Results of studying of the Brucella suis 245 strain vaccine's reactogenic properties in an experiment on reindeer

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Abstract. Living vaccines are not widely used in practice to prevent and control brucellosis in domesticated reindeer. Brucellosis vaccines from strains 19 or 82 are characterized by a high level of reactogenicity and lead to complications in vaccinated animals. When studying the reactogenic properties of the vaccine from the B. suis 245 strain in the experiment on reindeer, the reaction of the body of domesticated reindeer to the subcutaneous injection of brucella from the B. suis 245 strain was considered. At the same time, no significant differences were observed when the vaccine was administered at a dosage of 10 and 50 billion microbial cells, and the size of edema in millimeters was fixed at the level of 39.0 ± 3.5 and 44.1 ± 2.6, respectively (P> 0.05). The dynamics of the body temperature of animals depending on the method of administration and dose of the vaccine, regardless of the dosage, body temperature, like other indicators of a physiological nature, remained within the normal range. It was found that in the early stages after vaccination, the indicators of the physiological state of the animal's body are determined by whether the oral or subcutaneous method of administration of the vaccine was used. When vaccinated by the subcutaneous method, reactogenicity was less pronounced with the introduction of 5 billion microbial cells, compared with a dose of 50 billion microbial cells.

1 Introduction

Reindeer husbandry is a socially significant branch of animal husbandry in the northern regions of Yakutia. A limiting factor in the development of reindeer husbandry is brucellosis.

In Yakutia, brucellosis of domesticated reindeer is widespread. This nosological unit is a significant limiting factor for the further development of reindeer husbandry, in addition, it poses a serious social danger. Currently, brucellosis of domesticated reindeer in the Russian Federation is recorded in the Republic of Sakha (Yakutia), Yamalo-Nenets and Chukotka Autonomous Districts, Khabarovsk, Krasnoyarsk, Tyumen, Magadan,

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Kamchatka and Amur regions (Gordienko L.N. et al. 2017; Layshev K.A. et al. 2018; Sleptsov E.S. et al. 2013).

In Yakutia, brucellosis of domestic rerindeer was first recorded in the Oymyakonsky district in 1955, and then in 1958 - in Tomponsky and in 1961 - in Allaikhovsky districts [8]. In subsequent years, this disease was also established in other reindeer herding farms of the republic.

In five districts of Yakutia, as of 01.01.2020, 35 points with an unfavorable situation for brucellosis of domestic reindeer were identified during the spring, spring-summer and autumn coralizations on the territory of the republic. In 2020, 117,877 animals were examined for brucellosis in the republic (more than 75% of the total livestock as a whole), a positive reaction was detected in 0.1 percent of the studied individuals (109 animals).

According to Vasilyeva A.A., the most widespread brucellosis of northern domestic reindeer was in the farms of Ust-Yansky, Tomponsky, Oleneksky, Eveno-Bytantaysky, Bulunsky, Momsky and Nizhne Kolyma districts. The disease had an uneven spread. Thus, according to serological and clinical studies of reindeer from farms in 12 districts of the republic, a high incidence of brucellosis of deer was established in Bulunsky, Verkhoyansky, Allaikhovsky, Tomponsky and Oymyakonsky districts.

The experience of fighting reindeer brucellosis, which has been practiced for many years by carrying out general economic and special veterinary and sanitary measures, has shown their insufficient effectiveness. The fight against this disease is also complicated by the presence of natural foci of brucellosis among populations of wild reindeer (Pavlovsky E.N., 1948; Poltoratskaya L.F., 1988, etc.).

Many researchers in Taimyr, Yamal, Yakutia and the Magadan region (Zabrodin V.A. et al., 2015; Laishev K.A., 2018; Khoch A.A., 2001; Sleptsov E.S., 2018, etc.) studied the epizootic situation, diagnostic methods, means and methods for specific prevention of brucellosis in reindeer and, in particular, work was carried out to test vaccines Brucella abortus strains 19 and 82, as well as 75 / 79 AB.

In Yakutia, for a long period of time, which lasts from 1955 to 2019, despite the annual planned anti-epizootic measures, such as diagnostic tests, isolation and slaughter of reindeer of patients or positively reacting, they could not achieve all the benefits of improving the herds from brucellosis of reindeer in any reindeer husbandry.

A number of experts pointed to the insufficient level of effectiveness of the measures taken to prevent and combat the disease in the territories of reindeer herding farms in Tyumen and Taimyr [1,2,7,10]. At the same time, in the available literature sources, we have not found clear statements on the high effectiveness of the measures taken to prevent the widespread spread of the disease in the fight against brucellosis of domestic reindeer.

Thus, due to the lack of effective measures to combat brucellosis of domestic deer and to a certain extent the complexity of the epizootic situation in the regions of the Far North of Russia, these tasks are among the priorities in domestic veterinary medicine, which are associated with the search for effective vaccines, i.e. specific means of protection that can provide a guaranteed cure and prevention of the disease.

Back in the 60s of the twentieth century, a number of steps were taken in the USSR and foreign countries to find means of specific protection against brucellosis of domestic reindeer.

In the 70s and 80s of the last century, when it became quite obvious that it was impossible to eradicate reindeer brucellosis in the Far North only by general measures, the issue was intensively addressed [1,2,6]. Thus, agglutinogenic and weakly agglutinogenic live vaccines from attenuated strains of B. abortus 19, 82, B. melitensis Rev-1 were tested for immunization of domestic reindeer. In addition, experiments were conducted with vaccines from homologous strains of Brucella B-209 and OT-47 [5,9,10,11,12].

Russian scientists have tested a vaccine from the B. abortus 19 strain on brucellosis-healthy reindeer in doses of 65 and 30 billion. microbial cells during subcutaneous administration. As a result of studies, it was found that the vaccine is harmless to this animal species and causes immunobiological changes in the body, accompanied by the production of agglutinins in high titers (1800-3200 IU/ml) and complement-binding antibodies [3, 4, 6]. During the entire observation period (5 months) after immunization, the number of animals showing resistance to infection with the virulent Brucella 010 culture was 80% of reindeer. Based on these data, the authors believe that immunization of reindeer with the B. abortus 19 vaccine will have a positive value.

A number of other authors noted that the majority of reindeer vaccinated with the B. abortus 19 vaccine at doses of 60, 15 and 6 billion mc, after vaccination, depression and an increase in temperature by 40-41 ° C and inflammatory edema at the injection site of the vaccine were observed, and some animals had abscesses [7,8]. Some veterinary specialists noted lameness in animals, which disappeared in most after 15-20 days. In some reindeer, bursitis of the premaxillary mucous bags appeared after 2-5 months.

It is generally accepted that Brucella cultures, the main carrier of which is deer, in terms of their cultural, morphological and biochemical properties, belongs to type 4 of the suis biovar.

Despite many years of experience in the fight against brucellosis of reindeer, it is impossible to achieve high efficiency and obtain stable results of recovery with the use of "heterogeneous" vaccines from B. abortus 19 and 82 strains.

To combat brucellosis of domestic reindeer, to reduce the recovery time of reindeer farms and to protect healthy reindeer from infection, there is an urgent need for the use of means, specific prevention of brucellosis of this type of animal. This is evidenced by the data obtained by a number of domestic and foreign researchers, as well as the results of our observations. As mentioned above, previously tested vaccines from the B. abortus strain 19, 82 and B. melitensis Rev-1 have certain disadvantages. In common practice, heterogeneous vaccine strains are used to prevent brucellosis of domestic reindeer and to combat this disease.

Considering that until now there are no sufficiently effective means of specific protection of domestic reindeer from brucellosis, the role of searching for vaccine strains from cultures homologous to this type of brucella is significantly increasing.

In our opinion, vaccines from homologous strains, in particular Brucella suis 245, from the collection of museum strains of brucella of the Federal State Budgetary Scientific Institution "Federal Scientific Centre VIEV" (FSC VIEV), are promising for study and are of some interest and its antiepizootic effectiveness in the system of measures for the prevention and control of brucellosis of reindeer [2,7].

Therefore, the development of means of specific prophylaxis against brucellosis in domestic reindeer is a topical issue.

The goal of this work was to study the reactogenic effects of the B. suis 245 vaccine on domesticated reindeer.

At the same time, the task was set to study the reactogenic properties of the vaccine from the B. suis 245 strain on domestic reindeer of the Republic of Sakha (Yakutia).

2 Methods

The work was carried out in the period from 2014 to 2019 in the laboratory of chronic infections of FSC VIEV, on the experimental base of the Vyshne-Volotsk department of FSC VIEV, in the laboratory of reindeer husbandry and traditional industries of the North, in the Agricultural production cooperative - the nomadic tribal community "TURVAURGIN" (SPK KRO "TURVAURGIN Rodina") and Koche small peoples of the

north - Chukchi "TEVR" (KRO MNS (Ch) "TEVR") Nizhnekolymsky ulus of the Republic of Sakha (Yakutia).

To study the reactogenic properties, a 2 day old agar culture of the B. suis 245 strain of the second generation was taken. Cultures of the reference strains of the species B. abortus 54 and B. suis 1330 grown under similar conditions were used as controls, as well as vaccine strains used for the prophylaxis of brucellosis in cattle and small ruminants - B. abortus 19, 82 and B melitensis Rev-1.

In an experiment on 72 reindeer, the reactogenicity, the timing of settlement and survival of brucella culture from the strain B. suis 245, as well as the immunological response of the animal organism (according to serological and allergic studies) in dynamics at various times after immunization were studied.

For 6 days, the general and local reactions of the body to the vaccine were determined by subcutaneous administration. The studies were carried out by monitoring the condition of experimental animals, measuring body temperature, as well as measuring (for two weeks) inflammatory edema at the site of inoculation of the vaccine.

3 Results

From the data presented in the specialized literature, it can be concluded that to this day live vaccines are not used on a large scale in practice to prevent and control brucellosis in domesticated reindeer. This is mainly because for this animal species the previously proposed vaccines (from strains 19 or 82) are characterized by a high level of reactogenicity and often lead to complications in vaccinated animals, manifested in the form of abscesses and tumors at the injection site of the vaccine. And, due to damage to the joints of the extremities (bursitis, tendovaginitis, etc.), to the appearance of lameness in animals.

We took into account the reaction of the body of domesticated reindeer (both local and general) to the subcutaneous injection of brucella from the B. suis 245 strains. For this purpose, within 14 days after the vaccination, the body temperature was measured, the magnitude of the inflammatory edema (oval shape) at the injection site was measured, and its consistency was also taken into account. In addition, we monitored the general condition of the vaccinated animals, the presence of depression, respiratory rate, appetite, etc.

The diagram in Figure 1 shows data on the local reaction of the animal organism. Taking into account the oval shape of inflammatory edema, the average sizes were used according to the formula: A+B/2; where A is the length, B is the width.

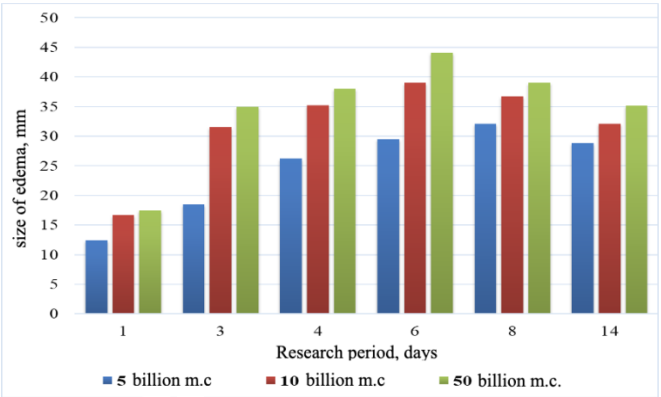

Fig. 1. Local reaction of the body of domesticated reindeer after injection of different doses of Brucella from strain B. suis 245.

A day after the injection of the vaccine by the subcutaneous method at the injection site, considering the reaction of animals to vaccination, the appearance of pronounced swelling, hot, painful, and rather dense consistency (with an organoleptic examination) was noted, which then took the form of diffuse edema, reaching its maximum value on the sixth day after vaccination. At the same time, no significant differences were observed when the vaccine was administered at a dosage of 10 and 50 billion microbial cells, and the size of the edema in millimeters was recorded at the level of 39.0 ± 3.5 and 44.1 ± 2.6, respectively (P> 0.05). Two vaccinated olene patients which received a dose of 50 billion microbial cells developed purulent abscesses at the injection site, which persisted for more than 14 days.

In response to the administered dose of 5 billion microbial cells, edema was less pronounced at the injection site in animals of group 1, compared with similar indicators of animals in groups 2 and 3 (on the sixth day - 29.5 ± 1.5, P <0.05). Over the next day, the accounting revealed a reduction in the magnitude of the edema; on the 14th day, it was 28.8 ± 2.3 at a dosage of 5 billion microbial cells; at a dosage of 10 billion microbial cells - 32.1 ± 1.8; at a dosage of 50 billion microbial cells - 35.1 ± 1.7 mm.

Only when using the subcutaneous method of vaccination was the general reaction of the animals' bodies noted, which was expressed in the suppression of the state and a decrease in appetite. For 10-12 days, 3 individuals vaccinated subcutaneously at a dosage of 50 billion microbial cells showed a limp on the forelimbs. During the entire control period (fifteen days), abortions were not observed in pregnant females.

At the same time, in the case of using the subcutaneous route of administration, the body temperature in animals rose to 40.5-40.8°C after a day. The greatest rise in temperature was recorded on the second day (from 40.7 to 41.9°C); further on, the temperature began to decrease, reaching the standard values on the sixth day.

Table 1. Temperature reaction in animals immunized by different methods and doses of vaccine from B. suis 245 strains.

Period before and after vaccination, days	**Body temperature - °C (M±м)**					
	Vaccination method and dose billion m.c.					
	Subcutaneously			**Orally**		
	5 bn. m.c.	**10 bn. m.c.**	**50 bn. m.c.**	**5 bn. m.c.**	**10 bn. m.c.**	**50 bn. m.c.**
before	37,8 + 0,2	37,6 ±0,3	37,3 ±0,2	37,8 ±0,2	37,7 ± 0,4	37,8 ± 0,4
1	40,5 ±0,1	40,8 ±0,2	40,8 ± 0,3	37,0 ±0,2	37,4 ±0,5	37,2 ± 0,1
2	40,7 ± 0,3	41,6 ±0,9	41,9 ±0,6	38,0 ±0,1	38,1 ±0,3	37,6 ± 0,2
4	40,8 ±0,1	40,7 ± 0,3	41,2 ± 0,1	38,5 ±0,1	37,6 ± 0,2	37,4 ±0,1
5	38,7 ± 0,1	39,2 ±0,1	39,3 ±0,3	38,2 ±0,2	37,7 ± 0,2	37,8 ± 0,8
6	38,4 ±0,5	38,3 ±0,2	38,2 ±0,2	38,5 ±0,7	37,8 ± 0,6	37,8 ± 0,1

The dynamics of the body temperature of animals depending on the method of administration and dose of the vaccine is presented in Table 1. The data given in the table indicate that in the case of the oral administration of the vaccine in animals, regardless of the dosage, the body temperature, as well as other indicators of a physiological nature (general condition, appetite, etc.), remained within the normal range (approximately 38.0 ± 0.5°C).

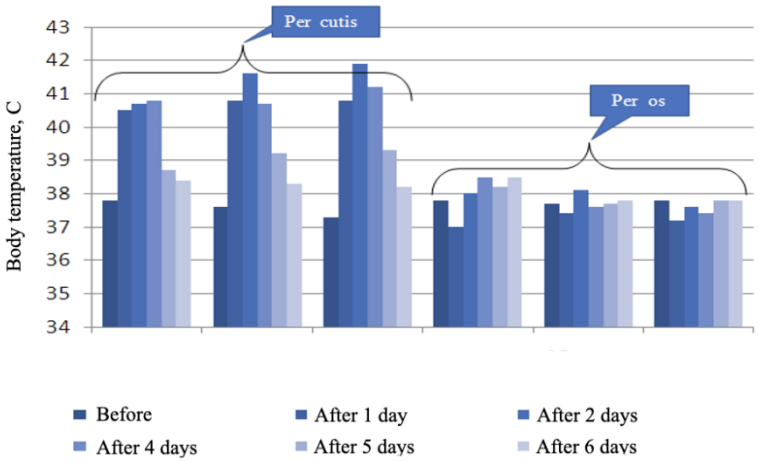

Fig. 2. Dynamics of the body temperature of domesticated reindeer, depending on the dose and method of use of the vaccine preparation from the B. suis 245 strains.

The diagram (Fig. 2) clearly shows the dependence of the temperature reaction of deer on the method of administration and the dose of the vaccine preparation.

As can be seen from the diagram, the difference in indicators for subcutaneous and oral immunization methods is significant ($P<0.05$), while there was no statistically significant difference in indicators depending on the dose of the vaccine ($P> 0.05$).

4 Discussion

It was found that in the early stages after vaccination, the indicators of the physiological state of the animal's body are determined by whether the oral or subcutaneous method of administration of the vaccine was used. When vaccinated by the subcutaneous method, reactogenicity was less pronounced with the introduction of 5 billion mc, compared with a dose of 50 billion mc.

The results of our studies on the reactogenic properties of the vaccine from the strain B. suis 245 have shown that with the subcutaneous method of its administration, it is also not devoid of the above disadvantages. At the same time, applying this vaccine to the root of the tongue allows you to eliminate all side effects (there is no depression of the general condition, body temperature remains within normal limits, animals do not limp, tendovaginitis, arthritis, etc. do not develop).

Oral administration of the vaccine, in our opinion, is the most technologically advanced, does not present any difficulty in comparison with the subcutaneous and the conjunctival method and, moreover, it is more preferable due to the absence of post-vaccination complications observed with other methods of immunization.

5 Conclusions

With the oral method of immunization, in contrast to the subcutaneous method, the culture of brucella from the B. suis 245 strain does not spread beyond the lymph nodes of the head and is completely eliminated 10 days after administration. In addition, this method allows you to eliminate all side effects (depression of the general condition, fever, lameness, tendovaginitis, arthritis, etc.). The oral method of using the vaccine from the B. suis 245 strain for the specific prevention of brucellosis in domesticated reindeer is theoretically and experimentally substantiated, given that mucous membranes are the main gateways of

infection in brucellosis. This method is the most promising, technologically advanced, is not difficult compared to the subcutaneous and, in particular, the conjunctival method and, moreover, is more preferable due to the absence of post-vaccination complications observed with other methods of immunization.

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