 1-6, 2-7, 3-8, 4-9, 5-10 were challenged respectively at 3-5. 5-7. 5-10 and 13 months post–vaccination. The vaccinated animals were challenged with 200 MLD and the controls with 1 MLD of a virulent strain of **B.anthracis** (strain C2). One MLD of the challenge dose contained 30000 spores. The body temperatures of the animals were daily recorded for twelve days. The inoculation sites of the vaccination were measured. They varried from a lentil to the size of a hazelnut.

RESULTS

Tables 1 and 2 show the results of the challenge with virulent C_2 strain. The live sporulated vaccine conferred immunity for, at least, 13 months (the end point of the experiment) in sheep and goats.

In the first challenge test, two sheep and three goats died in group 1 and many colonies of **B.anthracis** were isolated from bone marrows whereas only one female goat died in group 6 and colonies were obtained from bone marrow culture in the latter case.

In the second challenge test, 5.5 months after the vaccination, two goats and one sheep died from group 2 but only one sheep died in group 7. **Bacillus anthracis** was isolated from all of the dead animals.

In the third challenge test, 7.5 months after the vaccination, one sheep and one goat died in group 3 with posotive cultures from bone marrow, but all the animals in group 8 resisted the challenge test.

In the 4th challenge test, 10 months after the vaccination, one female goat from group 4 and one female sheep from group 9 died and **B.anthracis** was isolated from the dead animals. The rest of the animals resisted.

In the 5th challenge test, 13 months after the vaccination, all the sheep in groups 5 and 10 resisted the challenge but a goat from each of the two groups died from anthrax infection.

All the control animals which were challenged with 1 MLD of C2 strain

died and **B.anthracis** was isolated from their bone marrows. Severity of the reaction in S/C vaccinated groups were apparently less than the I/D vaccinated groups in this experiment.

DISCUSSION

Anthrax avirulent spore vaccine is used in most of infected countries and it is very effective and protective against anthrax disease (5,6,7). In this survey, a vaccinal dose induced a high degree of immunity in sheep and goats for a period of, at least, 13 months. Subcutaneous route is more practical and easier than intradermal route and it is recommended as the method of choice for vaccination against anthrax.

At the present time, preventive and control measures are tending to the mass vaccination programs with research being conducted to prepare more effective bacterial and viral vaccines. Mass vaccinations using the separate vaccines against different diseases are on economical point of view, not recommended, therefore attentions are now focussing onto preparation and use of combined vaccines.

Since anthrax spore vaccine is commonly and extensively used in Iran, we decided to replace the S/C vaccination route for the old I/D method in order to make the production and usage of the combined vaccines feasible. Some experiments are being carried out to prepare the combined anthrax and clostridial liquid vaccines. The results will be published in future.

SUMMARY

Anthrax spore vaccine attenuated by Sterne method was prepared with a local strain. For determination of the duration of the immunity, filty seven sheep and fifty seven goats were vaccinated and challenged with virulent C2 strain. Half of the animals were vaccinated subcutaneously and the other half intradermally. The immumity engendered by these two methods of the vaccination was compared. The results showed that, S/C route was more practical than I/D route and a vaccinal dose conferred a high degree of immunity in animals for, at least, one year.

Table 1–Intradermally vaccinated sheep and goats that were challenged 3-5. 5-7. 5-10 and 13 months after the vaccination.

Group No	Goat	Sheep	Vaccinal dose	Route	Interval between vaccination and the challenge (months)	Challenge dose	Number of surviving per No. of challenged	Percentage of the immune
1	5	5	0.2	I/D*	3	200 MLD	5/10	50
2	5	5	0.2	I/D	5.5	200 MLD	7/10	70
3	6	6	0.2	I/D	7.5	200 MLD	10/12	83
4	5	5	0.2	I/D	10	200 MLD	9/10	90
5	5	5	0.2	I/D	13	200 MLD	9/10	90
Controls	2	3	–	–	–	1 MLD	0/5	–

I/D intradermal

Table 2 – Subcutaneously vaccinated sheep and goats that were challenged 3-5. 5-7. 5-10 and 13 months after the vaccination.

Group No.	Goat	Sheep	Vaccinal dose	Route	Interval between vaccination and challenge (months)	Challenge dose	No.of surviving per No. of challenged	Percentage of the immune
6	5	5	0.5	S/C*	3	200 MLD	9/10	90
7	5	5	0.5	S/C	5.5	200 MLD	9/10	90
8	6	6	0.5	S/C	7.5	200 MLD	12/12	100
9	5	5	0.5	S/C	10	200 MLD	9/10	90
10	5	5	0.5	S/C	13	200 MLD	9/10	90
ontrols	2	3	–	–	–	1 MLD	0/5	–

S/C Subcutaneous

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