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Testing the Biological Properties of the Vaccine from Strain Brucella Abortus 75/79-Ab on Reindeer

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Abstract | The research was aimed at studying the biological properties of the vaccine from strain *Brucella abortus* strain 75/79-AB in reindeer. Here we describes a series of experiments on the reactogenic properties of a weakly agglutinogenic vaccine from strain B. abortus 75/79-AB. The spread rate and colonization rate of a culture from strain B. abortus 75/79-AB in the animal organisms were studied based on the dosage of the preparation. Analysis of the results of the experiment for studying the immunological reactivity of animals after the administration of various dosages of the vaccine from strain B. abortus 75/79-AV shows that there is some dependence of the level of specific antibodies in the blood serum of reindeer on the dosages of the vaccine used. The results of the research show that the experimental animals inoculated with this strain were able to resist experimental infection with the reference culture B. abortus 1330. The dynamics of antibody titers after the administration of the vaccine from strain B. abortus 75/79-AV are the evidence of the fact that this strain is actually weakly agglutinogenic, and diagnostic titers fall out after 70 days in the blood serum of the vaccinated reindeer.

Keywords | Brucellosis, Vaccine, Strain, Reindeer, Antibodies, Immunogenicity, Immune reactivity

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INTRODUCTION

Reindeer brucellosis in the Far North of the Russian Federation is widely spread, and is a significant limiting factor for further development of reindeer breeding; it keeps posing serious social danger. Holding anti-brucellosis events requires continuous improvement in order to increase their efficiency and reliability. With that, along with the general preventive measures, a priority area in this system is creating high specific protection against brucellosis in the animals.

However, to date, live vaccines have not been widely used for preventing and fighting reindeer brucellosis. The main disadvantages of the live vaccines proposed before (strain 19 B. abortus, B. suis 61) are that they have very high reactogenicity, and often cause complications, such as abscesses and edema at the location of injection, lameness caused by lesions of the joints on rear and front limbs (bursitis, tendovaginitis, etc.). Of all known vaccines, only the vaccine from strain B. abortus 82 is widely used in the specific prevention of brucellosis in reindeer.

In the Republic of Sakha (Yakutia), its use is rather efficient; however, some series of this vaccine have increased pregnancy-terminating ability. Therefore, there is a need appeared to produce the vaccine from the weakly agglutinogenic strain B. abortus 75/79-AV (Fyodorov, 2006; Ignatov, 2010; Albertyan et al., 2013; Godfroid, 2017; Kanouté et al., 2017; Fedorov et al., 2018). This

study address this need and proposes the application of such vaccine to safeguard animals from this deadly disease.

MATERIALS AND METHODS

The research was aimed at studying the biological properties of the vaccine from strain B. abortus 75/79-AB in the organism of reindeer. The work was performed in the reindeer herd of the Integrated Agricultural Production Centre (IAPC) Tompo in the Tompo district, Sakha Republic (Yakutia) of the Russian Federation. In experiment I, the authors studied the reactogenic properties of the vaccine from strain B. abortus 75/79-AB on 40 animals of all age groups. Two weeks after the immunization, the overall and local reactions to the vaccine were determined by measuring the body temperature, monitoring the general condition of the organism, and determining the size and consistency of the inflammatory edema in the area of vaccine injection. In experiment II, the time of colonization, migration, and elimination of this strain in the organisms of animals were tested. In experiment III, 27 reindeer were used, which were divided into three groups, and subcutaneously immunized at the dosage of 25, 50, and 100 billion m.c. The vaccine was subcutaneously injected into the upper third of the neck, according to the standard practice. In the immunized reindeer, the authors studied the dynamics of serological reactions by Rose Bengal test (RBT), agglutination test (AT) and complement-fixation test (CFT) on days 7, 15, 30, 45, 70, 90, and 120 after the vaccination, and the allergic reaction to RIEV brucellin (of the Russian Research Institute of Experimental Veterinary n.a. Y.R. Kovalenko) on day 30 after the immunization.

RBT is a system for the rapid diagnosis of animal brucellosis, containing a brucella antigen – a suspension of inactivated brucella of the species B. abortus in a lactic acid buffer solution of pH 3.65 with 0.5% of phenol, colored with Bengal pink. When contacting a positive serum of an animal tested for brucellosis, flakes are formed (Vinokurov et al., 2017).

AT is one of the main methods for the diagnosis of animal brucellosis in acute and subacute cases. Diagnosticum is diluted with saline according to instruction. 0.5 ml is added to all tubes except the control. After the addition of diagnosticum, the dilution of the serum is doubled respectively, i.e. 1:50, 1:100, etc. Serum with antigen is mixed by shaking and placed in a thermostat at 37°C for 18-20 hours. After incubation, the tubes are kept for 1-2 hours at room temperature. The reaction is described. The description is carried out based on the turbidity standard, depending on the degree of precipitation of the agglutinate and the clarification of the liquid (Vinokurov et al., 2017). CFT for chronically ill with brucellosis animals is related to the fact that antigens and antibodies, when correlating, form an immune complex. A complement (C) is attached to this complex via an Fc-fragment of antibodies. This results in binding of the complement to the antigen-antibody complex. If the antigen-antibody complex is not formed, then the complement remains free. CFT is carried out in two phases: the 1st phase–incubation of a mixture containing antigen + antibody + complement; the 2nd phase (indicator) – detection of a free complement in the mixture by adding to it a hemolytic system, consisting of sheep erythrocytes and hemolytic serum containing antibodies to them. In the 1st phase of the reaction, the formation of an antigen-antibody complex results in the binding of the complement to it. In the 2nd phase, hemolysis of the erythrocytes, sensibilized by antibodies, does not occur (positive reaction). If the antigen and the antibody do not match each other (there is no antigen or antibody in the sample), the complement remains free and in the 2nd phase, joins the erythrocyte-anti-erythrocyte antibody complex, causing hemolysis (negative reaction) (Vinokurov et al., 2017).

RESULTS AND DISCUSSION

The state of reindeer organisms' local reaction before the experiment, and after subcutaneous injection is depicted in Table 1. Inflammatory edema was started appearing after 24 minutes in the area of injection; a slight swell was observed with dense consistency and being warm to the touch. After that, an edema formed, which reached the maximum volume on the seventh day after the immunization, compared to other observation periods (Albertyan et al., 2013).

Table 1: Indicators of the local reaction of the reindeer vaccinated subcutaneously to various dosages of the vaccine from strain B. abortus 75/79-AB.

Time after vaccination (days)	Dosage of vaccination (billion m.c.)		
	25	50	100
	Inflammatory edema size, mm (M ± m)		
Before vaccination	0	0	0
1	8.1 ± 1.1	12.1 ± 2.1	17.5 ± 1.5
2	11.0 ± 1.2	14.8 ± 1.8	19.0 ± 2.3
3	15.4 ± 1.6	16.1 ± 1.4	23.5 ± 1.1
4	18.4 ± 1.6	19.5 ± 1.3	26.0 ± 1.4
7	22.1 ± 1.7	25.0 ± 1.5	31.1 ± 1.9
14	20.1 ± 1.3	23.0 ± 1.1	28.8 ± 3.0

On day seven, the edema reached the maximum size; in the groups, it reached: 22.1 ± 1.7 mm, 25.0 ± 1.5 mm, 31.1 ± 1.9 mm, respectively. With that, no significant difference was noted in the size of edema in the reindeer vaccinatedwith the dosages of 25 and 50 billion m.c.

Table 2: The results of the experiment for studying the rate of colonization and elimination of the vaccine from strain B. abortus 75/79-AB from reindeer organisms.

No. of groups	Vaccination method	Dosage in billion m.c.	Time of slaughtering after the vaccination (days)	Number of slaughtered animals	Infected	
					Total animals	Including generalized
1	subcutaneously	25	15	3	3	1
	subcutaneously	25	75	3	0	0
	subcutaneously	25	90	3	0	0
2	subcutaneously	50	15	3	3	2
	subcutaneously	50	75	3	0	
	subcutaneously	50	90	3	0	0
	subcutaneously	100	15	3	3	
	subcutaneously	100	75	3	2	
3	subcutaneously	100	90	3	0	1 (from the spleen)

(the size of edema was 22.1 ± 1.7 mm and 25.0 ± 1.5 mm, respectively, $P < 0.05$). With the dosage of 100 billion m.c., it reached 31.1 ± 1.9 mm.

On day 14, the size of the edema with the dosage of 25 billion m.c. reached 20.1 ± 1.3 mm; with the dosage of 50 billion m.c.–23.0 ± 1.1 mm; and with the dosage of 100 billion m.c.–28.8 ± 3.0 mm. Neither deterioration in the state of reindeer, nor loss of appetite were observed.

In this way, the results of studying the reactogenic qualities of the vaccine from strain B. abortus 75/79-AV confirm that the characteristics of the physical condition of the body in case of subcutaneous immunization depend on the dosage (reactogenicity is less manifested with the dosages of 25 and 50 billion m.c. than with the dosage of 100 billion m.c. injected) Fyodorov (2006).

The periods of strain B. abortus 75/79-AB colonization, migration, and elimination from the organism of the animals showed that with subcutaneous injection in the dosages of 25, 50 and 100 billion m.c., the organism recovered on day 15.

It should be noted that in the experiment, the cultures of vaccinal strain were isolated from all animals inoculated subcutaneously at various dosages. In most cases, the vaccinal process proceeded in a generalized manner. Brucellae of the vaccine strain after reindeer slaughtering in all groups at later periods after vaccination (75 and 90 days) were almost completely eliminated from the organisms of these animals.

It was only from one of three animals immunized with the dosage of 100 billion m.c. that a single culture was obtained from a slice of the liver on day 75 (Table 2).

In studying the reindeer blood serum immunological reactivity in RBT after the subcutaneous vaccination at the dosages of 25, 50, and 100 billion m.c., positive reactions were noted in all reindeer as early as on day seven, which persisted for up to 45 days (Table 3).

Table 3: Dynamics of the level of agglutinating antibodies in blood serum.

Time after the vaccination (days)	The percentage of positively reactive/average antibodies' titer		
	Method and dosage of vaccination (billion m.c.)		
	25	50	100
Before immunization	0/0	0/0	0/0
7	0/14.1	0/10.4	36.2/27.2
15	66.6/54.5	75/72.9	100.0/6.0
30	0/12.1	25.4/14.6	66/58.3
45	0/12.1	25/14.6	66/58.3
70	0/10.1	0/12.1	18.1/15.9
90	0/4.1	0/8.3	18.1/15.9
120	0/4.1	0/8.3	9.1/13.6

The number of reacting reindeer gradually decreased, and by day 70 after the vaccination, it reached zero. Upon comparison of the data for this group of animals to the data obtained in groups of other reindeer, veracious ($P < 0.05$) was the difference between antibodies' levels.

A decreased (below the diagnostic) level of agglutinating antibodies was observed in the blood of animals inoculated subcutaneously at the dosage of 100 billion m.c. after 70 days, and disappearance of agglutinins from the reindeer inoculated with the dosages of 25 and 50 billion m.c. started 30 days after the immunization.

It should be noted that complement-fixing antibodies (IgG) were detected in the CFT on the seventh day after the injection, the maximum level was reached by day 30,

and the lowest levels were reached by day 90 after the immunization (Vinokurov et al., 2017) (Table 4).

Table 4: Dynamics of the level of complement-fixing antibodies

Time after the vaccination (days)	The percentage of positively reactive/average antibodies' titer		
	Method and dosage of vaccination (billion m.c.)		
	25	50	100
Before immunization	0/0	0/0	0/0
7	8.3/0.4	45.4/3.2	80.02/6.0
15	8.3/0.4	46.6/4.8	80.0/6.0
30	33.3/2.1	45.4/4.8	100.0/16.0
45	50.0/2.9	36.3/3.1	100.0/8.0
70	16.6.08	18.1/2.9	40.0/3.0
90	8.3/0.4	0/0	40.0/3.0
120	0/0	0/0	20.0/2.0

However, antibodies' titer in the reindeer vaccinated with the dosage of 100 billion m.c. was much higher than that in the reindeer vaccinated with the dosages of 25 and 50 billion m.c. ($P > 0.05$).

Therefore, the results of studying the immunological reactivity of the vaccine from strain B. abortus 75/79-AB injected to animals have shown that there is some dependence of the level of specific antibodies in the serum of the studied reindeer on the dosages of the vaccine from strain B. abortus 75/79-AV.

With that, the dynamics of antibody titers after the administration of the vaccine from strain B. abortus 75/79-AV are an evidence of the fact that this strain is actually weakly agglutinogenic, and the diagnostic titers in the blood serum of vaccinated reindeer fall out mainly after 70 days.

Detecting reactions of delayed-type hypersensitivity (DTH) in reindeer in vivo is based on the use of allergic testing by a specific allergen on day 70 after the vaccination.

Hence, titers of complement-fixing antibodies and agglutinins in the blood serum of animals vaccinated subcutaneously at the dosage of 100 billion m.c. were significantly higher ($P<0.05$), and remained for a longer period than in the animals vaccinated in the dosages of 25 and 50 billion m.c. Based on the allergic study performed with the use of RIEV brucellin, it is clear that the percentage of positively reacting reindeer on day 70 after the vaccination was equal to 22.2 – 25.0 % and, generally speaking, did not depend on the dosage of initial strain introduced.

One of the conditions in the process of experimental work involving determination of the duration and intensity of immunity created in reindeer by a specific vaccine preparation is the determination of the minimal infecting dosage of the virulent strain that causes infection in 100 % of intact reindeer in similar physiological condition and age.

The experiments performed by the authors earlier have shown that in reindeer, the dosage of 2.5 million m.c. causes a generalized form of infection, and this dosage generates a minimal infecting dosage of reference strain B. suis 1330.

Five months after the vaccination, for checking the immunity level, nine experimental reindeer (three reindeer in each group) and three intact (reference) reindeer were infected with the dosage of 25 billion m.c. using the conjunctival method.

Thirty (30) days after the infection, the animals were slaughtered, and bacteriological examination of the reindeer was performed. For the bacteriological research, 15 – 18 objects were taken from each reindeer, and inoculations were made onto special nutrient media Ignatov (2010).

CONCLUSION

Based on the results of checking the immunity level of the animals, it was apparent that in groups of the animals vaccinated subcutaneously with the vaccine from strain B. abortus 75/79-AB in various dosages, Brucella cultures were not identified (100 % immune).

Thus, the experimental animals vaccinated with this strain withstood the experimental infection with the reference culture of B. abortus 1330 in the amount of 25 million m.c. five months after the immunization with the vaccine in the dosages of 25, 50 and 100 billion m.c.

Based on the results of studying the immunological reactivity of animals after injecting the vaccine from strain B. abortus 75/79-AB, one can draw a conclusion that there is some dependence between the level of specific antibodies in the blood serum of the studied reindeer and the dosages of the vaccine from strain B. abortus 75/79-AB. With that, the dynamics of antibodies' titers after the injection of the vaccine from strain B. abortus 75/79-AB show that this strain is actually weakly agglutinogenic, and after 70 days in the blood serum of vaccinated reindeer, diagnostic titers fall out, which fact indicates the efficiency of the vaccine against brucellosis in reindeer in the Far North of the Russian Federation.

AUTHORS CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IAPC: Integrated Agricultural Production Centre
RBT: Rose Bengal test
CFT: Complement-fixation test
RIEV: The Russian Research Institute of Experimental Veterinary n.a. Y. R. Kovalenko

REFERENCES

- Albertyan MP, Iskandarov MI and Fedorov AI (2013). Immunobiological properties of the weakly agglutinogenic vaccine against brucellosis in farm animals. Vet. Sci. Feed. 4: 20–23.
- Fedorov VI, Sleptsov ES, Vinokurov NV, Grigoriev II, Zakharova OI and Maksimova AN (2018). On adapting domestic even reindeer to the mountain-taiga zone of the North-East of Russia. J. Pharm. Sci. Res. 10(7): 1660-1662.
- Fedorov VI, Stepanov AI, Sleptsov ES, Vinokurov NV, Bochkarev II and Maksimova AN (2018). Northern domestic reindeer husbandry of the republic of Sakha (Yakutia): Retrospective analysis and development trends. J. Pharm. Sci. Res. 10(10): 2559-2563.
- Fyodorov AI (2006). The value of humoral and cellular immunity during brucellosis. Physiol. Pathol. Immune Syst. 10(8): 3–7.
- Godfroid J (2017). Brucellosis in livestock and wildlife: zoonotic diseases without pandemic potential in need of innovative one health approaches. Arch. Public Health. 75(1): 34. https://doi.org/10.1186/s13690-017-0207-7
- Ignatov PE (2010). Dialogues about the insidious brucellosis. Moscow. 103.
- Kanouté YB, Gragnon BG, Schindler C, Bonfoh B and Schelling E (2017). Epidemiology of brucellosis, Q fever and Rift Valley fever at the human and livestock interface in northern Côte d'Ivoire. Acta Trop. 165: 66–75. https://doi.org/10.1016/j.actatropica.2016.02.012
- Vinokurov NV, Sleptsov ES, Layshev KA, Fedorov VI, Iskandarov MI and Fedorov AI (2017). Diagnosis of reindeer brucellosis in the Far North of the Russian federation. Novosibirsk: ANS Sibak.