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# *Study on a Combined Anthrax-Tetanus Vaccine for Immunization of Equines*

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**Abstract:** In a study on a combined anthrax-tetanus vaccine, a group of horses and mules was vaccinated and subsequently challenged with 250 MLD anthrax virulent C2 strain. All animals were resistant to the challenge test, while one unvaccinated horse-used as control-died of 1 MLD challenge dose and culture examination confirmed the presence of B.anthracis.Serum antibody of vaccinated animals were detected by sero neutralization assay. Sufficient amount of tetanus antitoxin in vaccinated horses and mules indicated the presence of prolonged immunity in the tested animals.

**Keywords:** *Anthrax vaccine / Tetanus vaccine / Vaccines / Combined vaccines / Equine immunization*

## Introduction

Anthrax and tetanus are considered as serious diseases of equines in Iran. These diseases are often seen in horse-breeding centers which partly belong to the army bases. Equines, particularly horses are sensitive to these infections, that cause mortality among them.

In a preliminary study, Delpy and Mirchamsy *(2)* reported the efficacy of a combined anthrax-tetanus vaccine prepared with local C5 anthrax strain. This vaccine has been used intradermic in 16000 horses due to army unit. In 1984,

attenuated 34F2 strain has been replaced by Sotoodehnia and Aarabi *(7)* for anthrax spore vaccine production, according to W.H.O requirements.

Following some technical modifications in anthrax and tetanus vaccines production, a new study was required to determine the safety and immunogenicity of the mentioned combined vaccine in horses and mules. In this study protection was evaluated by challenge test for anthrax and sero neutralization test for tetanus vaccines *(5,4,3)*.

## Materials and Methods

*VACCINE:*

Each vaccine was individually prepared based on its standard protocol *(3,5)*. They were mixed in a way that each dose of 2 ml. contained approximately $16x10^6$ viable anthrax spores and 30 Lf tetanus toxoid. The vaccine was administered subcutanously in the area behind the shoulder.

*ANIMALS:*

Five healthy horses of about two-year-old and five mules of the same age were selected and vaccinated with the combined anthrax-tetanus vaccine. A one year-old horse was also chosen as unvaccinated control.

*SAFETY TESTS:*

Three groups of guinea-pigs, each with ten guinea-pigs was injected by one of the routes of subcutanous, intramuscular and intraperitoneal. Two groups received 2 ml. vaccinal dose and the third group (intraperitoneal) 4 ml. double dose.

Safety test was also done in three groups of mice, each with five mice. All groups with the mentioned similar routes of injection, received 2 ml. vaccinal dose.

The animals were kept for five weeks and their weight variations were checked every week. Neither death nor significant side effect was observed in guinea-pigs and mice (6).

*POTENCY TESTS:*

*a-Anthrax challenge test:*

The vaccinated horses and mules were challenged in three weeks-interval post vaccination with 250 MLD anthrax virulent C2 strain. MLD was calculated $10^5$ viable spores and control animal died when received 1 MLD, four days after injection.

*b-Potency test of tetanus vaccine:*

The same batch of combined vaccine was prediluted 1:50 and 1 ml were inoculated subcutanously to ten guinea-pigs. Second injection was performed 28 days after the first injection and serum samples were collected two weeks after the last injection. Sera were pooled and sero neutralization test carried out for antibody titration on mice. Tetanus antitoxin was measured about 10 IU (international unit) in vaccinated guinea-pigs (1). Serum samples were collected from the vaccinated horses and mules, and each was individually tested for antibody titration by sero neutralization test. The average antitoxin unit obtained was 10 IU by sero neutralization assay *(1,4)*.

## Results and Discussion

All vaccinated horses and mules survived the challenge with anthrax virulent strain (Table 1). Control animal died after a period of four days with the appearance of systemic infection and a widespread oedema at the site of injection. Bacillus anthracis was isolated from the bone marrow.

Assessment of antitoxin in vaccinated horses and mules showed a high degree of protection by serological tests.

In general, the combined anthrax-tetanus vaccine could be able to confer immunity in horses and mules as much as single vaccine might induce.

*Table 1.* Potency test of combined anthrax-tetanus vaccine in horses and mules.

| Number of animals | Species | Vaccinal dose | Route | Day intervals between vaccination & challenge | Challenge dose | Results |
|---|---|---|---|---|---|---|
| 1 | Mule | 2 ml. | S/C* | 21 | 250 MLD** | Resisted |
| 2 | " | " | " | " | " | " |
| 3 | " | " | " | " | " | " |
| 4 | " | " | " | " | " | " |
| 5 | " | " | " | " | " | " |
| 6 | Horse | " | " | " | " | " |
| 7 | " | " | " | " | " | " |
| 8 | " | " | " | " | " | " |
| 9 | " | " | " | " | " | " |
| 10 | " | " | " | " | " | " |
| Control | " | - | - | - | 1 MLD | Died |

* S/C = Subcutaneous

** MLD = $10^5$ spores of B. anthracis, virulent strain C2

## References


1 - *Code of federal regulations 21 food and drug parts 620. 62 revised 1974.*

2 - *Delpy, L, P.Mirchamsy, H.Immunisation des chevaux contre la fievre charbonneuse et le tetanos, par vaccins associes. Bulletin de l'academic veterinaire de France, 1951, vol 24,62-65.*

3 - *Manual of details of test required on final vaccine. W.H.O, BLG/UNDP/1982.*

4 - *Manual of Manufacturing and testing procedures for D.T.P vaccine and therapeutic sera. Razi Institute. Revised 1974, Page 106.*

5 - *Requirements for anthrax spore vaccine (live-for veterinary). W.H.O. Technical report series. No. 361. 1967.*

6 - *Requirement for biological substances Nos.8.10 revised 1978.*

7 - *Sotoodehnia, A.Aarabi, I.Comparision of two anthrax spore vaccines prepared with Sterne 34F2 and native C5 strains in sheep and goats in iran. 1984, Arch. Inst. Razi, 34,35,51-54.*