Experimental substantiation of the use of a small dose of the vaccine to prevent the specific brucellosis of cattle

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Abstract. The expansion of zones with the cattle without anti-brucellosis immunity in healthy areas increases the risk of cattle infection from neighboring infected regions and states. There is an urgent problem of developing the anti-brucellosis immunity in the adult cattle. The initial full-dose vaccination with the vaccine from strain B. abortus 82 is fraught with post-vaccination abortion. The study is aimed at the exploration of the immune response and immunity in cows vaccinated against brucellosis according to different schemes with small doses of vaccines from B. abortus strains 19 and 82. The results of the experiment showed that the small-dose revaccination with a vaccine from strain 82 is the most promising, because without any harm to the formed immunity, the problem associated with the reactogenicity and agglutinogenicity of the vaccine can be solved. In addition, a ten-time reduction in the vaccine dose can reduce vaccine production volumes and decrease financial expenses of the farmers.

1 Introduction

The scientifically-based system of special preventive measures against cattle brucellosis was developed at the end of the 20th century by several research institutes: the Federal Center for Toxicological, Radiation and Biological Safety in Kazan; the Institute of Experimental Veterinary Medicine of Siberia and the Far East; the All-Russian Research Institute of Brucellosis and Animal Tuberculosis, which made it possible to radically improve the epizootic brucellosis-related situation in Russia and classify this disease as a controlled infection.

First of all, it was possible due to the strict schedule of specific preventive measures with the use of the anti-brucellosis vaccine made from B. abortus 82 strain. As a result, the number of infected areas was sharply reduced, and most regions and republics were found to be brucellosis-free. However, the epizootologically justified mass immunization of cattle with a live anti-brucellosis vaccine from strain 82 in infected territories during the period of widespread brucellosis cases became no longer adequate for healthy regions from both an environmental and economic points of view. In this regard, in some healthy areas, the specific preventive measures against brucellosis were completely stopped or conducted only in relation to young animals.

At the same time, appreciating the improvement of the epizootic situation in relation to cattle brucellosis in the whole country, we cannot but pay attention to the fact that the expansion of areas with animals that have not been vaccinated against brucellosis increases the risk of infection because of neighboring infected territories whose farming business is closely related. This circumstance justifies the need for further specific preventive measures in regions with short healthy periods or in regions bearing a risk of infection from neighboring regions or countries [1–3].

The reduction in immunization, both for environmental reasons and due to a sharp decrease in the number of livestock, has made some changes not only in the system of special anti-brucellosis measures, but also in the output plans of biological enterprises specializing in the production of vaccines, in particular, the vaccine from strain 82. The rise in the price of the vaccines has become another reason for farmers to cut down the vaccine purchasing volume or even to refuse to buy it.

Based on the above mentioned circumstances, we define the purpose of our study as follows: to test the scheme of special preventive measures against cattle brucellosis with the use of the vaccine made from strain 82 in small doses, which allows minimizing negative side effects after vaccination, without any immunity decrease. Also, there are some financial advantages of the suggested scheme for the farmers due to the use of the vaccine in small doses.

One of the reasons for reducing the dose of the vaccine is the proliferation ability of live vaccines. To some extent, this ability makes it possible to exclude a strict correlation between the dose of the injected vaccine determined by the number of microbial cells (m.c.) and the formed immunity level. The doses of vaccines from strains 19 and 82 used in the veterinary practice,
regardless of the puberty of the immunized cattle, are 80
and 100 billion m.c., respectively. In the available
literature and in regulatory documents, there is no clear
justification for such doses.

In the 1980s, much research was done aimed to test a
small dose of the vaccine from strain 19 [4-11]. First of
all, there was a need to solve the problem of prolonged
seropositivity in animals vaccinated with this vaccine,
which complicated the post-vaccination diagnosis of
brucellosis in cattle. Currently, in the specific anti-
brucellosis vaccination schemes, only young cattle is
allowed to be vaccinated with the vaccine from strain 19
due to the fact that the synthesis of post-vaccination
antibodies is extinguished in the 5-month-old young cattle
[12, 13]. At the same time, vaccination with a small dose
of the vaccine (3 billion m.c.) allowed the reduction of the
age up to 3 months old. Positive results were obtained
from the immunization of animals with a small dose of
vaccine from strain 19 (4 billion m.c.) using the
conjunctival method.

3 Results

Preliminary study of heifers showed that a full dose of the
vaccine from strain 82 stops antibody synthesis by 3, and a
small dose by 2 months.

The dynamics of post-vaccination reactions in cows of a
successful herd is shown in table 2.

From the above data, a significant difference in the
degree of manifestation of the compared immunological
reactions is visible. In almost all groups of animals and in
all periods of the study, the Agglutination Test (AT)
indicators were prevailing. Out of 1305 blood serum
samples studied, AT indicators were found in 409 cases
(31.3%), Complement Fixation Test (CFT) indicators
were found in 203 cases (15.5%) and almost the same
number of Rose-Bengal Test (RBT) indicators were found
in 229 cases (17.5%). It should be noted that the AT
indicators completely included the RBT indicators, and
there was no particular correlation between the AT titer
and positive RBT.

The immune response in each group of animals
differed. The cattle that were immunized only with the
vaccine from strain 82 had the fastest immune response
(Schemes 1 and 2). Antibodies were recorded in all
animals only on the 14th day. The level of agglutinin
production was much higher in cows vaccinated
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animals only on the 14th day. The level of agglutinin
production was much higher in cows vaccinated
according to Scheme 1.

Other results were obtained in groups where heifers
were primarily vaccinated with a vaccine from strain 19,
and then revaccinated before mating and after calving
with a vaccine from strain 82, and the last vaccination
was also carried out with different doses (Schemes 3 and 4).

The primary sensitization after the injected vaccine
from strain 19 was expressed by a more active immune
response, if compared with the two previous schemes. In
some animals, antibodies were recorded before the 33rd
day and with weak indicators even before the 60th day.
As expected, the maximum immune response was with
the full dose of the vaccine. It should be noted that if we
take into account AT in the titer of 50 IU, then in the both
groups, 100% cows had the immune response. Thus, the
re-immunization of cows both with the full and small
doses of the vaccine from strain 82 provided a short-term
immune response detected by serological methods.

### Table 1. Cattle vaccination schemes with different doses of vaccines

| Scheme No | Age, strain, dose | 3-5 months old | 16-18 months old | After calving |
|-----------|------------------|----------------|-----------------|--------------|
| 1         | 82 f.d.          | 82 f.d.        | 82 f.d.         |
| 2         | 82 f.d.          | 82 f.d.        | 82 sm.d.        |
| 3         | 19 f.d.          | 82 f.d.        | 82 f.d.         |
| 4         | 19 f.d.          | 82 f.d.        | 82 sm.d.        |
| 5         | 19 f.d.          | 19 sm.d.       | 19 sm.d.        |
| 6         | 19 f.d.          | 19 sm.d.       | 19 sm.d.        |
| 7 control |                  |                |                 |

Note: f.d.- full dose of vaccine, sm.d. - small dose of vaccine
The table shows that for the early diagnosis of brucellosis in cows of the control groups, the most preferable is AT: the infection caused a primary immune response in cows. On the 7th day, 40% of animals vaccinated with a dose of 100 million m.c. had diagnostic titers with negative indicators of CFT and RBT. Also, more animals (30%) vaccinated with a larger dose had the immune response during this period versus 10% and 20% of animals.

In total, over the entire indicated period of the study in all groups, 79.2% of animals had the immune response detectable by AT, 66.1% of animals had the immune response detectable by CFT and 71.7% of animals had the immune response detectable by RBT. For comparison, it should be noted that in four groups of cows that were revaccinated with the vaccine from strain 82, the diagnostic indicators of AT, CFT and RBT were, respectively, in 83.2%; 89.1%; 78.2% of animals.
Table 3. The sensitivity of serological methods in cows immunized with various anti-brucellosis vaccines after vaccination.

| Vaccination scheme | Dose of infection, mln m.c. | Testing period, in days | AT, IU Had reactions, % | CFT positive RBT |
|-------------------|-----------------------------|-------------------------|-------------------------|-----------------|
|                   | 100                         |                         | 100 and more 50         |                 |
| Control           | 100                         | 7                       | 20 20 -                | 60 60          |
|                   | 15                          | 80 20                   | 100                    | 100            |
|                   | 21                          | 80 20                   | 100                    | 100            |
|                   | 29                          | 100 -                   | 100                    | 100            |
|                   | 41                          | 60 40                   | 100                    | 100            |
| Control           | 200                         | 7                       | 20 10 -                | 60 60          |
|                   | 14                          | 100 -                   | 100                    | 100            |
|                   | 28                          | 86.7 13.3              | 100                    | 100            |
|                   | 39                          | - 100                  | 100                    | 100            |
| No1               | 200                         | 7                       | 50 25                  | 75 75          |
|                   | 14                          | 75 25                  | 100                    | 100            |
|                   | 28                          | 75 25                  | 100                    | 100            |
|                   | 39                          | 50 25                  | 75                    | 100            |
| No 2              | 200                         | 7                       | 80 -                   | 60 60          |
|                   | 14                          | 80 20                  | 100                    | 100            |
|                   | 28                          | 100 -                  | 100                    | 100            |
|                   | 39                          | - 20                   | 100                    | 100            |
| No 3              | 100                         | 7                       | 20 40                  | 80 80          |
|                   | 15                          | 20 40                  | 80                    | 100            |
|                   | 21                          | 60 40                  | 100                    | 60            |
|                   | 29                          | 20 80                  | 100                    | 80            |
|                   | 41                          | 40 40                  | 80                    | 60            |
| No 3              | 200                         | 7                       | 40 20                  | 60 60          |
|                   | 14                          | 80 20                  | 60                    | 100            |
|                   | 28                          | 60 20                  | 100                    | 100            |
|                   | 39                          | 20 40                  | 100                    | 60            |
| No 4              | 200                         | 7                       | 20 -                   | 60 60          |
|                   | 14                          | 80 -                   | 80                    | 40            |
|                   | 28                          | 60 20                  | 100                    | 100            |
|                   | 39                          | 20 20                  | 100                    | 60            |
| No 5              | 200                         | 7                       | 40 40                  | 100 100       |
|                   | 14                          | 80 20                  | 100                    | 100            |
|                   | 28                          | 80 20                  | 100                    | 100            |
|                   | 39                          | - 20                   | 60                    | 100            |
| No 6              | 200                         | 7                       | 80 20                  | 100 100       |
|                   | 14                          | 100 -                  | 100                    | 100            |
|                   | 28                          | 80 20                  | 100                    | 100            |
|                   | 39                          | 20 20                  | 100                    | 100            |

Table 4. Schemes for immunizing cows before infection

| Scheme / Number of cows | Vaccination scheme (Age, strain, vaccination) |
|-------------------------|-----------------------------------------------|
| 3-6 months old          | 18-20 months old | After 1st calving |
| 1st / 5                 | 19              | 19                  | 19* |
| 2nd / 5                 | 19              | 19*                 | 19* |
| 3rd / 5                 | 19              | 19*                 | 19* |
| 4th / 5                 | 19              | 82                  | 82 |
| 5th / 4                 | 82              | 82                  | 82 |
| 6th / 5                 | 82              | 82                  | 82 |
| 7th / 3                 | control         |                     |     |

Note: * - a small dose of the vaccine from strain 82 - 10 bln m.c., vaccine from strain 19 - 3 bln m.c.
The most important stage was to check the immunity level in cows after the vaccination, pre-immunized according to the following schemes (Table 4).

For the experiment, all animals were selected in brucellosis-free farms and they were vaccinated with anti-brucellosis vaccines according to the indicated schemes.

Before the first vaccination, the animals had a negative reaction. After reimmunization of heifers before insemination, a negative result was also obtained. The examination of the heifers before the third vaccination (after 10 months from the previous vaccination) showed weak reactions only in animals vaccinated according to Schemes 1 and 2; the vaccine from strain 19 was used in the both schemes. Only two cows vaccinated according to Scheme 1 had the immune response detected by AT in the titer of 50 IU and one cow vaccinated according to Schemes 1 and 2: the vaccine from strain 19 was used in the both schemes. The vaccine from strain 82 is the following: it reduces its reactogenic and agglutinogenic properties, and in addition, it allows cutting down vaccine production volumes and financial expenses of the farmers.

The data presented in Table 5 show that there is no significant difference in the immune resistance in animals vaccinated according to different schemes. Obviously, triple immunization, regardless of the vaccine and dose, provided approximately equal immune protection on average in 60% of animals. It is generally accepted that 50-70% of the vaccinated animals in the herd are considered a high indicator.

| Vaccination scheme (vaccine strain) | Number of cattle | Infected | Number of immun. cattle (%) | Index of infection |
|------------------------------------|------------------|----------|-----------------------------|-------------------|
| 19 f.d.+19 f.d.+19 sm.d.           | 5                | 2 0 2   | 60                          | 10.4              |
| 19 f.d.+19 sm.d. +19 sm.d.        | 5                | 1 0 1   | 80                          | 3.2               |
| 19 f.d.+82 f.d.+82 f.d.           | 5                | 1 1 2   | 60                          | 2.4               |
| 19 f.d.+82 f.d.+82 sm.d.          | 5                | 1 1 2   | 60                          | 2.4               |
| 82 f.d.+82 f.d.+82 f.d.           | 4                | 1 1 2   | 50                          | 3.0               |
| 82 f.d.+82 f.d.+82 sm.d.          | 5                | 1 1 2   | 60                          | 3.2               |
| Control                           | 3                | 3 0 3   | 0                           | 24.0              |

Note: S – Systemic form of infection, R - Regional form of infection

Table 5. The results of immunity testing in cows vaccinated against brucellosis according to different schemes

4 Conclusion

The experimental materials of the study prove the advisability of using small doses of vaccines from strain 82 in the cattle vaccination schemes against brucellosis. The study proves that revaccination of cows with a small dose of vaccine from strain 82, regardless of the previous vaccination schemes, ensures approximately the same immunity as the full-dose vaccination schemes. The advantage of the scheme with a ten-time reduced dose of the vaccine from strain 82 is the following: it reduces its reactogenic and agglutinogenic properties, and in addition, it allows cutting down vaccine production volumes and financial expenses of the farmers.

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