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PRELIMINARY TRIALS OF ORAL IMMUNIZATION OF WILDLIFE AGAINST ANTHRAX

J. Rengel and H. Boehnel

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DEPARTMENT OF THE ARMY
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INSTITUTE OF INFECTIOUS DISEASES
FREDERICK, MARYLAND

Translated by

SCITRAN COMPANY
1482 EAST VALLEY ROAD
SANTA BARBARA, CALIFORNIA 93108
(805)969-2413
FAX (805)969-3439

PRELIMINARY TRIALS OF ORAL IMMUNIZATION OF WILDLIFE AGAINST ANTHRAX

J. Rengel and H. Boehnel

Institute of Horticulture and Animal Health in the Tropics and Subtropics, Georg August University, Goettingen, and Institute of Applied Biotechnology in the Tropics, Georg August University, Goettingen.

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Summary

In pilot trials relating to the vaccination of wild animals in African game reserves, guinea pigs were vaccinated against anthrax. The vaccine was prepared in suspension using the Goettingen IBT Bioreactor method. Guinea pigs immunized orally or subcutaneously survived infection by 1000 spores from a field strain isolated from an elephant in the Luangwa Valley Animal Reserve in Zambia. The animals immunized orally or subcutaneously and infected with 2500 spores died. A technique was developed using gas chromatography to identify B. anthracis organisms excreted in the feces.

Anthrax as a zoonosis has lost much of its terror in Europe, although there continue to be sporadic reports of human infections in which the pathogen was brought in from a tropical country (1,3,11).

Anthrax in animals is a soil disease in which the pathogen is usually ingested orally as a spore, penetrates the mucous membrane and by means of vegetative reproduction causes septicemia and rapid death. In the case of humans it is a

contact disease. The organism is usually transmitted to humans by contact with products such as wool, meat or skins from animals that died of anthrax; depending on the localization of the infection site it causes dermal, pulmonary or intestinal anthrax (Fig. 1).

In humans the clinical course is generally acute or highly acute in the case of pulmonary and intestinal anthrax and the disease symptoms are not specific, so that therapy is usually impossible. In the case of dermal anthrax, on the other hand, there are good chances of recovery. In many countries chemical therapy is legally prohibited for animals.

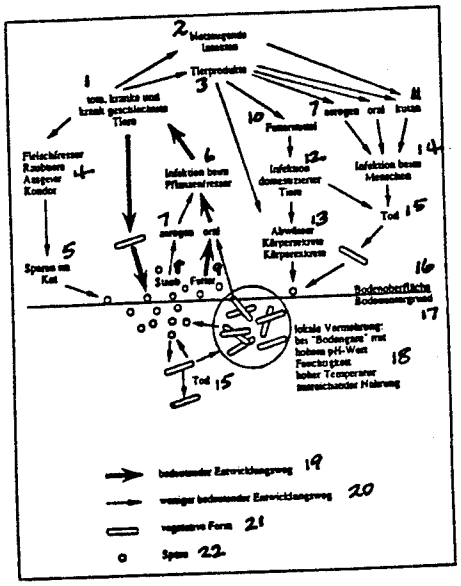

Fig. 1. Schematic representation of epizootiology of anthrax. (13, mod. using 18 and 14).

Key: 1 dead or sick animals or animals sick when slaughtered; 2 blood-sucking insects; 3 animal products; 4 carnivores, beasts of prey, vultures, condors; 5 spores in feces; 6 infection in herbivores; 7 aerogenic; 8 dust; 9 feed; 10 feedstuffs; 11 cutaneously; 12 infection of domesticated animals; 13 sewage, bodily secretions and excretions; 14 infection in humans; 15 death; 16 ground surface; 17 underground; 18 local reproduction: in the case of "soil refining" if high pH and temperature, moisture and sufficient nutrients are present; 19 important path of development; 20 less important path of development; 21 vegetative form; 22 spore.

Metaphylactic treatment of the other animals in the same herd or of people who are also at risk of infection, using tetracycline or other antibiotics, is normally successful (4,14,22).

Except in the case of dermal anthrax in humans, diagnosis is normally only possible after death. The organisms cannot be detected in the peripheral blood until about 12 hours after the death of the animals (7). Uncoagulated blood, severe spleen tumor with liquefaction of the spleen pulpa, and a grey musculature that appears cooked are pathognomonic. Naturally, the pathogen should be identified in the laboratory in each case.

In the tropics, mixed infections may occur in animals in conjunction with clostridia. This confuses the picture and makes the laboratory diagnosis much more difficult (15).

The pathogen of anthrax is B. anthracis. It occurs throughout the world as only one type, although there are forms with varying degrees of virulence, such as the highly pathogenic Vollum strain. The main pathogenic mechanisms are present in the exotoxins coded by plasmides (pX01) and the bacterial D-glutamine polypeptide capsule (pX02) (5).

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In addition to management measures such as fencing, reducing occupancy density or reforestation (14), immunization is the method of choice to prevent diseases in domesticated animals. Since 1939 the so-called "Sterne strain $34F_2$" has been used with great success all over the world to prepare spore vaccines that are administered to the animal subcutaneously (about 10 to 20 x 10^6 spores/dose) (17,5).

In humans the use of a living spore vaccine is associated with a risk of side-effects and so the attempt has been made

to build up a similar immunity to the disease using toxin vaccines. In animal tests it has been possible to achieve essentially 100% immunity in guinea pigs using the Sterne vaccine, but the toxin vaccines demonstrated a much lower degree of protection (8).

Anthrax is not limited to domesticated animals; wild animals also become sick and die of the disease. The various species of animals have varying susceptibilities to infection by B. anthracis. The risk is

- severe for cattle, sheep, buffalo, reindeer, elk, llamas, camels, elephants, rhinos, antelope, zebras;
- moderate for pigs, beasts of prey kept in captivity;
- low for dogs, cats, mink and foxes.

Birds (except for the ostrich), hyenas, wild dogs and carrion eaters are almost resistant.

Significant outbreaks of the disease occur repeatedly in wildlife reserves in Africa as a result of the high concentration of susceptible animals in a small area. This results in a local accumulation of anthrax spores in African game parks, so that the disease also demonstrates a rising trend (3,10,12,19,20,21,22). In the Luangwa Valley National Park in Zambia the losses caused recently by anthrax far exceed the losses resulting from poaching. In addition, this produces a considerable risk to the local population, as the neighboring people eat the meat of fallen animals regardless of the cause of death.

Because of the tenacity of the spores, once a habitat has been infected by spores it remains infected for years or decades (9,7).

The immunization that is customary for domesticated animals is not feasible for wild animals. The large number of animals makes it impossible to finance the administration of the vaccine by shooting the vaccine from helicopters.

Therefore studies were set up to prepare a "swallowed vaccine" for wild animals similar to the natural form of infection and to test its effectiveness in the laboratory. The long-term goal was to immunize the elephants in the Luangwa Valley National Park in order to limit the increasing epizooty there.

Personal studies

Our studies using guinea pigs had the following objectives:

- to build up immunity following the oral adminstration of living spores and challenge tests using pathogenic field organisms;
- to detect vaccine bacteria excreted fecally;
- to determine the bacteriological stability of the vaccine strain after several animal passages.

Our studies were carried out cooperatively with the Balmoral Central Veterinary Research Laboratory, Lusaka/Zambia.

Immunization tests

For the vaccine we used "Sterne" spores that had been obtained in the Goettingen IBT bioreactor and had already been kept at room temperature for 15 months.

The guinea pigs were given varying amounts of spores, in some cases by means of a feeding tube and in other cases packed in alginate pellets (16). A booster was given after two weeks. The alginate pellets given to animals 32, 33 and

34 were prepared one day ahead, while those given to animals 29, 30 and 31 had been kept for a month in a refrigerator and had partly liquified again. Animal 32 ate only half of the alginate pellets, while animals 29, 30 and 31 ate only an undetermined portion of the spores offered. As a control one animal group was immunized s.c.

The challenge was given i.m. using spores from a field strain that had been isolated in a dead elephant in the Luangwa Valley National Park. In preliminary tests we selected 1000 to 2500 spores as the dose (Tab. 1).

For technical reasons (number of isolation cages) it was not possible to keep the time between the last immunization and the challenge constant.

Table 2 shows the survival figures and the time until death after infection with 1000 spores. Table 3 shows the results of the challenge test using 2500 spores.

In the first test series 2 of the 3 animals immunized i.m. survived. They had not received any booster. The animals immunized by means of a feeding tube all survived; only a few of those that had eaten (some of) the alginate pellets survived. In the second test all the animals died; the time until death occurred was much longer for the animals immunized i.m. Probably the number of spores selected for the challenge was too high.

Detection of the excretion of vaccine bacteria in the feces

The feces of 14 animals immunized orally were quantitatively collected, weighed, mixed and analyzed for the presence of B. anthracis.

Figure 2 shows the analysis process (13). The clear classification of the isolates, especially of B. cereus and B. anthracis, was done by means of gaschromatographic analysis of the cell composition (6) (Fig. 3).

Six hours after the oral administration of 2.3×10^8 anthrax spores it was already possible to detect the pathogen in the excreted feces. The number of positive findings dropped consistently up to the sixth day after administration. After that it was not possible to detect it in any of the animals. One day after the last positive finding in the feces it was also not possible to find any spores in gastric juice removed by stomach probe. The animals that ate only a small amount of food and thus excreted a small amount of feces excreted bacteria for the longest time.

Six animals that had received an oral immunization six times at three week intervals in each case excreted anthrax bacteria in the feces for the first two days after administration every time.

None of the five animals that had received 2.3×10^7 spores s.c. excreted any bacteria in the feces.

In one test 2 sheep were fed enriched feed that contained 10^8 spores. It was not possible to detect any B. anthracis in their feces.

Bacteriological stability

Since orally immunized animals may excrete living vaccine bacteria, we investigated whether a change in immunity occurs in several animal passages.

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Tab. 1

Intramuscular challenge of unimmunized guinea pigs using a varying number of B anthracis spores (Zambia field strain) (TTD = time till death)

Tiernummer 1	intramuskuläre 2 Belastung mit „Sambia" (Sporen/Dosis)	TTD (h)
35	250	überlebt 3
36	500	76
37	1000	70
41	1000	42
42	1000	48
43	1000	51
44	1000	58
45	1000	67
38	1500	überlebt 3
39	2500	50
40	5000	46

Key: 1 animal no.; 2 intramuscular challenge using "Zambia" (spores/dose); 3 survives.

Tab. 2

Survival and time till death of immunized guinea pigs after challenge using 100 B.anthracis spores (Zambia field strain)

Tier-Nr. 1	Impfstoffgabe „Sterne" (Sporen/Dosis) 2	1. Boosterung „Sterne" (Sporen/Dosis) 3	Zeitpunkt der Belastung nach letzter Impfstoffgabe 4 (Wochen)	i.m. Belastung „Sambia" (Sporen/Dosis) 5	TTD (h)
	6 oral mittels Schlundsonde				
17	3×10^7	3×10^8	5	1000	überlebt
18	3×10^7	3×10^8	5	1000	überlebt
19	3×10^7	3×10^8	5	1000	überlebt
	7 oral über Alginatkugeln				
29	$2{,}3 \times 10^8$	$2{,}3 \times 10^8$	4	1000	53
30	$2{,}3 \times 10^8$	$2{,}3 \times 10^8$	4	1000	82
31	$2{,}3 \times 10^8$	$2{,}3 \times 10^8$	4	1000	83
32	$6{,}9 \times 10^8$	$2{,}3 \times 10^8$	3	1000	52
33	$6{,}9 \times 10^8$	$2{,}3 \times 10^8$	3	1000	überlebt
34	$6{,}9 \times 10^8$	$2{,}3 \times 10^8$	3	1000	überlebt
	8 subkutan				
26	$2{,}3 \times 10^7$		3	1000	112
27	$2{,}3 \times 10^7$		3	1000	überlebt
28	$2{,}3 \times 10^7$		3	1000	überlebt

Key on following page.

Key: 1 animal no.; 2 "Sterne" vaccine given (spores/dose); 3 "Sterne" booster (spores/dose); 4 time of challenge after last vaccine given (weeks); 5 i.m. challenge using "Zambia" (spores/dose); 6 orally by feeding tube; 7 orally with alginate pellets; 8 subcutaneously; ueberlebt = survives.

Tab. 3

Time till death of immunized guinea pigs after challenge using 2500 B. anthracis spores (Zambia field strain)

Tier-Nr. 1	Impfstoffgabe „Sterne" (Sporen/Dosis) 2	1. Boosterung „Sterne" (Sporen/Dosis) 3	2. Boosterung „Sterne" (Sporen/Dosis) 4	Zeitpunkt der Belastung nach letzter Impfstoffgabe 5 (Wochen)	i.m. Belastung „Sambia" (Sporen/ Dosis) 6	TTD (h)
	7 oral mittels Schlundsonde					
14	$1{,}5 \times 10^7$	3×10^8	3×10^8	3	2500	50
15	$1{,}5 \times 10^7$	3×10^8	3×10^8	3	2500	68
16	$1{,}5 \times 10^7$	3×10^8	3×10^8	3	2500	50
20	2×10^8	3×10^8		2	2500	50
21	2×10^8	3×10^8		2	2500	65
22	2×10^8	3×10^8		2	2500	68
	8 subkutan					
23	$2{,}3 \times 10^7$	$2{,}3 \times 10^8$		2	2500	89
24	$2{,}3 \times 10^7$	$2{,}3 \times 10^8$		2	2500	86
25	$2{,}3 \times 10^7$	$2{,}3 \times 10^8$		2	2500	120

Key: 1 animal no.; 2 "Sterne" vaccine given (spores/does); 3 first "Sterne" booster (spores/dose); 4 second "Sterne" booster (spores/dose); 5 time of challenge after last vaccine given (weeks); 6 i.m. challenge using "Zambia" (spores/dose); 7 orally by feeding tube; 8 subcutaneously.

B. anthracis was isolated in the feces of three guinea pigs orally immunized using 7×10^8 spores. A new batch of vaccine was prepared from this mixture and thus three guinea pigs were immunized again orally using the same dose. Bacteria were isolated again in their feces and a subsequent vaccine was prepared and administered orally to three animals again.

The bacteria retrieved from the last three animals were compared with the initial bacteria using gas chromatography. We were not able to detect any changes in the fatty acid level using the BIS computer program.

Three guinea pigs were immunized s.c. with the initial bacteria and three with the final bacteria (3×10^7 spores in each case). All the animals susrvived; they were then, as shown above, challenged using 1000 spores each. All the animals survived.

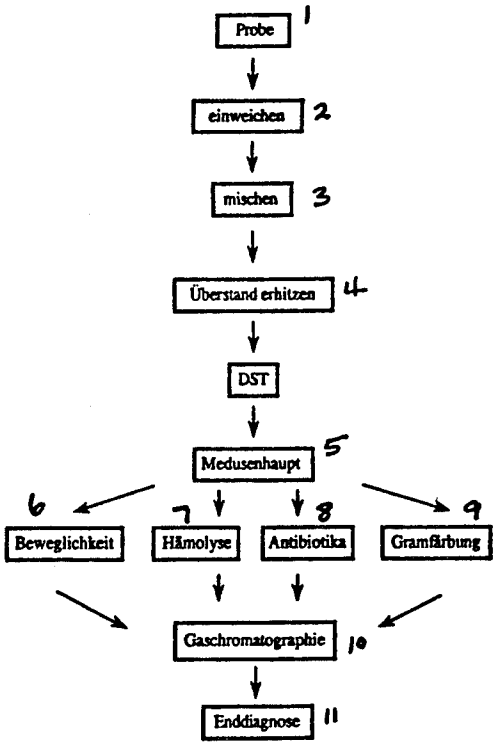

Fig. 2. Analysis process for detecting B. anthracis in the feces (DST = disc sensitivity test agar/oxoid) (13)

Key: 1 specimen; 2 soaking; 3 mixing; 4 heating of residue; 5 head of Medusa; 6 mobility; 7 hemolysis; 8 antibiotics; 9 gram staining; 10 gas chromatography; 11 final diagnosis.

Discussion

In the tests described we were able to demonstrate for the first time that it is possible to immunize guinea pigs orally against anthrax. In the literature available to us Ebedes (2) quoted his colleague Bergmann with the remark that the latter had succeeded in producing an oral vaccine that protected guinea pigs against repeated challenges using virulent anthrax spores. No further information was given.

The vaccine bacteria excreted in the feces did not show any changes in virulence or immunogenity.

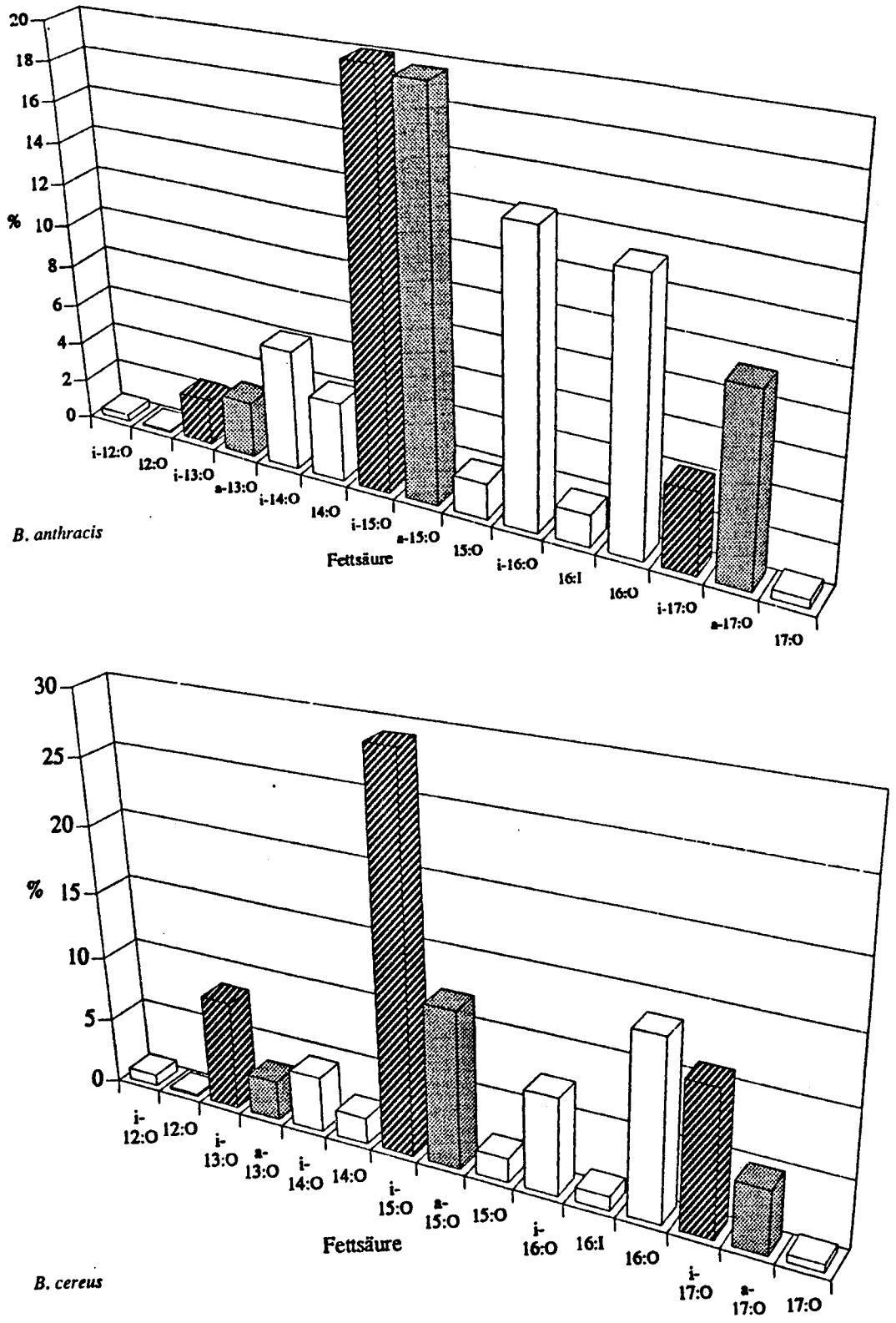

Fig. 3. Gas chromatograms of B. anthracis and B. cereus (fatty acid methyl ester).
Key: i = iso; a = antiiso; 12 = no. of carbon atoms; :O = saturated fatty acid; :I = unsaturated fatty acid; Fettsaeure = fatty acid.

The use of live vaccines from generally weakened or modified field pathogens should basically only be undertaken in the case of all diseases in the areas where the disease occurs. Otherwise it may result in unwarranted dispersion of disease bacteria.

The tests in guinea pigs that we have described should be repeated in the the animal species that are being considered for immunization. Restriction to individual animal species, such as elephants, could be achieved by deliberately putting out their favorite food (in this case ears of corn or melons). It would be advisable to have game wardens monitor the bait at least at the start of an immunization campaign, as the number of spores ingested would need to be adjusted for the species of animal being treated.

Critics have taken a decisive stand against this kind of immunization (Turnbull, personal communication). In our opinion, because of the massive losses of rhinos, buffalo, antelopes and elephants the Luangwa Valley National Park must be considered extremely contaminated. Since, as already mentioned, the meat of dead animals is eaten by the local population, the disease bacteria have also spread beyond the confines of the reserve. The number of people who have become sick or died is not known.

Any "Sterne" bacteria excreted by immunized animals would, according to the epizootiology of anthrax, would to some extent compete immunologically with existing highly pathogenic local strains. The vaccine bacteria would be ingested again with food and the animals would be orally immunized. In the literature available to us we cannot find any indication that the "Sterne" strain is not stable and has reverted to its original strain.

The studies described should encourage researchers to set up systematic field studies in order to reduce the risk of anthrax to humans and animals in and near tropical animal reserves.

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