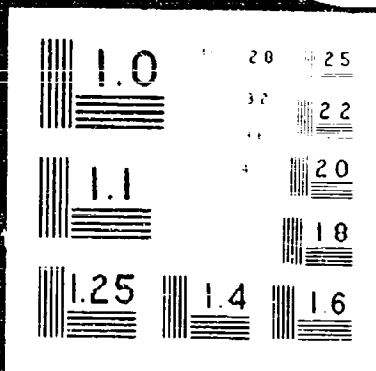


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1 91

KOLPSOV, S. G. (Professor), and SEMAILOV, N. A.
(Candidate of Veterinary Sciences)

Issledovanie immunogennykh svoystv gidroksis' -aluminievoy
vaksiny protiv sibirskoy levy i ispytanie ee v shirokoi praktike

/Studies of the immunogenic properties of the aluminum hydroxide
vaccine against anthrax and testing it at-large in the practice

Gosudarstvennyi Nauchno-Kontrol'nyi Institut Veterinarnykh
Preparatov. Trudy v.8. (Biopreparaty, virusy, mikroby).
p.250-255. 1956. Moscow. 41.9 Un39

(In Russian)

Scientific study of the immunogenic properties of the

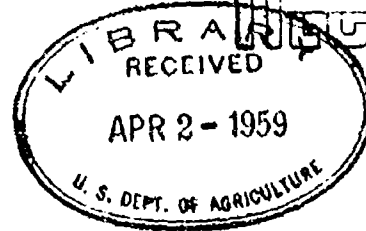
variant of the vaccine

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STUDIES OF THE IMMUNOGENIC PROPERTIES OF THE ALUMINUM
HYDROXIDE VACCINE AGAINST ANTHRAX AND TESTING IT
AT-LARGE IN THE PRACTICE

1956

... against anthrax, the effec-



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9.1

KOLYSOV, S. G. (Professor), and NIKHAILOV, N. A.
(Candidate of Veterinary Sciences)

Issuchenie immunogennykh svoistv gidroksid'-aluminievoy
vaksiny protiv sibirskoi iasvy i ispytanie ee v shirokoi praktike

/Studies of the immunogenic properties of the aluminum hydroxide
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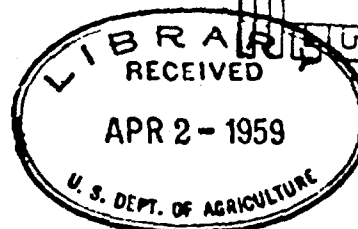
Gosudarstvennyi Nauchno-Kontrol'nyi Institut Veterinarnykh
Preparatov. Trudy v.6: (Biopreparaty, virusy, mikroby),
p.250-255. 1956. Moscow. 41.9 Un39

(In Russian)

Officially issued for the purpose of

Variant 6

Isolated



STUDIES OF THE IMMUNOGENIC PROPERTIES OF THE ALUMINUM
HYDROXIDE VACCINE AGAINST ANTHRAX AND TESTING IT
AT-LARGE IN THE PRACTICE

1956-1959

For prophylactic vaccination of animals against anthrax, the effec-
tive Tschenkivskii's and STI vaccines were applied. However, the mentioned
vaccines are capable of producing in the animals postvaccinal complications.

This was the reason for the development of a better antianthrax vaccine.

The search for methods of obtaining anthrax vaccinal strains
possessing highly immunogenic properties of a low virulence is of greatest
theoretical and practical importance.

By his numerous investigations, the Russian scientist I. V. MICHURIN
had proved that living organisms are easily changed under the influence of
the outer habitat, and that there is nothing unchangeable in the nature of
living organisms. Thus, I. V. MICHURIN's teachings confirm the fact that
the nature of living substances (be they complicated or protozoan organisms)



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Roy H. Chapman
ROY H. CHAPMAN
Director
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Army Biological Labs.

Studies of Immunogenic Properties of Aluminum Hydroxide Vaccine Against Anthrax and Testing it at-large in Practice., by Kolesov, S. G. and Mikhailov, N. A., Trudg Gos. Nauch-Kontrol. Inst. Vet. Prep, 6:250-5, 1956

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can be changed in accordance with the will of the experimentator. The basic method, used in microbiology for the obtaining of vaccinal strains, is the method for a directed action of microorganisms.

In 1946 through 1949, one of the authors of this article (S. G. KOLESOV) conducted extensive studies on the obtaining and selection of antianthrax cultures with useful properties, and applied the method of directed action. For these purposes a great number of anthrax strains were many times periodically seeded on serous media and on apopton agar. The result was, the variant Sh-15 of a low virulence was obtained from the strain "Shuia-2" isolated from a cadaver of a swine in 1933.

Later on, during the studies of the cultural, virulent and immunogenic properties of this strain, it was established (KOLESOV, and BORISOVICH, 1951-1952; KOLESOV and NIKHAILOV, 1953-1954) that the vaccine prepared from strain Sh-15 possesses immunogenic properties of a low virulence; this was proved by tests on laboratory animals.

Experiments of 1954. The investigation of the cultural and biological properties of the vaccine were studied in 1954, and it was established that the vaccine preserves its initial properties.

A total of 653 liters of 2 experimental and 3 productional series of the aluminum hydroxide vaccine was prepared. /Begin. p.252/ During the control tests it was observed that the vaccine of all series contained a pure anthrax culture with viable spores in the concentration of 45 to 48 millions per 1 ml.

Testing the vaccine for harmlessness to rabbits and for virulence to

guinea pigs, it was established that the vaccine at a dose of 2 to 3 ml is harmless to the former and of a low virulence to the latter (infection at doses of 1 ml produced death in approximately 10 per cent of the guinea pigs). The new vaccine is less virulent than the 2nd Teankovskii's vaccine and the STI vaccine. In cases when under similar conditions, the STI vaccine produced death in 60 per cent of the guinea pigs, whereas the new vaccine (table 1.) caused death only in 11.5 per cent, in spite of the fact that in the new vaccine the number of viable spores was higher by 84.4 per cent than in the STI vaccine.

During the control tests of the immunogenic properties of the aluminum hydroxide vaccine on guinea pigs, it was established that the vaccine possesses high immunogenic properties which at a dose of 0.5 ml are capable to prevent the disease in 66 to 100 per cent of the guinea pigs, and at a dose of 1 ml in 69.5 to 100 per cent guinea pigs infected with 10 to 15 lethal doses of the 2nd Teankovskii's vaccine 14 to 19 days postvaccination. At a dose of 1 ml the vaccine protected 60 per cent guinea pigs vaccinated 46 days prior to the control infection. We consider that the difference in the resistance of the animal's organism was the cause of the fluctuation of the percentage indicating the survival of the guinea pigs, since the experiments were conducted at different seasons of the year. /Begin. p.253/

In October and November of 1964, experimental tests were conducted on sheep, and the duration of the stability of immunity produced by the aluminum hydroxide vaccine against anthrax was checked. The tests were commissioned;

Table 1.

Testing vaccines for virulence and harmfulness on rabbits and guinea pigs (1954)

Name of vaccine	Date of experiment	Series No. of vaccines	Number and kind of animals	Dose of vaccine in ml	No reaction	Local reaction						died	survived	Per cent of survivors
						Value of reaction								
						+	++	+++	+++	+++	+++			
Aluminum hydroxide	Jan. 1954	18	10 rabbits	2	1	9	-	-	-	-	3	10	100	
Glycerin		20	10 guin. pigs	1	1	-	-	-	-	-	-	3	7	70
		19	5 rabbits	2	-	4	-	-	-	-	-	-	5	100
STI		19	10 guin. pigs	1	1	1	1	-	-	-	-	3	7	70
		6/3	10 "	1	-	3	2	-	-	-	-	8	2	20
Aluminum hydroxide	April 1954	21	5 rabbits	2	2	2	-	-	-	-	1	5	100	
STI		21	5 guin. pigs	1	-	2	-	-	-	-	-	-	4	80
		22	5 rabbits	3	2	2	-	-	-	-	-	-	5	100
STI		10	10 guin. pigs	1	-	-	-	-	-	-	-	10	-	-
		272	"	1	2	4	11	3	1	1	1	3	22	88
Aluminum hydroxide	May 1954	13	"	0.5	1	3	12	1	-	-	1	19	95	
Aluminum hydroxide		18	"	3	7	1	1	-	-	-	-	-	9	100
		2,3 and 4	9 rabbits	2	5	1	1	-	-	-	-	-	6	100
STI		6	33 guin. pigs	1	-	10	14	15	1	-	-	2	31	94
		6	10 "	0.5	-	-	-	-	-	-	-	-	10	100
			30 "	1	-	-	4	5	-	-	-	15	80	
Total on new vaccine			40 rabbits 113 guin. p.	17	17	20	2	1	14	5	-	13	49	100
Total on STI vaccine			50 " pigs	-	-	21	45	23	-	-	-	103	103	83.5
						-	6	8	22	14	33	17	34	34

Testing the vaccine for its immunogenic properties on guinea pigs (1954)

Name of vaccine	How many series of the vaccine	How many experiments	How many animals	Dose of the vaccine (in ml)	Control infection			
					After how many days	Died	Survived	Percent of survival
Aluminum hydroxide (series no. 13, 18, and 21)	3	3	16	1	19	-	16	100
	2	2	9	0.5	19	-	9	100
	2	2	9	1	14	-	9	100
	2	2	8	0.5	14	-	8	100
Aluminum hydroxide (Productional series 2, 3 and 4)	3	3	15	0.5	15	2	13	86.7
	3	3	17	0.5	19	6	11	65
	3	3	13	1	17	4	9	69.3
	3	3	17	1	45	7	10	60
Total	6	7	104	-	-	19	85	81.8
Control of Zerkhovskii's 2nd breeding					-	29	-	-

they were conducted by S. O. KOLASOV, A. V. KACHAMIDZE, S. G. ARZIANI, V. A. MENTESHASHVILI and other physicians of the Tabakhmolsk Almoombine.

Castrated rams that had been vaccinated with the aluminum hydroxide vaccine at the dose of 0.3 ml in the fall of 1953, were infected also rams vaccinated at a dose of 0.3 ml 7 to 14 days prior to the infection. The standard spore virus of anthrax, series no. 16, issued on April 17, 1954, was used for the infection. The virus was pretitrated on rams at a dose of 1 ml in dilution 1:25, 1:100 and 1:250 (2 head per dilution). All infected animals died of anthrax.

The control tests were conducted on 33 sheep. The latter were infected subcutaneously with the virus of series no. 16 at a dose of 1 ml in dilution 1:300. The following experimental results were obtained:

a) out of 10 castrated rams, that had been subjected to the infection $12 \frac{1}{2}$ months postvaccination, 3 died; one of them died 3 days later than the controls. Seven head survived; 4 of them had reacted to the anthracis virus, whereas the fifth showed slightly increased body temperature one time;

b) all 5 rams, vaccinated with the vaccine 14 days prior to the infection survived and showed no reaction at all;

c) all 5 rams vaccinated with the combination (vaccine and serum) 14 days prior to the infection, survived; they showed a slight thermal reaction of a short duration;

d) out of the 4 rams, vaccinated with the vaccine 7 days prior to the infection, one died; the others had not reacted to the virus;

e) all 4 rams vaccinated with the vaccine without aluminum hydroxide 7 days prior to the infection, survived and had not reacted to the virus;

f) 5 non-vaccinated control rams, died of anthrax; 3 died $2\frac{1}{2}$ days after the infection, another died 3 days postinfection, and a third died 6 days postinfection.

Thus, in crucial experiments on sheep, tests of the immunogenic properties of the aluminum hydroxide antianthrax vaccine have proved that the vaccine is capable of producing stable and longlasting immunity (not less than for one year).

In 1954, the aluminum hydroxide vaccine was tested against anthrax on the livestock of large farms.

In the spring, the following animals of kolkhoses of the Krasnodar Krai were vaccinated with the mentioned vaccine: 1634 head of cattle (including 1108 head of young animals); 2021 head of sheep (including 726 4 months old lambs); 185 horses and 3520 swine (including 2337 younglings); all in all 7160 animals were vaccinated. No complications have occurred. It must be also noted, that /Bogin. p.254/ 600 calves were simultaneously vaccinated with the aluminum hydroxide vaccine and with the vaccine "ankar" /emphysematous carbuncle/. Cases of complications were not observed either.

In the fall, local veterinary workers conducted vaccination of animals on kolkhoses of the Stavropol' and Krasnodar Krai and in the Rostov Oblast'. We instructed the leading veterinary surgeons and gave them the directions for the conduction of these vaccinations. The aluminum hydroxide vaccine against anthrax, series no. 2, issued on June 20, 1954, series no. 3 issued on April 27, 1954 and series no. 4, issued by the Kaluga Bioplant on June 29, 1954 was used for the vaccination. It was proved that the vaccine

was pure, harmless to rabbits, and immunogenic in tests on guinea pigs.

The vaccine was injected subcutaneously to one-year old animals and to older ones at the following doses: to cattle--1 ml; to horses--0.75 ml; to swine--0.5 ml; to sheep--0.3 ml; to goats--0.2; to younglings under 3 months of age: to cattle and horses--0.3 ml to 0.5; to swine--0.3 ml; to sheep--0.1 to 0.2 ml, and to goats--0.1 ml.

During the months of October and November there were vaccinated 141,254 head of cattle, 31,734 horses; 468,630 sheep; 6,361 goats and 15,590 swine. In the fall of 1954, all in all 648,499 animals were vaccinated.

Analysing the local reports and the material obtained, the following results concerning the application of the vaccine had been obtained:

The reaction to the vaccine of the majority of animals was either insignificant or mild. The reaction in horses and cattle was manifested by the appearance of a swelling at the spot of the injection of the vaccine in the size of 3 x 4, 4 x 5 less frequently, 6 x 7 cm. After 3 to 4 days, the swelling decreased, became small or disappeared entirely. A temperature rise of 0.5 to 0.8 to 1.0 degrees was observed; in some animals it lasted for 1, 2 or 3 days, thereafter it returned to normal. In sheep and goats, a small swelling occurred on the 2nd day on the spot of the injection of the vaccine; it was of the size of 1 x 2 or 2 x 3 cm; it decreased after 3 to 4 days and sometimes dissolved completely. Some of the sheep were limping on that extremity into which the vaccine was injected. On the second day, the body temperature rose by 0.3 to 0.7 to 1 degree, very seldom by 1.2 or 1.5 degrees. The reaction in swine was insignificant.

On farms of the Stavropol' and Krasnodar Krai, several thousand head of 4-year old and younger cattle were subjected to simultaneous vaccination with the aluminum hydroxide vaccine and the "amkar" vaccine. No complications and losses occurred. After the vaccination, the horses and oxen were not freed from work. Neither complications nor losses occurred.

During the mass vaccinations in the fall, there were only /Begin. p.255/ 3 cases of complications with a fatal outcome. On one of the kolkhozes of the Krasnodar Krai, 5 young sheep died.

In the "aul" Khabez /mountain village in the Caucasus/ of the Cherkessy Autonomous Oblast', complications had occurred in 2 goats, one of them died. In the Asov Raion, Rostov Oblast', one foal died on the kolkhoz imeni Kalinin on the 5th day. One young ox died on the 2nd day in the kolkhoz imeni "Pol'shevik". In the Matveevo-Kurgan Raion complications had occurred in 23 goats; 13 of them died. Exhaustion was the cause of the animals' death. Thus, the number of losses of animals in proportion to the number of vaccinated animals constituted only minimal fractions (tenths, hundredths, thousandths and ten-thousandths parts) of one per cent.

Consequently, as the result of the mass vaccination of agricultural animals against anthrax, conducted for prophylactic purposes, it was established that the aluminum hydroxide vaccine causes only in single animals a local and thermal reaction and that as a rule, the vaccine is approved being a harmless biopreparation.

According to the data of our experimental tests on laboratory animals and on cattle of farms, we consider that the aluminum hydroxide vaccine

against anthrax is less harmful than the 2nd Tsenkovskii's vaccine and the vaccine STI. Tsenkovskii's 2nd vaccine, when inoculated at minimal doses, causes death in all guinea pigs, and sometimes in 25 per cent of rabbits.

The STI vaccine, at a dose of 25 to 30 million viable spores, causes death in 40 to 60 per cent of guinea pigs and at a dose of 125 to 150 million spores--death in some rabbits. Whereas the aluminum hydroxide vaccine, that was introduced by us, is less harmless, as evident from the data mentioned above. On the average, it produced death in not more than in 10 per cent guinea pigs when injected at a dose of 45 million viable spores, and does not cause death in rabbits when injected at a dose of 120 to 150 million spores.

Tests of the vaccinal strain Sh-15 for the stableness of its properties during 5 years have proved that the strain Sh-15 is capable of preserving its cultural and biological properties.

Conclusion

1. Testing the vaccine in crucial experiments, it was established that it possesses well-expressed immunogenic properties and produces in sheep immunity that lasts not less than for one year.

2. The harmlessness of the vaccine was proved by tests on 656,659 agricultural animals of various raions of 2 kraia and of 1 oblast'.

3. We consider that it is expedient to recommend the application of the aluminum hydroxide vaccine against anthrax for prophylactic vaccinations of agricultural animals in wide productional experiments.