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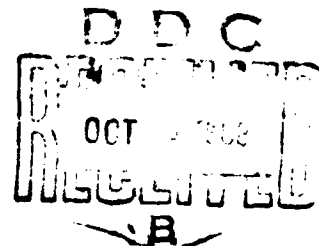
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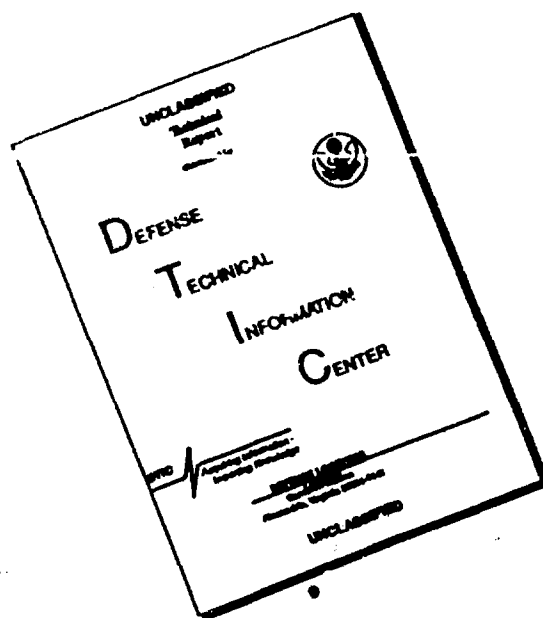


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IMMUNIZATION EFFICIENCY IN VARIOUS MODES OF ADMINISTRATION  
OF THE LIVE BRUCELLOSIS VACCINE

[Following is the translation of an article by A. Ye. Khayev, Military-Medical Order of Lenin Academy imeni S. M. Kirova and the Severo-Osetinskiy Medical Institute, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 11, 1966, pages 34-37. It was submitted on 17 Dec 65. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Immunogenesis in the subcutaneous administration of live brucellosis vaccine has been studied quite sufficiently (Zdrodovskiy, 1938, 1953; Vershilova, 1954 and 1961, and others), but immunogenesis during the cutaneous method of vaccination has been investigated less (Vershilova, 1961, and others). Problems of immunogenesis during the intracutaneous method of vaccination and its variants, in spite of the use of this method in the vaccinothrapy of brucellosis (Alisov, 1943; Bilibin, 1947; Tselishchev, 1956; Pushintseva, 1960, and others), have been investigated insufficiently up until the present time.

The mission of this investigation was the study of the influence on immunogenesis of the various routes of administering small doses of the brucellosis antigen.

The investigations were carried out on 150 guinea pigs weighing 250-350 grams. The animals were vaccinated with the dry brucellosis vaccine from the Br. abortus No. 19 strain (product of the Kashinskaya Biologicals Manufacturing Plant, Series 1907, expiration date 31 August 1965), which is most common both in the Soviet Union and abroad.

In a preliminary test we determined the minimum immunizing dose during the intracutaneous administration of vaccine in one point by the method of Alisov. For this purpose 20 guinea pigs were immunized with the vaccine in various doses: 100, 1000, 10,000; and 100,000 microbial cells (based on the seric turbidity standard). On the 30th day after immunization we noted in the animals the following immunological shifts, expressed in average titers of the Wright Reaction: 1st group - 0; 2nd group - 1:10; 3rd group - 1:20; and 4th group - 1:160. Since a dose of 100,000 microbial cells caused expressed immunological shifts in all the guinea pigs, it was accepted by us as the minimum immunizing dose and used in the subsequent experiments.

In the 1st series of tests 130 pigs were divided into 6 groups (22-24 pigs each) and immunized in accordance with the following arrangement: The animals of 3 groups received 100,000 brucellae each intracutaneously; the

pigs in the 1st group were injected with the entire dose in 1 point, the 2nd group in 2 points, and the 3rd group in 4 points; the animals in the 4th and 5th groups were immunized subcutaneously with 100,000 and 500 million microbial cells respectively; the animals of the 6th group (control) were not subjected to the influence of immunization. For the intracutaneous immunization in 1 point the vaccine was administered in a volume of 0.1 ml, in 2 points in a volume of 0.2 ml, and in 4 points - in a volume of 0.4 ml, and for the subcutaneous immunization - in a volume of 0.5 ml.

For the purpose of evaluating the immunological shifts taking place in the bodies of the animals we carried out serological and bacteriological investigations, and also allergy investigations by the generally accepted methods.

The results of studying the general condition and the dynamics of weight of the animals following immunization showed that the intracutaneous immunization of the animals with small doses did not influence these indices. As a rule no changes were detected at the site of administration of the vaccine (the blisters formed immediately after intracutaneous injection of the vaccine resorbed in the course of 10-15 minutes). The subcutaneous administration of vaccine in the same, and especially in a larger dose (500 million microbial cells), led to a certain slowing down of growth, and a lowering of weight and general flabbiness.

C In the course of the first 10 days after vaccination in all of the immunized animals the number of leukocytes in the blood increased on the average by 2000-3000 cells in  $1 \text{ mm}^3$  in comparison with the control group (Figure 1).

It is known that immunization with live brucellosis vaccine is accompanied by the spreading of the microbes throughout the organs and tissues, which causes an immunological reaction on the part of the lymphoid tissue. Bacteriological investigations, conducted by us in various periods after the administration of the vaccine (after 30 minutes, 2, 4, 24 hours, and 2, 5, 10, and 15 days), showed that in the group of animals, inoculated intracutaneously in 1 point, brucellae were seeded out after 15 days from the regional inguinal node, in those inoculated in 2 points - from the right and left nodes, liver and spleen, and in animals, vaccinated in 4 points - the brucellae were isolated already in 24 hours from the inguinal lymph nodes, liver and spleen, and later from other organs and tissues. The subcutaneous administration of the vaccine in the amounts of 100,000 and 500 million cells was accompanied by the rapid dissemination of brucellae throughout the organs and tissues. After 30 minutes - 2 hours, brucellae were detected in the inguinal lymph nodes, spleen, liver, and sometimes in the blood, lungs and marrow. After 15 days brucellae were seeded out in large quantities from the inguinal lymph nodes, spleen, liver and blood.

The results of studying the immunological reorganization of the animal organism, based on the Wright Reaction (Figure 2), showed that with the intracutaneous administration of the vaccine in 1 point, specific agglutinins were ascertained in the blood of the animals only on the 15th day, and their average titer equaled 1:13 (from 1:5 to 1:40), by the 30th day the amount of agglutinins increased - the average titer reached 1:37 (from 1:20 to 1:160); subsequently a gradual lowering took place in the titer of agglutinins and on the 45th day the average titer comprised 1:6 (from 1:5 to 1:20).

Following the intracutaneous administration of the same dose of vaccine in 2 points, an earlier immunological reaction was observed on the part of the macroorganism: Specific antibodies appeared on the 10th day after immunization; after 15 days the average titer of agglutinins reached 1:51 (from 1:10 - 1:160), by the 30th day the extent of the agglutinin titer was maintained at approximately the same level, with a certain tendency for lowering; by the 45th day the antibody titers were reduced to 1:24.

The administration of the same dose of brucellae in 4 points was accompanied by a still earlier and more expressed immunological reaction. Already on the 10th day after injection of the vaccine the average antibody titer equaled 1:10, and on the 15th day it comprised 1:64 (from 1:2 - 1:160); subsequently there was a lowering in the titer of agglutinins, and by the 45th day it equaled an average of 1:18.

The resulting data testified to the fact that with the same dose of antigen the effectiveness of intracutaneous vaccination is directly proportional to the number of points in which the antigen is applied; this was confirmed by statistical processing.

During the subcutaneous vaccination with doses of 100,000 and 500 million brucellae, expressed immunological shifts were noted already on the 10th day after administration of the antigen. The average titers of antibodies in both groups were approximately the same and comprised 1:68-1:76. After 15 days the titers of antibodies reached the maximum value, and in the group inoculated with the large dose they were somewhat higher. Thirty days after the vaccination a lowering of titers began. It became equal in the animals of both groups, comprising on an average 1:30 and 1:33.

The study of the allergic reorganization of the organism showed (see the table) that during the intracutaneous administration of brucellae the Burnet test was negative in the animals of both groups 15 days after vaccination. On the 30th day after immunization in 1 point the Burnet test was positive in 6 out of 8 guinea pigs, and after immunization in 2 and 4 points - in all the animals. Here the positive Burnet reaction was expressed more clearly in the pigs immunized in 4 points, and less clearly - in those immunized in 1 point. The positive allergic Burnet test was

preserved after 45 days. In the group of animals, inoculated subcutaneously with 100,000 brucellae, the Burnet allergic test was negative for the entire period of observation (45 days), but with the administration of 500 million brucellae, just as with the intracutaneous vaccination, a positive Burnet reaction was noted on the 30th day, but was less expressed. Thus, the most expressed allergic reorganization in the animals was observed following the intracutaneous administration of the vaccine in a quantity of 100,000 cells in 4 and 2 points and then in 1 point, and also during the subcutaneous administration in a dose of 500 million. The subcutaneous injection of 100,000 microbial cells did not cause the allergization of the guinea pig organism. This evidently testified that the intracutaneous method, connected with the implication in the immunization process of a large number of skin receptors, is more effective than subcutaneous.

For the purpose of checking the intensity of immunity, 45 days after vaccination the guinea pigs were infected with 1 Dlm of brucellae from the Br. abortus No. 19 vaccine strain. This dose comprised 2 million cells in 0.25% agar. The infection was performed intraperitoneally after the animals were treated with cortisone (0.2 mg per 1 g of weight) in order to lower their resistance. As a result of infection 3 out of 4 pigs in the control group died in a period of 10 days. The test animals, inoculated by the intracutaneous method in 2 and 4 points, and also subcutaneously, turned out to be resistant to the stated dose and remained alive. No disturbances in the general condition were noted.

#### Conclusions

1. Intracutaneous immunization of guinea pigs with small doses (100,000 microbial cells) of live brucellosis vaccine from the Br. abortus No. 19 strain caused a more expressed immunization process than the subcutaneous immunization with the same doses, which was confirmed by the accumulation of specific antibodies and the allergic reorganization of the animal organism.
2. The injection of a minimum immunizing dose under the skin did not stimulate the allergization of the guinea pigs. This was probably connected with the insufficient involvement of the skin covering in the immunization process.
3. The effectiveness of the intracutaneous vaccination of animals with live brucellosis vaccine depended not only on the dose and site of administration of the antigen, but also on the number of points of application of the antigen; administration of the minimum immunizing dose (100,000) of brucellae in 2 and 4 points caused a more active immunization process than the administration of the same dose of vaccine in 1 point.

#### Literature

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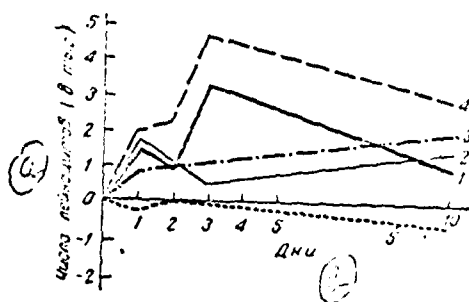


Figure 1. Changes in the number of leukocytes in the blood of animals, immunized intracutaneously in a dosage of 100 000 microbial cells in 1 point (1), in 2 points (2), in 4 points (3), subcutaneously in a dosage of 500 million microbial cells (4); control (5). Initial is accepted as zero.

a - number of leukocytes (in thousands); b - days.

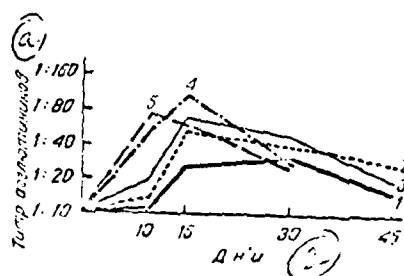


Figure 2. Immunological reorganization, based on the Wright reactions, of animals, immunized intracutaneously in a dosage of 100 000 microbial cells in 1 point (1), in 2 points (2), in 4 points (3), subcutaneously in a dosage of 100 000 microbial cells (4), and in a dosage of 500 million microbial cells (5).

a - titer of agglutinins; b - days.

Results of the allergic test in immunized animals

| Method of Immunization | Total dose (in microbial cells) | Number of animals in group | Burnet reaction |               |               |
|------------------------|---------------------------------|----------------------------|-----------------|---------------|---------------|
|                        |                                 |                            | after 15 days   | after 30 days | after 45 days |
| Intracutaneously       | in 1 point 100 000              | 8                          | 0/8             | 6/2           | 6/2           |
|                        | in 2 points 100 000             | 6                          | 0/6             | 6/0           | 6/0           |
|                        | in 4 points 100 000             | 6                          | 0/6             | 6/0           | 6/0           |
| Subcutaneously         | 100 000                         | 6                          | 0/6             | 0/6           | 0/6           |
|                        | 500 mil.                        | 6                          | 0/6             | 0/6           | -             |
| Control                |                                 | 6                          | 0/6             | 0/6           | 0/6           |

Note: Numerator - positive reaction, denominator - negative.