Arch. Inst. Razi, 1984, 34,35, 51-54

THE COMPARISION OF TWO ANTHRAX SPORE VACCINES PREPARED WITH STERNE 34F2 AND NATIVE C5 STRAINS IN SHEEP AND GOATS IN IRAN

by:
Sotoodehnia, A., Aarabi, I.

SUMMARY

Two groups of sheep and goats were vaccinated with two batches of anthrax spore vaccines. One batch had been prepared with native (C5) and the other with (34F2) uncapsulated attenuated strains. At the same time a group of guinea-pigs was also vaccinated with Sterne strain. Vaccinated sheep and goats each were divided into two groups and were challenged with 100 or 200 MLD of virulent strain (C2) but guinea-pigs with 100 and 200 MLD of virulent strain (17JB). All sheep, goats and guinea-pigs survived the challenge test whereas the controls except one guinea-pig, died of anthrax infection.

Introduction

Upto 1973, anthrax spore vaccine was prepared in pepton free agar medium with avirulent C5 strain. In this year N. Z. Case medium was used for vaccine production with good results. A few years later in 1978, native strain was replaced by Sterne strain and in this experiment we have compared and evaluated both native and Sterne vaccine strains by challenge tests.

Materials and methods

Animals:

Twenty healthy sheep and goats were divided into two groups. No anthrax vaccine had previously been used to these animals. One group was vaccinated subcutaneously with O.5 ml of anthrax spore vaccine prepared from Sterne strain and the other group was vaccinated S/C with O. 5 ml of anthrax vaccine by using the native strain. Both groups were challenged with a virulent strain three weeks later.

Vaccine preparation:

Two batches of anthrax spore vaccines were respectively prepared with uncapsulated avirulent strain C5 and strain 34F2*. 24 hours broth cultures were seeded into Roux flasks that contained N. Z. Case solid medium and kept for 4 days at 37°C. The formula of the medium is as follows.

	gram
Pancreatic digest of casein (N-Z Case, TT)	5
Yeast extract (Difce)	3
Dipotassium hydrogen phosphate (K2HP04)	5
Potassium dihydrogen phosphate (KH2P04)	1
Calcium chloride (Cac12, 6H2O)	0.1
Ferrous sulfate (FeSO4, 7H2O)	0.01
Manganese sulfate (Mn SO4, 4H2O)	0.03
Magnesium sulfate (MgSO4, 7H2O)	0.05
Granular agar	25
Distilled water	1000

Sterilize in autoclave (final ph = 7.2)

To obtain a better growth, Roux bottles were maintained at room temperature for four more days after the initial incubation period. Pure cultures were washed off by physiological saline, using glass beads to remove the organisms from the surface of the agar, into flasks to contain 45 Roux bottle cultures. Merthiolate 1/20000 was added to the saline in order to kill the vegetative forms. These flasks were sampled for the purity test and maintained at + 4°C until the results were obtained The pure harvests were pooled and the viable spore count was carried out in the final bulk by plating suitable dilutions on an appropriate medium and also by the coulter counter equipment and using a Thoma's counting chamber. The suspension was adjusted in a way to cantain 4-5 millions viable spores per ml. To this Saponin* was added to make a final concentration of 0.1 percent. The final bulk underwent purity test as well. If the tests were satisfactory, 0.5 ml of this final bulk was used as a vaccinal dose.

Tests for bacterial contamination:

Tests were made by Microscopic examination of stained smears and by inoculation into nutrient broth, nutrient agar and thioglycollate media.

Safety tests:

Safety test for each batch of vaccine was carried out by subcutaneously inoculation of suitable dilutions of a thick suspension into 12 healty guinea-pigs and 4 sheep and goats which were checked for two weeks. In both groups, no death occurred and no sever reactions were observed during that period.

---

* Sterne strain 34F2 was obtained from International laboratory for biological standards, Central veterinary laboratory, Weibridge, England.

* Saponine MT obtained from Merck Company.

Vaccination:

Ten healthy sheep and goats were vaccinated S/C with 0.5 ml of strain 34F2 and also ten sheep and goats were vaccinated S/C with a vaccinal dose (0.5ml) of strain C5.

Ten healthy guinea-pigs between 300 and 500 g. of weight were each inoculated with a dose of vaccine (0. 5ml) of Sterne strain 34F2. All of the vaccinated sheep, goats and guinea-pigs were observed for 21 days. No death or ill reaction occurred during this period.

Challenge test:

Each vaccinated group of sheep, goats and guinea-pigs was divided into two subgroups. Subgroups were challenged with 100 or 200 MLD of virulent strain. Vaccinated sheep and goats were challenged with the virulent local strain (strain C2) but guinea-pigs were challenged with the Pasteur strain No. II (17Jb)*. In each group of sheep and goats, one control sheep was inoculated with 1 MLD of virulent strain that contained 30000 viable spores. In the guinea-pig group, 5 control guinea-pigs were challenged with 1 MLD of virulent strain. One MLD contained 100-1000 viable spores (1). Animals were kept and observed for a further period of two weeks. Table 1 and 2 show the results of challenge tests in guinea-pigs, sheep and goats.

Results

1 – All of the vaccinated guinea-pigs inoculated with anthrax spore vaccine (strain 34F2) survived and resisted the challenge test (100 and 200 MLD of strain 17JB) whereas 4 control guinea-pigs died between 3-5 days post challenge and one resisted during the challenge test. Anthrax colonies were isolated from internal organs of dead g-pigs.

2 – Both groups of vaccinated sheep and goats completely resisted the challenge test (100 and 200 MLD of strain C2) whereas control animals died 3 days post challenge with systemic infection of **Bacillus anthracis.**

Discussion

Strain 34F2 has been shown to be immunogenic and safe in animals (1). Strain C5 could produce a degree of immunity in sheep and goats as high as that produced by Sterne strain, but the latter had a better growth rate and the previlege of being a standard strain. In a field trial, a flock of 220 sheep and goats in an area (Hoseinabad Kushkizar) near Karadje city were vaccinated S/C with 0.5 ml. of anthrax vaccine (strain 34F2). Animals were observed for two weeks, no untoword reaction appeared but a transient oedema at the sites of inoculation.

Following this experiment and the field trial strain 34F2 replaced the strain C5 for production of anthrax vaccine at the Razi Instirute.

*Strain 17JB was obtained from Weibridge, England.

Table 1. Challenge test in guinea-pigs with virulent strain (17Jb).

Animal No.	Species	Vaccinal dose	Poute	Vaccine strain	Challenge dose	Survived animals %
5	guinea-pig	0.5	S/C	Sterne	100 MLd	100
5	guinea-pig	0.5	S/C	Sterne	200 MLD	100
5	guinea-pig (controls)	–	–	–	1 MLD	20

******

Table 2. Challenge test in sheep and goats with virulent strain (C2).

Animal No.	Species	Vaccinal dose	Route	Vaccine strain	Challenge dose	Survived animals %
5	sheep and goats	0.5	S/C	Sterne	100 MLD	100
5	sheep and goats	0.5	S/C	Sterne	200 MLD	100
1	sheep (control)	–	–	–	1 MLD	0
5	sheep and goats	0.5	S/C	native (C5)	100 MLD	100
5	sheep and goats	0.5	S/C	native	200 MLD	100
1	sheep (control)	–	–	–	1 MLD	0

*****

References

1 – Requirments for anthrax spore vaccine (live-for veterinary use). W. H. O. Technical report series, 1967, No.361.