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On the question of further improvement of the technology of producing dry live vaccine (brucellosis), by P. A. Vershilova, N. S. Samchava and E. P. Fanderlit (Institute of Epi-Microbiology, Gamalei, USSR)

Live dry brucellosis vaccine, prepared from blood strain BA in the IEI, USSR, proved to be of high epidemiological effectiveness during its use in a center of sheep brucellosis. However other regions have various indexes of its effectiveness. But the decrease of infections with brucellosis of immunized subjects is 60, 25, 12 and 6 times. This data can mean many things, but we believe it is the quality of vaccine.

It is known that for a durable and long lasting immunity, there is needed a specific number of live brucella, which will cause a marked vaccine process. Therefore the vaccine used in practice must contain the required number of live brucella in one injection dose. As test of mass-produced vaccine showed, there is not always obtained a standard quantity of live brucella. Many times a series of vaccine prepared in the same conditions does not compare, according to live brucella content. Much of the fault in losing these live brucella comes in the drying of the vaccine in chamber apparatuses.

Hoping to obtain a live dry vaccine of high quality, we studied the first stages of the preparation of this vaccine, which evidently hold great importance in regard to the quality, and also the preparation of other vaccines. Also, it was necessary to determine the retention of the quantity of live brucella and humidity during storage at various temperatures and in various conditions.

The first phase of our study was a test of the ability of the vaccine to maintain itself in stabilized conditions (gelatin 3%, saccharose 10%) in storage -- room temperatures, in refrigerators +6-8 and also in freezing -20, 40 and 70
and drying with the latter.

The determining of the live brucella was done by sowing 100 microbe cells (cultivation of dry vaccine by ordinary intestinal standards) on a solid nourishing medium in a Peter type container and tabulating the grown colonies. 100 microbe cells of brucella usually reproduce to 300 colonies.

As Table 1 (not translated) shows, storage at room temperature for 3 hours did not cause any apparent effect on the vitality and stability of the brucellas. A 70% death rate appeared after 6 hours. So it is recommended that the preparation and pouring into ampules of the serum be less than 3 hours after its removal from the refrigerator.

Storage of the microbe suspension in a refrigerator for 5 days caused a commencement of death of the cells. Recommended that if conditions necessitate, the suspension can be stored in a refrigerator to 3 days (72 hours).

Freezing temperatures (-20, 40 or 70) allowed us to store the brucella for 24-48 hours without loss.

Tests indicated that thawing and secondary freezing of frozen brucella caused a decrease in the number of live brucella.

Tests with two types of drying apparatus showed that the chamber apparatus, in contrast to the collective apparatus, caused a 35 to 50% greater death rate. However, the chamber apparatus is more economical. So we tried to establish means of insuring a greater rate of survival in the chamber apparatus type of drying. We used various compositions of serum (dense, lean) but results were still a 39 to 53% death, which left live brucella not sufficient to meet standards.

Next we studied the effect of regimen on the survival rate of drying
brucella. We tried 3 regimens, varying in the heat applied to the vaccine. The first test was with heat applied, equal to room temperature. The second test was the same as the first or the first 10 hours, then we applied an electric element which raised the temperature to 25-27 by the end of the drying process. The third test was the same as the second except that the electric element was applied 3 hours after the beginning of the drying process. The temperature rose to 28-29. The length of the drying process for the first test was 42-49 hours, second 28-30 and third 24-26 hours.

The first and second of these tests proved most suitable according to the percentage of survival.

As is known, live dry vaccines must be kept in a vacuum if they are to be stored for a long time. We at the institute use high frequency currents. We tested this current to study its effect on the survival of the live brucella. Five series of serums, with a 1.7 to 1.9% humidity, were subjected to high frequency currents for 15-30 and 60 minutes. Then they were sowed again on a solid nourishing medium and the outgrown colonies were tabulated.

Results showed that the subjection to 60 minutes of high frequency current of the serum caused a quantitative death, so we recommend the 15-30 minute subjection period.

We studied the effect of the density of the original vaccine suspension on the survival rate after a year's storage. We used bacterial suspensions containing 5, 7, 8, 9 and 10 milliard microbic bodies in 1 milliliter. They were stored in a refrigerator for 1 year to 6-8°C. They were then sowed on a solid medium. The suspension containing 10 milliard cells proved to be best, losing only about 0.1% of vitality.
Tests with 10 and 15 milliard microbe cells, conducted as above, gave the same results as above, the vaccine was retained in its original form.

Four series of vaccines were tested in various temperatures, for 4 months. Their humidity content at the beginning of the test was 1.3-1.5%. The tests were at 4-6° in a refrigerator, 37° in a thermostat, and at room temperature. Sowing and tabulation of the cells was done as above. Results indicated that the death rate was 3 times greater in room conditions and 10-13 times greater in thermostat conditions, in comparison with refrigerated conditions.

Next we studied the effect of humidity content on the rate of survival of the cells in live dry vaccine. We tested 6 series of vaccines, containing 0.7-1%, 1.2-1.5% and 2.2-3% humidity, storing them for 9 months in a refrigerator.

Results showed that the most favorable results were obtained with a humidity content of 1.2, 2.2, and 3%. This is the required percentage used in preparation at the present. The great rate of death of the stems containing 0.7-1% humidity can possibly be explained by the longer drying process connected with it.

We also tested serum storage at low temperatures (-40) and discovered no loss in vitality.

As a result of our tests we recommend the following conditions:

1. The original live vaccine should contain 12-15 milliard microbe bodies in 1 milliliter.

2. The vaccine can be stored in a refrigerator (4-8°) for 1, 2 or 3 days before pouring out and should be poured before 3 hours.

3. After pouring of the vaccine, it can be stored at -20 to -70 for 24-48 hours.

4. During drying of the live brucellosis vaccine in chamber apparatus,
additional heat should be applied after 10 hours, to attain a maximum of 25-27°.

5. The humidity content should be 2-3%.

6. The high frequency current of the vacuum in the ampules should be for less than 30 minutes.

NOTE: Tables not translated.