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AD815200

Translation No 1675

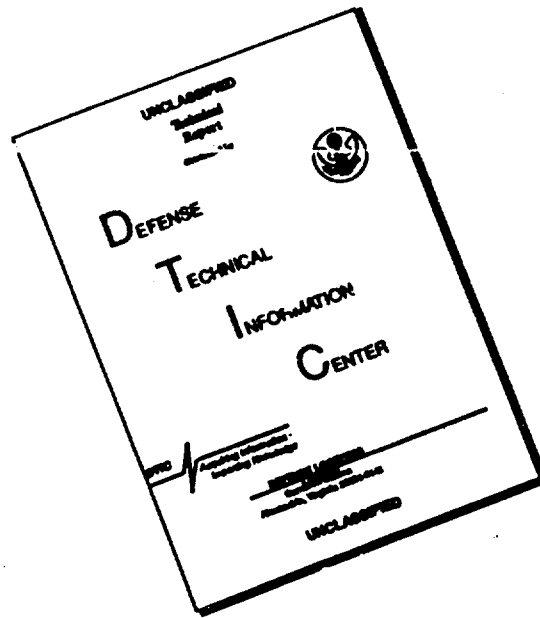
April 1966

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THE RESULTS OF STUDYING THE BRUCELLA VACCINE FROM THE BR. ABORTUS 104-M STRAIN

[Following is the translation of an article by I. F. Taran, Ye. I. Zamakhayeva, S. V. Abakin, A. M. Polyakova and N. M. Nelyapin, Kavkaz and Zakavkaz Scientific-Research Antiplague Institute, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Vol 42, No 8, 1965, pages 99--104. It was submitted on 1 Jul 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

In the Soviet Union the specific prophylaxis of brucellosis infection has occupied one of the leading places in the complex of antibrucellosis measures. In the last two decades extensive work has been carried out for the purpose of obtaining highly immunogenic strains for vaccination. As a result, foreign and Soviet scientists have proposed a number of vaccine strains, however the majority of them have not found subsequent application. The Br. abortus 19 vaccine strain has received international recognition. The relative intensity of immunity, created by this vaccine, caused the necessity for further searches.

As a result of the work which was carried out in the laboratory of P. F. Zdrovovskiy, Kotlyarova (1950) proposed Br. abortus 104-M as a vaccine strain. It was distinguished by a somewhat greater residual virulence and a greater immunogenicity than the vaccine from the Br. abortus 19 strain. This was our basis for carrying out the present investigation.

The work was made up of three sections: 1) a study of the harmlessness, immunological effectiveness and intensity of immunity, created by the Br. abortus 104-M vaccine in tests on guinea pigs; 2) a study of its harmlessness, immunological and epizootological effectiveness in tests on sheep; 3) a study of immunological and epidemiological effectiveness of the vaccine on humans.

The investigations showed that following the administration of 10 and 100 microbial cells to guinea pigs, the insemination of the internal organs of animals which received Br. abortus 19 was 4 time less intense, and those which received Br. abortus 104-M -- 2 times less intense than following the administration of a virulent culture. The 104-M vaccine culture took root in the organism of man better than the culture of 19 following administration in doses of from 100 up to 1 million microbial cells. The intensity of immunity was more expressed in guinea pigs inoculated with the 104-M vaccine (table 1).

In the group of guinea pigs which were inoculated with the 104-M vaccine, specific morphological changes developed following the administration of 10 million microbial cells and were characterized by a proliferative inflammation in the form of hyperplasia of the reticular cells and the Kupffer cells in the liver, and the formation of granulomas out of the epithelioid cells in isolated lymph nodes and the spleen. In animals which were vaccinated with strain 19, all these symptoms were more weakly expressed, even following the administration of 100 million and 1 billion microbial cells.

In the tests for studying the vaccine from the 104-M strain during cutaneous application, it was established that the minimum dose which conditioned a generalized infection equaled 1 million microbial cells. However, a well expressed immunological reaction after a month following inoculation was caused by doses of no less than 100 million brucella.

As a result of the morphological investigations it was established that the mechanism of development of the vaccine process during cutaneous application was analogous to its development during subcutaneous administration.

Immunity in guinea pigs which were inoculated with 1 billion and 5 billion microbial cells developed in the period from the 3rd through the 9th months. By 12 months the intensity of immunity was noticeably lowered, and this lowering was more expressed in pigs which were inoculated with 5 billion microbial cells.

The immunity which emerged following administration of vaccine from the 104-M strain developed slowly, and nonsusceptibility to infection with a virulent culture was achieved at the price of significant qualitative changes in the lymph nodes and internal organs. Even in optimum periods (6 months) the intensity of immunity remained relative and bore a barrier nature. Thus, although strain 104-M was more immunogenic than strain 19, for the successful struggle with brucellosis in foci one specific prophylaxis is insufficient, and it is necessary that the entire complex of antibrucellosis measure be carried out along with it.

Taking into consideration the considerable reactogenicity of vaccine from the 104-M strain, we undertook the mission of studying the bacteriology and morphological picture of the vaccinal process and also the intensity of immunity under the conditions of numerous revaccinations. As a result, it was established that in guinea pigs after a month following vaccination with 5 billion microbial cells a generalized process was noted with the abundant seeding of brucella from the lymph nodes and internal organs with an average titer of agglutinins up to 1:160 and an index of enlargement of the spleen up to 2 (in healthy guinea pigs from the same batch the index fluctuated from 0.8 up to 1.2). Following the first cutaneous revaccination with 2.5 billion microbial cells a generalization of the vaccine process was not observed. An insignificant increase in the titers of agglutinins and an increase of the spleen index up to 2.2 were noted. With the subsequent second and third revaccination there were

more guinea pigs with a generalized process than following the first vaccination. There was a lowering in the titers of agglutinins and the enlargement index of the spleen (1.3).

In the lymph nodes and internal organs of the guinea pigs following repeated revaccination there were intensive changes in the form of necroses and abscesses, intensive eosinophilia in the lymph nodes and spleen, growth of scar tissue, atrophy of the Malpighian bodies of the spleen and necrobiosis of the hepatic cells, especially expressed following triple revaccination.

Thus, it was established in the experimental section of our investigations that the 104-M vaccine was more reactogenic and immunogenic than the generally recognized 19 vaccine. The first one is capable of causing significant morphological shifts, which also guarantees its immunogenicity. The main conclusions, characterizing the 104-M vaccine in the tests on guinea pigs, served as the basis for testing this vaccine on sheep.

We used 185 healthy sheep for a comparative study of the two vaccine strains. As a result it was established that strain 104-M possessed more expressed immunogenic properties than the vaccine from strain 19. Thus, out of 23 sheep inoculated with the first preparation in a dose equal to 10 billion microbial cells, immunity developed in 22. In one animal there was a regional infection following infection after 11 months following vaccination. Out of 29 sheep inoculated with the second vaccine in the same dose and in the same periods, only 13 turned out to be immune. Six animals developed a regional infection and 5 animals a generalized infection.

The study of the harmlessness of vaccine from strain 104-M was carried out on 56 pregnant sheep in the 2nd--3rd month of pregnancy.

The sheep were vaccinated subcutaneously with 8 billion microbial cells based on the optical standard. Lambing on the part of the vaccinated animals proceeded normally, there were no abortions, and 63 healthy lambs were born.

There was no less interest in studying immunity in sheep which were inoculated with 10 billion microbial cells with a subsequent revaccination. As a result, after 11 months following the first revaccination with strain 104-M immunity was obtained against 50 generalized doses of a virulent strain in 60% of the test animals. Following revaccination with strain 19 such an immunity was obtained in only 20% of the sheep. After 11 months following the second revaccination complete immunity had developed in 40 and 20% of the sheep respectively.

The 104-M vaccine strain was stable and preserved its properties with great persistence. This is testified to by the tests on sheep and guinea pigs which were conducted in our laboratories for the last 6 years. All of these observations served as the basis for studying the new vaccine strain in an industrial experiment.

Beginning with 1956 we, jointly with the veterinarians from the state and collective farms, inoculated 335,437 sheep with vaccine from

strain 104-M. They came from unsafe flocks, where prior to the vaccination up to 25% of the sheep had brucellosis. The animals were vaccinated subcutaneously with 8--10 billion microbial cells. The sheep were vaccinated once with a subsequent second and third revaccination. The complex examination of the inoculated sheep was carried out in various periods following the vaccination and revaccination. The sheep were revaccinated after 10--11 months and several flocks of ewes were left without vaccination for 2--3 years.

As a result of vaccination with strain 104-M abortions of a brucellosis etiology in sheep on brucellosis affected farms ceased, and there was an increase in the output of livestock from 89 up to 100-110 lambs per 100 ewes.

It should be noted that in individual years on the farms a significant drop-out of ewes and lambs was observed, and also extensive sterility by unsatisfactory feeding and the presence of other diseases among the animals. Along with this the epizootological nature of the farms in respect to brucellosis turned out satisfactorily. In the aborted fetuses in several flocks, brucellosis cultures of the melitensis type were isolated only in the first 2 years following the onset of vaccination. Consequently brucellosis infection among immunized sheep was cut. Following the immunization of the animals with the 104-M vaccine the farms were economically stronger, they accomplished their production plans, and 110,960 healthy ewes were reared from female sheep which were derived from vaccinated sheep.

During the study of the duration of immunity (table 2) it was established that immunological reactions in sheep which were vaccinated with strain 104-M were more expressed than in animals which were inoculated with strain 19, especially the allergic test. Together with this, by the end of the first year the postvaccinal reactions in animals of both groups has almost completely died away.

After 2 years following immunization 25 sheep out of the group of animals which reacted positively were subjected to a bacteriological and biological investigation. No brucella cultures were isolated. Thus, the high immunogenicity of vaccine from strain 104-M creates a foundation for recommending it for the extensive vaccination of sheep. The experience of the mass vaccination of sheep with this vaccine on 8 farms in Stavropolskiy Krai confirmed its harmlessness and high epizootological effectiveness.

At the same time we studied the feasibility of using the vaccine from Br. abortus strain 104-M for the specific prophylaxis of brucellosis infection in man. The study of the harmlessness and immunological effectiveness of the vaccine during subcutaneous and cutaneous application was carried out by means of vaccinating the adult population (from 17 to 55 years old) in brucellosis foci. All told 4392 persons were under observation. Of these, 612 were inoculated subcutaneously and 3730 cutaneously.

The following doses were used for subcutaneous inoculation: 100 million (545 men), 250 million and 300 million (all told 67 men) microbial cells. The larger doses were used for vaccination in the most active foci of brucellosis, where infection of the professional group of the population reached 29%. In these groups people with negative immunological reactions for brucellosis were inoculated.

It was established that during subcutaneous administration to man the vaccine from strain 104-M in quantities of 100 million microbial cells was harmless, caused an immunological reconstruction in the organism, and turned out to be completely acceptable based on reactogenicity. This supported the data which was obtained earlier by other investigators (Kotlyarova et al., 1959). In foci of brucellosis the subcutaneous administration of large doses of vaccine (250--300 million microbial cells) caused a significant general and local reaction, proceeding like a type of allergy, in 54% of those inoculated. This prevented the recommendation of the stated doses for the vaccination of man, though they did not lead to brucellosis infection.

When studying the harmlessness and immunological effectiveness of vaccine from strain 104-M during cutaneous application, it was administered in the following doses: 1.5 billion, 3 billion, 5 billion, 7 billion, and 10 billion microbial cells. It was established that after 1--2½ months following inoculation among those who were inoculated with 1.5 billion microbial cells 64% reacted weakly and positively, among those inoculated with 3 billion -- 79% among those inoculated with 5 billion -- 80-90%, and among those inoculated with 7--10 billion -- 73%. The degree of expression of the postvaccinal reactions depended on the professional composition of those inoculated. Thus, in groups of farmers the reactions were more expressed than in students. This may be explained by the presence among the inoculated farmers of persons who were sensitized to brucellosis infection and who were not detected during a single preliminary check.

During the comparative study of the immunological effectiveness of the vaccines when administered cutaneously in a dose of 5 billion microbial cells, it was established that strain 104-M is more immunogenic than strain 19.

Thus, the test of cutaneous vaccination of persons with vaccine from strain 104-M showed its acceptable reactogenicity, harmlessness, and immunological effectiveness in doses of 100 million microbial cells during subcutaneous and 5 billion microbial cells during cutaneous application.

The epidemiological effectiveness of the cutaneous method of immunization of persons with the 104-M vaccine was checked in 1959--1960 in 4 rayons of Stavropolskiy Kray in the most active fresh foci of brucellosis among sheep and goats. Brucellosis was recorded in 40 out of the 56 sheep farms which were located in the rayons. Infection of animals reached 40--90% and mass abortions in sheep were noted. The brucellosis etiology of these abortions was confirmed bacteriologically. All told 51,446 men were

inoculated. A vaccine was used which was prepared by the Scientific-Research Antiplague Institute for Kavkaz and Zakavkaz. One human dose comprised 3--5 billion microbial cells based on the optical standard. The inoculations were carried out from August through March, which was specified as the extended period for insemination and lambing for sheep according to economic plans. The effectiveness of the inoculations was taken into consideration over a period of 15 months following the immunization.

Among 88 persons who became sick, 44 men were inoculated by the cutaneous method against brucellosis with vaccine from strain 104-M, 4 men -- with vaccine from strain 19, and the remaining 40 men turned out to be unvaccinated. Of the 44 men in the group inoculated with the 104-M vaccine who became sick, 11 had been inoculated more than once in the past with vaccine from strain 19. In all those who became sick the diagnosis was confirmed clinically and by immunological reactions, and in 27 men -- bacteriologically. In all cases the brucellosis causative agent of the goat-sheep type was isolated. Based on severity the clinical course of brucellosis in those who had been inoculated and those who had not was different.

The disease in those who had been inoculated set in after 15 (1 man), 30 (3 men), 60 (4 men), 90 (7 men), 120-150 (16 men), 180--210 (10 men), and 240 (3 men) days.

When calculating the effectiveness of inoculations by regions regardless of profession when comparing morbidity among non-inoculated and inoculated persons in the optimum periods (2--3 months prior to the beginning of lambing) the index of effectiveness turned out to be equal to 4, that is, there was 4 times less sickness among those inoculated than among the non-inoculated. When calculating the effectiveness of inoculations individually by foci the disease was noted in 3.3% of those vaccinated in the optimum periods, and in 6.7% of those not inoculated. The index of effectiveness equaled 2. This very clearly showed the dependency of the effectiveness of inoculations on the epizootological situation on the farms.

Thus, the experience of working in the foci showed that in the struggle with brucellosis foremost importance belongs to the specific prophylaxis of this disease in animals against a background of a rise in the public and sanitary culture on the farms. For this purpose vaccine from Br. abortus strain 104-M may be used with the greatest effectiveness. The presence of still another vaccine strain, which based on the main properties does not yield to the Br. abortus 19 strain, makes it possible to steadily equip the antiepidemic and antiepzootic service with a full-value vaccine.

#### Conclusions

1. An experimental study of the vaccine strain of Br. abortus 104-M on guinea pigs supported its fine immunogenic properties during subcutaneous and cutaneous application, its ability to "take" and the ability to cause an
- 6.

active immunological reorganization.

2. During experimental testing on sheep, vaccine from the Br. abortus strain 104-M turned out to be harmless in doses of 8--10 billion microbial cells, it survived for a sufficiently long time in the organism, and it created an immunity which was more intense than that of Br. abortus 19. On the farms where the sheep were treated with the vaccine from this strain a sharp reduction was noted in the number of abortions, there was an increase in the output of healthy young stock, and a significant lowering of morbidity with brucellosis among the persons who take care of these animals.

3. Strain Br. abortus 104-M may be recommended for the prophylactic vaccination of sheep for the purpose of forming healthy livestock.

#### Literature

1. Kotlyarova, Kh. S., Zh. mikrobiology, 1950, No 6, page 13.
2. Kotlyarova, Kh. S., Polyakova, A. M., Lukashova, L. V., Vestn. AMN, USSR, 1959, No 2, page 42.

Table 1

Intensity of immunity in guinea pigs, infected with Br. melitensis 548 after 3 months following vaccination with Br. abortus

Dose of vaccine (in microbial cells)	19				104-M			
	Number of animals	Not infected	Infected		Number of animals	Not infected	Infected	
			Regional infection	Generalized infection			Regional infection	Generalized infection
100	12	0	1	11	10	1	3	6
1000	12	2	2	8	12	4	2	6
10 000	12	1	3	8	12	5	6	1
100 000	11	4	2	5	11	7	4	0
1 000 000	11	2	4	5	12	10	2	0
10 000 000	12	2	4	6	11	7	3	1
100 000 000	12	4	6	2	12	6	5	1
1 000 000 000	12	8	2	2	12	10	2	0
Total	94	23	24	27	92	50	27	15

Table 2

Results of the immunological investigation of sheep in various periods following vaccination

Name of vaccine	Name of reaction	Number of positively reacting animals (im %) in various periods of investigation following vaccination (in days)									
		10	20	30	60	100	225	270	330	360	510
104-M	Wright	100	100	90	60	54	13	0.8	2	0.8	0
	Complement fixation	80	100	99	80	68	15	0.8	0.2	0.5	0
	Burnet	0	0	100	100	81	50	0.2	0.2	1	0
19	Wright	100	100	85	60	50	5	0.3	0.3	0.3	0
	Complement fixation	75	100	100	65	45	2.5	0	0.2	0	0
	Burnet	0	0	0	40	50	17	0	0.2	0	0